Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 6927–6931, November 1983
Genetics

Transformasomes: Specialized membranous structures that protect DNA during *Haemophilus* transformation

(bacterial transformation/*Hin*dIII restriction endonuclease/membrane–DNA complex)

MARC E. KAHN, FRANCIS BARANY, AND HAMILTON O. SMITH

Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Contributed by Hamilton O. Smith, August 4, 1983

ABSTRACT **The mechanism by which *Haemophilus* protects donor DNA from cellular restriction and degradative enzymes during transformation is unclear. In this report, we demonstrate that donor DNA enters *Haemophilus influenzae* through specialized membranous extensions, which we have termed "transformasomes." DNA within transformasomes is in a protected state—resistant to external DNase and cellular restriction enzymes, although remaining unmodified and double-stranded. The ability of donor DNA to exit from transformasomes is dependent on its topological conformation. Circular DNA remains intact within transformasomes, while linear DNA rapidly exits and undergoes homologous recombination. Protected donor DNA can be preferentially removed from the surface of competent cells by extraction with organic solvents. Structurally intact transformasomes containing donor DNA could be partitioned into the organic layer and can be further purified by density centrifugation.**

Haemophilus influenzae is a Gram-negative bacterium that can be induced to high levels of competence for transformation under conditions of slowed growth (1). Competent cells efficiently take up homologous donor DNA from the medium and integrate it into the chromosome to yield transformation rates of 1–3% for various genetic markers (for reviews, see refs. 2–5). Although various changes in outer and inner membrane composition have been observed during competence induction (6–9), little is known about the actual biochemistry and mechanics of DNA uptake. Furthermore, it is puzzling how donor DNA, which remains duplex after uptake, escapes restriction and degradation by cellular nucleases while awaiting integration. Recently, Kahn *et al.* (10) have suggested that specialized membranous extensions on the surface of competent cells might be responsible for capturing and protecting the donor DNA after uptake. By comparative morphological studies, these membranous structures were shown to appear on the surface of *H. influenzae* and *Haemophilus parainfluenzae* as they become competent (10, 11). The structures extend ≈35 nm from the cell, average 20 nm in diameter, are located at points of fusion between the inner and outer membrane, and are composed of a lipid bilayer—predominantly outer membrane proteins (10, 12) and lipopolysaccharide. Pore structures with an opening of 5 nm are localized at points of attachment of the membranous extension to the outer membrane. (An electron micrograph of this structure is seen in Fig. 5.)

Two lines of evidence implicate the membranous extensions as being the organelles responsible for DNA binding and uptake during transformation. First, membranous extensions are internalized as a consequence of the addition of homologous donor DNA to competent *H. parainfluenzae* cells, and membrane–donor DNA complexes have been isolated from mechanically disrupted cells (10). Second, studies with competence-deficient vesicle-shedding mutants of both *H. influenzae* and *H. parainfluenzae* strongly suggest that the DNA binding activity associated with competent cultures resides exclusively on membranous extensions (9, 12). From these observations, it has been proposed that during binding and uptake, donor DNA is in some way packaged into this unique membrane body, which we have renamed the "transformasome."

In this study, we follow the fate of cloned homologous DNA molecules during uptake and integration and characterize an intermediate in the transformation pathway—"the protected state." DNA in the protected state appears to be physically protected from cellular restriction and degradative enzymes, although unmethylated and double-stranded. Based on our ability to preferentially reisolate protected-state DNA as a DNA–membrane complex by extraction of cells with organic solvents, we present additional evidence that protected-state DNA resides in transformasomes.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *H. influenzae* Rd, KW22 and KW23 (13), were grown in 2.5% heart infusion broth (Difco), supplemented with 10 μg of hemin (Eastman) and 2 μg of NAD (Sigma) per ml. Competent cells, routinely able to transform at 3% for chromosomal markers, were prepared in MIV media as described (1). Plasmids pPUP3 (pBR322 containing an 11-base-pair uptake site in the *Pst* I site) (14) and pCML6 were isolated from *Escherichia coli* strains HB101 (r^-, m^-, *recA*) and MM294 (*endA hsdR*) by the procedure of Birnboim and Doly (15) and were purified further by CsCl/ethidium bromide centrifugation (16). Plasmid pCML6, provided by D. Danner, contains 10-kilobases (kb) of *H. influenzae* Rd DNA inserted into the *Bam*HI site of pEUP1 (14).

^{32}P Labeling of DNA. Nick-translation of 1 μg of *Cla* I-linearized DNA was carried out in a volume of 20 μl containing 50 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM EDTA, 7 mM mercaptoethanol, 300 μM dNTPs (dATP, dGTP, and dTTP), and 20 μCi (1 Ci = 37 GBq) of [α-^{32}P]dCTP (3,000 Ci/mM, Amersham) and was incubated with 10^{-5} mg of pancreatic DNase (Bethesda Research Laboratories) per ml at 23°C for 1 min. One unit of *Micrococcus luteus* polymerase (Miles) was added, and the mixture was incubated for 5 min at 15°C; this was followed by the addition of 600 μM ATP, 300 μM dCTP, and 1 Weiss unit of T4 ligase (New England BioLabs) for 2 min at 15°C. Unincorporated ^{32}P label was removed by chromatography with Sephadex G-25 preequilibrated with M21 media (1). Specific activity of DNA averaged 2×10^7 cpm/μg. Nick-translation of covalently closed circular (ccc) and open circular (oc) forms of pPUP3 will be described (unpublished data).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ccc DNA, covalently closed circular DNA; oc DNA, open circular DNA; kb, kilobase(s).

Purification of Discrete Size Classes of *Haemophilus* Chromosomal DNA. KW23 chromosomal DNA (100 μg) was cleaved with *Eco*RI, the digest was electrophoresed with a 1% low-melting agarose gel (Bethesda Research Laboratories) run at 1.5 V/cm overnight at 23°C, and the gel was stained with ethidium bromide. Prominant bands of differing size classes were excised from the gel by using fluorescent monofilament fishing line, 0.2 mm in diameter, and the DNA was recovered by heating to 65°C for 5 min, followed by two extractions with phenol, two extractions with *n*-butanol, and precipitation with 3 vol of ethanol. DNA was then nick-translated and used as donor in our uptake assay.

Donor DNA Uptake Assay. Nick-translated pCML6 DNA was added to competent cells in MIV media (10 ng of DNA per 10^9 cells or 0.65 molecules per cell), and the mixture was allowed to incubate for various times at 37°C. Under these conditions our cells would take up ≈70% of the added DNA molecules. Uptake was terminated by chilling to 0°C, and unbound DNA was removed by washing twice in 10 mM Tris·HCl, pH 8.0/10 mM EDTA/1.5 M CsCl at 4°C. This washing procedure removes essentially all DNase I-sensitive donor DNA. DNA was isolated by lysing cells in 1% $NaDodSO_4$ at 23°C, treating with proteinase K (1 mg/ml; Boehringer Mannheim) for 1 hr at 37°C, and extracting each twice with phenol and butanol. The aqueous phase was made 300 mM in Na acetate and precipitated with 3 vol of ethanol at −20°C. DNA was analyzed on 0.8% agarose gels (Sigma) in 0.2 M glycine·NaOH (pH 8.5) run for 2 hr at 6 V/cm. Gels were dried and autoradiographed using Kodak XAR-5 film. Restriction endonucleases used to analyze reisolated DNA were purchased from either New England BioLabs or Boehringer Mannheim and used as recommended.

Phenol and Phenol/Acetone Extraction of Competent Cells. Nick-translated pCML6 DNA was added to competent *H. influenzae* cells and allowed to incubate for either 5 min (donor DNA in protected state) or 60 min (donor DNA incorporated into the chromosome). Cells were washed at 4°C in the Tris/EDTA/CsCl buffer as described above and resuspended in 0.5 ml of either that buffer or 10 mM Tris·HCl, pH 8.0/10 mM EDTA in a 1.5-ml Eppendorf centrifuge tube. An equal volume of phenol or phenol/acetone, 1:1 (vol/vol), was added, and the mixture was shaken gently by hand for 1 min, followed by centrifugation. Radioactivity contained in the organic phase, aqueous phase, and cell pellet was determined by Cerenkov radiation, and the material in each phase was dialyzed against 10 mM Tris·HCl, pH 8.0/10 mM EDTA/0.25 M NH_4 acetate and examined by electron microscopy and agarose gel electrophoresis.

Electron Microscopy. Purified DNA was examined by the formamide technique of Davis *et al.* (17), whereas membranous material was visualized by negative staining with 1% phosphotungstic acid (pH 7.0). Photographs were taken on a Zeiss EM10 electron microscope at 60 kV.

Sucrose Gradient Centrifugation. After dialysis, material extracted from 100 ml of competent cells by either phenol or phenol/acetone was layered onto a discontinuous sucrose gradient consisting of 2.4 ml each of 2.2 M, 1.76 M, 1.32 M, and 0.88 M sucrose in 20 mM Tris·HCl, pH 7.4/50 mM NaCl/8 mM $MgCl_2$/2 mM mercaptoethanol and centrifuged in an SW 41 rotor at 39,000 rpm for 18 hr at 10°C. Fractions were collected from the bottom of the tube, and the radioactivity and absorbance at 280 nm of each fraction were determined.

RESULTS

Plasmid pCML6 is a derivative of pBR322 with a 10-kb insert of *H. influenzae* Rd DNA. A map of pCML6, established by multiple restriction endonuclease digestions is presented in Fig. 1.

Protection of Donor DNA from Restriction During Transformation. Our initial experiment was a time course of donor DNA uptake and integration during transformation. DNA was extracted from competent *H. influenzae* cells at various times after the addition of nick-translated linear pCML6. Analysis by autoradiography revealed that, at early time points, the majority of donor label appeared in a band indistinguishable from donor DNA (Fig. 2, lanes A–C). At subsequent time points, donor label was present in a region of the gel that corresponded to chromosomal DNA (Fig. 2, lane D). Because reisolated donor DNA remained significantly larger than pCML6 *Hin*dIII fragments (compare lanes B and F), we concluded that donor DNA was not restricted *in vivo* [some reisolated DNA was smaller than full-length donor DNA, probably because of degradation from an end but not from restriction (18)]. The inability of *Haemophilus* to restrict unmodified donor DNA may have been due to (*i*) *Hin*dIII methylation immediately upon uptake, (*ii*) conversion of donor DNA to a single-stranded form upon uptake, (*iii*) the absence of restriction enzyme activity in competent cells, (*iv*) protection of donor DNA by a DNA binding protein, or (*v*) protection by a cellular structure.

The first two possibilities were ruled out because reisolated DNA of donor size remained sensitive to *in vitro Hin*dIII digestion (Fig. 2, lanes E–H) (single-stranded DNA is resistant to *Hin*dIII). *In vitro* sensitivity to *Hin*dIII served as a convenient assay, allowing us to distinguish donor unmethylated DNA from chromosomal DNA. Because cell-free extracts of competent *H. influenzae* Rd, prepared by sonication, contained over 100-fold the activity needed to cleave donor DNA (Fig. 2, lane I), we also were able to eliminate the possibility that restriction enzyme activity was absent during competence development. Based on these experiments, we have concluded that, immediately after binding, donor DNA was protected from restriction by either DNA binding proteins or a discrete membrane structure. We have defined this intermediate in the transformation pathway as the "protected state." Properties of DNA in the protected state include (*i*) resistance to external DNase and high-salt washing and (*ii*) resistance to cellular restriction enzymes although unmethylated and double-stranded.

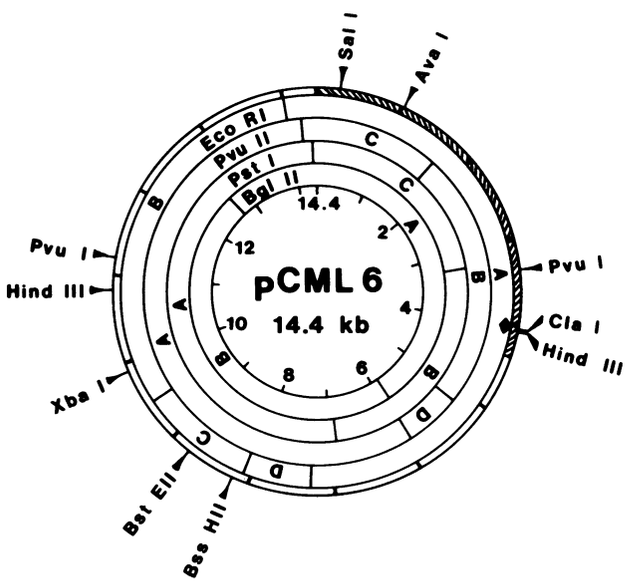

FIG. 1. Restriction map of plasmid pCML6. Plasmid pCML6 contains a 10-kb *Sau*3A fragment of *H. influenzae* Rd DNA cloned into the *Bam*HI site of pEUP1 (14). pBR322 sequences are indicated by the shaded region. ◆, Synthetic 11-base-pair "uptake sequence" in the *Eco*RI site.

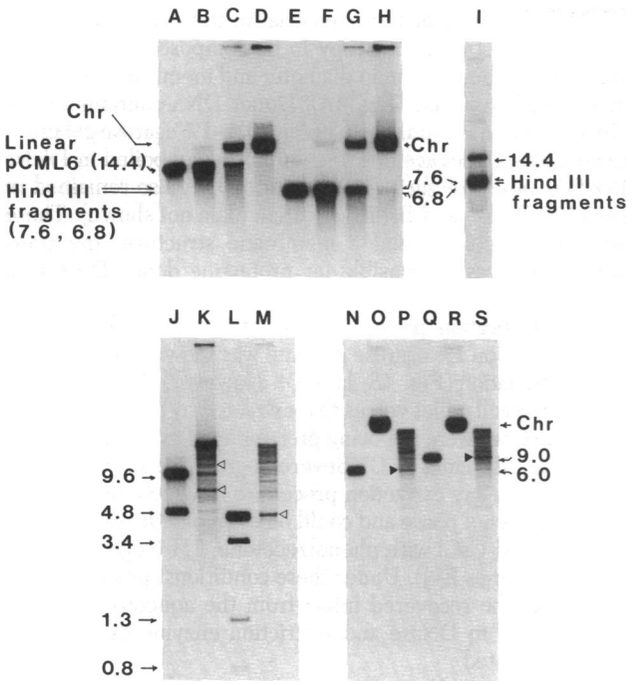

FIG. 2. Autoradiogram showing fate of unmodified and modified donor DNA after uptake in *H. influenzae*. Nick-translated *Cla* I-linearized pCML6 was added to competent *H. influenzae*, and DNA was reisolated after various times as described. Lanes: A–D, input *Cla* I-linearized pCML6 (lane A) and reisolated DNA after donor *Cla* I pCML6 uptake for 2 min (lane B), 15 min (lane C), and 40 min (lane D); E–H, input and reisolated DNA (lanes A–D, respectively) digested by *Hin*dIII *in vitro* (chromosomal DNA is methylated and hence resistant to *Hin*dIII); I, pCML6 DNA digested with the crude sonicate of competent *H. influenzae* KW22, demonstrating presence of endogenous *Hin*dIII; J and K, *Bst*EII digest of input *Cla* I linearized pCML6 (lane J) and reisolated DNA (lane K) after 40 min uptake; L and M, *Eco*RI digest of input (lane L) and reisolated (lane M) DNA (△, chromosomal bands that were labeled through homologous integration of donor DNA); N–S, examination of integration efficiencies of KW23 DNA (methylated at *Hin*dIII sites) with purified fragments (generated by *Eco*RI) that were nick-translated and added to competent KW22. Lanes N–S include: 6.0-kb (lane N) and 9.0-kb (lane Q) input *Eco*RI fragments, reisolated DNA after uptake of 6.0-kb (lane O) and 9.0-kb (lane R) fragments, and the *Eco*RI-digest of reisolated DNA after uptake of 6.0-kb (lane P) and 9.0-kb (lane S) fragments (▲, extent of homologous integration).

Exit of Donor DNA from the Protected State. After a 40-min incubation (Fig. 2, lane D), the majority of the donor [^{32}P]DNA label was localized in the chromosome. Because only a single strand becomes integrated (19) and homologous *Haemophilus* sequences comprise approximately 70% of pCML6, as much as 35% of the total radioactivity taken up by cells could become integrated into the chromosome. Therefore, upon digestion with a restriction enzyme, this integrated material should be entirely contained in either internal restriction fragments of pCML6 or in junction fragments in which one cleavage site is in pCML6 and the other is in the chromosome. However, after digestion with either *Bst*EII (Fig. 2, lanes J and K) or *Eco*RI (Fig. 2, lanes L and M), 85–90% of the donor label was found to be randomly incorporated into chromosomal restriction fragments, and only 10–15% was incorporated into fragments representative of proper integration. These results suggest degradation of donor DNA and efficient random reincorporation of donor label into the chromosome.

In order to exclude the possibility of artifact due to our donor DNA having been grown in *E. coli* or the presence of heterologous pBR322 sequences on pCML6, various size classes of *Eco*RI-digested *H. influenzae* chromosomal DNA were isolated from low-melting agarose gels, nick-translated, and used as donor DNA in our uptake assay. Reisolated chromosomal DNA from cells allowed to take up this material for 60 min was cut with *Eco*RI and analyzed by autoradiography (Fig. 2, lanes N–S). Our results indicate that the amount of ^{32}P label representing proper integration increased linearly with the size of the donor starting material. Degradation of donor DNA and efficient random reincorporation of label was observed and, therefore, was biologically significant.

Circular Molecules Remain in the Protected State. Because transformation using plasmid DNAs is significantly less efficient than transformation using linear DNA (20), we compared the fate of ccc, oc, and linear forms of plasmid pPUP3 in our radioactive-uptake assay. Consistent with our previous findings, linear pPUP3 molecules were entirely degraded after 60 min, and radioactivity associated with these molecules was reincorporated randomly into the chromosome (Fig. 3, lanes M–O). In contrast, a large percentage of the ccc DNA molecules taken up by the cells remained in the protected state, intact, after 60 min (Fig. 3, lanes G–I). Radioactivity that was randomly incorporated into the chromosome was probably due to the degradation of linear and oc DNA molecules present in our preparation. The majority of oc DNA molecules was degraded and label was reincorporated into the chromosome; however, an appreciable percentage of molecules failed to exit from the protected state after 60 min (Fig. 3, lanes J–L). These results indicate that ccc and some oc DNA molecules, although taken up efficiently by the cell, are unable to undergo further processing and exit from the protected state. This topological constraint suggested that circular DNA remained protected within a cellular structure.

DNA in the Protected State Is Enclosed in a Membrane Structure, the Transformasome. Reasoning that protected-state DNA may represent DNA on the surface of cells within transformasomes, we attempted to preferentially remove these structures by extraction of cells with a variety of organic solvents and detergents. We observed that phenol extraction of cells in the presence of 1.5 M CsCl specifically released protected-state DNA into the organic phase. Migration of pro-

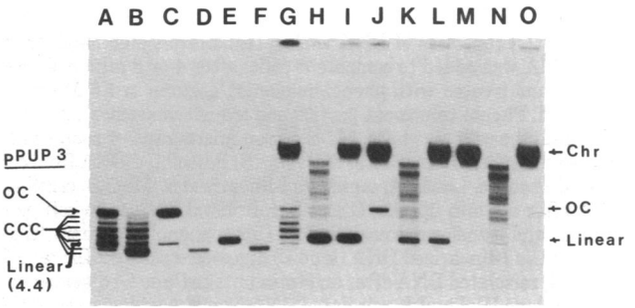

FIG. 3. Autoradiogram showing the fate of ccc, oc, and linear DNA after uptake. Transformation with nick-translated pPUP3 DNA (50 ng = 5×10^5 cpm), reisolation of, and electrophoresis of DNA was as described. Lanes: A, ccc DNA [topoisomers form when sealing nicked DNA; about half of the DNA could not be sealed (21)]; B, denatured ccc DNA (note that about half of the ccc DNA is oc; C, oc DNA; D, denatured oc DNA; E, *Pvu* I-linearized DNA; F, denatured *Pvu* I linearized DNA; G–O, reisolated DNA after a 1-hr uptake. Lanes G–O include: ccc DNA uptake (lane G) [about half of the DNA remained within transformasomes and the rest was incorporated into the chromosome, presumably from oc DNA present in the input DNA (lane A)], digested with *Pvu* II (single site in plasmid) (lane H), and digested with *Hin*dIII (lane I); oc DNA uptake (lane J), digested with *Pvu* II (lane K), and digested with *Hin*dIII (lane L); linear DNA uptake (lane M), digested with *Pvu* I (lane N), and digested with *Hin*dIII (lane O). Results essentially identical to those in lanes G–L were obtained when ccc or oc DNA uptake was for 30 min instead of 1 hr.

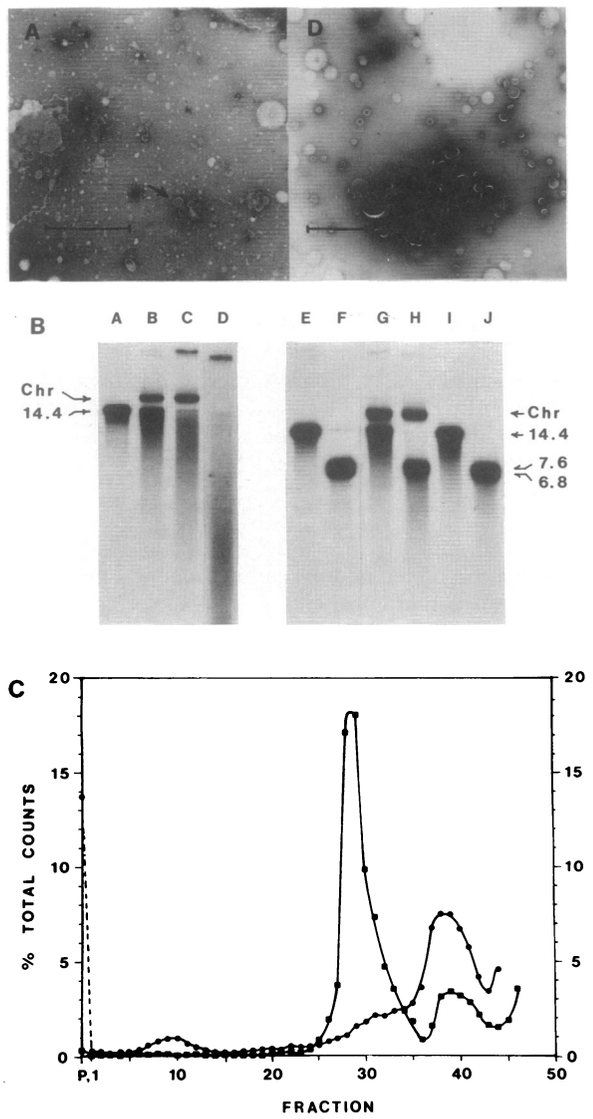

FIG. 4. Properties of DNA within transformasome. Nick-translated DNA was added to competent cells; after 4 or 8 min, cells were washed and treated with phenol or phenol/acetone in 1.5 M CsCl as described. Phenol treatment partitioned transformasomes containing DNA into the organic phase. (*A*) Electron micrograph of such phenol-treated transformasomes after dialysis. (*B*) Reisolated DNA from cells treated as above. Lanes: A, input *Cla* I-linearized pCML6; B, reisolated DNA after a 4-min uptake; C and D, reisolated DNA from cell pellet (lane C) and transformasomes (lane D) after phenol treatment; E and F, input *Cla* I-linearized DNA (lane E) digested with *Hin*dIII (lane F); G and H, reisolated DNA after an 8-min uptake (lane G) digested with *Hin*dIII (lane H); I and J, reisolated DNA from the aqueous layer after phenol/acetone treatment (lane I) digested with *Hin*dIII (lane J). Analysis of DNA from lane I by electron microscopy demonstrated donor length and smaller DNA with minimal chromosomal contamination. (*C*) Sucrose gradient of donor DNA reisolated (and dialyzed) after treatment with phenol (●) or phenol/acetone (■). P, pellet. A portion of donor DNA label from phenol-treated cells was pelleted. Analysis by electron microscopy revealed essentially homogeneous transformasomes (*D*), which still had DNA trapped within. A small peak around fraction 10 in *C* corresponds to the outer membrane and some free transformasomes, whereas the broad peak at the top of the tube (lane D in *B*) represents naked degraded DNA. The peak at fraction 30 corresponds to intact donor DNA released from transformasomes by phenol/acetone treatment (lane I in *B*). (Bars in electron micrographs = 1 μm.)

tected-state DNA into the organic phase was dependent on the presence of 1.5 M CsCl (see Table 1), presumably because of the greater solubility of lipopolysaccharide in phenol than in 1.5 M CsCl. After dialysis, material in the organic phase was shown by electron microscopy to be composed of membrane vesicles averaging 85 nm in diameter and membrane fragments heterogeneous in size (Fig. 4*A*). Donor DNA contained in the phenol fraction was unable to enter a 0.4–1% agarose gel unless treated with either acetone or chloroform and boiled in 1% Na-DodSO_4. Much of the protected-state DNA also remained resistant to DNase and *Eco*RI digestion (data not shown). Therefore, we concluded that a membrane structure, the transformasome, was responsible for protecting donor DNA from digestion during transformation.

After purification, protected-state DNA from the phenol phase was analyzed on 1% agarose gels and was shown to have undergone degradation (Fig. 4*B*, lane D). However, a comparison of DNA in the cell pellet versus that extracted by phenol indicated that, in fact, we were removing preferentially the protected-state DNA (Fig. 4*B*, lanes A–C). Severe degradation of donor DNA resulted from any extraction procedure in which the DNA entered the phenol phase and could be overcome by extraction of cells in 1.5 M CsCl with phenol/acetone, 1:1 (vol/vol), (Table 1; Fig. 4*B*, lanes E–J). Under these conditions, protected-state DNA could be recovered intact from the aqueous phase and was sensitive to DNase and restriction enzyme digestion (no longer protected).

The sedimentation properties of protected-state DNA contained in phenol and phenol/acetone extracts of competent cells were compared (Fig. 4*C*). The majority of donor [^{32}P]DNA label contained in the phenol/acetone extract migrated as a sharp band containing intact donor pCML6 and a slower sedimenting band containing partially degraded pCML6 (as determined by gel electrophoresis and electron microscopy). In contrast, donor [^{32}P]DNA label contained in the phenol extract could be localized in a broad peak representing degraded donor DNA and in a pellet, which was shown by electron microscopy to contain membrane vesicles, homogeneous in size (100 nm) (Fig. 4*D*). DNA associated with these vesicles remained resistant to DNase and was unable to enter a 0.4% agarose gel (data not shown).

DISCUSSION

We present evidence for a mechanism by which *H. influenzae* is able to protect double-stranded DNA from restriction and other intracellular degradative enzymes during transformation. Immediately after uptake, donor DNA appears to be in a pro-

Table 1. Distribution of released donor DNA label after extraction of competent *H. influenzae* with organic solvents

Aqueous buffer with cells	Organic solvent	Pellet	Organic phase	Aqueous phase
TE	Phenol	31 *C*	1	68 *D*
TE/1.5 M CsCl	Phenol	23 *C*	69 *D*	8
TE	Phenol/ acetone, 1:1*	53 *C*	47 *D*	0.1
TE/1.5 M CsCl	Phenol/ acetone, 1:1†	47 *C*	1	52 *D*
TE/1.5 M CsCl	Acetone	87 *C* and *D*	—	13

Nick-translated pCML6 was added to competent *H. influenzae* for 5 min at 37°C, and cells were chilled and washed as described. About 25% of donor DNA label was incorporated into the chromosome. Cells were extracted with 1:1 ratios of aqueous to organic solvents by gentle shaking. Identity of DNA partitioning into the phases was determined by gel electrophoresis and by electron microscopy. TE, 10 mM Tris·HCl, pH 8.0/10 mM EDTA; *C*, chromosomal DNA; *D*, majority of donor DNA.
* After mixing, acetone partitioned mainly into the aqueous phase.
† After mixing, acetone partitioned mainly into the organic phase.

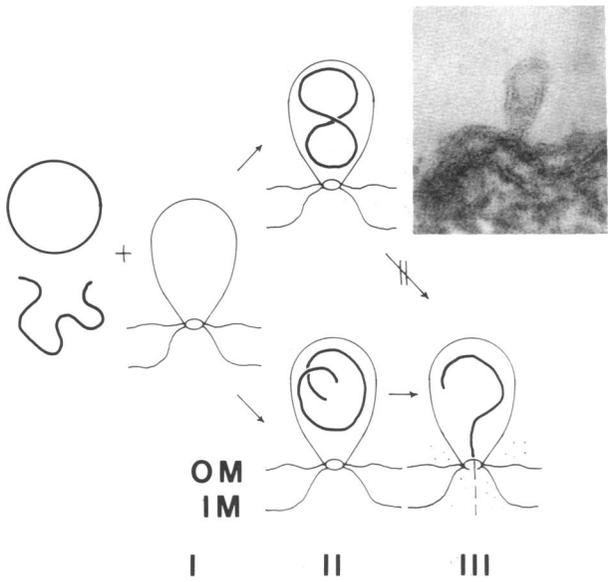

FIG. 5. Hypothetical model for DNA uptake in *H. influenzae*. Schematic drawing of transformasomes on the surface of competent *H. influenzae*. OM, outer membrane; IM, inner membrane. (*Inset*) Thin section of competent *H. influenzae* cell surface with transformasome.

tected state characterized by (*i*) resistance to external DNase and salt washing and (*ii*) resistance to *in vivo* restriction although unmethylated and double-stranded. Kinetic uptake experiments demonstrate that linear donor DNA remains in the protected state for a short time (<5 min), after which donor [^{32}P]DNA label can be detected in the chromosome. Exit of linear DNA from the protected state is accompanied by appreciable degradation of the molecule and rapid, random reincorporation of donor label into the chromosome. It is of interest that plasmid ccc DNA molecules are unable to rapidly exit the protected state. This result supports the concept of physical protection of donor DNA and may account for the inefficiency of plasmid molecules to transform in *H. influenzae* (20).

What physical properties allow donor DNA to avoid restriction and cellular degradative enzymes during transformation? Previous evidence based on morphological features of competent cells (10) and our present finding that protected-state DNA can be preferentially extracted as a rapidly sedimentating DNA–membrane complex are consistent with a hypothetical model presented in Fig. 5. Donor DNA is rapidly internalized into a unique membrane structure, the transformasome, where it is physically protected from restriction and cellular degradative enzymes. The mechanism of internalization is unknown but may be analogous to phage packaging. If linear, the molecule exits the transformasome as a single strand that immediately integrates into the chromosome. Because we are unable to observe a single-stranded intermediate in the cell and all protected-state DNA is extractable by phenol, presumably in transformasomes, it is tempting to speculate that recombination must take place in close proximity to the transformasome. ccc DNA molecules remain trapped indefinitely in the transformasome, possibly due to topological constraints or the absence of a free end.

We have recently analyzed by polyacrylamide gel electrophoresis the polypeptide composition of structurally intact transformasomes purified by gradient centrifugation. Preliminary results show an enrichment of competence-specific proteins and various outer membrane proteins, some of which are similar in molecular weight to those reported by Concino and Goodgal (12), by using transformasome-shedding mutants of *H. influenzae*. These results suggest that transformasomes are unique specialized structures that are formed *de novo* during competence development and are responsible for selective uptake and protection of transforming DNA.

We thank Riva Eichner, Sam Kelly, Richard Moxon, Marilyn Pifer, Eric Spitzer, and Bernard Weiss for helpful discussion and Mildred Kahler for expert typing. This work was supported by National Institutes of Health Grant 5-P01-CA16519. F.B. is supported by a Helen Hay Whitney Postdoctoral Fellowship, M.E.K. is supported by National Institutes of Health Postdoctoral Fellowship 5-T32-CA09139, and H.O.S. is an American Cancer Society Research Professor.

1. Herriott, R. M., Meyer, E. M. & Vogt, M. (1970) *J. Bacteriol.* **101,** 517–525.
2. Smith, H. O., Danner, D. B. & Deich, R. A. (1981) *Annu. Rev. Biochem.* **50,** 41–68.
3. Goodgal, S. H. (1982) *Annu. Rev. Genet.* **16,** 169–192.
4. Lacks, S. A. (1977) in *Microbial Interactions, Receptors and Recognition*, ed. Reissig, J. L. (Chapman & Hall, London), Vol. 3, pp. 177–232.
5. Notani, N. K. & Setlow, J. K. (1974) *Prog. Nucleic Acid Res. Mol. Biol.* **14,** 39–100.
6. Concino, M. F. & Goodgal, S. H. (1981) *J. Bacteriol.* **148,** 220–231.
7. Zoon, K. C. & Scocca, J. J. (1975) *J. Bacteriol.* **123,** 666–677.
8. Zoon, K. C., Habersat, M. & Scocca, J. J. (1976) *J. Bacteriol.* **127,** 545–554.
9. Kahn, M., Concino, M., Gromkova, R. & Goodgal, S. (1979) *Biochem. Biophys. Res. Commun.* **87,** 764–772.
10. Kahn, M. E., Maul, G. & Goodgal, S. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6370–6374.
11. Kahn, M. (1981) Dissertation (Univ. of Pennsylvania, Philadelphia).
12. Concino, M. F. & Goodgal, S. H. (1982) *J. Bacteriol.* **152,** 441–450.
13. Wilcox, K. W. & Smith, H. O. (1975) *J. Bacteriol.* **122,** 443–453.
14. Danner, D. B., Smith, H. O. & Narang, S. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2393–2397.
15. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7,** 1513–1523.
16. Radloff, R., Bauer, W. & Vinograd, J. (1967) *Proc. Natl. Acad. Sci. USA* **57,** 1514–1521.
17. Davis, R., Simon, M. & Davidson, N. (1971) *Methods Enzymol.* **21,** 413–430.
18. Barany, F., Kahn, M. E. & Smith, H. O. (1983) *Proc. Natl. Acad. Sci. USA*, in press.
19. Notani, N. & Goodgal, S. H. (1966) *J. Gen. Physiol.* **49,** 197–209.
20. Gromkova, R. & Goodgal, S. (1981) *J. Bacteriol.* **146,** 79–84.
21. Lindquist, R. C. & Olivera, B. M. (1982) *Cell* **31,** 53–60.