Motor subunit of ecor124i and ecoa1 functions

Function of motor subunit of EcoR124I and EcoA1 restriction-modification systems

Introduction

Effects of the Type I restriction-modification systems were described in the early 1950s, constituting the ability of certain bacterial strains to restrict or enlarge the host range of viruses after only one growth cycle. This phenomenon was called ‘ host-controlled variation in bacterial viruses’ [1-3]. A decade later it was discovered to be the result of DNA methylation and degradation occurring in bacterial cell [4, 5]. It was learned that restriction (R) of bacteriophage host range was caused by host cell’s ability to degrade unmodified viral DNA. Modification (M), in contrast, widens host range protecting phage DNA by methylation.

Type I Restriction-Modification Enzymes

Characteristics and Function

Type I R-M systems are encoded by three genes: *HsdM* , *HsdS* and *HsdR* . The products of these genes, referred to as HsdM, HsdS and HsdR, or simply M, S and R, constitute multifunctional enzymatic complexes that are able of both restriction and modification activity. HsdM and HsdS subunits are able to form a trimeric complex called Mtase with stoichiometry of M_2S_1 , which is capable of recognizing specific DNA sequences and methylation of adenine residue within this sequence. However, methylation occurs only if the target sequence is already modified on one strand (this state is referred to as ‘ hemimethylated’). In case if both DNA strands are unmodified within the

target sequence, the DNA is identified as foreign, which triggers further addition of two HsdR subunits and formation of pentameric complex, followed by restriction of foreign DNA. It was found out that, unlike type II restriction enzymes, type I R-M systems do not cleave DNA at predictable distances from recognition sequence []. Instead, HsdR subunits initiate ATP-driven translocation of DNA, pulling DNA through themselves in both directions until some kind of stalling happens.

The ability to discern foreign DNA from cell’s own through methylation makes RM systems act as bacterial ‘ immune system’, protecting bacterial cell from viruses and other mobile genetic elements. It is however unclear whether defense from invading DNA is the primary function of type I R-M systems: there is some evidence that supports the view that RM systems may themselves act as selfish mobile genetic elements [].

Families

EcoR124I = IC

EcoAI = IB

Overall structure and subunit activities

Function and Importance

Genes that code for EcoR124I and EcoA1: location, expression and its regulation

Operon structure

EcoR124I is plasmid-encoded,

EcoR124 vs EcoA1

Control of gene expression

Structure and function of HsdR subunit

Methods

The sheer complexity of RM systems necessitates using a wide array of approaches to study them. These approaches range from classical microbiological assays, routines of molecular biology and protein expression to protein modeling and molecular dynamics *in silico* . The methodology described below focuses on restriction assay *in vivo* , though first implemented in 1960s but often poorly explained; we then shift our attention to gel mobility shift assay used to evaluate complex assembly on DNA *in vitro* . Lastly, we give a detailed explanation of measuring translocation speed RM system on DNA using stopped flow technology.

Other methods that are often exploited to study RM systems deserve a brief mention here. Standard technique of studying properties of any enzyme in relation to the role that certain residues play is site-directed mutogenesis. Most often, PCR mutogenesis is employed when a primer with desired mutation (often single nucleotide substitution) is used. RM system genes are usually cloned into a suitable plasmid that is capable to maintain itself inside *E. coli* . Special plasmids might be used for purposes of protein overexpression or propagation of RM system inside cells for *in vivo*

experiments. Many of these plasmids have been constructed over the years (see table 1 for EcoR124 as an example).

It is often possible to test different enzymatic activities of RM systems *in vitro* , working with purified proteins. Detailed protocols have been developed that allow fast and efficient purification of HsdR and Mtase []. HsdR purification involves one step ion-exchange chromatography [], while purification of Mtase requires an additional step of affinity chromatography where heparin attached to chromatography column imitates DNA and prompts assembly of M and S subunits, forming M_2S complex, or Mtase [].

With purified subunits of an RM system readily available, it is possible to evaluate its activities *in vitro* , such as their ability to cleave and translocate DNA. Cleavage assay is done by simply mixing DNA substrate, Mtase and HsdR with buffer containing ATP and S-adenosylmethionine and taking out aliquots of reaction mix over a period of time, normally starting with seconds and going up to an hour. DNA products are then visualized on agarose gel, allowing to distinguish between intact DNA substrate and products of enzymatic reaction (see picture for an example of such gel).

Accessing translocation activity of RM complex is often done indirectly by monitoring ATP consumption during translocation because the latter is ATP-dependent, with one molecule of ATP consumed to advance DNA by one base pair. ATP consumption is measured either photometrically or using radiolabeled ATP. In first case, the ability of malachite green to form a colored complex with inorganic phosphate (one of the products of ATP hydrolysis) is exploited [].

Restriction assay in vivo

When RM systems were first discovered [1-3], it was done indirectly by observing how phenotype of bacterial viruses (bacteriophages, or simply phages) is changed in response to their growth in certain mutant strains of bacteria, affecting the ability of virus to reproduce in some strains but not the others. The change appeared to be transient, as the phenotype was readily reverted to its original state by growing the phage in a suitable strain of bacteria. These simple observations that subsequently led to discovery of RM systems were possible thanks to development in bacterial virology techniques [6].

A modification of that early assay is still relevant today and allows for fast screening of mutated HsdR subunits in relation to their restriction capability. It was later developed into what is known today as spot tests for restriction and modification [7-8]. Below we describe a variant of restriction test for EcoR124II system that we use in our research.

Strains and plasmids

The test is performed in *E. coli* stain *JM109(DE3)* (Promega), that lacks genes of *E. coli* K RM system and RecA-, thus preventing undesirable restriction and recombination with host chromosomal DNA of transformed plasmids. The strain allows for easy transformation with plasmids containing genes of RM system EcoR124II (listed in the table 2).

Bacteriophages

Phage λ was grown on appropriate *E. coli* strain to achieve phage modification. Phage grown on strain containing EcoR124I receives modification on EcoR124I recognition site (λvir. R124I, or λ_{I}), on strain containing EcoR124II is modified on EcoR124II (λvir. R124I, or λ_{I}). Non-modified phage is referred to as λvir. 0 or λ_{0} and is obtained from *E. coli* strain without RM system.

Media and solutions

Standard LA and LB media were used to grow bacteria; along with soft agar medium (LA with 1. 5% agar). Phage buffer was used for phage storage and dilutions (22 mM KH_2PO_4, 49. 1 mM Na_2HPO_4, 85. 6 mM NaCl; pH adjusted to 7. 2; after autoclaving: 1 mM $MgSO_4$, 0. 1 mM $CaCl_2$, 0. 001% gelatine).

Table 2.
Plasmids used for restriction assay.

Plasmid	RM genes (EcoR124II)	Restriction phenoty	Antibiotic resistan

		pe	ce
pACMS 124II	*HsdM, HsdS*	–	Cm
pPKF650 124II	*HsdM, HsdS, HsdR*	+	Cm
pTRC R124	*HsdR*	–	Am

Phage manipulations

1. Phage lysate preparation. To multiply phage λ, 0. 1 ml of stock phage lysate was mixed with 0. 5 ml of overnight culture of appropriate bacterial strain and 3 ml of soft agar preheated to 45° C

Unknowns

Mix

The difference between EcoR124I and II is and additional 12bp repeat in non-specific spacer that separates two TRDs [“ from x to Nanodevice”]

References

1. Luria, S. E. and Human, M. L. (1952) F nonhereditary, host-induced variation of bacterial viruses. J. Bacteriol., 64, 557-569.
2. Anderson, E. S. and Felix, A. (1952) Variation in Vi-phage II of Salmonell typhi. Nature, 170, 492-494.

3. Bertani, G. and Weigle, J. J. (1953) Host controlled variation in bacterial viruses. J. Bacteriol. 65, 113-121.
4. Dussoix, D. and Arber, W. (1962) Host specificity of DNA produced by Eschirichia coli. II. Control over acceptance of DNA from infecting phage lambda. J. Mol Biol., 5, 37-49.
5. Arber, W. and Dussoix, D. (1962) Host specificity of DNA produced by Echerichia coli. I. Host controlled modification of bacteriophage lambda. J. Mol Biol., 5, 18-36
6. ADAms, M. H. 1950 Methods of study of bacterial viruses, pp. 1-73. In Methods in medical research. Vol. II. Year Book Publishers, Chicago, Ill.
7. Colson, Clover, Symonds, Stancey (1965), Genetics.
8. Hubacek, J. and Clover, S. W. (1970). Complementation analysis of tempetature-sensitive host specificity mutation in Eshcerichia coli. J. Mol. Biol., 50, 111-127.