Hfr Strains of *Escherichia coli* K-12

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MODES OF Hfr FORMATION

Integration of F

Since the first reports by Cavalli-Sforza (9) and Hayes (24) of strains of *Escherichia coli* K-12 which are conjugational donors of chromosomal markers at high frequency (Hfr strains), many other Hfr strains have been isolated and our understanding of the mechanisms involved in Hfr formation has increased considerably. All Hfr strains arise from the integration of a conjugative plasmid into the bacterial chromosome by one of several possible types of recombination events. The most commonly used Hfr strains have been formed by either spontaneous or UV-induced integration of the *E. coli* F factor (see chapter 126). F integration can also be selected for at high temperature by the use of *E. coli* mutants which are defective in initiation of DNA replication at high temperature (28, 41, 47). The wild-type F factor has been found to integrate at at least 20 different sites on the *E. coli* K-12 chromosome (37); however, these sites are very nonrandom, and the spectrum of possible sites may vary from strain to strain (13). Different investigators have independently isolated Hfr strains with similar, if not identical, insertion sites (points of origin). As reviewed by Davidson, Deonier, Ohtsubo, and others (16, 18, 27, 48, 49; see chapters 111 and 129), the repeated integration of F at certain sites of the chromosome has been shown in at least some cases to be due to recombination events between an insertion sequence (IS element) on the F factor and a homologous IS element on the chromosome. Some of the chromosomal IS elements probably involved in the formation of certain Hfrs are indicated in Fig. 1. Note that for some Hfrs, there is no known IS element at a chromosomal position near the observed point of origin. The mechanism of F integration at these sites is not clear. Both integration and excision of F at IS sequences are considerably reduced in *recA* mutants (12, 19), but this is not true in the case of certain R factors (25, 26). In some cases integration has involved recombination between a larger transposable element, γδ (= Tn*1000;* see chapters 124, 126, and 140), and an integrated copy of γδ in the *E. coli* chromosome, which does not normally carry γδ (22, 34).

Integration of Other Sex Factors

Other conjugative plasmids have also been used to derive Hfr strains, notably ColV (30) and certain R factors (40, 45; see chapter 129). It is unclear at present whether the mechanisms of integration of these plasmids are similar to those involved in F-factor integration, and it is also unclear whether all F-factor integration events involve an IS, transposon, or homologous recombination event. A review of various possible integration mechanisms is given by Reimmann and Haas (45).

Integration of an F-Prime Factor

The integration of derivatives of the F factor has also produced Hfr strains. In particular, the use of an F-*lac* factor (see chapter 129), which is temperature sensitive for replication (i.e., F_{ts}-*lac* = F42-114; see reference 37), carried in a strain the chromosomal *lac* genes of which are deleted permits the selection of Lac^+ derivatives at high temperature which result from the integration of the F_{ts}-*lac* (transposition Hfrs) (3, 4, 8, 15). Alternatively, acridine orange has been used to select for stably integrated F-prime factors (5). The spectrum of integration sites obtained in this way is quite different from the spectrum obtained with wild-type F (37).

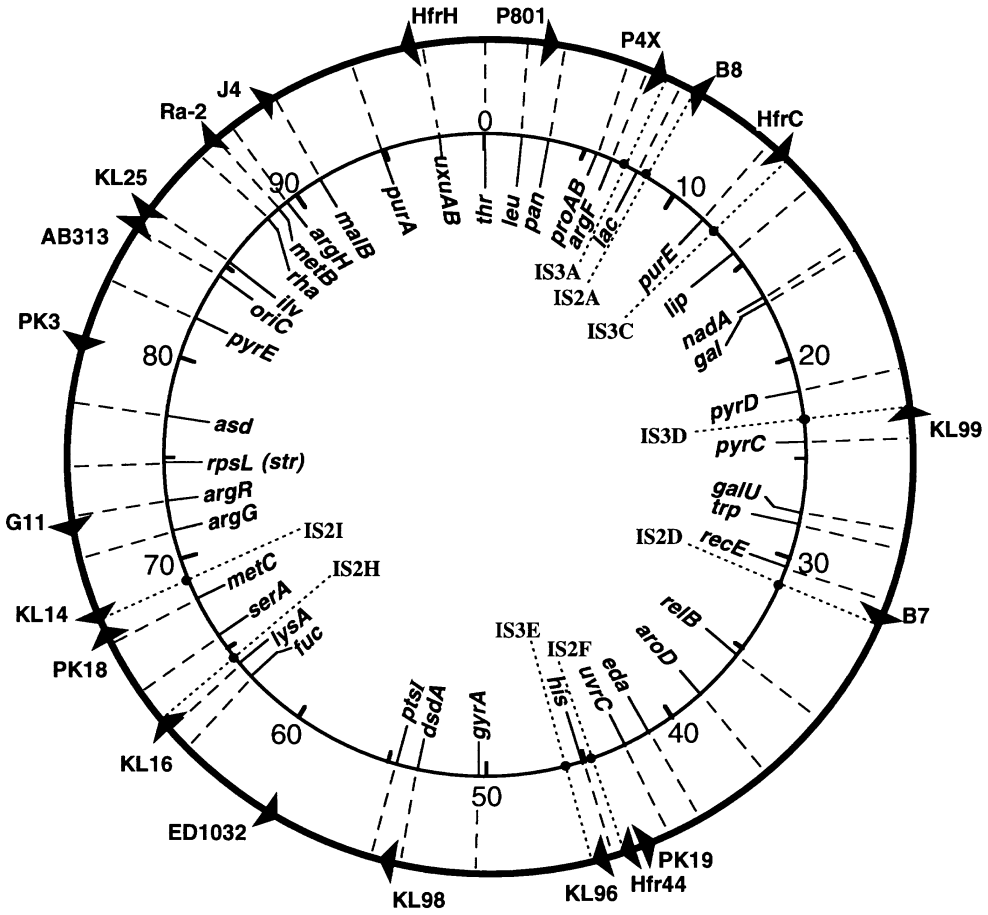

FIGURE 1 Approximate map positions of integrated sex factors (F, F_{ts}-lac, of ColV) for some Hfr strains. See Table 1 for commonly used derivatives of these strains. The sequence of chromosomal genes transferred from a given strain begins behind the arrowhead; e.g., HfrH transfers genes in the order *uxu*AB, *thr*, *leu*, etc. The positions of the IS sequences which appear to correlate with the sites of F insertion for some of the Hfrs are indicated and can be found on the physical map in chapter 129.

Another type of Hfr variant (inversion Hfr) was obtained from a conventional Hfr strain by Berg and Curtiss (5) by the use of a fluctuation experiment. Rare derivatives of the parental Hfr were found which transfer chromosomal markers in reverse order compared with the original. These strains presumably arose by inversion of the region carrying the F factor.

If an F-prime factor is introduced into a normal haploid *E. coli* strain, the extensive homology in the resulting partially diploid region allows frequent recombination events by which the F-prime factor is integrated into (and subsequently excised from) the chromosome (14, 43, 44). The process of "chromosome mobilization" in the transitory Hfr cells of such a strain is convenient for the conversion of a recipient strain into a high-frequency donor, with an Hfr-like polarity of transfer which depends on the particular F-prime used. In at least one case, the transitory Hfr state in this type of donor was stabilized by an inversion of one of the copies of the merodiploid region (1, 6). Oriented chromosomal transfer can be induced by an

F-prime (e.g., F-*lac*) even when the region of chromosomal homology (e.g., *lac*) has been transposed from its normal site as a result of gene fusion events (39). Certain R factors have also been shown to cause oriented chromosomal transfer in a *recA*-independent process (25, 26; see also chapter 129).

Directed-Transposition Hfr Strains

The transposition approach to Hfr strain isolation was further developed by selection of a chromosomal mutation concomitant with selection for F-prime integration (3, 4). This allows the isolation of rarer and more specifically designed transposition Hfr strains with particular desired properties (21).

This directed transposition approach was even further extended with *Salmonella typhimurium* (official designation, *Salmonella enterica* serovar Typhimurium) by Chumley et al. (10), who used an F_{ts}-*lac* which also carried a composite transposon, Tn*10.* By using bacterial mutants which carried chromosomal Tn*10* elements at various sites, it was found that the F_{ts}-*lac*::Tn*10* factor predictably integrated by recombination with the particular Tn10 carried in the chromosome. This enables a much more directed approach to integration of F at particular chromosomal sites, if necessary. This approach is equally applicable to *E. coli*, provided that the chromosomal *lac* region has been deleted in order to prevent preferential integration at the *lac* region by homologous recombination (32).

Double Hfr Strains

By crossing two different Hfr strains, Clark (11) constructed a strain which contained two integrated F factors with different points of origin. Both of the F factors in this "double male" strain were active, i.e., could transfer the chromosomal genes adjacent to them from a high proportion of cells. The chromosome in this type of strain appears to be a single linkage group (J. 0. Falkinham and A. J. Clark, *Genetics* **68:**s18, 1971). Other examples of double Hfr strains have also been found (31, 51; A. R. Kaney, Ph.D. thesis, University of Illinois, Urbana, 1966; C. W. Vermeulen, Ph.D. thesis, University of Illinois, Urbana, 1966).

The insertion of still another F factor into the chromosome of a double Hfr strain was also reported (2). The addition of this third F, on an F-prime factor, produced a "triple male" strain in which at least some cells could transfer chromosomal markers by the point of origin characteristic of the F-prime factor.

REPRESENTATIVE Hfr STRAINS

The availability of Hfr strains with integration sites (points of origin of transfer) distributed around the *E. coli* K-12 map greatly facilitates strain construction and crude mapping by conjugational recombination (see chapter 137; reference 37 gives a review of most of the known F integration sites [and some indication of Hfr stability]). The points of origin of a group of particularly useful Hfr strains are shown in Fig. 1, and these strains are listed in Table 1. These strains include a set (Hfr kit [Table 1]) composed of strains with very few chromosomal mutations, thus allowing selection for wild-type recombinants when crossed with mutant recipient strains which have a counterselectable phenotype.

Hfr strains which transfer a transposon (and thus a selectable drug resistance) are often useful in mapping and strain construction, and a series of these derivatives, constructed by Wanner (50), is also given in Table 1 (Hfr::Tn*10* kit).

TABLE 1 Useful *E. coli* K-12 Hfr strains

Hfr strain	Ancestral Hfr strain	PO no.[a]	Earliest marker known	Latest marker known	Site of Tn*10*[b]	Comment(s)	Reference(s)
3000[c] (= HfrH Thi$^{-\lambda-}$)	HfrH	1	*uxuBA*	*valS*			24, 33; chapter 133
NK6051[d]	HfrH	1	*uxuBA*	*valS*	*purE*		50
P801[c]	P801	120	*leu*	*pan*			B. J. Bachmann, personal communication from F. Jacob
BW6165[d]	P801	120	*leu*	*pan*	*argE*		50
BW113[c]	P4X	3	*argF*	*lac*			17, 36
BW6156[d]	P4X	3	*argF*	*lac*	*zje*		50
B8[c]	B8	118	*tsx*	*lac*			7
BW6160[d]	B8	118	*tsx*	*lac*	*zdh*		50
KL226[c]	HfrC	2A	*gsk*	*fep*			29, 36, 42
BW7261[d]	HfrC	2A	*gsk*	*fep*	*leu*		50
KL99[c]	KL99	42	*pyrC*	*pryD*			36
BW7620[d]	KL99	42	*pryC*	*pyrD*	*zed*		50
KL208[c]	B7	43	*rac*	*trg*		F deleted from 33–43 kb	7, 36, 49
BW7623[d]	B7	43	*rac*	*trg*		F deleted from 33–43 kb	50
PK191[c]	PK19	66	*supD*	*cheC*		Fertility factor is ColV	30, 36
BW5660[d]	PK19	66	*supD*	*cheC*	*srlC*	Fertility factor is ColV	50
KL96[c]	KL96	44	*his*	*purF*			35, 36
BW7622[d]	KL96	44	*his*	*purF*	*trpB*		50
KL983[c]	KL98	53	*dsdA*	*supN*			36
BW5659[d]	KL98	53	*dsdA*	*supN*	*zdh*		50
ED1032	ED1032	201	*thy*	*his*		Transposition of F42-114; transfers *tra* genes early	8
KL16[c]	KL16	45	*lysA*	*serA*			35, 36
BW6163[d]	KL16	45	*lysA*	*serA*	*zed*		50
PK18	PK18	132	*metC*	*arG*		Fertility factor is ColV	23, 30
KL14[c]	KL14	68	*cca*	*tolC*			20, 36
BW6159[d]	KL14	68	*cca*	*tolC*	*ilv*		50
G11	G11	124	*str*	*arG*			38
KL800[c]	PK3	131	*xyl*	*malA*		Fertility factor is ColV	30; K. B. Low, unpublished data
BW6175[d]	PK3	131	*xyl*	*malA*	*argE*	Fertility factor is ColV	50
KL228[c]	AB313	31	*rbs*	*ilvE*			36
BW6169[d]	AB313	13	*rbs*	*ilvE*	*argA*		50
KL25[c]	KL25	46	*ilvE*	*pyrE*		Very unstable Hfr	35, 36
Ra-2[c]	Ra-2	48	*metB*	*rha*		Very unstable Hfr	33, 34, 36
BW6164[d]	Ra-2	48	*metB*	*rha*	*thr*	Very unstable Hfr	50
KL209[c]	J4 (= P10)	18	*argE*	*purA*		F factor in *malB*	36, 46
BW6166[d]	J4 (= P10)	18	*argE*	*purA*	*zhf*	F factor in *malB*	50

[a]Point of origin (PO) numbers were arbitrarily assigned to distinguish independent F-factor insertion events in the formation of Hfr strains. These numbers do not relate to map position (see Fig. 1).
[b]Sites of Tn*10* insertion in these strains lie within the range of 10 to 30 min from the points of origin. Some of these strains also carry a deletion of the *lac* region, as well as certain other markers. The "zxy" system for naming the sites of unknown transposon insertions is given in reference 10. The second letter (x) indicates the appropriate 10-min interval of the map (a = 0 to 10, b = 10 to 20, etc.), and the third letter (y) indicates the particular minute within that interval (a = 0 to 1, b = 1 to 2, etc.). All other gene symbols are as given on the *E. coli* K-12 map (see chapter 109).
[c]Strain is included in the Hfr kit available from the *E. coli* Genetic Stock Center, c/o M. Berlyn, Department of Biology, Osborn Laboratory, Yale University, P.O. Box 6666, New Haven, CT 06511-7444.
[d]Strain is included in the Hfr::Tn*10* kit from the address given in footnote *c*.

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