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Editing DNA Replication and Recombination by Mismatch Repair: From Bacterial Genetics to Mechanisms of Predisposition to Cancer in Humans

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Editing DNA replication and recombination by mismatch repair: from bacterial genetics to mechanisms of predisposition to cancer in humans

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SUMMARY

A hereditary form of colon cancer, hereditary non-polyposis colon cancer (HNPCC), is characterized by high instability of short repeated sequences known as microsatellites. Because the genes controlling microsatellite stability were known in bacteria and yeast, as was their evolutionary conservation, the search for human genes responsible for HNPCC became a 'targeted' search for known sequences. Mismatch-repair deficiency in bacteria and yeast produces multiple phenotypes as a result of its dual involvement in the editing of both replication errors and recombination intermediates. In addition, mismatch-repair functions are specialized in eukaryotes, characterized by specific mitotic (versus meiotic) functions, and nuclear (versus mitochondrial) localization. Given the number of phenotypes observed so far, we predict other links between mismatch-repair deficiency and human genetic disorders. For example, a similar type of sequence instability has been found in HNPCC tumours and in a number of neuro-muscular genetic disorders. Several human mitochondrial disorders display genomic instabilities reminiscent of yeast mitochondrial mismatch-repair mutants.

In general, the process of mismatch repair is responsible for the constant maintenance of genome stability and its faithful transmission from one generation to the next. However, without genetic alteration, species would not be able to adapt to changing environments. It appears that nature has developed both negative and positive controls for genetic diversity. In bacteria, for example, an inducible system (SOS) exists which generates genetic alterations in response to environmental stress (e.g. radiation, chemicals, starvation). Hence, the cost of generating diversity to adapt to changing conditions might be paid as sporadic gene alterations associated with disease.

1. INTRODUCTION

> Perhaps the most telling lesson of the past forty years has been the recognition that very different forms of life are built around essentially similar mechanisms. All species are discovered to have more in common with each other than their differences would suggest.
>
> (John Maddox in 'News and Views' *Nature, Lond.* 1993 **363**, 13.)

The identification of two genes (and other unpublished candidates) (Fishel *et al.* 1993; Leach *et al.* 1993; Bronner *et al.* 1994; Papadopoulos *et al.* 1994) linked to a predisposition to a hereditary form of colon cancer, hereditary non-polyposis colon cancer (HNPCC), was made possible due to the strong conservation among DNA repair functions between prokaryotes such as *E. coli* and eukaryotes such as the yeast *S. cerevisiae* (Reenan & Kolodner 1992*b*). The two genes discovered are human homologues to two components, *mutS* and *mutL*, from *E. coli*, of the so called mismatch-repair system (MRS). Indeed, the crucial observations which led to the discovery of two genes involved in HNPCC were clearly reminiscent of the known phenotypes of such genes in other organisms (see below) with *E. coli* being the best studied. Linkage was detected using a 'microsatellite marker' on chromosome 2 revealing a previously unknown location for a colon cancer gene (Peltomaki *et al.* 1993). Such probes were designed to detect changes in microsatellite DNA characterized as short (1–6 base pair [b.p.]), repetitive DNA sequences occurring on average every 10^5 b.p. in genomic DNA. Surprisingly, the microsatellite linkage markers varied in size throughout the genome in most of the HNPCC tumours examined, indicating that nucleotides were both being added and deleted in microsatellite blocks (RER^+ phenotype for replication error) (Aaltonen *et al.* 1993; Thibodeau *et al.* 1993).

During DNA replication, DNA polymerases frequently misalign the newly synthesized template strand on short, tandemly repeated sequences creating small 'loop-outs' of unpaired bases that are normally

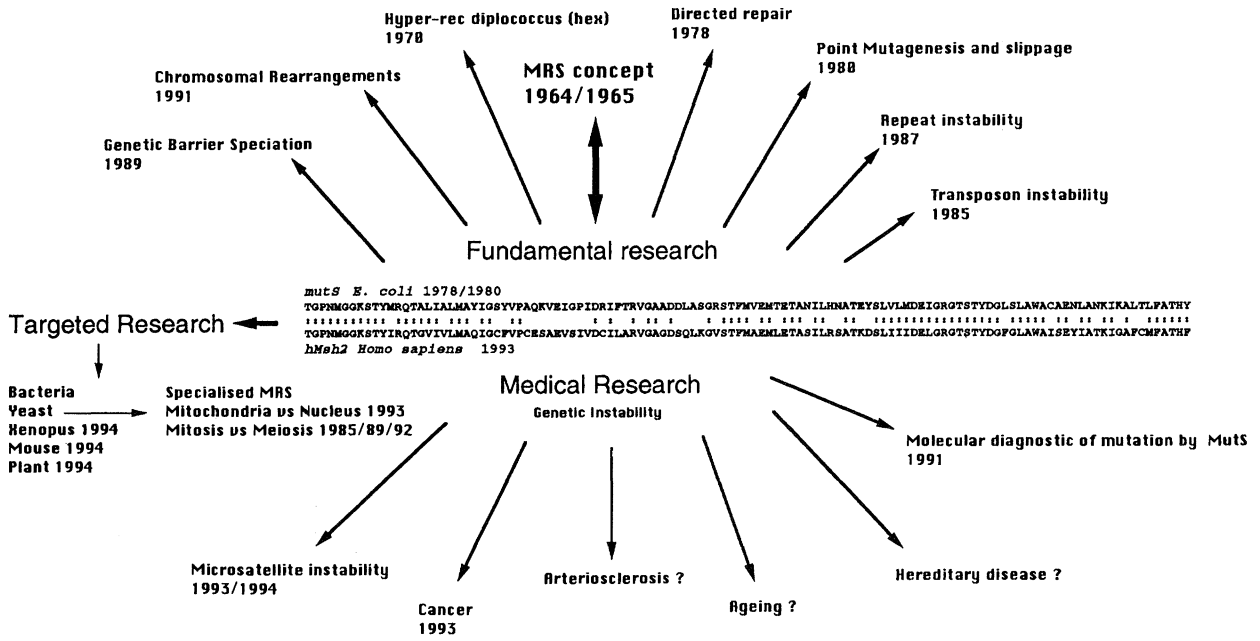

Figure 1. From bacterial mismatch repair system (MRS) to human genetic disease: a chronology. The centre of the figure presents the alignment of a well conserved region (including the ATP binding site) of the *E. coli mutS* gene with its human homologue *hMSH2*. The ':' indicate amino acid similarities. The years indicated provide the dates of the most important observations in the development of this field (see text for references). The association between MRS and arteriosclerosis is hypothetical but is suggested from studies on mutagenesis and clonality of arteriosclerosis plaques (Benditt & Benditt 1973). The possible link between the MRS, genetic disease and ageing is discussed in the text.

recognized and corrected by mismatch-repair enzymes (Dohet *et al.* 1986). Specific gene products encoded by the *mutS*, *mutL*, *mutH* genes are thought to operate on replication errors as a complex (Modrich 1991). Mutation in any of these three genes produces a strong mutator phenotype characterized by enhanced levels of base-substitution events, frameshift mutations at repetitive sequences, and recombination between homeologous (diverged) DNA sequences (Radman & Wagner 1986; Modrich 1991; Radman & Wagner 1993). As a result, it was thought that the genetic instability observed in human colon cancer cells probably reflected a defect in the maintenance of DNA integrity. This prediction was reinforced by the demonstration of a similar instability in $(CA)_n$ repeats, the most frequent form of microsatellite DNA in eukaryotes, in *E. coli mutL* and *mutS* mutants (Levison & Gutman 1987) and *S. cerevisiae msh2* (*mutS* homologue) *pms1* and *mlh1* (*mutL* homologues) mutants (Strand *et al.* 1993).

The striking sequence homology between components of the *E. coli* (MutS,L) and yeast (MSH2, MLH1, PMS1) mismatch-proteins was exploited and used to clone the first human homologue *hMSH2* and more recently, *hMLH1*, as well as homologues in *Xenopus* and mice (I. M. Varlet, M. Radman & N. D. Wind, unpublished results) (see figure 1). The Hopkins group speculated that approximately 40% of HNPCC non-hMSH2 tumours might be accounted for by another component of the mismatch-repair complex. To this end, a search for potential *mutL* homologues was initiated, using the expressed sequence tag method (EST) sequence data base which contains sequence information for approximately 10^5 cDNA fragments. Three candidates were found: one with homology to the yeast *MLH1* counterpart and the other two with homology to the other yeast *mutL* homologue *PMS1*. Confirmation that the two genes *hMSH2* and *hMLH1* were indeed responsible for the majority of HNPCC cases was provided by substantial evidence. This included the mapping of these two genes to the regions of chromosomes 2 and 3 known to contain the HNPCC loci, the finding of mutations in these two genes in HNPCC-afflicted individuals (as heterozygotes in somatic cells and homozygotes in tumours) and the hypermutability of microsatellite-like $(CA)n$ repetitive sequences in tumour cell lines compared to somatic cell lines (Fishel *et al.* 1993; Ionov *et al.* 1993; Leach *et al.* 1993; Bronner *et al.* 1994; Papadopoulos *et al.* 1994), and from the failure of nuclear extracts to repair known heteroduplex DNA substrates for mismatch-repair enzymes (Parsons *et al.* 1993).

2. AN ARRAY OF DIFFERENT DISORDERS

HNPCC has been hallmarked as one of the most common genetic diseases of man, affecting as many as one in 200 individuals. In addition, as judged by microsatellite instability, afflicted individuals appear to manifest an increased susceptibility to other epithelial cancers such as endometrial cancer. However, microsatellite changes have also been detected at significantly high incidence in sporadic cases of colon, bladder (Gonzalez-Zulueta 1993), pancreas and gastric carcinomas (Han *et al.* 1993) and to a much lesser extent in breast and ovarian cancers (Wooster *et al.* 1994). These findings demonstrate that this type of genetic instability is not

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unique to HNPCC and suggests that sporadic tumours can acquire this phenotype during tumour initiation and/or progression.

In addition to a likely association of a mismatch-repair-deficient phenotype with the above mentioned cancers, a genetic instability that produces an expansion of 5′CXG 3′ trinucleotide repeat sequences has been observed in seven other hereditary neurological and neuromuscular diseases, including Huntington's disease and Fragile-X syndrome (Nelson & Warren 1993). Although at present the mechanism underlying the relatively large expansions observed for these syndromes is not known, it is possible that the instability associated with these syndromes results from a compromised DNA-editing function.

As the search for further human DNA repair homologues continues a number of cancer and hereditary disease-associated loci may emerge. For example, some mitochondrial disorders characterized by gene deletions, duplications and the accumulation of point mutations (reviewed by Schapira 1993) could be linked to mismatch-repair deficiencies. As described below, yeast *msh1* mutants display a 'petite' phenotype due to mtDNA mutations resulting in the loss of mitochondrial functions (Reenan & Kolodner 1992*a*). Because of the apparent specialization of eukaryotic mismatch-repair components (described below) in terms of compartmentalization (nuclear versus mitochondrial), life cycle (mitotic versus meiotic functions) and repair substrates recognized (replication versus recombination mismatches), malfunctions in components of the mismatch-repair machinery can be predicted to manifest an array of different disorders.

3. MULTIPLE PHENOTYPES OF MISMATCH REPAIR

Mismatch repair was first postulated thirty years ago to explain particular aspects of genetic recombination including non-mendelian segregation of genetic markers (e.g. gene conversion) and excess recombination between closely linked markers (Holliday 1964; Ephrussi-Taylor *et al.* 1965). The molecular evidence confirming that such marker effects result from two different mechanisms of mismatch repair was obtained in 1987 using a phage lambda assay for the repair of specific mismatches (Jones *et al.* 1987). The characterization of pneumococcal *hex* mutants which display a mutator phenotype and no marker effects (Lacks 1966; Lacks 1970; Tiraby and Fox, 1973) raised the question of how the repair of replication errors could be directed to the newly synthesized strand. In 1975, Meselson proposed that the transient hemi-methylated state of newly replicated DNA could provide the signal to differentiate nascent and template strands (for a historical review, see Radman & Wagner 1988). One year later this was demonstrated experimentally using hemi-methylated heteroduplex molecules (Radman *et al.* 1978, 1980). The isolation and characterization of *E. coli mutS*, *mutL*, and *mutH* mutants soon confirmed the first observation (Glickman & Radman 1980) and since then many laboratories have further characterized the specificity of the generalized MRS (Radman & Wagner 1986; Claverys & Lacks 1986; Modrich 1991). *In vitro* repair assay has been developed (Lu *et al.* 1983) and the repair proteins have been purified facilitating *in vitro* reconstitution of mismatch-repair (Lahue *et al.* 1989).

The multiple phenotypes of *mutS* and *mutL* mutants in *E. coli* suggest that the MRS is the most versatile and multifaceted DNA-editing system known (see figure 2). The system involves mismatch (base–base or small insertion/deletion DNA loops) detection by MutS and MutL proteins and mismatch-stimulated unwinding by a helicase (helicase II, encoded by the *mutU* gene also known as *uvrD*). *E. coli* deficient in MutS, MutL, or MutU functions are genetically destabilized and show large increases in: (i) deletion events resulting from excision of transposable elements (Tex phenotype) (Lundblad & Kleckner 1985); (ii) spontaneous base substitution and frameshift mutagenesis (mutator phenotype) (Levison & Gutman 1987; Modrich 1991); (iii) large chromosomal rearrangements resulting from crossovers between diverged DNA repeats (chromosomal instability phenotype) (Petit *et al.* 1991; Radman & Wagner 1993*b*; Radman *et al.* 1993); (iv) recombination of genetic markers (hyper-rec phenotype) (Feinstein & Low 1986) and (v) recombination with related species such as *Salmonella* (disrupted genetic barrier phenotype) (Rayssiguier *et al.* 1989). The increases relative to wild-type bacteria range from ten-fold to 10^3-fold, depending on the particular phenotype measured. All of these phenotypes result directly from the failure of the MRS or its components to operate on mismatched DNA. Deletions result from the replicative bypass of secondary structures (e.g. hairpins) in template strands which, when mismatch repair is functional, are presumably melted by the mismatch-stimulated helicase activity because such structures generally contain mispaired or unpaired bases. MRS controls the fidelity of DNA replication by repairing the newly synthesized strands and restores the parental sequence; strand direction occurs via adenine methylation of GATC sequences or strand discontinuities (for a review, see Modrich 1991). The chromosomal instability, hyper-rec and disrupted genetic barriers phenotypes all result from the failure of the disabled MRS to disrupt mismatched recombination-intermediates.

As previously mentioned, it appears that the bacterial MRS may also be well-conserved functionally in eukaryotes ranging from yeast to man and possibly plants as well (G. Cerovic & M. Radman, unpublished results) (see figure 1). The main difference is that mismatch repair genes in eukaryotes appear to be specialized, interacting with either nuclear or mitochondrial DNA. In yeast, *msh1* mutants (*mutS* homologue 1) accumulate mitochondrial DNA deletions and rearrangements and therefore display a 'petite' phenotype even when the wild type *MSH1* gene is subsequently reintroduced (Reenan & Kolodner 1992). *msh2* and *pms1* mutants show both mitotic mutator and meiotic hyper-rec phenotypes (Williamson *et al.* 1985; Kramer *et al.* 1989; Reenan & Kolodner 1992*a*,*b*). *msh3* mutants show a weak mutator phenotype but show an increase in recombination between

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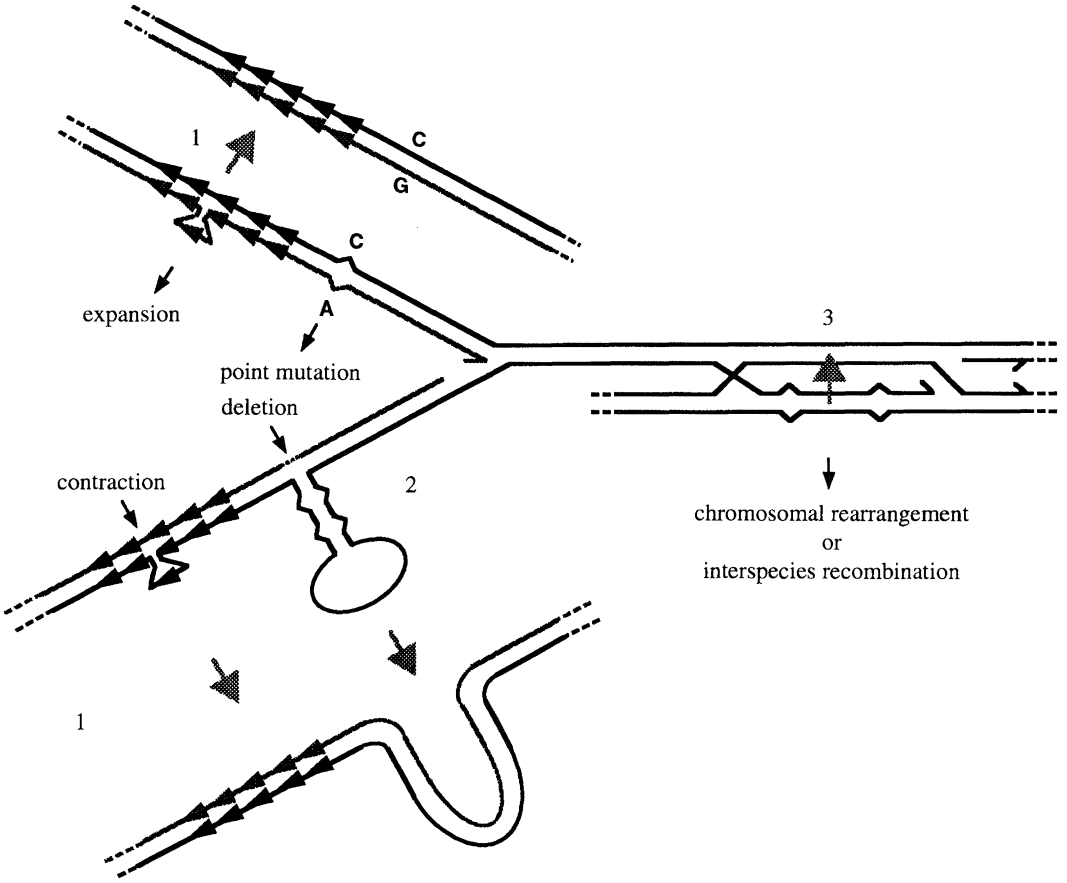

Figure 2. The maintenance of DNA integrity by the mismatch repair system (MRS).
Array of arrows indicate repeats of mono-, di-trinucleotides. Mismatched bases are indicated by opposing arrowheads.

1. Editing of the newly synthesized strand. Avoidance of point mutations and small insertions and deletions by the excision of the nascent DNA strand containing mismatched or unpaired bases.

2. Editing of the template strand. Avoidance of the formation of deletions by melting of deletogenic secondary structures containing mismatched base pairs.

3. Editing of recombination intermediates. Prevention of the formation of recombination intermediates between diverged sequences, thus avoiding chromosomal rearrangements and interspecific recombination. The implication for speciation and the prevention of horizontal gene transfer are discussed in Radman & Wagner 1993*b*.

The MutS protein detects mismatched and non-paired bases. The MutL protein binds to the complex to which helicase II (encoded by *mutU*) associates. The MutH protein of *E. coli* cleaves the newly synthesized strand at hemimethylated GATC sites.

diverged repeated sequences (New *et al.* 1993). The *MSH4* gene is expressed only during meiosis and *msh4* mutant phenotypes are under investigation (S. Roeder, personal communication).

4. MODEL ORGANISMS AND GENETIC DISEASE

Because of the overall conservation of the MRS between prokaryotes and eukaryotes and the fact that phenotypes of mismatch repair mutants in model prokaryotic and lower eukaryotic organisms are known, a number of predictions can be made concerning the implications for human genetics. To begin with, one would predict that a mismatch-repair deficiency could be responsible for predisposition to a number of human genetic disorders as for the predicted and recently demonstrated mutator phenotype in human colon tumours. Furthermore, since the MRS is also involved in the editing of recombination intermediates, a link between enhanced frequencies of chromosome rearrangements (as observed for such disorders as Fanconi's anemia or breast cancer) and a deficiency in mismatch repair could be predicted. Deletions between inverted repeat elements (such as Alu sequences or LINE elements) could be linked to a 'Tex-like' phenotype as described above. Ageing, in particular cases like progeria, could be linked to a mitochondrial mismatch repair deficiency, as characterized for yeast *msh1* 'petites'. The existence of a yeast gene (*MSH-4*) expressed only during meiosis and the hyper-rec effect of *msh2*, *pms1* and *mlh1* suggest a role for mismatch repair in fertility, as exemplified by bacteria in the maintenance of the genetic barrier between closely related species.

The specificity of mismatch recognition by the MutS protein facilitates, on one hand, the diagnostic identification of mutation (based on the formation of heteroduplex DNA between normal and test DNA (Wagner *et al.* 1995)) and, on the other hand, provides an explanation for the instability of micro-

satellite sequences. The microsatellite sequences monitored during the mapping of the human genome are found to be more or less stable. In 'normal' cells, tetranucleotide repeats are apparently the most unstable (Weber & Wong 1993). Yet it is well known that polymerase slippage occurs more frequently at mono-, di-, and trinucleotides (as seen in HNPCC tumours) whereas the MutS protein has been shown to efficiently recognize small base loop-outs up to three bases in size. The degree of the 'accordion' effect observed at microsatellites in normal cells is therefore due both to polymerase slippage errors and the specificity of recognition of such errors by MutS. Therefore, repetitions of mono-, di- and trinucleotides are highly destabilized as seen in HNPCC tumours where the mismatch repair system is defective. Larger repeats, not well recognized by the MutS protein, are much less destabilized but for the same reason, appear as the most unstable microsatellites in cells with functional mismatch-repair.

5. EVOLUTIONARY IMPLICATIONS

In general, the MRS is an essential negative regulator of genetic alterations (see figures 2 and 3) linked to genetic disease (germinal mutation) or sporadic cases (somatic mutation). To avoid the toll of deleterious mutations, all organisms have evolved highly sophisticated mechanisms of mutation avoidance. However, without genetic diversity it would be impossible to adapt to a changing environment. Undesirable genetic alterations therefore can be seen as the price paid for the capacity to evolve. To minimize this cost to the organism, evolution has selected inducible systems for the creation of genetic diversity, i.e. positively regulated systems for the generation of genetic alterations (see figure 3). The induction of a mutator system, like the sos system in *E. coli* (Radman 1974; Walker 1984), increases on demand, the plethora of genetic alterations that the MRS normally suppresses (see figure 2). An inducible system is advantageous: in a favourable environment the mutation rate is maintained at a low level as compared to conditions of environmental stress (as a response to radiation, chemical agents, conditions of starvation etc.) when the sos-system is induced and variability enhanced. In this way, an inducible system, like the bacterial sos system, responding to environmental factors could be responsible for sporadic as well as hereditary forms of a number of human diseases. Bloom's syndrome, which constitutively synthesizes a number of proteins which are inducible in normal cells, could be an example of an unregulated sos-like response in humans which in turn could explain the associated enhanced genetic instability and elevated frequency of cancers characteristic of this syndrome (Mallick *et al.* 1982; Herrlich *et al.* 1984). Analogously to research on mismatch repair, pursuit of the mechanisms of inducible genetic alteration systems like the bacterial sos system should provide an understanding of the potential link between environment and genetic disease.

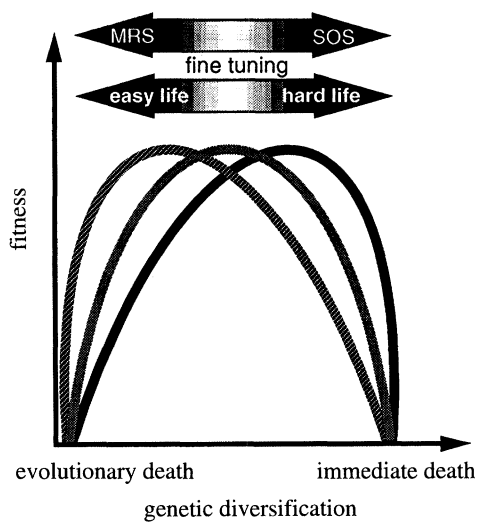

Figure 3. Genetic alterations and evolution. Too many mutations cause immediate death by error catastrophe. Too few mutations prevent adaptation to changing environments and lead to evolutionary death. The mutation rate which maximizes population fitness varies with adaptation to the environment, hence the necessity for fine tuning of the rate of genetic variability. Genetic diversification is fine tuned by an antagonistic control couple: mismatch repair and sos systems. The MRS appears to be constitutive and prevents all aspects of genetic diversification; whereas the sos system, which creates variability, is inducible and allows adaptation to adverse conditions. Genetic alterations, cancers and genetic diseases could appear as a price to pay for evolution.

We have speculated that clones presenting a mutator phenotype in nature (found in approximately 1% of bacterial and human populations (Tröbner & Piechocki 1984; Peltomaki *et al.* 1993)) may represent population 'hotspots' for genetic defects and speciation events (Rayssiguier *et al.* 1989; Matic *et al.* 1994). Now we suggest that, in addition, conditions causing sos induction provide time 'hotspots' for the population of cells or organisms to produce sporadic genetic defects and stimulate speciation events. These considerations further weaken the notion of random genetic alterations. Untangling genetic control mechanisms involved in the timing of DNA sequence evolution will be instrumental to our understanding of the origins of species and of genetic diseases. The presence of mutator genes in the human population, even in a heterozygous state, raises a question of major concern: what is the impact of this subpopulation on the overall incidence of genetic diseases?

Thanks to the considerable conservation of systems responsible for the maintenance of DNA integrity from bacteria to man, bacterial genetics remains a source of new paradigms piloting research into the causes of genetic alterations in man. The finding that a defect in the human MRS provides a predisposition to colon cancer is the most recent example of such a 'targeted' research.

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