STRAND-SPECIFIC TRANSFER OF DONOR DNA DURING CONJUGATION IN *E. COLI**

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Abstract.—After conjugation of *E. coli*, only the preformed donor DNA strand with a leading 5' end can be recovered from the recipient. DNA that was transferred to recipients from radioactive-labeled donor strains lysogenic for λ bacteriophage was recovered as radioactive material in mature phage particles. The radioactivity was recovered in strand *l* when gene transfer was in the order gal- λ -bio, and in strand *r* when the order of transfer was bio- λ -gal. The assignment of the 5' end as the origin of the transferred strand follows directly from the known orientation of prophage λ in the chromosome.

Mating between bacteria involves the transfer of DNA from donor to recipient bacteria. The mechanism of this transfer has aroused considerable interest, especially since DNA synthesis has been shown to be somehow involved in this process. Thus, Jacob, Brenner, and Cuzin¹ and Ptashne² have found that if the DNA in the donor was labeled in both strands with heavy isotopes prior to mating, the transferred DNA isolated from the recipient was hybrid DNA, composed of one heavy and one light strand. Gross and Caro³ also have shown by autoradiography that the transferred donor DNA consists of equal amounts of DNA existing before the mating and DNA synthesized during the mating.

In this report we wish to consider whether both the complementary DNA strands existing before mating can be transferred or whether only a particular one of the two strands is transferred to the recipients.

Materials and Methods.—(a) *Media:* Z medium contains Tris (pH 7.4), 0.1 M; caseamino acids, 0.125%; ammonium chloride, 0.1%; NaCl, 0.01 M; MgSO₄, 2 mM; CaCl₂, 0.2 mM; KH₂PO₄, 0.32 mM; and glucose, 0.5%. We used two L-broths⁴—one supplemented with 0.01 M phosphate (pH 7.4) and one supplemented with 100 μ g/ml thymidine—and a suspending buffer for λ containing 1 mM MgSO₄, 100 mM NaCl, 0.1% gelatin, and 10 mM Tris adjusted to pH 7.4 with HCl.

(b) *Organisms:* The bacterial strains and their relevant characteristics are presented in Table 1. In this paper, λ always refers to λ ind.⁻⁵ Other λ derivatives used are the thermoinducible λ ind ts857⁶ and λ vir.⁷

The F'Gal used in these studies is the one described by Jacob and Wollman⁸ that contains the attachment site for bacteriophage λ . It was derived from a Cavalli-like Hfr and thus presumably injects genes in the order bio-att λ -gal. To confirm this assumption, we performed an interrupted mating which was scored for the transfer of chromosomal markers that is promoted by interaction with this F'. The media and methods were based on those of Adelberg and Burns.⁹ The results in Figure 1 show that the transfer of chromosomal markers is in the order pro-thr-his, as expected if the F'Gal transfers in the order bio- λ -gal.

(c) *Preparation of uniformly labeled λ :* Strain GI1000, lysogenic for the thermoinducible λ ind-ts857, was grown to $2-4 \times 10^8$ cells/ml at 30°C. The culture was then placed in a 42° water bath and incubated with aeration until lysis occurred. The phages were purified as described below in (d). For labeling phages with H³, Z medium was supplemented with threonine, leucine, thiamine, 0.01 M phosphate, and thymidine (3 and 5

FIG. 1.—Time of entry of chromosomal markers transferred from F'-Gal (λ) GI57 to GI157. The formations after interrupted mating of Pro⁺Sm^R, Thr⁺Sm^R, and His⁺Sm^R recombinants were scored on the appropriate minimal plates, using methods and media described by Adelberg and Burns.⁹

$\mu\text{g/ml}$). P³²-labeled phages were prepared in Z medium supplemented with threonine, leucine, thiamine, thymidine (20 $\mu\text{g/ml}$), and mM phosphate (25 $\mu\text{g/ml}$).

(d) *Mating procedure and purification of zygotically induced phages:* The donor strain was grown to $1-2 \times 10^8$ cells/ml in 50 ml of Z medium containing P³²-phosphate (20 $\mu\text{g/ml}$) or H³-thymidine (10 $\mu\text{g/ml}$). The labeled cells were washed three times with either phosphate-supplemented L-broth for P³²-labeled cells or thymidine-supplemented L-broth for H³-thymidine-labeled cells, resuspended in 50 ml of the respective L-broth for 25 min, and shaken at 37° before mating. The recipient strain was grown in 50 ml L-broth supplemented with either 0.01 M phosphate or 100 $\mu\text{g/ml}$ thymidine to about 2×10^8 cells/ml. The donors and recipients were then mixed in a 2-liter flask to incubate without shaking for 30 min (60 min for the HfrH). At this time, 300 ml of supplemented

TABLE 1. Bacterial strains and their relevant characteristics.

Strain	Relevant properties	History
EC2702 Hfr	F inserted in <i>gal</i> , transfers <i>attλ</i> early	From J. Beckwith
GI1009 Hfr	EC2702(λ)	Lysogen of EC2702
GI1010 Hfr	GI1009/ λ	From GI1009 by resistance to λvir
MM28 F ⁻	Sm ^R	From M. Meselson
GI29 F ⁻	MM28(λ)	Lysogen of MM28
GI31 F'/Gal(λ)	GI29 containing F'/Gal(λ)	GI57 $\times$ GI29
GI32 F'/Gal(λ)	GI31/ λ	From GI31 by resistance to λvir
MM56 F'/Gal	This F' also contains <i>attλ</i> and <i>bio</i> ⁺ .	From M. Meselson
GI57 F'/Gal(λ)	Pro ⁺ Thr ⁺ His ⁺ Sm ^S . Prophage present on F'.	Lysogen of MM56
MM94 F ⁻	Formerly called C600.4	From M. Meselson ¹²
GI95 F ⁻	MM94/ λ	From MM94 by resistance to λvir
GI96 F ⁻	MM94(λ)	Lysogen of MM94
AB259 HfrH	Hayes-type Hfr	From P. Howard-Flanders
GI140 HfrH	AB259(λ)	Lysogen of AB259
AB1157 F ⁻	<i>proA2</i> , <i>leu-8</i> , <i>thr-4</i> , <i>thi</i> , <i>argE3</i> , <i>his-4</i> , Sm ^R	From P. Howard-Flanders
GI157 F ⁻	AB1157(λ)	Lysogen of AB1157
GI100 F ⁻	CR63(λind^- ts 858). Thr ⁻ Leu ⁻ Thi ⁻ Thy ⁻	From D. Kaiser via F. Blattner

Genotypic symbols are those of Taylor and Trotter.²⁰ Other symbols are as follows: F'/Gal(λ), λind^- prophage inserted in F'/Gal episome; F, sex factor; (λ), lysogenic for λind^- ; / λ ; resistant to λvir ; Sm^R, resistant to streptomycin; Sm^S, sensitive to streptomycin; Pro, proline; Thr, threonine; His, histidine; Leu, leucine; Thi, thiamine; Thy⁻, requires thymine or thymidine for growth.

L-broth was added, and the culture was agitated vigorously for 80 additional min to allow development of progeny phages. After the addition of chloroform, the phage particles were concentrated by two cycles of differential centrifugation. CsCl was added to the phage suspension in the buffer (described in (a) above) to give a density of 1.50 gm/cc, and the mixture was centrifuged for 16 hr in a SW65 Spinco rotor. The phage band (visible to the eye) was collected and dialyzed for 30 min or more against the buffer to remove CsCl. About 1 ml of the phage suspension was then layered on a CsCl step gradient (1.3 ml each of CsCl solutions made up in the buffer to densities of 1.3, 1.5, and 1.7 gm/cc in a $\frac{1}{2} \times 2''$ tube) and centrifuged for 1 hr at 35,000 rpm in a SW65 rotor. The phage band was collected, dialyzed, and further purified on a second step gradient run under identical conditions. At this stage, all detectable radioactivity was present in the phage band.

(e) *Separation of λ strands*: Our procedure for strand separation was based on that of Hradecna and Szybalski.¹⁰ About 10^{11} viable phage particles were used in 1 ml of 1 mM Tris pH 7.9, 1 mM NaCl, and 10^{-2} EDTA (pH 8.0 at 0.1 M). To this were added 0.04 ml of 2% Sarkosyl NL97 (Geigy Chemical Co.) and 50 μ g of poly (U,G) (Miles Chemical Co.). The mixture was then heated at 94° for 2.5 min and cooled in ice water. Four ml of 1 mM EDTA and 6 gm CsCl (Harshaw) were added, and the refractive index at 25° was adjusted to a value between 1.4020 and 1.4065, depending on the particular lot of poly (U,G) being used. The solution was pipetted into a cellulose nitrate tube previously boiled in EDTA, and light mineral oil was added to fill the tube. Centrifugation in the Spinco 65 fixed-angle rotor was for 40 hr at 35,000 rpm and 12°C. Drops were collected into vials containing 10 ml scintillation fluid—1000 ml toluene, 0.2 gm 1,4-bis [2-(4-methyl-5-phenyloxazolyl)]benzene, 4 gm 2,5-diphenyloxazole, 500 ml Triton X-100 from Packard Instruments, and 167 ml distilled water—and counted in a scintillation counter.

Results.—In the mating experiments, we confine our attention to the radioactivity label that is initially incorporated equally into both DNA strands of donor cells prior to mating. The problem then is to determine the amount of radioactivity found in each strand after transfer into the recipient. At present, the best procedure for isolating the separated strands of DNA is that of Szybalski and co-workers^{10, 11} who find that usually one of the two strands will bind more poly (U,G) than the other and will consequently have a heavier buoyant density in CsCl. *E. coli* DNA strands, however, do not separate well,¹¹ possibly because the DNA is fragmented during isolation, resulting in a population of strands with nonuniform distributions of poly (U,G) binding sites. Bacteriophage DNA strands, however, can easily be separated by this technique. Partly for this reason, we did not examine the transferred *E. coli* DNA directly, but rather the DNA of phage λ transferred as a prophage.

The donor strain, lysogenic for λ (in which state the λ DNA is inserted in the bacterial chromosome), is mated with nonlysogenic recipients. Owing to the absence of λ repressor in the recipient, the λ prophage induces and detaches itself from the bacterial DNA and begins autonomous replication. Ultimately, the recipient cells are lysed, and some of the transferred DNA is recovered in the progeny phage particles. We first show that the latter part of this process—DNA replication and maturation—does not result in an unequal recovery of λ strands.

Equal recovery of the two lambda strands after infection from without: Bacteria were infected with λ phages uniformly labeled in both strands with H^3 , and the progeny phages were isolated and purified after one cycle of growth. These progeny phages were mixed with phage labeled uniformly with P^{32} , and the strands were separated as described in a previous section. As shown in Figure 2a,

the H^3 and the P^{32} activities are nearly superimposable, forming two approximately equal bands corresponding to the two λ strands. This demonstrates that both strands are recovered equally after a lytic cycle of growth.

We have not done an experiment with density label to demonstrate that the infecting phage DNA actually replicated. However, to ensure at least that the DNA had in fact entered the bacterial cells and had re-emerged in a new protein coat, the original infecting phage particles were also labeled with S^{35} in their protein coats. From the recovery of H^3 cpm relative to S^{35} cpm in the progeny phages, less than 2.5 per cent of the labeled DNA in this experiment was derived

FIG. 2.—Density gradient profiles of λ DNA strands separated with poly (U,G).

(a) DNA from phage obtained after one-cycle growth of H^3 -labeled parental phage infecting from without (●) compared with DNA uniformly labeled with P^{32} in both strands (○). The cpm in the peaks are: H^3 , $r = 2107$, $l = 1787$; P^{32} , $r = 608$, $l = 499$.

(b) DNA from phage obtained after mating P^{32} -labeled GI1010, which transfers markers in the order *gal- λ -bio*, with GI95 (○) compared with DNA uniformly labeled with H^3 (●). The cpm in the peaks are: H^3 , $r = 4060$, $l = 4300$; P^{32} , $r = 1030$, $l = 4330$.

(c) DNA from phage obtained after mating H^3 -labeled GI32, which transfers F' Gal(λ) in the order *bio- λ -gal*, with GI95 (●) compared with DNA uniformly labeled with P^{32} (○). The cpm in the peaks are: H^3 , $r = 2420$, $l = 314$; P^{32} , $r = 608$, $l = 499$.

To facilitate comparison of the gradients shown in the graph, strand r was aligned in each of the three gradients, and the fractions beyond the peaks were omitted, since they contained negligible radioactivity. Generally there were about 65 fractions per gradient, of which 34 are shown.

from unadsorbed or uninjected parental phage particles. The multiplicity of infection in this experiment was 1.3 viable phages per bacterium. Similar results were obtained at a multiplicity of 0.01.

Unequal recovery of the two lambda strands after mating: The lysogenic donor strain GI1010 derived from Hfr EC2702 was labeled with P^{32} and mated with the recipient and the progeny phages purified after zygotic induction. These phages were mixed with phages uniformly labeled with H^3 , and the strands were separated as described above. The H^3 activity from the uniformly labeled phages again forms two approximately equal bands. The P^{32} activity originally trans-

ferred from the donors, however, is found predominantly in the lighter of the two strands (Fig. 2b).

Transfer in the opposite direction: There are two possible explanations for the experimental result discussed above. Preferential transmission or recovery may be intrinsic to one of the two strands of the prophage (and of the bacterial chromosome). Alternatively, the chemical polarity of the transferred strand may be the important element. These alternatives may be distinguished by repeating the experiment with reversed direction of transfer.

In this experiment (Fig. 2c) we have used an F'Gal that transfers in the order *bio*- λ -*gal*, in contrast to the Hfr previously used, which transfers in the order *gal*- λ -*bio*. Comparison of Figures 2b and 2c, reveals that opposite strands are preferentially recovered in these two experiments. This result rules out explanations which assume that asymmetric transfer is uniquely a property of one particular strand.

Is there any transfer of the complementary strand? The most asymmetric result that we obtained is a 7.7:1 ratio of strands. The presence of a small amount of radioactivity in the minor band might indicate transfer of both strands, but with greatly unequal probabilities. However, it seems more likely to us that one strand is exclusively transferred. Radioactive label may have found its way into the minor band as a result of degradation of some of the transferred bacterial and phage DNA and subsequent reincorporation randomly into progeny phage DNA. Supporting this interpretation is the finding that, if the origin of transfer is moved further from the prophage, the fraction of radioactive label found in the minor band is larger. When the amount of bacterial chromosome between the origin and lambda was increased from 1 to 2 per cent (GI1010 and F'Gal) to 20 per cent (Hfr Hayes), we found 773 cpm in *r* and 1531 cpm in *l*.

Discussion.—This report establishes that only a particular one of the two DNA strands of prophage λ is recovered from recipient cells following transfer from lysogenic donor bacteria. Furthermore, opposite strands are recovered when donors injecting in opposite directions are employed.

The polarity of the recovered strand can be determined from the known orientation of the λ -prophage genes in the *E. coli* chromosome¹³ and the known 3' to 5' direction of the strands relative to the λ gene order.¹⁴ The 5' end of strand *l* is toward *gal*, and the 5' end of strand *r* is toward *bio*. Our experimental finding is that strand *l* is recovered when the order of injection is *gal*- λ -*bio*, showing that transfer proceeds in the direction 5' to 3'. When the order of injection is reversed, strand *r* is recovered, again showing 5' to 3' transfer. Ohki and Tomizawa¹⁵ have independently reached the same conclusion.

Implications for the mechanism of DNA transfer: The sex factor is an autonomous DNA element that can be inserted into the bacterial chromosome. It is currently thought that the orientation of the insertion determines whether the chromosome is transferred clockwise or counterclockwise with respect to the standard circular map order.^{16, 17} This model, together with our finding that the transferred DNA strand always enters beginning with the 5' end, leads us to conclude that a unique strand of the sex factor enters the recipient, regardless of the direction of chromosomal transfer. Since the transferable strand of the

sex factor can be connected to either chromosomal strand at the time of integration, the direction of transfer and the chromosomal strand transferred with the sex-factor strand are both determined by the relative orientation of the sex-factor and chromosomal strands at the time of integration.

There are two reasonable explanations for the absence of the strand complementary to the one recovered in the recipient. Either it was destroyed by a nuclease or it remained intact, in which case it must still be in the donor, for it was not recovered from the recipient. If it is intact, we can imagine that a strand is made available for transfer from the donor by a single-chain scission, presumably at a specific site in the unique strand of the sex factor that is transferred. This scission creates the asymmetric structure needed and accounts for the polarity of gene transfer and the transfer of opposite λ strands when the direction of gene transfer is reversed.

DNA synthesis in the donor may be the means by which the single strand is made available for transfer. After the single-strand break is made, nucleotide addition to the free 3' end by DNA polymerase could displace single-stranded DNA with a 5' leading end, freeing it for transfer into the recipient. This would conform to the model of continuous synthesis from a circle, as proposed by Fulton,¹⁸ and is in fact the rolling circle model of DNA synthesis proposed by Gilbert and Dressler.¹⁹

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¹ Jacob, F., S. Brenner, and F. Cuzin, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 329.

² Ptashne, M., *J. Mol. Biol.*, **11**, 829 (1965).

³ Gross, J. D., and L. Caro, *J. Mol. Biol.*, **16**, 269 (1966).

⁴ Luria, S. E., and J. W. Burrous, *J. Bacteriol.*, **74**, 461 (1957).

⁵ Jacob, F., and A. Campbell, *Compt. Rend.*, **248**, 3219 (1959).

⁶ Lieb, M., *J. Mol. Biol.*, **16**, 149 (1966).

⁷ Jacob, F., and E. L. Wollman, *Ann. Inst. Pasteur*, **87**, 653 (1954).

⁸ Jacob, F., and E. Wollman, *Sexuality and the Genetics of Bacteria* (New York: Academic Press, 1961).

⁹ Adelberg, E. A., and S. N. Burns, *J. Bacteriol.*, **79**, 321 (1960).

¹⁰ Hradecna, Z., and W. Szybalski, *Virology*, **32**, 633 (1967).

¹¹ Kubinski, H., Z. Opara-Kubinska, and W. Szybalski, *J. Mol. Biol.*, **20**, 313 (1966).

¹² Meselson, M., *J. Mol. Biol.*, **9**, 734 (1964).

¹³ Rothman, J. L., *J. Mol. Biol.*, **12**, 892 (1965).

¹⁴ Wu, R., and A. D. Kaiser, these PROCEEDINGS, **57**, 170 (1967).

¹⁵ Ohki, M., and J. Tomizawa, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 33 (1968), in press.

¹⁶ Scaife, J., and J. D. Gross, *Genet. Res.*, **4**, 328 (1963).

¹⁷ Beckwith, J. R., and E. R. Signer, *J. Mol. Biol.*, **19**, 254 (1966).

¹⁸ Fulton, C., *Genetics*, **52**, 55 (1965).

¹⁹ Gilbert, W., and D. Dressler, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 33 (1968), in press.

²⁰ Taylor, A. L., and C. D. Trotter, *Bacteriol. Rev.*, **31**, 332 (1967).