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Specific Labeling of the *Bacillus subtilis* Chromosome Terminus

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The deoxyribonucleic acid labeled by a procedure described previously for labeling the chromosomal terminus of *B. subtilis* 168 was substantially enriched for sequences homologous to bacteriophages SPβ and ϕ3T, which integrate in the terminal region.

Although the circularity and bidirectional replication of chromosomes of *Escherichia coli* and *Bacillus subtilis* are well established, the process of termination remains obscure (7, 11). The terminus may be a specific nucleotide sequence at which replication forks stop during normal growth and which may be concerned with attachment of the chromosome to the membrane. Alternatively, the sequence at the terminus may be nonspecific and merely the point at which the replication forks meet. There are reports of a specific point of termination in *E. coli,* but it clearly does not significantly impede replication during conjugation (6, 7).

I have recently described a method by which the terminus can be specifically labeled (10). This relies on the observation that spores of *B. subtilis* contain completed chromosomes and that in the presence of an inhibitor of DNA synthesis, i.e., 6-(hydroxyphenylazo)uracil (HPU), the only spores formed are those whose chromosomes are complete. Thus, if sporulating cells are pulse-labeled with [^{3}H]thymidine and then treated with HPU, the only labeled spores formed will have chromosomes completed during the pulse period and will therefore be labeled in the terminal region.

The last section of the *B. subtilis* chromosome to replicate lies between *ilvA* and *gltA* (3) and includes the prophage of SPβ (8, 14), which is 6 $\times 10^7$ daltons long (12). Genetic mapping studies using transduction suggest that there is a section of the chromosome of similar size between *gltA* and *ilvA* in addition to SPβ. The whole region is probably about 5% of the chromosome (8, 12).

The specificity of the terminus labeling procedure, described previously (10), can be assessed by determining the degree of homology of this DNA with DNA of phage SPβ or with ϕ3T, which has an attachment site within the same region (13).

Pairs of strains, one lysogenized with and the other lacking the phage, were labeled under sporulation-inducing conditions (Table 1). The percentage of radioactive DNA in the spores that was homologous with the appropriate phage was measured by DNA-DNA hybridization (Table 1).

When bacterial strains carrying either SPβ or ϕ3T were pulse-labeled for 10 min as described in Table 1, the radioactive DNA isolated from spores was greatly enriched for sequences homologous to SPβ or ϕ3T. Thus, 20 and 2% of terminus-labeled DNA of SPβ^+ and SPβ^- strains, respectively, hybridized with SPβ DNA. This must be a minimum figure as under the same conditions 62% of pure ^{3}H-labeled SPβ DNA (3 μg) was bound to filters.

Most strains of *B. subtilis* 168 contain phage SPβ. The Thy$^-$ strain (168TT) may be an exception as infective particles of SPβ cannot be detected in mitomycin C-induced lysates (1). Although DNA from vegetative cells of this strain has very little homology with SPβ compared with an SPβ^+ strain, terminus-labeled DNA does have homology with SPβ (Table 1). This may represent part of the SPβ prophage or part of another SPβ-like phage possibly derived from strain W23 (2).

Phage ϕ3T, which has substantial homology with SPβ, also has homology with the "SPβ-like" DNA of 168TT; i.e., about 12% of terminus-labeled DNA of 168TT hybridizes with ϕ3T. However, when the strain is lysogenized with ϕ3T, about 30% of terminus-labeled DNA hybridizes with ϕ3T.

Short periods of labeling followed by HPU treatment should label only the terminal region, whereas longer pulses should label parts of the chromosome distal to the terminus. The effect of pulse length on the percentage of labeled DNA hybridizing with the appropriate phage is illustrated in Fig. 1. Using 168 (SPβ^+), the percentage of labeled DNA homologous with SPβ is highest during the last 10-min period before the addition of HPU and decreases continuously with longer pulse length (Fig. 1A). With the SPβ^- strain there is a low level of hybridization

TABLE 1. *Percentage of terminus-labeled DNA homologous with appropriate phage*[a]

Strain	Genotype	Phage DNA on filter	% of ^{3}H-labeled spore DNA bound to filter
168	*trpC2* (SPβ$^+$)	SPβ	20
RB1124	Su^{3+} (SPβ$^-$)	SPβ	2
MS144	*trpC2 thyA thyB* (φ3T$^+$)	φ3T	31
168TT	*trpC2 thyA thyB* (φ3T$^-$)	φ3T	12
BD280	*hisA thyA thyB* (φ105)	φ105	1.0
168TT	*trpC2 thyA thyB* (φ105$^-$)	φ105	0.8
RB1030	*trpC2* (SPO2$^+$)	SPO2	2
168	*trpC2* (SPO2$^-$)	SPO2	2

[a] Sporulation was induced in all strains except RB1124 by method A (10). Sporulation was induced in RB1124 by method B (10). [*methyl*-^{3}H]thymidine (specific radioactivity, 52 Ci/mmol) was added (1 μCi/ml) 110 min after the start of sporulation. HPU was added after 10 min. The spores were purified and the DNA was extracted as described previously (9a, 10). The percentage of this DNA homologous with the appropriate phage was measured exactly as described by Kourilsky et al. (5). The nitrocellulose filters used (type HAWP; Millipore Corp., Bedford, Mass.) contained 5 μg of denatured DNA, whereas the hybridization mixture contained approximately 1 μg of [^{3}H]DNA and at least 3,000 cpm in 0.6 ml of hybridization mixture. Samples were incubated for 5 days at 35°C. A blank filter was included in each reaction mixture and gave less than 30 cpm above background. Phages were prepared by single-burst experiments at a multiplicity of 10 and were purified with cesium chloride. All DNA was purified with phenol and ethanol precipitation after pronase and sodium dodecyl sulfate treatment.

which does not change appreciably with length of pulse period (Fig. 1A). Similar experiments with 168TT confirm that the highest percentage of homology for SPβ or φ3T is found in spores pulse-labeled for 10 min before the addition of HPU. The same strain lysogenized with φ3T gives a similar pattern but with a peak of about 30% (Fig. 1B).

The decreasing proportion of hybridizing DNA in longer pulses occurs because the prophage DNA is diluted by DNA from sections of the chromosome distal to the terminus (Fig. 1A and B). The apparent peak in the percentage of DNA homologous with either phage at 10 min before HPU addition is in contrast to the genetic evidence, which suggests that the prophages are located very close to the terminus. However, an analysis of restriction fragments produced by *Bam, Sal, Eco*RI, and *Xba* from spores labeled for 10 min as in Table 1 suggest that the total length of DNA synthesized is no more than 10^8 daltons. This can be explained in two ways: (i) the terminal region of the chromosome replicates more slowly than other parts, and (ii) the effective pulse length is in fact shorter than 10

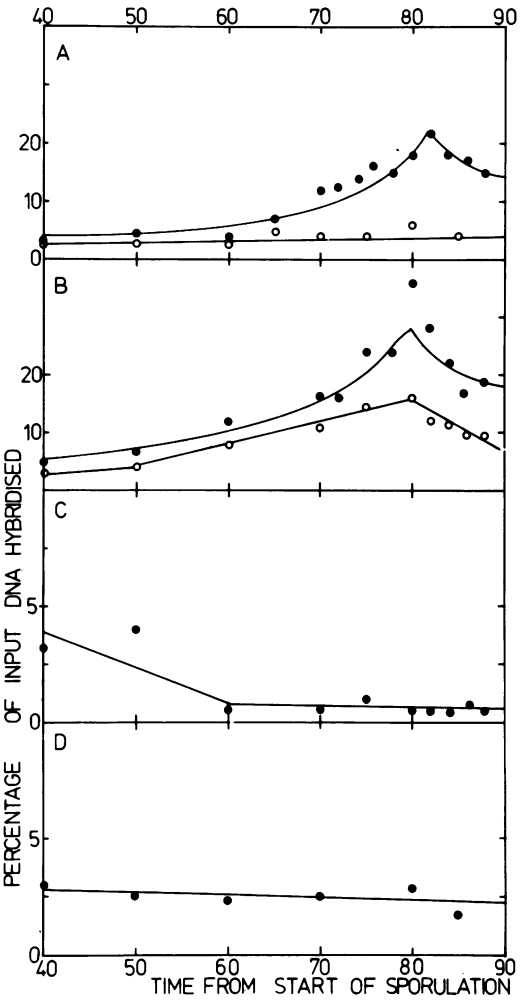

FIG. 1. *Effect of pulse length on percentage of labeled spore DNA homologous with phage DNA. Sporulating cells prepared as in Table 1 were pulse-labeled with [^{3}H]thymidine at the times corresponding to each point, and HPU (50 μg/ml) was added at 90 min to all cultures. The percentage of labeled DNA homologous to the appropriate phage DNA was determined as described in Table 1. Symbols: (A) ●, 168 (SPβ$^+$); ○, RB1124 (SPβ$^-$) hybridized with filters of SPβ. (B) ●, 144 (φ3T$^+$); ○, 168TT (φ3T$^-$). (C) ●, BD280 (φ105$^+$). (D) ●, RB1030 (SPO2$^+$).*

min. Thus, if HPU inhibited sporulation for, say, 5 min after termination and if sporulating cells were pulse-labeled for 10 min before HPU treatment, the only labeled spores obtained would have been labeled between 10 and 5 min before addition of HPU. This is supported by a previously published observation (10) that shows that when the specific radioactivity of pulse-labeled

spores is plotted against pulse length, the line extrapolates to a point about 5 min before addition of HPU.

The prophages of SPO2 and ϕ105, which are located close to the origin and at about 0.6 on the replication map (4, 9), respectively, have been used in similar experiments (Table 1; Fig. 1). The prophages are not labeled significantly when lysogens are labeled for 10 min, but with longer pulses (30 to 40 min) there was a small increase in the amount of radioactive DNA that was homologous to ϕ105. However, there was no change with an SPO2 lysogen.

In summary, the high degree of homology between DNA labeled by the procedure described and the appropriate phage DNA indicates that the terminus is indeed labeled specifically. Support for this conclusion has also been obtained by T. Adams and R. G. Wake, who used a density-labeling technique (personal communication).

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