Newly Synthesized DNA and *Bacillus subtilis* DNA Incorporated into Transformable *Diplococcus pneumoniae* (37486)

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The uptake of DNA by a variety of procaryotic and eucaryotic genetically transformable cells is nonspecific. Heterologous and homologous DNA are taken up by the cells equally well (1–3). Due to host cell recognition, however, the biological activity of only homologous or closely related DNA, but not heterologous DNA, becomes hereditably manifest in the host by incorporation of a macromolecular segment of donor DNA into the host genome (3–8). Little or no new DNA synthesis is required in *Diplococcus pneumoniae* for recovery of biological activity of donor DNA from initial temporary inactivation (eclipse) within the host cell or for genetic integration with host DNA (9). Marked retardation in DNA synthesis in competent relative to noncompetent cells has been found in genetic transformation in *Bacillus subtilis* (10–12).

Using radioactivity, density and biological labeling techniques, we have previously demonstrated that *B. subtilis* DNA is taken up by transformable *D. pneumoniae* and that small elements, which may include oligodeoxynucleotides, are covalently incorporated into macromolecular host *D. pneumoniae* DNA (3). Deoxynucleosides are incorporated into the DNA of transformable *D. pneumoniae* with or without simultaneous incorporation of small elements of *B. subtilis* DNA (3). It is the purpose of this paper to report CsCl density gradient experiments with transformable *D. pneumoniae,* briefly alluded to previously (3), which indicate similarity with respect to buoyant density of newly synthesized pneumococcal DNA and DNA from *D. pneumoniae* containing incorporated elements of density and radioactivity labeled *B. subtilis* DNA.

Methods. The details of materials and methods used in this study have been previously described (3). A transformable culture of R1 *D. pneumoniae* was exposed to ^{3}H-thymidine for 20 min in the presence or absence of heavy labeled $^{32}P^{2}H^{15}N$-*B. subtilis* 168^{+} DNA. The DNA was then extracted from the pneumococci and subjected to CsCl equilibrium density gradient centrifugation with or without prior sonication to smaller molecular size or both sonication and heat denaturation.

Results. When ^{3}H-thymidine alone (Fig. 1) or ^{3}H-thymidine and heavy ^{32}P labeled *B. subtilis* 168^{+} transforming DNA (Fig. 2) were exposed for 20 min under transforming conditions to a culture of *D. pneumoniae,* both radioactivity labels were covalently incorporated into recipient *D. pneumoniae* macromolecular DNA, and banded with light native pneumococcal DNA in CsCl equilibrium density gradients (Figs. 1A, 2A, C). The two incorporated labels also behaved identically in CsCl equilibrium density gradients if the DNA was first subjected to shearing by sonication or to sonication and heat denaturation, in a manner expected for native light DNA. Sonication resulted in broadening of the DNA peak due to smaller molecular size and sometimes in a slight shift to the heavy side of the gradient relative to the biological label of recipient DNA, probably due to slight denaturation of the DNA during sonication (3) (Figs. 1B, 2B, D). After both sonication and heat denaturation the ^{3}H-thymidine label and the ^{32}P label derived from heavy *B. subtilis*

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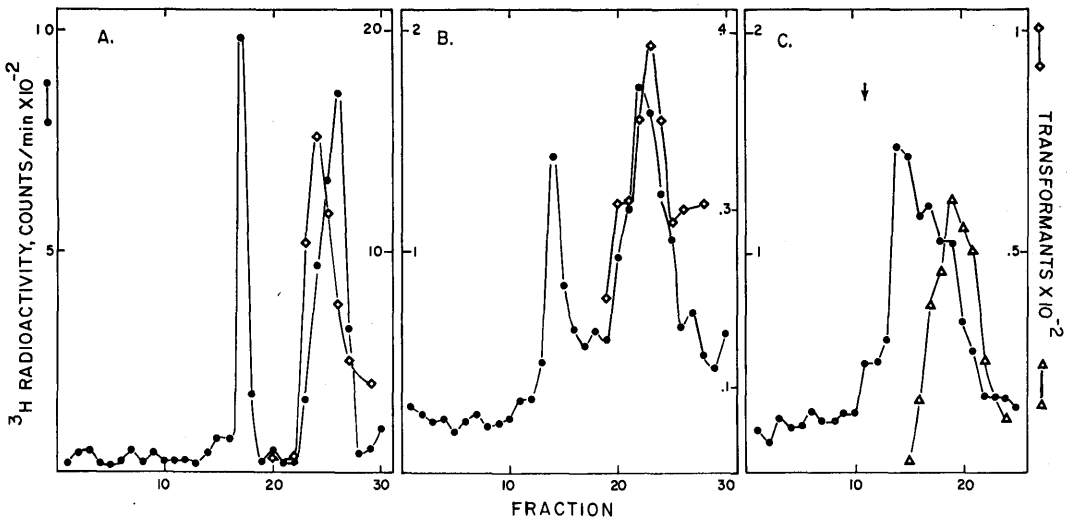

FIG. 1. CsCl equilibrium density gradient distribution of extracts of *D. pneumoniae* R1 obtained following 20 min exposure to ^{3}H-thymidine under transforming conditions. ^{2}H^{3}H^{15}N-*B. subtilis* 168$^+$ transforming DNA was added to the gradient as a heavy DNA position marker. (A) Unsonicated; (B) sonicated; (C) sonicated and heat denatured. (●) ^{3}H, counts/min/50 μl; (◇) transformants/10^{-2} ml to *p*-nitrobenzoate resistance (resident *D. pneumoniae* DNA marker); (△) transformants/10^{-2} ml to streptomycin resistance (*D. pneumoniae* R6 light position marker). Arrow in (C) indicates position expected for heavy *B. subtilis* DNA.

DNA assumed the position of denatured light DNA between native heavy and light DNA markers (Figs. 1C, 2E).

Discussion. These results demonstrate that monomer units are covalently incorporated into *D. pneumoniae* DNA with or without simultaneous covalent incorporation of small elements of *B. subtilis* DNA under the transforming conditions of these experiments. The newly synthesized DNA bearing the ^{3}H-thymidine label and the DNA bearing the ^{32}P label from *B. subtilis* DNA are similar to resident pneumococcal DNA with respect to buoyant density in the native and denatured states. The lack of distinction by buoyant density in these experiments between incorporation of elements of *B. subtilis* DNA and incorporation of nucleoside derived monomer units into *D. pneumoniae* DNA does not allow conclusion as to the nature of the small elements of *B. subtilis* DNA covalently incorporated into *D. pneumoniae* DNA since small oligonucleotides would not be expected to manifest their high density under the conditions of these experiments.

It is conceivable that the DNA synthesis in transformable *D. pneumoniae* in the presence of *B. subtilis* DNA observed in these experiments represents both replication and repair synthesis related to recombination and excision repair phenomena.

Summary. Both ^{3}H-thymidine and small ^{32}P-labeled elements of heavy density ^{32}P^{2}H ^{15}N-*Bacillus subtilis* 168$^+$ DNA were covalently incorporated into macromolecular DNA of genetically transformable R1 *Diplococcus pneumoniae*. The ^{3}H and ^{32}P markers in the DNA extracted from *D. pneumoniae* both assumed the position of light density doublestranded DNA on CsCl isopycnic equilibrium centrifugation when the DNA was untreated or shortened by sonication prior to centrifugal analysis, and of light density single-stranded DNA when first denatured by heat.

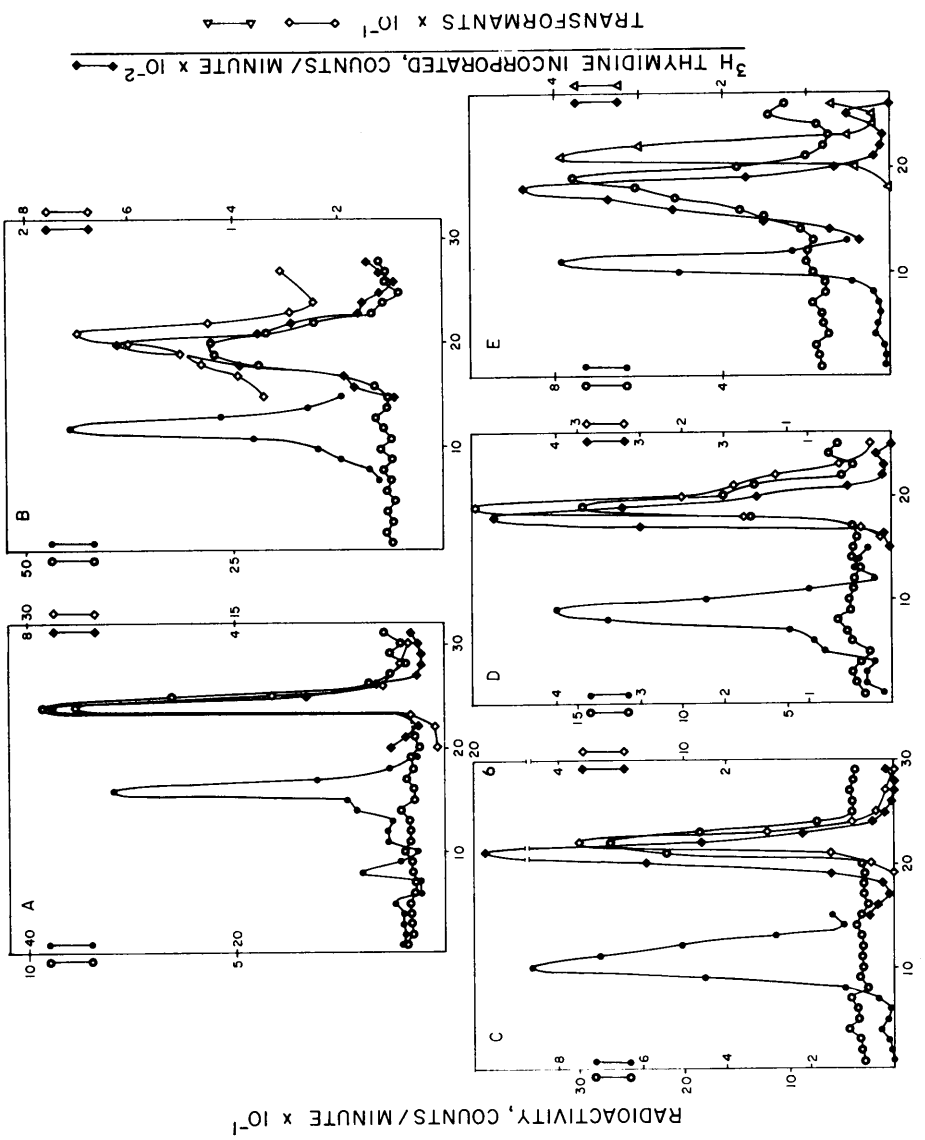

FIG. 2. CsCl equilibium density gradient distribution of extracts of *D. pneumoniae* obtained following 20 min exposure under transforming conditions to $^{32}P^{2}H^{15}N$-*B. subtilis* 168^{+} transforming DNA and ^{3}H-thymidine. (A, B) and (C, D, E) represent two different experiments. (A, C) Unsonicated; (B, D) sonicated; (E) sonicated and heat denatured. (○) ^{32}P, counts/min/50 μl; (●) ^{3}H, counts/min/50 μl ($^{2}H^{3}H^{15}N$-*B. subtilis* 168^{+} DNA position marker); (closed diamond) ^{3}H, counts/min/50 μl (^{3}H-thymidine derived radioactivity); (◇) transformants/10^{-2} ml to *p*-nitrobenzoate resistance (resident *D. pneumoniae* DNA marker); (△) transformants/10^{-2} ml to streptomycin resistance (added *D. pneumoniae* R6 light DNA position marker).

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