F-Mediated Conjugation, F^+ Strains, and Hfr Strains of *Salmonella typhimurium* and *Salmonella abony*

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INTRODUCTION

Some wild-type lines of *Salmonella* spp. contain plasmids which are self-transmissible by conjugation (25). However, most such plasmids have very low frequencies of self-transmission owing to repression of transfer functions; this situation has been extensively analyzed by Datta et al. (6). In addition, most plasmids give infrequent chromosome mobilization because of the low frequency of plasmid-chromosome recombination. A system for chromosome mobilization was established by Smith et al. (40) for *Salmonella typhimurium* (official designation, *Salmonella enterica* serovar Typhimurium) by using colicin factors. The system was based on their observation that conjugative colicin factors newly transmitted to a cell are transiently derepressed and give very high frequencies of subsequent transfer to recipient cells. This system of Col-factor-mediated conjugation (called HFC) used ColI and ColE1 factors and gave recombination frequencies of up to $1/10^6$ cells tested; this allowed the construction of a partial linkage map of *S. typhimurium* (41). Stably derepressed colicin factors have subsequently been isolated (25), and some stable insertions of colicin factors into the chromosome of *Escherichia coli* have been obtained.

However, the majority of genetic analysis by conjugation in both *E. coli* K-12 and *S. typhimurium* has been done using the F factor from *E. coli* K-12. This plasmid is well suited for genetic analysis because it is derepressed for F function and because homologous recombination between the plasmid and the chromosome gives rise to Hfr strains with the plasmid stably inserted in the chromosome.

The F factor of *E. coli* K-12 was transferred from *E. coli* to *S. typhimurium* LT2 by Zinder (51) and to *Salmonella abony* by Mäkelä et al. (23). Hfr strains of *S. typhimurium* (30, 35, 37, 51) and *S. abony* (22) were isolated. These strains of *Salmonella* are not hybrids between *E. coli* and *Salmonella* spp. for chromosomal genes; they carry the F factor from *E. coli*, but all the data indicate that they carry no chromosomal genes from *E. coli*.

Merodiploids are common among the progeny of *S. typhimurium-E. coli* intergeneric crosses. This merodiploidy is usually maintained through F′ factors, but in some cases, the extra DNA is inserted into the recipient chromosome (15, 16, 19, 29). Merodiploids of these types are not considered in this report.

REPRESSION AND DEREPRESSION OF THE F FACTOR IN *S. TYPHIMURIUM*

The F factor of *E. coli* K-12 is a conjugative plasmid which determines the presence of F pili, conjugal transfer of F and the chromosome, surface exclusion of the entry of related plasmids, incompatibility of related plasmids, and inhibition of the replication of certain female-specific phages (for reviews, see references 10, 13, and 49 and chapter 126 in this volume). In most F-like plasmids, the *tra* gene is negatively regulated by the *finO* and *finP* gene products (*fin* for fertility inhibition). Mullineaux and Willetts (26) speculate that a complex of *finP* RNA and the FinO protein, when both are present, can interact with the leader portion of the *traJ* transcript to prevent its transcription and/or translation. However, the F factor shows no fertility inhibition, because the *finO* gene is inactivated by insertion of an IS*3* element (50). Since F function in *E. coli* is not repressed, up to 100% of cells have F pili, participate in conjugation, and adsorb male-specific bacteriophages. Numerous other plasmids determining sex pili and conjugal transfer have these functions repressed, and only a small fraction of the cells (from 10^{-1} to 10^{-4})

have pili and can transfer plasmid or chromosome (6). In some cases, these other plasmids also inhibit F fertility (i.e., they are Fin^+); some others do not (these are called Fin^-) (6). Gasson and Willetts (11) described five systems inhibiting F function; one of these, the FinOP system, is responsible for the often poor expression of F fertility functions in many commonly used *S. typhimurium* lines. This system requires three genes: *finO*, the *trans*-acting product of which acts on many different plasmids; *finP*, which makes a plasmid-specific *trans*-acting product; and *traO*.

When F was transmitted into strains of *Salmonella*, the fertility of the resulting F^+ lines was usually much less than that of F in *E. coli* K-12, and the cultures did not show visible lysis by male-specific phage M13 or f2 when tested on solid media (23, 35, 51). Cells of *S. typhimurium* LT2 carrying the F′ factor F*lac*$^+$ had repressed F function (Fin^+), since only 0.001 to 0.007 Lac^+ transconjugant was formed per donor cell and only 0.02% to 0.05% of the cells formed plaque-forming centers with the male-specific phage f2 (34). In addition, phage f2 was propagated to only a low titer (about 10^6 to 10^7 PFU ml). The lack of visible lysis of these strains on agar plates by the male-specific phages f2 and M13 is a rapid and accurate test for the Fin^+ phenotype in strains known to be carrying F (Fin^- cells carrying F show visible lysis by these phages). This fertility inhibition is due to a 90-kb plasmid present in strain LT2 and many other *S. typhimurium* strains and referred to as the virulence plasmid, or pSLT (34, 39, 45). pSLT is stably replicated, and spontaneous curing occurs at a very low rate (45). pSLT is not self-transmissible, but it is mobilizable by plasmids such as F. Over half of the lines of *S. typhimurium* independently isolated from nature have a Fin^+ plasmid resembling pSLT (2). The Fin^+ property of pSLT is due to the FinOP fertility inhibition system (11).

F-derepressed (Fin^-) strains of *S. typhimurium* have been isolated, and the following evidence indicates that they are now as fertile as *E. coli* K-12 F^+ lines: in crosses of *S. typhimurium* F*lac*$^+$ Fin^- donors with *S. typhimurium* recipients, the yield was ca. 1.0 Lac^+ transconjugant per donor cell; there was close to 1.0 f2 infective center per donor cell; male-specific phages f2 and M13 were multiplied to titers of $>10^{10}$ PFU ml and gave visible lysis of these strains on agar; and almost 100% of cells had F pili which adsorbed f2 (34, 38).

There are four separate sources of F-factor-derepressed (Fin^-) strains in *S. typhimurium;* these are available from the *Salmonella* Genetic Stock Centre. The first is F42 *lac*+ plasmids with *finP* or *traO* mutations, which were described by Finnegan and Willetts (9). F42 *finP301 lac*$^+$ was transferred by conjugation to *S. typhimurium* with selection for the Lac+ phenotype (all *Salmonella* spp. are naturally Lac–) to yield strain SA2197 [*purC7*(F42 *finP301 lac*+)]. Although strain SA2197 still carries pSLT, as do nearly all *S. typhimurium* LT2 strains, its phenotype is Fin– (33). Second, Fin^- strains may be obtained by using normal F factors in strains of *S. typhimurium* which have been cured for pSLT; these lines are rare. One such cured line was obtained from H. J. Whitfield (24). Other cured lines have been isolated by M. F. Edwards (Ph.D. thesis, Stanford University, Stanford, Calif., 1985), who has developed a general method of removing pSLT, as follows. A selectable tetracycline resistance marker is inserted into pSLT by using P22 phage to transduce Tn*10* from a line bearing a known Tn*10* insertion in pSLT. The cells are then treated with novobiocin to promote curing of pSLT. Strains which have lost the plasmid are selected by growing the cells in media (3) which inhibit the growth of tetracycline-resistant cells.

The third means of constructing Fin^- strains uses pKZ1, a plasmid identified by Smith et al. (39) as carrying the kanamycin resistance gene. pKZ1 is in the same *Inc* group as pSLT but is itself Fin^-, and although it is nonconjugative, it is efficiently mobilized by F. The pKZ1-carrying transconjugants are easily selected by kanamycin resistance, and pSLT is expelled from $pKZ1^+$ cells by incompatibility (34). A fourth source of Fin^- strains is a set of *S. typhimurium* F^+ and Hfr strains previously reported (35) and summarized here in Table 1. The F^+ strain SR297 (Table 1) and Hfr strains isolated by Zinder (51) are Fin^+ (SR305 and SR315), but F^+ strains derived from transfer of this F to other *S. typhimurium* lines (Table 1) are insensitive to repression by pSLT, probably owing to mutations in either *finP* or *traO*. The isolation of these Fin^- strains occurred after the maintenance of F in strain SR297 and its transfer to produce new F^+ lines. The mechanism is probably as follows. Maintenance of F in a culture during growth involves spontaneous

curing and reinfection; a derepressed F in a line of *S. typhimurium* carrying pSLT would have a greatly increased transfer frequency. Therefore, it is not surprising that derepressed mutants seem to achieve high frequency in the population and are isolatable without overt selection.

ROLE OF THE CELL SURFACE OF THE RECIPIENT STRAIN IN CONJUGATION

The composition of the cell envelope is important in determining the ability of a cell to act as a recipient in conjugation with a cell carrying either an F plasmid (ConF function) or an I plasmid (ConI function). All *ompA* mutants of *E. coli* (affected in the outer membrane protein OmpA) were inefficient recipients in matings with F-carrying strains ($ConF^-$) when the mating was done in liquid medium but were close to normal for ConF function when the mating was done on membrane filters (1). Similarly, *ompA* mutants of *S. typhimurium* are ineffective as recipients in F-mediated conjugation in liquid medium (33). Thus, the OmpA protein is necessary for stabilization of the mating aggregates but does not appear to be the sole receptor for the F pilus.

Since strains of *E. coli* (12, 28) and *S. typhimurium* (33, 47) with heptose-deficient lipopolysaccharide are also $ConF^-$, components of the lipopolysaccharide are also required for efficient conjugation. The most efficient *S. typhimurium* recipients are those having a complete or nearly complete core region without any O (somatic) side chains. The lipopolysaccharide compositions of these efficient recipients resemble that of *E. coli* K-12, all strains of which lack the O (somatic) side chain (43) found on wild-type *E. coli* and *Salmonella* lines.

Although core lipopolysaccharide is required for efficient recipient capacity in conjugation, the O (somatic) side chains of the lipopolysaccharide reduce the frequency of conjugation (14, 47). In crosses of *S. typhimurium* F *finP301 lac*$^+$ to F^- strains of *S. typhimurium* in which the cells were mated in broth, recipient strains which were rough mutants affected in the outer core region of the lipopolysaccharide gave ca. 1.0 Lac^+ recombinant per donor cell; smooth strains (with O side chains on the lipopolysaccharide) gave about 1/20 as many Lac^+ recombinants per donor cell (33). However, when strains were mated on membrane filters, both smooth and rough strains gave equally efficient levels of transfer (i.e., ca. 1.0 Lac^+ transconjugant per donor cell). Reduced fertility of smooth recipients may occur because the O side chains of the lipopolysaccharide shield the conjugation receptor and reduce the efficiency of stabilization of mating aggregates, since gradient-of-transmission experiments indicated that once mating aggregates are formed, they are equally stable in both smooth and rough recipients. Jarolmen and Kemp (14) and Watanabe et al. (47) also noted that F^+ transconjugants in a smooth recipient strain are frequently rough mutants. We also observed that many of the F^+ strains isolated upon transfer of F to smooth strains of *S. typhimurium* were rough mutants (Table 1). This result may be due to the increased efficiency of transfer to the rare spontaneous rough mutants present in the population of smooth recipient cells.

F-MEDIATED CONJUGATION CROSSES BETWEEN *E. COLI* K-12 AND *S. TYPHIMURIUM*

Intergeneric plasmid exchange is affected by host-mediated restriction-modification systems. Transfer of F*lac*$^+$ from *S. typhimurium* LT2 to *E. coli* K-12 is much more frequent than the reverse. The frequency of F*lac*$^+$ transfer from a derepressed F *finP301 lac*$^+$ *S. typhimurium* line to a rough *S. typhimurium* recipient was ca. 1.0 Lac^+ transconjugant per donor cell, and transfer to an *E. coli* K-12 F^- line (C600) was almost as high (0.46 Lac^+ transconjugant per donor cell) (33). However, whereas F*lac*$^+$ *E. coli* K-12 lines were very fertile in crosses to *E. coli* recipients (as expected), crosses from *E. coli* to smooth *S. typhimurium* recipient strains with wild-type host restriction genes gave only 1.6×10^{-5} Lac^+ transconjugants per donor cell. Rough recipient strains gave 10 to 14 times as many Lac^+ transconjugants as smooth strains (about the same increase as seen in crosses within *Salmonella* spp.), and strains with host restriction mutations *hsdL* and *hsdSA* gave ca. 100 times as many transconjugants as did strains with the wild-type genes. Thus, the *S. typhimurium* F^- strain SL1655, which has mutations in *hsdL hsdSA* (for host restriction) and *rfaG* (for

lipopolysaccharide synthesis), gave 0.037 Lac^+ transconjugant per donor cell in crosses with *E. coli* when mating was in broth and 0.11 Lac^+ transconjugant when mating was on membrane filters. Recipient strains with a mutation in the host restriction gene *hsdSB* as well as in *hsdL* and *hsdSA* have been isolated by Bullas and Ryu (4), and these strains (e.g., LB5000 and LB5010) are very effective recipients for conjugation from *E. coli.* Some of these strains also carry a *galE* (galactose epimerase) mutation that gives rough lipopolysaccharide, which enhances the frequency of transformation of plasmid DNA (21), and they are very efficient recipients of transforming plasmid DNA isolated from either *Salmonella* spp. or *E. coli.* Thus, they are very useful in manipulations with recombinant DNA.

The frequency of transconjugants in crosses of *E. coli* Hfr strains with *S. typhimurium* F^- when selection is for chromosomal genes from the donor strains is usually very low (8). This is because of the barriers to conjugation described above, which are due to O (somatic) side chains on the lipopolysaccharide and to host restriction, and also because recombination between the nonhomologous chromosomes of the two genera to give rise to stable transconjugants occurs at very low frequency. Many of the recombinants for chromosomal genes are due to recombination at duplicated and highly conserved genes such as *rrn* (19). The frequency of recombinants due to homologous recombination in crosses of *E. coli* Hfr into *S. typhimurium* F^- was increased from 10^{-8} to 10^{-4} by using *mutH, mutS,* or *mutL* mutations in the *S. typhimurium* F^- strain; this suggests that the mismatch error repair system prevents recombination between DNA with substantial divergence but that when the system is missing, homologous recombination is much more tolerant of divergence (27).

F^+ AND Hfr STRAINS OF *S. TYPHIMURIUM* AND *S. ABONY*

The F factor of *E. coli* K-12 was transmitted into *S. typhimurium* to produce SR297 F^+, and strain SR305 (HfrA) was isolated from this line (Table 1) (51). However, strain SR297 F^+ is not a good source of different Hfr strains because it produces Hfr strains almost all of which are the HfrA type (Table 1, Fig. 1) (35). As a result, strain SR297 was postulated to contain a sex factor affinity region (*sfa*) on the chromosome at the site of the origin of chromosome transfer in HfrA (35). To provide a wider range of Hfr strains, F was transmitted from SR297 into a number of other lines of *S. typhimurium* LT2. Those F^+ strains from which Hfr strains were isolated are listed in Table 1.

The F factor was similarly transmitted from *E. coli* K-12 into an *S. abony* strain to produce the auxotroph SW1363 F^+ and the prototroph SW803 F^+ (22, 23), and Hfr strains were isolated from both of these. The Hfr strains of *S. typhimurium* and *S. abony* listed in Table 1 were isolated as described by Sanderson et al. (35). The points of origin of the Hfr strains are illustrated in the partial linkage map in Fig. 1, with *S. typhimurium* strains outside the circle and *S. abony* strains inside the circle. The data from which the points of origin were derived were presented by Sanderson et al. (35) or are, in some cases, discussed in the footnotes to Table 1.

USE OF TRANSPOSONS IN ISOLATION OF Hfr STRAINS

Although many Hfr strains are available, mapping by conjugation may be limited by lack of a donor with a convenient point of origin and orientation of transfer. Chumley et al. (5) developed a system using the transposable drug resistance element Tn*10* (17) that permits the isolation of an Hfr strain with an origin at any site of the chromosome at which a Tn*10* insertion has been isolated. This system also permits selection of the direction of chromosome transfer. This method requires the directed insertion of an F′ *ts*114 lac^+ plasmid into the chromosome by homologous recombination between a Tn*10* sequence carried on the plasmid and a second Tn*10* sequence located on the chromosome.

TABLE 1 F^+ and Hfr strains of *Salmonella*

Strain	Sex type	Infectious F	Source	Genotype[a]	Fin phenotype[b]	Phage sensitivity phenotype[c]	Point of origin of transfer[d]		Genes transferred
							Min	Direction	
S. typhimurium									
F^+ strains									
SR297	F^+-*sfa*	+	N. Zinder	*hisD23 gal-50 sfa-1*	+	Smooth	82	CW	*O-ilv-metA-thr-pyrE*
SA27	F^+	+	SA1383	*purE8 rfx-3057*	–	Rough			
SA28	F^+	+	SA1384	*serA13 rfa-3058*	–	Rough			
SA607	F^+	+		*serA15*	–	Smooth			
SA622	F^+	+	SA1386	*serA150*	–	Smooth			
SA627	F^+	+		*thrA9*	–	Smooth			
SA991	F^+	+		*thrB49 leuBCD39 ara-7*	–	Smooth			
Hfr strains									
SR305	HfrA		N. Zinder	*hisD23 gal-50*	+	Smooth	82	CW	*O-ilv-metA-thr-pyrE*
SR315	FS21		N. Zinder	*leu-256*	+	Smooth	30	CW	*O-pyrF-trp-his-pur*
SU354	HfrB2			*metA22* (P22)$^+$	–	Smooth	35	CW	*O-tre-his-purC-trp*
SU418	HfrB2			*proA26* (P22)$^+$	–	Smooth	35	CW	*O-tre-his-purC-trp*
SU436	HfrB3		SR297	*hisD23 gal-50*	+	Smooth	72	CCW	*O-argE-serA-cysC-aroC*
SA458	HfrK1–1		SA28	*serA13 rfa-3058*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA464	HfrK1–2		SA28	*serA13 rfa-3058*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA486	HfrK3		SA28	*serA13 rfa-3058*	–	Rough	61	CW	*O-argE-ilv-leu-cysC*
SA534	HfrK4		SA28	*serA13 rfa-3058*	–	Rough		CW	*O-pyrB-thr-trp-purA*
SA535	HfrK5		SA28	*serA13 rfa-3058*	–	Rough		CW	*O-metG-aroD-purG-his*
SA536	HfrK6		SA28	*serA13 rfa-3058*	–	Rough	76	CW	*O-xyl-cysE-ilv-malA*
SA537	HfrK1–3		SA27	*purE8 rfx-3057*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA538	HfrK1–4		SA27	*purE8 rfx-3057*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA539	HfrK1–5		SA27	*purE8 rfx-3058*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA540	HfrK2–1		SA27	*purE8 rfx-3057*	–	Rough	79	CCW	*O-cysE-xyl-malA-pyrE*
SA639	HfrK1–6		SA622	*serA150*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA640	HfrK2–2		SA622	*serA150*	–	Rough	79	CCW	*O-cysE-xyl-malA-pyrE*
SA642	HfrK1–7		SA622	*serA150*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA653	HfrK2–3		SA627	*thrA9*	–	Rough	79	CCW	*O-cysE-xyl-malA-pyrE*
SA654	HfrK9		SA627	*thr-9*	–	Smooth	40	CW	*O-his-metG-purC-tre*
SA722	HfrK10		SA607	*serA15 pur-268*	–	Smooth	84	CCW	*O-ilv-pyrE-xyl-metE-cya*
SA828	HfrK25		SA804	*serA15 rfx*	–	Rough	30	CW	*O-trp-his-purC-gal*
SA949	HfrK7		SA991	*thrA49 leuBCD39 ara-7*	–	Smooth	76	CW	*O-xyl-cysE-ilv-malA*
SA952	HfrK14		SA991	*thrA49 leuBCD39 ara-7*	–	Smooth	80	CW	*O-pyrE-ilv-metA-rfa*
SA955	HfrK20		SA28	*serA13 rfa-3058*	–	Rough	15	CCW	*O-purE-pro-leu-gal*
SA962	HfrK16		SA991	*thrA49 leuBCD39 ara-7*	–	Smooth	41	CW	*O-his-metG-purC-tre*
SA965[e]	HfrK17		SA991	*leuBCD39 ara-7*	–	Smooth	10	CCW	*O-pro-metA-ilv-purE*
SA966[e]	HfrK19		SA991	*leuBCD39 ara-7*	–	Smooth	98	CCW	*O-purA-metA-ilv-purB*
SA967	HfrK1–8		SA991	*thrB49 leuBCD39 ara-7*	–	Rough	79	CW	*O-pyrE-ilv-thr-cysE*
SA969[e]	HfrK15		SA991	*leuBCD39 ara-7*	–		90	CCW	*O-thi-argF-ilv-purD*
SA970	HfrK11		SA28	*serA13 rfa-3058*	–	Smooth	80	CCW	*O-pyrE-cysE-xyl-ilv*
SA975	HFrK13		SA991	*thrA49 leuBCD39 ara-7*	–	Rough	78	CW	*O-cysE-pyrE-ilv-xyl*
SA977	HfrK8		SA991	*thrA49 leuBCD39 ara-7*	–	Smooth	76	CW	*O-xyl-cysE-ilv-malA*
SA978	HfrK12		SA991	*thrA49 leuBCD39 ara-7*	–	Smooth	80	CCW	*O-pyrE-cysE-xyl-ilv*
S. abony									
F^+ strains									
SW803	F^+	+			+				
SW1363	F^+	+		*met-1511 aro-851 st4–501*	+				
Hfr strains									
SW1444	HfrH1	–	SW1391	*met-1151 aro-851 str-501*	+	Smooth	96–98	CW	*O-pyrB-pro-purA*
SH81	HfrH1	–		*rfb-3798*	+	Rough	96–98	CW	*O-pyrB-pro-purA*
SH461	HfrH1	–	SH81	*pyrA216 rfb-3798*	+	Rough	96–98	CW	*O-pyrB-pro-purA*
SW1403	HfrH2	+	SW1363 F^+	*met-1151 aro851*	+	Smooth	53–60	CCW	*O-purC-his-serA*
SH566	HfRH3	+	SW1452	*str-501*	+		20–32	CCW	*O-gal-pro-trp*
SW1446	HfrH4	+	SW803 F^+		+		90–94	CCW	*O-metA-ilv-pyrB*
SH472	HfrH4	+	SW1446	*pur-259*	+		90–94	CCW	*O-metA-ilv-pyrB*
SW1462	HfrH5	+	SW803 F^+		+		35–40	CW	*O-his-purC-trp*
SH465	HfrH5	+	SW1462	*thi-53*	+	Rough	35–40	CW	*O-his-purC-trp*
SH473	HfrH7	+	SW803 F^+	*cys-1187*	+		80–82	CW	*O-ilv-pyrB-pyrE*
SH650	HfrH10	+	SW803 F^+		+		90–98	CCW	*O-metA-ilv-pyrB*

TABLE 1 *Continued*

Strain	Sex type	Infectious F	Source	Genotype[a]	Fin phenotype[b]	Phage sensitivity phenotype[c]	Point of origin of transfer[d]		Genes transferred
							Min	Direction	
SH474	HfrH10	+	SH650	*thi-51*	+		90–98	CCW	*O-metA-ilv-pyrB*
SH462	HfrH12	+	SH671	*cys-1189*	+	Rough	50–60	CCW	*O-aroC-his-serA*

[a]Genetic nomenclature is according to the system of Demerec et al. (7).
[b]+, Fertility inhibition. Strains with this phenotype inhibit the fertility of F because of the Fin^+ plasmid pSLT (see text). –, fertility of F is not inhibited.
[c]Phage sensitivity is an indicator of the lipopolysaccharide content of the cell. Some of the tests were done by B. A. D. Stocker at Stanford University. The smooth phage sensitivity phenotype, which indicates the presence of normal lipopolysaccharide in the cell wall, is that of sensitivity to the O-specific phages P22.c2, P22h.c2, and 9NA as well as to phage FO and resistance to the rough-specific phages 6SR, Ffm, and Br60. Deviations from the smooth phage sensitivity pattern are called rough. Among the rough phenotypes, several subgroups can be distinguished. Methods for phage sensitivity tests and definitions of phage sensitivity phenotypes are provided by Wilkinson et al. (48).
[d]Min, inferred point of insertion of F into the chromosome; corresponds to minutes of transfer time in interrupted conjugation experiments with Hfr strains (Fig. 1). CW, clockwise direction of transfer; CCW, counterclockwise direction of transfer. The earlier description of Hfr strains (35) showed a linkage map inferred to be 138 min in length; this was later revised to 100 min to correspond with the linkage map of *E. coli* K-12 (36).

Wild-type *S. typhimurium* lacks the genes for the utilization of lactose, but it is Lac^+ when it harbors a plasmid carrying the *lac* operon of *E. coli.* F′ *ts*114 lac^+ is temperature sensitive for replication and is lost during growth at 42°C. When Lac+ survivors are selected at 42°C, the most common class results from integration of the F′ *ts*114 lac^+ plasmid into the chromosome (5).

Derivatives of F′ *ts*114 lac^+ that carry a Tn*10* insertion in the F-derived part of the plasmid in the two possible orientations were constructed. When *S. typhimurium* strains with a Tn*10* insertion in the chromosome and carrying the plasmids described above were grown at 42°C and Lac^+ colonies were selected, Hfr formation occurred by homologous, *recA*-dependent recombination between plasmid-borne and chromosomal Tn*10* elements. All Hfr strains isolated from a given merodiploid showed the same direction of transfer. Depending on the orientation of Tn*10* in the F′ plasmid, Hfr strains transferring in either orientation could be obtained for any chromosomal Tn*10* insertion.

Hfr strains generated by this method are sufficiently stable for genetic mapping crosses if they are used shortly after isolation. However, they have proven to be rather unstable during storage and thus are difficult to maintain. Most investigators reisolate the desired Hfr strain rather than attempting to maintain it. Reisolation is easily done by maintaining the donor in the F′ form in a –70°C freezer. When the Hfr form is required, the freezer stock can then be streaked to minimal medium containing lactose and grown at 42°C. The large, fast-growing colonies are then picked for immediate use in crosses (J. R. Roth, personal communication).

One important use of Tn*10*-directed Hfr strains has been to locate unmapped Tn*10* insertions with respect to other markers. Lac^+ Hfr insertions are selected in a strain with a chromosomal Tn*10* insertion and either F′ *ts*114 lac^+ *zzf-20*::Tn*10* (in one orientation) or F′ *ts*114 lac^+ *zzf-21*::Tn*10* (in the opposite orientation); the F′ elements with Tn*10* in the opposite orientation yield Hfr strains with an opposite direction of transfer. The determination of frequency of formation of transconjugants with various mutant genes shows the position of the point of origin of transfer and thus the location of the Tn*10* insertion (5). If Tn*10* is inserted into a gene, thus resulting in an altered phenotype, the position of the gene is determined.

Strains of *S. typhimurium* with Tn*10* (or Tn5) insertions at many different sites on the chromosome are listed by Sanderson and Roth (36) and are available from the original investigator or the *Salmonella* Genetic Stock Centre at the University of Calgary. For a detailed list, see chapter 141 in this volume. In conjunction with the *zzf-20* and *zzf-21* Tn*10* insertions in F, these chromosomal Tn*10* insertions have been used to map any known gene as either clockwise or counterclockwise from a known point of insertion (e.g., see reference 42).

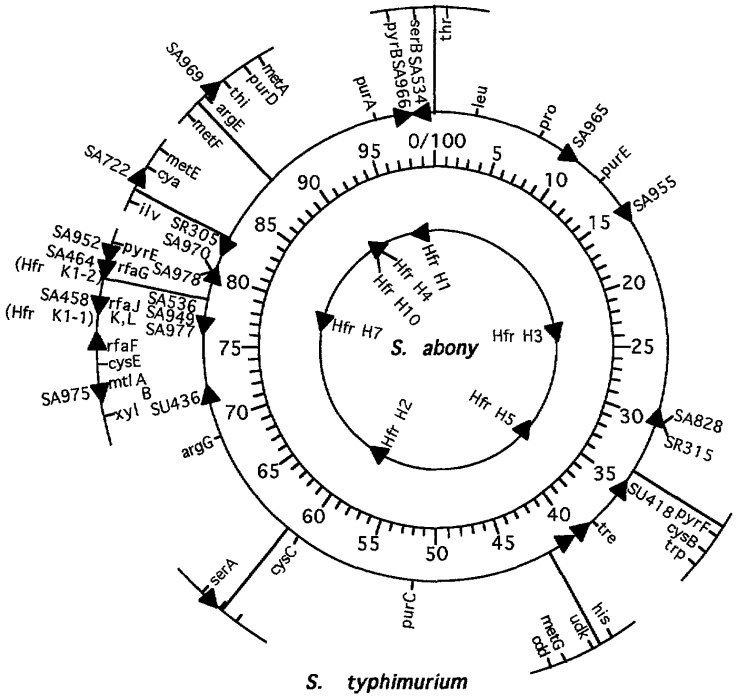

Figure 1 Hfr strains of *S. typhimurium* (outside the circle and *S. abony* (inside the circle). The chromosome is divided into 100 units, starting at *thr* (unit 0), as shown by numbers on the circle. The point of origin and orientation of transfer of each strain are indicated by arrows. For *S. typhimurium*, the strain number (e.g., SR305) is given, and Table 1 gives the Hfr designation (HfraA) and the genotype of each strain. In those cases in which F is inserted into a known transduction linkage group, the Hfr is displayed on an arc outside the main circle. Where more than one Hfr strain number is shown in a gene interval (e.g., SA536, SA949, and SA977, shown within a bracket), the numbers represent independent isolates for which the points of origin have not been proven different, though differences may exist. Not all strains with points of origin in the *rfa* genes (HfrK1 and HfrK2 at 79 min) are shown. For more details, see Table 1. discussions of experiments revealing points of origin and other properties of Hfr strains are provided by Sanderson et al. (35), and a few corrections in the original data are provided in the footnotes of Table 1. Only genes whose positions are known with respect to the points of origin of the Hfr strains are shown here; the complete linkage map is shown elsewhere (36) (see chapter 110 also).

F′ STRAINS IN *S. TYPHIMURIUM*

A small number of F′ strains carrying segments of the chromosome of *S. typhimurium* along with the F factor have been reported (e.g., F′ *trp-cysB-pyrF* [31] and F′ *his* [46]). My own efforts to isolate F′ strains have frequently been unsuccessful. Formation of F′ strains is normally due to homologous recombination at insertion sequences in an Hfr strain (44). *S. typhimurium* LT2 lacks many of the insertion sequences present in *E. coli;* it has six copies of IS*200* (18). This lack may explain the small number of F′ strains isolated.

Hfr strains isolated by the methods of Chumley et al. (5) have been used to isolate F′ strains (J. A. Pogliano and S. R. Maloy, unpublished data). First, *S. typhimurium* Hfr strains were constructed by selecting for recombination between Tn*10* insertions in opposite orientations on a temperature-sensitive F′ and a chromosomal *melB*::Tn*10* insertion (5). F′ derivatives were selected by mating the Hfr strains with *S. typhimurium metA recA* F^-, with selection for Met+ transconjugants. Hfr strains oriented in one direction gave no F′ strains, but those oriented in the other direction yielded F′ strains carrying the genes ace^+, $iclR^+$, and $metA^+$; these F′ strains were used in complementation studies (Pogliano and Maloy, unpublished data).

A set of F′ strains carrying all parts of the chromosome of *E. coli* is available (20). Many of these strains have been transferred into *S. typhimurium* (32) and are available from the *Salmonella* Genetic Stock Centre.

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