


Phage Origin of Mitochondrion-Localized Family A DNA Polymerases in Kinetoplastids and Diplonemids

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Abstract

Mitochondria retain their own genomes as other bacterial endosymbiont-derived organelles. Nevertheless, no protein for DNA replication and repair is encoded in any mitochondrial genomes (mtDNAs) assessed to date, suggesting that the nucleus primarily governs the maintenance of mtDNA. As the proteins of diverse evolutionary origins occupy a large proportion of the current mitochondrial proteomes, we anticipate finding the same evolutionary trend in the nucleus-encoded machinery for mtDNA maintenance. Indeed, none of the DNA polymerases (DNAPs) in the mitochondrial endosymbiont, a putative α -proteobacterium, seemingly had been inherited by their descendants (mitochondria), as none of the known types of mitochondrion-localized DNAP showed a specific affinity to the α -proteobacterial DNAPs. Nevertheless, we currently have no concrete idea of how and when the known types of mitochondrion-localized DNAPs emerged. We here explored the origins of mitochondrion-localized DNAPs after the improvement of the samplings of DNAPs from bacteria and phages/viruses. Past studies have revealed that a set of mitochondrion-localized DNAPs in kinetoplastids and diplomemids, namely PolIB, PolIC, PolID, PolI-Perk1/2, and PolI-dipl (henceforth designated collectively as “PolIBCD+”) have emerged from a single DNAP. In this study, we recovered an intimate connection between PolIBCD+ and the DNAPs found in a particular group of phages. Thus, the common ancestor of kinetoplastids and diplomemids most likely converted a laterally acquired phage DNAP into a mitochondrion-localized DNAP that was ancestral to PolIBCD+. The phage origin of PolIBCD+ hints at a potentially large contribution of proteins acquired via nonvertical processes to the machinery for mtDNA maintenance in kinetoplastids and diplomemids.

Key words: DNA replication, DNA repair, autographivirus, Euglenozoa, lateral gene transfer, mitochondria.

Significance

Multiple types of DNA polymerases (DNAPs) localized in mitochondria have been identified among eukaryotes, but their precise origins have yet to be elucidated. This study reports that a type of mitochondrion-localized DNAP in kinetoplastids and diplomemids was derived from a DNAP of a particular group of phages. The results presented here suggest that the machinery for DNA maintenance in the mitochondria of kinetoplastid/diplonemid has been remodeled by the proteins acquired via nonvertical genetic processes.

Introduction

Mitochondria in the extant eukaryotes are the descendants of an endosymbiotic α -proteobacterium in the last eukaryotic common ancestor (Roger et al. 2017). The mitochondrial (mt) proteins, which are localized in mitochondria, are almost entirely nucleus-encoded and evolutionarily multifarious

(Gabaldón and Huynen 2007; Wang and Wu 2014; Gray 2015). Only 10–20% of mt proteins were predicted to be of the α -proteobacterial origin, suggesting that the original proteome of the mitochondrial endosymbiont has been remodeled largely (Gray 2015). There are three possible evolutionary paths that co-opt non- α -proteobacterial proteins

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into the molecular machinery in mitochondria. The non- α -proteobacterial mt proteins could emerge 1) de novo, 2) by recycling of the pre-existing eukaryotic proteins, or 3) via lateral gene transfer. Mitochondria, in principle, retain their own genomes that have been descended from the mitochondrial endosymbiont, albeit the entire set of proteins required for mtDNA maintenance (replication and repair) is nucleus-encoded with some exceptions. To our knowledge, the genes encoding proteins for DNA maintenance have been identified rarely in mtDNAs and are regarded as the product of a lineage-specific horizontal gene transfer (Bilewicz and Degnan 2011) or the reminiscent of linear plasmids in mitochondria (Fricova et al. 2010; Swart et al. 2012; Nishimura et al. 2019). Thus, as a part of the mitochondrial proteome, the machinery for mtDNA maintenance may be dominated by non- α -proteobacterial proteins. Indeed, none of the known DNA polymerases (DNAPs) localized in mitochondria is most unlikely the direct descendants of the DNAPs in the α -proteobacterial endosymbiont that gave rise to the ancestral mitochondrion (see below).

Phylogenetically diverse eukaryotes possess family A (famA) DNAPs that are evolutionarily related to DNA polymerase I (Poll) in bacteria (Jung et al. 1987; Moriyama et al. 2011; Moriyama and Sato 2014). Some of famA DNAPs in eukaryotes are known to be localized in mitochondria (Krasich and Copeland 2017). So far, four distinct types of mitochondrion-localized famA DNAP have been identified. First, “plant and protist organellar DNA polymerase (POP)” appeared to be broadly distributed among eukaryotes (Moriyama et al. 2011; Hirakawa and Watanabe 2019). Second, animals and fungi are known to use DNA polymerase gamma (Pol γ) for mtDNA maintenance (Graziewicz et al. 2006). The third type of mitochondrion-localized famA DNAP is “PollA” that is shared among the members of the classes Kinetoplastea, Diplonemea, and Euglenida, which comprise the phylum Euglenozoa (Klingbeil et al. 2002; Harada et al. 2020). The members of Kinetoplastea and Diplonemea possess the fourth type of mitochondrion-localized famA DNAP. “PollB,” “PollC,” and “PollD” were originally reported from a model kinetoplastid *Trypanosoma brucei*, and later identified in the broad members of Kinetoplastea (Klingbeil et al. 2002; Harada et al. 2020). The three DNAPs were shown to be closely related to one another in phylogenetic analyses. A recent study further identified multiple DNAPs, which are closely related to but distinct from PollB, C, or D, in an early-branching kinetoplastid *Perkinsella* sp. and diverse diplomonads (Poll-Perk1/2 and Poll-dipl; Harada et al. 2020). PollB, C, D, and their related DNAPs were derived from a single molecule and, thus, can be regarded collectively as the fourth type of mitochondrion-localized famA DNAP (henceforth termed as “PollBCD+” in this study). Pioneering studies have not considered any of the known mitochondrion-localized famA DNAPs as the direct descendant of Poll in the mitochondrial endosymbiont, but have failed to clarify

how and when POP, Pol γ , PollA, and PollBCD+ were established in the eukaryotic evolution (Moriyama et al. 2011; Hirakawa and Watanabe 2019; Harada et al. 2020).

In this study, we explored the origins of mitochondrion-localized famA DNAPs by analyzing an improved data set wherein the sequence sampling from bacteria and phages was improved drastically. We recovered the intimate affinity between PollBCD+ and the famA DNAPs of a particular group of phages in the phylogenetic analyses. Furthermore, these DNAPs appeared to share a unique insertion of consecutive eight amino acid (aa) residues. Altogether, we conclude that the extant DNAPs belonging to PollBCD+ were derived from a single phage famA DNAP acquired by the common ancestor of Kinetoplastea and Diplonemea. We also propose that PollA in Euglenozoa emerged from a type of cytosolic famA DNAP (Pol θ). The emergences of PollA and PollBCD+ may represent a tip of remodeling the machinery of mtDNA maintenance undergone in Kinetoplastea and Diplonemea.

Results

Prior to this study, the origin of none of the four types of mitochondrion-localized famA DNAPs (i.e., Pol γ , POP, PollA, and PollBCD+) has been elucidated in detail. This study successfully clarified the origin of PollBCD+ by analyzing phylogenetic alignments that are much richer in bacterial and phage famA DNAPs than those analyzed in the past studies. The sampling of the bacterial homologs was insufficient to reflect the diversity of bacteria in the previously published phylogenies of famA DNAPs (Moriyama et al. 2011; Hirakawa and Watanabe 2019; Harada et al. 2020). Furthermore, only a few famA DNAPs of phages have been included in the phylogenetic analyses. In this study, we prepared the “global famA DNAP” alignment by incorporating diverse bacterial and phage sequences (446 in total) deposited in public databases and 27 sequences that represent the four mitochondrion-localized types of DNAPs (Pol γ , POP, PollA, and PollBCD+), a single cytosolic DNAP (Pol θ), and a single plastid-localized DNAP found exclusively in apicomplexans and chrompodellids (PREX).

The global famA DNAP phylogeny reconstructed four clades, with all exclusively comprising the eukaryotic homologs: 1) POP, 2) PollA plus Pol θ , 3) PREX, and 4) Pol γ (shaded in blue in fig. 1A; see the [supplementary materials](#) for the tree with sequence names; Inagaki and Harad [dataset] 2020). The maximum likelihood bootstrap values (MLBPs) for the four clades varied from 69% up to 100%. The POP, PollA plus Pol θ , or Pol γ sequences showed no clear affinity to any bacterial or phage famA DNAPs, leaving their origins uncertain. The PREX sequences grouped with bifunctional 3′–5′ exonuclease/DNA polymerases found in phylogenetically limited bacteria as previously reported (Janouškovec et al. 2015; Hirakawa and Watanabe 2019; Harada et al. 2020). Curiously, the PollBCD+ sequences were paraphyletic but

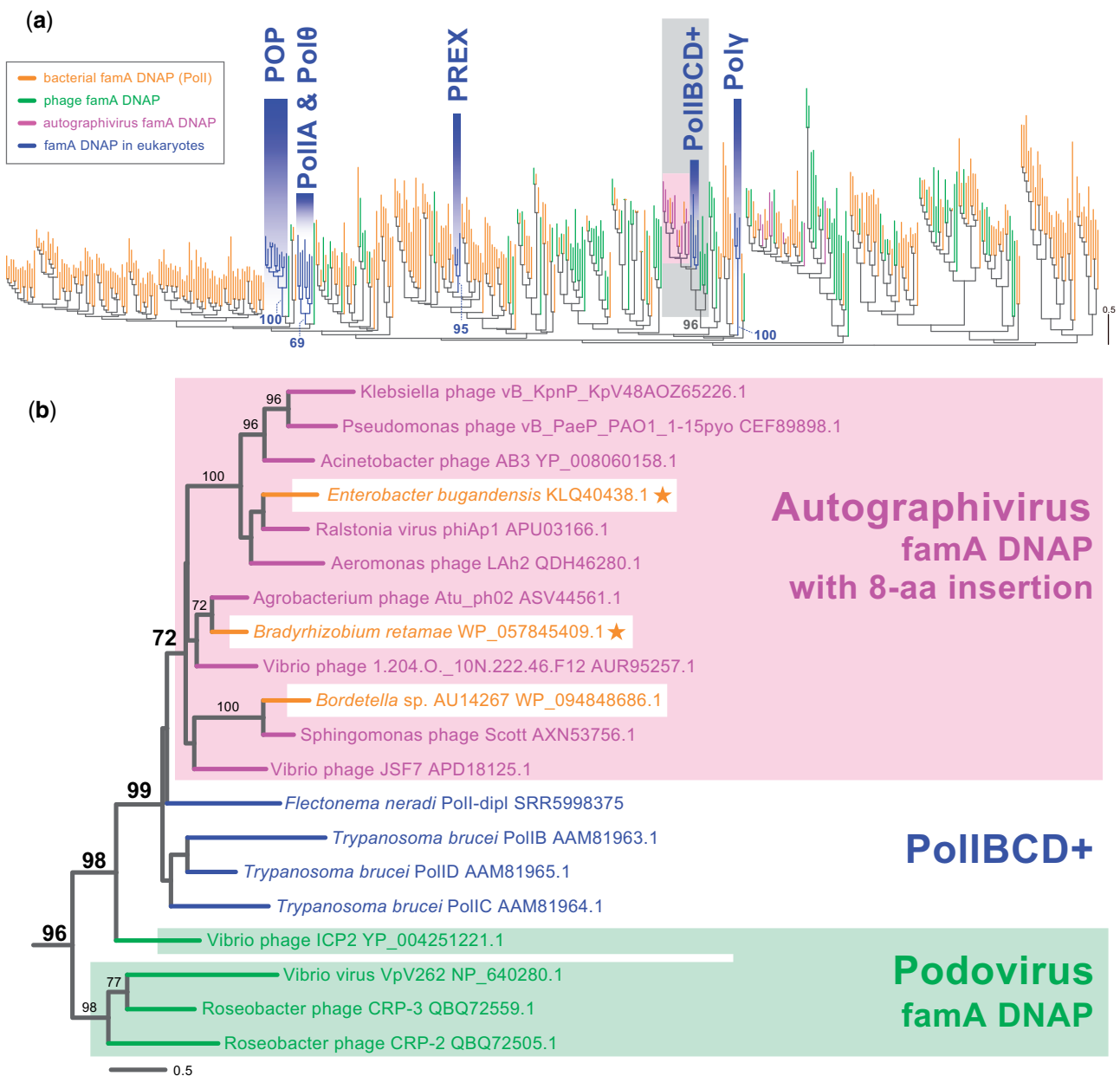


Fig. 1.—Maximum likelihood (ML) phylogenetic tree inferred from an alignment of the famA DNAP sequences of bacteria, phages/viruses, and eukaryotes. (A) Overview of the entire ML tree. All of the sequence names are omitted. The bacterial and eukaryotic sequences are shown in orange and blue, respectively. The sequences of autographiviruses are shown in magenta. A subset of autographiviruses possesses famA DNAPs in the pink-shaded clade bears the characteristic insertion of eight amino acid residues (AGV^{+ins} famA DNAPs; see the main text for the details). Other phage/viral sequences are shown in green. Only ML bootstrap values of interest are shown. The subtree containing PolIBCD⁺ and AGV^{+ins} famA DNAP sequences (shaded in gray) is enlarged and presented as (B). ML bootstrap values greater than 70% are shown. AGV^{+ins} famA DNAP sequences marked by stars are of the putative lysogenic phages in bacterial genomes.

nested within a robustly supported clade mainly comprising the famA DNAP homologs of phages belonging to families Autographiviridae and Podoviridae (fig. 1B; this figure corresponds to the portion shaded in gray in fig. 1A). The famA DNAP homologs of autographiviruses and three bacteria formed a subclade with an MLBP of 72%. The coding regions of two out of the three bacterial famA DNAP homologs in this

subclade (marked by stars in fig. 1B) are flanked by phage-like open reading frames (ORFs) in the corresponding genome assemblies deposited under the GenBank accession numbers LEDQ01000001.1 and NZ_LLYA01000167.1. Phage-like ORFs including that of famA DNAP encompass more than 40 kb consecutively in the two bacterial genomes. Thus, the two “bacterial famA DNAPs” are most likely of lysogenic

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autographiviruses in the bacterial genomes. On the other hand, no phage-like ORF was found around that of *famA* DNAP in the genome of *Bordetella* genomosp. 9 strain AU14267 (NZ_CP021109.1), suggesting that this bacterium horizontally acquired a *famA* DNAP gene from an autographivirus. The four PollBCD+ sequences were positioned at the base of the Autographiviridae clade described above and the grouping of PollBCD+ sequences and autographivirus *famA* DNAPs as a whole received an MLBP of 99% (fig. 1B). The global *famA* DNAP phylogeny strongly suggests an intimate evolutionary affinity between PollBCD+ and autographivirus *famA* DNAPs.

The members of Autographiviridae commonly display head-to-tail capsid structures and possess double-stranded linear DNA genomes of approximately 41 kb in length. This viral family comprises nine subfamilies and 132 genera (Lavigne et al. 2008; Adriaenssens et al. 2020). We searched for autographivirus *famA* DNAPs in the GenBank nr database and detected 175 homologs of 99 members belonging to 57 genera and 76 unclassified members. Each of the 175 members of Autographiviridae seemingly possesses a single *famA* DNAP. Intriguingly, the autographivirus *famA* DNAPs were split into two types based on the presence/absence of “8-aa insertion” in the polymerase domain (supplementary fig. S1 and table S1, Supplementary Material online). In this study, we designate autographivirus *famA* DNAPs with 8-aa insertion as “AGV^{+ins} *famA* DNAPs.” Each AGV^{+ins} *famA* DNAPs was predicted to possess only polymerase domain by InterProScan5 with the Pfam database (Jones et al. 2014; El-Gebali et al. 2019) (supplementary table S2, Supplementary Material online). AGV^{+ins} *famA* DNAPs were found in 40 members belonging to 23 genera, and 51 unclassified members (supplementary fig. S1, Supplementary Material online). Although only a subset of the 175 autographivirus *famA* DNAPs were included, the global *famA* DNAP phylogeny demonstrated the distant relationship between AGV^{+ins} *famA* DNAPs and other autographivirus *famA* DNAPs lacking 8-aa insertion (fig. 1A).

To re-examine the phylogenetic affinity between PollBCD+ and AGV^{+ins} *famA* DNAPs, we selected nonredundant sequences from the 91 AGV^{+ins} *famA* DNAPs and aligned with 24 PollBCD+ sequences and four *famA* DNAPs of phages belonging to a family Podoviridae as the outgroup. The second *famA* DNAP alignment was subjected to both ML and Bayesian methods. In the second phylogenetic analyses, AGV^{+ins} *famA* DNAPs and PollBCD+ sequences formed a clade supported by an MLBP of 100% and a Bayesian posterior probability (BPP) of 1.0 (fig. 2). PollBCD+ sequences appeared to possess eight amino acids that are most likely homologous to 8-aa insertion in AGV^{+ins} *famA* DNAPs (fig. 2), strengthening the phylogenetic affinity between PollBCD+ and AGV^{+ins} *famA* DNAPs. Besides PollBCD+ and AGV^{+ins} *famA* DNAPs, 8-aa insertion was found solely in the *famA* DNAP homolog of *Vibrio* phage ICP2 placed at the basal

position of the clade of PollBCD+ and AGV^{+ins} *famA* DNAPs (fig. 2). In the analyses of the second alignment, AGV^{+ins} *famA* DNAPs grouped together with an MLBP of 92% and a BPP of 0.99, excluding PollBCD+ sequences that formed a clade with an MLBP of 72% and a BPP of 0.66 (fig. 2). The weak statistical support for the monophyly of PollBCD+ sequences is not incongruent with their paraphyletic relationship reconstructed in the global *famA* DNAP analysis (fig. 1B).

Discussion

The phylogenetic analyses of the global alignment of *famA* DNAPs (fig. 1A and B) and the second alignment rich in AGV^{+ins} *famA* DNAP homologs (fig. 2) consistently recovered the specific affinity between PollBCD+ and AGV^{+ins} *famA* DNAPs. These results strongly suggest that PollBCD+ in the extant kinetoplastids and diplomonids can be traced back to a single autographivirus *famA* DNAP, particularly the one with 8-aa insertion. In other words, PollBCD+ is a typical example of non- α -proteobacterial mt proteins acquired laterally from a phage. Prior to this study, the phage origins of mitochondrion-localized RNA polymerase (RNAP) and Twinkle mtDNA helicase/primase (simply termed Twinkle below) have been known (Shutt and Gray 2006). Unlike PollBCD+ of which distribution is restricted to Kinetoplastea and Diplonemea, the mitochondrion-localized RNAP and Twinkle are ubiquitous among eukaryotes, implying that the last eukaryotic common ancestor (LECA) had already used the two proteins for transcription and replication in the mitochondrion, respectively. The phage origins of the mitochondrion-localized RNAP, DNAP, and helicase/primase prompt us to propose that individual eukaryotic lineages possess unique mt proteins acquired from phylogenetically diverse phages.

We failed to pinpoint the exact origin of PollBCD+ even by analyzing the second alignment, wherein the known diversity of AGV^{+ins} *famA* DNAPs was covered (fig. 2). We might be able to find an AGV^{+ins} *famA* DNAP homolog that branches PollBCD+ sequences directly in a future phylogenetic study covering the true diversity of phage *famA* DNAPs. In particular, we regard that autographivirus *famA* DNAP genes in bacterial genomes are significant. To our knowledge, no autographivirus has been reported to infect eukaryotes. Thus, the common ancestor of kinetoplastids and diplomonids may have acquired the *famA* DNAP gene from a lysogenic autographivirus in a bacterial genome. If so, the bacterial genomes harboring AGV^{+ins} *famA* DNAP genes are critical in investigating the origin of PollBCD+ at a finer level than that in this study.

Members of classes Kinetoplastea and Diplonemea, together with Euglenida, share another type of mitochondrion-localized *famA* DNAP, namely PollA (Harada et al. 2020). It is reasonable to postulate that the common ancestor of the three classes—most likely the ancestral

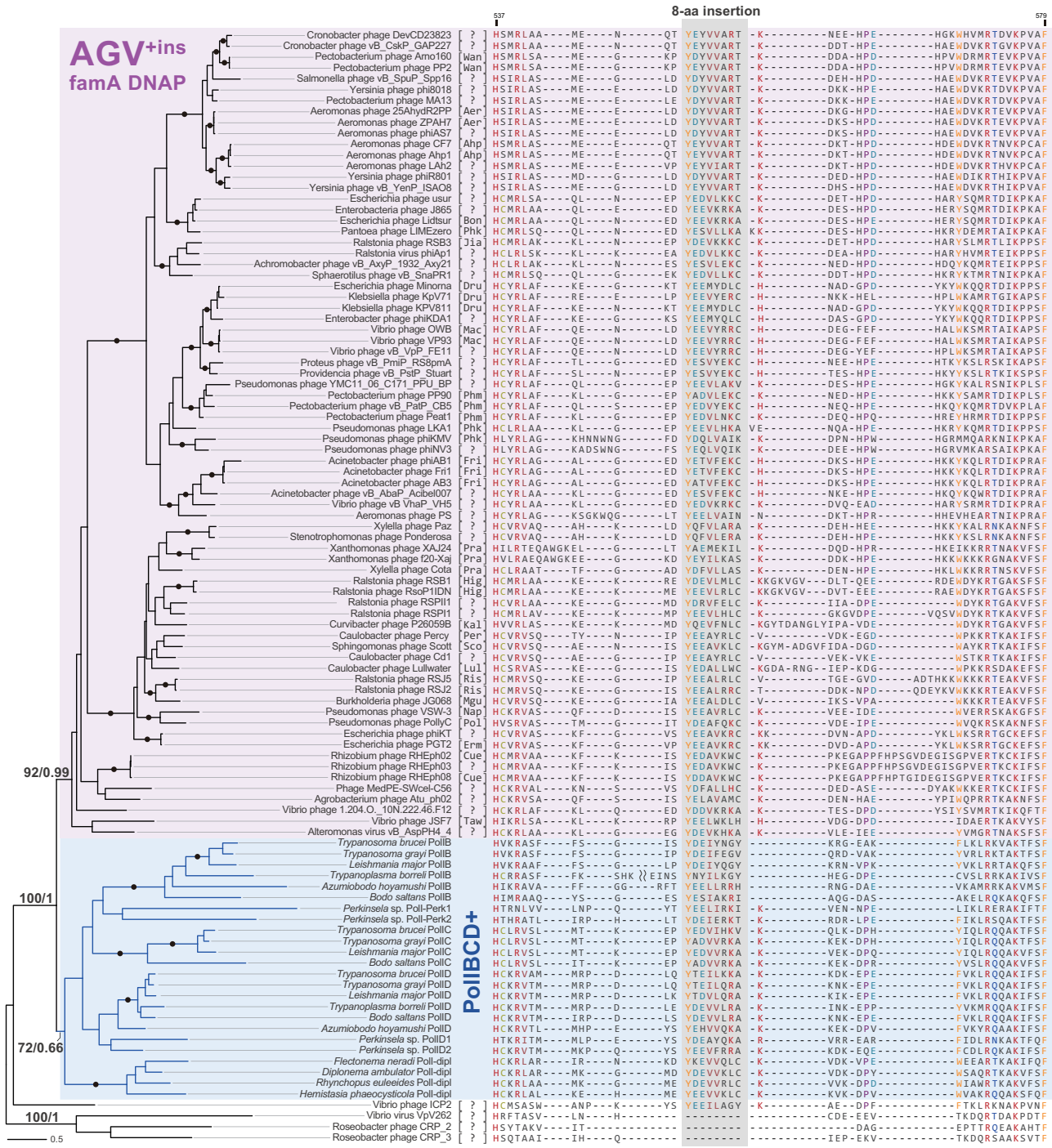


FIG. 2.—Phylogenetic relationship among 74 AGV⁺ins famA DNAP and 24 PolIBCD⁺ sequences that share a unique insertion of eight amino acid residues (8-aa insertion). The tree topology and branch lengths inferred by the maximum likelihood (ML) method are shown on the left. ML bootstrap values (MLBPs) and Bayesian posterior probabilities (BPPs) for only the nodes critical to infer the origin of PolIBCD⁺ are shown. As ML and Bayesian analyses reconstructed the essentially same tree topology, only BPPs for the selected nodes are presented. The nodes supported by an MLBP of 100% and a BPP of 1.0 are marked by dots. The genus names of the autographiviruses (and podoviruses), from which famA DNAPs were sampled, are given in brackets. Abbreviations are follows: Aer, Aerosvirus; Ahp, Ahphunavirus; Bon, Bonnellvirus; Cue, Cuernavacavirus; Dru, Drulivivirus; Erm, Ermolevavirus; Fri, Friunavirus; Hig, Higashivirus; Jia, Jiaoyazivirus; Kal, Kalpattivirus; Lul, Lullwatervirus; Mac, Maculivirus; Mgu, Mguivirus; Nap, Napahaivirus; Per, Percyivirus; Phk, Phikmvirus; Phm, Phimunavirus; Pol, Pollyceevirus; Pra, Pradovirus; Ris, Risjevirus; Sco, Scottvirus; Taw, Tawavirus; Wan, Wanjuvivirus; ?, unclassified. The amino acid sequences of 8-aa insertions and their flanking regions are shown on the right. 8-aa insertions are shaded in gray. The residues are colored according to their degrees of conservation. The amino acid residue numbers shown on the left and right edges of the alignment are based on the famA DNAPs of *Cronobacter* phage DevCD23823 (YP_009223394.1).

euglenozoan—had established the ancestral PollA. Although the origin of PollA has not been addressed explicitly, past studies recovered the phylogenetic link between PollA and Pol θ , a type of famA DNAP operated in the cytosol of eukaryotic cells. The original study reporting PollA, B, C, and D in *Trypanosoma brucei* has hinted at the phylogenetic affinity between PollA and Pol θ (Klingbeil et al. 2002). A recent phylogeny including famA DNAPs sampled from eukaryotes and limited bacteria (note that no phage homolog was included) reconstructed a clade of PollA and Pol θ sequences with high statistical support (Harada et al. 2020). The PollA–Pol θ affinity persisted even after the sampling of famA DNAPs from bacteria and phages was improved drastically in this study (fig. 1A). We here propose that the ancestral PollA was derived from a Pol θ homolog followed by the change in subcellular localization from the cytosol to the mitochondrion. Noteworthy, the evolutionary processes yielded PollA and PollBCD+ are different substantially from each other. The former emerged through the recycling of a pre-existing eukaryotic protein, whereas the latter is of phage origin (see above).

The repertoires of mitochondrion-localized DNAPs in euglenozoans appeared to be more complex than those in the majority of other eukaryotes in which a single type of mitochondrion-localized DNAP (i.e., POP or Pol γ) seemingly operates. The complexity in the repertoire of DNAPs in euglenozoan mitochondria seems to coincide with that in the structure of their mtDNAs (Lukeš et al. 2002; Roy et al. 2007; Spencer and Gray 2011; Dobáková et al. 2015; Yabuki et al. 2016; Burger and Valach 2018). Nevertheless, it is unlikely that the non- α -proteobacterial background is restricted to PollA and PollBCD+ among the proteins involved in mtDNA maintenance. Rather, the machinery for mtDNA maintenance in the common ancestor of kinetoplastids and diplomonids (and its descendants) are heavily remodeled by both incorporating exogenous proteins via lateral gene transfer and recycling the pre-existing nucleus-encoded proteins. The above conjecture can be examined only after we identify the major proteins involved in DNA maintenance in kinetoplastid/diplomonid mitochondria and their evolutionary origins.

Finally, we here explore briefly the early evolution of mitochondrion-localized DNAP. None of the known types of mitochondrion-localized famA DNAP showed any affinity to Poll of the extant α -proteobacteria (fig. 1A), suggesting that the famA DNAP operated in the mitochondrial endosymbiont was discarded in the early eukaryotic evolution. It is intriguing to point out that POP has been identified in phylogenetically diverse groups, whereas Pol γ , PollA, and PollBCD+ appeared to be specific to Opisthokonta, Euglenozoa, and a subclade in Euglenozoa (i.e., Kinetoplastea and Diplonemea), respectively (Moriyama et al. 2011; Moriyama and Sato 2014; Hirakawa and Watanabe 2019; Harada et al. 2020). The parsimonious interpretation of the distribution of POP, Pol γ , PollA, and PollBCD+ in the tree of eukaryotes nominates POP to be

the most ancient mitochondrion-localized DNAP. If so, the switch of mitochondrion-localized DNAP from POP to Pol γ may have occurred in the ancestral opisthokont species. In contrast, it is not straightforward to hypothesize how the inventory of mitochondrion-localized famA DNAPs in euglenozoans had been shaped. Recent phylogenomic studies unified jakobids, heteroloboseans, *Tsukubamonas globosa*, and euglenozoans into one of the major eukaryotic assemblages Discoba (Rodríguez-Ezpeleta et al. 2007; Yabuki et al. 2011, 2018; Kamikawa et al. 2014). Nevertheless, to our knowledge, none of POP, Pol γ , PollA, or PollBCD+ has been found in the discobid members except euglenozoans. Thus, even though euglenids use mitochondrion-localized POPs (POP_e1 and POP_Rhabd; Harada et al. 2020), it is uncertain whether the euglenid POPs were the direct descendants of the hypothetical POP established in the early eukaryotic evolution. Unfortunately, there are many lineages for which the information of mitochondrion-localized DNAPs is absent and we are currently not in the position to propose any scenarios for the early evolution of mitochondrion-localized DNAP (including that in the LECA) with confidence. To address the above issue appropriately, we need to understand the true diversity and distribution of mitochondrion-localized famA DNAPs in eukaryotes in the future.

Materials and Methods

Global Phylogeny of famA DNAPs

We searched for the amino acid (aa) sequences of bacterial and phage famA DNAPs in the NCBI nr database as of March 6, 2020, by BlastP using the polymerase domain of *Escherichia coli* Poll (KHH06131.1; the portion corresponding to the 491st–928th aa residues) as a query (Camacho et al. 2009; Sayers et al. 2020). We retrieved the sequences matched to the query with E values $\leq 1 \times 10^{-4}$ and covered more than 200 aa in the polymerase domain. Note that the sequences derived from metagenome analyses were excluded from this study. The redundancy within famA DNAP sequences was removed by a cluster analysis using CD-HIT v4.7 with a threshold of 40% (Li and Godzik 2006; Fu et al. 2012). We finally selected 119 and 327 aa sequences of phage and bacterial famA DNAPs, respectively, for the downstream analyses (see below).

The bacterial and phage famA DNAP aa sequences (446 in total) were aligned with those in eukaryotes (27 in total), namely 1) mitochondrion-localized famA DNAPs in Kinetoplastea and Diplonemea (PollA, B, C, D, and Poll-dipl), 2) mitochondrion-localized famA DNAPs in animals and fungi (Pol γ), 3) Pol θ localized in the cytosol, 4) mitochondrion and/or plastid-localized famA DNAPs in diverse eukaryotes (POP), and 5) plastid-localized famA DNAPs in apicomplexan parasites and their relatives (PREX). The aa sequences were aligned by MAFFT v7.455 with the L-INS-i model (Katoh and

Standley 2013). Ambiguously aligned positions were discarded manually, and gap-containing positions were trimmed by using trimAl v1.4 with the -gt 0.95 option (Capella-Gutiérrez et al. 2009). The final “global famA DNAP” alignment comprised 473 sequences with 316 unambiguously aligned aa positions. The final global famA alignment is provided as a part of the [supplementary materials](#) (Inagaki and Harad[dataset] 2020). We subjected this alignment to the ML phylogenetic analysis by IQ-TREE v1.6.12 using the LG + Γ + F + C60 + PMSF model that was selected by ModelFinder with -madd option (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017; Wang et al. 2018). The guide tree was obtained using the LG + Γ + F model, which was selected by ModelFinder with -m option. The statistical support for each bipartition in the ML tree was calculated by a 100-replicate nonparametric bootstrap analysis.

Phylogenetic Analyses of an Alignment Rich in Autographivirus famA DNAPs

We retrieved 175 famA DNAP aa sequences of autographiviruses from the NCBI nr database. The details of the survey were the same as described above. The 175 famA DNAPs were sampled from 99 members belonging to 57 genera and 76 unclassified members in the family Autographiviridae. The autographivirus famA DNAPs were found to comprise two types based on the presence/absence of an insertion of eight aa residues (8-aa insertion; see above). The famA DNAPs with 8-aa insertion (AGV^{+ins} famA DNAPs) appeared to be closely related to PollBCD+, mitochondrion-localized famA DNAPs in kinetoplastids (PollB, C, D, Poll-Perk1/2) and that in diplomonads (Poll-dipl). The redundancy among the AGV^{+ins} famA DNAPs was reduced by a cluster analysis using CD-HIT v4.7 with a threshold of 90%. Finally, we aligned the aa sequences of 74 AGV^{+ins} famA DNAPs, 24 PollBCD+, and famA DNAPs of four members of Podoviridae by MAFFT v7.455 with the L-INS-i model. Ambiguously aligned positions were discarded manually, and gap-containing positions were trimmed by using trimAl v1.4 with the -gt 0.9 option. The final version of the second alignment is provided as a part of the [supplementary materials](#) (Inagaki and Harad[dataset] 2020). The final alignment containing 102 sequences with 581 unambiguously aligned aa positions was subjected to both ML and Bayesian phylogenetic analyses. The ML and ML bootstrap analyses were performed as described above. For Bayesian analysis using Phylobayes v4.1 (Lartillot et al. 2009), we ran four Markov Chain Monte Carlo chains for 100,000 cycles with burn-in of 25,000 (maxdiff = 0.09472) and calculated the consensus tree with branch lengths and BPPs from the remaining trees. The aa substitution model was set to CAT + GTR in Phylobayes analysis described above.

Supplementary Material

[Supplementary data](#) are available at *Genome Biology and Evolution* online.

Acknowledgments

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Data Availability

The [supplementary data](#) are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.9kd51c5fv>.

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