

# Division Site Regulation in a Temperature-Sensitive Mutant of *Bacillus subtilis*

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Division site location was measured in *Bacillus subtilis* clones grown from spores at 30 and 45 C. Regulation of division location is lost in mutant 168ts-151 at 45 C.

Although much remains to be clarified about the genetic control of cell division, the analysis of temperature-sensitive mutants has proved valuable in demonstrating that certain processes of the cell cycle need not be dependent upon one another absolutely (3, 6, 8-11). Cell division may be dissociated from deoxyribonucleic acid (DNA) synthesis in certain mutants (5, 7). Genome segregation can occur in the absence of DNA synthesis or cell division, and under certain conditions even in the absence of cell elongation (4, 6).

One aspect of the cell cycle that has received little attention is the location of the division site along cell length. The normal partitioning of parent cell into daughter cells usually results in two cells of nearly equal length. Somehow the division location is coordinated with the segregation of daughter DNA molecules into distinct spatial regions. In this way each daughter cell is assured a copy of the cell's genetic material. In *Bacillus subtilis* the regulation of division site location along cell length may be lost by mutation. The present report describes aberrant division in a temperature-sensitive DNA<sup>-</sup> mutant able to continue cell division after DNA synthesis has ceased at the restrictive temperature. The tendency of daughter cells to remain together in chains, made possible the analysis of cross-wall location in clones derived from single spores.

When germination and outgrowth of 168ts-151 spores occur at permissive temperature (30 C), cross walls are produced at very regular length intervals resulting in cells of near uniform length. On the other hand, germination and outgrowth at 45 C results in a population of very different size cells. The basis for this distinction is apparent at the time of the second division during outgrowth. Two hundred clones, each consisting of a chain of four cells grown from a spore at 30 C and 200

clones grown from spores at 45 C, were studied and measured in the phase contrast microscope. The contour length of each clone was measured and the location of each cross wall was recorded as a fraction of the total length from one end. Cross-wall location was then plotted as shown in Fig. 1. These data represent the statistical locations of cross walls along clone length in populations grown at 30 C (above) and 45 C (below). The regular locations found in populations at 30 C is indicative of near uniform cell lengths. In contrast, clones grown at 45 C produce cross walls over a broad distribution of locations along clone length. The locations of outside cross walls in clones at 45 C even overlap the position at which the center cross wall is found in clones grown at 30 C. As a result of irregular cross-wall location, clones grown at 45 C usually contain one or more very short cells. Some typical clones grown at 30 and 45 C are shown in Fig. 2.

<sup>3</sup>H-thymine incorporation in cells of 168ts-151 has been studied as an index of DNA synthesis and the cellular location of DNA. During outgrowth at 45 C, DNA synthesis stops before cell elongation ceases. Autoradiographs indicate that both daughter cells produced by the first cell division at 45 C contain DNA, but frequently the smallest cell produced by the second division does not. As four-cell clones continue to elongate and divide at 45 C, additional anucleate cells are produced. Although a relationship of genome location to cell division site has been sought in these populations, no statistical correlation has yet been detected. Further studies of mutants such as 168ts-151 should provide new insight into the manner in which division site is regulated.

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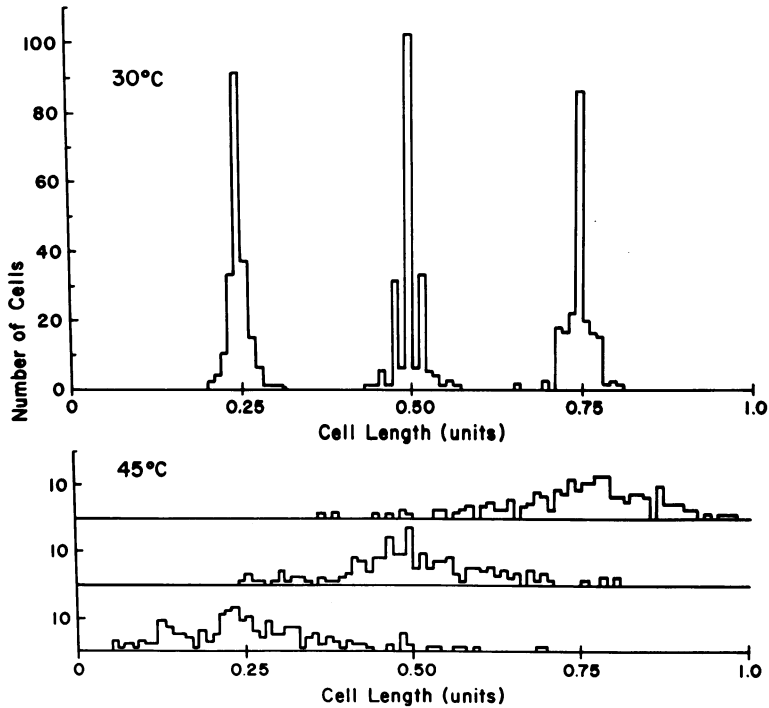


FIG. 1. Cross wall location in four-cell clones of mutant 168ts-151 grown from spores at 30 C (above) and 45 C (below). Purified spores were heat-shocked in distilled water at 70 C for 15 min, cooled to 26 C (room temperature), and inoculated into enriched minimal medium (7) at an  $OD_{680nm}$  of 0.1 to 0.2. The cultures were grown with bubbling aeration at 30 and 45 C. Samples were withdrawn periodically and fixed in Formalin or processed for kinetic and autoradiographic studies. Populations were examined and measured with a Wild M20 phase contrast microscope. Clone lengths are normalized to 1.0. Clones at 30 C ranged from 12 to 26  $\mu$ m in length; average length was 16  $\mu$ m. Clones at 45 C ranged from 10 to 58  $\mu$ m in length; average length was 26  $\mu$ m. Due to the overlap of left, central, and right cross-wall site locations in the clone populations grown at 45 C, the data for each site are shown on separate axes below. If the data are transformed, grouping the smallest cells always towards one side of the graph, the scatter is not appreciably reduced.

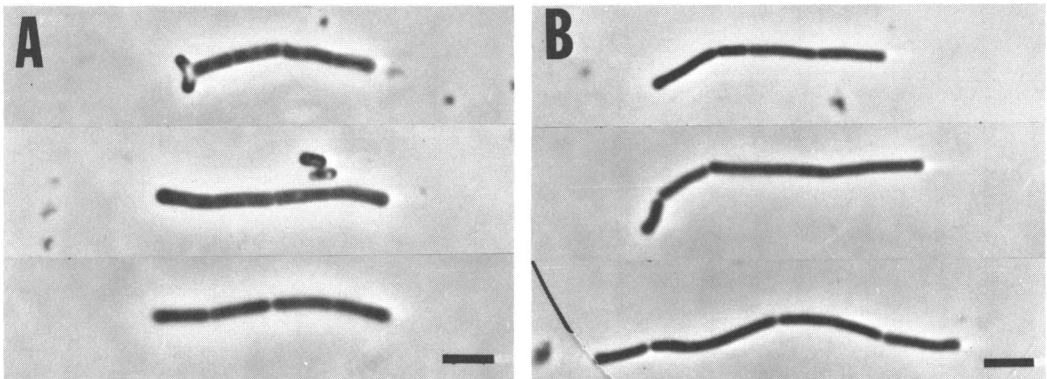


FIG. 2. Morphology of 168ts-151 clones grown from spores at 30 and 45 C. Phase contrast micrographs of typical four-cell clones found in (A) 30 C populations and (B) 45 C populations. Bar, 5.0  $\mu$ m.

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