Does RNA polymerase help drive chromosome segregation in bacteria?

Jonathan Dworkin and Richard Losick*

Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

Contributed by Richard Losick, September 5, 2002

In contrast to eukaryotic cells, bacteria segregate their chromosomes without a conspicuous mitotic apparatus. Replication of bacterial chromosomes initiates bidirectionally from a single site (*oriC*), and visualization of the region of the chromosome containing *oriC* in living cells reveals that origins rapidly move apart toward opposite poles of the cell during the cell cycle. The motor that drives this poleward movement is unknown. An attractive candidate is RNA polymerase, which is a powerful and abundant molecular motor. If, as has been suggested for other macromolecular complexes, the movement of RNA polymerase is restricted in the cell, then transcription would translocate the DNA template, thereby providing the motive force to separate replicating chromosomes. A coordinated effect of many transcribing RNA polymerases could result from the widely conserved global bias of gene orientation away from *oriC*. By using fluorescence microscopy of living *Bacillus subtilis* cells, we demonstrate that an inhibitor of RNA polymerase acts to inhibit separation of newly duplicated DNAs near the origin of replication. We propose that the force exerted by RNA polymerase contributes to chromosome movement in bacteria, and that this force, coupled with the biased orientation of transcription units, helps to drive chromosome segregation.

Chromosome segregation is fundamental to the transmission of genetic information. Although much is known about the underlying mechanisms of mitosis in eukaryotes, far less is understood about how a chromosome is partitioned into each daughter cell in bacteria. An early and influential proposal, the replicon model, suggested that attachment of newly duplicated chromosomal replication origins to the membrane followed by zonal growth between the attachment sites would progressively separate the origins and, subsequently, the entire chromosomes (1). More recent experiments employing time-lapse microscopy to visualize movement of chromosome origins in living cells demonstrated, however, that this movement is rapid and not dependent on cell growth (2–5). Rather, these experiments were consistent with the existence of a mitotic-like motor that would drive chromosome separation (6).

Previous work suggested several candidates for such a mitotic motor. One such candidate is MukB, the functional equivalent in *Escherichia coli* to the chromosome-condensing protein structural maintenance of chromosomes (SMC), which is found in eukaryotes and in many prokaryotes (but not in *E. coli*; ref. 7). However, recent evidence implicates MukB in supercoiling-dependent chromosome compaction (8), and the movement of the *oriC* region of the chromosome is not significantly impaired in the absence of SMC in *Bacillus subtilis* (9). Another proposed candidate for the mitotic motor is *B. subtilis* Spo0J (5), a member of the Par family of plasmid partitioning proteins, but Spo0J is also not needed for *oriC* movement (2) and seems instead to be involved in compaction of the origin-proximal region of the chromosome (10). Thus, although both of these proteins probably participate in some aspect of chromosome segregation, the phenotypes of the mutants of the genes encoding them make it unlikely that they serve as the motor driving chromosome movement.

The observation that the DNA replication machinery in *B. subtilis* is immobile and localizes to the mid-cell led to the proposal that extrusion of the newly replicated chromosomal DNA from a stationary replication factory (replisome) could be the force that powers *oriC* separation (11). One issue raised by this model is whether the intrinsic flexibility of DNA would interfere with the ability of a pushing force such as that provided by a centrally located replisome to drive *oriC* proximal regions to the poles. Although the persistence length of DNA *in vivo* is not known definitively, estimates are in the range of ≈ 50 nm, substantially shorter than the distances ($\approx 1 \mu\text{m}$) traversed by separating *oriC* regions (12). In *Caulobacter crescentus*, for example, the replisome initially localizes at one pole of the cell and, after the initiation of DNA replication, one of the origins moves across the entire length of the cell to the opposite pole (13). Coupling of the newly replicated DNA to a large macromolecular structure could increase its rigidity, as has been proposed for a SeqA polymer in *E. coli*, but there is no direct evidence for such a structure or for its role in DNA segregation (14).

Like other molecules involved in DNA transport in bacteria, the protein(s) that mediates the poleward movement of *oriC* is likely to interact directly with DNA (15). One of the best characterized molecular motors that acts on DNA is RNA polymerase, which can pull on DNA with a force >30 pN, making it one of the strongest motors yet observed (16). In *in vitro* assays, immobilized RNA polymerase drives translocation of its DNA substrate (17). If the movement of RNA polymerase in the cell is restricted, as has been proposed, then transcription would translocate the chromosome (18). In addition to its force-generating characteristics, RNA polymerase is a highly abundant protein present in many copies in the cell (upwards of 10,000 copies per cell for rapidly growing *E. coli*; ref. 19). Thus, RNA polymerase is an attractive candidate to serve as a motor contributing to *oriC* movement. Consistent with this idea, we find that application of an inhibitor of RNA polymerase to *B. subtilis* cells reduces separation of newly replicated *oriC*-containing regions of the chromosome.

Methods

Plasmid and Strain Construction. To create plasmid pBW2, the *tetO* cassette from p306tetO112 (20), which consists of 112 tandem *tetO* repeats, was subcloned into the *Bam*HI site of pDG364. Insertion into the *Bam*HI site placed the *tetO* cassette adjacent to a chloramphenicol-resistance (Cm^R) gene and between the front and back ends of the *B. subtilis amyE* gene. To create plasmid pBW3, the *tetR-gfp* sequence from plasmid p3524 (20) was amplified by PCR and cloned between the *Bsp*DI and *Sac*II sites of plasmid pAT19 (2), so that the *tetR-gfp* sequence was downstream of the *veg* promoter. To create plasmid pCB36, the front half of the *B. subtilis dacA* gene was cloned into the *Aat*II site, and the back half of the gene was cloned into the *Nco*I site

Abbreviation: Arg-HX, arginine hydroxamate.

*To whom correspondence should be addressed at: Biological Laboratories, Department of Molecular and Cellular Biology, 16 Divinity Avenue, Cambridge, MA 02138. E-mail: losick@mcb.harvard.edu.

of pER82. As a consequence, the two halves of *dacA* flanked a copy of the *amyE* gene, which was itself interrupted by a kanamycin-resistance (Kan^R) gene. Similarly, to create plasmid pCB37, the front half of the *B. subtilis hutM* gene was cloned into the *AatII* site and the back half into the *NcoI* site of pER82. As a consequence, the two halves of *hutM* flanked the *amyE* gene, which was itself interrupted by the Kan^R gene.

To create strain JDB799, pBW3 was used to transform the prototrophic *B. subtilis* strain PY79, selecting for macrolide, lincomycin, and streptogramin B resistance (1 mg/ml erythromycin, 25 $\mu\text{g}/\text{ml}$ lincomycin). This transformation resulted in chromosomal integration of pBW3 through single reciprocal recombination at the *veg* locus, creating strain JDB697. Next, *dnaB134* was introduced into JDB697 by transformation with chromosomal DNA from a derivative of strain KI1258 (courtesy of A. Grossman, Massachusetts Institute of Technology, Cambridge, MA) that carries a spectinomycin-resistance (Spc^R) gene in the chromosome near the *dnaB* mutation followed by selection for Spc^R (100 $\mu\text{g}/\text{ml}$), resulting in strain JDB787. Then, we transformed JDB787 with chromosomal DNA from strain JDB773, which contained the *tetO* cassette integrated into the chromosome at *dacA*. Strain JDB773 was created by transforming PY79 with pCB36, followed by selection for Kan^R (10 $\mu\text{g}/\text{ml}$). Transformants were tested for amylase production to ensure that the plasmid had integrated at *dacA* and not at *amyE*. The *tetO* cassette then was introduced into a Kan^R Amy^+ transformant by transformation with pBW2, followed by selection for Cm^R (5 $\mu\text{g}/\text{ml}$). The Cm^R transformants were tested for kanamycin sensitivity and amylase production to confirm that pBW2 had integrated by double crossover at *dacA*. Finally, chromosomal DNA from strain JDB773 was used to transform JDB787, selecting for Cm^R , resulting in JDB799. Strain JDB800, the Str^R derivative of JDB799, was created by transforming JDB799 with chromosomal DNA from strain 1A405 (*rpoC10*; courtesy of A. Sonenshein, Tufts University School of Medicine, Boston, MA), and selecting for streptolydigin (Str^R) resistance (50 $\mu\text{g}/\text{ml}$). To create strain JDB792, we transformed JDB787 with chromosomal DNA from strain JDB781, which contained the *tetO* cassette integrated into the chromosome at *hutM*. Strain JDB781 was created by transforming PY79 with pCB37, followed by selection for Kan^R . Kan^R transformants were tested for amylase production to ensure that the plasmid had integrated at *dacA* and not at *amyE*. Then, the *tetO* cassette was introduced into a Kan^R Amy^+ transformant by transformation with pBW2, followed by selection for Cm^R . The Cm^R transformants were tested for kanamycin sensitivity and amylase production to confirm that pBW2 had integrated by double crossover at *dacA*. Finally, chromosomal DNA from strain JDB781 was used to transform JDB787, selecting for Cm^R , resulting in JDB792. Strain JDB871, the Str^R derivative of JDB799, was created by transforming JDB792 with chromosomal DNA from strain 1A405 and selecting for Str^R .

Growth Conditions. Strains JDB799 and JDB800 were grown in CH medium (CH = Sterlini–Mandelstam growth medium; ref. 21). Following established protocol (22), strains JDB792 and JDB871 were grown in S750 minimal medium and, to induce the stringent response, cells were grown for 60 min in the presence of arginine hydroxamate (Sigma) at 500 $\mu\text{g}/\text{ml}$. To relieve stringent arrest, cells were washed twice in fresh S750 medium and resuspended in an equal volume of S750 medium with excess arginine (250 $\mu\text{g}/\text{ml}$) and casamino acids (0.01%). For microscopy experiments, kanamycin (Sigma) was used at 100 $\mu\text{g}/\text{ml}$ and streptolydigin (Amersham Pharmacia) was used at 200 $\mu\text{g}/\text{ml}$.

Fluorescence Microscopy. The equipment and general conditions for microscopy of GFP were as described (23). To label mem-

branes, cells were incubated with the blue-emitting dye trimethylammonium diphenyl-hexatriene *p*-toluenesulfonate (Molecular Probes) at a final concentration of 1 mM and imaged with a W-NUA filter cube and neutral density filters for 1 s. Images were captured and processed with METAMORPH V.4.5 (Universal Imaging, Media, PA). To measure interfocal distances, the line-drawing tool of METAMORPH was used.

Results

To monitor movement in the *oriC*-proximal region of the chromosome, we created a strain (JDB799) in which a cassette containing tandem copies of the operator (*tetO*) for the tetracycline resistance repressor TetR was inserted into the chromosome at a site (*dacA*) located very close (≈ 17 kb) to the origin of replication. Visualization of the cassette was achieved by the presence in JDB799 of a construct that constitutively produced a fusion of the TetR repressor to GFP. The TetR-GFP-decorated *tetO* cassette in JDB799 was readily visualized as bright foci by fluorescence microscopy. So that a high degree of synchrony could be achieved, JDB799 contained a mutation (*dnaB134*) that causes the initiation of DNA replication to be temperature sensitive (Fig. 1A).

When cells of JDB799 were shifted to a restrictive temperature (45°C) for 60 min, almost all of the cells exhibited a single fluorescent focus, consistent with the idea that replication had been arrested at *oriC* (Fig. 1B). When, however, the temperature was shifted down from 45°C to 30°C for 45 min, a high proportion of the cells showed two well separated (>1 μm) *tetO* foci (Fig. 1B). We interpret this to indicate that replication had resumed at the permissive temperature, and that the newly duplicated origin regions had moved apart. Inhibition of RNA polymerase was achieved by the use of streptolydigin, which prevents transcription initiation and elongation through interaction with the β' -subunit of RNA polymerase (24). Streptolydigin was added after the temperature shift down to investigate the dependence of DNA separation on transcription.

A complication in interpreting the effect of the drug was the known requirement for RNA synthesis in replication initiation at *oriC* (25). Thus, an effect of streptolydigin could be attributed either to inhibition of replication initiation or to inhibition of the separation of newly duplicated origin regions or to both. Because we are unable to distinguish among these possibilities, we limited our analysis to cells with two distinct *tetO* foci (and hence, in which replication initiation had taken place after the temperature downshift), and we measured the effect of streptolydigin on the extent to which pairs of *tetO* foci had separated from each other. The interfocal distance observed when streptolydigin was added shortly after the downshift was markedly shorter than when the protein synthesis inhibitor kanamycin was added after the downshift (Fig. 1B and C). As a control, a mutation (*rpoC10*) in the gene for the β' -subunit of RNA polymerase that renders the enzyme resistant to streptolydigin was introduced into JDB799 (creating strain JDB800). When cells of the drug-resistant strain (JDB800) were exposed to streptolydigin, the magnitude of the separation between *tetO* foci was found to be similar to that observed for cells of the streptolydigin-sensitive parent that had been treated with kanamycin (Fig. 1B and C).

Because of the complication arising from the requirement for RNA synthesis in initiation at *oriC*, we adapted a previously devised protocol (22) as an independent strategy to investigate the effect of transcription on DNA segregation. Under conditions of amino acid starvation (the “stringent response”), DNA replication in *B. subtilis* is arrested at two sites, LSTer and RSTer, which are located on the left-hand and right-hand arms of the chromosome, respectively; each one is ≈ 130 kb downstream of *oriC* (26). This arrest can be induced by addition of the amino acid analogue arginine hydroxamate (Arg-HX) to the medium. The resumption of DNA replication after removal of Arg-HX is

Fig. 1. *oriC* movement in the presence of inhibitors of protein synthesis and transcription. (A) After a temperature shift from 45°C to 30°C, replication initiates at *oriC* (blue) and the *tetO* cassette at *dacA* (green dot) is replicated and the daughter copies undergo progressive separation. Note that the cartoon is a simplification of the *in vivo* situation where the replication forks colocalize in the replisome (as is illustrated in Fig. 3). (Ba) *tetO* foci in cells of strain JDB799 held at 45°C. (b) *tetO* foci in cells of JDB799 that had been downshifted to 30°C and incubated for 45 min in the absence of drug. (c) *tetO* foci in cells of JDB799 that had been downshifted to 30°C and incubated for 45 min in the presence of kanamycin (kan). (d) *tetO* foci in cells of JDB799 that had been downshifted to 30°C and incubated for 45 min in the presence of streptomycin (str). (e) *tetO* foci in cells of the *Str^R* strain JDB800 that had been downshifted to 30°C and incubated for 45 min in the presence of streptomycin. (Bar = 1 μm.) (C) Interfocal distances between *tetO* foci in kanamycin-treated cells of JDB799 (green bars, *n* = 244), streptomycin-treated cells of JDB799 (blue bars, *n* = 221), and streptomycin-treated cells of the *Str^R* strain JDB800 (red bars, *n* = 117) as described above for B.

known not to require protein or, importantly, RNA synthesis (27), an observation that we have confirmed by showing that streptomycin does not interfere with incorporation of [³H]thymidine into DNA after the removal of Arg-HX (data not shown).

To monitor DNA separation in cells that had been released from replication arrest at LSTer, we created a strain (JDB792) in which a *tetO* cassette was inserted into the chromosome at a site (*hutM*) just downstream (with respect to the direction of replication) of the LSTer site. As before, visualization of the cassette was achieved by the presence in JDB792 of a construct that constitutively produced a TetR-GFP fusion (Fig. 2A). Finally, so that a high degree of synchrony could be achieved, JDB792 contained the mutation *dnaB134* (above), which causes the initiation of DNA replication to be temperature sensitive.

When cells of JDB792 were synchronized by growth at 45°C followed by a shift to 30°C, many of the cells were found to exhibit a conspicuous pattern in which fluorescent foci were

present in closely opposed pairs or doublets (Fig. 2B). We interpret these doublets as representing *tetO* cassettes that had recently undergone duplication as a result of replication through the LSTer site. In the presence of Arg-HX, however, most cells contained either a single focus or two well spaced (>1 μm) foci, a result consistent with the idea that in drug-treated cells, replication was arrested at LSTer (Fig. 2B). Drug-treated cells with multiple, well spaced foci likely represented cells in which initiation of a second round of replication had commenced before cell division. When the Arg-HX-treated cells were washed to remove Arg-HX and then incubated for 30 min in the presence of excess arginine, many cells were observed that once again exhibited doublets of the fluorescent foci (Fig. 2B).

Addition of streptomycin strongly inhibited the formation of doublets (Fig. 2B). Because the resumption of DNA replication after arrest at LSTer does not require RNA synthesis (data not shown and ref. 27), we believe that many of the single foci

Fig. 2. *LSTer* movement in the presence of inhibitors of protein synthesis and transcription. (A) After a temperature shift from 45°C to 30°C in the presence of arginine hydroxamate (Arg-HX), replication initiates at *oriC* (blue) but then stalls at *LSTer* (orange) and *RSTer* (not shown). Removal of Arg-HX allows replication of the *tetO* cassette at *hutM* (green dot) and separation of the daughter copies. (Ba) *tetO* foci in downshifted cells of JDB792. (b) *tetO* foci in downshifted cells of JDB792 that been treated with Arg-HX; (c) *tetO* foci in downshifted- and Arg-HX-treated cells of JDB792 that were washed to remove Arg-HX and then incubated for 30 min. (d) *tetO* foci in downshifted- and Arg-HX-treated cells of JDB792 that were washed to remove Arg-HX and then incubated for 30 min with kanamycin (kan). (e) *tetO* foci in downshifted- and Arg-HX-treated cells of JDB792 that were washed to remove Arg-HX and then incubated for 30 min with streptomycin (str). (f) *tetO* foci in downshifted- and Arg-HX-treated cells of the *Str^R* strain JDB871 that were washed to remove Arg-HX and then incubated for 30 min with streptomycin. (Bar = 1 μ m.) Listed below the images are the corresponding percentages of cells exhibiting doublets of *tetO* foci (defined as having an interfocal distance of <1 μ m), based on the numbers of cells indicated.

observed in the presence of streptomycin were due to inhibition of foci separation rather than a block in replication of the *tetO* cassette. Addition of kanamycin after relief of arginine starvation had no significant effect on the frequency at which doublets were observed (Fig. 2B). Thus, the streptomycin-dependent inhibition of foci separation was evidently not a consequence of a secondary inhibition of protein synthesis. The fraction of cells that contained doublets of the *tetO* foci was more than threefold higher in the presence of kanamycin than in the presence of streptomycin (Fig. 2B). To rule out effects of streptomycin unrelated to the inhibition of RNA polymerase, we used a derivative of JDB792 (JDB871) that contained the streptomycin-resistance mutation *rpoC10*. When this streptomycin-resistant strain was exposed to drug, the magnitude of separation of the *tetO* cassettes was similar to that observed for its untreated streptomycin-sensitive parent (Fig. 2B). Thus, streptomycin inhibited the segregation of newly duplicated DNA proximal to *oriC* both after release from a block in the initiation of replication at *oriC* (Fig. 1C) and after release from a replication block at the *LSTer* site (Fig. 2B).

Discussion

We propose that the pulling force exerted by RNA polymerase plays a critical role in chromosome segregation in bacteria, at least in the initial separation of replication origin regions (Fig.

3). If RNA polymerase is stationary, or even if it is only partially immobilized, then transcription would result in movement of the DNA template within the cell (18). For example, when RNA polymerase is attached to a glass slide, transcription causes translocation of the DNA template (16). In the cytoplasm, viscosity might not ordinarily be expected to impose significant frictional drag on RNA polymerase. However, the cytoplasm may not be porous to large protein complexes (28, 29). As precedents for immobile complexes in bacteria, the replisome, as noted above, is stationary in *B. subtilis* (11), and the tetrameric enzyme β -galactosidase, which has a molecular mass similar to that of RNA polymerase (≈ 500 kDa), exhibits little diffusion in the cytoplasm of *E. coli* (30). In addition, cotranscriptional synthesis of membrane proteins that would tether RNA polymerase to the membrane (31) or, in the case of rRNA genes, the assembly of nascent ribosomes on incipient ribosomal transcripts, might contribute to restricting the movement of RNA polymerase.

Because the majority of genes in bacterial chromosomes, including the highly expressed rRNA genes, are oriented away from *oriC* (32–34), the net bias of the action of transcribing RNA polymerases would be to cause movement of the chromosome, and because of the large number of transcribing RNA polymerases, the force generated thereby would be considerable. A transcription model based on gene orientation away from the

Fig. 3. RNA polymerase-mediated DNA movement during chromosome segregation. The biased orientation of transcription units away from the origin (exaggerated in the cartoon) drives movement of newly synthesized daughter DNA molecules away from the replisome.

replication origin predicts that large inversions that include *oriC* and both arms of the chromosome should not impede chromosome segregation, but that large inversions within a single chromosomal arm should be disallowed. Indeed, most reported examples of large inversions encompass *oriC* and flanking regions from both arms of the chromosome (35, 36). A conspicuous exception is a large inversion in *E. coli* that is entirely contained within one chromosomal arm and does not include *oriC*. However, in this case, the inversion is associated with a severe defect in chromosome segregation, involving the high frequency generation of anucleate cells (37).

A precedent for the involvement of RNA polymerase in DNA movement is the infection of *E. coli* by bacteriophage T7, in which the transcription machinery of the host and late phage-encoded RNA polymerase drive the transfer of phage DNA from the virion into the cell (38). Interestingly, the rapid movements of interphase chromosomes in eukaryotes has been attributed to active RNA Pol II (39), and the mobility of a particular chromosomal locus within the nucleus has been correlated with its transcriptional activity (40). Of course, RNA polymerase need not be the only motor that helps drive chromosome segregation. It may act in concert with other motors, such as the replisome (22), which could contribute both to the separation and to the

orientation of replicating chromosomes (41) through the proposed back-to-back organization of the replicative helicases (42).

Finally, RNA polymerase is an unusually powerful motor, generating >30 pN of force as compared with 6 pN for the muscle protein myosin (16). If our hypothesis about the nature of the mitotic motor in bacteria is correct, then the significance of the high level of force generation by RNA polymerase may lie as much in its role in chromosome segregation as in gene transcription. Intriguingly, one consequence of this mechanism is that the control of transcription of rRNA genes by the concentration of nucleotide triphosphates, which is thought to couple growth rate and ribosome synthesis (43), also may couple growth rate and chromosome segregation and, by inference, cell division.

We thank P. Model for bringing to our attention the T7 experiments, A. Grossman and L. Sonenshein for strains, J. Wang, H. Berg, and A. Grossman for helpful discussions, and A. Wright, A. Murray, and members of the lab for comments on the manuscript. Plasmids pBW2 and pBW3 were constructed by W. Winston and P. Graumann. This work was supported by National Research Service Award Fellowship 5F32-GM19729 (to J.D.) and by National Institutes of Health Grant GM18568 (to R.L.).

- Jacob, F., Brenner, S. & Cuzin, F. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **23**, 329–348.
- Webb, C. D., Graumann, P. L., Kahana, J. A., Teleman, A. A., Silver, P. A. & Losick, R. (1998) *Mol. Microbiol.* **28**, 883–892.
- Lin, D. C., Levin, P. A. & Grossman, A. D. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 4721–4726.
- Webb, C. D., Teleman, A., Gordon, S., Straight, A., Belmont, A., Lin, D. C., Grossman, A. D., Wright, A. & Losick, R. (1997) *Cell* **88**, 667–674.
- Glaser, P., Sharpe, M. E., Raether, B., Perego, M., Ohlsen, K. & Errington, J. (1997) *Genes Dev.* **11**, 1160–1168.
- Sharpe, M. E. & Errington, J. (1999) *Trends Genet.* **15**, 70–74.
- Hiraga, S. (2000) *Annu. Rev. Genet.* **34**, 21–59.
- Sawitzke, J. A. & Austin, S. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 1671–1676.
- Graumann, P. L. (2000) *J. Bacteriol.* **182**, 6463–6471.
- Lin, D. C. & Grossman, A. D. (1998) *Cell* **92**, 675–685.
- Lemon, K. P. & Grossman, A. D. (1998) *Science* **282**, 1516–1519.
- Bellomy, G. R. & Record, M. T., Jr. (1990) *Prog. Nucleic Acid Res. Mol. Biol.* **39**, 81–128.
- Jensen, R. B. & Shapiro, L. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 10661–10666.
- Sawitzke, J. & Austin, S. (2001) *Mol. Microbiol.* **40**, 786–794.
- Errington, J., Bath, J. & Wu, L. J. (2001) *Nat. Rev. Mol. Cell. Biol.* **2**, 538–545.
- Wang, M. D., Schnitzer, M. J., Yin, H., Landick, R., Gelles, J. & Block, S. M. (1998) *Science* **282**, 902–907.
- Gelles, J. & Landick, R. (1998) *Cell* **93**, 13–16.
- Cook, P. R. (1999) *Science* **284**, 1790–1795.
- Bremer, H. & Dennis, P. P. (1999) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC).
- Michaelis, C., Ciosk, R. & Nasmyth, K. (1997) *Cell* **91**, 35–45.
- Nicholson, W. L. & Setlow, P. (1990) in *Molecular Biological Methods for Bacillus*, ed. Harwood, C. R. & Cutting, S. M. (Wiley, New York), pp. 391–450.
- Lemon, K. P. & Grossman, A. D. (2000) *Mol. Cell* **6**, 1321–1330.
- Eichenberger, P., Fawcett, P. & Losick, R. (2001) *Mol. Microbiol.* **42**, 1147–1162.
- Yang, X. & Price, C. W. (1995) *J. Biol. Chem.* **270**, 23930–23933.
- Baker, T. A. & Kornberg, A. (1988) *Cell* **55**, 113–123.
- Levine, A., Autret, S. & Seror, S. J. (1995) *Mol. Microbiol.* **15**, 287–295.
- Levine, A., Vannier, F., Dehbi, M., Henckes, G. & Seror, S. J. (1991) *J. Mol. Biol.* **219**, 605–613.

28. Bray, D. (2001) *Cell Movements: From Molecules to Motility* (Garland, New York), pp. 303–305.
29. Luby-Phelps, K. (2000) *Int. Rev. Cytol.* **192**, 189–221.
30. Elowitz, M. B., Surette, M. G., Wolf, P. E., Stock, J. B. & Leibler, S. (1999) *J. Bacteriol.* **181**, 197–203.
31. Lynch, A. S. & Wang, J. C. (1993) *J. Bacteriol.* **175**, 1645–1655.
32. Brewer, B. J. (1988) *Cell* **53**, 679–686.
33. McLean, M. J., Wolfe, K. H. & Devine, K. M. (1998) *J. Mol. Evol.* **47**, 691–696.
34. Lopez, P. & Philippe, H. (2001) *C. R. Acad. Sci. Ser. III* **324**, 201–208.
35. Anagnostopoulos, C. (1990) in *The Bacterial Chromosome*, eds. Drlica, K. & Riley, M. (Am. Soc. Microbiol., Washington, DC), pp. 361–371.
36. Hughes, D. (1999) in *Organization of the Prokaryotic Genome*, ed. Charlebois, R. (Am. Soc. Microbiol., Washington, DC), pp. 109–128.
37. Niki, H., Yamaichi, Y. & Hiraga, S. (2000) *Genes Dev.* **14**, 212–223.
38. Zavriev, S. K. & Shemyakin, M. F. (1982) *Nucleic Acids Res.* **10**, 1635–1652.
39. Gasser, S. M. (2002) *Science* **296**, 1412–1416.
40. Buchenau, P., Saumweber, H. & Arndt-Jovin, D. J. (1997) *J. Cell Biol.* **137**, 291–303.
41. Dingman, C. W. (1974) *J. Theor. Biol.* **43**, 187–195.
42. Fang, L., Davey, M. J. & O'Donnell, M. (1999) *Mol. Cell* **4**, 541–553.
43. Gaal, T., Bartlett, M. S., Ross, W., Turnbough, C. L. & Gourse, R. L. (1997) *Science* **278**, 2092–2097.