1

OF/DE

3

21221

National Library
of CanadaBibliothèque nationale
du CanadaCANADIAN THESES
ON MICROFICHETHÈSES CANADIENNES
SUR MICROFICHE

NAME OF AUTHOR/NOM DE L'AUTEUR Gregory Seldon Bezanson

TITLE OF THESIS/TITRE DE LA THÈSE "dna843, a chromosomal mutation affecting F
prime plasmid determined properties: further
characterization of its action on superinfection immu
in Escherichia coli."

UNIVERSITY/UNIVERSITÉ Carleton University

DEGREE FOR WHICH THESIS WAS PRESENTED/
GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE Ph.D

YEAR THIS DEGREE CONFERRED/ANNÉE D'OBTENTION DE CE DEGRÉ 1974

NAME OF SUPERVISOR/NOM DU DIRECTEUR DE THÈSE Dr. V. N. Iyer

Permission is hereby granted to the NATIONAL LIBRARY OF
CANADA to microfilm this thesis and to lend or sell copies
of the film.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈ-
QUE NATIONALE DU CANADA de microfilmer cette thèse et
de prêter ou de vendre des exemplaires du film.

The author reserves other publication rights, and neither the
thesis nor extensive extracts from it may be printed or other-
wise reproduced without the author's written permission.

L'auteur se réserve les autres droits de publication; ni la
thèse ni de longs extraits de celle-ci ne doivent être imprimés
ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED/DATE 25/07/74 SIGNED/SIGNÉ Gregory S. Bezanson

PERMANENT ADDRESS/RÉSIDENCE FIXE P.O. Box 118
Berwick Kings Co
Nova Scotia

dnaB43, A CHROMOSOMAL MUTATION AFFECTING F PRIME
PLASMID DETERMINED PROPERTIES: FURTHER CHARACTERIZA-
TION OF ITS ACTION ON SUPERINFECTION IMMUNITY IN
Escherichia coli.

BY

Gregory Seldon Bezanson, M.Sc.

A Thesis submitted to the Faculty of
Graduate Studies in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy.

Department of Biology

Carleton University

Ottawa, Ontario

July 8, 1974

The undersigned recommend to the Faculty
of Graduate Studies acceptance of the thesis,
"dnaB43, a chromosomal mutation affecting F
prime plasmid determined properties: further
characterization of its action on superinfection
immunity in Escherichia coli".

Submitted by Gregory Seldon Bezanson, M.Sc. in
partial fulfilment of the requirements for the
degree of Doctor of Philosophy.

Thesis Supervisor

Chairman, Department of Biology

External Examiner

CARLETON UNIVERSITY

1

TABLE OF CONTENTS

	PAGE
Contents	i
Abstract	iv
List of Abbreviations	vi
Acknowledgements	vii
Introduction	1
Literature Survey	5
Materials and Methods	32
(a) Bacterial Strains	32
(b) Bacteriophage	35
(c) Media	36
(d) Reagents for isolation of F plasmid DNA	38
(e) Growth and Mating Conditions	39
(f) Measurement of Effective Mating Pairs	42
(g) Characterization of Presumptive Double Males	44
(i) Sucrose density gradient centrifugation	44
(ii) Assortment of plasmid associated marker genes	45
(h) Growth in the Presence of Salt	47
(i) Formation of Bacteriophage P1 Lysogens	50
(j) Nomenclature	53

Experimental and Results

PAGE

54

(a)	Superinfection Immunity in Rec ⁺ and Rec ⁻ F prime derivatives of CR34	54
(b)	Properties of F ⁻ Strains Bearing the Mutation <u>dnaB43</u> .	57
	(i) Cell growth: generation times	57
	(ii) Recipient Ability	59
	(iii) Formation of mating pairs	61
(c)	Properties of F Prime Strains Bearing <u>dnaB43</u>	63
	(i) Plasmid stability	65
	(ii) Phage sensitivity	69
	(iii) Donor ability	73
(d)	Correction for the influence of <u>dnaB43</u> on F ⁻ Recipient Ability.	76
(e)	Specificity of <u>dnaB43</u> Action.	79
(f)	Lifting of Superinfection Immunity and Temperature Sensitivity: Both Effects of <u>dnaB43</u> ?	86
	(i) Phenotypic suppression of <u>dnaB43</u>	87
	(ii) Construction of PlCM <u>bac</u> lysogens	94
	(iii) Construction of <u>dnaB</u> perodiploids	97
	(iv) Removal of <u>dnaB43</u> to a different genetic background.	103
(g)	Division Rates and Recipient Efficiencies of F Prime Cells: Influence of <u>dnaB43</u>	106
(h)	Influence of Altered Conjugation Conditions on <u>dnaB43</u> Action.	110

	PAGE
(i) Evidence Indicating that the DnaB ⁻ Effect Is Not Limited to <u>dnaB43</u> .	114
(j) Characterization of Presumptive Double Male Strains.	118
(i) Cell growth: generation times	120
(ii) Velocity sedimentation of cell lysates of presumptive double males	121
(iii) Assortment of F plasmid associated markers	125
(iv) Altered incompatibility or a numbers effect?	130
(v) F plasmid assortment from purified clones	133
(vi) Time rate of F prime plasmid loss from double males.	135
(vii) Summary.	140
DISCUSSION	141
(a) General Characterization of <u>dnaB43</u> action	142
(i) Influence of <u>dnaB43</u> on entry exclusion and establishment	144
(ii) Influence of <u>dnaB43</u> on intracellular incompatibility.	149
(iii) The altered superinfection immunity phenotype is directly attributable to <u>dnaB43</u> .	158
(iv) Evidence that the effect is gene specific rather than allele specific	163
(b) A Possible Mechanism of <u>dnaB43</u> Action	165
(c) Other Effects of <u>dnaB43</u>	186
(d) Comparison of <u>dnaB43</u> to other Incompatibility Mutations.	193
REFERENCES	198
GLOSSARY	220

ABSTRACT

Escherichia coli cells bearing the chromosomal mutation dnaB43 are unable to continue DNA synthesis upon an increase in incubation temperature to 43°C. The presence of this lesion also effects several changes in cell properties at the permissive temperature of 30°.

DnaB⁻ cells devoid of extrachromosomal plasmid elements (F⁻) are more efficient conjugational recipients and form 4 to 6 times as many effective mating-pairs as do isogenic DnaB⁺ controls. Strains carrying one fertility plasmid (F) are usually poor recipients for the conjugal introduction of a second homologous F factor. Inclusion of dnaB43 however, results in a significant decrease in such superinfection immunity. Relative to the comparable non-mutant organism, reductions in this barrier of 3 to 9 fold are observed depending upon the particular F plasmid being examined. Both the entry exclusion and intracellular incompatibility components of superinfection immunity are affected. Mutant cells carrying F' plasmids are also 60 to 90% less sensitive to infection by the male-specific bacteriophages R17 and M13 than are isogenic DnaB⁺ strains. In contrast, the former display an enhanced ability to serve as hosts for the propagation of the female-specific phage ØII (4 to 10 fold increase over DnaB⁺).

The altered superinfection immunity phenotype is directly attributable to the dnaB43 lesion. Bacteriophage P1 transduction

V

indicates it is cotransducible with dnaB43. Mutant cells carrying the dnaB⁺ product or its virus-determined analog display wild-type levels of immunity. This action of dnaB43 is independent of the presence of recA-coded functions and is neither salt corrected nor plasmid specific.

Double male strains containing the two F' plasmids F42 and F101 are more stable in the presence of dnaB43. Velocity sedimentation analysis of lysates of such double males in alkaline sucrose indicates the two F primes exist as autonomous units independent of both each other and the chromosome. Marker genes associated with each plasmid demonstrate different patterns of assortment in mutant and wild-type backgrounds during growth in medium non-selective for plasmid retention. This behavior is used to develop models for explaining dnaB43 action.

Evidence is presented which suggests the DnaB⁻ effect is not peculiar to the dnaB43 allele but also occurs in organisms bearing separate mutations in this gene.

LIST OF ABBREVIATIONS

CM	CHLORAMPHENICOL
DNA	DEOXYRIBONUCLEIC ACID
EexI	ENTRY EXCLUSION INDEX
EstI	ESTABLISHMENT INDEX
F'	F PRIME PLASMID
F-DNA	F PRIME PLASMID DNA
h	HOUR
log.	LOGARITHMIC
μ CI	MICRO-CURIE
μ g	MICROGRAM
ml	MILLILITRE
min	MINUTES
RNA	RIBONUCLEIC ACID
S	SEDIMENTATION COEFFICIENT
TR	TEMPERATURE RESISTANT
TS	TEMPERATURE SENSITIVE
UV	ULTRAVIOLET
<u>M</u>	MOLAR
ATP	ADENOSINE TRIPHOSPHATE
dATP	DEOXYADENOSINE TRIPHOSPHATE
CFU	COLONY FORMING UNITS
PFU	PLAQUE FORMING UNITS

ACKNOWLEDGEMENTS

These investigations were conducted under the thoughtful supervision of Dr. V.N. Iyer. His suggestions, criticism and aid in designing the experiments and preparation of this manuscript are most gratefully acknowledged.

Special thanks are due to the other members of "C.I.M.B." for their constant interest and more than occasional assistance. For the support of graduate fellowships from the National Research Council and Carleton University, the author is indebted.

To my wife, for her unceasing patience and encouragement, a simple but sincere thank you.

I N T R O D U C T I O N

In addition to their chromosomes, many Enterobacteriaceae carry smaller DNA - containing elements known as plasmids. These extrachromosomal factors are self - replicating and usually exist as autonomous units from the larger genetic entity. Their presence is not essential for cell survival. The plasmids that have been studied most extensively are those capable of promoting their own inter - cellular transfer. Included among this group are the antibiotic resistance (R), colicinogenic (Col), and fertility (F) factors.

The F plasmids are of main concern in the present study. Although characterized by its autonomous existence (Hayes, 1953; Lederberg, 1958), a fertility plasmid may upon occasion integrate with the chromosome. Because resultant strains demonstrate an increased capacity for inter - cellular transmission of chromosome marker genes, they are referred to as Hfr, for high frequency of recombinants, cells (Jacob and Wollman, 1958). However, this union can be unstable. The F plasmid is able to return to its autonomous state, and, in so doing either of two types of plasmid will be formed (Sharp et al, 1972). If during excision, only F DNA is liberated, a plasmid similar to the original results. But, if a portion of F is exchanged for chromosomal material, a substituted plasmid is generated. The latter are termed F prime (F') factors. Since integration is

dependent on chromosomally determined recombination functions, the formation of Hfr cells is drastically inhibited in the presence of mutations in the recA locus (Bastarrachea and Clark, 1968).

The process whereby inter - cellular transfer of plasmid and chromosome associated genes is accomplished, is called conjugation. Cells lacking F plasmid DNA are referred to as recipients or females and are designated as F^- ; those positive for such material are known as donors or males. Matings between Hfr and F^- cells result in high conjugal transfer of chromosomal markers with little exchange of F DNA (Cavalli et al, 1953). In contrast, crosses involving F^+ or F' donors display a low level of chromosome mobilization, but efficient plasmid transmission (Jacob and Wollman, 1956). DNA transfer under these conditions requires the presence of a specific, F - determined, filamentous, surface appendage termed the sex or F pilus (Curtiss, 1969; Brinton, 1971).

Cells bearing integrated or autonomous F - DNA are usually poor recipients for the conjugal introduction of another F plasmid. This resistance to the entrance of a second homologous factor manifest by plasmid containing cells is known as superinfection immunity (Watanabe, 1963). It is usually described as being subsumed by two distinct, but not always separable, phenomena (Novick, 1969; Meynell and Datta, 1969). Entry or surface exclusion functions to inhibit uptake into the cell of a second

3

superinfecting plasmid. Having gained admission, the incoming factor must next contend with intracellular incompatibility which acts to prevent stable co-habitation of the same cell by the two like plasmids. Such superinfection immunity is also observed between R (Hashimoto and Hirota, 1966) and Col (Kahn and Helinski, 1964) factors. In general, plasmids belonging to the same incompatibility group show entry exclusion towards each other but not towards those from other groups (Watanabe and Fukasawa, 1962; Meynell, 1969).

Entry exclusion may be diminished to minimal levels (F^- phenocopy) by extended cell growth (Lederberg et al, 1952), starvation for essential amino acids (Curtiss et al, 1969), inclusion of a second, compatible, plasmid (Willetts and Finnegan, 1970), and, in certain instances, by genetic modification of the resident plasmid (Achtman et al, 1971). Intracellular incompatibility unfortunately, is not dealt with as easily. So called double male strains constructed under the preceding conditions still continue to display an asymmetric distribution of F^- DNA to daughter cells (Dubnau and Maas, 1968; Achtman et al, 1972). Both Willetts (1972) and Achtman (1973) have suggested that incompatibility (which precludes complementation studies) is a major contributing factor to the present scarcity of information regarding the genetics of plasmid replication and function. Hence, any alteration in cell behavior that serves to lift superinfection immunity

barriers would be of value in this investigation.

A number of authors have reported the isolation of genetically determined variants in this property. In particular, Palchaudury and Iyer (1971) have observed the presence of a mutation rendering replication of the Escherichia coli K12 chromosome sensitive to elevated temperatures (dnaB43) to also effect a decrease in superinfection immunity barriers at the permissive temperature. Mutant cells were altered in both entry exclusion and intracellular incompatibility functions.

The following represents an extension of these studies and an attempt at discerning the basis of dnaB43 action.

L I T E R A T U R E S U R V E Y

The genetics, molecular structure and replication of bacterial plasmids have been the subjects of a number of recent reviews (Willetts, 1972; Achtman, 1973; Clowes, 1972; Helinski, 1973).

In addition, two rather exhaustive articles have dealt with conditional lethal chromosome mutations (eg. dna⁻) and prokaryote DNA replication in general (Gross, 1972, Smith, 1973).

Because of their existence, the work to be considered here is only that pertaining directly to transmissible plasmid - determined superinfection immunity or that allowing for a better interpretation of the data to follow.

In Escherichia coli, superinfection immunity occurs between F^+ and F' , F' and F' , F' and Hfr, and, Hfr and Hfr cells (Scaife and Gross, 1962; De Haan and Stouthamer, 1963; Dubnau and Maas, 1968; Bastarrachea and Clark, 1968). No combination of F DNA's is exempt from its action.

As indicated earlier, entry exclusion operates to inhibit the uptake of superinfecting plasmids. The presence on the recipient of plasmid determined sex pili is not an essential condition for exclusion. Cells depiliated by high speed blending followed by suspension in saline were still able to resist the entrance of F DNA. (Curtiss et al, 1969). Further,

mutant F primes defective in pilus production were still poor recipients in matings with donors of wild-type F plasmids (Achtman et al, 1971).

Echols (1963), as a result of studies on the induction of plasmid linked enzymes in F prime double males, concluded that exclusion was due to the failure to acquire plasmid genes rather than the failure to be inherited. Falkow et al (1971) provided indirect evidence in support of this view using a system that permits specific radioisotopic labelling of recently transferred plasmid DNA (see Freifelder and Freifelder, 1968). They observed that entry exclusion as it exists between the derepressed R plasmid (high transfer frequency), R₁, and an unsubstituted F plasmid results in a decrease in the uptake of ³H-thymine from the environment. Subsequent studies have indicated that reductions in incorporation as great as 80-90% occur in matings between R₁d_{rd} donors and F⁺ or RTF (resistance transfer factor) recipients relative to non-excluding controls (LeBlanc and Falkow, 1973).

A more direct approach has been to monitor the uptake and fate of tritiated superinfecting DNA in matings involving F prime or R containing minicell recipients (Sheehy et al, 1972).

7

Although the latter carry extremely little DNA of chromosome origin and are incapable of division (Adler et al, 1967), they continue to exert entry exclusion (Cohen et al, 1968). Because of their small size, such recipients can be conveniently separated from donors following mating and the products of conjugation examined in the absence of further cell growth.

Sheehy et al noted that very little Hfr or R DNA was transferred to these minicells. Sucrose density gradient analysis of radioactive DNA present in the recipients indicated superinfecting material is not degraded and that exclusion does not result in transfer of small fragments of DNA from donor organisms. For these reasons, the authors concluded that entry exclusion operates to prevent physical entry of DNA into cells expressing this property. The moot question is how far do minicells go in portraying the behavior of their chromosome containing, septating progenitors.

One manner in which this inhibition might be accomplished would be to reduce the number of mating pairs formed upon mixing of plasmid-bearing cells. Achtman et al (1971) have observed a correlation between exclusion and mating pair formation. F prime plasmids defective in entry exclusion formed large numbers of mating pairs, whereas, those harboring wild-type plasmid entered into significantly fewer of these liaisons.

Such, then, would be essentially a numbers effect rather than a direct inhibitory action. Because fewer cells form mating pairs, fewer express a potential for DNA transfer (see Vapnek and Rupp, 1971). The overall result, a decrease in the number of cells bearing markers formerly associated with both donor and recipient, would be the same as that expected had mating pairs been formed but transfer prevented.

All these studies have centered on recipient behavior. Other investigations suggest the donor may have a role to play in determining entry exclusion as well. For instance, Meynell and Ewins (1973) have reported that alterations in the structure of donor sex pili result in a significant lifting of exclusion barriers.

In E coli, the type of sex pilus appended to the cell surface is dependent upon the type of plasmid present in its cytoplasm. Type -F pili are determined by the classical fertility factor F (or F', Hfr). F-like pili are found on cells carrying R factors of the fi^+ type. This structure is similar but not identical to the F pilus. Finally, I-like pili are produced by transmissible plasmids like ColI or certain fi^- R factors. (The latter are not to be confused with Type-I or common pili which are coded for by the chromosome and are therefore born by F^+ and F^- cells alike). The three types of sex pilus are distinguishable on the basis of their serological (Lawn and Meynell, and sedimentation (Beard et al, 1972) properties and to a lesser extent by their phage specificities (Salzman, 1971).

Both Rfi^- and Rfi^+ factors are compatible with autonomous or integrated F plasmids. In the former situation, both F and I-like pili are formed as physically discrete structures. In the latter, a mixed type of pilus carrying subunit material from both F and F-like type pili predominates (Lawn et al, 1971).

Meynell and Ewins (1973) found that substitution of an Hfr/R^+ donor bearing the mixed-type sex pili for an isogenic strain bearing discrete F and I-like appendages in matings with an F^+ recipient produced a 50 to 150 fold increase in the number of progeny containing chromosomal donor markers. Since progeny frequencies were restored to control levels in recipients bearing mixed pili, it was suggested the operation of exclusion requires the presence of identical sex pili. Hence, if this barrier can be avoided simply by altering the structure of the sex pili used in conjugation, the latter apparently has a direct role in governing entry exclusion. Such is not inconsistent with the strict requirement for sex pili for DNA transmission in E^+/F^- matings (Brinton, 1971).

Recently, Goldfarb et al (1973) described the partial purification of a proteinaceous material occurring in filtrates of Hfr C cultures which when added to mixtures of Hfr and F^- cells increased the yield of conjugal recombinants by 10 to 12 fold. This substance, dubbed RSF for recombination stimulating factor, is determined by the integrated F plasmid. It apparently acts at the surface

of the recipient cell requiring the presence of phospholipids for its operation.

Since it is donor-determined, it would be interesting to assess the influence of RSF on progeny frequency during a cross with an excluding (F^+) cell.

Intracellular incompatibility apparently acts to prevent the stable inheritance of superinfecting plasmids. There is an unequal apportionment of factors to daughter cells usually with bias towards retention of the resident plasmid.

deHaan and Stouthamer (1968) were successful in introducing the F prime plasmid F42 (F'_{lac^+}) into a strain already carrying the factor F8 (F'_{gal^+}). The majority of Lac^+ progeny were also Gal^+ but all strains were unstable quickly segregating out both $Lac^+ Gal^-$ and $Lac^- Gal^+$ cells. Especially, $Lac^- Gal^+$ cells were found in relatively high numbers. The authors implied that such assortment into two different cell lines was necessary for the continued presence in the culture of each F prime.

Similarly, Scaife and Gross (1962) constructed F42/ F^+ double male strains. These too were very unstable. Unlike progeny from F^- recipients, subculturing produced a large proportion of variegated Lac^+ colonies indicating that such cultures were not pure for F42. They concluded, therefore, that in the presence of the second plasmid, F42 was prevented from multiplying and was thus severely restricted in its infectivity.

An analogous effect was observed in F42/Hfr double males by Dubnau and Maas (1968). Donors of the plasmid F42 were mated with stationary phase Hfr recipients to yield a high frequency of Lac⁺ progeny (15-30% transfer). However, their numbers steadily decreased upon growth in enriched medium giving rise to 60 to 80% Lac⁻ cells within three generations. To assess the integrity of the lac⁺ gene in such double males, newly mated recipients were suspended in chloramphenicol containing (20µg/ml) medium for 4 hours. The concentration of antibiotic was sufficient to inhibit growth but had no effect on cell viability. Since the proportion of Lac⁺ organisms in the culture remained constant throughout the entire incubation period, the authors were led to conclude that inhibition of F42 replication rather than DNA degradation was responsible for its dilution from double male strains. Their ability to induce the synthesis of β-galactosidase under these conditions argues against chloramphenicol mediated inhibition of nuclease production and/or activation and confirms the intactness of the lac⁺ gene.

A direct measurement of incompatibility determined replication inhibition was provided by Falkow et al. (1971). Analysis of the sedimentation properties of recently transferred plasmid DNA in R1/F⁺ (or RTF) crosses revealed the accumulation of forms not normally seen in control matings (RX F⁻) conducted under the same conditions.

The sedimentation values were such that it was suggested these new species represented an accumulation of replicative intermediates or aberrant replication forms. Subsequent studies (LeBlanc and Falkow, 1973) verified this conjecture and have indicated that replication of the incoming R plasmid does occur but at a greatly reduced rate. Conversion to the closed-circular, 75S, end-product was reduced some 11 fold relative to the compatible condition.

Novick and Brodsky (1972) used ^{32}P labelled penicillinase plasmids to arrive at a similar conclusion in staphylococcal strains. Temporary inhibition of plasmid replication occurred in both compatible and incompatible recipients, but, the delay was substantially longer in the latter instance. In plasmid minus cells, replication began within a single generation of its introduction as compared to upwards to four generations in plasmid positive recipients. This delay may be all that is necessary for the resident plasmid to establish its favored position within the cell.

To date no such direct studies have been completed for superinfecting F plasmids.

Rather than observing the expected asymmetric assortment of plasmid DNA, some authors have reported the formation of stable double male strains. Thus Scaife and Gross (1962) and Dubnau and Maas (1968) were able to obtain long-lived Lac^+ offspring from F_42/Hfr crosses. While Clark (1963) has described a viable double male resulting from the mating of two Hfr strains. And Press et al (1971) and Willetts and Bastarrachea (1972) have isolated plasmid positive strains stably carrying markers characteristic of each factor as a result of conjugation between two F' prime bearing cells. Thus there must be some mechanism of escape from incompatibility operating in these cells.

During the characterization of presumptive double male strains resulting from a $F57 (F^{his^+}/Hfr$ cross Bastarrachea and Clark (1968) noticed that only 1 out of 80 of these cells was able to transfer the his^+ marker to a recombination defective recipient. Since stable inheritance of chromosomal genes in such instances is dependent upon their integration with the recipient genome, and, whereas acquisition of those associated with autonomous, self-replicating, F plasmids is not so restricted, the authors concluded that $F57$ had escaped incompatibility by union with the chromosome to form a Hfr derivative. This was confirmed by Maas and Goldschmidt (1969) who demonstrated a greater than 10^{-4} fold reduction in the frequency of F_42/Hfr double male formation in the presence of the mutation recA1.

This would explain the high incidence of double males reported by Scaife and Gross (1962) Dubnau and Maas (1968); Clark, (1963) and others who have used recombination proficient cells as hosts. But, because those employed by Press et al (1971) and Willetts and Bastarrachea (1972) bore the recA⁻ lesion, an alternative method of escape must exist.

Presumptive double male strains from both these sources displayed high levels of cotransfer of all F prime markers in conjugation with appropriate RecA⁻ cells. Further, markers associated with each plasmid were simultaneously lost upon the spontaneous or acridine orange-induced generation of F⁻ segregants. Willetts and Bastarrachea investigated the sedimentation properties of DNA isolated from these cells and found that the few double males examined contained a single F plasmid of molecular weight greater than or intermediate to that of the individual parental F primes (F57, F101). Together, this information suggested that the F prime plasmids had circumvented incompatibility barriers by fusion to form a single plasmid.

The existence of such fused factors was subsequently verified by Palchauduri et al (1972). Utilizing the electron microscope and the cytochrome-C spreading technique, they were able to visualize the F plasmids present in three ex-conjugants from a F'trp⁺/F'arg⁺ cross. Measurements of their contour lengths indicated considerable variation in the size of these fused plasmids. They were, however always shorter than the sum

of the parental F Primes. Thus, different fused factors are formed from the same parents and always (within limits of the number examined) with a loss of significant amounts of plasmid material. An obvious candidate for the DNA lost is the region (s) determining F plasmid incompatibility.

The mechanics of this union are as yet unknown. Since the host cells were recombination defective, it must be mediated by a recA⁻ independent system. Or, alternatively, it must depend on a F plasmid determined function. If the former, its operation would constitute a type of illegitimate recombination as postulated by Franklin (1971). Although the analogy is limited, it is interesting to note that other "infectious" particles, for example, phages λ and Mu, carry their own integration systems (See E. Signer, 1968; Bukhari and Zipser, 1972). Because fused F plasmids occur in very low numbers ($<10^{-4}\%$) such events are rare.

In summary, then, entry exclusion appears to act by preventing the physical entry of superinfecting DNA. This, in part, may be manifest in a reduction in mating pair formation. Both donor and recipient seem to have a role in determining its operation. Intracellular incompatibility functions mainly to inhibit the replication of the incoming plasmid. As a secondary effect it might also determine the unequal apportionment of plasmid DNA to daughter cells. Since replication

inhibition would produce effectively the same result no clear distinction can be drawn between these two processes (if indeed one exists). Incompatibility may be avoided by integration into the chromosome or fusion to form a single plasmid.

Willetts (1974) has very recently reported the mapping of the plasmid coded entry exclusion and intracellular incompatibility determinants traS and inc. A series of Hfr strains carrying deletions extending varying distances into the integrated F factor were used to place traS between two other transfer loci traG and traD and inc between ϕII^R and the origin of transfer on the F plasmid map (See Willetts, 1972; Achtman, 1973).

The exclusion determinant forms part of a large operon believed to be under the general control of the traJ gene. The location of inc means it is one of the earliest markers transferred during conjugation.

Two major schemes have been devised to explain the operating mechanics of intracellular incompatibility. Both are derived from the model of replication control proposed by Jacob and Brenner (1963). It suggests that each genetic entity be considered as an

autonomous, circular structure (the replicon) carrying at least two loci (the initiator and the replicator) whose interaction serves to control its own duplication. To account for their regulation and stable inheritance, it was postulated that each replicon was attached to its own specific site on the cell membrane.

In the maintenance - site model (Jacob et al, 1963) incompatibility is explained on the basis that two homologous plasmids both require the same membrane attachment site at the same time for their replication. Therefore, because of the ensuing competition for the single membrane position only one plasmid is duplicated and passed on to both daughter cells. The other, less competitive, plasmid will always be transmitted to but one progeny cell and hence be diluted out of the population.

Considerable evidence has accumulated in support of this hypothesis. Among these is the observation that nonreplicating F prime plasmids cosegregate with a specific strand of chromosomal DNA (Cuzin and Jacob, 1967 a; Hohn and Korn, 1969). Another is the demonstration of associations between conjugally transferred F^+ DNA and minicell membranes (Shull et al, 1971) and replicating R plasmids and membrane containing cell particulates from both R^+ and R^- recipients (Hershfield et al, 1973).

If competition for a special site on the membrane is all that is involved in incompatibility, one is hard put to explain its persistence upon integration of one of the plasmids into the chromosome (Hfr) where it comes under the control of the larger replicon's replicating system. Another inconsistency is that although the colicinogenic factor ColV2 is incompatible with autonomous F prime plasmids and segregates F⁻ cells, it can stabilize in an Hfr strain (MacFarren and Clowes, 1967). Moreover its replication and the transfer properties of both it and the chromosome appear to be unchanged (Clowes, 1972).

The second explanation of incompatibility is the so-called repressor-molecule model of Pritchard et al (1969). Their interpretation of the replicon model implies the existence of some mechanism for specifying when each new round of replication should be initiated. To allow for this, a repressor of initiation is postulated which is active at one concentration but inactive at a two fold lower concentration and therefore permits a new cycle of synthesis to begin. A burst of inhibitor transcription occurs immediately after the start of replication thus producing enough material to prevent further initiations until its concentration is halved by subsequent cell division. If two F plasmids were present, twice as much repressor-molecule would be made and two doublings in cell mass would be required for its dilution to levels allowing new rounds of replication. It is presumed that during this time the F plasmids would segregate into different cell lines.

This model would account for the apparent inhibition of autonomous F multiplication by Hfr cells. It also implies that incompatible plasmids would be assorted in a random fashion with no bias existing towards either the incoming or resident factor. This behavior would, in turn, indicate a lack of specificity in repressor-molecule action. One would, therefore, expect the replication of both plasmids to be restricted in double male strains. Exceptions to both predictions have been recorded (Echols H, 1963; Frydman and Meynell, 1969; Novick and Brodsky, 1972).

Implicit in the maintenance - site model is the assumption that the attachment, maintenance and segregation sites all occupy the same position on the cell membrane. However, mention must be made of two recent reports which suggest that with certain plasmids actual events are not quite as simple as this system would have them be.

Novick and Brodsky (1972) have concerned themselves with penicillinase plasmid establishment and incompatibility in cultures of Staphylococcus aureus. Bacteriophage ϕ 11 was used to introduce ^{32}P -labelled plasmids into exponential phase plasmid containing and plasmid negative cells. By following the kinetics of their establishment, they were able to detect a significant temporal separation between the onset of plasmid replication

and the achievement of hereditary stabilization; the latter being manifest by the lack of asymmetric segregation of plasmids in favor of the preexisting one. In plasmid negative transductional recipients replication began within one generation after entry but stabilization did not occur until 2 to 3 generations later. In the case of plasmid positive cells delays of up to 4 generations were observed prior to the initiation of plasmid replication. It did not become hereditarily stable for a correspondingly longer time.

As with the F plasmid in E coli, the number of copies of penicillinase plasmid per Staphylococcus cell is severely limited (1-2 copies/chromosome). Because of this, the authors have interpreted these results as indicating that penicillinase plasmid establishment involves two distinct steps: replication, followed, after a delay, by stabilization. The replicon model is based on establishment being a one step event. The former requires the presence of two membrane attachment sites; one for replication (ie: maintenance) and another for segregation. As mentioned, the latter uses only one site for replication, maintenance and segregation.

Accordingly, Novick and Brodsky have divided intracellular incompatibility into two components. Upon entry of the superinfecting plasmid, there is a temporary inhibition of its replication and a much longer inhibition of its hereditary stabilization. Only when the latter is achieved may the incoming plasmid compete on an equal footing with the resident factor. The authors themselves point out that, although unlikely, they cannot completely rule out the possibility of these effects being merely a result of the transient integration of two plasmids. Further, it should be noted that the superinfecting plasmids examined by them were introduced via transduction as double-stranded, covalently-closed, circular particles. Most interpretations regarding the maintenance-site model have arisen from the behavior of plasmids introduced by conjugation.

LeBlanc and Falkow (1973) have provided information that may be interpreted as support for the Novick-Brodsky observations. They followed the replication of a superinfecting R plasmid in RTF-containing, ultraviolet-irradiated E coli K12 recipients. Since in their system neither donor nor recipient was capable of chromosomal DNA synthesis, the uptake and incorporation of ³H-thymine was used as a means of monitoring R duplication. Its progress was determined on neutral sucrose gradients.

Replication of Rldrd19 occurred at a much reduced rate in RTF recipients. Whereas in the R^- cell, a single cycle was estimated to take but 2.5 to 3.0 minutes, introduction of RTF lengthened this to 28 to 33 minutes. As a result, the 75S end-product did not occur in detectable amounts in the incompatible host until 60 to 90 minutes after mating. Similar replicative intermediates were observed in both R^- and R^+ recipients.

In a subsequent report, these authors (see Hershfield et al, 1973) confirmed the involvement of the cell membrane in this replication. Rldrd19 DNA sedimented directly onto a 60% sucrose shelf ("rapidly sedimenting cell component") for varying lengths of time during a 90 minute mating. From 20 to 90 minutes, the amount of DNA associated with this fraction decreased by over 50% in compatible recipient but in R^+ cells, the change was only 13%. They conclude this prolonged attachment to membranous material to constitute the action of RTF determined incompatibility on R1 replication.

The reduction in the rate of incoming R plasmid synthesis is formally analogous to the temporary inhibition of penicillinase plasmid replication noted by Novick and Brodsky. Because incompatible recipients were irradiated immediately prior to being mixed with donor cells, the possibility of plasmid

integration is greatly reduced (See Cole, 1971).

Further, since maximal plasmid replication times obtained in RFT recipients were but half the generation time of the host cell (65 minutes) it seems unlikely this effect alone can account entirely for R plasmid incompatibility. As in other systems, an unequal partition of plasmids to daughter cells is generally seen (Meynell and Datta, 1969; LeBlanc and Falkow, 1973). This might require a second attachment site (or, a second kind of attachment).

To sum, the data collected by these two groups of authors from two different systems tend to indicate that establishment of an incoming plasmid is not a unitary event as implied by the replicon model in its simplest form.

Several mutants of E coli are known in which the capacity to synthesize chromosomal DNA has become thermosensitive (Bonhoeffer and Schaller, 1965 ; Kohiyama et al, 1966; Fangman and Novick, 1968; Hirota et al, 1968; Carl , 1970; Wechsler and Gross, 1971). An increase in incubation temperature, usually to 39-43°, results in an inhibition of further DNA replication. In those cells where RNA and protein synthesis continue at near normal levels such cessation may be immediate (Bonhoeffer , 1966; Carl , 1970, Wechsler and Gross, 1971), or occur only gradually (Kohiyama et al, 1960; Fangman and Novick, 1968; Hirota et al, 1968).

The residual DNA synthesis observed at nonpermissive temperatures suggests that the round of replication already in progress at the time of the shift runs to completion but that new cycles can no longer be initiated (Kohiyama et al, 1966). Consequently, this type of lesion has been termed an initiation mutation. Accordingly, those belonging to the immediate-stop type are described as elongation mutations (Hirota et al, 1968).

To date, seven mutant classes, dna A through G, have been mapped in E coli K12 (Hirota et al, 1968; Carl, 1970; Wechsler and Gross, 1971). Two additional lesions dnaH and dnaI have been reported in E coli C (Komano and Sakai, 1972) and E coli B/r (Beyersman et al, 1974) respectively. Most prevalent among the elongation types are those located in the malB region (81 minutes) of the standard chromosome map (Wechsler and Gross, 1971; Beyersman et al, 1974). Cells bearing mutations from this subclass, in particular dnaB43, dnaB107 and dnaB279, are the chief object of the present study. (The allelic assignments are in keeping with those of Wechsler and Gross, 1971).

Temperature sensitive or, more accurately, DnaB⁻ mutants display a heterogenous mixture of properties. Ricard and Hirota (1969) reported that those bearing dnaB43, 70 and 313 were salt or sugar correctable, while strains carrying dnaB59 and 266 were not.

Gross (1972) likewise found cells bearing dnaB107 and 279 to be sugar remedial. (He also indicated that four of the five mutations designated non-suppressible by Ricard and Hirota could be corrected with high glucose concentrations). The lesions dnaB43 107 and 266 are recessive to the wild-type allele but dnaB313 is dominant (Siccardi et al, 1971; Wechsler and Gross, 1971). Analogous behavior was obtained with certain DnaA⁻ strains. In E coli K12 all dnaA⁻ mutations tested were recessive in F' dnaA⁺ merogenotes (Wechsler and Gross, 1971) whereas those isolated in E coli B/r were dominant (Beyersman et al, 1974). The latter authors suggestion that the dnaA⁺ product is composed of several subunits which upon mixing with mutant subunits gives rise to an inactive product is equally applicable in the dnaB situation.

Cells harboring dnaB6 , 22, 43 and 70 have been shown to support T4, T6 or P1 development at both permissive and non-permissive temperatures, but, phage lambda only at low temperatures. (Carl, 1970; Fangman and Novick, 1968; Hirota et al, 1968; Lanka and Schuster, 1970). Those bearing dnaB22, and, probably some other DnaB⁻ mutants, contain a specific sub-group (groP) that is unable to propagate phage λ even at 30° (Georgopoulos and Herskowitz, 1971). Still other mutants (dnaB279, 1029) are unable to facilitate phage ϕ X174 or M13 replication at high temperatures (Schekman et al, 1972; Olsen et al, 1972). This latter behavior is believed to be a property of other DnaB⁻ mutants as well.

The duplication of other extrachromosomal elements is similarly affected by dnaB⁻ mutations. Cells bearing dnaB43 and dnaB266 permit the synthesis of plasmid ColE1 DNA at both permissive and nonpermissive temperatures (Goebel, 1970, 1971). However, the replication of the colicinogenic and hemolytic factors ColV and Hly is severely restricted at elevated temperatures (Goebel and Schrempf, 1972). As with phage particles, certain plasmids are incapable of multiplication in the absence of dnaB⁺ products. It is perhaps significant that those plasmids insensitive to heat are of small molecular weight (4.2×10^6), are found in large amounts in the cell (20-25 copies/chromosome), and, are non-infectious. In contrast, those dependent on dnaB determined functions are of large molecular weights ($60-140 \times 10^6$), are found in small amounts (1-2 copies/chromosome) and are transmissible. Replication of large and small plasmids may involve different mechanisms. ColE1 synthesis is also dependent on DNA polymerase I activity (dnaE) and dnaA gene initiation products but ColV and Hly are not (Kingsbury and Helinski, 1970; Goebel, 1973).

As part of their replicon hypothesis Jacob and Brenner (1963) proposed that the transfer of the Hfr chromosome to the F⁻ recipient during conjugation is a consequence of DNA replication that is triggered by mating. Strains bearing dnaB⁻ mutations have been used to particular advantage in studies aimed at testing this supposition. Bonhoeffer (1966) employed an Hfr strain carrying the lesion dnaB43 to monitor the effect of high temperature on conjugal donor efficiency. He found that, although chromosome replication had ceased, its transfer to F⁻ cell was normal. If the same mutation was present in the recipient, recombinant formation was markedly reduced at the non-permissive temperature.

Subsequent experiments with the same mutation in different genetic backgrounds indicated that, although vegetative DNA replication was blocked, synthesis of new material did occur during mating at 43° (Bresler et al, 1968). This led these and other authors to distinguish between normal vegetative and transfer replication (Curtiss et al, 1970; Marinus and Adelberg, 1970). Similar behavior was observed in conjugations involving autonomous F plasmids (Vapnek and Rupp, 1971). Moreover, cesium chloride poly (U.G.) gradients indicated that the DNA synthesis occurring at non-permissive temperatures was asymmetric. The plasmid strand synthesized in the donor had the same polarity as the strand transferred to the recipient.

Most recently, Sarathy and Siddiqi (1973) utilizing the Bonhoeffer donor (thy⁻dnaB43) have determined that, although F mediated synthesis does normally take place at elevated temperatures, it can be effectively blocked by thymine deprivation without affecting chromosome transfer. Thus, the requirement for replication to drive DNA transfer remains an open question.

The nature of the thermolabile step(s) in DnaB⁻ mutants is still largely unknown. All mutants so far examined display normal levels of DNA polymerase I (Bonhoeffer, 1966; Kohiyama et al, 1966; Hirota et al, 1968; Carl, 1970; Wechsler and Gross, 1971) and polymerases II and III (Geffert et al, 1971) activity at the non-permissive temperature. Ligase activity remains unaltered (Gellert et al, 1968), and deoxynucleoside triphosphate precursors are synthesized in normal amounts (Bonhoeffer, 1966; Fangman and Novick, 1968; Carl, 1970; Wechsler and Gross, 1971). Further, preliminary screening of the E series of dnaB⁻ mutations produced by Hechery and Goldfine (1971) failed to reveal any defects in ribonucleotide reductase activity (Wechsler and Gross, 1971). Strains bearing dnaB43 were also able to repair ultraviolet - irradiation damage at elevated temperatures (Couch and Hanawalt, 1967).

By taking advantage of the in vitro dependence of ØX174 replication on dnaB gene activity, Wickner et al (1974) have been able to isolate the wild-type dnaB gene product. This material has a molecular weight of about 250,000. Highly purified preparations of it contained a ribonucleoside triphosphatase activity (ATPase) that was stimulated 10 fold by DNA. Further, gel electrophoresis showed a single band of protein coincident with the ØX174 complementing and nucleoside triphosphatase activities. Extensive characterization studies have indicated the dnaB⁺ product to be associated with two distinct triphosphatase activities; one DNA dependent, another independent.

Attempts at the isolation and purification of mutant dnaB⁻ products from thermosensitive cells were less successful (3-5% recovery of that for dnaB⁺). However, material obtained from a strain bearing dnaB1029 sedimented on glycerol gradients with a molecular weight of 125,000. This protein was extremely heat labile but its enzymic activity has not been characterized.

In their speculations on the role played by the dnaB⁺ gene product in DNA replication, these authors cite as yet unpublished data indicating it may be concerned in the initiation of RNA or DNA primer strands. Replacement of ØX174 single stranded DNA by ØX174 DNA-RNA hybrids abolished the requirement for the dnaB⁺ product for phage replication.

In addition, Hurwitz and Wickner (1974, in press) have found ATP or dATP is needed for the elongation of DNA and RNA primers catalyzed by DNA polymerase III. This requirement for ATP may somehow be coupled with the ATPase activities of the dnaB product. It is interesting to note that Hayes and Szybalski (1973) have demonstrated a similar need for the presence of dnaB⁺ for the initiation of phage λ primer RNA synthesis.

If DNA replication were to proceed in a discontinuous manner as some workers have suggested (Okazaki et al, 1968; Gilbert and Dressler, 1968), an ever constant synthesis of RNA primer material would be necessary (Sugino et al, 1972; Sugino and Okazaki, 1973). Failure to provide these could conceivably result in an immediate stop in chromosome replication. If the dnaB⁺ gene product were indeed involved in primer formation, mutants thermosensitive for this activity would also display an immediate cessation in DNA duplication at non-permissive temperatures. In an earlier study, Moses and Richardson (1970) noted that the presence of dnaB266 abolished ATP - stimulated DNA synthesis in toluene - treated cells at high temperatures.

Since transfer replication occurs in DnaB⁻ strains during conjugation at non-permissive temperatures, the dnaB⁺ gene is not involved in transfer synthesis (Bresler et al, 1968; Vapnek and Rupp, 1971). On the other hand, the finding

that at elevated temperatures Hfr DNA passed to DnaB⁻ recipients remains single stranded (Vapnek and Rupp, 1971) implies dnaB⁺ is required to make a complement along the transferred strand. For these reasons, certain authors have suggested that the dnaB⁺ product is involved in some function for the operation of which the two chromosome strands must temporarily behave in a non-identical manner (Gross, 1972, Siddiqi and Fox, 1973).

M A T E R I A L S & M E T H O D S

(a) Bacterial Strains

The origins and relevant properties of the Escherichia coli K12 strains employed in the present study are summarized in Tables 1 and 2. Organisms obtained outside this laboratory and utilized without modification retain the designation used by their source. Variants derived within Carleton University are labelled with a C123 number.

Strains CR34(B) and CR34G, although both Thy⁻ derivatives of C600 (Appleyard, 1954), differ somewhat in their sedimentation properties (see Results sec. (1)) and hence have been considered as distinct organisms. Strain KL110/F103 was received from B. Bachmann (1972).

CR34 cells defective in recombination (Rec⁻) were constructed by interrupted mating with KL16-99 an Hfr strain transferring thy+ and recA1 as early markers (Low, 1968). Selection was for streptomycin resistant, thymine independent progeny followed by secondary screening for ultraviolet - hypersensitivity. To test the latter, approximately 10^3 cells from exponential broth cultures were spread on nutrient agar and exposed to 20 ergs per second of short - range U.V. irradiation for 6 seconds. The U.V. source was a 115 volt, 0.2 amp "Mineralight" lamp from Ultraviolet Products Inc., California (Model U.V.S. - 11). Under these conditions recombination proficient (Rec⁺) strains

showed nearly 100% survival. Ex-conjugants displaying less than 20% survival were considered to harbor recA1.

Temperature sensitivity (TS) was confirmed by spreading cells on enriched agar and incubating overnight at 30 and 42°. The growth of cells bearing dnaB⁻ was severely restricted at the higher temperature. Most displayed an intermediate sensitivity to 37°.

The locations on the standard E coli chromosome map of genes utilized as markers in this study are indicated in Figure 1.

TABLE 1: PROPERTIES AND ORIGIN OF F⁻STRAINS USED

STRAIN	GENOTYPE 1.	ALTERNATE DESIGNATION	ORIGIN	REFERENCE
1) CR34	<u>thr</u> ¹ <u>leu</u> ⁶ <u>thi</u> ¹ <u>lac</u> ^{Y2} <u>thy</u> ^{A6}	CR34B	F. Ryan	Bonhoeffer & Schaller, 1965
2) CR34TS ⁺	as (1) but <u>dnaB</u> ⁴³ <u>str</u>	CR34TS43	F. Bonhoeffer	Bonhoeffer, 1966
3) TG149	as (2) but <u>his</u> ⁻	CR34TS74	F. Bonhoeffer	Masker & Hanawalt, 1973
4) CR34G	as (1)	(parent of strains 5&6)	H. Goldfine	Hechemy & Goldfine, 1971
5) E107	as (4) but <u>dnaB</u> ¹⁰⁷	CR34GTS107	H. Goldfine	Wechsler & Gross, 1971
6) E279	as (4) but <u>dnaB</u> ²⁷⁹	CR34GTS279	H. Goldfine	Wechsler & Gross, 1971
7) C09	as (1) but <u>thy</u> ⁺ <u>recA</u> ¹	CR34Rec ⁻ *	KL16-99X(1)	This Thesis
8) C050	as (7) but <u>dnaB</u> ⁴³	CR34TSRec ⁻	KL16-99X(2)	This Thesis
9) C099	<u>thr</u> ⁻ <u>leu</u> ⁻ <u>lac</u> ⁻ <u>pil</u> ⁻ <u>fla</u> ⁻ <u>Amp</u> ^{A1}	JE2571AmpA	V.N. Iyer	Iyer et al, 1974
10) C101	as (9) but <u>dnaB</u> ⁴³	JE2571AmpTS43	V.N. Iyer	Iyer et al, 1974
11) C027	<u>pro</u> ⁻ <u>his</u> ⁻ <u>thi</u> ⁻ <u>lac</u> ⁻ <u>recA</u> ¹ <u>spc</u> ^r	MA3050F ⁻	Spontaneous curing of MA3050	This Thesis
12) JC4121	<u>leu</u> ¹ <u>his</u> ¹ <u>arg</u> ⁶ <u>lac</u> ^{Y1} <u>recA</u> ¹ <u>str</u> ⁻ <u>spc</u> ⁻	-	N. Willetts	Willetts & Bastarrachea, 1972

1. Symbols as in Taylor and Trotter (1972).

+ : TS; growth sensitive to temperatures above 37°.

* Rec⁻ : hypersensitive to UV irradiation; X: conjugal cross

TABLE 2: PROPERTIES AND ORIGIN OF F PLASMID CARRYING STRAINS USED

STRAIN	GENOTYPE	ALTERNATE DESIGNATION	ORIGIN	REFERENCE
1) C05	<u>thr</u> 1 <u>leu</u> 6 <u>thi</u> 1 <u>lacY</u> 1 <u>thyA</u> 6 <u>str</u> r/ <u>lac</u> +	CR34/F42	(18) ⁺ X CR34	This Thesis
2) C07	as (1) but <u>thr</u> 1 <u>leu</u> +	CR34/F101	(16) ⁺⁺ X CR34	This Thesis
3) C034	as (1) but <u>dnaB</u> 43	CR34TS/F42 ⁺	V.N. Iyer	Palchaudury & Iyer, 1971
4) C047	as (2) but <u>dnaB</u> 43	CR34TS/F101	" "	" "
5) C010	as (1) but <u>thy</u> 1 <u>recA</u> 1	CR34Rec ⁻ /F42*	(18) X C09	This Thesis
6) C011	as (2) but <u>thy</u> 1 <u>recA</u> 1	CR34Rec ⁻ /F101	(16) X C09	" "
7) C059	as (5) but <u>dnaB</u> 43	CR34TSRec ⁻ /F42 ⁺	(18) X C050	" "
8) C061	as (6) but <u>dnaB</u> 43	CR34TSRec ⁻ /F101	(16) X C050	" "
9) C025	as (4) but <u>his</u> 1	TG149/F101	(16) X TG149	" "

1. Symbols as in Taylor and Trotter (1972). Genetic loci located on chromosome are indicated to left of / and those on F prime plasmid to its right.

+, *: See Table 1, ++: See Table 2 (cont'd) X: conjugal cross.

TABLE 2 (CONTINUED)

STRAIN	GENOTYPE	1.		
		ALTERNATE DESIGNATION	ORIGIN	REFERENCE
10) C014	as 4 ⁺⁺ but/thr+leu+	CR34G/F101	(16) X CR34G	This Thesis
11) C029	as (10) but dnaB107	E107/F101	(16) X E107	" "
12) C039	as (9) ⁺⁺ but/lac+	JE2571 AmpA/F42	(18) X C099	" "
13) C043	as (12) but/thr+leu+	JE2571 AmpA/F101	(16) X C099	" "
14) C044	as (12) but dnaB43	JE2571 AmpATS/F42 ⁺	(18) X C101	" "
15) C045	as (13) but dnaB43	JE2571 AmpATS/F101	(16) X C101	" "
16) C076	thr1 leu6 lacY1 recA13 str ^r /thr+leu+	AB2463/F101	V.M. Iyer	Palchaudury & Iyer, 1971
17) C077	as C027 but/his+	C027/F103	KL110/F103XC027	This Thesis
18) MA3050	as C027 but/lac+	C027/F42	W. Hayes	Devries & Maas, 1971
19) JC1553/ Fl34-1	leu1 his1 arg6 recA1 str ^r / thr+ leu+ dnaB+ met+	-	K.B. Low	Low, 1972
20) KL132/ Fl18	thr1 leu6 thyA25 pyrB31 recA1 str ^r malB ⁺ purA+ pyrB+	-	K.B. Low	Wechsler & Gross, 1971
21) CSH61	thi ⁻ str ^r HfrC	(O...pure lac+ leu+...F)	Cold Spring Harb. Collection	Miller, 1971

1: See Table 2 +: See Table 1 ++: See Table 2 X: Conjugal Cross

34

FIGURE 1: GENETIC MAP OF Escherichia coli K12 SHOWING THE LOCATION OF GENES USED AS MARKERS AND THE APPROXIMATE SIZE OF CHROMOSOMAL REGIONS CARRIED BY F PRIME PLASMIDS EMPLOYED IN THE PRESENT STUDY. Each F prime is depicted as an arc which has an arrowhead drawn to indicate the point of origin of its ancestral Hfr strain (inner circle). With the latter, the arrowhead shows the origin and direction of chromosome transfer during conjugation.

U

(b) Bacteriophage

Plasmid specific phages, R17, M13 and ØII, were maintained in phosphate - buffered saline (Paranchych and Graham, 1962). Transducing phage, P1 kc , P1CM, and P1CM bac, were stored in modified L Broth (sec. (d)) supplemented with 0.05 M calcium chloride and 0.01 M magnesium sulphate. The latter two phages were kindly provided by M. Yarmolinsky.

Measurement of phage titres was obtained using the standard agar overlay method (Adams, 1959) and modified L medium. A second assay procedure was developed to expediate the testing of the R17 and M13 sensitivities of large numbers of cells. Exponential test strains were pipetted onto sterile 12.7 mm Schleicher and Schull filter paper discs (0.1 ml per disc) and the latter placed well spaced on Eosine Methylene Blue agar for 2 minutes (12 per plate including controls). A loopful of phage (10^8 to 10^{10} pfu/ml) was then placed at the centre of the impression remaining on the medium surface upon removal of the discs. When dry, the plates were inverted and incubated overnight at 37° . The growth of cells bearing plasmid F101 was markedly inhibited at the region of virus/cell contact. Strains containing factor F42 displayed much less susceptibility to lysis but did give rise to a distinct change in colony color (lighter) in this area. Similar behavior occurred with both R17 and M13.

(c) Media

The medium utilized in agar overlay assays of phage R17, M13, and ØII was modified L - Broth supplemented with 0.05 M calcium chloride (Scott, 1968) and 0.1% (w/v) glucose and containing 0.8 and 1.5% Bacto - Agar (Difco) for top and bottom agars respectively. If P1 were in use, the glucose was replaced by 0.01 M magnesium sulphate. Efficient plaque formation was achieved only at 37° thus necessitating an increase in sodium chloride concentration to 0.25 M in experiments involving the use of strains bearing dnaB43.

Modified L Broth (Lennox, 1955) consisted of 10 gm. Bacto - Tryptone, 8 gm. sodium chloride and 1 gm. Yeast Extract (Difco) per liter of distilled water.

Unless otherwise indicated, all growth and mating was conducted in Difco Nutrient Broth (NB). In experiments with recombination proficient CR34 strains 10 µg per ml of thymine was added to this. NB plus 1.5% Bacto - Agar served as an enriched agar. Penassay Broth (Antibiotic Medium No. 3) was from Difco. Tris Maleate Medium (Paranchych, 1966) was used as liquid minimal medium while that of Davis and Mingioli (1950) was employed in plates (1.5% agar). Eosine Methylene Blue (EMB) agar minus carbon source was from Baltimore Biological Supplies.

Supplemental L amino - acids, thiamine hydrochloride, and thymine were added at final concentrations of 20, 10 and 2 μg per ml respectively. The antibiotics streptomycin (Calbiochem) and spectinomycin (Upjohn) were included at levels of 50 μg per ml each while only 12.5 μg per ml of chloramphenicol (Sigma Chemical) were used in P1 lysogeny studies. These were filter sterilized then added to autoclaved basal medium. Carbohydrates, glucose and lactose, were added to minimal medium and EMB agar at levels of 0.5 and 0.8% (w/v) respectively. When employed in the determination of phage sensitivity, EMB agar was further supplemented with 0.25 M sodium chloride and 0.05 M calcium chloride.

In a limited number of experiments, growth was carried out in a modified Curtiss synthetic medium (Curtiss et al, 1968). Cells destined to serve as conjugational recipients were incubated in a minimal salts solution supplemented with 0.5% Casamino Acids (Difco), 3% glycerol (v/v) and 100 μg per ml of gelatin (Rozen et al, 1971). Those to be employed as donors were grown in the same minimal salts solution but containing 1.5% Casamino Acids and 0.5% glucose (Sheehy et al, 1972). When necessary, thiamine and thymine were added at the previously indicated levels. The minimal salts solution consisted of 0.5% ammonium chloride, 0.1% ammonium nitrate, 0.9% sodium sulphate, 0.3% potassium phosphate (dibasic) and 0.01% magnesium sulphate ($7\text{H}_2\text{O}$). In this series of experiments all dilutions were carried out with buffered saline-gelatin solution (Curtiss et al, 1969).

(d) Reagents For Isolation Of F Plasmid DNA

Methyl - labelled ^3H - thymidine was purchased from Nuclear Dynamics Incorporated, El Monte, California.

The following reagents are essentially those of Freifelder (1968)

- 1) Deoxyadenosine (Calbiochem, Grade A) solution: 25 mg per ml.

- 2) Wash Buffer: KH_2PO_4 0.01 M
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.001 M
 CaCl_2 0.0001 M
 NaCl 0.1 M

pH 7.0

- 3) Lysis Buffer: NaCl 0.05 M
 EDTA (ethylenediaminetetraacetic acid) 0.02 M
Tris Base 0.02 M

- 4) Alkaline SDS: NaOH 0.8 M
 SDS (sodium dodecyl sulphate) 1% (w/v)

- 5) Alkaline Sucrose: EDTA 0.02 M
 NaOH 0.3 M
 NaCl 0.5 M

pH 12.0

Add sucrose to 5 or 20% (w/v)

(e) Growth And Mating Conditions

Unless given as otherwise, all experiments were completed at 30°C.

For the assay of phage sensitivity by the agar overlay method, test organisms were shaken for 6 hours in Tris Maleate Medium (1 ml per 15x125 mm culture tube) selective for retention of plasmid - associated marker genes. When assayed according to the filter disc method, cells were aerated in 1 ml NB for 4 hours.

For conjugation experiments, cells were grown in 10 ml of medium held in a 250 ml side - arm flask (Bellco Nephelo Culture Flask 516). Growth was monitored turbidimetrically using a Klett Summerson Colorimeter with a 500 - 570 nm filter. Exponential phase cultures were obtained by 4 - 5 hour incubations (minimal medium 6 hours) with moderate agitation (55 - 65 strokes per minute in a reciprocal shaking water bath) after a twenty - fold dilution from overnight cultures. Male derivatives of CR34 strains with optimal recipient ability (F⁻ phencopy) were achieved by growth in still culture for 16 hrs. (minimal medium, 18 hrs.). Although such cells have a greatly reduced rate of growth - generation times from 150 to 350 minutes, they are not truly in stationary phase (no growth) and will henceforth be referred to as overnight (ON) cultures.

After adjustment of cell numbers to approximately $1.0 - 3.2 \times 10^8$ per ml by addition of fresh medium, strains to be mated were mixed in a predetermined ratio in a 50 ml Erlenmeyer flask to give a final volume of 3 ml. The latter was then placed in a water bath and subjected to very mild agitation (35 - 40 strokes/min.) for 30 to 60 minutes. Mating pairs were subsequently separated by a ten - fold dilution in saline solution (0.85% w/v) followed by vortexing at approximately 1500 rpm for 45 seconds.

When matings were to be carried out on a membrane filter template according to the procedure of Sheehy et al (1972), the participants were grown to mid log phase ($2 - 5 \times 10^8$ cfu/ml) in modified Curtiss synthetic medium (Methods sec. (c)) then gently mixed in equal volumes (5 ml each) and collected on a 0.45μ pore filter (HA type 47 mm, Millipore Corp.) that had been prewet with 0.6 ml of fresh medium.

The membranes were then placed cell side up on prewarmed Penassay plates previously overlaid with 2.5 ml of top L agar and incubated at 30° for sixty minutes. At the end of this time the filters were removed from the plates put in 102 x 28 mm polyethylene centrifuge tubes containing 3 ml of chilled (4°) buffered saline - gelatin (BSG) solution and vortexed at top speed for 2 minutes. Cells recovered in this manner were subsequently diluted in chilled BSG.

With both mating procedures progeny were isolated by spreading appropriately diluted conjugation mixtures on solid media designed to permit the survival of only one member of any mating pair. The particular medium used was determined by the purpose of the experiment. Cells so selected for are henceforth referred to as the recipients. Those whose survival is militated against are accordingly designated as the donors. Such discrimination was achieved with auxotrophic and/or antibiotic sensitivity markers.

To construct F42, F101, F118 and F134-1 derivatives of CR34F⁻ strains (see Fig. 1), exponential phase cells were mated for 30 minutes at a donor to recipient ratio of 10:1. To measure entry exclusion, a F prime containing derivative of the strain being examined was mated in exponential phase with the appropriate F prime bearing donor - also in log. phase. Intracellular incompatibility functions were monitored by crossing exponential F prime donors with F plasmid containing recipients in the overnight ("stationary") phase of growth. In both instances the donor to recipient ratio was 10:1 and conjugation permitted to take place for 60 minutes. Preliminary tests revealed that little or no lethal zygosis took place in CR34 recipients under these conditions (Skurray and Reeves, 1973).

(f) Measurement Of Effective Mating Pairs

The formation of conjugational mating pairs was measured using the dilution method of deHaan and Gross (1962). Cells were mated in liquid culture according to previously described procedure in a donor to recipient ratio of 10:1, but at a ten - fold greater concentration of cells ($10^9:10^8$ cfu/ml). Equal volumes (1 ml) each of participants were mixed in a 50 ml Erlenmeyer flask and incubated with mild shaking for 8 minutes to allow formation of mating pairs. At this time, a 0.05 ml portion was removed from the mating mixture and diluted 500 fold into a 300 ml Erlenmeyer flask by gentle addition of fresh medium. Because of the change in cell densities, this step insures against any further mating pair formation. The resultant solution was returned to incubate another 27 minutes in order to facilitate completion of F plasmid transfer (total: 35 minutes). A 0.1 ml aliquot was withdrawn at this time, vortexed for 30 seconds, diluted - if necessary , and then spread on agar selective for markers associated with the incoming fertility factor.

Exponential and overnight phase recipients were prepared in NB as described in the Methods, section (e). The increase in donor numbers (to 10^9) was achieved by centrifugation at 5 for 20 minutes at 8,000 rpm, followed by resuspension in one-tenth volume (2.5 ml) of preutilized medium from the supernate.

To permit temperature equilibration and regeneration of sex pili possibly lost as a result of pellet formation (Branton, 1971), the suspension was reincubated an additional 20 - 30 minutes at growth temperature.

(g) Characterization Of Presumptive Double Males

The status of F prime plasmids in presumptive Lac+Thr+Lac+ double males obtained as a result of F42 x F101 crosses was determined in one of the following manners.

(i) Sucrose density gradient centrifugation of [^3H]- labelled DNA.

Lysis of radioisotopically labelled test cells and subsequent alkaline sucrose analysis of the lysates were carried out largely according to the procedure of Freifelder (1968) as modified by Palchaudury and Iyer (1969). Strains were grown without agitation for 14 - 16 hours (overnight) in 30 ml of Tris Maleate Medium selective for retention of plasmid associated markers. At this time, methyl - labelled [^3H]- thymidine and sterile deoxyadenosine were added to the culture to give final concentrations of $10\mu\text{Ci}$ ($0.09\mu\text{g}$) and $200\mu\text{g}$ per ml respectively. After moderate shaking at 30° for an additional 4 - 5h, the cells were harvested by centrifugation at 8,000 rpm for 20 min. The pellet was washed twice with one-third volume of wash buffer then gently resuspended in 1 ml of lysis buffer. Lysis was achieved by slow addition of 0.5 ml of alkaline SDS accompanied by gentle, continuous shaking at room temperature. The resultant viscous solution was then vortexed at about 1500 rpm for 30 seconds.

A portion of this lysate (0.12 ml) was subsequently layered onto 5 ml of a 5.0 to 20.0% linear alkaline - sucrose gradient and centrifuged at 40,000 rpm for 25 minutes at 23° in a Spinco SW65 rotor of the Beckman L2-65 ultracentrifuge. Nine drop fractions were collected from the bottom of the gradient on Whatman 3mm filter paper discs and oven dried. These were then incubated with three 15 minute changes of cold 5% trichloroacetic acid, followed by washing with ethanol (3x) and acetone (2x) in that order. Upon air drying, the discs were counted in a Beckman Model LS-230 liquid scintillation spectrometer. The scintillation fluid contained 4 grams of Omnifluor (New England Nuclear) dissolved in one litre of toluene.

(ii) Assortment Of Plasmid Associated Marker Genes

Presumptive double males were grown in medium nonselective for retention of F prime DNA. A determination of the frequency of plasmid associated markers in resultant populations afforded information as to the condition of conjugational plasmids in these strains.

Colonies suspected of being F prime double males were picked from the appropriate minimal agar and suspended in nutrient broth to a final concentration of approximately 10^6 cells per ml. These were then treated in either of two manners.

(a) Short incubation, rapid growth: tubes of suspended cells were shaken at 30° for 5 to 6 hours, then diluted and spread directly on minimal media selective for the growth of either Lac⁺, Thr⁺Leu⁺ or Lac⁺Thr⁺Leu⁺ colonies and left at 30° for 48 h. Each culture was also tested for its sensitivity to phage R17 by the filter disc assay described in section (b) of the methods. (b) Long incubation, slow growth: after incubation in still culture for 14 - 16 hours (overnight), a portion of each suspension was diluted, spread on nutrient agar and kept at 30° for 16 h. Colonies arising on these plates were then tested for the presence of markers initially associated with each F prime plasmid by simultaneous replication in 100-member lots to the appropriate minimal agar. Determination of the number of Thr⁺Leu⁺ colonies also fermenting lactose allowed the calculation of double male frequencies.

The prolonged growth period of method (b) assures full expression of incompatibility functions. It has a further advantage in that it discriminates against the selection of mixed clones of Thr⁺Leu⁺Lac⁻ and Thr⁻Leu⁻Lac⁺ cells which would give rise to sham double male colonies by crossfeeding.

(h) Growth In The Presence Of Salt

Several types of conditional lethal mutations which occur in E coli are osmotically and/or ionically remedial. Inclusion in the growth medium of particular concentrations of salt (eg NaCl, $MgCl_2$) or sugar (eg: sucrose) allows for cell survival under normally nonpermissive conditions. The particular lesion involved is said to be phenotypically suppressed. Among the defects remedied in this manner are those rendering chromosome replication sensitive to elevated temperatures; the dna⁻ mutations. The lesion dnaB43 is one such member of this group (Ricard and Hirota, 1969).

Early in the present studies, it was observed that incubation at 37° was an essential condition for infection and lysis of F prime cells by bacteriophage R17 and M13. To facilitate an examination of the phage sensitivities of F plasmid bearing DnaB⁻ strains, the amount of sodium chloride (NaCl) present in the top agar used in agar overlay assays was varied.

Cells carrying dnaB43 were incubated in standard glass culture tubes containing 2 ml of nutrient broth for 5 hours at 30°. Upon appropriate dilution, these were mixed in duplicate 0.1 ml portions with 2.5 ml of salt - modified top agar containing 0.1 ml of phage buffer. The mixture was then poured over a

standard bottom agar (0.80% NaCl) plate and when hardened these were inverted and placed at 30 and 37° overnight. Survival at the elevated temperature was determined from the resultant cell counts. Preliminary experiments indicated that a NaCl concentration of 0.25 M (1.5% w/v) gave optional growth (93 - 100% survival). Such top agar was employed in all subsequent assays.

To achieve phenotypic suppression of the dnaB⁻ mutation in liquid medium, mutant strains were shaken at 55 - 65 cycles per minute in 250 ml Bellco side - arm flasks containing 10 ml of NB supplemented with various concentrations of NaCl. Inoculae for these (1:20) were taken from NB cultures incubated overnight without aeration at 30°. Growth at 30, 37 and/or 42° was monitored turbidimetrically over a six hour incubation period (Klett-Summerson Spectrophotometer). Generation times were determined by withdrawing 0.1 ml aliquots from each culture at 1.5 and 4 hours and spreading subsequently diluted material on complete minimal medium.

The influence of NaCl on growth on solid media at restrictive temperatures was also determined. Cells incubated as previously described in NB plus salt for 4 hours at 42° were, after appropriate dilution, spread in duplicate on either complete minimal medium or EMB-lactose agar containing differing amounts

of salt. The plates were kept at 30 and 42° for 72 and 96 hours respectively. Calculation of the survival at the elevated temperature enabled a choice as to which concentration gave most effective suppression of dnaB⁴³. Sodium chloride displayed no remedial activity when included in Davis minimal agar. Indeed, on this medium, the presence of low levels of NaCl slightly inhibited growth of both wild-type and temperature sensitive cells even at permissive temperatures. Addition of 0.42 M salt (2.5%, w/v) to EMB-lactose agar, however, resulted in a $3 - 5 \times 10^3$ fold increase in survival at 42° (for strains C050 and C061 respectively). This medium was employed in all subsequent studies conducted at 42°.

In experiments designed to determine the effect of phenotypic suppression of dnaB⁴³ on superinfection immunity functions, cultures (both wild type and DnaB⁻) were incubated at 30 and 42° in nutrient broth containing 0.42 M NaCl. The procedure used for construction of double males was slightly modified from that previously described in Section (c). Overnight cultures were obtained by an 11 - 12 hour incubation in mildly agitated culture (35 strokes/min.) followed by a 2 - 3 hour period of still culture (total: 14h). Exponential recipients were achieved by a 1:10 subculture of this followed by 4 - 5 hours shaking at 55 - 65 cycles per minute. Conjugation was permitted to occur for 60 minutes.

(1) Formation Of Bacteriophage P1 Lysogens

Phage P1CM is a plaque-forming derivative of P1kc (Lennox, 1955) carrying a gene conferring resistance to chloramphenicol (CM) acquired through recombination with a CM^R plasmid (Kondo and Mitsuhashi, 1964). A plaque-forming mutant of this, P1CM bac, which is constitutive for the activation of the normally repressed virus determined dnaB analog protein (ban) has been isolated recently by D'Ari et al (1974, in press). Bacteriophage P1CM and P1CM bac lysogens of CR34 strains were constructed largely according to the procedure of Rosner (1972).

Exponential cultures of female and F prime cells being treated with P1 phage were adjusted to a density of $3 - 9 \times 10^7$ cfu per ml in modified L Broth supplemented with 0.01 M magnesium sulphate and 0.05 M calcium chloride. Then 0.1 ml of each was spread on Penassay agar containing 12.5 µg per ml of CM and left to stand for 10 minutes at room temperature. At this time a loopful of either P1CM or P1CM bac ranging in titre from $3.2 - 4.8 \times 10^9$ pfu per ml was cross-streaked over the surface of the Penassay plates. The latter were subsequently incubated at 30° for 96 hours at which time the number of chloramphenicol resistant colonies was determined.

When cells bearing dnaB43 were treated with P1 phage colonies of two distinct types arose. Upon testing the yellowish, rough (Rec⁺) and smooth, crusty (Rec⁻) colonies proved to belong

to the CR34 background.

Chloramphenicol resistant CR34 derivatives occurred with frequencies of from 10^{-4} to 10^{-5} percent of the number of cells originally placed on the plate. In agreement with Rosner (1972), fewer lysogens were formed in Rec^- than in Rec^+ cells. The number of CM resistant colonies formed in the former expressed as a percentage of the wild-type ranged from 6.8% for P1CM bac on strain C050 (F^-) to 50% for the same phage on C061 (F101). In general, more lysogens were formed in the absence of F plasmid DNA. Antibiotic resistant colonies were found in greater abundance in DnaB^- cells treated with P1CM bac than with wild-type phage. This is presumably related to the derepressed activation of dnaB⁺ analog in these particles.

All P1CM bac lysogens of C050 (7/7) and C061 (10/10), but only 50% of P1CM lysogens of the same strains (4/8 and 2/4 resp.) tested, grew at 37° in the presence of chloramphenicol. In addition, 71% of the C050/P1CM bac lysogens also survived at 42° . None of the untreated C050 and C061 colonies (CM^S) examined grew under such conditions.

Final confirmation that the newly acquired resistance to elevated temperature and/or chloramphenicol was attributable to the presence of lysogenic P1 phage was obtained by examining the supernatants from broth grown cultures of these strains. If P1 were maintained within the cell one might expect a low

level of spontaneous induction to lytic growth to occur. And, therefore, it should be possible to detect so released particles by testing the ability of cleared culture fluids to plaque K12 indicator cells in agar overlay assays. Such supernates did contain low concentrations of lytic phage (3 - 170 pfu/ml) displaying typical P1 plaque morphology. Greater release of phage occurred in overnight phase cultures. Supernates from cultures bearing P1CM gave rise to 10 to 20 fold more plaques than did P1CM bac lysogens.

(j) Nomenclature

The nomenclature used for phenotypes and genotypes follows the recommendations of Demerec et al (1966). Thus DnaB⁻ refers to the temperature sensitive phenotype displayed by cells carrying the mutation dnaB43. Lac⁺ colonies is an abbreviation for lactose fermenting colonies. Conjugational F prime plasmids are designated according to the numerical classification system described by Low (1972). Hence, the F plasmid bearing a lac⁺ mutation (F'lac⁺) is referred to as factor F42, while F'thr⁺leu⁺ becomes factor F101, and F'his⁺, factor F103. The use of this system has the advantage that it enables ready access to information regarding the plasmid's origin.

Presumptive double male cells are labelled in a manner indicating their derivation. Strains C09DML6 and C050DMS2 are F prime double males numbers 6 and 2 obtained from log. and overnight (stationary) phase C09 and C050 parents respectively.

EXPERIMENTAL and RESULTS

(a) Superinfection Immunity in Rec^+ and Rec^- F Prime Derivatives of CR34.

The presence in a F prime derivative of strain CR34 of the chromosomal mutation dnaB43 was reported to result in a lifting of the barriers to infection of such cells by a second F plasmid (Palchaudury and Iyer, 1971). Similar experiments were carried out in an effort to confirm this observation. Further, to determine whether this outcome was dependent upon recombination proficiency in the recipient, they were completed using both recombination proficient (Rec^+) and deficient (Rec^-) strains of CR34.

Matings were conducted as described in the Methods section.

Strain C076 served as a donor of factor F101 while F42 derivatives of various CR34 strains were employed as recipients. Progeny were selected on two types of minimal media: 1) containing thymine, thiamine and glucose (for markers on F101) and 2) as (1) but with lactose substituted as carbon source (for markers on both F101 and F42).

Results typical of such experiments appear in Table 3. The operation of superinfection immunity functions was assessed by determining the ratio of the frequency of $\text{Thr}^+ \text{Leu}^+$ progeny

obtained with overnight to that from exponential phase recipients (Palchaudury and Iyer, 1971). Frequencies are expressed as a percentage of the minority parent (recipient cells).

The presence of dnaB43 does indeed lead to an alteration in immunity functions. In the particular set of data given here, the DnaB⁻ recombination proficient strain, C034, displayed a 2.2 fold decrease in immunity against factor F101 relative to the non-mutant, wild-type, strain C05 (expts. 1 & 2 column V). Further, its action appears to persist in the recA1⁻ background. The dnaB43 bearing strain, C059, also showed an enhanced capacity for accepting a second F plasmid when compared to the C010 control (expt. 3 and 4 column V). It is to be noted that the switch from Rec⁺ to Rec⁻ recipients was accompanied by only a very slight decrease in the extent to which the dnaB43 mutation altered immunity functions. Indeed, in Table 3 the frequency with which Thr⁺ Leu⁺ colonies were formed was actually greater in recombination defective F prime cells than it was in isogenic Rec⁺ strains (cf. expt. 1 and 3, 2 and 4; column III and IV). With these strains and under these particular conditions, the presence of the recA⁻ lesion appears to limit the operation of superinfection immunity.

The frequencies of conjugal plasmid transfer resulting from these studies varied slightly with each experiment. However, in all cases examined the strain carrying the dnaB⁻ mutation

was the better recipient by at least six fold. Thus, for example, the frequencies ranged from 0.1332 to 0.2463% for exponential RecA^- , DnaB^- , C059 cells and from 0.0094 to 0.0402% for the isogenic DnaB^+ strain, C010, in the same phase. Such fluctuation is most likely due to spontaneous generation of F^- segregants which then act as normal (non-excluding) recipients. When F42 was the factor in residence, the percentage of female cells in the recipient population was consistently less than 15%.

TABLE 3: INFLUENCE OF dnaB43 ON THE ABILITY OF WILD-TYPE (C05, C010) AND DnaB⁻ (C034, Q059) CR34(P42) DERIVATIVES TO ACCEPT FACTOR F101 FROM STRAIN C076

EXPT. NO.	RECIPIENT	1. FREQUENCY OF DONOR MARKERS IN PROGENY EXPONENTIAL (LOG)	2. RATIO ON/LOG OVERNIGHT (ON)
1	C05 (Rec ⁺)	0.0054	0.0129 2.38
2	C034 (")	0.0235	0.0251 1.06
3	C010 (RecA ⁻)	0.0096	0.0332 3.45
4	C059 (")	0.1332	0.2100 1.58

1. Matings conducted as described in Methods Section. Donor/Recipient: 10:1,
NB at 30° for 60 minutes.

2. Number of Thr+Leu+ colonies per 100 recipient cells.

(b) Properties of F⁻ Strains Bearing the Mutation dnaB⁴³.

To establish a basis for intended studies of the relationship between the presence of dnaB⁴³ and the diminution of superinfection immunity in F prime plasmid carrying recipients, attempts were made to assess what effects, if any, the mutation might have on properties pertinent to this question in female (F⁻) strains. The rates of growth, recipient abilities, and capacities of wild-type and DnaB⁻ cells to form mating pairs were compared.

(i) Cell growth: generation times.

Exponential phase cells were obtained in the same manner as described for conjugational recipients in the Methods' section (e). Growth was monitored by determining changes in optical density. Generation times (T) were calculated from viable cell counts according to the formula $\log N_2 - \log N_1 / t = \log 2/T$; where N_1 is the population size at the first sample time and N_2 the total cell number determined t minutes later (Dawes, 1967). Aliquots for plating on complete minimal medium were removed between 2 and 5 hours.

Recombination defective strains generally grew less rapidly than did isogenic recombination proficient strains. Turbidimetric readings also indicated differences in the growth rates of wild and mutant - type cells. Generation times typical of these

strains are given in Table 4. The inference made from optical density measurements is supported. RecA^- cells divided at a slightly slower rate than did their Rec^+ counterparts, and the presence of dnaB43 (strains CR34TS and C050) effected a similar small change in generation times. However, the latter was very minimal (column IV), regardless of the presence or absence of the recA1 mutation.

**TABLE 4: DIVISION RATES OF VARIOUS
CR34 WILD-TYPE (CR34, C09)
AND DnaB⁻ (CR34TS, C050) F⁻
STRAINS DURING EXPONENTIAL
GROWTH IN NUTRIENT BROTH AT 30°**

STRAIN RECOMBINATION^{1.} GENERATION TIME (MINS)^{2.} EFFECT OF dnaB43^{3.}

CR34	+	33.9	1.08
CR34TS	+	36.8	
C09	-	35.7	1.08
C050	-	38.7	

1. +: Recombination proficient; -: defective
2. Average of at least 2 measurements
3. Ratio: DnaB⁻/wild-type

(11) Recipient ability.

The increased capacity for accepting a second F prime plasmid may be due to a generalized effect of dnaB43 on the strain's ability to function as a recipient in conjugation. If so, this might be reflected in the frequency with which F associated markers occur in progeny from F' x F⁻ type crosses. To assess the recipient ability of a number of CR34 derivatives, plasmids F42 and F101 were introduced by conjugation from strains MA3050 and C076 respectively.

Matings were conducted as described in the Methods section. Transfer of F42 was determined by spreading exconjugants on appropriately supplemented minimal agar containing lactose as carbon source; F101 transfer on selective plates having glucose substituted for lactose. All progeny tested were streptomycin resistant, histidine independent (except when TG149 was used) and threonine or lactose requiring.

The frequencies of colony formation in these crosses varied slightly with each experiment but remained within a range consistent for F plasmid transfer to such recipients at 30° (Table 5). The presence of dnaB43 did not adversely affect recipient ability in these strains. Indeed, in most instances, the frequencies were slightly enhanced relative to those of the non-mutant strain for both F101 and F42 associated markers. The increase in recipient ability ranged from 1.4 (F101) to 2.5

fold (F42) in Rec⁺ strains (expts. 1 & 2, 5 & 6). A slightly greater effect was noted in recombination defective DnaB⁻ cells where the increment ranged from 2.6 (F101) to 5.0 times (F42). This is seen in experiments 10 through 13. The increased acceptance of donor markers also occurred in the histidine auxotroph of the dnaB43 carrying organism, strain TG149 (expt. 9). However, it did not persist in two other DnaB⁻ mutants, E107 and E279, when the donor plasmid was F42 (expt. 7 and 8). Here the acquisition of mutations dnaB107 and dnaB279 was accompanied by a reduction in recipient ability. In keeping with an earlier observation (Results sec (a)) recombination defective strains were consistently more efficient recipients than isogenic Rec⁺ cells.

As far as can be determined, all the strains listed in Table 5 are derived from the E coli K12 strain CR34. However, the mutants E107 and E279 were isolated in a different laboratory and at a different time than CR34TS. It is therefore possible that the differences observed above regarding the recipient abilities of these two strains are not specifically due to their different dnaB alleles but rather to some other genetic difference between the two groups.

TABLE 5: ABILITY OF VARIOUS CR34 F⁻STRAINS TO ACCEPT FACTORS
F101 AND F42 FROM C076 AND MA3050

EXPT. NO.	DONOR PLASMID	RECIPIENT	1. FREQUENCY OF PLASMID MARKERS IN PROGENY		2.
			MEAN	3.	RANGE
1	F101	CR34 (<u>dnab⁺</u>)	0.0250		0.0196-0.0314
2	"	CR34TS (<u>dnab43</u>)	0.0349		0.0296-0.0402
3	"	E107 (<u>dnab107</u>)	0.0554		0.0456-0.0653
4	"	E279 (<u>dnab279</u>)	0.3017		0.1307-0.4726
5	F42	CR34	0.1492		0.0736-0.1912
6	"	CR34TS	0.3861		0.1780-0.4227
7	"	E107	0.0646		0.0466-0.0830
8	"	E279	ND ⁺		ND ⁺
9	"	TG149 (<u>dnab43</u>)	0.2457		0.0845-0.4069
10	F101	C09 (<u>dnab</u>)	0.1877		0.1302-0.2875
11	"	C050 (<u>dnab43</u>)	0.4880		0.3443-0.6647
12	F42	C09	0.1371		0.1218-0.1540
13	"	C050	0.6021		0.3542-0.8501

1. Strains 1-9, Rec⁺ 10-13, RecA⁻

Matings as described in the Methods. Donor/Recipient: 10:1, in NB at 30° for 60 minutes

2. Number of Thr⁺ Leu⁺ (Lac⁻) colonies per 100 recipient cells.

3. Average of at least 2 determinations

(111) Formation of mating pairs.

In part (11) it was shown that CR34 cells bearing dnaB43 are frequently better recipients than their non-mutant counterparts. Since it appears to be an essential condition for any exchange of plasmid DNA (Jacob and Wollman, 1961, Brinton, 1971), it was wondered whether the dnaB43 lesion might be exerting its effect at the level of the formation of mating pairs. Consequently, the facility with which wild-type (C09) and mutant (C050) recombination defective F⁻ strains entered into pair formation with strain MA3050 was monitored using the dilution method described in the Methods section (f).

Data obtained from this type of study is presented in Table 6. Since detection is dependent on the transfer and subsequent establishment of factor F42, this method allows the determination of only effective and not total mating pairs (Walmsley 1973). To correct for differences in the number of cells available for participation in conjugation, values are expressed as the number of Lac⁺ progeny achieved per 100 recipients.

Exponential females carrying dnaB43 (strain C050) are 4 times the more efficient in forming effective mating pairs (Table 6 column II). Growth into overnight phase produces a generalized increase in efficiency values (column III). However, this change is greater in recipients bearing dnaB43 than it is in non-mutant cells (1.84 vs 1.25 x increase). Thus the

difference in efficiencies of mating pair formation between DnaB⁻ and wild type strains (C09) is lifted to 6 fold. It would be of interest to determine whether this effect persists upon addition of a F plasmid to the recipient. Unfortunately, information obtained in this manner from cells expressing intracellular incompatibility is not easily interpreted.

**TABLE 6: FORMATION OF EFFECTIVE MATING PAIRS
BY WILD-TYPE (C09) AND DnaB⁻ (C050)
CR34 Rec⁻ F⁻ STRAINS DURING CONJUGATION
WITH THE F42 DONOR MA3050.**

RECIPIENT ^{1.}	EFFICIENCY OF MATING PAIR FORMATION ^{2,3.}	
	EXPONENTIAL	OVERNIGHT
C09	0.1657	0.2079
C050	0.7124	1.3118

1. Matings as described in the Methods. Donor/Recipient: 10:1, NB at 30 for 35 minutes.
2. Number of Lac⁺ colonies per 100 recipients after 8 minutes mixing followed by 500 X dilution (see text).
3. Average of at least 2 measurements. Percent error: 8-10%.

(c) Properties of F Prime Strains Bearing dnaB43.

Generation times typical of several CR34 F prime strains during growth in enriched medium are given in Table 7. Exponential phase recombination defective cells carrying plasmid F101 grew more slowly than their corresponding Rec⁺ derivatives. Further, DnaB⁻ cells (C047, C061) were duplicated more slowly than wild-type strains (C07, C011). However, as with F⁻ cultures (Table 4), the influence of dnaB43 on these values remained very small (column IV).

The presence of factor F101 in recombination proficient cells had only a slight affect on division rates (column IV). In RecA⁻ strains, however, doubling times were increased by 20 to 30 minutes. Introduction of F prime plasmid F134-1 produced just the opposite result. Mean generation times were decreased by 15 and 8 minutes in the wild-type (C049) and dnaB43 carrying (C060) strains respectively (Table 7).

Such behavior implies the existence of some form of interaction between chromosome and plasmid which is apparently more sharply revealed in the presence of the recA1 mutation. What its basis is in F101 cells is unknown, but the F134-1 effect is presumably related to the presence of greater than normal amounts of the dnaB gene product in cells merodiploid for this region of the chromosome (F' dnaB⁺). It is interesting to note that in certain instances physical union of plasmid and

chromosome is also associated with increased generation times ("integrative suppression", Nishimura et al, 1971)

The influence of dnaB43 on those properties associated with the presence of F plasmids was determined as well. Among these were plasmid stability, sensitivity to phages R17, M13 and ϕ II, and conjugal donor efficiency. F42, F101, and F134-1 derivatives of recombination deficient strains were examined. The F134-1 strains C049 (dnaB⁺) and C060 (dnaB43) were constructed as described in section (f-iii) of the Results from CR34RecA⁻ F⁻ cells.

TABLE 7: CELLULAR DIVISION RATES OF VARIOUS WILD-TYPE (C07, C011, C049) AND DnaB⁻ (C047, C061, C060) CR34 F PRIME STRAINS DURING GROWTH IN NUTRIENT BROTH AT 30° EFFECT OF dnaB43.

STRAIN	F PLASMID	RECOMBINATION	1. GENERATION TIME (MINS.)	2. EFFECT OF <u>dnaB43</u>	3.
C07	F101	+	37.9		
C047	F101	+	41.9		1.09
C011	F101	-	55.2		
C061	F101	-	69.2		1.27
C049	F134-1	-	21.0		
C060	F134-1	-	30.9		

1. +: Recombination proficient; - defective

2. Average of at least 2 determinations

3. Ratio (DnaB⁻/wild-type)

(i) Plasmid stability.

In E coli the presence in the cytoplasm of extrachromosomal DNA is generally not an essential condition for cell survival. One property of F prime cells that results from this dispensibility is the spontaneous generation of a sub - population of F⁻ strains through plasmid loss during growth in media non - selective for F retention. Each F plasmid differs in the degree to which it is stable in a particular genetic environment. One manner in which dnaB43 might possibly function to alleviate immunity barriers would be to increase the ease with which F or F prime factors are maintained within mutant cells. If so, the effect ought to occur in single as well as double male strains. Its action should therefore be reflected in the rate at which F associated markers are lost from 'DnaB⁻' single males grown in enriched medium.

After overnight incubation in minimal medium selective for the retention of plasmid markers, the F42, F101 and F134-1 derivatives of strains C09 and C050 were diluted 20 fold into fresh NB (approx. 10^6 cfu/ml) and shaken at 30° for six hours. At regular intervals during this period, 0.1 ml aliquots were withdrawn from each culture, diluted, then spread on appropriately supplemented sets of minimal agar that allowed determination of both total and F prime cell numbers. The results, expressed as the time rate of F plasmid loss, for each strain are illustrated in Figure 2. Variation due to differences in initial

population size were corrected for by letting the frequency of F prime cells obtained at zero time equal 100. All other values are expressed as a percentage of this number.

The presence of dnaB⁴³ in strain C059 had little effect on the stability of the plasmid F⁴². Virtually identical patterns of plasmid marker assortment were found in wild-type (C010) and DnaB⁻ cells (Fig. 2-a). Plasmid F101, however, showed a different pattern (Fig. 2-b). It was consistently lost from cells carrying dnaB⁴³ (C061) more rapidly than from wild-type strains. In Figure 2-b, for instance, at four hours, when cultures were well into exponential phase only 23% of the mutant population was Thr⁺ Leu⁺ as compared with 80% in strain C011. The slight increase in F plasmid frequency occurring after 5 hours incubation is presumably a result of secondary matings in the high concentration solution (10^8 - 10^9 cfu/ml).

That this behavior is directly attributable to dnaB⁴³ is suggested by Figure 2-c. It depicts the plasmid assortment pattern for strains containing factor F134-1. These strains are merodiploid for the dnaB region (dnaB⁺ / F' dnaB⁺). Once into exponential growth, there was no distinguishable difference in plasmid stability between C049 (dnaB⁺) and C060 (dnaB⁴³).

FIGURE 2: TIME RATE OF F PRIME PLASMID LOSS FROM
WILD-TYPE (0-0-) AND dnaB43 BEARING
(-0-0-) CR34 RecA^- STRAINS DURING
EXPONENTIAL GROWTH IN NUTRIENT BROTH
AT 30°.

Cells were subcultured (1:20) from overnight growth in selective minimal medium into fresh NB and shaken at 30° for 6 h. Each hour, 0.1 ml aliquots were withdrawn and tested for total and Lac^+ (or $\text{Thr}^+ \text{Leu}^+$) cell numbers by plating on appropriate selective agar. Arrow indicates beginning of log. phase.

The effect of dnaB43 on the stable maintenance of F101 is, on first consideration, opposite to what was expected. In view of its ability to promote the stable coexistence of F42 and F101, (Table 3, expts. 2 & 4), one might have expected each of the two plasmids to be individually at least as stable in the mutant as in the wild-type strain.

One further observation might be made. As the genetic (and presumably, physical) length of the chromosomal fragment appended to plasmid DNA increases, the stability of the resultant F prime element is diminished. Factor F42 with a molecular weight of 52×10^6 daltons (Clowes, 1972) carries approximately 0.5 minutes of chromosomal DNA as measured by the time required for conjugal entry of genetic markers associated with it. Using the same criterion, the factors F101 and F134-1 carry about 5 and 15 minutes respectively (see Figure 1). F⁻ segregants are generated very slowly from non-mutant cultures bearing F42 (Fig. 2-a). Factor F101 is less stable (Fig. 2-b), but is not lost as rapidly as F134-1 (Fig. 2-c). Within the limits of the number of F plasmids examined, the larger F plasmids are not as easily maintained in the absence of forces selective for their retention.

(ii) Phage Sensitivity

The presence within E coli cells of F plasmid DNA is usually accompanied by the appearance at the cell surface of long threadlike appendages termed sex pili (1-4 per cell). These, function as sites of absorption for the bacteriophage R17 and M13. R17 is an icosahedral RNA-phage that attaches to the sides of sex pili, while the filamentous DNA-phage M13 attaches exclusively to the tip of the pilus. (Paranchych and Graham 1962; Caro and Schnos, 1966). R17 and M13 do not infect F⁻ cells and hence are termed male specific.

The recombination proficient DnaB⁺ strain C034 exhibited a decreased capacity for plaquing R17 and M13. That this behavior was not due to defects in plasmid DNA was demonstrated by the return of phage sensitivity upon transfer of F42 to a different host (Palchaudury and Iyer, 1971). Recent studies indicate that this effect is due primarily to a reduction in the number of piliated cells in the population (Iyer et al, 1974).

The recombination defective DnaB⁻ strains C059, C061 and C060 were assayed with R17 and M13 to determine whether the effect persisted in this background.

After overnight growth in NB at 30°, test cells were subcultured 1:10 in 1 ml of liquid minimal medium selective for

retention of plasmid markers and incubated with mild aeration for six hours. At this time 0.1 ml samples from each culture were measured by the agar overlay method (see Methods sec.(b)) for their abilities to plaque an equal volume of R17 or M13 solution containing $10^8 - 10^{10}$ particles per ml. A second portion of the culture was used for determining total and F prime cell numbers on minimal agar. The resultant data were corrected for differences in test cell numbers by determining the ratio of plaque forming units (pfu) to colony forming units (cfu) for each strain. Values obtained in this manner for wild-type controls were considered to represent maximal plaquing efficiency (ie: 100%). All other values are expressed as a percentage of these. Thus, such units provide an estimate of the relative plaquing efficiency of mutant strains. When male specific phage sensitivity was being determined only F prime cell numbers were used for correction. Information derived from such experiments is presented in Table 8.

The diminished capacity for plaquing R17 and M13 persisted in recombination deficient dnaB43 strains (Table 8). F101 containing cells (C061) were generally more sensitive than their isogenic F42 cultures (C059). In the present instance, increases of 4 to 5 fold were obtained with the former. However, both F101 and F42 derivatives plaque R17 more efficiently than M13 (cf. columns III & IV). This may merely be a reflection of differences in the availability of attachment sites (side versus tip).

A number of bacteriophage particles display a decreased capacity for multiplication within cells bearing F plasmid DNA. They are characterized by the ability to form large plaques on F⁻ strains and either none or a reduced number of smaller plaques on F prime cells. Among these phages is included ϕ II. Its propagation is restricted in the presence of plasmid DNA and is hence referred to as being female specific (Cuzin, 1965). Studies of the ability of DnaB⁻ F prime strains to restrict ϕ II were conducted in parallel with the R17 and M13 experiments. The results appear in Table 9. Correction for differences in cell numbers was made by dividing plaque numbers by total cell numbers.

One expects to observe a greatly reduced plaquing efficiency for ϕ II on F101 and F42 derivatives of CR34. This, indeed, occurred in the non-mutant strains (Table 9, strains 1-4). With DnaB⁻ cells (strains 5-8), however, the plaquing efficiency was enhanced. The mutant strain harboring factor F42 (C059), for example, showed an increase in plaquing efficiency of some ten fold (column III).

Columns IV to VI describe the plaques obtained with ϕ II on the various test strains. Non-restricting (F⁻) cells gave rise to typical large, haloed zones of lysis. Such plaques indicate rapid, followed by more slow, lysis of cells. In the presence of a F prime plasmid there was a dramatic alteration in plaque morphology. In wild-type strains (eg. C011) the change was

classic: plaques characteristic of F⁻ strains were reduced in size and number; the majority of plaques being clear and small in diameter. The presence of dnaB₄₃, however, altered this pattern. DnaB⁻ cultures bearing F42 or F101, strains C059 and C061 respectively, continued to produce haloed-type plaques in majority. Although their size was generally reduced relative to the F⁻ control, they were larger than those achieved on isogenic wild-types.

Such alterations in plaque morphology are in keeping with changes in plaquing efficiency noted in column III. For instance, the near complete lack of restriction in strain C059 is accompanied by a near complete dominance of F⁻ type plaques (haloed). In F101 cultures where some inhibition does occur, the proportion of haloed plaques is correspondingly reduced. Taken together, such observations suggest that the presence of dnaB₄₃ serves to diminish the capacity of F prime strains to inhibit multiplication of phage ØII.

The F134-1 derivatives C049 and C060, displayed a relatively poor capacity for preventing the propagation of ØII (Table 9, column III). In addition, the dnaB₄₃ bearing strain failed to yield significant proportions of F prime-type plaques (clear). Thus, making the cell merodiploid for the dnaB region has not fully restored the wild-type phenotype. In agreement with this finding is the observation in Table 8 that the strains heterodiploid in the dnaB region were still largely insensitive to male specific phage as well.

**TABLE 8 : SENSITIVITY OF VARIOUS DnaB⁻
CR34 Rec⁻ F PRIME STRAINS TO
THE MALE SPECIFIC PHAGES R17
AND M13.**

STRAIN 1.	F PLASMID	RELATIVE PLAQUING EFFICIENCY 2.3.	
		R17	M13
C050	F ⁻	00.0	00.0
C059	F42	7.42	5.13
C061	F101	33.8	19.7
C060	F134-1	29.6	16.2

1. Grown to log. phase at 30° in minimal media selective for the retention of plasmid markers.
2. Percentage of corresponding non-mutant strain (see text).
3. Average of three trials. For strain C059 values ranged from 5.68-11.2 (R17), 2.79-9.86 (M13); for C061 from 25.2--38.1 (R17), 15.5-24.4 (M13); for C060 from 24.7-33.2 (R17), 13.2-18.6 (M13).

TABLE 9: RESTRICTION OF BACTERIOPHAGE ϕ II PROPAGATION BY VARIOUS
CR34 $\text{Rec}^+ \text{F}$ PRIME STRAINS

STRAIN	F PLASMID	RELATIVE PLAQUING EFFICIENCY 2.3.	PLAQUE MORPHOLOGY		
			TYPE	DIAMETER (mm)	ABUNDANCE (%)
1) C09	F ⁻	100	a (haloed) b (clear)	3.0-5.5 -	100 -
2) C010	F42	7.65	a b	1.8-2.8 0.4-2.0	0-10 90-100
3) C011	F101	5.95	a b	1.2-2.1 0.4-2.1	0-5 95-100
4) C049	F134-1	12.0	a b	2.0-4.0 0.8-1.4	0-20 80-100
5) C050	F ⁻	100	a b	3.0-5.0 -	100 -
6) C059	F42	81.0	a b	2.0-5.0 0.8-1.3	90-97 3-10
7) C061	F101	25.0	a b	1.3-3.5 0.6-2.8	70-90 10-30
8) C060	F134-1	33.7	a b	1.2-3.6 0.7-1.3	80-97 3-20

1. Strains 1 - 4: dnaB^+ ; strains 5 - 8: dnaB43^- . Grown to exponential phase in liquid minimal media selective for retention of plasmid markers.

2. This value defined in text.

(111) Donor ability.

A decrease in the sensitivity of F prime strains carrying dnaB⁴³ to bacteriophages that require the presence of functionally and structurally intact F pili for cell infection was observed in section (11). The occurrence at the cell surface of intact sex pili is also an essential condition for transfer of genetic material from donor to recipient during conjugation. Thus F⁺ or F prime cells suffering prolonged exposure to high shear forces will not pass plasmid DNA to F⁻ recipients (Brinton, 1971). The following experiments were carried out in order to assess whether dnaB⁴³ might also influence this aspect of pilus function.

The donor abilities of F42 and F101 derivatives of wild-type and DnaB⁻ recombination deficient CR34 strains were examined. If F42 transfer was to be followed, strains C027 and C077 (C027/F103) served as recipients, while strain JC4121 (SPC^R) was used to monitor F101 transmission. All cells were grown overnight in Nutrient Broth then diluted 20 times into either fresh broth or TMM medium selective for retention of plasmid associated markers and grown to exponential phase at 30°. Participants were mixed in a donor to recipient ratio of 1:10 and mated for 60 minutes. Lac⁺ progeny were selected on minimal agar containing proline, histidine, thiamine and lactose. Lac⁺ His⁺ progeny were isolated on lactose agar in the absence of histidine. Transfer of thr⁺ leu⁺ was assayed on glucose

minimal agar supplemented with histidine, arginine, methionine and 50 µg per ml of spectinomycin. The number of Lac⁺ (or Lac⁺ His⁺) or Thr⁺ Leu⁺ colonies formed per 100 minority parents was used as a measure of donor efficiency. These values, corrected for variation due to inherent differences in plasmid stability, are presented in Table 10. Each is an average of at least two separate determinations.

Using this protocol, it was not possible to detect significant differences in the donor abilities of wild-type (C010, C011) and mutant (C059, C061) strains (Table 10, column V). The donor efficiencies of all strains were enhanced by growth in selective minimal medium (expts. 2 & 4). This likely reflects the increased proportion of F⁺ cells in the donor population. It is possible that under these conditions, matings continue after plating thus making the procedure inadequate for the detection of the differences that are sought. Before concluding that dnaB43 does not effect donor proficiency, a more sensitive measurement of the latter would have to be made.

As an extension of this study, the effect upon donor ability of encountering an excluding recipient (ie; F prime) was tested. It is conceivable that the dnaB43 effect, if any, is noticeable only under such circumstances. Strains C010 and C059 were mated with exponential cultures of strain C077 and the frequencies of lac⁺ transfer compared (Table 10, expt. 5). Exclusion indices (column VI) were computed as described in the methods section

using values for F⁻ recipients from experiment 1. Again little difference in donor efficiencies was noted. Direct selection for double males tended to verify this (column VII).

The small values obtained for Eex I appear to result, at least in part, from the change in minority parent. A control experiment performed with donor and recipients in a 10:1 ratio gave an index ten fold greater in magnitude (data not given).

Meynell and Ewins (1973) reported that a significant reduction in entry exclusion occurred in matings where the donor bore structurally altered sex pili (different protein subunits). The presence of dnaB₄₃ apparently does not result in a similar effect in CR34 strains.

2 3

OF/DE

TABLE 10: TRANSFER OF F PRIME PLASMIDS FROM VARIOUS WILD-TYPE (C010, C011) AND DnaB⁻ (C059, C061) ~~C034~~ ⁸ STRAINS

EXPT. NO.	DONOR ¹	RECIPIENT ¹	GROWTH MEDIUM ²	TRANSFER FREQUENCY ³	Text 4. FREQUENCY Lac ⁺ His ⁺ PROGENY 2.
1	C010(F42)	C027	NB	0.0575	-
	C059 "	"	NB	0.0718	-
2	C010(F42)	"	TMM	0.4355	-
	C059 "	"	TMM	0.3434	-
3	C011(F101)	JC4121	NB	0.0854	-
	C061 "	"	NB	0.0962	-
4	C011(F101)	"	TMM	0.2820	-
	C061 "	"	TMM	0.2374	-
5	C010(F42)	C077	NB	0.0244	2.35
	C059 "	"	NB	0.0264	2.73
					0.0016
					0.0017

1. Donors & Recipients grown to log. phase then mixed 1:10 for 60 minutes at 30°.
2. NB: Nutrient Broth; TMM: Tris Maleate Minimal Medium.
3. Number of Lac⁺ (Thr⁺Leu⁺) or Lac⁺ His⁺ colonies per 100 donor cells; corrected for differences in cell numbers.
4. Entry Exclusion Index: Freq. of progeny from F' x F⁻ cross divided by same from F' x F' cross.

(d) Correction for the Influence of dnaB43 on F⁻ Recipient Ability.

Since dnaB43 bearing strains are the more efficient F⁻ recipients (Results, sec. (b)), the question arises as to whether this alone is sufficient to account for the lifting of immunity barriers noted in mutation-carrying F prime cells. In short, if the transfer frequencies obtained with F prime recipients were corrected for alterations in the recipient ability of isogenic F⁻ strains, would the DnaB-effect persist? Table 11 presents data typical of experiments bearing on this question.

The resistance to uptake of an incoming F plasmid manifested by exponential phase F prime recipients is expressed as the entry exclusion index (EexI). This value is determined by dividing the frequency at which conjugally transferred F prime markers - here thr⁺ leu⁺ - occur in progeny from an F' X F⁻ cross by that obtained with an isogenic F prime recipient; that is, F' X F', under identical mating conditions. Thus, when no entry exclusion is exerted; that is with "F⁻" recipients, the value of EexI is 1. When a similar correction is applied to data from overnight recipients, the number obtained is termed the establishment index (EstI). The computation of this ratio has a second advantage in that it normalizes for daily fluctuations in the mating efficiencies of both wild

type and dnaB⁴³ - bearing strains (see Results sec. (a)).

The dnaB⁴³ effect persisted even after this correction was made (Table 11, strains C034 & C059). With exponential recombination proficient F prime cells (expts. 1 & 2), the mutant strain was the better recipient by 4.0 fold (column III). A similar enhancement was observed in the presence of the recA1 mutation (Expts. 3-4). The effect was diminished, but still detectable, in overnight cultures where the influence of entry exclusion barriers was reduced by placing cells in F⁻ phenocopy (column IV). That phenocopy had been achieved was attested to by computing the ratio of the two indices column VI). With wild-type F primes (strains C05 & C011), the increase in recipient efficiency was about 3 fold. Growth of mutant strains to stationary phase had little effect on their recipient ability. However, this is not unexpected in view of the influence the presence of dnaB⁴³ has on the capacity of exponential cells to accept a second F plasmid.

Additional evidence in support of the dnaB⁻ mutation exerting an influence on post-entry immunity functions is given in column VI of Table 11. Ex-conjugants from matings involving overnight F prime recipients were spread on a medium that permitted the growth only of cells simultaneously carrying factors F101 and F42. Comparison of the frequencies of Thr⁺Leu⁺Lac⁺ progeny resulting from wild-type and DnaB⁻ parents reveals

that the latter are better able to support formation of double males. Indeed, in the wild-type recombination deficient strain C011, such double males were not observed at all ($\leq 0.0001\%$). With Rec⁺ strains, the data presented in experiments 1 and 2 indicate a 5 fold increase in the mutation bearing strain.

TABLE 11: INFLUENCE OF dnaB43 (STRAINS C034, C059) ON THE ABILITY OF F42 DERIVATIVES OF STRAIN CR34 TO ACCEPT FACTOR F101 FROM C076: CORRECTION FOR VARIATION IN RECIPIENT EFFICIENCY OF F⁻ CELLS

EXPT. NO.	RECIPIENT	1. Mex1 ^{2.}	Esti	2. EexI/Esti	FREQUENCY Lac ⁺ Thr ⁺ Leu ⁺ PROGENY 3.
1	C05 (Rec ⁺)	5.12	1.48	3.46	0.0046
2	C034 (")	1.26	1.12	1.12	0.0226
3	C011 (RecA ⁻)	12.6	3.66	3.44	ND ⁺
4	C059 (")	2.87	2.18	1.32	0.0466

1. Matings as described in Methods. Donor/Recipient: 10:1; 60 minutes in NB at 30°.
 2. EexI: Entry Exclusion Index; Esti: Establishment Index. Both obtained from ratio of frequency of Thr⁺Leu⁺ progeny from F'X F⁻ cross to that from F'XF⁻ cross; for EexI, recipients in log. phase; for Esti, all in overnight phase of growth.
 3. Number of Lac⁺Thr⁺Leu⁺ colonies per 100 overnight recipient cells.
- + : Not detected.

(e) Specificity of dnaB43 Action

The presence in the genome of numerous CR34 strains of the mutation dnaB43 has been shown to result in the formation of F101/F42 double males at an increased efficiency relative to the wild-type at the permissive temperature of 30° (Results, sec. (d)). In these experiments, the plasmid F101 was conjugally introduced into strains already harboring factor F42.

Is the DnaB effect specific for entry exclusion and incompatibility functions exerted by F42 on F101 only, or is it more universal in its action? If this order of entry were reversed, would the lifting of immunity barriers still be observed? And if so, is the effect peculiar to these two particular factors? Would it also occur upon the use of a different set of F plasmids? The following series of experiments were performed in an attempt to answer these questions.

Matings were carried out as described in the methods section. F and F101 derivatives of CR34 strains were employed as exponential and overnight recipients. Strain MA3050 served as a donor of F42. Lac⁺ progeny were selected on lactose minimal agar supplemented with threonine, leucine thymine and thiamine (plus histidine where strain C025 was utilized). Lac⁺Thr⁺Leu⁺ cells were isolated on lactose medium containing only thymine and thiamine. Counter selection of donor cells was completed by inclusion of 50mg per ml of streptomycin.

TABLE 12: SPECIFICITY OF dnaB43 ACTION: (A) REVERSE ORDER OF F⁻ PLASMID ENTRY; (EXPTS 1-5) (B) INTRODUCTION OF FACTOR F103 (EXPT. 6)

EXPT. NO.	RECIPIENT	1. Exi ² .	Esti	2. Exi/Esti	FREQUENCY OF DOUBLE MALE COLONIES 3.
1	C07 (Rec ⁺)	67.8	47.7	1.42	(a) Lac ⁺ Thr ⁺ Leu ⁺ progeny 0.0022
2	C047 (")	7.35	6.19	1.18	0.0260
3	C011 (RecA ⁻)	110.7	33.4	3.31	ND ⁺
4	C061 (")	14.7	12.9	1.13	0.00068
5	C025 (Rec ⁺)	17.2	13.4	1.28	0.0030 ⁺ His ⁺ Thr ⁺ Leu ⁺ progeny
6	C025 (")	8.11	7.79	1.04	0.0024

1. Strains C07 and C011: dnaB^+ ; strains C047, C061 & C025: dnaB43 . Matings as described in methods. Donor/Recipient: 10:1 in NB for 60 minutes at 30°. Donor for crosses 1-5: MA3050, (F42); for cross 6, strain C077 (F103).

2. Exi: Entry Exclusion Index; Esti: Establishment Index. Both obtained from the ratio of frequency Lac⁺ (His⁺) progeny from F' X F⁻ cross to that from F' X F⁺ cross; for Exi recipients in log phase; for Est I, in overnight phase of growth.

3. Number of Lac⁺ (His⁺) Thr⁺ Leu⁺ colonies per 100 overnight phase recipient cells.

+: Not detected.

Entry exclusion and establishment indices typical of these reverse order of entry experiments are given in Table 12 (expts. 1, 2, 5 & 6: Rec^+ ; 3 & 4: RecA^-). Examination of columns III and IV, reveals dnaB43 still to influence immunity functions, despite the change in resident F plasmid. Indeed, the difference in index values between wild-type (C07, C011) and mutant recipients (C047, C061) was even more marked. Here, decreases in exclusion of 7 to 9 fold were noted as compared to half that much in the earlier circumstances (Table 11).

The actual frequencies of Lac^+ progeny with which these values are calculated, fluctuated from experiment to experiment. Thus, with exponential phase, recombination defective wild-type recipients (C011) they ranged from 0.0009 to 0.0044% while with Rec^- , DnaB^- cells (C061) it varied from 0.0108 to 0.0724%. The mutant strain was invariably the better recipient, by at least ten fold. As before, the most likely explanation for such variation, is the spontaneous loss of plasmid DNA. In general, the proportion of females in the recipient population ranged from 1 to 35%.

Further corroboration of the influence of dnaB43 in overnight cultures was obtained from the frequencies of $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ progeny. (Table 12, column VI). Here again, the DnaB^- strain consistently displayed at least a ten-fold enhancement in recipient efficiency.

That the lifting of immunity barriers is not restricted to this particular pair of F plasmids was determined by introduction of factor F103 (from C077) into a F101 derivative of the Rec^+ , DnaB^- histidine auxotroph of CR34, strain C025 (Table 12, expt. 6). Values obtained for EexI and EstI consistently fell within the range displayed by the original dnaB43-bearing strain, C047 (expt 2). The frequency of double male formation, as determined by simultaneous selection for markers associated with both plasmids, is given in column VI. It is to be noted that the frequency is only slightly enhanced in this strain (cf, expt. 1, 2 and 6). Experiment 5, included as a control, indicates that similar behavior occurs when factor F42 is introduced. Hence, this property is strain rather than plasmid specific.

The parent for the C025 strain, is somewhat leaky for its histidine requirement. Thus, selection of His^+ recombinants becomes rather difficult (P. Hanawalt, personal communication). However, cells merodiploid for his (ie: $\text{his}^- / \text{Fhis}^+$) grow much more quickly on selective medium (histidine minus) than do isogenic female strains. Hence, it was possible to distinguish true progeny on the basis of colony size after short periods of incubation on minimal agar at 30° . All His^+ colonies examined were sensitive to F specific bacteriophage.

One further aspect of the present series of experiments warrants comment. A significant increase in the magnitude of the exclusion and establishment indices occurred upon reversal of the order of plasmid entry. When factor F101 was resident in non-mutant cells (expts. 1 and 3, Table 12), values some 8 to 30 fold larger than those resulting when F42 was resident (expts 1 and 3, Table 11) were noted for these two numbers. This difference is a reproducible one and persists, although to a lesser degree, in the presence of dnaB43 (Tables 11&12 expts. 2 and 4).

This observation suggests that factor F101 is more proficient in exerting superinfection immunity functions against F42 than the latter is against F101. If such were actually the case, it ought to be evident in the progeny frequencies obtained from a series of reciprocal F'X F' crosses where the recipients are isogenic for all markers save those located on the resident F plasmid (DeVries and Maas, 1973). The information presented in Table 13 was derived from this type of experiment. To normalize for any inherent differences in the rates of transfer of the two F prime factors themselves, the values obtained were divided by the progeny frequency occurring in the appropriate F'X F' cross.

TABLE 13:

ACCEPTANCE OF FACTORS F42 AND F101 BY EXPONENTIAL (a) AND
OVERNIGHT (b) F⁻ (C09) AND F⁺ PRIME (C010, C011) DERIVATIVES
OF WILD-TYPE CR34 ROG

EXPT. NO.	DONOR	1. RECIPIENT	1. FREQUENCY OF PROGENY BEARING DONOR MARKERS		2. F ⁺ X F ⁺ PROGENY	
			Lac ⁺	Thr ⁺ Leu ⁺	F ⁺ X F ⁺ PROGENY	F ⁺ X F ⁺ PROGENY
a-1	C076	C09	-	0.1877		0.132
	"	C010 (F42)	-	0.0248		
a-2	MA3050	C09	0.1371	-		0.008
	"	C011 (F101)	0.0011	-		
b-1	C076	C09	-	0.1495		0.271
	"	C010 (F42)	-	0.0406		
b-2	MA3050	C09	0.0467	-		0.029
	"	C011 (F101)	0.0014	-		

1. Matings as described in Methods. Donor/Recipient: 10:1; NB, 60 minutes at 30°.
2. Number of Lac⁺ (Thr⁺Leu⁺) colonies per 100 recipient cells.

The data confirm our suspicion. There is a marked difference in the degree to which the two factors inhibit each other's acceptance into the cell (Table 13, column VI). Factor F101 is the more efficient; Thr⁺Leu⁺ colonies occur in greater relative frequency in F42 containing recipients (C010) than do Lac⁺ progeny in the isogenic F101 bearing strain, C011 (9-16X increase). This occurs regardless of whether the recipients were originally in exponential or overnight phases of growth (cf. expts. a&b).

A similar situation was observed to exist in recombination proficient CR34 strains. In these cells, the "pecking order" in terms of proficiency of expression of immunity functions was F101 > F42 > F103. Identical patterns were found when these calculations were carried out using data from DnaB⁻ F prime cells (data not given).

Such variation in the degree to which two functionally similar (same pilus, compatibility types) F prime factors exert superinfection immunity, is not easily explained. This differential behavior is carried through to other plasmid determined properties.

Exponential cultures of F42 derivatives of both wild-type and mutant cells are 4 to 5 times less sensitive to male specific phage than their corresponding F101 derivatives (Data for the strain carrying dnaB43 given in table 8). These same strains are less adept in restricting the propagation of phage ϕ II (Table 9). During conjugation in NB, cells of the CR34 line transfer factor F42 at slightly lower frequencies than they do F101 (Table 10 expt. 1 and 3). Finally F42 displays unusually high degree of stability in single male strains in enriched liquid medium. Data obtained in Results section (c) reveal that cultures of C010 (wild-type) and C059(dnaB⁻) both contained less than 2% Lac⁻ cells even after 6 hours incubation in NB (Figure 2-a). In contrast, the wild-type and DnaB⁻ F101 derivatives, strains C0II and C061, segregated 20 and 60% F⁻ cells respectively (Fig. 2-b).

It may be that expression of certain plasmid functions is partially suppressed in the CR34 line. But why does this inhibition affect factor F42 only? Further, behavior identical to that noted in Tables 11 and 12 is observed when F42 and F101 were transferred to strain C099 an ampicillin-resistant P1 transductant of JE2571 (Table 17). Its persistence in a different genetic background is inconsistent with the suggestion that this differential expression of immunity functions is strain specific.

Alternatively, this behavior may be related to the origin and genetic structure of these plasmids. Factor F42 may be defective in certain F specified functions. This plasmid was originally isolated from the Hfr strain P804 by interrupted mating with a Rec⁺ recipient (Jacob and Wollman, 1961). If during the excision process, a portion of F-DNA remained with the chromosome in exchange for the lac⁺ region (Type I Excision; Sharp et al, 1972), genes critical to expression of superinfection immunity, phage sensitivity etc. may have been lost. And if during the formation of factor F101, these same regions were retained by the plasmid, F prime factors with different F-DNA contents, and hence different properties, would have resulted. Assessment of the validity of this proposal awaits measurement of the degree of sequence homology existing between F, F42 and F101. (Hsu, 1974, has indicated that less than 4% of the F-DNA was deleted during formation of factor F42).

(f) Lifting of Superinfection Immunity and Temperature Sensitivity: Both Effects of dnaB43?

The mutation dnaB43 resulted from nitrosoguanidine (NTG) treatment of strain CR34 (Bonhoeffer, 1965). Evidence has since accumulated which indicates that the action of NTG is such that it tends to induce multiple lesions in the same chromosome. Regions particularly susceptible to this activity are those lying near the replication fork at the time of mutagen addition (Guerola et al, 1971). The possibility therefore exists that the temperature sensitivity and immunity effects are results of independent mutations occurring in different areas of the one chromosome. Palchaudury and Iyer (1971) had previously reported that a spontaneous revertant of strain CR34TS which was insensitive to elevated temperatures also displayed a wild-type level of superinfection immunity. A number of different approaches have been utilized to determine whether the altered immunity phenotype can be specifically attributed to dnaB43 in recombination defective strains as well.

(1) Phenotypic suppression of dnaB43.

As an initial approach to testing the relationship between dnaB43 and superinfection immunity, the effect of salt on the recipient abilities of F plasmid containing cells was monitored. Inclusion of sodium chloride (NaCl) in culture broths has been reported to effect phenotypic suppression of the dnaB⁻ mutation thus permitting cells to grow at normally restrictive temperatures (Outram, unpublished data; Ricard and Hirota, 1969, 1973). Siccardi *et al* (1971) employed such conditions in their efforts to determine the immediate effects of expression of a dnaB⁻ type mutation (dnaB313) in *E coli* strain BT313. One result of incubation at non-permissive temperatures was an alteration in membrane - associated proteins. Since this change occurred even under conditions of phenotypic suppression, the authors concluded that such must be the primary effect of expression of this particular lesion.

Continuing this argument we reasoned that if the lifting of immunity barriers in F prime cells were a primary effect of dnaB43 in the CR34 background, then, it too should persist under salt-induced phenotypic suppression. To this end the influence of different concentrations of NaCl upon the capacity of strains carrying dnaB43 to multiply at normally restrictive temperatures was determined.

Test cells were subcultured from overnight cultures (30°, NB) into NB containing 0, 1.5, 2.0, 2.5, and 30% (w/v) of NaCl and shaken at 42° for seven hours. Growth was followed by measuring increases in absorbance at 550 nm. Material used for determining generation times was withdrawn at 1.5 and 4 hours. Data derived from these studies are illustrated in Figures 3 and 4.

Figure 3 depicts the influence of various NaCl concentrations on growth after four hours incubation at 42°. Mutant cells placed in 0.42M NaCl (2.5%) were least affected by high temperature. Such cultures appear to be phenotypically suppressed since removal of salt by centrifugation and washing in distilled water resulted in a return to temperature sensitivity. Strains bearing F' plasmid DNA were more resistant to the action of NaCl.

In Figure 4 (a & b) are compared the growth curves of wild-type and mutant cells in the presence and absence of 0.42M NaCl at 42°. Upon addition of salt, female dnaB⁻ cells (C050) showed a rate of increase in optical density nearly equal to that of the corresponding wild type (C09, Fig. 4-a). A similar, though not quite as dramatic, rise in absorbance occurred when the F101 derivative, strain C061, was incubated in the presence of salt (Fig. 4-b). With female temperature sensitive strains the increase over a five hour period regularly amounted to over 70% of that noted in the wild type; for F101 cultures the figure ranged between 55 to 65%.

FIGURE 3: EFFECT OF NaCl ON THE ABILITY OF F⁻
(-0-0-) AND F101 (-●-●-) DERIVATIVES
OF CR34 RecA⁻ CELLS CARRYING THE dnaB43
MUTATION TO GROW AT A NORMALLY NON-
PERMISSIVE TEMPERATURE.

Cells subcultured (1:20) from overnight growth at 30° in Nutrient Broth into fresh NB supplemented with various concentrations of NaCl and incubated with aeration at 42° for 4h at which time optical density was determined.

FIGURE 4: INFLUENCE OF 0.42M NaCl ON THE GROWTH OF F⁻ (a) AND F101 (b) DERIVATIVES OF WILD-TYPE (C09, C011) AND dnaB43 CARRYING (C050, C061) CR34RecA⁻ STRAINS IN NUTRIENT BROTH AT 42°.

-X-X-: C09, C011 minus salt; -O-O-: C050, C061 minus salt; -●-●-: C050, C061 plus salt.

Cells subcultured (1:20) from overnight growth in NB at 30° into fresh broth and incubated with aeration at 42°.

Addition of NaCl to NB cultures of wild-type cells growing at 42° slightly enhanced their cell division rates. The mean generation times of strains C09 and C011 under these conditions were 75 and 128 minutes. Those for isogenic strains carrying dnaB43 were 85 and 198 minutes. Thus, dnaB⁻ to wild-type ratios, 1.13 and 1.54 for F⁻ and F101 respectively, comparable to those observed at 30° (Table 7) were obtained, indicating phenotypic suppression had indeed occurred.

With this information in hand, it was possible to determine what effect transient phenotypic suppression of dnaB43 had on superinfection immunity. Strains C011 and C061 plus their isogenic F⁻ derivatives were tested for their abilities to take up factor F42 from MA3050 at 30 and 42° after extensive growth in broth containing 0.42 M NaCl. Matings at 30° were conducted as described in section (e) of the methods. The modified conjugation procedure necessitated by incubation at 42° was outlined in Section (h) of the same. Selection for Lac⁺ and Lac⁺ Thr⁺ Leu⁺ progeny at the lower temperature was accomplished on both minimal agar and EMB-lac agar plus 0.42 M NaCl (Lac⁺ only). At 42° only lac⁺ transfer was monitored using EMB-lac agar containing streptomycin (50 µg/ml) and 0.42 M NaCl. Reversion to temperature resistance was monitored by spreading unmated dnaB⁻ recipients on EMB-lac in the absence of salt followed by incubation at 42°. The rate was too low to have any influence on the results given in Table 14.

TABLE 14: PHENOTYPIC SUPPRESSION OF dnaB43: INFLUENCE ON THE ABILITY OF THE DnaB⁻ F101 BEARING CR34 Rec DERIVATIVE, C061, TO ACCEPT FACTOR F42 FROM MA3050.

EXPT. NO.	RECIPIENT	1. GROWTH CONDITIONS	Eexl 2.	Estl 2.	Eexl/Estl	FREQUENCY OF Lac ⁺ PROGENY ALSO Thr ⁺ Leu ⁺ 3.
1	C011	NB ⁺ , 30°	110.0	33.7	3.31	-
2	C061	" "	14.6	12.9	1.13	-
3	C011	NB+0.42M NaCl, 30°	123.4	23.6	5.22	29.4
4	C061	NB+0.42M NaCl, 30°	18.8	5.80	3.24	42.2
5	C011	NB+0.42M NaCl, 42°	260.8	19.6	13.3	5.18
6	C061	NB+0.42M NaCl, 42°	15.8	2.74	5.76	18.1

1. Matings as described in Methods, Recipient cell numbers adjusted to 1-5-3.6 x10⁷ cfu/ml before mixing with 10-fold excess of the donor for 60 minutes.
 2. Eexl: Entry Exclusion Index; Estl: Establishment Index (See Table 12).
 3. Lac⁺ colonies from EMB-lactose replicated to minimal agar in the absence of threonine and leucine.
- + : Nutrient Broth.

At 30° addition of 0.42 M NaCl produced a general increase in the recipient ability of all strains examined of around 10 to 20 fold, but led to no change in superinfection immunity (Table 14, expt. 1 to 4). Values obtained for Eex I and Est I in strains not carrying dnaB⁴³ (C011) were significantly greater in magnitude than those observed for cells that did harbor this lesion (strain C061).

Incubation at the higher temperature slightly diminished the recipient ability of all dnaB⁴³ - carrying strains. With cells bearing the wild-type dnaB allele, however, the frequency of Lac⁺ progeny was increased some 10 to 100 fold. Even with these changes and despite the fact that cells were mated under conditions of phenotypic suppression of temperature sensitivity, the dnaB⁴³ effect still persisted (expt. 5 and 6). Exponential recipients, for instance, displayed a 16 fold decrease in the resistance they offered to the uptake of the second F plasmid in the presence of dnaB⁴³. This is to be compared to the 7 fold decrease occurring at 30° (expts. 3 and 4).

Overnight incubation at 42° in the presence of NaCl likewise failed to restore the wild-type level of immunity in mutant strains (column V). Lac⁺ progeny from these crosses were tested for the presence of markers associated with the resident F plasmid (F101) by transfer to an appropriate minimal agar. The DnaB⁻ strain consistently gave rise to a greater number of Lac⁺ Thr⁺ Leu⁺ colonies than did the wild-type (column VII).

These data are reproducible and indicate that the lifting of superinfection immunity barriers that occurs in CR34 strains carrying dnaB⁴³ is not affected by phenotypic suppression of the latter to allow growth at elevated temperatures. The altered immunity is, therefore, very probably a primary effect of the presence of this mutation.

It is to be noted that the magnitude of the EexI/EstI ratio increased markedly in Experiments 1 through 6 of Table 14. Addition of salt and/or incubation at 42° had little effect on the entry exclusion exerted by strains bearing dnaB⁴³, but resulted in 2 (+NaCl, 30°) and 4 fold (+NaCl, 42°) decreases in the establishment index (cf. expt. 2, 4 and 6). Generally similar changes occurred in non-mutant cells. It would appear that conversion of plasmid containing cells to "F-" phenocopy is more easily accomplished at elevated temperatures. Altering the ionic environment by addition of NaCl also seems to help.

(11) Construction of P1CM and P1CM bac lysogens.

Several strains bearing dnaB⁻ mutations are capable of serving as hosts for the propagation of certain bacteriophages at temperatures non-permissive for bacterial chromosome replication (Fangman and Novick 1968; Hirota et al, 1968; Lanka and Schuster, 1970). During a study of phage P1 growth under such conditions in strain HfrH165/70 (dnaB70, Bonhoeffer, 1966), Beyersman and Schuster (1971) noted a transient recovery of chromosomal DNA syntheses in a number of phage producing cells. Their suggestion that P1 might be supplying the host with material similar to the product of the wild-type dnaB allele, has received support in the recent work of D'Ari et al (1974).

P1 determines a product that is functionally analogous to the protein specified by the dnaB allele in non-mutant bacterial cells. Its action is repressed in wild-type prophage, but D'Ari et al (1974) have isolated mutants that are constitutive for its expression. All DnaB⁻ cells lysogenized with such P1 bac mutants acquired a capacity to form colonies at normally non-permissive temperatures.

The question thus arose as to what affect construction of such lysogens would have on the reduced superinfection immunity phenotype herein observed in CR34 strains carrying the lesion dnaB43. If the latter were directly involved in determining

this effect, one would expect cells containing active analog to display greater entry exclusion and establishment barriers than those carrying repressed analog protein.

Bacteriophage P1 lysogens of the RecA^- , DnaB^- , strains C050 (F^-) and C061 (F101) were prepared as outlined in section (1) of the Methods. Primary selection of prophage - containing cells was conducted on the basis of resistance to low levels of chloramphenicol (CM). Two sets of lysogens were employed: cells carrying P1CM served as analog - repressed controls (temperature sensitive) and those harboring P1CM bac as analog - constitutive test strains (temperature resistant). Matings were carried out as described in Methods section (e) with the various lysogens functioning as exponential and overnight phase recipients for the F42 donor, strain MA3050.

Initially, donors and recipients were mixed in liquid cultures. But recipient efficiencies were so reduced in the presence of lysogenic phage that all subsequent conjugations were done on membrane filter templates. Female strains (F^-) displayed decreases in the frequency of Lac^+ progeny ranging from 30 to 250 fold, relative to non-lysogenic cells. Those carrying P1CM suffered most. The recipient abilities of isogenic F prime F101 cells were diminished 50 to 170 times, with P1CM bac strains being the more affected. The latter is not entirely unexpected, however, since such lysogens ought to contain derepressed dnaB⁺ analog protein.

Switching to a solid template produced increases in progeny frequencies of from 10 (P1CM bac) to 60 (P1CM) fold. Even then the numbers of Lac⁺ cells fell, at the least, 50 times below those obtained in the absence of P1 prophage. It is not yet known if this behavior is specific to temperature sensitive cells or whether it occurs in non-mutant strains as well.

The values obtained for entry exclusion and establishment indices are given in Table 15. They represent averages of data resulting from two independent experiments. Mutant cells lysogenized with P1 particles contributing derepressed dnaB⁺ analog (expt. 1) exert considerably higher levels of superinfection immunity than do those bearing wild-type (repressed) prophage (expt. 2). In the information presented, the resistance offered to the uptake of plasmid F42 by exponential phase C061/P1CM bac is some 9 times greater than that observed in strain C061/P1CM (column III). Similarly, overnight cultures of the former were approximately 8 fold more proficient in prohibiting the establishment of the incoming F prime (column IV). That incompatibility barriers were likewise affected by the presence of wild-type dnaB analog is implied by the frequencies of Lac⁺ Thr⁺ Leu⁺ colony formation found in column VI. These results thus support the suggestion of there being a direct causal relationship between the presence of dnaB⁴³ and the altered superinfection immunity phenotype.

TABLE 15: INFLUENCE OF P1 PROPHAGE ON THE ABILITY
OF THE DnaB⁻ F101 BEARING CR34 Rec⁺
DERIVATIVE, C061, TO ACCEPT FACTOR F42
FROM MA3050

EXPT. NO.	RECIPIENT	1. EexI	2. EstI	2. EexI/EstI	3. FREQUENCY OF PROGENY Lac ⁺ Thr ⁺ Leu ⁺
1	C061/PlCMBac	46.4	19.6	2.35	0.0158
2	C061/PlCM	5.02	2.54	1.96	0.0475

1. Donor/Recipient: 10:1; 60 minutes at 30° on membrane filters (See Methods).
2. EexI: Entry Exclusion Index; EstI: Establishment Index (See Table 12)
3. Number of Lac⁺ Thr⁺ Leu⁺ colonies per 100 overnight phase recipients.

(111) Construction of dnaB merodiploids.

A third approach has been to construct Rec⁻ derivatives that are merodiploid for the dnaB region. The F prime plasmids F134-1 (F' thr⁺ leu⁺ dnaB⁺ met⁺) and F118 (F' mal⁺ dnaB⁺ pyrB⁺) were introduced into strains C050 (F⁻, dnaB⁴³) & C09 (F⁻, dnaB⁺) by conjugation from donors JC1553/F134-1 and KL132/F118 respectively. F134 containing progeny were isolated by selection for threonine and leucine independence. Since colonies obtained with C050 recipients displayed from 60 - 90% survival at normally restrictive temperatures, the dnaB⁻ gene is complemented and must be trans-recessive. Similar conclusions were reached with merodiploid strains constructed by Wechsler and Gross (1971). Two such F134 derivatives, strains C049 (dnaB⁺/F' dnaB⁺) and C060 (dnaB⁴³/F' dnaB⁺) were employed in subsequent experiments.

Since factor F118 carried no markers easily identifiable in the CR34 background, it was not possible to isolate F prime cells directly on minimal agar. Instead, upon completion of mating all recipient cells were selected, then, tested by replication to nutrient agar for their capacity to grow at 37° and 42°. Seven of the 100 colonies examined grew at 37° while 3 grew well at 42°. Only one of the strains growing at 37°, C071-37, and all three that grew at 42° were R17 sensitive. One of the latter, strain C071-42, and C071-37 were employed in the following studies.

Merodiploids derived in the above manner were compared with an isogenic F101 carrying strain (C061) for their ability to accept a second F prime plasmid while in exponential or overnight phases of growth. Strain MA3050 served as a donor of plasmid F42. Matings were conducted in liquid culture at 30° as described in section (e) of the Methods.

When the F134-1 derivatives, strains C049 and C060, were recipients, production of Lac⁺ progeny was monitored on lactose minimal agar supplemented with threonine, leucine, thiamine and streptomycin (50 µg/ml). Lac⁺ Thr⁺ Leu⁺ colonies were isolated on lactose medium containing only thiamine and streptomycin. To achieve direct selection of double males from among the exconjugants of a cross involving strain C071-37, (F118⁺), appropriately diluted mating mixtures were spread on the same medium as used for selection of Lac⁺ single males, but were subsequently incubated at 37 rather than 30°.

Unlike C071-37, strain C071-42 was incapable of growth on minimal agar satisfying the auxotrophic requirements of the F⁻ parent. It flourished only when spread on enriched medium. The other two F118 derivatives growing at 42°, showed identical behavior. Acquisition of the ability to survive incubation at the elevated temperature was apparently accompanied by development of some yet undetermined auxotrophic requirement.

The frequency at which this occurs (3/100) in a RecA^- strain and the generation of temperature sensitive segregants during incubation in NB at 30° by C071-42 argued against possible integration of F-DNA into the chromosome (Strain KL132/F118, the original source of F118, behaved in a similar manner but continued to transfer its R17 sensitivity to the Rec^- recipient C050). Hence, for want of more unequivocal evidence, strain C071-42 was considered to harbor an autonomous F prime plasmid and therefore suitable for use in the present investigations.

In crosses involving this strain, lac^+ transfer was determined by spreading exconjugants on EMB-lactose agar supplemented with 0.4% (W/V) Nutrient Broth and 50 $\mu\text{g/ml}$ streptomycin. F42/F118 double males were selected by incubation of the same at 42° .

Information obtained from this type of experiment is illustrated in Table 16. Data presented in experiments 1 to 4 are averages of at least two different determinations. The presence of F118 in unmated recipients was determined by measuring their sensitivity to the F-specific phage R17.

TABLE 16: INFLUENCE OF THE dnaB⁺ ALLELE ON THE ABILITY OF WILD-TYPE (C049) AND dnaB43 - CONTAINING (C060, C071-37) Φ PRIME CR34 Rec⁻ STRAINS TO ACCEPT F42 FROM MA3050 : COMPARISON OF dnaB⁺ / F'dnaB⁺ MERODIPLOIDS (C049, C060, C071-37) WITH dnaB⁺/F'thr⁺leu⁺(C011) AND dnaB43/F'thr⁺leu⁺(C061).

EXPT. NO.	RECIPIENT	1. FREQUENCY OF Lac ⁺ PROGENY 2.3.		2.3. FREQUENCY DOUBLE MALES	
		EXPONENTIAL	OVERNIGHT	(a) Lac ⁺ Thr ⁺ Leu ⁺ progeny	(b) Lac ⁺ TR Progeny *
1	C061	0.0172	0.0320	0.0254	
2	C060	0.0012	0.0013	0.0010	
3	C011	0.0011	0.0014	ND ⁺	
4	C049	0.0010	0.0014	ND ⁺	
5	C071-37	(0.2375)	0.0066		0.0053

1. Matings as described in the Methods. Donor/Recipient: 10:1, 60 minutes in NB at 30°.
 2. Number of Lac⁺(Lac⁺thr⁺leu⁺) colonies per 100(overnight phase) recipient cells.
 3. Average of at least two determinations.
- + : Not detected.

The introduction of the wild-type allele had a dramatic effect on the dnaB43 recipients capacity to restrict the uptake of a superinfecting F prime plasmid. The recipient efficiencies of these cells was markedly reduced. In experiment 2 (Table 16) substitution of factor F134-1 for F101 (expt. 1) resulted in decreases in the frequencies of Lac⁺ progeny obtained with exponential (column III) and overnight (column IV) phase recipients of 14 and 25 fold respectively. The number of Lac⁺Thr⁺Leu⁺ cells was correspondingly diminished (column V). Values similar to these were noted for the acceptance of donor markers by non-mutant F101 (expt. 3) and F134-1 (expt. 4) derivatives.

Factor F134-1 carries non-F genetic markers running from metB to leuA and hence, accounts for approximately 15 minutes of the E coli chromosome. Cells bearing F134-1 are therefore diploid for a considerable portion of the fourth quadrant of the circular map (see Fig. 1). Thus experiment 2 of Table 16 does not allow the conclusion that complementation of dnaB43 by the wild-type allele specifically has resulted in the observed decrease in recipient ability. Ploidy for any gene within the region duplicated by F134-1 may just as well have produced these results (contribution of F134 minus that of F101 still leaves 10 minutes or, at least, 50 genes; Taylor and Trotter, 1972).

To lessen this possibility, a strain merodiploid for dnaB but carrying significantly less extraneous material (approx. 5 minutes) was formed by introducing factor F118. Information obtained from these recipients is given in Table 16, experiment 5.

As mentioned before, two types of F118 derivative were utilized. Strain C071-37 was sensitive to R17 and temperatures above 37°, while strain C071-42 was also R17 sensitive but grew well at 42°. The former possessed another distinguishing property. Its ability to plaque F-specific phage was unstable and quickly reduced to undetectable levels in rapidly growing NB cultures, even at 37°. For this reason, very little entry exclusion was observed in exponential C071-37 cells. Recipient efficiencies of the same order of magnitude as those noted in F⁻, DnaB⁻, strains were recorded (expt. 5, column III). Slower growing, overnight recipients did, however, retain some phage sensitivity and were thus capable of opposing the presence of factor F42. In experiment 5, a decrease in recipient efficiency of 5 fold, relative to the F101 control (expt. 1) was observed (column IV). Direct selection for F118/F42 double male strains by incubation at 37° gave a frequency of temperature resistant Lac⁺ colonies of 0.0053%.

In contrast to C071-37, both exponential and overnight cultures of strain C071-42 demonstrated a high degree of sensitivity to R17. However, progeny bearing F42 were not detected when the latter was used as a recipient in experiments identical to those mentioned above (0.001% level of detection). Strain C071-42 is either defective in its capacity to serve as a recipient or is able to exert an unusually high degree of immunity to superinfection by the second plasmid.

Repetition of these conjugations under conditions known to effect a 60 to 80 fold increase in progeny frequencies (ie; membrane matings, see Results sec. (h)) yielded similar results. And efforts to isolate temperature sensitive, R17 resistant, segregants of C071-42 meet with no success. All temperature sensitive strains tested (32/32) still plaqued male specific phage.

The information presented in Table 16 indicates that superinfection immunity functions are restored to a high level in F134-1 and F118 derivatives of the dnaB43 bearing strain, C050. These plasmids carry the wild-type allele of dnaB which is dominant. Thus, within the limits of the area covered by each F prime element, it appears that the reduced immunity phenotype occurring in DnaB⁻ cells in their absence is directly attributable to dnaB43 or a closely linked gene.

(iv) Removal of dnaB43 to a different genetic background.

Should the temperature sensitivity and immunity effects be due to the same or closely linked genes, one would expect these phenotypes to accompany each other upon transfer of the dnaB region to a new genetic milieu. As a by-product of an independent series of experiments conducted in this laboratory, P1 - mediated transduction of dnaB43 from CR34TS into the K12 strain JE2571 was achieved (Iyer et al., 1974). The availability of this strain (C101) thus, made such a test possible.

Preliminary screening of transductants was done on the basis of acquisition of resistance to low levels of ampicillin (Figure 1). Therefore, a temperature resistant, ampicillin resistant transductant, strain C099, was used as the control.

The maximum limit of any unknown genetic difference between C101 and C099 is therefore defined by the small distance between ampA1 and dnaB43. (2 mins.) F101 containing derivatives of C099 and C0101 were obtained by conjugation with strain C076.

The resultant F primes, strains C043 and C045, were mated in exponential and overnight phase with the F42 donor MA3050 according to previously outlined procedures (Methods, sec.(e)).

For reverse order of entry experiments, $\text{Lac}^+ \text{Thr}^- \text{Leu}^-$ progeny from these experiments, strains C039 and C044 (dnaB43) were mated with the F101 donor C076. Selection for Lac^+ , $\text{Thr}^+ \text{Leu}^+$, and $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ progeny was carried out on appropriately supplemented minimal agar. Values obtained for

EexI and EstI are presented in Table 17. These are averages of two independent experiments.

The altered immunity phenotype does appear to be co-transducible with the locus determining sensitivity to elevated temperatures. The dnaB43 carrying strain, C045, is significantly less proficient in inhibiting the uptake and establishment of factor F42 than is the isogenic DnaB⁺ strain C043 (Table 17, expt. 1 and 2). Acquisition of dnaB43 has resulted in a 5 fold decrease in the exclusion index and a 4 fold decrease in EstI (columns IV and V). Further, there is a noticeable increase in the frequency of double males in the dnaB43 - bearing culture upon direct selection for Lac⁺ Thr⁺ Leu⁺ progeny (column VI). Reversal of the order of plasmid entry yielded essentially similar results (expt. 3 and 4).

The degree to which entry exclusion and incompatibility barriers are lifted in these strains is not as great as was noted in the CR34 line (eg: Table 12, expt. 1 and 2). It should be noted, however, that the parent of the present line, strain JE2571 does not produce common (Type-I) pili (Nishimura et al., 1967), and that the role, if any, that these appendages play in conjugation is not yet known. In addition, it has been reported that development of resistance to ampicillin is often accompanied by changes in cell membrane properties (Monner et al., 1971).

TABLE 17: BACTERIOPHAGE P1 MEDIATED TRANSFER OF dnab43 TO
A DIFFERENT GENETIC BACKGROUND: EFFECT ON
SUPERINFECTION IMMUNITY.

EXPT. NO.	DONOR	1. RECIPIENT	1. EexI	2. EstI	3. FREQUENCY Lac ⁺ Thr ⁺ Leu ⁺ PROGENY
1	MA3050	C043	98.2	29.6	0.0106
2	"	C045	20.0	7.20	0.0315
3	C076	C039	11.6	4.25	0.0258
4	"	C044	6.08	2.45	0.0499

1. Strains C043 & C039: dnab⁺; strains C045 & C044: dnab43. Matings as described in the Methods. Donor/Recipient: 10:1, NB, 60 minutes at 30°; (All strains Rec⁺)
2. EexI: Entry Exclusion Index; EstI: Establishment Index (See Tables 11 & 12).
3. Number of Lac⁺Thr⁺Leu⁺ colonies per 100 overnight phase recipient cells.

The increase in E_{exI} and E_{spI} values which occurred in CR34 recipients upon replacement of F42 by F101 is also observed with the strains employed in Table 17, (expt. 1 and 3, 2 and 4), suggesting that this effect is strain independent (see Results, sec.(e)). F101 and F42 derivatives of C101 (strains C045 and C044 resp.) also display the altered sensitivity to male specific phage initially observed in $DnaB^-$ CR34 cells (Bezanson, data not given; and Iyer et al, 1974).

D.

Y*

(g) Division Rates and Recipient Efficiencies of F
Prime Cells: Influence of dnaB43

In section (a) of the Results, the efficiency with which F101 derivatives of wild-type and dnaB43 containing CR34 strains formed F42/F101 double male strains was determined. DnaB⁻ cells grown to exponential phase in NB at the permissive temperature of 30° exerted significantly lower levels of resistance to the uptake of factor F42 than did non-mutant strains (Table 12, expts 1-4, EexI). This enhanced capacity for accepting the second F prime plasmid is further illustrated in the frequencies with which Lac⁺ colonies were formed during matings involving dnaB43/F' dnaB⁺ merodiploids (Table 16, expts 1, 2 and 3). Colonies bearing F42 associated markers occurred in the DnaB⁻ strain (C061) at a level some 15 times greater than in the wild-type (strain C011, column III).

In Results section (b), the mean generation times of the recombination defective F101 strains, C011 and C061, during exponential growth in NB at 30° were calculated as 55.2 and 69.2 minutes respectively (See Table 7). Cells carrying dnaB43, that is, strain C061, therefore divide more slowly under the conditions used for measuring entry exclusion in Table 12. Hence, it is possible that the effect this mutation has on superinfection immunity stems in part from its action on cell growth.

Cultures duplicating at a slow rate do offer less opposition to the acceptance of a second F plasmid (Tables 11 and 12 exponential vs overnight recipients). The possibility that the presence of dnaB43 results in an analogous effect in exponential cultures was considered. It should, however, be noted that in RecA^+ strains, the difference in growth rate between F prime strains carrying or not carrying dnaB43 is minimal or absent (Table 7, This Thesis; Iyer et al, 1974). If this effect is important in RecA^- strains, any treatment that increases the difference in generation times between strains C011 and C061 would be expected to also enhance the recipient ability of the latter relative to the former. Data is available from previous studies in this section that allows a test of this premise.

In section (f-i) the effect of temperature on the recipient ability of these strains during growth in NB containing 0.42M NaCl was examined. Data concerning the division rates and frequencies of Lac^+ progeny formation generated during these studies are given in Table 18, experiments 2 and 3. Information obtained from cultures incubated in NB at 30° in the absence of NaCl is included for comparison (expt.1). Changes in the recipient ability of one strain relative to the other are expressed as the ratio of the number of Lac^+ progeny formed with DnaB^- recipients to that achieved non-mutant cells.

TABLE 18: EFFECT OF TEMPERATURE ON THE GROWTH RATES AND RECIPIENT EFFICIENCIES OF EXPONENTIAL CULTURES OF WILD-TYPE (C011) AND DnaB⁻ (C061) CR34 Rec⁻ STRAINS CARRYING FACTOR F101

EXPT. NO.	RECIPIENT	1. GROWTH CONDITIONS	GENERATION TIME (MINS.) ²	Lac ⁺ PROGENY Frequency ³	Ratio ⁴
1	C011	NB ⁺ , 30°	55.2	0.0011	37.2
	C061	NB ⁺ , 30°	69.2	0.0410	
2	C011	NB+0.42M NaCl, 30°	72.8	0.0257	2.77
	C061	NB+0.42M NaCl, 30°	91.9	0.0714	
3	C011	NB+0.42M NaCl, 42°	128.2	0.0714	1.46
	C061	NB+0.42M NaCl, 42°	198.5	0.1043	

1. All cells subcultured from overnight growth under same conditions. Recipient cell numbers adjusted to 1.5-3.6x10⁷ cfu/ml before mating with 10-fold excess of F42 donor, MA3050, for 60 minutes.

2. Average of at least two measurements.

3. Number of Lac⁺ colonies per 100 recipient cells.

4. Frequency of Lac⁺: C061/C011.

+ Nutrient Broth.

Addition of 0.42M NaCl at 30° resulted in a lengthening of the times between divisions for both wild-type (C011) and mutant (C061) cells (column IV, expt 1 and 2'). The number of Lac⁺ colonies formed with each recipient increased as well (column V). The greatest change occurred in strain C011 where the frequency rose some 23 fold. Under these conditions, the differences in generation times between wild-type and DnaB⁻ strains was 19.1 minutes (expt. 2, column IV). The ratio of Lac⁺ progeny was 2.77 (column VI).

Incubation at 42° in the presence of salt markedly lengthened the generation times of both strains (expt. 3, column IV). Lac⁺ colony frequencies were increased in both C011 and C061 recipients (column V). The difference in generation times at 30° and 42° between the two strains was extended from 19.1 to 70.3 minutes. At this higher temperature, the ratio of Lac⁺ progeny was 1.46 (column VI). Thus a 3.5 fold increase in the difference in mean generation times between C011 and C061 was accompanied by a 2 fold decrease in their recipient abilities relative to each other.

This reduction in relative recipient ability occurred because the elevation in incubation temperature enhanced the efficiency with which the wild-type strain could accept a second F plasmid to a greater extent than it did for the DnaB⁻ strain (cf: column V, expts. 2 and 3). Had dnaB43 acted to gain a lifting of superinfection immunity barriers indirectly through slowing growth rates in F prime cells, just the opposite would have been expected.

Rather, the present results indicate that the lengthening of generation times is non-specific in its effect upon recipient ability. An increase in the period of time between cell duplications was accompanied by a rise in the efficiency with which markers associated with a second F plasmid were accepted in both C011 and C061 (expt 3, column V).

Similar behavior was observed with the F⁻ derivatives C050 (dnaB43) and C09 (data not given.) It is interesting to note in this regard that the former organism has a mean generation time in NB at 30° (-NaCl) of 38.7 minutes; just 3 minutes longer than the wild-type (Table 4). Yet, it gives rise to Lac⁺ progeny during conjugation with MA3050 at a frequency five times greater than that observed for C09 (See Table 5, expt 12 and 15).

CR34 RecA⁻ cells merodiploid for the dnaB region (F'dnaB⁺) divide rapidly (Table 7; strains C049 & C060) and are poor recipients (Table 16). As with its other F prime derivatives the strain carrying dnaB43, C060, has a longer generation time than the isogenic non-mutant cell (C049). In this particular instance, the difference is 10 minutes. Yet, both form Lac⁺ progeny in matings with MA3050 at virtually identical frequencies ($1.0-1.2 \times 10^{-3}$). Here, then, is additional support for the effect of dnaB43 on generation times being independent of its action on recipient ability. Had changes in the latter simply and directly resulted from changes in the former, cells containing the wild-type dnaB allele would have shown equal division rates as well as recipient efficiencies.

(h) Influence of Altered Conjugation Conditions on dnaB43 Action

Sheehy et al (1972) have examined certain aspects of entry exclusion as it occurs in crosses between Hfr donors and F prime plasmid containing recipients. As part of this work, the effect of the presence of dnaB43 on recipient ability was monitored. Factor F101-containing minicells derived from such DnaB⁻ parents failed to show the reduction in entry exclusion previously reported by Palchaudury and Iyer (1971) to occur in CR34 strains bearing the same mutation (strain no. 7 of Bonhoeffer, 1965).

The conditions used by the two groups of investigators differed considerably. Whereas, Sheehy et al grew donors and recipients in a Casamino Acids-enriched minimal salts solution then mated them on a membrane filter, the second group incubated cells in Penassay Broth and carried out conjugation in liquid cultures. Further, Palchaudury and Iyer measured entry exclusion in F prime X F prime crosses where both donor and recipient contained chromosomal DNA (that is, non-minicells).

These differences may form the basis of Sheehy et al's inability to detect any lifting of exclusion barriers in their studies. Cells grown in a partially restrictive medium, then mixed on membrane filters, may not be in the same "conjugative state" as those grown and mated in enriched media. Alternatively, the dnaB43 effect might be specific for F prime/F Prime interactions and, hence, has no influence in the presence of Hfr donors.

Finally, the possibility exists that the dnaB43 product (s), if diffusible, is not segregated into the chromosome - less cells during their formation at the polar regions of the normal sized parent. Thus material essential for the dnaB43 action on entry exclusion would not be present in F prime bearing minicells.

To assess the effect of different growth and mating conditions on the altered immunity phenotype characteristic of CR34 DnaB⁻ cells, a typical set of F prime X F prime crosses were repeated using the procedure described by Sheehy et al (1972). The specificity of the dnaB43-mediated effect was also determined. Strains CSH61, a streptomycin sensitive, Cavalli-type Hfr that transfers lac⁺ as an early marker, was employed as a donor in crosses with F101 derivatives of wild-type (C07) and temperature sensitive (C047) recombination proficient strains of CR34. Should neither of these operations result in the restoration of "normal" (wild-type) levels of exclusion in dnaB43 bearing cells, then, the last explanation regarding minicell compartment-alization would gain support.

Overnight cells were subcultured (1:20) into Curtiss nutrient medium (Methods sec. (c)), then shaken at 30° until well into exponential growth (4-5 hours). Upon adjustment of cell numbers, donors and recipients were mixed, then collected on 0.45μ membrane filters as described in the Methods (sec. (e)). Conjugation was continued for 60 minutes. Data from these experiments appear in Table 19.

TABLE 19: INFLUENCE OF dnaB43 (C06L, C047) ON THE RECIPIENT EFFICIENCIES OF F101 BEARING RECOMBINATION PROFICIENT (C07, C047) AND DEFECTIVE (C011, C061) CR34 STRAINS DURING MATING ON MEMBRANE FILTERS.

EXPT. NO.	1. DONOR	2. RECIPIENT	3. FREQUENCY OF $\text{Iac}^+ \text{Thr}^+ \text{Leu}^+$ PROGENY
1	MA3050 (F')	C011	85.6
2	"	C061	15.5
3	CSH61 (Hfr)	C07	16.3
4	"	C047	1.35
			0.0372
			0.8253
			0.0364
			0.4261

1. All cells grown to log. phase in Curtiss Nutrient Medium (See Methods), Donor/Recipient: 10:1, 60 minutes at 30°.
2. Expt: Entry Exclusion Index (See Table 12).
3. Number of $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ colonies per 100 exponential phase recipients.

The influence of the presence of dnaB43 on entry exclusion persisted despite these changes. In the F prime X F prime crosses (Table 19, expts 1 and 2), the RecA⁻ DnaB⁻ strain C061 displayed an EexI value 6 fold less in magnitude than that obtained for non-mutant cells (column IV). The frequencies at which Lac⁺Thr⁺Leu⁺ colonies occurred also attest to this enhanced capacity for accepting a second F plasmid. The mutant strain gave rise to 13 times as many doubles (column V). Wild-type cells (C011) grown in Curtiss nutrient medium offered less resistance to the entry of factor F42 than did NB cultures (see Table 12, expt. 3). A similar decrease in EexI was observed when this type of cross was repeated using Tris Maleate Minimal medium grown cultures (data not given).

Substitution of an Hfr donor (CSH51) did not alter the difference in entry exclusion indices between Rec⁺ wild-type and mutant strains (Table 19 expts 3 and 4). That bearing dnaB⁺ (C07) was some 12 fold more proficient at exerting exclusion functions (column IV). Similar variation was noted in the frequencies of Lac⁺Thr⁺Leu⁺ progeny formation (column V). Thus, neither the change in

culturing and mating conditions nor in donor-type appear to account for Sheehy et al's failure to detect any lifting of entry exclusion in the presence of dnaB43.

Although not evident in Table 19, the shift from a liquid (NB) to a solid (membrane) conjugation template produced a generalized increase in progeny frequencies of from 60 to 80 fold. The greatest increase occurred in F prime ~~to~~ F prime type matings.

(i) Evidence Indicating That the DnaB⁻ Effect Is Not Limited to dnaB43.

The bulk of the information provided so far tends to indicate a direct role for the dnaB43 lesion in the lifting of superinfection immunity barriers seen in temperature sensitive CR34 strains. It is desirable to determine whether this effect is peculiar to dnaB43 or is of more general occurrence in the dnaB set of conditional lethal mutations. Time and circumstances have permitted the examination of only one other DnaB⁻ mutant.

Strain E107 (F⁻) bearing the mutation dnaB107, was isolated from strain CR34G by ethylmethane sulfonate mutagenesis at 30° (Hechemy and Goldfine, 1971). Like the Bonhoeffer mutant (CR34TS43), E107 displays an immediate stop in chromosome replication followed by DNA breakdown upon incubation at non-permissive temperatures (Wechsler and Gross, 1971). Further, it is recessive to the wild-type allele and sodium chloride remedial (Bezanson, data not given). The presence of dnaB107 also results in altered recipient abilities. As in recombination proficient CR34 strains, E107 displays an increased capacity for accepting factor F101 (Table 5, expts 1&3). However, its ability to take up F42 is significantly reduced (expts. 5&7).

A F101 derivative of E107, strain C029, was utilized as a recipient in a cross with the F42 donor, MA3050, in order to determine whether the presence of dnaB107 exerted any influence over superinfection immunity functions in this organism. Data resulting from such experiments is given in Table 20. All matings were conducted at 30° in liquid cultures as described in the Methods. Media employed for progeny selection was identical to that used in Table 12 for Rec⁺ CR34(B) strains.

During a study of the sedimentation properties of total cell lysates of a number of the E series of DnaB⁻ mutants, Wang, (1974) detected the presence of a previously unreported extrachromosomal DNA element in F⁻ derivatives of strains E107 and E279. This factor carries no known genetic markers and in E107 accounts for approximately 0.9% of the total acid insoluble radioactivity. A similar element was subsequently found in lysates of the parental strain CR34G. It was, however, absent in strain CR34(B); as obtained from F. Bonhoeffer. Thus, it was felt necessary to use a F101 carrying derivative of CR34G, strain C014, as the control in Table 20 (expt. 1).

F prime strains bearing the dnaB107 lesion do appear to offer less resistance to the uptake and establishment of a second F plasmid (Table 20, expt 2).

TABLE 20: INFLUENCE OF dna.B107 ON THE ABILITY OF WILD-TYPE (C014) AND dnaB⁻(C029) CR34G F101 DERIVATIVES TO ACCEPT FACTOR F42 FROM MA3050.

EXPT. NO.	RECIPIENT	1. ExI	2. EstI	3. FREQUENCY Lac ⁺ Thr ⁺ Leu ⁺ PROGENY
1	C014	75.9	44.4	0.0015
2	C029	16.2	13.2	0.0040

1. Matings as described in the Methods. Donor: Recipient: 10:1, NB, 60 minutes at 30°. (All strains Rec⁺)
2. ExI: Entry Exclusion Index, EstI: Establishment Index (See Table 12).
3. Number of Lac⁺Thr⁺Leu⁺ colonies per 100 overnight phase recipients.

The entry exclusion index obtained with the DnaB^- strain, C029, was approximately 5 times smaller than that of the wild-type (column III). Similarly, overnight cultures of C014 were the more proficient in their opposition to the incoming F42 factor (column IV). Direct selection for $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ doubles also indicated a preference for their formation in the mutant recipient (column V). The values given are averages of two independent experiments.

Thus it seems that strains bearing dnaB107 too do express an altered superinfection immunity phenotype. With the results in Table 20, its effect upon EexI and EstI (4.7 & 3.4X reductions) is not as great as that of dnaB43 (Table 12, 7-9X reductions). This may, in part be related to the reduced capacity of F^- cells carrying the first mutation to accept F42 (Table 5). Strain E107 does however possess an enhanced ability, relative to wild-type CR34(B) cells, for taking up factor F101. It would be of interest, then, to conduct a series of reverse order of entry experiments since these would be more comparable to the studies with organisms harboring dnaB43.

The present data clearly does not permit any decision as to the universality of the DnaB^- effect. It is of value, though, in that it indicates that two independent dnaB $^-$ mutations achieved (from nearly identical parents) by treatment with two different chemical mutagens do produce the same effect on F prime plasmid

determined superinfection immunity. This supports a belief that that dnaB⁻ effect is gene specific rather than allele specific.

In passing, it may be worth mentioning that these studies (Table 20) indicate the presence of the cryptic DNA-containing factor in strain CR34GF⁻ to have no adverse effect on its conjugational recipient ability. Rather, the frequency of Lac⁺ and Thr⁺Leu⁺ progeny is somewhat enhanced over that observed with CR34(B)F⁻ recipients (0.1886 vs 0.1492% and 0.0828 vs. 0.0250% for Lac⁺ and Thr⁺Leu⁺ respectively). Hence, it is unlikely that the new extrachromosomal element is of the same incompatibility set as the F plasmids (Novick, 1969).

(j) Characterization of Presumptive Double Male Strains.

Determination of the frequencies at which progeny carrying marker genes initially associated with two independent F plasmids are formed in F⁻ phenocopied cells suggests that CR34 strains bearing the mutation dnaB⁴³ possess an increased capacity for permitting the coexistence of normally incompatible F prime factors. This behavior is independent of which factor (F101 or F42) is resident in recipient cells (Tables 11 & 12, expt. 1 and 2), and does not require the presence of recombination functions (Tables 11 & 12, expt. 3 and 4). Since this lifting of incompatibility barriers persists under conditions of phenotypic suppression of temperature sensitivity (Table 14, expt. 5 & 6), but is diminished in strains merodiploid for the dnaB region (Table 16, expt. 1 and 2), it is very likely a primary effect of the presence of dnaB⁴³.

Assuming the lac⁺, thr⁺ leu⁺ markers still to be physically attached to F DNA, the question arises as to the condition of the F plasmids in these double male strains. Since the DnaB⁻ effect is retained upon introduction of recA1, the possibility of escape from incompatibility functions by integration of plasmid and chromosome is greatly reduced, if not eliminated. This leaves two possible alternatives. The two F prime plasmids must either continue to exist as independent, autonomous units or unite to form a single plasmid element (Press et al., 1971).

Because host cells are RecA^- , the latter would involve either plasmid determined or so called "illegitimate" recombination events (Franklin, 1971). Whether the two factors remain independent or not, immunity functions could be overcome either by suppression or deletion of the incompatibility (inc) region in one (or both) of the F plasmids. The velocity sedimentation properties of lysates of presumptive double male strains and the assortment patterns of plasmid markers were examined in an effort to evaluate these possibilities.

The majority of strains for use in these studies were to be taken from a medium selective for the retention of markers associated with both F42 and F101. However, as is evident in Tables 11 and 12 (expt. 3), few such $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ colonies were available in recombination deficient, DnaB^+ strains. To remedy this, several of these matings were repeated using a 10 fold increase in cell numbers. The latter was accomplished using the procedure outlined in section (f) of the Methods. This allowed the isolation of sufficient numbers of wild-type presumptive double males for testing.

The difference in recipient efficiency between non-mutant and DnaB^- strains was maintained under these circumstances. The frequency of $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ progeny achieved with the former ranged from 0.0079 to 0.0120% while values of 0.0139 to 0.0579% were recorded for the dnaB43 carrying strains.

(1) Cell growth: generation times

Cellular division rates typical of wild type and dnaB⁴³ containing recombination deficient "double male" strains are presented in Table 21. The generation time of the DnaB⁻ presumptive double male strain, C050DMS2, was 12.2 minutes longer than that of the non-mutant, C09DML6. Introduction of the second F plasmid (F42) has added about 10 minutes to the time between cell divisions in both types of cell (cf: Table 7). Such an effect further supports the earlier contention of the existance of some form of interaction between F plasmids and the cellular duplication machinery (see Results, sec.(c)).

Table 22 summarizes the changes in cell division rates obtained upon sequential addition of F plasmid DNA to wild-type and DnaB⁻ cells. The values given express the increase in generation times relative to that observed for the F⁻ condition. The bracketed number is the ratio of the generation times of double males (DM) and single males (SM). Introduction of factor F101 followed by F42 had its greatest effect on the division rate of the dnaB⁴³ strain, C050. Doubling the number of F plasmids in the cytoplasm also doubled the period between cell duplications. The bulk of this change came with addition of the first plasmid, F101 (cf: ratio SM/F⁻ vs. DM/SM).

**TABLE 21: CELLULAR DIVISION RATES OF
WILD-TYPE (C09) AND DnaB⁻ (C050)
CR34Rec⁻F101/F42 DOUBLE MALES
DURING EXPONENTIAL GROWTH IN
NUTRIENT BROTH AT 30°.**

STRAIN	GENERATION TIME (MINS)	1.	2.
		EFFECT OF <u>dnaB43</u>	
C09DML6	66.6		1.18
C050DMS2	78.8		

1. Average of at least two measurements.

2. Ratio: DnaB⁻/wild-type.

**TABLE 22: INFLUENCE OF F PRIME PLASMIDS
ON THE GENERATION TIME OF
WILD-TYPE (C09) AND dnaB⁻ (C050)
CR34 Rec⁻ STRAINS**

STRAIN 1.	RELATIVE GENERATION TIME 2.		
	F ⁻	SM	DM
C09	1.00	1.55	1.86 (1.20) +
C050	1.00	1.79	2.03 (1.18) +

1. During log. growth in NB at 30°.

2. Relative to F⁻ condition; SM: single male (F101);
DM: double male (F101/F42)

+: Ratio: DM/SM.

(11) Velocity sedimentation of cell lysates of presumptive double males.

The sedimentation properties in alkaline sucrose gradients of DNA present in total lysates from strains C050, C059, C061 and the presumptive double males C09DML6 and C050DMS2 are depicted in Figure 5 (a-d). Cells were incubated overnight in TMM medium selective for retention of plasmid associated marker genes, then labelled with [^3H]- thymidine and lysed according to the procedure described in the Methods. Immediately prior to lysis, a portion of each culture was tested for the presence of F - associated genetic markers spreading on the appropriate selective agar.

The female DnaB⁻ strain showed a complete absence of supercoiled extrachromosomal DNA (C050, fig. 5-a). Its F⁴² and F101 derivatives, strains C059 and C061, each had a single plasmid band peaking at fractions 18 and 13 respectively (fig. 5 b & c). In C059, the amount of plasmid DNA extracted equalled 0.82% of the total DNA present in the lysate; for F101 cells the value was 0.61%. Similar patterns occurred in lysates from DnaB⁺, Rec⁻, cells (not shown). In these cultures F⁴² accounted for 1.91% of the total acid insoluble counts while F101 amounted to 1.01%.

Lysates of DnaB⁻ and wild-type presumptive double males both revealed the presence of two distinct plasmid bands (fig. 5-d).

FIGURE 5: ALKALINE SUCROSE DENSITY GRADIENT ANALYSIS OF SDS TOTAL CELL LYSATES OF F⁻ (a), F42 (b) F101 (c) AND F42/101 (d) DERIVATIVES OF dnaB43 BEARING (C050, C059, C061, C050DMS2) AND WILD-TYPE (C09DML6) CR34RecA⁻ STRAINS. -0-0-1 DnaB⁻; -0-0-: WILD-TYPE.

Cells labelled with [³H]- thymidine for 4h. in the presence of deoxyadenosine on selective minimal medium, concentrated, lysed, then placed on a 5-20% sucrose gradient and centrifuged for 25 minutes at 40,000 rpm in a SW65 rotor of a Beckman. Model LS-230 centrifuge. Nine drop fraction were fixed on 3mm filter paper discs by treatment with cold 5% Trichloroacetic Acid.

As in single males the two bands peaked at fractions 13 and 18. In C09DML6 (dnaB⁺) the peaks were shifted slightly downwards to fractions 12 and 17. However, they still remained five fractions apart. This shift most likely resulted because of technical reasons since the chromosome peak was also slightly shifted towards the bottom of the gradient. In the mutant double male, C050DMS2, F42 and F101 each contributed 0.46 and 0.35% of the total DNA content giving a lac⁺ to thr⁺ leu⁺ ratio of 1.31 not unlike that of 1.34 found in single males. Values of 0.68 and 0.57% were calculated for factors F42 and F101 respectively in the wild-type double male resulting in a ratio of 1.19 (cf: 1.89 for single males). From 95 to 100% of both double male cultures were Thr⁺Leu⁺ at the time of lysis. In the non-mutant strain, 59% and in C050DMS2, 74% of these were also Lac⁺.

These results are interpreted as implying the existence of two independent and autonomous plasmids in a large majority of cells from both wild-type and DnaB⁻ presumptive double male strains that were selected in this manner (see also stability studies, Table 23). Although the amount of each plasmid present in lysates decreased in double male strains, their proportions relative to each other remained fairly constant, especially in the strain bearing dnaB⁴³. It is interesting to note that the sum of the contributions made by each factor in the latter nearly equals that made by F42 alone in the single

male. In non-mutant double males, the sum of the two accounts for only 65% of the contribution made by the single factor. Finally, since the plasmid bands peaked at similar positions in single and double males, the molecular weight of each F plasmid must have remained unchanged in the latter.

(iii) Assortment of F plasmid associated markers.

Velocity sedimentation analysis of lysates of two randomly selected F⁴²/F¹⁰¹ double male strains suggested that in the majority of cells sampled, the plasmids exist as two distinct units, each quite autonomous from the chromosome (See (j) sub-section ii). Efforts were made to determine whether such behavior is of general occurrence. The effect of growth in an environment non-selective for plasmid retention upon the assortment and loss of plasmid associated markers from a number of wild-type and DnaB⁻ presumptive F prime double males was monitored. Three types of behavior might be anticipated. Strains with plasmid DNA physically associated (integrated) with the chromosome might be expected to show minimal loss of plasmid markers, even upon prolonged incubation. Cells harboring plasmids independent of the chromosome would display much lower levels of marker stability. Among these, markers associated with a single combined plasmid would fail to show independent patterns of assortment.

Presumptive double males were picked directly from the appropriate minimal agar and grown for varying lengths of time in NB at 30°. The frequency of occurrence of Lac⁺, Thr⁺ Leu⁺ and Lac⁺Thr⁺Leu⁺ cells in the resultant cultures was then determined using procedures previously described in the Methods section (g). Results typical of such studies are

presented in Tables 23 and 24. All strains tested displayed good to excellent growth on selective minimal medium at the time of inoculation.

In Table 23, a number of progeny resulting from introduction of factor F101 into a F42 bearing recipient were examined. These colonies were picked from agar selecting directly for Lac⁺ Thr⁺ Leu⁺ cells, shaken in broth culture for six hours (4 - 5 generations, see Table 21) and then plated on selective minimal media.

The frequencies at which double male colonies occurred are given in Column IV. The Lac⁺ Thr⁺ Leu⁺ phenotype was unstable. Since similar results were obtained with both exponential and overnight (stationary phase) recipients, plasmid loss was unaffected by the physiological condition of the host at the time of mating. In general, F101/F42 double males appeared in greater numbers in DnaB⁻ strains. The average frequency for the non-mutant strains (C09) in Table 23 was 0.34% whereas that for the five in the dnaB43 background (C050) was 0.48%. Although not large, this difference does lend some support to the contention that operation of intracellular immunity functions is altered in cells carrying dnaB43.

All markers appear to assort independently of each other, regardless of the presence of dnaB43. Those originally attached

to factor F101 (thr⁺leu⁺) were considerably less stable than lac⁺ (Table 23; cf: columns III and IV). Strain C09DMS7 represents an extreme case. Here the ratio of Lac⁺ to Thr⁺ Leu⁺ colonies was 75.4. The more rapid loss of F101-associated markers agrees with results obtained in single males (Results sec (c)).

Fourty presumptive double males from the dnaB⁻ background and fifty from the wild-type were examined in this fashion. In every instance, behavior similar to that depicted in Table 23 was observed. All strains retained a capacity for plaquing the F - specific phage, R17, and were hypersensitive to U.V. irradiation (ie RecA⁻).

The behavior of progeny from matings where factor F42 was transferred into a F101 bearing recipient is illustrated in Table 24. These strains were either taken directly from Lac⁺ Thr⁺ Leu⁺ agar or from Lac⁺ medium by way of Thr⁺ Leu⁺ agar and incubated in NB without agitation for 14 to 16 hours before being spread on nutrient agar plates (equivalent to 4 - 8 generations). Colonies arising on the latter were then tested for the presence of F42 and F101 by replication to selective minimal medium. It was hoped that the lengthened growth period would allow for fuller expression of incompatibility functions and, thus, serve to enhance the differences in double male stability noted between C09 and C050 cultures in Table 23.

**TABLE 23: ASSORTMENT OF PLASMID
ASSOCIATED MARKERS IN WILD-TYPE
(C09) AND DnaB⁻(C050) CR34 Rac⁻
F42/F101 DOUBLE MALE STRAINS; I:
RAPID GROWTH.**

STRAIN ^{1,2.}	FREQUENCIES OF PLASMID MARKERS ^{3.}		
	<u>Lac</u> ⁺	<u>Thr</u> ⁺ <u>Leu</u> ⁺	<u>Lac</u> ⁺ <u>Thr</u> ⁺ <u>Leu</u> ⁺
C09DML2	72.7	1.52	0.14
C09DML6	52.2	4.34	0.34
C09DMS1	65.4	2.36	0.25
C09DMS6	39.2	3.45	0.83
C09DMS7	46.8	0.62	0.15
C050DML1	59.2	4.26	0.21
C050DML3	22.8	2.14	0.32
C050DMS2	68.8	6.06	0.91
C050DMS4	44.2	2.50	0.16
C050DMS5	12.0	2.40	0.79

1. Lac⁺thr⁺Leu⁺ colonies from C076 X C010 (C059) cross removed from minimal agar, suspended in NB and shaken 5-6 h. at 30°.
2. C09DML2: double male #2 fr. log. phase wild-type recipient.
C050 DMS2: double male #2 fr. stationary phase DnaB⁻ recipient.
3. Per 100 cells in population at sample time.

**TABLE 24: ASSORTMENT OF PLASMID ASSOCIATED
MARKERS IN WILD-TYPE (C09) AND
DMS⁻ (C050) CR34 Rec⁻ F42/F101
DOUBLE MALE STRAINS; II: SLOW GROWTH**

STRAIN 1.2.	SOURCE 4.	FREQUENCIES OF PLASMID MARKERS 3.		
		<u>Lac⁺</u>	<u>Thr⁺Leu⁺</u>	<u>Lac⁺Thr⁺Leu⁺</u>
C09DML1	A	1.00	72.0	1.00
C09DML2	"	0.00	78.6	0.00
C09DML3	"	8.16	68.4	4.08
C09DML4	"	2.05	38.4	1.00
C09DMS1	"	0.00	57.0	0.00
C09DMS2	"	0.00	80.6	0.00
C09DMS3	"	19.1	48.5	2.41
C09DMS4	"	17.3	43.4	2.79
C09DMS5	B	70.0	0.00	0.00
C09DMS6	"	69.4	1.02	0.00
C09DMS7	"	85.0	2.00	1.00
C09DMS8	"	78.0	1.02	0.00
C050DML1	A	35.7	64.8	8.25
C050DML2	"	26.0	83.8	6.00
C050DML3	"	50.8	67.8	20.0
C050DML4	"	40.6	62.5	4.68
C050DMS1	"	40.0	69.0	11.0
C050DMS2	"	26.5	79.6	6.12
C050DMS3	"	31.3	68.6	2.02
C050DMS4	"	43.3	66.6	10.0
C050DMS5	B	83.8	4.08	4.08
C050DMS6	"	71.8	11.1	3.03
C050DMS7	"	6.00	62.0	1.00
C050DMS8	"	14.0	84.0	4.00

1. Lac⁺Thr⁺Leu⁺ colonies from MA3050 X C011(C061) cross removed from minimal agar and incubated in still NB cultures for 14-16 hours at 30°.
2. & 3: See Table 23.
4. A: Davis minimal agar + lactose, thiamine, strept; B: as A plus threonine and leucine.

This does indeed seem to be the case. After 14 - 16 hours in still culture, it was possible to detect $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ colonies in only half of the C09 cells examined, whereas all DnaB^- strains gave rise to significant numbers of double males (Table 24, column V). Further, those wild-type strains that continued to display the $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ phenotype did so at a markedly lower frequency than the mutant strains. For the data given in Table 24, the average frequency of double male formation in the DnaB^+ line was 2.04% as compared to 6.68% in C050 cells. Here again, the operation of this effect was independent of whether recipient cultures were in logarithmic or stationary phases of growth. F42/F101 double males are more stable and exist for longer periods in the presence of dnaB^{43} .

The presumptive double male strains examined in Table 24 were isolated in two different manners. The majority were obtained by direct selection for $\text{Lac}^+ \text{Thr}^+ \text{Leu}^+$ cells on lactose minimal agar in the absence of threonine and leucine (column II, source A). Overnight strains S5 to S8, however, were obtained by initial selection for lactose utilization followed by replication to a different medium to test independence from threonine and leucine (column II, source B). Regardless of which procedure was employed, the results were the same: F42/F101 double males were more stable in the strain carrying dnaB^{43} .

The markers thr^+ , leu^+ and lac^+ assorted independently of each other (Table 24, columns III & IV). Had they been united in a single plasmid, they would have been lost together from the

presumptive double males. No strains failed to segregate either Lac⁻ or Thr⁻ Leu⁻ colonies. Such behavior would have been indicative of chromosomal integration. Strain C09DMS7 gave rise to 85% Lac⁺ colonies of which only 1.00% were also Thr⁺ Leu⁺. Replication of the former to fresh minimal agar resulted in a 21% decrease in lactose fermenting cells. Thus F42 had remained independent of the chromosome. Similar results were obtained for strain C050DMS5.

Colonies bearing lac⁺ occurred in majority in double males resulting from the direct selection procedure (Table 23, columns II & III). In these experiments factor F42 was resident in the recipient at the time of mating. In crosses where the order of entry was reversed (i.e. F101 resident) as in Table 12 Thr⁺ Leu⁺ colonies were predominant. However, the overnight wild-type strains S5 to S8 and strains C050DMS5 and S6 did not show such behavior (Table 24, column III). These cells were initially selected on lactose agar in the presence of threonine and leucine, so that retention of factor F42 was favored. Had no such bias existed, assortment patterns similar to the others may have resulted (See also sub-section (VI)).

The 10 to 100 fold increase in the frequency of Lac⁺ Thr⁺ Leu⁺ colonies obtained in Table 24 relative to Table 23 may be due, in part, to differences in growth conditions (still versus aerated). In general markers associated with the second F plasmid were less stable in rapidly growing double males (Table 23, column III) than in those with slower rates of increase (Table 24, column III).

130

(iv) Altered incompatibility or a numbers effect?

The foregoing studies were carried out during an increase in cell numbers from 10^6 to 10^8 cfu per ml. Under these conditions female cells bearing dnaB⁴³ are often better recipients than the corresponding wild-type (Results sec.(b), Table 5). Consideration of the values given in Table 13 (expts. 1 and 3) suggest the same to be true of F plasmid containing cells (Results sec.(f)). Because of this, the difference in double male frequencies seen between wild-type and DnaB⁻ strains in subsection (iii) may be an artifact. Rather than an altered intracellular incompatibility, it may represent an enhanced capacity for engaging in secondary matings with females and F prime single males generated during the incubation of such large numbers of presumptive double male cells in enriched medium (Stadler and Adelberg, 1972).

To test this possibility, a number of the rapid-growth type experiments (Table 23) were repeated using significantly lower cell densities. Concentrations were reduced 100 fold so that assortment of F plasmid markers was monitored during rises from 10^4 to 10^6 cfu per l. Similar steps have been taken to limit the formation of mating pairs in high concentration mixtures of donors and recipients (de Haan and Gross, 1962; Curtiss and Stallions, 1967).

Mutant and wild-type colonies were removed from minimal agar selective for retention of both F⁴² and F101 markers and suspended in 1ml of NB. After vortexing to insure equal distribution, an 0.05 ml aliquot was withdrawn from each solution and diluted 100 times in a 50 Erlenmeyer flask. The latter were then agitated at 55 - 65 cycles per minute in a 30° water bath for 7 hours.

At the end of this time, portions of the cultures were diluted and spread on media allowing the determination of total, Lac⁺, Thr⁺ Lac⁺ and Lac⁺ Thr⁺ Leu⁺ cell numbers. Appropriate controls were performed to confirm the presence of F⁴² and F101 at zero time. The results are presented in Table 25. The order of plasmid entry during construction of these double male strains was F⁴² into F101-containing recipients.

The difference in frequencies of double males persisted despite the use of lower cell densities. Lac⁺ Thr⁺ Leu⁺ colonies occurred in greater abundance in the presence of dnaB⁴³ (Table 25, column IV). In this table, the average value for the mutation carrying strains, 0.220%, was 4 fold larger than that calculated for the non-mutant cells (0.056%). It is to be noted that, in comparison to values obtained using higher cell densities: (Table 24), the frequencies of Lac⁺ and Thr⁺ Leu⁺ colonies were generally reduced in the present circumstances (Table 25, columns II & III). This may be taken as support for

the assumption that a decrease in cell concentration would likewise result in a lowering of the incidence of secondary matings in such cultures.

It is evident, therefore, that the differences previously seen between C09 and C050 with regard to F42/F101 double male stability are due to altered intracellular incompatibility functions not high densities and/or differential mating abilities. Ironically, it is the non-mutant strain (C09) that has shown the greater fall in Lac⁺ Thr⁺ Leu⁺ colony frequency as a result of the decrease in cell numbers.

TABLE 25: ASSORTMENT OF PLASMID ASSOCIATED MARKERS IN WILD-TYPE (C09) AND DnaB⁻ (C050) CR34 Rec⁻ F42/F101 DOUBLE MALE STRAINS DURING RAPID GROWTH IN NUTRIENT BROTH: LOW CELL DENSITIES

STRAIN	1.2. FREQUENCIES OF PLASMID MARKERS			3.
	<u>Lac⁺</u>	<u>Thr⁺ Leu⁺</u>	<u>Lac⁺ Thr⁺ Leu⁺</u>	
C09DML3	5.37	35.7	0.064	
C09DMS3	1.75	8.36	0.036	
C09DMS4	2.58	10.0	0.067	
C050DML3	1.92	7.48	0.286	
C050DMS1	1.82	2.00	0.154	
C050DMS4	7.62	14.3	0.219	

1. Lac⁺Thr⁺Leu⁺ colonies of same origin as in Table 24 suspended in NB and shaken at 30° for 6 hours.

2.3: See Table 23.

(v) F plasmid assortment from purified clones.

To carry out the characterization described in parts (iii) and (iv) of this section, presumptive F₄₂/F₁₀₁ double male colonies were taken directly from primary selection plates and used as inoculae for NB cultures. No attempts were made at their purification by replication to fresh restrictive medium. As a result, the question arises as to the clonal nature of these test colonies. Rather than being composed entirely of true double males, the original colony may just as well have contained a mixture of crossfeeding Lac⁺ Thr⁻ Leu⁻ and Lac⁻ Thr⁺ Leu⁺ cells. Indeed, the percentage of Lac⁺ Thr⁺ Leu⁺ organisms in the population immediately upon suspension of such colonies in individual broth tubes (Oh, Tables 23 & 24) ranged from 0.96 - 1.58 for the wild type to 3.70 - 8.05 for DnaB⁻ strains. Thus data regarding double male stability obtained in parts (iii) and (iv) is quite probably significantly influenced by the presence of mixed clones of cells. Since the prolonged incubation on liquid and solid enriched - media, followed by simultaneous replication to test minimal-agar was, in part, carried out to militate against this, the information contained in Table 24 is less likely to be artifactual.

In an effort to enrich for true F₄₂/F₁₀₁ double male cells, a single Lac⁺ Thr⁺ Leu⁺ colony from each presumptive double male

having been treated as in Table 24 was replicated to fresh selective medium and incubated for 72 hours (2X purification). Of the wild-type double males transferred, only 30% (4/12) continued to be able to utilize lactose in the absence of threonine and leucine, whereas nearly 75% (9/12) of the cells bearing dnaB43 remained stable.

The plasmid marker stabilities of a few of the surviving colonies were determined by the slow growth method described in subsection (iii). The results are given in Table 26. The percentage contribution of each type of cell has risen significantly. In the majority of cases, the second F plasmid to enter the cell (F42) occurred in fewer numbers (cf: columns II and III). Each F prime plasmid assorted independently. Upon replication to fresh media, strains C050DML1 and C050DMS2 displayed 23 and 35% losses in their independence from threonine and leucine and 12 and 20% decrease in their abilities to ferment lactose.

As seen in column IV the purification steps markedly enriched for the occurrence of Lac⁺ Thr⁺ Leu⁺ double males in both wild type and dnaB43 backgrounds (frequencies some 4 - 30 fold greater than in non-purified clones, Table 24). The average of the values presented for the four double males occurring in the DnaB⁻ strain C050, is 76.2%. In comparison, that for the four C09 strains is only 36.0%; 2 fold less (cf: 3X in Table 24). Thus it seems that the effect of the presence of dnaB43 on F prime double male stability and hence intracellular incompatibility persists in these purified cultures.

TABLE 26: ASSORTMENT OF PLASMID ASSOCIATED MARKERS FROM WILD-TYPE (C09) AND DnaB⁻ (C050) CR34 Rec F42/F101 DOUBLE MALE STRAINS AFTER A TWO STEP PURIFICATION ON SELECTIVE MINIMAL AGAR.

STRAIN	1.2.	FREQUENCIES OF PLASMID MARKERS ^{3.}		
		Lac ⁺	Thr ⁺ Leu ⁺	Lac ⁺ Thr ⁺ Leu ⁺
C09DML3		35.7	80.6	29.6
C09DML4		34.3	75.7	31.3
C09DMS3		70.0	55.0	45.0
C09DMS4		40.0	52.5	38.3
C050DML1		93.0	86.0	84.0
C050DML4		73.3	92.0	73.3
C050DMS1		69.0	97.0	68.0
C050DMS2		81.0	90.8	79.6

1. Lac⁺Thr⁺Leu⁺ colonies from Table 24 replicated to identical medium (2X purification); incubated 72 hours, then suspended in NB and shaken at 30° for 6 hours (See Text).

2.3.: See Table 23.

(vi) Time rate of F prime plasmid loss from double males.

In the preceding portions of this section, the capacity of wild-type and DnaB⁻ cells to facilitate the coexistence of two autonomous, normally incompatible, F prime plasmids was scrutinized. The stability of F42/F101 double male cells is significantly increased in the dnaB43 bearing strains (Tables 23 - 26). To gain additional insight into the workings of intracellular incompatibility, the rate of assortment of F plasmid associated markers from such presumptive double male cells was studied.

The time rate of F prime plasmid loss from recombination defective wild-type and mutant single male strains was monitored in Section (c) of the Results. In these studies, cells bearing factor F101 displayed a greater degree of plasmid loss than did isogenic F42 derivatives, suggesting the latter to be better able to maintain itself than is F101 (Fig. 2-a). This difference in F prime stability was further enhanced in the presence of the mutation dnaB43 (Fig. 2-b). It was wondered whether this behavior was retained in F42/F101 double males. Does the presence of a second plasmid in any way alter the segregation pattern of the other?

Two RecA⁻ double male strains resulting from introduction of factor F42 into exponential phase F101 recipients (Table 12) were enriched for Lac⁺ Thr⁺ Leu⁺ colonies by a single step

purification on selective minimal agar, then suspended in NB (approx. 10^6 cfu/ml) and grown into log. phase according to procedures described in the Methods section (e). At regular intervals during the 6 hour incubation, samples were removed from each culture and used to determine total, Lac⁺ and Thr⁺ Leu⁺ cell numbers. The results, plotted as percent plasmid associated markers versus number of generations, are depicted in Figure 6. To compensate for possible distortions due to initial differences in plasmid marker frequencies, the value obtained at 0 hours was taken to be 100. All other values are expressed as a percentage of this.

Panels (a) and (b) of Figure 6 represent a replotting of data presented for single males in Figure 2. For convenience, only the generation times of F101 single males were used in determining the abscissa. Figures 6-c and 6-d result from wild-type (strain C09DML18) and DnaB⁻ (strain C050DMS13) cells respectively. The presence of a second F prime plasmid decreased the stability of the other plasmid relative to the single male condition. This effect was most dramatic in the case of factor F42. Whereas in single males it continued to permeate a very large proportion of the total population, in the presence of F101 the smaller plasmid became fairly unstable (Fig. 6-c). The introduction of dnaB43 enhanced this effect (Fig. 6-d).

FIGURE 6: TIME RATE OF F PRIME PLASMID LOSS FROM
SELECTED WILD-TYPE (a,c) AND dnaB43
BEARING (b,d) CR34 RecA^- SINGLE (a,b)
AND DOUBLE (c,d) MALE STRAINS DURING
GROWTH IN NUTRIENT BROTH AT 30°.
-0-0-: Lac^+ (F42: C010, C059, C09DML18,
C050DMS13); -0-0-: $\text{Thr}^+ \text{Leu}^+$ (F101: C011,
C061, C09DML18, C050DMS13).

Double Male Cells removed from selective
minimal agar, suspended in NB ($\approx 10^6$ cfu/ml)
and shaken for 7 h. At each hour, 0.1 ml
was removed, diluted and spread on agar
permitting the determination of total,
 Lac^+ and $\text{Thr}^+ \text{Leu}^+$ cell numbers. Data for
single males replotted from Fig. 2. Generation
times were determined in separate experiments
as described in the text.

136

The wild-type double male displayed a pattern of plasmid loss similar in essence to that found in both non-mutant and DnaB⁻ single male strains (cf: Fig. 6, a, b & c). Factor F101 was several degrees less stable than F42. For instance, at the completion of four cell duplications, cultures of strain C09DML18 contained only 14% Thr⁺ Leu⁺ cells as compared to 57% Lac .

In double male strains bearing dnaB43 this pattern was markedly altered (Fig. 6-d). Both Fprime factors showed the same level of instability, disappearing from the culture at nearly identical rates. The latter being very similar to that observed for factor F101 in the wild-type double male or alone in strain C061 (cf: Fig. 6, b, c & d). At four generations the difference in Lac⁺ and Thr⁺ Leu⁺ cell frequencies in C050 DMS13 was only 1.3 fold.

Data resulting in graphs virtually superimposable upon those presented here were obtained when the above experiments were repeated using a 100 fold decrease in cell densities and two different double males.

By and large the information contained in Tables 23 through 26 indicates that the F plasmid not resident in the recipient at the time of double male construction is the less stable. It

occurs in a smaller portion of the cells present after 6 or 16 hours incubation in NB. In experiments where factor F⁴² was introduced into a F101 carrying recipient one would thus expect to observe fewer Lac⁺ colonies. This is confirmed in Tables 24 and 25. However, the present results obtained from double males of the same origin indicate the opposite to occur (Fig. 6 c & d). As in wild-type and dnaB⁴³ bearing single males, factor F101 (carrying thr⁺ leu⁺) is the less stable in F⁴²/F101 double males. Because this data takes into account differences in F prime population size at 0 time, it is most likely the more accurate measurement of plasmid stability. Hence, care must be taken in interpreting the stability relationships indicated in Tables 23 - 26.

(vii) Summary

To summarize this rather lengthy section, the information presented in Tables 23 - 26 is in full agreement with data concerning the sedimentation properties of total cell lysates illustrated in Figure 5.) Factors F42 and F101 remained autonomous in all wild-type and ~~Dna~~ double male strains examined (> 50 each); existing independently of both each other and the chromosome.

Data provided in previous sections have indicated that Lac^+ Thr^+ Leu^+ double males are formed more readily in the presence of the mutation dnaB43 (Tables 11, 12 & 14). The present studies of F plasmid behavior in established double males suggest that this may be due, at least in part, to the greater stability of this phenotype in DnaB^- cells (Table 23 - 26). This, in turn, may be related to the altered patterns of F prime assortment observed in a selected number of double male strains (Fig. 6).

DISCUSSION

The mutant CR34TS43 was originally recognized as such because of its inability to survive incubation at elevated temperatures (Bonhoeffer and Schaller, 1965). Cells bearing the lesion dnaB43 displayed an abrupt cessation of DNA synthesis following a shift of the culture from 30 to 43° (Bonhoeffer, 1966). Recent work in this laboratory has revealed that the presence of this mutation also effects changes in cell behavior at the permissive temperature. DnaB⁻ cells carrying a F prime plasmid were less sensitive to male specific phage and less adept at exerting superinfection immunity against a second F prime factor attempting to gain entry into the same cell (Palchaudury and Iyer, 1971). The present studies have confirmed and extended these observations.

(a) General Characterization of dnaB43 Action

The earlier results of Palchaudury and Iyer (1971) are confirmed in Table 3. CR34TS strains carrying the F prime plasmid F42 exert considerably less superinfection immunity towards factor F101 than do isogenic non-mutant cultures (expts. 1 and 2). The organisms utilized in this earlier investigation were wild-type for chromosomally determined recombination functions (Rec^+). The presence of dnaB43 does not effect the operation of these genes, even at non-permissive temperatures (Joshi and Siddiqi, 1968). Hence, it has been suggested that the observed reduction in superinfection barriers may represent an enhanced capacity for F prime/chromosome integration rather than an alteration in the DnaB⁻ strain's immunity system (Achtman, 1972). Accordingly, these experiments were repeated using recA1 derivatives of all recipients (Maas and Goldschmidt, 1969). The influence of dnaB43 persisted despite this change (Table 3, expts. 3 & 4), indicating that a true lifting of superinfection immunity had occurred.

The reduction in immunity barriers, (relative to the control, obtained here (2.2X) is smaller than that reported by Palchaudury and Iyer (15X). This may, in part, reflect alterations in culture conditions (Nutrient Broth vs. Penassay Broth) but is more likely due to differences in control cells. Since there

was some indication in preliminary experiments that temperature resistant strains isolated from cells bearing dnaB43 are not always true revertants (data not given), the isogenic non-mutant, wild-type CR34 strain rather than CR34TR was employed as a control in the present study.

(i) Influence of dnaB43 on entry exclusion and establishment

Where conjugative plasmids are concerned, the term superinfection immunity subsumes two distinct processes: entry exclusion and intracellular incompatibility (Meynell and Datta, 1969; Novick, 1969). As discussed in the introductory sections, the former acts to inhibit the conjugal uptake of a superinfecting plasmid; the latter to prevent its prolonged co-habitation with the resident plasmid of the same cell. An attempt was made at obtaining separate measurements of these phenomena by varying the physiological condition of the plasmid positive recipient.

The efficiency with which exponential phase F prime cultures accepted a second F prime plasmid was used as an estimate of entry exclusion. Growth of F prime (or F^+ , Hfr) cells to late stationary phase results in a lifting of exclusion barriers (Lederberg et al, 1952). The recipient abilities of such cultures were determined as a means of monitoring F prime incompatibility. Because this procedure involves conjugation as well, the parameter actually being measured is the facility with which the second factor is established in its new environment. Thus, these manipulations allow only an operational

discrimination between entry exclusion and intracellular incompatibility. The numbers resulting after the efficiencies obtained in this manner were corrected for inherent differences in the recipient abilities of isogenic F⁻ cells (see Table 5) are termed the entry exclusion (EexI) and establishment (EstI) indices. Donor cultures were always in exponential phase in these experiments.

Values obtained for the entry exclusion and establishment parameters upon introduction of factor F101 into CR34 strains carrying factor F42 were diminished some 2 to 4 fold in the presence of dnaB43. This effect was reproducible and, moreover, persisted when the order of plasmid entry was reversed (Tables 11 and 12). Thus, it is not restricted to the immunity exerted by F42 against factor F101. That such behavior is not peculiar to F42/F101 interaction is indicated by its retention after substitution of factor F103 for F42 (Table 12). The increased capacity for accepting a second F plasmid observed in exponential and stationary phase F prime DnaB⁻ strains is independent of the presence of the recA1 lesion; a mutation that virtually abolishes the integration of an F plasmid into the chromosome (Clark and Margulies, 1965).

The recipient efficiency of DnaB⁻ cells containing F42 is enhanced, relative to non-mutant controls, to a greater degree in exponential than in stationary phase cultures (Table 3). The implication that dnaB43 acts preferentially to alter entry exclusion is supported by the changes in EexI and EstI produced by this mutation in Table 11. Cells bearing the latter show 4 to 6 fold reductions in entry exclusion but only 1 to 2 fold decreases in establishment values. Similar behavior is demonstrated by F101-harboring recipients (Table 12).

Reversal of the order of F prime plasmid entry resulted in a substantial increase in the magnitude of the entry exclusion and establishment indices observed in both wild-type and DnaB⁻ strains (8-30 increase). It seemed that factor F101 was more proficient in exerting superinfection immunity towards F42 than vice versa. A series of F'XF' crosses involving recipients isogenic for all markers except those on the resident plasmid confirmed this. Significantly fewer Lac⁺ colonies (F42) were formed in F101 recipients than were Thr⁺Leu⁺ progeny (F101) in the corresponding strain bearing F42. In recombination defective (RecA⁻) strains the decrease ranged from 9 to 16 fold (Table 13).

It is possible that F42 is defective in certain of its F- determined functions. However, the observation that it, in turn, exerts a greater degree of immunity than does factor F103 suggests that this behavior is, rather, a property peculiar to each particular F prime plasmid. Further, Hsu (1974) has indicated that less than 4% of the original F-DNA was lost during the formation of F42.

Sheehy et al (1972) failed to observe behavior similar to that noted here when an Hfr donor was mixed with F prime-containing minicells derived from exponential phase, dnaB43 - bearing parents. Repetition of a number of the present experiments using their procedure did not alter our results. Further, the DnaB⁻ strain continued to show a marked reduction in exclusion (12X) upon substitution of an Hfr donor (Table 19). Thus these author's results must be peculiar to the minicell system.

Because minicells obviously cannot (and do not, Cohen et al, 1968) carry a full complement of normal-size cell components, one possible explanation may be that the dnaB43 product is not segregated into such DNA-less cells. Minicells are able to replicate ColE1 (Inselburg, 1970) and T4 (Roozen et al, 1971) and transfer F prime DNA (Kass and Yarmolinsky, 1970) but indications are that the dnaB product is not required for any of these processes (Goebel and Schrempf, 1972; Lanka and Schuster, 1970; Vapnek and Rupp, 1971).

Sheehy et al have not indicated whether they performed similar conjugations using the dnaB43 bearing minicell producer.

During the course of the present investigations, attempts were made at introducing factor F42 into a F101 containing DnaB⁻ derivative of the minicell producing strain P678-54 (Adler et al, 1967). Disappointingly, such cells were found to be highly resistant to the conjugal entry of this F plasmid, even in the absence of F101. This behavior was independent of the presence of dnaB43 (Data not given).

(ii) Influence of dnaB43 on intracellular incompatibility

Throughout the present study, an effort was made to determine whether the action of dnaB43 included an effect on F-determined incompatibility functions. In one type of experiment ex-conjugants from matings involving stationary phase recipients (E_{ex}^-) were spread on minimal media selective for the growth of progeny harboring both F42 and F101. Comparison of the frequencies at which such $Lac^+ Thr^+ Leu^+$ colonies arose in wild-type and $DnaB^-$ backgrounds provided a measure of the relative ease with which F prime double males could be formed. In a second series of experiments, the stability of double male strains isolated in this manner was monitored by following the assortment of plasmid associated markers during incubation in non-selective, enriched medium.

In general, $Lac^+ Thr^+ Leu^+$ presumptive double males were formed in significantly greater frequencies in the presence of dnaB43 (Tables 11, 12, 15, 16 & 17). With $RecA^-$ cells carrying factor F101 increases greater than 20 fold were noted in $DnaB^-$ recipients relative to non-mutant controls (Table 19). When selection was for markers associated with both plasmids, normal-sized (for $DnaB^-$) and smaller-sized (for wild-type) colonies appeared after 5 to 6 days incubation at 30° . In comparison, selection for single

(or double markers) as done in measuring entry exclusion and establishment indices, resulted in normal-sized colonies at 4 days. If recA1 were present, double male colonies occurred at frequencies equivalent to 40-60% (DnaB⁻) and 6-28% (wild-type) of those observed when selection was for a single plasmid.

Such differences in colony size and number imply the existence of an increased capacity for facilitating the presence of, a second homologous plasmid in strains containing dnaB43.

To determine the influence of the presence of dnaB43 on plasmid stability in the double male condition (and hence on F prime incompatibility), presumptive F42/F101 double males were suspended in Nutrient Broth and incubated at 30°. These conditions of incubation were varied so that the effect of rapid or slow growth (Tables 23 & 24) at high or low cell densities (Tables 24 & 25) could be monitored. In all cases, double male strains carrying the wild-type allele of dnaB displayed the greatest instability. The numbers of Lac⁺Thr⁺Leu⁺ colonies present in DnaB⁻ cultures after 6 hours agitation or 16 hours of still growth were consistently some 30 to 70% greater than in those containing non-mutant cells. In certain instances, the presence of double male cells was not detected at all in wild-type cultures (Table 24). The difference between wild-type and DnaB⁻ strains was observed regardless of the order of plasmid entry or the physiological condition

(exponential or stationary growth phase) of the recipient at the time of double male construction.

This enhanced stability does not stem from any effect dnaB43 may have on the recipient ability of F^- (Table 5) or F' prime (Table 16) strains. Dilution of suspensions of double males to achieve low cell concentrations followed by growth for 6 hours produced no significant changes in the above mentioned behavior (Table 25). Had a high rate of secondary F' prime transfer (Stadler and Adelberg, 1972) been responsible for the difference between wild-type and mutant strains, the latter would have displayed a substantial decrease in $Lac^+Thr^+Leu^+$ cell numbers. It should also be recalled that, in these and all other experiments, the incubation temperature was 30° , a condition under which matings are inherently less efficient than at, say, 37° . Nor does the apparent reduction in incompatibility seem to arise from a high incidence of mixed clones of $Lac^+Thr^-Leu^-$ and $Lac^-Thr^+Leu^+$ cells in the presence of dnaB43.

Colonies enriched for $\text{Lac}^+\text{Thr}^+\text{Leu}^+$ doubles by a 2 step purification on minimal agar were unchanged in their stability characteristics (Table 26). Cultures of mutant cells carried 53% more F42/F101 double males; only their individual frequencies were altered (upwards).

Thus, two normally incompatible F prime plasmids are found together in the same cell more often in a DnaB^- strain than in the wild-type. The force(s) usually operating to prevent the development of this condition; that is, intracellular incompatibility, is significantly reduced in the presence of dnaB43 .

A study was made of the rate of segregational loss of individual F plasmids from two randomly selected double male strains (RecA^-) originating from the introduction of factor F42 into F101 containing recipients. Surprisingly different patterns of assortment were obtained in wild-type and DnaB^- backgrounds. In the former, (strain C09DML18, Fig. 6-c) there was a preferential maintenance of factor F42 . After only three generations cultures of this strain contained but 19% Thr^+Leu^+ cells as compared to 52% Lac^+ . This behavior is remarkably similar to that noted in non-mutant (Fig. 6-a) and DnaB^- (Fig. 6-b) single males; the major difference being that the presence of the second factor alters the stability of the other considerably.

3

OF/DE

3

In contrast, F42 and F101 were lost at nearly equal rates from cultures of DnaB⁻ double males (strain C050DMS13, Fig. 6-d). There was no preferential retention of one particular plasmid. Since third generation cultures carried only 13% Thr⁺Leu⁺ and 17% Lac⁺ cells, both factors seem to have acquired the instability previously characteristic of F101 only.

These patterns are reproducible. Similar curves were obtained at low cell densities and with two different wild-type and dnaB43 - bearing double males (data not given). Thus, two types of F prime plasmid assortment occur in CR34 Rec⁻ double male strains. In the wild-type background one factor is maintained in preference to the other. The one most readily segregated is that showing the lesser stability in single males (here, F101). In DnaB⁻ mutants, this bias is abolished. Both F primes disappear at a rate similar to that observed for the less stable plasmid in mutant single males or wild-type doubles. The presence of dnaB43 allows the two factors to compete on a more equal footing.

The preferential loss of factor F101 is an interesting aspect of these studies. Since it had the advantage of being the resident plasmid during the construction of these particular double males, one might have expected the opposite behavior. As well, previous studies had judged F101 to be dominant in establishing itself in

such double male cells (Table 13, expt b). On the other hand, it had already indicated its greater instability in single males (Fig. 2-b). Other examples of such discrimination in plasmid maintenance can be found in the literature (Echols, H. 1963; MacFarren and Clowes, 1967; Frydman and Meynell, 1969).

As part of the investigation of presumptive F42/F101 double male stability discussed earlier, the frequency of Lac^+ and Thr^+Leu^+ colonies was also determined. This information permitted some speculation as to the condition of each F prime in such cells. Because, in the hundred-odd strains examined, the frequency of Lac^+ colonies was significantly different from that obtained for Thr^+Leu^+ , it was concluded that factors F42 and F101 had remained independent of one another. Moreover, since each showed a detectable degree of loss from the culture, it was assumed that both plasmids had remained independent of the chromosome as well.

There was no evidence of fusion between F42 and F101. Such may have indeed occurred, but was not detectable because of the frequencies at which doubles were found in the present system. Press *et al* (1971) achieved the union of two F primes carrying trp^+ and arg^+ markers at levels of $2 \times 10^{-4}\%$. While Willetts and Bastarrachea (1972) have reported values even less than this for the fusion of F57 (F'_{his^+}) and F101. In contrast, $\text{Lac}^+\text{Thr}^+\text{Leu}^+$ colonies occur at frequencies of 10^{-2} to $10^{-3}\%$ for wild-type CR34 RecA^- and $10^{-2}\%$ in its dnaB43 derivative (values for matings in liquid cultures). Hence, the rare fused particle would have easily been masked.

To confirm these conclusions, total cell lysates of selected, recombination deficient, double male strains were subjected to centrifugational analysis in alkaline sucrose. The DNA of these organisms had been previously labelled with (^3H)-thymidine in liquid minimal medium. The resultant sedimentation patterns substantiate that, at least in these particular double males, the fertility plasmids have remained independent of both each other and the chromosome. Two plasmid determined peaks were observed in both wild-type (C09DML6) and mutant (C050DMS9) double males (Fig. 5). The contribution made by each to the total acid-insoluble radioactivity was approximately half that made in its corresponding single male.

Further, both F primes continued to band at positions identical to those noted in lysates of the appropriate single male strain. For this reason, it is concluded that the molecular weights of the two plasmids have remained unchanged in the double males.

It would appear, then, that incompatibility barriers have not been overcome by fusion and/or deletion of the region in F determining this function from either plasmid. Forms displaying greater or intermediate sedimentation values would have been detected if union had occurred (Willetts and Bastarrachea, 1972; Palchauduri et al, 1972). Similarly, a shift in the position of one of the plasmid bands toward the top of the gradient would have been expected in the latter instance.

Throughout this section, it has been assumed that the behavior being examined was that of true F prime double males. The presence of recA1 and our failure to find evidence to the contrary (segregation patterns and physical characterization of plasmid DNA) argue against fusion of the two plasmids or integration of any plasmid into the chromosome at detectable frequencies. The existence of more transient unions is not as easily dismissed. The presence of mixed clones of cells exerted little, if any, influence during the stability studies as indicated by the experiments in which colonies were purified before releasing the selection pressure.

Two other bits of information further support this last conclusion. Dramatic changes in F plasmid assortment patterns were observed upon turning from single to presumptive double male strains (Fig. 6). If the behavior of mixed clones were under scrutiny, one would have expected no such alteration in plasmid stability. Moreover, had this condition prevailed during the examination of the sedimentation properties of double male cell lysates, the sum of the amounts of plasmid DNA present should have been near equal to that achieved by adding the values obtained in single males (Fig. 5). In point of fact, their sums are equivalent to or less than the number noted for the largest single male contributor alone (F42). Because the number of copies of F plasmid per chromosome is apparently severely restricted (Jacob *et al*, 1963; Vapnek *et al*, 1971; Frame and Bishop, 1971), this behavior is entirely in keeping with the existence of true double males.

Taken together, the bulk of information considered here indicates that, F42/F101 double males are more easily formed in strains bearing the mutation dnaB43; that their persistence under non-selective conditions may be due to an increased capacity in such cells for tolerating the presence of a second autonomous plasmid (Lac⁺Thr⁺Leu⁺ colonies more stable); and that this tolerance, in turn, may be associated with an altered mechanism of F prime plasmid assortment. The end result is an apparent lifting of intracellular incompatibility barriers.

- (iii) The altered superinfection immunity phenotype is directly attributable to dnaB43.

Because the mutant bearing dnaB43 was isolated after nitrosoguanidine treatment of strain CR34, the possibility existed that the altered superinfection immunity and temperature sensitivity phenotypes were the products of two independent lesions induced in the same chromosome (Guerola *et al*, 1971). Several approaches were taken to determine the relatedness of these two effects. Firstly, the influence of sodium chloride mediated phenotypic suppression of dnaB43 was monitored. Secondly, recombination defective cells bearing dnaB43 were "genotypically" suppressed by constructing strains carrying (a) wild-type dnaB analog protein (PlCMBac lysogens) and (b) wild-type dnaB product protein (dnaB43/F' dnaB⁺ merodiploids). Finally, phage P1 transduction was used to remove dnaB43 from CR34 and place it in a new genetic background.

• Addition of 0.4M NaCl to Nutrient Broth cultures of recombination deficient strains carrying dnaB43 (0.25M for Rec⁺) enables them to grow at temperatures that are otherwise non-permissive (Fig. 3&4). Similar observations have been made for this and other DnaB⁻ mutants by Ricard and Hirota (1969, 1973); Sicarrdi *et al* (1971) and Gross (1972).

Removal of the salt resulted in a return to temperature sensitivity. Further, no significant difference in mean generation times could be detected between wild-type and mutant cells under these conditions (ratio:1.13-1.54). Therefore, these cultures must have been only phenotypically corrected.

However, such phenotypic suppression does not have a like effect on the altered immunity trait. At 42° in the presence of salt the exclusion index of the non-mutant control was some 16 times larger than the value for the DnaB⁻ mutant; the establishment index was increased 7 fold (Table 14). The lifting of superinfection immunity must therefore be a primary effect of the dnaB43 lesion.

D'Ari et al (1974) have isolated a plaque-forming mutant of phage PlCM which is constitutive for the activation of a virus determined protein that facilitates the survival of dnaB⁻ cells at elevated temperatures. This material apparently substitutes for the defective chromosome product and hence is termed dnaB analog protein. DnaB43 bearing strains lysogenized with these particles were also resistant to a temperature that is not permissive for the non-lysogen. A Fl01 derivative of this was compared with an isogenic strain carrying wild-type prophage (analog repressed; temperature sensitive) for its ability to limit the conjugal entrance of factor F42. If the two phenotypes are attributable to the same lesion, the temperature resistant cells would be expected to display diminished recipient efficiencies as well.

Lysogens containing activated dnaB⁺ analog were the poorer recipients. Rises in entry exclusion and establishment indexes of 8 and 9 fold relative to the temperature sensitive control were observed (Table 15). Like the thermosensitivity character, the altered immunity is recessive in the presence of wild-type analog.

Because cells made merodiploid for the dnaB region by introduction of the F prime factors F134-1 and F118 also grew at high temperatures, it was suspected that the presence of the actual wild-type product would likewise produce a decrease in progeny frequencies. Indeed such results were obtained. The efficiency of F42 uptake into these cells was reduced to a level almost identical to that observed in F101 and F134-1 derivatives of the wild-type strain (Table 16). Similar behavior occurred in F118-containing strains which carried much less DNA of chromosomal origin (5vs15 minutes). Combined with the preceding studies, these results indicate a direct causal relationship between dnaB⁺ and the altered superinfection immunity phenotype.

Bacteriophage P1 had been used to transduce dnaB43 from a CR34 strain to a different genetic background (Iyer et al, 1974). When this new strain was tested for its ability to accept a second F prime plasmid, F101 derivatives were 4 to 5 times more efficient recipients than an isogenic, dnaB⁺ control (Table 17). Hence the thermosensitivity and immunity phenotypes are cotransducible. In the extensive work of Taylor and Trotter (1967) the longest distance for cotransduction of two markers is 2.2 minutes. The lesion determining the relaxed immunity and dnaB43 are either extremely closely linked or are one in the same mutation. P1 transductional derivatives that had received the closely neighbouring ampA mutation but not dnaB43 did not show the altered immunity pattern. They served as controls in these experiments.

The cell transduced in this series of experiments was the flagella/pili-minus mutant of Nishimura et al (1967), strain JE2571. The decrease in the extent to which entry exclusion and establishment are altered in its F prime derivatives relative to CR34 strains is probably related to the absence of Type-I (common) pili. Their role in conjugation is not yet understood. (See discussion Sec. (c) ✓.

In summary, the lifting of superinfection immunity barriers observed in DnaB⁻ CR34 strains appears to be a primary effect of the presence of the mutation dnaB43. Since introduction of a wild-type allele or a Pl-determined analog increases the level of immunity expressed in such cells, dnaB43 is very likely ~~the~~ direct determinant of this behavior. It is also cotransducible with dnaB43.

- (iv) Evidence that the effect ~~is~~ gene specific rather than allele specific

A limited number of experiments were performed to test whether the lifting of superinfection immunity barriers reported here was peculiar to the dnaB43 allele or extended to other mutations in this gene.

One other strain, E107, bearing the lesion dnaB107 was examined (Wechsler and Gross, 1971). Both mutations occur in the CR34 background; result in an immediate stop of DNA synthesis at elevated temperatures; are recessive to the wild-type allele; are phenotypically suppressible and map near malB (Wechsler and Gross, 1971; Gross, 1972). Unlike dnaB43 however, the mutation dnaB107 resulted from ethylmethane sulfonate treatment of temperature resistant cells (Hechemy and Goldfine, 1971).

The presence of dnaB107 does seem to be associated with a lifting of immunity. A F101 derivative of E107 displayed 3 to 5 fold decreases in its entry exclusion and establishment indices relative to a non-mutant control (Table 20). Reductions of twice this magnitude were observed in the comparable dnaB43 mating. Thus, this mutation is not quite as effective. The results are, however, significant in that the two mutations derived from independent events by the use of two different mutagens have the same overall effect on superinfection immunity functions.

These two dnaB⁻ mutations have many properties in common.

A comparison of the properties of strains bearing different mutant dnaB alleles suggests that there may be significant physiological and genetic differences between them.

Georgopoulos and Herskowitz (1971) found that some dnaB mutations are groP⁻ and others groP⁺. The groP⁻ genotype prevents the propagation of wild-type phage λ even at 30° but will allow the growth of λ that are mutant in gene P. Ricard and Hirota (1969) have reported that some dnaB⁻ mutants are phenotypically suppressible (by salts or sugar) and that others are not. It has also been reported (Siccardi et al, 1971) that one allele, dnaB313, is dominant to the wild-type. Before one begins to make generalizations about the influence of dnaB on superinfection immunity, it is essential that these as well as the many other dnaB⁻ alleles be examined.

(b) A Possible Mechanism of dnaB43 Action

The preceding, plus other relevant data, will now be used in an attempt to suggest the underlying mechanism of dnaB43 action.

Since most of the discussion will pertain to recombination defective cells, one early observation requires some elaboration. The data presented in Tables 3 and 5 indicate that strains carrying the lesion recA1 are more efficient in the uptake and establishment of F plasmids than are Rec^+ strains. This occurs with both F^- and F prime recipients but is statistically significant only in cases where the donor plasmid is F101. Although the presence of recA⁻ mutations is reported to have "little or no effect on plasmid inheritance" (Clark, 1973), one would expect the potential for stabilization by integration with the chromosome to favor acceptance by recombination-proficient cells, particularly in the case of F plasmid containing recipients.

Recombination defective F^- cells grow more slowly at 30° than do isogenic Rec^+ strains, but the increase in generation time is limited to 2 to 3 minutes (Table 4). The addition of factor F101 however, increases this difference to a more dramatic 20 to 30 minutes (Table 7). The question arises as to the existence of some special form of interaction between F101 and the chromosome. F101 affects the division rates of Rec^+ cells as well, but its occurrence is brought into sharper focus

by the presence of recA1 (Rec^+ vs. Rec^- : 5 vs. 30 minutes). The enhanced conjugal uptake of plasmid F101 may constitute another manifestation of this interaction.

It is worth recording the speculations of Moody and Hayes (1972) in this regard. They have observed that the capacity of certain plasmids (ie: ColIb Δ rd & Rldrd19) to mediate transfer of chromosomal genes is unchanged by the introduction of a recA mutation. To explain this, they postulate the existence of some mechanism of plasmid/chromosome interaction the operation of which may require no physical contact between them. The level of efficiency at which it functions is possibly determined by the particular plasmid and host involved. Alternatively, the observation of Capaldo et al (1974) that cultures of RecA^- cells may contain upwards to 33% nonviable cells, may bear some relationship to this behavior. At any rate, because the wild-type and DnaB^- strains employed here are isogenic for this lesion, the contribution its presence may make to recipient efficiencies can be regarded as essentially a background effect.

The salt correction studies have indicated that the altered superinfection immunity phenotype is a primary effect of the presence of dnaB43 and is independent of its reaction to elevated temperatures.

Female (F^-) and F' prime cells carrying dnaB43 divide at slightly slower rates than do non-mutant strains, but are generally the more efficient recipients. Organisms harboring plasmid DNA are usually better able to receive a second homologous factor upon growth to stationary phase (Lederberg et al, 1952). It therefore seemed possible that the presence of dnaB43 was having an analogous effect in mutant cultures. However, the change in the generation time of F^- cells is rather small (1.08X) and hence unlikely to account for the observed 2 to 5 fold increase in Thr^+ Leu^+ and Lac^+ progeny in these organisms.

F101 derivatives of $DnaB^-$ cells show an increase of 1.27 fold in the time between duplications relative to control strains. These same cells when grown under identical conditions demonstrated a 37 fold rise in Lac^+ progeny frequencies (Table 18, expt. 1). As with F^- strains, it is difficult to imagine how this

small change in generation time can produce such great increments in recipient abilities. Experiments designed to deliberately lengthen the division times of cells bearing dnaB43 failed to yield corresponding increases in recipient efficiencies (Table 18, column IV).

More revealing perhaps, is the finding that recombination defective F⁻ strains harboring dnaB43 form 4 times as many mating pairs as do non-mutant controls when mixed with cells carrying factor F42 (Table 6). The assay procedure utilized in measuring this parameter is based on the detection in transconjugants of genetic markers inherited from donor organisms. For this reason, the action of dnaB43 at this level may be to increase the proportion of effective mating pairs in mixtures of F prime and F⁻ cells. Indeed, it is rather significant that under identical conditions of growth, these same cells displayed an enhancement in recipient efficiency very similar in magnitude (Table 5, 5X) to that noted above for the change in mating pair formation. The action of dnaB43 on female cell recipient ability may stem entirely from its effect on mating pair formation. Growth of recipient cells to stationary phase raised this difference to 6 fold, implying that the capacity for forming mating pairs varies with the cell cycle.

Because of incompatibility considerations it was not possible to extend these studies to F prime recipients. One might, however, be able to measure total (effective + ineffective) mating pairs by using a physical assay method (Walmsley, 1973). Achtman et al (1971) used an electronic particle counter to determine the effect of several F plasmid-linked mutations on mating pair formation. Recipient cells carrying wild-type F prime DNA formed small numbers of these. But certain strains harboring mutant plasmids displayed an enhanced capacity for entering into such liaisons. Since they were also unable to inhibit the entrance of a superinfecting F prime, the authors have suggested that entry exclusion operates, at least in part, to decrease the formation of total mating pairs.

This proposal is not inconsistent with the present observations regarding the influence of dnaB43 on mating pair formation (at least in F⁻ cells) and the recipient ability of exponential F prime cultures. Because substantial differences in plasmid acceptance occurred between wild-type and mutant cells even after correction for the behavior of F⁻ recipients (entry exclusion index), the effect of dnaB43 on mating pair formation in F prime recipients may be fairly significant.

The stability of F42 and F101 single males was considered briefly in the foregoing section. The behavior of F134-1 derivatives of wild-type and DnaB⁻ strains is illustrated in Figure 2-c. F42 is much more stable than F101 and it, in turn, is more stable than F134-1. Addition of dnaB43 alters the stability of F101 considerably but has little effect on F42 or F134-1. F134-1 is relatively unstable, even in the absence of the dnaB43 mutation. There are two possible explanations for this behavior.

The preferential retention of F42 may not result from differential stability but rather from different transfer abilities. F prime plasmids capable of efficient reinfection of F⁻ segregants would quickly permeate the entire culture, thus, giving an appearance of high stability. However, if the data presented in Tables 10 (expt. 183) and 13 is to be believed this cannot be the case. CR34 cells grown in Nutrient Broth, both transfer and accept factor F101 more efficiently than they do F42. On this basis behavior opposite to that observed is expected.

More likely, it is the size of the F prime plasmid that determines its stability. Factors F42, F101 and F134-1 carry non-F DNA complementing approximately 0.6, 6.0 and 16% of the E coli chromosome. This increase also represents the degree of instability.

Clark et al (1969) reported isolation of an F prime factor carrying some 27 minutes of chromosomal DNA (30%). Its segregation frequency was so high the authors were unable to test the influence of acridine orange on plasmid elimination.

Invoking for the moment, the replicon model for plasmid maintenance (Jacob et al, 1963), one may speculate that factor F₄₂, which is predominantly F-DNA, possesses a high affinity for the F attachment site on the cell membrane. Upon the steady addition of DNA originating with the chromosome, the configuration and/or physical composition of F might be altered to such an extent that its accessibility to this site is diminished. Hence, the larger the F prime factor the less rigorous its interaction with the F maintenance site and the greater its instability. Because the chromosome is RecA⁺ and occupies its own specific attachment site, these larger F plasmids cannot be rescued by chromosomal integration or interaction with the second, non-F, maintenance site. The presence of dnaB43 somehow acts to enhance this instability (see subsequent paragraphs).

Alternatively, because F42 is composed almost entirely of DNA of F origin, the significance of each nucleic acid sequence with respect to plasmid survival is expected to be very great. Sharp et al (1972) have estimated that only about 16% of F-DNA is dispensable and can be lost without detriment to plasmid function. (Since approximately 1/5 of this region is already missing in F42, it is even more restricted). Addition of material of chromosome origin would increase the load of non-essential DNA. One conceivable consequence of this might be the acquisition of a new flexibility with regards to intra - and/or (because there may be 2 copies/cell) -inter-plasmid molecule recombination - like interactions. Since non-reciprocal exchanges would be less likely to result in losses of essential material, the F plasmid becomes more free to explore other molecular combinations. Increased participation in this activity may, however, lead to an overall reduction in plasmid stability; possibly because of intermediates being separated from the cell membrane, or, perhaps because a majority of the resultant new forms are "nonviable" or lack parental affinities for maintenance sites. At any rate, as the amount of non-F DNA is increased, one would expect a concomitant increase in instability. Inclusion of dnaB43 serves to enhance this type of interaction.

175

Plasmid stability is influenced by a number of parameters including culture medium (Results sec. (c)), temperature (Stadler and Adelberg, 1972) and the host Macrina and Balbinder, 1972). It is governed by both plasmid (Cuzin and Jacob, 1967b; Macrina and Balbinder, 1972) and chromosomal (Wada and Yura, 1971; Yamagata and Uchida, 1972; Stadler and Adelberg, 1972; Hathaway and Berquist, 1973) genes. Mutations in the latter have been mapped at 1 (maf), 64 (eps) and 0 (seg2) minutes (see papers cited in previous sentence). The action of dnaB43 mapping at 81 minutes constitutes a fourth entry to this group.

Presumptive F42/F101 double male strains grow rather slowly (Table 21). It has been noted that addition of factors F42 and F101 resulted in an almost 2 fold rise in the generation time of the F⁻ parent in both DnaB⁻ and DnaB⁺ backgrounds (Table 22). Doubling the F plasmid content has doubled generation times. (This may be construed as further evidence in support of the contention that true double males rather than mixed clones are being examined). In view of the suspected interaction of F101 and host chromosome, it may be significant that the greater change was wrought by the introduction of this plasmid (Table 22).

Despite the slow rate of increase these doubles display a high degree of plasmid instability. The pattern of F prime-associated marker loss demonstrated by wild-type cells is, to a first approximation, very similar to that seen in wild-type and mutant single males (Fig. 6). That observed in selected DnaB⁻ F42/F101

174
double males differs markedly (Fig. 6-d). The preferential maintenance of factor F42 has been lifted. Both F plasmids show a degree of stability characteristic of factor F101 in DnaB⁻ single and wild-type double males. The mechanism allowing discrimination between F42 and F101 has been overcome by the inclusion of dnaB43.

Extending the conjectures made regarding single plasmid stability, it may be that the action of dnaB43 has been to alter the F attachment site. In single males the maintenance of F42 is little affected, but F101 is even less able to enter into a stabilizing interaction with it. Upon being placed in the same cell however, both plasmids compete for the one site on an equal footing. Since DnaB⁻ double males lose the two F primes at the F101 rate, its presence in combination with the altered attachment site radically decreases F42 stability. (The difference in F42 stability in wild-type and DnaB⁻ cells may measure the contribution of the attachment site modification). The stability of Lac⁺Thr⁺Leu⁺ double male strains was not monitored in this particular series of experiments. However, in view of the data concerning this parameter presented in Tables 23 through 26, one can predict the overall effect of this alteration would be to increase the fraction of cells carrying both factors F42 and F101 in DnaB⁻ cultures over that observed in cultures of non-mutant cells.

Or applying the plasmid interaction model, the action of dnaB43 might be to increase the number of recombination - like liaisons between F42 and F101. The existence of composite plasmid molecules could certainly be used to account for the similarity in Lac⁺ and thr⁺leu⁺ assortment patterns observed in DnaB⁻ double males (Fig. 6-d). Again, the overall result, relative to the non-mutant condition, would be an increase in the frequency of cells bearing both sets of markers.

The occurrence of such fused F prime plasmids has been reported by several authors (Press et al, 1971; Willetts and Bastarrachea, 1972; Palchauduri et al, 1972). However, the instability of these structures is such that their isolation is a rather rare event (frequency: less than $10^{-4}\%$). In contrast, Lac⁺Thr⁺Leu⁺ double males were regularly formed in dnaB43 - bearing strains at frequencies of $10^{-2}\%$. This, plus the high level of plasmid marker loss observed in DnaB⁻ doubles in enriched medium, argues against a fusion mechanism being the major contributor to the present phenomenon. Further, since assortment studies and physical characterization in alkaline sucrose indicate an independent existence for F42 and F101, these unions would need be fairly transient (rapidly de-fused).

Sedimentation analysis of two fused F prime plasmids isolated by Willetts and Bastarrachea (1972) showed they were of larger or intermediate size to the parental plasmids, F57 and F101. The finding that strains carrying the smaller fused factor segregated F⁻ cells more rapidly than those bearing the other three F plasmids, while the larger was just as stable as either F57 or F101, may be taken as evidence for or against either of the foregoing models.

In section (f) of the Results it was reported that F⁻, DnaB⁻ strains lysogenized with wild-type and mutant (constitutive activation of dnaB⁺ analog protein) phage P1 demonstrate a general decrease in recipient efficiency relative to non-prophage-carrying cells. Among these, strains bearing wild-type phage (analog repressed) formed 5 to 6 fold fewer Lac⁺ progeny than those containing mutant particles. Since all previous experiments had indicated an enhancement of recipient ability in the presence of dnaB43 this behavior was unexpected. However, this can now be explained.

As elaborated in the altered maintenance-site model, cells bearing the lesion dnaB43 may possess a modified F plasmid membrane attachment site. It is now equally accessible to factors F42 and F101. Bacteriophage P1 is carried in the lysogenic state as an autonomous circular DNA molecule (Ikeda and Tomizawa, 1968).

Although the prophage presumably has its own particular region of interaction with the membrane (one copy per chromosome; expresses superinfection immunity; Rosner, 1972), the change effected in the F site might have made it accessible to P1 as well. Thus a culture lysogenized with wild-type particles would be less able to accept factor F42 because a significant proportion of its constituents have their F-site predisposed to P1 maintenance. In cells restored to a non-mutant phenotype by the presence of active dnaB⁺ analog (mutant phage), this behavior is not observed because normal F prime affinities have been reinstated. In the studies carried out to determine the effect of dnaB⁺ analog on superinfection immunity (discussion sec. (a)-(iii)), cultures of F101 bearing strains lysogenized with wild-type phage generally carried fewer Thr⁺Leu⁺ cells than those harboring mutant prophage. Since preliminary studies have indicated DnaB⁻ cells to be as responsive to P1 transduction as the wild-type (data not given), the presence of dnaB43 has not altered the phage attachment site.

With the superimposition of incompatibility functions, this effect is abolished (at least not detectable) in F prime derivatives of these lysogens. As expected, those bearing wild-type phage were the better recipients by 4 to 9 fold (see also Table 15.)

Phage P1 is capable of recombination with R plasmid DNA (Kondo and Mitsuhashi, 1964) and is able to circularize to form its prophage state, even in the absence of recA functions (Rosner, 1972). Hence the plasmid-interaction model can be just as easily invoked to

account for this behavior of P1 lysogens of DnaB⁻ cells.

Thirdly, P1 determines its own particular system of DNA restriction and modification. Lysogens of it are poor hosts for a number of heterologous phages (Lederberg, 1957). As well, they are poor recipients for conjugal entry of F plasmids. Glower *et al* (1963) observed 100 fold decreases in Lac⁺ progeny in crosses between a F42 donor and F⁻P1-lysogens. Thus, the behavior noted here may merely reflect the operation of such a system in dnaB43 cells carrying P1CM prophage. It may be significant, in this regard, that conjugations conducted under conditions of high F transfer such cells still formed Lac⁺ progeny at frequencies some 50 fold lower than corresponding non-lysogens. For a review of this topic see Boyer (1971)

The altered attachment-site model implies that cells bearing dnaB43 are changed in certain properties associated with the cell wall/membrane complex, even at permissive temperatures. Specifically, the nature of the interaction between F-DNA and the complex is modified. Although there is no direct evidence in support of this, certain of the data presented herein tend to point in that direction.

Variation of their ionic environment (0.42M NaCl) produced as much as a 5 fold increase in the recipient efficiencies of exponential phase F⁻ and F prime derivatives of the DnaB⁻ strain at 30°. Moreover, it permitted them to grow at normally non-permissive temperatures (Table 14). Incubation of E coli cells in the presence of similar high concentrations of sodium chloride (0.20M) has been reported to decrease their susceptibility to lysozyme action (Yoshinaga *et al*, 1974). These three effects may be related through the common medium of the cell wall.

Female strains bearing dnaB43 form greater numbers of effective mating pairs than isogenic wild-type cells. Just as some changes in cell membrane lipopolysaccharide composition result in a decreased ability to form mating pairs (Monner *et al*, 1971), other changes may produce an increase.

Exponential phase cultures of DnaB⁻ F prime cells offer less resistance to the entrance of a second F plasmid than do non-mutant controls. Since entry exclusion appears to be largely a surface phenomenon (Falkow et al, 1971; Sheehy et al 1972; LeBlanc and Falkow, 1973), its lifting in cells bearing dnaB43 bespeaks of an effect on the cell coat.

Finally, as will be discussed in a latter section, cells bearing the dnaB43 mutation are less capable of plaquing the male specific phages R17 and M13. In recombination proficient cells this behavior is apparently due to a reduction in adsorption sites. Cultures of F prime strains bearing dnaB43 contained significantly fewer F piliated cells than were seen in the wild-type (Iyer et al 1974). Since both growth and attachment of F pili occur via the cell membrane (Brinton, 1971), a change in its properties is indicated.

Evidence from outside the present study also suggests a membrane involvement in the temperature sensitivity phenotype. Siccardi et al, (1971) completed a study of the effect of incubation at both permissive and non-permissive temperatures upon the protein composition of cell membranes from strains bearing the lesions dnaB313, dnaB42 and dnaB266.

Preparations from these mutants displayed a deficiency of one protein component (MW60,000) and an increase in another (MW 30,000) relative to the wild-type when incubated at high temperatures. In addition, cells carrying the dnaB42 mutation which, significantly, is like dnaB43 in being both salt correctable and recessive showed similar changes when grown at permissive temperatures. Roughly analogous behavior was observed in strain MX742ts27 (dnaB27) when it was examined in a similar manner (Inouye and Gurthie, 1969).

It is rather obvious that this author prefers the replicon (maintenance-site) model (Jacob and Brenner, 1963) over the repressor-molecule model (Pritchard et al, 1969) of replication control as a basis for interpreting data concerning intracellular incompatibility. In addition to those cited at the beginning, there are some observations emanating from the present study that might be taken as support for this position.

In the repressor-molecule model incompatibility is explained as a random assortment of F prime plasmids with no bias being exerted towards either the incoming or resident factor.

Such behavior is not detected in the CR34 line. Wild-type F42/F101 double males display a preferential maintenance of factor F42.

Moreover, this occurs despite the fact that factor F101 is dominant in establishing itself in both exponential and overnight cultures of the same organism (Table 13). By this model, F101 is the more efficient producer of repressor, yet, it segregates more rapidly than F42. Finally, the action of P1 prophage on the dnaB43 - bearing cells (F⁻) recipient ability is not so neatly explained by the Pritchard model as it is by the use of maintenance sites.

Accordingly then, the terminology used throughout this discussion is that arising from the membrane attachment model of DNA apportionment (Jacob et al, 1963). Thus it was assumed that attachment, maintenance (or replication) and segregation sites all referred to the same position on the cell membrane. However recent reports have indicated that with certain plasmid systems, the actual behavior is not quite this simple (Novick and Brodsky, 1972; LeBlanc and Falkow, 1973; Hersfeld et al, 1973).

In brief, the establishment of an incoming plasmid does not appear to be an unitary event. Rather, there is an immediate, but temporary, delay in its replication followed by a longer delay in its hereditary stabilization. Incompatibility acts to inhibit both these processes. Since its effect on the latter is manifested through an asymmetric distribution of plasmids to daughter cells, two different membrane attachment sites must exist. (See Literature Survey for more detailed consideration).

The present investigations have concerned themselves with the stability of F prime plasmids in established double male strains. (Colonies arising upon prolonged incubation on selective media). Therefore, interpretations drawn from them refer to segregation and segregation sites only. No consideration has been given to replication (and replication sites). However, the existence of two separate sites would explain an apparent inconsistency in the altered attachment-site model proposed for dnaB43 action. Strains bearing this lesion are 3 to 7 times more efficient than non-mutant cells in their uptake of factor Fl01 (Stationary phase, RecA⁻). Yet, this same F prime plasmid is very unstable in established cultures. If dnaB43 were to alter the segregation site and not the replication site, such behavior could be accounted for.

In closing, two attempts at characterizing the action of dnaB43 on incompatibility - determined replication inhibition have met with only minor success. Stationary phase Fl01 derivatives of recombination defective CR34 cells were mixed with a F42 donor for 7 minutes then diluted 500 fold into fresh medium and incubated a further 60 minutes. In this manner, we sought to measure F42 establishment under conditions of minimal entry exclusion.

In one experiment the frequency of Lac^+ cells obtained with wild-type recipients increased for 15 minutes then slowly declined. Presumably because of its failure to replicate, factor F42 was diluted from the culture. The rise in DnaB^- cells was less rapid but continued for a full 35 minutes, after which it too displayed a slow decline. With F^- controls the number of Lac^+ progeny rose unchecked for upwards to 55 minutes. The generation times of cells under these conditions ranged from 106 to 125 for F^- and 170 to 200 for F prime recipients. These data can be interpreted as indicating a reduction of forces acting to inhibit replication of the incoming F plasmid in the presence of dnaB43. Disappointingly, they suffer from a low degree of reproducibility and hence have not been mentioned in the Results section.

Secondly, P1 was employed to introduce factor F42 into exponential cultures of the same strains described above (Willetts and Achtman, 1972; Novick and Brodsky, 1972). Unfortunately, this line seems to be highly refractive to infection by this phage ($10^2 \times$ fewer plaques than isogenic Rec^+ strains). Therefore, extremely low numbers of Lac^+ transductants were obtained.

Of those resulting with F^- recipients, 25 to 33% were resistant to R17 (data not given).

To summarize, the action of dnaB43 appears to be localized within the cell wall/membrane complex. Its functioning to lift entry exclusion barriers possibly results in increased effective mating pair formation. Its effect upon intracellular incompatibility seems to stem from a removal of the force (s) determining the maintenance of one F prime plasmid in preference to another. Two distinct mechanisms have been proposed to account for the latter. Of these, the one postulating an alteration (either structural or enzymatic) in the F plasmid attachment site so that it becomes more accessible to the incoming factor is more compatible with the observed behavior of DnaB⁻ cells.

Wickner et al (1974) have reported a purification of the dnaB⁺ product protein. A DNA - dependent and a DNA - independent ribonucleoside triphosphatase activity was associated with this material. These activities are only presumably a property of the dnaB⁺ protein itself. The various physiological effects of the dnaB43 mutation cannot readily be seen simply as resulting from temperature sensitive deficiencies in these activities.

One possibility that suggests itself is that of protein - protein interaction playing a physiologically determinative role. The indirect evidence concerning growth of phage lambda (Georgoulas and Herskowitz, 1971; Hayes and Szybalski, 1973) suggests that the λ P protein can interact with the dnaB protein and the products of other genes at a specific site or sites. That the dnaB product might be composed of several subunits has been put forward as a possible explanation for the dominance of certain dnaB⁻ alleles over the wild-type (see Literature Survey). An interaction of the dnaB⁻ protein with other proteins or at sites other than the replication complex could therefore be envisioned as the reason for its effect on other complex processes (entry exclusion, incompatibility, F piliation) besides DNA synthesis.

(c) Other Effects of the Presence of dnaB43

In confirmation of a previous report (Palchaudury and Iyer, 1971), F prime derivatives of CR34 strains bearing dnaB43 display a reduced sensitivity to the male specific phages R17 and M13. This effect is independent of the presence of recombination functions. Mutation bearing Rec^- cells carrying F plasmid DNA form some 65 to 90% fewer plaques with these phages than do wild-type strains (Table 8). Those harboring factor F101 are affected to a lesser degree than are F42 derivatives (4-5X less sensitive). This may indicate a defect in F42 function or, more likely, reflect an inherent difference peculiar to these two F plasmids.

Iyer et al (1974) have conducted an extensive study of this behavior in $\text{Rec}^+ \text{DnaB}^-$ cells. It was cotransducible with dnaB43 and appeared to be a secondary effect of its presence since addition of potassium chloride (0.15M) restored normal levels of sensitivity. Analysis of electron micrographs indicated dnaB43 acts to reduce the number of F- piliated cells in mutant cultures (6-fold less than wild-type) and, as well, the number of sex pili per cell. It is not yet known whether the formation or stability of attached pili is altered.

It is very likely that dnaB43 acts in the same manner in Rec⁻ strains. Since F pili are not needed for entry exclusion (Curtiss et al, 1969; Achtman et al, 1971) and dnaB⁻ cells are not known to have acquired a change in donor abilities (Table 10), this action on phage sensitivity is probably independent of dnaB43 effects on conjugation properties.

F prime derivatives of DnaB⁻ strains also display an altered sensitivity to the female specific phage ϕ II. Such cells are usually poor hosts for ϕ II propagation. However, in the presence of dnaB43 4 to 10 fold increases in plaquing efficiency were noted relative to non-mutant cells (Table 9). In keeping with the earlier observation, factor F42 was less able to restrict this phage than F101. Because male and female-phage specificity functions are different (Willettts and Finnegan, 1970; Achtman et al, 1971) this effect cannot be due to the absence of F pili.

Linial and Malamy (1970) used ³²P/- labelled ϕ II to determine the fate of phage DNA upon its infection of restricting hosts. ϕ II DNA is injected into F prime cells and becomes membrane attached. Although very little replication occurred, there was no detectable degradation of phage DNA. In subsequent studies involving restriction - defective mutants of F, Morrison and Malamy (1971) concluded that its inhibition of ϕ II (and T7) development operated at the level of translation by preventing the synthesis of several essential phage proteins.

In its attachment to the cell membrane and failure to replicate in the absence of DNA degradation, ϕ II behaves similarly to a superinfecting F plasmid. This comparison is extended by their like response to dnaB43: (a) enhanced infectivity, (b) decreased intracellular restriction. Hence in view of the Morrison-Malamy observations, it would be of interest to reconsider evidence in support of the Pritchard model of incompatibility. Alternatively, as with phage P1, a modification in the F maintenance site may make it available to ϕ II as well. Thus DnaB⁻ cells would be capable of accomodating twice as many phage particles. At any rate, the presence of this mutation has increased the CR34 cells capacity to serve as host for female specific phage growth.

Other reports have implied a role for chromosomal genes in moderating F plasmid determined phage sensitivities. E coli K12 strains that had acquired resistance to high levels of ampicillin also displayed a decreased sensitivity to the male specific phage MS2 and an increased ability to plaque female specific ϕ W (Monner et al, 1971). Such changes were correlated with alterations in cell membrane composition. Interestingly enough, one of the genes involved in determining this behavior (Amp^R) maps near dnaB43 at 83 minutes (Boman et al, 1968). In contrast to the dnaB43 situation, the Amp^R related changes in phage sensitivity were accompanied by a reduction in mating pair formation.

One additional point must be mentioned here. Mutant cells made merodiploid for the dnaB region by introduction of the factor F134-1 (F'dnaB⁺) acquired a number of wild-type traits (resistance to temperature and F42 uptake, increased division rate). Yet, when tested for their ability to plaque R17, R13 or ØII they failed to demonstrate a return to wild-type sensitivities (Tables 8&9). The implication that this behavior stems from a second site mutation, outside the area complemented by F134, is difficult to accept in view of its cotransduction with dnaB43 (Iyer et al, 1974).

Although not directly related to studies on dnaB43 action, one further observation will be discussed. In addition to sex specific (F,F-like & I-like) Enterobacteriaceae bear a second class of pilus termed Type-I or common pili (Meynell and Lawn 1967). These are shorter, more numerous, and plasmid independent structures (see Meynell and Data, 1969; Brinton 1971). In Salmonella and Shigella, their presence is apparently necessary for the optimal recipient ability of a plasmid - negative cell (Meynell and Lawn, 1967, Mulczyk and Duguid, 1968). In contrast, E coli mutants defective in common pili production seem to retain wild-type levels of recipient efficiency (Nishimura et al, 1967)

However, Ou (1973) has recently intimated that Type-I pili may indeed have a role to play in determining recipient efficiencies. Citing as yet unpublished evidence obtained while conducting an examination of recipient behavior during a HfrXF⁻ cross, he indicated common pili to function in inhibiting mating pair formation. Information resulting from the present investigations regarding the behavior of P1 - transduced derivatives of the common pili minus mutant of Nishimura tend to support this suggestion.

In Table 17 are compared the recipient abilities of exponential phase common pili minus strains carrying factors F101 and F42. Strain C043 harbors F101 and displays an entry exclusion index (EexI) 8.46 fold greater than its isogenic F42 derivative, strain C039. Likewise the DnaB⁻ strain bearing F101 (C045) is 3.30 times more proficient in limiting the uptake of a superinfecting factor than its corresponding F42 derivative (C044).

The lesion born by the Nishimura strain (pil⁻) has been mapped at 88 minutes (Maccàcaro & Hayes, 1961). Hence, according to Low (1972), the F prime factor F101 (derived from HfrH) must carry chromosomal DNA complementary to the pil region.

If the wild-type allele were dominant, Nishimura strains carrying this plasmid would also bear Type-I pili. Moreover, because these cells are more resistant to the conjugal entrance of a second F prime plasmid than those containing F42 (Pil⁻), the newly acquired common pili must play a role in entry exclusion. The absence of these appendages had no effect on the recipient efficiencies of F⁻ strains (data not given).

Similar reasoning can be applied to explain the difference in entry exclusion noted between F101 and F42 derivatives of CR34 strains (Results sec. (e)). Cells harboring factor F101 would be expected to show higher resistances to superinfection because they carry a double complement of pil determined common pili. Correspondingly, since they carry only a single dose of pil, strains bearing F42 would be less proficient at exclusion. Exactly this sort of behavior was observed (Tables 11 and 12).

For this reason, the ratio of entry exclusion indexes for F101 and F42 - bearing CR34 derivatives ought to be nearly twice that determined for the analogous Nishimura derivatives. The values calculated for wild-type and DnaB⁻ CR34 cells were 13.2 and 5.80 respectively. Comparison with the numbers obtained by doubling the

previously given values for Nishimura strains revealed them to be 79 and 88% equivalent. The 20% error may stem from cell - line determined differences. Since no such correlation could be demonstrated in overnight recipients, common pili must exert the bulk of their effect in rapidly growing cultures.

The presence of common pili on F101 derivatives of the Nishimura strain could be confirmed by electron microscopy.

(d) Comparison of dnaB43 to Other Incompatibility Mutations.

The isolation of a number of chromosomally determined incompatibility mutants has been reported in the recent literature. Hrebenda and Kwiatkowski (1972) treated F Plasmid containing 200PS cells (Rec^+) with nitroguanidine to obtain five so called Imm-mutants that facilitated the uptake of factor F42. SanBlas, Thompson, and Broda (1974) also used NTG to retrieve a derivative of the F^- strain ED134 (RecA^-) that enhanced the stable coexistence of two F prime plasmids. A strain (MA109I) permitting the continued presence of factor F101 was isolated by DeVries and Maas (1973) as a result of a cross between a F prime donor and a RecA^- Hfr recipient.

Three of the five imm⁻ mutations appear to be chromosome linked. One of these, imm9, has other properties in common with dnaB43. F plasmid containing cells bearing the former display a reduced sensitivity to male specific phage but are normal in their donor capacities. Just as certain F prime elements show an increased instability in CR34 DnaB^- strains, F42 derivatives of this mutant are more sensitive to acridine orange elimination. Unlike dnaB43, however, imm9 does not render chromosome replication sensitive to elevated temperatures. One eagerly awaits the results of mapping studies.

The "Broda" incompatibility mutation increases yields of double male cells during tri - parental matings with donors of factors F8 (F'gal⁺) and F30 (F'his⁺). Such doubles show independent transfer of plasmid markers to suitable recipients and grow at rates equivalent to the F⁻ parent. Sucrose density gradient analysis confirms the presence of two autonomous F plasmids. However, in contrast to dnaB43 this lesion is specific for F8/F30 interactions. Substitution of F101 for either of these effected a return to normal incompatibility levels. Indeed, its action is so refined that even the slightest modification in plasmid structure (F' gal⁺ → F'Δp gal⁺) was detectable. Although no comparable studies have been made with DnaB⁻ strains, it is worth noting that prolonged storage of F8/F30 double males results in a high incidence of his⁺ integration into the chromosome with a concomitant loss of male specific phage sensitivity. Again, the exact chromosomal location of this mutation has not been reported.

The Hfr strain described by DeVries and Maas differs somewhat from the foregoing incompatibility mutants. Although its lesion is born on the chromosome, it appears to be located within the integrated F-DNA. However, since F prime plasmids derived from this Inc⁻ mutant continue to demonstrate normal levels of incompatibility towards superinfecting factors, its expression is confined to the Hfr condition. In contrast to cells bearing dnaB43 and previous mutants of this type (Maas and Goldschmidt, 1969), the present strain remains proficient in entry exclusion.

The inc⁻ mutation does not affect donor ability. inc⁻/F101 merodiploids are very sensitive to acridine orange killing.

The authors have not been able to determine the basis of this integrated versus autonomous behavior. The high instability of double males harboring two above derived F primes argues against the wild-type product being dominant. Because it is operational only in the integrated state the inc⁻ mutation may prevent autonomous replication. Thus, excision from the chromosome results in the selection of either inc⁺ revertants or plasmids with repressed inc⁻ functions. In support of the latter, there are preliminary indications that such F primes regain the Inc⁻ phenotype upon their reinsertion into the chromosome (Maas, private communication).

This latest observation, if substantiated, is most interesting since it implies the existence of two different F - determined incompatibility systems. A primary, physical or sterical, system would operate to preclude the prolonged coexistence of autonomous F primes. A secondary, biochemical, system could function to inhibit Hfr/F prime stability. The former is designated primary because F primes derived from Inc⁻ Hfr's are Inc⁺.

The operation of two independent systems would easily explain behavior presently inconsistent with the maintenance - site model of incompatibility. Despite the presumed availability of an attachment site, Hfr cells still inhibit the stable establishment of superinfecting F plasmids. In cells bearing autonomous F primes, the primary F'/F' system acts. It results in segregation because of unequal sharing of the single maintenance site. Upon coming under the governance of the larger replicon, the Hfr/F' system prevails. The autonomous factor attaches to its site but is subsequently lost from the culture because a chromosome product inhibits its replication.

The non-isogenic plasmid ColV2 is incompatible with F', yet, can stabilize in Hfr cells. In F42/ColV2 - incompatibility, factor F42 is preferentially lost (MacFarren and Clowes, 1967) thus ColV2 must possess the greater affinity for the attachment site. With its insertion into the chromosome, F produces a protein which because of its lack of homology with F has no effect on ColV2 replication (approximately 30% homology between unsubstituted F and ColV-K94, Sharp et al, 1973).

If such behavior did occur and two incompatibility systems do exist, one would then expect to be unable to detect such Inc⁻ mutants in the integrative suppression system of Nishimura et al (1971). It would be of value in future studies to determine the influence of dnaB43 on F prime/Hfr incompatibility. Although its presence reduces incompatibility as it occurs between two F primes, such may not be the case in this instance.

REFERENCES

- Achtman, M.: Genetics of the F sex factor in Enterobacteriaceae. Curr. Topics Microb. Immun. 60: 70 (1973).
- Achtman, M., N. Willetts and A.J. Clark: Beginning a genetic analysis of conjugational transfer determined by the F factor in Escherichia coli by isolation and characterization of transfer deficient mutants. J. Bact. 106: 529 (1971).
- Achtman, M., N. Willetts and A.J. Clark: A conjugational complementation analysis of transfer - deficient mutants of F⁻ lac in Escherichia coli. J. Bact. 110: 831 (1972).
- Adams, M.: Bacteriophage, Interscience Publishers, New York (1959).
- Adler, H., W. Fisher, A. Cohen, and A. Hardigree: Miniature Escherichia coli cells deficient in DNA. Proc. Nat. Acad. Sci. U.S.A. 57: 321 (1967).
- Appleyard, R.K.: Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from Escherichia coli K12. Genetics: 39: 440 (1954).
- Bachmann, B.J.: Pedigrees of some mutant strains of Escherichia coli K12. Bact. Rev., 36: 525 (1972).
- Bastarrachea, F. and A.J. Clark: Isolation and characterization of an Escherichia coli strain harboring three sex factors. Genetics, 60: 641 (1968).

Zoyer, H.W., DNA restriction and modification mechanisms in bacteria. *Ann. Rev. Microb.*, 25: 153 (1971)

Beard J., J. Howe and M.H. Richmond: Centrifugation properties of sex pili. *J. Mol. Biol.* 66: 311 (1972)

Beyersmann D., and H. Schuster: DNA synthesis in P1 infected Escherichia coli temperature - sensitive in DNA replication. *Molec. Gen. Genetics*, 114: 173: (1971)

Beyersmann, D., W. Messer and M. Schlicht: Mutants of Escherichia coli B/r defective in deoxyribonucleic acid initiation: dnaI, a new gene for replication. *J. Bact.* 118: 783 (1974).

Boman, H.K. Eriksson - Greenberg, S. Normark and E Matsson: Resistance of Escherichia coli to penicillins. IV. Genetic study of mutants resistant to DL - ampicillin concentrations of 100 µg/ml. *Genet. Res. (Camb)* 12: 169 (1968)

Bonhoeffer, F.: DNA transfer and DNA synthesis during bacterial conjugation. *Z. Vererbungsl.* 98: 141 (1966).

Bonhoeffer, F., and H. Schaller: A method for selective enrichment of mutants based on the high UV sensitivity of DNA containing 5 - bromouracil. *Biochem. Biophys. Res. Comm.* 28: 93 (1965).

Bresler, S., V. Lanzov and A. Lukjaniec - Blinkova: On the mechanism of conjugation in Escherichia coli K12: *Molec. Gen. Genetics* 102: 269 (1968).

Brinton C.C., Jr.: The properties of sex pili, the viral nature of "conjugal" genetic transfer systems, and some possible approaches to the control of bacterial drug resistance. *Critical Rev. Microbiol.* 1: 105 (1971).

Capaldo, F., G. Ramsey, and S. Barbour: Analysis of the growth of recombination - deficient strains of Escherichia coli K12. J. Bact. 118: 242 (1974)

Carl, P.L.: Escherichia coli mutants with temperature-sensitive synthesis of DNA. Molec. Gen. Genetics. 109: 107 (1970).

Caro, L and M. Schnös: The attachment of the male-specific bacteriophage F1 to sensitive strains of Escherichia coli Proc. Nat. Acad Sci U.S.A. 56: 126 (1966).

Cavalli, L., J. Lederberg and E. Lederberg: An infective factor controlling sex compatibility in Bacterium coli. J. Gen. Microbiol. 8: 88 (1953).

Clark, A.J.: Genetic analysis of a "double male" strain of Escherichia coli K12. Genetics, 43: 105 (1963).

Clark, A.J.: Recombination deficient mutants in Escherichia coli and other bacteria. Ann. Rev. Genetics 7: 67 (1973).

Clark, A., and A. Margulies: Isolation and characterization of recombination - deficient mutants of Escherichia coli K12. Proc. Nat. Acad Sci U.S.A. 53: 451 (1965)

Clark, A., W.K. Maas and K.B. Low: Production of a merodiploid strain from a double male strain of Escherichia coli K12. Molec. Gen. Genetics 105: 1 (1969).

Clowes, R.C.: Molecular structure of bacterial plasmids. Bact. Rev. 36: 361 (1972).

Cohen A., W. Fisher, R. Curtiss III and H. Adler: DNA isolated from Escherichia coli minicells mated with F⁺ cells. Proc. Nat. Acad. Sci. U.S.A. 61: 61 (1968)

Cole, R.S.: Properties of F' factor DNA transferred from ultraviolet - irradiated donors: photoreactivation in the recipient and the influence of recA, recB, recC and Uvr genes, J. Bact. 106: 143 (1971).

Couch, J., and P. Hanawalt: DNA repair replication in ~~the~~ temperature - sensitive DNA synthesis deficient bacteria. Biochem. Biophys. Res. Commun. 29: 779 (1967)

Curtiss, R. III: Bacterial conjugation. Ann. Rev. Microb. 23: 69 (1969).

Curtiss, R. III and D. Stallions: Energy requirements for specific pair formation during conjugation in Escherichia coli K12. J. Bact. 94: 490 (1967).

Curtiss, R. III, L. Caro, D. Allison and D. Stallions: Early stages of conjugation in Escherichia coli, J. Bact. 100 1091 (1969).

Curtiss, R. III, L. Charmella, D. Stallions and J. Mays: Parental functions during conjugation in Escherichia coli K12. Bact. Rev. 32: 320 (1968).

Curtiss, R.III, R. Siegel, D. Stallions and G. Vandenbos :
Bacteriol. Proc. p.15 (1970).

Cuzin, F.: Un bacteriophage specifique du type sexuel F⁻
d' Escherichia coli K12. C.R. Acad. Sci. (Paris)
260: 6482 (1965)

Cuzin, F., and F. Jacob: Existence chez Escherichia coli
d'une unite genetique de segregation formee de differents
replicons. C.R. Acad.Sci. (Paris) 260: 5411. (1967a).

Cuzin, F., and F. Jacob: Mutations de L'episome F D'
Escherichia coli K12.II. Mutants a replication
thermosensible. Ann. Inst. Pasteur 112: 39 (1967 b).

D'Ari, R., A. Jaffe - Brachet, D. Touati - Schwartz and M.
Yarmolinsky: A dnaB analog specified by bacteriophage
P1. submitted to J. Mol. Biol, (1974).

Davis, B., and E. Mingoli: Mutants of Escherichia coli
requiring methionine or vitamin B₁₂. J. Bact. 60: 17
(1950).

Dawes, E. "Quantitative Problems in Biochemistry" Livingstone
Ltd. London, (1967).

Demeric, M., E. Adelberg, A. Clark and P. Hartman: A
proposal for a uniform nomenclature in bacterial genetics.
Genetics 54: 641 (1966).

de Haan, P., and J. Gross: Transfer delay and chromosome withdrawal during conjugation in Escherichia coli: Genet. Res. (Camb) 3: 251 (1962).

de Haan, P., and A. Stouthamer: F prime transfer and multiplication of sexduced cells. Genet. Res. (Camb) 4: 30 (1963).

deVries, J., and W.K. Maas: Chromosomal integration of F' factors in recombination deficient Hfr strains of Escherichia coli: J. Bact. 106: 150 (1971).

deVries, J. and W.K. Maas: Description of an incompatibility mutant of Escherichia coli: J. Bact. 115: 213 (1973).

Dubnau, E. and W.K. Maas: Inhibition of replication of an F' lac episome in Hfr cells of Escherichia coli. J. Bact. 95: 531 (1968).

Echols, H: Properties of F prime strains of Escherichia coli superinfected with F' lac and F' gal episomes. J. Bact. 85: 262 (1963).

Falkow, S., L. Tompkins, R. Silver, P. Guerry and D. LeBlanc: The replication of R - factor DNA in Escherichia coli K12 following conjugation. Annals. N.Y. Acad. Sci. 182: 153 (1971).

Fangman, W., and A. Novick: Characterization of two bacterial mutants with temperature - sensitive synthesis of DNA. Genetics 60: 1 (1968).

Frame, R., and J. Bishop: The number of sex-factors per chromosome in Escherichia coli. Biochem. J. 121: 93 (1971).

Franklin, N.C.: "Illegitimate Recombination" in "The Bacteriophage Lambda". ed. A. Hershey p. 175, Cold Spr. Harb. Lab. New York (1971).

Freifelder D.: Studies on Escherichia coli sex factors. III. Covalently closed F'lac DNA molecules. J. Mol. Biol. 34: 31 (1968).

Freifelder, D.R., and F. Freifelder: Studies on Escherichia coli sex factors: I. Specific labelling of F'lac DNA. J. Mol. Biol. 32: 15 (1968).

Frydman, A., and E. Meynell: Interactions between derepressed F-like R factors and wild-type Colicin B factors: Superinfection immunity and repressor susceptibility. Genet. Res. (Camb) 14: 315 (1969).

Gefter, M., Y. Hirota, T. Kornberg, J. Wechsler and C. Barnoux: Analysis of DNA polymerase II and III in mutants of Escherichia coli thermosensitive for DNA synthesis. Proc. Nat. Acad. Sci. U.S.A. 68: 3150 (1971).

Gellert, M.J. Little, C. Oskinsky and S. Zimmerman: Joining of DNA strands by DNA ligase of Escherichia coli. Cold Spr. Harb. Symp. Quant Biol. 33: 677 (1968).

- Glower, S., J. Schell, N. Symonds and K. Stacey: The control of host induced modification of phage ϕ 1., *Genet. Res. (Camb.)*; 4: 480 (1963)
- Georgopoulos, C., and I. Herskowitz: Escherichia coli mutants blocked in lambda DNA synthesis in "The Bacteriophage Lambda" ed. A.D. Hershey p. 553 Cold Spr. Harb. Lab. New York (1971).
- Gilbert W. and D. Dressler: DNA replication: The rolling circle model. Cold Spr. Harb. Symp Quant. Biol. 33: 473 (1963).
- Goebel, W.: Studies on extrachromosomal DNA elements: replication of the colicinogenic factor ColE1 in two temperature sensitive mutants of Escherichia coli defective in DNA replication. *Eur. J. Biochem.* 15: 311 (1970)
- Goebel, W.: Incorporation of deoxyribonucleoside triphosphates into plasmid DNA by toluenized cells of thermosensitive DNA replication mutants of Escherichia coli. *FEBS Letters* 14: 121 (1971).
- Goebel, W.: The influence of dnaA and dnaC mutations on the initiation of plasmid DNA replication: *Biochem. Biophys Res. Commun.* 51: 1000 (1973).
- Goebel, W., and H. Schrempf: Replication of plasmid DNA in temperature sensitive DNA replication mutants of Escherichia coli. *Biochim. Biophys. Acta* 262: 32 (1972).
- Goldfarb, D., G. Goldberg, L. Chermin, L. Gukova, I. Avdienks, B. Kuznetsova and I. Kushner: A protein produced by male strains of Escherichia coli K12 which increases the yield of recombinants in conjugation: Its nature and mode of action. *Molec. Gen. Genetics*, 120: 211 (1973).

Gross, J.D.: DNA replication in bacteria. Curr. Topics Microb. Immun. 57: 39 (1972).

Guerola, N.J. Ingraham and E. Cerda - Olmedo: Induction of closely linked multiple mutations by Nitrosoguanidine: Nature N.B. 230: 122 (1971).

Hathaway, B., and P. Bergquist: Temperature sensitive mutations affecting the replication of F prime factors in Escherichia coli K12. Molec. Gen. Genetics 127: 297 (1973).

Hashimoto, H., and Y. Hirota: Gene recombination and segregation of resistance factor R in Escherichia coli J. Bact. 91: 51 (1966).

Hayes, W.: Observations on a transmissible agent determining sexual differentiation in Bact. coli. J. Gen. Microbiol. 8: 72 (1953).

Hayes, W. and W. Szybalski: Molecular Cytogenetic Symp. (eds. Hankalo, B., and J. Papaconstantinou) Plenum Pub. Co. New, York (1973).

Hechemy, K. and H. Goldfine: Isolation and characterization of a temperature - sensitive mutant of Escherichia coli with a lesion in acylation of lysophosphatidic acid. Biochem. Biophys. Res. Comm. 42: 245 (1971).

Helinski, D.R.: Plasmid determined resistance to antibiotics: Molecular properties of R Factors. Ann. Rev. Microb. 27: 437 (1973).

- Hershfield, V., D. LeBlanc and S. Falkow: Membrane attachment of R. Factor DNA in compatible and incompatible cell pairs following conjugation. J. Bact 115: 1208 (1973).
- Hirota Y., A. Ryter and F. Jacob: Thermosensitive mutants of Escherichia coli affected in the processes of DNA synthesis and cellular division. Cold Spr. Harb. Symp. quant. Biol. 33: 677 (1968).
- Hohn, B., and D. Korn: Cosegregation of a sex factor with the Escherichia coli chromosome during curing with acridine orange. J. Mol. Biol. 45: 385 (1969).
- Hrebenda, J. and Z. Kwiatkowski: Mutations affecting superinfection immunity in Escherichia coli. Acta Microbiol Polonica IV: 145 (1972).
- Hsu, Ming-Pa: Electron microscope analysis of partial denaturation of F factor deoxyribonucleic acid. J. Bact. 118: 425 (1974).
- Ikeda, H, and J. Tomizawa: Prophage Pl, an extrachromosomal replication unit. Cold Spr. Harb. Symp. Quant. Biol. 33: 791 (1968).
- Inouye M. and J. Guthrie: A mutation which changes a membrane protein in E coli. Proc. Nat. Acad. Sci. U.S.A. 64: 957 (1969).
- Inselburg, J.: Segregation into and replication of plasmid DNA in chromosomeless segregants of Escherichia coli. J. Bact. 102: 642 (1970).

- Iyer, V.N., R.V. Iyer, S. Palchaudury, S. Becker and I. Stevenson:
A chromosomal mutation affecting F piliation in Escherichia coli. communicated to Molec. Gen. Genetics (1974).
- Jacob, F., and S. Brenner: Sur la regulation de la synthese
de DNA chez les bacteries: I. Hypothese du replicon.
C.R. Acad. Sci. (Paris) 256: 298 (1963).
- Jacob, F., S. Brenner, and F. Cuzin: On the regulation of
DNA replication in bacteria. Cold Spr. Harb. Symp.
Quant. Biol. 28: 329 (1963).
- Jacob, F., and E. Wollman: Recombination genetique et
mutants de fertilite chez Escherichia coli. C.R. Acad.
Sci., (Paris) 242: 303 (1956).
- Jacob, F., and E. Wollman: Les episomes, elements genetiques
ajoutes. C.R. Acad. Sci., (Paris,) 247: 154 (1958).
- Jacob, F., and E. Wollman: Sexuality and the genetics of
bacteria; New York, Academic Press, 1961.
- Joshi, G., and O. Siddiqi: Enzyme synthesis following
conjugation and recombination in Escherichia coli.
J. Mol. Biol, 32: 201 (1968).
- Kahn, P., and D. Helinski: Relationship between colicinogenic
factors El, V and an F factor in Escherichia coli. J. Bact.
88: 1573 (1964).
- Kass, L. and M. Yarmolinsky: Segregation of functional sex factor
into minicells. Proc. Nat. Acad. Sci. U.S.A. 66: 815 (1970).

- Kinsbury, D., and D. Helinski: DNA polymerase as a requirement for the maintenance of the bacterial plasmid CoIE1. Biochem. Biophys. Res. Commun. 41: 1538 (1970).
- Kohiyama, M., D. Cousin, A. Ryter and F. Jacob: Mutants thermosensible d'Escherichia coli K12 isolement et characterization rapide. Ann. Inst. Pasteur 110: 465 (1966).
- Kondo, E., and S. Mitsuhashi: Drug resistance of enteric bacteria. IV, Active transducing bacteriophage PlCM produced by the combination of R factor with bacteriophage Pl. J. Bact. 88: 1266 (1964).
- Komano, I., and H. Sakai: A temperature sensitive mutant in the initiation of DNA replication and ϕ X174 DNA synthesis in the cell. Molec. Biol. Meeting, Japan, (1972) Nucleic Acids, biosynthesis and structure; Kyoritsu Shuppan Ltd. Tokyo.
- Lanka, E., and H. Schuster: Replication of bacteriophages in Escherichia coli mutants thermosensitive in DNA synthesis. Molec. Gen. Genetics; 106: 274 (1970).
- Lawn, A and E. Meynell: Serotypes of sex pili. J. Hyg. 68: 683 (1970)
- Lawn, A., E. Meynell and M. Cooke: Mixed infections with bacterial sex factors: sex pili of pure and mixed phenotype. Ann. Inst. Pasteur. 120: 3, 1971).
-

Lederberg, J., Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria., Viol. 3:496(1957)

LeBlanc, D., and S. Falkow: Studies on superinfection immunity among transmissible plasmids in Escherichia coli. J. Mol. Biol. 74: 689 (1973).

Lederberg, J.: Extranuclear transmission of the F compatibility factor in Escherichia coli. Abstr. Commun. 7th intern. Congr. Microbiol., Stockholm 59 (1958).

Lederberg, J., L. Cavalli and E. Lederberg.: Sex compatibility in Escherichia coli. Genetics 37: 720 (1952).

Lennox, E.S.: Transduction of linked genetic characters of the host by bacteriophage P1. Virology, 1: 190 (1955).

Linial, M., and M. Malamy: Studies with bacteriophage ϕ II. Events following infection of male and female derivatives of Escherichia coli K12. J. Virol. 5: 72 (1970).

Low, K.B.: Formation of merodiploids in matings with a class of Rec^- recipient strains of Escherichia coli K12. Proc. Nat. Acad. Sci. U.S.A. 60: 160 (1968).

Low, K.B.: Escherichia coli K12 F prime factors, old and new. Bact. Rev. 36: 587 (1972).

Maas, W.K. and A. Goldschmidt: A mutant of Escherichia coli permitting replication of two F factors. Proc. Nat. Acad. Sci. U.S.A. 62: 873 (1969).

Maccacaro, G., and W. Hayes: The genetics of fimbriation in Escherichia coli. Genet. Res. (Camb) 2: 394 (1961).

MacFarren, A., and R. Clowes: A comparative study of two F-like colicin factors ColV2 and ColV3 in Escherichia coli K12. J. Bact. 94: 365 (1967).

Macrina, F., and E. Balbinder: Genetic characterization of a stable F' lac plasmid. J. Bact 112: 503 (1972).

Marinus, M. and E. Adelberg: Vegetative replication and transfer replication of DNA in temperature-sensitive mutants of Escherichia coli K12: J. Bact. 104: 1266 (1970).

Masker, W. and P. Hanawalt: Ultraviolet stimulated synthesis in toluenized Escherichia coli deficient in DNA polymerase I. Proc. Nat. Acad. Sci. U.S.A. 70: 129 (1973).

Meynell, G.G.: Exclusion, superinfection immunity and abortive recombinants in I^+ X I^- crosses. Genet Res. (Camb.) 13: 113 (1969).

Meynell, E., and N. Datta: in "Bacterial Episomes and Plasmids" Ciba Foundation Symposium; (eds. G. Wolstenhome and N.O'Connor) p.120; Churchill, London (1969).

Meynell, E., and A. Twins: Effect on exclusion of alterations to the sex pilus. J. Bact. 113: 71 (1973).

Meynell, G. and A. Lawn: Sex pili and common pili in the conjugational transfer of colicin factor Ib by Salmonella typhimurium. Genet. Res. (Camb) 9: 359 (1967)

Miller, J.H.: Experiments in Molecular Genetics, Cold Spr. Harb. Lab., New York (1972).

Monner, D., S. Jonsson and H. Brown: Ampicillin - resistant mutants of Escherichia coli K12 with lipopolysaccharide alterations affecting mating ability and susceptibility to sex-specific bacteriophages. J. Bact. 107: 420 (1971).

Moody, E. and W. Hayes: Chromosome transfer by autonomous transmissible plasmids: the role of the bacterial recombination (rec) system. J. Bact. 111: 80 (1972).

Morrison, J., and M. Malamy: T7 translational control mechanisms and their inhibition by F factors. Nature N. Biol. 231: 37 (1971).

Moses, R., and C. Richardson: Replication and repair of DNA in cells of Escherichia coli treated with toluene. Proc. Nat. Acad. Sci. U.S.A. 67: 674 (1970).

Mulczyk, M. and J. Duguid: Influence of the state of fimbriation on transmission of the colicinogenic factor Col1 between strains of Shigella flexneri J. Gen. Microbiol. 45: 459 (1968).

Nishimura, Y., L. Caro, C. Berg and Y. Hirota: Chromosome replication in Escherichia coli.IV. Control of chromosome replication and cell division by an integrated episome J. Mol. Biol. 55: 441 (1971).

Nishimura, Y., M. Ishibashi, E. Meynell and Y. Hirota: Specific piliation directed by a fertility factor and a resistance factor of Escherichia coli. J. Gen. Microbiol 49: 89 (1967).

Novick, R.P.: Extrachromosomal inheritance in bacteria. Bact. Rev. 33: 219 (1969).

Novick, R, and R. Brodsky: Studies on plasmid replication. I. Plasmid incompatibility and establishment in Staphylococcus aureus. J. Mol. Biol. 68: 285 (1972).

Okazaki, R., J. Okazaki, K. Sakabe, K. Sugimoto and A. Sugino: Mechanism of DNA chain growth.I.Possible discontinuity and unusual secondary structure of newly synthesized chains. Proc. Nat. Acad.Sci. U.S.A., 59: 598 (1968).

Olsen, W., W. Staudenbauer and P. Hofschneider: Replication of bacteriophage M13: specificity of the Escherichia coli dnaB function for replication of double stranded M13 DNA. Proc. Nat. Acad. Sci. U.S.A. 69: 2570 (1972).

Ou, J.,: Effect of Zn^{2+} on bacterial conjugation: increase in ability of F^- cells to form mating pairs. J. Bact. 115 684 (1973).

Palchaudury, S., and V.N. Iyer: Loss of an episomal fertility factor following the multiplication of coliphage M13. *Molec. Gen. Genetics*: 105: 131 (1969).

Palchaudury, S., and V.N. Iyer: Compatibility between two F' factors in an Escherichia coli strain bearing a chromosomal mutation affecting DNA synthesis. *J. Mol. Biol.* 57: 319 (1971).

Palchauduri, S., A. Mazaitis, W.K. Maas and A. Kleinschmidt: Characterization by electron microscopy of fused F prime factors in Escherichia coli. *Proc. Nat. Acad. Sci. U.S.A.* 69: 1873 (1972).

Paranchych, W.: Stages in phage R17 infection: the role of elivalent rations. *Virology* 28: 90 (1966).

Paranchych, W. and F. Graham: Isolation and properties of an RNA - containing bacteriophage. *J. Cell. Comp. Physiol.* 60: 199 (1962).

Press, R., N. Glansdorff, P. Miner, J. DeVries, R. Kadner and W.K. Maas: Isolation of transducing particles of ϕ 80 bacteriophage that carry different regions of the Escherichia coli genome. *Proc. Nat. Acad. Sci. U.S.A.* 68: 795 (1971).

Pritchard, R., P. Barth and J. Collins: Control of DNA synthesis in bacteria. *Symp. Soc. Gen. Microb.* 19: 263 (1969).

Ricard, M. and Y. Hirota: Effet des sels sur le processus de division celulaire d'Escherichia coli C.R. Acad. Sci. (Paris) 268: 1335 (1969).

Ricard, M. and Y. Hirota: Effet des sels et autres composés sur le phenotype de mutants thermo-sensibles d'Escherichia coli. *Ann. Microbiol. (Inst. Pasteur)*. 129: 29 (1973).

- Roozen, K., R. Fenwick and R. Curtiss III: Synthesis of ribonucleic acid and protein in plasmid containing minicells of Escherichia coli K12. J. Bact. 107: 21 (1971).
- Rosner, J.L.: Formation, induction, and curing of bacteriophage P1 lysogens. Virology 49: 679 (1972).
- Salzman, T.C. : Co-ordination of sex pili with their specifying R factors. Nature N. Biol. 230: 278 (1971).
- San Blas, F., R. Thompson and P. Broda: An Escherichia coli K12 mutant apparently carrying two autonomous F- prime factors. Genet. Res. (Camb) in press (1974).
- Sarathy, V., and O. Siddiqi: DNA synthesis during bacterial conjugation.II. Is DNA replication in the Hfr obligatory for chromosome transfer? J. Mol. Biol. 78: 443 (1973).
- Scaife, J. and J. Gross: Inhibition of multiplication of an F' lac factor in Hfr cells of Escherichia coli K12. Biochem.Biophys.Res. Commun. 7: 403 (1962).
- Schekman, R., W. Wickner, O. Westergaard, D. Brutlag, K. Geider, L. Bertch and A. Kornberg: Initiation of DNA synthesis: synthesis of ϕ X174 replicative form requires DNA synthesis resistant to rifampicin Proc. Nat. Acad. Sci. U.S.A. 69: 2691 (1972).
- Scott, J.R.: Genetic studies on bacteriophage P1. Virology 36: 564 (1968).
- Sharp, P., M-J. Hsu, E. Ohtsubo and N. Davidson: Election microscope heteroduplex studies of sequence relations among plasmids of Escherichia coli. I. Structure of F prime factors. J. Mol.Biol. 71: 471 (1972).

Sharp, P., G. Cohen and N. Davidson: Election Microscope Heteroduplex studies of sequence relations among plasmids of Escherichia coli.II. Structure of drug resistance (R) factors and F factors.J. Mol. Biol. 75: 235 (1973).

Sheehy, R., J. Orr and R. Curtiss III: Molecular studies on entry exclusion in Escherichia coli minicells. J. Bact. 112: 861 (1972).

Shull, F., J. Fralick, L. Stratton and W. Fisher: Membrane association of conjugally transferred DNA in Escherichia coli minicells. J. Bact. 106: 626 (1971).

Siccardi, A., B. Shapiro, Y. Hirota and F. Jacob: On the process of cellular division in Escherichia coli.IV. Altered protein composition and turnover of the membranes of thermosensitive mutants defective in chromosome replication. J. Mol. Biol. 56: 475 (1971).

Siddiqi, O, and M. Fox: Integration of donor DNA in bacterial conjugation. J. Mol. Biol. 77: 101 (1973).

Signer, E,: Lysogeny: the integration problem. Ann. Rev. Microb. 22: 451 (1968).

Skurray, R., and P. Reeves: Characterization of lethal ~~zygosis~~ associated with conjugation in Escherichia coli K12. J. Bact. 113: 58 (1973).

Smith, D.W.: DNA synthesis in prokaryotes: Replication.
Prog. Biophys. Molec. Biol. 26: 321 (1973).

Stadler, J., and E. Adelberg: Temperature dependence of
sex factor maintenance in Escherichia coli K12:
J. Bact. 109: 447 (1972).

Sugino, A. and R. Okazaki: RNA - linked DNA fragments in
vitro. Proc. Nat. Acad. Sci. U.S.A. 70: 88 (1973).

Sugino, A., S. Hirose and R. Okazaki: RNA - linked nascent
DNA fragments in Escherichia coli. Proc. Nat. Acad.
Sci. U.S.A. 69: 1863 (1972).

Taylor, A and C. Trotter: Revised linkage map of Escherichia
coli. Bact. Rev: 31: 331 (1967).

Taylor, A., and C. Trotter: Linkage map of Escherichia coli
strain K12. Bact. Rev. 36: 504 (1972).

Vapnek, D. and W. Rupp: Identification of individual sex-factor
DNA strands and their replication during conjugation in
thermosensitive DNA mutants of Escherichia coli. J. Mol.
Biol. 60: 413 (1971).

Vapnek, O., M. Lipman, and W. Rupp: Physical properties and
mechanism of transfer of R factors in Escherichia coli
J. Bact. 108: 508 (1971.)

- Wada, C., and T. Yura: Phenethyl alcohol resistance in Escherichia coli. II. Replication of F factor in the resistant strain C600. Genetics 69: 275 (1971).
- Walmsley, R.,: Physical assay of competence for specific mating pair formation in Escherichia coli. J. Bact. 114: 144 (1973).
- Wang, P: The effect of some R plasmids on DNA replication in E coli strains mutant in the dnaB function. Masters Thesis, Carleton University (1974).
- Watanabe, T.,: Infective heredity of multiple drug resistance in bacteria. Bact. Rev. 27: 87 (1963).
- Watanabe, T., and T. Fukasawa: Episome-mediated transfer of drug resistance in Enterobacteriaceae. IV. Interaction between resistance transfer factor and F factor in Escherichia coli. J. Bact. 83: 727 (1962).
- Wechsler, J., and J. Gross: Escherichia coli mutants temperature-sensitive for DNA synthesis. Molec. Gen. Genet. 113: 273 (1971).
- Wickner, S., M. Wright and J. Hurwitz: Association of DNA - dependent and - independent ribonucleoside triphosphatase activities with dnaB gene product of Escherichia coli. Proc. Nat. Acad. Sci. U.S.A., 71: 783 (1974).
- Willetts, N.S.: The genetics of transmissible plasmids. Ann. Rev. Genetics 6: 257 (1972).

Willetts, N.S.: Mapping loci for surface exclusion and incompatibility on the F factor of Escherichia coli K12: J. Bact 118: 778 (1974).

Willetts, N., and M. Achtman: A genetic analysis of transfer by the Escherichia coli sex factor F using P1 transductional complementation. J. Bact. 110: 843 (1972).

Willetts, N., and F. Bastarrachea: Genetic and physiochemical characterization of Escherichia coli strains carrying fused F' elements derived from KLF1 and F57. Proc. Nat. Acad. Sci. U.S.A. 69: 1481 (1972).

Willetts, N., and D. Finnegan: Characteristics of Escherichia coli K12 carrying both an F prime and an R factor. Genetic Res. (Camb) 16: 113 (1970).

Yamagata and H. Uchida: Chromosomal mutations affecting the stability of sex-factors in Escherichia coli. J. Mol. Biol. 63: 281 (1972).

Yoshinoga, K., H. Nakamura and M. Shimomura: Mild and efficient solubilization of Escherichia coli cells for the analysis of chromosomal DNA by sucrose gradient centrifugation. Biochim. Biophys. Acta 340: 109 (1974).

GLOSSARY

Several reviewers have suggested it would be helpful to include a glossary of the terms of microbial genetics used in this Thesis. The following list is offered in the hope that it will prove useful. An additional list may be found in chapter 8 of Methods In Microbiology Vol. 3A(1970).

allele: A particular form of a gene, that is a unique sequence of nucleotides, occupying a defined position, or locus, on the chromosome.

assortment: The separation to different cells or organisms of members of a set of genetic markers initially present in the same cell or organism. (also referred to as segregation.)

clone: The descendants of a single cell or individual by a process of nuclear and cell division not involving genetic recombination between cells..

conjugation: A parasexual mode of unidirectional (polarized) transfer of genetic information involving direct physical contact between a donor (donor) and a recipient (female) cell..

cotransduction: The transduction of more than one genetic marker in a single event.

double male: A general term for describing microbial cells bearing two distinct fertility (F) plasmids in their cytoplasm. Thus F^+/F^+ , F'/F' , Hfr/Hfr , and Hfr/F' cells are all double males.

F^+ , F' , F^- , Hfr : Different fertility types of microbial cells. F^+ , F' , and Hfr possess the fertility factor (F) and are male; in F^+ and F' cells, the factor is autonomous; in Hfr it is integrated with the chromosome. F^+ carries only F genes; F' , genes substituted from the chromosome. F^- cells lack F and are female.

genetic markers: The genes that differentiate parents in a cross, which allow the inheritance of particular chromosomal or plasmid regions from one or other parent by the progeny to be experimentally determined.

genotype: The sum total of the genetic information (genes) contained in the linkage structures (chromosome, plasmid) of a bacterial organism. The genetic constitution with respect to the region under examination.

illegitimate recombination: Recombination occurring in bacterial cells in the absence of the chromosomal gene recA. Recombinants are formed at low frequencies (0.0001%) and their formation does not depend on structural homology between the participants. (see also recombination)

lysogeny: The condition in which the DNA of a temperate bacteriophage (prophage) forms part of the make-up of the host-bacterium such that prophages are inherited by the descendant of the originally infected cell.

modification: The host controlled structural variation in DNA particles usually alien to its cytoplasm.

mutant: An individual or strain differing from the wild-type by one or more mutations.

phenotype: The characters expressed by an organism.

recombination: The sequence of processes resulting in the combination in the same genetic element of nucleotide sequences initially present in different genetic elements. In E. coli, such events are dependent on the presence of a functional recA gene.

restriction: The reduction in the tolerance of genetic elements such as phage, P plasmids, and bacterial chromosome fragments donated by one bacterial cell to another.

salt remedial: Term used to describe a mutation phenotypically suppressible by inclusion of salts or sugars in the growth medium (also referred to as sugar correctable)

supercoiled DNA: covalently-closed circular DNA twisted around itself.

temperate bacteriophage: A bacteriophage capable of establishing a lysogenic relationship with the bacterium it infects.

transduction: The transfer of bacterial DNA from one organism (donor) to another (recipient) by the agency of a bacteriophage, resulting in the production from the recipient of recombinant progeny.

wild-type: Usually the starting strain of an organism, isolated from nature, from which mutant individuals or organisms are subsequently derived.

END

26

03

7

FIN