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Review

Mutations in DNA polymerase gamma cause error prone DNA synthesis in human mitochondrial disorders★

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This paper summarizes recent advances in understanding the links between the cell's ability to maintain integrity of its mitochondrial genome and mitochondrial genetic diseases. Human mitochondrial DNA is replicated by the two-subunit DNA polymerase γ (pol γ). We investigated the fidelity of DNA replication by pol γ with and without exonucleolytic proofreading and its p55 accessory subunit. Pol γ has high base substitution fidelity due to efficient base selection and exonucleolytic proofreading, but low frameshift fidelity when copying homopolymeric sequences longer than four nucleotides. Progressive external ophthalmoplegia (PEO) is a rare disease characterized by the accumulation of large deletions in mitochondrial DNA. Recently, several mutations in the polymerase and exonuclease domains of the human pol γ have been shown to be associated with PEO. We are analyzing the effect of these mutations on the human pol γ enzyme. In particular, three autosomal dominant mutations alter amino acids located within polymerase motif B of pol γ. These residues are highly conserved among family A DNA polymerases, which include T7 DNA polymerase and *E. coli* pol I. These PEO mutations have been generated in pol γ to analyze their effects on overall polymerase function as well as the effects on the fidelity of DNA synthesis. One mutation in particular, Y955C, was found in several families throughout Europe, including one Belgian family and five unrelated Italian families. The Y955C mutant

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Abbreviations: mtSSB, mitochondrial single-stranded DNA binding protein; PEO, progressive external ophthalmoplegia; pol γ, DNA polymerase γ.

pol γ retains a wild-type catalytic rate but suffers a 45-fold decrease in apparent binding affinity for the incoming dNTP. The Y955C derivative is also much less accurate than is wild-type pol γ, with error rates for certain mismatches elevated by 10- to 100-fold. The error prone DNA synthesis observed for the Y955C pol γ is consistent with the accumulation of mtDNA mutations in patients with PEO. The effects of other pol γ mutations associated with PEO are discussed.

The human mitochondrial DNA sequence was elucidated in 1981 (Anderson *et al.*, 1981) and is a 16 569 bp circular, double-stranded molecule that encodes 13 protein subunits with roles in electron transport and oxidative phosphorylation, and 22 tRNAs and 2 rRNAs required for mitochondrial protein synthesis. One noncoding segment, the displacement loop, contains several *cis*-acting elements required for initiation of transcription and replication. Individual cells have up to 10 000 discrete mitochondrial genomes distributed within 10–100 organelles. Point mutations or deletions in mtDNA generates mitochondrial genetic diversity within a single cell, and this heteroplasmy usually increases exponentially with age (Cortopassi *et al.*, 1992; Michikawa *et al.*, 1999). Disruption of mitochondria function by exceeding the functional threshold for mutation of mitochondrial DNA causes a wide range of respiratory and tissue degeneration diseases (Wallace, 1994). In addition, mitochondrial functions are linked to the aging process, apoptosis, sensitivities to HIV drugs, and possibly some cancers.

The mutation rate of mitochondrial DNA is estimated to be about 10-fold higher than that of nuclear DNA (Brown *et al.*, 1979), and this difference is generally attributed to increased DNA damage from elevated concentrations of endogenous reactive oxygen species produced as by products of oxidative phosphorylation (Richter *et al.*, 1988; Shigenaga *et al.*, 1994). Additionally, mitochondrial DNA is not protected by histones and mitochondria appear to lack some DNA repair systems present in the nucleus. Thus, mutations can result from unrepaired replication errors or exogenous DNA damage.

Mitochondrial DNA is replicated in an asymmetric fashion where H-strand synthesis is primed by transcription in the D-loop (Schmitt & Clayton, 1993). After two-thirds of the H-strand is replicated, the L-strand origin is exposed, permitting initiation of the L-strand synthesis. Mitochondrial DNA is replicated by an assembly of proteins and enzymes including pol γ, single-stranded DNA binding protein (mtSSB), DNA helicase, and a number of accessory proteins and transcription factors (Schmitt & Clayton, 1993; Shadel & Clayton, 1997). DNA pol γ is the sole cellular DNA polymerase present in the mitochondria, and therefore necessitates its role in both mtDNA replication and repair. Because pol γ is associated with DNA in the mitochondrial matrix, it is a target for oxidative damage by reactive oxygen species which may impair DNA replication and repair (Graziewicz *et al.*, 2002). The cDNA for the single-stranded DNA binding protein that functions in mitochondria has been cloned and predicts a molecular mass of 15 kDa for each monomer (Tiranti *et al.*, 1993). The human mtSSB is 31% identical to *E. coli* SSB. A replicative helicase was identified last year as encoded by the Twinkle gene (Spelbrink *et al.*, 2001), and a topoisomerase I gene has been identified to function in mitochondria (Zhang *et al.*, 2001).

POL γ STRUCTURE AND SUBUNIT COMPONENTS

Mitochondrial DNA accounts for approximately 1% of the cellular DNA, and pol γ activity comprises only 1–5% of the total cellular DNA polymerase activity (Fry & Loeb, 1986; Kornberg & Baker, 1992). The mitochondrial DNA polymerase is a nuclear-encoded protein with both a polymerase domain and a 3′→5′ exonuclease domain. Animal cell pol γ is com-

posed of two distinct subunits whereas only one polypeptide exists in yeast. Animal cell pol γ was shown unequivocally in *Drosophila melanogaster* by Kaguni and colleagues to contain two subunits of 125 and 35 kDa (Wernette & Kaguni, 1986). Pol γ from *Xenopus laevis* contains two subunits of 140 kDa and 50 kDa (Insdorf & Bogenhagen, 1989), and an initial report on human HeLa cell pol γ identified 140 kDa and 54 kDa polypeptides in the most purified fraction (Gray & Wong, 1992). Originally cloned from *Saccharomyces cerevisiae* (Foury, 1989), the coding sequences for the larger, catalytic subunit have been isolated from human, mouse, chicken, *Xenopus laevis*, *Drosophila melanogaster*, *Schizosaccharomyces pombe*, and *Pychia pastoris* (Lewis *et al.*, 1996; Ropp & Copeland, 1995; 1996; Ye *et al.*, 1996). The predicted proteins encoded by these genes range from 115 kDa for *S. pombe* to 143 kDa for *S. cerevisiae*, and all the genes contain conserved sequence motifs for polymerase and 3′→5′ exonuclease functions. Based on extensive homology alignments (Ito & Braithwaite, 1991), pol γ has been grouped with *E. coli* pol I in the family A DNA polymerase class.

The gene for the smaller subunit from *Drosophila* was first isolated by Kaguni and colleagues (Wang *et al.*, 1997). A BLAST search of the *Drosophila* polypeptide sequence identified a partial cDNA clone of the human pol γ accessory subunit (Wang *et al.*, 1997). Amino acid alignment of the *Drosophila*, human, and *Xenopus laevis* accessory subunits revealed significant homology to class II aminoacyl-tRNA synthetases, although the ATP binding site and the anticodon binding site are impaired (Carrodeguas *et al.*, 1999). The mouse pol γ accessory subunit was crystallized as a dimer and the crystal structure indicated structural similarity to glycyl-tRNA synthetase (Carrodeguas *et al.*, 2001). The human accessory subunit is a 55 kDa protein (Fig. 1) and is required for highly processive DNA synthesis (Carrodeguas & Bogenhagen, 2000; Johnson *et al.*, 2000; Lim *et al.*, 1999).

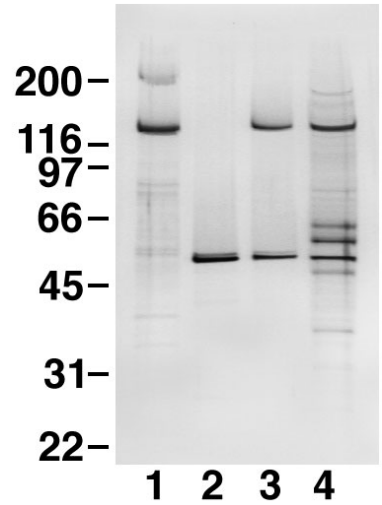

Figure 1. Preparation of human pol γ subunits.

Purified pol γ polypeptides were resolved on a 4–20% SDS/polyacrylamide gel and stained with ammoniacal silver: lane 1, 150 ng p140; lane 2, 40 ng p55; lane 3, about 120 ng reconstituted p140·p55 complex; lane 4, about 200 ng native HeLa pol γ complex. The positions of molecular mass standards (kDa) are indicated (from Lim *et al.*, 1999, with permission).

The p55 accessory subunit forms a high affinity, salt-stable complex with p140, and gel filtration and sedimentation analyses reveal a 190 kDa complex indicative of a native heterodimer (Lim *et al.*, 1999). Reconstitution of p140·p55 (Fig. 1) raises the salt optimum of p140, stimulates the polymerase and exonuclease activities, and increases the processivity of the enzyme by several 100-fold. Similar to p140, isolated p55 binds DNA with moderate strength and specificity for double stranded primer-template DNA. However, the p140·p55 complex has a surprisingly high affinity for DNA, and kinetic analyses indicate p55 enhances the affinity of p140 for primer-termini by two orders of magnitude. Thus the enhanced DNA binding caused by p55 is the basis for the salt tolerance and high processivity characteristic of pol γ.

THE FIDELITY OF DNA POL γ

Pol γ purified from chicken embryos or from pig liver mitochondria is highly accurate *in vi-*

tro, with these enzymes exhibiting error frequencies at a three nucleotide mutational target of $< 3.8 \times 10^{-6}$ per nucleotide and $< 2.0 \times 10^{-6}$ per nucleotide, respectively (Kunkel & Mosbaugh, 1989; Kunkel & Soni, 1988). Both enzymes contain intrinsic 3′ to 5′ exonuclease activities that prefer mispaired 3′-termini. Partial inhibition of these exonuclease activities with 20 mM dGMP increases the frequency of errors, suggesting the exonucleases proofread replication errors *in vitro* (Kunkel & Mosbaugh, 1989; Kunkel & Soni, 1988; Wernette *et al.*, 1988). Several lines of evidence indicate the exonuclease contributes to replication fidelity *in vivo*. Disruption of the exonuclease motifs in the yeast MIP1 gene generates a mutator phenotype, as exhibited by a several hundred-fold increase in the spontaneous frequency of forming mitochondrial erythromycin-resistant mutants (Foury & Vanderstraeten, 1992). Expression of exonuclease-deficient pol γ fusion proteins in cultured human cells also resulted in the accumulation of point mutations in mitochondrial DNA (Spelbrink *et al.*, 2000). Also, the loss of pol γ's exonuclease function in transgenic mice resulted in the rapid accumulation of point mutations and deletions in cardiac mtDNA, and the mutagenesis was accompanied by cardiomyopathy (Zhang *et al.*, 2000).

The human catalytic subunit of pol γ has high base substitution fidelity that results from high nucleotide selectivity and exonucleolytic proofreading (Johnson & Johnson, 2001; Longley *et al.*, 2001). Pol γ is also relatively accurate for single-base additions and deletions in non-iterated and short repetitive sequences. However when copying homopolymeric sequences longer than four nucleotides, pol γ has low frameshift fidelity, suggesting that homopolymeric runs in mtDNA may be particularly prone to frameshift mutation *in vivo* due to replication errors by pol γ. Pol γ also generates base substitutions inferred to result from a primer dislocation mechanism. Inclusion of the 55 kDa accessory subunit, which confers processivity to the pol γ catalytic subunit, decreases frameshift and base substitution fidelity. Kinetic analyses indicate that p55 lowers fidelity of replication by promoting extension of mismatched termini (Longley *et al.*, 2001).

POL γ IN BASE EXCISION REPAIR

Although mitochondria lack nucleotide excision repair (Clayton *et al.*, 1975) mitochondria have the capacity to repair damaged bases, (Croteau *et al.*, 1997; Croteau & Bohr, 1997; Croteau *et al.*, 1999; Driggers *et al.*, 1993; LeDoux *et al.*, 1999; LeDoux *et al.*, 1992; Sawyer & Van Houten, 1999). Pinz & Bogenhagen (1998) have reconstituted the minimum complement of repair proteins needed for base excision repair from *Xenopus laevis*, and Bohr and colleagues have isolated specific glycosylases which remove 8-oxo-dG and other damaged bases (Croteau & Bohr, 1997; LeDoux *et al.*, 1992; Stierum *et al.*, 1999). Additionally, the identification of the Msh1 gene suggests mismatch repair may occur in yeast mitochondria (Chi & Kolodner, 1994a; 1994b; Sia *et al.*, 2000).

As the only DNA polymerase present in mitochondria, pol γ is necessarily implicated in all such repair processes. Excision repair of a damaged base requires the concerted activities of a glycosylase to remove the damaged or inappropriate base, a class II AP endonuclease to incise the DNA 5′ to the AP site, a lyase activity to remove the 5′-terminal 2-deoxyribose-5-phosphate (dRP) sugar moiety from the downstream DNA, resynthesis, and ligation (Friedberg *et al.*, 1995). DNA repair enzymes that have been isolated from mitochondria include uracil-DNA glycosylase (Domena & Mosbaugh, 1985), AP endonuclease (Tomkinson *et al.*, 1988), an 8-hydroxydeoxyguanine specific endonuclease (Croteau *et al.*, 1997), and DNA ligase (Pinz & Bogenhagen, 1998). Pol γ can participate in uracil-provoked base excision repair reconstituted *in vitro* with purified compo-

nents, where subsequent to actions of uracil-DNA glycosylase and AP endonuclease, pol γ can fill a single nucleotide gap in the presence of a 5′ terminal deoxyribose phosphate (dRP) flap (Longley *et al.*, 1998). The removal of the dRP moiety can proceed *via* simple hydrolysis or by enzyme catalyzed β-elimination (Friedberg *et al.*, 1995). Reconstitution experiments with *Xenopus laevis* mitochondrial proteins localized the dRP lyase function to either the mtDNA ligase or pol γ (Pinz & Bogenhagen, 1998). The catalytic subunit of human pol γ was subsequently shown to catalyze the release of the dRP residue from incised apurinic/apyrimidinic sites to produce a substrate for DNA ligase (Longley *et al.*, 1998). The dRP lyase activity does not require divalent metal ions, and the ability to trap covalent enzyme-DNA complexes with $NaBH_4$ strongly implicates a Schiff base intermediate in a β-elimination reaction mechanism. The dRP lyase reaction proceeds by formation of a covalent enzyme-DNA intermediate that is converted to an enzyme-dRP intermediate following elimination of the DNA (Pinz & Bogenhagen, 2000).

DEFECTS OF POL γ IN MITOCHONDRIAL DISEASES

Mitochondrial genetic diseases are caused by point mutations and deletions in mitochondrial DNA (Wallace, 1992). Mutations can result from spontaneous errors of replication or from unrepaired chemical damage to DNA, such as oxidation or exposure to UV-radiation. To date, three mitochondrial afflictions have been attributed to alterations in pol γ: progressive external ophthalmoplegia, Alper's syndrome, and male infertility. Progressive external ophthalmoplegia is a rare disease characterized by the accumulation of point mutations and large deletions in mtDNA. Patients suffering from generalized PEO display ocular myopathies, ptosis, and muscle weakness as the major clinical findings. Sequence analysis through the pol γ gene (Ropp & Copeland, 1996) in a Belgian pedigree with dominant PEO identified a heterozygous A to G mutation at codon 955 (Y955C) and three autosomal recessive mutations in single and compound form (Van Goethem *et al.*, 2001). More recently, Zeviani and coworkers have reported eight new mutations in the pol γ gene sequence that cause heritable PEO, but the functional consequences of these mutations are unknown (Lamantea *et al.*, 2002). The mutations in pol γ that are associated with PEO and other mitochondrial disorders are shown in Fig. 2. Interestingly, the autosomal dominant mutations all map in the polymerase domain while recessive mutations map elsewhere, near the 3′–5′ exonuclease region and spacer regions. Errors of DNA synthesis are detected by the polymerase domain and transferred to the exonuclease domain for exonucleolytic proofreading. Faliure to excise mispaired termini results in dissociation of the polymerase from DNA. Thus, the dominant mutations may be altering DNA synthetic fidelity of the pol γ by altering base selection. Although the gene for the p55 accessory subunit of pol γ was also analyzed no mutations were identified in any of the PEO families (Lamantea *et al.*, 2002).

DNA polymerase γ is a family A DNA polymerase which is a class of DNA polymerases represented by the well studied *E. coli* DNA polymerase I and T7 DNA polymerase (Delarue *et al.*, 1990; Ito & Braithwaite, 1990; 1991). The crystallographic structure of T7 DNA polymerase with primer-template DNA and ddNTP as well as the *E. coli* DNA polymerase I have been solved at high resolution (Doublie *et al.*, 1998; Kiefer *et al.*, 1998; Ollis *et al.*, 1985). Located in the active site of pol γ, residues Arg943, Tyr955, and Ala957 are highly conserved residues among a wide variety of DNA polymerases. These residues which are found mutated in different PEO families, participate in recognition of the incoming nucleoside triphosphate (Fig. 3). The analogous residues in T7 DNA polymerase are

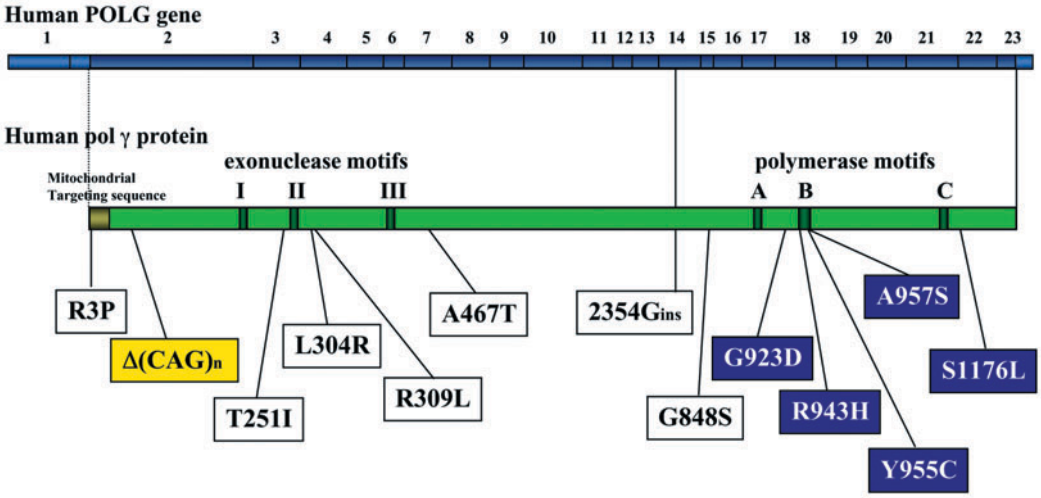

Figure 2. Mutations in the pol γ gene and amino acid changes in the protein that cause mitochondrial disorders.

Amino acid substitutions and mutations in the gene are indicated below the pol γ protein. Mutations in clear and blue boxes are recessive and dominant PEO mutations, respectively, while the mutation in the yellow box is associated with male infertility. The numbers of the exons in the pol γ gene are indicated on top.

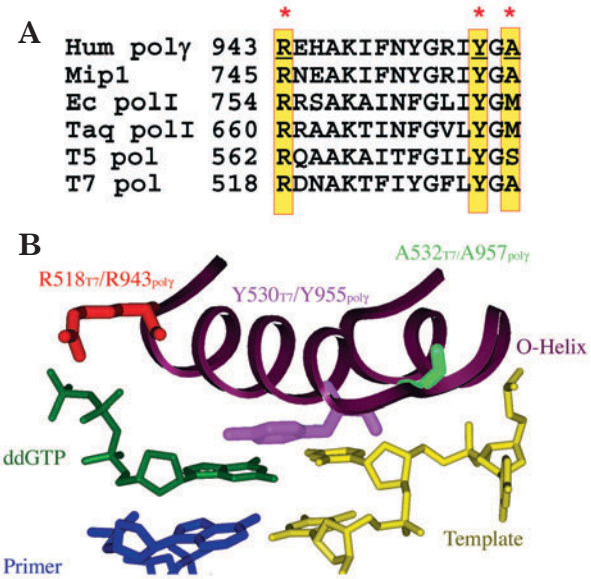

Figure 3. Sequence alignment and nucleotide binding pocket of pol γ.

A. Alignment of amino-acid sequence of the polymerase motif B from family A polymerase with the PEO mutations indicated by asterisk. Amino-acid sequences are from human pol γ (Hum polγ), *Saccharomyces cerevisiae* pol γ (Mip1), *E. coli* pol I (Ec polI), *Thermus aquaticus* pol I (Taq polI), T5 phage DNA polymerase (T5 pol), and T7 phage DNA polymerase (T7 pol). B. The nucleotide binding pocket of T7 DNA polymerase ternary complex showing the amino-acid residues that are altered in PEO. The relative positions of the primer, the DNA template, the incoming ddGTP, and the three residues in motif B that are mutated in different PEO patients.

part of the O-helix in the three dimensional structure, making up part of the fingers domain which is responsible for interaction with the incoming dNTP (Doublie *et al.*, 1998). Alteration of these residues in PEO should have significant effects on the selection and incorporation of nucleotides into DNA. We have overproduced several of the mutant pol γ proteins to analyze the biochemical effects of the PEO mutations on the function and fidelity of pol γ.

The Y955C mutation was found in several families throughout Europe, including one Belgian family and five unrelated Italian families, suggesting that the pol γ Tyr955 codon is a hot spot for the A to G transition that converts this codon to cysteine. The analogous residue in *E. coli* pol I is Tyr766. Alteration of Tyr766 in *E. coli* pol I to serine causes a subtle effect on $K_{m(dNTP)}$ and a slight decrease in k_{cat} attributed to a 2.5 fold increase in $k_{D(DNA)}$ (Polesky *et al.*, 1990). The three dimensional structure of T7 DNA polymerase in a ternary complex with DNA and nucleotide triphosphates places this residue in close contact with the incoming dNTP. Tyr530 hydrogen bonds to Glu480 to form part of the binding pocket for the incoming dNTP and to help

discriminate against ribonucleotides (Doublie *et al.*, 1998). Serine and alanine substitutions at the Tyr766 position in Klenow produces a mutator polymerase due to decrease stringency for selecting dNTP (Bell *et al.*, 1997; Carroll *et al.*, 1991). Although the Y766F substitution in Klenow fragment does not show appreciable increases in misinsertion, the alanine or serine substitutions at this position cause a 10-fold increase in base pair substitution and a 17-fold increase in deletions (Bell *et al.*, 1997). Interestingly, this 17-fold increase in deletions occurred between direct repeats. Biochemical analysis of the Y955C mutant polymerase indicates that it retains wild-type catalytic turnover efficiency but suffers a 45-fold decrease in affinity for dNTPs (Ponamarev *et al.*, 2002). The results we observed for the Y955C mutation in pol γ were consistent with the bacterial DNA polymerases containing the analogous mutation. The Y955C mutation increases the mutation rate at a single base pair by over 50-fold (Ponamarev *et al.*, 2002). The enhanced base substitution error rate of Y955C pol γ may promote deletions between direct repeats in mtDNA through a misinsertion event following correct synthesis through a direct repeat sequence. Failure of the polymerase to proofread the error or to extend the mismatch favors a slippage event between the direct repeats that creates a matched DNA terminus at a downstream template sequence. The Y955C study represents the first biochemical analysis of the mechanisms by which a mutator DNA polymerase can generate the mutations observed *in vivo* with certain mitochondrial diseases.

Arg943 in human pol γ is analogous to arginine residues 518 and 754 in T7 DNA polymerase and *E. coli* pol I, respectively. The structure of the T7 DNA polymerase predicts Arg943 to interact with the γ-phosphate group of the incoming nucleotide and may assist in the removal of the pyrophosphate leaving group (Astatke *et al.*, 1995; Doublie *et al.*, 1998). Alteration of this residue in *E. coli* pol I to alanine shows a 100-fold increase in $K_{m(dNTP)}$ and at least a 10-fold decrease in k_{cat} for insertion (Astatke *et al.*, 1995). Fidelity studies of R754A in Klenow showed a slight antimutator effect in this highly compromised mutant polymerase due to the enhanced discrimination against extending certain mismatches (Minnick *et al.*, 1999). Alteration of this residue to histidine as in PEO would most likely cause a decrease in affinity for the incoming nucleotide and possible alter fidelity.

Ala957 in human pol γ is analogous to Ala532 of T7 DNA polymerase, and this residue links α-helices O and O1 in T7 DNA polymerase. Changing this residue to Ser in PEO may alter the conformation of these two helices and change the affinity for dNTP as well as the fidelity by pol γ. Clinical analysis of PEO patients has revealed additional amino acid changes which predicts altered function for pol γ (Lamantea *et al.*, 2002; Van Goethem *et al.*, 2001). The other mutations found in PEO have not been studied with the bacterial DNA polymerases and the predicted effect is less obvious for the human pol γ, which stresses the need for biochemical analysis of these mutations. Table 1 lists the amino-acid mutations in PEO and their predicted effect on the pol γ function.

The gene for human pol γ contains a CAG trinucleotide tract that encodes a polyglutamine stretch near the N-terminus of the mature protein (Ropp & Copeland, 1996). Loss of the common ten-repeat allele (Fig. 2) is associated with male infertility and loss of sperm quality in different population groups (Rovio *et al.*, 2001). Although the deletion of this allele has no detectable effect on mitochondrial function in tissue culture cells (Spelbrink *et al.*, 2000), alteration of the CAG repeat may contributes to as much as 5–10% of infertility cases in the European population (Rovio *et al.*, 2001). Polyglutamine tracts in proteins can be sites for protein–protein interactions and this alteration in pol γ may result in suboptimal mtDNA replication.

Alper's syndrome is a rare but severe heritable, autosomal recessive disease that afflicts young children. Within the first few years of life, patients exhibit progressive, spastic paresis of the extremities and progressive mental deterioration leading to seizures, blindness, deafness, and eventual death. Naviaux reported an Alper's patient with reduced electron transport chain function, dicarboxylic aciduria, fulminant hepatic failure, and lactic acidosis which resulted in death at 42 months (Naviaux *et al.*, 1999). Skeletal muscle biopsy indicated a reduction of mtDNA content to 30% of normal with no detectable pol γ activity (Naviaux *et al.*, 1999). Although a heritable deficiency in mtDNA replication is suggested, the genetic defect(s) causing Alper's syndrome remain unreported.

Defects in other nuclear genes controlling maintenance of mtDNA have also been associated with mitochondrial diseases, including the genes for adenine nucleotide translocator 1 (ANT1) at locus 4q34-35 (Kaukonen *et al.*, 2000), thymidine phosphorylase at locus 22q13.32-qter (Nishino *et al.*, 1999), a putative mitochondrial helicase (Twinkle) at locus 10q24 (Spelbrink *et al.*, 2001), and an unidentified gene at locus 3p14-21 (Kaukonen *et al.*, 1996). Multiple mutations within the "Twinkle" gene encoding a putative mitochondrial helicase are causally linked to dominant PEO with mtDNA deletions (Spelbrink *et al.*, 2001), and mutations in the nuclear genes for ANT1 or thymidine phosphorylase also induce pathogenic mutation of mtDNA (Kaukonen *et al.*, 2000; Nishino *et al.*, 1999). Although predicting the ability of a defective mitochondrial helicase to interfere with mitochondrial DNA maintenance is fairly straightforward, models of mitochondrial DNA mutagenesis for ANT1 and TP mutants are more complex and involve a restriction or unbalancing of the available intra-mitochondrial pool of deoxynucleoside triphosphates. Nucleotide pool imbalance is known to enhance base substitution errors by pol γ (Kunkel & Soni, 1988;

Table 1. Conservation of PEO mutations in pol γ and the predicted effects

Mutation	PEO[1]	Conserved in pol γ	T7 DNA pol homology	Structural data	Predicted effect
R3P	R	No	–	–	Mitochondrial import
T251I	R	Only in animals	–	–	Structural, p55 interaction?
L304R	R	V, M	–	–	exo function? structural?
R309L	R	Yes	R145?	Surface aa in exo domain	DNA binding? exo function?
A467T	R	I in S.c.	–	–	exo function? structural
G848S	R	Yes	–	–	?
G923D	D	Yes	G503?	On surface between α-helices	Structural?
R943H	D	Yes	R518	H-bonds to γ-phosphate	$\uparrow K_{m(dNTP)}$ $\downarrow k_{cat}$
Y955C	D	Yes	Y530	Interacts w/ Incoming dNTP	$\uparrow K_{m(dNTP)}$ $\downarrow$ fidelity
A957S	D	Yes	A532	Links α-helices O & O1	Structural, DNA binding?
S1176L	D	N in S.c.	?	?	?

[1] R designates recessive and D designates dominant phenotype mutation.

Wernette *et al.*, 1988). Additionally, Wallace observed mtDNA rearrangement and increased production of reactive oxygen species in the mitochondria of ANT1$^{-/-}$ knockout mice, suggesting pathogenesis may result from enhanced oxidative damage to mtDNA (Esposito *et al.*, 1999).

In summary, autosomal defects in genes responsible for the maintenance of mtDNA have recently been shown to cause heritable mitochondrial diseases. Recently, mutations in the gene for the catalytic subunit of pol γ have been shown to cause PEO. Biochemical analysis of the autosomal dominant Y955C mutation indicates a clear effect on dNTP affinity and the fidelity of DNA replication. We predict the other autosomal dominant mutations in pol γ may have similar detrimental effects on DNA replication. *In vitro* analysis of pol γ proteins harboring these other mutations will certainly help elucidate the *in vivo* biochemical consequences of these PEO mutations.

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