Role for the Female in Bacterial Conjugation in

Escherichia coli

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Hfr and F′ Lac male strains of Escherichia coli were mated with purine-requiring females which had been starved for purine. These females formed mating pairs with the males. However, a mating in the absence of purine markedly reduced the yield of recombinants. Transfer of F′ Lac or of λ prophage also occurred infrequently. It was concluded that deoxyribonucleic acid transfer from male to female requires some, as yet unknown, function of the female.

When the male cells of Escherichia coli come into contact with female cells, deoxyribonucleic acid (DNA) is transferred from the male donor to the female recipient. The mechanism by which this transfer occurs is not known, although it is well established that it is an energy-requiring process (3, 4) and that DNA synthesis is somehow involved (7). Some years ago, Fisher (3, 4) reported that starvation for a carbon source prior to mating markedly reduced the number of recombinants formed if it was the male strain that was starved, but had little effect if the female was starved. From this it was concluded that an energy supply to the male is more critical than to the female. In the ensuing years this conclusion was continually restated until it became common to consider the female as a strictly passive partner which might be ignored in any consideration of the mechanism of transfer. As a result, the principal effort in the study of the transfer mechanism has gone into detail studies of the precise role of the male. Only recently has the lore of the field been questioned by two sets of experiments which suggested that the female may play an active role (1, 2).

One of these experiments was performed in an attempt to test the theory of transfer proposed by Jacob, Brenner, and Cuzin (19). In this theory, it was proposed that (i) under the influence of a DNA replication system controlled by the sex factor, F, the transferred DNA is synthesized in the male during mating and (ii) the only DNA which is transferred is this newly replicated DNA. The female was assumed to be passive insofar as DNA replication is concerned, although whether the female might carry out some other essential function was not discussed. In the past 2 years, many experiments have been described which strongly support but in no way prove this theory.

The experiments of Bonhoeffer (1), however, strongly contradict this theory. Bonhoeffer isolated two mutant strains, one Hfr male and one female, which were incapable of DNA synthesis at 42 C. These strains were separately mated with normal males and females at temperatures which either permitted or prevented DNA synthesis in the temperature-sensitive strain. When DNA synthesis was prevented in the male, normal recombination frequencies were observed; however, when prevented in the female, no recombinants were found. Thus, he concluded that DNA synthesis is required only in the female and not in the male. One possible defect in these experiments is that DNA synthesis was not measured during the mating, which is an important control since the temperature-sensitive system may not be the replication system involved in mating. Secondly, recombinant formation and not DNA transfer was actually measured.

A second set of experiments implicating the female are those of Curtis and Charmella (2).

These two sets of experiments have prompted a reinvestigation of the role of the female. In this paper, experiments are described which demonstrated that the female is indeed an active partner in the DNA transfer process. Several possible roles for the female will be eliminated. However, these experiments do not give any positive information about the female-controlled process.

Materials and Methods

Abbreviations. Thy−, requiring thymine (2 μg/ml); Pro−, requiring proline; StrS and StrR, susceptible and resistant to streptomycin (500 μg/ml), respectively; ΔS, susceptible to phage λ; λimm434, phage λ containing immunity locus of phage 434; Pur−, requiring a purine; Lac+ and Lac−, ability or inability, respectively, to utilize lactose as a sole carbon source;
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(F' lac), possessing an F' sex factor containing the lactose operon; pro, thr, thi, genes determining synthesis of proline, threonine, and thiamine, respectively; lac, gene controlling ability to utilize lactose.

**Strains employed.** The strains used in genetic crosses have the following properties. DF47: Hfr, Thy-, StrS; direction of injection, origin, pro, thr, . . . lac, F. DF87: F', (F' Lac), Thi-, Pro-, StrS. DF105: Hfr, Lac+, Thy-, StrS, lysogenic for ximm434; direction of injection, origin, thr, pro, . . . thi, F. DF107: F', Lac-, Pro-, Thy-, Pur-, XS, StrR. (The purine locus is located roughly between the histidine and streptomycin loci.)

The strain (DF3) used as an indicator for λ was resistant to streptomycin (500 µg/ml) and harbored F' Lac.

**Medium.** The medium consisted of 0.08 m NaCl, 0.02 m KCl, 0.02 m NH₄Cl, 0.001 m MgCl₂, 0.0002 m CaCl₂, 0.02 m Na₂SO₄, 0.001 m KH₂PO₄, 0.12 m tris(hydroxymethyl)aminomethane (pH 7.5), and 0.2% glucose. It was supplemented when necessary with thymine, 5 µg/ml; thiamine, 5 µg/ml; Casamino Acids, 0.1%; L-proline, 20 µg/ml; hypoxanthine (for the purine requirement), 20 µg/ml; and streptomycin, 500 µg/ml.

To detect recombinants synthesizing proline and resistant to streptomycin, the medium was solidified with 1.2% agar.

For determination of viable counts, Difco Nutrient Agar was used. Plates for assay of λ and λ infective centers consisted of 1% Difco tryptone, 0.5% NaCl, and 1.2% agar; wells were made in agar consisting of 0.8% Difco Nutrient Broth, 0.5% NaCl, 500 µg of streptomycin per ml, and 0.8% agar.

Ability to ferment lactose was assayed on Difco EMB Agar, supplemented with thiamine, thymine, and, when appropriate, streptomycin (200 µg/ml).

All growth was at 37 C.

**Mating.** Crosses were performed as follows. Male and female cells were mixed in appropriate concentrations to make 6 ml in a 125-ml Erlenmeyer flask and were swirled at speed 3 on a New Brunswick Gyrotary Shaker. Interruption of mating was accomplished by shaking a 10-ml sample of a 100-fold dilution on a Vortex Junior mixer (Scientific Industries, New York, N.Y.) for 1 min.

**RESULTS**

The basic program pursued in the present investigation was to determine whether the ability of the female to serve as a recipient of transferred DNA depends upon metabolism or macromolecular synthesis of some type. The approach was to use a female which, during mating, would be starved of a required purine, in this case, hypoxanthine, in order that synthesis of protein, ribonucleic acid (RNA), and DNA, as well as any metabolic function involving adenosine triphosphate (ATP), be substantially reduced. Because of the large pools of purine-containing compounds present in E. coli, it was actually necessary to prestarve the cells. The extent to which these pools can be eliminated in this way is shown in Fig. 1, in which the effect of hypoxanthine starvation (in the presence of amino acids) on DNA synthesis, as determined by the incorporation of 14C-thymine, is demonstrated. Under these conditions, DNA synthesis stopped within 10 min. When required amino acids were absent, thymine incorporation continued slowly, presumably because adenosine monophosphate (AMP) and guanine monophosphate (GMP) were slowly added to the pools by degradation of RNA without resynthesis.

In all of the following mating experiments, the female, DF107, was starved of hypoxanthine, in the presence of both thymine and Casamino Acids, for 30 min prior to mating.

**Appearance of recombinants in hypoxanthine-starved females.** A culture of DF107 was starved of hypoxanthine for 30 min as described above. At the end of this period, the Hfr strain, DF47, was added, and the cell density was adjusted to 5 × 10⁷/ml (male) and 2 × 10⁷/ml (female). The culture was immediately divided in half; hypoxanthine was added to one half, and both portions were gently shaken. At various times, samples were taken, vortexed, and plated on agar plates selective for Pro+StrR recombinants. The results of this experiment are shown in Fig. 2, in which it is clear that the number of recombinants occurring in the mating without hypo-
xanthine was considerably less than that found in the presence of hypoxanthine. Hence, the female appears to play some role in the mating process, although it is not clear whether the interference caused by hypoxanthine starvation is at the level of recombination, DNA transfer, or, trivially, pair formation.

**Zygotic induction in hypoxanthine-starved cells.** To determine whether the decrease in the number of recombinants in hypoxanthine-starved cells is a result of a reduction in integration efficiency (i.e., recombinant formation), a system in which DNA transfer can be assayed without the necessity of recombination is needed. Hence, the effect of the starvation on prophage transfer or zygotic induction was studied. The Hfr strain DF105, lysogenic for λimm434, was mated with the prestarved female, DF107, in the presence and absence of hypoxanthine, as above. Samples were taken at various times, vortexed, and plated on the streptomycin-resistant male indicator, DF3, on streptomycin-containing plates. (A male indicator was used to prevent the Hfr cells from mating with the indicator.) The results of such an experiment are shown in Fig. 3. The maximal amount of transfer varied from one experiment to another (up to 80%), but the ratio of plaques found in the presence and absence of hypoxanthine remained essentially constant. It is clear that the appearance of infected centers is strongly inhibited by the hypoxanthine starvation, so that the effect described above for Pro+StrR recombinants is unlikely to be a result of reduced integration efficiency.

In several zygotic induction experiments, Pro+StrR recombinants were simultaneously selected. The result was similar to that in Fig. 2.

**Effect on pair formation.** To test the effect of hypoxanthine starvation on pair formation, the technique of Wollman and Jacob (10) was used. Males (DF105) and females were mixed under mating conditions. At various times, samples were taken and gently diluted 1,000-fold into warm broth which had been diluted to one-tenth the normal concentration. At 60 min after initiation of mating, the samples were plated for infective centers. In this period of time, all males which formed pairs should have transferred the prophage. The result of such an experiment is shown in Fig. 5. By this assay, 70 to 80% of the males formed pairs with unstarved females and transferred λ in 40 min.

Measuring pair formation with the hypoxanthine-starved cells is more complicated than the above for the following reason. In the assay just described, those cells which formed pairs before dilution resulted in the production of infectious
centers only if the prophage was transferred before spontaneous breakage of the pair occurred. However, little chromosome transfer occurred in the absence of hypoxanthine, and \( \lambda \) prophage required at least 25 min (Fig. 3) to be transferred by DF105. Thus, the duration of the mating time for transfer of \( \lambda \) was less in a mating with hypoxanthine-starved cells than with unstarved cells, whereas the time for spontaneous separation of the pairs was the same. Hence, when the extent to which pairs are formed with hypoxanthine-starved cells is assayed in this way, the measured value must be taken as a minimum. The result of the assay is also shown in Fig. 5, and, as expected, pair formation appears to be decreased. However, it is clear from these results that even if this decrease in pair formation were real (maximally, a factor of 2), it could not account for the much larger decrease in the number of zygotes formed (ca. 30-fold) seen in Fig 3.

In several experiments the effect of hypoxanthine starvation on both pair formation and the appearance of zygotic induction was measured simultaneously by mating males with prestarved females for 50 min and plating the infectious centers before and after vortexing. The results of these experiments are shown in Table 1, in which it is shown that pair formation was nearly normal in the absence of hypoxanthine and that transfer occurred inefficiently in the absence of hypoxanthine, even between cells which had paired. These results again demonstrate that pair formation plays little or no role in the observed decrease in zygotic induction.

A similar study was carried out for \( F' \) Lac transfer. In this case, the assay of pair formation was nearly equally sensitive for mating in the presence and absence of hypoxanthine, since the mating period was shorter (30 min) and it was possible to plate the mating mixture under nonselective conditions (i.e., without streptomycin). The experiment was done as follows. Males (DF87) were mated with hypoxanthine-starved females for 30 min, in the presence and absence of hypoxanthine. Samples were taken and either (i) vortexed and plated on an indicator plate containing streptomycin or (ii) not vortexed and plated on a Nutrient Agar plate without streptomycin. In the second case, pairs in which \( F' \) Lac had not yet transferred could complete transfer on the plate. Furthermore, if a pair separated spontaneously on the plate before \( F' \) Lac had been transferred, the progeny of both male and female cells would mate on the plate with high

Fig. 4. Transfer of \( F' \) Lac in a mating between a \( F' \) Lac male, DF87, and a hypoxanthine-starved female, DF107, in the presence (solid circles) and absence (open circles) of hypoxanthine, as a function of time after mixing. See text for details.

Fig. 5. Pair formation between the lysogenic male, DF105, and female, DF107. In the + hypoxanthine case (open circles), the females were not prestarved for hypoxanthine, and the mating took place in the absence of hypoxanthine. In the - hypoxanthine case, the females were prestarved of hypoxanthine, and the mating took place in the absence of hypoxanthine. The time axis represents the time at which the mating mixture was diluted 1,000-fold into 0.08% Difco Nutrient Broth. All platings at \( t = 60 \) min, at which time samples were plated for infectious centers. See text for details.
probability. (To avoid spurious mating of individual cells on the plates, the maximal number of colonies per plate was kept below 50.) After 16 hr of incubation, all Lac+ colonies were picked and streaked on Lac indicator plates containing streptomycin to avoid counting colonies from the unmutated StrS males. The results of such an experiment are shown in Table 2. In the nonvortexed samples (pairing assay), the number of Lac + StrR recombinants was independent of the presence of hypoxanthine, whereas the vortexed samples (transfer assay) showed the decrease seen before in the absence of hypoxanthine. Hence, again the decrease in F-ductants in hypoxanthine-starved cells cannot be due to a decrease in pair formation.

Presence of lysogens after mating. In studies of genetic transfer, zygotic induction is preferred to recombinant formation in order to avoid considering complications resulting from varying efficiencies of integration. However, even with such an experiment, it is possible to have prophage transfer without apparent expression (although this is rare), in that the zygote could either become lysogenic or be killed. Therefore, an apparent decrease in zygotic induction might result from an increase in the number of lysogens or from death of potential infective centers.

This possibility becomes more important in the present case, since Goldthwait and Jacob (6) showed that some purine-like compound is involved in lysogenic induction. To test this, the following experiment was done. DF105 at 2 x 10^6 and DF107 (prestarved for 30 min) at 1.3 x 10^6 were mated in the presence and absence of hypoxanthine. The high ratio of males to females was used to ensure that nearly all females were participating. After 60 min, both cultures were vortexed and plated both for infectious centers and for colonies. A colony could result only from a female which had either not received a prophage or had become lysogenic. Several hundred colonies were picked and tested for lysogeny. The results were the following. In the cultures with and without hypoxanthine, the percentage of the females yielding infected centers was 70 and 2.3%, respectively. The number of females converted to lysogens was less than 0.4% in each case, showing that the decrease in infected centers is not compensated for by the appearance of lysogens. The total female cell count was also compared for zero time and 60 min after mating for the hypoxanthineless case. No decrease in cell number was seen, indicating that prophage transfer followed by cell death did not occur.

Table 2. Effect of hypoxanthine starvation of the female on the transfer of F' Lac between paired cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Prophage transfer (no. of plaques per 100 Hfr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. +H +h −Vortex</td>
<td>77</td>
</tr>
<tr>
<td>B. −H +h +Vortex</td>
<td>40*</td>
</tr>
<tr>
<td>C. +H +h +Vortex</td>
<td>70</td>
</tr>
<tr>
<td>D. −H −h +Vortex</td>
<td>3</td>
</tr>
</tbody>
</table>

* The females, DF107, were starved for 30 min before addition of the DF87 males. The males were added, and the culture was divided into two portions. One was adjusted to 20 μg of hypoxanthine per ml (+H); to the other, nothing (−H) was added. After 30 min of mating, the cultures either were vortexed and plated on indicator agar selecting against the males or were not vortexed and were plated on Nutrient Agar. The colonies which grew on the Nutrient Agar Plates were picked and tested for the Lac+ character on streptomycin-Lac indicator plates.

b C/A = 115% of pairs yielded Lac+StrR colonies in +H case. D/B = 2.3% of pairs yielded Lac + StrR colonies in −H case.

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Ability of hypoxanthine-starved cells to support growth of λ. It is possible that males transfer the prophage to the hypoxanthine-starved female,
but for some reason these bacteria can neither support the growth of λ nor be lysogenized. To test this, the following experiment was done. The female, DF107, was starved for hypoxanthine for 30 min. The culture was then divided into two portions. To one was added 5 µg of hypoxanthine per ml; to the other, nothing was added. Each was incubated for 25 min, after which phage was added at a multiplicity of 0.2; the cultures were then further incubated for 35 min. This procedure simulates a 60-min mating with a 25-min time of entry.

At the end of the final incubation, each culture was divided into two portions. To one was added λ antiserum (kindly provided by Lawrence Levine). After a 5-min incubation, this was assayed for infectious centers and for lysogenic bacteria. The other fraction was shaken with a few drops of a 1:1 mixture of xylene-chloroform to inactivate infectious centers and was then assayed for free phage. In the case of the culture incubated with hypoxanthine, the absorbed phages were distributed as follows: 39% as infectious centers, 60% as lysogens, and 1% unaccounted for. For the culture without hypoxanthine: 29% appeared as infectious centers; 50% as lysogens; and 21%, unaccounted for.

Since 79% of the phages absorbing to hypoxanthine-starved cells can function, it is clear that a reduced ability of these cells to support the growth of phage cannot account for the reduction in infected centers produced by zygotic induction. Once again, we are led to believe that DNA transfer is reduced to hypoxanthine-starved females.

DISCUSSION

When female cells are starved of a required purine prior to mating, their ability to serve as a recipient of genetic material, whether it be episomal DNA, chromosomal DNA which need not be integrated (i.e., prophage), or chromosomal DNA which is normally found in recombinants is substantially reduced. Since pair formation has been shown to occur normally or at least near normally, one must conclude either that DNA is not transferred or that DNA is transferred but not expressed. The latter is unlikely, since expression of λ development is not inhibited by hypoxanthine starvation—that is, although the cells are not converted to infectious centers by mating, they do become infectious centers if exposed to free λ phage. A more powerful argument is that if DNA were transferred but destroyed in the female, this would show itself as a large decrease in pair formation; i.e., if DNA were transferred and destroyed during the period of mating without hypoxanthine, then when mating pairs were plated without pair separation they would not give rise to infectious centers or colonies of Lac+StrR recombinants, as was seen in Fig. 4. Therefore, it is probable that DNA is not transferred to a purine-starved female. Hence, the evidence points to a positive role of the female in the actual DNA transfer process.

Since hypoxanthine starvation inhibits synthesis of DNA, RNA, and protein, and probably all metabolic processes involving ATP, the precise role of the female is not indicated by any of these experiments. Furthermore, it is not possible to state whether all females are active partners since hypoxanthine starvation does not reduce transfer to zero but only inhibits it about 20- to 25-fold. We assume, though, that this residual transfer is a result of a continual supply of purine compounds provided internally by RNA turnover.

Recently, a role has been ascribed to the female by Bonhoeffer (1), who has argued that DNA synthesis is necessary in the female, and by Curtis and Charamella (2), who have proposed that the female pulls the DNA in. The present experiments are not inconsistent with their ideas, but give no further information.

These experiments raise an important question about the results of Gross and Caro (8), whose work has provided the most powerful evidence in favor of the hypothesis of Jacob, Brenner, and Cuzin (9) that only DNA newly replicated in the male is transferred and that this is synthesized concomitantly with transfer. In the experiments of Gross and Caro, the extent to which radioactively labeled DNA is transferred to nonradioactive females is determined autoradiographically by a grain count over the females. Clearly, it is important that the female itself does not synthesize DNA in these experiments. To avoid this, adenine-requiring or UV-killed females were used. In view of the present experiments, one might explain the work of the Gross and Caro by assuming that, whenever a female contained transferred radioactive DNA from the male, that female must also not have been totally starved. Thus, some of the grains in the females might have represented DNA synthesis in the female.

However, Gross and Caro carried out controls which suggested that, in the absence of adenine, mating with the adenine-requiring female was normal and DNA synthesis in the female was prevented. This difference between their results and the present ones might be due to strain differences (they used E. coli strain C whereas a derivative of K-12 was used in the present work). Alternatively, different genetic blocks in the purine
pathway might have different effects on transfer. A third possibility is that, in their experiments, the cells were not really starved of adenine and that the apparent reduction in DNA synthesis was a result of enlargement of endogen pools and dilution of the label. This latter point seems unlikely. At any rate, since the interpretation of the Gross-Caro experiments is based upon careful quantitative considerations, they should probably be re-evaluated.

One year ago an experiment asking the same questions as the Gross and Caro experiment was reported (5), in which F' Lac males were mated with hypoxanthine-starved females in the presence of 5-bromouracil. Hypoxanthine starvation was used to prevent the transferred F' Lac from replicating with the female, for such replication would make the experiments ambiguous. Without giving the details of the experiment, let it suffice to say that the experiment was used to show that fewer than 1% of the F' Lac particles were transferred without replication. In these experiments, the transfer frequencies were quite low, and it was assumed that this was a result of the 5-bromouracil. In the light of the present experiments, this work suffers from the same criticism applied to that of Gross and Caro and must be re-evaluated. Nonetheless, work from several other laboratories (11) suggests that the conclusions are probably valid.

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