SHORT COMMUNICATION

***Haemophilus influenzae* and *Neisseria gonorrhoeae* Recognize Different Specificity Determinants in the DNA Uptake Step of Genetic Transformation**

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Cross-transformation and quantitative competition experiments showed that *Neisseria gonorrhoeae* and *Haemophilus influenzae* do not interact with each other's DNA in transformation. These organisms must interact with different recognition sequences during DNA uptake.

INTRODUCTION

The Gram-negative transformable bacteria *Haemophilus influenzae* (Scocca *et al.*, 1974) and *Neisseria gonorrhoeae* (Dougherty *et al.*, 1979) interact productively with DNA derived from members of their own genus; DNA prepared from distantly related organisms (e.g. Gram-positive bacteria or vertebrate tissues) does not bind to the DNA uptake system of competent recipient cells, nor compete with homologous DNA for uptake and transformation. In the case of *H. influenzae*, specificity for DNA uptake involves the recognition by the competent cell of an eleven-base-pair sequence which is present at a frequency of approximately one per 4000 base pairs in DNA from members of the genus *Haemophilus*, but which is present infrequently in DNA from other sources (Danner *et al.*, 1980). It is probable that specificity in the DNA uptake system of *N. gonorrhoeae* involves recognition of sequence information also.

We wished to determine if DNA from either of these genera would interact with recipient cells from the other genus. Inter-generic cross-reactivity would, if present, have important implications for the evolution of transformation in Gram-negative organisms; its absence would serve to emphasize that transformation in these organisms occurs only in the presence of a taxonomically appropriate donor.

To answer this question, we investigated the ability of DNA prepared from streptomycin-resistant strains of *N. gonorrhoeae* and *H. influenzae* to transform recipient cells of both organisms to antibiotic resistance. The capacity of heterologous wild-type DNA to competitively inhibit transformation of competent recipient cells by the appropriate homologous DNA preparations was also determined.

METHODS

Bacterial strains. *Haemophilus influenzae*, strain Rd, both wild-type and streptomycin-resistant, were from the collection of R. M. Herriott. *Neisseria gonorrhoeae* wild-type (FA19) and streptomycin-resistant (FA130) strains were kindly provided by P. F. Sparling.

Growth of bacteria. Wild-type *H. influenzae* cells were grown and made competent for transformation by the method of Herriott *et al.* (1970). Cells of *N. gonorrhoeae* FA19 type 2 were harvested for transformation assays from plates of GC medium (Difco) containing 1% (v/v) IsoVitalax (BBL) after growth for 18 h at 37 °C in an atmosphere of 5% (v/v) CO₂ in air.

For the preparation of DNA, *H. influenzae* cells were grown and harvested by the method of Scocca *et al.* (1974); *N. gonorrhoeae* type 2 cells were grown as described above and the growth was scraped off the plates, suspended in 10 mM-Tris/HCl, 1 mM-EDTA, pH 7.6, and harvested by centrifugation for 10 min at 5000 g.

Preparation of DNA. *Haemophilus influenzae* DNA was prepared as previously described (Scocca *et al.*, 1974). In the preparation of *N. gonorrhoeae* DNA, cell lysis was by the method of Sox *et al.* (1978), and deproteinization of the DNA was by the method cited above for *H. influenzae* DNA.

Transformation assay. Transformation of *H. influenzae* was measured as described by Herriott *et al.* (1970). Transformation of *N. gonorrhoeae* was determined by the method of Dougherty *et al.* (1979). Competent recipient cells were added to the transformation assay at 5.5×10^9 colony-forming units (c.f.u.) ml⁻¹ for *N. gonorrhoeae* or at 9.0×10^8 c.f.u. ml⁻¹ for *H. influenzae* except where noted. The concentration of DNA was saturating (at least 1 µg ml⁻¹) in all experiments.

RESULTS AND DISCUSSION

To determine whether DNA from either *H. influenzae* or *N. gonorrhoeae* would interact with recipient cells from the other organism, we first tested the ability of DNA prepared from streptomycin-resistant strains of *N. gonorrhoeae* and *H. influenzae* to transform recipient cells of both organisms to antibiotic resistance. The transformation frequencies were 0.7% for *H. influenzae* and 0.01% for *N. gonorrhoeae* using homologous DNA preparations. Neither organism was transformed to streptomycin resistance by high concentrations of heterologous DNA under conditions in which high levels of transformation were achieved using homologous DNA preparations. This result could be due to either the failure of heterologous DNA to interact with the uptake system of the recipients, or the inability of the selected phenotype to be expressed because of the lack of sequence homology in the genetic markers used. To distinguish between these alternatives, the capacity of heterologous wild-type DNA to competitively inhibit transformation of competent recipient cells by the appropriate homologous DNA preparations was investigated.

Competent recipient cells were incubated with a saturating concentration of homologous transforming DNA bearing the marker for streptomycin resistance; the concentration of either homologous or heterologous wild-type DNA was varied as indicated in Fig. 1. Incubation mixtures were diluted, plated and scored for transformants. The data are presented in the form of reciprocal plots, using the equation:

$$\frac{\text{No. of transformants in control}}{\text{No. of transformants with competing DNA}} = k \times \frac{\text{Concentration of competing DNA}}{\text{Concentration of transforming DNA}} + 1$$

in which the slope of the line affords an estimate of the relative affinities of the transforming and competing DNAs for the uptake system of the competent cells (Sisco & Smith, 1979).

The results of such a competition experiment using *H. influenzae* as recipient are shown in Fig. 1(a); competition data for a similar experiment using *N. gonorrhoeae* as recipient are presented in Fig. 1(b). It is clear from the results of these studies that transformable *H. influenzae* and *N. gonorrhoeae* show no appreciable interaction with each other's DNA, since transformation by homologous DNA was not affected by up to a 10-fold excess of heterologous competing DNA. We conclude that *H. influenzae* and *N. gonorrhoeae* interact with different specificity determinants in the DNA uptake step of genetic transformation. These organisms appear to have evolved different recognition sequences within their chromosomes to enable them to identify and interact with suitable donor DNA molecules during transformation. The question remains as to how these sequences are accommodated within

Fig. 1. Competitive inhibition of the transformation of *H. influenzae* and *N. gonorrhoeae* by homologous or heterologous DNA. Competent recipient cells were added to the transformation assay and incubated with a saturating concentration ($1 \mu\text{g ml}^{-1}$) of transforming DNA bearing the streptomycin resistance marker and a varying concentration of homologous or heterologous wild-type DNA. (a) Competitive inhibition of the transformation of *H. influenzae*: competent recipient cells were added to the assay at 1.4×10^9 c.f.u. ml^{-1} . (b) Competitive inhibition of the transformation of *N. gonorrhoeae*: competent recipient cells were added to the assay at 4.1×10^9 c.f.u. ml^{-1} . The competing DNA used was wild-type *H. influenzae* DNA (O) or wild-type *N. gonorrhoeae* DNA (●).

Replication of the experiments with homologous competing DNA yielded plots with slopes that differed by less than 10% from those presented here. In experiments with heterologous competing DNA, the numbers of transformants were consistently indistinguishable from those of the control.

the genome, and also whether these sequences serve any function beside their role in maintaining the integrity of the genome of an organism which exchanges genetic information by means of transformation.

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