JOURNAL OF BACTERIOLOGY, Nov. 1972, p. 751-760
Vol. 112, No. 2

Molecular Events Accompanying the Fixation of Genetic Information in *Haemophilus* Heterospecific Transformation

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Received for publication 4 August 1972

Heterospecific transformation between *Haemophilus influenzae* and *H. parainfluenzae* was investigated by isopycnic analysis of deoxyribonucleic acid (DNA) extracts of ^{3}H-labeled transforming cells that had been exposed to ^{32}P-labeled, heavy transforming DNA. The density distribution of genetic markers from the resident DNA and from the donor DNA was determined by transformation assay of fractions from CsCl gradients, both species being used as recipients. About 50% of the ^{32}P atoms in *H. parainfluenzae* donor DNA taken up by *H. influenzae* cells were transferred to resident DNA, and only a small amount of the label was lost under conditions of little cell growth. There was less transfer in the reciprocal cross, and almost half of the donor label was lost. In both crosses, the transferred donor material transformed for the donor marker considerably more efficiently when assayed on the donor species than on the recipient species, indicating that at least some of the associated ^{32}P atoms are contained in relatively long stretches of donor DNA. When the transformed cultures were incubated under growth conditions, the donor marker associated with recipient DNA transformed the donor species with progressively decreasing efficiency. The data indicate that the low heterospecific transformation between *H. influenzae* and *H. parainfluenzae* may be due partly to events occurring before association of donor and resident DNA but results mostly from events that occur after the association of the two DNA preparations.

Some transfer of genetic information from donor deoxyribonucleic acid (DNA) to competent recipient cultures has been observed in various heterospecific transformation systems (12), but the extent of fixation of this genetic information is much lower than that observed in homospecific transformation. Even the transformation levels of markers representing conserved gene loci are sometimes drastically reduced, and the transformation of other markers is still lower (7).

Heterospecific DNA that has entered the recipient cell may be prevented from transforming the cell for various reasons. (i) Immediately after entry, the heterospecific DNA may be recognized as foreign and thus be subjected to the degradative action of restriction enzymes (10, 20), which can recognize the DNA as foreign. (ii) Because of differences in the base sequences of the incoming DNA and the resident DNA, the overall integration of heterospecific DNA may be impaired (18). (iii) If integration does occur, portions of the integrated regions may be mispaired with their opposite strands because of local inhomologies, making single-strand parts of the hybrid DNA susceptible to the degradative action of nucleases specific for such structures. The last hypothesis assumes that heterospecific transformation takes place, as does homospecific transformation, by the insertion of single-strand DNA segments of donor DNA (3, 9, 16). (iv) When the genome containing heterospecific single-strand regions replicates, and these regions become double-stranded, they may contain the specific sites for the restriction enzyme (13), resulting in degradation after integration. (v) Even if the integrated donor DNA is not degraded, it may prove lethal for the recipient cell, thus preventing the realiza-

tion of genetic information. Killing of recipient cells by heterospecific DNA has indeed been observed (1, 22).

To examine these possibilities, we investigated the fate of ^{32}P-labeled, heavy transforming DNA in homospecific and heterospecific *Haemophilus influenzae* and *H. parainfluenzae* transformations. The input DNA was followed by isopycnic analysis and by assays of the donor and resident DNA genetic markers on both of the species. We found that biologically active unintegrated DNA is recoverable in heterospecific transformation as it is in homospecific transformation. There was considerable transfer of donor *H. parainfluenzae* DNA label to *H. influenzae* resident DNA, but not so much in the case of the reciprocal cross. In both crosses, resident DNA molecules that had donor DNA label associated with them produced a much larger number of donor marker transformants when assayed on the donor species. Thus, the associated molecules contain considerable genetic information as well as label from both the DNAs. As a function of time, however, the efficiency of these molecules for transformation of the donor marker decreased when assayed on the donor species, and increased when assayed on the recipient species. Such a change takes a considerable time, and even after about 120 min of growth the change was not complete. The ratio of transformation by the associated donor marker on the two species was about one at this time, whereas DNA extracted from transformed colonies transformed the original recipient species for the donor marker more efficiently (2). Some loss of donor genetic information both before and after association between donor DNA and recipient DNA was also observed, as measured by specific biological activity.

MATERIALS AND METHODS

Bacterial strains. *H. influenzae* strain Rd (wild type) and strains *str* (resistant to 250 μg of streptomycin/ml) and *nov* (resistant to 2.5 μg of novobiocin/ml) were originally obtained from Roger M. Herriott. *H. parainfluenzae* was obtained from John W. Bendler. The resistant strains of *H. parainfluenzae*, *str* and *nov*, were derived by mutation (2). In both strains, the two drug markers are linked, although the linkage is looser in *H. parainfluenzae* (2).

Growth medium for cells. The growth medium was as previously described (19).

Preparation of ^{32}P-, ^{2}H-, ^{13}C-, and ^{15}N-labeled DNA. Heavy *H. influenzae* DNA was prepared as described earlier (17). *H. parainfluenzae* cells grew poorly at D_2O concentrations higher than 70 to 80%. A heavy DNA could, however, be obtained either (i) by growing the cells in the synthetic medium (10) with 70% D_2O and [^{13}C]glucose substituted for deuterated glucose, or (ii) by growing the cells in an 80% D_2O-Neopeptone medium consisting of the following additions to each 100 ml of 1.75% Neopeptone: 4.4 ml of 20% [^{13}C]glucose, 10 ml of concentrated (10 times) Earle's solution (8) without phosphate or carbonate, 12 μliters of 0.5 M cysteine-HCl, 1.25 ml of 2.5 mg/ml uridine, 0.12 ml of 10 mg/ml hemin plus 10 mg/ml L-histidine, and 0.25 ml of 1 mg/ml nicotinamide adenine dinucleotide. The DNA extracted from such cells has a buoyant density in CsCl which is 0.022 g/ml greater than that of light *H. influenzae* DNA (Fig. 1).

Preparation of ^{3}H-labeled, competent recipient cells. The procedure was as previously described (17).

Transformation of labeled cells. ^{3}H-labeled competent cells were exposed to homospecific or heterospecific ^{32}P-labeled, heavy transforming DNA at 37 C for 5 min in MIV medium (21). Deoxyribonuclease (5 μg/ml) was added, and the mixture was incubated for another 3 min. A sample was withdrawn at this time and designated as the time zero sample. Incubation of the transforming cells was continued, and additional samples were withdrawn at various times. These samples were then lysed before the transformation assays or equilibrium centrifugation. The *H. parainfluenzae* cells used in these

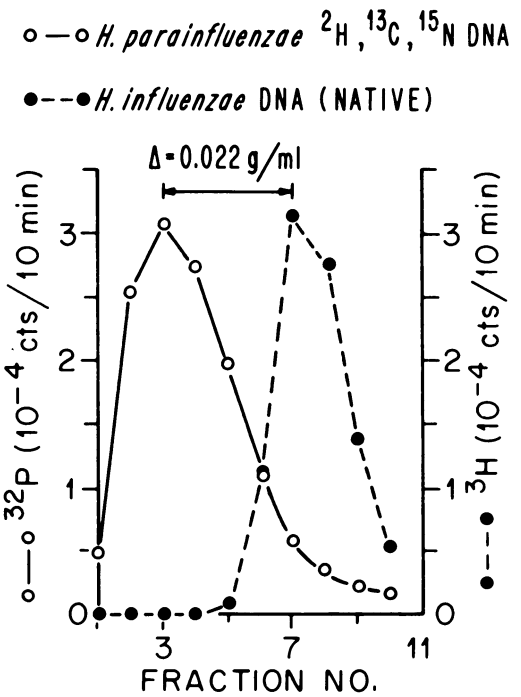

FIG. 1. *Equilibrium sedimentation profiles of ^{32}P-labeled, heavy H. parainfluenzae DNA and ^{3}H-labeled, light H. influenzae DNA.*

experiments were transformed at a frequency of about 0.2% with *H. parainfluenzae* DNA. The *H. influenzae* cells were transformed homospecifically with a frequency about five times higher.

Transformation of labeled cells followed by growth. The above procedure was used, except that the total incubation in MIV medium was for 17 min. The culture was then diluted 10-fold into warm growth medium, and 10-ml samples were withdrawn after a further 0, 40, 80, or 120 min of incubation and concentrated 10-fold in 0.15 M NaCl plus 0.015 M trisodium citrate (SSC) before lysis.

Lysis of transforming cells and preparation of DNA extracts. It was shown earlier that treatment of *H. influenzae* transforming cultures with digitonin followed by low-speed centrifugation permits physical separation of most of the unintegrated DNA from resident DNA and the associated donor atoms (17). The lysates containing DNA can then be banded, with or without deproteinization, in CsCl gradients. After treatment of *H. parainfluenzae* with digitonin, however, there was no such release of unintegrated DNA. Accordingly, in the present experiments, *H. parainfluenzae* as well as *H. influenzae* cells were lysed with 1% Sarkosyl at 37 C. The lysates were then extracted with chloroform-amyl alcohol (24:1), and the water layers obtained after centrifugation were treated with 100 μg of ribonuclease/ml before equilibrium centrifugation in CsCl.

Equilibrium centrifugation. The method of Brunk and Leick (5) was used. Details were as previously described (17).

Collection and assay of fractions. About 65 five-drop fractions (around 0.06 ml) were collected and mixed with 0.4 ml of SSC. Part of the sample (generally 0.12 ml) was placed on paper strips, processed, and counted according to the procedure of Carrier and Setlow (6). About 10 fractions per gradient were assayed for transformation as described earlier (17) on both *H. influenzae* and *H. parainfluenzae* recipient cells.

RESULTS

DNA uptake and loss of donor DNA label after uptake. Data on the extent of uptake of ^{32}P-labeled DNA in homospecific and heterospecific transformations are shown in Tables 1 and 2. Uptake by *H. parainfluenzae* cells was about one-third that of *H. influenzae* cells, whether the DNA was homospecific or heterospecific. These data indicate that neither *H. influenzae*, as previously shown (22), nor *H. parainfluenzae* discriminates between heterospecific and homospecific DNA in uptake.

The loss of donor DNA label, measured as the fraction of acid-insoluble donor DNA radioactivity remaining as a function of time after uptake, is also shown in Tables 1 and 2. It was previously observed (17), and is observed here (Table 2), that in homospecific transformation of *H. influenzae* there is no loss of donor DNA label. On the other hand, we found that there is a measurable small loss of donor DNA label in homospecific *H. parainfluenzae* transformation (Table 1). Furthermore, donor DNA label is lost in both heterospecific crosses, although more is lost by the *H. parainfluenzae* recipients.

Donor DNA label associated with resident DNA. Since in all of the experiments reported here heavy transforming DNA was used, it was possible to separate the donor DNA label associated with resident DNA during transformation from unassociated donor DNA. Such associated donor DNA label may not necessarily represent only macromolecular integration. Tables 1 and 2 show the fractions of donor label associated with recipient DNA at various times during homospecific and heterospecific transformations. These data were obtained from the distributions of label in CsCl gradients shown in Fig. 2, 3, and 4, and from a CsCl gradient of *H. influenzae* homospecific transformation (not shown). The homospecific transformations have similar kinetics and extents of association. The two heterospecific crosses show both different kinetics and different extents of association. With *H. parainfluenzae* as the recipient cells, the fraction of associated *H. influenzae* label did not exceed 20% in 30 min (Table 2), whereas in the reciprocal cross there was more than 50% association of donor label (Table 1).

Tables 1 and 2 also present the specific biological activity of associated and unassociated donor DNA. These specific biological activities, calculated from the assay of donor DNA on the species from which it came, provide a measure of the genetic integrity of heterospecific DNA in the heterospecific crosses. The specific biological activity of *H. parainfluenzae* DNA in *H. influenzae* cells (Table 1) was initially higher than in *H. parainfluenzae* cells, presumably reflecting the somewhat slower association kinetics of the heterospecific cross, since association is considered to result in the formation of nontransforming broken molecules (15, 17). However, the specific biological activity of *H. influenzae* DNA in *H. parainfluenzae* cells was not markedly different from that in *H. influenzae* cells (Table 2).

Distribution of homospecific donor and resident DNA radioactivity and biological activity in CsCl gradients. We already have a considerable amount of information on the mechanism of *H. influenzae* transformation (15–17). Very little is known about the molecular events in *H. parainfluenzae* homospecific

TABLE 1. *Uptake and distribution of H. parainfluenzae DNA ^{32}P label and genetic information in H. parainfluenzae and H. influenzae cells*

Recipient cell	Uptake[a]	Sampling time[b] (min)	Loss of donor label (% loss of initial counts bound)	Acid-insoluble donor DNA label associated with resident DNA (% of total)[c]	Specific biological activity of donor DNA[d]
H. parainfluenzae	950 (100%)	0	0	28	2.8
		10	8	70	1.8
		40	14	79	1.4
H. influenzae	3,480 (366%)	0	0	19	5.1
		10	10	25	3.0
		40	20	55	1.9

[a] Acid-insoluble ^{32}P counts per 10 min in 0.05 ml of competent cell lysate. Input DNA: 2×10^4 counts per min per ml of cell suspension.

[b] After DNA uptake and deoxyribonuclease digestion.

[c] Calculated from data of Fig. 2 and 3.

[d] Transformations of total donor DNA marker assayed on *H. parainfluenzae* per ^{32}P counts per 10 min, calculated from data of Fig. 2 and 3.

TABLE 2. *Uptake and distribution of H. influenzae DNA ^{32}P label and genetic information in H. influenzae and H. parainfluenzae cells*

Recipient cell	Uptake[a]	Sampling time[b] (min)	Loss of donor label (% loss of initial counts bound)	Acid-insoluble donor DNA label associated with resident DNA (% of total)[c]	Specific biological activity of donor DNA[d]
H. influenzae	11,500 (100%)	0	0	35	3.0
		10	0	68	1.9
		30	0	76	1.6
H. parainfluenzae	3,860 (100%)	0	0	9	2.4
		10	19	15	2.2
		30	43	18	1.1

[a] Acid-insoluble ^{32}P counts per 10 min in 0.05 ml of competent cell lysate. Input DNA: 4×10^4 counts per min per ml of cell suspension.

[b] After DNA uptake and deoxyribonuclease digestion.

[c] Calculated from data of Fig. 4 and similar data for *H. influenzae* homospecific transformation (not shown).

[d] Transformations of total donor DNA marker assayed on *H. influenzae* per ^{32}P counts per 10 min, assayed from digitonin lysates of *H. influenzae* cells or calculated from data of Fig. 4.

transformation. CsCl gradient profiles of donor and resident DNA radioactivity and biological activity from such transformation are shown in Fig. 2. The data exhibit an unforeseen complexity in that the lighter fractions contain a larger amount of resident DNA biological activity than do the heavier fractions. The *str nov* recombinants are obtained from fractions which are one to two fractions heavier than the peak of the resident DNA label. The two markers are known to be less tightly linked in *H. parainfluenzae* than in *H. influenzae* (2, 14), so that there are fewer recombinants (double transformants) in homospecific *H. parainfluenzae* transformation than in homospecific *H. influenzae* transformation. The position of the recombinant maximum in Fig. 2 probably reflects both the weak linkage and density heterogeneity in the region of these two markers in *H. parainfluenzae*. The data indicate that the recombinants are almost 50% heavy, and therefore that there is material transfer of relatively long segments of donor DNA to resident DNA in the transfer of genetic information in *H. parainfluenzae*.

Most of the unassociated donor DNA in *H. parainfluenzae* transformation is recoverable in a biologically active form. However, the specific biological activity corresponding to the donor label associated with resident DNA is

relatively low (Fig. 2). This can be explained by assuming that completion of integration requires considerable time, and that transformation by incompletely integrated, associated molecules may favor integration of resident rather than donor genetic material. Alternatively, the structure of the hybrid molecules may be such that their entry into the cell is prevented.

Distribution of heterospecific donor and resident radioactivity and biological activity in CsCl gradients. Figures 3 and 4 show the results of equilibrium sedimentation of DNA from heterospecific transformations. One of the most striking properties of the DNA representing donor-resident association is that it transforms for donor marker with a much higher efficiency on the donor species. For example, the donor *H. parainfluenzae* marker from *H. influenzae* cells yielded about 50-fold more transformants when assayed on *H. parainfluenzae* that when assayed on *H. influenzae* (Fig. 3). In the reciprocal cross, the same phenomenon was observed (Fig. 4). These data suggest that at early times the donor DNA associated with resident DNA is in long pieces, so that there is more homology of the single-strand region surrounding the donor marker with the donor species than with the recipient species. However, the associated donor transforming activity when assayed on the donor species decreased somewhat with time of incubation of the heterospecific transformation mixture, whereas it increased when it was assayed on the recipient species (Fig. 3 and 4).

Results (not shown) similar to those of Fig. 3 were obtained with *nov H. parainfluenzae* DNA transforming *str H. influenzae* cells. However, since the *nov* marker transforms in heterospecific crosses with much less efficiency than the *str* marker (2), the results were not as clear as those in Fig. 3.

It should be noted that in both heterospecific transformations the donor DNA biological activity was recoverable immediately after

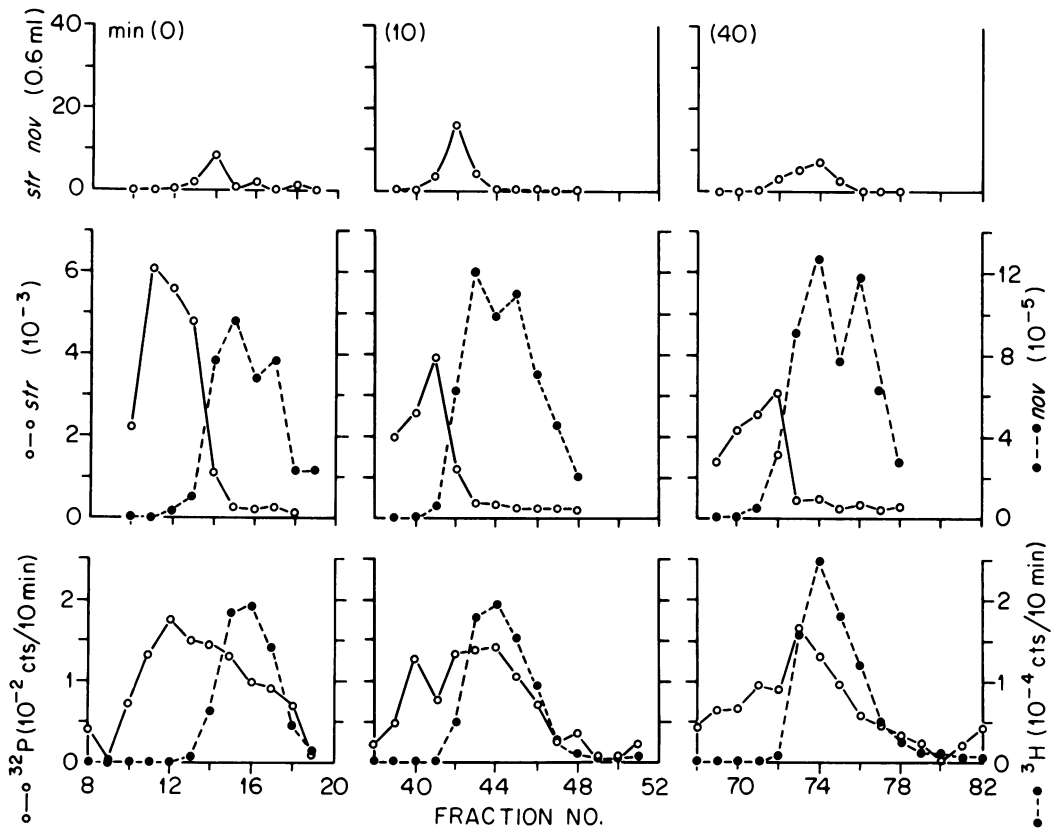

FIG. 2. *Distribution of radioactivity and transforming activity of lysates of ^{3}H-labeled H. parainfluenzae nov cells exposed to ^{32}P-labeled, heavy H. parainfluenzae str DNA and centrifuged to equilibrium in CsCl gradients. Times shown are after 5 min of DNA uptake and 3 min of incubation with deoxyribonuclease.*

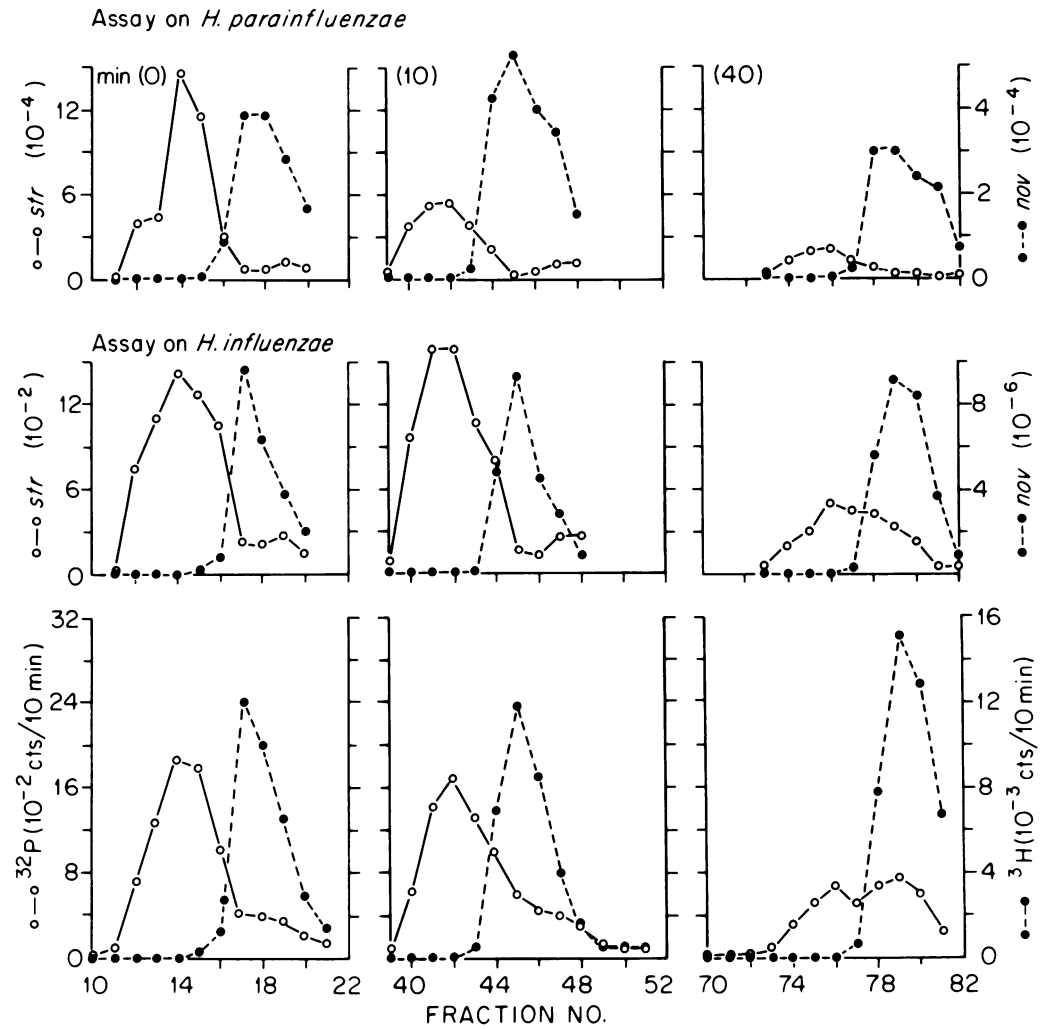

FIG. 3. *Distribution of radioactivity and transforming activity of lysates of ^{3}H-labeled H. influenzae nov cells exposed to ^{32}P-labeled, heavy H. parainfluenzae str DNA and centrifuged to equilibrium in CsCl gradients. Times shown are after 5 min of DNA uptake and 3 min of incubation with deoxyribonuclease.*

uptake. This seems to be a characteristic of *Haemophilus* transformations. The native state of transforming DNA after entry into the cell may be important for the action of the restriction endonucleases that have been discovered in *H. influenzae* (20) and *H. parainfluenzae* (11), especially as the *H. influenzae* enzyme has been shown to be specific for native DNA (13).

Fate of associated H. parainfluenzae DNA during growth of H. influenzae recipient cells. Since there is an appreciable fraction of *H. parainfluenzae* DNA label associated with *H. influenzae* resident DNA in this heterospecific cross (Fig. 3), and since transformation by the donor marker is considerably more efficient when assayed in *H. parainfluenzae* than in *H. influenzae*, it was of interest to determine the effect of growth of the transformed culture on the relative efficiency of transformation by associated donor DNA assayed in the two species. The results of such an experiment are shown in the radioactivity and transformation profiles after equilibrium centrifugation (Fig. 5) and can be summarized as follows.

(i) Initially, all associated or unassociated *H. parainfluenzae* DNA had a higher biological activity when assayed on *H. parainfluenzae* than on *H. influenzae*.

(ii) The associated donor DNA marker assayed on the donor species gradually decreased, whereas there was an increase in the

corresponding transformation of the recipient species.

(iii) After 120 min in growth medium, the associated donor DNA transformed both species with about the same efficiency.

(iv) After 80 min of growth, there was loss of associated donor ^{32}P label. There was a smaller, but significant, loss of resident ^{3}H label as well. These data are in accord with the previous observation (22) that part of an *H. influenzae* competent culture exposed to *H. parainfluenzae* DNA in nongrowth medium and later transferred to growth medium begins to lyse about 100 min after the start of the exposure. It is reasonable that more ^{32}P label than ^{3}H resident label should be lost, since there is evidence that only those *H. influenzae* cells in which more of the *H. parainfluenzae* DNA is integrated become subject to its lethal effect (2).

All of these observations suggest that eventual fixation of heterospecific DNA information is not a very efficient process and requires a much longer time than does the fixation of homospecific DNA. The conversion to resident DNA-type information is gradual.

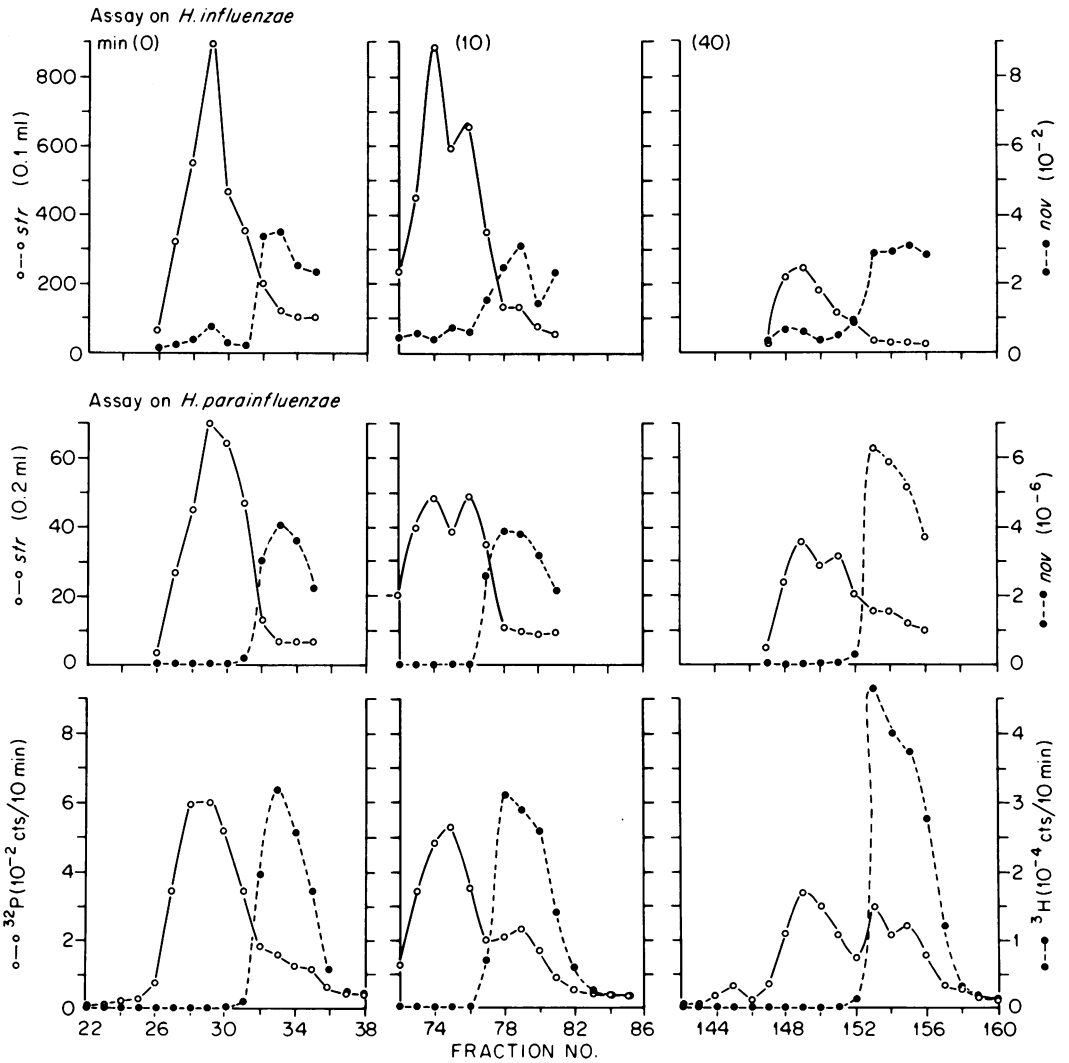

FIG. 4. *Distribution of radioactivity and transforming activity of lysates of ^{3}H-labeled H. parainfluenzae nov cells exposed to ^{32}P-labeled, heavy H. influenzae str DNA and centrifuged to equilibrium in CsCl gradients. Times shown are after 5 min of DNA uptake and 3 min of incubation with deoxyribonuclease.*

DISCUSSION

It was shown earlier than competent *H. influenzae* takes up *H. parainfluenzae* DNA as well as it does *H. influenzae* DNA (22). We have also presented evidence that competent *H. parainfluenzae* does not discriminate in uptake of DNA from the two species. These observations indicate that DNA uptake is not a factor in the low transformation between these species, and that other steps in the transformation process must be considered.

One possible factor in low heterospecific transformation is degradation by restriction enzymes (11, 20), which could make some of the incoming heterospecific DNA too short for efficient pairing. Our measurements of association between heavy transforming DNA and light recipient DNA indicate that about 50% of the *H. parainfluenzae* DNA label taken up by *H. influenzae* cells becomes associated with the *H. influenzae* genome, whereas there is only about 20% association in the reciprocal cross. These data suggest that the action of restriction enzymes in preventing association of donor and recipient DNA does not alone account for the low heterospecific transformation. The *nov* marker, for example, is transformed three to five orders of magnitude less efficiently in heterospecific than in homospecific transformation between these species, and the corresponding difference in transformation of the *str* marker is about two orders of magnitude (2).

The observed decrease in the amount of donor DNA label association in heterospecific as compared with homospecific transformation (about 30% for *H. parainfluenzae* donor DNA and about 75% for *H. influenzae* donor DNA) could also result from the inhomology between the two DNAs. The homology between the DNAs of the two species has been found by hybridization measurements to be about 50% (4). However, the lack of information on the distribution of the nonhomologous regions makes it difficult to correlate the amount of homology with the amount of DNA-DNA association of label. The fact that association of donor DNA label with resident DNA is reduced below the level of homology in heterospecific transformation of *H. parainfluenzae* but not of *H. influenzae* may reflect a greater efficiency of the *H. parainfluenzae* restriction enzyme for certain DNAs (11) or, alternatively, a failure of *H. parainfluenzae* cells to utilize the degradation products of donor DNA.

On the other hand, the depression of association of DNA label in heterospecific transformation of *H. influenzae* cells is *less than* the level of inhomology. This might indicate that the associated DNA label includes the sum of the macromolecular DNA hybrids and resynthesis with donor atoms from degraded incoming DNA. Alternatively, nonhomologous regions of donor DNA may become associated with resident DNA when these regions adjoin homologous regions of donor DNA. The latter hypothesis is in accord with the observations of the lethal effect of *H. parainfluenzae* DNA on competent *H. influenzae* cells (1, 2, 22).

Association of donor DNA label may or may not represent completed integration of single strands of heterospecific donor material. Integration of *H. parainfluenzae* DNA into the *H. influenzae* cell DNA has previously been measured from the amount of resident DNA label released to the medium, presumably as a consequence of the completion of integration (2, 22). Steinhart and Herriott (22) presented evidence that the amount of resident DNA label released is equivalent to the amount of donor DNA integrated. From such measurements, these authors reported the ratio of heterospecific to homospecific integration as 60%. Beattie and Setlow (2) reported a value of 20%. The decrease with time in the biological activity of associated donor *H. parainfluenzae* marker in *H. influenzae* cells, assayed on *H. parainfluenzae* (Fig. 3), may represent the elimination of incompletely integrated donor material, or some of the integrated stretches of DNA may contain mispaired regions that become susceptible to degradation.

It is remarkable that at early times heterospecific donor DNA associated with resident DNA yields many more donor marker transformants on the donor DNA species than on the recipient DNA species (Fig. 3 and 4). This suggests that, at least initially, the genetic information transferred to resident DNA behaves more like the donor type. We infer from this that relatively long stretches of donor DNA material are initially associated with resident DNA. (Resident DNA transforms the resident DNA species much more efficiently.) When the *H. influenzae* culture which had been exposed to *H. parainfluenzae* DNA was grown for several hours, much of the associated donor marker activity was lost, although it still gave about as many transformants on the donor species as on the recipient species (Fig. 5). Many of the cells containing donor marker may cease to be viable, in accordance with the experiment reported previously (2), in which it was shown that *H. influenzae* donor DNA *str* marker extracted from surviving viable *H. parainfluenzae* cells gives about 30 times more

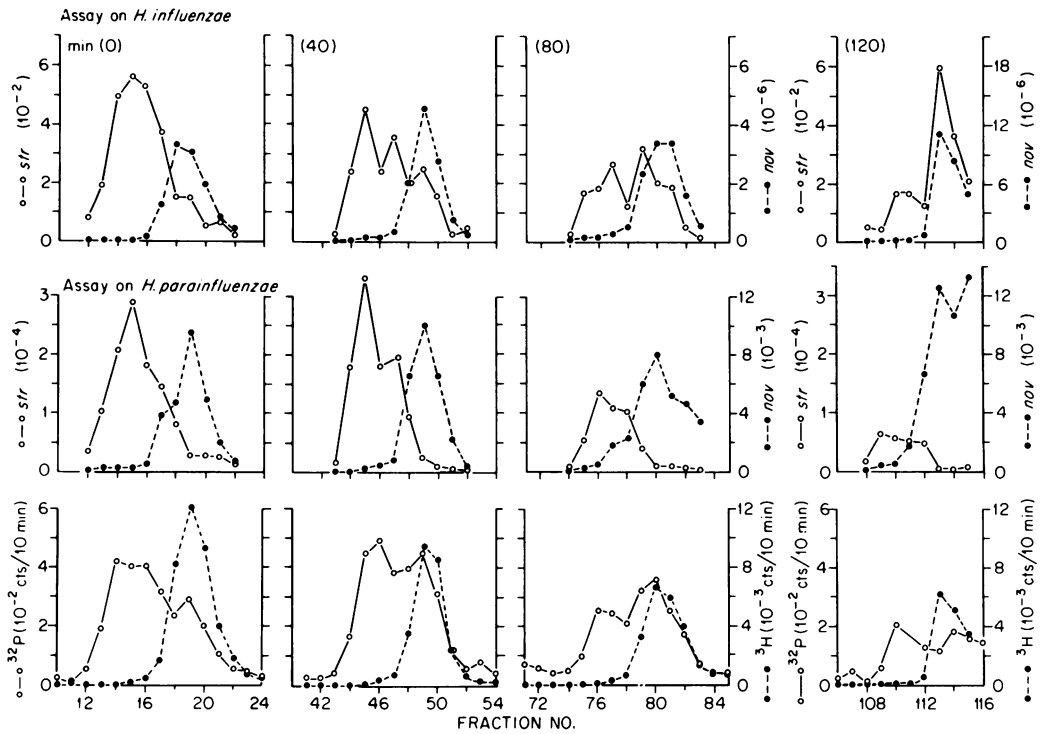

FIG. 5. *Distribution of radioactivity and transforming activity of lysates of ^{3}H-labeled H. influenzae nov cells exposed to ^{32}P-labeled, heavy H. parainfluenzae str DNA for 5 min in MIV medium at 37 C, followed by 12 min of incubation with deoxyribonuclease. The culture was diluted into warm growth medium and further incubated for the times shown before lysis and equilibrium centrifugation.*

efficient transformation on *H. parainfluenzae* than on *H. influenzae*. However, the efficiency of transformation by this DNA on *H. influenzae* was higher than by DNA extracted from *H. parainfluenzae* cells made *str* by mutation. Thus, it appears that the surviving heterospecific transformants contain even less DNA homologous to the original donor species than do transforming cells grown several hours.

ACKNOWLEDGMENTS

This investigation was sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corp. N. K. Notani was supported by a National Science Foundation Senior Foreign Scientist Fellowship and was on leave of absence from Bhabha Atomic Research Centre, Bombay, India. We thank R. F. Grell, R. F. Kimball, and R. B. Setlow for helpful comments on the manuscript.

LITERATURE CITED

1. Beattie, K. L., and J. K. Setlow. 1969. Killing of *Haemophilus influenzae* cells by integrated ultraviolet-induced lesions from transforming deoxyribonucleic acid. J. Bacteriol. **100**:1284-1288.
2. Beattie, K. L., and J. K. Setlow. 1970. Transformation between *Haemophilus influenzae* and *Haemophilus parainfluenzae*. J. Bacteriol. **104**:390-400.
3. Bodmer, W. F., and A. T. Ganesan. 1964. Biochemical and genetic studies of integration and recombination in *Bacillus subtilis* transformation. Genetics **50**:717-738.
4. Boling, M. E. 1972. Homology between the deoxyribonucleic acid of *Haemophilus influenzae* and *Haemophilus parainfluenzae*. J. Bacteriol. **112**:745-750.
5. Brunk, C. F., and V. Leick. 1969. Rapid equilibrium isopycnic CsCl gradients. Biochim. Biophys. Acta **179**: 136-144.
6. Carrier, W. L., and R. B. Setlow. 1971. Paper strip method for assaying gradient fractions containing radioactive macromolecules. Anal. Biochem. **43**:427-432.
7. Dubnau, D., I. Smith, P. Morell, and J. Marmur. 1965. Gene conservation in Bacillus species. I. Conserved genetic and nucleic acid base sequence homologies. Proc. Nat. Acad. Sci. U.S.A. **54**:491-498.
8. Earle, W. R. 1943. Production of malignancy *in vitro*. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Nat. Cancer Inst. **4**:165-212.
9. Fox, M. S., and M. K. Allen. 1964. On the mechanism of deoxyribonucleate integration in pneumococcal transformation. Proc. Nat. Acad. Sci. U.S.A. **52**:412-419.
10. Goodgal, S. H., and E. H. Postel. 1967. On the mechanism of integration following transformation with single-stranded DNA of *Hemophilus influenzae*. J. Mol. Biol. **28**:261-273.
11. Gromkova, R., and S. H. Goodgal. 1972. Action of Haemophilus endodeoxyribonuclease on biologically active deoxyribonucleic acid. J. Bacteriol. **109**:987-992.
12. Hotchkiss, R. D., and M. Gabor. 1970. Bacterial transformation with special reference to recombination process. Annu. Rev. Genet. **4**:193-224.

13. Kelly, T. J., Jr., and H. O. Smith. 1970. A restriction enzyme from *Hemophilus influenzae*. II. Base sequence of the recognition site. J. Mol. Biol. **51**:393–409.
14. Nickel, L., and S. H. Goodgal. 1964. Effect of interspecific transformation on linkage relationships of markers in *Haemophilus influenzae* and *Haemophilus parainfluenzae*. J. Bacteriol. **88**:1538–1544.
15. Notani, N. K. 1971. Genetic and physical properties of unintegrated donor DNA molecules during *Hemophilus transformation*. J. Mol. Biol. **59**:223–226.
16. Notani, N. K., and S. H. Goodgal. 1966. On the nature of recombinants formed during transformation in *Hemophilus influenzae*. J. Gen. Physiol. **49** (part 2): 197–209.
17. Notani, N. K., J. K. Setlow, V. R. Joshi, and D. P. Allison. 1972. Molecular basis for the transformation defects in mutants of *Haemophilus influenzae*. J. Bacteriol. **110**:1171–1180.
18. Ravin, A. W., and K.-C. Chen. 1967. Heterospecific transformation of Pneumococcus and Streptococcus. III. Reduction of linkage. Genetics **57**:851–864.
19. Setlow, J. K., D. C. Brown, M. E. Boling, A. Mattingly, and M. P. Gordon. 1968. Repair of deoxyribonucleic acid in *Haemophilus influenzae*. J. Bacteriol. **95**:546–558.
20. Smith, H. O., and K. W. Wilcox. 1970. A restriction enzyme from *Hemophilus influenzae* I. Purification and general properties. J. Mol. Biol. **51**:379–391.
21. Steinhart, W. L., and R. M. Herriott. 1968. Fate of recipient deoxyribonucleic acid during transformation in *Haemophilus influenzae*. J. Bacteriol. **96**:1718–1724.
22. Steinhart, W. L., and R. M. Herriott. 1968. Genetic integration in the heterospecific transformation of *Haemophilus influenzae* cells by *Haemophilus parainfluenzae* deoxyribonucleic acid. J. Bacteriol. **96**:1725–1731.