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RESTRICTED CHROMOSOME-MEMBRANE ASSOCIATION IN A STABLE L-FORM OF BACILLUS SUBTILIS.

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ABSTRACT

A stable L-form (sal-1) of Bacillus subtilis BR151 was found to retain a chromosome-membrane association. The attachment of the chromosome to the membrane in the L-form is specific for the replication origin but not for the replication terminus. These results are in contrast to those obtained with the parental, cell-walled strain in which both the origin and terminus of chromosomal replication are preferentially attached to the membrane.

INTRODUCTION

Attachment of the chromosome to the cytoplasmic membrane and to the cell wall has been well demonstrated in cell-walled prokaryotes and is postulated to be instrumental in DNA segregation and division control [1,2,3,4]. This attachment is specific for the origin and terminus of chromosomal replication and for the replication point [1,4,5]. In Mycoplasma the chromosome was also found to be attached to the membrane [1,6]. Studies of unstable L-forms, utilizing electron microscopy, have also revealed chromosome-membrane association [7]. Yet, the genetic specificity of the chromosomal attachment in cell wall-deficient prokaryotes has not been studied. In this report, we demonstrate that a stable L-form (sal-1) of B. subtilis BR151, which has no cell wall polymers and divides aberrantly [8,9, R.W. Gilpin, personal communication], retains a chromosome-membrane association. The attachment is preferential for the region at the origin of chromosomal replication, but the specific attachment of the region close to the replication terminus has been lost.

MATERIALS AND METHODS

Bacterial strains. The stable L-form (sal-1) of B. subtilis BR151 [8,9] was kindly provided by R. W. Gilpin and retains the metB10 marker. The parental strain, B. subtilis BR151 lys-3, trpC2, metB10, was transformed to prototrophy for

lys-3 and trpC2 with B. subtilis W168 DNA to maintain the same auxotrophic background as the L-form. All strains used as recipients in the transformation experiments are listed elsewhere [10].

Media and growth conditions. The L-form (sal-1) was grown in sal-1 medium [10] which contains 1.2M NaCl for stabilization, according to a modification of the technique described by Gilpin et al [9], in the presence of deoxyadenosine (200 µg/ml) and ^{14}C-thymidine (0.1 µCi/ml, 53 mCi/mmole) for about two generations. The culture was washed in NCP buffer [11] plus 1.2M NaCl and lysed in NCP buffer by osmotic shock. The parental strain, B. subtilis BR151 metB10, was grown and labeled under the same conditions as the L-form but without the NaCl, washed in NCP buffer, and lysed by addition of lysozyme (0.5 mg/ml).

Renografin gradients. Lysates of the L-form and its parental strain were applied onto 0-38% linear Renografin density gradients [11] and were centrifuged, fractionated and counted according to a modification of the technique described by Ivarie and Péne [11].

Transformation procedures. The procedures for the development of competence and transformation were according to those described by Boylan et al [12]. Dilutions of the peak fractions from the Renografin gradients served as donor DNA.

RESULTS

Attachment of the chromosome to the membrane. Uniformly labeled DNA from an unsheared lysate of the L-form sal-1 (Materials and Methods) forms one distinct band at the lower part of the Renografin gradient. This band corresponds to the membrane-DNA (mDNA) peak which is found in the untreated lysate of the parental strain B. subtilis BR151 metB10 (results not shown). The DNA attached to the membrane of both the L-form lysate and the lysate from the parental strain contains about 80% of the label incorporated. Shearing the L-form lysate by vortexing for 30 seconds at setting no. 6 causes a decrease in the amount of labeled DNA which appears in the m-DNA peak and generates a slower sedimenting peak which bands at the upper part of the Renografin gradient. This new peak corresponds to the free DNA (f-DNA) peak of the parental strain (results not shown). The distribution of the uniformly labeled DNA from the L-form sheared lysate consists of about 55% label which appears as free DNA and about 30% which appears as firmly attached membrane associated DNA. This distribution is similar to that of the uniformly labeled DNA from the sheared

lysate of the parental strain [10].

Genetic analysis of the specificity of chromosome attachment. Since the DNA isolated in the Renografin gradients can be used in transformation assays, genetic analysis for the specificity of DNA attached to the membrane was performed, using peak fractions from the sheared lysate of the L-form and the parental strain as donor DNA. Genetic markers were tested throughout the chromosome and included markers at the origin and terminus regions of chromosomal replication as well as internal loci. The specificity of the attachment was determined by the membrane enrichment index (MEI), calculated according to Sueoka and Quinn [2] using leuA8 as a standard. Several transformation experiments were performed for each marker with both the L-form and the parental strain DNA peak samples, and the average MEI was calculated. An MEI value which equals or exceeds 1.40 was taken to indicate that the marker is enriched in the DNA attached to the membrane, hence preferentially bound. An MEI value close to 1.00 was accepted to show no specificity for the attachment to the membrane. The results from the transformation experiments are shown in Figure 1. None of the internal markers that we have examined has any specificity for association with the membrane in both the L-form and the parental strain. The origin region of chromosomal replication (purA16 and cysA14) in the L-form is preferentially attached to the membrane as is the case in the parental strain. However, the region close to the replication terminus (trpC2, thyB, citK5, gltA292, and thyA) does not appear to be attached to the membrane of the L-form although it is preferentially attached to the membrane in the parental strain.

DISCUSSION

This study demonstrates that the L-form, sal-1, retains a membrane-chromosome association in both sheared and unsheared lysates. This attachment is quantitatively similar to that of the parental strain and may indicate the importance of maintaining such an association for normal cell growth.

Genetic analysis for the specificity of the membrane-chromosome association in the L-form shows that the preferential attachment of the origin of chromosomal replication is retained. However, specificity for the attachment of the terminus region has been lost, when compared to the parental strain. This finding suggests that the two sites of attachment are different, and that the attachment of the origin is of primary importance. The loss of association of the terminus to the membrane in the L-form could be the

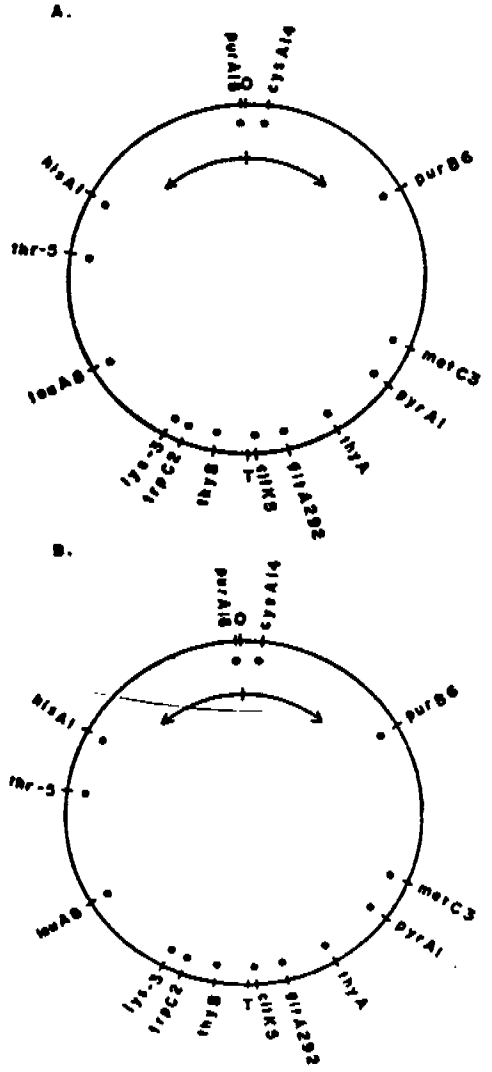

Figure 1. Genetic analysis of m-DNA from the L-form and its parental strain. Genetic markers along the entire chromosome of both the L-form and its parental strain were examined for specific enrichment in membrane samples (MEI) by transformation (Materials and Methods). Represented is the genetic map of the L-form sal-1 (A) and of the parental strain BR151 metB10 (B). The genetic map of Bacillus subtilis was constructed from those of Lepesant-Kejzlarova et al [13] and Young and Wilson [14]. Each marker is designated as enriched ( ) or non-enriched (o) in m-DNA samples. Replication origin (O); Replication terminus (T).

result of one or more of the following physiological and/or genetic alterations. First of all, the loss of the cell wall may have removed an outer surface site for terminus attachment [15,16]. Secondly, a loss or alteration of a binding protein(s) which aids in the specific attachment of the chromosome to the membrane (or cell wall) could be reflected in the loss of association of the terminus region. Also, a temperate bacteriophage which inserts in this area of the chromosome [17] could participate in the binding of the genome. The L-form could have lost such a bacteriophage. Finally, the presence of a high concentration of salt (1.2M NaCl) throughout the growth cycle of the L-form could be responsible for the dissociation of specific, bound regions of the chromosome [3,9]. We are presently investigating all of these possibilities.

The chromosome of B. subtilis has been found to bind to the cell wall polymer [4,15]. It is attractive to speculate that any alteration of the integrity of the cell wall-protein-membrane-DNA complex would result in aberrant division and DNA segregation patterns. Our observation that the L-form, sal-1, of B. subtilis is altered in a part of the replication complex, the attachment of the terminus of chromosome replication, provides initial support for such a speculation. It also provides opportunity for further studies on the role of the bacterial outer surface in DNA replication and segregation, and cell division.

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