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Supercoiling of the DNA template during transcription

(DNA topology/topoisomerases/transcriptional swivel/translocation along DNA/gene regulation)

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ABSTRACT **Transcription of a right-handed double-helical DNA requires a relative rotation of the RNA polymerase and its nascent RNA around the DNA. We describe conditions under which the resistance to the rotational motion of the transcription ensemble around the DNA can be large. In such cases, the advancing polymerase generates positive supercoils in the DNA template ahead of it and negative supercoils behind it. Mutual annihilation of the positively and negatively supercoiled regions may be prevented by anchoring points on the DNA to a large structure, or, in the case of an unanchored plasmid, by the presence of two oppositely oriented transcription units. In prokaryotes, DNA topoisomerase I preferentially removes negative supercoils and DNA gyrase (topoisomerase II) removes positive ones. Our model thus provides an explanation for the experimentally observed high degree of negative or positive supercoiling of intracellular pBR322 DNA when DNA topoisomerase I or gyrase is respectively inhibited. We discuss the implications of our model in terms of supercoiling regulation, DNA conformational transitions, and gene regulation in both prokaryotes and eukaryotes.**

Plausible effects of transcription on the dynamics of the template DNA have been recognized for some time. Maaløe and Kjeldgaard (1) pointed out that as transcription proceeded along the helical template, it might be difficult for the RNA polymerase and its nascent RNA chain to turn around the DNA; thus the DNA might turn around its axis instead as the transcription apparatus tracks along.

With the discovery of *Escherichia coli* DNA topoisomerase I, the possible requirement of a transcriptional "swivel" in the DNA was again raised (2). Such a swivel would allow a segment of the DNA being actively transcribed to rotate without turning the entire DNA molecule around its axis. Indirect evidence favoring such a possibility includes the finding that eukaryotic DNA topoisomerase I is often found to be associated with transcriptionally active genes (3–6).

Whereas the possibility of turning the DNA during transcription is well-recognized, transcription is rarely viewed as a force that might actively supercoil DNA. Only special cases, in which the polymerase is interacting with a DNA-bound regulatory protein, or when both the polymerase and the DNA are anchored on a cellular structure, have been considered in terms of DNA supercoiling driven by transcription (7, 8). In this communication, we describe several cases in which transcription may lead to DNA supercoiling. We believe that the concepts presented here can explain a number of observations in the literature that have not been adequately interpreted. These and other implications of template supercoiling by transcription are discussed.

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THEORY AND MODELS

Basic Considerations. Fig. 1 illustrates the basic mechanics of transcription. An RNA polymerase, together with its nascent RNA and RNA-bound proteins (ribosomes and newly synthesized proteins in prokaryotes and ribonucleoprotein complexes in eukaryotes), are tracking along a right-handed double-helical DNA segment with its ends anchored on a cellular structure. The DNA segment is thus a loop topologically and is a reasonable representation of a chromosomal loop. We consider first the case in which no topoisomerase is present and the DNA is relaxed at t_0 (Fig. 1 *a* and *b*). For simplicity, we assume further that at t_0 it is much easier to rotate the DNA around its helical axis than to rotate the transcription apparatus R (including the polymerase, RNA, and RNA-bound proteins) around the DNA. Thus as R advances, the DNA in front of it becomes positively supercoiled, whereas the DNA behind it becomes negatively supercoiled (Fig. 1*c*). As the degree of supercoiling builds up, however, it becomes increasingly difficult to turn the DNA. A point is therefore reached at time t when R must rotate around the DNA. The system reaches a balanced point when the frictional torque on R is equal to the supercoiling torque in the DNA.

We show in the *Appendix* that the supercoiling torque τ_s is

$$\tau_s = 1.4 \times 10^{-12} (|\sigma_+| + |\sigma_-|) \text{ dyne cm}, \quad [1]$$

where σ_+ and σ_- are the specific linking differences or superhelical densities of the positively and negatively supercoiled loops, respectively. Thus to supercoil DNA significantly to a $|\sigma| \geq 0.01$, the frictional torque should be of the order of 10^{-14} dyne cm (1 dyne = 1.000×10^{-5} newtons) or greater. As estimated in the *Appendix*, in a dilute aqueous solution the frictional torque in dyne cm is usually $\approx 10^{-17}$ to 10^{-16}, which is much smaller than what is needed to cause significant supercoiling. For a very long transcript with many ribosomes or ribonucleoprotein particles dotted along it, however, the frictional torque can be significant.

There are also several situations that may greatly increase the effective frictional torque: (*i*) Any part of the transcribing polymerase and macromolecules associated with it is attached to a large cellular structure. This situation is similar to the case discussed previously (7, 8) for the anchoring of the polymerase. In prokaryotes, a case of particular interest is the transcription of genes encoding membrane proteins. As transcription and translation are coupled in prokaryotes, anchoring of the N-terminal portion of a newly-synthesized peptide chain would force the rotation of the DNA to a much higher degree of supercoiling. (*ii*) Any part of the transcribing polymerase and macromolecules associated with it interacts with a site on the DNA in addition to the site at which RNA chain synthesis occurs. This is again similar to the case

Abbreviation: R, transcription ensemble including RNA polymerase, nascent RNA, and RNA-bound protein.

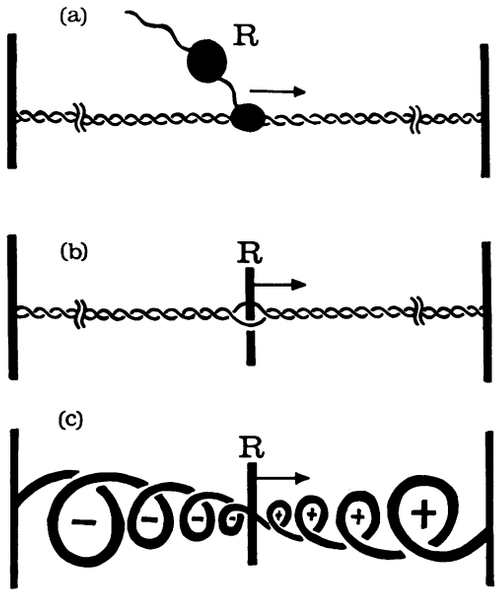

FIG. 1. A graphical illustration of the mechanics of transcription. (*a*) A transcription ensemble R including the polymerase, the nascent RNA, and proteins bound to the RNA is moving in the direction of the arrow along a DNA segment; the ends of the DNA segment are anchored on a large structure represented by the solid bars. (*b*) The transcription ensemble can be viewed as a divider separating the helical DNA into two parts. (*c*) If R is moving from left to right without turning around the DNA, the DNA in front of the polymerase becomes overwound, or positively supercoiled; the DNA behind the polymerase becomes underwound, or negatively supercoiled.

discussed previously for the interaction between the polymerase and a DNA-bound regulatory factor (7, 8). Two additional examples in this category are the invasion of the DNA by a part of the nascent RNA chain and the pairing between two divergent transcripts sharing complementary sequences. (*iii*) In the cellular milieu the motion of a nascent RNA chain with proteins associated with it might be severely hindered, so that the effective frictional drag could be much larger than the values estimated for a dilute solution. In contrast to the two possibilities described above, the third possibility should apply in general, rather than only in special cases.

Transcription on a Plasmid. When there is a single transcriptional unit on a circular plasmid, the positively and negatively supercoiled domains can cancel each other by rotating the entire DNA around its helical axis, even if the RNA polymerase is immobile (9). The frictional torque against a DNA turning around its helical axis is, however, usually small even if the DNA is decorated with nucleosomes or similar protein–DNA complexes (see the *Appendix*). Thus we expect little effect on supercoiling in this case. The situation could be very different, however, when there are more than one transcriptional unit per plasmid; this is discussed below.

Effects of Multiple Transcription Units. When two or more polymerases are transcribing an anchored DNA segment or loop, the situation is not very different from the case with a single polymerase. Suppose two divergently transcribing polymerases, rather than a single enzyme, are moving toward the ends of the anchored DNA segment shown in Fig. 1*a*. The two polymerases would divide the DNA segment into three regions: the central one would be negatively supercoiled, whereas the flanking regions would be positively supercoiled. Similar to the case with a single transcriptional unit, Eq. **1** can be used to estimate the degrees of supercoiling of the various regions if the viscous drag on each transcription ensemble can be calculated. For the case when the polymerases are moving in the same direction at the same speed, the middle portion would be relaxed. The supercoiling effects on the flanking regions would be increased by roughly a factor of two, but the case is qualitatively similar to the single transcription unit case.

In contrast, two polymerases moving in opposite directions on a circular plasmid are very different from the case with a single polymerase: the two opposing polymerases divide the circular DNA into a positively and a negatively supercoiled region that cannot annihilate each other without turning at least one of the transcribing ensembles. Thus, the case of two opposing polymerases on a circular template is similar to the case of transcription on a loop with its base anchored. On the other hand, when two polymerases are moving in synchrony in the same direction on a circular DNA, the supercoiled regions can again cancel each other by turning the connecting DNA, and the situation is similar to the one in which a single polymerase is tracking along a ring.

The Effects of the Topoisomerases. In cases where the effective frictional torques are large, the transcriptional process would generate positively and negatively supercoiled regions. In prokaryotes, it is well known that DNA topoisomerase I can relax only negatively supercoiled DNA (10) in the absence of single-stranded regions (11). DNA gyrase, on the other hand, negatively supercoils DNA in the presence of ATP (12, 13). In other words, the action of the gyrase is to reduce the linking number of a DNA ring or loop; it can relax a positively supercoiled loop effectively, but it maintains, rather than relaxes, a negatively supercoiled loop. Thus DNA topoisomerase I and DNA gyrase form an opposing pair; each can relax efficiently only one kind of supercoiled regions. This dichotomy has interesting consequences, which we will discuss later.

In eukaryots, DNA topoisomerase I, as well as II, can relax both positively and negatively supercoiled loops (14, 15); differential actions, if any, would be dependent on the locations of binding sites and/or other features of the various regions that might influence the distributions of the enzymes.

DISCUSSION

Generation of Positively and Negatively Supercoiled Domains by a Tracking Process. We have considered a number of conditions under which the transcription process can lead to the supercoiling of the template. Some of the same principles are applicable in other situations in which a macromolecular assembly translocates along the DNA: the tracking model for DNA supercoiling was first discussed as a possible model for DNA gyrase (16, 17), and was invoked again for the action of the *Eco* R·K type I restriction enzyme (18). The possibility of localized supercoiling by a tracking process was also recognized (19).

The unique feature of the model is the simultaneous generation of positively and negatively supercoiled regions. In prokaryotes, the existence of two diametric topoisomerases, one (DNA topoisomerase I) that cannot relax positively supercoiled loops and the other (gyrase) that cannot relax negatively supercoiled ones, has set the stage for differential effects of topoisomerase mutants on the degree of supercoiling of intracellular DNAs. We discuss below our interpretations of some of the puzzling observations in the literature.

Effects of Inactivation of a DNA Topoisomerase on the Degree of Supercoiling of Intracellular DNAs. The generally accepted picture on the regulation of DNA supercoiling in prokaryotes is that there is a dynamic balance between the actions of DNA topoisomerase I and DNA gyrase (13, 15). Several observations are, however, difficult to explain based on this mechanism.

First, Lockshon and Morris (20) showed that a significant fraction of the plasmid pBR322 isolated from *E. coli* cells

after inhibition of gyrase by drugs is positively supercoiled. Although binding of catalytically inactive gyrase to DNA can increase the linking number of the DNA when it is relaxed, the effect is a small one and amounts to <1 supercoil per bound gyrase (13, 17). The degree of positive supercoiling observed in the experiments of Lockshon and Morris was high; 20 or more positive supercoils per plasmid were observed. Second, in *E. coli topA* mutants, but not in its wild-type control, pBR322 was found to be much more negatively supercoiled than its derivatives, missing various regions in a segment encoding resistance against tetracycline (21, 22). It is particularly striking that inactivation of the *tet* promoter of the plasmid is sufficient to abolish the high degree of negative supercoiling of the plasmid in *topA* mutants.

These results can be explained by the model that the transcription process under certain conditions is contributing significantly to, or may even dominate, the steady-state level of supercoiling. Because the model involves the simultaneous generation of positively and negatively supercoiled loops, inhibition of gyrase in a $topA^+$ strain would yield positively supercoiled molecules; the functional topoisomerase can only relax the negatively supercoiled loop. Similarly, in a *topA* strain a negatively supercoiled region would not be relaxed effectively. The experiments of Pruss and Drlica (21, 22) provided strong evidence that the higher degree of negative supercoiling of pBR322 in a *topA* mutant compared with some of its derivatives is related to transcription. As we have described in the earlier sections, a number of conditions could lead to significant template supercoiling by transcription. Further experiments are needed to determine whether it is the *tet* gene in particular, or the divergent transcription of the β-lactamase gene and the *tet* gene on pBR322, that sets apart pBR322 from its derivatives that lack parts of the tetracycline-resistance transcription unit. We favor the latter possibility.

Rate Considerations. In *E. coli*, there are of the order of 500 gyrase molecules per cell (13), which comes to an average of 1 gyrase per 6 kilobases (kb) of DNA. Therefore, on a plasmid the size of pBR322, there is ≈1 gyrase molecule per plasmid. The turnover number of gyrase has been estimated to be 0.5–1 supercoils per sec (23–25). Thus the rate of removal of positive supercoils on a small plasmid by gyrase is of the order of 0.5–1 supercoils per sec. This rate is rather slow. As described in the *Appendix*, if the effective frictional torque on the transcription ensemble is large, positive and negative supercoils are generated at a rate of four each per sec per transcript. Thus even in $topA^+$ gyr^+ strains, transcription may significantly affect the degree of supercoiling of regions of intracellular DNA. The turnover number of DNA topoisomerase I has not been measured, but it is known to be strongly dependent on the degree of negative supercoiling and on temperature (10). Similarly, no reliable estimates can be made for the eukaryotic topoisomerases *in vivo*.

Biological Implications. The twin transcriptional-loop model has a number of implications. As discussed in the two sections above, under a number of conditions transcription might be an important determinant of the steady-state degree of supercoiling of intracellular DNA. Our model also suggests that because of the presence of multiple domains of different degrees and signs of supercoiling in an intracellular DNA ring or loop, the average degree of supercoiling of the entire DNA ring or loop, which can be determined by linking number measurements or titrations with intercalating dyes, may not be an adequate indicator of the topological state of DNA *in vivo*.

Whereas the differential actions of bacterial topoisomerase I and gyrase make it ideal to test the twin transcriptional-loop model in prokaryotes, the basic concepts are valid in both prokaryotes and eukaryotes.

Localized supercoiling of DNA by transcription and, perhaps, by other tracking processes may extend the range of the degree of supercoiling of intracellular DNA that may be utilized to drive structural transitions. Sequences that can assume helical forms that differ from the canonical B-structure, including alternating purine-pyrimidine and oligopurine-oligopyrimidine stretches, are often present in the regulatory regions of genes (see, for example, ref. 26 and the references therein).

Finally, the twin transcriptional-loop model raises the possibility that the process of transcription may affect a distal point in cis through template supercoiling. For example, through such a mechanism the transcription of a gene may activate or deactivate an adjacent gene. Further experimentation is needed to test the validity of the various implications. The basic concept that transcription is one of the determinants of the topological state of DNA *in vivo* is, however, likely to be correct.

APPENDIX

Estimation of the Magnitudes of the Supercoiling and Frictional Torques in a Dilute Aqueous Solution. Imagine that one end of a DNA segment N base pairs (bp) in size is anchored on a wall and the other end is being turned. We define the linking number α of the DNA at any time as the linking number of the ring formed by joining the appropriate ends of the segment without rotating them appreciably around the helix axis. When the DNA is in its most stable structure, $\alpha = \alpha^\circ = N/h^\circ$, where h° is 10.5 bp, the number of bp per DNA helical turn in solution (27–31). As one end is being turned, α changes and the segment becomes supercoiled. The free energy ΔG_τ of supercoiling (32–36) is:

$$\Delta G_\tau = K(\alpha - \alpha^\circ)^2. \quad [2]$$

The supercoiling torque Γ_s is the change in this free energy when the end is turned by 1 radian:

$$\Gamma_s = d\Delta G_\tau/d(2\pi\alpha) = K(\alpha - \alpha^\circ)/\pi \quad [3]$$

When N is larger than 2000 bp, K is ≈1100 RT/N (36) and

$$\begin{aligned}\Gamma_s &= 1100\ RT(\alpha - \alpha^\circ)/\pi N \\ &= 1100\ RT(\alpha - \alpha^\circ)/10.5\pi\alpha^\circ \\ &= 33\ RT(\alpha - \alpha^\circ)/\alpha^\circ \\ &= 33\ RT\sigma, \end{aligned} \quad [4]$$

where σ is defined as $(\alpha - \alpha^\circ)/\alpha^\circ$ and is termed the specific linking difference, or superhelical density (19, 37).

In the model considered in the paper, a positively supercoiled domain with a specific linking difference σ_+ and a negatively supercoiled domain with a specific linking difference σ_- are generated. We also change Γ_s, the torque for one mole of molecules, to τ_s, the supercoiling torque for one molecule, by changing the gas constant R to the Boltzmann constant k:

$$\tau_s = 33\ kT(|\sigma_+| + |\sigma_-|) \quad [5]$$

The absolute values of the specific linking differences are used in Eq. 5 to avoid the need of keeping track of their signs. Eq. 5 is Eq. 1.

The rotational frictional coefficient f_r of a DNA was considered in Meselson (38), in which f_r is taken to be that of

a cylinder of a radius r of 10 Å:

$$f_r = 4\pi r^2 L\eta = 4V\eta \quad [6]$$

where L is the length, η the solvent viscosity, and V the hydrated volume. At the maximal rate of transcription, RNA is being synthesized at a rate of 40 nucleotides/sec, and therefore the DNA needs to be turned at an angular velocity ω of 4 turns per sec, or 8π rad·sec^{-1}. The frictional torque τ_s is therefore

$$\tau_s = \omega f_r = 32\pi V\eta \quad [7]$$

In a dilute aqueous solution, $\eta = 0.01$ dyne sec/cm^2, and τ_s is readily estimated to be 10^{-17} to 10^{-16} dyne cm for DNAs with sizes between 10 and 100 kb, the size of a typical large plasmid or a chromosomal loop. Nucleosome formation along the DNA does not change V very much, and the frictional torque is not grossly altered.

For rotating the transcription ensemble, *E. coli* RNA polymerase has a hydrated volume $\approx 10^{-18}$ cm^3, and a sphere of this size turning at an angular velocity of 8π rad sec^{-1} is readily calculated to be $8\pi \cdot 6 \cdot 10^{-18} \cdot \eta$ or about 10^{-18} dyne cm in a dilute aqueous solution. When RNA is being synthesized, a nascent RNA 1000 nucleotides in length can stretch to a distance as far as 5000 Å. The average dimension, however, is expected to be much smaller, and a reasonable guess of the average location of a ribosome or a ribonucleoprotein particle would be of the order of 200 Å from the helical axis of the DNA. We approximate a ribosome by a sphere of a radius of 100 Å and, thus, a translational friction coefficient of about $2 \cdot 10^{-7}$ dyne sec/cm in a dilute aqueous solution. The frictional force on the particle is $200 \cdot 10^{-8} \cdot 8\pi \cdot 2 \cdot 10^{-7}$, or 10^{-11} dyne. The corresponding torque is $10^{-11} \cdot 200 \cdot 10^{-8}$ or $2 \cdot 10^{-17}$ dyne cm. This term is also small. For a very long transcript, such as 20 ribosomes dotted along the message with an average distance of 1000 Å, the frictional torque is estimated as $\approx 5 \times 10^{-15}$ dyne cm, which would give a fairly significant sum ($|\sigma_+| + |\sigma_-|$) of ≈ 0.003.

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1. Maaløe, O. & Kjeldgaard, N. O. (1966) *Control of Macromolecular Syntheses* (Benjamin, New York).
2. Wang, J. C. (1973) in *DNA Synthesis in Vitro*, eds. Wells, R. D. & Inman, R. B. (University Park Press, Baltimore), pp. 163–174.
3. Higashinakagawa, T., Wahn, H. & Reeder, R. H. (1977) *Dev. Biol.* **55**, 375–386.
4. Bauer, W. R., Ressner, E. C., Kates, J. & Patzke, J. V. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1841–1845.
5. Fleischmann, G., Pflugfelder, G., Steiner, E. K., Javaherian, K., Howard, G. C., Wang, J. C. & Elgin, S. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6958–6962.
6. Gilmour, D. S., Pflugfelder, G., Wang, J. C. & Lis, J. T. (1986) *Cell* **44**, 401–407.
7. Wang, J. C. (1985) in *Interrelationship Among Aging, Cancer and Differentiation*, Jerusalem Symposium on Quantum Chemistry and Biochemistry, eds. Pullman, B., Ts'o, P. O. P. & Schneider, E. L. (Reidel, Holland), Vol. 18, pp. 173–181.
8. Wang, J. C. (1987) *Harvey Lect.* **81**, 93–110.
9. Gamper, H. B. & Hearst, J. E. (1982) *Cell* **29**, 81–90.
10. Wang, J. C. (1971) *J. Mol. Biol.* **55**, 523–533.
11. Kirkegaard, K. & Wang, J. C. (1985) *J. Mol. Biol.* **185**, 625–637.
12. Cozzarelli, N. R. (1980) *Science* **207**, 953–960.
13. Gellert, M. (1981) *Annu. Rev. Biochem.* **50**, 879–910.
14. Vosberg, H.-P. (1985) *Curr. Top. Microbiol. Immunol.* **114**, 19–102.
15. Wang, J. C. (1985) *Annu. Rev. Biochem.* **54**, 665–697.
16. Gellert, M., Mizuuchi, K., O'Dea, M. H., Ohmori, H. & Tomizawa, J. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 35–40.
17. Liu, L. F. & Wang, J. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2098–2101.
18. Yuan, R., Hamilton, D. L. & Burckhardt, J. (1980) *Cell* **20**, 237–244.
19. Wang, J. C. (1983) in *Nucleic Acid Research: Future Development*, eds. Mizobuchi, K., Watanabe, I. & Watson, J. D. (Academic, New York), pp. 549–566.
20. Lockshon, D. & Morris, D. R. (1983) *Nucleic Acids Res.* **11**, 2999–3017.
21. Pruss, G. J. (1985) *J. Mol. Biol.* **185**, 51–63.
22. Pruss, G. J. & Drlica, K. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8952–8956.
23. Maxwell, A. & Gellert, M. (1984) *J. Biol. Chem.* **259**, 14472–14480.
24. Sugino, A. & Cozzarelli, N. R. (1980) *J. Biol. Chem.* **255**, 6299–6306.
25. Baker, T. A., Funnell, B. E. & Kornberg, A. (1987) *J. Biol. Chem.* **262**, 6877–6885.
26. Evans, T. & Efstratiadis, A. (1986) *J. Biol. Chem.* **261**, 14771–14780.
27. Wang, J. C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 200–203.
28. Wang, J. C. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 29–33.
29. Peck, L. J. & Wang, J. C. (1981) *Nature (London)* **292**, 375–378.
30. Rhodes, D. & Klug, A. (1980) *Nature (London)* **286**, 573–578.
31. Rhodes, D. & Klug, A. (1981) *Nature (London)* **292**, 378–380.
32. Bauer, W. R. & Vinograd, J. (1970) *J. Mol. Biol.* **47**, 419–435.
33. Hsieh, T.-S. & Wang, J. C. (1975) *Biochemistry* **14**, 527–535.
34. Depew, R. E. & Wang, J. C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4275–4279.
35. Pulleyblank, D. E., Shure, M., Tang, D., Vinograd, J. & Vosberg, H. P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4280–4284.
36. Horowitz, D. & Wang, J. C. (1984) *J. Mol. Biol.* **173**, 75–91.
37. Wang, J. C., Peck, L. J. & Becherer, K. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 85–91.
38. Meselson, M. (1972) *J. Mol. Biol.* **71**, 795–798.