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Minicell Production and Bacteriophage Superinducibility of Thymidine-Requiring Strains of *Haemophilus influenzae*

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Aminopterin- or trimethoprin-resistant thymidine-requiring strains of *Haemophilus influenzae* produce minicells, and the ratio of minicells to cells increases during the stationary phase of growth. Strain LB11, isolated after mutagenesis of a thymidine-requiring strain (*Rd thd*), produces more minicells than the parent strain. The mutations involved in high frequency minicell production have been transferred into the wild type (strain *Rd*) by transformation. The thymidine requirement in the resulting strain, MC1, is essential for minicell production, since spontaneous revertants of MC1 to prototrophy do not produce minicells. The ratio of minicells to cells was increased more than 10^3-fold by differential centrifugation. The minicells contain little or no deoxyribonucleic acid (DNA). Phage HP1*c*1 apparently cannot attach to minicells. Competent cells of LB11 and its thymidine-requiring parent strain produce defective phage as a result of exposure to transforming DNA, whereas only LB11 produces many defective phage in response to the competence regime alone. Competent HP1*c*1 and S2 lysogens of MC1 and *Rd thd* are also superinducible by transforming DNA, but competent LB11 lysogens produced about the same amount of HP1*c*1 or S2 phage with or without exposure to transforming DNA possibly because of competition between the induced defective phage and Hp1*c*1 or S2 phage.

Minicell-producing strains of *Escherichia coli* (1, 11), *Bacillus subtilis* (16), and *Salmonella typhimurium* (23) have been isolated and studied. Minicells are produced as a result of cell division occurring at sites near the cell poles as opposed to the normal midcell division site (1, 16). Minicells from these bacteria are approximately spherical and contain little or no deoxyribonucleic acid (DNA) (1, 16, 23). Several methods have been developed for the separation of minicells from cells, and the purified minicells have been used to study a number of biological problems (5).

We have found that all aminopterin- or trimethoprin-resistant, thymidine-requiring mutants of *Haemophilus influenzae* can produce minicells. The introduction of at least one other mutation results in an increased yield of minicells in strain LB11, and these mutations have been transferred into the wild-type strain. LB11 is superinducible for defective phage during transformation (17). This characteristic is associated with the thymidine requirement. The competence regime alone induces many phage in LB11 but in the parental thymidine-requiring strain.

MATERIALS AND METHODS

Bacteria. Other thymidine-requiring strains (Table 1) were isolated by plating strain *Rd* on $M1_c$ agar (10) containing thymidine (50 μg/ml) and aminopterin (20 μg/ml) or trimethoprin (10 μg/ml). Single colony isolates were subcultured into $M1_c$ medium with and without thymidine to test the thymidine requirement. Lysogens of the strains in the table that carry prophages of HP1*c*1 and S2 bacteriophages were isolated by previously described methods (17).

Media, transformation, and phage assays. Growth media and growth conditions (18), $M1_c$ minimal medium (10), MIV competence medium (21), transformation methods, and the assay of phage released from lysogens during transformation (17) have been described.

Screening for minicell production. Cultures grown in brain heart infusion (BHI) liquid medium were examined for minicells when in the late stationary phase or after being spread on BHI agar so as to produce a dense growth after incubation for 2 days. The cells were examined under a phase contrast microscope, and the ratio of minicells to cells was determined by counting in a hemocytometer.

Attempts at mapping of minicell mutations in LB11. Competent cells of LB11 or LB11 sensitive to streptomycin and novobiocin were exposed to transforming DNA prepared from a wild-type strain resist-

TABLE 1. *Strains of Haemophilus influenzae*[a]

Strain	Genotype, isolation, and characteristics	Reference
Rd	Wild type	
Rd thd	*Rd str nov vio thd* isolated by selection for spontaneous aminopterin-resistant mutant of *Rd str nov vio*	2
LB11	A slightly ultraviolet-sensitive mutant of *Rd thd* isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. A streptomycin and novobiocin-sensitive variant was obtained after transformation by DNA from a kanamycin-resistant strain, these three markers being closely linked on the *H. influenzae* genetic map (9).	17
LB11 *rec1*	Recombination defective strain isolated by transformation of streptomycin-sensitive LB11 with DNA from a *rec1 str* strain. The procedure has been described previously (17).	This paper
MC1	Rd transformed to *ery str stv thd* by a mixture of two lysates prepared from LB11 *ery* and LB11 *stv*. MC1 was selected for its high minicell yield. The lysates were prepared as described earlier (12).	This paper

[a] Abbreviations: ery, erythromycin; nov, novobiocin; str, streptomycin; stv, streptovaricin; thd, thymidine; vio, viomycin.

ant to streptovaricin (8 μg/ml), novobiocin (25 μg/ml), kanamycin (7 μg/ml), streptomycin (250 μg/ml), erythromycin (10 μg/ml), spectinomycin (15 μg/ml), and nalidixic acid (2 μg/ml). Around 150 LB11 transformants of each drug-resistant type were selected and screened for minicell production.

Purification of minicells. Strain LB11 was grown to stationary phase in BHI growth medium and 6 ml were centrifuged for 40 s in a multispeed attachment of an International centrifuge at 14,000 × *g*. The supernatant was retained, and the pellet was resuspended in 6 ml of BHI, recentrifuged, and the second supernatant was combined with the first one. The remaining cells and most of the minicells in the supernatants were pelleted by centrifugation at 14,000 × *g* for 15 min and resuspended in 1 ml. More of the contaminating cells were removed with a final centrifugation in a Misco centrifuge for 1.5 min at 9,300 × *g*. The resulting suspensions contained less than one cell/10^3 minicells. Since stationary cultures usually contain less than one minicell/cell (Table 2), the final suspensions contained minicells purified by more than a factor of 10^3. In some experiments the suspension was further treated with around 2 × 10^8 HP1c1 phage/ml, which lysed the few remaining cells, while the minicells remained intact. The suspension was then incubated with 200 μg of deoxyribonuclease per/ml and 1 mM Mg^{2+} to degrade the remaining cell DNA.

Radioactive labeling of DNA of strain LB11. Cells were grown to saturation in BHI growth medium in the presence of 16 μCi of [^{3}H]thymidine per ml and 1 mg of inosine per ml.

Measurement of defective phage induced during transformation. Two milliliters of competent cells were incubated with or without transforming DNA at 37 C with shaking for 30 min. Four milliliters of growth medium were then added, the culture was incubated for a further 120 min and then put on ice. A mixture of 0.2 ml of culture, 0.2 ml of 1% aqueous phosphotungstic acid, and 0.04 ml of latex spheres (final concentration 3.5 × 10^{10}/ml) was sprayed onto carbon-coated parladion films supported on 300-mesh copper grids. The dried microdroplets were examined and the defective phage and spheres were counted with the electron microscope, as described previously (17).

TABLE 2. *Ratio of minicells to cells*[a]

Strain	Culture conditions		
	Exponential phase	Deep stationary phase	Dense growth on BHI agar
Rd thd	0.04	0.06	0.2
LB11	0.06	0.6	1.0
MC1	0.06	0.7	1.2

[a] Cultures were grown in BHI growth medium. Ratios were determined from liquid cultures of 0.4 optical density units at 675 nm (exponential phase), after overnight growth, 1.0 optical density units at 675 nm (deep stationary phase), and after incubation on agar for 2 days.

RESULTS

Minicell-producing strains of H. influenzae. LB11 cultures examined by light microscopy, reveal cells longer than wild-type cells, wild-type size cells and minicells. Figure 1a is a low magnification electron micrograph of cells from an exponential culture of LB11. The cell in the upper left is beginning a midcell division, whereas the cell in the lower center appears to be undergoing invagination near the end of the cell. The latter will result in an asymmetrical division and the production of a minicell. A cell dividing to yield a minicell is also shown in Fig. 1b.

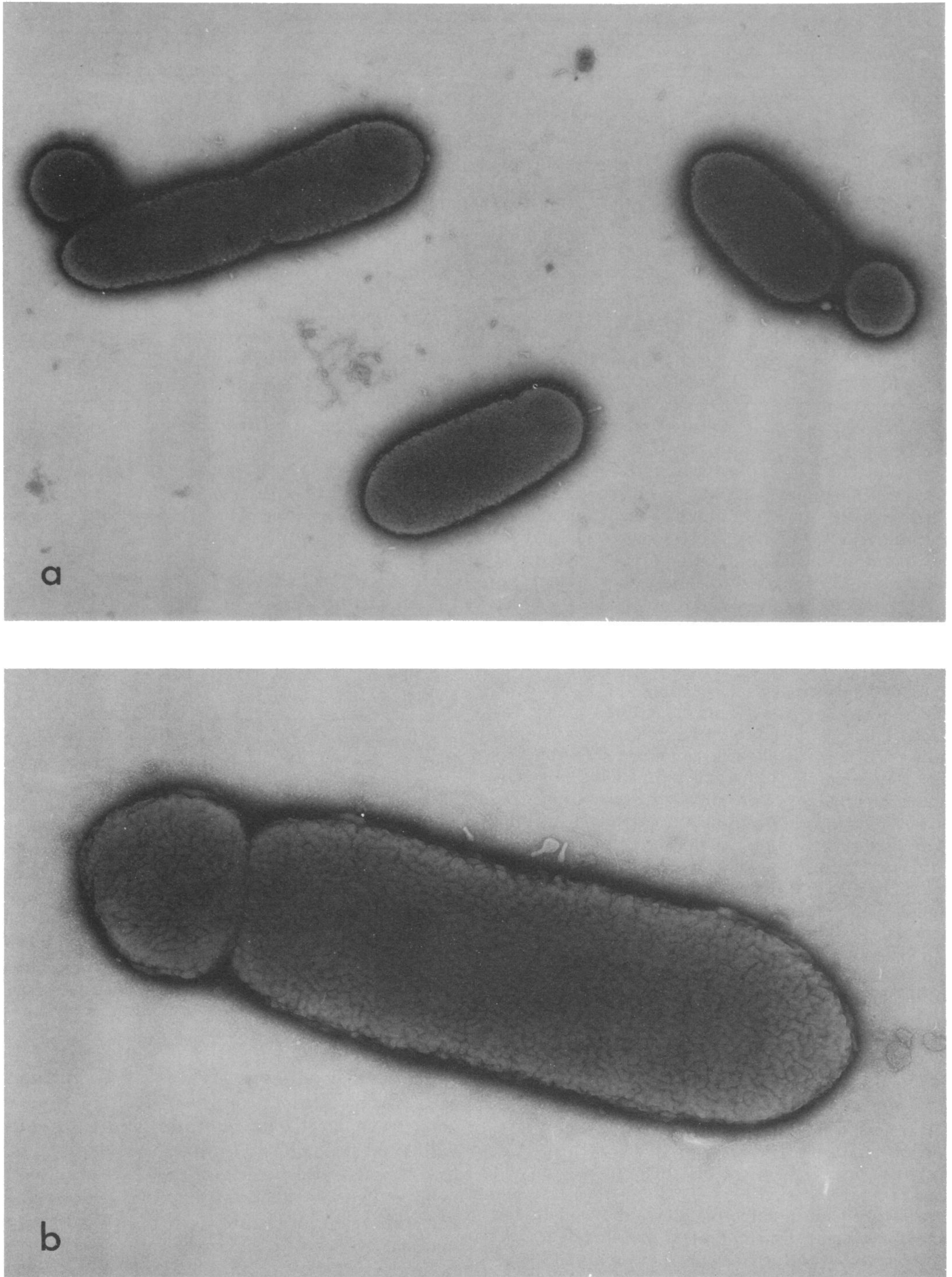

FIG. 1. *Electron micrographs of an exponential phase culture of LB11 showing cells and minicells. The samples were stained with phosphotungstic acid. (a) ×16,000; (b) ×36,400.*

During exponential growth the ratio of minicells to cells increases and reaches a value of about 0.6 in late stationary phase (Table 2). When a dense inoculum of LB11 is spread on a BHI agar plate so that the cells grow in a thick streak, filamentous cells, wild-type size cells and minicells are produced (Fig. 2). The ratio of minicells to cells is about 1.0 in such a preparation (Table 2), which is greater than in the liquid stationary-phase cultures. Examination of a dense growth of cells on BHI agar was found to be the best method for determining whether other strains of *H. influenzae* could produce minicells.

Rd thd, the parent strain of LB11, was found to produce long cells and minicells when plated in the above manner (Fig. 2), although few minicells were present in the stationary liquid culture (Table 2). The minicell to cell ratio for *Rd thd* was 10 times less than that of LB11. This suggests that LB11 contains at least one other mutation (besides the thymidine requirement), which was induced by the mutagenic treatment, and results in an increased yield of minicells and the production of filaments when the cells are plated on agar.

Strain *Rd thd* was isolated by growing *Rd* in the presence of aminopterin (2). Aminopterin is an inhibitor of dihydrofolate reductase, and has been previously used to isolate spontaneous thymine-requiring mutants of *Escherichia coli* (20). More thymidine-requiring strains of *H. influenzae* were isolated by this method to determine whether all such strains produce minicells. Strain *Rd* was grown in the presence of aminopterin (20 μg/ml) or trimethoprin (10 μg/ml), another inhibitor of dihydrofolate reductase (20). Thirty-seven aminopterin-resistant colonies and 31 trimethoprin-resistant colonies were isolated and all were found to require thymidine and produced a few minicells when grown on BHI agar, the ratio of minicells to cells being the same as the original *Rd thd* strain. We therefore conclude that all thymidine-requiring mutants of *H. influenzae*, isolated in this way, can produce minicells. The frequency of transformation for a single genetic marker under the conditions we have used is usually between 1 and 2%. Competent *Rd* exposed to a lysate of an aminopterin-resistant mutant was transformed with a frequency of 2%. The aminopterin-resistant transformants were thymidine requiring and produced minicells. These three characteristics must therefore result from the same mutation.

Transfer of the minicell mutations into the

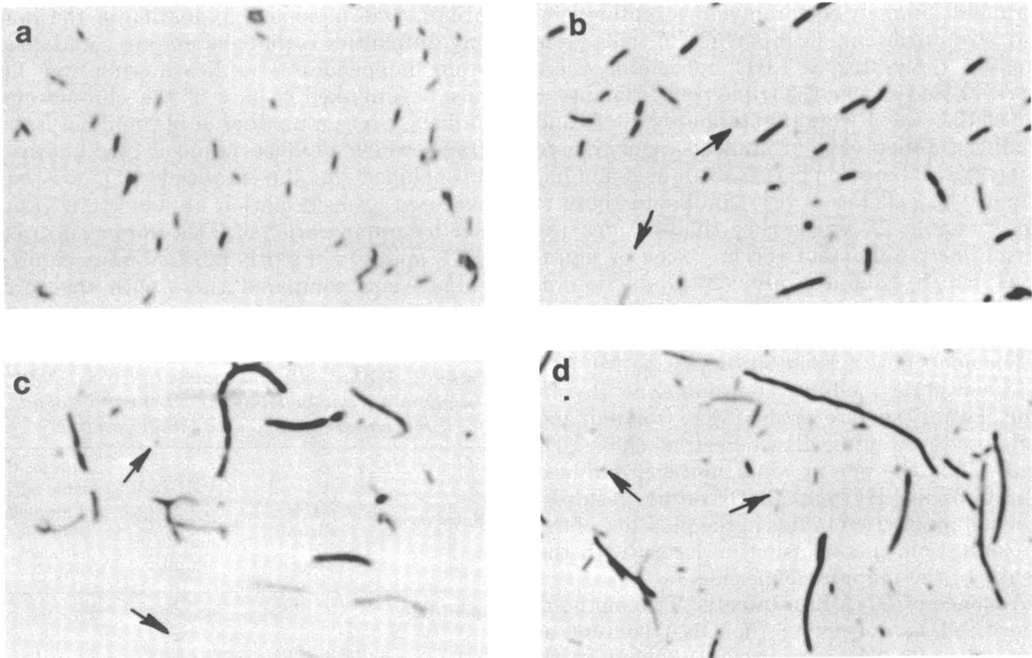

FIG. 2. *Photomicrographs of strains: (a) Rd, (b) Rd thd, (c) LB11 and (d) MC1. Cultures were streaked on BHI agar and incubated at 37 C for 2 days. The cells were stained with crystal violet. The arrows point to minicells.* ×250.

wild-type strain. Attempts were made to map the mutations in LB11 which result in minicell production. Competent LB11 cells were exposed to transforming DNA carrying seven drug resistance markers. The LB11 drug-resistant transformants were selected and screened for minicell production and thymidine requirement to determine the linkage of these mutations to the drug markers. The only transformants which had lost the ability to produce minicells had also lost the thymidine requirement. The highest co-transformation of drug resistance and thymidine independence and inability to produce minicells was obtained with the erythromycin marker (8/112, or 7% of erythromycin-resistant transformants being wild type with respect to thymidine requirement and minicell production). Among all the other transformants the percentage of prototrophic co-transformants which did not produce minicells was 1.5 to 3. Subsequent attempts to demonstrate some linkage of the erythromycin marker and the *thd* locus by transforming *Rd thd* with wild-type DNA carrying the erythromycin resistance marker were unsuccessful. Nevertheless an attempt was made to transfer the minicell mutations into the wild-type strain with the use of three drug markers which had yielded the highest co-transformation in the mapping experiments, namely erythromycin, streptomycin, and streptovaricin. Competent *Rd* cells were exposed to lysates of LB11 containing these markers, and among the triple transformants, 6 out of 136 (4.6%) were thymidine requirers and produced a high yield of minicells. Only one of these (MC1) has been closely examined and has minicell to cell ratios very similar to those of LB11 (Table 2), suggesting that besides the thymidine requirement the mutation or mutations which enhance minicell production in LB11 have also been transferred to MC1.

Spontaneous revertants of MC1 to thymidine independence were isolated by plating MC1 on $M1_c$ synthetic medium containing no thymidine. Twenty such revertants were isolated, and none produced minicells when streaked on BHI agar. Thus the one or more mutagen-induced mutations in LB11 apparently do not result in minicell production unless in the presence of the *thd* mutation, a conclusion in accord with the results of the mapping experiments.

Absence of DNA in minicells. Two methods were used to determine the DNA content of minicells in LB11 cultures labeled with [^{3}H]thymidine: (i) measurement of the ratio of viable cells and radioactivity in DNA of supernatant and pellet after centrifugation of the culture for 2 min at 11,500 rpm; (ii) measurement of acid-insoluble radioactivity in a highly purified preparation of minicells. The results of the first method are presented in Table 3. Dividing minicells have not been observed in electron micrographs. The colony counts are therefore an estimate of the number of viable cells only. Although there was some variability from experiment to experiment, the data indicate that all the radioactive counts in the supernatant can be accounted for as cells in the supernatant. Radioactive counts of purified minicell preparations yielded a maximum of 7.5 $\times 10^{-6}$ acid-insoluble counts/min per minicell. Since the amount of label per cell in the original culture was about 10^{-3} counts/min and assuming that all cell DNA was eliminated from the purified minicell preparations and that no phage DNA became labeled during the final purification step, the amount of DNA per minicell could be no more than about 10^7 daltons. We conclude that there is either no DNA in minicells, or at the most one or two small plasmids.

The genetic marker conferring resistance to 150 μg of viomycin per ml (8) has not been located on the *H. influenzae* chromosome, although a number of mapping attempts have been made (J. W. Bendler, personal communication). One possible explanation of the mapping difficulties is that the marker could reside on an independently replicating plasmid, and thus be unlinked to any of the chromosomal markers. Since a number of plasmids in *E. coli* strains which produce minicells are known to be included in the minicells (5), we have measured transformation to viomycin resistance by a preparation of DNA from a culture of LB11, and from a partly purified minicell preparation, and compared these with the corresponding transformations to streptomycin re-

TABLE 3. *Viable cells and radioactivity in DNA of supernatant and pellet after centrifugation of an LB11 culture labeled with [^{3}H]thymidine*[a]

Substance	Viable cells/ml	Acid-insoluble radioactivity (counts/min per ml)	Ratio: viable cells per ml/acid-insoluble radioactivity
Supernatant	3.4×10^5	8.3×10^2	2.4×10^{-3}
Pellet	8.9×10^8	2.2×10^6	2.5×10^{-3}

[a] The supernatant contained around 10^8 minicells/ml, as determined by microscopy count, and the pellet around 10^9 minicells/ml.

sistance, since the streptomycin marker has been well mapped on the *H. influenzae* chromosome (7, 15). The results are presented in Table 4. It is clear that there is no relative increase in transformation to viomycin resistance in the minicell preparation, and thus it is improbable that the viomycin marker is on plasmids included in the minicells. The transformation by the lysed minicell preparation is presumably due to the DNA from the remaining contaminating cells.

Lack of attachment of HPlc1 phage to minicells. Table 5 shows an experiment in which attachment was measured by determining the decrease in the phage titer in the supernatant when cells or minicells were sedimented by centrifugation. By this criterion, attachment could be detected with as few as 10^7 cells/ml, but not with 40 times this many minicells. The surface area of the cell is about four times greater than that of the minicell (calculated from measurements on electron micrographs). The surface area of the total minicells available for phage attachment was therefore 10 times greater than in the cell suspension.

TABLE 4. *Transformation to viomycin and streptomycin resistance by DNA purified from an LB11 culture and by a lysed preparation of minicells partially purified from an LB11 culture*

Source of DNA	Transformants/ml		Ratio of transformants
	Streptomycin	Viomycin	(Viomycin-streptomycin)
Cells and minicells	4.9×10^7	1.1×10^8	2.2
Minicell preparation	4.0×10^2	9.0×10^2	2.2

TABLE 5. *Evidence that HPlc1 phage does not attach to minicells*[a]

Cells/ml	Unattached phage/ml ($\times 10^{-4}$)	Minicells/ml	Unattached phage/ml ($\times 10^{-4}$)
0	1.8	0	2.0
10^5	2.2	4×10^5	1.9
10^6	1.8	4×10^6	2.2
10^7	0.74	4×10^7	2.4
10^8	0.28	4×10^8	1.9

[a] Phage at around 2×10^4/ml were incubated for 15 min at 37 C with various concentrations of wild-type cells or purified minicells. The mixtures were centrifuged, and the phage remaining in the supernatant were assayed.

It is thus suggested that HPlc1 phage do not attach to minicells.

Induction of minicell production by mitomycin C. When mitomycin C (0.33 μg/ml) was added to a culture of the wild-type strain in BHI growth medium, the defective phage was induced (3), the cells filamented, and minicells were produced. Mutation-to-thymidine requirement and treatment with mitomycin C result in minicell production. Other treatments which interfere with DNA replication, such as nalidixic acid and ultraviolet irradiation, might also result in minicell production.

Phage superinducibility. It has been previously reported (17) that an average of about 11% of competent *Rd* cells are killed after exposure to homologous transforming DNA due to the induction of defective phage. Competent cells of LB11 were shown to be superinducible under the same conditions resulting in 50 to 80% killing. More recent measurements of the amount of killing have been in the range of 20 to 60%. The explanation for this change is unknown, but the decreased killing may have resulted from changes taking place in strain LB11 during growth and subculturing. A large killing effect can be observed easily as a decrease in the optical density of the culture due to cell lysis 70 to 80 min after treatment with transforming DNA (Fig. 3). Competent cells of MC1 and *thd* were also superinducible for defective phage on exposure to transforming DNA (Fig. 3), resulting in 20 to 60% killing. The amount of killing was variable, but was always greater than that of the wild-type strain. The amount of killing was dependent on the DNA concentration, and is related to the amount of transformation (Fig. 4).

The lysis of cells that had been through the competence regime but had not been exposed to transforming DNA appeared to be greater for LB11 than for MC1 or *Rd thd* (Fig. 3). LB11 may therefore contain yet another mutation which was not transferred to MC1 and results in an increased sensitivity to induction of defective phage in competent cells. This sensitivity of competent LB11 cells to induction presumably accounts for our observation that competent LB11 plated on BHI agar gives lower colony counts than the other strains.

The defective phage released from LB11 and *Rd thd* after the competence regime and transformation were counted by electron microscopy (Table 6). In the absence of transforming DNA more phage were released from competent LB11 cells than from competent *Rd thd* cells, as expected from the previous observations of

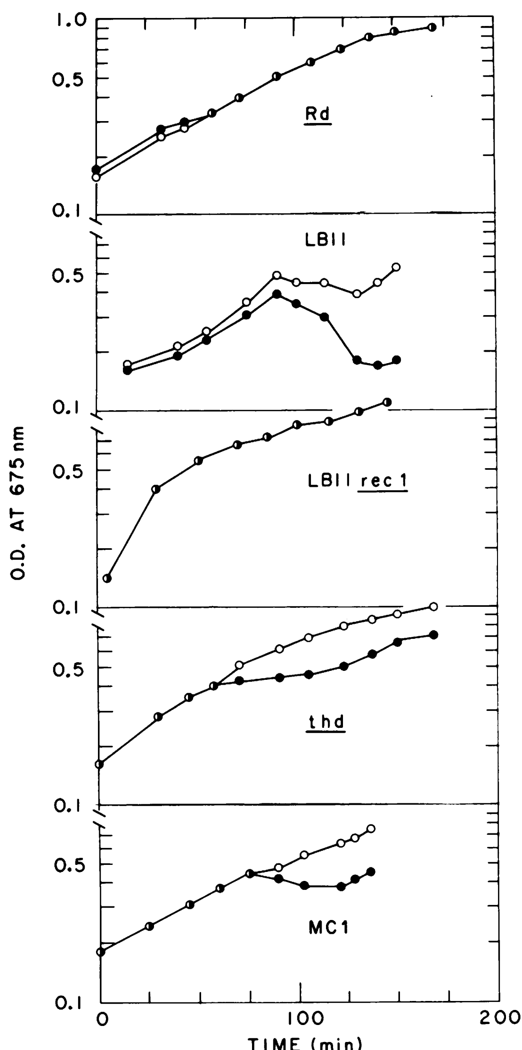

FIG. 3. *Optical density of cell cultures in growth medium following the competence regime. Two milliliters of competent cells were incubated with (●) or without (O) 16 µg of transforming DNA per ml at 37 C with shaking for 30 min. Four milliliters of growth medium were then added, the incubation continued and optical density was measured at intervals with a Bausch and Lomb Spectronic 20 spectrophotometer.*

optical density (Fig. 3). However, after the addition of transforming DNA the increase in the number of phage released was less for LB11 than for *Rd thd*, possibly because more competent LB11 cells were lysed even in the absence of transforming DNA.

The competence regime causes induction of HP1c1 in lysogens and transformation induces even more phage (17). Lysogens of the minicell-producing strains were isolated after infection with HP1c1 or S2 phages. The lysogens were examined to determine whether compared with the wild-type lysogens their prophages were also superinducible during transformation. The phage released were assayed by plaque formation (Table 7). The number of plaque-forming units released per milliliter of competent lysogen was of the order of 10^4 to 10^5 for MC1, *Rd thd*, and LB11, but was only 10^2 to 10^3 for the wild type. After exposure to transforming DNA the number of plaque-forming units from *Rd thd* and MC1 increase twofold or more but there was no increase in the number released from LB11. This may result from a greater competition between the defective and nondefective phages in LB11 than in MC1 and *Rd thd*.

The induction of phage (defective or nondefective) thus appears to be associated with the thymidine requirement in strains *Rd thd* and MC1, and LB11 may contain yet another mutation which leads to an even greater susceptibility to phage induction during the competence regime alone.

The induction of HP1c1 and S2 prophages after exposure of competent *Rd* lysogens to transforming DNA has been considered to involve a recombination event (17). LB11 *rec1*, which is transformed with a very low frequency (around 10^{-8}) is not superinducible for defective phage (Fig. 3), suggesting that the superinducibility of LB11 probably also involves a recombination event.

There is some evidence that thymineless death is in part due to phage induction (4, 13, 14, 19). If thymidine starvation were involved in the superinducibility of thymidine-requiring strains, addition of thymidine to the MIV competence medium would be expected to give a reduced amount of killing. However, an increase in both transformation and killing was observed in *Rd thd* when thymidine was present in the competence medium (Table 8).

DISCUSSION

Aminopterin- or trimethoprin-resistant thymidine-requiring strains of *H. influenzae* have been found to produce some minicells. The ratio of minicells to cells was so low in the exponential and stationary phases of growth in liquid culture that the minicells were not noticed until *Rd thd* had been examined after 2 days of incubation on BHI agar. It is possible that minicell production by thymidine-requiring strains is a unique characteristic of *H. influenzae*; alternatively, thymidine-requiring strains of other bacteria may also produce a few minicells, but they have not been noticed. The

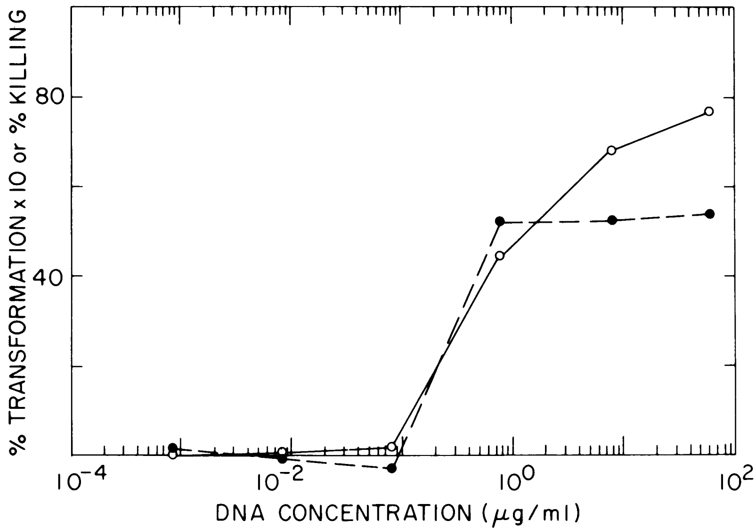

FIG. 4. *Transformation* (O) *and killing* (●) *of Rd thd as a function of the concentration of transforming DNA. Competent cells were incubated with various concentrations of transforming DNA at 37 C with shaking for 30 min. The cells were then plated to determine the number of transformants and the total number of viable cells.*

TABLE 6. *Electron microscope counts of defective phage released following the competence regime in LB11 and Rd thd with and without exposure to transforming DNA*

Strain	Transforming DNA	Phage counts	Latex sphere counts	Phage released/ml of culture
LB11	–	249	1500	1.3×10^{10}
	+	545	1500	2.8×10^{10}
Rd thd	–	4	1034	3.0×10^{8}
	+	115	1005	8.8×10^{9}

connection between thymidine requirement and minicell production is unclear. Thymidine-requiring mutants of *E. coli* also have abnormal division patterns resulting in filamentation (22). The fact that mitomycin C induces filaments and minicells in wild-type *H. influenzae* suggests the possibility that many agents which interfere with the normal cell division cycle may cause minicell production in *H. influenzae* and possibily also in other bacteria.

The ratio of minicells to cells in various strains of *H. influenzae* increased during the stationary phase of growth in liquid cultures. *B.*

TABLE 7. *Induction of phage from H. influenzae lysogens during transformation*[a]

Lysogen		Transformation (%)	PFU/ml ($\times 10^{-3}$)		Increase in PFU/ml due to addition of DNA ($\times 10^{-3}$)
Strain	Phage		–DNA	+DNA	
Rd (wild type)	HP1c1	0.8	0.08	0.52	0.44
	S2	0.5	0.31	0.84	0.53
MC1	HP1c1	1.4	4	30	26
	S2	1.2	11	58	47
Rd thd	HP1c1	0.4	120	210	90
	S2	2.3	68	103	35
LB11	HP1c1	1.2	59	41	
	S2	0.5	14	15	

[a] After exposure of competent cells to transforming DNA (8 μg/ml), the cultures were diluted to approximately 10^{4} cells/ml in growth medium and incubated for 2.5 h at 37 C with shaking. The cultures were centrifuged to remove the remaining cells and cell debris, and the supernatants were assayed for plaque-forming units (PFU).

TABLE 8. *Effect of 50 μg of thymidine per ml on the killing of competent cells by exposure to transforming DNA (8 μg/ml)*

Strain	Thymidine in competence medium			
	−		+	
	Transformation[a] (%)	Killing (%)	Transformation[a] (%)	Killing (%)
Rd thd	2.9	19	9	52
MC1	0.9	28	5	39

[a] Transformation to nalidixic acid resistance (2 μg/ml).

subtilis and *E. coli* minicells are also most numerous in the stationary phase (5). The increased ratio of minicells in the stationary phase is not because the cells are more prone to autolysis than the minicells. The optical density and the viable cell count of LB11 and *Rd thd* liquid cultures remains approximately constant for more than 3 h into the stationary phase, and we have observed an increase in the ratio of minicells to cells at the end of this time. Thus, the increase in the ratio cannot be the result of cell lysis. Khachatourians (5) found that the doubling time for the minicell number was less than the doubling time for the cell number in the *E. coli* minicell-producing mutant, and minicells continued to be produced and increased in number for as long as 7 h into the stationary phase. Preliminary results of minicell and cell counts during the growth cycle suggest that this may also be the case in *H. influenzae* minicell-producing cultures. When the cells are in restrictive growth conditions, as when entering the stationary phase, or during dense growth on BHI agar, there is apparently an increased probability of a cell undergoing a division to produce a minicell rather than normal division.

Several treatments which inhibit bacterial DNA synthesis result in filamentation and phage induction, such as ultraviolet radiation, mitomycin C, and thymidine starvation (13). Although we have shown that the superinducibility of thymidine-requiring strains of *H. influenzae* is not the result of thymidine starvation, it may result from abnormalities in DNA synthesis caused by the peculiarities of nucleotide metabolism in such strains, and be comparable to the effects of ultraviolet radiation, mitomycin C, and thymidine starvation.

The amount of killing induced by transforming DNA is correlated with the amount of transformation, and there is little or no killing in *H. influenzae* strains defective in genetic recombination (17; Fig. 3 and 4). This suggests that a recombination event is involved in the induction of defective phage during transformation, possibly at a prophage insertion site, as proposed by Garro (6), who observed the induction of a lysogen of *B. subtilis* during transformation.

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LITERATURE CITED

1. Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree. 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. U.S.A. **57**:321–326.
2. Beattie, K. L. 1972. Breakage of parental DNA strands in *Haemophilus influenzae* by 313 nm radiation after replication in the presence of 5-bromodeoxyuridine. Biophys. J. **12**:1573–1582.
3. Boling, M. E., D. P. Allison, and J. K. Setlow. 1973. Bacteriophage of *Haemophilus influenzae* III. Morphology, DNA homology, and immunity properties of HP1c1, S2, and the defective bacteriophage from strain *Rd*. J. Virol. **11**:585–591.
4. Ephrati-Elizur, E., D. Yosuv, E. Shmueli, and A. Horowitz. 1974. Thymineless death in *Bacillus subtilis:* correlation between cell lysis and deoxyribonucleic acid breakdown. J. Bacteriol. **119**:36–43.
5. Frazer, A. C., and R. Curtiss III. 1974. Production, properties, and utility of bacterial minicells. Curr. Top. Microbiol. Immunol. **69**:1–84.
6. Garro, A. J. 1973. DNA-mediated prophage induction in *Bacillus subtilis* lysogenic for φ105c4. J. Virol. **12**:18–24.
7. Goodgal, S. H. 1961. Studies on transformations of *Hemophilus influenzae* IV. Linked and unlinked transformations. J. Gen. Physiol. **45**:205–228.
8. Goodgal, S. H., and R. M. Herriott. 1961. Studies on transformations of *Hemophilus influenzae* I. Competence. J. Gen. Physiol. **44**:1201–1227.
9. Herriott, R. M. 1971. Effects on DNA: transforming principle, p. 175–217. *In* A. Hollaender (ed.), Chemical mutagens: principles and methods for their detection, vol. 1. Plenum Publishing Corp., New York.
10. Herriott, R. M., E. Y. Meyer, M. Vogt, and M. Modan. 1970. Defined medium for growth of *Haemophilus influenzae*. J. Bacteriol. **101**:513–516.
11. Hirota, Y., A. Ryter, and F. Jacob. 1968. Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677–693.
12. Kimball, R. F., and J. K. Setlow. 1974. Mutation fixation in MNNG-treated *Haemophilus influenzae* as determined by transformation. Mutat. Res. **22**:1–14.
13. Medoff, G., and S. Overholt. 1970. Thymineless death in *Escherichia coli* 15T⁻ and recombinants of 15T⁻ and *Escherichia coli* K-12. J. Bacteriol. **102**:213–216.
14. Medoff, G., and M. N. Swartz. 1969. Induction of defective phage and DNA methylation in *Escherichia*

coli 15T⁻. J. Gen. Virol. **4**:15-27.
15. Michalka, J., and S. H. Goodgal. 1969. Genetic and physical map of the chromosome of *Hemophilus influenzae*. J. Mol. Biol. **45**:407-421.
16. Reeve, J. N., N. H. Mendelson, S. I. Coyne, L. L. Hallock, and R. M. Cole. 1973. Minicells of *Bacillus subtilis*. J. Bacteriol. **114**:860-873.
17. Setlow, J. K., M. E. Boling, D. P. Allison, and K. L. Beattie. 1973. Relationship between prophage induction and transformation in *Haemophilus influenzae*. J. Bacteriol. **115**:153-161.
18. Setlow, J. K., D. C. Brown, M. E. Boling, A. Mattingly, and M. P. Gordon. 1968. Repair of deoxyribonucleic acid in *Haemophilus influenzae*. I. X-ray sensitivity of ultraviolet-sensitive mutants and their behavior as hosts to ultraviolet-irradiated bacteriophage and transforming deoxyribonucleic acid. J. Bacteriol. **95**:546-558.
19. Sicard, N., and R. Devoret. 1962. Effets de la carence en thymine sur des souches d'*Escherichia coli* lysogènes K12T et colicinogène 15 T. C. R. Acad. Sci. **255**: 1417-1419.
20. Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554-555.
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. Suit, J. C., T. Barbee, and S. Jetton. 1967. Morphological changes in *Escherichia coli* strain C produced by treatments affecting deoxyribonucleic acid synthesis. J. Gen. Microbiol. **49**:165-173.
23. Tankersley, W. G., and J. M. Woodward. 1974. Induction, isolation and characterization of a minicell-producing strain of *Salmonella typhimurium*. Proc. Soc. Exp. Biol. Med. **145**:802-805.