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Cell Division and Transcription of *ftsZ*

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For normal cell division, the *ftsZ* gene must be transcribed from a number of promoters that are located within the proximal upstream genes (*ddlB*, *ftsQ*, and *ftsA*). We show that the main promoters have identical responses to changes in growth rate, i.e., under all conditions, the frequency of transcription per septum formed is approximately constant and independent of cell size or growth rate per se. We also show that transcription from these promoters is independent of stationary-phase transcription factor σ^s.

The Mg^{2+}-dependent GTPase FtsZ (10, 20, 21) is a key cell division protein in *Escherichia coli* and probably in all eubacteria (8). Five thousand to twenty thousand molecules of FtsZ are distributed throughout the cytoplasm of nondividing cells, but these aggregate into a ring around the center of the cell when division begins (5). During subsequent ingrowth of the peptidoglycan septum, this ring decreases in diameter and finally disappears when the septum is complete (5). FtsZ is essential for cell division (9), but excess FtsZ can cause the formation of extra divisions, and at still higher excess it can inhibit all division (3, 4, 30). Regulation of the level of FtsZ activity is therefore likely to be important to the progress of the cell cycle. In this report, we describe the relationship between growth rate and transcription from two of the three main sets of promoters (lying within upstream genes *ddlB*, *ftsQ*, and *ftsA* [Fig. 1]) which are together required for full expression of the *ftsZ* gene (9). This confirms previous reports (2, 12, 15, 16) that, at all growth rates, transcription from *ftsZ* promoters is approximately constant per cell (although cell size itself increases exponentially with growth rate) and shows that each of the three sets of required promoters shows a similar response. We also show here that this behavior is independent of the stationary-phase sigma factor σ^s (6, 17, 18).

The procedure of Simons et al. (26) was used to construct two new λ bacteriophages (λRWS100 and λRWS200) in which *lacZYA* is transcribed from promoters within the *ftsQ* and *ftsA* genes. Figure 1 shows that λRWS100 has all of these promoters (*ftsZ4p3p2p1p*) within *ftsA* (2, 19, 23, 24, 27, 31) and the very weak promoter within *ftsQ* (11), while λRWS200 lacks the proximal promoters (*ftsZ2p*$_1$*p*). The promoters cloned into λRWS200 are the same as those in the λJFL100 phage originally used by us (12), but the new phage has four transcriptional terminators immediately upstream of the cloned segment and also lacks all but 70 bp of the *trp* DNA which is located between the promoters and *lacZ* in λJFL100. Expression of *lacZ* in the new phages cannot be due to readthrough from promoters (in the phage or in the chromosome) upstream of the *ftsQA* DNA (as has been suggested as a possible criticism of results obtained with λJFL100 [2]); it also does not arise in the residual *trp* fragment, because an otherwise identical phage (λRWS1) which lacks the *ftsQA'* DNA does not express *lacZ*. λRWS100 expresses *lacZ* from all of the *ftsZ* promoters, except those within *ddlB*. A construct similar to λRWS100 has been briefly described in an earlier report (1) and was reported to show increased transcription in the stationary phase.

We infected a Δ*lac-proB* strain (TP8503 [23]) with each of the λRWS phages and screened for monolysogens (26). These strains were grown in different media (Vogel-Bonner [VB] salts-glycerol, VB-glucose, VB-Casamino Acids, L broth, and L broth-glucose) at 37°C to obtain a range of growth rates and cell sizes. The cells were maintained in exponential-phase growth for several generations before and during the assay period by regular dilution with fresh medium at 37°C. Figure 2 shows the average specific β-galactosidase activities (measured at intervals during exponential-phase growth, when growth rate and cell size were both constant) as a function of average cell size (measured in the same cultures at the same times). Average cell size increases as an exponential function of growth rate (13, 25), and the specific activities (β-galactosidase activity per optical den-

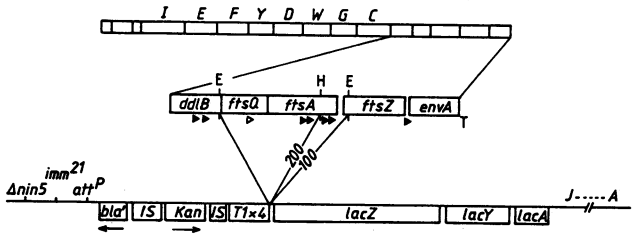

FIG. 1. Organization of the *mra* gene cluster, location of promoters in the *ddlB-ftsZ* region, and structures of λRWS100 (*ftsQ ftsA ftsZ'::lacZ*) and λRWS200 (*ftsQ ftsA'::lacZ*). The top line shows the coding sequences in the *mra* cluster. The letters refer to the following known genes: I, *ftsI*; E, *murE*; F, *murF*; Y, *mraY*; D, *murD*; W, *ftsW*; G, *murG*; C, *murC*. The middle line (to larger scale) shows the *ddlB*, *ftsQ*, *ftsA*, *ftsZ*, and *envA* genes at the end of the operon, with the locations and directions of promoters (arrowheads) and transcription terminator (T) in this region indicated. (The solid arrowheads are the strong promoters [*ftsQ2p1p*, *ftsZ4p3p2p1p*, and *envAp*; 2, 19, 23, 27, 31], and the open arrowhead is the very weak promoter [*ftsAp*; 11, 19, 23, 24, 27, 31].) The locations of the *Eco*RI (E) and *Hin*dIII (H) restriction sites are shown. The E-E segment was cloned at the site shown in λRS45 (bottom line, same scale) to give λRWS100, and the E-H segment was cloned at the same site to give λRWS200. λRS45 (26) (bottom line) contains a fragment of a β-lactamase gene (*bla'*), a kanamycin resistance determinant (*Kan*) between two IS*903* ends (*IS*), four copies of a strong transcription terminator (*T1×4*), a cloning site, 70 bp of the *trpB* gene (too small to show), and the ribosome-binding site and complete *lacZYA* coding sequences.

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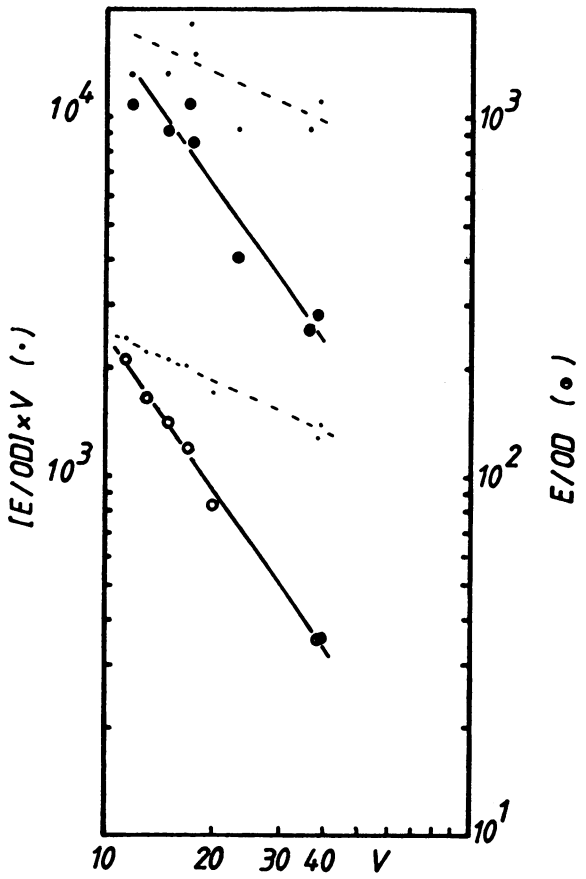

FIG. 2. Differential expression of different sets of promoters (E/OD) as a function of cell size (V; measured as median cell volume in arbitrary units with a Coulter Particle Analyzer). Estimates of E/OD were made from the differential rates of β-galactosidase activity and OD increase during log-phase growth in cultures in different growth media at 37°C. Symbols: ●, TG8503(λRWS100); ○, TG8503(λRWS200). Also shown (dashed lines and dots) is the equivalent of the enzyme/cell ratio ([E/OD]×V) for both strains.

sity [OD] unit [E/OD]) of both sets of promoters show a strong negative dependence on cell size. The amount of transcription per cell from these promoters, however, changes much less, as reported previously for λJFL100 and similar constructions (Fig. 2) (2, 12).

The two constructions used here monitor the activities of promoters in *ftsQ* and *ftsA*, but there are additional promoters, required for full expression of *ftsZ*, within the *ddlB* gene (9). The activities of these have been measured in cells growing at different growth rates (28). To compare those results with ours, the published data for one of these promoters (*ftsQ2p*) were replotted in Fig. 3, which shows specific activity (E/OD) against 2^R (where R is the growth rate in doublings per hour), which is proportional to average cell volume (13, 14). Transcription from the remaining promoter (*ftsQ1p*) within *ddlB* is stimulated by the product of the *sdiA* gene; however, transcription from this promoter does not seem to be essential, because the *sdiA* gene can be deleted without detectable effects on cell division (29). Therefore, most of the promoters which are required for full expression of *ftsZ* show identical responses to changes in growth rate and cell size.

In a previous report (12), we showed that the rate of transcription from *ftsZ4p*$_3$*p* (in λJFL100) reflected the frequency of cell division rather than the overall rate of mass and protein synthesis during the transition period between one growth rate and another. In consequence, the amount of β-galactosidase per cell did not change during the transition from one growth rate to the next. Figure 4 shows that transcription from λRWS100 in TP8503 mirrors the increase in cell numbers during the transition from slow to fast growth, so that as in our previous study of λJFL100, β-galactosidase per cell remains nearly constant. We obtained similar results with λRWS200 (data not shown).

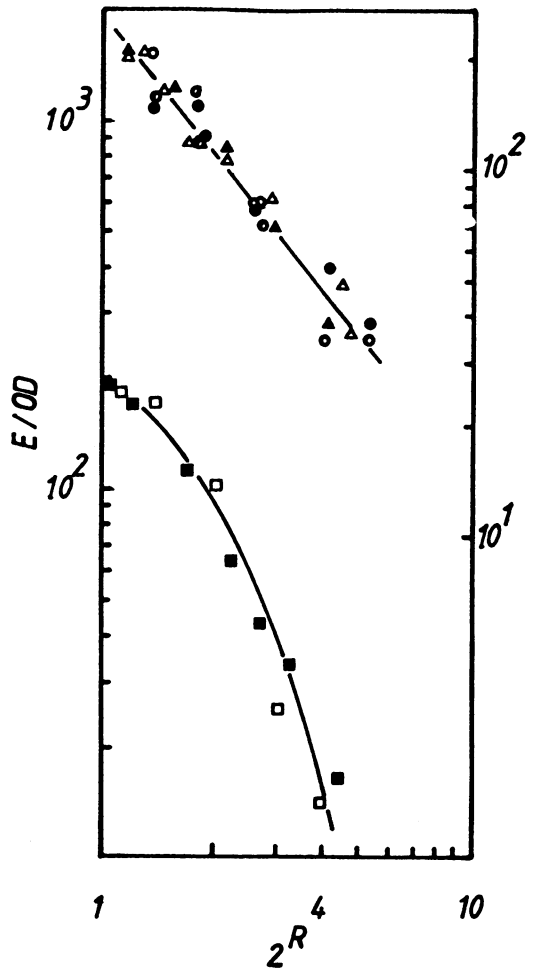

FIG. 3. Differential expression of different sets of promoters (E/OD) as a function of estimated average cell size (V), calculated from the growth rate (R, doublings per hour) by assuming that $V = k \cdot 2^R$ (13, 14). Data for TG8503(λRWS100) (●; left scale) and TG8503(λRWS200) (○; right scale) are the same as in Fig. 2, except that V was calculated from the growth rate rather than measured directly, to allow comparison of our data with those reported for other promoters (28). Data for the *ftsQ2p*$_1$*p* promoters [▲, △] were obtained from reference 28 and replotted against $V = k \cdot 2^R$ on a vertical scale chosen to superimpose these points on those of the two TG8503 strains (representing the behavior of *ftsZ4p3p* and *ftsZ4p3p2p1p*). The relationship between transcription and cell size (or growth rate) is closely similar for all three sets of promoters. Also shown are datum points for the *bolA1p* promoter [■, □] replotted from reference 28 as before. The relationship between transcription and growth rate appears to be different from that of the *ftsZ* promoters.

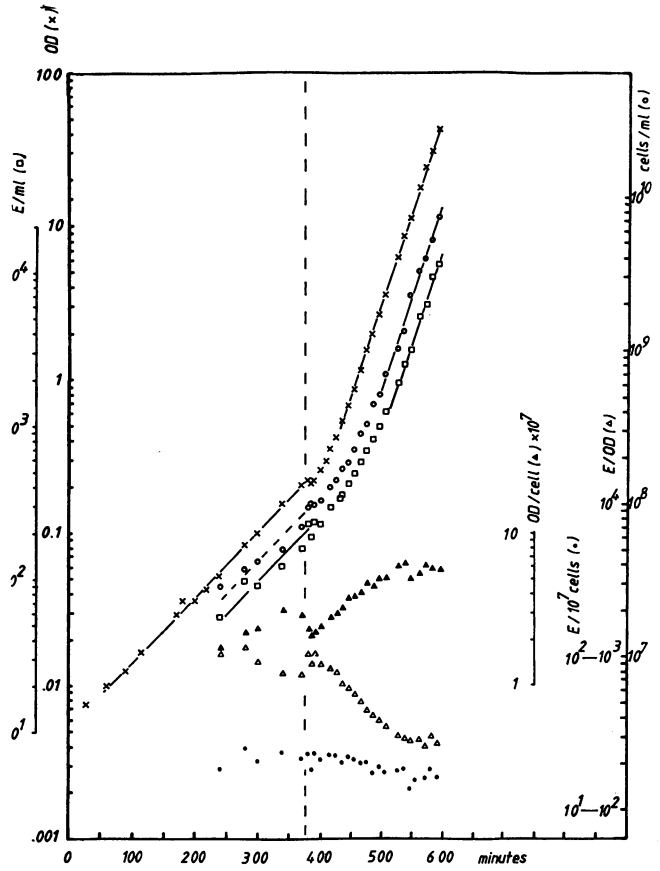

FIG. 4. Effects of a shift-up from poor medium to rich medium on cell growth and division and transcription from the *ftsZ4p3p2p1p* set of promoters. Cells of TG8503(λRWS100) were maintained in the log phase by periodic dilution with fresh medium (minimal medium-glycerol) and then shifted to L broth-glucose (dashed line at 375 min) and maintained thereafter below an OD of 0.2 by periodic dilution with fresh broth. (The datum points have been corrected for these dilutions.) The primary measurements (upper three curves) are OD (×), cells per milliliter (○), and enzyme activity per milliliter (□). Calculated from these were OD per 10^7 cells (▲), enzyme activity per OD unit (△), and enzyme activity per 10^7 cells (●). Although growth rate, cell size, and enzyme activity per OD unit all changed after the shift, enzyme activity per cell remained almost constant throughout.

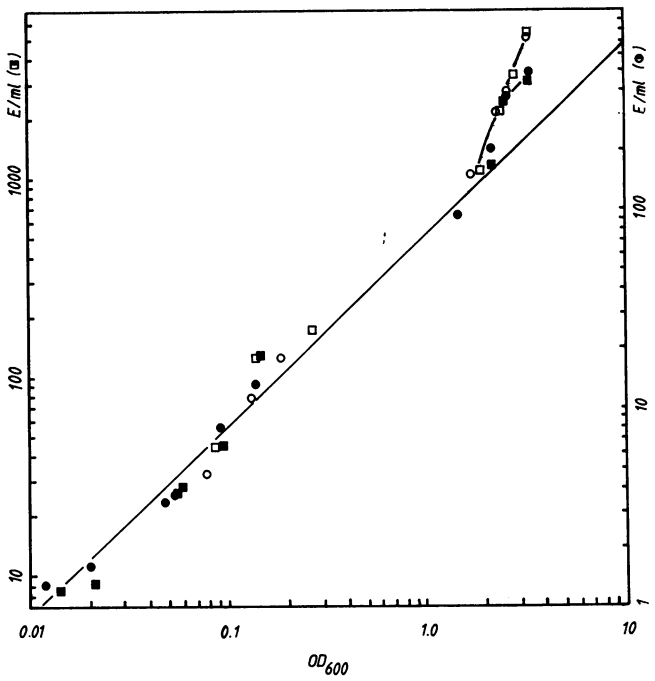

FIG. 5. Relative increase in β-galactosidase activity per milliliter and OD units per milliliter of batch cultures of TG8503(λRWS100) (■), TG8503(λRWS100)*rpoS* (□), TG8503(λRWS200) (●), and TG8503 *rpoS*(λRWS200) (○) in VB salts-glucose-Casamino Acids. The right-hand scale shows activities for λRWS200 strains, and the left-hand scale shows activities for λRWS100 strains. Enzyme activities are in standard Miller units per milliliter, and ODs were measured at 600 nm in a 1-cm light path. (ODs were measured with appropriately diluted samples to obtain values in the range in which cell mass and OD are proportional, and these values were corrected for dilution.) The rate of enzyme activity was approximately proportional to the rate of cell mass increase at low OD values (balanced growth in the log phase) but increased at higher OD values (entry into the stationary phase).

In contrast, another report (22), on transcription of *lacZ* from *ftsZ4p3p* in λJFL100 in a different strain of cells (GC3439), suggested that β-galactosidase per cell increases after a similar medium shift (at least initially). The difference between our results and these appears to be that strain GC3439 shows prolonged inhibition of cell division (i.e., constant cell numbers) after a shift up in growth rate, in contrast to the behavior of most *E. coli* strains (25). Perhaps cell separation is delayed after a medium change in strain GC3439, while chromosome replication and septum initiation proceed normally; despite this, the change in E/OD after the shift appears to be very similar to that seen in our strains.

Our results can be summarized by saying that the frequency of transcription of *ftsZ* seems to be closely tied to the frequency of cell division (or septum initiation) under most growth conditions, but we still do not know how this is achieved. An extreme condition in which this behavior is seen is during entry into the stationary phase; as the rate of mass increase slows down, the rate of cell division remains unchanged for a period, so that cell size progressively diminishes (25). Under these conditions, the rate of transcription from the set of *ftsZ* promoters remains linked to the rate of division, so that the rate of transcription of *ftsZ* increases relative to the rate of overall protein synthesis (2, 28; Fig. 5). The *rpoS* gene, which codes for a novel sigma factor (σ^s), is required for transcription of some genes (e.g., *bolA* [1]) which are preferentially expressed during entry into the stationary phase (18). Moreover, the *rpoS* gene itself appears to be expressed at a rate which is inversely proportional to growth rate, in a way similar to that of *ftsZ* (18). We therefore examined the effect of inactivation of *rpoS* on transcription of *ftsZ*. The *rpoS*$^+$ allele was replaced by P1 transduction of *rpoS*::Tn*10* (18) into our lysogenic strains. Inactivation of the *rpoS* gene had no detectable effect on growth rates during the log phase. Figure 5 shows the increase in β-galactosidase activity versus the increase in cell mass (OD) in *rpoS*$^+$ and *rpoS*::Tn*10* cultures of λRWS100 and λRWS200 lysogens in minimal medium-Casamino Acids. In all four cultures, the ratio of enzyme to cell mass remained constant during log-phase growth and increased on entry into the stationary phase (OD greater than about 1.8). Inactivation of *rpoS* had no effect on the E/OD ratio in either lysogen and no effect on the culture OD

at which the increased relative rate of transcription commenced. The only detectable effect of *rpoS* inactivation is that the E/OD ratio may reach a higher level during the stationary phase. This effect, which is not large, may be correlated with the prolonged division of *rpoS*::Tn*10* cells during entry into the stationary phase (18). No effects of *rpoS* inactivation on transcription were seen in any of the other growth media (data not shown).

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