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The Plant and Protist Organellar DNA Replication Enzyme POP Showing Up in Place of DNA Polymerase Gamma May Be a Suitable Antiprotozoal Drug Target

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1. Introduction

Mitochondria and plastids are eukaryotic organelles that possess their own genomes. The existence of organellar genomes is explained by the endosymbiotic theory [1], which holds that mitochondria and plastids originated from α -proteobacteria-like and cyanobacteria-like organisms, respectively [2,3]. Organellar genomes are duplicated by the replication machinery, including DNA polymerase, of the each organelle. The enzymes involved in the replication of organellar genomes are thought to be encoded by the nuclear genome and transported to the organelles after synthesis [4].

DNA polymerase γ (Pol γ) is the enzyme responsible for replicating the mitochondrial genome in fungi and animals [5,6]. Pol γ belongs to family A DNA polymerases, which share sequence similarity to DNA polymerase I (PolI) of *Escherichia coli*. Animal Pol γ consists of two subunits: a large subunit with DNA polymerase and 3'-5' exonuclease activities, and a small subunit that enhances processivity and primer recognition. The activity of Pol γ is inhibited by *N*-ethylmaleimide (NEM) and dideoxy nucleotide triphosphate (ddNTP).

In the late half of the 1960s, the presence of organellar DNA polymerase was confirmed by the measurement of DNA synthesis activity in isolated plant chloroplasts [7,8] and mitochondria of yeast and animals [9,10]. Since the 1970s, DNA polymerases have been purified from the chloroplasts and mitochondria of various photosynthetic organisms (Table 1), with biochemical data suggesting that plant organellar DNA polymerases and γ -type DNA polymerases share similarities with respect to optimal enzymatic conditions, resistance to aphidicolin (an inhibitor of DNA polymerase α , δ , and ϵ), sensitivity to NEM, molecular size, and template preference. Despite such observation, no gene encoding a homolog of Pol γ has

been found in the sequenced genomes of bikonts, including plants and protists. Therefore, the DNA polymerase of both mitochondria and plastids in photosynthetic organisms had remained unidentified. Sakai and colleagues [11-13] isolated nucleoid-enriched fractions from chloroplasts and mitochondria of tobacco leaves. They detected DNA synthetic activity in the nucleoid fraction and showed that the apparent molecular mass of the polypeptide exhibiting the activity was similar to Klenow fragment of DNA polymerase I (PolI) in *E. coli*. After their suggestion, it was found that the genomes of bikonts, consisting of plants and protists, encode one or two copies of genes encoding a DNA polymerase having distant homology to *E. coli* PolI. Homologs of this polymerase have been isolated in several plants, algae, and ciliates. Because genes encoding this type of enzyme are present in both photosynthetic eukaryotes and protists, we proposed to call this type of DNA polymerase POP (plant and protist organellar DNA polymerase).

| Year | Organism (organelle) | Mr (kDa) | Optimal condition for enzymatic activity | | | Inhibition by NEM (mM) | 3'-5' Exo-nuclease activity |
|------|---|--------------------|--|------------------------|------------------|------------------------|-----------------------------|
| | | | pH | MgCl ₂ (mM) | NaCl or KCl (mM) | | |
| 1973 | <i>Euglena gracilis</i> (cp) ^a | | 7.2 | 6 | 10-15 | | |
| 1979 | Wheat (mt) ^b | 110 ^m | 7 | 5 | 150 | 5 | yes |
| 1980 | Cauliflower (mt) ^c | | | | 150 | 1 | |
| 1980 | Spinach (cp) ^d | 105 ⁿ | 8-9 | 0.1-1 | 100 | 2 | |
| 1981 | Wheat (mt) ^e | 180 ^m | 8 | | | no | |
| 1984 | Pea (cp) ^f | 87 ^m | | 12 | 120 | 1 | no |
| 1990 | Soybean (cp & mt) ^g | 85-90 ⁿ | 8 | | 125 | strongly | |
| 1991 | Spinach (cp) ^h | 105 ⁿ | | | | 1 | yes |
| 1991 | <i>Chlamydomonas</i> (cp) ⁱ | 110 ⁿ | | | 100 | 2 | no |
| 1993 | Chenopodium (mt) ^j | 80-90 ⁿ | | 10 | 125 | 1 | yes |
| 1995 | Soybean (cp) ^k | | | | | | yes |
| 2002 | Pea (cp) ^l | 70 ⁿ | 7.5 | 8 | 125 | partially | yes |

Table 1. Previous studies on organellar DNA polymerases with no gene identification in plants and algae. cp, chloroplast; mt, mitochondrion; NEM, *N*-ethylmaleimide. a-l: references [14-25]. ^mdetermined by gel filtration; ⁿdetermined by glycerol density gradient. Reproduced from [26].

2. Enzymatic characteristics of POPs

The isolation of POP was first reported in rice (*Oryza sativa*) [27,28] and later in several higher plants and algae, including thale cress (*Arabidopsis thaliana*) [29,30], tobacco (*Nicotiana tabacum*) [31], red alga (*Cyanidioschyzon merolae*) [32], and a ciliate (*Tetrahymena thermophila*)

[33]. POPs typically consist of 900-1050 amino acid residues and contain 3'-5' exonuclease and DNA polymerase domains (Figure 1). In addition, POPs have an organellar targeting peptide at the *N*-terminus.

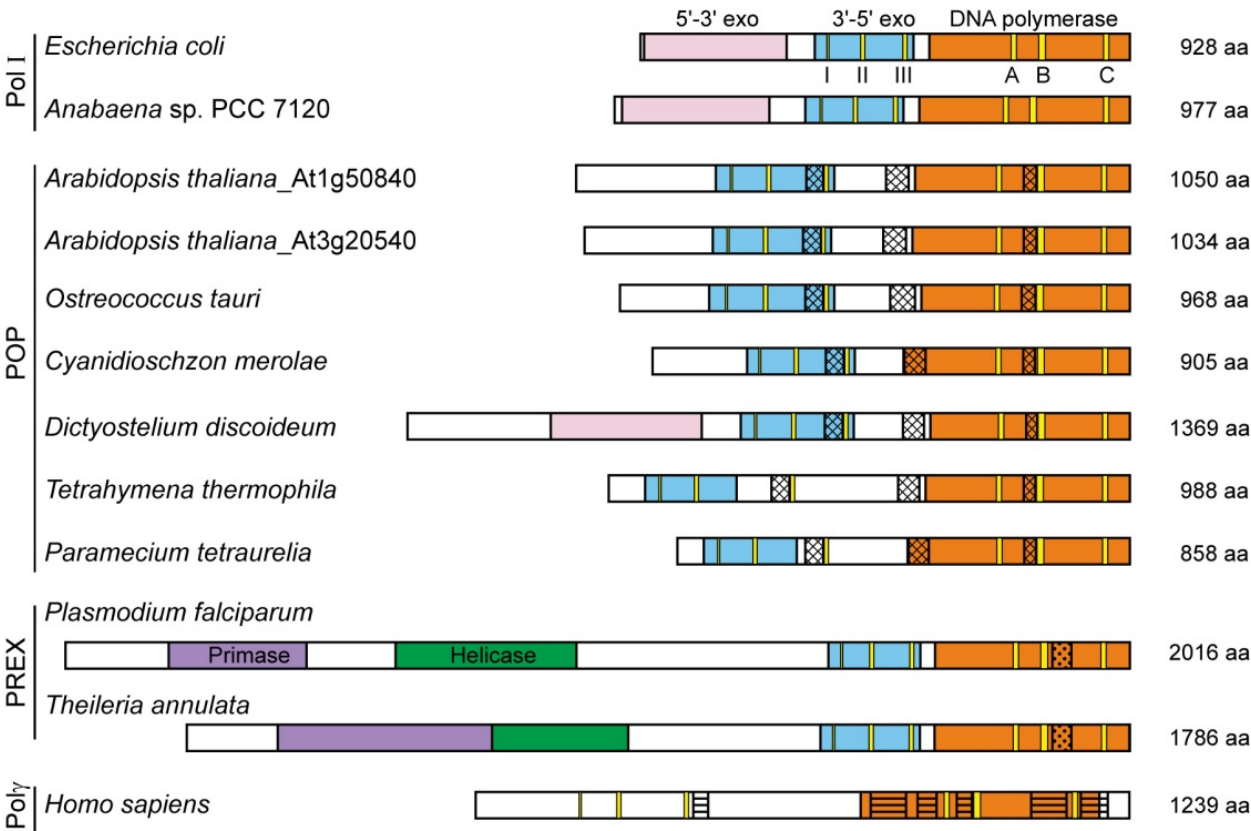


Figure 1. Schematic comparison of the structure of family A DNA polymerases. The colored boxes indicate domains estimated from the Pfam database: pink, 5'-3' exonuclease domain; blue, 3'-5' exonuclease domain; orange, DNA polymerase domain; purple, primase domain; green, helicase domain. Yellow boxes indicate characteristic motifs in the 3'-5' exonuclease and DNA polymerase domains. Thatched boxes represent conserved sequences in POPs. Dotted and striped boxes indicate conserved sequences in PREX and Poly, respectively. In Poly of *Homo sapiens*, a 3'-5' exonuclease domain was not found by Pfam, although 3'-5' exonuclease activity was reported for Poly [6]. This figure was modified from [32] with permission of the publisher.

2.1. Properties of DNA polymerase activity

The properties of DNA polymerase activity of POPs have been examined using recombinant [27,28,31] or native proteins purified from *Cyanidioschyzon* and *Tetrahymena* cells [32,33]. The optimal concentrations of KCl and MgCl₂ for DNA polymerase activity are 50-150 and 2.5-5 mM, respectively, which roughly coincide with the values reported in previous studies for organellar DNA polymerases in plants (Table 1). POPs display the highest activity with Poly(dA)/oligo(dT) as a template. Poly(rA)/oligo(dT) could also serve as a template, indicating that POPs have reverse transcriptase activity. Poly also exhibits reverse transcriptase activity, although the physiological importance of this activity remains to be elucidated.

2.2. Processivity

Processivity is defined as the number of nucleotides added by a DNA polymerase per one binding with the template DNA. POPs, in general, have high processivity values; for example, the processivity of rice recombinant GST-POP and *Cyanidioschyzon* POP is 600-900 nt and 1,300 nt, respectively [28,32]. In comparison, the Klenow fragment of *E. coli* PolI has mid-range processivity of <15 nt [28]. POPs contain three additional internal sequences relative to other family A DNA polymerases (Figure 8). The role of the two extra sequences, amino acid residues 635-674 (Insert I) and 827-852 (Insert II) positioned before motif A (Figure 8-1) and between motif A and motif B (Figure 8-2), were examined in rice POP [28]. DNA binding was decreased in Insert I and II deletion-mutant proteins, while DNA synthesis activity and processivity were decreased only in the POP protein lacking Insert I. These findings suggest that the high processivity of POPs may be due to the existence of the inserted sequences. In animals, Pol γ consists of two subunits, a large subunit (Pol γ A) having DNA polymerase and 3'-5' exonuclease activities and a small subunit (Pol γ B) that enhances processivity and primer recognition [34]. Processivity of the *Drosophila* Pol γ A subunit is <40 nt, whereas that of Pol γ holoenzyme (Pol γ A and Pol γ B) is >1,000 nt [35]. In contrast to animal Pol γ , POPs display high processivity as a single subunit, and no accessory subunits of POP have been identified to date [28,32].

2.3. Sensitivity to inhibitors

The effects of inhibitors, such as aphidicolin, NEM, dideoxyTTP (ddTTP), and phosphonoacetate (PAA), on the DNA synthesis activity of POPs were evaluated [27,31-33]. Aphidicolin is a specific inhibitor of DNA polymerases α , δ , and ϵ and acts through competition with dCTP or dTTP [36,37]. The sulfhydryl reagent NEM inhibits DNA polymerases α , γ , δ , and ϵ [38], and has a half maximal inhibitory concentration (IC₅₀) of <0.1 mM for Pol γ . PAA is an analog of pyrophosphate and interacts with viral DNA polymerases and reverse transcriptases at pyrophosphate binding sites to create an alternative reaction pathway [39,40]. ddTTP severely inhibits DNA polymerases β and γ , but only weakly impairs the activities of DNA polymerases δ and ϵ [41]. POPs are not inhibited by aphidicolin or NEM. The inhibitory effect of ddTTP differs depending on the organism, with the IC₅₀ ranging from 4-615 μ M for POPs (Figure 2A). The activity of POPs is severely inhibited by PAA, as demonstrated by IC₅₀ values of 1-25 μ M for several POPs (Figure 2B, C). In contrast, other family A DNA polymerases, including PolI and Pol γ , are not markedly inhibited by PAA, suggesting that PAA is a useful marker for the classification of organellar DNA polymerases in unsequenced eukaryotes. T4 DNA polymerase and DNA polymerase δ of *Saccharomyces cerevisiae*, which are both family B DNA polymerases, are also not sensitive to PAA, but the respective Motif A mutants of each protein, L412M (T4 DNA polymerase) and L612M (DNA polymerase δ of *S. cerevisiae*), are inhibited by PAA [42,43]. The mechanism of inhibition by PAA has not been studied in detail for family A DNA polymerases, and the critical amino acid residues involved in sensitivity to PAA in POPs are unknown due to the limited similarity of family A and B DNA polymerases in the Motif A region.

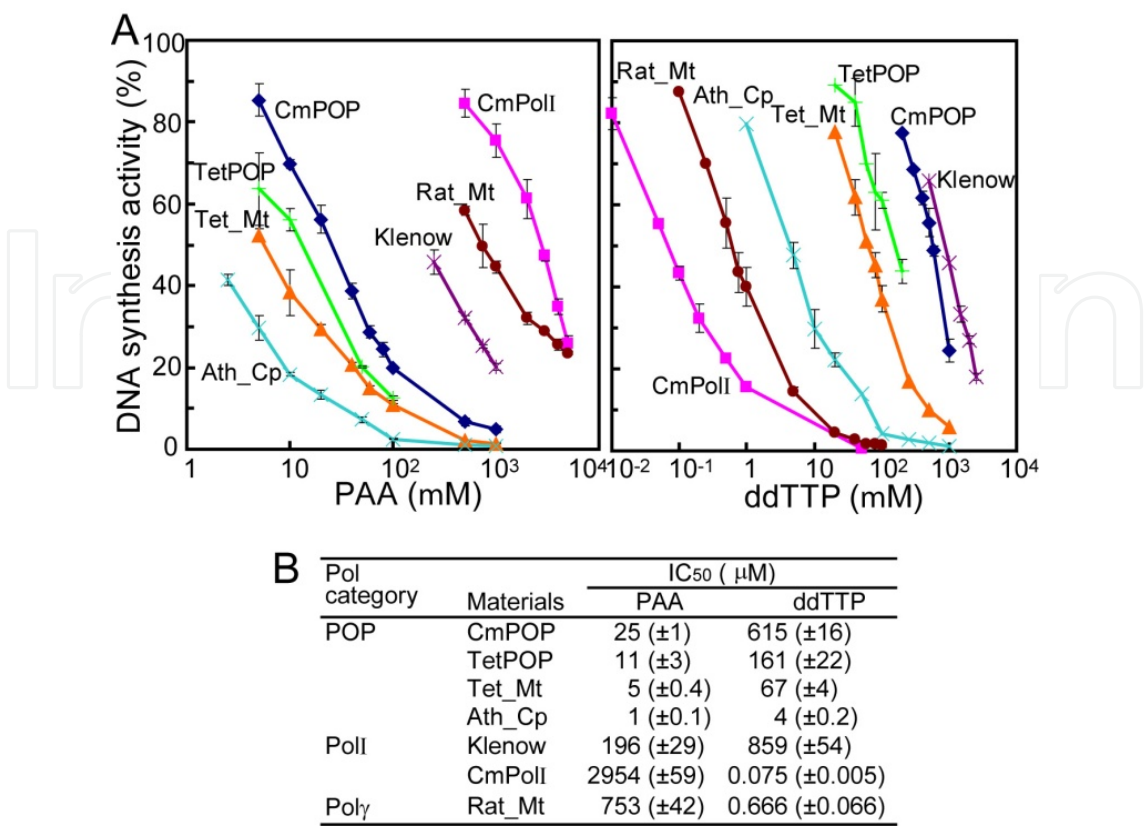


Figure 2. Effect of inhibitors, phosphonoacetic acid (PAA), and dideoxy TTP (ddTTP) on DNA synthesis activity (A). Half maximal inhibitory concentration (IC₅₀) for PAA or ddTTP (B). Tet_Mt, *Tetrahymena thermophila* mitochondria; Ath_Cp, *Arabidopsis thaliana* chloroplasts, Klenow, *Escherichia coli* Poll Klenow fragment; CmPoll, *Cyanidioschyzon merolae* Po-II; Rat_Mt, rat liver mitochondria. Reproduced from [33] with permission.

2.4. 3'-5' Exonuclease activity

POPs have a 3'-5' exonuclease domain containing three conserved regions, Exo I, Exo II, and Exo III (Figure 1), and this exonuclease activity has been demonstrated in rice [28] and *Cyanidioschyzon* [32]. In rice POP, replacement of Asp365 with Ala in the Exo II domain abolishes nuclease activity, but has no effect on DNA polymerase activity. With regard to 3'-5' exonuclease proofreading activity, POP shows relatively high fidelity for base substitutions (10⁻⁴ to 10⁻⁵; [28]). The primary structure of Poly appears to lack a 3'-5' exonuclease domain, as indicated by the low E-value of 0.17 for this domain in human Poly determined using the motif search software Pfam (<http://pfam.sanger.ac.uk/>). However, Poly possesses Exo I, Exo II, and Exo III motifs in the N-terminus (Figure 1), and exhibits 3'-5' exonuclease activity and high replication fidelity [6].

2.5. Subcellular localization

POP was first isolated as a plastidial DNA polymerase in rice, and its localization was confirmed by immunoblot analysis using isolated plastids [27]. Subsequent studies using GFP-fusion proteins and/or immunoblotting with isolated plastids and mitochondria

demonstrated that POPs are localized to both plastids and mitochondria in *Arabidopsis* and tobacco [31,44], and in the alga *Cyanidioschyzon* [32]. The mitochondrial localization of POP in the ciliate *Tetrahymena* was also determined by immunoblotting [33]. Figure 3 shows all of the known DNA polymerases found in the model plant *A. thaliana* and in humans. The nuclear-localized DNA polymerases involved in genome replication, DNA polymerase α , δ , and ϵ , are conserved in bikonts and opisthokonts, whereas the nuclear polymerases related to DNA repair differ between organisms. POP and Pol γ are the sole replicational DNA polymerases in bikont or opisthokont organelles, where they also act as DNA repair enzymes.

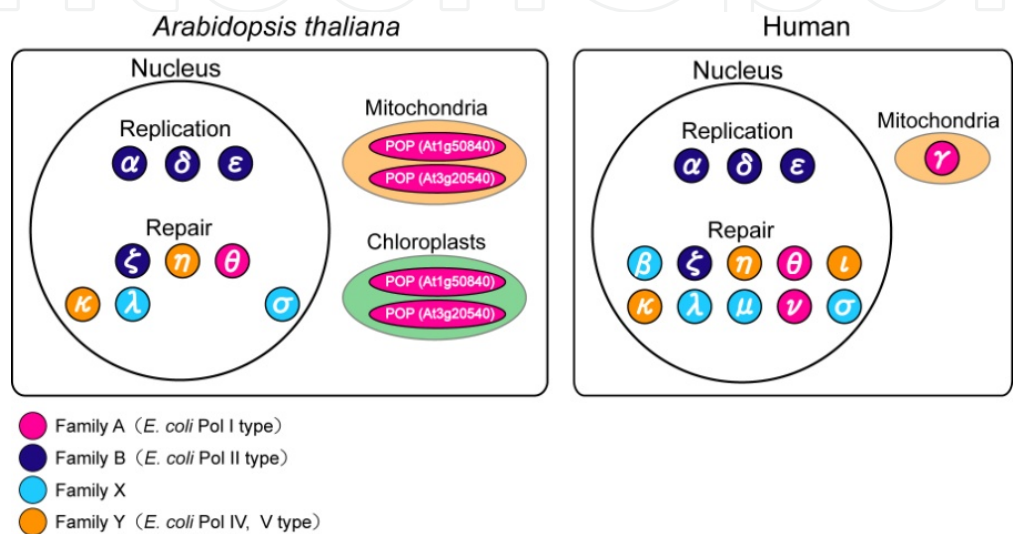


Figure 3. DNA polymerases of a model plant and human. Greek letters in colored circles corresponding to families indicate eukaryotic DNA polymerases alpha to sigma.

2.6. The role of POP *in vivo*

POPs exhibit high processivity and 3'-5' exonuclease activity, and were originally thought to function as organellar DNA replicases. This speculation was verified by analyzing *POP* mutant of *Arabidopsis* [30], whose genome encodes two *POP* genes, *At1g50840* and *At3g20540*, whose protein products are each localized to both plastids and mitochondria (Figure 3). The *At1g50840-At3g20540* double mutant was lethal, while each single mutant had a phenotype characterized as reduced DNA levels in plastids and mitochondria. In addition, only the *At3g20540* mutant displayed elevated sensitivity to ciprofloxacin, which is an inducer of DNA double-strand breaks (DSB). Together, these results show that two distinct POPs are involved in genome replication for plastids and mitochondria, and that the product of *At3g20540* also functions as a DNA repair enzyme in both organelles. In rice, the repair activity of POP was examined by a base excision repair (BER) assay using a recombinant protein, revealing that POP has 5'-deoxyribose phosphate (dRP) lyase activity [28]. Pol γ also displays this repair activity [45].

3. Role of POPs in cell-cycle regulation

3.1. Organellar genome replication in plant tissues

Nuclear genomes are replicated during the DNA synthesis phase (S phase), with the daughter genomes being distributed at the mitotic phase (M phase) to maintain ploidy levels. Observations of mitochondrial DNA stained with 4',6-diamidino-2-phenylindole (DAPI) and microautoradiography using [³H]thymidine have demonstrated that the DNA content and synthesis activity in mitochondria change dramatically during cell proliferation. In the root apical meristem of geranium (*Pelargonium zonale*), mitochondrial DNA in the promeristem, which is located just above the quiescent center, maintain high levels of DNA. However, in the upper root region, located immediately below the elongation zone, mitochondria contain small amounts of DNA [46]. Similar results were reported for the root apical meristem of *Arabidopsis* [47], tobacco [48], and rice [49], shoot apical meristem of *Arabidopsis* [50], and cultured tobacco cells [48,51], in addition to plastids. In *Avena sativa*, plastid DNA is extensively replicated in small cells of shoot apical meristem. Subsequently, as the cells increase in size, plastid numbers increase, while the DNA levels within plastids decrease [52-54]. These results suggest that organellar DNA is predominantly replicated in the meristem, and that the subsequent partition of organellar DNA to daughter cells does not coincide with the synthesis of organellar DNA in cells outside of the meristem center. In multicellular plants, therefore, the replication of organelle genome is not synchronized with the cell cycle or even organellar division.

3.2. Expression of POP in plants

The spatial expression patterns of POPs were analyzed in *Arabidopsis* and rice by *in situ* hybridization, which revealed that *POP* genes are strongly expressed in the apical meristem of roots and shoots, leading to high POP protein levels in these tissues [27,29]. In cultivated tobacco BY-2 cells, the amount of POP transcripts and proteins increases at the initiation of plastidial and mitochondrial DNA replication [31]. These results indicate that POPs function as the organellar genome replicase.

3.3. Red algal cell cycle

The unicellular red alga *C. merolae* contains a single plastid and mitochondrion [55], which both have division cycles that are synchronous with the cell cycle. Synchronous cultures of *Cyanidioschyzon* have been obtained by light-dark cycles [56]. Our group has also performed synchronous culture of *C. merolae* [32,57] using an initial long dark period (30 h) to force the cells into the G1 phase (Figure 4), followed by a 6-h light/18-h dark regime with bubbling with ordinary air. However, due to the low nutrient levels, the conditions were not sufficient to drive the cell cycle (Figure 4A). Two subsequent cycles of 6-h light/18-h dark with a supply of 1% CO₂ enabled the cells to accumulate enough photosynthetic products to allow progression of the cell division cycle, resulting in the synchronous division of cells 4-5 h after the start of the dark period (Figure 4B, C). Therefore, this culture method can discrimi-

nate the effects of light from those of the cell cycle in photosynthetic eukaryotes, and contains the cycle in which cellular nutrient level transitions from low to high (Figure 4B).

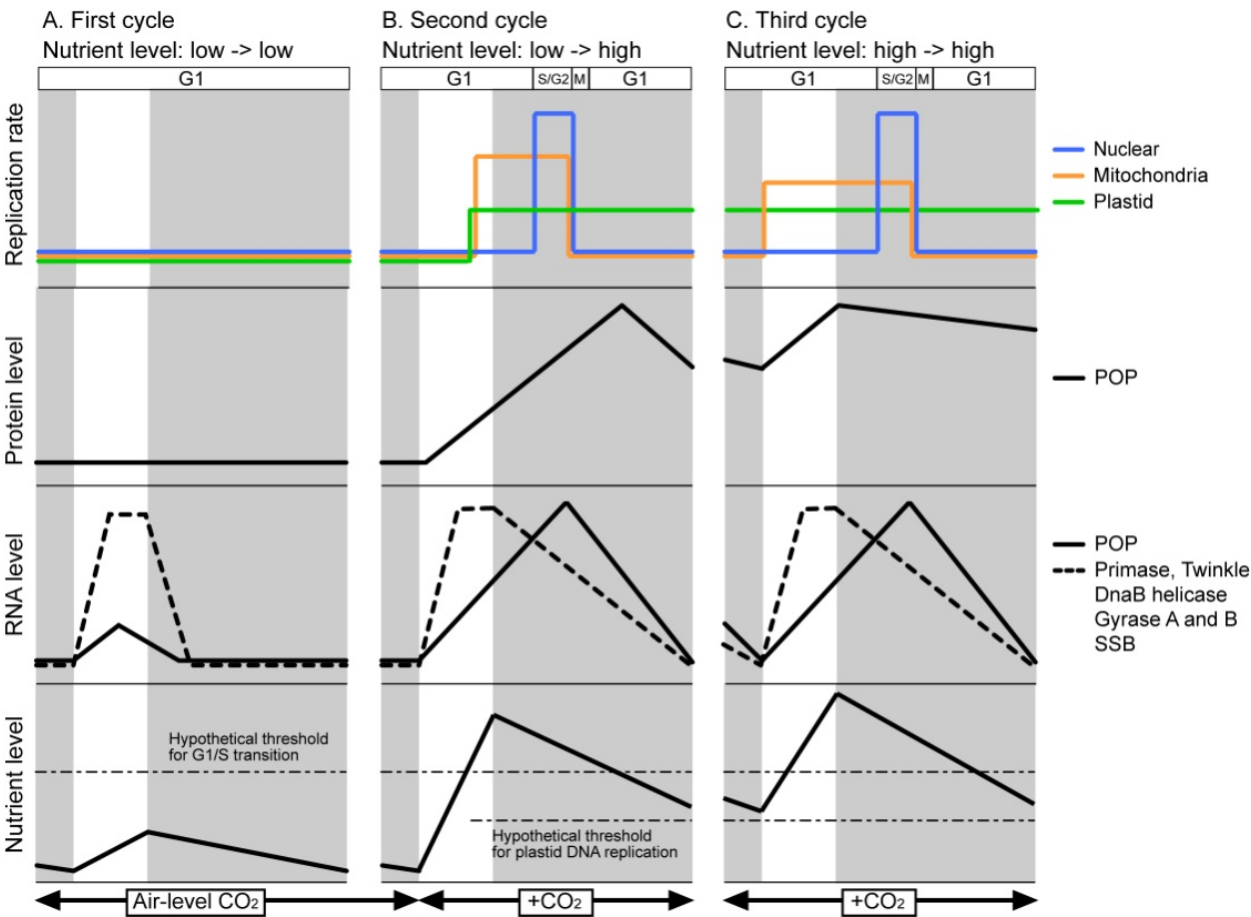


Figure 4. Cell cycle of *Cyanidioschyzon* and the expression of protein or mRNA related to organellar DNA replication. Three cell cycle patterns (A, B, and C) with respect to the nutrient level are shown. The nutrient level was controlled by aeration with or without the addition of CO₂. These drawings are based on the data taken from [57]. The shaded and white areas indicate dark and light cycles, respectively.

We have also determined the replication phases of nuclear, plastid, and mitochondrial DNA by quantitative PCR using cyanobacterial DNA as an internal standard to estimate the absolute amount of DNA (Figure 4, [57]). In the first cell cycle pattern, the level of nuclear and organellar DNA was unaltered (Figure 4A). Nuclear DNA replicated at or near the M-phase in the second and third cycles (Figure 4B, C). The replication of the mitochondrial genome was synchronized with the cell cycle to some extent, with mitochondrial DNA beginning to increase from the middle (second cycle) or beginning (third cycle) of the light phase, and doubling at or near the M-phase, as was observed for nuclear DNA (Figure 4B, C). In contrast, plastid DNA replication continued throughout the entire cell cycle, even after cell division was complete (Figure 4B, C). These results suggest that the replication of nuclear and organellar DNA is initiated after the accumulation of sufficient nutrients by photosynthesis, and that light alone does not serve as a replication signal for nuclear or organellar genomes.

Therefore, *C. merolae* cells may have two checkpoints (or thresholds) based on their nutritional state. The first checkpoint occurs during the G1/S-phase transition. Once cells overcome this point, the nuclear and organellar genomes are targeted for replication. The second threshold is specific for plastid DNA replication. After passage of the first checkpoint for G1/S transition, plastid DNA replication proceeds if the cellular nutrient level exceeds the nutritional threshold required for the replication process.

3.4. Expression of POP in the red algal cell cycle

We determined the expression of POP in synchronous culture of *C. merolae*. The protein level of POP was very low in the first non-dividing cycle (Figure 4A), but continued to increase from the second light period, and subsequently decreased during the dark period (Figure 4B). In the third cycle, the protein level of POP appeared constitutive during the cell cycle, although slight increases in the light phase and decreases in the dark phase were observed (Figure 4C). A small peak in the *POP* mRNA level was detected during the first light period (Figure 4A), with larger peaks appearing soon after entering the dark cycle (Figure 4B, C). The large peaks of *POP* mRNA levels correlated with the rise in mitotic indices.

The transcript level of other possible genes related to organellar DNA replication in *C. merolae* was also examined (Figure 4). Gyrase A and B, which are types of bacterial topoisomerase II, are related to both plastid and mitochondria genome replication in *C. merolae* [58] and *A. thaliana* [59]. SSB is a bacterial single-stranded DNA binding protein that is localized to mitochondria in *A. thaliana* [60]. In plants, DNA primases have not yet been isolated, although primase activity was detected in the chloroplasts of pea and the green alga *Chlamydomonas reinhardtii* [61,62]. DnaB is a bacterial replicational helicase that is encoded in the plastid genome of *C. merolae*. Twinkle is a replicational helicase and is localized to mitochondria in animals. Animal twinkle has only helicase activity; however, it is predicted that twinkle in plants and protists might have both helicase and primase activities [63]. Changes in the expression of these genes were qualitatively similar with each other, and were mainly stimulated by light. The expression pattern of these genes was also similar to that of genes related to photosynthesis, respiration, nuclear DNA repair, and ubiquitin in *C. merolae* [57]. In contrast, the expression pattern of POP transcripts was similar to that of cell cycle regulatory genes, including nuclear replicational DNA polymerase, mitotic cyclin, and mitotic cyclin-dependent kinase (CDK). Based on these findings, it appears that the replication of organellar genomes might be controlled by the expression of POP rather than that of other proteins related to organellar genome replication. Notably, the kinetics of replication differed for plastid and mitochondrial genomes; however, the regulatory mechanisms controlling the replication of the two organelles remain to be elucidated.

4. Possible evolutionary history of organellar DNA polymerases in eukaryotes

POP belongs to family A DNA polymerases, consisting of polymerases harboring sequence similarity to bacterial PolII, such as Pol γ , DNA polymerase θ (Pol θ), DNA polymerase ν (Pol ν), and PREX (plastid replication and repair enzyme complex, [64]). Pol θ and Pol ν are DNA repair enzymes and are localized to the nucleus [65,66]. PREX is an apicoplast (plastid like organelle)-localized DNA polymerase in the malaria parasite *Plasmodium falciparum* and contains a DNA polymerase domain, as well as helicase and primase domains (Figure 1 and [67]). Figure 8 shows the alignment of the DNA polymerase domain of several family A DNA polymerases. Although bacterial PolII, POP, and PREX share some homology, POP and PREX contain specific sequences, and the domain structure is clearly different in each polymerase (Figure 1 and Figure 8). Pol γ shows low similarity to other family A DNA polymerases, and has many Pol γ specific sequences.

Figure 5 shows a phylogenetic tree of family A DNA polymerases. From the tree, it is clear that POPs belong to a well-defined clade that is evolutionarily separated from bacterial PolII. Therefore, it can be concluded that POPs did not originate from PolII of cyanobacteria nor α -proteobacteria. Although PREX may have originated from a red algal secondary endosymbiont, their origin remains unclear, because PREX do not contain POP-specific sequences (Figure 8). POPs are widely conserved in eukaryotes, including amoebozoans, that have a close relationship with opisthokonts in phylogenetic analyses, but POPs have not been detected in opisthokonts, including animals and fungi (Figure 6). This suggests that POP might have originated before the diversification of photosynthetic eukaryotes. Pathogenic protists of animals, including *Blastocystis hominis* and *Perkinsus marinus*, possess POP, while genome-unsequenced pathogens, such as the green alga *Prototheca*, are likely to have POP. Therefore, POP is expected to be a suitable target for killing these pathogens.

From the phylogenetic tree, we proposed an evolutionary model of organellar DNA polymerases (Figure 7). Initially, when the ancestor of eukaryotes acquired mitochondria, the elementary mitochondrial replicase was likely bacterial DNA polymerase III (PolIII) (1 in Figure 7A). PolIII was then replaced by a POP, and the host cell then used POP for the replication of organellar genomes (2 in Figure 7A). We presume that PolIII must have been introduced upon the endosymbiosis event, but another possibility is that an endosymbiont or a host cell had already possessed POP before endosymbiosis. But this idea is considered unlikely because no bacteria having POP have been found so far. In this respect, it is of interest to note that, based on phylogenetic analysis in family A DNA polymerases, it has been postulated that Pol γ is of phage origin [69]. POP could also have been acquired from a virus. In effect, the ultimate origin of the ancestral POP is still unknown. The phylogenetic tree (Fig. 5) suggests that the closest relative of POP is Pol ν or Pol θ , which are present in various eukaryotes. It is not impossible then that an ancestral polymerase in eukaryotic host diverged into POP, Pol ν and Pol θ .

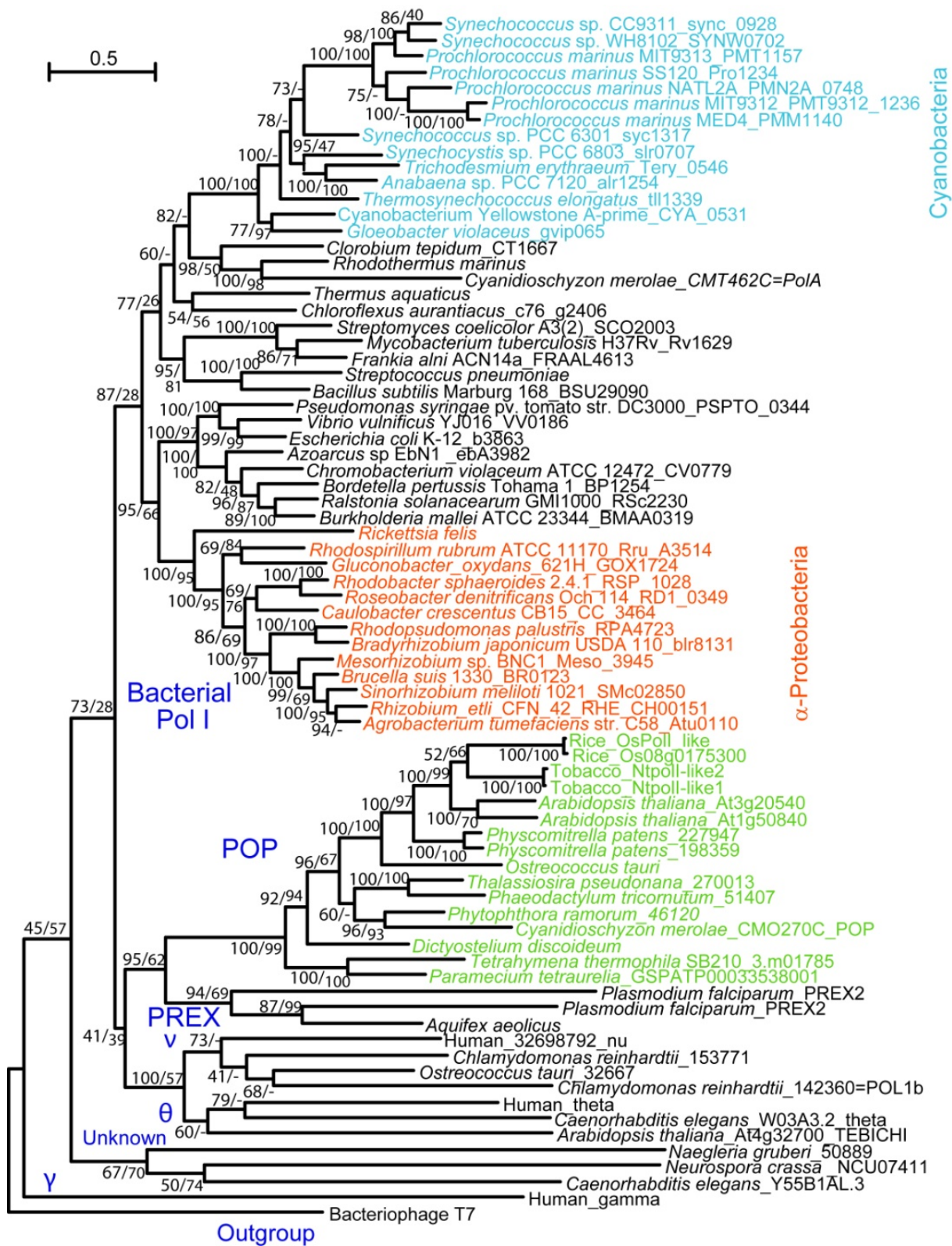


Figure 5. Phylogenetic tree of POPs and other family A DNA polymerases. Reproduced from [32] with permission.

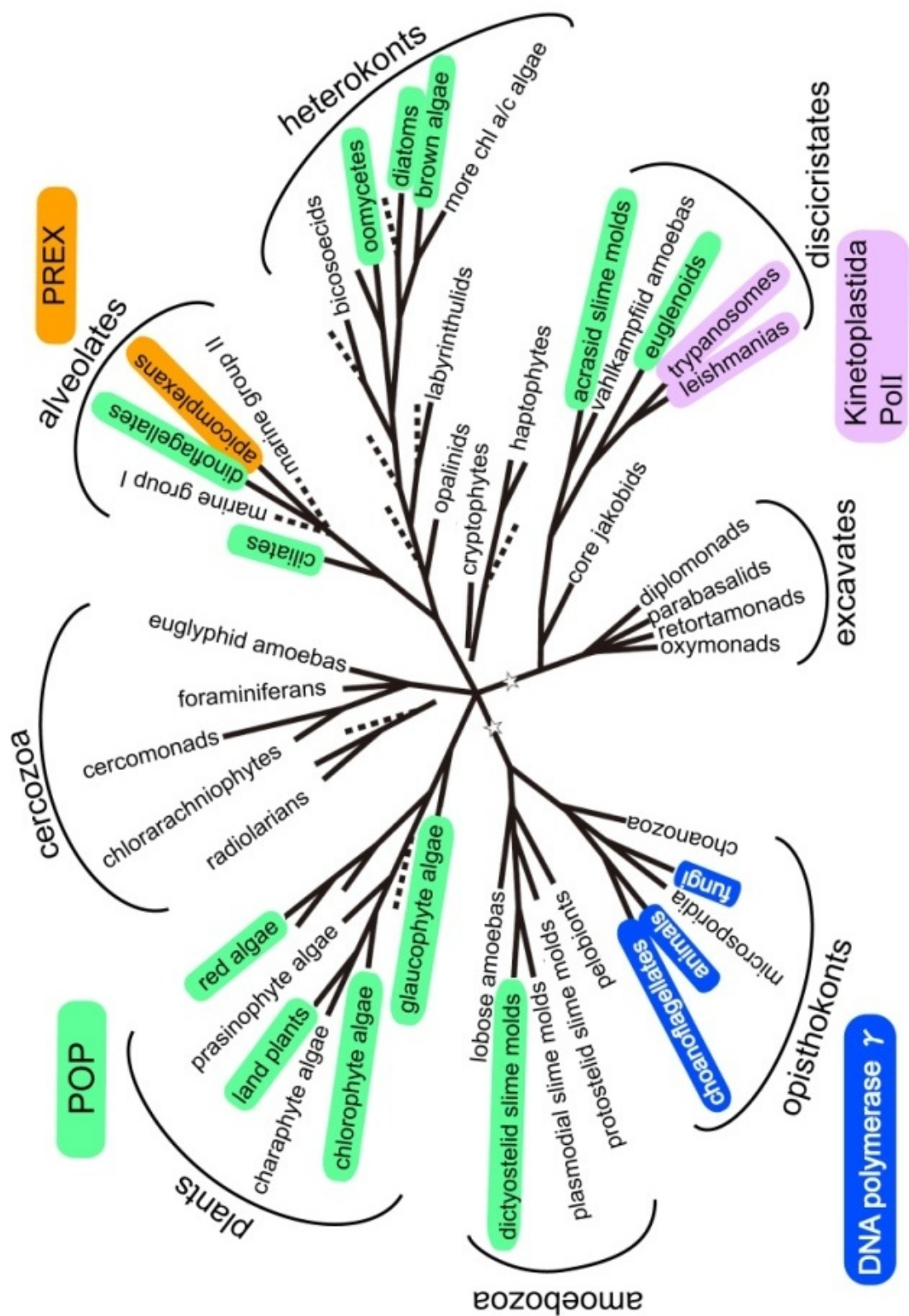
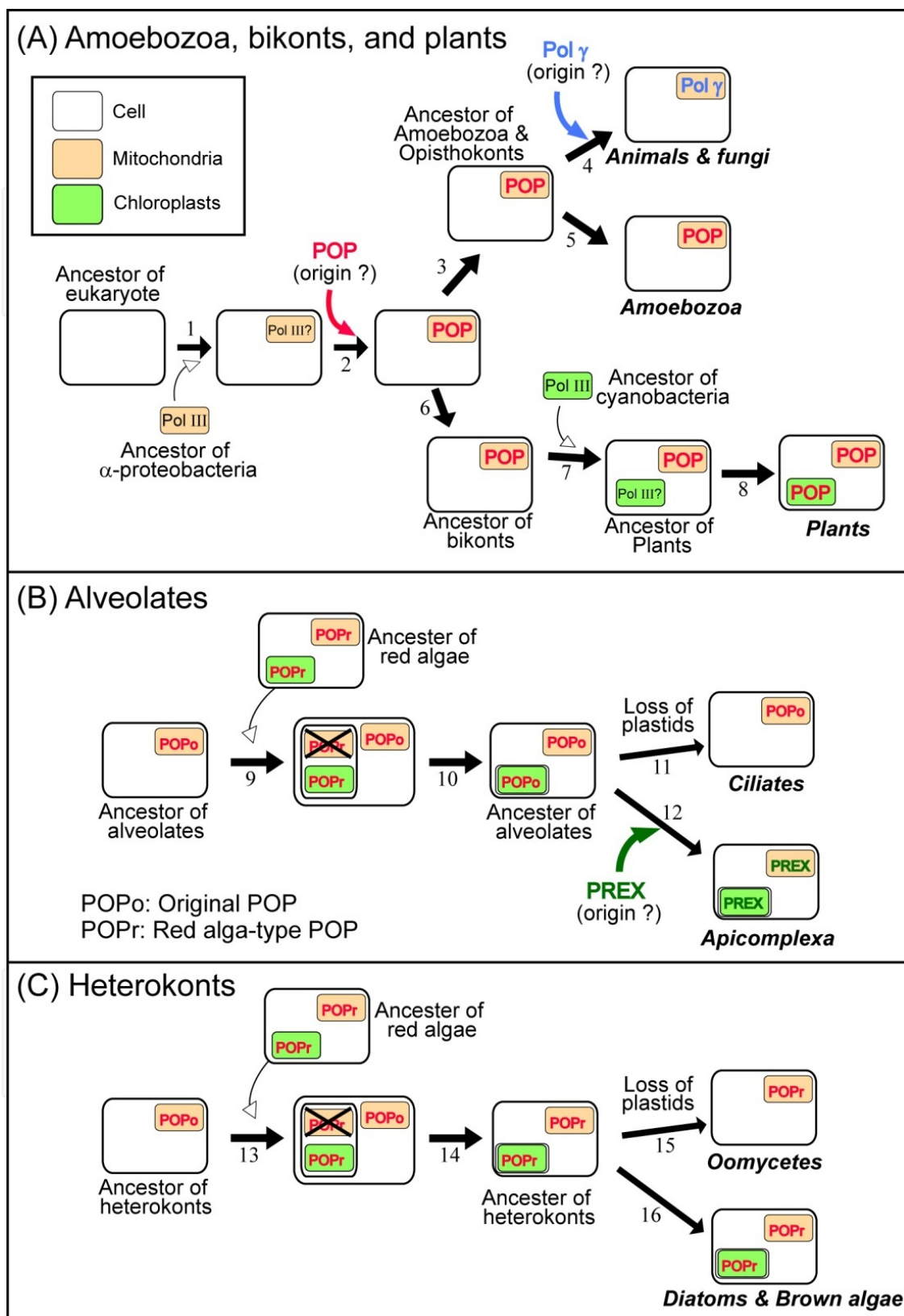


Figure 6. Distribution of organellar DNA polymerases in eukaryotes. Taxons containing POP, Poly, PREX, and kinetoplastida Poll are enclosed in light green, blue, orange, and purple boxes, respectively. The tree topology in this figure was adapted from [68], and the figure was modified from [33] with permission.



In the plastids of plants and algae, POP also replaced PolIII, and thus POPs are presently found in most eukaryotes (3-5 and 6-8 in Figure 7A). In opisthokonts, however, POP was replaced by Pol γ , whose origin is also unknown (4 in Figure 7A). Chromalveolates, consisting of alveolates and heterokonts such as diatoms, must have had a POP for mitochondrial replication before the occurrence of secondary endosymbiosis. Phylogenetic analysis suggests that the POPs of diatoms are more closely related to red algal POP than the POPs of ciliate *Tetrahymena* (Figure 5). The original POP might have been replaced by the POP of a red algal endosymbiont in diatoms (13-16 in Figure 7C), whereas in ciliates, the original POP has been retained (9-11 in Figure 7B).

Based on the genomic data obtained to date, Pol γ is found only in opisthokonts, indicating that two different polymerases cannot co-exist, at least over a long evolutionary span. The catalytic subunit of bacterial PolIII is also not encoded by eukaryotic genomes, although the PolIII gamma subunit, which functions as a clamp loader in bacteria, is conserved in land plants, such as *A. thaliana*, which has three gamma subunits, At1g14460, At2g02480, and At4g24790 [13]. One of the possible reasons why PolIII was replaced by POP may be the fact that POP is a single polypeptide enzyme, whereas PolIII consists of ten subunits. Therefore, the nuclear control of organellar DNA replication would be easier with nuclear-encoded POP. This also raises the question: why was POP replaced with Pol γ ? Unfortunately, although we do not have a clear answer for this question, the replacement event might be related to the mechanism of organellar genome replication. In animals, three replication modes have been proposed: the classical strand-displacement replication mode, a strand-coupled mode, and a RITOLS (ribonucleotide incorporation throughout the lagging strand) mode [70]. This contrasts with plant plastids, for which at least two modes of replication have been proposed, namely rolling circle replication via a D-loop and recombination-dependent replication [71]. Although the proposed replication modes in animals and plants remain to be confirmed, it is likely that the type of replication mode is different in the organelles of animals (opisthokonts) and plants (bikonts). In opisthokonts, the replication mode of organellar genomes of animals may have arisen before the replacement of POP with Pol γ , with Pol γ being a suitable enzyme for the replication process of animals. Secondary or tertiary endosymbionts do not exist among opisthokonts, a fact that may be due to differences in the organellar genome replication mode or organellar DNA polymerase type.

5. Conclusion and prospects

POPs have been isolated as organellar-specific DNA polymerases in a number of photosynthetic eukaryotes and ciliates. As the majority of biologists still believe that all mitochondrial replication enzymes are Pol γ , the primary objective of this review was to introduce POP to the wider research community. Although both POP and Pol γ are family A DNA polymerases, their primary structures are quite different from one another. However, POP and Pol γ display similar DNA polymerase activities that are characteristics of replicases, including high processivity, 3'-5' exonuclease activity, and reverse transcriptase activity. Eukaryotes containing a POP gene do not have a gene for Pol γ , and *vice versa*. In our hypothesis con-

cerning the transitional evolution of organellar DNA polymerase in eukaryotes, POP was proposed to be the primary organellar replicase and was then replaced by Poly in opisthokonts. POP might have been replaced by PREX and kinetoplastida PolI in apicomplexa and trypanosomes, respectively. Phylogenetic evidence suggests that organellar DNA polymerases are easily replaced, unlike nuclear replicational DNA polymerases, which are conserved in all eukaryotes.

The sensitivity of POP to DNA polymerase inhibitors clearly differs from that of Poly. To date, POPs have been shown to be commonly inhibited by phosphonoacetate. The inhibition mechanisms remained unclear for family A DNA polymerases, including POP, although it was reported that motif A in the polymerase domain of family B DNA polymerases is involved in the sensitivity to phosphonoacetate [42,43]. The detailed study of the inhibitory mechanisms and structural analysis of POP are needed, although POP is likely to be conserved in pathogenic bikonts, such as the green alga *Prototheca* and chromalveolata *Blastocystis*. Determining the structural differences in essential enzymes between a pathogen and host, and identifying pathogen-specific enzymes with no homologues in a host may identify suitable targets for chemotherapy. Such an approach is needed for targeting the malaria parasite. Chloroquine, mefloquine, and quinine have been used as antimalarial drugs. These reagents inhibit the production of the malarial pigment hemozoin. In addition, dihydrofolate reductase (DHFR) of malaria parasite is inhibited by proguanil and pyrimethamine. However, drug-resistant mutants of the parasite have emerged, and a new drug and enzyme target are therefore needed [72]. An apicoplast is non-photosynthetic plastid-like organelle that contains 27-35 kb of DNA in apicomplexa, and DNA replication within apicoplasts may be a good drug target, because apicoplasts harbor various essential metabolic pathways, such as those involving fatty acids, isoprenoid, and heme [73]. In plants and protists, our knowledge of the supporting players of organellar DNA replication, such as primase, helicase, topoisomerase, and single-stranded DNA binding protein (SSB), are limited. To understand the mechanism and regulation of replication in plastids and mitochondria, it is necessary that the composition of these enzymes in each organelle be determined. In addition, reconstitution of the replicational machinery of each organellar genome should be attempted. In humans, successful *in vitro* reconstitution of the mitochondrial DNA replisome, including Poly, twinkle helicase, and SSB, was demonstrated [74]. The further development of organellar replisome models in plants and protists may pave the way for greater understanding of the replication mode and discovery of new antiprotozoan reagents.

In multicellular plants, genomes of organelles are replicated in meristematic tissues, but the process is not synchronous with the cell cycle or even with organellar division. In the unicellular red alga *C. merolae*, which contains a single plastid and mitochondrion, the expression of POP appears constitutive during the cell cycle. POP is localized in both organelles, but the kinetics of replication differs for plastid and mitochondrial genomes. Replication of the mitochondrial genome is synchronous with the cell cycle to a certain extent, whereas replication of the plastid genome continues throughout the entire cell cycle. The organellar replication is regulated by cellular nutrient levels, and POP protein levels are closely correlated with nutrient levels. The mechanisms regulating the replication of plastids and mitochondria represent a new and exciting area of research in cell biology.

Appendix

| | | |
|-------------|--|--|
| DP01_Eco | LAELEKKAHEIAGEFNLS | SHDEVLVPEKLFKVPNIDKVIIEGK |
| DP01_S8102 | LERLETEAKAAAGVDNLAS | CNDEDLPEKLFKVPNDKVIIEGK |
| DP01_G1V | LESLEQEAATLAGSKFNLS | ARGHTWPKFKVPNDGATIEGK |
| DP01_A7120 | LAKLEQEAATLAGSKFNLS | ADKPGVELVREFTOSTEWLANDAA |
| DP01_Tel | LAALQQAQDANDVGPFNLS | KTDLLPAAERTFEVENTEQVVESAG |
| POP2_Ath | FRWASKHCPD-AKYMVGSDTIRLFFGGISN | RTDDELLPAAERTFEVENTEQVVESAG |
| POP2_Ath | FRWASKHCPD-AKYMVGSDTIRLFFGGISN | RTDDELLPAAERTFEVENTEQVVESAG |
| POP_Osa | FRWASKHCPD-AKYMVGSDTIRLFFGGISN | RTDDELLPAAERTFEVENTEQVVESAG |
| POP_Ostu | FRWASKHCPD-AKYMVGSDTIRLFFGGISN | RTDDELLPAAERTFEVENTEQVVESAG |
| POP_Cme | FREWASQCAD-AIHMAESQNRKUFFAPVRN | RTDDELLPAAERTFEVENTEQVVESAG |
| POP_Tet | FREWASQCAD-AIHMAESQNRKUFFAPVRN | RTDDELLPAAERTFEVENTEQVVESAG |
| POP_Pte | FIKWOTQEGDVSQCSQLOLFFAPCK | RTDDELLPAAERTFEVENTEQVVESAG |
| POP_Ddi | FINWAKTHSPD-AENMAPVDASIQULFFAPQNR | RTDDELLPAAERTFEVENTEQVVESAG |
| PREX_Pbe | NVERDKLKKELKNDINNVS | RTDDELLPAAERTFEVENTEQVVESAG |
| PREX_Pfa | NIKDNILKKLKNENINVS | RTDDELLPAAERTFEVENTEQVVESAG |
| PREX_Tan | QEIISNTLYSOLNQSEINLS | RTDDELLPAAERTFEVENTEQVVESAG |
| Nu_Human | LKELEQEAHFVAG-ERFLIE | RTDDELLPAAERTFEVENTEQVVESAG |
| Nu_Mouse | LKELEQEAHFVAG-ERFLIE | RTDDELLPAAERTFEVENTEQVVESAG |
| Nu_Danio | LKELEQEAHFVAG-ERFLIE | RTDDELLPAAERTFEVENTEQVVESAG |
| Nu_Ostu | VEELQNRADYVGGVEINIL | RTDDELLPAAERTFEVENTEQVVESAG |
| Theta_Osa | LKEL-REATKLAGSFSLNA | RTDDELLPAAERTFEVENTEQVVESAG |
| Theta_Ath | LRSEKKAFAELAGMTFSLSN | RTDDELLPAAERTFEVENTEQVVESAG |
| Theta_Ppt | LRSEKKAFAELAGMTFSLSN | RTDDELLPAAERTFEVENTEQVVESAG |
| Theta_Human | LDATETQAYGLAGHSFSTS | RTDDELLPAAERTFEVENTEQVVESAG |
| Gamma_Mouse | VVWVAMPQGLISGERYKE | RTDDELLPAAERTFEVENTEQVVESAG |
| Gamma_Human | LMTDLANDACGLISGERYKE | RTDDELLPAAERTFEVENTEQVVESAG |
| Gamma_Tru | LMTDLANDACGLISGERYKE | RTDDELLPAAERTFEVENTEQVVESAG |
| Gamma_Xtr | LMTDLANDACGLISGERYKE | RTDDELLPAAERTFEVENTEQVVESAG |
| Gamma_Sce | IVQILKIDVILLNDKDFYLDPWLS | RTDDELLPAAERTFEVENTEQVVESAG |
| Gamma_Spo | LSQVAKAKDLINTKDTVLNDPWLR | RTDDELLPAAERTFEVENTEQVVESAG |
| DP01_Eco | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA |
| DP01_S8102 | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA |
| DP01_G1V | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA |
| DP01_A7120 | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA |
| DP01_Tel | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA | PKQQLTILFEKQGI-KPLKKTGGG-PSTSEEV-LEELA |
| POP1_Ath | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD |
| POP2_Ath | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD |
| POP_Osa | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD |
| POP_Ostu | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD | KPTKFRNKLHRSIDPSTENFTASGMPVSGDV-LKELAKVSAEYDPMDDVSDISLEEVVEDDD |
| POP_Cme | REGAPKQKVALHGIVTPEIPIQVTFATGLPVPSSV-LRSLAKPGGAARAIMDQTLSEED | REGAPKQKVALHGIVTPEIPIQVTFATGLPVPSSV-LRSLAKPGGAARAIMDQTLSEED |
| POP_Tet | KPKKRTIQISGLG-LPPVAFASGMPACTAV-LRSLAKPGGAARAIMDQTLSEED | KPKKRTIQISGLG-LPPVAFASGMPACTAV-LRSLAKPGGAARAIMDQTLSEED |
| POP_Pte | KPKKRTIQISGLG-LPPVAFASGMPACTAV-LRSLAKPGGAARAIMDQTLSEED | KPKKRTIQISGLG-LPPVAFASGMPACTAV-LRSLAKPGGAARAIMDQTLSEED |
| POP_Ddi | KPKKRTIQISGLG-LPPVAFASGMPACTAV-LRSLAKPGGAARAIMDQTLSEED | KPKKRTIQISGLG-LPPVAFASGMPACTAV-LRSLAKPGGAARAIMDQTLSEED |
| PREX_Pyo | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF |
| PREX_Pbe | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF |
| PREX_Pfa | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF |
| PREX_Tpa | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF |
| PREX_Tan | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF | QOQILKALQNNVRDLSN-KLIDNTSDANLKNF |
| Nu_Human | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Nu_Mouse | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Nu_Danio | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Theta_Osa | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Theta_Ath | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Theta_Ppt | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Theta_Human | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Gamma_Mouse | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Gamma_Human | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Gamma_Tru | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Gamma_Xtr | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Gamma_Sce | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |
| Gamma_Spo | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD | SNQUREILFKGLHLSQRNSLPRGLQKAYFSSEAVNALRD |

| | | | |
|-------------|--------------------------------------|----------------------------------|----------------------------------|
| DP01 Eco | LDYPLPKVLEVRGLAKLSTY | DKLPLMINTKTRVH | TSVHNAVAGRLSTDPNLTNPVNEGRRI |
| DP01_88102 | DDHPFVLELHVRKLSUKSTY | IDLAPOLVEATGRVH | DDFNAVAGRLSSNPNLONIPVREISGRI |
| DP01 Glv | DDHPFVLELHVRKLSUKSTY | VDALPILVPRDTRGVH | TDFFVTVAGRLSSNPNLONIPVREISGRI |
| DP01 A7120 | DDGFPVALLSHRTLUKSTY | VDALPILVPRDTRGVH | TDFFVTVAGRLSSNPNLONIPVREISGRI |
| DP01 Tel | DDGFPVALLSHRTLUKSTY | VDVLPTRVPTQGRVH | TEFNAVAGRLSSNPNLONIPVREISGRI |
| POP1 Ath | TSAYGTAYAFGGGKEKACHAIALCVCSDISLINF | TLPLQSNVSGKDRGVH | CSLN-INTEGRLARAPLONQPALEKDRYKI |
| POP2 Ath | TSAYGTAFADFGGSEKACHAIALCVCSDISLINF | TLPLQSNVSGKDRGVH | CSLN-INTEGRLARAPLONQPALEKDRYKI |
| POP Osa | TSYPGTAYAFGGGKREKACHAIALCVCSDISLINF | TLPLQSNVSGKDRGVH | CSLN-INTEGRLARAPLONQPALEKDRYKI |
| POP Ostu | KKCGGAFADFGGGQVQACADALDAAVADITLLSFG | TLPLQSDTRGPEGRVH | GALN-INTEGRLARAPLONQPALEKDRYKI |
| POP Cme | RESADALAAKAAVALLACAGVETLLDSF | TLPLQ--KMDVKNHRH | ASLN-INTEGRLARAPLONQPALEKDRYKI |
| POP Tet | YNOIGSYVKQDEMGKASHAISELILQKOVETLLTF | TLPLQ--ELADQGRH | CSLN-MNTEGRLARAPLONQPALEKDRYKI |
| POP Pte | LYYHDFKAGEPKGACDAVINALLEKISLEVLLHTE | TLPLQ--ELADQGRH | CSLN-MNTEGRLARAPLONQPALEKDRYKI |
| POP Ddi | ECYHDFKPERGKREASTAINALLEKISLGTLLHTE | TLPLQ--KLADNSRLH | TSLN-INTEGRLARAPLONQPALEKDRYKI |
| PREX Pyo | INHNNAVILLENBEVKLYLXAF | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| PREX Phe | INHNNAVILLENBEVKLYLXAF | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| PREX Pfa | INHNNAVILLENBEVKLYLXAF | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| PREX Tan | MSNPILISLREYKANKALAF | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| Nu Human | HPHUKLILLEYVQHKIKSTYFDG | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| Nu Mouse | LHPLUKLILLEYVQHKIKSTYFDG | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| Nu PanLo | HPHUKLILLEYVQHKIKSTYFDG | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| Nu Ostu | HPHUKLILLEYVQHKIKSTYFDG | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| Theta Osa | QHPFVILKEHRTLAKLINGTLG | SIGSLAQ--LCSSGR | YIIOGNMLTSAAGRLSAPVQVQGRG |
| Theta Ath | QHPFVILKEHRTLAKLINGTLG | SIGSLAQ--LCSSGR | YIIOGNMLTSAAGRLSAPVQVQGRG |
| Theta Ppt | HPHUKLILLEYVQHKIKSTYFDG | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| Theta Human | HPHUKLILLEYVQHKIKSTYFDG | YKLPILHINTKTKIH | TTTFVGLKFSGRFSEKPLAQIPOQKN |
| gamma Mouse | RLGQFSTSDINKLKLHLPGLG | LIEMERIRINAIVKVPFLQKSHLNP | LIEMERIRINAIVKVPFLQKSHLNP |
| gamma Human | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Ttu | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Xtr | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Sca | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Spo | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| DP01 Eco | QAFIAP--EDYIVIVSADYSIELIRIMAHLSRDLG | LTFAEGKDIHRAFAEVLGFLP | LTFAEGKDIHRAFAEVLGFLP |
| DP01_88102 | RRAPLPO--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Glv | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 A7120 | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Tel | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP1 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP2 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ostu | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Cme | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Tet | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Pte | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ddi | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pyo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Phe | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pfa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Tan | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Mouse | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu PanLo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ppt | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| gamma Mouse | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Human | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Ttu | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Xtr | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Sca | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Spo | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| DP01 Eco | QAFIAP--EDYIVIVSADYSIELIRIMAHLSRDLG | LTFAEGKDIHRAFAEVLGFLP | LTFAEGKDIHRAFAEVLGFLP |
| DP01_88102 | RRAPLPO--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Glv | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 A7120 | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Tel | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP1 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP2 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ostu | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Cme | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Tet | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Pte | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ddi | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pyo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Phe | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pfa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Tan | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Mouse | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu PanLo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ppt | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| gamma Mouse | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Human | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Ttu | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Xtr | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Sca | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Spo | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| DP01 Eco | QAFIAP--EDYIVIVSADYSIELIRIMAHLSRDLG | LTFAEGKDIHRAFAEVLGFLP | LTFAEGKDIHRAFAEVLGFLP |
| DP01_88102 | RRAPLPO--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Glv | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
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| DP01 Tel | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP1 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP2 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ostu | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Cme | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Tet | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Pte | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ddi | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pyo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Phe | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pfa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Tan | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Mouse | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu PanLo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ppt | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| gamma Mouse | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Human | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Ttu | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Xtr | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Sca | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Spo | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| DP01 Eco | QAFIAP--EDYIVIVSADYSIELIRIMAHLSRDLG | LTFAEGKDIHRAFAEVLGFLP | LTFAEGKDIHRAFAEVLGFLP |
| DP01_88102 | RRAPLPO--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Glv | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 A7120 | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Tel | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
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| POP2 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ostu | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Cme | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Tet | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
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| PREX Phe | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Pfa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| PREX Tan | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu Mouse | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Nu PanLo | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Ppt | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| Theta Human | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID-- | QEAIRGDDVHALRALLID--KDEVSA |
| gamma Mouse | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Human | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Ttu | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Xtr | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Sca | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| gamma Spo | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP | YNDVNIPLGQWFKPLPHKDNQSNVSPKADFLP |
| DP01 Eco | QAFIAP--EDYIVIVSADYSIELIRIMAHLSRDLG | LTFAEGKDIHRAFAEVLGFLP | LTFAEGKDIHRAFAEVLGFLP |
| DP01_88102 | RRAPLPO--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Glv | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 A7120 | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| DP01 Tel | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP1 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP2 Ath | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Ostu | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Osa | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Cme | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALRALLID--KDEVSA | QEAIRGDDVHALRALLID--KDEVSA |
| POP Tet | RRAPLPE--QWTLILSADYSIELIRILTHLSGEEVL | QEAIRGDDVHALR | |

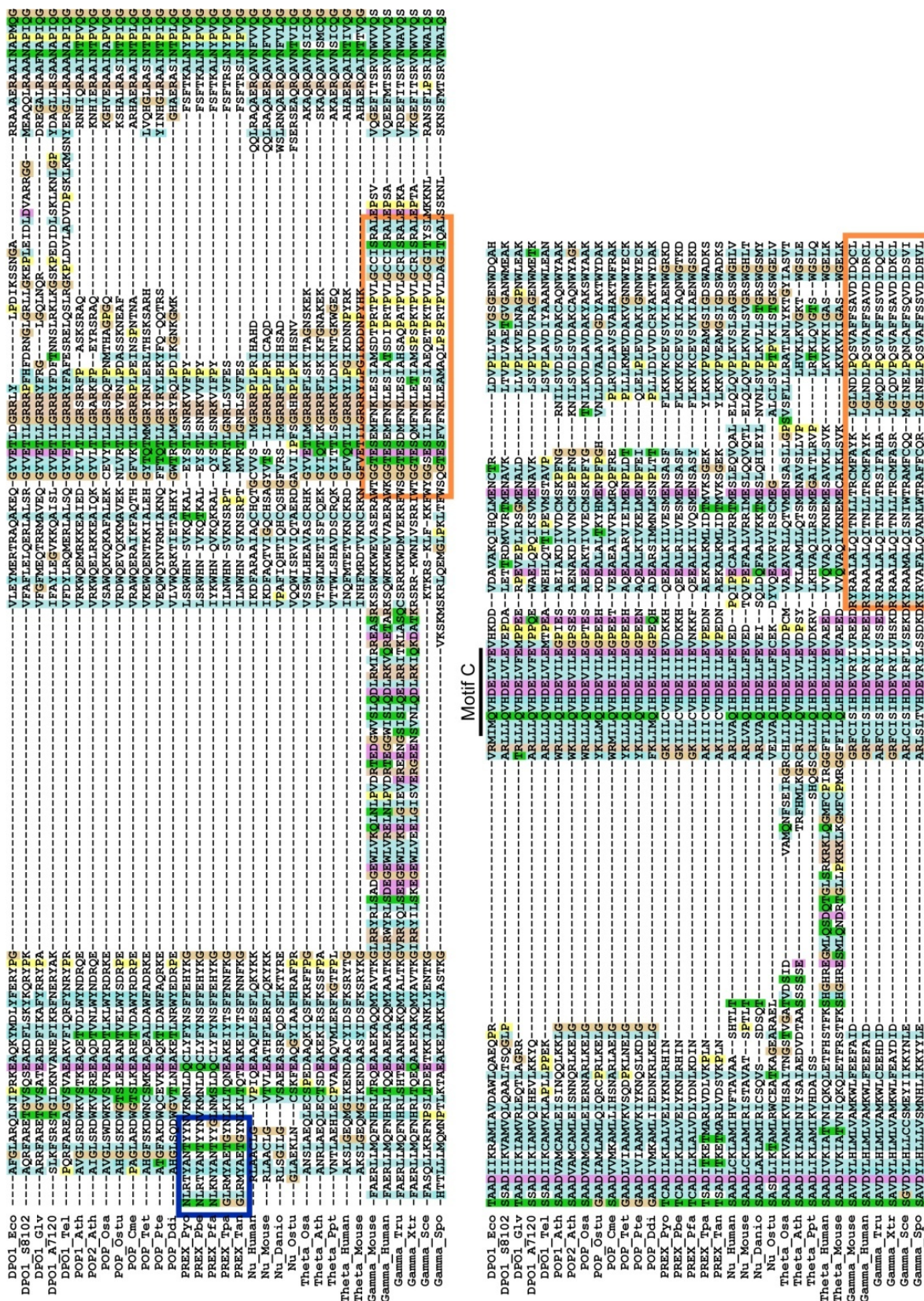


Figure 8. Alignment of the DNA polymerase domain of family A DNA polymerases. Green, blue, and orange boxes show specific sequences of POPs, DNA polymerase nu, and DNA polymerase gamma, respectively. Eco, *Escherichia coli*;

S8102, *Synechococcus* sp. WH8102; Glv, *Gloeobacter violaceus*; A7120, *Anabaena* sp. PCC 7120; Tel, *Thermosynechococcus elongates*; Ath, *Arabidopsis thaliana*; Osa, *Oryza sativa*; Ostu, *Ostreococcus tauri*; Cme, *Cyanidioschyzon merolae*; Tet, *Tetrahymena thermophila* SB210; Pte, *Paramecium tetraurelia*; Ddi, *Dictyostelium discoideum*; Pyo, *Plasmodium yoelii*; Pbe, *Plasmodium berghei*; Pfa, *Plasmodium falciparum*; Tpa, *Theileria parva*; Tan, *Theileria annulata*; Danio, *Danio rerio*; Ppt, *Physcomitrella patens*; Tru, *Takifugu rubripes*; Xla, *Xenopus tropicalis*; Sce, *Saccharomyces cerevisiae*; Spo, *Schyzosaccharomyces pombe*. Reproduced with permission [33].

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