UDC 577.213.6:575.224:575.8 *review*
ISSN 1330-9862

(FTB-1185)

The Role of Mismatch Repair in Bacterial Evolution

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Received: October 7, 2002
Accepted: April 24, 2003

Summary

Experimental (directed) evolution is a study of evolution under defined and reproducible conditions, particularly on model laboratory populations of bacteria. Recently, remarkable success of directed evolution has been reported, ranging from industrial enzymes, with substantially improved activities and thermostabilities, to vaccines and pharmaceuticals as well as a generation of novel microorganisms with desired properties. It has become clear that the major process influencing evolution is DNA Mismatch Repair (MMR). The MMR system controls genome stability of the species and is highly conserved from bacteria to humans. It maintains the integrity of DNA by repairing errors made during the replication process and by preventing genetic recombination between diverged DNAs. Inactivation of MMR results in the generation of hereditary mutators with highly increased mutation rates as well as in abolishment of genetic barriers between species. Most of the mutations are deleterious, but some of them are beneficial and enable mutators to survive environmental stress. In the stable environment mutators lose their advantage because of accumulating deleterious mutations. Strains with beneficial mutations could survive by reacquiring MMR wild type alleles in horizontal gene transfer through hyperrecombination phenotype of MMR mutators. During evolutionary history, MMR functions have been repeatedly lost and reacquired by horizontal gene transfer, which gives rise to the mosaic gene structure of MMR genes. This mosaicism is a hallmark of the evolutionary process.

Key words: MMR system, mutator bacteria, adaptive mutagenesis, evolution

Introduction

Evolution is the change in the frequencies of gene alleles in response to natural selection affecting processes of DNA replication and repair (*1*). Evolution, especially microevolution, will be profoundly important for biotechnology of the next generation. New technologies have emerged using enhancers of natural processes of genetic evolution within the host. They have been applied to microorganisms, plants and mammals to yield genetically diverse offspring suitable for biotechnological, agricultural or pharmaceutical development. These new technologies generate organisms with new output traits that can be directly used for commercial application without time-consuming need for gene isolation and characterisation. Recently, remarkable success of directed evolution has been reported ranging from industrial enzymes with substantially improved activities and thermostabilities to vaccines, pharmaceuticals as well as a generation of novel microorganisms with desired properties (*2*).

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New biotechnology companies employ technologies of directed evolution *in vitro* and *in vivo* (Fig. 1). In directed evolution experiments *in vitro*, pioneered by Stemmer (*3*) and Arnold (*2*), the processes of natural evolution are accelerated in a test tube using cyclical application of mutagenesis, screening and recombination. Protocols for random recombination of homologous genes *in vitro* are technically simple in bacteria and are now being patented by major companies (*e.g.* Caltech, Maxygen) (*4*) and used for a wide range of new products.

Another strategy is directed evolution *in vivo* developed by Radman (Mixis technology) (*5*). It generates diversity within a host and employs knowledge concerning the role of MMR system in evolution by controlling mutation rates and interspecies recombination. Inhibition of MMR allows *in vivo* recombination between diverged DNA fragments and elevates mutation rates to create *de novo* variation. This technology easily and rapidly allows recombination of diverged genes and genomes to increase biodiversity and generate novel biosynthetic compounds. The advantage of this *in vivo* technology is the ability to produce diversity within complex metabolic pathways without requiring a detailed knowledge of the genes involved and it is a powerful tool for compound discovery and developmental processes.

Expected progress in applied evolutionary biology will be accompanied by progress in basic experimental research. Experimental evolution refers to experiments designed to study evolution under defined and reproducible conditions. These experiments generally employ closed population systems, particularly laboratory populations of model organisms. Laboratory populations of bacteria whose large population sizes and short generation times facilitate direct observation of long term evolutionary processes are mostly used to study mutation and recombination.

Mechanisms of Adaptive Mutagenesis in Bacteria

Bacteria are constantly confronted by variable and stressful environments and the ability for genetic adaptation by affected bacteria is essential for their evolutionary success. The probability of generation of strains to adapt better to a new environment depends on bacteria's capacity to produce genomic diversity. Biological evolution results from changes in the genetic constitution of species. Genetic variation arises through two processes: mutation and recombination. Recent studies have shown that bacterial mutation rate is essential for the process of evolution (*6*). Mutation occurs when DNA is imperfectly copied during replication leading to difference between a parent gene and that of its offspring.

Most mutations are likely to be deleterious and so the spontaneous mutation rate is generally held at a very low value. For example, mutation rate in *Escherichia coli* is approximately $5 \cdot 10^{-10}$ per base pair per generation (*7*). Such a low rate is due to several cellular mechanisms that control the fidelity of replication to preserve genetic information (*8*). Numerous enzymes have evolved for both protection and repair of DNA from various damaging agents (*9*). Also, there are several steps of controlling the DNA replication process (*i.e.* equilibration and correction of nucleotides before using them in DNA synthesis, proof-reading activity of DNA polymerasing enzyme to ensure correct incorporation of nucleotides) as well as different steps of correcting replicative errors. The post replicative MMR system is one of the most important ones. Inactivation of any of these »housekeeping« systems can lead to increased mutation rate. Strains with higher spontaneous mutation rate than the wild type strains are called mutators (*10*). Mutators have been found in the natural population and isolated in the laboratory (*11-13*).

Transient mutators are those that have an increase in the mutation rate depending on environmental conditions and once the environmental conditions are stabilised the mutator state will revert to normal (*7,14*). Temporary state of hypermutability can arise through an increase in the rate of DNA polymerase errors (which may or may not be triggered by DNA damage). The mechanisms of stress-induced mutagenesis can be different (*8*). There are various enzyme systems that respond to stress conditions and DNA damage (oxidative damage, alkylating agents, phototoxic agents, heat, starvation) and all of them may generate mutations (*9*). Adaptive mutagenesis is a process that produces mutations to relieve the selective pressure. The inducible SOS response in bacteria turns on otherwise repressed wild-type mutator genes (*umuC*, *umuD* and *dinB*) and upregulates a number of recombination genes (*e.g. recA*, *recN*, *recQ*, *ruvA*, *ruvB*) when bacteria undergo a genotoxic or a metabolic stress. These bacteria mutate at increased rates only under such selective pressure. As soon as growth conditions are restored (either by genetic adaptation or by a favourable environmental change), the mutator and hyperrecombination activities are repressed by the LexA repressor. The ultimate proof of the SOS hypothesis is the identification of several enzymes with the mutation producing function (*15*). These enzymes, DNA mutases, all belong to a special group of DNA polymerases. The selective pressure gives rise to mutations in the repair genes, resulting in fixation of heritable mutators. Those affecting postreplicative MMR are the most important for evolutionary process.

Mutators of the Mismatch Repair System

The best known class of mutation-rate mutants are general mutators, which have increased mutation rates throughout their genome (*8,9*). Its mutator activity is due to defects in DNA proofreading and repair functions. In contrast to transient mutators, which revert to non-mutator state when selection pressure is removed, these mutants have constitutively higher mutation rates – they are hereditary mutators. Constitutive mutators are found among both natural and laboratory populations of bacteria. Most spontaneous mutator mutants appear to be defective in MMR pathway (*11-18*). Depending on the type of function that is defective, mutators can have mutation rates that are moderately (~10-fold) to strongly (100–1000-fold) increased. The MMR system is involved in control of replication fidelity and inactiva-

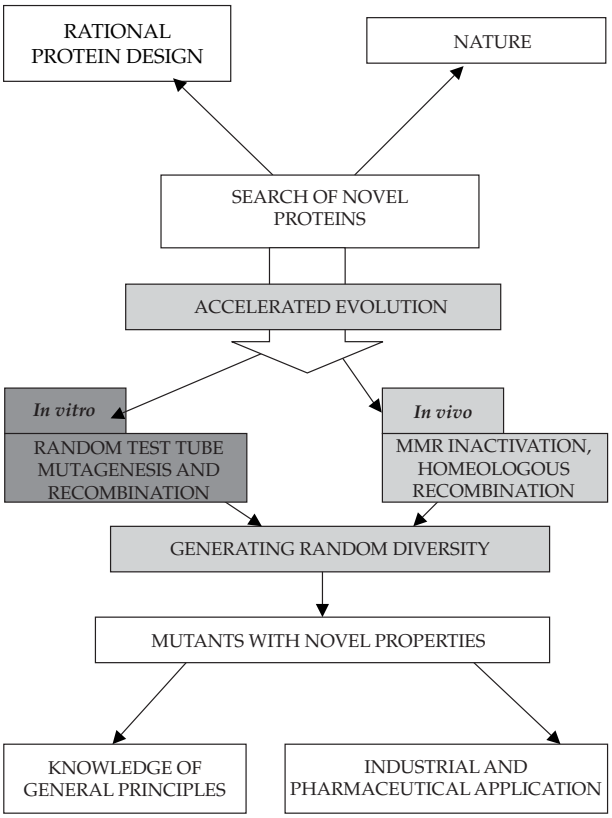

Fig. 1. Application of experimental evolution to the new biotechnologies

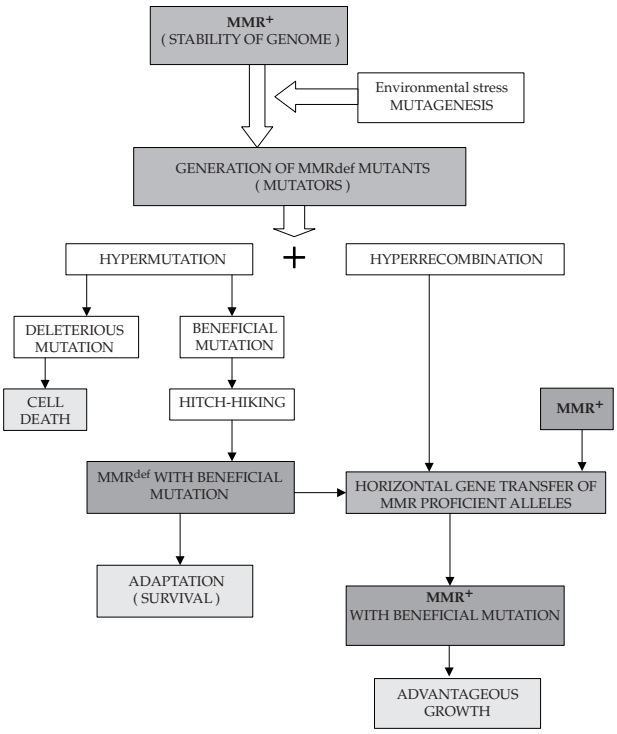

Fig. 2. The role of mismatch repair in bacterial evolution

tion of any of MMR genes that increase mutation rates 10^2 to 10^3 fold.

The best characterised MMR system is the methyl-directed MMR system of *E. coli* and it is well characterised genetically as well as at the biochemical level (*19-22*). It corrects replication errors by removing impaired and unpaired bases from newly synthesised DNA. MMR operates also on mismatched heteroduplexes formed during the processes of genetic recombination and gene conversion (*23*) and on the mismatches formed after spontaneous deamination of 5-methyl cytosine (*24*). The basic mechanism of MMR involves three steps; recognition of the mismatch, excision of the misincorporated base and DNA surrounding the mismatch, and as the last step, repair synthesis to replace the excised DNA (see 22 for review). The mismatched base is recognised by the MutS protein. In a reaction requiring ATP hydrolysis, MutL, together with the MutS-mismatched DNA complex, stimulate strand scission by MutH, opposite a *dam* methylated GATC parental DNA sequence, ensuring that the DNA excised is the newly replicated unmethylated daughter DNA. DNA polymerase III and SSB proteins perform repair synthesis to replace the excised DNA. Adenine methylation at GATC sites of a parent DNA strand serves to target repair to the daughter strand by an excision/resynthesis mechanism. As it has been shown to depend on the *mutH*, *mutL*, *mutS*, *mutU* and *dam* gene products, this repair pathway is known as mutHLS pathway. The genes are called *mut* because they were identified as mutations that lead to increased levels of spontaneous mutation. Although the mutation rate of a cell is controlled by multiple pathways, any of which can lead to a mutator phenotype when defective, of particular significance is the fact that all mutators identified among natural isolates are defective in methyl-directed MMR. Apart from controlling fidelity of DNA replication MMR controls recombination as well (*25*). The MMR proteins recognise mismatched bases in the DNA and abort recombination process between diverged DNAs. Thus, MMR is also a potent inhibitor between non-identical DNA sequences. Inactivation of MMR genes (mutator phenotype) greatly increases the frequency of mutations as well as horizontal gene transfer among diverged species (*26*).

Generation of the MMR Mutators

The bacterial mutators are frequently found in nature (successful pathogenic and commensal bacteria) (*11,16,18*), as well as in experimental cultures (survivors of long-term laboratory culture and selection under laboratory conditions) (*1,27*). Most of them have been shown to be defective in MMR. The occurrence of MMR mutators among isolates of pathogenic *E. coli* and *Salmonella enterica* is over 1 %. *Pseudomonas aeruginosa* mutator strains obtained from patients with cystic fibrosis were over 10 % of MMR mutator type. Most of these natural isolates are pathogens and investigations have revealed a link between hypermutability and evolution of antibiotic resistance (*28*).

The spread of mutators occurs because they can create or acquire a beneficial mutation (*e.g.* antibiotic resistance) that gives them advantage over non-adapted bacteria. In an asexual population, the mutator may then spread with the advantageous gene, by a phenomenon called »hitch-hiking« (*29*) and increasingly fitter individuals will arise in the population. Strong mutator genes, such as those that increase mutation rates by 1000-fold, like MMR *mutS* gene, can accelerate adaptation, even if the mutator gene remains at a very low frequency (*11*).

Experimental data have confirmed that hypermutability is really beneficial and could be a target of positive selection itself (*1*). When 12 independently propagated clonal populations of *E. coli* were serially cultured over 10 000 generations in a nutrient limited environment, most of them retained the ancestral mutation rate, but three populations displayed mutation rates one or two orders of magnitude higher than those of the ancestor. These whole populations have become mutators due to the defects in MMR genes.

The indirect selective benefit of mutators depends on opportunities for adaptation (*28*,*30*,*31*). The fraction of mutations that improve adaptation depends on the evolutionary history of the population in the new environment. If the population is already well adapted, then most if not all mutations will have negative effects on fitness, and the mutator subpopulation will not have opportunity for »hitch-hiking«. Instead, it will suffer because of the increased production of deleterious mutations. However, if the environment is in some respect novel, adaptation is not perfect and the mutator population may outcompete the wild-type majority by its association with beneficial mutations. »Hitch-hiking« of mutator alleles with beneficial mutations depends on the physical linkage between mutator and beneficial mutation (*32*). If mutator and beneficial mutation are not separated regularly, as in asexual bacterial populations, mutators will profit from their indirect beneficial effect. The final consequence of the »hitch-hiking« process will be the spread of a mutator allele in the population, causing the entire population of cells to become a mutator.

The evolutionary fate of such a mutator population is insecure (*33*). It must restore the low mutation rate in order to save itself from extinction because of lots of deleterious and lethal mutations. If a successful mutator culture grows in nature it can exchange DNA with members of a similar bacterial culture and reacquire the functional *mut* gene. This adapted nonmutator will now overgrow the adapted mutator and nonadapted wild type because it does not produce any more deleterious mutations. The same will occur with a reversible *mut* mutation: a rare back mutation to nonmutator phenotype will give it selective advantage. Alternatively, the adaptive mutation may be transferred from the mutator to a nonmutator and a stable genome will be created.

Restoration of a low mutation rate by back-mutation is not very likely because these gene inactivations are mostly frameshift mutations and are difficult to revert (*13*). A more likely route to re-establish a low mutation rate is by horizontal transfer of a functional copy of the MMR gene from a related population.

Reverting from Hypermutability by Horizontal Gene Transfer

Barriers to chromosomal gene transfer between bacterial species control their genetic isolation. The major barrier is genomic sequence divergence (*25*). The ability of related DNAs to undergo recombination decreases with the increased sequence divergence. On the molecular level it is due to the action of MMR proteins. MutS and MutL proteins bind to non-homologous regions of heteroduplex recombinational intermediates formed between the strands of diverged DNAs and prevent homeologous recombination. It was shown that MMR cells lacking either the MutS or MutL function carry out homeologous recombination resulting from crosses between diverged species *(S. enterica* and *E. coli)* three orders of magnitude more frequently than MMR^+ strains (*26*).

Because inactivation of MMR greatly increases recombination rates between related species, MMR deficient strains might have contributed considerably to recombination that has given rise to the observed genomic sequence mosaicism of *E. coli* natural isolates (*34*). Particularly, genes of the MMR show a highly mosaic sequence structure. Comparison of *mutS* phylogeny against predicted *E. coli*, the whole chromosome phylogenies revealed striking levels of phylogenetic discordance among *mutS* alleles and their respective strains (*35*). This is a result of frequent recombinational exchanges leading to the replacement of MMR mutator genes by non-mutator ones. In general, the sequence mosaicism of MMR genes may be a hallmark of a mechanism for adaptive evolution that involves modulation of mutation and recombination rates by recurrent losses and reacquisitions of MMR gene functions (*35*,*36*).

Sequence of MMR genes is a mixture of short sequences that had diverged in the different *E. coli* strains before being assembled by recombination. This apparent horizontal gene transfer correlates with hyperrecombination phenotype of MMR-deficient mutators. Naturally occurring mutators most often carry a defect in MMR that may help in their own restoration by horizontal transfer from a divergent partner.

Association of the hyperrecombination and the mutator phenotypes of MMR-deficient bacteria may allow them to play an important role in bacterial evolution (Fig. 2), permitting the rare beneficial mutation to become separated from frequent deleterious mutations. This unique role of MMR defects in promoting homeologous recombination-gene exchange between and among species, along with mutator capability to associate different, favourable mutations in the same genome, may account for the general mosaic structure of bacterial genomes. Thus, the mosaicism results from horizontal transfer of chromosomal sequences between closely related but divergent species (*37*).

The MMR system constitutively controls the genetic stability of species and is expected to decrease the frequency of horizontal gene transfer, especially in well adapted bacteria. By inhibiting all the mechanisms of genetic alterations, MMR reduces the rate of evolution. By creating the same kinds of genetic alterations the SOS system accelerates the rate of diversification. After

a period of high mutation rates due to MMR deficiency and the SOS induction through environmental stress, population is stabilised by return of MMR proficiency and repression of SOS response. Accumulated DNA sequence divergence becomes a structural element for genetic stability and leads to the establishment of multiple genetic barriers within highly diverged population (*38*).

Conclusion

Experimental evolutionary biology provides increasing evidence that new beneficial mutations which have evolved in the MMR mutators can be rescued through homeologous recombination and serve for adaptation of a bacterial population. Genetic barriers are almost eliminated in MMR deficient mutants, indicating that *mutS* and *mutL* mutator genes may be essential in the divergent evolution of bacteria. The facility for recombining homeologous genes from different species and even crossing entire genomes, offers opportunity for directed evolution of new mosaic genes, which could be a source of novel enzymatic activities and new metabolites for applications in biotechnology and medical therapy (*6*).

Acknowledgments

We thank Prof. Dr. Marija Alačević and Dr. William Ragland for critical reading of the manuscript. The work was supported by the grants of Croatian Ministry of Science and Technology No. 000980071 and No. 0058013.

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Uloga staničnog popravka krivo sparenih baza u evoluciji bakterija

Sažetak

Eksperimentalna (usmjerena) evolucija obuhvaća evolucijske procese u definiranim i reproducibilnim uvjetima, posebno na modelnim laboratorijskim populacijama bakterija. Najnovija primjena usmjerene evolucije u području biotehnologije pokazala se vrlo uspješnom u proizvodnji industrijskih enzima s poboljšanom aktivnošću i termostabilnošću, te cjepiva i lijekova, a i u stvaranju novih sojeva mikroorganizama sa željenim svojstvima. Nedavno je postalo jasno da popravak krivo sparenih baza (eng. »Mismatch Repair«, MMR) u DNA ima glavnu ulogu u procesu evolucije. MMR sustav kontrolira stabilnost vrsta i vrlo je sačuvan od bakterija do čovjeka. MMR održava stabilnost DNA popravljajući greške nastale tijekom replikacije te sprečava genetičku rekombinaciju između različitih (divergentnih) DNA sekvencija. Inaktivacijom MMR nastaju nasljedni mutatori s velikom frekvencijom mutacija i rekombinacija između divergiranih DNA sekvencija. Takvi mutatori većinom nakupljaju štetne i letalne mutacije, ali mogu stvoriti i povoljne mutacije koje im omogućavaju preživljavanje u nepovoljnim uvjetima okoliša. U stabilnim uvjetima mutatori gube prednost nad stanicama divljega tipa zbog nagomilanih štetnih mutacija. Mutator s povoljnom mutacijom može preživjeti ako ponovno stekne normalne MMR funkcije. Hiperrekombinacijski fenotip MMR mutatora omogućuje da se MMR funkcije vrate horizontalnim prijenosom gena iz stanica u okolišu. Tijekom bakterijske evolucije, ovisno o uvjetima okoliša, MMR su se funkcije neprekidno gubile mutacijom i ponovno vraćale horizontalnim prijenosom gena između divergentnih DNA. Tako nastala mozaična struktura MMR gena pokazatelj je evolucije u bakterija.