

Regulation of DNA supercoiling in *Escherichia coli*: genetic basis of a compensatory mutation in DNA gyrase

Fiona McEachern and L. Mark Fisher

Department of Cellular and Molecular Sciences, Division of Biochemistry, St George's Hospital Medical School, University of London, England

Received 23 May 1989; revised version received 15 June 1989

Bacterial DNA supercoiling is controlled by balancing the supercoiling activity of DNA gyrase and the relaxing activity of DNA topoisomerase I. We have characterized the *gyrB* gene from a *topA* deletion mutant of *Escherichia coli* (DM800) that has a compensatory mutation in *gyrB*, lowering the activity of gyrase 10-fold, and thereby redressing the intracellular level of supercoiling. The mutant gene differs from the wild type in carrying three rather than two direct tandem repeats of a 6 bp sequence encoding Ala-Arg. We suggest this novel mutation affects domain spacing and was generated by an unequal crossing over event, possibly involving gyrase.

DNA supercoiling; DNA gyrase; DNA topoisomerase I; Compensatory mutation; (*Escherichia coli*)

1. INTRODUCTION

DNA supercoiling is important in many DNA transactions including DNA replication, transcription and recombination [1]. In *E. coli*, the level of supercoiling is controlled by regulating the opposing enzyme activities of DNA gyrase and DNA topoisomerase I, which introduce and remove DNA supercoils, respectively. Evidence for this idea has come from studies of *E. coli* mutants lacking *topA*, the gene for topoisomerase I [2,3]. These $\Delta topA$ strains are viable because they have acquired changes in other genes, termed compensatory mutations, that readjust the level of supercoiling. For strains DM750 and DM800, the compensatory mutation has been mapped to *gyrA* and *gyrB* genes, respectively, which encode the A and B subunits of the A₂B₂ gyrase complex [2,3].

The mutant A or B proteins reconstitute a defective gyrase activity: for example DM800 gyrase B protein is 10 times less active than the wild type subunit [4]. Given the interest in compensatory mutations as an adaptive response and their value in probing gyrase structure-function, we report here the first analysis of such a mutation, in the DM800 *gyrB* gene.

2. EXPERIMENTAL

2.1. Southern blot analysis and DNA cloning

Chromosomal DNA from *Escherichia coli* K-12 strains DM800 [$\Delta(topA\ cysB)204\ acrA13\ gyrB225$] and RW1053 [*recA* $\Delta(bio\ attB\ gal)$] was prepared by a standard method [5]. The wild-type *gyrB* gene in plasmid pFM1 was obtained by subcloning a 5.5 kb *EcoRI*-*PstI* fragment from pMK47 [6] into plasmid pUC9. To isolate the DM800 *gyrB* clone, genomic DNA from DM800 was cut with *EcoRI* and *PstI*, fractionated on a 1% agarose gel, and DNA in the 5–6 kb size range was purified and used to construct a pUC9 plasmid library in *E. coli* host HB101 *recA13*. The library was replicated onto Hybond-N (Amersham) and the membrane probed at high stringency with the *gyrB* insert from pFM1, radiolabeled with ³²P by random prim-

Correspondence address: L.M. Fisher, Dept of Cellular and Molecular Sciences, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, England

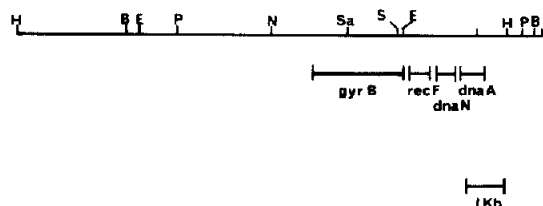


Fig.1. The *gyrB* locus in *Escherichia coli* K-12. B, E, H, N, P, S, and Sa denote sites for *Bam*HI, *Eco*RI, *Hind*III, *Nru*I, *Pst*I, *Sma*I and *Sal*I. An *Eco*RI site is at the 5'-end of the *gyrB* gene.

ing using the Multiprime kit (Amersham) and [α - 32 P]dCTP. Southern blotting and hybridization to genomic DNA was carried out similarly.

2.2. DNA sequence analysis

Chemical sequencing of DNA restriction fragments was done as described previously [7]. Fragments were labeled at 5'- or 3'-ends using polynucleotide kinase and [γ - 32 P]ATP or reverse transcriptase and [α - 32 P]dCTP, recut with a second restriction enzyme, and the singly end-labeled fragments separated and purified by electrophoresis in low gelling agarose. For dideoxy sequencing, selected restriction fragments were cloned into M13mp10 and M13mp11 and used to transform *E. coli* XL-1 Blue *recA*. Preparation of single-stranded DNA templates and DNA sequencing have been described [8].

3. RESULTS

3.1. Isolation of the DM800 *gyrB* gene from an enriched plasmid library

The *gyrB* locus in DM800 and in wild-type *E. coli* K-12 gave essentially identical genomic restriction maps by Southern blot analysis (fig.1) [4]. A

size selected plasmid library of DM800 DNA enriched for the *gyrB* gene was screened by colony hybridization to a *gyrB* probe. Four independent positive clones were obtained carrying plasmids with 5.5 kb *Eco*RI-*Pst*I *gyrB* inserts, and the nucleotide sequence of one was determined (fig.2).

3.2. Structure of the DM800 *gyrB* gene: the molecular basis of the compensatory mutation affecting gyrase B subunit activity

The nucleotide sequence of the DM800 *gyrB* gene was compared to its counterpart in wild type *E. coli* K-12 (fig.3) [9]. The sequences are identical except for the presence of a 6 bp insertion GCCCGT in the 2.6 kb DM800 *gyrB* gene immediately following a direct repeat of the same sequence (fig.3a, arrows). This insertion in DM800 *gyrB* and its absence in the wild type gene is clearly visible on inspection of M13 sequencing products for a 1.2 kb *Cla*I fragment (fig.3b,c; brackets). These results were confirmed by Maxam-Gilbert sequencing of the complementary strand in a 750 bp *Cla*I-*Sal*I fragment labeled at the 3'-*Cla*I end (fig.3a, data not shown). Interestingly, a second directly repeated sequence CCCGTG (lines in fig.3a) overlaps the GCCCGT motif and is also present as a triple repeat in DM800. Irrespective of which hexameric motif is considered to have been introduced into the *gyrB* gene, the mutation results in the insertion of the sequence Ala-Arg into the gyrase B protein, following a direct repeat of the same residues (fig.3a).

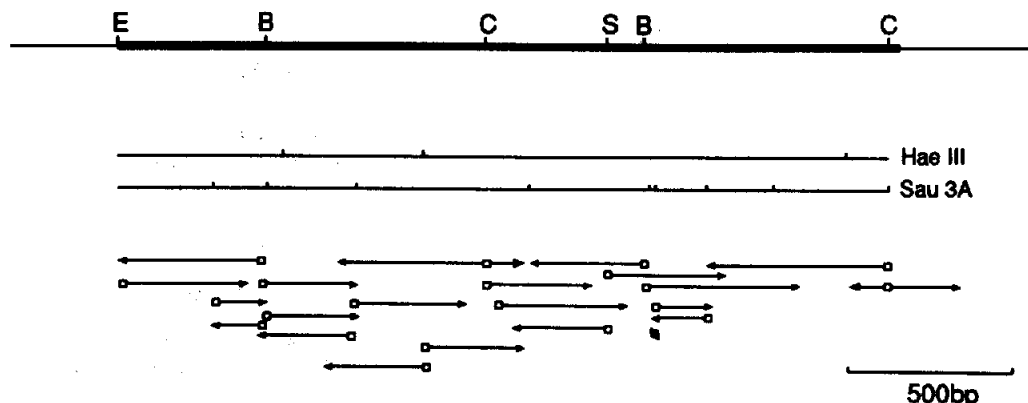


Fig.2. DNA sequencing strategy for the DM800 *gyrB* gene. B, C, E and S denote sites for *Bgl*II, *Cla*I, *Eco*RI and *Sal*I. Open and filled arrows indicate sequences determined by the dideoxy and chemical sequencing methods, respectively.

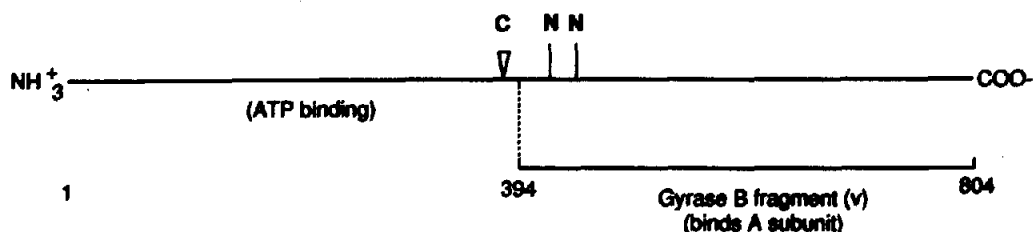
Gyrase B protein

Fig.4. Position of the compensatory Ala-Arg insertion (C) in the gyrase B protein. N denotes two nalidixic acid mutations in *gyrB* that map at codons 428 and 447.

recognize GAA(N₆)RTCG and GAA(N₇)RTCG: the *hdsS* genes (determining specificity for the two restriction systems) differ only in the presence of a 12 bp sequence directly repeated twice in the RI24 gene and three times in the RI24/3 gene [13]. It is suggested that the Hsd S polypeptide has two DNA-binding domains, each recognizing one half of the recognition sequence and separated by a spacer whose length is dependent on the direct repeats. For DM800 gyrase B protein, the cloned *gyrB* gene should allow the overexpression of mutant protein and examination of its enzymatic properties.

We suggest that the DM800 compensatory mutation arose by unequal crossing over. Given the similarity between the direct repeat and the gyrase cleavage consensus sequence [14], this event may have been mediated by gyrase. Further work is in progress to analyze the likely mechanism responsible for these mutational events.

Acknowledgements: We thank Martin Gellert for *E. coli* strains and Martin Cullen, Mike Clemens, Edward Margerrison and Stephen Halford for helpful discussion. This work was supported by an SERC Postgraduate Studentship, by the Gunnar Nilsson Cancer Research Trust Fund and the Douglas Hems Memorial Fund.

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