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Mechanism of Acquisition of Chromosomal Markers by Plasmids in *Haemophilus influenzae*

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The hybrid plasmid pNov1 readily acquired genetic information from the chromosome of wild-type, but not *rec-2*, cells. Most of the recombination had taken place 1 h after entrance of the plasmid into the cell, as judged by transformation of *rec-2* by lysates made from wild-type cells exposed to pNov1. Measurement of physical transfer from radioactively labeled cellular DNA to plasmids recombining in wild-type cells failed, since there was little more radioactivity in plasmids from such cells than from labeled *rec-2* recipients, in which no recombination took place. *Eco*RI digestion of pNov1 divided the DNA into a 1.7-kilobase-pair fragment containing the novobiocin resistance marker and a 13-kilobase-pair fragment containing all of the original vector and considerable portions homologous to the chromosome. Transformation by the large fragment alone resulted in a plasmid the size of the original pNov1. Our hypothesis to explain the data is that genetic transfer from chromosome to plasmid took place by a copy choice mechanism.

The recombinant plasmid pNov1 carries an ampicillin resistance marker from the parent plasmid RSF0885 (3) and a novobiocin resistance marker from a cloned piece of chromosomal DNA (15). When pNov1 enters a competent cell, two types of transformation may occur, resulting in three types of transformants. In the most frequent type, the novobiocin marker integrates into the chromosome, and the plasmid is lost from the cell, so that the recipient is novobiocin resistant and ampicillin sensitive. The plasmid may also become established, conferring ampicillin resistance on the cell, and sometimes also novobiocin resistance. However, most of the ampicillin transformants are novobiocin sensitive, and the novobiocin resistance marker cannot be detected by transformation of novobiocin-sensitive strains by lysates of these cells (15). The loss of the novobiocin resistance marker from pNov1 has been postulated to result from a recombination event between chromosome and plasmid, in which chromosomal genetic information for novobiocin sensitivity is acquired by the plasmid. The evidence for this conclusion is that (i) Rec^+ genes are required for the plasmid alteration, (ii) there is no change in the plasmid when the recipient already carries a novobiocin resistance marker, and (iii) purified plasmids from wild-type novobiocin-sensitive ampicillin transformants readily transform novobiocin-resistant cells to sensitivity (15).

In this paper we use the term recombination to mean transfer of genetic information from one DNA molecule to another, without specifying a mechanism. Since the recombination (gene transfer from chromosome to plasmid) is extraordinarily high, involving up to 70% of the plasmid transformants, it seemed feasible to investigate the mechanism of this process by physicochemical combined with biological techniques. One approach was to allow plasmids to enter competent, radioactively labeled cells, incubate the cells in growth medium, and then remove the intracellular plasmids from the cells for transformation or radioactivity assays. A second approach was to cut out the novobiocin marker from the plasmid with a restriction endonuclease, transform with the remaining linear portion missing the novobiocin marker, and examine the nature of the plasmid of the transformant. The results of these experiments suggested that the transfer of genetic information from chromosome to plasmid took place in the absence of physical transfer of DNA, by a copy choice mechanism.

MATERIALS AND METHODS

Microorganisms and transformation. The wild-type *H. influenzae* strain Rd and BC200, the recombination-defective *rec-1* and *rec-2* mutants of these strains, and growth media have been described previously (1, 12, 13). The plasmid pNov1 (15) confers resistance to 25 μg of novobiocin per ml and 5 μg of ampicillin per ml. Transformation was carried out in MIV medium by the method of Steinhart and Herriott (17).

Plasmid purification. Plasmid preparations, either as crude cleared lysates or highly purified DNA, were made as described previously (9).

Restriction mapping and separation of fragments from gels. Double restriction endonuclease digests were used for mapping. Fragments were electroeluted from gels by a standard method (6).

Determination of transforming activity of intracellular plasmids. The basic plan of the experiments is shown in Fig. 1. Two sets of cells were used, wild-type Rd (Rec^+) and *rec-2* (Rec^-). Competent cells (24 ml), Rec^+ or Rec^-, were exposed to plasmid DNA for 10 min in MIV medium (17) and then centrifuged and suspended at the same concentration in growth medium. They were incubated with vigorous shaking for various times, 0.1-ml samples were taken for transformation assays, and 6 ml was used to prepare a cleared lysate. Each of the lysates was assayed for transformation on Rec^+ and Rec^- cells. In some experiments ampicillin-resistant colonies from the first transformation were picked, grown up in ampicillin, and spotted on plates containing ampicillin or ampicillin and novobiocin to determine the fraction of the plasmid transformants that had recombined with the chro-

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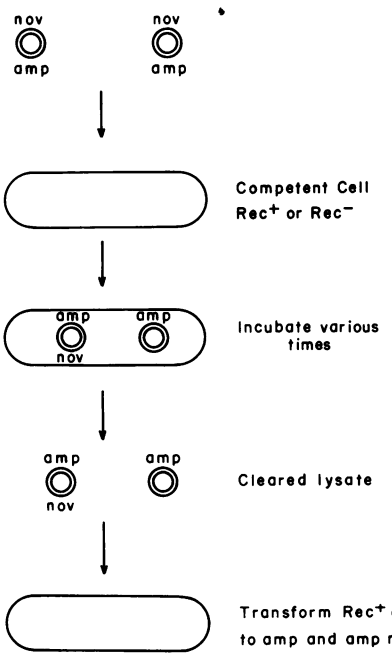

FIG. 1. Scheme for measuring transforming activity of intracellular plasmids. In the example given, one of the two original plasmids carrying a novobiocin (nov) resistance marker has lost this marker, and it is known from previous work (15) that the plasmid has acquired the information for novobiocin sensitivity in place of novobiocin resistance. amp, Ampicillin.

mosome to produce pNov1s carrying novobiocin sensitivity information.

Attempt to show physical transfer of DNA from chromosome to plasmid. The basic plan of the experiments was similar to that of Fig. 1, except that the cells were radioactively labeled and also contained a chromosomal genetic marker, and the plasmids of the cleared lysate were further purified by two ethidium bromide-cesium chloride equilibrium sedimentations to look for transfer of the label from chromosome to plasmid. The *rec-2* strain was used as a control, since no evidence of chromosome-to-plasmid genetic transfer was observed in this strain (Table 1), and since there is no more DNA degradation in this strain than in the wild type (4).

Streptomycin-resistant Rd or *rec-2* cells (120 ml) were grown with aeration in supplemented brain heart infusion (13) containing 2 mg inosine per ml and 2 mCi of [^{3}H]thymidine (72 Ci/mmol). When the cells reached a concentration of about 2×10^8/ml, the culture was centrifuged twice, and the cells were suspended in MIV medium at the same concentration and incubated for 100 min without radioactivity. The fully competent culture was then exposed to purified plasmid DNA (around two plasmid molecules per cell) for 20 min, centrifuged, suspended at the same concentration in growth medium, and incubated for 1 h, again without radioactive label. The specific activity at the end of this time was 2.7×10^5 cpm/μg of DNA, based on trichloroacetic acid-insoluble counts from samples, the DNA content per cell (Roger M. Herriott, personal communication), and the measured number of CFU (around 2×10^9 CFU/ml of both Rec$^+$ and Rec$^-$ cells).

A cleared lysate was made from the culture, eliminating about 99% of the radioactive counts along with most of the chromosomal DNA. The lysate was then centrifuged to equilibrium in ethidium bromide-cesium chloride (10). Since no plasmid band could be observed by visual inspection of the centrifuge tube, five fractions were taken and assayed on the Rec$^+$ strain for transformation to ampicillin resistance (a measure of the amount of plasmid DNA present) and to streptomycin resistance (a measure of the amount of chromosomal DNA present). The radioactivity of the fractions was also measured on filter paper disks processed through trichloroacetic acid with final washes in alcohol and acetone and counted in Betafluor (National Diagnostics). Fractions that transformed to ampicillin resistance were dialyzed and centrifuged in ethidium bromide-cesium chloride, and fractions were collected and assayed as before.

RESULTS AND DISCUSSION

Transforming activity of intracellular plasmids during pNov1 transformation. The experiments testing the transforming activity of intracellular plasmids (Fig. 1) were undertaken to gain information on the intracellular kinetics of the change of pNov1 to the novobiocin-sensitive form of the plasmid. This information was essential for deciding when to take samples in the experiments with radioactively labeled chromosomes (see below).

Table 1 shows the results of lysate experiments with the recombination-defective *rec-2* strain and the wild-type Rd strain. In the case of *rec-2* cells, the transforming activity of pNov1 showed little or no change with time of incubation, judged by ampicillin transformation from the *rec-2* lysates as assayed on *rec-2* or Rd cells. It is also seen from lysates of *rec-2* assayed on *rec-2* cells that the ratio of double ampicillin-novobiocin transformants to single ampicillin transformants remained at about 1, showing that pNov1 did not lose its novobiocin marker in *rec-2* cells. This confirms previous results indicating that the recombination defect of *rec-2* eliminates transfer of information from chromosome to plasmid (15). However, when the *rec-2* lysates were assayed on the wild type a decrease in the ratio occurred. This decrease was even more dramatic in other similar experiments carried out for longer incubation times, in which the loss of novobiocin resistance assayed in Rd cells from the

TABLE 1. Transformation by cleared lysates of cells being transformed by pNov1[a]

Original recipient	Recipient for lysate assay	Time of incubation in growth medium before lysis (min)	No. of transformants per ml (10^2)		Ampicillin-novobiocin/ Ampicillin
			Ampicillin	Ampicillin-novobiocin	
rec-2	*rec-2*	0	0.89	0.93	1.0
		10	1.1	1.2	1.1
		30	1.3	1.1	0.9
		60	1.4	1.4	1.0
	Rd	0	437	97	0.22
		10	547	133	0.24
		30	468	87	0.19
		60	401	52	0.13
Rd	Rd	0	1,250	460	0.37
		10	1,170	390	0.33
		30	750	250	0.33
		60	610	190	0.31
	rec-2	60	3.0	1.4	0.47

[a] See Fig. 1. The frequency of transformation to ampicillin resistance in the original *rec-2* recipient was 5×10^{-7}; in the wild-type Rd original recipient it was 8×10^{-4}, calculated from the number of transformants divided by the total number of viable cells. For the *rec-2* transformations, from 10 to 30 plates per point were used to obtain adequate numbers of transformants.

rec-2 lysates was well over 90% after 2 h of incubation, whereas there was little change in Rd assays of Rd lysates at that time (data not shown). Since there was no recombination taking place in the *rec-2* strain (Table 1), the apparent decrease in the ratio (increase in recombination) must have been due to stimulation of recombination in the second (Rd) recipient because of a change in the plasmids inside *rec-2* cells. This change probably is a conversion to relaxed (or nicked) form. Evidence for this assertion is that lysates of *rec-2* cells incubated for 1 h after exposure to pNov1 and centrifuged to equilibrium in cesium chloride-ethidium bromide caused transformation to ampicillin resistance mostly from the position of open circular DNA (data not shown).

A complication in the experiments with Rd cells as both the original recipient and the recipient for the lysate assay is that recombination can occur during each of the two transformations. Assay of the Rd lysates on *rec-2* cells makes it possible to assess the kinetics of the transfer of novobiocin sensitivity from chromosome to plasmid in Rd, since in the *rec-2* recipient there is no transfer (Table 1) (15). From the data of the 60-min lysate of Rd cells titered on *rec-2* cells (Table 1), the ratio of ampicillin-novobiocin transformants to ampicillin transformants is 0.47, indicating that there had been substantial recombination (100 − 47, or 53%) by 60 min. The final amount of recombination in Rd was 64%, as judged by assay of ampicillin-resistant colonies resulting from the first transformation of Rd cells. Thus the data of Table 1 show that 53 of 64 or 83% of the total recombination was already completed in the Rd strain by 60 min.

We have attempted to obtain data from the earlier time points of Rd lysates assayed on *rec-2* cells, but even with 40 plates per point the statistics were very poor. However, it was clear that both the single and double transformations increased markedly with time before lysis, by approximately a factor of 30 over a 60-min period (data not shown). This is in contrast to the decrease in transformation seen when the same lysates were assayed on Rd cells (Table 1). The latter data are in accord with the degradation of radioactively labeled plasmid DNA after entrance into the competent Rd cell, as seen by conversion of trichloroacetic acid-insoluble to trichloroacetic acid-soluble radioactive counts (15). The difference in the transformation by lysates of Rd assayed on Rd cells and on *rec-2* cells reflects the greater difficulty in establishment of plasmids in *rec-2* cells (15). The increase in *rec-2* transformation with time of lysis may mean that the plasmids must first be processed in some way inside the Rd strain to be able to transform *rec-2* cells.

Inability of pNov1s carrying novobiocin sensitivity to recombine. Since the transfer of novobiocin sensitivity from chromosome to plasmid has been seen to occur with high frequency (60 to 70%), it would be expected that similarly there would be acquisition of novobiocin resistance from the chromosome of a resistant cell by the incoming plasmid pNov1s carrying information for novobiocin sensitivity. However, such was not the case. Rd cells were exposed to pNov1s for 20 min, followed by incubation for 1 h in growth medium before a cleared lysate was made. The lysates as assayed on Rd yielded 5.4×10^4 ampicillin transformants per ml, 9.4×10^5 novobiocin transformants per ml, and 1.5×10^2 ampicillin-novobiocin transformants per ml. The frequency of double transformants among the ampicillin-resistant transformants was no more than would be expected from the frequency of novobiocin transformants in the total population. Thus the double transformants are considered to be mostly the result of independent transformation events, from pNov1s for ampicillin resistance and from chromosomal DNA in the cleared lysate for novobiocin resistance. We cannot exclude the possibility that there was a very small fraction of the plasmids that had acquired the novobiocin resistance marker from the chromosome. However, it is clear that if such transfer took place at all, it did so at a very low frequency compared with the frequency with which pNov1 acquires novobiocin sensitivity (15).

Chromosomal novobiocin sensitivity may be dominant over the lower-level novobiocin marker (conferring resistance to 2.5 μg/ml) on the plasmid pDM2 (14). However, this possible dominance cannot explain the present results because the high-level novobiocin resistance marker used here is expressed on a plasmid even when the recipient's chromosome codes for novobiocin sensitivity (15).

The inability of pNov1s to undergo chromosome-to-plasmid gene transfer could have resulted from selective pressure on the Rd strain carrying the plasmid pNov1. Cells carrying pNov1 contain somewhat more DNA gyrase activity than do cells with pNov1s (16). Elevated gyrase can cause induction of defective phage in Rd and thus outgrowth of cells lacking the plasmid (16). To test the hypothesis of selective pressure, we have used as a recipient a novobiocin-resistant strain, BC200, which lacks an inducible defective phage (11). After exposure of the cells to pNov1s, they were grown, and a cleared lysate was made. Novobiocin-sensitive BC200 cells were exposed to the lysate and transformed to ampicillin resistance. Three percent (6 of 174) of these transformants were also novobiocin resistant, as determined by growth of cultures of the transformants on plates containing both novobiocin and ampicillin. To eliminate the possibility that these clones were also the result of two independent recombination events, i.e., a double transformation from a chromosomal novobiocin marker and the plasmid ampicillin marker, lysates were made from 18 independently obtained novobiocin- and ampicillin-resistant transformants and assayed on BC200*rec-1*. This strain is extremely defective in chromosomal transformation (with frequencies about 10^{-6} those of the parent BC200). Since all of the lysates yielded approximately the same number of ampicillin transformants as ampicillin-novobiocin transformants, we concluded that gene transfer from the chromosome to pNov1s did occur in this strain, but at a much lower level (3%) than the 60 to 70% observed with pNov1 in BC200.

The fact that pNov1s does not readily recombine to become pNov1 distinguishes this particular type of recombination from chromosomal recombination, since pNov1s readily donates the novobiocin sensitivity information to the chromosome of novobiocin-resistant wild-type cells of the same strain as used in the present experiments (15).

Physical transfer of DNA from chromosome to plasmid? Radioactively labeled Rd (Rec^+) or *rec-2* (Rec^-) cells were exposed to pNov1 and incubated for 1 h before lysis, a time shown (see above) to allow most of the gene transfer from chromosome to plasmid to take place in the Rec^+ strain. The lysate was then fractionated by equilibrium centrifugation, and fractions were analyzed for radioactivity and chromosomal and plasmid DNA. A second centrifugation was performed with fractions containing plasmid DNA and analyzed as before. The data from these analyses of the fractions containing plasmid DNA are shown in Table 2. The radioactivity in these fractions, containing a large part of the plasmid DNA and a small amount of chromosomal DNA, represented an exceedingly small part of the 2×10^7 cpm incorporated into DNA before the start of the plasmid purification. Thus there had not been a substantial amount of breakdown of chromosomal DNA and incorporation of

TABLE 2. Calculation of the maximum number of bases in plasmid DNA that could have come from the radioactive chromosome[a]

Phenotype	Ampicillin transformants per ml	Plasmid DNA[b] (μg/ml)	Streptomycin transformants per ml	Chromosomal DNA[b] (μg/ml)	Observed cpm/ml	cpm/ml in chromosomal DNA[c]	cpm/ml in plasmid DNA if fully labeled[c]	Maximum fraction of plasmid DNA labeled[d]	Maximum no. of bases in plasmid DNA that could have come from the chromosome[e]
Rec^+	8×10^4	0.3	1.6×10^4	1.6×10^{-4}	375	48	0.9×10^5	0.003	90
Rec^-	1.2×10^5	0.4	6.1×10^4	6.1×10^{-4}	385	183	1.2×10^5	0.002	60

[a] Based on the data of the plasmid fraction from the second cesium chloride-ethidium bromide centrifugation.
[b] The amount of plasmid or chromosomal DNA was calculated from the number of ampicillin- or streptomycin-resistant transformants, respectively, with the use of calibration curves of number of transformants as a function of concentration of pNov1 or chromosomal DNA containing a streptomycin resistance marker (data not shown).
[c] The product of the calculated amount of chromosomal or plasmid DNA and the specific activity (2.7×10^5 cpm/μg). In the case of plasmid DNA, fully labeled means that the DNA had the same specific activity as the total DNA.
[d] The actual counts per minute in plasmid DNA were taken to be the observed counts minus the calculated counts of chromosomal DNA. The maximum fraction of labeled plasmid DNA was then the ratio of actual counts to counts if fully labeled.
[e] The product of the maximum fraction of labeled plasmid DNA and the number of bases in pNovI (29,400).

radioactivity into plasmid DNA. There was also no appreciable plasmid replication during the hour of incubation after plasmid DNA uptake, since we showed previously (15) that the amount of degradation of radioactively labeled pNov1 DNA after this time is approximately the same as the decrease in ampicillin transformation of strain Rd by this DNA.

The numbers in Table 2 representing calculated maximum physical transfer are approximately the same for Rec^+ and Rec^- cells, but only the Rec^+ cells undergo chromosome-to-plasmid gene transfer (Table 1) (15). Therefore we conclude that the plasmid radioactivity in both strains must have arisen from a mechanism other than physical transfer of DNA, such as a very small amount of chromosomal breakdown and resynthesis into plasmid DNA. The calculated net number of radioactive bases in plasmid DNA from the Rec^+ cells (30 with the control *rec-2* counts subtracted) was equivalent to only about 0.002 times the 18,000 bases in the homologous portion of the plasmid available for recombination (15). This value we consider to be much too low to account for the observed recombination frequency of 53% obtained with only one part of the available homologous DNA.

Recombination of transforming DNA in *H. influenzae* has been estimated to include an average of around 9,000 bases (7). Thus the gene transfer we have observed could have included as much as half the homologous region, and the genetic data suggested that more than half of the molecules took part. Our estimate of the number of radioactive bases in the plasmids if there had been physical transfer is 9,000/4, or more than 2,000, several orders of magnitude greater than the observed value.

Transformation by pNov1 cleaved with restriction enzymes. If transfer of genetic information from chromosome to plasmid occurs by a copy mechanism rather than by physical transfer, it might be expected that ampicillin transformation could be carried out by plasmids with deletions in the parts homologous to the chromosome, and that plasmids from such transformants would be the same size as the original intact plasmids. On the other hand, cleavage in the parent plasmid portion with no chromosome homology would be expected to eliminate ampicillin transformation.

Figure 2 shows a restriction map of pNov1. *Bst*EII is one of the two restriction enzymes we have investigated that makes a cut in the RSF0885 portion of pNov1, but not anywhere else. *Hae*II, *Sst*II, *Xho*I, *Cla*I, and *Eco*RI cleave in the portion homologous to the chromosome, but not in the RSF0885 portion. We also found that *Thh*III, *Sma*I, *Sal*I, and *Sal*II made no cleavages in pNov1, and *Hpa*II made five.

Treatment with *Bst*EII or *Pvu*I caused the ampicillin transformation by pNov1 to drop more than 2 orders of magnitude (Table 3). We suspect that the residual transformation resulted from a few uncut molecules (which could not be seen on our gels). However, *Eco*RI and *Xho*I considerably increased the ampicillin transformation over that of the control (Table 3). These results are unlikely to have been caused by reduction of gyrase expression, since the largest effect was obtained with *Xho*I, which makes a single cleavage a considerable distance from the gyrase gene located between the *Eco*RI sites (Table 4). We interpret the transformation data of Table 3 as a reflection of the greater probability of pairing with the chromosome by the linear forms of the plasmid, since this pairing apparently protects some of the plasmid DNA from degradation (15). The stimulation is lower for *Eco*RI degradation, presumably because it cuts out

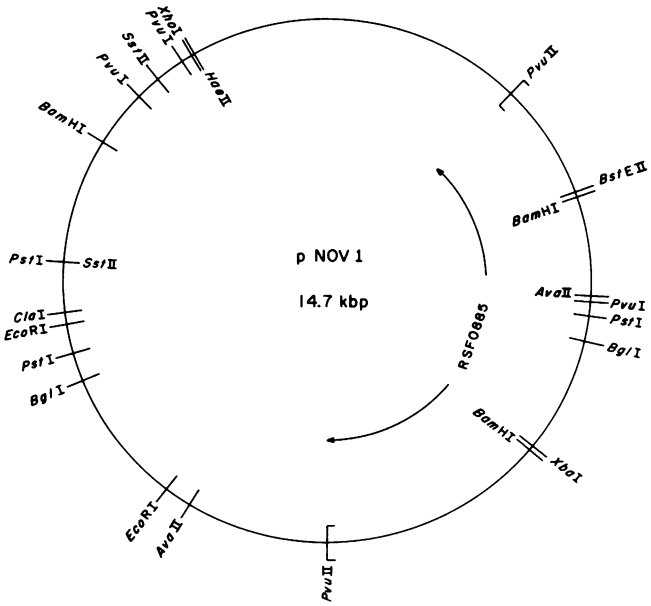

FIG. 2. Restriction endonuclease map of pNov1. The portion consisting of the parent plasmid RSF0885 is shown, and the remainder is a single cloned fragment of *H. influenzae* chromosomal DNA, containing the novobiocin resistance marker.

TABLE 3. Transformation of strain Rd by pNov1 treated with restriction endonucleases[a]

Restriction enzyme	Relative transformation (%)		Total ampicillin transformants tested	% Novobiocin-sensitive ampicillin transformants
	Ampicillin	Novobiocin		
None	100	100	90	68
*Eco*RI	536	5	80	100
*Xho*I	888	200	33	61
*Pvu*I	0.3	ND[b]	ND	ND
*Bst*EII	0.8	ND	ND	ND

[a] Ampicillin-resistant colonies were picked, grown in ampicillin, and spotted on plates containing ampicillin and novobiocin.
[b] ND, Not done.

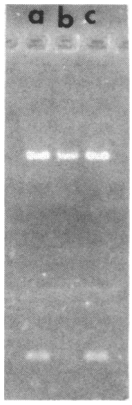

FIG. 3. Agarose gel electrophoresis of pNov1 cut with *Eco*RI (a), the electroeluted large fragment from *Eco*RI digestion (b), and the *Eco*RI-digested plasmid of a transformant by the large fragment (c). The large fragment is 13 kbp, and the small fragment is 1.7 kbp (Fig. 2).

a 1.7-kilobase-pair (kbp) piece from the plasmid (Fig. 2). Thus there is somewhat less continuous DNA available for pairing.

The novobiocin transformation decreased after *Eco*RI digestion, but not after *Xho*I digestion. The interaction between plasmid and recipient chromosome resulting in recombination was similar after *Xho*I digestion and in the control uncleaved plasmid, but ampicillin transformants from the *Eco*RI-digested molecules had all lost the novobiocin resistance. These data suggested that the novobiocin resistance marker might be close to one or both of the *Eco*RI sites. To test this idea, we separated the large and small *Eco*RI fragments and transformed with them separately. The results given in Table 4 (experiment I) show that the novobiocin resistance marker was on the 1.7-kbp fragment. The separation of the fragments was not perfect, with more contamination of the 13-kbp fragment by the 1.7-kbp fragment than the reverse, as would be expected from trailing of the small fragment on the gel, or from contamination of the large fragment with incompletely cleaved plasmid.

Ampicillin transformation by a preparation of large *Eco*RI fragment resulted in a plasmid that was the same size as the original uncleaved plasmid (Fig. 3). The plasmid of the transformant can be cleaved by *Eco*RI into two fragments the same size as the two fragments of the original *Eco*RI-cleaved plasmid. The data of Table 3 show that *Eco*RI-cleaved pNov1, the same as used for the experiment of Fig. 3, produced no novobiocin-resistant clones among the ampicillin-resistant transformants. This would not be the case if there were appreciable numbers of transformants from uncleaved molecules remaining after *Eco*RI digestion. Therefore we conclude that the transformant used for the gel of Fig. 3 is representative of the class of transformants from the large fragment alone.

The data in Table 3 suggested that when *Eco*RI-digested pNov1 without fragment separation was used to transform

TABLE 4. Transformation of strain Rd by large and small *Eco*RI fragments of pNov1 and pNov1s

Expt	Fragment source (kbp)	Transformants/ml		Ampicillin/ novobiocin
		Ampicillin	Novobiocin	
I	pNov1 (13)	4.5×10^6	1.2×10^4	375
	pNov1 (1.7)	1.4×10^3	1.9×10^6	0.0007
II	pNov1 (13)	8.4×10^6		
	pNov1 (1.7)	1.0×10^3	8.8×10^5	
	Large and small fragments together	8.5×10^6	2.1×10^5	

recombination proficient hosts, there was little or no contribution of genetic information from the small fragment to the established plasmid. A further test of this conclusion was experiment II of Table 4. To avoid any novobiocin resistance transformation by contamination of the 13-kbp fragment preparation, we used the 13-kbp fragment obtained from the plasmid carrying novobiocin sensitivity (pNov1s) and the 1.7-kbp fragment from pNov1. Each cell was exposed to around 15 fragments of each type. The presence of the small fragment did not affect the ampicillin transformation by the large fragment, but the large fragment depressed novobiocin transformation by the small fragment, presumably because of competition by the larger homologous portion. Eighty-three ampicillin-resistant transformants by the two fragments together were picked, grown in ampicillin, and then tested for novobiocin resistance. Since all were sensitive to that antibiotic, it was concluded that the small and large fragments rarely or never combined inside a recipient by pairing next to each other on the homologous portion of the chromosome to produce plasmid progeny carrying genetic information from both fragments.

Are the results peculiar to pNov1? Since there is some selective pressure against pNov1, because of the increased probability of induction of defective phage (16), we consider here whether our results have any general application. Several other plasmids, approximately the same size as pNov1 but carrying different antibiotic resistance markers, lost their antibiotic resistance markers upon transformation into the wild type with approximately the same high frequency as did pNov1 (J. K. Setlow, unpublished data). Furthermore, it was rare that the homologous chromosomal resistance marker was transferred from the chromosome to the plasmid carrying the information for sensitivity to the antibiotic. In all cases transformation of the resistance marker from the plasmid to the wild-type chromosome took place with high frequency from a saturating amount of plasmid. Thus in all these respects these other plasmids behaved like pNov1.

Comparison with other plasmid systems. In several other systems it has been reported that plasmids can acquire homologous genetic information from chromosomes. In *Bacillus subtilis* the frequency of this type of recombination is considerably lower than for pNov1 (2), but as in *H. influ-*

enzae there is a strict requirement for Rec^+ genes. In *Streptococcus pneumoniae* there is also such recombination, and it is possible to regenerate a plasmid by transformation when the incoming molecules have had portions removed by restriction endonucleases (5). Among several models proposed to explain these data is the copy mechanism we favor as an explanation for our results. Since the DNA is single stranded after entry into *S. pneumoniae* (8), this model does not require any extra assumptions about why the information from the second strand of the intact recombining plasmid is not preserved in the cell. However, since DNA enters *H. influenzae* in double-stranded form (7), and since no novobiocin resistance marker is found in lysates of ampicillin transformants of pNov1 where chromosome to plasmid gene transfer has taken place (15), we assume that one of the two strands is not paired with the chromosome. We further postulate that when part of the chromosome is copied during plasmid replication, the unpaired strand is broken down by nucleolytic action, and the plasmid is then replicated to completion from the already copied strand containing chromosomal genetic information.

Comparison of chromosome-to-plasmid and plasmid-to-chromosome genetic transfer in *H. influenzae*. The transfer of information from plasmid to chromosome differs from the chromosome-to-plasmid transfer in two respects. (i) The novobiocin resistance marker readily enters the chromosome from the plasmid, but we have seen that it rarely went from chromosome to plasmid, even under nonselective conditions. (ii) When the chromosome was transformed for a novobiocin marker by the plasmid and there was no selection for novobiocin, only about half of each transformant colony isolated contained the novobiocin marker (J. K. Setlow, unpublished data), suggesting that the mechanism of this transfer is a single-strand insertion like that of transformation of chromosomal DNA, known to involve physical transfer of DNA (8). Our hypothesis is that plasmid-to-chromosome transfer of information goes by a physical transfer mechanism, whereas chromosome-to-plasmid transfer goes by a copying mechanism.

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