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Inversion of Transfer Modes and Sex Factor-Chromosome Interactions in Conjugation in *Escherichia coli*

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ABSTRACT

A study was made of the mating properties of an unusual system of interconvertible donor strains of *Escherichia coli* K-12: Ra-1, Ra-2, and RaF+. The Ra-1 and Ra-2 strains are Hfr strains whose origins are widely separated on the chromosome and whose transfer modes proceed in the opposite direction from one another. When Ra-1 cells were mated with females, a small fraction of the donors transferred markers via the Ra-2 mode. This effect was enhanced by preconjugal ultraviolet (UV) treatment of the Ra-1 cells. Among the survivors of UV-treated Ra-1 cells, a few stable Ra-2 cells were found. When Ra-2 cells were used as the donors, some of them were found to mate via the Ra-1 mode, in analogy with the Ra-1 to Ra-2 alteration with inversion of F mentioned above. Related experiments suggested that the inversion occurs by detachment of the F factor from one Hfr origin locus, followed by reassociation of the F factor with the other Hfr origin locus. Both the Ra-1 and Ra-2 strains reverted spontaneously to an F+ strain, called RaF+. Cultures of RaF+ cells were found to mate primarily according to the Ra-1 and Ra-2 transfer modes, with smaller contributions also coming from transfer modes with origins elsewhere on the chromosome in a way which is similar to the transfer of markers from a normal F+ strain. The RaF+ sex factor was found to be wild type, whereas the chromosome was found to carry irregularities (sex factor affinity loci) at the locations of the Ra-1 and Ra-2 origins. Only about 10% of the donor capacity of the RaF+ strain was due to stable spontaneous Hfr cells in cultures of RaF+ cells.

Conjugation in *Escherichia coli* K-12 results in the transfer of chromosomal material from a donor (male) cell into a recipient cell, to give rise to one or more recombinant genotypes which characterize the daughters of the recipient cell. For a cell to be a donor, it must contain an element called the sex factor (F factor). The F factor is a small piece of deoxyribonucleic acid (DNA) which is about 2% as large as the *E. coli* chromosome. About 40% of the DNA of the F factor is hybridizable with chromosomal DNA (4). Various types of donor strains have been reported which differ in the manner and efficiency of chromosomal transfer during conjugation. The differences in these donor types are due to differences in the state of the F factor within the donor cell. The donor types reported so far are: F+, Hfr, F′, *sfa*, and double male.

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The F factor in F+ cells exists independently of the chromosome and may be readily transferred to recipient cells during conjugation, even though chromosomal markers are in general not transferred (8). Approximately one of every 10^4 F+ cells transfers some chromosomal markers during conjugation. The number of male markers which appear in recombinants is roughly independent of position on the male chromosome.

In Hfr cells, the F factor is integrated into the chromosomal linkage group. Various Hfr strains have been isolated which differ in the chromosomal location of the sex factor. When mixed with F− cells, almost all of the cells in an Hfr culture are able to transfer chromosomal markers to recipients. Transfer of markers proceeds in a polarized fashion, beginning always at the location of the sex factor (the "origin") and proceeding sequentially along the linkage group until the integrated sex factor is reached and is itself trans-

ferred. Random chromosomal breakage during transfer terminates the transfer process and results in a higher number of early markers (i.e., near the origin) transferred as compared to late markers (8).

An F′ strain is similar to an F^+ strain except that the F′ sex factor carries a duplication of one or more chromosomal loci in addition to its normal complement of DNA. F′ cells can readily transfer the F′ sex factor to recipient cells during conjugation. Transfer of chromosomal markers by an F′ strain occurs with a higher efficiency than by an F^+ strain. The chromosomal markers which are transferred first (and most efficiently) are those near the chromosomal segment which is homologous with the extra DNA carried on the F′ sex factor. Pairing between these homologous segments is believed to be responsible for the preferential region for the origin of chromosomal transfer (14).

The F factor in an *sfa* (sex factor affinity) donor strain carries no extra DNA but nevertheless exhibits the same type of affinity for one chromosomal location (as indicated by relatively high efficiency of transfer, beginning at one chromosomal location), as observed for an F′ strain (1). In the case of the *sfa*, this is presumably due to the presence in the chromosome of a segment of extra DNA which is homologous with sex factor DNA. A reasonable scheme for conversion between F^+, Hfr, F′, and *sfa* donor types is presented in a paper by Adelberg and Pittard (2). An essential aspect of this scheme is that the formation of an F′ factor or *sfa* locus can occur as a result of a single crossover event which converts a single circular Hfr chromosome into separate chromosomal and F factor parts, such that either the chromosome or F factor emerges with more than its usual complement of DNA. In some cases, the crossover event can occur in such a way that the F factor receives chromosomal DNA and at the same time the chromosome is left with some DNA from F. The resulting F factor is called an F_2.

The double-male strain resembles an Hfr strain except that in the double male an F factor is integrated at each of two separate chromosomal locations. When a double-male cell mates, either one F factor or the other is active in promoting transfer of the chromosomal segment adjacent to it. Each double-male cell appears to have an equal probability of carrying out chromosome transfer via either of the two transfer modes (3).

In this paper, the results of a study of the mating behavior of a new variety of donor are presented. In this donor, the Ra-1 Hfr strain, the origin of transfer can be induced to change as a result of preconjugal irradiation treatment (9). The new "inverse" mode of transfer induced by radiation has its origin at a chromosomal location which is different from the normal Ra-1 origin, and the inverse mode proceeds in the opposite chromosomal direction as compared to the Ra-1 mode. Some stable Hfr cells of this inverse type (designated Ra-2) are found among the survivors of irradiated Ra-1 cells. The present work constitutes a further study of the Ra-1 and Ra-2 strains and of the F^+ strain (RaF^+) which arises spontaneously from either the Ra-1 or the Ra-2 strain. Comparison of the behavior of the RaF^+ strain with a normal F^+ strain has led to some findings which are of relevance to the nature of sex factor-chromosome interactions and the manner of chromosome transfer by F^+ strains.

MATERIALS AND METHODS

The isogenic donor strains Ra-1, Ra-2, and RaF^+ are genetically prototrophic and sensitive to streptomycin and to infection by T6 bacteriophage. The Ra-1 strain was derived by Joset (9) by ultraviolet (UV) treatment of *E. coli* strain W1485F^+ (obtained from the Institut Pasteur). The Ra-2 and RaF^+ donor strains were derived from the Ra-1 strain as mentioned above. A "normal" F^+ strain (HF^+) was obtained as a spontaneous revertant of the standard Hayes Hfr strain. This strain is sensitive to streptomycin and phage T6 and auxotrophic for thiamine.

Two recipient (F^-) strains were used. Their pertinent auxotrophic characters are as follows. PA309 (from H. Marcovich): *arg*$^-$, *thr*$^-$, *trp*$^-$, *his*$^-$, *xyl*$^-$, *str-r*. KL63 (derived in this laboratory): *arg*$^-$, *his*$^-$, *lys*$^-$, *str-r*. The genetic symbols are explained in Fig. 1, which is a genetic map of *E. coli* [modified from Taylor and Thoman (15)] showing the chromosomal locations of the various markers used here.

Cells were grown and mated (in a 1:10 male to female cell ratio) in nutrient broth with gentle shaking at 37 C. Matings were ordinarily allowed to continue for 90 min, after which portions of the mating mixture were spread onto recombinant-selective media (12). Interrupted matings were performed with a vibratory blending device (11).

UV irradiations were carried out by chilling exponentially growing cells and then exposing them to a General Electric germicidal lamp whose radiation intensity was peaked at a wavelength of 2,537 A. The dose rate was 25 ergs per mm^2 per sec.

RESULTS

Location of the Ra-1 and Ra-2 origins of transfer. Mating experiments with unirradiated Ra-1 cells showed that the Ra-1 origin lies between the *lys* and *serA* markers (Fig. 1). The *lys* marker, which had an entry time of 5 min as shown by the interrupted-mating results (Fig. 2A), is probably less than 0.5 min from the Ra-1 origin. This is indicated by the relatively low level of *lys* recombinants in standard matings (about one-third) as compared to the level for the *his* marker which is transferred later. This is an example of the depression of recombination frequency (relative to the frequency expected from the simple gradient of

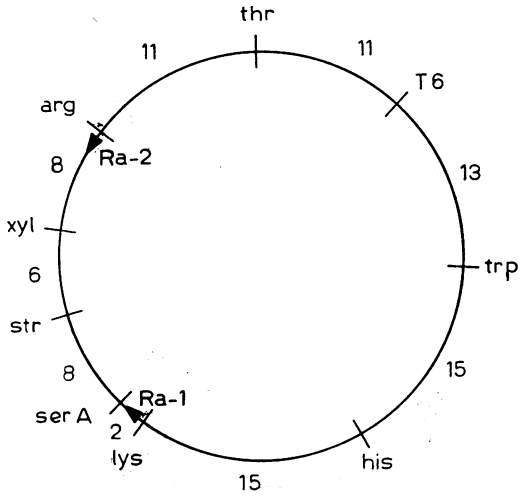

FIG. 1. *Genetic map of Escherichia coli. Nutritional requirements: arg, arginine; thr, threonine; trp, tryptophan; his, histidine; lys, lysine; ser A, serine. Ability to utilize sugars: xyl, xylose. Resistance to agents: str, streptomycin; T6, T6 bacteriophage. The loci for the origins of transfer are indicated for the Ra-1 and Ra-2 strains.*

transfer) which occurs for markers located very near the origin of transfer (10).

The Ra-2 strain, which was isolated from irradiated Ra-1 cultures as mentioned previously (9), exhibits transfer kinetics as seen from the interrupted-mating results shown in Fig. 2B. The *arg* marker had an entry time of about 5 min, followed by *thr* at 14 min, etc. The *arg* marker shows a low level relative to the later markers, which in this case indicates that the *arg* marker is about 1 min from the Ra-2 origin (10).

Inversion of transfer modes exhibited by the Ra-1 and Ra-2 strains. The earlier report (9) indicated that preconjugal irradiation of Ra-1 cells induces a fraction of them to transfer markers via the Ra-2 mode during conjugation. Subsequent work with the Ra-1 strain revealed that it gives rise to F^+ cells (RaF^+) spontaneously at a relatively high rate. (Stock cultures of Ra-1 cells were found to contain a minimum of 5% RaF^+ cells.) It was therefore necessary to determine whether the induction of the Ra-2 transfer mode in irradiated Ra-1 cultures was due to the presence of RaF^+ cells. Accordingly, very pure Ra-1 cultures were grown from fresh single-colony isolates of the Ra-1 strain, and their mating behavior after irradiation was compared to that of a pure culture of RaF^+ cells. Parallel interrupted-mating experiments were performed with the pure Ra-1 culture and the RaF^+ culture, both with and without a preconjugal low dose of UV irradiation. The *arg* marker was assayed, since its early transfer is indicative of how much inversion of the Ra-1 cells to the Ra-2 mode is present; *arg* enters at 5 min via the Ra-2 mode and at 78 min via the Ra-1 mode. Figure 3A shows the results of such an experiment. In this case, the Ra-1 culture was found to be more than 98% pure, i.e., less than 2% RaF^+. Figure 3A shows that the RaF^+ strain was induced to transfer the Ra-2 mode. The Ra-1 strain was also induced to transfer the Ra-2 mode, to approximately the same extent as for the RaF^+ strain. Hence, Hfr cells in the Ra-1 culture must account for the majority of the Ra-1 inversion to

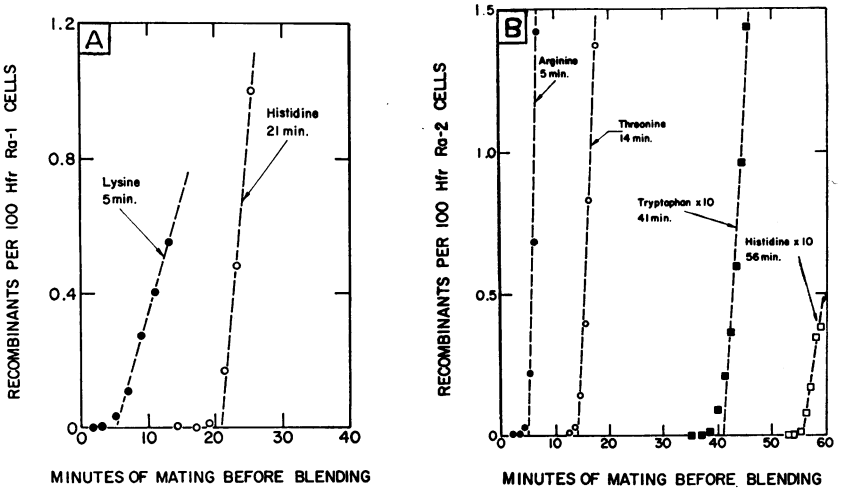

FIG. 2. (A) *Interrupted-mating experiment, unirradiated Ra-1 donor cells × KL63.* (B) *Similar experiment, Ra-2 donor cells × PA309.*

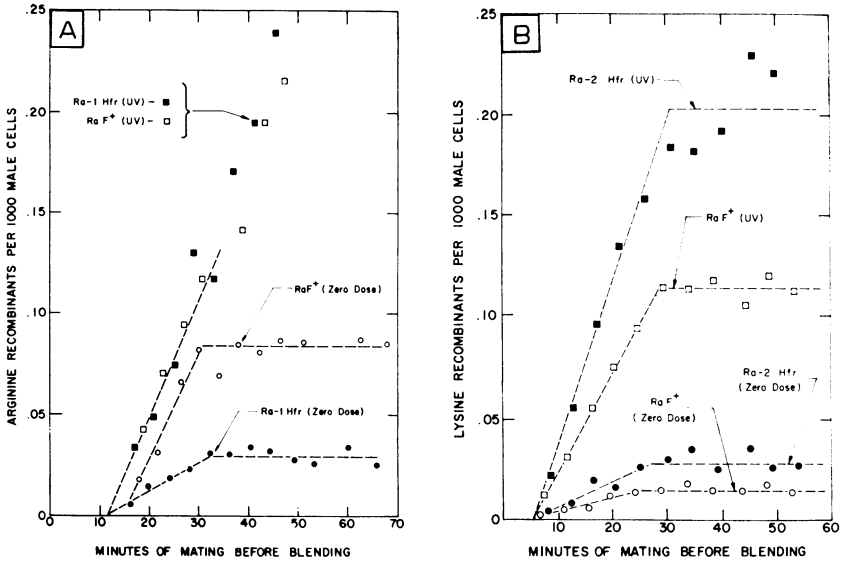

FIG. 3. (A) *Parallel interrupted-mating experiments showing transfer of the Ra-2 mode (arg near the origin) by Ra-1 and RaF+ cells, with and without a low dose of UV (40% survival) prior to mating with PA309. The recombination frequencies were all reduced to correct for growth of the females during the experiment, relative to the female titer at time 0. (B) Similar experiment showing transfer of the Ra-1 mode (lys near the origin) by Ra-2 and RaF+ cells mated with KL63.*

the Ra-2 mode. Figure 3A also shows that, even without preconjugal irradiation, the Ra-1 and RaF+ strains both transfer markers via the Ra-2 mode to some extent.

When stable Ra-2 Hfr cells were found to be present in irradiated Ra-1 cultures, interest was aroused as to what would happen to Ra-2 cells after preconjugal irradiation. Interrupted-mating experiments with irradiated Ra-2 cells were carried out, and it was found that the Ra-2 strain was induced to transfer markers via the Ra-1 mode in a manner analogous to the Ra-1 inversion to the Ra-2 mode. As with Ra-1, the Ra-2 strain was found to give rise spontaneously to RaF+ cells. These RaF+ cells have been indistinguishable from those arising from the Ra-1 strain. To demonstrate the role of Ra-2 Hfr cells in the inversion to the Ra-1 mode, parallel interrupted-mating experiments were carried out by use of a very pure Ra-2 culture and also an RaF+ culture, both with and without preconjugal irradiation. The *lys* marker was selected for assay of the inverse mode, since the Ra-2 cells do not normally transfer *lys* until about 72 min after the start of mating whereas *lys* has an entry time of about 5 min via the Ra-1 mode. Figure 3B shows typical results of this experiment. The Ra-2 culture was found to be more than 95% Hfr, i.e., less than 5% RaF+. Thus, the Ra-2 strain was induced by irradiation to transfer markers via the Ra-1 mode, to at least as great an extent as with RaF+ cells. Even with no irradiation, small numbers of Ra-2 and RaF+ cells transfer markers via the Ra-1 mode.

The Ra-1 and Ra-2 strains form an unusual system of Hfr types, wherein either one is induced to some extent by irradiation to transfer markers via the other mode. Some normal Ra-1 cells were found in irradiated Ra-2 cultures, just as Ra-2 cells were found in irradiated Ra-1 cultures.

Abnormalities in the Ra chromosome. Since the relationship between the sex factor and the chromosome is known to be important in determining the manner of chromosome transfer by donor cells, it was hoped that an investigation of the nature of the Ra sex factor would help to clarify the situation existing in the Ra system. Accordingly, the RaF+ strain was "cured" of its sex factor by acriflavine treatment (7), and the resulting F− strain was made F+ by infection with the F factor from a presumably normal F+ strain (HF+). The resulting hybrid F+ strain was found to have the same mating characteristics as the RaF+ strain (B. Low, Thesis, Univ. of Pennsylvania, Philadelphia, 1965). Furthermore, when the normal F+ strain (HF+) was treated with acriflavine to yield an F− derivative, followed by infection of this F− with the sex factor from the RaF+ strain, the resultant hybrid F+ strain was found to have the same mating properties as the normal F+ strain (HF+). (The

HF^+ strain gives recombination frequencies roughly one-tenth as high as the RaF^+ strain, and the ratios of recombination frequencies for different markers are somewhat different for the two strains.)

This whole series of experiments was repeated with a second independently obtained HF^+ revertant (from the Hayes Hfr strain) as the "normal" F^+ strain. The results of all the hybrid tests in this second series were equivalent to those obtained with the first HF^+ isolate. These experiments indicate that the RaF^+ strain (and thus also the Ra-1–Ra-2 system) carries a normal sex factor but possesses chromosomal irregularities which give rise to the mating behavior characteristic of the Ra system.

Kinetics of marker transfer by the RaF^+ strain; comparison with a normal F^+ strain. The interrupted-mating experiments indicated that some cells in an RaF^+ culture transfer markers via the Ra-1 mode and some transfer via the Ra-2 mode. To study these transfer kinetics in more detail, interrupted-mating experiments with RaF^+ cells were carried out with simultaneous assay of several different markers. Results of such an experiment are shown in Fig. 4A, which indicates the entry kinetics of the *arg*, *thr*, *his*, and *trp* markers. Straight dashed lines are drawn through the linear portions of the data, even though each marker was transferred to some extent earlier than would be expected from extrapolations of the straight lines. This early transfer of markers is seen most easily for the markers with the later extrapolates, i.e., *his* and *try*.

The straight-line extrapolates of Fig. 4A support the conclusion that RaF^+ cultures mate primarily according to two modes of transfer, the Ra-1 and Ra-2 modes. The *arg* and *thr* extrapolates of 7 and 16 min, respectively, indicate the presence of the Ra-2 mode, and the *his* extrapolate of 24 min indicates the presence of the Ra-1 mode. The *trp* extrapolate of 36 min is consistent with the Ra-1 mode but is presumably also contributed to by the Ra-2 mode. The observed predominance of the Ra-1 and Ra-2 modes in the RaF^+ mating cultures is in accord with the finding that all of more than a dozen Hfr strains which have been isolated from the RaF^+ strain (both with and without prior UV treatment) have been of either the Ra-1 or Ra-2 type. (We should add, however, that the origins of transfer active in an F^+ culture need not all necessarily correspond to suitable loci for the stable insertion of F to form Hfr cells.)

To complete the analysis of Fig. 4A, we need to consider the early transfer of markers entering before the straight-line extrapolates corresponding to the Ra-1 and Ra-2 modes. Evidently, other modes of transfer are active in the population of mating RaF^+ cells, even though the Ra-1 and Ra-2 modes predominate. To compare this observed RaF^+ behavior with that of a normal F^+ strain, interrupted-mating experiments with a normal F^+ strain (HF^+) were performed.

Figure 4B shows typical results of an inter-

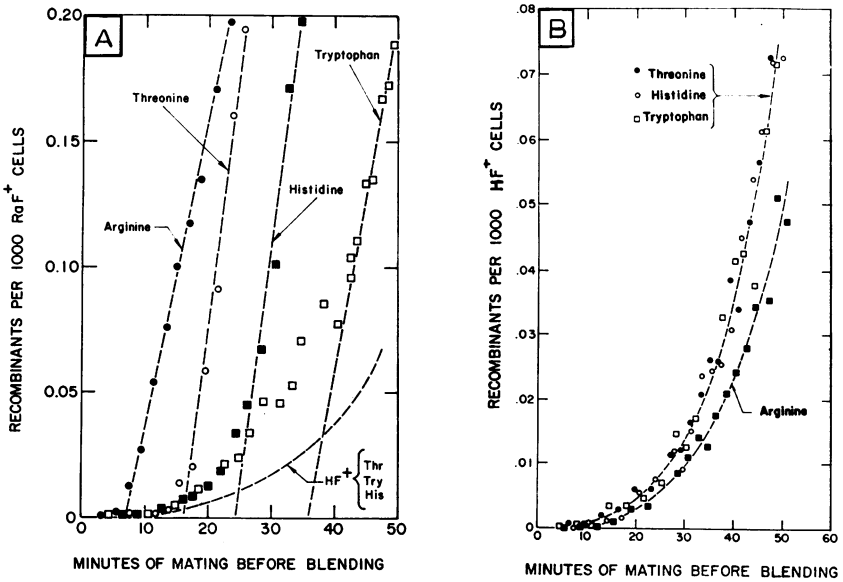

FIG. 4. (A) *Interrupted-mating experiment, RaF^+ cells × PA309. (B) Similar experiment, with the normal F^+ strain (HF^+) as the donor. The recombinant level from this experiment is also indicated in Fig. 4A.*

rupted-mating experiment with HF^+ as the donor strain. Each of the four markers assayed (*arg*, *thr*, *trp*, and *his*) began to be transferred less than 10 min after mixing male and female cells. If we take into account the lapse of (approximately) 5 min between mixing of cells and transfer of the earliest chromosomal marker by any transfer mode (10), we conclude that there are origins of transfer less than 5 min distant from each of the four markers shown in Fig. 4B. It should be noted that these four markers are widely separated on the chromosomal map. Furthermore, these blendor curves do not have linear portions characteristic of single modes of transfer; instead, the curves rapidly increase in slope as time progresses. This suggests that a given marker is transferred at different times as a result of the presence of many different transfer modes active in the population of HF^+ cells. Because of the nearly identical blendor curves exhibited by the four widely spaced markers, we are led to the conclusion that in these F^+ cells the sex factor associates itself with many more or less uniformly distributed loci on the chromosome, so as to result in conjugational transfer modes having many differently situated origins (within a large population of F^+ cells). If we estimate from these data that loci for chromosome mobilization for transfer in a F^+ strain are uniformly distributed around the map and are separated by less than 3 min (which is the approximate limit of resolution for Fig. 4B), we can therefore estimate that along the entire 90 min of chromosomal length there must be at least 30 (and perhaps vastly more) such loci.

We can now compare the behavior of the RaF^+ strain with that of the normal F^+ strain, HF^+. The magnitude of the blendor curves for the HF^+ strain (Fig. 4B) has been shown on Fig. 4A for comparison with the RaF^+ results. We see here that the "normal" HF^+ marker transfer kinetics give rise to blendor curves with the same shape and order of magnitude as does the early transfer observed with the RaF^+ strain, e.g., the early entries of the *trp* and *his* markers prior to the extrapolated times corresponding to the major (Ra-1 and Ra-2) modes of transfer. We can therefore surmise that, although the sex factor in the RaF^+ strain associates itself most often with one of the two primary sites on the chromosome (i.e., the locations of the Ra-1 and Ra-2 origins), it also associates itself with other chromosomal loci, in a fashion similar to that which occurs in a "normal" F^+ strain (HF^+).

Chromosome transfer in (Ra)F^+ cultures: estimation of the contribution due to stable Hfr cells. It became apparent from the results discussed above that matings with the RaF^+ strain involve chromosomal transfer predominantly according to two modes, corresponding to Ra-1 and Ra-2. This situation made possible an investigation of the question: do stable Ra-1 and Ra-2 donors exist in an RaF^+ culture at the level necessary to account for the numbers of recombinants obtained from $RaF^+ \times F^-$ matings? To evaluate the relative contributions of Ra-1 and Ra-2 modes to RaF^+ matings, parallel crosses were performed as described below.

Freshly isolated cultures of Ra-1 and Ra-2 cells were grown for mating, concurrently with a culture of RaF^+ cells. The three male types were mated in parallel experiments with identical portions of the female strain, PA309. In each case, *arg*, *thr*, *trp*, and *his* recombinants were assayed. The resulting recombination frequencies are plotted in Fig. 5 as a function of the marker entry times as measured for the Ra-1 mode. With both the Ra-1 and Ra-2 Hfr gradients now available, as well as the RaF^+ recombination level, it was possible to find the effective fractions of Ra-1 and Ra-2 donors present in the F^+ mating culture. This was done by multiplying the Ra-1 and Ra-2 gradients by trial-and-error multipliers such that, by adding a certain amount of Ra-1 to a certain amount of Ra-2, the sum would be at the level given by the RaF^+ recombination curve. The best fit to the RaF^+ curve was obtained by adding approximately 1.0% of the Ra-1 recombination level to 0.9% of the Ra-2 level (Fig. 5).

From these results we can see that the desired check of the presumed amount of Ra-1 and Ra-2 Hfr cells in the F^+ culture amounts to a search for about 1 Hfr cell per 50 cells in an RaF^+ culture, i.e., 1% Ra-1 and 1% Ra-2. This kind of search is well within practical limits of feasibility with the use of the replica-plating technique for testing fertility (1). Accordingly, RaF^+ cells were grown to exponential phase and plated onto nutrient agar plates. The colonies which appeared were tested for the Hfr state by the replica-plating method. (The replica-plating method was also checked for efficiency of detection of Hfr clones by doing the same test on clones which were known to be Hfr, i.e., clones obtained from a pure culture of Hfr cells. The efficiency of Hfr detection was better than 90%.)

The result of this test of RaF^+ clones is surprising. Of a total of 1,776 colonies tested (comprising four separate experiments), only 3 were Hfr (one of the Ra-1 type and two of the Ra-2 type). This is in marked contrast to the numbers which would have been present (about 18 Ra-1 type and about 16 Ra-2 type) if the RaF^+ donor potential were due entirely to Hfr cells in the RaF^+ culture, as calculated from the results in Fig. 5. A direct comparison of the number of

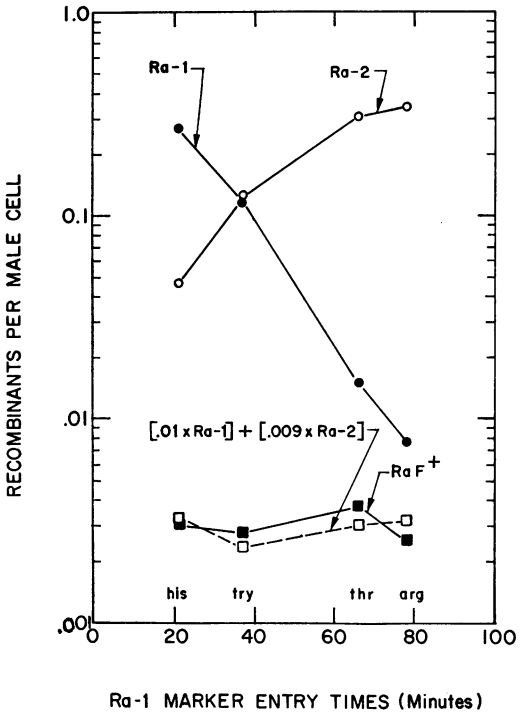

FIG. 5. *Recombination frequencies from standard matings of Ra-1, Ra-2, and RaF+ cells with the PA309 recipient strain.* (□) *Values calculated by combining fractions of the Ra-1 and Ra-2 values as indicated.*

stable Hfr cells with the number of effective donors in an RaF+ population thus reveals that only about 10% (3 of 34) of the donor ability of this F+ strain is due to the presence of stable Hfr cells which spontaneously arise in the F+ culture.

DISCUSSION

The basic nature of the RaF+–Ra-1–Ra-2 system of strains is indicated by four of the results discussed above: (i) the RaF+ strain transfers chromosomal markers with a much higher frequency than does a normal F+ strain; (ii) this relatively high frequency of chromosomal transfer occurs primarily according to two specific transfer modes, Ra-1 and Ra-2, which are about equal in strength; (iii) the RaF+ strain sex factor is normal and the Ra chromosome carries irregularities which result in the high frequency of transfer according to the Ra-1 and Ra-2 modes; and (iv) relatively pure cultures of the Ra-1 or Ra-2 Hfr types can be isolated. [This is to be contrasted with a double-male strain, wherein each cell has has approximately the same potential for transfer of either of the two transfer modes which characterize the strain (3).]

These four characteristics are just those observed for *sfa* strains, except that in the usual *sfa* strain there is only one chromosomal site for preferential F attachment instead of two (1, 13). It therefore seems appropriate to depict the RaF+–Ra-1–Ra-2 system as a *double sex factor affinity system.* In this system, there are presumably small pieces of DNA present in the chromosome at the the Ra-1 and Ra-2 origins which are homologous with sex factor DNA and which therefore lead to preferential attachment of F at these sites, followed by chromosomal transfer during conjugation. This conclusion is given support by the following finding: when *arg* recombinants were obtained from an Ra-1 × PA309 mating and were made into F+donors by infection with RaF+ sex factors, most of these donors in subsequent matings showed a gradient of recombination frequencies which decreased from *arg* to *his.* This suggests that the *sfa* near *arg* was incorporated into the original *arg* recombinants, whereas the *sfa* at the Ra-1 origin was not. This is reasonable, since a marker at the origin is expected to have a low probability of integration into recombinants (10). Similarly, *his* recombinants from Ra-2 × PA309 matings were sometimes found to have inherited the *sfa* at the Ra-1 origin but not the one at the Ra-2 origin.

It should perhaps be mentioned that the term *sfa* as defined earlier and as used here may tend to oversimplify the picture of the RaF+ system as compared to other *sfa* systems. It is not known whether the Ra-1 and Ra-2 *sfa* loci arose according to the simple exchange scheme mentioned before (2) or whether they evolved via some more complicated process. Also, owing to the limited number of markers used in the present study, there remains the possibility that other (perhaps much "weaker") *sfa* loci exist on the Ra chromosome, for example in the *xyl-ser* region.

Because of the relatively high RaF+ donor ability and the predominance of two particular transfer modes (Ra-1 and Ra-2) in a mating involving RaF+ cells, it became possible to establish the extent to which spontaneous stable Hfr cells contribute to this F+ mating efficiency. The result indicated that only about 10% of the RaF+ donor ability is due to the presence of Hfr cells; the majority of the RaF+ fertility results from some association of the F factor with the Ra-1 and Ra-2 origin loci in a way which is not stably manifested in the F+ culture. (One can imagine, for example, that the presence of female cells, making contact with the F+ cells in a mating mixture, might trigger some more stable chromosomal attachment of F which does not exist in the F+ culture alone.) We see from these results that both stable and unstable associations of the F factor can occur with the same chromosomal

locus (two loci, in the case of the RaF$^+$ strain) in a culture of F$^+$ cells.

Let us next summarize the situation regarding the "inversion" of transfer modes observed in the Ra-1 and Ra-2 strains. When a pure culture of Ra-1 Hfr cells is mated with females (Fig. 3A), some of the Hfr cells do not transfer the chromosome with the Ra-1 origin but instead transfer markers according to the Ra-2 mode, which proceeds in the opposite chromosomal direction from the Ra-1 mode and which starts with an origin located far from the Ra-1 origin on the genetic map. This inversion of modes was found to be greatly enhanced by preconjugal UV treatment of the Ra-1 cells. The analogous situation was found to hold for Ra-2; in a culture of conjugating Ra-2 Hfr cells, some of the Ra-2 cells transfer DNA according to the Ra-1 mode (Fig. 3B). Here again, a low UV dose greatly enhances the inversion of modes. In both Ra-1 and Ra-2, the amount of the "opposite" mode present in a given Hfr culture is of about the same magnitude as the amount of that mode present in the RaF$^+$ strain (Fig. 3A and 3B). This indicates that the Ra-1 Hfr state can go to a (not necessarily stable) Ra-2 donor state without first existing in the RaF$^+$ state (and similarly for Ra-2 going to an Ra-1 donor state), for, if the small fraction of Ra-1 cells involved in inversion did first go to the RaF$^+$ state, an even smaller fraction would be observed to transfer in the inverse mode. [Recall that only about 1% of RaF$^+$ cells can mate at all via either mode (Fig. 5).]

The implications of the RaF$^+$–Ra-1–Ra-2 results are summarized in the schematic relationships shown in Fig. 6. The species of Ra-1 and Ra-2 shown in brackets are intended to represent states which result in transfer of a particular mode but which are not stable Hfr states.

A further conclusion can be drawn from these results with regard to the effect of UV on the mating system in *E. coli*. It is well known that UV stimulates the production of Hfr cells from F$^+$ cells. In view of the relationships discussed regarding the F$^+$ and Hfr states, we can say that UV thus stimulates the *attachment* of the sex factor to the chromosome, leading to transfer of chromosomal material into recipient cells. One of the implications of the Ra-1–Ra-2 inversion study is that UV can also stimulate the *detachment* of F from its chromosomal Hfr locus (with subsequent reassociation with the other *sfa* locus, resulting in the inverse transfer mode). This conclusion depends on the assumption that there is only one F factor per chromosome in the Ra-1 and Ra-2 Hfr states; for inversion of modes to occur, F must physically move from one Hfr locus to the other. This assumption seems reasonable since, when arginine recombinants from Ra-1 matings are checked for mating ability, most of them are females. (Since *arg* is very closely linked to the Ra-2 Hfr locus, the presence of an F factor at that locus would result in mostly male *arg* recombinants.) The analogous test of recombinants from Ra-2 matings has also been carried out (by use of the lysine marker, which is very closely linked to the Ra-1 Hfr locus). The results again indicated the absence of an extra sex factor.

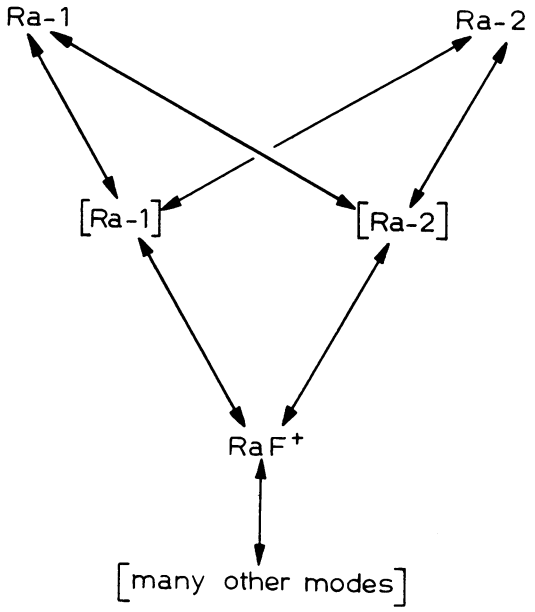

FIG. 6. *Suggested relationships among donor states in the Ra-1–Ra-2–RaF$^+$ system. The brackets are intended to indicate transient donor states.*

It should also be mentioned that the UV stimulation of an inverse transfer mode, described above, may be associated with the stimulation of DNA synthesis at a chromosomal site which is different from the usual origin of DNA synthesis. This possibility arises from evidence recently obtained by Gross and Caro (5) that one strand of the DNA transferred from male to female during conjugation is synthesized at the time of transfer. UV stimulation of a new transfer mode in the Ra-1–Ra-2 system may thus imply that DNA synthesis is initiated at the new transfer origin. Even in nonmating *E. coli* cells, UV irradiation stimulates relocations of the site of DNA synthesis on the chromosome (6). The results presented herein suggest specifically that, after UV irradiation in the Ra-1–Ra-2 system, DNA synthesis associated with transfer can begin with a new *direction*, as well as a new origin.

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