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# Highly Specific Labeling of the *Bacillus subtilis* Chromosome Terminus

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By making use of the sporulation process, the terminus region of the *Bacillus subtilis* chromosome has been labeled with [$^3$H]thymine in a highly specific manner. The result achieved supports the view that *B. subtilis* spores contain only completed chromosomes.

Both the origin and the terminus regions of the bacterial chromosome show a specific interaction with the cell surface, probably the membrane (see reference 5), and it is possible that these interactions reflect a role for both regions in the process of nucleoid segregation or the division process, or both. Considerable progress has been made in the chemical characterization of the chromosomal origin of *Escherichia coli* and *Bacillus subtilis* (6, 7, 10, 12). The successful approach to labeling the origin in some of these studies took advantage of the highly synchronous initiation of a round of DNA replication that can be achieved by the use of a thymine starvation approach. We report here an approach that has been successful in labeling specifically the terminus region of the *B. subtilis* chromosome. It should enable further progress on the characterization of this region. The approach makes use of the sporulation process, and the result achieved provides additional support for the view that *B. subtilis* spores contain only completed chromosomes (2, 8). M. Sargent (personal communication) has used much the same approach to successfully label the chromosome terminus of *B. subtilis*, but his method for identifying the region has been different.

When an exponential culture of *B. subtilis* is suspended in a poor (starvation) medium, the process of sporulation is quickly induced (11). Recently, Dunn et al. (4) investigated the relationship between DNA replication and the induction of sporulation in this starvation system. The DNA replication inhibitor 6-(*p*-hydroxyphenylazo)uracil (HPUra) was found to have a marked effect on the level of sporulation achieved when added up to 2 h after resuspension. This correlated with the time needed for the cell population, as a whole, to complete all rounds of replication. The reduction in spore yield when HPUra was added before 2 h was considered to result from only a portion of the population having completed its last round of replication at the time of addition of the drug. Those still replicating when HPUra was added would not sporulate. Clearly, if only completed chromosomes are incorporated into spores, it should be possible to specifically label the terminus region (by density or radioactivity) by adding the appropriate DNA label during the initial stages of sporulation when rounds of replication are completing and, shortly afterwards, arresting replication with HPUra. Spore chromosomes containing label should be those that completed the last round of replication between the times of addition of the label and HPUra.

The approach outlined was first applied in an attempt to density label the terminus with 5-bromouracil (BU). However, all chromosomes labeled with BU in the starvation medium were excluded from the spores that formed, even in the BU-tolerant strain BUt-32 (3).

In subsequent experiments [$^3$H]thymine was used to label the temperature-sensitive *dna-1* strain (13) under permissive conditions. (The *dna* mutation is of no significance in this particular study. *dna-1* has been used because of other studies being pursued with terminus-labeled chromosomes and for which use of this strain is necessary). *B. subtilis* (*dna-1*) was grown at 34°C in a rich casein hydrolysate medium (plus 10 μg of thymine per ml) and suspended in a poor minimal salts medium (plus 5 μg of thymine per ml) as previously described (10). The effect of HPUra (100 μM), added between 30 and 90 min after suspension, on spore yield was examined and found to be in accordance with previous observations (4); when HPUra was added at 60 min the yield was reduced to approximately 20% of the control ($4.7 \times 10^8$ spores per ml). This indicates that much of the chromosome population was still replicating at this time. In subsequent experiments, [$^3$H]thymine was added at 60 min and HPUra was added at 70 min. After purification of the spores that formed, the chromosomal location of the label was established in a conventional density transfer experiment involving germination of the spores in the presence of BU (9).

Figure 1 shows the replication behavior of the

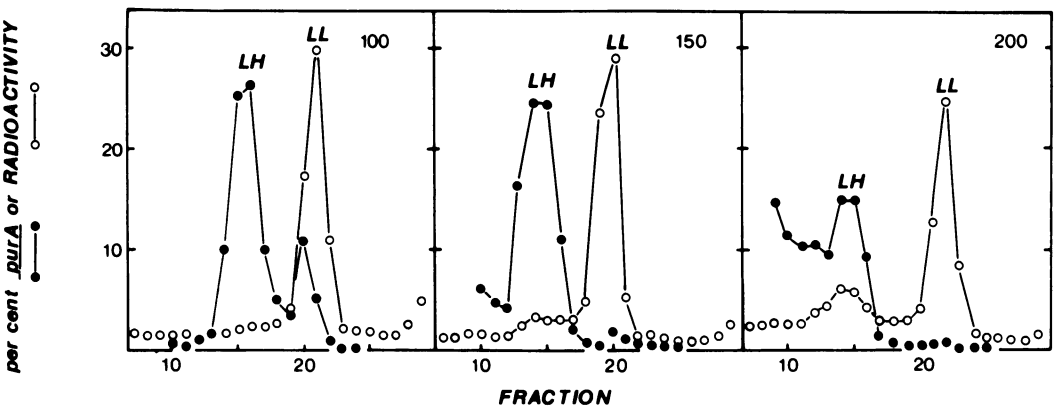


FIG. 1. *Late replication of radioactivity upon germination of [$^3$H]thymine-labeled spores. For the labeling of spores, B. subtilis (dna-1) was grown at 34°C (see text). At midexponential growth (absorbance at 590 nm = 0.7) the culture (75 ml) was centrifuged and suspended in the same volume of supplemented minimal salts medium. At 60 min after resuspension, [methyl-$^3$H]thymine (40 μCi/ml) was added, followed by HPUra (100 μM) at 70 min. After 20 h the spores were collected and cleaned in the usual way (1). The spores were germinated at 34°C (3 × 10$^8$ per ml) in 30 ml of 60% strength casein hydrolysate medium plus glucose (1.2%, wt/vol). At 60 min a mixture of BU and thymine (final concentrations, 50 and 5 μg/ml, respectively) was added. Samples (10 ml) were taken at 100, 150, and 200 min for DNA extraction. The extracts were prepared and fractionated in CsCl gradients, and the fractions were analyzed for purA activity and radioactivity as previously described (1). The total radioactivity recovered from the gradients after germination for 100, 150, and 200 min (left, middle, and right panels) were 7,200, 12,200, and 9,600 cpm, respectively.*

origin marker, *purA*, and the [$^3$H]thymine upon germination and outgrowth of labeled spores in the presence of BU for 100, 150, and 200 min. By 100 min most of the *purA* had replicated (shifted to the LH position), whereas the radioactivity was unreplicated (LL position). Even at 200 min, by which time a second round of replication had started, only a minor portion of the radioactive DNA had replicated. The LL and LH fractions were pooled in each case and analyzed for the relative amounts of the midreplicating and terminus markers, *leuA* and *metB*, respectively. Figure 2 summarizes the complete data from analysis of the three samples in Fig. 1, along with those from a completely independent experiment, expressed as percent replication versus time after germination. It is clear that there was no significant replication of the radioactivity before *leuA*, and the bulk of it (>80%) replicated after *metB*. Thus, it is located exclusively in the terminal region of the chromosome.

From the 50% replication levels of *purA* and [$^3$H]thymine in Fig. 2 it can be calculated that the average time for replication of the chromosome in the presence of BU is about 130 min. *metB* (50% level) is replicated about 12 min before the [$^3$H]thymine. Current maps of the *B. subtilis* chromosome place *metB* at about 10% of one replicating arm from the terminus. The 10% replication time separating *metB* and [$^3$H]thymine, therefore, means that at least 50% of the latter is very close indeed to the terminus. One other aspect of Fig. 2 worthy of note is the

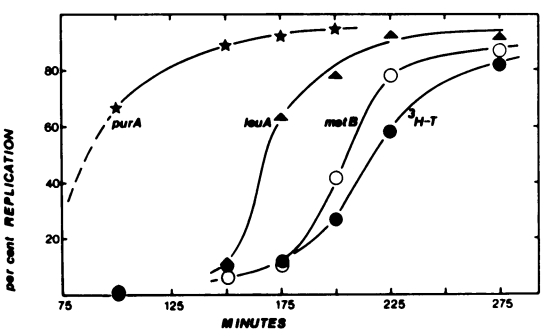


FIG. 2. *Chromosomal terminus location of radioactivity in [$^3$H]thymine($^3$H-T)-labeled spores. From the data in Fig. 1 (in addition to those from a completely independent labeling experiment) and further analysis of pooled LH and LL material for leuA and metB activities, the percent replication of the genetic markers indicated, as well as the [$^3$H]thymine region of the chromosome, at various times after spore germination were calculated as shown.*

flatter curve describing the replication behavior of the radioactive DNA in comparison with those for the genetic markers. This would be expected because varying lengths of the terminal region would be labeled in the approach used. After the [$^3$H]thymine was added to the starvation culture, individual rounds of replication would have completed at different times during the subsequent 10 min. Presumably, the specificity of labeling at the terminus could be increased by using a labeling period of <10 min in the starvation medium.

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