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DNA Replication Fidelity: Proofreading *in Trans*

Proofreading is the primary guardian of DNA polymerase fidelity. New work has revealed that polymerases with intrinsic proofreading activity may cooperate with non-proofreading polymerases to ensure faithful DNA replication.

Tina M. Albertson[1,2] and Bradley D. Preston[1]

Normal cells replicate their DNA with remarkable fidelity, accumulating less than one mutation per genome per cell division [1]. It is estimated that replicative DNA polymerases make errors approximately once every 10^4–10^5 nucleotides polymerized [2,3]. Thus, each time a mammalian cell divides approximately 100,000 polymerase errors occur, and these must be corrected at near 100% efficiency to avoid deleterious mutations. This is accomplished through the combined actions of 3′→5′ exonucleolytic proofreading and post-replication mismatch repair [2].

Proofreading is the primary guardian of DNA polymerase fidelity (Figure 1). Eukaryotes encode three DNA polymerases with intrinsic 3′→5′ exonucleolytic proofreading activity: polymerases δ and ε in the nucleus, and mitochondrial polymerase γ [3,4]. Polymerases δ and ε, together with polymerase α (primase), are essential replicative enzymes functioning at DNA replication forks [5]. Point mutations that selectively inactivate the exonuclease domains of polymerases δ or ε confer mutator phenotypes in yeast [6] and mammalian cells [7], and there is good evidence that these exonucleases correct replication errors on opposite DNA strands [8]. It is not clear, however, whether polymerases δ and ε correct only their own errors. In yeast, the combined disruption of polymerase δ and polymerase ε proofreading confers a synergistic increase in spontaneous mutation rate [6]. This suggests that polymerases δ and ε cooperate to suppress DNA replication errors by proofreading for each other. A new study by Pavlov *et al*. [9], recently published in *Current Biology*, has revealed that polymerase δ proofreading also cooperates with polymerase α, indicating that proofreading *in trans* may be a general property of polymerase δ and perhaps other eukaryotic 3′→5′ exonucleases.

The ability of one polymerase to proofread for another is particularly relevant to polymerase α which lacks intrinsic exonuclease activity and is error-prone [3]. The main function of polymerase α is to prime DNA synthesis [5]. This involves templated synthesis of ~20 base pairs of DNA twice at each replication origin to initiate leading-strand DNA synthesis and repeatedly (every ~200 base pairs) to sustain lagging-strand synthesis. After primer synthesis, a switch occurs from polymerase α to the principal replicative polymerases δ and ε. Assuming polymerase α synthesizes 5% of the diploid genome, an error rate of 10^{-4} per nucleotide [3] would generate 30,000 mutations each time a mammalian cell divides. This is substantially higher than the observed rate of <1 mutation per genome per cell division [1].

The idea that an extrinsic exonuclease may proofread for polymerase α first came from biochemical studies of Perrino and Loeb [10,11]. Using purified proteins, these investigators showed that a proofreading exonuclease from *Escherichia coli* (DnaQ) significantly increased the fidelity of polymerase α [10]. This suggested that a mechanism for proofreading could exist in eukaryotic cells involving polymerase α and a separate 3′→5′ exonuclease. Perrino and Loeb [11] then searched in cell extracts for a mammalian exonuclease that can function in this capacity. They purified a proofreading 3′→5′ exonuclease that, upon further characterization, was shown to be polymerase δ. These data provided strong biochemical evidence that a single proofreading exonuclease can be shared by polymerases α and δ. Subsequent studies showed that other purified exonucleases can also proofread for polymerase α *in vitro* [4].

Pavlov *et al*. [9] have now addressed the key question of whether shared proofreading occurs *in vivo*. This new study took advantage of a novel yeast polymerase α allele (*pol1-L868M*)

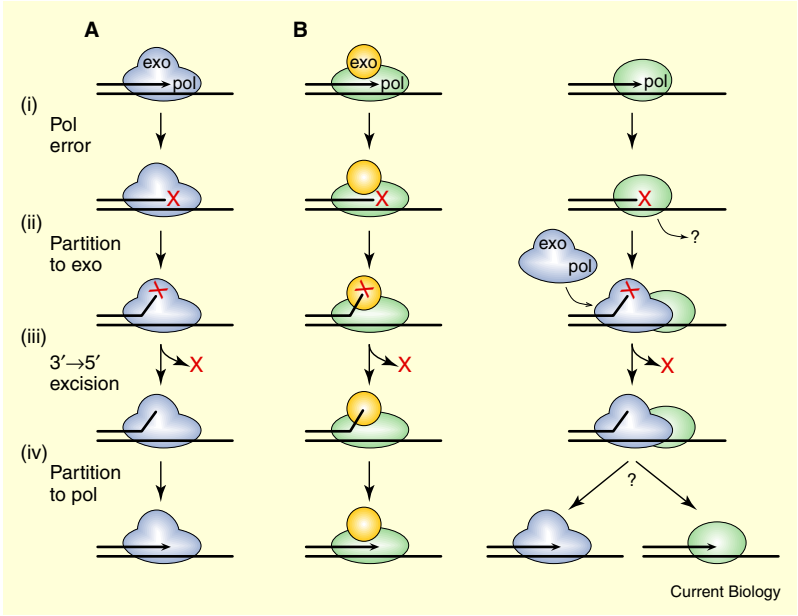

Figure 1. DNA polymerase proofreading by 3′→5′ exonucleases.

When DNA polymerases make a mistake (red cross), DNA synthesis stalls to allow excision of the misincorporated nucleotide by an associated 3′→5′ exonuclease. (A) Intrinsic proofreading is performed by DNA polymerases that have both polymerase (pol) and exonuclease (exo) domains in the same polypeptide molecule (blue). Proofreading is intramolecular and involves partitioning of the nascent DNA strand between the two active sites present in a single protein. (B) Extrinsic proofreading is catalyzed by exonucleases that cooperate with discrete DNA polymerases (green) in multi-protein complexes. Proofreading is intermolecular and requires partitioning of the nascent DNA strand between the polymerase and exonuclease proteins. Extrinsic proofreading can occur by simple exonucleases (yellow) or by the exonuclease domain of a proofreading polymerase (blue) which may trigger a polymerase switch. Some proofreading polymerases can engage two primer-template DNA molecules simultaneously: one bound to the polymerase domain while the exonuclease domain proofreads a second [20].

recently described by the Suzuki group [12]. *Pol1-L868M* encodes a single amino acid substitution at a position in the dNTP binding pocket known to influence polymerase fidelity. Cells expressing the *pol1-L868M* allele grow normally but exhibit about a two-fold increase in spontaneous mutation rate. Consistent with this phenotype, purified L868M polymerase α protein is five-times more error-prone than wild type polymerase α but retains normal processivity and catalytic activity.

To determine whether polymerase δ's exonuclease cooperates with polymerase α *in vivo*, Pavlov *et al*. [9] introduced the polymerase α allele into a strain deficient for polymerase δ proofreading (*pol3-5DV*) and looked for synthetic mutator phenotypes. The results were striking. When expressed alone, the polymerase α and polymerase δ alleles were relatively weak mutators (two-fold and nine-fold, respectively, at the *CAN1* locus). When combined, however, these two alleles strongly synergized to affect a greater than 100-fold increase in spontaneous mutation rate. Similar results were observed at other reporter loci (*trp1-289* and *his7-2*) and reflected synergistic increases in both base substitution and frameshift mutations. Interestingly, this cooperation appears to be specific to polymerase δ, as no synergy occurred when the polymerase α allele was expressed in a strain defective for polymerase ε proofreading (*pol2-4*). Pavlov *et al*. [9] also report experiments showing that polymerase α/δ synergy is largely independent of polymerase δ's role in mismatch repair [13]. Taken together, these data support the model of Perrino and Loeb [11] and strongly suggest that polymerase δ can proofread polymerase α's errors *in vivo*.

An alternative interpretation considered by Pavlov *et al*. [9] is that L868M polymerase α may help extend mispairs made by the proofreading-deficient polymerase δ enzyme. This is supported by their observation that L868M increases polymerase α's ability to extend 3′-terminal mispairs. Binding of polymerase α to polymerase δ could facilitate this polymerase switch [14]. These two models (proofreading *in trans* by polymerase δ and mispair extension by polymerase α) are not mutually exclusive. Both are expected to rescue unextended 3′ mispairs, which may explain the greater-than-multiplicative increase in mutation rate observed in *pol1-L868M pol3-5DV* double mutants.

The discovery that polymerase δ can share its proofreading exonuclease has broad implications with potential relevance to human disease. Proofreading *in trans* may underlie the high skin cancer incidence in mice defective for polymerase δ proofreading [7]. This unusual tissue-specific phenotype is not readily explained by polymerase δ's role in normal DNA replication. An intriguing possibility is that polymerase δ proofreads for DNA polymerase η, a specialized translesion DNA polymerase that is defective in the human skin cancer syndrome, xeroderma pigmentosum variant [15]. Polymerase η is highly error-prone *in vitro* [3,16] but not *in vivo* [17]. Moreover, polymerase η binds polymerase δ's processivity factor PCNA [18] and cooperates with polymerase δ proofreading to faithfully bypass UV dimers in cell-free systems [19]. Collectively these data suggest that proofreading *in trans* by polymerase δ may be a protective mechanism to remove mispairs generated during error-prone bypass of DNA damage.

In broader terms, the studies of Pavlov *et al*. [9] significantly advance the idea that eukaryotic polymerases function in concert with separate proofreading proteins. Twelve of the 15 known human DNA polymerases have

no proofreading activity and are error-prone [3]. While low-fidelity polymerases may serve specific cellular needs [3], most processes involving DNA synthesis must occur faithfully to maintain genome stability. Proofreading *in trans* is one possible mechanism to increase the fidelity of error-prone polymerases. To date, there is good evidence that polymerase δ proofreads for polymerase ε [6] and polymerase α [9] and compelling data linking polymerase δ proofreading with polymerase η [7,15,19]. Other mammalian 3′→5′ exonucleases could also function as extrinsic proofreaders [4]. Specialized DNA polymerases and exonucleases may play important tissue-specific roles to suppress distinct types of spontaneous or environmentally induced mutations. An exciting challenge will be to identify these roles and the polymerase/exonuclease partners that are involved.

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Plant GTPases: Regulation of Morphogenesis by ROPs and ROS

Polarized cell growth in plants is controlled by Rho-like small GTPases (ROPs), not only through the canonical WAVE/Arp2/3 pathway, but also through newly defined plant-specific pathways involving the regulated release of reactive oxygen species (ROS).

Joachim F. Uhrig and Martin Hülskamp

Small GTPases of the Rho family are universal master regulators, which have been shown to transmit signals from outside the cell to intracellular signalling cascades in many contexts in both animals and yeast, potentially affecting a wide variety of cellular processes. These GTPases cycle between the active GTP-bound and inactive GDP-bound form in steps that are regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) [1] (Figure 1).

Plants do not have clear orthologues of the Rho/Rac/Cdc42 GTPases, but they do have a distinct class of Rho-like proteins known as Rho-like small GTPases (ROPs) [2]. The regulators of ROPs in plants are also markedly different, with the exception of three GDI homologs which have been identified by sequence similarity and shown to interact with ROPs [3]. Most ROP-GAPs contain a distinctive Cdc42/Rac-interactive binding (CRIB) domain. CRIB domains are also found in Cdc42/Rac effectors, where they mediate binding to the active GTPase.

How ROPs are activated by GEFs had been unclear until very recently, as the *Arabidopsis* genome does not appear to encode GEFs of the usual type — with the characteristic tandem arrangement