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nov: a new genetic locus that affects the response of *Escherichia coli* K-12 to novobiocin

Jasna Rakonjac,[1†] Mirjana Milic,[1] Dragana Ajdic-Predic,[1§] Diogenes Santos,[2] Radmila Ivanisevic[1] and Dragutin J. Savic[1]*

[1]*Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 282, PO Box 794, 11000 Belgrade, Yugoslavia.*

[2]*Imperial Cancer Research Fund, Institute of Molecular Medicine, University of Oxford, Oxford OX2 9DU, UK.*

Summary

We have identified a new gene locus (*nov*) affecting the resistance of *Escherichia coli* K-12 to novobiocin. The gene also affects, although to a lesser extent, tolerance to another gyrase inhibitor coumermycin. Transductional and complementation analysis show that *nov* is located between *attØ80* and the *osmZ* (*hns*) genes at minute 27 of the *E. coli* K-12 genetic map. In standard laboratory strains of *E. coli* K-12 *nov* exists at least in two allelic forms.

Introduction

The antibiotics novobiocin (Nov) and coumermycin (Cou) inhibit DNA replication by blocking the β subunit of DNA gyrase (Drlica and Franco, 1988). Most *Escherichia coli* mutants conferring resistance to Nov and Cou map within the *gyrB* gene (Gellert *et al.*, 1976). However, mutations in *dnaA* and *rpoB* can also change the bacterial response to these antibiotics. A decrease in hypersensitivity to Nov and Cou in *dnaA*(Ts) mutants by a class of *rpoB* mutants may be explained by a bypass of the requirement of supercoiling in the initiation of DNA replication, although other explanations unrelated to DNA supercoiling are feasible (Filutowicz and Jonczyck, 1981; 1983).

E. coli K-12 has high natural resistance to Nov due to the low permeability of the outer membrane lipopolysacharide (LPS) layer (Nikaido and Vaara, 1987). Mutations that impair synthesis or stability of LPS (e.g. *acrA* and *rfaD*) increase sensitivity to Nov about 100-fold (Coleman and Leive, 1979). Unexpectedly, *E. coli* is much less resistant to Cou, which is essentially a dimer of Nov (Gellert *et al.*, 1976). The molecular basis of this difference remains unknown.

In this work we report identification, characterization and mapping of a new genetic locus (*nov*) whose product(s) influences the tolerance of *E. coli* to gyrase inhibitors novobiocin and coumermycin. The gene is located between *attØ80* and the *osmZ* genes at minute 27 of the *E. coli* K-12 genetic map immediately clockwise from the *opp* operon.

Results

Novobiocin-resistant recombinants in the trp *region of* E. coli

An analysis of Trp^+ transductants in a P1-mediated cross between the widely used *E. coli* K-12 laboratory strains, C600 (donor) and SY209, *trp* derivative of AB1157, (recipient) (Table 1), yielded a proportion of transductants (36%) significantly more resistant to Nov than either of the parental strains (Table 2). In a control experiment, His^+ and Pro^+ transductants obtained from the same transductional mixtures showed the same level of resistance to Nov as the native resistance of the recipient strain (100 μg ml^{-1}). This indicated that a locus linked to *trp* has an effect on Nov resistance. Very similar results were obtained with other donor strains and one additional recipient (Table 2). Each of the parental strains, and approximately 60% of the transductants, failed to grow on plates supplemented with 300 μg ml^{-1} of Nov. C600 and SY209 did not grow on concentrations higher than 75 and 100 μg ml^{-1}, respectively (Table 2). In contrast, approximately 40% of the Trp^+ recombinants grew well on such plates. Furthermore, the Nov resistance (Nov^R) character could be crossed out with P1 phages grown on the original, Nov sensitive (Nov^S) recipient. In these backcross transductions (SY333 × SY353 and AB1157 × SY339) the percentage of Nov^S recombinants among Tet^R and Trp^+ transductants was similar to the percentage of Nov^R transductants in the original crosses (34% and 38% respectively).

Novobiocin resistance is not due to suppressor mutations

Several *sup* genes are linked to the *trp* operon (Bachmann, 1990). Although neither the donor or recipient

Received 20 July, 1991; revised 1 February, 1992; accepted 20 February, 1992. Present addresses: †The Rockefeller University, New York, New York 10021, USA; §Department of Microbiology and Immunology, University of Oklahoma, Oklahoma City, Oklahoma 73190, USA. *For correspondence. Tel. (11) 491 395; Fax (492) 492 397.

Table 1. Bacterial strains.

Strain	Genotype	Source/Construction
Escherichia coli		
C600	*thi-1, thr-1, leuB6, lacY1, tonA21, supE44, rfbD1,* λ*-nov*^{R}	Appleyard (1954)
W3110	*thyA36, deoC2,* IN*1,* λ*-nov*^{R}	Bachmann (1972)
CY10	W3110, *tna trp*::Tn*10*	C. Yanofsky
MC4100	*araD39,* Δ*(argF–lac) U169, rpsL150, relA1, deoC1, ptsF25, rbsR, flbB5301, nov*^{R}	Casadaban (1976)
GM128	MC4100, φ*(proU–lacZ), hyb2* (λ*plac*Mu*15), zch-97*::Tn*10 osmZ200*	Higgins *et al.* (1988)
KO635	Δ*lac3(X74)tna7*::Tn*10 nov*^{R}	DiNardo *et al.* (1982)
KL701	*pyrD34, trp-45, his-68,recA1, thi-1, galK35, malA1, xyl-7, mtl-2, rpsL118/*F'*recE, cysB, topA, trp, galU, nov*^{R}*, osmZ*(F'123)	Low (1972)
JC10240	HfrKL16 *thr-300, ilv-318, spoT1, relA1, rpsE2300, thi-1, recA56, srl-300*::Tn*10*	Csonka and Clark (1980)
Wild type	*nov*^{R}	Laboratory collection
X478	*purE42, proC32, leu-6, mtl-1, xyl-5, ara-14, lacZ36, azi-6, rpsS109, tonA23, tsx-67, supE44*	Berg *et al.* (1967)
LA101	C-600, *hsdS, supF58*	Laboratory collection (from F. Galibert)
CBK130	*proC*::Tn*5*	Shaw and Berg (1979)
SD108	*trpE63, pyrF287, acrA13*	DiNardo *et al.* (1982)
BRE2076	MC4100, φ*(proU::lacZ) hyb2,* (λ *plac*Mu*15) osmZ203*	Higgins *et al.* (1988)
AB1157	*thi-1, thr-1, leuB6,* Δ*(gpt-proA), 62, argE3, hisG4, lacY1, galK2, ara-14, xyl-5, mtl-1, tsx-33, rpsL31, supE44, rfbD1, mgl-51, kdgK51,* λ$^-$*, rac*$^-$ *nov*^{S}	DeWitt and Adelberg (1962)
SY209	AB1157, *trp nov*^{S}	Savic (1979)
SY333	AB1157, *trp*::Tn*10 nov*^{S}	P1/CY10 × AB1157 this paper
SY339	AB1157, *trp*::Tn*10 nov*^{R}	P1/CY10 × AB1157, this paper
SY353	SY209, *trp nov*^{R}	P1/C600 × SY209, this paper
SY382	SY209, *cysB*(Ts) *nov*^{S}	Rakonjac *et al.* (1991)
SY385	GM128, Δ*(zch-97*::Tn*10) nov*^{R}	this paper
SY387	SY209, *trp zch-97*::Tn*10 nov*^{S}	P1/GM128 × SY209, this paper
SY388	SY387, *trp*	P1/SY387 × SY209, this paper
SY389	SY385, *zch-97*::Tn*10 trp nov*^{S}	P1/SY388 × SY385, this paper
SY416	SY209, *proC*::Tn*5*	P1/CBK130 × SY209, this paper
SY417	SY416, *proC*$^+$*, acrA13*	P1/SD108 × SY416, this paper
SY418	SY353, *proC*::Tn*5*	P1/CBK130 × SY353, this paper
SY419	SY418, *proC*$^+$*, acrA13*	P1/SD108 × SY418, this paper
SY420	SY209, Δ*lac*	P1/MC4100 × SY209, this paper
SY421	SY353, Δ*lac*	P1/MC4100 × SY353, this paper
SY422	SY420, φ*(proU::lacZ) hyb2* (λ *plac*Mu*15*)	P1/BRE2076 × SY420, this paper
SY423	SY421, φ*(proU::lacZ) hyb2* (λ *plac*Mu*15*)	P1/BRE2076 × SY421, this paper
SY424	SY209/pACYC184	This paper
SY425	SY353/pACYC184	This paper
SY426	SY209, *recA56 srl300*::Tn*10*	P1/JC10240 × SY209
SY427	SY353, *recA56 srl300*::Tn*10*	P1/JC10240 × SY353
Plasmid		
pBR322	Hymeric cloning vehicle	Bolivar *et al.* (1977)
pMS421	Hymeric cloning vehicle	Grana *et al.* (1988)
pACYC184	Hymeric cloning vehicle	Chang and Cohen (1978)

strains used are known to contain *supF* and/or *supC* suppressor alleles (Table 1), the possibility that suppressor mutations in this region influence tolerance to Nov was investigated. λ phage mutant A_{227}, which is dependent for growth on active *supF* or *supC*, failed to grow on any of the tested strains, including the donors, recipients, and a number of Trp$^+$ Nov^S and Trp$^+$ Nov^R transductants. As expected, λ mutants depending on active *supE* (N7, N213, R216, and R221) grew successfully on AB1157, SY209 and its derivatives (results not shown). Thus, *supF* and *supC* are not involved in the Nov^R phenotype.

nov *is located between* trp *and* supF *on the* E. coli *chromosome*

The *nov* locus in the *trp* region of the *E. coli* chromosome was mapped more accurately by transductional crosses between strains LA101 (a *supF* derivative of C600), and a *cysB* derivative of SY209. When constructing this latter strain, several independently isolated *cysB* mutants showed unexpected high resistance to Nov relative to SY209. We bypassed this problem by using a *cysB*(Ts) mutation in SY209 (strain SY382; Rakonjac *et al.*, 1991), which at 30°C exhibits a CysB$^+$ phenotype and has equal resistance to Nov as the parental strain. Thus, selection for CysB$^+$ was performed at 42°C, the temperature at which SY382 exhibits a distinct CysB$^-$ phenotype. Results of this analysis verified previous mapping data in this region (Higgins *et al.*, 1988), and showed that *nov* is located between *trp* and *supF* (Fig. 1).

Table 2. Cotransduction of the Nov^R character with *trp*.

Donor		Recipient		% of Nov^R clones among *trp*$^+$ or Tet^R transductants	
C600	(75)	SY209	(100)	36	(300)
MC4100	(150)	SY209	(100)	38	(300)
W3110	(50)	SY209	(100)	25	(300)
KL701	(50)	SY209	(100)	50	(300)
KO635	(150)	SY209	(100)	36	(300)
wt	(50)	SY209	(100)	50	(300)
CY10	(150)	AB1157	(100)	40	(300)
CY10	(150)	X478	(150)	43	(350)

a. In each analysis, 50 transductants were analysed per cross. Numbers in the body of the table represent mean values of two independent analyses. Tet plates contained 20 μg of tetracycline per ml.
b. Numbers in parentheses give native strain and transductants acquired resistance to novobiocin (μg ml^{-1}).

Fig. 1. Genetic map of the *trp–supF* region including the *nov* locus derived from the cross LA101 × SY382. Cotransduction frequencies and the relative location of each gene are indicated. Position of *nov* was computed from the analysis of *nov* as an unselected marker among selected transductants, *cysB*$^+$ (24%), *trp*$^+$ (50%), and *trp*$^+$*supF* Thr$^+$ (93%; *thr-1* of SY209 is suppressible by *supF*). Presence of active *supF* among *trp*$^+$ Thr$^+$ transductants was verified by using them as indicators for *supF*-dependent λ phage mutant A_{227}. Approximately 60 transductants were analysed for each selected marker. *Percentage of *osmZ200* transductants among *zch97*::Tn*10* *nov*^{R} cotransductants selected on tetracycline. Position of *osmZ* is based on mapping results presented in Fig. 2A. **Percentage of *supF* (Thr$^+$) among *trp*$^+$ *nov*^{R} cotransductants (selection for *trp*$^+$). ***Percentage of *nov*^{R} among *trp*$^+$ *supF* (Thr$^+$) cotransductants (double selection for *trp*$^+$ Thr$^+$). Vertical line = selected marker; arrowhead = unselected marker. The map is not drawn to scale.

nov *is distinct from* osmZ (bglY, pilG)

The *osmZ* gene (Higgins *et al.*, 1988, 1990; Hulton *et al.*, 1990) is a pleiotropic locus between *trp* and *supF*. Additional mapping was undertaken to determine whether or not *nov* is an allele of the *osmZ* (also called *bglY*, *pilG*, *drdX*; Higgins *et al.*, 1990). *osmZ* codes for the neutral, abundant DNA-binding protein H-NS and *osmZ* mutations are highly pleiotropic and affect a range of chromosomal and plasmid functions (Higgins *et al.*, 1988; 1990; Hulton, *et al.*, 1990; May *et al.*, 1990; Goransson, *et al.* 1990). The data (Fig. 2) show that *nov* and *osmZ* occupy distinct positions on the genetic map. Strain GM128, a derivative of MC4100, harbours the *nov*^{R} form of the *nov* locus (Table 2), the *osmZ200* mutation which strongly induces the *bgl* operon (Higgins *et al.*, 1988), and the closely linked *zch*::Tn*10* (Fig. 2). Among Tet^R transductants, the overwhelming majority (98%) were Nov^R indicating the transfer of *nov*^{R}; among those, 79% also expressed the Bgl$^+$ phenotype, suggesting a more distal location of *osmZ* relative to *zch*::Tn*10* (Fig. 2A). Interestingly, all *nov*^{R} *osmZ200* transductants demonstrated increased resistance to Nov (400 μg ml^{-1}) relative to *nov*^{R} *osmZ*$^+$ transductants which exerted the expected increase of resistance to Nov (300 μg ml^{-1}).

The only *nov*^{S} *osmZ*$^+$ (Nov^S Bgl$^-$) transductant from the first cross (SY387) was used as a donor in the second transduction with SY385 (GM128 ΔTn*10*) as recipient (Fig. 2B). In this case, 98% of Tet^R transductants displayed a Nov^S phenotype, lowering the natural level of resistance to Nov of strain SY385 from 150 μg ml^{-1} to only 30 μg ml^{-1}. Among this recombinant class, 85% were *nov*^{S} *osmZ*$^+$ (Nov^S Bgl$^-$), while 13% were *nov*^{S} *osmZ200*. Only one was *nov*^{R} *osmZ200* (Nov^R Bgl$^+$). In contrast to the first cross, *osmZ* alleles in this case (*nov*^{S} *osmZ200* versus *nov*^{S} *osmZ*$^+$) did not contribute to the overall resistance of recombinants to Nov; in this background (GM128), resistance was affected solely by the *nov* alleles. These data, and the phenotypes of *nov* and *osmZ* alleles and the recombination frequencies, lead us to conclude that *nov* and *osmZ* are not alleles of the same locus.

To confirm this, the hybrid clone λ252 from the *E. coli* W3110 library (Kohara *et al.*, 1987), was used. The DNA insert of λ252 harbours the distal portion of the *trp* operon (*trpABC*), and extends 17.5 kb counter-clockwise encompassing *tonB* and *attØ80*, but not *osmZ* which is located on the neighbouring clone λ251 (Goransson *et al.*, 1990; May *et al.*, 1990; Fig. 3). Transductional crosses λ252 × SY209 and λ252 × SY388 produced approximately 40% and 2% *nov*^{R} recombinants respectively among selected Trp$^+$ transductants. As expected, no *osmZ*$^+$ recombinants were found in the control cross λ252 × SY389 (*osmZ200*). All Trp$^+$ transductants were tetracycline sensitive (Tet^S), indicating occurrence of double recombination events. Finally, a more accurate location of *nov* was determined by complementation analysis. A 12 kb *Eco*RI–*Pst*I fragment from λ252, subcloned into the low copy-number plasmid pMS421 or into pBR322, produced Nov^R transformants when introduced into Nov^S recipient

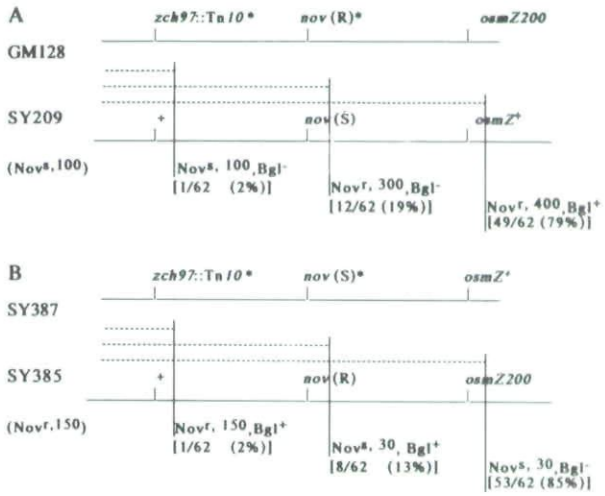

Fig. 2. Transductional mapping of *nov* and *osmZ*. In each cross, A and B, 62 Tet^R recombinants were tested for resistance to Nov (*nov*^{R} versus *nov*^{S}), and induction of the silent *bgl* operon (*osmZ*$^+$ versus *osmZ200*). For the sake of clarity, phenotypic designations for both characters (Nov^S/Nov^R; Bgl$^-$/Bgl$^+$) are given next to the strain number of recipients, and for each recombinant class. Designations for resistance to Nov (Nov^S = Nov-sensitive; Nov^R = Nov-resistant) describe resistance levels relative to recipients. In addition, numbers in superscripts (e.g. Nov^{R,100}), specify absolute resistance levels to Nov on plates. Bgl$^-$ = uninduced *bgl* operon; Bgl$^+$ = induced *bgl* operon. Given data do not permit a distinction between the presented order and the order *nov-zch97*::Tn*10-osmZ*. The asterisks placed at both loci (*zch97*::Tn*10** and *nov**) indicate that fact. The map is not drawn to scale.

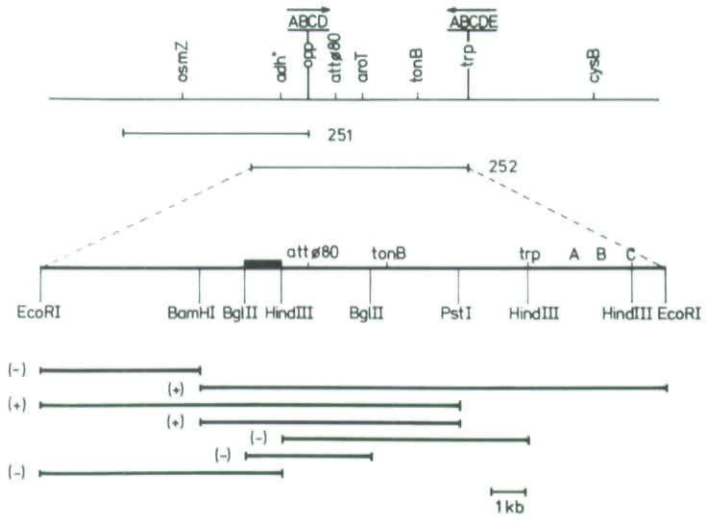

Fig. 3. Genetic location of the *nov* locus in the *trp–osmZ* region of the *E. coli* chromosome. Section of the linkage map (upper line) drawn after Bachmann (1990). The horizontal bars below show the extent of *Eco*RI chromosomal DNA fragments contained in λ vectors (clones 251-252) or restriction fragments subcloned into pMS421 and/or pBR322 plasmids. *Eco*RI–*Pst*I, *Bam*HI–*Eco*RI, and *Bam*HI–*Pst*I fragments have been cloned and tested in both vectors, while *Eco*RI–*Bam*HI, *Hin*dIII–*Hin*dIII, *Bgl*II–*Bgl*II, and *Eco*RI–*Hin*dIII fragments have been cloned only into pBR322 plasmid. Distribution of genes and restriction sites in the clone 252 compiled after Postle and Reznikoff (1978), Kohara *et al.* (1987), Goransson *et al.* (1990), and our restriction enzyme analysis. *Bgl*II restriction site within *nov* has been discovered in this analysis. The signs (+) and (–) denote positive or negative complementation for Nov^R character of the corresponding subclones. Thickened bar denotes minimal space occupied by *nov*.

SY209 (Fig. 3). Positive complementation was also obtained with *Bam*HI–*Eco*RI and *Bam*HI–*Pst*I fragments, but not with *Eco*RI–*Bam*HI, *Eco*RI–*Hin*dIII, *Hin*dIII–*Hin*dIII, and *Bgl*II–*Bgl*II fragments. In a control experiment, the same clones did not produce any change in phenotype of Nov^R recipient SY353. The same pattern of complementation was obtained when $RecA^-$ variants of SY209 and SY353 (SY426 and SY427) were used as recipients. On the basis of these results we conclude that *nov* is situated between the *Bam*HI site and *attØ80* (Fig. 3).

Finally, different phenotypes of the *osmZ* and *nov* were demonstrated in two additional experiments: (i) neither nov^S nor nov^R exerted any effect on the expression of *proU* (strains SY422 and SY423; results not shown), an operon known to be strongly affected by *osmZ* mutation (Higgins *et al.*, 1988; Hulton *et al.*, 1990); (ii) neither allele of *nov* affected motility (strains SY209 and SY353, results not shown) which is reduced by *osmZ* mutation (cited in Hulton *et al.*, 1990).

Effect of nov *on DNA supercoiling*

The effects of Nov are exerted through inhibition of DNA gyrase (Gellert *et al.*, 1976). Thus, we considered the possibility that *nov* may affect cellular tolerance to Nov by influencing the control of DNA supercoiling. We studied this possibility by measuring expression of the supercoiling-sensitive *lac* and *bgl* promoters (Borowiec and Gralla, 1985; Borowiec *et al.*, 1987; Higgins *et al.*, 1988; Sternglanz *et al.*, 1981). The different *nov* alleles did not influence *lac* and *bgl* expression when LacZ and BglB activities were tested on indicator plates. In addition, enzymatic activities of β-galactosidase and phospho-β-glucosidase B (Miller, 1972; Reynolds *et al.*, 1981) was measured in SY353 nov^R and SY209 nov^S. The results (Table 3) suggest that *nov* does affect the *lac* and *bgl* operons, although to a much lesser extent than *osmZ* affects *bgl*. (Higgins *et al.*, 1988). The effect disappears when catabolite repression is removed, in agreement with the previous finding that cessation of catabolite repression makes *lac* promoter less dependent on supercoiling changes (Sternglanz *et al.*, 1981). We also measured supercoiling of the plasmid pACYC184 isolated from SY209 and SY353 cultures. The results (Fig. 4) show that nov^R slightly increases negative supercoiling of

Table 3. Measurements of β-galactosidase and phospho-β-glucosidase B activities in nov^S and nov^R strains

Strain	Medium	No. of isolates	Gal units	Gal units nov^S/nov^R
SY209	LB	3	6.36	1.31
SY353		3	4.85	
SY209	LB+IPTG	3	1184	1.56
SY353		3	760	
SY209	Minimal (A)+ glucose+IPTG	3	1470	1.72
SY353		3	851	
SY209	Minimal (A)+ succinate+IPTG	3	4677	1.06
SY353		3	4413	
			PGB units	PGB units nov^S/nov^R
SY209	LB	3	0.049	0.53
SY353		3	0.093	

a. Assays were performed as described by Miller (1972); and Reynolds *et al.* (1981).
b. PGB = phospho-β-glucosidase B.
c. Minimal medium (A) was prepared as described by Miller (1972).

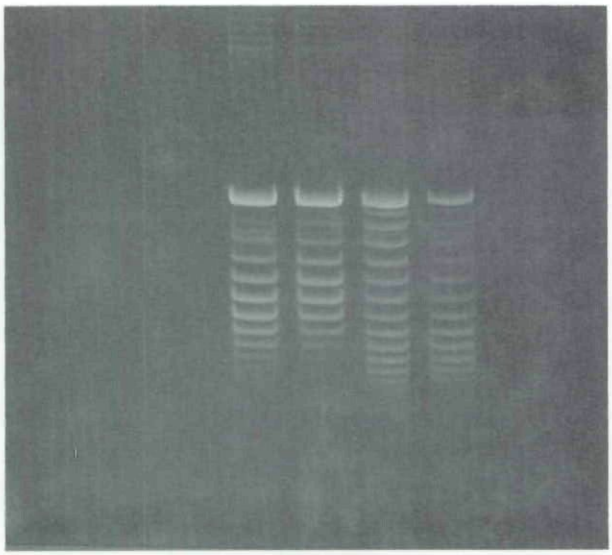

Fig. 4. Effect of *nov* alleles on *in vivo* plasmid supercoiling. Strains SY424 (SY209 pACYC184) and SY425 (SY353 pACYC184) were grown to stationary phase in LB and NB medium. Plasmid DNA was extracted and topoisomers were separated by electrophoresis as descibed in the *Experimental procedures*. (Numbering from the left) lane 1, SY424 LB; lane 2, SY425 LB; lane 3, SY424 NB; lane 4, SY425 NB. Plasmids extracted from cultures in the exponential phase of growth did not show any difference in supercoiling (results not presented).

pACYC184, but only in the stationary phase of growth. These effects point to a possible influence of the *nov* gene with DNA supercoiling, although the nature of the influence remains obscure.

Effect of nov *on transport of novobiocin through the outer membrane*

We considered the possibility that *nov* affects the permeability of the outer membrane to novobiocin by monitoring survival and efficiencies of plating the strains SY209 (Nov^S) and SY353 (Nov^R) in the presence of Nov, as well as Cou which is essentially indifferent to the LPS barrier. Increased resistance of SY353 to novobiocin was reproduced on LA plates, and to a lesser extent in LB medium (Fig. 5). Higher levels of resistance to Nov of Nov^R transductants obtained in the first analysis (Table 2) are the consequence of the self- protecting effect observed when cells are crowded (i.e. when they grow as streaks). The effect of the nov^R allele on *E. coli* tolerance to Cou was clearly present, but in comparison with the tolerance to Nov, was less pronounced on solid medium (Fig. 5). The possibility that *nov* alters outer membrane permeability was tested in an experiment with Nov^S and Nov^R strains harbouring an *acrA* mutation. Mutations in the *acrA* gene result in supersensitivity to Nov as a result of increased permeability of the outer membrane (Coleman and Leive, 1979). As shown (Fig. 6), the increase in sensitivity to Nov of *acrA* mutants was not accompanied by a change of a curve profile relative to those obtained with Acr^+ mutants (Fig. 5).

Discussion

The results presented in this paper identify a new gene in the *trp–osmZ* region of the *E. coli* chromosome that affects cellular tolerance to Nov and to a lesser extent Cou. The nature of the gene is, at present, obscure. The results show that among standard laboratory *E. coli* K-12 strains this gene exists in at least two allelic forms.

Genetic linkage and complementation analysis show that *nov* is located somewhere between *attØ80* locus and the *opp* operon (Fig. 3). It seems unlikely that Nov^R shares a common physiological step with the transport of oligopeptides. The distinction between *nov* and *opp* is confirmed as unique *Bam*HI site on λ252 is located 681 bp 5' to the termination codon of the last gene of the *opp* operon and 712 bp upstream of the typical transcription terminator (S. A. Short, personal communication). Thus, one may assume that *nov* is located between *opp* operon and the *attØ80* locus.

This study shows that resistance of strains to novobiocin does not exhibit any direct relationship to the allelic form of the *nov* locus (Table 2), and reflects the complex nature of bacterial resistance to Nov. It also points to the possibility that the effect of different alleles of that gene

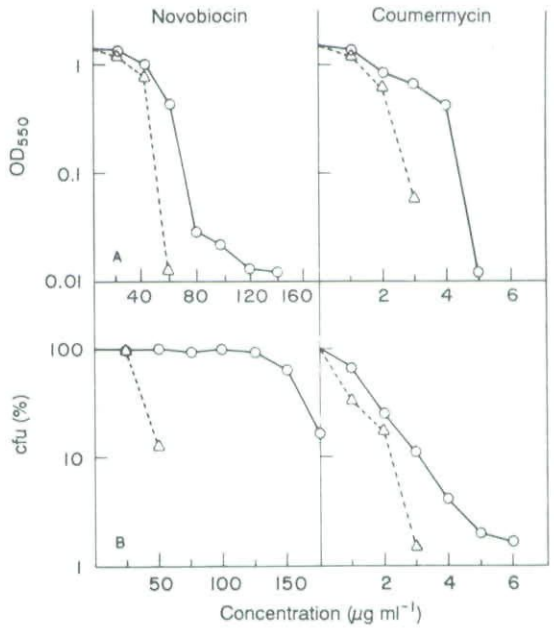

Fig. 5. Survival on novobiocin and coumermycin in LB medium (A), and efficiency of plating on LA plates (B) of SY209 (Nov^S) and SY353 (Nov^R) strains expressed as colony forming units.
SY209 = Δ; SY353 = O.

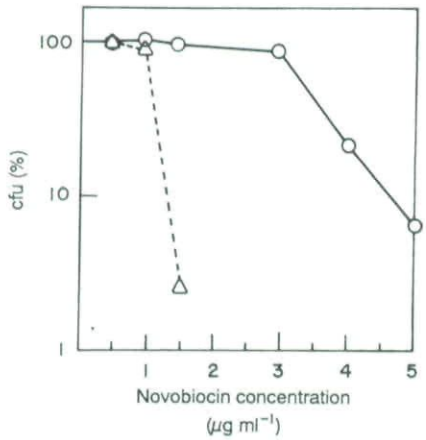

Fig. 6. Efficiency of plating of SY417(*nov*^{S} *acrA13*) and, SY419 (*nov*^{R} *acrA13*) on novobiocin. Δ, SY417; O, SY419.

may be hidden by counterbalancing mutation(s) at another locus (loci). Our conjecture is that this equilibrium is disrupted by the transductional replacement of the 'sensitive' allele with 'resistant' one, bringing to recombinants a substantial increase in resistance to Nov. Recent results which demonstrate that *cysB* and *cysE* genes affect *E. coli* resistance to Nov (Rakonjac *et al.*, 1991), also illustrate the complexity of this phenotypic trait.

The data available do not point to any substantial role of *nov* in the transport of Nov. On the other hand, the effect of *nov* on the supercoiling-sensitive promoters is slight (Table 2) and could be a consequence of *nov* primary effect at other site(s). For instance, shuffling of *nov* alleles might simulate fluctuations of environmental factors which may influence DNA supercoiling (Higgins *et al.*, 1988). The unequal pattern of differential survival of the tested strains in liquid and on solid medium could be an illustration of such an effect; further study of *nov* will help in understanding its role in cellular metabolism.

Experimental procedures

Bacterial strains and media

All bacterial strains listed in Table 1 are *E. coli* K-12. Novobiocin was purchased from Sigma, and coumermycin (Sigma), and azaserine (Sigma) were generous gifts from A. Pedrini and P. E. Hartman. Liquid medium was Luria broth (LB) solidified when necessary with 1.5% agar (LA). Minimal medium was the minimal A medium supplemented when necessary with amino acids as described by Miller (1972). The concentration of tetracycline in the plates was 20 μg ml^{-1}. Novobiocin and coumermycin were added to media or plates as described below. McConkey plates were used for measuring β-galactosidase ativity, and McConkey plates supplemented with salicin (0.5%) and bromthymolblue (0.1%) were used for detecting activity of the *bgl* operon (Defez and De Felice, 1981).

Genetic manipulations

All transductions with P1 *vir* were carried out as described elsewhere (by Miller (1972). Introduction of the active *supF* allele from LA101 into SY382 (*thr-1*) was done by scoring unlinked *supF*-suppressible Thr$^+$ clones among selected *trp*$^+$ transductants, or directly selecting *trp*$^+$Thr$^+$ transductants. Transductions with λ *E. coli* phage clones 251 and 252 were performed as described by Phadnis *et al.* (1991). Isolation of *cys*$^-$ mutants was performed by selection to azaserine resistance (Hulanicka *et al.* 1979). Selection of *cysB* mutants from among other cysteine-requiring mutants was achieved by complementation with F'123 (*cysB*$^+$). Curing of Tn*10* was conducted as described by Maloy and Nunn (1981).

Experiments with novobiocin and coumermycin

E. coli when plated in high numbers grows successfully even at 300 μg Nov ml^{-1}. Therefore, all transductional manipulations and mapping of the *nov* alleles were performed non-selectively using nearby markers (*trp*,*trp*::Tn*10*,*cysB*) for selection. All screenings for *nov*^{S} and *nov*^{R} alleles were performed by streaking undiluted overnight cultures of purified selected clones onto LA plates containing various amounts of Nov. Plates were inspected after 24 h and 48 h of incubation at 37°C. Efficiencies of plating were expressed as colony forming units (cfu). Overnight cultures grown in LB were diluted and plated on LA plates supplemented with increasing amounts of antibiotics. Presented percentages were computed from efficiencies of plating on LA plates without antibiotics. Survival curves in LB supplemented with different amounts of antibiotics were obtained in the following way: overnight cultures in LB were diluted to 400 cells ml^{-1} in LB supplemented with different amounts of the antibiotic. After 18 h of incubation with aeration, differential survival was monitored by measuring optical density (OD) at 550 nm.

Biochemical analyses

For the DNA supercoiling assay, plasmids were isolated on Qiagen columns. Electrophoresis was run for 16 h at 3 V cm^{-1} in Tris-phosphate buffer (TPE) buffer containing 20.5 μg ml^{-1} of chloroquine. After electrophoresis, the gel was stained with ethidium bromide (1.5 μg ml^{-1}), rinsed and photographed on Polaroid type 665 film. β galactosidase and β-D-glucosidase B assays were done as described by Miller (1972) and Reynolds *et al.*, (1981).

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