

Short Communication

Isolation of a Non-Classical Mutant of the DNA Recognition Subunit of the Type I Restriction Endonuclease *R.EcoR124I*

Marie Weiserova¹ and Keith Firman^{2,*}

¹ Czech Academy of Sciences, Institute of Microbiology, Videnska 1083, 14220 Prague 4, Czech Republic

² Biophysics Laboratories, School of Biological Sciences, University of Portsmouth, St. Michael's Building, White Swan Road, Portsmouth, PO1 2DT, UK

* Corresponding author

We have used deletion mutagenesis and PCR-based misincorporation mutagenesis to produce a collection of mutations in the central conserved region of the DNA binding subunit of the type IC restriction endonuclease *EcoR124I*. It has been proposed that this domain is involved in protein-protein interactions during the assembly of the endonuclease. While a large percentage of these mutations gave a classical *Res⁻Mod⁻* phenotype, one mutant was isolated with a non-classical *Res⁻Mod⁺* phenotype. The loss of restriction activity, but retention of the ability to modify indicates that this mutation cannot affect DNA binding and must alter the assembly of the endonuclease in such a way as to prevent DNA cleavage but allow methylation. This mutant resulted from a single amino acid change *Trp²¹²→Arg*. The location of the single amino acid change is at the border of the central conserved region and the second target recognition domain (TRD2) and suggests that this region is extremely important for the assembly of the methylase with the HsdR subunit into a functional restriction endonuclease.

Key words: Misincorporation mutagenesis / PCR / Protein-protein interactions / Restriction-Modification / Subunit assembly.

Type I restriction and modification systems are encoded by three genes. All three genes are required for production of the restriction endonuclease; *hsdR* is absolutely required for restriction and is transcribed from its own promoter (P_{RES}), while *hsdM* and *hsdS* are transcribed from a separate promoter (P_{MOD}) and together are required for modification [for reviews see Bickle and Krüger (1993) and Wilson and Murray (1991)]. The *hsdS* and *hsdM* genes can also produce an independent methylase with a stoichiometry of $HsdM_2:HsdS_1$ (Dryden *et al.*, 1993; Taylor *et al.*, 1992).

The type I restriction and modification systems are divided into four families (type IA e.g. *EcoKI*, type IB e.g. *EcoAI*, type IC e.g. *EcoR124I* and type ID e.g. *StySBLI*) based on gene order, amino acid conservation, complementation assays and enzymatic properties (Bickle and Krüger, 1993; Redaschi and Bickle, 1996; Wilson, 1991; Titheradge *et al.*, 1996). Within each family there are distinct regions of the HsdS subunit in which amino acid identities are strongly conserved. One such region lies about midway between the C- and N-termini and is known as the central conserved region, while the other region is at the C-terminus (Cowan *et al.*, 1989; Kannan *et al.*, 1989; Murray *et al.*, 1982). In addition, type IC HsdS subunits have a conserved region at the N-terminus. This region is normally present at the C-terminus of other type I systems and is, therefore, part of a 'split-repeat' (Kneale, 1994). Outside of these conserved regions the amino acid sequences are highly variable even between members of the same family and these variable regions are responsible for DNA recognition (Figure 1). These two regions have been named TRD1 and TRD2 (for target recognition domains) and can be 'swapped' between related systems to generate novel DNA specificities (Fuller-Pace *et al.*, 1984; Gann *et al.*, 1987).

Classically, mutations within the *hsdS* gene produce a *Res⁻Mod⁻* phenotype, as do classical mutations within the *hsdM* gene. It was from a complementation analysis of these mutants that the existence of the *hsdS* gene was confirmed (Glover, 1970; Hubacek and Glover, 1970). Since the HsdS subunit is responsible for DNA recognition the *Res⁻Mod⁻* phenotype is presumed to reflect a loss of this ability; the inability to bind DNA would prevent both restriction and modification functions. However, the HsdS subunit must also interact with HsdM (to produce the methylase) and possibly the HsdR subunit in order to assemble into the endonuclease. In addition, it has been proposed that the conserved amino acid domains present in HsdS are responsible for such protein-protein interactions (Cowan *et al.*, 1988). We have previously described a series of deletion mutants of the C-terminus of the HsdS subunit which have been shown to directly affect the ability of the HsdS subunit to interact with the HsdM subunit (Abadjieva *et al.*, 1994) and we have used these mutants to identify a repeated amino acid sequence required for such interactions (Figure 1). The anti-restriction protein Ard also possesses a consensus motif, derived from protein-sequence comparisons of this region of HsdS, and