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

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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 *ΔtopA* 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 A_2B_2 gyrase complex [2,3].

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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 [*Δ(topA cysB)*204 *acrA13 gyrB225*] and RW1053 [*recA Δ(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 *Eco*RI-*Pst*I fragment from pMK47 [6] into plasmid pUC9. To isolate the DM800 *gyrB* clone, genomic DNA from DM800 was cut with *Eco*RI and *Pst*I, 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 ^{32}P by random prim-

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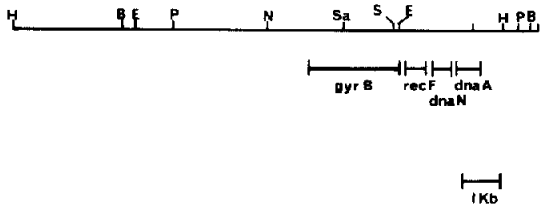

Fig.1. The *gyrB* locus in *Escherichia coli* K-12. B, E, H, N, P, S, and Sa denote sites for *Bam*HI, *Eco*RI, *Hin*dIII, *Nru*I, *Pst*I, *Sma*I and *Sal*I. An *Eco*RI site is at the 5′-end of the *gyrB* gene.

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).

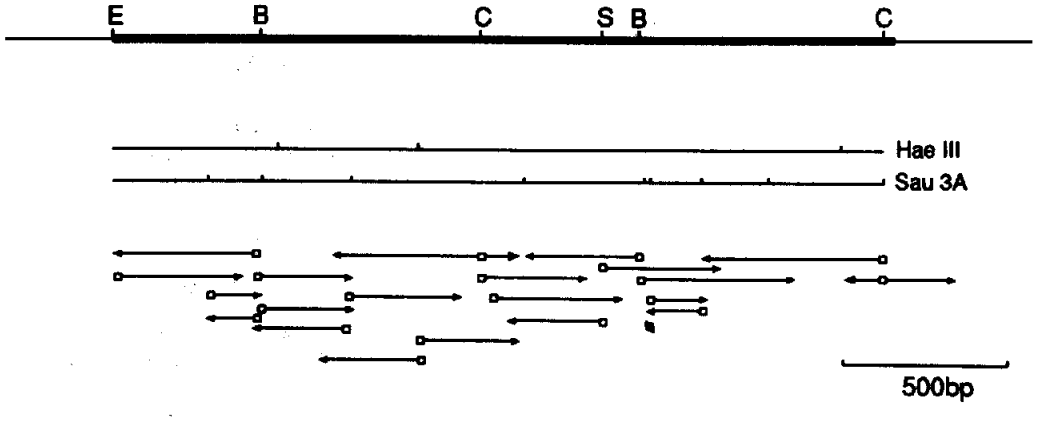

Fig.2. DNA sequencing strategy for the DM800 *gyrB* gene. B, C, E and S denote sites for *Bgl*II, *Cla*I, *Eco*RI and *Sal*. Open and filled arrows indicate sequences determined by the dideoxy and chemical sequencing methods, respectively.

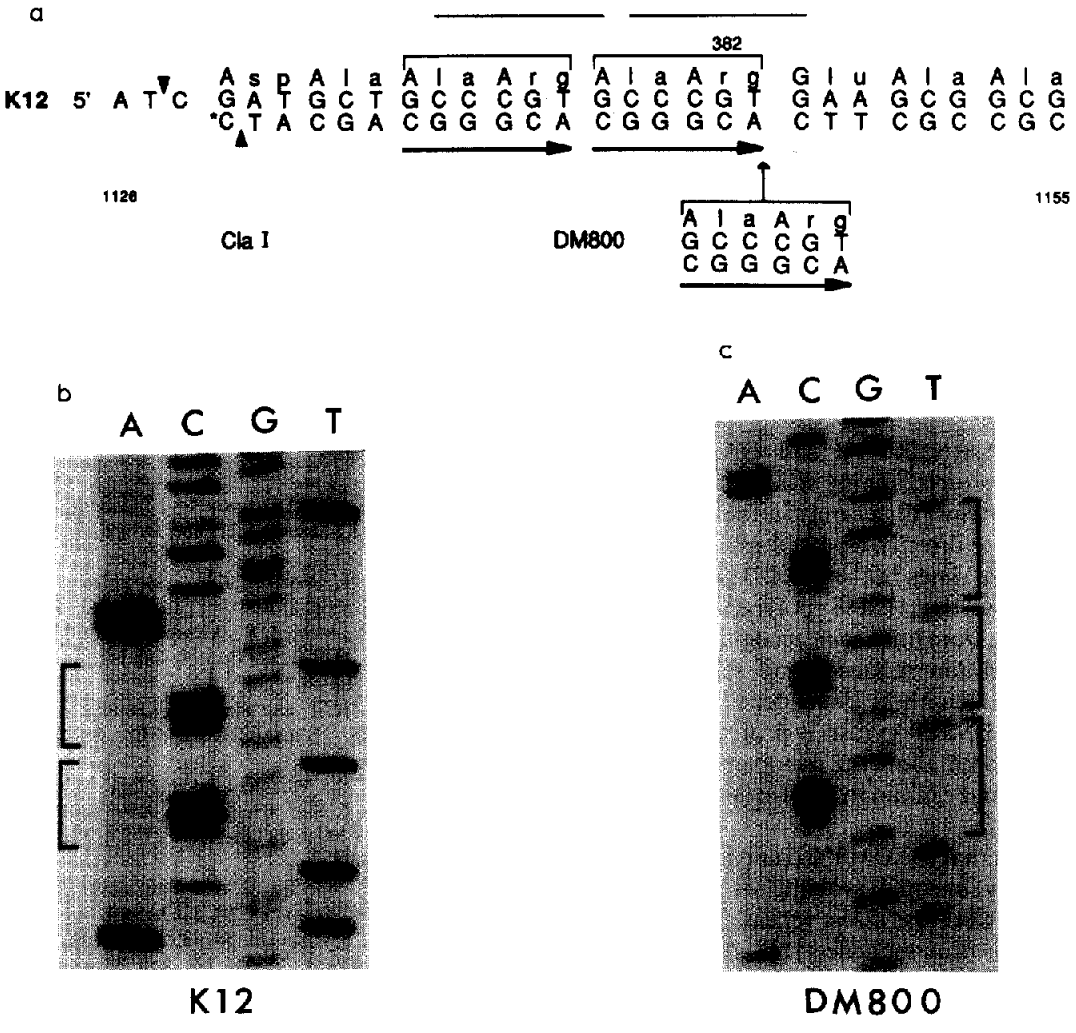

Fig.3. Structure of the DM800 *gyrB* gene. (a) Comparison of the *gyrB* genes of wild type K-12 and DM800 *E. coli* strains. Triangles indicate sites of cleavage by *Cla*I; asterisk denotes the 3'-labeled end used for Maxam-Gilbert sequencing (see text). (b and c) Dideoxy sequencing gels showing top strand DNA sequence (see a).

4. DISCUSSION

DNA gyrase catalyzes ATP-dependent DNA supercoiling by a double-strand break mechanism [10,11]. The two A subunits mediate transient DNA breakage-reunion whereas the B subunits bind ATP and engage in energy transduction [1]. The Ala-Arg insertion in the DM800 gyrase B protein occurs after residue 382 (figs 3,4) and markedly diminishes the activity of the protein. How might this loss of activity occur? A fragment of the gyrase B protein called ν (residues 394–804) has been isolated which can activate the gyrase A subunit functions, but seems to have lost the ATP-binding domain of the B protein (fig.4) [12]. The Ala-Arg repeats lie just outside the *gyrB* fragment region in a possible spacer region connecting the A subunit-binding- and ATP-binding domains of the B subunit. An extra Ala-Arg repeat would alter the spacing of the domains and thereby interfere with efficient subunit coupling. A similar domain spacing mechanism has recently been proposed as the basis for changes in DNA recognition by *Eco*RI24 and *Eco*RI24/3 type I restriction enzymes which

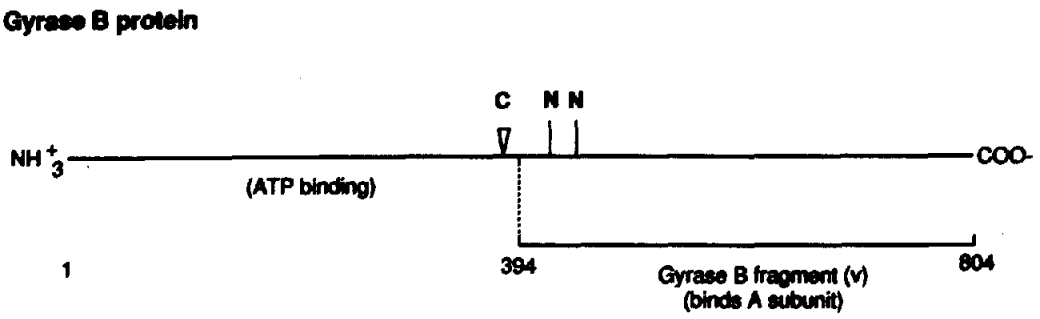

Fig.4. Position of the compensatory Ala-Arg insertion (C) in the gyrase B protein. N denotes two nalidixic acid mutations in *gyrB* that map at codons 428 and 447.

recognize GAA(N_6)RTCG and GAA(N_7)RTCG: the *hsdS* 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.

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