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Heterospecific transcription of the *Escherichia coli rpo*B-3 allele in Gram-negative bacteria

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Summary

*rpo*B is the structural gene for the β-subunit of *E. coli* RNA polymerase. The *rpo*B-3 allele confers resistance to the antibiotic rifampicin and is unusual in being dominant to the wild-type allele. We used the plasmid pZD23, a derivative of the broad host range conjugative plasmid RP4, to introduce the *rpo*B-3 allele into a range of bacterial species. Species belonging to the same family as *E. coli* (*Enterobacter aerogenes*, *Citrobacter freundii*, *Hafnia alvei møller*, *Klebsiella pneumoniae*, *Salmonella typhimurium*) expressed *rpo*B-3 to give a rifampicin resistant phenotype; this demonstrated heterospecific transcription. The transfer of pZD23 to the non-Enterobacteriaceae species *Azotobacter vinelandii* and *Rhizobium leguminosarum* did not result in rifampicin resistance. In the former case this was due to non-expression of the *rpo*B-3 resistance phenotype, in the latter case the dominant resistance phenotype had been lost from pZD23. Heterospecific transcription can be used as a criterion for the investigation of genetic relatedness between bacterial species.

1. Introduction

The RNA polymerase enzymes (nucleoside triphosphate RNA nucleotidyl transferase, EC 2.7.7.6) of most bacteria have a similar multimeric structure (Burgess, 1976) consisting of four subunits (α, β, β' and σ); the sizes of the subunits (except σ) are conserved among eubacterial species. It is sometimes possible to obtain functional RNA polymerase enzymes by combining subunits from different bacterial species *in vitro* (Lill *et al.* 1975). These sorts of studies are interesting both for helping to understand the functions of the different subunits and for indicating how conserved the enzyme is among bacteria. However, such an approach is not possible when screening strains for relatedness, because reconstitution experiments are time consuming and require considerable biochemical expertise (Zillig *et al.* 1976).

In this paper we describe a genetic method of testing for the formation of functional RNA polymerase enzymes on combining subunits from different species. Mutations in the gene coding for the β-subunit (*rpo*B) in *E. coli* can give resistance to the antibiotic rifampicin; most such alleles are recessive to the sensitive wild type allele, but the allele *rpo*B-3 is dominant (Kirschbaum & Konrad, 1973). We have constructed a derivative of the broad host range plasmid RP4 which carries *rpo*B-3 (Al-Doori *et al.* 1982). pZD23 (Fig. 1) carries, in addition, the gene for the β'-subunit (*rpo*C), but not the genes for the other two subunits. As pZD23 is an RP4 derivative, it is possible to use it to introduce *rpo*B-3 into practically any Gram-negative species of bacteria. In this paper we show that heterospecific transcription gives rise to rifampicin resistance in some, but not all, species when *rpo*B-3 is introduced.

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2. Materials and Methods

(i) *Bacterial strains and plasmids*

The plasmid-containing *E. coli* K12 strains ZD7 (*met*B *thi rec*A56 (RP4 λ *att*)) and ZD2723 (F$^-$ *his trp*A9761 *arg*E171 *rec*A56 (pZD23)) and the strain ZD162 (*met*B *thi rec*A56 *rps*L) were constructed by the author (see Al-Doori *et al.* 1982). The *E. coli* K12 strain AW1 (*met*B *thi rec*A56) and the *arg*$^-$ *Salmonella typhimurium* strain used came from the collection of J. Scaife. The *Enterobacter aerogenes*, *Citrobacter freundii* and *Hafnia alvei møller* strains came from the collection of J. Fleming. *Rhizobium leguminosarum* (*phe*-1 *trp*-12 *str*-37) was provided by J. Beringer and *Azotobacter vinelandii* AVM100 was provided by R. Olsen.

In *E. coli*, the plasmids pZD23 and RP4λ*att* confer

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resistance to tetracycline (10 μg/ml) and kanamycin (25 μg/ml). pZD23 also confers resistance to rifampicin (100 μg/ml).

(ii) *Media*

The following media were used for growth and conjugation: glucose minimal medium (Clowes & Hayes, 1968); SY minimal medium (Sherwood, 1970; Beringer, 1974); TY broth (Beringer, 1974); L-broth (Lennox, 1955).

(iii) *Microbiological tests*

The different Enterobacteriaceae strains were distinguished from each other using the following criteria: citrate utilization, methyl red test, Voges Proskauer test, H_2S production, gelatin hydrolysis and sorbitol utilization. These tests were performed as described in Cruickshank *et al.* (1975).

(iv) *Conjugation experiments*

Liquid culture matings (Miller, 1972) were normally used to transfer RP4*λatt* and pZD23. Five-hour matings were used for transfer from *E. coli* to enterobacterial recipients and 2 h matings were used when *E. coli* (strain AW1) was the recipient. Eight-hour matings were needed when *A. vinelandii* was the recipient. It was necessary to use filter matings (Towner & Vivian, 1976) to transfer pZD23 into *S. typhimurium* and *R. leguminosarum*.

Transconjugants (except for *Rhizobium*) were selected on glucose minimal medium supplemented with kanamycin (25 μg/ml) (and arginine (20 μg/ml) for *S. typhimurium*). SY minimal medium supplemented with phenylalanine (50 μg/ml) was used for *R. leguminosarum*. The species of purified transconjugants was checked by microbiological tests. Fifty transconjugants from each mating were patched onto pairs of plates, one containing rifampicin (100 μg/ml) to test for rifampicin resistance. Ten transconjugants were also mated with *E. coli* strain AW1 and the resulting transconjugants were checked for tetracycline and rifampicin resistance.

3. Results

It was essential to distinguish between the different Enterobacteriaceae species employed in this work. The criteria used are summarized in Table 1. The strains were tested before conjugation and the identity of the transconjugants was also confirmed. All species were sensitive to kanamycin (25 μg/ml) and rifampicin (100 μg/ml).

We transferred pZD23 from *E. coli* into all the species shown in Table 2 by conjugation. The transconjugants were selected on the basis of their resistance to kanamycin (25 μg/ml) and the *E. coli* donor strain was counter-selected by plating on minimal medium (see Materials and methods). It was found that pZD23 could be successfully transferred to most species by liquid matings (Table 2). However, in the cases of *S. typhimurium* and *R. leguminosarum* it was necessary to use filter matings. These two species also showed very low transfer frequencies for the RP4 *λ att* parent of pZD23 (Table 2).

For the Enterobacteriaciae species tested (Table 2, lines 1–6) all of the kanamycin resistant transconjugants (50 tested for each species) proved to be resistant to rifampicin. Plasmids from such transconjugants (10 tested in each case) were transferred back to *E. coli* and proved to have retained all the antibiotic resistance markers (tetracycline, kanamycin and rifampicin).

Neither *R. leguminosarum* nor *A. vinelandii* (Table 2, lines 7, 8), became rifampicin resistant on receiving pZD23. However, when pZD23 was transferred back to *E. coli* from *Rhizobium* (10 independent pZD23-containing clones used as donors), all transconjugants were rifampicin sensitive, but kanamycin resistant and tetracycline resistant. In contrast, when pZD23 was transferred from *A. vinelandii* to *E. coli* the transconjugants were resistant to all three antibiotics confirming retention of a functional *rpo*B-3 allele.

4. Discussion

We successfully transferred the *E. coli rpo*B-3 allele into all of the species used except for *Rhizobium leguminosarum*. The most striking result was that all five of the enterobacterial species that we tested (Table 2, lines 2–6) became resistant to rifampicin.

Table 1. *Differentation between various members of the Enterobacteriaceae*

Bacterial species	Citrate utilization	Methyl red	Voges proskauer	H_2S production	Gelatin hydrolysis	Sorbitol (acid & gas)
E. aerogenes	+	−	+	−	−	+
E. coli	−	+	+	−	−	+
C. freundii	+	+	−	+	−	+
H. alvei møller	−	−	−	−	−	−
K. pneumoniae	+	−	−	−	−	+

+, Positive result such as utilization of citrate as sole carbon source or the production of acid and gas from sorbitol fermentation.
−, Negative result such as the inability of the bacterial species to hydrolyse gelatin or produce H_2S.

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Table 2. *Heterospecific Expression of* E. coli rpo*B-3 gene in some Gram-negative Bacteria*

Bacterial Strain	RP4 λatt	pZD23	Rif-resistant hybrids (%)
E. coli	$2 \cdot 6 \times 10^{-3}$	$5 \cdot 2 \times 10^{-3}$	100
E. aerogenes	$4 \cdot 5 \times 10^{-4}$	$4 \cdot 5 \times 10^{-4}$	100
C. freundii	$1 \cdot 4 \times 10^{-4}$	$1 \cdot 1 \times 10^{-2}$	100
H. alvei møller	$4 \cdot 7 \times 10^{-6}$	$1 \cdot 1 \times 10^{-5}$	100
K. pneumoniae	$6 \cdot 3 \times 10^{-6}$	$1 \cdot 1 \times 10^{-4}$	100
S. typhimurium	$5 \cdot 3 \times 10^{-7}$	$7 \cdot 6 \times 10^{-3}$ [a]	100
R. leguminosarum	1×10^{-6}	$1 \cdot 4 \times 10^{-5}$ [a]	0
A. vinelandii	$2 \cdot 8 \times 10^{-5}$	$3 \cdot 6 \times 10^{-5}$	0

[a] Millipore mating technique was employed in these matings.

This table illustrates the frequencies of transfer (per donor) of the plasmids RP4 λatt and pZD23. It differentiates between the alien species which have recognized *E. coli rpo*B-3 and thus become rifampicin resistant (100 μg/ml) and other bacterial genera in which no expression of the same gene is detected.

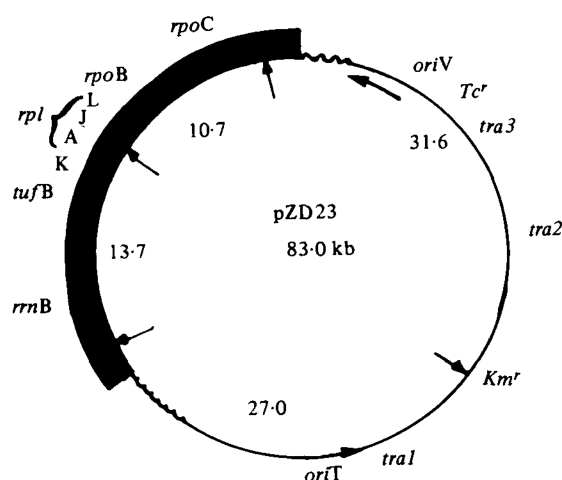

Fig. 1. Genetical and physical map of plasmid pZD23 (Al-Doori *et al.* 1982). ↑, *Hin* dIII restriction sites. ▬, *E. coli* chromosomal region containing *rpo*B. *rpo*C is the structural gene for the β' subunit; *rpl* genes are ribosomal protein genes; *tuf*B encodes protein chain elongation factor Tu; *rrn*B is a ribosomal RNA operon. These genes lie between about 89′ and 89·5′ on the genetic map of *E. coli* (Bachmann & Low, 1980). ∿∿, Sequences derived from phage λ.

This shows that the α and σ RNA polymerase subunits of these species must interact successfully with the *E. coli* β-subunit coded for by *rpo*B-3. We do not know the source of the β'-subunit in the hybrid polymerases as the *E. coli rpo*C gene is also carried by pZD23 (Fig. 1).

In the case of *R. leguminosarum*, there is no evidence that we have examined the effect of an intact *rpo*B-3 gene in this species, as all ten transconjugants tested failed to transfer a rifampicin resistance phenotype back to *E. coli*. Presumably, the mutations had occurred after transfer to *Rhizobium* as the same *E. coli* donor culture was used successfully to transfer *rpo*B-3 to other species. However, it is not known whether the inactivation of the *rpo*B-3 allele represents selection against this gene (e.g. if the gene product interacted with other *Rhizobium* RNA polymerase subunits to form an inactive product, then it might have a very deleterious effect) or represents genetic instability in *Rhizobium* such as that seen with RP4 in *R. lupini* (Puhler & Burkhardt, 1978). It is known that *E. coli trp* genes can be expressed in *Rhizobium* (Nagahari *et al.* 1979).

In the case of *Azotobacter vinelandii*, a functional *rpo*B-3 allele had been transferred, as shown by successful transfer back to *E. coli*. The *A. vinelandii* RNA polymerase enzyme has identical molecular-weight subunits to the *E. coli* enzyme (Burgess, 1976) and recognizes the same promoter and terminator sites in phage T7 (Wiggs *et al.* 1979). Despite this, the rifampicin resistance phenotype of *rpo*B-3 is not expressed. It was noticed that *A. vinelandii* (pZD23) derivatives grew poorly at 37 °C producing very small colonies, whereas they grew normally at 30 °C. It is possible that this temperature-sensitivity is caused by interactions with *E. coli* RNA polymerase subunits (see Scaife, 1976), but other explanations cannot be ruled out.

Using pZD23, it is easy to transfer *rpo*B-3 into almost any Gram-negative bacterial species. The ability to engage in heterospecific transcription shows a very close relationship of the transcriptional apparatus of the species concerned to that of *E. coli*. The data presented here are compatible with classical bacterial taxonomy (Krieg & Holt, 1984) in that the *Enterobacteriaceae* form a family in the group of 'Gram-negative facultative anaerobes', whereas *Rhizobium* and *Azotobacter* are two families in the group of 'Gram-negative aerobic rods and cocci'. Comparison of rRNA sequences (Stackebrandt & Woese, 1981) suggests that *Azotobacter* is much more closely related to *E. coli* than *Rhizobium* is. Thus, pZD23 is likely to prove most useful in investigating bacteria closely related to the enterobacteria.

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