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Genetic Analysis of DNA-Surface Interactions in *Bacillus subtilis*

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In a paper which has provided the foundation for many productive experiments in molecular biology and bacterial cell division, Jacob, Brenner, and Cuzin (11) proposed the "replicon" model to explain some of the mechanisms of DNA initiation and replication. One major concept of their proposal was that portions of the cellular genome were attached to the cytoplasmic membrane. That assumption made it possible to account for proper segregation of the genome into daughter cells concomitant with cell growth.

Subsequently, research in several laboratories demonstrated that the chromosome of various bacteria was indeed attached to the surface. Moreover, the attachment was at the origin and terminus of replication and at the replication point, as well as at several nonspecific points along the chromosome (4, 5, 9, 13, 17, 18). The specific association of the chromosome at sites involved in DNA replication reinforces the concept of a causal relationship among DNA-surface attachment, cell division, and chromosome segregation. To study this relationship further, we have investigated DNA-surface attachments in *Bacillus subtilis* under conditions in which normal cell division and DNA segregation are disturbed.

MEMBRANE-DNA COMPLEX IN A STABLE L-FORM

A stable L-form from *B. subtilis*, *sal-1*, has been propagated in liquid media since 1969 (19). The L-form grows in the absence of any cell wall and is stabilized by 1.2 M NaCl. *sal-1* and its salt-independent derivative, *sig-1*, have been shown to divide aberrantly, producing progeny with altered cytoplasm and DNA contents (6, 7). In this respect, these two L-forms are similar to other L-forms isolated from *B. subtilis* (14). We have used *sal-1* as a model for an organism which has lost proper cell division and DNA segregation control.

To determine whether the DNA-membrane attachment remained intact in these aberrantly dividing cells, we isolated DNA-membrane complexes by use of Renografin gradients (9). The complexes were then assayed for DNA content by transformation, and membrane enrichment indices were calculated (9). As shown in Fig. 1, when compared to spheroplasts (panel A), the L-form (panel B) retained enrichment for genetic markers close to the origin of replication, *purA16* and *cysA14*. Several internal markers were not enriched in either sample. However, selective enrichment for genes close to the terminus of replication, such as *trpC2*, *gltA292*, *citK5*, and *thyB*, was found to be lost in the L-form but not in spheroplasts. It is attractive to speculate that this loss of replication terminus attachment contributes to the loss of division control in the L-form.

There are several factors which could have contributed to the loss of replication terminus binding (9). Among the most probable alternatives are the absence of a cell wall or the presence of high salt (1.2 M NaCl) in the growth medium. If the loss of cell wall contributed to the dissociation of the terminus of replication from the surface, then it follows that the cell wall contributes to the makeup of the normal DNA-surface complex.

CELL WALL-DNA COMPLEXES

To examine the possibility that the cell wall is involved in attaching the chromosome to the surface of *B. subtilis*, we isolated cell walls at various stages of growth and examined the preparations for transforming activity (1, 15; R. J. Doyle et al., submitted for publication). Not only did isolated cell walls possess transforming activity, but the transformation was specifically enhanced for genetic markers around the replication origin and terminus (Fig. 1C). The extent of enrichment in the cell wall differs from that observed with membrane-DNA complexes. Thus, *hisA1*, *pyrA1*, and *sacA* were enriched in the cell wall samples, whereas the same markers were not enriched in membrane-DNA complexes (9, 15, 18). In contrast, *cysA14*, a marker which was shown to be membrane-enriched, was not found to be specifically attached to the cell wall (9, 15, 16). The significance of these differences is not obvious at the present time. There is an overall symmetry ap-

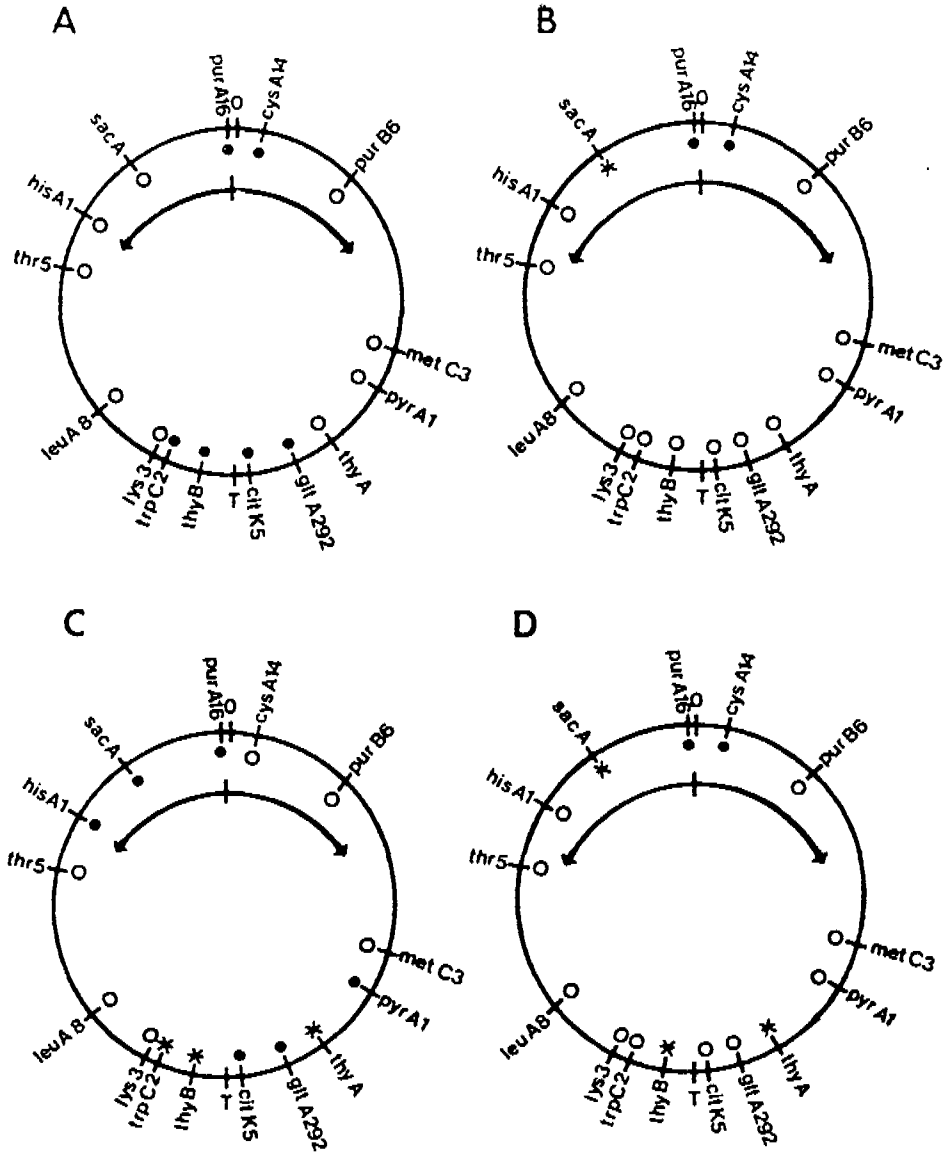

FIG. 1. *Genetic analysis of surface-DNA complexes in* Bacillus subtilis. *Genetic markers on the chromosome of* B. subtilis *and its L-form,* sal-1, *were examined for enrichment in membrane and wall preparations (9, 15). Represented are the enrichment maps for spheroplast membrane-DNA (A), L-form* (sal-1) *membrane-DNA (B), cell wall-associated DNA (C), and membrane-DNA from 1.2 M NaCl-treated cells (D). The genetic map of* B. subtilis *is that presented by Young and Wilson (20). Each marker is designated as enriched (●), nonenriched (○), or not examined (*). Replication origin (O), replication terminus (T).*

parent in the cell wall-DNA profile (Fig. 1C), which is not present in the membrane-DNA preparations (Fig. 1A and B). This loss of symmetry could be due to changes in the topography of the in vivo DNA-surface complex after removal of the cell wall. Along with these alterations it is possible that prolonged growth in the absence of a cell wall could also result in

the dissociation of the terminus from the membrane.

EFFECT OF SALT ON MEMBRANE-DNA COMPLEXES

An alternate possibility for the loss of attachment of the replication terminus by the L-form is the presence of 1.2 M NaCl in the growth medium of this organism. A high concentration of salt may destroy the integrity of the surface-DNA complex. The effect of salt on morphology has been documented (3, 12).

We grew *B. subtilis* BUL 404 (*metB10*) in 1.2 M NaCl and isolated membrane-DNA complexes at various intervals. In Fig. 1D, we show the membrane-DNA profile from cells grown in 1.2 M NaCl for 120 min. It is obvious that growth in salt dissociated the replication terminus from the membrane in the bacterial strain. Recent experiments suggest that replication terminus dissociation occurs within the first 30 min after addition of 1.2 M NaCl to the culture growth medium. Moreover, after 120 min of growth in 1.2 M NaCl, the entire population of cells has assumed abnormal morphological characteristics, suggestive of changes in cell division control. Thus, we have been able to emulate the L-form state of replication terminus detachment and induce abnormal cell division by growing *B. subtilis* cells in high salt. Presumably, plasmolysis has occurred in these cells. Therefore, it is attractive to postulate that the replication terminus site may be labile to plasmolytic detachment of the membrane from the wall. Such removal could destroy the integrity of this surface-DNA site. In contrast, the replication origin-surface complex appears to be inviolate to the effects of high salt concentrations. Origin of replication binding has also been maintained in the L-form. This suggests that the replication origin-surface complex may be of primary and vital importance for cell survival.

DISCUSSION

On the basis of the foregoing series of experiments, several conclusions can be drawn concerning the surface-DNA complex in *B. subtilis*. First, the in vivo complex contains not only the chromosome and membrane, but also peptidoglycan. Second, of all the binding sites, the attachment of the origin of replication is of primary importance. This attachment is maintained in all samples assayed to date. Third, the replication terminus attachment appears to be labile to high salt and perhaps prolonged growth in the absence of the cell wall. Thus, the L-form and cells grown in 1.2 M NaCl have lost preferential attachment at the terminus of replication. Terminus attachment and normal morphology can be restored after removal of the 1.2 M NaCl (S. Horowitz et al., submitted for publication). Finally, once the attachment in the terminus region is lost, cells are observed to have altered morphology and division patterns.

These data suggest that the DNA-surface complex is a vital structure for the procaryotic cell and has a dynamic role in the maintenance of the cell cycle. The complex may regulate not only DNA replication but also DNA segregation and cell division events. If the complex is disturbed, then aberrancies in cell division processes occur. It is possible that information for the proper maintenance of the complex and its functions resides in the proteins found in both the cell wall and cell membrane (2, 8, 10). Such interactions, however, remain uncharacterized at the present time. The ability to manipulate the constitution of the DNA-surface complex and the morphology of cells leads to the possibility that the individual functional components may be isolated and studied.

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