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Directional transport and integration of donor DNA in *Haemophilus influenzae* transformation

(bacterial transformation/transformasomes/Southern hybridization/middle-labeled DNA/covalently closed termini)

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ABSTRACT **DNA transport and integration in *Haemophilus influenzae* transformation was studied with a plasmid clone of homologous DNA (pCML6). Our results indicate that: (i) donor DNA enters specialized membranous extensions on the cell surface, which we have termed "transformasomes"; (ii) linear DNA undergoes degradation upon exiting transformasomes; and (iii) DNA without a free end remains within transformasomes and is not degraded. By comparing the fate of label from uniformly labeled versus middle-labeled DNA, it appears that donor DNA undergoes degradation from an end prior to recombining with the chromosome. Using donor DNA with covalently closed termini (hairpin ends) prevents efficient exit from transformasomes. When one hairpin is removed, exit of donor DNA is shown to be directional from the free end, with preferential homologous integration of the 3′ strand from that end.**

The transport and efficient integration of donor DNA is a fundamental process that has evolved in several transformable bacteria (for reviews, see refs. 1–4). In gram-positive *Streptococcus pneumoniae*, double-stranded DNA is bound to cells, and a membrane-bound endonuclease degrades one strand while the other strand is internalized (2, 5, 6). The internal single-stranded DNA then undergoes homologous integration over a period of about 15 min (7). In gram-negative *Haemophilus influenzae*, cells take up double-stranded DNA (8, 9), and there is no detectable single-stranded DNA intermediate, although only a single strand is thought to integrate (10, 11). We have demonstrated that donor DNA is first "captured" within outer-membrane extensions on the cell surface (12) (transformasomes), where it is in a protected state, untouched by external DNase or internal restriction and modification enzymes (13). The process whereby DNA exits from transformasomes, continues to elude restriction, and finally integrates into the chromosome is not understood. One clue to this process is the influence of topological constraints in determining the fate of donor DNA. Circular DNA remains within transformasomes, while linear DNA rapidly exits (13).

In this work, we have constructed donor DNAs with various topological conformations and distribution of label. Based on the fate of donor label after uptake, we propose a model for the mechanism of DNA transport from the transformasomes into the chromosome.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media. *H. influenzae* Rd strain KW22 (14) was grown in heart infusion broth (Difco) supplemented with hemin at 10 μg/ml and NAD^+ at 2 μg/ml (Sigma). *Escherichia coli* strains HB101 (*hsdS recA*), and MM294 (*endA hsdR*) containing plasmids were grown in L broth (15). Plasmids were prepared by the alkaline procedure of Birnboim and Doly (16) and further purified by one or two CsCl/ethidium bromide centrifugations (17). Plasmid pCML6 contains a 10-kilobase (kb) insert of *H. influenzae* Rd DNA in the *Bam*HI site of a pBR322 derivative (13, 18).

Preparation of ^{32}P-Labeled DNA. DNA was nick-translated with *Micrococcus luteus* polymerase, and nicks were sealed with T4 ligase to a specific activity of 2×10^7 cpm/μg as described (13). For preparation of middle-labeled DNA, pCML6 (7 μg) was cleaved at the unique *Bst*EII site, treated with calf intestine alkaline phosphatase (Boehringer Mannheim), and 5′-end-labeled in a reaction mixture (20 μl) containing 50 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM EDTA, 7 mM mercaptoethanol, and 200 μCi of [γ-^{32}P]ATP (5,200 Ci/mmol, Amersham; 1 Ci = 37 GBq). After 45 min at 37°C, ATP was added to 1 mM, and incubation was continued an additional 2 min at 37°C, after which the kinase was heat inactivated (63°C for 5 min) (see structure II in Fig. 4*C*). The volume was then increased to 40 μl by addition of buffer containing 1 mM ATP and 3 units (Weiss) of T4 ligase (New England BioLabs). Ligation to form circles and multimers proceeded at 23°C for 2 hr and was terminated by heat inactivation. Monomeric, linear middle-labeled DNA was obtained by cleaving the above multimers with 16 units of *Cla* I (Boehringer Mannheim) at 37°C for 30 min (Fig. 4*C*, structure III). For construction of hairpins, 10 μg of poly(dG-dC) (about 400 bases, Boehringer Mannheim) was heat-denatured, chilled at −50°C for 3 min, and phosphorylated with kinase (New England BioLabs). Hairpin ends (5 μg = 90-fold excess) were ligated to middle-labeled DNA (2 μg) in the presence of *Cla* I by addition of 3 units of ligase (1 hr at 23°C) in the above buffer. Gaps and nicks were sealed with 0.7 unit of polymerase I large fragment (New England BioLabs) in the presence of 250 μM of all four dNTPs (23°C for 15 min) and 0.03 unit of *Exo* III at 37°C for 10 min followed by 15 min at 23°C. Remaining nicked molecules were destroyed by addition of 60 units of *Exo* III at 37°C for 30 min, and all enzymes were heat-inactivated at 63°C for 10 min (Fig. 4*C*, structure IV). Radioactive ^{32}P from unligated ends was removed by two treatments with 4 units of calf intestine alkaline phosphatase for 30 min at 37°C. One hairpin could be selectively removed by cleaving with 20 units of *Sal* I (Fig. 4*C*, structure V). All DNAs constructed were purified over a G-25 column as described (13), yielding a specific activity of $1–2 \times 10^6$ cpm/μg.

Restriction Enzyme Digestions, Gel Electrophoresis, and Blot Hybridizations. Restriction endonucleases were from New England BioLabs or Boehringer Mannheim and were used as recommended. *Exo* III was a generous gift of B. Weiss. Genomic and plasmid DNA restriction fragments were separated

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Abbreviations: ccc, covalently closed circular; oc, open circular; kb, kilobases.

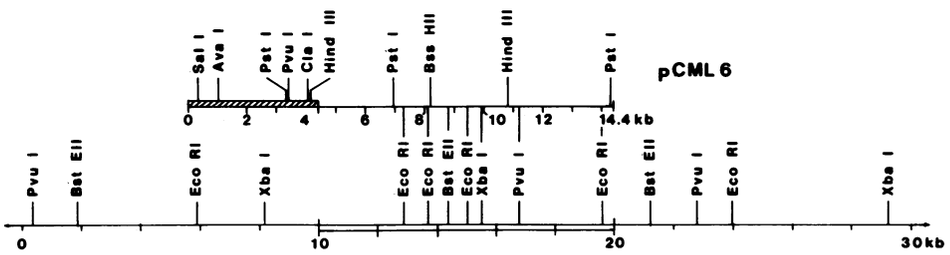

FIG. 1. Restriction map of plasmid pCML6 and surrounding regions in the chromosome. Plasmid pCML6 contains a 10-kb *Sau*3A fragment of *H. influenzae* Rd DNA cloned into the *Bam*HI site of pEUP1 (18). On integration, donor DNA becomes flanked by adjacent chromosomal sequences. Restriction analysis then yields junction fragments having one cut in the donor region and one cut in the adjacent chromosomal region. Relative orientation of junction fragments in the chromosome was determined by double-digest Southern analysis and was confirmed by redistribution of labeled donor pCML6 into various junction fragments.

by electrophoresis on 0.8% agarose gels in 0.2 M glycine·NaOH (pH 8.5) at 6V/cm for 2 hr. Gels were dried and autoradiographed on Kodak XAR-5 film. Hybridization was performed as described by Southern (19), with fragments transferred to nitrocellulose as described by Smith and Summers (20).

Donor DNA Uptake and Reisolation of DNA. Competent *H. influenzae* were prepared by the MIV procedure of Herriott *et al.* (21). Less than one DNA molecule per cell was added to competent cells (50 ng to 2 ml = 3.0×10^9 molecules to 4.6×10^9 cells) at 37°C, and transformation was terminated by chilling to 0°C. After removal of soluble radioactivity by centrifugation, loosely bound excess DNA was removed by washing cells in buffer containing 1.5 M CsCl as described (13). Cells were lysed in 1% $NaDodSO_4$ and treated with proteinase K (1 mg/ml), and DNA was isolated by two extractions with phenol, two extractions with butanol, and ethanol precipitation (13).

RESULTS

In the experiments described below, labeled cloned DNA was added to competent *H. influenzae* cells, and the fate of that label was determined. A restriction map of pCML6 and the surrounding chromosomal DNA is shown in Fig. 1. Orientation of chromosomal fragments was determined by means of Southern hybridizations and middle-labeling experiments described below.

Early Fate of Linear Donor DNA. Nick-translated linear pCML6 was added to competent *H. influenzae* cells, and total DNA was extracted at various times. Analysis by autoradiography revealed that at early time points (up to 2 min), the majority of donor label appeared in a band indistinguishable from donor DNA (Fig. 2*A*). At subsequent time points, the label was chased into a band corresponding to chromosomal DNA. We have demonstrated (13) that donor DNA remained unmethylated, as evidenced by its *in vitro* sensitivity to the restriction endonuclease *Hin*dIII (Fig. 2*B*). Resistance of donor DNA to external DNase and internal restriction and modification enzymes is a consequence of being protected within transformasomes on the surface of competent *H. influenzae* (13).

The kinetics of donor DNA redistribution of label was determined by excising and assaying the radioactivity in discrete regions of gels (Fig. 2 *A* and *B*). In this experiment, DNA uptake was virtually complete in 1–2 min, followed by a release

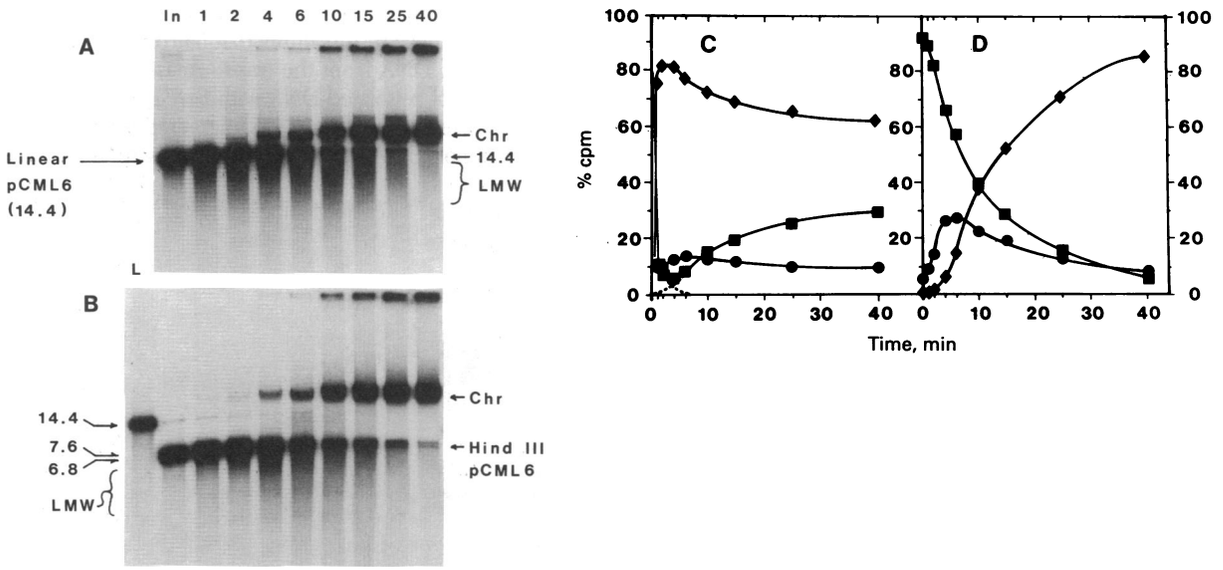

FIG. 2. Autoradiogram showing kinetics of linear DNA uptake and integration in *H. influenzae*. Nick-translated *Cla* I-linearized pCML6 DNA (300 ng = 6×10^6 cpm) was added to 10 ml of freshly grown competent KW22 cells at 37°C. Samples (1 ml) were removed at 1, 2, 4, 6, 10, 15, 25, and 40 min, chilled to 0°C, and washed, and DNA was reisolated as described. Electrophoresis was in 0.8% agarose with 22,000 cpm loaded per lane. (*A*) Reisolated DNA. (*B*) Reisolated DNA digested with *Hin*dIII. Chromosomal (Chr) DNA is methylated and hence resistant to *Hin*dIII cleavage. In, input DNA; L, linear *Cla* I pCML6; LMW, lower molecular weight form. (*C*) Fate of total DNA from kinetics as described above. ◆, DNA uptake (cells); ■, soluble radioactivity; ●, loosely bound radioactivity (removable by washing cells with 1.5 M CsCl). Maximum DNA uptake was 82%. (*D*) Fate of label inside cells. Bands were excised from the gels and assayed for radioactivity. Percentages = cpm in band/total cpm in lane and were not corrected for the slight decline of total label associated with cells at the later time points. The graph is from the gel in *B*; a similar one was obtained from *A* (not shown). ◆, Chromosomal DNA; ■, *Hin*dIII fragments of input DNA; ●, *Hin*dIII fragments of lower molecular weight intermediates. Zero time point indicates values for input DNA.

of label from the cells into the media (Fig. 2*C*). Shortly after uptake, there was a decrease in full-length donor DNA, followed by the transient appearance of a lower molecular weight form and finally integration (or reincorporation) of label into the chromosome (Fig. 2*D*). The lower molecular weight form of donor DNA is visualized as a smear beneath the intact donor DNA band and is shown to be double-stranded by its *in vitro* sensitivity to *Hin*dIII. Donor DNA and the lower molecular weight intermediate have been shown to be associated with transformasomes after treatment of *H. influenzae* with organic solvents that remove these structures (13). We believe the lower molecular weight form represents donor DNA undergoing degradation from an end as it leaves the transformasomes and enters the cytoplasm (ref. 13 and this work).

Homologous Integration of Donor DNA into the Chromosome. We examined the ability of linear pCML6 DNA to undergo homologous integration into the chromosome. The extent of this homologous integration (see Fig. 2*A*) was analyzed by digestion with the restriction endonuclease *Bst*EII (Fig. 3*A*). Because *Bst*EII cleaves pCML6 only once, digestion of reisolated chromosomal DNA should yield two higher molecular weight "junction" fragments that are homologous to pCML6 (12.5- and 7.0-kb fragments; see Fig. 1). Radioactive pCML6 undergoing homologous integration will incorporate label into these junction fragments. The resulting autoradiogram is analogous to a Southern hybridization, except the probe is the donor DNA and "hybridization" occurs *in vivo*. The percentage of label undergoing homologous integration (Fig. 3*A*) was quantitated by excising and assaying the junction fragment bands for radioactivity and correcting for random background reincorporation. Only 13% of the chromosomal label was contained in homologous junction fragments, whereas the majority of donor label (87%) was randomly reincorporated. A similar result was obtained with homologous DNA grown in *H. influenzae* (containing the proper methylation pattern) as donor DNA (13), indicating that massive degradation was not due to restriction after leaving the transformasomes. These results suggested that donor DNA underwent extensive degradation, perhaps from the end(s), and that this released label was randomly and efficiently reincorporated into the chromosome.

In an effort to minimize random reincorporation of label putatively released from the ends, pCML6 DNA was internally labeled at its unique *Bst*EII site. Restriction endonuclease *Bst*EII cleaves the middle base degenerate site 5′ G↓G-T-N-A-C-C (22). Therefore, *Bst*EII-cleaved 5′-end-labeled molecules would be expected to religate preferentially in a head-to-tail fashion. Upon cleavage with a second enzyme, such DNA would be uniquely labeled at an internal restriction site (see diagram in Fig. 4*C*). The majority (ranging from 75% to 90% in different experiments) of *Bst*EII middle-labeled molecules were of the correct structure (Fig. 3, lanes A–C). When this DNA was used for uptake, a significantly greater percentage of label appeared at the proper junction fragments (Fig. 3, lanes E and F; confirmed by Southern analysis, lanes G–J) than appeared when using nick-translated DNA (Fig. 3*A*, 40-min time point). Similar results were obtained by using *Xba* I middle-labeled DNA (data not shown). These results suggested that donor DNA was degraded from the end(s), thus accounting for the high degree of random reincorporation of label when using uniformly labeled donor DNA.

Processing of Donor DNA Containing Covalently Closed Termini. The above result led us to question whether a free end was required for donor DNA exit from the transformasomes. Both covalently closed circular (ccc) and open circular (oc) DNA remain within transformasomes for an extended time (60 min; ref. 13); this could be due to either a topological constraint, absence of a free end, or both. Ultrastructural studies of transformasomes indicate the presence of a pore structure of 30 Å in diameter located at the point of membrane attachment (12). The effective diameter of double-stranded circular DNA would be in excess of 30 Å, possibly preventing exit through this pore structure. To distinguish between a topological barrier and a requirement for a free end, we constructed middle-labeled DNA with covalently closed termini (hairpin ends) (see Fig. 4*C*). Such DNA was added to competent cells, and its fate was determined after 20- and 60-min uptake (Fig. 4*A*). Linear DNA with free ends was chased rapidly into the chromosome (Fig. 4*A*, lanes C–J), whereas hairpin DNA was sluggish in exiting from the transformasomes (Fig. 4*A*, lanes K–N). When one of the hairpins was removed, transport out of the transformasomes was almost as rapid as with linear DNA (Fig. 4*A*, lanes O–R). These results imply that hairpin ends block (or slow down) exit of DNA from the transformasomes.

Digestion of reisolated DNA with *Bst*EII demonstrated about equal representation of junction fragments when using middle-labeled donor DNA containing free ends or both ends cova-

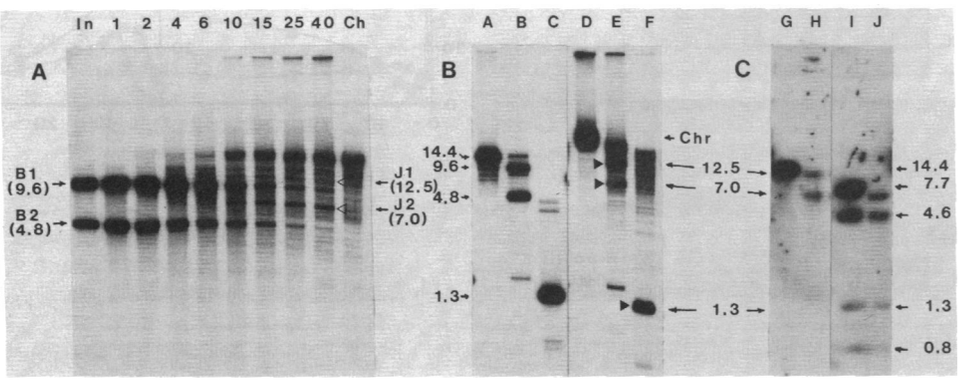

FIG. 3. Autoradiogram demonstrating homologous integration of DNA into the chromosome. (*A*) Reisolated DNA (from kinetics experiment in Fig. 2) digested with *Bst*EII. In, input DNA treated with the same enzyme; Ch, *Bst*EII digest of reisolated DNA from an unrelated (14 kb) fragment uptake—a control for random incorporation at the same bands as the correct junction fragments. J1 and J2 are chromosomal junction fragments of 12.5 and 7.0 kb, respectively (see Fig. 1); B1 becomes J2 on integration, and B2 becomes J1. (*B*) *Cla* I-linearized *Bst*EII-middle-labeled pCML6 (lane A), digested with *Bst*EII (lane B) or *Eco*RI (lane C). Faint bands represent minor aberrant products of the middle-labeling procedure. Lanes D–F show reisolated DNA after uptake (lane D), digested with *Bst*EII (lane E) or *Eco*RI (lane F). Background chromosomal banding pattern seen in the autoradiogram was identical to that observed by ethidium staining of the gel (data not shown). (*C*) Southern blots of KW22 DNA probed with pCML6 to confirm the identification of junction fragments. Lanes: G, *Bst*EII-digested pCML6; H, *Bst*EII-digested KW22 DNA; I, *Eco*RI-digested pCML6; J, *Eco*RI-digested KW22 DNA. △, Junction fragments labeled when using nick-translated DNA; ▲, fragments labeled with *Bst*EII-middle-labeled DNA.

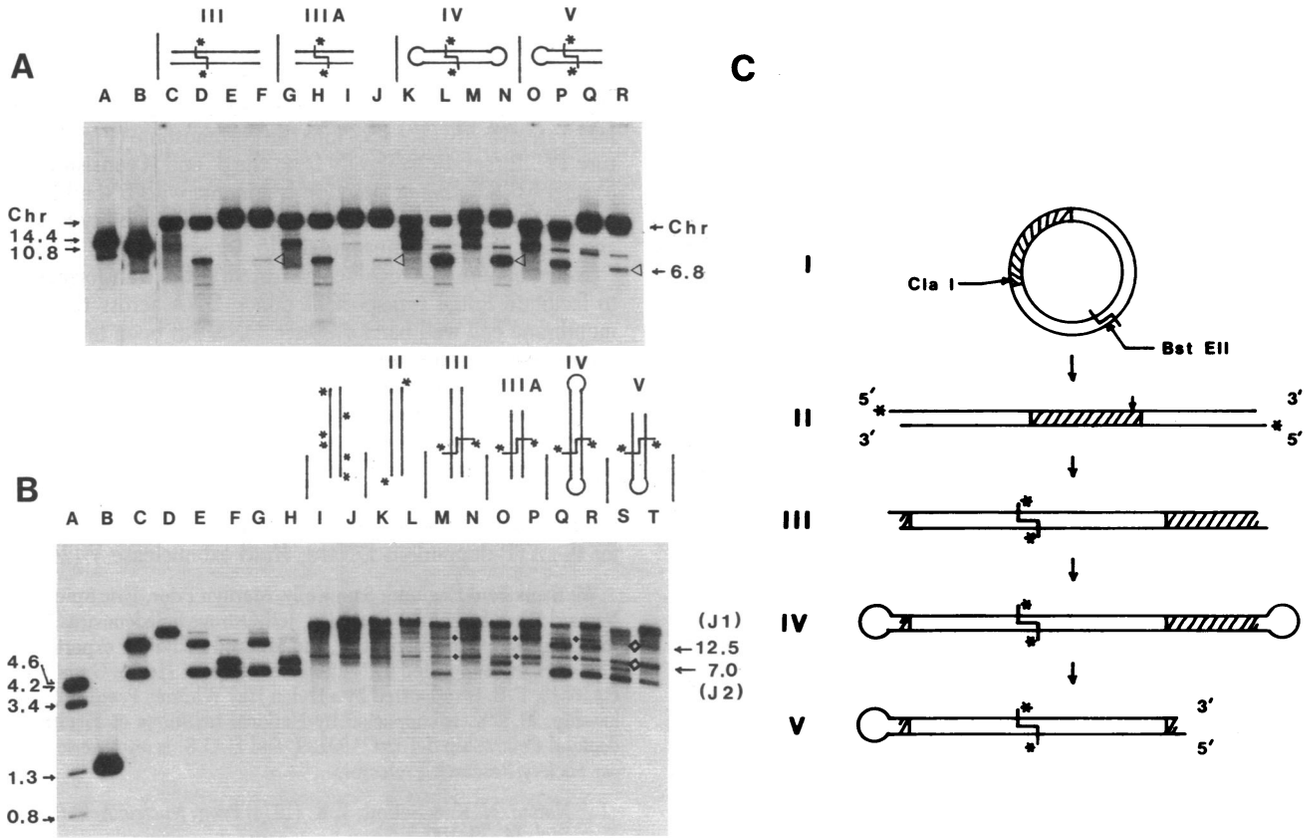

FIG. 4. Autoradiogram showing the fate of *Bst*EII-middle-labeled DNA with covalently closed termini. Transformation with *Bst*EII-middle-labeled pCML6 for 20 or 60 min, reisolation, and electrophoresis of DNA (2,000 cpm per lane) was as described in Fig. 3. (*A*) Uptake of DNA. Lanes: A, input *Cla* I-linearized DNA; B, input *Cla* I/*Sal* I-linearized DNA; C–R, reisolated DNA after uptake of the DNA structure pictured above the lanes. In groups of four lanes: first group of four lanes, 20-min uptake; second group, *in vitro Hin*dIII digest of 20-min uptake; third group, 60-min uptake; fourth group, *in vitro Hin*dIII digest of 60-min uptake. △, Position of 6.8-kb middle-labeled fragment generated by *in vitro Hin*dIII digestion, representing the portion of donor DNA still protected within transformasomes; *, position of radioactive labeling. (*B*) Fate of label within the chromosome. Lanes: A and B, *Eco*RI digest of nick-translated (lane A) and *Bst*EII-middle-labeled (lane B) *Cla* I-linearized pCML6 DNA; C–H, *Bst*EII digest of input DNA *Cla* I-linearized nick-translated pCML6 (lane C), structure II (lane D), structure III (lane E), structure IIIA (lane F), structure IV (lane G), and structure V (lane H); I–T, *Bst*EII digest of reisolated DNA after uptake of DNA structure pictured above the lanes. In groups of two lanes: first group of two lanes, 20-min uptake; second group, 60-min uptake; third to sixth groups are as in *A*. ◇, Preferential labeling of the 7.0-kb junction fragment when donor DNA contained only one hairpin; compare to approximately equal labeling with other middle-labeled donor DNA [◆ (between lanes)]. (*C*) Schematic drawing of DNA constructions. Structures: I, ccc pCML6 DNA; II, *Bst*EII-end-labeled pCML6; III, *Cla* I-linearized middle-labeled pCML6; IIIA, *Cla* I/*Sal* I-linearized pCML6 (not drawn); IV, *Cla* I-linearized pCML6 with hairpin DNA at both ends; and V, *Cla* I/*Sal* I-linearized pCML6 with hairpin DNA at only the *Cla* I end.

lently closed (Fig. 4*B*, lanes I–R). When donor DNA contained only one hairpin at the *Cla* I end, the 7.0-kb junction fragment was almost exclusively represented (Fig. 4*B*, compare lanes S and T to lanes O and P); the faint band at 12.5 kb is equivalent to the amount of integration with both ends in the hairpin configuration (Fig. 4*B*, lanes Q and R). The same results were obtained in three different experiments, including conditions where chromosomal DNA replication (but not repair) was inhibited (data not shown). We concluded that DNA exit from the transformasome was directional, starting from the free end. These results imply that the 5′ strand was preferentially destroyed while the 3′ strand became integrated.

Table 1. Fate of donor DNA after uptake by *H. influenzae*

Donor DNA conformation	Remains in transformasomes	Rapidly exits transformasomes
Circular		
Covalently closed	+	−
Open	+	+
Linear		
Blunt ends	−	+
5′ overhang	−	+
3′ overhang	−	+
Ad5 DNA (5′ protein)	+	−
Hairpin on both ends	+	−
Hairpin on one end	−	+

Ad5, adenovirus 5.

Fate of Other Donor DNAs. Table 1 summarizes the fate of various DNA conformers after uptake. ccc and some oc DNA remain within transformasomes (13). Linear DNAs, whether containing a blunt end, 5′ overhang, or 3′ overhang (up to 2 kb of single-stranded overhang generated with phage λ exonuclease or *Exo* III) were all rapidly processed out of the transformasomes. However, blockage of the 5′ strand with a protein or amino acid (adenovirus 5 DNA with or without treatment with proteinase K; data not shown), inhibited processing of donor DNA out of the transformasomes. These findings are consistent with the hypothesis that exit of donor DNA from the transformasome requires a free 5′ end and proceeds by preferential degradation of the 5′ strand.

DISCUSSION

The mechanism of donor DNA uptake has been extensively studied in *H. influenzae*. Early work demonstrated that DNA

remained double-stranded after uptake (8, 9) and, through unknown intermediate steps, only a single strand integrated into the chromosome (10, 11).

This work describes the biochemical fate of donor DNA. One striking characteristic of DNA processing in *H. influenzae* was the obligatory degradation of DNA from an end. Analysis (by electron microscopy) of DNA molecules reisolated from transformasomes indicated that many were smaller than full-length donor DNA (unpublished data). Our results indicate that the 5′ strand is preferentially degraded from an end, but the surviving (single-stranded) 3′ end is subsequently also degraded—unless it undergoes proper homologous integration. Indeed, internal heterologous sequences (pBR322 within pCML6 linearized at the *Xba* I, *Bst*EII, or *Bss*HII site) appear to be degraded and have a minor effect on proper integration of homologous DNA (data not shown). When one considers loss of label from degradation of (*i*) one strand, (*ii*) an end, and (*iii*) heterologous DNA, calculations with middle-labeled and nick-translated DNA indicate that portions of at least half of the donor molecules participate in integration.

From our data we present a hypothetical model for donor DNA transport and integration. Donor DNA is rapidly taken up into transformasomes on the surface of competent *H. influenzae* (Fig. 5, structure I). Those molecules with a blocked or inaccessible 5′ end (ccc, some oc, hairpin, and adenovirus 5 DNAs) remain within the transformasomes. Those molecules with a free 5′ end (linear, blunt, 3′ or 5′ overhang, and some oc and nicked DNAs) can exit in a directional process through a 30 Å pore at the bottom of the transformasome. Exit from the transformasomes is accompanied by: (*i*) release of soluble counts from the bacteria into the medium [this work; and also demonstrated by Stuy (23)], (*ii*) appearance of a lower molecular weight intermediate within the transformasomes, and (*iii*) degradation of both strands of DNA from one end, with preferential degradation of the 5′ strand. We speculate that double-stranded donor DNA within the transformasome is converted to a single-stranded form (the 3′ strand surviving) upon exiting through the pore, thus avoiding restriction (Fig. 5, structure III). If homologous, this hypothetical 3′ single strand would integrate directly (3′ → 5′) into the chromosome (Fig. 5, structure IV). Integration directly from the 3′ end is consistent with (*i*) unequal labeling of junction fragments with 5′-end-labeled donor DNA (Fig. 4*B*, lanes K and L) and (*ii*) inability to detect any single-stranded intermediate (8, 9).

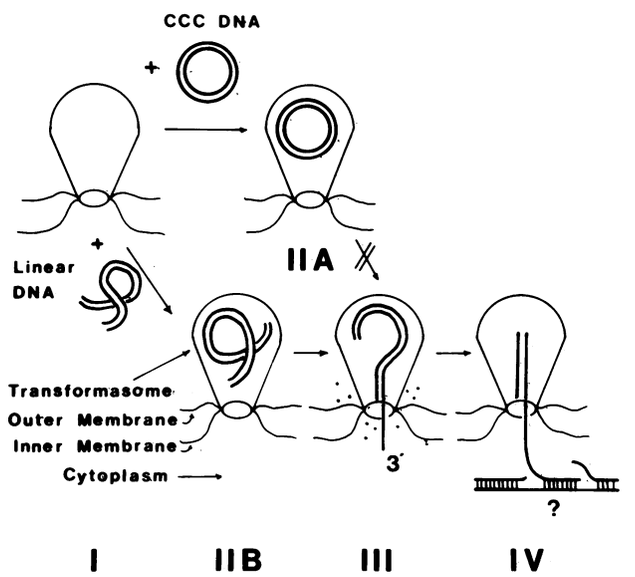

FIG. 5. Hypothetical model for DNA uptake in *H. influenzae* that contain transformasomes on their surface. Uptake of DNA into the transformasomes where it is resistant to external DNase and internal restriction and modification enzymes is seen in diagrams II. If the ends are blocked, DNA remains within the transformasome (diagram IIA), but if there is a free end (diagram IIB), DNA can exit through a pore at the point of membrane–transformasome attachment. Exit from the transformasome (diagram III) is accompanied by degradation from that end and preferential survival of the 3′ strand. The 3′ strand may integrate in the homologous region of the chromosome (diagram IV).

Haemophilus have evolved a structure, the transformasome, to facilitate initial transport of donor DNA across the outer-membrane cell wall barrier. Once DNA has been taken up by *Haemophilus*, transport across the inner membrane may be similar to that described in gram-positive bacteria (2, 5, 6): A membrane-bound endonuclease degrades one strand of donor DNA, while the other strand is internalized (2, 5, 6). Although no analogous enzyme has been implicated in *H. influenzae*, it is intriguing that the conformational requirements for exit from the transformasomes is identical to the substrate requirements for the ATP-dependent DNase, *Hind* exonuclease V (24–27).

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1. Notani, N. K. & Setlow, J. K. (1974) *Prog. Nucleic Acid Res. Mol. Biol.* **14**, 39–100.
2. Lacks, S. A. (1977) in *Microbial Interactions, Receptors and Recognitions*, ed. Reissig, J. L. (Chapman & Hall, London), Vol. 3, pp. 177–232.
3. Smith, H. O., Danner, D. B. & Deich, R. A. (1981) *Annu. Rev. Biochem.* **50**, 41–68.
4. Goodgal, S. H. (1982) *Annu. Rev. Genet.* **16**, 169–192.
5. Lacks, S. & Greenberg, B. (1973) *J. Bacteriol.* **114**, 152–163.
6. Lacks, S., Greenberg, B. & Neuberger, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2305–2309.
7. Fox, M. S. (1960) *Nature (London)* **187**, 1004–1006.
8. Voll, M. J. & Goodgal, S. H. (1961) *Proc. Natl. Acad. Sci. USA* **47**, 505–512.
9. Stuy, J. H. (1965) *J. Mol. Biol.* **13**, 554–570.
10. Notani, N. & Goodgal, S. H. (1966) *J. Gen. Physiol.* **49**, 197–209.
11. Goodgal, S. H. & Postel, E. H. (1967) *J. Mol. Biol.* **28**, 261–273.
12. Kahn, M. E., Maul, G. & Goodgal, S. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6370–6374.
13. Kahn, M. E., Barany, F. & Smith, H. O. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6927–6931.
14. Wilcox, K. W. & Smith, H. O. (1975) *J. Bacteriol.* **122**, 443–453.
15. Miller, J. H. (1972) in *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523.
17. Radloff, R., Bauer, W. & Vinograd, J. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 1514–1521.
18. Danner, D. B., Smith, H. O. & Narang, S. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2393–2397.
19. Smith, G. E. & Summers, M. D. (1980) *Anal. Biochem.* **109**, 123–129.
20. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
21. Herriott, R. M., Meyer, E. M. & Vogt, M. (1970) *J. Bacteriol.* **101**, 517–525.
22. Lautenberger, J. A., Edgell, M. H. & Hutchison, C. A., III (1980) *Gene* **12**, 171–174.
23. Stuy, J. H. (1974) *J. Bacteriol.* **120**, 917–922.
24. Friedman, E. A. & Smith, H. O. (1972) *J. Biol. Chem.* **247**, 2859–2865.
25. Orlosky, M. & Smith, H. O. (1976) *J. Biol. Chem.* **251**, 6117–6121.
26. Wilcox, K. W. & Smith, H. O. (1976) *J. Biol. Chem.* **251**, 6122–6126.
27. Wilcox, K. W. & Smith, H. O. (1976) *J. Biol. Chem.* **251**, 6127–6134.