Lateral transfer of restriction-modification systems and their components

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Restriction-modification (RM) systems of bacteria and archaea restrict invasions of phages and other foreign DNAs. In addition, they play several other biological roles reviewed in [1]. RM systems are considered mobile genetic elements despite the absence of own mechanisms of translocation. Many cases of lateral transfer of RM-systems were reported, however we do not know publications studying their evolution using all available data. This is why we have started investigation of RM-systems' evolution in a large scale [2]. Fortunately, the information about all known RM-systems is stored in the REBASE (http://rebase.neb.com/rebase/) curated by R.Roberts and D. Macelis for several decades.

We have downloaded all data on RM-systems encoded in completely sequenced genomes from REBASE. Key activities of RM-systems are double strand cleavage of DNA in or near of specific sites and methylation certain bases of DNA in the same sites, preventing DNA cleavage by the cognate restriction endonucleases. The third obligatory function is recognition of specific DNA sequences. RM-systems are classified into four types and a number of subtypes depending on distribution of

activities between RM genes and other their features. Type I RM-systems typically consist of DNA methyltransferase (MTase) gene, restriction endonuclease (RE) gene and gene of DNA recognition protein (S-protein) . Orthodox Type II RM-systems consist of MTase gene and RE gene, target recogbition domains (TRD) are in both proteins. Type III systems also consist of MTase gene and RE gene, but TRD present in MTase gene only. Type IIG systems include one gene merging all three activities. Type IIM and Type IV RM systems consist of one gene encoding endonuclease, which cleaves only modified (mainly, methylated) sites in DNA; they arose against phages, that modify own DNA to prevent cleavage by host RM-systems.

We classified all Types of RM-systems into homologous classes according Pfam families of catalytic domains of MTases and REs. We calculated RM-systems of each class per phylums of bacteria and archaea. Examples are shown in Table 1.

Table 1. Distribution of certain classes of homologous RM-systems per phyla of prokaryotes (fragment of the complete table).

Class	Type RM	Archaea total	Euryarchaeota	Thaumarchaeota	Bacteria total	Actinobacteria	Bacteroidetes	Chlorobi	Cloacimonetes	Cyanobacteria	Firmicutes	Proteobacteria	Spirochaetes
A-archea, B-bacteria		A	A	A	B	B	B	B	B	B	B	B	B
N6_Mtase/ResIII	I	212	205	4	3356	223	162	18	4	93	636	1967	38
N6_N4_Mtase/ResIII	III	28	26	2	1382	78	49	8	2	11	127	1008	6
Mrr_cat	IV	47	47	0	1138	165	20	1	0	32	190	683	14
DNA_methylase/EcoRII-C	II	0	0	0	135	8	16	0	0	1	9	98	1
DNA_methylase/RE_NgoPII	II	0	0	0	57	0	3	0	0	4	8	38	1
N6_N4_Mtase/RE_TdeIII	II	2	1	1	19	0	2	2	2	0	2	2	3
DNA_methylase/RE_TdeIII	II	0	0	0	21	0	0	0	0	8	8	1	0

We are interested in reconstruction of RM-system evolution within each homologous class. Two circumstances should be taken into account. First is exchange MTases and REs genes among RM systems. Indeed, in our data 6 of 7 MTase catalytic domains were found in combination with different REs catalytic domains and 54 of 72 RE

catalytic domains were found with different MTase catalytic domains. Second, Mtases are much more conserved than REs.

Sequences of MTases' and REs' catalytic domains of each class were aligned for further phylogenetic analysis. However not all alignments look reasonably. In addition, RM system annotations could be erroneous in a number of cases. This is why we present here data for two classes of RM-systems. First is N6_Mtase/ResIII class of RM systems of Type I including 3854 systems in almost all phyla of Archea and Bacteria. It is the most represented class of RM systems. Initially we expected that this class evolved mainly vertically. Currently we restricted our study of this class to Cyanobacteria (93 RM systems of N6_Mtase/ResIII class). Phylogenetic trees for REs and MTases were constructed separately. Branches with bootstrap support less than 80 were collapsed. These trees were compared to each other and with taxa of cyanobacteria. We found a number of cases of lateral transfer of both genes simultaneously as well as some cases of lateral transfer of separate genes. DNA recognition S-proteins of these RM systems revealed high variability. Putatively, variability of S-proteins is main mechanism of host adaptation to invasions of new phages and phage adaptation to RM systems. This assumption meats several confirmations in published works.

The same approach was applied to study two classes of orthodox Type II RM systems, DNA_methylase/RE_TdeIII (19 RM systems) and N6_N4_Mtase/RE_TdeIII (21 RM systems), see Fig. 1-3.

Multiple events of lateral transfer of RM systems were detected. Moreover three cases of putative gene exchange between two classes of RM systems were found.

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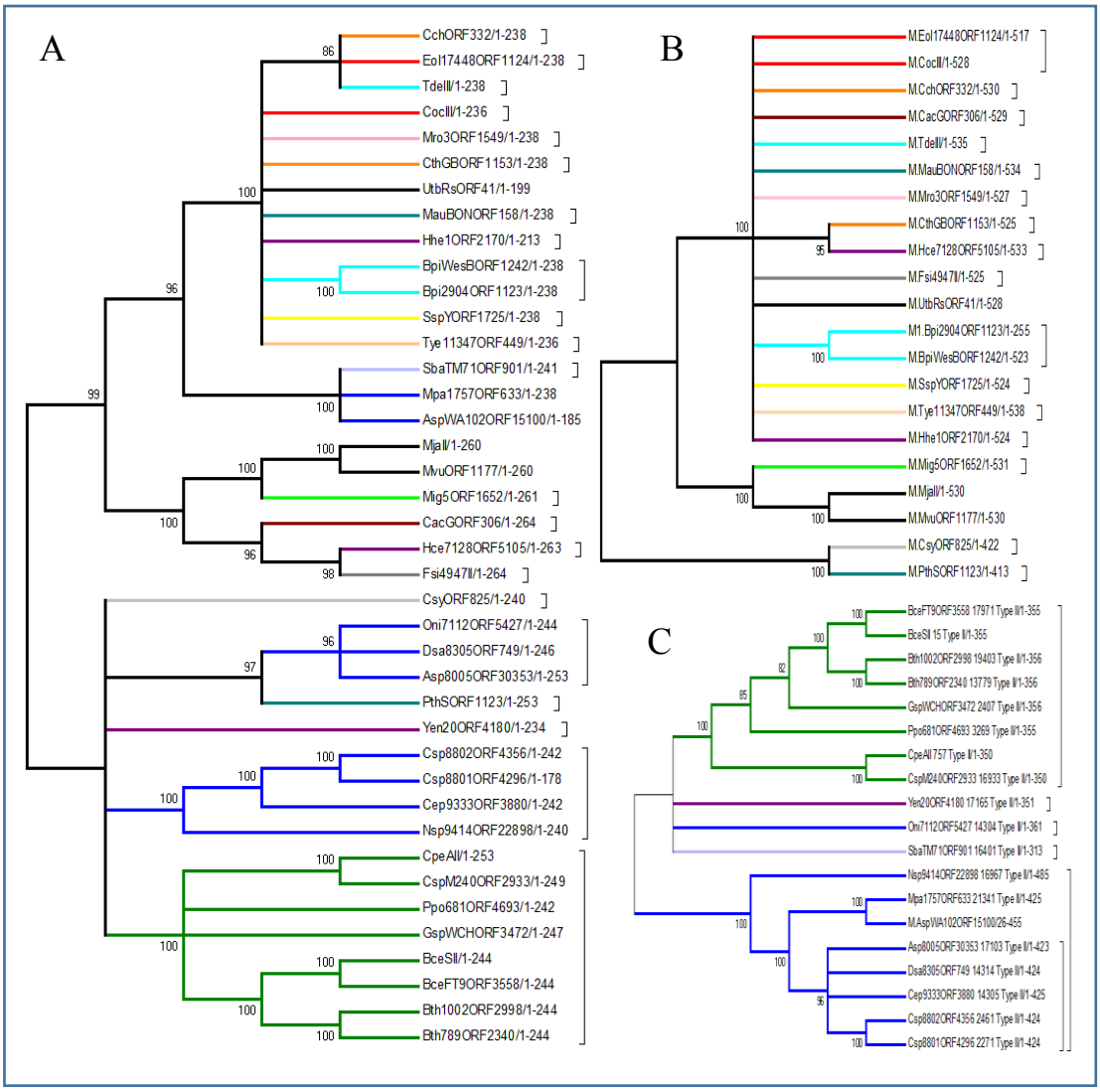

Fig. 1. Phylogenetic trees of REs , Pfam domain RE_TdeIII (A), MTases, Pfam domain N6_N4_Mtase (B) and MTases, Pfam domain DNA_methylase (C). Branches with bootstrap less than 80 are collapted. Branch colors correspond to phyla of bacteria and archaea. Each RE (Fig.1A) form RM system with the MTase (Fig.1B or C) having similar name.

1. A.Ershova, et al. (2015) Role of Restriction–Modification Systems in Prokaryotic Evolution and Ecology, *Biochemistry (Moscow),* **80** :1373-1386

2. O.I. Bezsudnova et al., (2016) Evolution of restriction-modification systems in large scale, *Abstracts of BGRS\SB-2016, Novosibirsk, Russia, 29 Aug.- 2 Sept.,*40.