Axial distortion as a sensor of supercoil changes: a molecular model for the homeostatic regulation of DNA gyrase

SHYAM UNNIRAMAN[1] and VALAKUNJA NAGARAJA[1,2,*]

[1]*Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India*
[2]*Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560 064, India*

Abstract

Negative supercoiling stimulates transcription of many genes. In contrast, transcription of the genes coding for DNA gyrase is subject to a novel mechanism of autoregulation, wherein relaxation of the template DNA stimulates their transcription. Since DNA gyrase is the sole supercoiling activity in the eubacterial cell, relaxation-stimulated transcription (RST) could reflect an autoregulatory mechanism to maintain supercoil levels within the cell. Extensive deletion and mutational analyses of *Escherichia coli gyrA* promoter have shown that the –10 region is essential for RST; however, a molecular model has proved to be elusive. We find a strong bend centre immediately downstream of the –10 region in the *gyrA* promoter. On the basis of analysis of various mutants in the –10 region, we propose a model where axial distortion acts as a sensor of topological changes in DNA. Our model is consistent with earlier data with *E. coli gyrA* and *gyrB* promoters. We also extrapolate the model to explain the phenomenon of RST of *gyr* promoters in other organisms and contrast it with promoters induced by supercoiling.

[Unniraman S. and Nagaraja V. 2001 Axial distortion as a sensor of supercoil changes: a molecular model for the homeostatic regulation of DNA gyrase. *J. Genet.* **80**, 119–124]

Introduction

Transcription from many promoters in *Escherichia coli* is sensitive to topology of the DNA. Since negative supercoiling can provide the energy for melting the template DNA, it is not surprising that, in most cases, supercoiling stimulates transcription initiation (Wang and Lynch 1993). The supercoiled status of DNA inside the cell is determined by the supercoiling activity of DNA gyrase and the relaxing activities of topoisomerases I and IV (Zechiedrich *et al*. 1997).

DNA gyrase is a heterotetramer composed of two polypeptides each of GyrA and GyrB. The steady-state levels of the enzyme increase by three-fold to four-fold when cells are treated with drugs that inhibit the enzyme (Menzel and Gellert 1983). Inhibition of DNA gyrase leads to a global relaxation of the genome, which, in turn, induces transcription of both *gyrA* and *gyrB* genes. Since DNA gyrase is the sole supercoiling activity in the eubacterial cell, relaxation-stimulated transcription (RST) probably reflects a homeostatic mechanism for the regulation of supercoiling within physiological limits.

Although RST provides the cell with a convenient method for regulation of DNA topology, the mechanism underlying the phenomenon is unclear. Deletion analysis of the promoter regions of both *gyrA* and *gyrB* genes of *E. coli* defined a short region around the transcriptional start site, including the –10 region, that is necessary and sufficient for conferring RST to a reporter gene (Menzel and Gellert 1987a,b). Furthermore, extensive mutagenesis of the *gyrA* promoter shows that the –10 region is responsible for both promoter strength and supercoil-sensitive behaviour (Straney *et al*. 1994). However, the *gyrA* promoter region harbours a sequence that matches the *E. coli* consensus for extended –10 promoters (TGNTATAAT, see figure 1a). Since most extended –10 promoters do not show RST, it appears unlikely that the sequence of the –10 region alone is responsible for RST.

*For correspondence. E-mail: vraj@mcbl.iisc.ernet.in.

Keywords. DNA curvature; supercoiling; DNA topology; gyrase; relaxation-stimulated transcription.

In the present study, we provide a molecular model for the autoregulation of DNA gyrase in *E. coli* and other species, wherein intrinsic curvature in the vicinity of the –10 element is proposed to act as a sensor of DNA topology and thereby modulate transcription initiation.

Methods

The curvature propensity of the sequence was calculated using the bend.it server (http://www2.icgeb.trieste.it/~dna/bend_it.html), with the DNAase I-based bendability parameters of Brukner *et al*. (1995) and the consensus bendability scale (Gabrielian and Pongor 1996). Both these trinucleotide models for predicting DNA curvature produce results that are in close agreement with NMR and X-ray crystallographic data. We obtained very similar results with both algorithms. Since the consensus scale algorithm includes both the DNAase I sensitivity and nucleosome positioning-based bendability parameters, in the discussion that follows only the results with the consensus algorithm are presented. A window of 10 nucleotides (slid one base pair at a time) was used; however, similar results were obtained with a window size of 5, 21 or 31 base pairs. The extent of curvature was plotted as the degree of bending normalized to a single helical turn (or 10.5 base pairs). Curvature was also calculated by two other models—the CURVATURE algorithm (Bolshoy *et al*. 1991; Shpigelman *et al*. 1993) and the AAWedge program (http://lfd.uiuc.edu/staff/gohlke/curve/). In both cases, a window of 21 nucleotides was used, which was slid one nucleotide at a time. The extent of curvature was plotted as curvature units versus the base position.

Results and discussion

Intrinsic curvature in the gyrA promoter

Many non-B DNA structures are preferentially formed in a supercoil-dependent manner (Palecek 1991; Dai and Rothman-Denes 1999); therefore the *gyrA* promoter region of *E. coli* was analysed for presence of such structures. The *gyrA* promoter harbours a consensus –10 element (figure 1a), and we find the entire promoter region to be devoid of features that would predispose it to the formation of cruciforms (palindromic stretches), triplex (polypurine–polypyrimidine repeat sequences) or Z-DNA (alternating purine–pyrimidine stretches). We therefore scanned the region for more subtle changes in topology such as curvature using the consensus scale algorithm developed by Gabrielian and Pongor (1996).

We now define the terms used for axial distortion throughout the discussion that follows. In the strict sense, bending is the 'tendency of adjacent base pairs to be non-parallel in an additive manner over several base pair

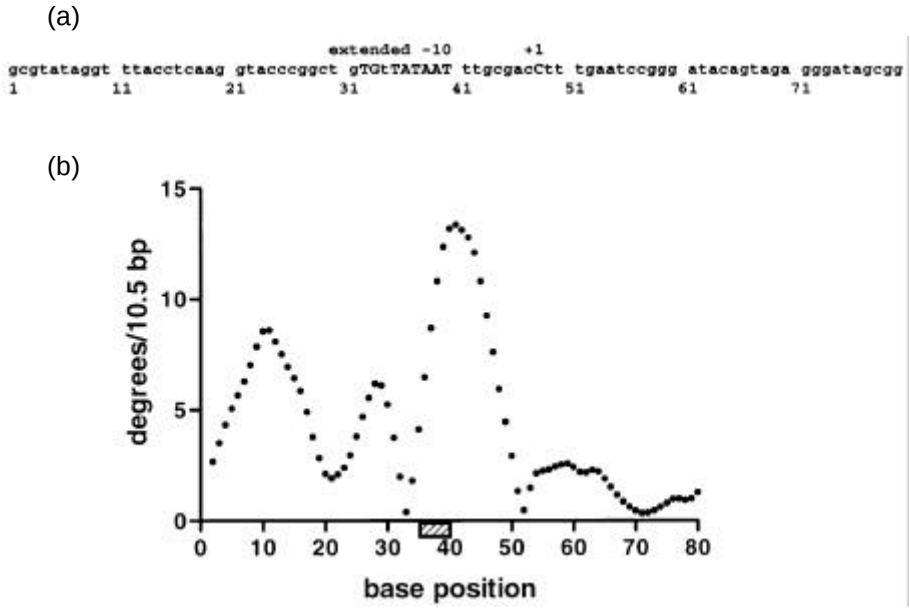

Figure 1. Curvature in the promoter region of *gyrA* from *E. coli*. (a) The sequence in the promoter region is shown with the extended –10 region and the transcription start site (+1) in upper case. (b) The curvature propensity of the promoter region. The hatched box represents the –10 hexamer. Intrinsic curvature was calculated on the bend.it server using the consensus scale algorithm with a window size of 10 nucleotides. An axial distortion less than 5 degrees per 10.5 bp is considered straight DNA.

steps' while curvature is 'the tendency of the helix axis to follow a non-linear pathway over an appreciable length' (Goodsell and Dickerson 1994). Since our analysis is restricted to the short promoter region, we assume that there is a direct effect of bending on curvature and, therefore, use these two terms interchangeably. Our analysis reveals the presence of a strong centre of curvature at the 3′ end of the –10 element in the *E. coli gyrA* promoter. Figure 1b shows the curvature propensity plot for the *gyrA* promoter region. Since this window would include contributions from the –10 region itself, one would expect mutations in the –10 region to alter curvature.

Deletion analysis by earlier workers implies a primary role for the region in the vicinity of the –10 element (Menzel and Gellert 1987a,b). Furthermore, Menzel and coworkers (Straney *et al*. 1994) specifically mutagenized the –10 region of the *gyrA* promoter and generated 30 different mutants that are affected in both basal activity and supercoil sensitivity. These mutants cover a wide range in basal activities and inducibility. To understand the role, if any, played by curvature in modulating transcription, we tested the intrinsic curvature of these mutants. In the following analysis, we have excluded one of the mutants (TTTACT) that shows a >100-fold reduction in basal promoter activity. Although most mutants retain the curvature downstream of the –10 region, many show a dramatic change in the amplitude of the bend (figure 2a). Furthermore, there is a direct linear correlation (*P* value = 0.0014) between the extent of curvature and the level of RST shown by the mutants (figure 2b). It is noteworthy that there is a statistically significant correlation between curvature and RST in the entire region encompassing the peak. Figure 2b shows a representative plot at base position 41, which corresponds to the nucleotide immediately downstream of the –10 element and also to the bend centre in the wild-type promoter. On the basis of earlier analysis of mutants, the presence of a TAHAAT sequence in the –10 region was believed to be necessary for RST (Straney *et al*. 1994). If curvature was the primary determinant of RST, we would expect a decrease in curvature in mutants lacking this stretch. In agreement with this expectation, most mutations away from this consensus appear to reduce the intrinsic curvature of the region and probably thereby lead to a decrease in RST.

It should be noted here that these results are corroborated qualitatively by the CURVATURE (Bolshoy *et al*. 1991; Shpigelman *et al*. 1993) as well as the AAWedge algorithms. A representative curvature propensity plot using the latter algorithm is shown in figure 3.

Intrinsic curvature as a sensor of supercoiling

DNA curvature is known to work in conjunction with supercoiling in many cases (Figueroa *et al*. 1991; Owen-Hughes *et al*. 1992; Perez-Martin and de Lorenzo 1997). Therefore it is not surprising that presence of a bend centre near the –10 region modulates the supercoil-sensitive behaviour of the *gyrA* promoter. However, since the geometry of bent DNA is similar to that adopted by supercoiled DNA, bending is usually invoked to assist or accentuate the effect of supercoiling. Our model is the first wherein bending plays a role antagonistic to supercoiling.

Axial distortion could modulate transcription either directly or indirectly. In a direct role, axial distortion could optimally align promoter elements to facilitate any of the various steps in transcription initiation on the relaxed template. The superpositioning of supercoiling in such a context would misorient the promoter elements and reduce transcription initiation. Since the centre of curvature is in a region downstream of the –10 region, it is plausible that bending modulates either promoter melting or clearance by the polymerase rather than initial recruitment of the polymerase.

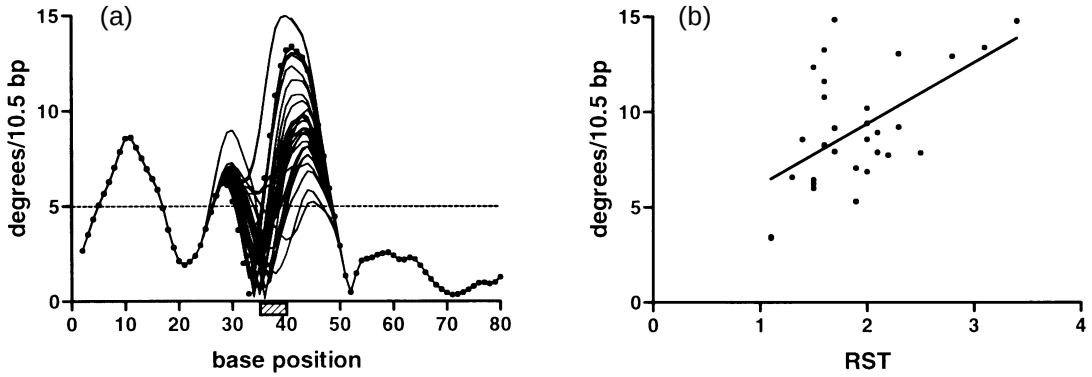

Figure 2. Curvature in various mutants of *gyrA* promoter from *E. coli*. (a) Composite curvature propensity plots of all mutants. The plot for the wild-type promoter is indicated by the dotted line. The hatched box represents the –10 hexamer. (b) Linear correlation of curvature at position 41 as a function of RST in various mutants. The Pearson's coefficient of correlation is 0.5572 (*P* value = 0.0014).

In an indirect role, intrinsic curvature of DNA could facilitate the binding of a global repressor. Bent DNA is known to attract many architectural proteins, such as H-NS, HU, IHF or FIS (Figueroa *et al*. 1991). Supercoil-sensitive binding of such proteins would allow them to act as classical repressors, however functioning in a supercoil-dependent manner. Specifically, if the repressor were to bind preferentially to supercoiled DNA, it would repress transcription only in a supercoiled context. Two evidences support an indirect role for curvature. Firstly, Carty and Menzel (1990) had implicated a titratable *trans* factor in the autoregulation of gyrase under *in vitro* conditions. Secondly, recent work has shown that binding of HU to DNA is supercoil dependent (Kobryn *et al*. 1999) in a manner required by our model. Sequence analysis of the *gyrA* promoter reveals absence of IHF or FIS recognition sequences overlapping the –10 region. However, since H-NS and HU show low sequence specificity in their interaction with DNA, it is difficult to rule out their role in RST.

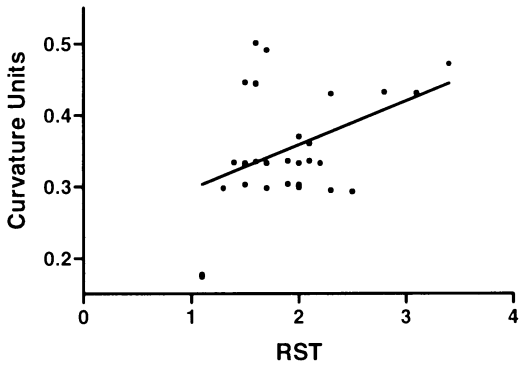

Figure 3. Correlation of curvature predicted by AAWedge algorithm as a function of RST. Curvature propensity was calculated with a sliding window of 21 nucleotides. The correlation is shown at base position 41. The Pearson's coefficient of correlation is 0.4095 (*P* value = 0.0246).

A global model for RST

The *gyr* genes are subject to autoregulation in all bacteria that have been tested so far. Therefore, is the molecular mechanism of RST also conserved throughout the prokaryotic kingdom? To test this possibility, first we analysed the *gyrB* promoter from *E. coli* for intrinsic curvature. Unlike *gyrA*, the *gyrB* promoter resembles a classical s^{70}-dependent promoter with a –10 and a –35 element. However, the presence of a strong curvature in the spacer region immediately upstream of the –10 region (table 1) implies that the underlying mechanism for RST is conserved at least in *E. coli*. Our model is further strengthened by the recent characterization of the *gyrA* gene in *Streptococcus pneumoniae* (Balas *et al*. 1998). The promoter appears to incorporate features from both *gyr* promoters in *E. coli* with a bend centre present upstream of an extended –10 element (Balas *et al*. 1998; table 1). In contrast, *Klebsiella pneumoniae gyrA* promoter (Dimri and Das 1990) is very similar to that of *E. coli*.

Table 1. Analysis of curvature in supercoil-sensitive promoters.

Promoter	Position of bend centre[1]	Magnitude (°/10.5 bp)	Accession number/Source
Gyrase promoters and promoters induced by relaxation			
E. coli gyrA	41*	13.4	Menzel and Gellert 1987a
Klebsiella pneumoniae gyrA	41*	13.4	KPGYRA; Dimri and Das 1990
Streptococcus pneumoniae gyrA	27*	11.8	AF053121; Balas *et al*. 1998
E. coli gyrB	37	10.7	Menzel and Gellert 1987a
Streptomyces sphaeroides gyrB^r	31	5.7	SSGYRBNR
Borrelia burgdorferi gyrB	–	–	BBU04527
Mycobacterium smegmatis gyr[2]	–	–	MSGYRAB; Unniraman and Nagaraja 1999
Streptomyces sphaeroides gyrB^{s2}	–	–	SSGYRBNS
E. coli recA	39	9.3	ECRECA; Menzel and Gellert 1983
Promoters induced by supercoiling			
E. coli topA P1	–	–	ECTOPA
E. coli topA P2	–	–	ECTOPA
E. coli topA P3	40	8.2	ECTOPA
E. coli topA P4	–	–	ECTOPA
Salmonella typhimurium hisR	36	6.2	STHISR
E. coli proUV	–*	–	ECPROV
E. coli galP1	–*	–	AE000178

[1]The 3′ end of the –10 regions aligned at base position 40.
[2]Minimal promoter does not show RST.
*Extended –10 promoters.
'–', Indicates no significant bond centre in the vicinity of the –10 region.

In *Mycobacterium smegmatis* the *gyr* genes are part of a single operon with transcription initiating from a solitary promoter present upstream of *gyrB* (Unniraman and Nagaraja 1999). Analysis of the putative promoter region reveals a distinct lack of any axial distortion upstream of the +1 start site (table 1). In accordance with our model, we find that regions up to 1 kb downstream of the promoter are necessary for RST in *M. smegmatis* and the promoter does not play a primary role in the phenomenon. To further substantiate this demarcation, we analysed all known gyrase promoters for presence of curvature in the vicinity of the –10 region (±5 nucleotides). It is noteworthy that roughly half of these show a significant curvature in this region while others do not (table 1). Interestingly, both position and extent of curvature are conserved between the *E. coli* and *K. pneumoniae gyrA* (Dimri and Das 1990) promoters.

Thus, it appears that there are at least two distinct mechanisms for RST in bacteria. The first class consists of genes where the promoter region itself carries all the cues for RST, and employs curvature as a sensor of changes in supercoiling. The second class uses a distally placed sensor that transduces the signal to the promoter, thereby modulating transcription. However, in the absence of a detailed characterization of RST in various species, we cannot derive a direct relationship between presence of bending and the role of the promoter in RST. Analysis of other bacterial species would reveal the extent to which one or the other mechanism is preferred throughout the kingdom. We also conducted a complementary study of promoters that are induced by supercoiling. The majority of these promoters lack any bend centre in this region (table 1), implying that presence of a curvature in the vicinity of the –10 element is characteristic of promoters induced by relaxation. Interestingly, the promoter of the *E. coli recA* gene, which is subject to RST (Menzel and Gellert 1983), reveals a strong bend centre overlapping the –10 region (table 1).

Conclusions

Gyrase genes are subject to autoregulation as a function of the global supercoil status of the genome. Transcription of the *gyr* genes is induced when the template DNA is relaxed. Since negative supercoiling assists in the melting of DNA, it is difficult to understand the repressive influence of supercoiling on transcription of the *gyr* genes.

We propose a novel role for axial distortion as a sensor of DNA topology. DNA curvature could operate in either of two ways. Directly, curved DNA overlapping the –10 region could enhance promoter melting or clearance by RNA polymerase on a relaxed template; or indirectly it could attract a repressor such as HU or H-NS in a supercoil-dependent manner. There are several lines of evidence in support of a model associating bending with supercoil sensitivity. First is the presence of a strong centre of curvature in the promoter region of several *gyr* promoters (table 1). Secondly, mutations in the –10 region of the *gyrA* promoter that reduce RST show a correlated decrease in curvature (figure 2b). Thirdly, a *trans* factor has earlier been implicated in RST in *E. coli* (Carty and Menzel 1990). Lastly, HU has recently been shown to bind to DNA in a supercoil-dependent manner (Kobryn *et al*. 1999).

The most attractive aspect of the model is that it is imminently testable. In the *E. coli* or *K. pneumoniae gyrA* promoters, mutations downstream of the –10 region specifically designed to reduce the intrinsic curvature are predicted to decrease RST without affecting strength of the promoter. In the case of *E. coli gyrB* and *S. pneumoniae gyrA* promoters, similar mutations would need to be made upstream of the –10 element, in the spacer region. In addition, it would be interesting to test if RST is retained in the absence of H-NS or HU function, paving the way to resolving the question of direct versus indirect role of axial distortion.

Acknowledgements

The work is supported by a grant from the Council of Scientific and Industrial Research, Government of India.

References

Balas D., Fernandez-Moreira E. and de la Campa A. G. 1998 Molecular characterization of the gene encoding the DNA gyrase A subunit of *Streptococcus pneumoniae*. *J. Bacteriol.* **180**, 2854–2861.

Bolshoy A., McNamara P., Harrington R. E. and Trifonov E. N. 1991 Curved DNA without A-A: experimental estimation of all 16 DNA wedge angles. *Proc. Natl. Acad. Sci. USA* **88**, 2312–2316.

Brukner I., Sanchez R., Suck D. and Pongor S. 1995 Sequence dependent bending propensity of DNA as revealed by DNAse I: Parameters for trinucleotides. *EMBO J.* **14**, 1812–1818.

Carty M. and Menzel R. 1990 Inhibition of DNA gyrase activity in an in vitro transcription-translation system stimulates *gyrA* expression in a DNA concentration dependent manner. Evidence for the involvement of factors which may be titrated. *J. Mol. Biol.* **214**, 397–406.

Dai X. and Rothman-Denes L. B. 1999 DNA structure and transcription. *Curr. Opin. Microbiol.* **2**, 126–130.

Dimri G. P. and Das H. K. 1990 Cloning and sequence analysis of *gyrA* gene of *Klebsiella pneumoniae. Nucl. Acids Res.* **18**, 151–156.

Figueroa N., Wills N. and Bossi L. 1991 Common sequence determinants of the response of a prokaryotic promoter to DNA bending and supercoiling. *EMBO J.* **10**, 941–949.

Gabrielian A. and Pongor S. 1996 Correlation of intrinsic DNA curvature with DNA property periodicity. *FEBS Lett.* **393**, 65–68.

Goodsell D. S. and Dickerson R. E. 1994 Bending and curvature calculations in B-DNA. *Nucl. Acids Res.* **22**, 5497–5503.

Kobryn K., Lavoie B. D. and Chaconas G. 1999 Supercoiling-dependent site-specific binding of HU to naked Mu DNA. *J. Mol. Biol.* **289**, 777–784.

Menzel R. and Gellert M. 1983 Regulation of the genes for *E. coli* DNA gyrase: homeostatic control of DNA supercoiling. *Cell* **34**, 105–113.

Menzel R. and Gellert M. 1987a Fusions of the *Escherichia coli gyrA* and *gyrB* control regions to the galactokinase gene are inducible by coumermycin treatment. *J. Bacteriol.* **169**, 1272–1278.

Menzel R. and Gellert M. 1987b Modulation of transcription by DNA supercoiling: a deletion analysis of the *Escherichia coli gyrA* and *gyrB* promoters. *Proc. Natl. Acad. Sci. USA* **84**, 4185–4189.

Owen-Hughes T. A., Pavitt G. D., Santos D. S., Sidebotham J. M., Hulton C. S., Hinton J. C. and Higgins C. F. 1992 The chromatin-associated protein H-NS interacts with curved DNA to influence DNA topology and gene expression. *Cell* **71**, 255–265.

Palecek E. 1991 Local supercoil-stabilized DNA structures. *Crit. Rev. Biochem. Mol. Biol.* **26**, 151–226.

Perez-Martin J. and de Lorenzo V. 1997 Cues and consequences of DNA bending in transcription. *Annu. Rev. Microbiol.* **51**, 593–628.

Shpigelman E. S., Trifonov E. N. and Bolshoy A. 1993 CURVATURE: software for the analysis of curved DNA. *Comput. Appl. Biosci.* **9**, 435–440.

Straney R., Krah R. and Menzel R. 1994 Mutations in the –10 TATAAT sequence of the *gyrA* promoter affect both promoter strength and sensitivity to DNA supercoiling. *J. Bacteriol.* **176**, 5999–6006.

Unniraman S. and Nagaraja V. 1999 Regulation of DNA gyrase operon in *Mycobacterium smegmatis*: a distinct mechanism of relaxation stimulated transcription. *Genes Cells* **4**, 697–706.

Wang J. C. and Lynch A. S. 1993 Transcription and DNA supercoiling. *Curr. Opin. Genet. Dev.* **3**, 764–768.

Zechiedrich E. L., Khodursky A. B. and Cozzarelli N. R. 1997 Topoisomerase IV, not gyrase, decatenates products of site-specific recombination in *Escherichia coli*. *Genes Dev.* **11**, 2580–2592.

Received 14 December 2001