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Mismatch repair as an important source of new mutations in non-dividing cells

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Abstract. This paper describes a mechanism which permits somatic cells to generate random mutations in the complete absence of cell proliferation. Knowledge of the existence of this mechanism should provide us with the basis for a better understanding of a number of important biological phenomena, and in particular may help to explain the origins of many human cancers.
Key words. Somatic mutations; mismatch repair; DNA damage; endogenous mutations; human cancer.

It can be argued that serious attempts to explore the possibility that living cells can generate new mutations independently of the processes involved in genomic replication are long overdue. This paper takes up the challenge, primarily by describing mechanisms which clearly ought to permit somatic cells to generate random mutations in the complete absence of cell proliferation. The mechanisms involved are remarkably simple; they are commonly referred to as post-replication mismatch repair (MMR) processes, and are virtually universal among cellular organisms[1]. Such mechanisms are normally assumed to be primarily concerned with mutation avoidance, of course, but it is axiomatic that they are also capable of generating mutations whenever they operate in circumstances which are not perfectly configured for mutation avoidance.

Thus, although MMR systems can indeed act in a strand-specific manner to replace bases which have been inserted inappropriately at particular sites in newly-synthesised sections of DNA, the ability of these systems to correct mismatches appears to be strictly limited to the most recently replicated sections of genomic DNA. Such sections of DNA usually appear to be labelled or 'tagged' for short periods of time immediately following their passage through the replication fork by cellular programmes which (a) allow the relevant parental and newly-synthesised DNA strands to be distinguished from one another, albeit transiently, and (b) are beginning to be well understood in organisms ranging from bacteria such as *Escherichia coli* to insects (*Drosophila melanogaster*) and humans[2]. In all other situations, MMR systems are at least formally capable of operating in non-instructed or randomly-templated ways, and hence of *generating* mutations (see fig. 1, lower right).

When they do operate in a non-instructed manner, MMR systems appear to be concerned with restoring the structural integrity of DNA duplexes rather than with maintaining their informational integrity. Thus they appear to be *obliged* to generate new and complete mutations, doing so on every occasion upon which they remove 'correct' (i.e. parental) bases rather than 'incorrect' bases from mismatched pairs. On average, therefore, this will be the outcome of about half of their interactions with mismatches in 'untagged' sections of DNA.

Recognition of this potentially key role of randomly-templated MMR (RT-MMR) mechanisms in mutation *formation* may well shed some new light on several important biological phenomena. For example, given that the mutations generated by mismatch repair are likely to be time-dependent (rather than replication-dependent) in origin, their study is likely to be crucial to our understanding of the origins of the multiple mutations which have now been found in association with the cancer genotype (and phenotype) in several different types of human cancer.

Mismatch repair and error-avoidance

Although there are bound to be differences of detail among the vast numbers of different forms of life which exist and have yet to be considered in detail, the basic tasks and requirements for post-replicative MMR systems seem to be more or less identical in all cellular organisms from bacteria to humans[1-4]. In part this assumption is a function of the universality of the DNA duplex among living organisms, since the main problem which has to be solved by mismatch repair must always take the same basic form, namely the continued presence of mismatched base pairs in the new DNA duplexes created by the replication process itself. In a simplistic sense, the primary task of mismatch repair is the error-free correction of these mismatches, and it is clear that the goal of maximum fidelity in all DNA

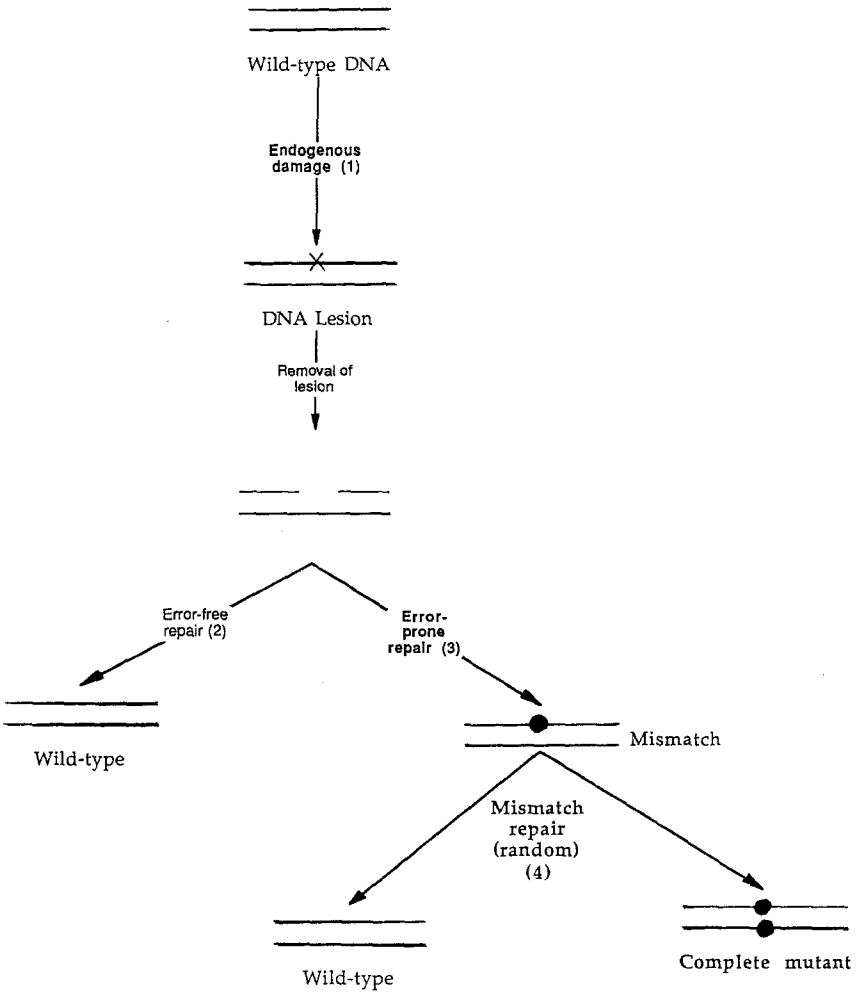

Figure 1. Pathway leading to the formation of spontaneous mutations in the absence of genomic replication. In step (1), an endogenous DNA-damaging agent generates a lesion which then provokes either error-free repair (EFR, step 2) or error-prone repair (EPR, step 3) of the lesion in question. The errors generated by EPR are assumed to include the formation of mismatched base pairs, which are almost certainly the penultimate premutational structures for base-pair substitution mutations. Resolution of these mismatched base pairs by randomly-templated mismatch repair (4) will then lead with roughly equal probability to mutations being fixed or wild-type sequences being restored. All of these steps can take place in a single cell, and at no stage is there a requirement for genomic DNA replication. Thus an individual cell can either remain wild-type or acquire a fully-mutant genotype, depending entirely upon the particular path which is followed at points (2), (3) and (4). It should also be noted that although the actual mutation-generating step (4) is (by definition) very highly error-prone in its own right, the numbers of mismatches which feed into this pathway can be controlled by ensuring that the ratio of EPR to EFR is low. Thus the actual numbers of spontaneous mutations which can be expected to result from the operation of a pathway of this sort will depend upon a combination of the amounts of endogenous DNA damage experienced by the cell in a given set of conditions and the relative amounts of EFR and EPR activity which are available to the cell at a given point in time. Both of these variables may be subject to change in changing environmental conditions, so that metabolically inert cells with relatively low levels of endogenous DNA-damaging agents may yield significantly fewer spontaneous mutants per unit of time than will cells which are fully engaged in oxidative metabolism. There is both direct and indirect evidence available to support this suggestion.

replication events can only be attained if this is done very consistently and in a manner which is strongly biased in favour of wrong-base removal[1,4]. Thus in all properly functioning mismatch correction systems, it is crucial for cells to be able to distinguish between a newly synthesised strand containing a recently inserted but inappropriate base on the one hand and its parental or template strand which by definition contains the correct one on the other.

How is this outcome achieved? In *E. coli*, the crucial distinction between the two component strands of a DNA duplex depends upon the post-synthetic methylation of adenine in a four-base (GATC) sequence in their DNA[1]. Methylation is carried out by a specific enzyme known as the *dam* methylase, which acts on the newly-synthesised strand. The net effect is that there is always a short time-period in the immediate post-synthetic phase when a short section of DNA is hemi-methylated, with the parental chain being fully methylated and the nascent chain unmethylated. This time period or 'window of opportunity' appears to be sufficient to allow the MMR system to monitor the newly-synthesised strand

for incorrect or mismatched bases, to cut them out, and to replace them with their correct equivalents. The replacement step is usually assumed to involve an accurate DNA polymerase acting in the appropriate manner to fill in the gap left following removal of the incorrectly-paired base and some of its immediate neighbours. The human MMR system appears to operate in more or less the same general fashion, except that the signal which is used to distinguish the nascent and old strands appears to be simply an appropriately-placed single-strand nick (rather than an unmethylated adenine) in the nascent strand[2].

Several interesting points can be made about the importance for living organisms of mechanisms such as the one just outlined. Firstly, the uncorrected error-rates associated with normal DNA replication processes appear to be too high to be tolerated by cells in almost any conceivable circumstances[1,3]. That MMR makes an important contribution to improved copying fidelity in bacteria can be illustrated by noting that it is widely believed to be responsible for improving the accuracy of the outcome of DNA replication events by anywhere between one and five orders of magnitude[5,6]. Secondly, although the details may differ from organism to organism, mismatch repair processes as such appear to be universal among cellular organisms, and indeed many organisms appear to possess more than one type of MMR system (*E. coli*, for example, has at least five)[1,2,4]. Also, organisms that lose the capacity to remove mismatched bases from their DNA by mutation usually acquire some dramatic new properties, including significantly increased spontaneous mutation rates. In bacteria, the relevant loss mutants are *mutH*, *L* and *S*, all of which do indeed exhibit pronounced mutator activity[1]; a minority of humans carry inherited mutations (h*MSH2* or h*MLH1*) in genes which are directly homologous to the bacterial *mutS* and *mutL* genes. These mutations all confer pronounced mutator activities on the cells of affected individuals, and in humans the markedly increased susceptibility to colon and other forms of cancer which characterise patients with hereditary non-polyposis colon cancer (HNPCC)[2,7,8].

Mismatch repair as a cause of mutations

Thus it is now widely accepted that MMR systems have a vital role to play in mutation avoidance in all cellular organisms which have DNA as their genetic material, and indeed that they may also be important in cancer avoidance in humans and other animals[2,7,8]. The obvious corollary of the first of these points does not appear to have been sufficiently explored as yet, however. This is that there must be quite lengthy periods of time when it is simply not possible for cells to distinguish between the two strands of any heteroduplex DNA molecules which they happen to contain, in which case any mismatch repair reactions which they do carry out will undoubtedly be unguided. The possible consequences are not trivial, since the most likely outputs of MMR reactions under these circumstances will be a fifty/fifty mixture of (i) accurately resolved mismatches and (ii) wrongly resolved mismatches.

In other words, randomly-templated MMR (henceforth RT-MMR) systems necessarily generate complete, fixed, mutations on approximately 50% of the occasions on which they are called upon to operate. Potentially unguided (or randomly-templated) activities of suitable MMR systems therefore appear to endow cells with a simple and straightforward way of generating mutations which is totally independent of genomic replication. This is an important point, if only because current dogma does not acknowledge the existence of any truly replication-independent form of mutagenesis[9]. Indeed, genomic or chromosome replication is usually assumed to be an essential element in the formation of spontaneous mutations by all mechanisms and in all species examined so far[9]. This assumption is so widespread that rates of mutation are routinely expressed in units such as 'per cell per generation' or 'per cell division'. There is simply no other widely accepted way of expressing them at present. I believe that this particular paradigm is overdue for change.

Only two assumptions are necessary for my basic argument to be sustained. The first is that mismatches can be formed in DNA molecules at times and in ways which have nothing whatsoever to do with genomic replication. The second is that mismatches can be removed from DNA molecules at times or in places other than those in which a 'window of opportunity', such as that offered by differential DNA methylation patterns in prokaryotes or nicked single-strands in eukaryotes, is available to guide the removal process. Conditions in which these two key assumptions are valid are certainly available in cellular organisms from time to time. One direct piece of evidence for this proposition stems from the existence of the well-known phenomenon of gene conversion. This phenomenon appears to be widespread among eukaryotic organisms, and is called gene *conversion* specifically because it involves the resolution of mismatches which are inevitably present in the heteroduplex regions of the recombinant DNA molecules *without regard for directionality* (see ref. 10). Thus the existence of the phenomenon known as gene conversion very clearly establishes that mismatches can be formed in DNA molecules by mechanisms that are independent of genomic replication, and that when this happens they may well be resolved by MMR enzymes in circumstances in which strand discrimination is all but impossible (e.g. at locations downstream of replication forks).

It does not appear to have been widely recognised as yet that mismatches formed in DNA by mechanisms which do not directly involve the genomic replication process

may also be susceptible to resolution by the so-called post-replicative MMR systems. Indeed it is difficult to imagine what barriers there might be to completion of MMR reactions in non-replicating cells in most cell types, especially since many of the organisms which have been examined in sufficient detail have been shown to harbour more than one type of MMR system. This observation applies even to the simplest of organisms, apparently. (As noted above, even *E. coli* appears to have at least five distinct MMR systems[1].) For resting cells to be able to generate new mutations in fully-replicated DNA molecules, therefore, they may merely have to acquire a few mismatches (e.g. in the course of a DNA damage-induced repair reaction) and then wait for the most suitable available RT-MMR system to convert roughly one-half of them into two-stranded mutant base pairs.

Error-prone DNA repair as a source of mismatched bases

Genomic replication and recombination aside, there is only one other way for mismatches to be introduced into DNA molecules – during repair replication. This process, often also called unscheduled DNA synthesis or repair resynthesis, occurs in a wide variety of situations (including both recombination and so-called recombinational repair events), and is usually associated in people's minds with cellular responses to the sorts of DNA damage which can be generated when cells are exposed to external mutagenic agent (e.g. radiation or any of a wide range of mutagenic chemicals)[11].

Most currently-favoured models for mutagenesis by radiation and chemicals make provision for the introduction of errors into DNA molecules at some stage during repair of the particular type(s) of damage that the mutagens in question have caused, and many of these models also make provision for what is usually called an error-prone repair step. This step is often either assumed or known to be inducible, as it is in the case of the well-documented SOS response of *E. coli*, for example[11].

Some models of induced and/or SOS mutagenesis make provision for a process called 'lesion bypass', in which one strand of the duplex contains a non-coding, mutagen-induced, lesion of one sort or another, opposite which a cellular DNA polymerase can somehow insert bases at random during attempts to effect a complete repair[12]. It is not difficult to envisage some of these randomly-inserted bases being tolerated in much the same way as mismatches are, i.e. by being allowed to persist until they can be converted into double-stranded mutations at the next round of genomic replication. Mutations generated in this particular way are usually classified as replication-dependent, of course, and will almost certainly represent the majority class in most situations.

There may be another way of handling non-coding DNA lesions, however. Suppose that certain of the hypothetical 'lesion + randomly-inserted-base' structures described above are dealt with by an MMR system as if they are mismatches. Given sufficient time, many such structures can be expected to arise in fully-replicated regions of the DNA (i.e. regions for which guidance with respect to strand identity is unavailable); if and when resolution of these structures does take place, the outcomes will presumably involve either (i) the re-generation of further hybrid structures (if indeed this is possible in such circumstances), or (ii) the formation of new structures in which the non-coding lesions have been removed, and the sets of bases which were inserted at random opposite them during the initial attempts to copy them therefore remain to act as the coding templates for what must in most cases automatically thereby become complete (i.e. double-stranded) MMR-generated mutations. Mutations which are generated in this way will of course be fully independent of genomic replication for their fixation.

Inducible error-prone repair mechanisms may well cause occasional slippage events to occur during DNA synthesis and hence may also lead to the formation of frameshift mispairs[2]. Once again, resolution of these frameshift mispairs in areas which are outside the 'windows of opportunity' for methylation-instructed MMR (i.e. most of them) may occur, and by definition should lead to the formation of complete mutations approximately 50% of the time (with restoration of the *status quo* being assured on the other 50% of occasions)[1].

With the above background in mind, it does not appear to be too difficult to explain, at least in principal, how classical mutagenic agents such as UV and some chemicals might generate mutations by first provoking the activities of a number of DNA repair transactions, one or more of which can then cause the occasional mismatch to be formed as the cell attempts to finish off a repair tract. The mismatches so formed can presumably then be resolved to yield complete (i.e. two-stranded) mutations, either (i) during the next scheduled replication cycle or (ii) in a replication-independent manner, by courtesy of RT-MMR.

Exactly the same principles should apply to the generation of mutations during attempts made by cells to repair (and hence survive) damage to their DNA caused by *endogenous* agents, some of which may be very important causes of what have often been referred to as 'spontaneous' mutations[9]. The sorts of 'natural' damage to DNA envisaged here include depurination, depyrimidination, deamination, single-strand breaks, double strand breaks, base modification and protein-DNA crosslinks; these may be caused by thermodynamic decay processes as well as reactive molecules formed from metabolic processes leading to free radicals such as OH, peroxides and sundry reactive oxygen species, and a

variety of other chemicals capable of producing lesions in DNA (e.g. endogenous alkylating agents). As with externally applied agents, the pathways to mutagenesis following the induction of DNA damage by these endogenous processes will almost certainly involve the formation of mismatched base pairs; in some cases this may happen directly because of base modification reactions, but in others it is more likely to happen in the course of attempts to repair the relevant damage. It also seems likely that a clear majority of the mismatched base pairs which are formed in the course of any of the above mentioned 'spontaneous' processes will be generated at random in those lengthy regions of the genome which are well-removed from the immediate passage of a replication fork, in which case the overall outcome of mismatch formation and resolution will normally be manifest in the form of a 50/50 mixture of wild-type and randomly-distributed mutant sequences, more or less exactly as illustrated in figure 1.

Generating mutations in non-dividing cells

There appear to be no strong reasons for the amounts and types of endogenous damage inflicted on the DNA of resting cells to differ greatly from those inflicted on the DNA of the dividing cells used for most experimental purposes in most laboratories. (Some quantitative differences can perhaps be expected in situations where resting cells are participating in fewer metabolic activities of the sorts which lead to the formation of free radicals and chemicals capable of causing oxidative damage, though.) Nevertheless, given that the average mammalian cell can expect to experience around 10,000 DNA modification events per cell *per hour* from purely endogenous sources[13], and that these events can be expected to engender comparably large numbers of DNA repair events, it may well be that there are plenty of opportunities for misrepair-engendered mismatches to be formed during the lifetime of such a cell.

Generally speaking, therefore, the cycles involving DNA damage, repair, misrepair and hence mismatch acquisition which we expect to occur in growing cells can also be expected to occur in resting cells. Assuming that these resting cells do not replicate their DNA for considerable periods of time (a not unreasonable assumption in many situations), it seems more likely than not that any mismatches which they do acquire in the course of error-prone repair, for example, will remain in their DNA until they can somehow be resolved. One way in which they can be resolved is of course by RT-MMR, in which case 50% of the mismatch-containing cells themselves should become fully mutant as a direct consequence of MMR-determined *structural* repairs to the appropriate DNA molecules.

Until now, most workers appear to have been assuming that any mismatches which do succeed in arising in non-dividing cells will be likely to persist more or less indefinitely in their DNA in the absence of a replication cycle to separate them into the anticipated 'one-normal-plus-one-mutant' daughter molecules; this is of course consistent with the prevailing belief that complete mutations only became 'fixed' (i.e. double-stranded) when replication occurs. With the benefit of hindsight, I believe that this should always have been seen as an impossible expectation, since there clearly are a number of well-documented instances of situations in which mutations virtually *must* have been generated in non-dividing cells if the data are to make any sense at all. (Some of these situations are mentioned below.) At the outset of this study, I was primarily concerned with one such problem, which relates to the formation of new mutations in stationary phase bacteria in circumstances which seemed to rule out replication events from any involvement in their formation (see refs. 14, 15 and 16). Interestingly, the very existence of a pathway which is capable of generating mutations in stationary phase cells of bacteria clearly implies that there may have been an early evolutionary origin for a process which may well prove to be of considerable relevance to the primarily eukaryotic phenomena which make up the tumour cell genotype (especially with regard to their acquisition of multiple mutations). Since the key features of tumourigenesis appear to be common to all multicellular organisms, insofar as we can tell, it would certainly make sense for their underlying cause to have had an early evolutionary origin – probably as a prokaryotic function, although an origin at the prokaryotic/eukaryotic transition may be worthy of further consideration.

One difficulty with the scenario for mutation-generation by RT-MMR outlined above is suggested by the observation that fully-methylated DNA does not appear to be a substrate for the *E. coli* MutH,L and S mismatch repair gene products in vitro[1]. This may not be a major problem, though, since there are almost certain to be many situations in resting cells in which some of the bases whose methylation status is critical to the MMR recognition process have either been temporarily removed from the DNA in the course of an ongoing repair reaction (and are therefore unmethylated), or else have undergone a spontaneous depurination or depyrimidination onslaught and hence have lost their methylation protection. Alternatively, a minor adjustment (perhaps mediated by a co-factor) to the recognition site of the relevant enzyme, or indeed the presence of a second, methylated sequence-insensitive, recognition/incision pathway (e.g. the VSP repair pathway[1]), will readily allow this particular problem to be overcome in vivo. Furthermore, the very existence of the phenomenon of gene conversion in eukaryotic organisms implies that eukaryotic cells are not normally prevented from carrying out unbiased mismatch repair in vivo.

Discussion

Having noted the existence in cellular organisms of a mechanism which supplies them with the ability to generate mutations in fully-replicated DNA *without any involvement of the normal genomic replication machinery*, it soon becomes apparent that any cells which have this capacity may also be able to adjust their own intrinsic mutability without having to make concomitant and permanent alterations to their future capacity to transmit genetic information to their progeny in the normal accurate manner (in other words, without having to bring about permanent alterations in any part of their genomic replication machinery). Thus for example subsets of cells which temporarily increase their levels of oxidative activity in response to environmental signals (e.g. those mediated by small molecular weight messengers or hormones) can certainly be expected to subject their genomes to higher levels of oxidative stress, and hence it is not at all unreasonable to expect them also to exhibit increased levels of 'spontaneous' mutability.

There are several other important implications. Some of these are:

(i) The long-established tradition of describing mutation rates (and mutation frequencies) in units such as 'per cell per generation' or 'per cell division' is simply not relevant to mutations generated by randomly-templated mismatch repair. Thus it may make more sense to describe mutation rates in terms such as 'mutations per cell per unit of time', rather than 'mutations per cell per generation' . With this in mind, it should perhaps be acknowledged that many cell populations are likely to consist of mixtures of growing and non-growing cells for much of the time, and that most such populations can presumably therefore be expected to generate mixtures of replication-dependent and replication-independent mutations, each at their own, idiosyncratic, rates. On the evidence available to date, the former class are likely greatly to outnumber the latter class, certainly in the short term, and it may only be after lengthy periods of time that the replication-independent class begin to make a significant contribution to overall mutation yields.

(ii) The question of multiple mutations and their expected frequencies in individual cells now appears to be wide open to re-evaluation, this time from first – and quite different – principles.Thus the proposed role of RT-MMR in generating mutations in resting cells may well help to explain the otherwise troublesome finding that clones of cells from many different types of human tumour routinely contain several more mutations than can possibly be accounted for on a 'per generation' basis[4,9,17]. A randomly-targeted time-dependent MMR mechanism may also provide a plausible explanation for the origins of tumours in primarily non-mitotic tissues such as brain and muscle.

(iii) The existence of a pathway for generating mutations in non-dividing cells in a time-dependent manner rather than in a replication-dependent manner means that much of the information we have about the age-dependent incidence of human cancers[18] (and also its multi-stage nature[9,17]) now makes very good sense.

Some very specific phenomena which can be explained more readily in the light of the RT-MMR model for mutation generation include: (i) the time-dependent appearance of most of the spontaneous mutants arising in chemostat cultures of a tryptophan-requiring strain of *E. coli* growing on limiting tryptophan (or conversely, their demonstrated independence of growth rates and hence of numbers of replication events)[19]; (ii) the recovery of spontaneous mutants in non-dividing cells of *E. coli*[20]; (iii) the recovery of the reciprocal products of mitotic exchange in experiments involving UV-irradiation of the *inos-2* mutant of *Ustilago maydis*[21]; (iv) the pure clones of lactose-negative mutants obtained in *E. coli* after treatment with 5-bromouracil[22]; (v) the additional mutations generated in *Drosophila* sperm during long-term storage in the seminal receptacle of the female[23]; (vi) the frequency of pure mutant clones arising in a non-selective forward mutation assay system involving repair-competent and repair-deficient strains of the yeast *Saccharomyces cerevisiae*[24]; (vii) the additional mutants arising in supposedly non-dividing (stationary phase) *Escherichia coli* and yeast cultures which have variously been described as directed, selection-induced or adaptive mutants[14,15,16]; (viii) the numerous cumulative changes in the chromosomes of *Drosophila* which are said to be associated with ageing[25]; and (ix) some well-known, ageing-related phenomena in humans, including perhaps some autoimmune diseases – which may well result from the formation of mutant or 'rogue' antibody-producing clones – and the plaques of mutant endothelial cells which have been implicated in atherosclerosis[26]. On a larger evolutionary scale, the model involving RT-MMR also provides support (and perhaps a mechanism) for the suggested association of rates of DNA evolution with metabolic rates put forward by Martin and Palumbi in 1993 on the basis of their findings with a wide range of eukaryotic species[27].

Somewhat ironically, it may well be precisely because the RT-MMR mechanism is so decidedly error-prone that its potential contribution to mutagenesis – usually regarded as an outcome of extremely rare events, and certainly not of events which occur roughly 50% of the time – has been overlooked by us all for so long. However, when the relatively low rates of mismatch formation by EPR (misrepair) mechanisms are taken into account, the final numbers of complete mutations which are likely to be generated in a replication-independent manner no longer seem to be improbably large.

Overall, it seems likely that the numbers of mismatches arising in the DNA of resting cells will be quite low

under 'normal' conditions; the actual numbers will be open to influence by a wide range of factors, however, including for example the presence or absence of applied mutagens, or the presence or absence of high levels of any of the many endogenous oxidative and other chemicals which have the capacity to cause DNA damage[28]. Moreover, the levels of the most significant potentially mutagenic endogenous chemicals can themselves often be influenced by a wide range of more general environmental factors, including temperature, pH and the osmotic and gaseous environments.

It may seem ironic that it is the very properties of mismatch repair systems which make them invaluable to cellular organisms as mutation-reduction systems which simultaneously endow them with the capacity to *generate* mutations whenever they are confronted with the need to resolve mismatches in fully-replicated DNA molecules. But this may be inevitable, given that MMR systems have two distinct tasks to perform when they deal with DNA molecules which contain mismatched base pairs. One of these tasks is relatively straightforward, involving only restoration of the *structural* properties of a DNA molecule from a mismatch-containing (heteroduplex) state to the fully base-paired (and hence structurally normal) homoduplex state; the second task has a great deal more demanded of it, since it involves (perfect) restoration of the *informational* content of the molecule as a sine qua non, and it is therefore not at all surprising that this task should have much more complex requirements (including making use of hemi-methylated DNA molecules as substrates, for example).

Thus the widespread distribution of cancer in multicellular organisms may simply reflect the fact that the same mismatch repair systems which serve to reduce the genetic variability of cell populations when they are transmitting their genetic material from generation to generation also provide them with the capacity to carry out purely structural repairs to fully-replicated DNA molecules, thereby (but incidentally) ensuring that they will possess a significant capacity for post-replication variability.

Clearly there are also situations in which the operation of randomly-templated MMR systems can turn out to be advantageous for their host organisms. Such situations may well arise when unicellular organisms find themselves in changing environments, and could become especially apparent when the usable and available sources of energy are greatly restricted. Thus, the ability of non-growing cell populations to continue to generate variants in a time-dependent manner may well prove to be very advantageous in such circumstances.

There are also some specialised situations in higher organisms when the capacity to generate mutations in a replication-independent manner could be considered extremely valuable (e.g. in the immune response, where B lymphocytes appear to generate antibody diversity and promote affinity maturation by mechanism(s) which clearly require some form of somatic hypermutation[29,30]). Situations such as these notwithstanding, most of the mutational events which arise in a time-dependent manner in eukaryotes may well prove to be harmful for individual organisms (as is likely to be the case with most tumours, for example). If the views presented here are generally accepted, it may become possible to regard cancer as simply a non-adaptive or maladaptive corollary of a predominantly adaptive feature, namely the well-known ability of mismatch repair to minimise the genetic variability resulting from DNA copying systems *before* the genetic material which has just been duplicated has been transmitted from one generation to the next.

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