FtsZ Regulates Frequency of Cell Division in *Escherichia coli*

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Cell division is regulated so that it occurs only once per cell cycle. In *Escherichia coli*, a rod-shaped bacterium, division normally takes place at the center of the long axis of the cell; however, in the minicell mutant, division can also take place at the cell pole. Such divisions take place at the expense of normal divisions, resulting in an overall increase in nucleated cell length. We report here that increasing the level of FtsZ can completely suppress the cell length increase of the minicell mutant by increasing the frequency at which cell division events take place. This result suggests that the level of FtsZ controls the frequency of cell division in *E. coli*.

The two major periodic events common to all cell cycles are DNA replication and cell division. In *Escherichia coli*, DNA replication is controlled at the level of initiation and the timing of initiation is correlated with a doubling of cell mass (4). Regulation of cell division probably also occurs at the level of initiation which coincides with the attainment of a critical cell length (5). How these events are regulated at the molecular level and integrated into the cell cycle is a central problem in cell biology. It appears that in *E. coli*, two genes have primary roles in regulating the initiation of these two events.

The frequency of initiation of DNA replication appears to be regulated through the *dnaA* gene product (15). Biochemical and physiological studies indicate that initiation is dependent on DnaA (6, 15) and that the initiation potential correlates with the level of the DnaA protein (13). The leading candidate for a similar role in cell division is the *ftsZ* gene (12, 17). Increasing the level of FtsZ results in a hyperdivision activity that is expressed as a minicell phenotype (16). Additional support for FtsZ having a key role in cell division is provided by the finding that FtsZ is the target for the cell division inhibitor, Sula, a component of the SOS response that is induced after DNA damage (7–10).

Insight into the regulation of cell division has been gained by study of the minicell mutant (1). This mutant displays an increased and heterogeneous cell length, along with the production of minicells. A model that explains this observation has been proposed (14). It states that in the minicell mutant, the cell poles become available for septation and that when the cell doubles in size, its division potential is used randomly at any available site (polar as well as internal) but there is only sufficient potential to utilize one site. As a result, a cell undergoing a polar division, yielding a minicell, wastes its division potential and will be longer. Thus, the overall increase in the average cell length reflects the number of divisions that result in minicell formation. This phenotype can be contrasted with the minicell phenotype induced by increasing the level of FtsZ, which does not increase the average cell length (16).

Since we had observed that increasing the level of FtsZ in wild-type strains resulted in an increase in division activity, we reasoned that increasing the level of FtsZ in a minicell mutant, and therefore increasing its division activity, should

result in a decrease in the average cell length, with the increased division activity compensating for the availability of the polar division sites. To increase the level of FtsZ, various plasmids containing the *ftsZ* gene (Fig. 1) were introduced into the minicell mutant strain PB114, which has the *min* locus deleted (3). Introduction of the plasmids resulted in various levels of FtsZ due either to different copy numbers or to different amounts of DNA (and promoters) on the 5' side of the *ftsZ* gene (11, 18). Western immunoblot analysis of the minicell mutant strain carrying these various plasmids revealed that the levels of FtsZ increased between two- and sevenfold, depending on the plasmid introduced (Fig. 2).

The effect of increasing FtsZ on the cell length distribution of the minicell mutant strain was determined by photographing cells during exponential growth and measuring the cell lengths. The cell length distributions were determined in two ways. In the first, septa were ignored and attached cells were scored as one cell (chain length). In the second, septa were taken into account because they represent division activity (cell length). Figure 3B shows the typical cell length distribution of the minicell mutant strain containing a control plasmid (minicells are not counted). The cell length distribution is quite broad compared with that of the parental wild-type strain (Fig. 3A) because of the presence of elongated cells.

The effect of increasing FtsZ on the cell length distribution of the minicell mutant strain was quite dramatic (Fig. 3C, D, and E). Increases in FtsZ within the range of two- to sevenfold suppressed the appearance of elongated cells in the population and resulted in a shift in the cell length distribution so that it resembled that observed with *min*⁺. A twofold increase in FtsZ, which was seen with plasmid pBEF0, resulted in an almost complete return to a wild-type cell length distribution (Fig. 3C). An increase in FtsZ of three- to fourfold, which was seen with pBS58 (Fig. 3D), resulted in a population that contained predominantly small cells. In addition, many free minicells were observed—more than were seen with either of the other two plasmids. A larger increase in FtsZ, which was seen with pZAQ (Fig. 3E), resulted in a cell length distribution that resembled that of the pBEF0 population. To confirm these results, pBS58 was introduced into the original minicell mutant, P678-54 (1). A similar reduction in cell length was observed (data not shown).

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FIG. 1. Plasmids that lead to altered levels of FtsZ. A restriction map of a portion of transducing phage λ 16-2 and the location of the *ftsZ* gene are shown. —, Chromosomal DNA; —, λ DNA. pZAQ contains a *PstI*-*ClaI* fragment cloned into the high-copy-number plasmid pBR322, pBS58 contains a *PstI* fragment cloned into the low-copy-number plasmid pGB2 (2), and pBEF0 contains a *BamHI* fragment cloned into this vector.

Although plasmids with different genetic contents were used, we believe that their effect on the *min* mutant phenotype is due to the increased levels of FtsZ that they induce. All of these plasmids contained *ftsA* in addition to *ftsZ*; however, a control plasmid, pJW2, which contained the *ftsQ* and *ftsA* genes but not *ftsZ* and was previously shown to have no effect on the level of FtsZ (12), had no effect on the *min* mutant phenotype (data not shown).

These experiments demonstrate that increasing the level of FtsZ can suppress the broad cell length distribution of the minicell mutant strain. Thus, whatever limits septation in the *min* deletion mutant can be suppressed by increasing the level of FtsZ. It has been argued that the increased length associated with the broad cell length distribution is due to the activation of polar sites as possible division sites, which results in competition between the polar and nonpolar sites for the limited division capacity (14). Our results are consistent with the elevated level of FtsZ increasing the division capacity so that more than one division can take place per cell doubling. This increased capacity for division compensates for the availability of the poles as division sites. This is supported by the observation that cells containing increased

FtsZ, especially pBS58, continue to make minicells in addition to completing all the nonpolar divisions.

An increase in the level of FtsZ of three to fourfold (with pBS58) had the most dramatic effect on cell length distribution, decreasing the average cell length to less than wild-type length. This suggests that in the presence of this plasmid, cells are initiating cell division at a smaller cell length. A higher level of FtsZ did not suppress as well, and fewer minicells were present. However, if one takes visible septa into account, the suppression was still effective. At this higher level of FtsZ, it appeared that completion of the septum might take more time, as though something was limiting the process. This is consistent with the earlier observation that at even higher levels of FtsZ (>10-fold), septation is completely inhibited (16).

The possibility that the level of FtsZ dictates the division potential of a cell was first suggested by the observation that an increased gene dosage induces a minicell phenotype in a wild-type strain. Since it had been postulated that the *min* locus inhibits the polar sites, we suggested (16) that an increase in FtsZ is able to overcome this inhibition. de Boer et al. (3) have recently shown that the *min* locus is composed of three genes, of which two (*minCD*) act together to inhibit cell division and a third (*minE*) gives the inhibitor topological specificity. We have postulated that the target of the MinCD inhibitor is FtsZ, since alleles of *ftsZ* that were selected for resistance to the inhibitor SulA are also resistant to MinCD (9a).

One could imagine two models to explain how multiple division events per cell cycle are occurring. In the first, division is triggered at a unique time in the cycle and multiple division events occur simultaneously. In the second, cells divide whenever the level of FtsZ passes a threshold level. The dividing cells seen in this study seldom have more than one septum, even though multiple division events per cell cycle have to occur. This favors the second model and suggests that the level of FtsZ governs the frequency of cell division and that the cell has a mechanism for measuring the level of FtsZ.

From examination of the cell length distributions in strains that overproduce FtsZ, it appears that there is a clear distinction between minicells and the smallest cells; i.e., there is not a continuum of sizes. This suggests that FtsZ does not determine the location of the septa but merely the frequency at which septation occurs. This is consistent with

FIG. 2. FtsZ content of minicell strain PB114, which contains the plasmids indicated in Fig. 1. Samples of cultures growing exponentially in L broth were analyzed by immunoblot analysis, as described previously (16). To estimate the relative FtsZ content in the different strains, serial dilutions of samples containing the various plasmids were run and compared with the sample with the control plasmid.

FIG. 3. Distributions of cell lengths. Samples of strains growing exponentially in L broth were analyzed by photomicroscopy. Cell lengths were determined as described in the text.

the proposal of Teather et al. (14) that the cell must have distinct processes for controlling the localization and frequency of divisions.

The pattern of division observed in the minicell mutant suggested that a diffusible division factor was responsible for triggering cell division and that this division factor behaved as a quantum (14). The results presented here are consistent with FtsZ being this division factor.

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