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Escherichia coli K-12 F-Prime Factors, Old and New

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DEVELOPMENTS IN THE UNDERSTANDING AND USE OF F-PRIME FACTORS

The critical early history of sexuality in *Escherichia coli* is well known and has received extensive review, in particular by Jacob and Wollman (90) and Hayes (70). Reviews and articles which are particularly relevant to the concepts of F-prime and episome are given by Driskell-Zamenhof (47), Scaife (148), Sharp et al. (153), and Campbell (26, 27). In view of the depth and high quality of these treatises, only a skeletal summary of early F-prime history will be included here, and more recent aspects will be emphasized.

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Fertility; F⁺ and Hfr strains

Sexually mediated exchange of information between chromosomes in *E. coli* is dependent on the functions of a sex factor in the donor cell (68). The sex factor, F, is a small deoxyribonucleic acid (DNA) molecule (molecular weight $\sim 63 \times 10^6$) which, in the F⁺ or autonomous configuration, normally exists as a covalently closed circle (153), and which can cause its own epidemic spread by the transfer of one of its DNA strands into nearby F⁻ recipient cells (160) in a process about which much basic information is still lacking. F also effects the transfer of chromosomal DNA, either with high efficiency by virtue of its metastable insertion into the circular chromosome known as the Hfr configuration, or alternatively with low efficiency by chromosome mobilization when in

the F^+ configuration (see below). Recent work by Sharp et al. (153) has provided strong evidence that, in the formation of an Hfr strain, the circular F factor can recombine with the chromosome at either of at least two points along the F "map." There are at least 22 chromosomal sites where this recombination can occur (see below, and Fig. 2).

Sex-Factor Affinity

The first indication that the *E. coli* F factor could undergo rare reactions with the chromosome (in addition to the $F^+ \rightarrow$ Hfr $\rightarrow F^+$ processes referred to above) was reported in 1957 by Richter (142), who found that some F^- recombinants which were derived from a cross with a certain Hfr strain, Hfr_3, were converted with high efficiency to Hfr_3-like donor strains when infected with the wild-type F factor. This type of "memory" of the presence of F at a former Hfr point of origin, as indicated by the recovery of high frequency donors with that point of origin after re-infection with F, was also noticed in F^- strains derived from another Hfr by Adelberg and Burns (5), who used the term *sex-factor affinity* (*sfa*) to describe this phenomenon. More recently, strains which carry more than one chromosomal *sfa* locus have been described (102). In these strains, cells which are high-frequency donors with a point of origin corresponding to one *sfa* locus can be converted to donors which initiate chromosome transfer at another *sfa* locus.

The First F-Primes

The strain which Adelberg and Burns ultimately showed to carry an *sfa* locus carried at the same time an altered F factor (F2) which was also endowed with a "memory" of a chromosomal locus in the region where the original Hfr had its point of origin. Following the suggestion of Wollman that F2 was produced by a rare recombinational event between the integrated F factor and an adjoining region of the chromosome (5), Jacob and Adelberg isolated another altered F factor, F-*lac*, which carried chromosomal genes (*lac*) and which was phenotypically identifiable by transfer to an appropriate (Lac^-) recipient cell (85). F-*lac* was isolated by mating an Hfr strain (P804, which transfers the *lac* genes terminally in conjugation) with a Lac^- F^- recipient. Selection was made for early transfer of donor *lac* genes on the assumption that only *lac* genes linked to an autonomous F factor would enter the recipient cell at an early time.

The term "F-prime" at first was used to refer to any "altered" F factor. In present usage, F-prime refers to an altered F factor which carries genetic material acquired from the chromosome.

Deletion of Proximal or Distal Hfr Genes (or both) in the Formation of F-primes; Primary F-Prime Strains

Strong genetic evidence that chromosomal genes become incorporated into F-prime factors by a recombinational event was found by Scaife and his colleagues (23, 147, 151). They observed that the F factor of the F-prime, F13, was bracketed between the same genes as it had been in the Hfr strain (Hfr 13) in which F13 had been formed. Further, in the strain in which F13 was first detected, the chromosome appeared to be deleted for the genes carried on the episome (151), since the strain could not be cured of the episome, i.e., obtained free of the episome in a viable state (*see below*). This type of F-prime strain, in which a reciprocal recombinational event has preserved both the episome and the corresponding deleted chromosome, is called a "primary" F-prime strain and it exemplifies the Campbell model of episome integration and excision involving one circle $\leftrightarrow$ two circle conversions (26, 27). The direct isolation of primary F-prime strains from Hfr parent strains was achieved in a systematic way by Berg and Curtiss (17), who used replica plating techniques to test many colonies. There is no reason to suppose that the F-prime factors of primary F-prime strains are fundamentally any different from those of strains known as "secondary" F-prime strains, in which the F-prime confers diploidy for the chromosomal region that it carries. However, the isolation of primary F-prime strains is more difficult since direct selection (as was the case for F-*lac* which was isolated in a secondary F-prime strain) is not possible. The recovery of primary F-prime strains may also be intrinsically less frequent since it requires a reciprocal crossover, and the possibility exists that a transferable F-prime might be formed under conditions where the (deleted) chromosome is not restored to continuity.

Figure 1 illustrates the kind of recombinational event discussed above. Scaife (148) denoted F-primes which carry both proximal and distal regions of the parental Hfr chromosome as type II F-primes, as opposed to type I which are presumed to carry either a proximal *or* a distal Hfr region. In Fig. 1, the further distinction is made between F-primes carrying only early Hfr genes, type IA, and those carrying only late Hfr genes, type IB ("backsided"). As

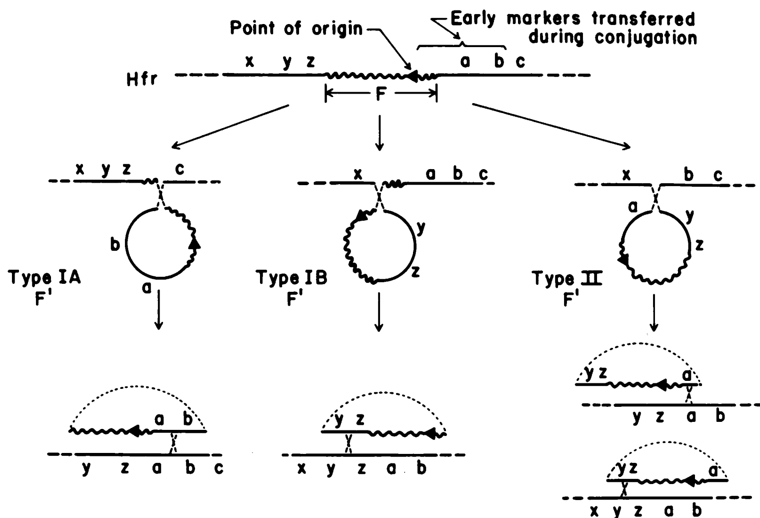

FIG. 1. *Variations in the topology of F-prime formation and chromosome mobilization. The line in the top portion of the figure represents part of the chromosome of a hypothetical Hfr strain which transfers the genetic markers a, b, and c early and x, y, and z late in conjugation. The middle part of the figure indicates the relative orientation of the F factor and chromosomal markers during the formation of the three types of F-primes shown. The bottom portions of the figure indicate regions of homologous pairing crossover with the chromosome when the various types of F-prime are in secondary F-prime strains.* (*See text.*)

will be seen below (see also Fig. 3 and 4), genetic evidence suggests that type I (A or B) F-primes are formed more frequently than type II. In a *rec*$^+$ strain, type II F-prime merodiploids give rise to Hfr haploid recombinants (18). A small deletion of the F factor is predicted in the formation of a type I F-prime (Fig. 1), and several possible examples of such a deletion are reported by Sharp et al. (153).

Chromosome Mobilization

Chromosome mobilization by an episome refers to the conjugational transfer of chromosomal genes which are not normally carried on that episome. Whereas chromosomal transfer from F$^+$ strains occurs very rarely, chromosomal transfer from secondary F-prime strains occurs with high frequency (134). Solid genetic evidence indicates that this is due to a high rate of recombination between the chromosome and the homologous region of the F-prime, which leads to integration of the F-prime into the chromosome and thereby produces many cells which transfer chromosomal markers with as high a frequency as do Hfr cells (132, 150). This is substantiated by the finding that primary F-prime strains do not mobilize the chromosome for transfer with high frequency (17, 151). Moreover, secondary F-prime strains which are recombination-deficient because of a *recA* (31, 103) mutation also fail to mobilize the chromosome for transfer with high frequency (164). In these cases where chromosome mobilization is minimal, the introduction of some special system for recombination between episome and chromosome restores chromosome mobilization, as in the case of recombination between integrated episomal and chromosomal phage ϕ80 genomes (154), or similarly, phage mu genomes (J. Zeldes, *personal communication*).

The bottom of Fig. 1 indicates that there should be a difference in the time of transfer of chromosomal markers between a type IB F-prime and the parental Hfr. The configuration of integrated type IB F-primes is always such that transfer of duplicated terminal genes always precedes the transfer of the normally proximal Hfr markers. This type of "delay" in entry times has been reported for F14, which is presumably a type IB F-prime (134). In contrast, there should not be any delay in marker entry times from a type IA F-prime strain (see Fig. 1). Type II F-prime strains would contain a mixture of integrated F-primes which result from crossovers either in the proximal or distal regions (Fig. 1, bottom right), and thus chromosomal marker entry times would appear to be similar to those from the parental Hfr strain (*see* reference 104 for a discussion of marker entry times). A sensitive interrupted mating experiment might therefore be useful in distinguishing between type IB and type II F-primes. Berg and Curtiss have confirmed the prediction

that in strains which bear an F-prime which simultaneously carries two different chromosomal segments, chromosome mobilization of both corresponding regions of the chromosome occurs at high frequency (17).

The nature of the low-frequency chromosome mobilization by F^+ (or haploid F-prime) strains is at present awaiting elucidation, and might represent a unique mode of recombination. This type of mobilization is stimulated by low doses of ultraviolet light (49, 137) and is strongly dependent on *recA* function (33, 119), and yet apparently does not lead to stable insertion of F into the chromosome.

Loss of F-Prime Factors; Curing

F-prime-bearing strains give rise to F-prime$^-$ segregants at a low rate (90). The rate of loss of episome (rate of curing) is greatly enhanced by exposure of cells (particularly at low cell densities where retransfer is prevented) to either acridine dyes at *p*H 7.6 (12, 75), rifampin (13, 145), elevated growth temperature (156), thymine deprivation (34), or infection by the filamentous phage M13 (128). F-prime$^-$ derivatives of F-prime strains are also obtained after prolonged incubation of cells in >1% sodium dodecyl sulfate (3). Evidence suggests that sodium dodecyl sulfate is more toxic for F-carrying cells than for F^- cells and thus causes enrichment of spontaneous F^- or defective F variants. Although laboratory methods for carrying out F-prime curing abound, the basis for the curing phenomenon is not understood in any case. This situation is further complicated by the existence of certain strains whose F-prime factors are resistant to curing by, e.g., acridine orange (12, 170), even though superinfection with a second F-prime results in exclusion of the first in the normal way (*see below*). The genes responsible for this resistance to curing have not been identified. In the case of curing by rifampin, it has been found that some functions determined by the F factor are selectively repressed by rifampin at concentrations which still allow cell growth and F-prime replication (145).

Derived F-Prime Factors and F-Prime Stability

Mutations in the sex factor. Three major classes of mutations involving F function have now been found. Mutations which result in thermosensitive replication of F-*lac* have been studied by Cuzin and Jacob (42, 86). Mutations (*tra*$^-$) which result in defective conjugational transfer and which fall into 11 different complementation groups have been discovered and mapped by Achtman et al. (1, 2), Willetts and Achtman (165), Ohtsubo (126, 127), and Ippen-Ihler et al. (82). Finally, mutations (*inc*$^-$) in F which result in loss of the normal Hfr F-prime incompatibility (see below) are under study by Maas and co-workers (108; W. K. Maas, *personal communication*). Analyses of the functions which are defective in all these types of mutants are eagerly awaited.

Mutations in the region of diploidy. In addition to their widespread use in testing for dominance and complementation, F-prime merodiploids are useful in detecting mutations which would be lethal in a haploid strain. F-prime merodiploids have been used to isolate recessive lethal amber and ochre suppressors (117, 155), mutations in an essential gene, *rif* (7, 8), phage mu-induced polar mutations in genes which code for ribosomal proteins (124), and fusions of operons (see below).

F-prime–chromosome recombination and segregation. The classic uses of F-primes for studying the nature of the genetic control of the *lac* operon (90) and for studying intracistronic complementation (61, 130) depended on the construction of heterozygous merodiploids of many types. The process of heterogenote → homogenote conversion, which gives rise to desired F-prime genotypes, is still not understood. The appearance of, for example, F-*lac*$^-$/*lac*$^-$ cells at a low level (e.g., 0.1 to 1%) (90, 168) in a culture of F-*lac*$^+$/*lac*$^-$ cells presumably requires recombination; it does not occur in *recA*$^-$ cells. However, it is not known whether the loss of the *lac*$^+$ allele is due to reciprocal recombination followed by abnormal segregation of episomes and chromosomes from the same multinucleate cell, or whether perhaps the initial recombinational events include gene conversion or "repair" of the *lac*$^+$ allele to *lac*$^-$. Analysis by Herman (72, 73), Meselson (116), and Berg and Gallant (18) of the products of recombination between episome and chromosome has established that when episome-chromosome recombination occurs, it is sometimes reciprocal but more often not. Non-reciprocality, therefore, clearly must be reckoned with in any eventual explanation of these events. Hall and Howard-Flanders (67) have detected recombinant F-prime formation at both short and long times after formation of a heterozygote, by transferring the recombinant episomes into a *recA*$^-$ tester strain. They found that recombinant episomes are formed in *recB*$^-$ or *recC*$^-$ heterozygotes with much higher frequency than are viable haploid recombinants from HfrxF$^-$ (*recB*$^-$ or *recC*$^-$) matings. They

also have found (J. Hall, *personal communication*) that the level of recombinant episomes present soon after the initial formation of the heterozygote is approximately the same as is found in heterozygotes which have been extensively subcultured. (This is in contrast to an initial report (67) based on results using a culture which was overgrown with variant merodiploid cells [J. Hall, *personal communication*].)

Whereas recombination between episome and chromosome is of intrinsic interest, it is at times a hindrance in studies which require very stable heterozygous merodiploids, in particular when dealing with large F-primes or type II F-primes. The use of *recA*$^-$ strains (103) obviates this difficulty, as does the use of *E. coli/Salmonella* merodiploids which are very stable, due presumably to nonhomology in DNA sequences even though functional homology exists (6, 21, 29, 54, 62, 99, 144).

F-prime–F-prime recombination within F. After transfer of one F-prime into a strain which carries a genetically different one, recombinant F-primes can be recovered in which crossovers have occurred between the F factors. As shown by Gottesman and Beckwith (65), this makes possible the transfer of useful mutations in the F factor from one F-prime to another, and also might be advantageous in mapping mutations in F (N. Willetts, *personal communication*).

Deletions of F-prime material. Deletion of parts of an F-prime has often been detected. This can happen in either *rec*$^+$ (11, 36, 88) or *recA*$^-$ strains (79; J. S. Parkinson, *personal communication*, B. Low, *unpublished data*). Whereas the occurrence of deletions, due to "illegitimate" recombination (58), probably occurs at a low rate in these strains, cells whose episomes have been shortened by deletion can, in some cases, grow much faster than the parent merodiploid (79; B. Low, *unpublished data*), and the occurrence of such variant diploids can interfere with accurate complementation analysis. This problem has been particularly vexing in the case of F-primes which bear the *uvrC-cheB* region of the chromosome. J. S. Parkinson (*personal communication*) has shown that F-prime strains which are diploid for this region are very mucoid and slow growing (the mucoidy is reduced in *galK*$^-$ strains) and are very susceptible to overgrowth by segregants which have deleted episomes. Examples of some deleted F-prime factors (which actually have advantages for certain studies) are given in Tables 2 and 3.

Transductional shortening. One of the more mysterious aspects of F-prime biology, at present, is the ability of P1 transducing phage to transduce a large F-prime factor such as F14 in its entirety, and also to produce a large family of transductants which carry deleted derivatives of the original F-prime. Transductional shortening was first observed by Pittard and Adelberg (132) and was used by Ramakrishnan and Adelberg (139) and by Marsh and Duggan (111) as a method for detailed deletion mapping of genes and mutations in the *ilv* operon (*see* Table 2 and Fig. 3, F16, F216, F25, F315). A similar set of deletions for the *gal* operon has been isolated and used by Ohtsubo (126, 127), and it is likely that F-primes carrying other regions of the chromosome could be deleted in a similar manner.

It has not been established whether the shortened episomes are produced in the donor strain and transduced as small intact circles, or whether the transducing particles carry linear segments from the original episome which are particularly prone to circularization in the recipient cells. Whereas transductional shortening of F14 has been observed by several investigators (see above), merodiploid transductants have not been detected when the P1 is grown on the Hfr parent of F14, i.e., AB313 (131). P1 transduction of F and neighboring genes from an Hfr strain to an F$^-$ strain resulting in a haploid Hfr recombinant has been observed with the P4X Hfr (46).

F-prime fusion. In the course of studies on F-prime incompatibility, Maas and co-workers observed that direct selection for the presence of two F-primes at once, in a *recA*$^-$ background, produced some episomes which had characteristics expected of tandem insertions of one of the parental F-primes into the other. Press et al. (136) describe a particularly useful variety of such fused F-primes which was obtained when one of the parental F-primes carried a phage attachment site, *att*80 (see Table 3 and Fig. 4, F155-105). In this case, it is possible to isolate transducing phages for genes on the other F-prime which had been transposed close to *att*80 as a result of the F-prime fusion (9, 136). Examination of the contour lengths of these (129) and of other fused episomes (166) revealed that they are shorter than the sum of the two parental F-primes. Thus, the process of F-prime fusion is thought to involve concomitant deletion of some of the genetic material. In at least some cases (136), loss of some F functions of one of the F-prime factors suggests that part of one F factor was deleted. It is not yet known if deletion of some F material is a requirement for stable F-prime

fusion. Other examples of the deletion of F-prime genetic material in conjunction with rare recombination events have been reported by Fan, who studied the integration of F13 at abnormal locations on the chromosome (52; see also below).

Loss of F. As Berg and Gallant have shown (18), the F factor on a type II F-prime is subject to loss in a *rec*$^+$ merodiploid, because two crossovers (one on each side of F) serve to integrate the F factor into the chromosome and presumably liberate the region of diploidy without means for replication. In a *recA*$^-$ strain, this insertion of F would not be observed; however, if the F-prime arose from an Hfr strain which was unstable (i.e., reverts to F$^+$ at high frequency), the tendency of F to be excised might persist in a type II F-prime *recA*$^-$ strain. No careful comparative studies of instabilities of F in Hfrs and corresponding type I or II F-prime strains have been reported, nor has the possible effect of *rec*$^-$ mutations on these instabilities been ascertained.

Chromosomal Integration of F-Primes; Directed Transposition

Although genetic transpositions in *E. coli* have been reported (45; also 90, p. 167), they have been rare. Cuzin and Jacob (41) discovered that, when the chromosome was deleted for a corresponding region on an F-prime, the F-prime (in this case F_{ts114} *lac*, whose replication is thermosensitive) integrated at low frequency at other chromosomal sites. The rare cells with stably integrated F_{ts114} *lac* produced homogeneous Lac$^+$ colonies at elevated temperature (42 C), and these colonies could be identified on indicator plates. Moreover, the strains carrying transposed F_{ts114} *lac* factors had become Hfr donors for markers near the (new) site of F. A large number of such transposition Hfr factors were found and analyzed by Beckwith et al. (14, 15), and some are indicated in Table 1 and Fig. 2 (EC1, EC2, etc.). When selection is made for F-prime insertions that inactivate a particular chromosomal gene by the insertion event, and which thereby result in, e.g., resistance to a phage or a metabolic inhibitor, specific sites of integration are found. This was shown by Beckwith et al. (14, 15), and by Gottesman and Beckwith (65) for the case of integration at the *tonB* locus (close to the ϕ80 attachment site; see EC15, EC45, EX3, on Table 1 and Fig. 2), and by Ippen et al. (81) for the case of integration in the *gal* operon (close to the λ attachment site; *see* EC2701 and EC2702 on Table 1 and Fig. 2). Other selectable integration regions are summarized by Franklin (58). The potential of recovering these "directed" transpositions of various chromosomal segments adjacent to phage attachment sites, either by the above method or by F-prime fusion (136), represents a significant advance because of potential isolation of desired genes on transducing phages. Voll has also used a selection for *tonB* insertions to isolate a transducing phage for (*his*) genes using an F-prime from *Salmonella* (161). Certain transposition Hfrs also permit the selection of a series of deletions and fusions of genes (118) for use in the study of gene function and regulation.

Another mode of selection of transposition Hfrs has been described by Nishimura et al. (123), who found that integration of a sex factor, or F-*lac*, into the chromosome of a mutant which is temperature sensitive for the initiation of DNA synthesis, allows the growth of the strain at the high (normally nonpermissive) temperature. This "integrative suppression" of the DNA$^-$ phenotype was not found to occur in *recA*$^-$ strains. This last result is somewhat puzzling in light of the studies of De Vries and Maas (44), who did obtain integrated episomes in *recA*$^-$ Hfr strains. In the latter studies, the F-prime generally became integrated in the region of the chromosome homologous to it. This is a somewhat unexpected indication that extended homology can influence the illegitimate recombination events (58) which occur in *recA*$^-$ cells. The method of formation of double Hfrs by F-prime insertion into *recA*$^-$ Hfrs might be of considerable use if certain double Hfrs are needed for the isolation of long F-primes (see below). Integration of F_{ts}*lac* into the chromosome of a *recA*$^-$ strain was also found by Broda and Meacock (24), who obtained directed transpositions in the *tsx* region.

A special class of transposition Hfr strains was isolated by Berg and Curtiss (17). In the course of their search for primary F-prime strains (see above), they isolated strains in which the F factor (and in some cases, neighboring chromosomal genes) had undergone an inversion.

Physical Properties of F-Primes

Relative sizes of F-prime factors have been estimated by Falkow and Citarella, by using DNA-DNA hybridization (51); by Freifelder, by using relative sensitivity to nicking by X rays (59, 60); by Matsubara, by using centrifugation gradients (114); and by Davidson and co-workers (153), by measuring relative contour lengths in electron micrographs. For epi-

TABLE 1. *Some Hfr strains of Escherichia coli K-12*

Strain name(s)	Approximate origin map location (min)[a]	Stability vs. reversion to F+[b]	References (isolation or characterization, or both)[c]	Comments[d]
A1	0		147, 151	Transposed F13
A4	8		147	Transposed F13
A5	56		147	Transposed F13
AB311	40	U	159	
AB312	60	S	159	
AB313	74	U	159	
B1	10		22	
B2	10		22	Similar to Hfrs B3, B4, B5
B7	29	U	22	Similar to Hfrs B9, B10
B8	11		22	Similar to Hfrs B11, B13
B11	11		22	Similar to Hfrs B8, B13
B12	40		22	
EC1	22		15	Transposed F42-114
EC2	63		15	Transposed F42-114
EC3	8		15	Transposed F42-114
EC6	56		15	Transposed F42-114
EC7	87		15	Transposed F42-114
EC8	25		14, 15	Transposed F42-114
EC9	56		15	Transposed F42-114
EC15	25		14, 15	Directed transposition of F42-114, at *ton B*
EC28	31		15	Transposed F42-114
EC29	14		15	Transposed F42-114
EC35	3		15	Directed transposition of F42-114, at *ton A*
EC40	3		15	Directed transposition of F42-114, at *ton A*
EC45	25		15	Directed transposition of F42-114, at *ton B*
EC102	12		14, 15	Directed transposition of F42-114, at *tsx*
EC2701	17		81	Directed transposition of F42-114, at *gal K*
EC2702	17		81	Directed transposition of F42-114, at *gal K*
ED1032	50		25	Transposed F42-114; transfers *tra* genes early
ED1039	37		25, 112	Transposed F42-114
EX3	25		65	Directed transposition of F'-*thr-ara* from *E. coli* B, at *tonB*
G1	40		113	
G6	60	S	113	
G11	62		113	
Hfr3	79	U	143, 152	F factor inserted within *malB*; carries sex-factor affinity locus
Hfr6	11		See reference 10	
Hfr13	11		18, 23, 77; see reference 10	
Hfr44	38		A, B	
Hfr Cavalli	14	VS	28	
Hfr H (Hayes)	87	S	69, 109	
IOR2	10		17	Inversion from OR11
IOR3	10		17	Inversion from OR11
J4 (= P10)	79	U	89, 90, 152	F factor inserted within *mal B*

[a] See Fig. 2.
[b] Abbreviations: VS, very stable; S, stable; U, unstable; VU, very unstable.
[c] Numbers refer to literature cited. Letters refer to the following: A, P. Cooper, *personal communication*; B, J. S. Parkinson, *personal communication*; C, B. Low, *unpublished data*; D, R. Curtiss III, *personal communication*; E, F. Jacob, *personal communication* to B. J. Bachmann; F, P. Kahn, *personal communication*.
[d] Symbols are as in Taylor (158).

TABLE 1—*Continued*

Strain name(s)	Approximate origin map location (min)[a]	Stability vs. reversion to F+[b]	References (isolation or characterization, or both)[c]	Comments[d]
KL14	60	VS	104	
KL16	56	S	101, 104	
KL19	22	U	C	
KL25	74	U	104	
KL96	40	U	104	
KL98	45	U	48, 104	
KL99	22	U	104	*pyrD-fabA*-origin-*pyrC*, (see 37)
OR1	10		39, D	F factor inserted in early part of *lac Z*
OR7	12		39	
OR11	10		17	
OR21	11		38, 39	
P3 (= 4000)	14		89, 90	
P4X (= J2)	10	S	89, 90	
P13 (= Type 6)	74		90, 162	
P72 (= Type 5)	76		63, 89, 90	
P804	10		88, E	
PK3	68		93	From integrated col V factor; non-colicin producing
PK19	37		93, B, F	From integrated col V factor; non-colicinogenic derivative = PK191 (very stable)
PK23	3		93	
PK30	40		93	
R1	74		101, 140	
R3	3		140	
R4	10		140	
R5	3		140	
Ra-1	56	VU	92, 102	Carries sex-factor affinity locus
Ra-2	77	VU	101, 102, 104	Carries sex-factor affinity locus
TOR13	56		17	Transposed F251
TOR36	85		16	Transposed F258
Type III	68		123	Integrative suppression of *dnaA*$^-$ by F
Type XI	62		123	Integrative suppression of *dnaA*$^-$ by F *lac* (i^-)

somes small enough to be isolated as covalently closed circles (less than 4–5 min in chromosome length; N. Davidson, *personal communication*), the latter method offers the least uncertainty.

Regulation of F-Prime Replication, and Expression of Diploid Genes

In the course of their studies on the *lac* operon, Jacob and Monod (87) measured β-galactosidase activities in *lacZ*$^+$/*lacZ*$^+$ diploids and in *lacZ*$^+$ haploids. The diploids had 1.5 to 2.5 times the enzyme activity of the haploid strains. This result is roughly consistent with what would be expected if each cell contained one episome per chromosome and if gene expression for both were the same. In contrast to this, the expression of the tryptophan synthetase gene on the *trp*$^+$ episome isolated by Fredericq (see 157) was found by Stetson and Somerville to be two to three times that of a *trp*$^+$ haploid strain (157). They also found that reversion of *trpA46* to wild type occurred three times as frequently on the episome as on the chromosome. Similar experiments carried out in various other laboratories also show episomal gene expression at levels between one and three times that of the corresponding chromosomal gene (60, 122, 135, 141). The number of copies of an F-prime factor per chromosome has been estimated to be between one and two, depending on the particular F-prime used (57, 80, 157). One reason that these parameters vary from episome to episome may be that the initiation of replication of an episome usually does not coincide with that for the chromosome, as found by Zeuthen and Pato (172) and

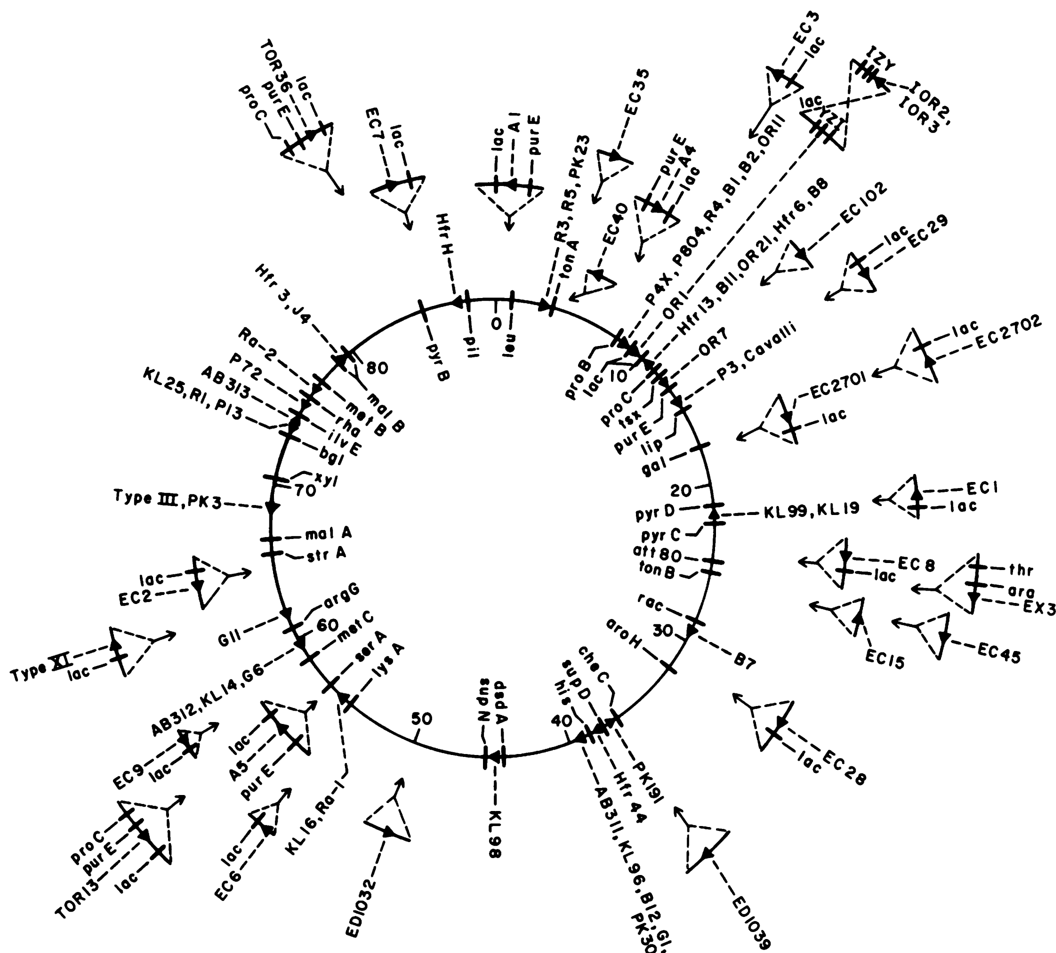

FIG. 2. *Genetic map of E. coli K-12 showing approximate locations of Hfr points of origin for strains listed in Table 1. Genetic loci and their positions are as given by Taylor (158). Two or more Hfrs assigned to the same arrowhead do not necessarily have identical points of origin. The arcs on the outer portions of the figure denote F-primes which have been integrated into the chromosome to form transposition Hfrs (see text) with point of origin locations approximately as shown.*

by Cooper (35). In spite of the apparent nonsynchrony of episome and chromosome replication, segregation of F-primes and chromosomes appears to occur in an orderly way. This is suggested by studies of segregation of parental and daughter strands of F-prime and chromosome after curing of a temperature-sensitive F-prime at high temperature (43) or after curing with acridine orange (80).

The gene functions involved in regulation of F-prime replication are obscure, but several steps have been taken toward their identification. Cuzin (42, 86) isolated a number of mutations in F-*lac* which confer temperature sensitivity of replication and which belong to several complementation groups. Genetic studies have also been initiated on another aspect of control of replication: incompatibility. It has been known for some time (106, 149) that F factors residing on two different linkage groups (e.g., two different F-prime factors in the same cell, or an F-prime factor in an Hfr cell) do not stably coexist. Maas et al. (108; and *personal communication*) have found mutations (*inc*$^-$) in the F-factor which result in loss of this incompatibility.

On the Nature of F-Prime Formation

If F-primes are formed in Hfr cells by a more or less randomly positioned "loop-out" of chromosomal material with F incidently included, then this mode of F-prime generation can

probably be thought of as equivalent to deletion formation, i.e., the process of illegitimate recombination between nonhomologous chromosomal segments (see discussion by Franklin, 58).

Several curious results have been reported which suggest that F-prime formation might sometimes involve other processes besides "classical" illegitimate recombination. One such result is the detection of shortened F-primes after P1 transduction, mentioned above. Another is the observation that most of the F-primes isolated from Hfrs using *recA*$^-$ recipients, i.e., so that types I and II F-primes should be stably recovered, appear to be type IA or IB (see Fig. 3). (There is no real indication of the exact genetic termini of most F-prime factors, so there is much room for doubt as to whether any given one is Type I or Type II.) In other cases, peculiar recombinants were isolated from matings involving the F factor. These include (i) the formation of Hfrs from $F^+ \times F^-$ matings (R5, Reeves, and P802, P804, and P808, Jacob; see reference 10); (ii) the isolation of F14, which apparently involved the transfer of most of the parental Hfr chromosome into a recipient cell, followed by retention of only the most distal region and the concomitant deletion of chromosomal material of approximately the length of the F-prime (F14) thus formed (134, 135); (iii) the large differences in frequency of F-prime formation (e.g., F-primes covering *lac*) from matings of Hfrs with very similar points of origin (e.g., P4X, c.f. P804) with appropriate (Lac$^-$) F^- strains (B. Low, *unpublished data*; E. Signer, *personal communication*); (iv) the large number of defective merodiploids (as detected by using male- and female-specific phages; see 103 and 169) formed from Hfr $\times$ F^- *recA*$^-$ crosses, which might arise via loss of essential F genes when the "loop-out" occurs in the Hfr cell, or possibly might arise after transfer by circular fusion of an early region normally transferred by the Hfr (R. Novick, see discussion in 167); and (v) the high frequency of generation of long merodiploids from matings of a mutant F_{ts} *gal* strain with an F^- strain as described by Bergquist and Adelberg (4, 19). Taken together, all of these results suggest that either F can stimulate illegitimate recombination in certain regions, or it might participate in some other rare mechanism of recombination.

In the case of F-prime formation from matings of double male strains with F^- recipient strains (see below), a straightforward mechanism can be suggested. The presumed section of one of the F factors (from the double male) which is transferred at the leading end during conjugation could conceivably recombine with the homologous region of the second F which is transferred, to form a large F-prime loop with one intact F factor. The "leading ends" of the two F factors in the double male would have to be close enough in length to allow a functional recombinant F to be formed. Whether or not this mechanism is valid could be tested by comparing the rates of F-prime formation from double males using *rec*$^+$ and *recA*$^-$ recipients.

ISOLATION OF NEW F-PRIME FACTORS AND THE USE OF recA$^-$

From Hfr Strains

As mentioned above (also see reference 103), F-prime transfer to a *recA*$^-$ strain results with high efficiency in formation of stable merodiploids. The use of *recA*$^-$ strains has facilitated the isolation of many type IA F-primes, i.e., F-primes which carry proximal Hfr segments and which could not be recognized in a normal Hfr $\times$ F^- (*rec*$^+$) cross because of the vast excess of haploid recombinants resulting from the cross. There is no evidence that the use of *recA*$^-$ increases the number of potential F-primes in any given cross; rather, *recA*$^-$ recipients simply allow stable and efficient recovery of the episomes. Even in the isolation of Type IB F-primes from Hfr strains, the use of *recA*$^-$ recipients is preferable to recover potentially unstable (e.g., very long) F-primes, or very rare F-primes which might not be detected because of a low-frequency background of haploid recombinant clones due to abnormal chromosome mobilization from the Hfr strain.

For some unexplained reason, the number of F-primes recovered from Hfr $\times$ F^- (*recA*$^-$) crosses varies tremendously with different Hfr strains and with different regions of the chromosome (103; and B. Low, *unpublished data*). For example, F-primes which cover the *thr-leu-proA* region of the map are easily obtainable from Hfr H derivatives (more than one F-prime recovered per 10^5 Hfr cells), in contrast to the selection for *aroD*$^+$ F-primes from Hfr B7, or *his*$^+$ F-primes from KL98, where less than one F-prime is recovered per 10^7 Hfr cells. In matings of Hfr strains with *recA*$^-$ strains, some of the rare "recombinants" are found not to carry normal F-prime factors but instead are defective (nonfertile) merodiploids which usually show evidence of the presence of part of the F factor as determined by male- or female-

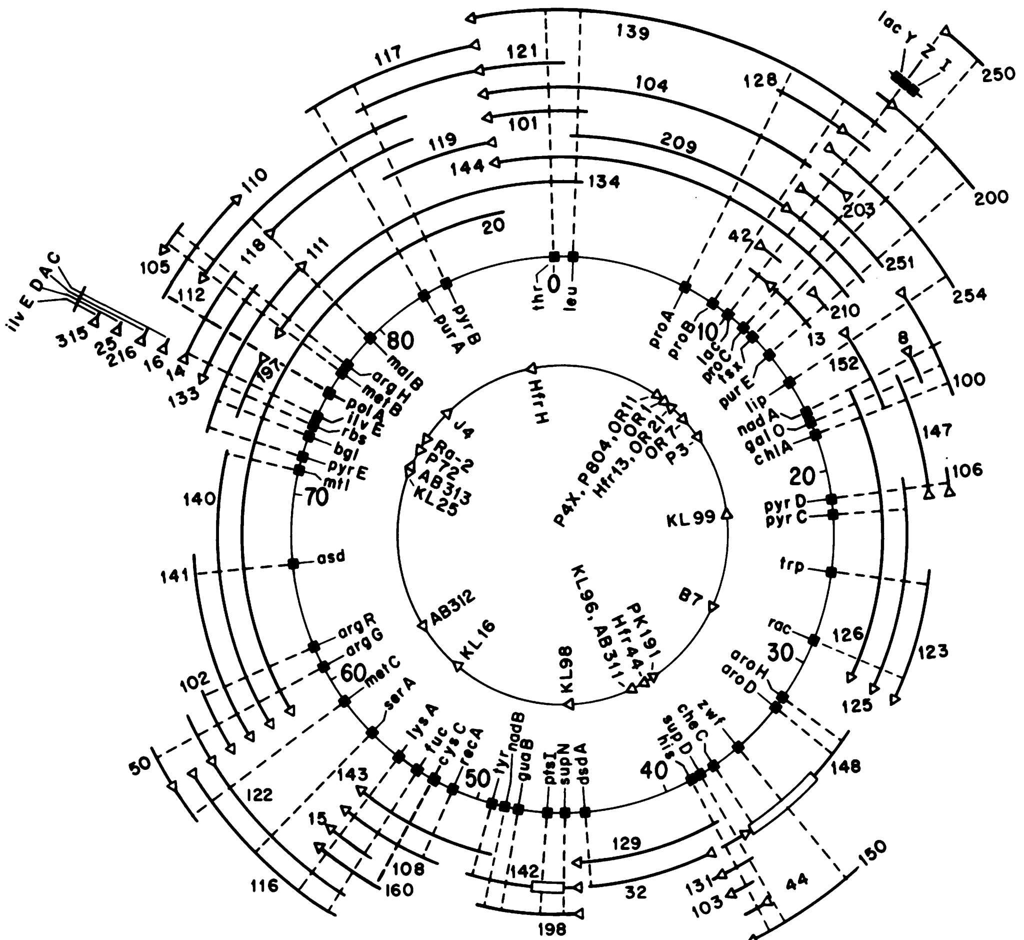

FIG. 3. *Genetic map of E. coli K-12 showing approximate chromosomal regions carried by F-prime factors listed in Table 2. Each F-prime is represented by an arc which has an arrowhead drawn to show the point of origin of the ancestral Hfr strain (see inner circle). The dashed lines, which extend radially from the genetic markers on the outer circle, indicate the approximate termini of the F-primes as far as they are known. Known deletions are indicated by narrow rectangles (e.g., F142, deleted for ptsI).*

specific phage sensitivity, or both (103). The frequency of formation of these "defective" merodiploids varies drastically from Hfr to Hfr.

Fiil (53) has shown that it is sometimes possible to isolate type IA F-primes from crosses of Hfr strains with *recA*$^+$ recipients. In his method, he reduced the background of haploid recombinants by employing Hfr strains with alternating "+" and "−" genetypes for several closely spaced markers in the proximal region. The (rec^+) recipient strain carried the corresponding opposite alleles ("−," "+," "−," etc.), and selection was made for all the "+" markers, thereby allowing only a small number of haploid recombinants (i.e., those few which resulted from the necessary combination of rare crossovers) to be formed.

From Double Male Strains

From crosses of one Hfr strain with another, strains which carry two integrated F factors have been obtained by Clark (30) and by Kaney and Atwood (96). Double male strains which have F factors in the same orientation on the chromosome have been used to donate one of the chromosomal regions between the F factors to recipient strains and allow selection of merodiploids which have long regions of merodiploidy (32, 95, 100, 107, 171). The potential usefulness of this method seems great, particularly for making F-primes carrying chromosomal regions which cannot easily be obtained by the method described above. It may also be possible to isolate F-primes from tran-

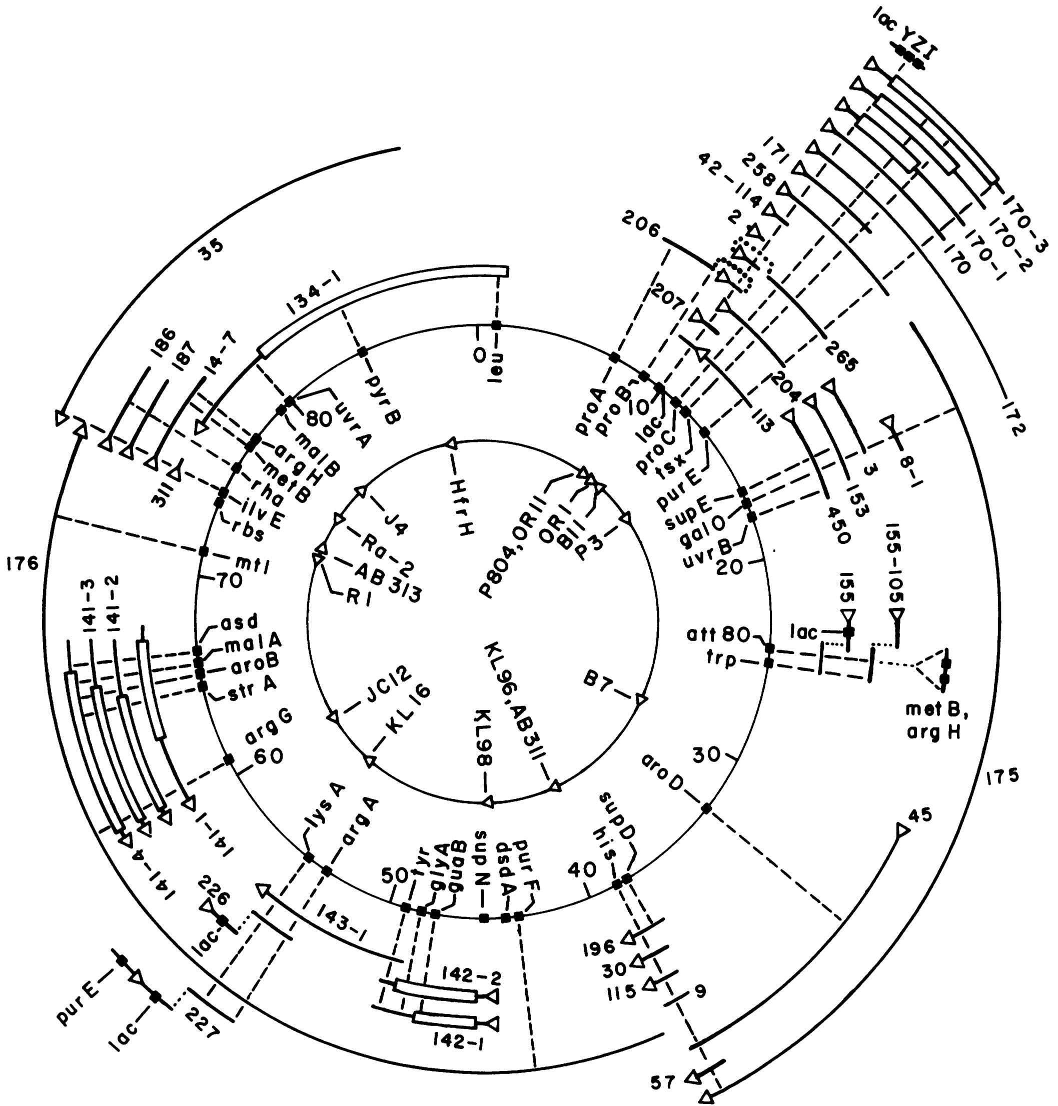

FIG. 4. *Genetic map of E. coli showing approximate chromosomal regions carried by F-prime factors listed in Table 3. F-primes are depicted as in Fig. 3. F-primes derived from transposition or inversion Hfrs (e.g., F155, F206) are depicted by two arcs which are connected by a short dotted line to indicate the unusual junction of two different chromosomal regions.*

sient double males formed after Hfr × Hfr (95) or F-prime × Hfr (J. S. Parkinson, *personal communication*) matings. In Fig. 3 and 4, the estimated lengths of several F-primes derived from double male strains are shown (F20, F35, F175, F176). The extent of diploidy and the behavior of the long F-primes derived from double males have not yet been studied in detail.

By Transduction?

Another possible method for isolation of F-primes is by transducing small F-prime-forming fragments from an Hfr strain to a recipient, as first suggested by Pittard (131). This approach might lead to much needed insight into the process of chromosome fusion (M. Stodolsky, *personal communication*).

NOMENCLATURE—HFR AND F-PRIME STRAINS AND F-PRIME FACTORS

The usefulness of results reported in the

TABLE 2. *Selected E. coli K-12 F-prime factors for general diploid analysis*

F-prime map no.	Original or alternate name	Strain from which F' was derived	Ancestral Hfr[a]	References (isolation or characterization, or both[b]	Comments
1	F, F^1			5, 90, 146, 153	Denotes wild-type F
8	F_8; F_8-*gal*	W3208 (Hfr 8)	W3208 (Hfr 8)	77, 153	Hfr 8 never isolated
13	F_{13}	W3213 (Hfr 13)	W3213 (Hfr 13)	10, 77, 151	Primary F'; carries *tsx*$^-$
14	F14	AB313	AB313	132–135	See text
15	F_{15}	W3201 (Hfr 15)	W3201 (Hfr 15)	83	Hfr 15 never isolated
16	F_{16}	AB1206 (F14)	AB313	132	From F14 by transduction
20	F20	JC182	AB312	32, 107	From double male donor
25	F25	AB1206 (F14)	AB313	G	From F14 by transduction
32	F32	EM2000	AB311	115	
42	F *lac*	P804	P804	85, 90	
44	F44	Hfr 44	Hfr 44	A	
50	F50	JC182	AB312	50	
100	F_1; F_1-*gal*; F "long"; f-200	P3	P3	153, E	Carries Su_{II}^+ (*supE*$^-$)
101	KLF1	3000	Hfr H	103	
102	KLF2	JC12	AB312	103	
103	KLF3	KL96	KL96	103	Carries *met G*$^+$ (see reference 20)
104	KLF4	3000	Hfr H	103	
105	KLF5	Ra-2	Ra-2	103	
106	KLF6	KL99	KL99	C	Replaces original KLF6 (reference 103)
108	KLF8	KL16	KL16	103	Carries *rel*$^-$
110	KLF10	J4	J4	7, 66, C	
111	KLF11	J4	J4	C	Different from F111 in reference 171
112	KLF12	Ra-2	Ra-2	C	
116	KLF16; F16	JC 12	AB312	C	
117	KLF17	3000	Hfr H	C	
118	KLF18	J4	J4	C	
119	KLF19	3000	Hfr H	C	
121	KLF21	3000	Hfr H	C	
122	KLF22	3000	Hfr H	C	
123	KLF23	B7	B7	C	
125	KLF25	B7	B7	C	
126	KLF26	B7	B7	C	
128	F *pro-lac*	P804	P804	H	
129	KLF29	KL183	KL98	C	
131	KLF3-1	KL173	KL96	C	Carries Su_I^+ (*supD*$^-$)
133	KLF33	KL25	KL25	C	
134	KLF34	Ra-2	Ra-2	C	
139	KLF39	3000	Hfr H	C	
140	MAF1	JC182	AB312	I	
141	KLF41	JC12	AB312	103	
142	KLF42	KL183	KL98	C	
143	KLF43	KL16-99	KL16	C	Carries *rel*$^-$; original *recA*$^-$ allele from KL16-99 reverted to *rec*$^+$
144	KLF44	3000	Hfr H	C	
147	KLF47	KL99	KL99	C	
148	KLF48	PK191	PK19	C	

[a] See Table 1.

[b] Numbers refer to literature cited. Letters refer to the following: A, P. Cooper, *personal communication*; C, B. Low, *unpublished data;* D, R. Curtiss III, *personal communication*; E, F. Jacob, *personal communication* to B. J. Bachmann; G, J. Pittard, *personal communication* to D. Duggan; H, E. Signer, *personal communication*; I, W. K. Maas, *personal communication*; J, W. Epstein, *personal communication*; K, E. Wollman, *unpublished data.*

TABLE 2—*Continued*

F-prime map no.	Original or alternate name	Strain from which F' was derived	Ancestral Hfr[a]	References (isolation or characterization, or both[b]	Comments
150	DFF1	KL96	KL96	56	
152	F_2-*gal*; F_2	P3	P3	71, K	
160	F.*rel*$^+$	NF56		53	
197	F185	P72	P72	162	
198	F198	KL98	KL98	J	
200	ORF200	χ225	OR1	D	Primary F'
203	ORF203	χ503	OR21	D	
209	ORF209	χ313	P4X	D	
210	ORF210	χ436	OR7	D	Primary F'
216	F2016	AB1206 (F14)	AB313	111, G	From F14 by transduction
250	ORF100	χ225	OR1	D	
251	ORF1	χ493	OR11	17	Primary F'
254	ORF4	χ536	OR11	17, 71	Primary F'
315	F310	AB1013 (F14-7)	AB313	111	From F14-7 by transduction

literature of bacterial genetics is diminished whenever the derivation of F-primes or Hfr points of origin cannot be traced. For Hfr strains not newly isolated, it is important to include the name of the original ancestral Hfr, e.g. one of those listed in Table 1. For new F-prime factors, reference to the parental Hfr as well as the ancestral Hfr is needed to enable accurate characterization at some future date.

F-prime factors are designated by numbers which are generally assigned by the investigators who isolate them. The map numbers used in Tables 2 and 3 for the various F-prime factors are, wherever possible, the same as the numbers originally reported. This procedure was not followed in cases where the same F number has been used for more than one F-prime, e.g., F1 (= wild-type F), F_1-*gal* (= F100), F2 (= F2 of Adelberg and Burns), F_2-*gal* (= F152), F3 (reference 134), and F3 (reference 125) (= F153). The effort to avoid overlapping F numbers has resulted in the assignment of certain groups of numbers to some investigators. At the *E. coli* Genetic Stock Center (Department of Microbiology, Yale University School of Medicine, New Haven, Conn.), the attempt is being made to keep a record of F numbers which have already been used, to avoid duplication.

F-prime factors which are derived from another F-prime have been numbered by a suffix following the original F-prime number, such as F14-7 which was derived by mutation from F14, and F170-1 which was derived by deletion from F170.

F-prime strains have been designated in various ways in the literature. The notation "F-prime number/host strain number," e.g., F13/AB2463, has the advantage that it indicates both the F-prime present and the carrier strain. Alternatively, a new strain number (e.g., JC5488 = F15/AB1157) is commonly assigned to represent the F-prime-bearing strain.

HFR STRAINS

As an aid in the isolation of F-primes from Hfr parent strains, a list of Hfrs of potential use is presented. In Table 1, 73 different Hfr strains are listed. The choice of these strains was somewhat arbitrary, based in part on their uniqueness of point of origin, their usage in the current literature, their isolation as special types of Hfrs, or their historical importance. Some of these Hfr strains may no longer exist in the laboratory, but are included here because they were used to sire well known F-primes. The approximate map location of each point of origin is listed in Table 1 and is indicated in Fig. 2. For other known Hfr strains, the reader is referred, in particular, to references 15, 22, 25, 40, 93, and 113.

F-PRIME FACTORS

Data on known F-prime factors derived from *E. coli* K-12 are summarized in Tables 2 and 3. Map position and extent of these F-primes are shown in Fig. 3 and 4. Table 2 contains a representative group of F-primes which are useful in dominance studies because almost all of the genes which they carry are wild type. Table 3 lists additional F-primes, many of which also carry only wild-type alleles. Some of the F-primes listed in Table 3 may no longer

TABLE 3. *Additional F-prime factors, including unusual types*

F-prime map no.	Original or alternate name	Strain from which F' was derived	Ancestral Hfr[a]	References (isolation or characterization, or both[b])	Comments
2	F^2; f-2	P4X	P4X	5, 59	Carries small uncharacterized chromosonal segment
3	F_3	AB257(= P3) (see reference 10)	P3	134	From P3, not Cavalli; cf. F153
8-1	F_{ts}-*gal*	AB2605	W3208 (Hfr 8)	19	Temperature sensitive for replication
9	F9	Not reported	Not reported	76	Orientation of F not reported
14-7	F14-7	AB1206 (F14)	AB313	111, 138	Carries *ilvO*$^-$; from F14
30	F30	G1	G1	12, 64	Carries *his-323*
35	F35	Not reported	R1	171	Constructed by using a double male
42-114	$F_{t.s.-114}$ *lac*; F_{ts} *lac*	200 PS (F *lac*)	P804	86, 42	Temperature sensitive for replication
45	F45	B7	B7	A	Poor replication and low fertility
57	F *his*	AB311	AB311	166, N	
113	FBB1	B11	B11	23	
115	KLF15	KL96	KL96	136, C	
134-1	F134-1	F134/JC1553	Ra-2	121	Spontaneous deletion from F134
141-1		(F 141)	AB312	79	Deletion from F141
141-2		(F141)	AB312	79	Deletion from F141; Similar deletion reported (11)
141-3		(F141)	AB312	79	Deletion from F141
141-4		(F141)	AB312	79	Deletion from F141
142-1	KLF42-1	F142/KL253	KL98	C	Spontaneous deletion from F142
142-2	KLF42-2	F142/KL253	KL98	C	Spontaneous deletion from F142
143-1	KLF43-1	F143/KL259	KL16	C	From F143; carries *rel*$^-$ and *recA*$^-$
153	F_3-*gal*; F *gal*$_3$	P3	P3	84, 125, K	Carries *att* λ
155	F *trp*	EC-8	P804 (transposed)	136	Carries *lac*$^+$, $F_{t.s.-114}$; *att 80*, and *trp*$^+$
155-105	F' *arg met trp att* 80	F105; F155/7	Ra-2; EC-8 (P804 transposed)	129, 136	Fusion of F155 and F105; some material deleted
170	F-*lac-pur*	P804	P804	88	
170-1	F-*lac-pur*, deletion type A	(F170)	P804	88	Deletion from F170
170-2	F-*lac-pur*, deletion type B	F(170)	P804	88	Deletion from F170
170-3	F d6	(F170)	P804	88, 110	Deletion from F170; *lac* and *purE* operons fused
171	F-*lac-tsx*	P804	P804	88	cf. F98 (reference 163)
172	F-*lac-gal*	P804	P804	88	
175	PD-1	KDM-3352	AB311	95	Some material probably deleted in *gal-trp* region; from double male
176	PD-3	KDM-3533	AB313	95	From double male
186	F186	AB313 Ilv$^-$ Pol$^-$	AB313	L	Carries *ilv*$^-$ *polA1*
187	F187	AB313 Ilv$^-$	AB313	L	Carries *ilv*$^-$
196	WH-1	KL96-B	KL96	78	Carries Su_1^+ (*supD*$^-$)
204	ORF104	χ225	OR1	D	
206	ORF206	χ535	IOR2, from OR11	17, D	In strain χ646; carries inversion
207	ORF207	χ629	IOR3, from OR11	17, D	In strain χ647
226	CB026	χ771	TOR 13, from F251	M	F' carries transposition
227	CB027	χ771	TOR 13, from F251	M	F' carries transposition
258	ORF 8; 306	χ562	OR11	17, 18, 163	Primary F'; similar to F251
265	ORF15	χ629	IOR3, from OR11	17	Primary F'; carries inversion
311	F311	AB1013 (F14-7)	AB313	111	Carries *ilv* 0_A^-; from F14-7 by transduction
450	F450	W3102 (F100)	P3	60, 153	Related to F100; carries Su_{II}^+ (*sup E*$^-$)

[a] See Table 1.

[b] Numbers refer to literature cited. Letters refer to the following: A, P. Cooper, *personal communication*; C, B. Low, *unpublished data*; D, R. Curtiss III, *personal communication*; L, J. Gross, *personal communication*; M, C. Berg, *personal communication*; K, E. Wollman, *unpublished data*; N, T. Takano, *unpublished data*.

exist in the laboratory but are included to illustrate possible configurations of derived F-prime factors, e.g., F170-1, F170-2, and F170-3, which are particular examples of deleted F-primes from a common ancestral F-prime, F170 (see above). The sequence of marker transfer by any given F-prime is simply predicted by assuming that the F-prime exists as a closed loop which contains the origin of transfer corresponding to the ancestral Hfr (see Fig. 1 and 2, and Table 1). It should be emphasized that the exact genetic length of all of the F-primes listed is unknown, and that the arcs drawn in Fig. 2 and 3 are based only on the genetic markers for which information is available. Spontaneous deletions of F-prime material are a common occurrence (see above), and some unknown deletions are very likely present in the F-primes listed here.

The F-prime lists in Tables 2 and 3 are representative rather than all-inclusive. Extensive sets of derived F-primes have been constructed for special purposes. Particularly detailed genetic analyses have been carried out with F-primes carrying *his*$^-$ alleles (62, 64), *ilv*$^-$ mutations and transductional deletions (111, 138), transductionally shortened derivatives of F8 (126, 127), *lac*$^-$ mutations and fusions with other operons (72, 73, 87, 88, 90, 118), and mutations of F carried on F42 (1, 2, 42, 86, 165).

CAN E. COLI BE MADE DIPLOID FOR ANY LOCUS?

It has already been pointed out that merodiploids have by now been constructed for almost every region of the chromosome where positive selection is possible for transfer of the region when carried by an episome (see above). However, there exists the possibility that some genetic locus has never been made diploid because the isolation of an episome thought to include that locus involved a deletion of the locus very shortly after (or even before) the episome-forming event in the parental Hfr cell. As mentioned above, the construction of appropriate double male strains might greatly increase the ease in which partial diploids for certain genetic regions are obtained.

An intriguing development which relates to the possibility of diploidy for the whole *E. coli* chromosome comes from studies of Kvetkas concerning a mutation in a gene denoted *lar* isolated in *E. coli* B (97, 98). The expression of *lar*$^-$ results in cells whose excessive size and DNA content are consistent with a possible diploid state. Preliminary results of Floyd (55) suggest that heterozygosity can be introduced into *lar*$^-$ strains by conjugation and maintained for many generations. Detailed characterization of mutants of this type by using presently available techniques might yield important information on the organization and control of segregation of the bacterial chromosome.

ACKNOWLEDGMENTS

Grateful thanks are extended to many colleagues who have informed me of their successes and failures in various episomic projects. B. J. Bachmann has been extremely helpful as the organizer of F-prime and other strains at the *E. coli* Genetic Stock Center, and I also salute her for courage and success in attacking the *E. coli* K-12 pedigree (*see* reference 10) and shaping it into a usable form. Unpublished work was supported by Public Health Service grants GM 06048 from the National Institute of General Medical Sciences and CA 06519 from the National Cancer Institute.

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