

A motif conserved among the type I restriction-modification enzymes and antirestriction proteins: a possible basis for mechanism of action of plasmid-encoded antirestriction functions

Anatol A. Belogurov* and Eugene P. Delver

Department of Genetic Engineering, Cardiology Research Center, Moscow 121552, Russia

Received November 14, 1994; Revised and Accepted January 19, 1995

ABSTRACT

Antirestriction proteins Ard encoded by some self-transmissible plasmids specifically inhibit restriction by members of all three families of type I restriction-modification (R-M) systems in *E.coli*. Recently, we have identified the amino acid region, 'antirestriction' domain, that is conserved within different plasmid and phage T7-encoded antirestriction proteins and may be involved in interaction with the type I R-M systems. In this paper we demonstrate that this amino acid sequence shares considerable similarity with a well-known conserved sequence (the Argos repeat) found in the DNA sequence specificity (S) polypeptides of type I systems. We suggest that the presence of these similar motifs in restriction and antirestriction proteins may give a structural basis for their interaction and that the antirestriction action of Ard proteins may be a result of the competition between the 'antirestriction' domains of Ard proteins and the similar conserved domains of the S subunits that are believed to play a role in the subunit assembly of type I R-M systems.

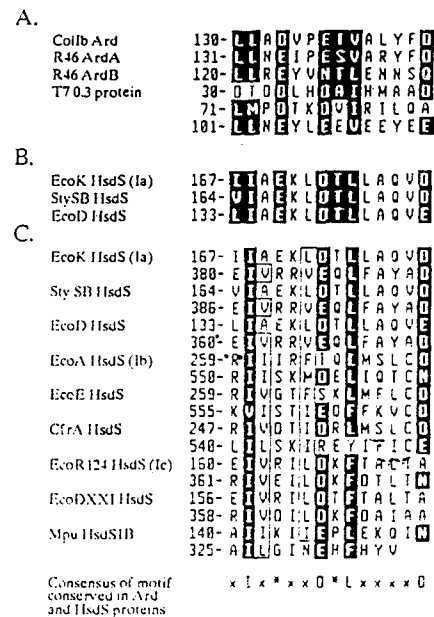


Figure 1. A motif conserved in the antirestriction proteins and DNA specificity subunit (HsdS) of type I R-M systems. Deduced amino acids from antirestriction proteins ('antirestriction' domain) (A) and HsdS polypeptides (the Argos repeat-like sequences) (B and C) were aligned using the CLUSTAL program. The sequences used for alignment were ColIb Ard (6), R46 ArdA (8), R46 ArdB (9), T7 0.3 protein (12), EcoK HsdS (18), EcoD HsdS (18), EcoA HsdS (30), EcoE HsdS (30), CfrA HsdS (17), EcoR124 HsdS (31), EcoDXXI HsdS (GenBank accession number X73984) and Mpu HsdS1B (32). The families to which the type I enzymes belong are indicated in parentheses. Note that the appropriate sequences of type Ia systems EcoB (18) and StySP (19) are identical to that of EcoK and therefore not shown. Black boxes represent similar (in one-letter notation, A.S.T; D.E.N.Q; H.K.R; F.L.L.M.V; Y.W) and identical amino acids in antirestriction proteins and their conservation in the HsdS polypeptides. Open boxes represent amino acid residues conserved only in the HsdS polypeptides. The numbering of residues of each polypeptide is shown on the left. Two Argos repeat-like sequences presented for each HsdS are located within its central (first line) and carboxyl (second line) conserved regions, respectively (see Fig. 2 for details). Symbols * and x in the consensus sequence represent polar amino acid residues and lack of consensus, respectively.

INTRODUCTION

Self-transmissible plasmids play an important role in the dissemination of variety of genes among different bacterial populations. However, DNA transfer mediated by these plasmids may be limited by host 'immigrant control', restriction endonucleases that recognize the 'molecular passport', the methylation pattern of guest DNA (see, for recent reviews 1-4). To overcome the host restriction barrier, some self-transmissible plasmids were found to encode antirestriction functions Ard (alleviation of restriction of DNA) (5-9). Recently, we have found that the broad-host-range IncN plasmid R46 encodes at least two types of antirestriction proteins, ArdA and ArdB. These proteins are non-homologous but functionally similar in that both efficiently inhibit restriction by members of all three families of type I systems in *E. coli* (EcoK, EcoB, EcoD, EcoA, and EcoR124). In addition, they slightly affect the type II (EcoRI) restriction and do

* To whom correspondence should be addressed