director of a well-orchestrated "tour de synapse" that integrates intra- and perhaps extracellular signals from other pathways to guide the Wg signal from cell to cell.

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Structure Casts Light on mtDNA Replication

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In this issue, Lee et al. (2009) present a crystal structure of the human mitochondrial DNA polymerase (POLγ). The structure of this heterotrimeric enzyme lays a foundation for understanding how POLγ mutations cause human mitochondrial disease and why some antiviral nucleoside analogs cause cellular toxicity.

In 1890, the German scientist Altmann observed mitochondria in tissue sections from mouse liver and coined the term *Elementarorganismen*, as he thought he had found free-living organisms inside the nucleated cell (Altmann, 1890). We know today that mitochondria are not independent at all, despite having their own genome. Instead, they rely on a large number of nuclear genes for their function (Falkenberg et al., 2007). Mammalian mitochondrial DNA (mtDNA) is a circular, double-stranded DNA molecule of ~16 kb that only encodes 13 proteins, essential subunits of the oxidative phosphorylation system. The remaining ~1500 mitochondrial proteins are encoded in the nucleus, including all proteins needed for replication and expression of the small mtDNA genome (Falkenberg et al., 2007). In this issue of *Cell*, Lee et al. (2009) present the structure of the human mitochondrial DNA polymerase (POLγ), a nuclear-encoded heterotrimeric enzyme that replicates the mtDNA genome. The structure of POLγ provides insight into the function of a unique spacer domain involved in processivity and subunit interaction. Mutations in the POLγA subunit are a frequent cause of disease, and the Lee et al. study now provides a basis for classifying and understanding such mutations. Finally, the structure will facilitate the development of antiviral nucleoside analogs that do not affect mtDNA maintenance.

The structure of human POLγ at 3.2 Å resolution (Lee et al., 2009) is a valuable advance as the molecular machinery required for mtDNA replication is not well understood (Figure 1). As the authors report, crystallization of POLγ was not straightforward and required the use of mutant versions of both the catalytic (POLγA) and accessory subunits (POLγB). The structure shows that POLγ is an asymmetric heterotrimer (AB_2), where only one of the POLγB subunits forms extensive contacts with the POLγA monomer. These findings are in good agreement with a previously published crystal structure of POLγB at 2.2 Å resolution (Carrodeguas et al., 2001) and with an electron microscopic surface map of the POLγ holoenzyme (Yakubovskaya et al., 2007). Although the POLγA subunit folds into a "right-hand" conformation, typical of other polymerases, it contains a unique spacer domain that is absent from other DNA POL I family members. The X-ray structure reveals that part of this spacer region belongs to the thumb subdomain of the right hand. The spacer folds into two subdomains: a globular domain associated with intrinsic processivity (IP) and an accessory subunit-interacting domain (AID). The IP subdomain interacts with the primer-template DNA complex and helps to explain why POLγA remains relatively processive even in the absence of the POLγB subunits. The AID contacts one of the two PolγB subunits.

Lee et al. provide modeling data that argue against direct contacts between the accessory factor POLγB and the primer-template DNA. Instead, the POLγB subunits increase processivity by affecting POLγA conformation so that it interacts with a longer stretch of template DNA.

POLγ cannot on its own replicate mtDNA, but requires a set of additional proteins. In vitro biochemistry has allowed the reconstitution of a minimal replisome that is able to carry out leading strand mtDNA replication (Falkenberg et al., 2007). In addition to POLγ, the minimal replisome contains the hexameric TWINKLE helicase, and the mitochondrial single-stranded DNA-binding protein (mtSSB) (Falkenberg et al., 2007). The mitochondrial RNA polymerase (POLRMT) provides the primers required for initiation of both leading- and lagging-strand DNA synthesis (Falkenberg et al., 2007; Wanrooij et al., 2008). The crystal structure reported by Lee et al. (2009) will facilitate a detailed structure-function analysis of the mitochondrial replisome. Interestingly, there is a possible TWINKLE-interacting surface in the POLγA spacer region and this predicted contact site is an obvious candidate for experimental validation by biochemical assays.

Mutations in mtDNA are a common cause of mitochondrial diseases. Affected patients present a variety of symptoms, and it is therefore not surprising that mutations in POLγ, the enzyme that copies mtDNA, can also cause disease phenotypes in humans such as cerebral cortical atrophy with liver failure (Alpers syndrome), necrotic lesions in the basal ganglia (Leigh syndrome), ataxia, neuropathy, epilepsy, myopathy, external ophthalmoplegia, and cognitive impairment, among others (Copeland, 2008). Many disease mutations map to the catalytic POLγA subunit, including the spacer region, with few mutations found in the POLγB accessory subunit (Copeland, 2008). The effects of mutations in the catalytic region have been readily elucidated on the basis of comparisons with other polymerase structures. However, because the spacer region is absent in other DNA polymerase I family members, the structural effects of mutations in the spacer have been difficult to predict. The Lee et al. study now allows the classification of mutations according to structural features and the authors present a first attempt along these lines. One example is the W748S mutation, which has been associated with autosomal-recessive ataxia in adults and the Alpers syndrome in children. The structure demonstrates that W^{748} is important in maintaining the local structure of the IP domain and that the residue forms stacking-interactions with F^{750} and H^{733}. Destabilizing this stack can undermine the POLγA interaction with template DNA and lead to lower polymerase activity.

Many of the proteins involved in replication and transcription of mtDNA, including POLγA, have homologs in bacteriophages (Falkenberg et al., 2007). This relationship to bacteriophage DNA replication proteins may provide an explanation for the mitochondrial toxicity caused by some antiviral nucleoside analogs, a well-known problem in the treatment of HIV infections (Lewis and Dalakas, 1995). The POLγ structure now opens up new avenues for the molecular design of nucleoside analogs that lack this side effect. Lee et al. found several important structural differences between human POLγ and the HIV reverse transcriptase, e.g., the nucleotide-binding sites formed between the palm and fingers subdomains are structurally distinct between the two polymerases, a feature that may be used for intelligent drug design.

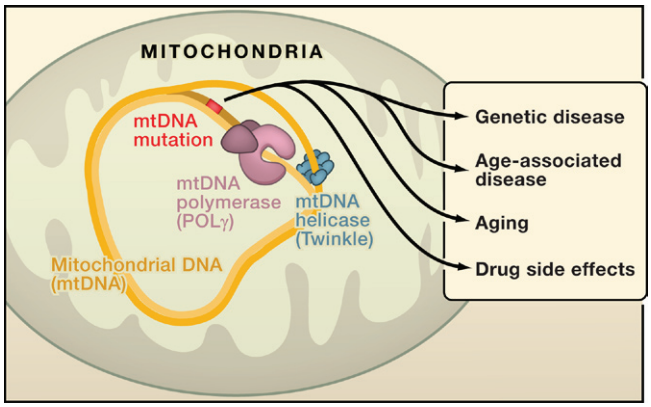

Figure 1. Mitochondrial Replication and Human Disease
The mitochondrial DNA polymerase (POLγ) is the only DNA polymerase in mammalian mitochondria. The heterotrimeric POLγ together with the mtDNA helicase (also denoted TWINKLE) and additional factors form the replisome responsible for replicating mtDNA. Mutations in the catalytic subunit of POLγ are a surprisingly common cause for human mitochondrial disease. Antiviral nucleoside analogs are sometimes recognized by POLγ and can cause drug side effects by interfering with mtDNA replication. POLγ replication error may be responsible for generating the majority of the mtDNA mutations implicated in age-associated disease and aging.

Somatic mutations in mtDNA are implicated in the aging process and there is biochemical evidence to suggest that endogenous replication errors by POLγ rather than unrepaired damage to mtDNA are the most important source of mtDNA mutations in human tissues (Zheng et al., 2006). Consistent with this idea, mtDNA mutator mice, which have a homozygous knockin mutation disrupting POLγA proofreading, accumulate substantial amounts of mtDNA point mutations and develop a premature aging syndrome (Falkenberg et al., 2007). Furthermore, maternal transmission of point mutations derived from these mtDNA mutator mice creates mouse lines with mtDNA mutation patterns that resemble those observed in natural populations of mice and humans (Stewart et al., 2008). It is thus possible that POLγ replication errors are an important source of mtDNA mutations in mammals. The POLγ structure should facilitate the development of a variant enzyme with increased proofreading capacity. Such an antimutator POLγ will be an important experimental test of the hypothesis that intrinsic replication errors rather than unrepaired DNA damage is the main explanation for sequence variations of mammalian mtDNA.

In summary, the elucidation of the structure of POLγ is an important milestone toward a molecular understanding of mtDNA replication, having in addition a number of exciting implications for human disease.

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