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Review

DNA polymerase gamma and mitochondrial disease: Understanding the consequence of *POLG* mutations

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ABSTRACT

DNA polymerase γ is the only known DNA polymerase in human mitochondria and is essential for mitochondrial DNA replication and repair. It is well established that defects in mtDNA replication lead to mitochondrial dysfunction and disease. Over 160 coding variations in the gene encoding the catalytic subunit of DNA polymerase γ (*POLG*) have been identified. Our group and others have characterized a number of the more common and interesting mutations, as well as those disease mutations in the DNA polymerase γ accessory subunit. We review the results of these studies, which provide clues to the mechanisms leading to the disease state.

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1. Introduction

Mitochondria have their own small 16.5 kb circular double-stranded DNA that encodes 22 tRNAs, 2 rRNAs and 13 polypeptides that are absolutely essential for electron transport and oxidative phosphorylation. Nuclear genes encode the other 1000–1500 proteins that are imported into the mitochondria [1]. These include the proteins involved in mitochondrial DNA (mtDNA) replication, which if defective can produce mtDNA mutations leading to mitochondrial dysfunction and disease [2] (Fig. 1).

MtDNA is replicated by an assembly of proteins and enzymes including DNA polymerase γ (pol γ) and its accessory protein, single-stranded DNA binding protein (mtSSB), mtDNA helicase (Twinkle), and a number of accessory proteins and transcription factors (reviewed in [3]). The minimal proteins needed for in vitro mtDNA replication include the two subunit pol γ, the mitochondrial helicase and mtSSB [4]. Two modes of DNA replication have been proposed, an asynchronous strand displacement model and a strand-coupled bidirectional replication model (reviewed in [5]). In the asynchronous strand displacement model mtDNA is replicated in an asymmetric fashion where DNA synthesis is primed by transcription through the H strand origin within the D-loop [6]. After two-thirds of the nascent H strand is replicated, the L strand origin is exposed, allowing initiation of nascent L strand synthesis. In the strand-coupled model bidirectional replication is initiated from a zone near OriH followed by progression of the two forks around the mtDNA circle [7]. In both models the DNA polymerization reaction is performed by pol γ.

Of the 16 DNA polymerases in the eukaryotic cell, only pol γ is known to function in the mitochondria [8–10]. The holoenzyme of human pol γ consists of a catalytic subunit (encoded by *POLG* at chromosomal locus 15q25) and a homodimeric form of its accessory subunit (encoded by *POLG2* at chromosomal locus 17q24.1) [11]. The catalytic subunit is a 140 kDa enzyme (p140) that has DNA polymerase, 3′–5′ exonuclease and 5′ dRP lyase activities. Alignments reveal that the catalytic subunit sequence is well conserved across species, with all genes containing conserved sequence motifs for DNA polymerase and 3′–5′ exonuclease functions (Fig. 2). The accessory subunit is a 55 kDa protein (p55) required for tight DNA binding and processive DNA synthesis [12]. In 2001, the first disease mutations were identified in *POLG* [13]. These mutations were associated with progressive external ophthalmoplegia (PEO). Subsequently, mutations in *POLG* were identified in patients with Alpers syndrome and other infantile hepatocerebral syndromes, ataxia–neuropathy syndromes, Charcot–Marie–Tooth disease, idiopathic parkinsonism, nucleoside reverse-transcriptase inhibitor (NRTI) toxicity, among others (for review see [2,3,14]). These diseases are characterized by mtDNA deletions or depletion in symptomatic tissues. To date, approximately 150 disease mutations in *POLG* have been identified, which places *POLG* as a major locus for mitochondrial disease (http://tools.niehs.nih.gov/polg/) (Fig. 3). *POLG2* mutations associated with

Abbreviations: mtDNA, mitochondrial DNA; PEO, progressive external ophthalmoplegia; pol γ, DNA polymerase γ; NRTI, nucleoside reverse-transcriptase inhibitor; ad, autosomal dominant; WT, wild-type; ROS, reactive oxygen species; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PTC, premature termination codon; NMD, nonsense-mediated mRNA decay pathway; NAS, nonsense-associated alternative splicing pathway

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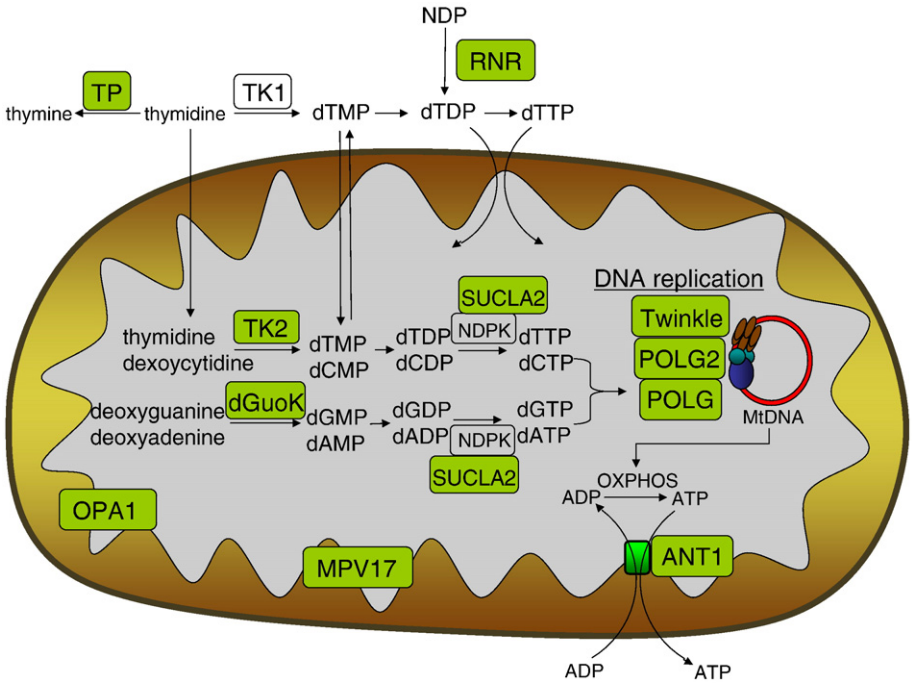

Fig. 1. The mitochondrion and some of the enzymes involved in mtDNA replication or nucleotide metabolism. Mutations in some of these genes (green boxes) are associated with mtDNA depletion or mutations in humans.

PEO have also been discovered (Fig. 1) [15]. Thus, our laboratory and others have been motivated to determine how mutations in both subunits of pol γ cause disease. Here is a comprehensive review of what is currently understood from experimental studies.

2. *POLG* mutations associated with autosomal dominant mitochondrial disease

PEO is the only mitochondrial disease that has cosegregated with autosomal dominant (ad) mutations in *POLG*. Nearly all of the adPEO mutations in *POLG* are located in the polymerase domain of pol γ (Fig. 4). One of the first adPEO mutations to be discovered was the Y955C mutation [13], and this was also the first to be biochemically characterized [16]. We found the Y955C p140 to be a mutator, causing a 10- to 100-fold increase in misinsertion errors, most likely as a consequence of a 45-fold decrease in binding affinity for the incoming nucleoside triphosphate. This enhanced mutagenesis is mitigated by a functional intrinsic exonuclease activity resulting in only a 2-fold mutator effect for base pair substitutions by the exonuclease-proficient Y955C enzyme. In a subsequent study of four adPEO mutant p140 variants, the Y955C and R943H substitutions were predicted to interact directly with the incoming dNTP by analysis of a structural model of the polymerase active site based upon the solved crystal structure of T7 DNA polymerase [17]. Recombinant proteins carrying these substitutions retain less than 1% of the wild-type (WT) polymerase activity and display a severe decrease in processivity. The significant stalling of DNA synthesis and extremely low catalytic activities of both enzymes are the two most likely causes of the severe clinical presentation in R943H and Y955C heterozygotes [17]. The G923D and A957S p140 proteins retained less than 30% WT polymerase activity. This is consistent with the reduced clinical severity of PEO in individuals heterozygous for the G923D and A957S mutations [17].

In a genetic model developed to evaluate the homologous PEO mutations in the yeast *MIP1* gene, we studied Y757C (Y955C in human *POLG*), as well as L260R (human L304R), I416T (human A467T), G725D (human G923D), R745H (human R943H), and A759S (human A957S) in *Saccharomyces cerevisiae* [18]. The amino acid codon changes were made directly into the chromosomal *MIP1* gene by *delitto perfetto* site-directed mutagenesis. The L260R, G725D, R745H and

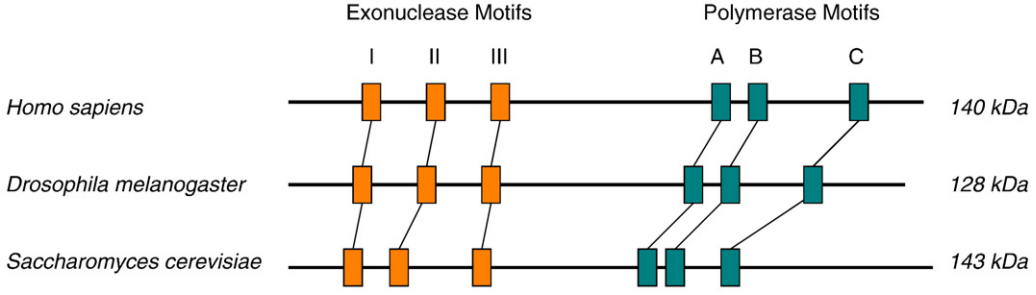

Fig. 2. Schematic linear organization of the pol γ catalytic subunit with conserved domains. The conserved 3′–5′ exonuclease domains (orange) are encoded by the three motifs I, II, and III while the DNA polymerase domains (green) are encoded by the three ABC motifs.