

Maintenance of species identity and controlling speciation of bacteria: a new function for restriction/modification systems?

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Abstract

Bacteria frequently exchange DNA among each other by horizontal gene transfer. However, maintenance of species identity and in particular speciation requires a certain barrier against an unregulated uptake of foreign DNA. Here it is suggested that formation of such a barrier is one important biological function of restriction/modification systems, in addition to the classical function of protection of bacteria against bacteriophage infection. This model explains the extreme variability and wide distribution of restriction/modification systems among prokaryotes, the prevalence of RM-systems in pathogenic bacteria and the existence of several RM-systems in single bacterial strains.

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1. Introduction

Restriction/modification (RM) systems (reviews: Wilson and Murray, 1991; Bickle and Kruger, 1993; Heitman, 1993; Raleigh and Brooks, 1998) consist of two activities: a methyltransferase (reviews: Cheng, 1995; Jeltsch, 2002) that modifies adenine or cytosine residues at certain recognition sites and a restriction endonuclease (reviews: Pingoud and Jeltsch, 1997, 2001) that recognizes the same sequence and cleaves the DNA if it is unmethylated. The restriction enzyme is not harmful to the host cell, because its DNA is protected from cleavage by methylation, but incoming DNA will be cleaved. RM-systems are extremely common in bacteria; up to date more than 200 different systems have been identified (Roberts and Macelis, 2001). Moreover, large numbers of different RM-systems occur in single species: 16 different RM-systems have been identified biochemically in *Neisseria gonorrhoeae* (Stein et al., 1995) and in the genomes of *Helicobacter pylori* and *Methanococcus jannaschii* there are 30 and 13 genes for DNA methyltransferases that could belong to an RM-system. Moreover, RM-systems cover an enormous range of

specificities: almost all palindromic sequences comprising 4 or 6 bp are recognized by at least one RM-system. What is the origin of this enormous variety and distribution?

2. Results and discussion

Traditionally, RM-systems are regarded as defense mechanism against bacteriophage infection. Indeed, experimental results demonstrate a 10^2 – 10^4 -fold protection of the host cell by different RM-systems. The efficiency of RM-systems against phages is also documented by the occurrence of various anti-restriction defense mechanisms in phages, like incorporation of modified nucleotides, phage encoded multispecific methyltransferases, and the reduction of the number of sites for RM-systems in the DNA of many phages (review: Bickle and Kruger, 1993). In addition, conjugating plasmids encode anti-restriction proteins like ArdA and ArdB, which are induced during conjugative transfer and inhibit the RM-systems of the acceptor cell (review: Velkov, 1999). On the other hand, the discovery of RM-systems was stimulated by the fact, that RM-systems never provide a full protection of a bacterial culture against bacteriophage infection (Arber and Dussoix, 1962). Later, population genetic experiments suggested that protection of bacteria is only a transient phenomenon and RM-systems provide a significant selective value only under certain

Abbreviations: RM-system, restriction/modification system.

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environmental conditions (Korona et al., 1993). Thus, it appears questionable if the evolutionary pressure due to the presence of bacteriophages would be sufficient to maintain the diversity of RM-systems. This scenario is even more questionable for RM-systems with recognition sequences comprising 8 bp. So far at least 12 of such systems have been found in nature, although these sites occur only all 65536 bp and therefore are hardly found on an average bacteriophage genome. Moreover, there are some unusual endonucleases which only cleave methylated DNA like McrBC (Raleigh and Wilson, 1986; Pieper et al., 1997; Panne et al., 1998), McrA (Raleigh et al., 1989) or mrr (Waite-Rees et al., 1991) (all from *Escherichia coli*, McrBC homologous enzymes have recently been identified in *Bacillus subtilis* and *Methanobacterium thermoautotrophicum*), DpnI from *Streptococcus pneumoniae* (de la Campa et al., 1988) and drg, a DpnI homologue from *Neisseria meningitidis* (Bucci et al., 1999). Since bacteriophage DNA has a low probability to carry that specific kind of methylation, which is recognized by these enzymes, functioning of these enzyme in phage protection is questionable. It should be noticed that these systems directed against modified DNA are the antipodes of conventional RM-systems, which employ DNA modification as protection against digestion. In fact, these systems exclude each other and cannot be present in one cell. Taken together, although RM-systems undoubtedly protect bacteria against bacteriophage infection to a certain degree, it remains questionable if this function can explain the enormous spreading of these biological systems in the prokaryotic world. So could there be other functions?

Interestingly, RM-systems are genetically very stable in bacteria, because if the genes of an RM-system are removed, the proteins remain in the cell. During time, the concentrations of both enzymes of the RM-system, DNA methyltransferase and restriction endonuclease, decrease due to dilution by cell divisions and protein turnover. Inevitably, somewhere along this way the activity of the methyltransferase does not suffice to modify all recognition sites on the genome. If at this time still some restriction activity is present, the endonuclease will cleave the genome of the cell and induce cell death. Such behavior has been shown experimentally, demonstrating that RM-systems are self-sustainable (Naito et al., 1995). Therefore, RM-systems can be considered selfish genes, which might not provide important advantages to the host, if any (Kobayashi, 2001). However, this model does not explain that the genes for the endonucleases alone are not lost or inactivated by mutations. The wide distribution of active endonucleases in RM-systems, therefore, demonstrates that a functional RM-system (containing an active endonuclease) must provide some selective value to the host.

Bacteria frequently exchange DNA among each other as well with organisms of different species (reviews: Davison, 1999; Dubnau, 1999; Jain et al., 1999). However, maintaining the genetic uniqueness of species including the adapta-

tions of each species to its special ecological niche requires genetic isolation to a certain degree. Furthermore, genetic isolation is a prerequisite to the evolution of new species, because only under conditions of genetic isolation a sub-population is able to develop new biological properties. Obviously, adaptation to a new ecological niche requires some mutations, which would be severely decelerated if not prevented in case of a massive back-crossing induced by horizontal gene transfer with the ancestor, which is adapted to a different environment. In higher organisms, genetic isolation is achieved by geographical or temporal isolation or a specialized sexual behavior. Moreover, hybrids are almost in every case sterile. In bacteria, one way to achieve a genetic isolation is to control the uptake of DNA from the environment (Tortosa and Dubnau, 1999). As an alternative, the intracellular fate of DNA taken up can be regulated—which is the unique function of RM-systems. The influence of restriction enzymes on DNA taken up by bacteria from the environment has been shown experimentally (McKane and Milkman, 1995; Milkman, 1997; Milkman et al., 1999), because incomplete digestion of incoming DNA leads to short fragments that are highly recombinogenic and the mosaic pattern of DNA integrated into the bacterial genome reflects the effect of restriction cleavage of incoming DNA. Therefore, it has been suggested that stimulation of homologous recombination might be an additional function of RM-systems (Price and Bickle, 1986). However, this model does not explain, why bacteria evolved so many different RM-systems with different DNA recognition specificities. Thus, stimulation of recombination appears to be an inevitable by-product of RM-activity rather than a genuine function of these systems.

The methylation pattern of the DNA is a strain specific bar code that is an integral part of the identity of each strain and allows a clear distinction between self and non-self DNA. The potential of RM-systems and endonucleases cleaving methylated DNA to establish a barrier against the uptake of DNA has been noticed long ago (Murray et al., 1975). For this reason, today, most high efficiency cloning strains of *E. coli* are devoid of the *E. coli* RM-systems and McrBC⁻. When considered from the view of the high incidence of lateral gene flow among bacteria, the genetic barrier against gene transfer build by RM-systems is likely to be of great importance to preserve the identity of bacterial species and allow speciation. In fact, this function may be the most important role of RM-systems in vivo.

A function of RM-systems in the maintenance of species identity, the model put forward in this paper, also explains why so many different RM-systems are found in single bacterial species, because the presence of different RM-systems in different lines of the same species divides the species into different biotypes, which do not exchange genes among each other. Such division of one species into different biotypes expressing mutually exclusive RM-systems is an ideal starting point for a rapid adaptation to different eco-

logical niches. This model is exemplified in *N. meningitidis*, which consists of two biotypes, one containing a dam methyltransferase which generates methylated G^mATC sites and another containing the *drg* restriction enzyme that cleaves these sites (Bucci et al., 1999). Whereas the *drg*-line causes severe bacterial meningitis, the dam-line lives as an apathogenic commensal. Another example is the *H. pylori* strain, which contains more than 20 RM-systems many of them being inactive (Lin et al., 2001). However, all strain-specific RM-genes are active, whereas most genes shared with other strains are inactive. This has been interpreted with the model that strain-specific genes have been acquired more recently. According to the model put forward here, these strain specific RM-systems guarantee the genetic identity of the strain and, therefore, they must have remained active.

Perhaps pathogenic microorganisms require a very efficient protection from foreign DNA, because prior to the infection of a new host many pathogens exist in free living forms and come into close contact to their apathogenic relatives. This would inevitably lead to genetic exchange and the dilution of special adaptations. In this model, the first step in the generation of a new bacterial species would be that one bacterium acquires a functional RM-system, which have been shown to have a high incidence of lateral gene transfer (Jeltsch and Pingoud, 1996). This RM-system would isolate the clone from most of the genetic exchange with conspecific bacteria and allow a rapid evolution, which occasionally may lead to the emergence of a new species or, more often, lead to extinction. It is interesting to note that under unfavorable environmental conditions, bacteria can shut down their RM-systems (review: Velkov, 1999), which stimulates the uptake and integration of DNA. This response could be considered as a “genetic reset” which replaces individual genetic alterations of the cell by the DNA sequence typical for the strain.

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