

## *Haemophilus influenzae* Periplasmic Protein Which Binds Deoxyribonucleic Acid: Properties and Possible Participation in Genetic Transformation

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Received for publication 20 June 1979

A protein which binds to either single-stranded or double-stranded deoxyribonucleic acid (DNA) but not to ribonucleic acid has been isolated by osmotic shock treatment of growing cells. This periplasmic protein differs from the principal intracellular binding protein in its greater thermolability and by the absence of salt-induced cooperativity in its interaction with single-stranded DNA. Certain mutant strains of *Haemophilus influenzae* defective in the DNA uptake steps of genetic transformation were found to be deficient in periplasmic DNA-binding protein, suggesting that this protein participates in the uptake of DNA in transformation.

Genetic transformation of bacteria requires that the recipient cells interact with external DNA molecules and transfer these external molecules across the cellular boundary in genetically intact form. The ability to carry out DNA transport is a transient property in most transformable species; cultures in which the capacity to be transformed has developed or has been induced are termed "competent" (10, 20).

Proteins which bind to DNA have been observed in a number of transformable bacteria. Wall-membrane complexes prepared from competent *Streptococcus sanguis* bound more DNA than did preparations from noncompetent cells, and the binding was inhibited by antibody to intact competent cells (3). A binding protein was observed in wall-membrane complexes of *S. pneumoniae*. Again, preparations from competent cells were more active in binding, but treatment of preparations from noncompetent cells with autolysin increased their activity to levels characteristic of preparations from competent cells (17). The pneumococcal binding protein was readily solubilized by washing wall-membrane preparations from competent cells; certain properties of the reaction of this protein with DNA, such as time and temperature dependence of the interaction, suggest that simple binding is not the only process occurring in the presence of the protein (18). Membrane vesicle preparations from both noncompetent and competent cultures of *Bacillus subtilis* were found to bind substantial quantities of DNA; there did appear to be a high-affinity binding component which was increased in preparations from competent

cells (6, 7). Competent cultures of *B. subtilis* synthesized a soluble binding protein specific for denatured DNA; this protein was missing in a mutant defective in transformation and was also absent in early sporulating cells (4).

Transformable *Haemophilus influenzae* can interact with external DNA in a number of ways, depending on the nature of the DNA and the physiological state of the cells. Log-phase cells can bind single-stranded DNA, but they do not take it up and are not genetically transformed by it. Competent cells can bind and be transformed by double-stranded DNA; the effective interaction of competent cells with single-stranded DNA to yield transformants requires pretreatment of the cells with EDTA at low pH (11). A degree of competition between single- and double-stranded DNAs in transformation of competent *H. influenzae* has been observed (11, 12), and mutants unable to take up either native or denatured DNA have been isolated (1), suggesting that uptakes of single and of double strands may share one or more components.

To understand how *H. influenzae* transports DNA, we have begun to study the DNA-binding proteins of this species and the possible association of any of these proteins with the cell surface. This paper reports the presence of a periplasmic protein in log-phase cultures of *H. influenzae* which can bind both single- and double-stranded DNAs. After competence development, this protein cannot be dissociated from the cells by osmotic shock treatment. Some properties of the periplasmic activity have been compared to those of an intracellular binding

protein preparation. The latter activity appears to be similar to the DNA-unwinding or helix-destabilizing protein described in *Escherichia coli* (for review, see reference 2). The periplasmic activity is more sensitive to heat and does not exhibit salt-dependent cooperativity in interaction with single-stranded DNA. Since the periplasmic DNA-binding protein is apparently absent in a mutant defective in transformation, it may be involved in some of the interactions of DNA with the cell surface which occur in the process of transformation in this species. (A preliminary report of these findings has been made [S. L. Sutrina and J. J. Scocca, Fed. Proc. Fed. Am. Soc. Exp. Biol., 37:1418, 1978].)

### MATERIALS AND METHODS

*H. influenzae* Rd was grown as previously described (16, 26) to a density of  $10^9$  cells per ml. Mutant strains of *H. influenzae* defective in the ability to take up transforming DNA (1) were the generous gift of S. H. Goodgal. The procedures for preparing and assaying cultures with high competence for transformation were those of Herriott et al. (5).

DNA from *H. influenzae* was labeled with  $^{32}\text{P}$  and purified as described (16). Solutions of DNA were diluted to ca. 50  $\mu\text{g}/\text{ml}$  in 0.15 M NaCl and denatured by heating to 100°C for 5 min, followed by quick cooling. Protein was determined by the method of Schaffner and Weissmann (15). Cyclic phosphodiesterase:3'-nucleotidase was assayed according to reference 14, with bis(*p*-nitrophenyl)phosphate as the substrate.

Osmotic shock treatment of cells was done according to the procedure of Neu and Heppel (9), as modified (14). Stage II treatment in the present experiments used 5 mM  $\text{MgCl}_2$  in place of water.

Extracts containing intracellular binding protein were prepared by suspending cells in a solution of 0.2 M potassium phosphate buffer, pH 7.4–0.13% Brij 35 for 20 min at 28°C. After centrifugation, the supernatant solution was partially purified by heating, and detergent was removed by adsorption of the protein to and elution from DNA-agarose (S. L. Sutrina and J. J. Scocca, unpublished data).

The assay used for the binding protein depends on the retention of protein-DNA complexes by nitrocellulose filters under conditions which allow DNA alone to pass through the filters. For double-stranded DNA, the conditions were essentially those of Riggs et al. (13). Assay mixtures (2.2 ml) contained 10 mM Tris chloride (pH 7.4), 10 mM KCl, 0.1 mM EDTA, 10 mM magnesium acetate, 0.1 mM dithiothreitol, 0.05 mg of bovine serum albumin per ml, 5% dimethyl sulfoxide, 0.05 to 0.5  $\mu\text{g}$  of [ $^{32}\text{P}$ ]DNA per ml, and 0 to 1.0 ml of shock fluid. Assay components were prewarmed to 23°C and mixed, and duplicate 1.0-ml portions were passed through filters (Schleicher & Schuell type B-85, soaked in a buffer containing 10 mM Tris chloride [pH 7.4], 10 mM KCl, 0.1 mM EDTA, 10 mM magnesium acetate, and 5% dimethyl sulfoxide) under negative pressure. Filters were washed once with 0.5 ml of the buffer used to soak the filters, and the filters were dried and counted.

Assays with single-stranded DNA were done similarly, except that denatured DNA was added and the concentrations of dimethyl sulfoxide in the assay, washing, and soaking solutions were increased to 20% (8).

Blank values, obtained by omitting shock fluid from the reaction mixtures, have been subtracted from all values presented here. With single-stranded DNA, the blank was between 1 and 2% of the input radioactivity. With double-stranded DNA, values between 5 and 20% were obtained; these values fluctuated from preparation to preparation, and several treatments of both the DNA and the filters failed to provide significant improvement. Since preliminary experiments showed that double- and single-stranded DNAs competed equally well for binding by the protein, we used single-stranded DNA as the substrate for most routine assays. In those experiments in which native DNA was used as the substrate, we used preparations which showed the lower background values.

### Spot tests for screening of cells for defects in transformation.

The methods to screen cells for defects in transformation used were adapted from the procedures of Weiss and Milcarek (21), which employ microtitration plates and multiprong transfer devices to facilitate the handling and transfer of large numbers of colonies. Appropriately diluted suspensions of streptomycin-susceptible bacteria were spread on the surfaces of complete brain heart infusion agar plates and grown overnight at 37°C. Single colonies were picked and inoculated into individual wells of microtitration plates; each well contained 0.2 ml of complete brain heart infusion medium. These master plates were incubated at 37°C for approximately 6 h, by which time the inoculated wells showed appreciable turbidity. To each well, 0.2 ml of complete medium containing 30% glycerol was added; the contents were mixed, and the plate was stored at -70°C until needed. For transformation, the cultures on the master plate were inoculated into microtitration wells which contained 0.2 ml of complete medium supplemented with 2 mM cyclic 3',5'-AMP (23, 24) and 100  $\mu\text{g}$  of DNA from a streptomycin-resistant strain per ml. These plates were incubated overnight at 37°C. The cultures on these transformation plates were then printed, 48 at a time, onto the surfaces of four agar plates. One of these contained complete growth medium, the second contained brain heart infusion without the required growth supplements hemin and NAD, and the third and fourth contained complete brain heart infusion agar plus streptomycin sulfate at a concentration of 100  $\mu\text{g}/\text{ml}$ . These plates were then incubated overnight at 37°C. Colonies of interest were selected according to the following criteria: (i) confluent growth on complete medium, (ii) absence of growth on unsupplemented brain heart infusion agar, and (iii) identical patterns of growth on both plates containing antibiotic. The patterns of growth on the latter pair of plates provided information as to the transformability of the original clones. Fully transformable wild-type clones gave a zone of confluent growth approximately 0.5 cm in diameter. A mutant (*com-58*) with a level of transformability of ca. 10% that of the wild type showed growth of single colonies within the zone of the original droplet. These patterns were highly repro-

ducible and readily distinguishable from each other. Approximately 200 colonies of a stock of the mutant strain *H. influenzae com-58* were screened in this fashion; of these, one colony was found which showed nearly confluent growth on streptomycin agar. The response of this isolate to competence development in liquid media showed that it had regained a level of transformability approaching that of the wild type; we designated this isolate *com-58-R*. In addition, two colonies were derived from strain *com-58* which gave no streptomycin-resistant colonies on screening; they were designated *com-58-A* and *com-58-B*. These isolates showed substantially reduced levels of transformation when tested under standard conditions (approximately 1% of that of the wild type and 10% of that of the parent strain, *com-58*).

## RESULTS

Osmotic shock treatment of gram-negative bacteria causes the release of a limited group of periplasmic proteins and enzymes (9); some of the binding proteins released by this treatment play a role in solute transport (22). *H. influenzae* cultures treated in this way release ca. 1% of the total cellular protein and 40 to 60% of the cyclic phosphodiesterase:3'-nucleotidase into the stage II supernatant solution (14). Assays of osmotic shock fluids from log-phase *H. influenzae* for the ability to bind DNA showed the presence of substantial activity in the stage II (cold water) supernatant fraction and negligible activity in the stage I (Tris-EDTA-sucrose) fraction.

Some properties of the reaction between the

shock fluids and single-stranded DNA are shown in Fig. 1. The extent of retention of DNA on filters was a linear function of the amount of shock fluid added (Fig. 1A); little dependence of binding activity on DNA concentration was observable in the linear portion of the assay curve. The extent of binding was only slightly dependent on temperature (Fig. 1B) and was quite rapid, since maximal values were obtained when samples were filtered immediately after mixing (Fig. 1C). These findings indicate that the interaction between the binding activity and the DNA is not due to enzymatic alteration of the DNA which might increase its affinity for the filters but that it is a binding reaction in the strict sense.

**Stability of binding activity.** Treatment of the active shock fluids with pronase (10  $\mu\text{g}$  of shock protein per  $\mu\text{g}$  of pronase) for 30 min at 37°C completely eliminated binding activity; treatment with RNase A had no effect. The binding activity was stable to 0.1 N HCl for 10 min at 0°C. Activity was stable to heating at 60°C for 60 min at neutral pH but was lost at 80°C with first-order kinetics (half-life, ca. 20 min). This inactivation at 80°C distinguished the periplasmic binding protein from the major intracellular DNA-binding protein, since the latter activity was completely stable to heating at 80°C for extended periods of time (unpublished data).

**Specificity of the interaction.** Competition

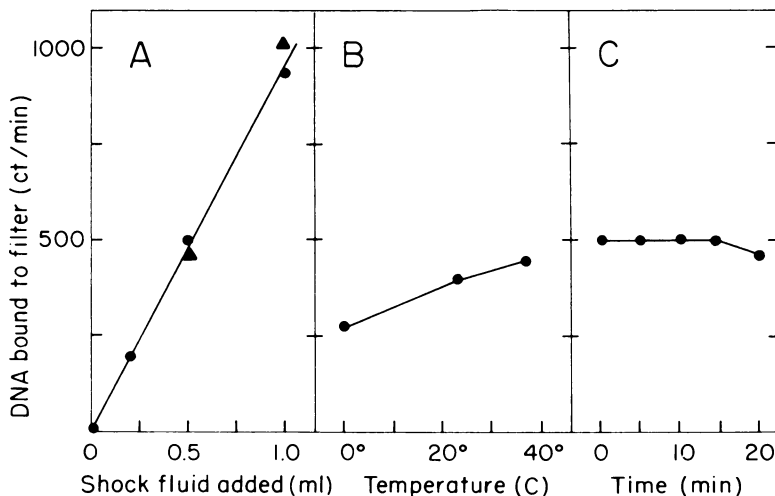


FIG. 1. Properties of the binding reaction. (A) Dependence of the retention of single-stranded DNA by filters upon the quantity of osmotic shock fluid added. The symbols represent measurements made at two concentrations of DNA: 0.01 (▲) and 0.03 (●)  $\mu\text{g/ml}$ . The temperature was 25°C. (B) Effect of incubation temperature on the binding reaction. Shock fluid (0.5 ml) and the assay mixture (containing 0.05  $\mu\text{g}$  of DNA per ml) were brought to the indicated temperature separately and then mixed, filtered, and washed immediately. (C) Time course of the reaction. Reaction mixtures prepared as in (B) were incubated at 25°C for the indicated times, filtered, and washed. All values are the averages of duplicate assays and have been corrected for blank values obtained by omitting shock fluid from the assay mixture.

experiments between radioactive and nonradioactive DNAs were conducted to determine if the periplasmic binding activity exhibited any preference for double-stranded DNA of homologous origin, since it has been shown that the interaction between *H. influenzae* cells and DNA in transformation is specific (16). The data from these experiments were plotted according to the method presented by Sisco and Smith (19), and the plots are presented in Fig. 2. Comparison of the slope values shows that *E. coli* DNA was only one-third as effective a competitor as was DNA from *H. influenzae*, indicating that the periplasmic protein exhibits an appreciable degree of specificity. However, the specificity seen here is an order of magnitude less than that seen with intact transformable cells (16).

Competition experiments between native and denatured DNA preparations from *H. influenzae* indicated that the two types of DNA were bound approximately equally on a weight basis. Addition of a 2.5-fold excess (by weight) of unlabeled native DNA reduced the binding of denatured DNA to 34% of the control value. Conversely, the presence of a 3-fold excess of unlabeled denatured DNA reduced the binding of native DNA to 22% of the control value. However, competition plots of data of this type show marked curvature. This curvature may be due to the production of more than two equivalents of single strands per equivalent of double-stranded DNA as a consequence of nicks in the strands and also as a consequence of the presence of residual double-stranded material in the denatured preparations. Better quantitative es-

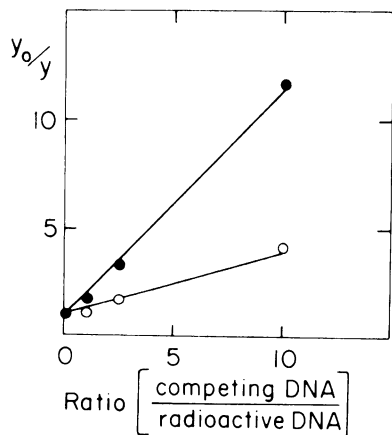


Fig. 2. Specificity of binding protein for native DNA preparations. The radioactive DNA was from *H. influenzae* (0.05  $\mu\text{g}/\text{ml}$ ), and the competing DNA was from either *H. influenzae* (●) or *E. coli* B (○). The slope with *H. influenzae* DNA was 1.0; with *E. coli* DNA, the slope was 0.3.

timates of the relative affinities of the binding protein for single- and double-stranded DNAs will require the use of DNA molecules of defined structure (e.g., phage DNA).

Competition experiments with purified bulk RNA from yeast showed that a 10-fold excess of RNA had no effect on the binding of either single- or double-stranded DNA.

**Effect of salts on binding reactions.** As indicated above, the periplasmic DNA-binding protein is more thermolabile than the major intracellular DNA-binding protein. Additional evidence that these two proteins are distinct entities was obtained in studies on the influence of salts on the association between the binding protein preparations and DNA. The periplasmic activity showed hyperbolic saturation curves at both low and high salt concentrations (Fig. 3). In contrast, increasing the concentration of salt in assays with the intracellular binding protein led to markedly sigmoidal saturation curves. Hill plots of these and other data gave a slope of 1.1 for the periplasmic binding protein independent of ionic strength; the Hill slope for the intracellular binding protein was a linear function of ionic strength, with values of 1.0 at 10 mM NaCl and 2.5 at 300 mM NaCl.

**Possible role in transformation.** To assess the protein's possible role in transformation, we assayed a series of mutants defective in DNA uptake (1) for DNA-binding activity in shock fluids. One mutant of this kind, *com-58*, showed markedly diminished levels of this protein in shock fluids; cyclic phosphodiesterase was also reduced in the shock fluids, but to a lesser extent (Table 1). The quantity of protein solubilized from this mutant was within the range expected for wild-type cells. Assays of the medium used to grow the mutant failed to reveal the presence of either the binding protein or the cyclic phosphodiesterase, suggesting that the lowered levels of these components in shock fluids was not due to release of them into the growth medium.

Several derivatives of strain *com-58* were isolated on the basis of their altered transformation properties by using the spot tests described in Materials and Methods. A summary of the transformation frequencies and the activities of the periplasmic DNA-binding protein preparations of these strains is presented in Table 2. There was a striking quantitative correlation between the transformabilities of these strains and the levels of DNA-binding activity which could be released from them by shock treatment. Strain *com-58-R*, which has regained 80% of the transformability of wild-type strains, had ca. 75% of the wild-type periplasmic DNA-binding activity. Strain *com-58-A*, in which transformability is

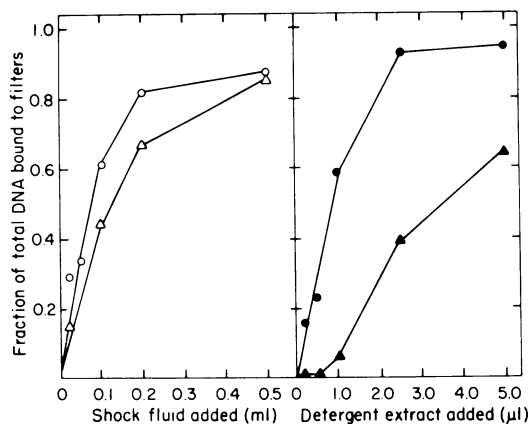


FIG. 3. Comparison of ionic strength effects on the interactions of periplasmic and of intracellular binding protein preparations with single-stranded DNA. Assays were conducted as described in the text except that sodium acetate or NaCl was added to the final concentrations indicated. Shock fluids were used as isolated; detergent extracts were prepared by suspending cells in a solution of 0.02 M potassium phosphate buffer (pH 7.4)-0.13% Brij 35 for 20 min at 28°C and centrifuging the suspension. The supernatant solution was partially purified by heating and DNA-cellulose chromatography (unpublished data). (Left) Periplasmic activity; (right) intracellular activity. Experiments contained 0.01 (●, ○), 0.25 (△), and 0.2 (▲) M [Na<sup>+</sup>].

more severely depressed than in the parent strain *com-58*, showed virtually no detectable DNA-binding activity in shock fluids.

The development of transformability had a profound influence on the extent to which the periplasmic DNA-binding protein was released by shock treatment. The shock fluids from competent cultures contained 6% of the DNA-binding activity, compared with log-phase cultures (Table 3). The quantity of cyclic phosphodiesterase released from competent cells was 36% of the control value, and the quantity of protein released was reduced by one-third. Again, the culture supernatants were devoid of DNA-binding activity. Mixing of shock fluids from competent cultures with active preparations did not diminish the binding activity, indicating that no inhibitor of the reaction was present in shock fluids from competent cells. It appears that the binding protein and other periplasmic proteins were poorly released by osmotic shock treatment of competent cells; this inaccessibility was more pronounced for the binding protein than for a hydrolytic periplasmic enzyme.

## DISCUSSION

Three lines of evidence indicate that the periplasmic DNA-binding protein is distinct from

the major intracellular DNA-binding protein. First, the periplasmic activity is more heat labile. Second, the periplasmic protein shows hyperbolic binding curves at all ionic strengths tested, whereas the intracellular binding protein exhibits marked cooperativity in binding as the ionic strength is increased. Third, a mutant strain of *H. influenzae* lacks the periplasmic activity but retains normal levels of the intracellular activity. Taken together, these findings indicate that the periplasmic DNA-binding activity is not the result of leakage of the intracellular binding protein from the cells but rather represents a novel type of DNA-binding protein located at the cell periphery.

The finding of a DNA-binding protein associated with the cell surface raises the attractive possibility that this protein may play a role in the uptake of DNA in genetic transformation. Evidence in favor of this possibility is based

TABLE 1. Release of periplasmic constituents from mutants of *H. influenzae* defective in DNA uptake<sup>a</sup>

Strain	Protein (μg/ 10 <sup>10</sup> cells)	Activity (% of wild type)	
		DNA binding	Cyclic phosphodiesterase
<i>com-2</i>	5	93	123
<i>com-6</i>	4	91	113
<i>com-51</i>	15	101	105
<i>com-58</i>	8	9	29
<i>com-62</i>	14	75	138
<i>com-68</i>	10	76	96

<sup>a</sup> Stage II supernatant fractions were prepared and assayed as described in the text.

TABLE 2. Transformation frequencies and activity of periplasmic DNA-binding protein in derivatives of *H. influenzae com-58*

Strain	Transformation frequency (%)	DNA-binding activity (% of wild type)
Wild type	1.0	100
<i>com-58</i>	0.15	11
<i>com-58-R</i>	0.8	73
<i>com-58-A</i>	0.009	<5

TABLE 3. Effect of competence development on release of components by osmotic shock<sup>a</sup>

Cell type	Activity (U/10 <sup>12</sup> cells)		Protein (mg/ 10 <sup>12</sup> cells)
	DNA binding	Cyclic phosphodiesterase	
Log phase	11 ± 4	14 ± 7	0.8 ± 0.3
Competent	0.7 ± 0.7	5 ± 2	0.6 ± 0.2

<sup>a</sup> Results given are the means ± standard deviations for eight consecutive experiments.

largely on genetic data. As shown here, a mutant strain defective in the uptake of DNA has markedly diminished levels of the binding protein. Further, derivatives of this mutant strain show a good quantitative correlation between the degree of transformability and the quantity of active DNA-binding protein in shock fractions. These results indicate that the binding protein must be present in the periplasmic space of log-phase cells for these cells to develop competence.

Two difficulties exist in assigning a precise role in transformation to this binding protein. The first is the difficulty in demonstrating its presence and location in competent cells. This may be due in part to the general resistance of competent cells to osmotic shock, but it may also be a consequence of a firmer association of the binding protein with the cell membrane. Proteins which are soluble in growing cells and membrane bound in competent cells have been identified in previous work from this laboratory (25), and the DNA-binding protein may be one of these.

The second difficulty is the reduced degree of specificity in the interaction between the periplasmic binding protein and DNA compared with that seen with intact cells. Since the interaction between the cells and transforming DNA is specific, a binding protein involved in the process would also be expected to show specificity. It is possible, however, that the specificity of the overall uptake process will only be reflected in the complete uptake apparatus, but not in the isolated constituents. Alternatively, the binding protein may participate in the formation of a DNA-protein complex distinct from that which is responsible for the specific interaction between transforming DNA and competent cells. Further clarification of these points will be assisted by the purification of the binding protein and the production of antibody specifically directed against it. These studies are in progress.

#### ACKNOWLEDGMENTS

We thank R. Deich, K. Sisco, and H. Smith for helpful discussions and for permission to cite unpublished data; L. Grossman and S. Krag for valuable advice and careful reading of the manuscript; and S. H. Goodgal for generous gifts of mutant strains.

This work was supported by Public Health Service grant GM-21890 from the National Institute of General Medical Sciences. S.L.S. is a Worthington-Kunitz Fellow.

#### LITERATURE CITED

- Caster, J. H., E. H. Postel, and S. H. Goodgal. 1970. Competence mutants: isolation of transformation deficient strains of *Haemophilus influenzae*. *Nature (London)* **227**:515-517.
- Champoux, J. J. 1978. Proteins that affect DNA conformation. *Annu. Rev. Biochem.* **47**:449-479.
- Deddish, P., and H. D. Slade. 1971. Binding of deoxyribonucleic acid by cell walls of transformable and non-transformable streptococci. *J. Bacteriol.* **105**:779-786.
- Eisenstadt, E., R. Lange, and K. Willecke. 1975. Competent *Bacillus subtilis* cultures synthesize a denatured DNA binding activity. *Proc. Natl. Acad. Sci. U.S.A.* **72**:323-327.
- Herriott, R. M., E. M. Meyer, and M. Vogt. 1970. Defined nongrowth media for stage II development of competence in *Haemophilus influenzae*. *J. Bacteriol.* **101**:517-524.
- Joenje, H., W. N. Konings, and G. Venema. 1974. Interactions between exogenous deoxyribonucleic acid and membrane vesicles isolated from *Bacillus subtilis* 168. *J. Bacteriol.* **119**:784-794.
- Joenje, H., W. N. Konings, and G. Venema. 1975. Interactions between exogenous deoxyribonucleic acid and membrane vesicles isolated from competent and noncompetent *Bacillus subtilis*. *J. Bacteriol.* **121**:771-776.
- Legault-Démare, J., B. Desseaus, T. Heyman, S. Sèror, and G. P. Ress. 1967. Studies on hybrid molecules of nucleic acids. I. DNA-DNA hybrids on nitrocellulose filters. *Biochem. Biophys. Res. Commun.* **28**:550-557.
- Neu, H. C., and L. A. Heppel. 1965. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroblasts. *J. Biol. Chem.* **240**:3685-3692.
- Notani, N. K., and J. K. Setlow. 1974. Mechanism of bacterial transformation and transfection. *Prog. Nucleic Acid Res. Mol. Biol.* **14**:39-100.
- Postel, E. H., and S. H. Goodgal. 1966. Uptake of single-stranded DNA in *Haemophilus influenzae* and its ability to transform. *J. Mol. Biol.* **16**:317-327.
- Postel, E. H., and S. H. Goodgal. 1967. Further studies on transformation with single-stranded DNA of *Haemophilus influenzae*. *J. Mol. Biol.* **28**:247-259.
- Riggs, A. D., H. Suzuki, and S. Bourgeois. 1970. *lac*-Repressor-operator interactions. I. Equilibrium studies. *J. Mol. Biol.* **48**:67-83.
- Rodden, J. L., and J. J. Scocca. 1972. Purification and properties of cyclic phosphodiesterase:3'-nucleotidase, a periplasmic enzyme of *Haemophilus influenzae*. *Arch. Biochem. Biophys.* **153**:837-844.
- Schaffner, W., and C. Weissmann. 1973. A rapid, sensitive, and specific method for the determination of protein in dilute solution. *Anal. Biochem.* **56**:502-514.
- Scocca, J. J., R. L. Poland, and K. C. Zoon. 1974. Specificity in deoxyribonucleic acid uptake by transformable *Haemophilus influenzae*. *J. Bacteriol.* **118**:369-373.
- Seto, H., R. Lopez, and A. Tomasz. 1975. Cell surface-located deoxyribonucleic acid receptors in transformable pneumococci. *J. Bacteriol.* **122**:1339-1350.
- Seto, H., and A. Tomasz. 1975. Selective release of a deoxyribonucleic acid-binding factor from the surface of competent pneumococci. *J. Bacteriol.* **124**:969-976.
- Sisco, K. L., and H. O. Smith. 1977. Sequence-specific DNA uptake in *Haemophilus* transformation. *Proc. Natl. Acad. Sci. U.S.A.* **76**:972-976.
- Tomasz, A. 1969. Some aspects of the competent state in genetic transformation. *Annu. Rev. Genet.* **3**:217-232.
- Weiss, B., and C. Milcarek. 1974. Mass screening for mutants with altered DNAses by microassay techniques. *Methods Enzymol.* **29**:180-196.
- Wilson, D. D. 1978. Cellular transport mechanisms. *Annu. Rev. Biochem.* **47**:933-965.
- Wise, E. M., S. P. Alexander, and M. Powers. 1973. Adenosine 3':5"-cyclic monophosphate as a regulator of bacterial transformation. *Proc. Natl. Acad. Sci. U.S.A.* **70**:471-474.

24. Zoon, Z. C., M. Habersat, and J. J. Scocca. 1975. Multiple regulatory events in the development of competence for genetic transformation in *Haemophilus influenzae*. *J. Bacteriol.* **124**:1607-1609.
25. Zoon, K. C., M. Habersat, and J. J. Scocca. 1976. Synthesis of envelope polypeptides by *Haemophilus influenzae* during development of competence for genetic transformation. *J. Bacteriol.* **127**:545-554.
26. Zoon, K. C., and J. J. Scocca. 1975. Constitution of the cell envelope of *Haemophilus influenzae* in relation to competence for genetic transformation. *J. Bacteriol.* **123**:666-677.