

Restriction alleviation and modification enhancement by the Rac prophage of *Escherichia coli* K-12

Gareth King and Noreen E. Murray*

Institute of Cell and Molecular Biology, University of Edinburgh, Darwin Building, Kings Buildings, Edinburgh EH9 3JR, UK.

Summary

Bacteriophage λ encodes an antirestriction function, Ral, which is able to modulate the activity of the *Escherichia coli* K-12 restriction and modification system, EcoKI. Here we report the characterization of an analogous function, Lar, expressed by *E. coli sbcA* mutants and the hybrid phage λ .reverse. *E. coli sbcA* mutants and λ .reverse both express genes of the Rac prophage, and we have located the *lar* gene immediately downstream of *recT* in this element. The *lar* gene has been cloned in an expression plasmid, and a combination of site-directed mutagenesis and labelling of plasmid-encoded proteins has enabled us to identify a number of translational products of *lar*, the smallest of which is sufficient for restriction alleviation. Lar, like Ral, is able both to alleviate restriction and to enhance modification by EcoKI. Lar, therefore, is functionally similar to Ral and the nucleotide sequences of their genes share 47% identity, indicating a common origin. A comparison of the predicted amino acid sequences of Lar and Ral shows only a 25% identity, but a few short regions do align and may indicate residues important for structure and/or function.

Introduction

Bacteriophages use numerous mechanisms to escape host-encoded restriction and modification (R-M) systems (see Bickle and Krüger, 1993, and references therein). The activity of the *Escherichia coli* K-12 R-M enzyme, EcoKI, is modulated by a number of anti-restriction systems, including the Ocr activity of phages T3 and T7 (Studier, 1975), and the Ral activity of phage λ (Zabeau *et al.*, 1980). Ocr has been shown to competitively bind EcoKI, and block both restriction and modification of the phage (Bandyopadhyay *et al.*, 1985). However, Ral is

different; it alters the activity of the restriction complex so that unmodified DNA that escapes restriction is efficiently methylated (G. King and N. Murray, unpublished). EcoKI is a member of the Type IA R-M enzymes, which are the only prokaryotic DNA methyltransferases (MTases) shown to have a strong preference for hemimethylated DNA *in vivo*, and are also the only systems in which restriction is known to be alleviated by Ral (Loenen and Murray, 1986). One *ral* homologue, that of phage P22, has been sequenced; the predicted amino acid (aa) sequences of the two gene products are almost identical (Semerjian *et al.*, 1989). *E. coli sbcA* hosts (Templin *et al.*, 1972) and a derivative of phage λ , designated λ .reverse (λ .rev; Zissler *et al.*, 1971), express a Ral-like activity (Simmon and Lederberg, 1972; Toothman, 1981). In *sbcA* mutants, the *recE* and *recT* genes, the general recombination functions of a defective lambdoid prophage, Rac, have been activated (Kushner *et al.*, 1974; Clark *et al.*, 1993; Kolodner *et al.*, 1994). λ .rev also expresses the *recE* and *recT* genes (Gottesman *et al.*, 1974) acquired by a recombination event between λ and the excised Rac prophage (Kaiser and Murray, 1979). The gene encoding the Ral-like activity of the Rac prophage has been termed *lar* (Toothman, 1981).

The Type IA R-M systems are encoded by three genes: *hsdR*, *hsdM*, and *hsdS*. The HsdM and HsdS subunits together form a MTase that methylates two adenine residues in complementary strands of a bipartite DNA recognition sequence. In the presence of the HsdR subunit the complex can also act as an endonuclease, binding to the same target sequence but cutting the DNA some distance from this site. Whether the DNA is cut or modified depends on the methylation state of the target sequence. When the target site is unmodified, the DNA is cut. When the target site is hemimethylated, that is when only one adenine residue in the target is methylated, the complex acts as a maintenance MTase modifying the DNA so that both strands become methylated. This maintenance MTase activity ensures that the pattern of DNA methylation is preserved after replication. The enzyme recognizes a completely modified target sequence, but dissociates from the DNA (Suri *et al.*, 1984; Dryden *et al.*, 1993; Powell *et al.*, 1993).

Experiments dependent on expression of *ral* from a multicopy plasmid suggest that Ral alleviates restriction by changing the activity of EcoKI from a maintenance MTase to a *de novo* MTase, which efficiently methylates

unmodified DNA (Loenen and Murray, 1986). The efficient methylation of unmodified DNA is also observed with Ral and the *EcoKI* MTase alone, and mutations have been isolated in *hsdM* which mimic the activity of Ral (Kelleher *et*

al., 1991). We are interested in the molecular mechanisms of these activities, and in defining important domains or residues within Ral and *EcoKI*.

Here we report the identification of *lar*, the gene

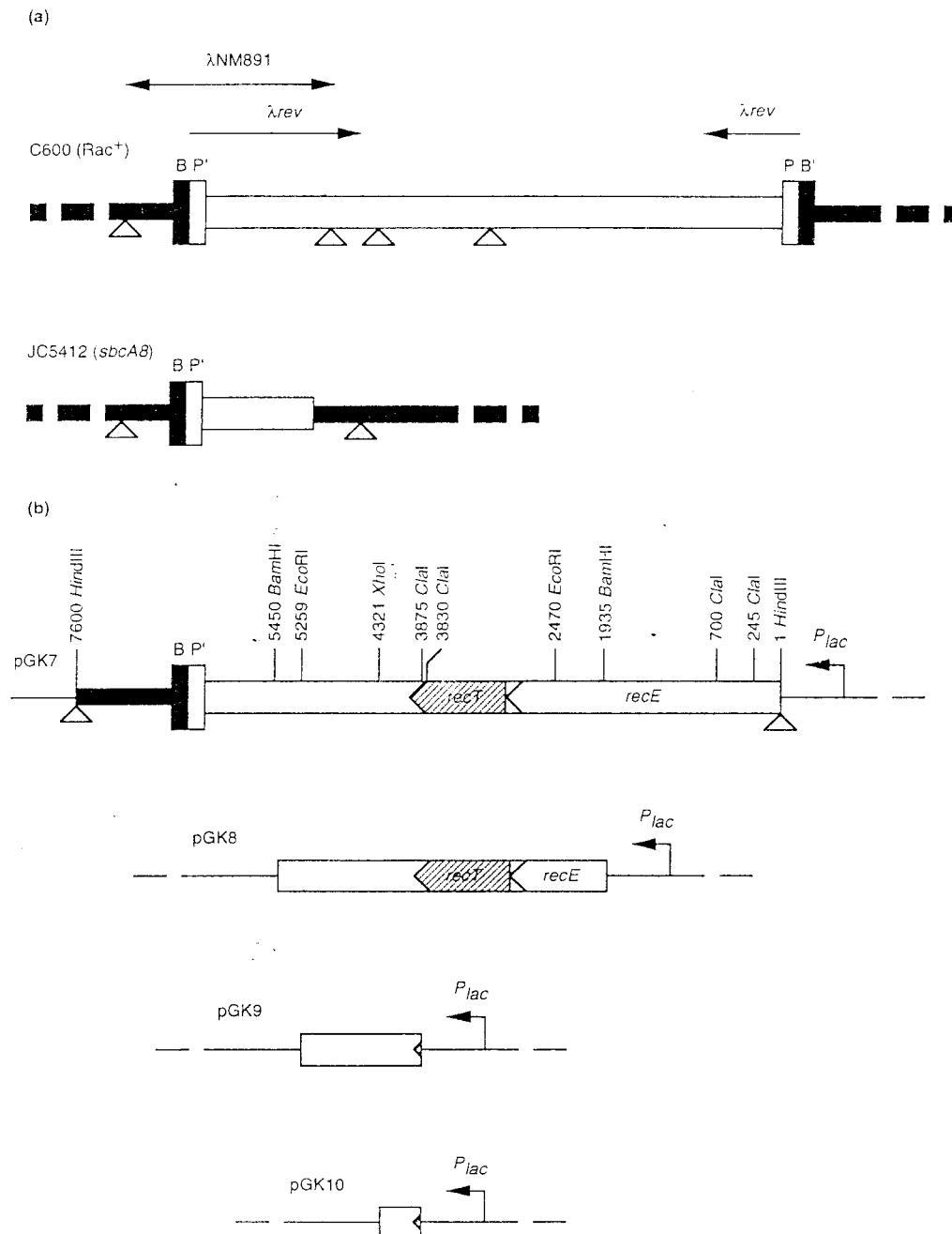


Fig. 1. Localization of the region encoding *lar*.

a. Origin of λ .rev, λ .NM891 and the Rac prophage integrated in the chromosome of the *E. coli* strain C600 and an *sbcA* mutant (*sbcA8*; JC5412). The DNA common to λ .rev and the *sbcA8* mutant is contained in λ .NM891.

b. The 7.6 kb *Hind*III fragment from λ .NM891 was cloned in pUC18 (Yanisch-Perron *et al.*, 1985) to generate pGK7. Segments of this 7.6 kb *Hind*III fragment were cloned and screened for restriction-alleviation activity. The *Bam*HI fragment was cloned in pUC18 to generate pGK8. The *Cla*I-*Eco*RI fragment was excised from pGK8, converted to a blunt-ended fragment using Klenow polymerase, and ligated to pUC18 cut with *Sma*I, to generate pGK9. pGK10 was similarly made with the 500 bp *Xho*I-*Eco*RI fragment from pGK9. Thick line, *E. coli* chromosome; thin line, pUC18 DNA; open box, Rac DNA; *PB*, hybrid Rac attachment site; open triangles, *Hind*III target sites; *P_{lac}*, *lacUV5* promoter in pUC18; DNA sequence numbering as Clark *et al.* (1993).