CMLS, Cell. Mol. Life Sci. 61 (2004) 2799–2811
1420-682X/04/222799-13
DOI 10.1007/s00018-004-4220-y

CMLS Cellular and Molecular Life Sciences

Review

Mitochondrial DNA mutators

F. Foury*, J. Hu and S. Vanderstraeten

Unité de Biochimie physiologique, Croix du Sud 2-20, 1348 Louvain-la-Neuve (Belgium), Fax: +32 10473872, e-mail: foury@fysa.ucl.ac.be

Received 25 May 2004; received after revision 21 June 2004; accepted 7 July 2004

Abstract. In this article we review our current knowledge of the mechanisms by which point mutations arise in the mitochondrial DNA (mtDNA) of *Saccharomyces cerevisiae* and discuss to what extent these mechanisms operate in human mtDNA mutagenesis. The 3′–5′ exonuclease proofreading activity of Pol γ ensures accuracy of mtDNA replication in both yeast and humans, while the role of base excision repair in mtDNA error avoidance remains debated. The mitochondrial mismatch repair Msh1 protein, which removes transitions in yeast, is absent in humans, a particularity that might cause accumulation of transitions, while the most frequent substitution in yeast mtDNA is A:T to T:A transversion. Proofreading-deficient mutator human cell lines and knockin mice have been created. They will be useful for studying the mechanisms by which mtDNA mutations accumulate in diseases, ageing, malignancy and drug therapy.

Key words. Base excision repair; mismatch repair; mitochondria; mutator; oxidative damage; PEO; Pol γ; yeast.

Introduction

Beside nuclear DNA the eukaryotic cell contains essential genetic information in the mitochondria. As any other DNA, mtDNA is prone to mutate. The human mitochondrial genome mutates at high rates, approximately 10 times faster than the nuclear genome. In the late 1980s mtDNA mutations causing mitochondrial disorders were identified in humans [1]. The mitochondrial genomes of the patients were shown to contain maternally inherited point mutations in the homoplasmic or heteroplasmic state, or deletions occuring sporadically or caused by mutations in nuclear genes. It is now estimated that the prevalence of mitochondrial diseases is 1 per 10,000 individuals. Moreover, the mitochondrial theory of ageing proposes that ageing is directly linked to progressive accumulation of mitochondrial mutations during life as a result of continuous exposure of mtDNA to reactive oxygen species (ROS) [2]. Point mutations may result from DNA replication errors, insufficient post-replicative error correction or error-prone repair of damaged DNA. Because the mitochondrial genome has unique features, in particular a small amount of genetic information borne by a multiplicity of identical DNA molecules (homoplasmy), the mechanisms by which these mutations arise and are fixed may be quite different from those described for nuclear genomes. As underscored by Bogenhagen in a review on mtDNA repair in vertebrates [3], it was postulated for many years that mtDNA replication was not faithful and mtDNA repair inexistent. The data accumulated in recent years show that this is not true and that mtDNA could not be maintained without systems correcting DNA replication errors and repairing DNA damage. However, our knowledge in this field is still fragmentary, and in many instances claims are not supported by solid experimental data. In particular, the role of oxidative damage in the accumulation of point mutations is not clear. Elucidation of the mutagenic mechanisms operating in the mitochondrial genome is therefore of great importance with regard to mitochondrial pathologies.

* Corresponding author.

Saccharomyces cerevisiae is considered a model organism for human housekeeping genes and more specifically for mitochondrial disease genes [4, 5]. The aim of this review is to describe the mechanisms by which mtDNA point mutations are generated in *S. cerevisiae*. We also discuss to what extent yeast is a model for humans.

The human and yeast mitochondrial genomes

Mitochondrial genomes are characterized by the coexistence of numerous identical molecules. The human mtDNA molecule is a supercoiled 16-kb circle, has high gene density and no introns [6]. The asymmetric strand-displacement model of mtDNA replication, which involves unique initiation sites on each strand and continuous DNA synthesis, has generally been accepted [7]. However, it was recently proposed that mtDNA replication is initiated at multiple sites, with the formation of Okazaki fragments and massive ribonucleotide incorporation [8]. The yeast 85.8-kb mtDNA molecule is characterized by a high A+T content (80%), introns and low gene density [9]. Though the genetic map of *S. cerevisiae* mtDNA is circular, mtDNA is mostly in the form of linear tandemly arrayed molecules [10]. Very active homologous recombination is a hallmark of the yeast mitochondrial genome [11] and plays an important role in replication and repair. The first mtDNA mutations (mit$^-$) causing respiratory defects were identified in *S. cerevisiae* in 1975 [12]. In this organism an astonishingly high number of nuclear alleles cause instability of the mtDNA [13], leading to the production of respiratory deficient mutants. These mutants, called cytoplasmic petites or rho$^-$, contain homoplasmic mtDNA molecules with large deletions, or may even be devoid of mtDNA [11]. In contrast, a direct role in accumulation of point mutations has been shown only for a few genes.

Mitochondrial DNA polymerase

The nuclear *MIP1* gene encoding the catalytic subunit of the mitochondrial DNA polymerase (Pol γ) was first identified in *S. cerevisiae* [14]. Mip1p, a 1354-residue polypeptide bearing homology to the prokaryotic Pol A family [15], is characterized by an N-terminal region containing the three typical motifs Exo1, Exo2 and Exo3 of the 3′–5′ exonuclease editing domain, and a C-terminal part containing the polymerase domain specific for the Pol A family. The polymerase domain of Pol A enzymes has been compared to a right hand, with a deep cleft accepting the template primer and surrounded by three subdomains [16]. The 'palm' at the bottom of the cleft is the site of the catalytic reaction and contains essential metal ions and conserved carboxylate residues, the 'fingers' interact with the template and incoming dNTP, and the 'thumb' makes essential contacts with duplex DNA.

The first Pol γ purified to homogeneity was obtained from *Drosophila* in 1986 and shown to be a heterodimer [17]. However, animal Pol γ genes were cloned and sequenced only many years later [18, 19]. Pols γ from higher eukaryotes share substantial amino acid sequence identity with Mip1p (~40%) [18]. Compared to prokaryotic Pol A, Pols γ contain a large novel domain of unknown function bridging the exonuclease and polymerase domains. The complexity and length of this spacer domain have increased during evolution from yeast to humans. Animal mtDNA polymerases contain an accessory subunit bearing homology to class IIa aminoacyl-transfer RNA (tRNA) synthetases that increases their catalytic activity and processivity [19]. This accessory subunit does not exist in fungi.

Pol γ proofreading-deficient mutants are strong mutators in mitochondria

The fidelity of DNA polymerases is based on both selectivity towards the incoming dNTP at the polymerization site and editing activity of the 3′–5′ exonuclease. It was first believed that Pol γ had no proofreading activity. However, in the late 1980s it was shown that Pol γ from *Drosophila*, pig and chicken had an in vitro 3′–5′ exonuclease activity editing mispaired bases [20–22]. However, the exonuclease activity could not be assigned to a specific subunit. Purified Mip1p has a 3′–5′exonuclease activity preferentially removing mismatches at the 3′ end of a partial duplex DNA [23, 24]. Interestingly, similarly to the human enzyme [25], the 3′–5′ exonuclease of Mip1p is poorly active on single-stranded DNA [F. Foury, unpublished observations].

The 3′–5′ exonuclease domain of all editing DNA polymerases contains invariant aspartate and glutamate residues coordinating two metal ions which play an essential role in the catalytic reaction [26]. Asp171 (Exo1), Asp230 (Exo2) and Asp347 (Exo3) of Mip1p have been substituted for glycine or alanine residues (fig. 1A) [23, 24]. The exonucleolytic activity of D171G, D230A and D347A Mip1 is ~10^4 times lower than wild-type. The doubly mutated D171G-D230A Mip1p has no measurable 3′–5′ exonuclease activity [24]. Proofreading-deficient Mip1 polymerases efficiently extend mismatches and have high misincorporation rates in vitro [24]. The frequency of misincorporation is the highest for the G:T mispair (also observed to a lesser extent with wild-type Mip1p), followed by C:A, C:T, A:A and T:T mispairs (table 1). The C:C mispair is negligible.

The singly mutated Exo$^-$ alleles introduced in *S. cerevisiae* result in a 100–200-fold increase in the frequency of mtDNA point mutations eliciting resistance to ery-

Table 1. Mip1p and Msh1p mismatch preferences.

A. Mispair	dNTP (μM)	% Misincorporation	
		Wild-type Mip1p	D171G-D230A Mip1p
T:G	0.25	0	51
	5.0	7	87
C:T	0.25		26
	5.0		50
C:A	0.25		72
	5.0		84
A:A	0.25		13
	5.0		64
C:C	25		19

B. Mismatch recognized by Msh1p	% Remaining radiolabelled duplex
T:G; G:T; A:C	15
G:G; A:A	40
T:C	50
G:A	55
A:G; T:T	60
C:C	80
G:C	100

(*A*) The rate of misincorporation is given for an in vitro assay using purified Mip1p and a partial DNA duplex in the presence of either dGTP, dTTP, dATP or dCTP at the indicated concentrations [24]. (*B*) The affinity of Msh1p for different mismatches is estimated by an in vitro assay using purified Msh1p, ATP, a radiolabelled homoduplex or heteroduplex DNA and unlabelled homoduplex or heteroduplex competitors that displace the radiolabelled DNA [45–46]. The affinity of Msh1p for a mismatch present in the unlabelled duplex is therefore inversely proportional to the amount of radiolabelled duplex that remains bound to Msh1p.

thromycin (E^R), while the increase reaches 1500-fold in the D171G-D230A mutant [23, 24] (table 2). Increased mutation rates are also observed for mitochondrial mutations eliciting resistance to chloramphenicol (C^R) and oligomycin (O^R). An in vitro random mutagenesis has led to the identification of several other residues playing an important role in the proofreading function of Mip1p in vivo [27] (fig. 1 A and table 2). These data have established for the first time that the proofreading activity of Pol γ plays a major role in accurate replication of mtDNA.

The D171G-D230A mutant accumulates cells devoid of mtDNA. Mitochondrial DNA instability might be explained by low polymerization processivity as observed in vitro with the purified mutant Pol γ, or by frequent DNA replication arrest resulting from the numerous replication errors.

The yeast studies have constituted a basis to develop human and mouse mutator Pols γ. Human cell lines overexpressing a proofreading-deficient Pol γ with the Exo1 D198A mutation were found to accumulate mtDNA mutations [28]. After 3 months of culture a plateau of approximately five mutations per 10 kb, mostly G:C to A:T transitions, was reached, followed by a decline in the mutation rate. This suggests that a threshold in the mutational load that could be tolerated by the cell was reached and followed by selection against mutations [28]. Moreover, it has very recently been reported that knockin mice expressing a proofreading-deficient Pol γ with the Exo2 D257A mutation accumulate somatic point mutations and deletions in their mtDNA [29]. The point mutations are evenly distributed in coding (cytochrome b) and non-coding regions of the mtDNA and affect all tissues. The young mice look normal, but they have reduced life expectancy. After 6 months of life, they start to show many signs of premature ageing (e. g. hair loss, curvature of the spine, loss of fertility, osteoporosis). They also exhibit a progressive reduction in respiration associated with focal cytochrome oxidase deficiency and increased mitochondrial mass. However, the symptoms of ageing are not associated with concomitant increase in the mutation rate. This suggests that somatic mtDNA mutations are produced very early, before birth, and the defects result from accumulation of physiological damage produced by the mutations during the life of mouse.

In recent years it has been shown that mutations in Pol γ [30] are a common cause of progressive external ophtalmoplegia (PEO), a mitochondrial disorder associated with multiple deletions of mtDNA [31]. Homozygous and compound heterozygous mutations in the 3′–5′ exonuclease domain of human Pol γ have been identified in recessive PEO (fig.1 A and table 2). The G268A and W312R mutations are deleterious at the homozygous state [32, 33]. Gly268 is a highly conserved residue in the Exo2 site, and it has been shown that the modification of its yeast equivalent Gly224 into aspartate gives a strong mutator phenotype [27]. The G268A mutation has also been found associated with A467T in the spacer region between Exo and Pol domains [33]. The common T251I mutation downstream of the Exo1 motif is compound heterozygous with a number of mutations in the exonuclease (R309L), spacer (A467T, P587L) or polymerase (G848S, H932Y) domains [34]. Data obtained from a small number of patient biopsy samples and cell cultures with the T251I, G268A and W312R mutations have revealed an increased mutation load both in coding and non-coding regions of mtDNA [33]. Although no familial history about these mutations is known that could firmly establish their mutator function, these data suggest that 3′–5′ exonuclease deficiency in humans decreases accuracy of mtDNA replication and may be a cause of disease.

Mutators in the polymerase domain may result from a decrease in dNTP selectivity or low expression of Pol γ

Increased mutation rates may be caused by decreased selectivity of the polymerase towards the incoming dNTP. A

Table 2. Mutations in the 3′–5′ exonuclease domain of yeast and human Pol γ.

A. Yeast mutation	Mutation rate	Human equivalent	Domain
Wild-type	1		
D171G	110	D198	Exo1
E173K*	210	E200	Exo1
S198L*	~5000	S225	between Exo1 and Exo2
G224D*	270	G268	Exo2
H225D*	495	H269	Exo2
D230A	220	D274	Exo2
S304L*	120	S355	between Exo2 and Exo3
D347A	90	D399	Exo3
T351I*	220	T403	Exo3
D171G–D230A	~1500		Exo1 + Exo2

B. Human mutation	Mutator	Homozygous	Domain	Yeast equivalent	Compound heterozygous	Domain	Yeast equivalent
T251I	yes	no	upstream Exo2	none	**R309L**	downstream Exo2	R265
					P587L	spacer	None
					R807P	spacer	R607
					G848S	Pol	G651
					H932Y	Pol	H734
G268A	yes	yes	Exo2	G224			
L304R	?	?	downstream Exo2	L260	A467T	spacer	I416
R309L	?	?	downstream Exo2	R265	T251I	downstream Exo2	None
W312R	yes	yes	downstream Exo2	F268			
D198A**	yes		Exo1	D171			

(*A*) The *mip1* mutations obtained by random mutagenesis are marked with an asterisk [27]. The relative frequency of E^R mutants in the mutator strains is normalized to wild-type strain. The human equivalent residue and localization are given. (*B*) Mutations in the 3′–5′ exonuclease domain of human Pol γ that cause PEO [32–34] are given as well as the yeast equivalent residue. The existing compound heterozygous mutations are also indicated as well as the domain where they are located.
The double asterisk indicates the mutation created in human mutator cell lines [28]. Residues in bold letters are conserved residues.

systematic search in yeast has revealed mutator alleles scattered along the polymerase domain, with a high spot between residues 714 and 724 (G714S, G715D, T716I, M721I and E724K) (fig. 1B) in the finger domain [27, 35]. Based on the structure of the Pol A family, these mutations have tentatively been localized between helices L and N, a region that is specifically conserved in Pol γ (fig. 1B). The modest increase in the frequency of point mutations is probably underestimated, since proofreading is functional. Interestingly, the human G923D mutation affecting a conserved glycine which is the immediate neighbour of the E724K mutator residue in yeast (fig. 1B) has been associated with PEO at the homozygous state [34]. The compound heterozygous H932Y mutation affecting a conserved histidine residue is also close to this region [34]. The purified G714S and T716I Mip1p exhibit the same apparent $K_{m(dNTPs)}$ and $K_{(DNA)}$ as wild type (table 3), an observation suggesting that decreased selectivity for the incoming dNTP is not the cause for increased mutability in vivo [35]. However, these yeast mutants are characterized by a thermosensitive phenotype with low amounts of mtDNA at 28 °C and total loss at 37 °C. This in vivo phenotype correlates with increased sensivity of the polymerization activity to elevated temperature in vitro and recovery of very low amounts of Pol γ in cell extracts, a trait suggesting that the region encompassing these mutations is important for proper folding. In addition, the mutant proteins have a low polymerization processivity and a high 3′–5′ exonuclease to polymerase activity ratio in vitro (table 3), suggesting that in vivo many mismatch excision events are not followed by forward polymerization but are associated with dissociation of the DNA-enzyme complex. Similarly, poorly expressed Pol γ and mutator activity were associated features in a *mip1* mutant whose enzyme was truncated at the 1006th codon [F. Foury, unpublished observations]. Thus these data suggest that the low capacity of DNA synthesis by the mutant mtDNA polymerases is associated with an increased frequency of point mutations. An explanation could be that low mtDNA load facilitates the propagation and fixation of the mutations. Moreover, combination of the truncation and Exo^- D171G mutations pre-

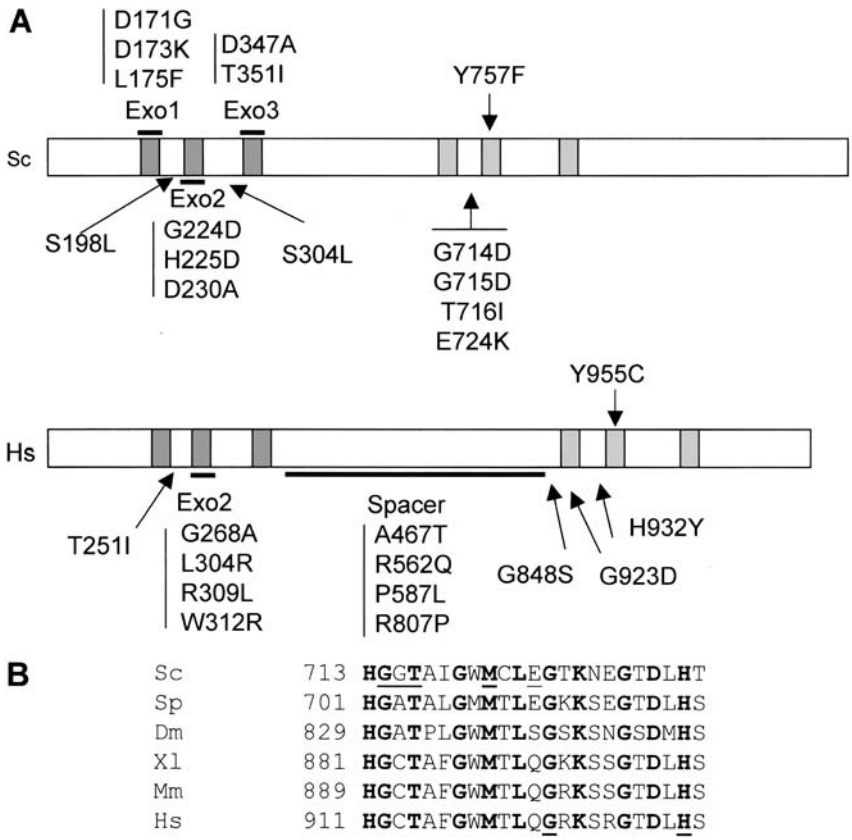

Figure 1. Mutator alleles in Pol γ. (*A*) Schematic linear representation of yeast (Sc) and human (Hs) Pols γ. The 3′–5′ exonuclease motifs Exo1, Exo2 and Exo3 and the three conserved motifs of the polymerase domain are represented by grey boxes. The human 3′–5′ exonuclease and compound heterozygous mutations are shown (see table 2). The G923D mutation in the human polymerase domain affects a conserved glycine whose yeast equivalent is Gly725, which is adjacent to mutator E724K. The Y955C mutation is responsible for a dominant form of PEO. (*B*) The Mip1p sequence from residue 713 to 732 is highly conserved in Pols γ. Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Dm, *Drosophila melanogaster*; Xl, *Xenopus laevis*; Hs, *Homo sapiens*. Conserved residues are in bold letters. Mutated residues in yeast or human are underlined.

Table 3. Steady-state kinetic constants of purified wild-type and mutant Mip1p.

Strain	$K_{(DNA)}$ (nM)	$K_{m(dCTP)}$ (μM)	$K_{m(dTTP)}$ (μM)	$K_{m(dATP)}$ (μM)	$K_{m(dGTP)}$ (μM)	Processivity	Exo/Pol
Wild-type	0.19 ± 0.02	1.8 ± 1.0	0.4 ± 0.1	1.2 ± 0.3	1.2 ± 0.5	1	1
G714S	0.22 ± 0.07	ND	ND	ND	ND	0.02	72
T716I	0.46 ± 0.06	2.6 ± 0.5	ND	ND	ND	0.07	5
Y757F	0.34 ± 0.10	16.8 ± 7.1	2.7 ± 0.4	2.4 ± 0.92	4.6 ± 0.3	0.91	1

The apparent K_m for dNTPs was determined using purified Mip1p, primed single-stranded M13 DNA and increasing dNTP concentrations [35]. Under the conditions used there was a linear relationship between time and DNA synthesis. The measurements were performed for two incubation times, 3 and 6 min. $K_{(DNA)}$ was measured using a gel mobility shift assay and a 68-mer hairpin oligonucleotide ^{32}P-radiolabelled at its 5′ end [35]. The binding assay was carried out at 4 °C for 15 min in a buffer containing 10 mM Tris-HCl pH 7.5, 10% glycerol and 0.05% poly(ethylene glycol) p-isooctylphenyl ether, in the absence of magnesium and in the presence of excess purified Mip1p and increasing concentrations of the hairpin substrate (from 0.02 nM to 1 nM). Before loading the samples onto an 8% polyacrylamide gel and running electrophoresis for 3 h at 4 °C in a buffer containing 7.5 mM Tris-borate, 0.1 mM EDTA and 5 mM dithiothreitol, a 1-h pre-electrophoresis was carried out. Processivity was estimated as the ratio of full-length single-stranded DNA to total DNA synthesis and normalized to wild-type [24].

cludes maintenance of the mitochondrial genome. This suggests that excess mtDNA errors cannot be tolerated below a certain threshold in mtDNA synthesis activity. As discussed below, mtDNA synthesis might be a limiting factor in mismatch repair.

Depletion of mtDNA might also be the cause for the increased accumulation of point mutations in human immunodeficiency virus (HIV)-infected patients treated with nucleoside analogues [36]. In these patients the nucleoside analogues constitute a severe block to mtDNA replication, as a result of their efficient incorporation by Pol γ into the nascent DNA chain and their poor excision by the proofreading activity [37].

Tyr757 Mip1p (fig. 1A) is a conserved residue in the Pol A and Pol γ families [13]. The three-dimensional structure of T7 DNA polymerase localizes the equivalent tyrosine residue in helix O (Tyr766 in *Escherichia coli* PolI), in close proximity to the incoming dNTP [38]. Y766S and Y766A PolI mutations increase the K_m for dNTPs and are associated with a mutator phenotype due to decreased selectivity for dNTP [39, 40]. Y757F and Y757S *mip1* genes were constructed by site-directed mutagenesis and introduced into yeast [35]. The apparent K_m for dNTPs is increased in the purified Y757F enzyme, specially for pyrimidine bases, with an 8-fold increase for dCTP and a 6-fold increase for dTTP [35] (table 3). The Y757F *mip1* mutant has a mild phenotype in vivo. The stability of the mitochondrial genome is not affected; however, a 4–5-fold increase in the frequency of E^R mutation accumulation is observed [F. Foury, unpublished observations]. In contrast, like the *E. coli* Y766S mutation, the Y757S *mip1* mutation is severe, with total loss of mtDNA [35]. Tyr757 is a residue of special interest. The modification of the human equivalent Tyr955 into a cysteine residue is a cause of dominant PEO [31]. The effects of the Y955C mutation have been characterized in vitro using recombinant purified Pol γ reconstituted with the p55 accessory subunit [41]. Though little effect is observed when the mutation is alone, the Y955C mutation in an exonuclease-deficient context results in a 45-fold decrease in the apparent binding affinity for the incoming dNTP, and a 10–100-fold increase in mismatches on primed single-stranded DNA. There is no evidence for increased frequency of point mtDNA mutations in PEO patients bearing an Y955C allele [32], an observation consistent with the small 2-fold increase in the in vitro mutation rate with proofreading-proficient Y955C Pol γ [41].

Mitochondrial mismatch repair

Twenty-five years ago, the first mtDNA mutators were isolated in yeast by two groups [42, 43]. Many years later, we found that our mutators mapped to two loci, *MIP1* and *MSH1*. The *MSH1* gene has been isolated by Kolodner and co-workers based on homology to the *E. coli* mismatch repair *MutS* gene [44]. *MSH1* encodes a mitochondrial protein which binds DNA and has an ATPase (dATPase) activity [45, 46]. The ATPase activity is stimulated by homoduplexes; however, Msh1p preferentially binds heteroduplexes, and there is an inverse correlation between ATPase activity and DNA affinity. It is believed that binding to mismatches is associated with a conformational change which attenuates the ATPase activity by decreasing the V_{max}. In contrast, ATP increases the specificity for the mismatch. Msh1p has the highest affinity for G:T and A:C mispairs, followed by G:G and A:A mispairs, and has very little affinity for the C:C mispair (table 1). The affinity depends, however, on the sequence context. Interestingly, the G:T and A:C mispairs, which are those preferentially extended by Mip1p, are also those which are best recognized by Msh1p, suggesting that they are efficiently corrected.

The deletion of the *MSH1* gene leads to high instability of the mtDNA, resulting in the loss of the mitochondrial genome after 20 generations [44, 47]. It has, however, been possible to isolate *msh1* mutants with high mutator activity (up to 100–500-fold increase of E^R mutations compared to wild-type) and which nevertheless maintain some mitochondrial DNA under selective pressure for respiration [24]. Diploid strains heterozygous for the *msh1* deletion show a 7-fold increase in the frequency of point mutations, indicating that Msh1p is a limiting factor for repair of mtDNA. Together, these data show that Msh1p plays an important role in the correction of DNA replication errors. Once Msh1p has bound to the mismatch, it is unknown by which mechanisms the mismatch is removed. As discussed below, Pol γ certainly plays an important role in mismatch repair. Interestingly, independent studies performed in humans and yeast [48, 49] have identified Mlh1p as a component of the mitochondrial proteome; moreover, yeast cells with an *mlh1* deletion have a growth defect on respiratory carbon sources. In the nucleus, Mlh1p forms a complex with Msh2p or Pms1p, and plays a major role in the processing of the mismatch [50]. Whether an Mlh1p isoform localizes to mitochondria requires further confirmation.

There is no *MSH1* homologue in the human genome, and it has been proposed that the high mutation rate of human mtDNA is due to the absence of mismatch repair. However, a mismatch-repair activity on G:T and G:G mispairs with no strand discrimination has recently been described in purified mitochondrial lysates shown to be devoid of MSH2 [51]. It has been hypothesized that this activity is involved in the repair of small mtDNA deletions at repetitive sequences, rather than in the correction of point mutations [52]. In addition, a proteomic approach has identified MSH5 and MLH1 in mouse mitochondria while neither MSH2 nor PMS1 were present [49], suggesting that the mitochondrial localization of MSH5 and MLH1

is not an artefact due to nuclear contamination. In the nucleus *MSH5* plays no role in mismatch correction but is required for meiotic crossing-over [53].

S198L Pol γ, a supermutator in the exonuclease domain with measurable 3′–5′ exonuclease activity

The yeast S198L mutant was isolated for its extremely high accumulation of E^R mutations, up to 5000 times higher than wild-type [27]. This phenotype is associated with instability of the mitochondrial genome, especially at high temperature. Ser198 is a conserved residue in Pol γ located slightly downstream of the Exo1 site (fig. 1A), in a region exposed to the surface of the protein corresponding to a loop between β-sheet 4 and helix B in the three-dimensional structure of the 3′–5′ exonucleolytic domain [16]. The biochemical properties of the S198L mutant are quite different from those of the other Exo⁻ mutants. Despite giving a strong mutator phenotype in vivo, S198L Pol γ has still ~10% of the wild-type 3′–5′ exonuclease activity [54]. Like wild-type, S198L Pol γ has a marked preference for mismatches and has only low mispairing and mismatch extension properties [54]. The S198L protein is poorly expressed and unstable, specially at 37 °C, suggesting a folding defect.

Interestingly, a large-scale screening for alleles decreasing the mutator activity of the S198L mutant has identified the intragenic E173K *mip1* mutation localizing to the Exo1 site [54]. All tested *mip1* mutations at Exo1, Exo2 and Exo3 sites also behave as partial suppressors of the S198L hypermutator phenotype (table 4). For instance, the mutation rate of the double S198L-D171G mutant is ~10-fold lower than that of the S198L mutant. In addition, we have found by Western blot analysis that the amount of the S198L-D171G Pol γ in mitochondria is ~5 times higher than that of the S198L Pol γ, suggesting that the Exo⁻ mutation stabilizes the S198L protein. S198L Pol γ stabilization is not dependent on a specific Exo⁻ mutation, but is linked to loss of the 3′–5′ exonuclease activity.

Mutational spectrum of 3′–5′ exonuclease *mip1* and mismatch-repair *msh1* mutants

A mutational spectrum has been determined using an in vitro assay consisting of a single-stranded DNA fragment of the *Lacz* gene copied by purified wild-type and mutant Mip1 Pol γ [55]. After DNA elongation, the gap-filled plasmids were transformed into an *E. coli mutS* strain, and colonies were screened for mutations in the *Lacz* gene by a blue/colorless test. The fraction of colorless to blue colonies was ~5 times higher for 3′–5′ exonuclease deficient Mip1p than for wild-type [54]. Transitions were two times more frequent than transversions both for wild-type and mutant Mip1p. Two mutational hot spots were observed. One is a sequence containing a stretch of five contiguous C and three T and accounts for the high frequency of transitions (C to T), as well as deletions and additions (fig. 2). All substitutions are C to T transitions except one G to C transversion. In contrast to transitions, transversions are scattered along the sequence. In view of the small number of DNA samples sequenced (~15 for each Mip1p), it could not be assessed whether the mutation spectrum is different in wild-type and mutant Mip1p. The type of substitutions suggests that Mip1p generates in vitro a high fraction of transitions at repetitive pyrimidine stretches as a result of transient misalignments. Similar substitutions by transient misalignment have been observed for mammalian Pol γ [53].

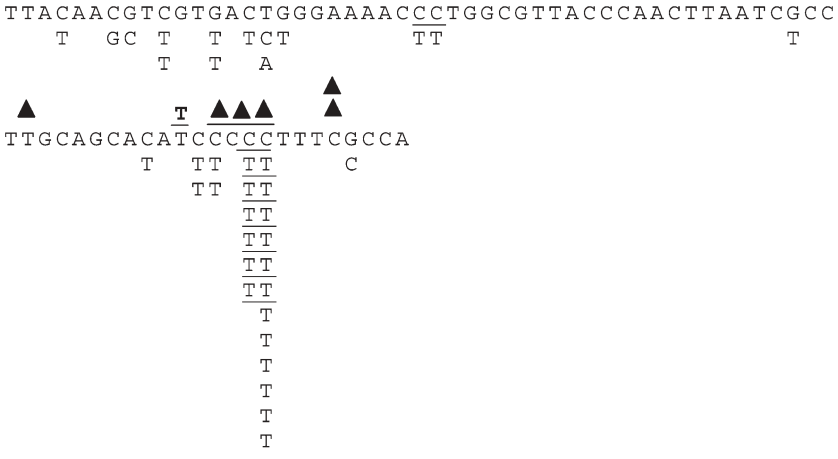

Figure 2. Spectrum of errors generated in vitro by purified yeast Pol γ in a fragment of *Lacz* gene. A linear sequence of the template strand containing the two mutation hot spots is shown. The single base substitutions are shown by letters below each line and represent the changes in the template strand. The base changes underlined represent simultaneous adjacent base substitutions in the same DNA sequence. Base additions and deletions are indicated above the sequence by a bold letter for additions and a black triangle for deletions.

Table 4. Mutator properties of S198L mutation and suppression by Exo⁻ mutants.

Strain	E^R
Wild-type	1
S198L	~5000
E173K	~100
D171G	~100
L175F	~25
D230A	~200
D347A	~100
E173K–S198L	~400
D171G–S198L	~650
L175F–S198L	~2000
D230A–S198L	~450
D347A–S198L	~500

The relative frequency of E^R mutations is normalized to wild-type strain [24, 54].

Table 5. Mutational spectra of mutator strains at the E^R locus.

Strain	Substitution	G^1	A^2	A^3
Wild-type	A	8		
	G		8	
	T	1	13	
	C		4	
Transitions (% total)	47			
D171G-D230A	A			
	G			2
	T		11	
	C		2	
Transitions (% total)	27			
S198L	A	6		
	G		8	1
	T		5	
	C		3	
Transitions (% total)	65			
msh1-1	A			
	G		11	
	T		2	
	C		3	2
Transitions (% total)	65			
msh1-2	A			
	G		14	1
	T		2	
	C		1	
Transitions (% total)	83			

The spectrum of yeast mtDNA mutations in vivo is quite different. We have determined the frequency of occurrence of several antibiotic resistant mutations [24]. Erythromycin is a particular suitable marker. Resistance to erythromycin can result from a change in the 21S ribosomal RNA (rRNA) gene at any of the three nucleotides marked in bold letters (5′AAGACG**G**^{1}**A**^{2}**A**^{3}AGACCC). Moreover, cellular resistance to erythromycin is similar in wild-type and mutator strains, so that no bias should be introduced in the selection of E^R clones. A roughly equal fraction of transitions and transversions was observed for the wild-type strain, with predominance of A:T to T:A transversions (~35%) (table 5). The frequency of A:T to T:A transversions is dramatically enhanced in the D171G-D230A mutant, reaching ~75% of the substitutions. In contrast, the frequency of A:T to G:C transitions reached ~65% in the S198L mutant. The two *msh1* mutants were also characterized by a high frequency of A:T to G:C transitions (61 and 82% for *msh1-1* and *msh1-2*, respectively), and a substantially decreased frequency of A:T to T:A transversions (12%). The fraction of A:T to C:G transversions was similar in all strains. The mutational spectrum of substitutions giving rise to oligomycin-resistant mutants at the *atp9* locus has also been analysed. The large majority of the mutations conferring oligomycin resistance are A:T to T:A transversions in all wild-type and mutant strains, with, however, a lower frequency for S198L *mip1* and *msh1* mutants [24]. Similarly, data reported in the literature show that the majority of the cytochrome b gene mutations are A:T to T:A transversions.

The extremely high frequency of A:T to T:A transversions in the exonucleolytic proofreading-deficient mutants indicates that in the absence of editing there is no system correcting the A:A (or T:T) mispairs that have been extended by Pol γ. The similarity of the mutation spectra for *msh1* and S198L *mip1* mutants suggests that the S198L mutant is defective in the correction of transitions as are *msh1* mutants. We propose that in addition to partial deficiency in proofreading S198L is defective in mismatch repair as a result of low DNA polymerization activity leading to saturation of the mismatch repair pathway. The high frequency of A:T to T:A transversions suggests that A:A and T:T mispairs are poorly recognized by Msh1p in vivo, while the G:T and C:A mispairs, which are the most frequently extended mispairs by Pol γ, are efficiently removed by the *MSH1*-dependent pathway. It is unlikely that A:T to T:A transversions result from misalignments by Pol γ, since it has been observed that poly(AT) sequences introduced into an artificially engineered *arg8* gene targeted to mtDNA are particularly stable [56]. Poly(GT) is much less stable with frequent two-base frameshifts [56, 57], and it has been shown that in *msh1* mutant strains, instability is 35-fold increased in heterozygous diploids, suggesting a role for mismatch repair in poly(GT) stability. However, the biological significance of this finding may be limited, since GT tracts are not common in yeast mtDNA.

Proofreading and mismatch repair play a major role in faithful maintenance of the yeast mitochondrial genome. The combination of specific proofreading and mismatch-repair deficient mutations that are not severe alone results

in rapid loss of the mitochondrial genome [24]. This error catastrophe shows that mismatch repair and proofreading act synergistically and have overlapping functions.

The mutation spectrum in humans is radically different. Substitutions are mostly A:G to C:T transitions both in the numerous mtDNA variants of the human population and in the mtDNA of mutator transgenic cell lines devoid of proofreading activity [28]. The current hypothesis is that no classical mismatch repair exists in human mitochondria that would correct the G:T mispair commonly produced by DNA polymerases.

Oxidative damage to mitochondrial DNA and mutagenesis

Oxidation of purine/pyrimidine bases and generation of abasic sites (AP) are major sources of lesions in the DNA [58]. These lesions constitute blocks to DNA replication, and their occasional bypass can be mutagenic. It has been calculated that 10,000 AP sites are produced per day and per nuclear genome in HeLa cells. Oxidative damage is so important that cells have evolved many overlapping specialized repair systems to eliminate the damage. A ubiquitous error-free repair system is base excision repair (BER) [59]. This system is mainly composed of DNA glycosylases that recognize and excise the modified bases, leaving AP sites. AP sites are removed by conjugated actions of specific endonucleases which incise on the 5′ side of the AP site, leaving a free 3′OH terminus for extension by DNA polymerase, and AP lyases which incise the DNA strand on the 3′ side of the AP site. The gap is filled up and closed by a DNA polymerase and a ligase.

A particularly debated and poorly understood aspect in mtDNA mutagenesis is the contribution of oxidative damage to the accumulation of point mutations. The mitochondrial DNA is particularly exposed to ROS (H_2O_2, $O_2^{-\bullet}$, $OH^{\bullet}$), which can be generated by incomplete reduction of oxygen during respiration. These ROS may generate several types of DNA lesions, including abasic sites and oxidized bases. However, reliable quantification of the mtDNA oxidative damage is difficult because base oxidation is readily produced during the isolation procedure of mtDNA, and indeed, the levels of oxidative damage in mtDNA vary considerably from one publication to another (see [60]). Even in the case of mtDNA, which is a multi-copy system, the inexistence of AP repair mechanisms could not be tolerated. BER is active in yeast mitochondria [3, 61]. Ogg1p, Ntg1p and Ung1p are DNA glycosylases localizing to both nucleus and mitochondria [62–65]. *OGG1*, cloned and sequenced first in *Saccharomyces cerevisiae*, encodes a DNA glycosylase removing 7,8-dihydro-8-oxyguanine (8-oxoG) paired with cytosine [62]. If 8-oxoG is not removed, this base can mispair with adenine during DNA replication, leading to increased frequency of G:C to T:A transversions. Ntg1p recognizes oxidized pyrimidine bases such as thymine glycol [63, 64]. Ung1p [65] removes uracil that has been produced by cytosine deamination or incorporated instead of thymidine. Ogg1p and Ntg1p have an associated AP lyase activity. Apn1p [66] is the major yeast AP endonuclease in the nucleus, but can be transported into mitochondria after physical interaction with Pir1p [67]. Pir1p probably masks the nuclear signal present in the C-terminal part of Apn1p. Apn1p incises DNA on the 5′ site of the AP site and also endows a 3′-phosphodiesterase activity removing the 3′-blocking groups generated by the AP lyase activity. The phenotype resulting from the deletion of these genes seems rather mild [59–65]. There is only a modest increase in mitochondrial petite frequency in *ogg1* and *ung1* deleted strains [68, 69], with no substantial increase in point mtDNA mutations (E^R). However, it has recently been reported that an *ogg1* deletion results in a 10-fold increase of mitochondrial O^R mutants as a result of an increased rate of G:C to T:A transversions [70]. This mutator effect is overcome upon overexpression of Msh1p, indicating that Msh1p might play a role in repair of oxidative damage [70]. The rather mild effects produced by BER gene deletions suggest that several repair mechanisms overlap, and it would be of interest to construct multiple deletion strains. In this respect, a remarkable synergetically deleterious effect on mtDNA stability has been observed in a strain deleted for both *NTG1* and *PIF1* [71]. *PIF1* encodes a DNA helicase [72] that plays a role in recombination and replication in mitochondria and telomere stability in the nucleus. A deleted *pif1* strain exhibits an increased load of E^R point mutations [71] that are mainly transitions [F. Foury, unpublished observations]. In conclusion, there is no clearly established relationship between oxidative damage to yeast mtDNA and increased frequency of spontaneous point mutations. However, our knowledge of the mechanisms involved in base damage, removal and fixation of the pre-mutagenic events in mitochondria is so limited that any conclusion is premature.

Human cells also possess genes that encode nuclear and mitochondrial forms of Ogg1, Ung1 and Ntg1 (called Nth1) [73, 74] (table 6). $OGG1^{-/-}$ null mice have been generated. They are viable and healthy, though they exhibit increased levels of 8-oxoG in their mitochondria [75]. There are probably more proteins involved in protection of mtDNA against oxidative damage in human than in yeast mitochondria. For instance, a homologue of *E. coli* MutY which has DNA glycosylase activity removing adenine mispaired with G, C and 8-oxoG [76] has a dual nuclear and mitochondrial localization in humans and is not present in *S. cerevisiae*. Moreover, Tgg1 is a new thymine glycol glycosylase activity in human mitochondria [77]. Human mitochondria also contain a bacterial MutT homologue (MTH1) absent from yeast, that de-

Table 6. Human and yeast genes of the mitochondrial BER pathway.

Gene	Function	*S. cerevisiae*	*H. sapiens*
OGG1	DNA glycosylase (8-oxodG.dC)	yes	yes
UNG1	DNA glycosylase (uracil)	yes	yes
NTG1 (NTH1)*	DNA glycosylase (oxidized pyrimidines)	yes	yes
APN1	AP endonuclease	yes	no
MUTY	DNA glycosylase (adenine)	no	yes
MTH1	8-oxo-dGTPase	no	yes

* The human gene is in brackets.

grades 8-oxo-dGTP [78]. In contrast to yeast Apn1, Ape1, the major AP endonuclease in humans, has no mitochondrial localization [74]. Therefore, human and yeast mitochondria share many enzymes involved in BER, though there are probably more repair enzymes in human mitochondria.

In vitro assays have shown that AP sites constitute an effective barrier to DNA synthesis by *Xenopus laevis* Pol γ [79]. In rare cases of translesional synthesis, dAMP is incorporated opposite the abasic site and is mutagenic. It has also been shown that nucleoside derivatives of benzo[a]pyrene are poorly elongated by human Pol γ in vitro and cause erroneous purine incorporation during translesion synthesis [80]. In contrast, Pol γ readily elongates 8oxo-dG, and it has been estimated that *X. laevis* Pol γ incorporates the correct dCMP opposite oxo-dG 73% of the time and misincorporates dAMP in 23% of cases [79]. Therefore, depending on the lesion there is a block in DNA synthesis or a pre-mutagenic event. However, whether oxidative damage is a source of accumulation of point mutations in the mtDNA is still a matter of speculation.

Yeast, a model organism for mtDNA mutagenesis in humans?

Though yeast is generally considered as a classical model organism for humans, the validity of this model for the study of the faithful maintenance of the mitochondrial genome may be questionable. This review has underlined the deep differences in organization of the yeast and human mitochondrial genomes and their response to mutagenic events. However, we have also shown that the two organelles share a number of basic enzymes involved in mtDNA replication and repair.

What is perhaps most important is that the yeast model has given a great impetus to the studies performed in humans. The gene encoding the catalytic subunit of the mitochondrial DNA polymerase was cloned and sequenced in yeast 7 years before publication of the sequence of the animal orthologues. The demonstration that the 3′–5′ exonuclease proofreading domain of Pol γ plays an important role in the fidelity of mtDNA replication was brought by studies in yeast with a panoply of mutator proofreading-deficient mutants. These data have encouraged scientists to produce mtDNA mutator human cell lines and mice. The knockin mutator mice created by Larsson and co-workers [29] have provided for the first time strong evidence that accumulation of somatic mtDNA mutations plays a role in ageing. The whole process is probably mediated through a cascade of events including apoptosis.With regards to the mitochondrial theory of ageing, it will be crucial to determine whether ROS are directly involved. These mutators should also be of particular importance to explore the mechanisms by which mtDNA mutations are fixed in human mitochondrial diseases [81]. There is now evidence for an increased accumulation of mtDNA point mutations in specific tumours [82, 83] and HIV-infected patients treated with nucleoside-analogue reverse-transcriptase inhibitors [36]. Moreover, in several patients with PEO, the 3′–5′ exonuclease domain of Pol γ contains mutations which are associated with an increased load of mtDNA mutations [32]. A very recent report has also shown that in the muscle of PEO patients with mutations in the mitochondrial helicase Twinkle or Pol γ, there is an age-dependent enhanced accumulation of point mutations in the control region of the mtDNA [84]. Yeast is a particularly powerful tool to search for suppressors of any genetic defect. It is thus probably not utopian to believe that genes suppressing the mutator activity of *mip1* mutants in yeast might also be present and effective in humans.

Acknowledgements. This work has been supported by the European Union MitAGE project, and by grants of the Belgian National Fund for Scientific Research and the Interuniversity Poles of Attraction Program (Belgian State, Prime Minister's Office, Federal Office for Scientific, Technical and Cultural Affairs).

1 Pulkes T. and Hanna M. G. (2001) Human mitochondrial DNA diseases. Adv. Drug Deliv. Rev. **49:** 27–43
2 Jacobs H. T. (2003) The mitochondrial theory of aging: dead or alive? Aging Cell **2:** 11–17
3 Bogenhagen D. F. (1999) Repair of mtDNA in vertebrates. Am. J. Hum. Genet. **64:** 1276–1281
4 Foury F. and Kucej M. (2002) Yeast mitochondrial biogenesis: a model system for humans? Curr. Opin. Chem. Biol. **6:** 106– 111
5 Steinmetz L. M., Scharfe C. and Deutschbauer A. M. (2002) Systematic screen for human disease genes in yeast. Nat. Genet. **31:** 400–404

6 Anderson S., Bankier A. T., Barrell B. G., de Bruijn M. H., Coulson A. R., Drouin J. et al. (1981) Sequence and organization of the human mitochondrial genome. Nature **290:** 457–465

7 Tapper D. P. and Clayton D. A. (1981) Mechanism of replication of human mitochondrial DNA. Localization of the 5′ ends of nascent daughter strands. J. Biol. Chem. **256:** 5109–5115

8 Bowmaker M., Yang M. Y., Yasukawa T., Reyes A., Jacobs H. T., Huberman J. A. et al. (2003) Mammalian mitochondrial DNA replicates bidirectionally from an initiation zone. J. Biol. Chem. **278:** 50961–50969

9 Foury F., Roganti T., Lecrenier N. and Purnelle B. (1998) The complete sequence of the mitochondrial genome of *Saccharomyces cerevisiae*. **440:** 325–331

10 Williamson D. (2002) The curious history of yeast mitochondrial DNA. Nat. Rev. Genet. **3:** 475–481

11 Dujon B. (1981) Mitochondrial genetics and functions. In: The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance, pp. 505–592, Strathern J. N., Jones E. W. and Broach J. R. (eds) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

12 Tzagoloff A., Akai A., Needleman R. B. and Zulch G. (1975) Assembly of the mitochondrial membrane system. Cytoplasmic mutants of *Saccharomyces cerevisiae* with lesions in enzymes of the respiratory chain and in the mitochondrial ATPase. J. Biol. Chem. **250:** 8236–8242

13 Contamine V. and Picard M. (2000) Maintenance and integrity of the mitochondrial genome: a plethora of nuclear genes in the budding yeast. Microbiol. Mol. Biol. Rev. **64:** 281–315

14 Foury F. (1989) Cloning and sequencing of the nuclear gene MIP1 encoding the catalytic subunit of the yeast mitochondrial DNA polymerase. J. Biol. Chem. **264:** 20552–20560

15 Braithwaite D. K. and Ito J. (1993) Compilation, alignment and phylogentic relationships of DNA polymerases. Nucleic Acids Res. **21:** 787–802

16 Beese L. S., Derbyshire V. and Steitz T. A. (1993) Structure of DNA polymerase I Klenow fragment bound to duplex DNA. Science **260:** 352–355

17 Wernette C. M. and Kaguni L. S. (1986) A mitochondrial DNA polymerase from embryos of *Drosophila melanogaster*. Purification, subunit structure and partial characterization. J. Biol. Chem. **261:** 14764–14770

18 Lecrenier N., Van Der Bruggen P. and Foury F. (1997) Mitochondrial DNA polymerases from yeast to man: a new family of polymerases. Gene **185:** 147–152

19 Kaguni L. S. (2004) DNA polymerase γ, the mitochondrial replicase. Annu. Rev. Biochem. **73:** 293–320

20 Kunkel T. A. and Soni A. (1988) Exonucleolytic proofreading enhances the fidelity of DNA synthesis by chick embryo DNA polymerase-gamma. J. Biol. Chem. **263:** 4450–4459

21 Kaguni L. S. and Olson M. W. (1989) Mismatch-specific 3′–5′ exonuclease associated with the mitochondrial DNA polymerase from *Drosophila* embryos. Proc. Natl. Acad. Sci. USA **86:** 6469–6473

22 Kunkel T. A. and Mosbaugh D. W. (1989) Exonucleolytic proofreading by a mammalian DNA polymerase. Biochemistry **28:** 988–995

23 Foury F. and Vanderstraten S. (1992) Yeast mitochondrial DNA mutators with deficient proofreading exonucleolytic activity. EMBO J. **11:** 2717–2726

24 Vanderstraten S., Van den Brule S., Hu J. and Foury F. (1998) The role of 3′–5′ exonucleolytic proofreading and mismatch repair in yeast mitochondrial DNA error avoidance. J. Biol. Chem. **273:** 23690–23697

25 Johnson A. A. and. Johnson K. A. (2001) Exonuclease proofreading by human mitochondrial DNA polymerase. J. Biol. Chem. **276:** 38097–38107

26 Bernad A., Blanco L., Lazaro J. M., Martin G. and Salas M. (1989) A conserved 3′–5′ exonuclease active site in prokaryotic and eukaryotic DNA polymerases. Cell **59:** 219–228

27 Hu J. P., Vanderstraeten S. and Foury F. (1995) Isolation and characterization of ten mutator alleles of the mitochondrial DNA polymerase-encoding MIP1 gene from *Saccharomyces cerevisiae*. Gene **160:** 105–110

28 Spelbrink J. N., Toivonen J. M., Hakkaart G. A., Kurkela J. M., Cooper H. M., Lehtinen S. K. et al. (2000) In vivo functional analysis of the human mitochondrial DNA polymerase POLG expressed in cultured human cells. J. Biol. Chem. **275:** 24818–24828

29 Trifunovic A., Wredenberg A., Falkenberg M., Spelbrink J. N., Rovio A. T., Bruder C. E. et al. (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature **429:** 417–423

30 Van Goethem G., Dermaut B., Lofgren A., Martin J. J. and Van Broeckhoven C. (2001) Mutation of POLG is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. Nat. Genet. **28:** 211–212

31 Van Goethem G., Martin J. J. and Van Broeckhoven C. (2003) Progressive external ophthalmoplegia characterized by multiple deletions of mitochondrial DNA: unraveling the pathogenesis of human mitochondrial DNA instability and the initiation of a genetic classification. Neuromol. Med. **3:** 129–146

32 Del Bo R., Bordoni A., Sciacco M., Di Fonzo A., Galbiati S., Crimi M. et al. (2003) Remarkable infidelity of polymerase gamma A associated with mutations in POLG1 exonuclease domain. Neurology **61:** 903–908

33 Di Fonzo A., Bordoni A., Crimi M., Sara G., Del Bo R., Bresolin N. et al. (2003) POLG mutations in sporadic mitochondrial disorders with multiple mtDNA deletions. Hum. Mutat. **22:** 498–499

34 Lamantea E., Tiranti V., Bordoni A., Toscano A., Bono F., Servidei S. et al. (2002) Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. Ann. Neurol. **42:** 211–219

35 Hu J. (1999) Biochemical characterization of mutants in the polymerization domain of the mitochondrial polymerase in *Saccharomyces cerevisiae*. Thesis, Catholic University of Louvain, Louvain-la-Neuve, Belgium

36 Martin A. M., Hammond E., Nolan D., Pace C., Den Boer M., Taylor L. et al. (2003) Accumulation of mitochondrial DNA mutations in human immunodeficiency virus-infected patients treated with nucleoside-analogue reverse-transcriptase inhibitors. Am. J. Hum. Genet. **72:** 549–560

37 Lee H., Hanes J. and Johnson K. A. (2003) Toxicity of nucleoside analogues used to treat AIDS and the selectivity of the mitochondrial DNA polymerase. Biochemistry **42:** 14711–14719

38 Kiefer J. R., Mao C., Braman J. C. and Beese L. S. (1998) Visualizing DNA replication in a catalytically active Bacillus DNA polymerase crystal. Nature **391:** 304–307

39 Carroll S. S., Cowart M. and Benkovic S. J. (1991) A mutant of DNA polymerase I (Klenow fragment) with reduced fidelity. Biochemistry **30:** 804–813

40 Bell J. B., Eckert K. A., Joyce C. M. and Kunkel T. A. (1997) Base miscoding and strand misalignment errors by mutator Klenow polymerases with amino acid substitution at tyrosine 766 in the O helix of the fingers subdomain. J. Biol. Chem. **272:** 7345–7351

41 Ponamarev M. V., Longley M. J., Nguyen D., Kunkel T. A. and Copeland W. C. (2002) Active site mutation in DNA polymerase γ associated with progressive external ophtalmoplegia causes error-prone DNA synthesis. J. Biol. Chem. **277:** 15525–15528

42 Johnston L. H. (1979) Nuclear mutations in *Saccharomyces cerevisiae* which increase the spontaneous mutation frequency in mitochondrial DNA. Mol. Gen. Genet. **170:** 327–331

43 Foury F. and Goffeau A. (1979) Genetic control of enhanced mutability of mitochondrial DNA and γ-ray sensitivity in *Sac-*

charomyces cerevisiae. Proc. Natl. Acad. Sci. USA **76:** 6529–6533
44 Reenan A. R. and Kolodner R. D. (1993) Characterization of insertion mutations in the *Saccharomyces cerevisiae* MSH1 and MSH2 genes: evidence for separate mitochondrial and nuclear functions. Genetics **132:** 975–985
45 Chi N. W. and Kolodner R. D. (1994) The effect of DNA mismatches on the ATPase activity of MSH1, a protein in yeast mitochondria that recognizes DNA mismatches. J. Biol. Chem. **269:** 29984–29992
46 Chi N. W. and Kolodner R. D. (1994) Purification and characterization of MSH1, a yeast mitochondrial protein that binds to DNA mismatches. J. Biol. Chem. **269:** 29993–29997
47 Koprowsk P., Fikus M. U., Mieczkowski P. and Ciesla Z. A. (2001) Dominant mitochondrial mutator phenotype of *Saccharomyces cerevisiae* conferred by msh1 alleles altered in the sequence encoding the ATP-binding domain. Mol. Genet. Genomics **266:** 988–994
48 Sickmann A., Reinders J., Wagner Y., Joppich C., Zahedi R., Meyer H. E. et al. (2003) The proteome of *Saccharomyces cerevisiae* mitochondria. Proc. Natl. Acad. Sci. USA **100:** 13207–13212
49 Mootha V. K., Bunkenborg J., Olsen J. V., Hjerrild M., Wisniewski J. R., Stahl E. et al. (2003) Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. Cell **26:** 629–640
50 Schofield M. J. and Hsieh P. (2003) DNA mismatch repair: molecular mechanisms and biological function. Annu. Rev. Microbiol. **57:** 579–608
51 Mason P. A., Matheson E. C., Hall A. G. and Lightowlers R. N. (2003) Mismatch repair activity in mammalian mitochondria. Nucleic Acids Res. **31:** 1052–1058
52 Mason P. A. and Lightowlers R. N. (2003) Why do mammalian mitochondria possess a mismatch repair activity? FEBS Lett. **554:** 6–9
53 Hollingsworth N. M., Ponte L. and Hasley C. (1995) MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in *Saccharomyces cerevisiae* but not mismatch repair. Genes Dev. **9:** 1728–1739
54 Vanderstraeten S. (1996) Fidelity of mitochondrial DNA replication in yeast. Thesis, Catholic University of Louvain, Louvain-la-Neuve, Belgium
55 Bebenek K. and Kunkel T. A. (1995) Analysing fidelity of DNA polymerases. Methods Enzymol. **262:** 217–232
56 Sia E. A., Butler C. A., Dominska M., Greenwell P., Fox T. D. and Petes T. D. (2000) Analysis of microsatellite mutations in the mitochondrial DNA of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **97:** 250–255
57 Koprowski P., Fikus M. U., Dzierzbicki P., Mieczkowski P., Lazowska J. and Ciesla Z. (2003) Enhanced expression of the DNA damage-inducible gene DIN7 results in increased mutagenesis of mitochondrial DNA in *Saccharomyces cerevisiae*. Mol. Genet. Genomics **269:** 632–639
58 Evans M. D., Dizdaroglu M. and Cooke M. S. (2004) Oxidative DNA damage and disease: induction, repair and significance. Mutation Res., http://www.mutationresearch.com
59 Boiteux S. and Guillet M. (2004) Abasic sites in DNA: repair and biological consequences in *Saccharomyces cerevisiae*. DNA Repair **3:** 1–12
60 Marcelino L. A. and Thilly W. G. (1999) Mitochondrial mutagenesis in human cells and tissues. Mutation Res. **434:** 177– 203
61 Croteau D. L., Stierum R. H. and Bohr V. A. (1999) Mitochondrial DNA repair pathways. Mutation Res. **434:** 137–148
62 Van der Kemp P. A., Thomas D., Barbey R., de Oliveira R. and Boiteux S. (1996) Cloning and expression in *Escherichia coli* of the OGG1 gene of *Saccharomyces cerevisiae*, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. Proc. Natl. Acad. Sci. USA **28:** 5197–5202
63 Alseth I., Eide L., Pirovano M., Rognes T., Seeberg E. and Bjørås M. (1999) The *Saccharomyces cerevisiae* homologues of endonuclease III from *Escherichia coli*, Ntg1 and Ntg2, are both required for efficient repair of spontaneous and induced oxidative DNA damage in yeast. Mol. Cell. Biol. **19:** 3779–3787
64 You H., Swanson R. L., Harrington C., Corbett A. H., Jinks-Robertson S., Sentürker S. et al. (1999) Ntg1p and Ntg2p: Broad specificity *N*-glycosylases for the repair of oxidative DNA damage in the nucleus and mitochondria of *Saccharomyces cerevisiae*. Biochemistry **38:** 11298–11306
65 Percival K. J., Klein M. B. and Burgers P. M. (1989) Molecular cloning and primary structure of the uracil-DNA-glycosylase gene from *Saccharomyces cerevisiae*. J. Biol. Chem. **264:** 2593–2598
66 Popoff S. C., Spira A. I., Johnson A. W. and Demple B. (1990) Yeast structural gene (APN1) for the major apurinic endonuclease: homology to *Escherichia coli* endonuclease IV. Proc.Natl. Acad. Sci. USA **87:** 4193–4197
67 Vongsamphanh R., Fortier P. K. and Ramotar D. (2001) Pir1p mediates translocation of the yeast Apn1p endonuclease into the mitochondria to maintain genomic stability. Mol. Cell. Biol. **21:** 1647–1655
68 Singh K. K., Sigala B., Sikder H. A. and Schwimmer C. (2001) Inactivation of *Saccharomyces cerevisiae* OGG1 DNA repair gene leads to an increased frequency of mitochondrial mutants. Nucleic Acids Res. **29:** 1381–1388
69 Chatterjee A. and Singh K. K. (2001) Uracil-DNA glycosylase-deficient yeast exhibit a mitochondrial mutator phenotype. Nucleic Acids Res. **29:** 4935–4940
70 Dzierzbicki P., Koprowski P., Fikus M. U., Malc E. and Ciesla Z. (2004) Repair of oxidative damage in mitochondrial DNA of *Saccharomyces cerevisiae*: involvement of the MSH1-dependent pathway. DNA Repair **3:** 403–411
71 O'Rourke T. W., Doudican N. A., Mackereth M. D., Doetsch P. W. and Shadel G. S. (2002) Mitochondrial dysfunction due to oxidative mitochondrial DNA damage is reduced through cooperative actions of diverse proteins. Mol. Cell. Biol. **22:** 4086–4093
72 Lahaye A., Stahl H., Thines-Sempoux D. and Foury F. (1991) PIF1: a DNA helicase in yeast mitochondria. EMBO J. **10:** 997–1007
73 Slupphaug G., Markussen F. H., Olsen L. C., Aasland R., Aarsaether N., Bakke O. et al. (1993) Nuclear and mitochondrial forms of human uracil-DNA glycosylase are encoded by the same gene. Nucleic Acids Res. **21:** 2579–2584
74 Takao M., Aburatani H., Kobayashi K. and Yasui A. (1998) Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. Nucleic Acids Res. **26:** 2917–2922
75 Klungland A., Rosewell I., Hollenbach S., Larsen E., Daly G., Epe B. et al. (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc. Natl. Acad. Sci. USA **96:** 13300–13305
76 Parker A. R. and Eshleman J. R. (2003) Human MutY: gene structure, protein functions and interactions, and role in carcinogenesis. Cell. Mol. Life Sci. **60:** 2064–2083
77 Takao M., Kanno S., Shiromoto T., Hasegawa R., Ide H., Ikeda S. et al. (2002) Novel nuclear and mitochondrial glycosylases revealed by disruption of the mouse Nth1 gene encoding an endonuclease III homologue for repair of thymine glycols, EMBO J. **21:** 3486–3493
78 Kang D., Nishida J., Iyama A., Nakabeppu Y., Furuichi M., Fujiwara T. et al. (1995) Intracellular localization of 8-oxo-dGTPase in human cells, with special reference to the role of the enzyme in mitochondria. J. Biol. Chem. **270:** 14659–14665
79 Pinz K. G., Shibutani S. and Bogenhagen D. F. (1995) Action of mitochondrial DNA polymerase gamma at sites of base loss or oxidative damage. J. Biol. Chem. **270:** 9202–9206
80 Graziewicz M. A., Sayer J. M., Jerina D. M. and Copeland W. C. (2004) Nucleotide incorporation by human DNA poly-

merase gamma opposite benzo[a]pyrene and benzo[c]phenanthrene diol epoxide adducts of deoxyguanosine and deoxyadenosine. Nucleic Acids Res. **32:** 397–405

81 Jacobs H. T. (2001) Making mitochondrial mutants. Trends Genet. **17:** 653–660

82 Bianchi N. O., Bianchi M. S. and Richard S. M. (2001) Mitochondrial instability in human cancers. Mutation Res. **488:** 9–23

83 Penta J. S., Johnson F. M., Wachsman J. T. and Copeland W. C. (2001) Mitochondrial DNA in malignancy. Mutation Res. **488:** 119–133

84 Wanrooij S., Luoma P., van Goethem G., van Broeckhoven C., Suomalainen A. and Spelbrink J. N. (2004) Twinkle and POLG defects enhance age-dependent accumulation of mutations in the control region of mtDNA. Nucleic Acids Res. **32:** 3053–3064