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Binding of Rabbit Gamma Globulin by Competent *Bacillus subtilis* Cultures

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Deoxyribonucleic acid (DNA)-mediated transformation of *Bacillus subtilis* can be inhibited by antibodies which specifically interact with single-stranded DNA. This inhibition occurs at a time when the transformation reaction is insensitive to deoxyribonuclease. Studies with radioactive proteins revealed that the maximal binding of gamma globulin occurs immediately preceding the development of maximal competence in the population. Other proteins, such as deoxyribonuclease cytochrome *c* and serum albumin also adsorb to the surface of the cell. After treatment with lysozyme, 67% of the radioactive gamma globulin remains associated with the cytoplasmic membrane. These findings suggest that the DNA is complexed in a deoxyribonuclease-insensitive form to the surface of the cell and is converted to a single-stranded state prior to transport past the membrane and integration into the chromosome.

Studies of the inhibition of bacterial transformation by nucleic acid-specific antibodies suggested that the rabbit gamma globulin must first interact with the recipient bacterial cells for maximal inhibition of genetic transformation (4). This conclusion was based upon the observations that: the extent of inhibition was independent of the concentration of extracellular transforming deoxyribonucleic acid (DNA); the preexposure of transforming DNA to the nucleic acid-specific antibodies did not enhance the inhibition; and the inhibitory activity of the antiserum fractions could not be completely removed by washing cells which had been incubated with antibody prior to contact with DNA (4). The availability of an efficient method of labeling the rabbit gamma globulin provided the possibility of a direct study of the physical association between antibody and recipient cell. Verification of the hypothesis that cell-antibody contact is required for effective inactivation of transforming DNA would be important, not only in elucidating the mechanism of inhibition by antibody, but also in providing information concerning some of the processes involved in the inital steps of transformation. The data to be presented clearly show that the insensitivity of newly acquired DNA to deoxyribonuclease does not necessarily prove that the DNA is in an intracellular compartment.

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MATERIALS AND METHODS

Bacterial strain. Strain 3014 was isolated by D. Birdsell and is a highly transformable phage-resistant mutant of *Bacillus subtilis* 168 (*try2gta*C14).

Transformation procedure. Strain 3014 was grown overnight on Tryptose Blood Agar Base (TBAB; Difco) and then transferred to Spizizen's minimal medium (25) supplemented with 0.5% glucose, 0.02% casein hydrolysate, 50 mM magnesium sulfate, and L-tryptophan (50 μg/ml). The cells were grown in this medium for 5 hr at 34 C on a gyrorotary shaker (110 rev/min), centrifuged, and resuspended in minimal medium in which the concentrations of casein hydrolysate and tryptophan were reduced to 0.01% and 5 μg/ml, respectively. DNA was added at various times during this growth period at 34 C, and the frequency of transformation from tryptophan auxotrophy to prototrophy was determined as described previously (25).

Preparation of DNA. DNA was isolated from wild-type cells (25). Radioactive DNA was isolated by similar procedures from an auxotroph that required tryptophan and thymine which was grown in minimal medium (25) supplemented with 50 μg of L-tryptophan per ml, 6 μg of thymine per ml, and 1.25 μc of ^{3}H-thymine per ml (specific activity, 15 mc/mmole). The final specific activity of the DNA was 28 μc/mg.

Preparation of nucleic acid-specific antibodies. Antiserum prepared against denatured calf thymus DNA (15) was fractionated by means of a slight modification of the method of Levy and Sober. Diethylaminoethyl (DEAE) cellulose (Cellex D; Bio-Rad Laboratories, Richmond, Calif.) was washed (14) and equilibrated in tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.4, 0.01 M) containing 0.03 M NaCl. After sizing

and deaeration, the DEAE was packed to a height of 10 cm in a column that had an inner diameter of 1 cm and a length of 30 cm. In most experiments, 0.2 ml of serum was added to the column directly. When the antiserum was purified for labeling with ^{131}I, the serum was first precipitated with $(NH_4)_2SO_4$ (40% of saturation), and the washed precipitate was dissolved in the starting buffer (Tris, *p*H 7.4, 0.01 M, containing 0.03 M NaCl). The column was eluted stepwise with increasing concentrations of NaCl (0.05, 0.10, and 0.15 M) in the same buffer. The optical density of the fractions was determined at 280 nm. Fractions under each peak were pooled, heated at 65 C for 60 min, sterilized by filtration (HA 0.45-μm filter; Millipore Corp., Bedford, Mass.), and stored at 4 C.

The specificity of the fractions of antiserum was determined by complement fixation tests (4). The analysis demonstrated that the first fraction eluted from the column (F1) contained both the complement-fixing antibodies and the factor which inhibited transformation.

Preparation of ^{131}I-labeled protein samples. Solutions of gamma globulin fractions and other proteins, containing from 0.5 to 5.0 mg/ml, were labeled by P. McConahey by use of a modification of the method of Hunter and Greenwood (6). After labeling, the samples were dialyzed against 0.05 M phosphate buffer for 24 hr at 4 C.

Assay of the binding of isotopically labeled material to cells. The recipient culture was incubated at 34 C with the labeled material for various intervals of time. The cells were sedimented at 8,000 rev/min at 4 C and then washed three times with minimal medium. After the last wash, the culture was resuspended to one-tenth its original volume, and radioactivity was determined in a Packard Tri-Carb scintillation spectrophotometer (23).

Chemical analysis. DNA and protein were determined by the methods of Burton (25) and Lowry et al. (12), respectively.

RESULTS

The dependence of inhibition of transformation by anti-DNA (F1) on the time of exposure of the antibody to the recipient cells is illustrated in Fig. 1. In this experiment, competence was developed in a one-step process (4). With the one-step method, the level of competence is lower and maximal competence develops at 90 min rather than in 60 min after transfer to the transformation medium. Efficiency of inhibition with constant amounts of antibody appears to be related to both the general level of transformation of the culture and the physiological conditions of recipient cells. This observation could be explained in two general ways. (i) Time is required to establish the most efficient association between the recipient cell and antibody. Assuming that all cells react with antibody to an equal extent, the decrease in inhibition observed after maximal competence is due to an increased

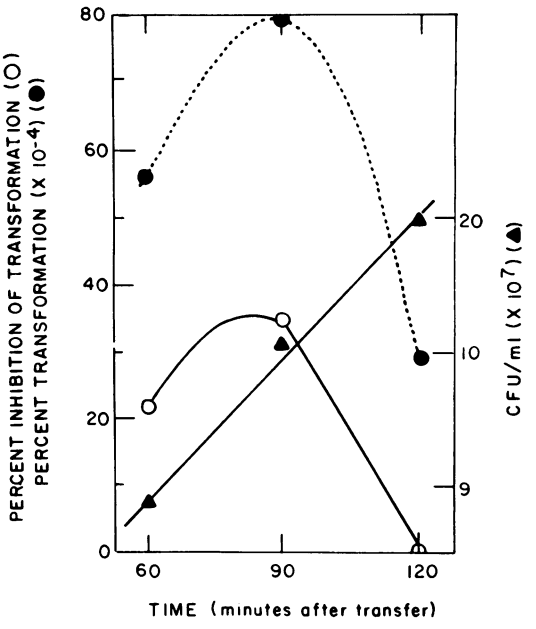

FIG. 1. *Relationship between inhibition of transformation by anti-calf thymus DNA antibodies and competence. Competence was developed as described in Materials and Methods. At various intervals after the transfer to the final growth medium, DNA (2 μg/ml) and anti-DNA F1 (20 μg) were added to the cultures. The per cent transformation and extent of inhibition were assayed as described in Materials and Methods.*

number of noncompetent cells (dividing cells) and a constant number of competent cells (nondividing or biosynthetically latent cells; 13). (ii) The lower inhibition of transformation by antibody observed in late stages of competence might be related to alterations in the cell wall or the cell membrane which influence the binding of antibody. Such alterations might be related to the electrokinetic properties of competent *B. subtilis* cells described by Jensen and Haas (8). To distinguish between these two possibilities, the binding of ^{131}I-labeled anti-DNA (F1) was studied during the development of competence in the transformation medium. As shown in Fig. 2, the binding of antibody decreases after the peak of maximal competence (60 min). The data suggest that the antibody does not become distributed among a greater number of cells during continued incubation, but, as shown by the total counts per milliliter of culture, is actually released back into the medium. This behavior of immediate binding and subsequent release is similar to the observations of Lerman and Tolmach on the binding and release of transiently bound transforming DNA by competent pneumococcal cultures (10).

Since evidence has been presented that it is

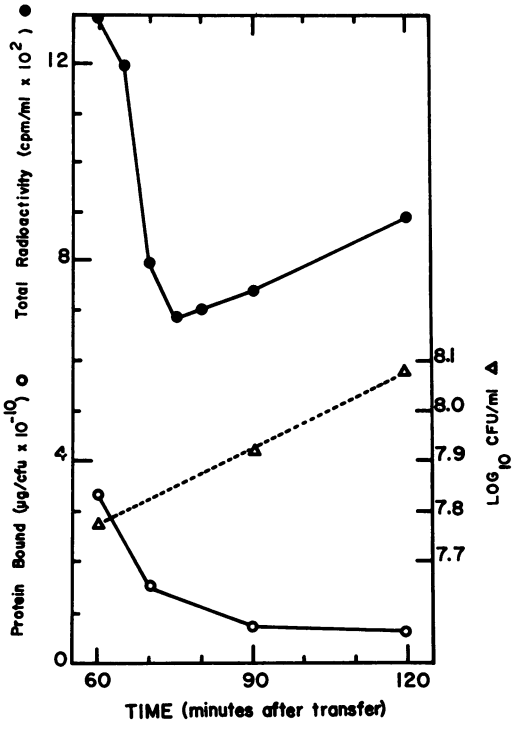

FIG. 2. *Binding of ^{131}I-labeled anti-DNA (F1) by a competent culture of B. subtilis. A culture of B. subtilis was grown for 5 hr in the growth medium, centrifuged, and resuspended in transformation medium containing 20 μg of anti-DNA (F1) per ml, specific activity, 6.65 × 10^3 counts per min per μg of protein. At various intervals, samples were removed and washed three times in minimal medium at 4 C; the radioactivity of the samples was determined in a scintillation counter.*

only the competent cells in a *B. subtilis* culture that bind highly polymerized DNA (3), it is possible that the binding of isotopically labeled transforming DNA by competent cultures would reflect the interaction of the active subset of the total bacterial population with the labeled macromolecule, and could therefore serve as a reference for similar studies with other compounds of interest. Therefore, ^{3}H-DNA was added to a competent culture at various times, and the reaction was stopped after 2 min by repeated washes at 4 C. Despite the brief exposure to DNA, high frequencies of transformation were obtained (Fig. 3).

The ability of a culture approaching the competent state to bind ^{131}I-labeled rabbit anti-DNA gamma globulin is illustrated in Fig. 4. The results indicate that the ability to bind the antibody and competence are related, but that the time of maximal binding of antibody *precedes* the time of maximal transformability.

The fact that the optimal time for binding the positively charged gamma globulin molecule is just prior to the peak of competence is strongly suggestive of an electrostatic interaction between the negatively charged competent cell (8) and the

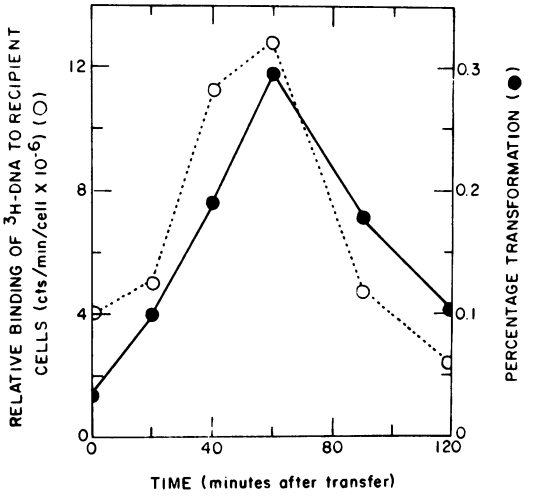

FIG. 3. *Binding of ^{3}H-DNA to competent B. subtilis. Cultures were grown for 5 hr in the growth medium, centrifuged, and suspended in transforming medium as described in Materials and Methods. At various intervals, the cells were exposed to ^{3}H-DNA (2 μg/ml) for 2 min prior to the addition of deoxyribonuclease (50 μg/ml). The procedures of washing and counting of samples were similar to those described in Fig. 2.*

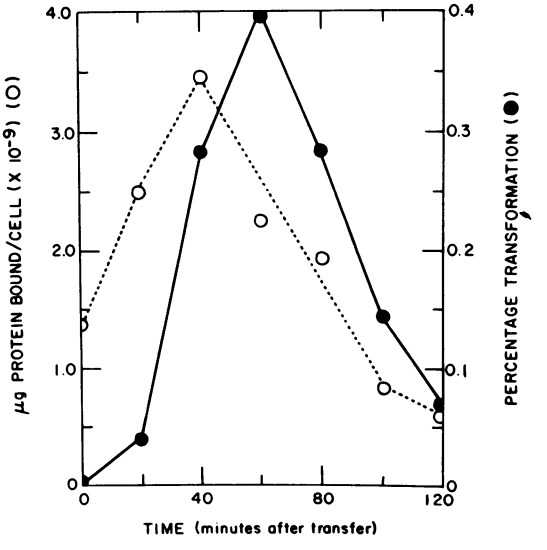

FIG. 4. *Binding of ^{131}I-labeled anti-DNA (F1) to competent cultures. Conditions for growth were similar to those described in Fig. 3. At various intervals, one sample of cells was incubated for 2 min with DNA (2 μg/ml) and another was incubated with 20 μg of anti-DNA per ml (specific activity 6.65 × 10^3 counts per min per μg of protein).*

antibody molecule. This type of interaction could explain the rapidity of binding and the demonstrated release of gamma globulin during subsequent growth. One method of decreasing the electro-negativity of the surface region of the bacterial cell is to increase the ionic strength of the medium. If the binding of antibody to the cell is ionic, the binding should be reversed by increasing the ionic strength. The data presented in Table 1 show this to be the case. Washing of the recipient cell-antibody complex in a concentrated salt solution removed 67% of the adsorbed antibody. In a similar experiment in which a competent culture exposed to bovine serum albumin (BSA) was used, 48% of the serum protein was removed after two washings in the concentrated media.

Further experiments were performed in an attempt to determine whether antibody DNA was associated with the cell wall or the protoplast membrane (23). Strain 3014 was grown as usual to a stage just prior to the peak of competence. One portion was exposed to ^{3}H-DNA and another portion was exposed to ^{131}I-labeled F1. After 10 min at 34 C, the cultures were washed three times in minimal medium and resuspended in standard saline citrate containing 20% sucrose and 10 μg of lysozyme per ml. This mixture was incubated for 10 min at 37 C and centrifuged at 18,000 rev/min for 10 min at 4 C; the radioactivity of the supernatant fraction and pellet was determined in the scintillation counter. The results showed that the majority of the DNA remained associated with the pellet. The DNA in the supernatant fractions is in the form of oligonucleotides (23). The antibody was distributed between the pellet and supernatant fluid in an identical manner (Table 2). In addition, the results of experiment B in Table 2 show that the presence of the antibody does not reduce the percentage of counts bound to the structures which are resistant to lysozyme.

TABLE 1. *Effect of exposure of cell-bound ^{131}I-serum protein to solutions of high ionic strength*

^{131}I-serum protein	Relative binding after treatment[a]		
	A	B	C
Anti-DNA (F1)	.75	.43	.25
Bovine serum albumin	.48	.23	.25

[a] Expressed as micrograms of protein bound per 10 ml of culture. Treatment was as follows: (A) washed three times in minimal medium (4 C); (B) washed once in 10× minimal medium (4 C); (C) washed twice in 10× minimal medium (4 C).

TABLE 2. *Distribution of ^{3}H-DNA and ^{131}I-anti-DNA (F1) between supernatant fluid and pellet after solubilization of the cell wall of B. subtilis with lysozyme*

Expt	Material assayed	Counts per min per culture	
		^{3}H-DNA	^{131}I-anti-DNA (F1)
A[a]	Whole cells	2,960	5,090
	Supernatant fluid	546	1,368
	Pellet	840	2,718
B[b]	Whole cells	2,150	9,790
	Supernatant fluid	342	2,040
	Pellet	859	3,905

[a] Results from two parallel cultures, exposed to labeled material 45 min after transfer to transformation medium; deoxyribonuclease was added 65 min after transfer. Cells were washed three times in minimal medium and samples were counted. The remainder of the sample was resuspended in 0.1 M NaCl + Na citrate, 20% sucrose, and 10 μg of lysozyme per ml, and was incubated for 10 min at 37 C. Cultures were then centrifuged at 18,000 rev/min and supernatant fluid and pellet were analyzed for radioactivity.

[b] Assay procedure same as above except ^{3}H-DNA culture was preexposed to unlabeled anti-DNA (F1) and ^{131}I anti-DNA (F1) culture was preexposed to unlabeled transforming DNA.

Additional experiments were designed to gain information regarding the mechanism of antibody inhibition of bacterial transformation. In one experiment, ^{3}H-labeled transforming DNA was added to a recipient culture that had been previously exposed to anti-DNA (F1) at the time of maximal binding of antibody, and the extent of DNA binding was compared to an untreated control. If the sites blocked by antibody were responsible for the binding of the transforming DNA (i.e., assuming that the binding sites are identical), then the extent of inhibition of transformation should be directly related to the reduction in DNA binding. On the other hand, if DNA can react with antibody that is already associated with the plasma membrane, then the presence of the antibody should not influence the extent of association between transforming DNA and the recipient cell. The results showed that there was no direct correlation between inhibition of binding and inhibition of transformation (Table 3).

The data presented in Fig. 5 show that deoxyribonuclease I is maximally bound by the recipient population at approximately the same time as the rabbit gamma globulin. Similar results were obtained with cytochrome *c*. On the other

hand, the binding of serum albumin is different. Thomas (18) suggested that the state of deoxyribonuclease insensitivity in bacterial transformation may reflect a differential ability of the recipient to take up the enzyme and the transforming DNA (i.e., a decrease in uptake of deoxyribonuclease and a concomitant increase in the ability to interact with the nucleic acid). If this were the case, then the inhibitory effect of the enzyme at this time should be similar to the action of the antibody, and the antibody should be unable to inactivate deoxyribonuclease-insensitive transforming DNA. The data presented in Table 4 show that the DNA rapidly escapes the action of the enzyme, but that this deoxyribonuclease-insensitive material remains sensitive to the inhibitory action of the antibody.

TABLE 3. *Effect of anti-DNA (F1) and of normal rabbit serum (F1) on the binding of isotopically labeled DNA to recipient cells*

Serum fraction employed[a]	Inhibition of uptake of labeled DNA	Inhibition of transformation[b]
	%	%
Anti-DNA (F1)	3	32
Anti-DNA (F1)	27	68
Normal rabbit (F1)	8	0

[a] An amount of 30 μg of protein was added to 1 ml of recipient cells.

[b] Based on results obtained with parallel cultures that had been exposed, under identical conditions, to unlabeled transforming DNA.

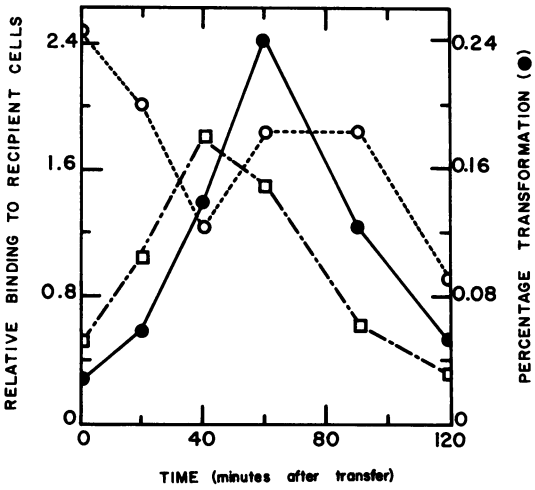

FIG. 5. *Binding of various macromolecules to cultures of competent B. subtilis. Samples of competent cultures were incubated with various macromolecules for 2 min at various intervals and washed; binding was determined as described in Fig. 2. Bovine serum albumin, ○; deoxyribonuclease, □; per cent transformation, ●.*

TABLE 4. *Inhibition of transformation by anti-DNA (F1)*

Time of DNA addition	Time of deoxyribonuclease addition[a]	Time of anti-DNA (F1) addition[b]	Inhibition of transformation
min	*min*	*min*	%
0	40	—	0
0	40	45	79
0	40 and 45	—	14
30	45	—	0
30	45	48	92
30	45 and 48	—	0

[a] Deoxyribonuclease concentration: 20 μg/ml.

[b] Antibody concentration: 30 μg/ml.

DISCUSSION

The observations reported in this paper, while not providing definitive answers concerning the precise nature of early steps in bacterial transformation, nevertheless suggest a number of previously unrecognized intermediate steps. It has usually been assumed that the transforming DNA which is "irreversibly" bound has reached an intracellular compartment, and thus is no longer available to the extracellular environment. The finding that transformation can be interrupted by antibodies to single-stranded DNA, even after exposure of the recipient cell-DNA complex to deoxyribonuclease, strongly suggests that this conclusion is not entirely correct (if one regards sites between the membrane and the wall as being not really intracellular). The possibility that antibodies are transported into the cell and inactivate intracellular transforming DNA is weakened by two observations. First, the present data have shown that alterations in the ionic environment release the cell-associated protein. Second, autoradiographic studies have shown that the transforming DNA remains at membrane sites for extended periods (21, 23), suggesting that the inactivation by antibody does not take place in a truly intracellular environment.

The observation that bacterial cells bind various macromolecules when grown under the described conditions poses several questions regarding the nature of the competent state in *B. subtilis*. If competence, as it has frequently been defined, is related solely to the recipient cells' ability to bind highly polymerized DNA irreversibly, then it appears that at different times the cells are capable of binding different macromolecules. These changing binding properties of

the recipient cells may reflect subtle changes both in the structure of the cell wall and in the plasma membrane, including change in the ionic nature of the cell periphery due to changing physiological conditions within the competent cells.

A number of different studies have demonstrated alterations in the properties of the surface of the competent cells. For instance, the work of Jensen and Haas (8) revealed variation in the capacity of competent cells to pass through membrane filters. Chemical analysis of the walls of *B. subtilis* revealed changes in the content of *N*-acyl galactosamine (22, 24) during growth. This amino sugar, which is confined to the teichoic acid fraction of the cell wall (F. E. Young and A. P. Jackson, Federation Proc. 27:436, 1968), occurs in greater amounts in the cell walls of the wild type under conditions of maximal competence. Recently, however, mutants with extensive alterations in teichoic acid have been isolated which have low levels of galactosamine in the cell wall but are still highly transformable (F. E. Young, *unpublished data*). Thus, many alterations of the cell wall may occur which influence the binding capacities of macromolecules in a fashion that is observed with purification of macromolecules by binding to ion-exchange resins. The relevance of such alterations to the development of competence is still unclear.

The observation that maximal binding of the rabbit gamma globulin precedes the period of maximal transformability (Fig. 1) explains the previously discussed enhancement of inhibition by anti-DNA (F1), when cells and antibody are preincubated prior to DNA addition (2, 4). The gamma globulin may be adsorbed rapidly to the bacterial surface, perhaps to the membrane, and then either may be more readily available at sites of DNA binding or may be slowly released after the transforming DNA has become bound to the cell membrane. Such a mechanism obviously would concentrate antibody and DNA at the bacterial surface.

The site of binding of both the rabbit gamma globulin and the transforming DNA appears to be the plasma membrane or, less likely, some other lysozyme-resistant portion of the cell wall. It has been suggested previously that the binding to the membrane may be facilitated by the action of an autolytic enzyme active on the surface of competent cells, creating "gaps" in the mucopeptide structure of the cell wall and exposing membrane to which the transforming DNA (26) and, as shown here, the gamma globulin and deoxyribonuclease may become electrostatically complexed. The finding that antibody which is specific for single-stranded DNA does not interfere with the initial interaction of transforming DNA and recipient cell, but inhibits the reaction with deoxyribonuclease-insensitive cell-associated DNA, suggests that the cell-associated DNA attains single-stranded characteristics, a property that might be a consequence of the binding to the plasma membrane. A single-stranded stage is known to exist in pneumococcal transformation (9) and has been suggested in the *B. subtilis* system (20). In addition, the observation that this membrane-bound DNA is resistant to degradation by deoxyribonuclease but is still reactive with the antibody might indicate that the configuration of the DNA membrane complex is such that the phosphodiester bonds between the bases are protected, whereas the hyrophobic bases are repelled by the outer membrane layer and are still susceptible to reaction with the antibody. Another possibility could be a geographic separation of binding sites for deoxyribonuclease and DNA on the membrane, thus preventing an interaction between the two.

Recent studies in a number of laboratories have suggested that the complexing of DNA with the cell may occur in specialized mesosomes or in mesosomes involved in cell division. For instance, Wolstenholme et al. indicated that the transforming DNA is located near the mesosomes (21). The studies of Javor and Tomasz (7) and Young (23) demonstrate that DNA is frequently bound near the tips of cells. The more recent study of Javor and Tomasz showed clearly that the DNA is also bound in the middle of cells (7). Whether the DNA penetrates through mesosomes that are associated with cell division or specialized mesosomes involved in sporulation must be resolved by future experiments.

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LITERATURE CITED

1. Akrigg, A., S. R. Ayad, and G. R. Baker. 1967. The nature of a competence-inducing factor in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. 28:1062–1067.
2. Braun, W., O. Plescia, M. Kohoutova, and J. Grellner. 1966. Inhibition of transforming activities by antisera against DNA and poly dAT, p. 181–185. *In* M. Kohoutová and J. Hubáček (ed.), The physiology of gene and mutation expression. Academia, Prague.
3. Cahn, F. H., and M. S. Fox. 1968. Fractionation of transformable bacteria from competent cultures of *Bacillus subtilis* on Renografin gradients. J. Bacteriol. 95:867–875.
4. Erickson, R. J., W. Braun, O. J. Plescia, and Z. Kwiatkowski. 1968. Inhibition of bacterial transformation by nucleic acid-specific antibodies, p. 201–221 *In* O. J. Plescia and W. Braun (ed.), Nucleic acids in immunology. Springer-Verlag, New York.
5. Fox, M., and R. D. Hotchkiss. 1957. Initiation of bacterial transformation. Nature 179:1322–1325.

6. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. Nature 194:495–496.
7. Javor, G. T., and A. Tomasz. 1968. An autoradiographic study of genetic transformation. Proc. Nat. Acad. Sci. U.S.A. 60:1216–1222.
8. Jensen, R. A., and F. L. Haas. 1963. Electrokinetics and cell physiology. II. Relationship of surface charge to onset of bacterial competence for genetic transformation. J. Bacteriol. 86:79–86.
9. Lacks, S. 1962. Molecular fate of DNA in genetic transformation of pneumococcus. J. Mol. Biol. 5:119–131.
10. Lerman, L. S., and L. J. Tolmach. 1957. Genetic transformation. I. Cellular incorporation of DNA accompanying transformation in pneumococcus. Biochim. Biophys. Acta 26:68–82.
11. Levy, H. B., and H. A. Sober. 1960. A simple chromatographic method for preparation of gamma globulins. Proc. Soc. Exp. Biol. Med. 103:250–252.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
13. Nester, E. W., and B. A. D. Stocker. 1963. Biosynthetic latency in early stages of deoxyribonucleic acid transformation in *Bacillus subtilis*. J. Bacteriol. 86:785–796.
14. Peterson, E. A., and H. A. Sober. 1962. Column chromatography of proteins: substituted celluloses, p. 3–27. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 5. Academic Press Inc., New York.
15. Plescia, O. J., W. Braun, and N. C. Palczuk. 1964. Production of antibodies to denatured deoxyribonucleic acid (DNA). Proc. Nat. Acad. Sci. U.S.A. 52:279–285.
16. Ravin, A. W. 1961. The genetics of transformation. Advan. Genet. 10:61–163.
17. Shockman, G. D. 1965. Symposium on the fine structure and replication of bacteria and their parts. IV. Unbalanced cell-wall synthesis: autolysis and cell-wall thickening. Bacteriol. Rev. 29:345–358.
18. Thomas, R. 1955. Recherches sur la cinétique des transformations bactériennes. Biochim. Biophys. Acta 18:467–481.
19. Tomasz, A. 1966. Model for the mechanism controlling the expression of competent state in pneumococcus cultures. J. Bacteriol. 91:1050–1061.
20. Venema, G., R. H. Pritchard, and T. Venema-Schröder. 1965. Properties of newly introduced transforming deoxyribonucleic acid in *Bacillus subtilis*. J. Bacteriol. 90:343–346.
21. Wolstenholme, D. R., C. A. Vermeulen, and G. Venema. 1966. Evidence for the involvement of membranous bodies in the processes leading to genetic transformation in *Bacillus subtilis*. J. Bacteriol. 92:1111–1121.
22. Young, F. E. 1965. Variation in the chemical composition of cell walls of *Bacillus subtilis* during growth in different media. Nature 207:104–105.
23. Young, F. E. 1967. Competence in *Bacillus subtilis* transformation system. Nature 213:773–775.
24. Young, F. E., I. P. Crawford, and J. Spizizen. 1963. Biochemical aspects of competence in the *Bacillus subtilis* transformation system. I. Chemical composition of cell walls. J. Biol. Chem. 238:3119–3125.
25. Young, F. E., and J. Spizizen. 1963. Incorporation of deoxyribonucleic acid in the *Bacillus subtilis* transformation system. J. Bacteriol. 86:392–400.
26. Young, F. E., and J. Spizizen. 1963. Biochemical aspects of competence in the *Bacillus subtilis* transformation system. II. Autolytic enzyme activity of cell walls. J. Biol. Chem. 238:3126–3130.