TRANSFER OF PHOSPHORUS-CONTAINING MATERIAL ASSOCIATED WITH MATING IN *ESCHERICHIA COLI*

ALAN GAREN* AND P. D. SKAAR**

Carnegie Institution of Washington, Department of Genetics, and The Biological Laboratory, Cold Spring Harbor, N.Y. (U.S.A.)

Genetic analyses of the mating process in *E. coli* have shown that genes are transferred unilaterally from one parental cell to the other[1-3]. The purpose of the present experiments is to determine whether genetic transfer can be correlated with the transfer of cellular material. Since genetic information in certain strains of bacteria resides in desoxyribonucleic acid (DNA)[4,5], the experiments were designed to detect transfer of DNA between mating cells. The technique used is based on the finding that, in the course of reproduction of coliphage T2, a large fraction of the atoms initially present in the DNA of the bacterial host is incorporated into particles of progeny phage[6]. This fact makes it possible to use T2 to scavenge for DNA that has been transferred between bacteria as a result of mating. In a bacterial mating experiment, a T2-resistant strain can be used as one of the parents and can be labeled with radiophosphorus ^{32}P, and the other parent can be sensitive to T2 and unlabeled. After mating has occurred, T2 can be added to infect the initially unlabeled sensitive cells. The ^{32}P activity appearing in T2 progeny can be used as a measure of the amount of DNA phosphorus transferred from resistant to sensitive cells. Preliminary results from this kind of experiment have been reported elsewhere[7].

MATERIALS AND METHODS

Bacterial strains

Most of the strains of *E. coli* K12 used have been described in a preceding publication[8]. Their pertinent characteristics for the present experiments are the following. W1895: *Hfr* (Cavalli) Lac_1+ V_2^s V_6^s; CS101: *Hfr* (Cavalli) Lac_1+ V_2^r V_6^s; CS2: $F-$ Lac_1+ V_2^s V_6^s; CS89: $F-$ Lac_1- V_2^r V_6^s; CS100: $F-$ Lac_1- V_2^s V_6^r. *Hfr* indicates the donor mating type, $F-$ the recipient type. Genetic transfer is observed to occur only in the direction from *Hfr* to $F-^{1,2,3,8}$. *Lac*+ and *Lac*− indicate ability and inability, respectively, to ferment lactose. V^r and V^s indicate resistance and sensitivity, respectively, to the T coliphage indicated by the subscript. Resistant strains do not adsorb the phage[9].

Phage strains

Wild-type strains of coliphages T2H and T6 were used. Stocks were prepared by growing the phage on *E. coli* B, and were titrated for plaque-forming activity by the agar-layer method[10].

* Fellow of the National Foundation for Infantile Paralysis. Aided by a grant (C2158) to Dr. A. D. HERSHEY from the National Cancer Institute of the National Institutes of Health, United States Public Health Service. Present address: Biology Department, Massachusetts Institute of Technology, Cambridge, Mass.

** This work was supported partly under contract by the U.S. Army Chemical Corps, Fort Detrick, Frederick, Md., and partly by a grant from the National Science Foundation. Present address: Department of Zoology and Entomology, Montana State College, Bozeman, Mont.

References p. 463.

Mating procedure and measurement of phosphorus transfer

The cells were prepared for mating in the following manner. Parental cultures were grown for 12 hours in Difco Penassay broth at 37° C without aeration, and then were washed and resuspended in Difco peptone broth of low phosphorus content at 1/20 of their original concentration. Where required, ^{32}P as neutral orthophosphate was added to the peptone medium in convenient tracer quantities (about 0.02 mc/ml). The cultures were aerated at 37° C for 2¼ hours (to give about $2 \cdot 10^8$ cells/ml), centrifuged, washed twice, and resuspended at a twofold dilution in Penassay broth not containing ^{32}P. The washed cultures were aerated at 37° C for 30 min, centrifuged, and resuspended in Penassay broth at a concentration of about 10^9 cells/ml.

At this time, the cultures either were mixed in appropriate ratios or kept separate, and in either case were aerated at 37° C for 60 min. The cell titers increased about twofold during this period. After 60 min, the cells were centrifuged and resuspended in an equal volume of T2 buffer[11], and infected with about 10 T2 particles per sensitive cell. Five minutes were allowed for adsorption of the phage, and then sufficient Penassay broth was added to yield a concentration of about $4 \cdot 10^8$ cells/ml. This phage-growth tube was aerated at 37° C (time 0). At 15 min, 10 T2 particles per sensitive cell were added to help produce lysis-inhibition[12]. At 80 min, 200 UV-irradiated T2 particles per cell were added to inhibit the readsorption of progeny phage, and at 90 min the cells were lysed by the addition of NaCN to a concentration of 0.015 *M*. Phage yields were about 250 particles per sensitive cell. The T2 progeny were purified by first filtering under suction through filter paper on which a thin layer of "Celite Filter-Aid" (Johns Mansville Co.) had been deposited, and then centrifuging the filtrate for two cycles in a Spinco ultra-centrifuge (15,000 r.p.m., head No. 30, for 45 min), and resuspending the phage pellet in T2 buffer. About 40% of the phage initially present in the lysate remained at the end of the purification steps. No corrections were introduced, since the loss of phage was approximately the same in all experiments. The amount of ^{32}P specifically associated with the purified T2 was determined by first adding T2-resistant *E. coli* B/2 cells and centrifuging at about 3000 *g* to remove non-specifically adsorbing radioactivity, and subsequently adsorbing phage to heat-killed T2-sensitive *E. coli* H[11]. The radioactivity adsorbing to the sensitive cells was counted, and this value was used as a measure of the amount of ^{32}P incorporated into T2 particles.

Measurement of genetic transfer

In mating experiments where genetic transfer was expected (*i.e.* with mixtures of *Hfr* and *F*— cells) the number of parental cells engaged in mating was measured. The procedure used has been described in detail in a previous publication[8]. In this procedure, an aliquot of the mixture of mating cells is exposed to phage (either T2 or T6) to which the *Hfr* parent is sensitive and the *F*— parent is resistant, and then samples are plated to yield isolated colonies on EMB-lactose medium[13]. With this medium it is possible to distinguish the colonies formed by *Lac*+ strains (always the *Hfr* parent) and by *Lac*— strains (always the *F*— parent). Colonies containing both *Lac*+ and *Lac*— cells form distinctive sectored colonies. The number of sectored colonies appearing after the parental cells have been exposed to phage provides a *minimal* estimate of the number of cells that mated[8].

In order to calculate the *fraction* of parental cells that mated, it is necessary to know the total number of *F*— and *Hfr* cells present when mating is measured. The number of *F*— cells is given by the number of *Lac*— type colonies appearing after exposure to the appropriate phage. The number of *Hfr* cells must be determined by plating before exposure to phage, since the *Hfr* strain is sensitive to the phage. Unpaired *Hfr* cells form *Lac*+ colonies; *Hfr* cells still paired with an *F*— partner form sectored colonies. However, sectored colonies also are formed by *F*— zygotes that have separated from their *Hfr* partners, and these must be subtracted from the total number of sectored colonies. It has been shown that at the time of sampling the mating mixtures, there is present about an equal number of unpaired *F*— zygotes and *Hfr*:*F*— pairs[8]. Therefore, the total number of *Hfr* parental cells is given by the number of *Lac*+ colonies *plus* one-half the number of sectored colonies.

Measurement of phosphorus in DNA

Cells labeled with ^{32}P were prepared in the manner described in a preceding section. The ^{32}P activity was fractionated in the following steps[14]. The cells were precipitated in ice cold 0.3 *M* trichloracetic acid (TCA). The TCA precipitate was suspended in 1 *M* KOH for 15 h at 37° C to solubilize RNA, and then the suspension was chilled, neutralized with HCl, and TCA was added to a final concentration of 0.3 *M* to precipitate DNA. The TCA precipitate was resuspended in 0.05 *M* phosphate buffer pH 7 containing 0.003 *M* $MgSO_4$ and 10 μg/ml of crystalline desoxyribonuclease, and kept 20 min at 37° C. The suspension again was precipitated in 0.3 *M* TCA and centrifuged. The amount of ^{32}P activity in the supernatant was measured, and this value was used as a relative measure of the amount of phosphorus contained in DNA.

References p. 463.

RESULTS

The ^{32}P transfer between cells was measured for three cases: for transfer from labeled *Hfr* to unlabeled *F*— cells, from labeled *F*— to unlabeled *F*— cells, and from labeled *F*— to unlabeled *Hfr* cells. (Mating occurs only between *Hfr* and *F*— cells, and the direction of genetic transfer is from *Hfr* to *F*—.) Each transfer experiment was run in three separate parts (A, B and C). As the final step in each part, T2 was added to the mixture of parental cells (the labeled strain being T2-resistant and the unlabeled strain T2-sensitive) and a determination was made of the amount of ^{32}P that was incorporated into T2 progeny.

Part A involved only T2-resistant strains labeled with ^{32}P. This part acts as a control to measure the amount of ^{32}P incorporation into T2 that does not depend on transfer between cells. Any incorporation found in this part probably resulted from the presence of T2-sensitive cells in the population.

Part B involved mixtures of labeled T2-resistant cells and unlabeled T2-sensitive cells. The incorporation detected in this part includes, in addition to ^{32}P transferred from resistant to sensitive cells, the background found in Part A. The difference between parts B and A, (B—A), is the amount of incorporation resulting specifically from the transfer of ^{32}P from resistant to sensitive cells.

Part C involved only T2-sensitive cells labeled with ^{32}P. The results in this part measure the maximum portion of the ^{32}P label in sensitive cells that can be incorporated into T2.

Transfer from Hfr to F— cells

Table I shows the results from experiments in which the *Hfr* strain CS101 was used as the T2-resistant parent and the *F*— strain CS100 as the T2-sensitive parent. The ratio of the numbers of *Hfr* to *F*— cells in the mating tube was varied from 0.08 to 4.4, and the fraction of *Hfr* cells found to have mated decreased as this ratio increased. Concomitantly, the amount of ^{32}P transferred from *Hfr* to *F*— cells, as indicated by the values of (B—A), tended to decrease. At low ratios of *Hfr* to *F*— cells (up to 0.4), the average value from nine measurements of (B—A) was 0.068% of the total ^{32}P initially present in *Hfr* cells.

Transfer from F– to F– cells

Table II shows the results from experiments in which both the T2-sensitive and T2-resistant cells were *F*—. Consequently, no mating took place when the strains were mixed in part B. Nevertheless, cell to cell transfer of ^{32}P was detected, as indicated by the values of (B—A) which averaged, at low ratios of labeled to unlabeled cells, 0.011% of the ^{32}P initially present in the labeled cells. This value is to be compared with 0.068% found as a result of transfer from *Hfr* to *F*— cells. It is clear that transfer associated with mating exceeded transfer between nonmating *F*— cells.

The basis for the small amount of ^{32}P transfer between *F*— cells may have been the release of ^{32}P-containing substances into the medium, followed by incorporation into sensitive cells and ultimately into T2. It is pertinent that all strains used in these experiments are lysogenic for λ phage[15], and that irradiation of lysogenic cells can induce lysis[16]. The ^{32}P radioactivity in labeled cells may have been of sufficient intensity to induce a significant amount of lysis.

References p. 463.

TABLE I

TRANSFER OF ^{32}P FROM *Hfr* TO *F*— CELLS: CS101 (*Hfr* V_2^r) × CS100 (*F*— V_2^s)

Ratio of the numbers of Hfr to F— cells	*Per cent of Hfr cells engaged in mating**	*Per cent of the ^{32}P in labeled cells incorporated in T2*			
		A labeled CS 101	*B mixture of labeled CS 101 and unlabeled CS 100*	*(B–A)*	*C Labeled CS 100*
0.11	46	0.012	0.075	0.063	2.2
0.11	19	0.007	0.051	0.047	2.9
0.50	7		0.046	0.039	
3.1	2		0.029	0.022	
0.10	50	0.023	0.155	0.132	2.4
0.41	27		0.108	0.085	
0.16	26	0.008	0.068	0.060	3.2
1.3	11		0.065	0.057	
4.4	5		0.035	0.027	
0.08	31	0.023	0.086	0.063	2.4
0.60	24		0.103	0.080	
2.2	10		0.100	0.077	
0.24	31	0.006	0.068	0.062	1.9
3.7	5		0.029	0.023	
0.15	not measured	0.016	0.072	0.056	2.7
0.40			0.059	0.043	
0.71			0.037	0.021	

* Determined by the transfer of the *Lac*+ gene from *Hfr* to *F*— cells, as described in the section on methods. This is a *minimum* value.

TABLE II

TRANSFER OF ^{32}P IN THE ABSENCE OF MATING: CS89 (*F*— V_2^r) AND CS2 (*F*— V_2^s)

Per cent of the ^{32}P in labeled cells incorporated in T2			
A labeled CS 89	*B* Mixture of labeled CS 89 and unlabeled CS 2*	*(B–A)*	*C labeled CS 2*
0.005	0.015	0.010	2.6
0.011	0.017	0.006	
	0.020	0.009	3.0
0.008	0.022	0.014	2.8
	0.019	0.011	
	0.019	0.011	
	0.013	0.005	
0.005	0.017	0.012	2.5
	0.012	0.007	
	0.016	0.011	
	0.011	0.006	

* The different values in the same experiment were obtained with different ratios of the numbers of CS89 to CS2 cells, varying from 0.2 to 4.

Transfer from F— to Hfr cells

Table III shows the results from experiments in which the *F*— strain CS89 was used as the T2-resistant parent and the *Hfr* strain WI895 as the T2-sensitive parent. The ratio of the numbers of *F*— to *Hfr* cells was varied from 0.08 to 4.6, and the fraction of *F*— cells found to have mated decreased as this ratio was increased. Concomitantly, the amount of ^{32}P transferred from *F*— to *Hfr* cells, as indicated by the values of (B—A), tended to decrease. This implies that the incorporation may have derived in part from ^{32}P that was transferred from *F*— to *Hfr* cells as a consequence of matings. At the three lowest ratios of *F* to— *Hfr* cells, where the fraction of *F*— cells engaged in mating was highest, the values of (B—A) averaged 0.018% of the total ^{32}P initially present in *F*— cells. Since more than half of this transfer probably was not associated with matings (Table II), we conclude that transfer from *F*— to *Hfr* cells is at most about 15% as efficient as transfer from *Hfr* to *F*—.

TABLE III

TRANSFER OF ^{32}P FROM *F*— TO *Hfr* CELLS: WI895 (*Hfr* V_2^s) × CS89 (*F*— V_2^r)

Ratio of the numbers of F— to Hfr cells	*Per cent of F— cells engaged in mating**	*Per cent of the ^{32}P in labeled cells incorporated in T2*			
		A labeled CS89	*B mixture of labeled CS89 and unlabeled W1895*	*(B–A)*	*C labeled W1895*
0.11	25	0.003	0.017	0.014	2.7
0.85	18		0.010	0.007	
2.1	8		0.002	(0)	
0.31	23	0.007	0.021	0.014	2.7
0.82	19		0.026	0.019	
1.9	14		0.012	0.005	
0.18	30	0.011	0.036	0.025	2.4
0.82	16		0.026	0.015	
1.9	12		0.021	0.010	
4.6	7		0.008	(0)	

* Determined by the transfer of the *Lac*+ gene from *Hfr* to *F*— cells, as described in the section on methods. This is a *minimum estimate.*

The relationship of transferred phosphorus to DNA

In the experiments just described, it was found that phosphorus was transferred predominantly in the direction from *Hfr* to *F*— cells, which also is the direction of transfer of genetic information. The correlation in the directions of transfer of phosphorus and genetic information suggests that the phosphorus was present in bacterial genetic material, DNA. Moreover, the method used to detect phosphorus transfer is especially sensitive for the phosphorus of DNA. Nevertheless, the possibility can not be excluded that other, presumably nongenetic, phosphorus compounds also were detected by the technique of scavenging with T2[6].

We now wish to estimate the fraction of the total DNA of an *Hfr* parent transferred during mating. Two assumptions will be made. First, that the transferred phosphorus measured in the determinations of (B—A) in Table I, after correction for transfer not associated with matings (Table II), is entirely DNA phosphorus. Second,

References p. 463.

that the DNA phosphorus transferred from *Hfr* to *F*— cells is incorporated into T2 with the same efficiency as the DNA phosphorus pre-existent in *F*— cells. Then:

$$\% \textit{Hfr} \text{ DNA transferred} = \frac{(\text{B}—\text{A}) - 0.011}{D} \times \frac{100}{E}$$

where D is the percent of the total phosphorus of an *Hfr* cell present in DNA, E is the efficiency with which T2 incorporates the DNA phosphorus in *F*— cells in a particular experiment, and (B—A) is the corresponding value listed in Table I for the percent of *Hfr* phosphorus incorporated into T2 as a result of transfer to *F*— cells. The value 0.011 is subtracted from (B—A) to correct for the average amount of transfer not associated with matings (Table II).

In order to evaluate D and E it is necessary to know the fraction of the phosphorus of *Hfr* and *F*— strains present in DNA. These values were measured by the procedure described in the section on methods, with the results that the percent phosphorus found in DNA was 8.6 for the *Hfr* strain CS101 and 8.9 for the *F*— strain CS100. Therefore:

$$D = 8.6 \text{ and } E = \frac{0.75(C)}{8.9}$$

where (C) is the percent of *F*— ^{32}P incorporated into T2 (as listed in Table I) and is multiplied by 0.75 to correct for the finding that about 25% of the bacterial material incorporated into T2 may have derived from sources other than DNA[6]. It should be noted that the values of (C) were measured on unmated *F*— cells, whereas those of (B—A) were measured on mated *F*— cells that contained, in addition to their own complement of DNA, also transferred *Hfr* DNA. It must therefore be considered that the incorporation of ^{32}P into T2 may have been less efficient in the measurements of (B—A) than of (C), due to the presence of excess DNA. However, this factor probably is not significant since, as will be shown, transferred *Hfr* DNA amounted to less than 10% of the total DNA.

In calculating percent DNA transfer, we will consider only the six experiments in Table I in which the ratio of *Hfr* to *F*— cells was less than 0.25, so as to be reasonably certain that in most matings only one *Hfr* cell conjugated with an *F*— partner. The results of the calculations are shown in Table IV. The Table also shows the percent DNA transferred per mating *Hfr* cell, based on estimates of the *minimum* frequency of mating (as described in the section on methods). We conclude that on the average a mated *Hfr* cell transferred less than 10% of its DNA.

TABLE IV

FRACTION OF *Hfr* DNA TRANSFERRED DURING MATING

Per cent of Hfr DNA transferred	*Per cent of Hfr cells engaged in mating*	*Per cent of DNA transferred per mating Hfr cell*
3.3	46	7.2
1.7	19	9.0
6.9	50	13.8
2.1	26	8.1
3.0	31	9.7
3.7	31	12.0
		Average 10

References p. 463.

Extent of transfer of the Hfr genome

The estimate made in Table IV, of the fraction of DNA transferred during mating, is pertinent to the problem of whether or not an *Hfr* parent transfers its complete genome. The available genetic evidence is not unambiguous; some experiments suggest complete transfer[17], others partial transfer[1,18,8]. In evaluating the present radiochemical evidence, it must be taken into consideration that the cellular DNA appears to reside in several "nuclei"[2,19]. Under the conditions for growth used in the present experiments, the number of nuclei should average about three per cell, and therefore a single nucleus should contain about 33% of the total DNA. Since this estimate is three times greater than the average percent DNA transfer calculated in Table IV, it would appear that an *Hfr* parent usually transferred less DNA than is contained in one of its nuclei, and therefore probably only part of its genome, to an *F*— mating partner.

It should also be noted that, for the calculations in Table IV, the estimate of the number of *Hfr* cells that had mated was based on the number of *F*— cells yielding recombinants containing the *Lac*+ gene of the *Hfr* parent. This must be a *minimum* estimate, since in some matings this gene may not have been transferred, while in others it may have been transferred but not incorporated into the *F*— genome[8]. There is evidence that the efficiency of mating may be close to 100%[3]. This consideration lowers, by a factor of about three, the estimate of the fraction of *Hfr* DNA transferred, and increases the likelihood that transfer of genetic information is incomplete.

ACKNOWLEDGEMENTS

We are grateful to Dr. JOSHUA LEDERBERG for furnishing strains of *Hfr* and *F*—.

SUMMARY

Transfer of phosphorus-containing material (probably DNA) has been detected between mating *Hfr* and *F*— cells of *E. coli* K12. The amount of transfer is greatest in the direction from *Hfr* to *F*—, which also is the direction in which genetic information is transferred. The average amount of phosphorus transferred in a single mating event appears to be less than the amount contained in the DNA of a cell nucleus. This implies that transfer of the *Hfr* genome usually is incomplete.

REFERENCES

1 W. HAYES, *J. Gen. Microbiol.*, 8 (1953) 72.
2 J. LEDERBERG, *J. Bacteriol.*, 71 (1956) 497.
3 E. L. WOLLMAN, F. JACOB AND W. HAYES, *Cold Spring Harbor Symposia Quant. Biol.*, 21 (1956) 141.
4 O. T. AVERY, C. M. MACLEOD AND M. MCCARTY, *J. Exptl. Med.*, 79 (1944) 137.
5 R. D. HOTCHKISS, *Harvey Lectures*, 49 (1954) 124.
6 A. D. HERSHEY, A. GAREN, D. K. FRASER AND J. D. HUDIS, *Yearbook Carnegie Inst.*, 53 (1954) 210.
7 P. D. SKAAR AND A. GAREN, *Genetics*, 40 (1955) 596.
8 P. D. SKAAR AND A. GAREN, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 619.
9 A. GAREN AND T. T. PUCK, *J. Exptl. Med.*, 94 (1951) 177.
10 M. H. ADAMS, *Methods in Med. Research*, 2 (1950) 1.
11 A. D. HERSHEY AND M. CHASE, *J. Gen. Physiol.*, 36 (1952) 39.
12 A. H. DOERMANN, *J. Bacteriol.*, 55 (1948) 257.
13 J. LEDERBERG, *Methods in Med. Research*, 3 (1951) 5.
14 A. D. HERSHEY AND N. E. MELECHEN, *Virology*, 3 (1957) 207.
15 J. LEDERBERG AND E. M. LEDERBERG, *Genetics*, 38 (1953) 51.
16 A. LWOFF, L. SIMINOVITCH AND N. KJELDGAARD, *Ann. Inst. Pasteur*, 79 (1950) 815.
17 T. C. NELSON AND J. LEDERBERG, *Proc. Natl. Acad. Sci. U.S.*, 40 (1954) 415.
18 E. L. WOLLMAN AND F. JACOB, *Compt. rend.*, 240 (1955) 2449.
19 C. F. ROBINOW, *J. Hygiene*, 43 (1944) 413.

Received August 23rd, 1957