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(RESEARCH ARTICLE)

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Polymerase and Proofreading Exonuclease Domains of the Nuclear-encoded DNA-dependent RNA Polymerase of Plant Mitochondria

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Abstract

Mitochondria, found in all eukaryotic cells, play a crucial role in generating much needed biological energy for the cells in the form of adenosine triphosphates (ATPs). It is a semi-autonomous organelle and is partly controlled by its own genome and mostly by the nuclear imports. To replicate its own genome, it uses two DNA polymerases, viz. polymerases IA and IB which are essentially similar to the *E. coli* DNA polymerase I. The nuclear-encoded RNA polymerase (NEP) (EC 2.7.7.6) is imported from the nucleus and involves in the transcription of all mitochondrial genes. In *Arabidopsis thaliana,* the mitochondrial NEP showed 59.05% identity to the NEP of the chloroplasts, but only 28.24% identity to the T7 RNA polymerase, suggesting the NEPs of mitochondria and chloroplasts are distinctly different. However, in both the plant NEPs, the polymerase catalytic core and proofreading (PR) exonuclease domains are completely conserved. The mitochondrial NEP's catalytic core from different plant sources is remarkably conserved and is in close agreement with other RNA polymerases reported already and possesses a typical template-binding pair (-YG-), a basic catalytic amino acid (K) to initiate catalysis and a basic nucleotide selection amino acid R at -4, from the catalytic K. The catalytic metal-binding motifs are identified based on sequence similarity and site-directed mutagenesis (SDM) experiments. The PR exonuclease of the mitochondrial NEP belongs to the DEDD-superfamily of exonucleases.

Keywords: Mitochondrial transcription; Nuclear-encoded RNA polymerase; RNA polymerase active site; Proofreading exonuclease; Exonuclease active site; Mechanism of action

1 Introduction

Mitochondria are double-membrane structures found in all eukaryotic cells and harbour the mitochondrial genomic DNAs (mtDNAs) and mitochondrial plasmids. The mitochondria are of sizes ranging from 0.5 to 4 μm and usually about dozens of mtDNA copies are found in a single mitochondrion and furthermore, as a single eukaryotic cell harbours 100s of mitochondria, the total number of copies of mtDNA exceeds > 1000 copies per cell. The copy number and shape of mitochondria vary considerably in different cell types and under different physiological conditions. They generate the much needed chemical energy for cellular activities in the form of ATPs by the process of aerobic respiration and hence, known as the power house of the cells. Thus, they are essential organelles found in all eukaryotes from yeasts to animals and plants.

Plant mitochondrial genomes are large and complex as compared to animal mitochondrial genomes. For example, nearly all animal mitochondrial genomes are ~16.5 kb in length with no or a few introns and possess little non-coding DNA, whereas plant mitochondrial genomes are quite large and their sizes range from 200 to 2500 kb. For example, the model plant *Arabidopsis thaliana*'s mitochondrial genome is of 3, 66,924 bp and codes for 33 proteins, 3 rRNAs and 21 tRNAs [1]. It is interesting to note that even though their genomes are much bigger in size, they do not contain significantly more genes than their animal counterparts. The larger size of plant mitochondrial genomes is mainly due to large numbers of introns, repeats and non-coding regions in their genomes. Using mostly nuclear-encoded proteins,

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mitochondria contain all of the machinery for transcribing and translating genes encoded by the mtDNA. Unlike the nucleus, a single mitochondrion possesses many copies of its own genome. For example, depending on the tissue type and age of the plant, the number of copies per mitochondrion can vary between 50 and 500 [2].

Replication of mtDNA utilizes a bacterial-like, single subunit DNA polymerases, viz. IA and IB. Thus, most plants possess two nuclear-encoded copies of the DNA polymerases, IA and IB. It is interesting to note that in all plants where localization has been examined, these enzymes are dual targeted to both mitochondria and chloroplasts [3]. Structural, phylogenetic and multiple sequence alignment (MSA) analysis of plant mitochondrial and chloroplast DNA polymerases reveal a similarity to bacterial DNA polymerase I [4,5]. mtDNAs play an important role in cytoplasmic male sterility and this property is attributed mainly to mtDNA rearrangements, causing plants to lose their ability to make pollen, a trait which is exploited by crop breeders for hybrid generation.

1.1 Transcription by Plant Mitochondrial RNA Polymerase

RNA polymerases are crucial enzymes of life as they control the gene expression at the transcription level. Therefore, understanding the structure, function, mechanism and regulation of RNA polymerases has been the primary goal of molecular biologists since its discovery. Interestingly, these polymerases are highly conserved in all kingdoms of life, from viruses to animals and plants. [6]. Whereas the DNA polymerases essentially involve in the replication and repair of genomes, the RNA polymerases are involved in transcription of genomes, i.e., involved in the flow of genetic information from DNA→RNA in both prokaryotes and eukaryotes.

Different types of RNA polymerases perform transcription in prokaryotes and eukaryotes. For example, viruses contain two types of RNA polymerases (RNAPs), viz. DNA-dependent RNAPs (DdRps) and RNA-dependent RNAPs (RdRps), which are single-subunit (SSU) types. Eubacteria and archaebacteria employ a single type of RNA polymerase for all their transcription needs, but it is of a multi-subunit (MSU) type. However, eukaryotes use 5 different types of RNA polymerases (I-V), which are also of MSU type [6]. The chloroplast transcription in higher plants is performed by two types of RNAPs, i) plastid-encoded RNA polymerase (PEP) and ii) nuclear-encoded RNA polymerase (NEP). PEP is essentially a eubacterial-type MSU enzyme whose catalytic core subunits are encoded by the chloroplast genome itself. However, the mitochondria use only one type of RNA polymerase that is also nuclear-encoded (NEP). Both the plant NEPs are SSU enzymes which are of T7 bacteriophage-type. [7]. Three different subtypes of NEPs are reported from mitochondria and chloroplasts in plants, viz. RPOT-1, RPOT-2 and RPOT-3. The RPOT-1 and RPOT-3 are imported into mitochondria and chloroplasts, respectively, whereas the RPOT-2 has dual targeting properties and transcribes genes in both the organelles [8].

1.2 PR Functions in RNA Polymerases

Both the DNA and RNA polymerases do make mistakes during the replication/transcription processes, but rarely [9]. However, the mistakes are corrected promptly by the PR enzymes associated with or part of these polymerases. Based on the active site structures, the PR enzymes are classified into two major groups, viz. DEDD- and PHP–superfamilies. The DEDD-superfamily is the most common in biological systems. There are two types of DEDD-superfamilies: One type of DEDD-superfamily is intrinsic type and is found in the same DNA/RNA polymerase polypeptide and functions as a multifunctional enzyme (e.g.), *E. coli* DNA pol I. In the second type of DEDD-superfamily is extrinsic type and the exonuclease function is performed by a tightly associated subunit along with the polymerase subunits in a multienzyme complex system (e.g.), ε-subunits of bacterial DNA pols III, ExoNs in the SARS-Coronaviruses, in the PA subunits of the polymerase in human influenza viruses and T4 DNA polymerase [7,10-13]. Again, the DEDD-superfamily of PR exonuclease consists of two subfamilies, viz. DEDD(Y) and DEDD(H), depending upon whether they employ an invariant Y or a H as the proton acceptor to initiate catalysis [14].

These two superfamilies, DEDD and PHP are invariably found/associated with the DNA/RNA replicases and transcriptases to repair any error that might occur during the replication/transcription processes [15]. However, the PHP-superfamily is not that common and has been reported only from the bacterial kingdom, but also has been reported from the viral kingdom by Palanivelu, recently [11]. The PHP-superfamily is found in the bacterial replicative DNA polymerases III (intrinsic) (the DNA pols III belong to the C-family polymerases), and in bacterial DNA polymerases X. (It is interesting to note, that the A- and B-families of the replicative polymerases are found both in prokaryotes and eukaryotes, but the C-family is found only in bacterial kingdom. Whereas the A–family polymerases possess two exonuclease domains (3→5' and 5'→3' exonucleases), the B-family polymerases possess only one exonuclease domain, i.e., 3'→5' PR exonuclease) [16]. All three families of DNA polymerases are mainly involved in genome replication and repair in biological systems.

The error rates of RNAPs are very minimal and generally in the range of 10^{-4} to 10^{-6} [7] and are corrected promptly during the transcription process itself. For error corrections, the RNAPs have evolved essentially two different mechanisms (Table 1). In the MSU-RNAPs of prokaryotes, eukaryotes, and chloroplasts, which are involved mainly in the transcription of mRNAs, the PR active site is embedded within the polymerase active site itself [6,9]. However, in other SSU RNAPs, the PR activity is either found on the same polypeptide as a separate domain (multifunctional enzyme type, MFE) or with a closely associated subunit of the enzyme (multienzyme complex type, MEC) as discussed earlier. The mitochondrial mRNAs have no 5' caps but have poly–A tails. Table 1 shows the types of PR activities and their localization in viruses, prokaryotes, eukaryotes and organelles.

Table 1 PR activities and their localization in viruses, prokaryotes, eukaryotes and organellar RNA polymerases

RNA/DNA Pols	Source(s)	PR active site	Reference(s)
DdRps-MSU-RNAP	**Prokaryotes**	Intrinsic within the RNAP active site itself	[6,9]
DdRps-MSU-RNAPs	**Eukaryotes**		
DdRps-MSU-RNAPs	**All eukaryotic (pols I-III)**	Intrinsic within the RNAP active site itself	[6,9]
DdRps-MSU-RNAPs	**Plants (pols IV & V)**	Intrinsic within the RNAP active site itself	[6,9]
RdRps-SSU Types	**(+) Strand RNA Viruses** (e.g., SARS-CoVs, SARS-related CoVs, Human-CoVs)	DEDD(**H**)-superfamily# (In the associated ExoN subunit)	[10]
RdRps-SSU Types	**(-) Strand RNA Viruses** (e.g., Human Influenza Viruses A, B & C)	DEDD(**H**)- superfamily# (In the PA subunit of the RNA polymerase)	[11]
DdDps-SSU Type	**T4 DNA pol** (*E. coli* Phage)	DEDD(**Y**) superfamily^ (In the same polypeptide)	[12]
DdDps-SSU Type	***E. coli* DNA pol I**	DEDD(**Y**) superfamily^ (In the same polypeptide)	[13]
	Plant (Chloroplasts)		
DdDps-SSU Types	**DNA pols IA & IB** (Plant chloroplasts)	DEDD(**Y**) superfamily^ (In the same polypeptide)	[5]
DdRps-SSU Type	(**NEP**) (Plant chloroplasts)	DEDD(**H**) superfamily# (In the same polypeptide)	[5]
	Plant (Mitochondria)		
DdRps-SSU Type	RNA Pol (**NEP**) (Plant Mitochondria)	DEDD(**H**) superfamily# (In the same polypeptide, MFE)	This work

Pols, Polymerases. ^*E. coli* DNA pol I and pol II types; # Similar to the ε-subunit of bacterial DNA pols III (DNA replicases).

The polymerase and PR exonuclease domains in the DNA polymerases IA, IB and Nuclear-Encoded RNA Polymerase (NEP) of the plant chloroplasts and the MSU eubacterial type PEP are already discussed in detail by Palanivelu [5 and references therein], and, therefore, the polymerase and PR exonuclease domains of the NEP of plant mitochondria are analyzed and reported in this communication.

2 Material and methods

The protein sequence data of the NEPs from various plant mitochondria were obtained from PUBMED and SWISS-PROT databases. The advanced version of Clustal Omega was used for protein sequence analysis. The polymerase and PR active sites are arrived at by sequence similarities, site-directed mutagenesis (SDM) and X-ray crystallographic data from other DNA and RNA polymerases, already reported.

3 Results and discussion

3.1 Analysis of Mitochondrial NEPs

Figure 1 shows the MSA of the mitochondrial NEPs from various plant sources. (Only the regions required for the discussions are shown here). The *A. thaliana* sequence is used as the reference and highlighted in yellow. The N-terminal region of ~150 amino acids is not conserved and showed many gaps in the alignment (not shown). After that, conservations are observed and a clear demarcation of the PR exonuclease domain is seen (highlighted in red). The PR exonuclease domain contains the typical DEDD-superfamily active site amino acids and is highlighted in light blue. The first conserved triad DV/I/TE of the DEDD-superfamily of exonucleases is seen after ~500 amino acids from the N-terminal. Again, after ~745 amino acids, a second demarcation in the sequences is seen and that contains the polymerase domain and the active site amino acids are highlighted in yellow. The polymerase core region is completely conserved in all NEPs from different plant sources and contains the template-binding -YG- pair, the catalytic amino acid K and the nucleotide discriminating amino acid R at -4 from the catalytic K. The active site, -^{746}VDR-^{4}KLVK^{752}Q^{1}TVMTSVY^{8}GVT-, is found to be very similar to the confirmed active site of *E. coli* DNA pol I, -QR-^{4}RSAK^{758}A^{1}INFGLIY^{8}GM- [13] and in close agreement to the active sites of the other DNA/RNA polymerases already reported (Table 2) [7]. The catalytic metal-binding Ds in –HQD^{677}- and –HD^{909}S- motifs are highlighted in dark green. Site-directed mutagenesis data have shown that the substitutions of the Ds in –AFD^{537}G- and -HD^{812}S motifs to D^{537}→N and D^{812}→N resulted in the complete loss of T7 RNAP activity [17,18]. An additional –DxD- type metal-binding motif is found in this domain and highlighted in light green. All the RNA polymerases invariably end in the tetrapeptide, -YFFN- and its significance is not clear now. Interestingly, a highly conserved decapeptide, –IPYL/I/VPML/I/VPP-, is found in all the mitochondrial NEPS at the N-terminal region (highlighted) and its significance is not clear now.

3.1.1 CLUSTAL O (1.2.4) MSA of plant mitochondrial RNA polymerases (NEPs)

```
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tr|S8EG02|S8EG02_9LAMI              HTSRTVCREK--RQTRRYGVVQCDPLVHKGLEKAARHMVIPYMPMLVPPVNWTGYDKGGY      412
tr|A0A0Q3EKS1|A0A0Q3EKS1_BRADI      HEMRTVTREQQ-KSSRRYGVIKCDPLIRQGLDRTAKHMVIPYMPMLIPPISWTGYDKGAH      438
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tr|A0A8J5SZQ4|A0A8J5SZQ4_ZIZPA      HVLRQPIVENG-RLKKKHWVIECDHLVHEGFESTARHVEIPYLPMLIPPKKWKSYDTGGY      394
                                    :  :       .  . :  : *. ::  *.  :.. : :**:***:**  * .*: *.:
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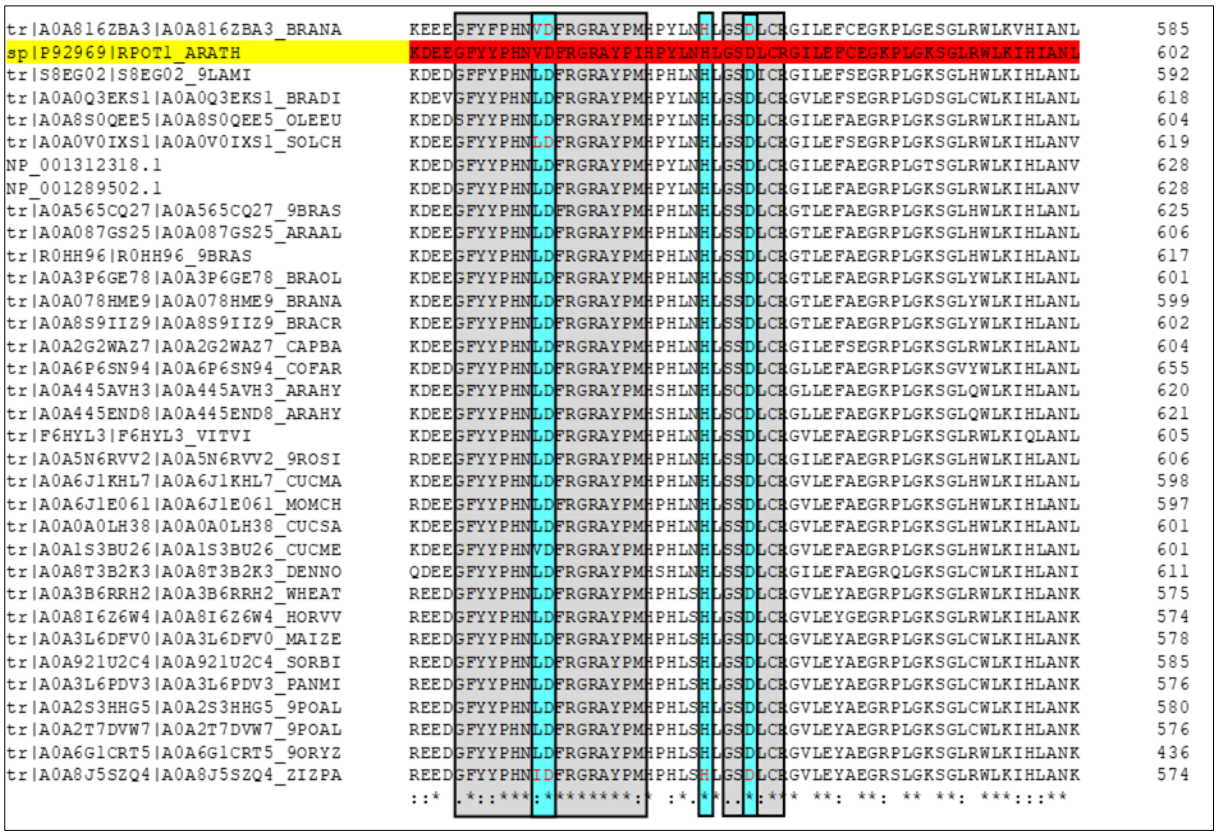
PR exonuclease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sp|P92969|RPOT1_ARATH KDEEGFYYPHNVDFRGRAYPIHPYLNHLGSDLCRGILEFCEGKPLGKSGLRWLKIHIANL 602
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tr|A0A6G1CRT5|A0A6G1CRT5_9ORYZ REEDGFYYPHNLDFRGRAYPMHPHLSHLGSDLCRGVLEYAEGRPLGKSGLRWLKIHLANK 436
tr|A0A8J5SZQ4|A0A8J5SZQ4_ZIZPA REEDGFYYPHNIDFRGRAYPMHPHLSHLGSDLCRGVLEYAEGRSLGKSGLRWLKIHLANK 574


PR Exonuclease
Polymerase
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sp|P92969|RPOT1_ARATH YAGGVDKLAYEDRIAFTESHLEDIFDSSDRPLEGKRWWLNAEDP FQCLAACINLSEALRS 662
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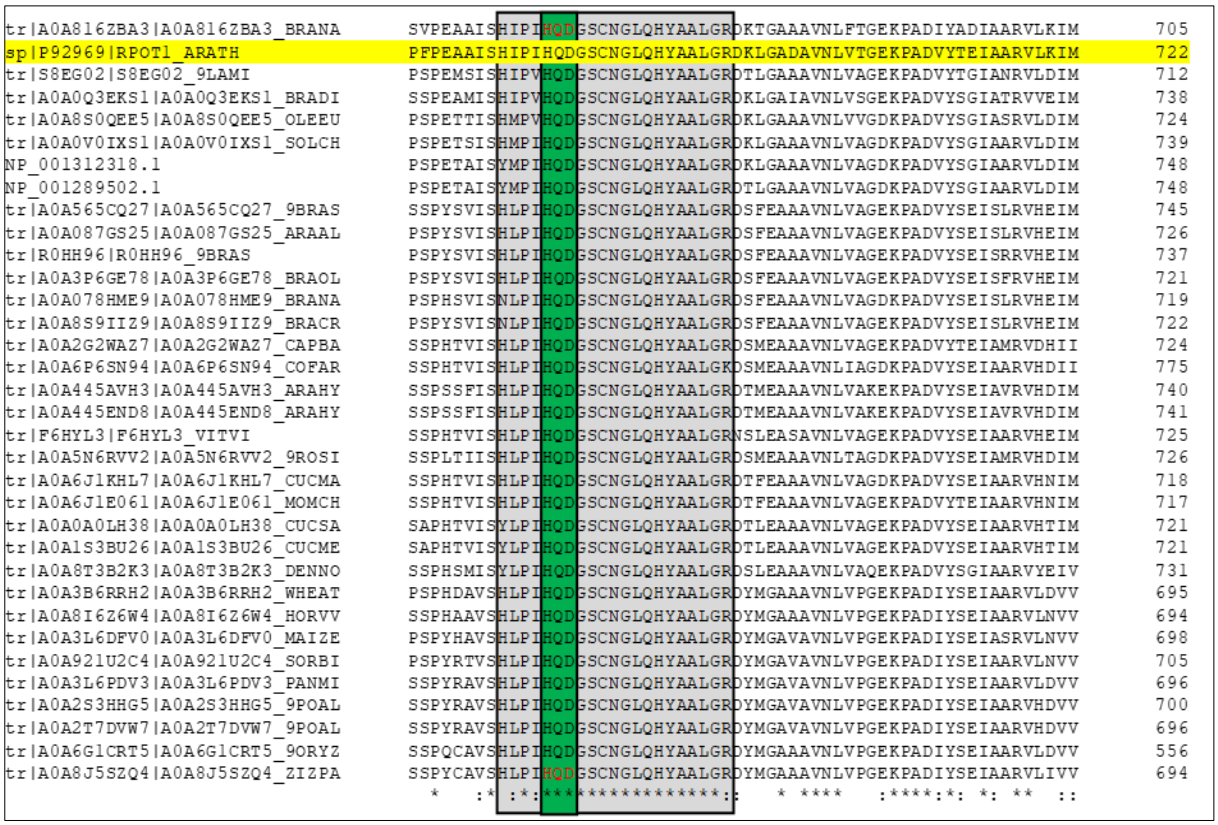
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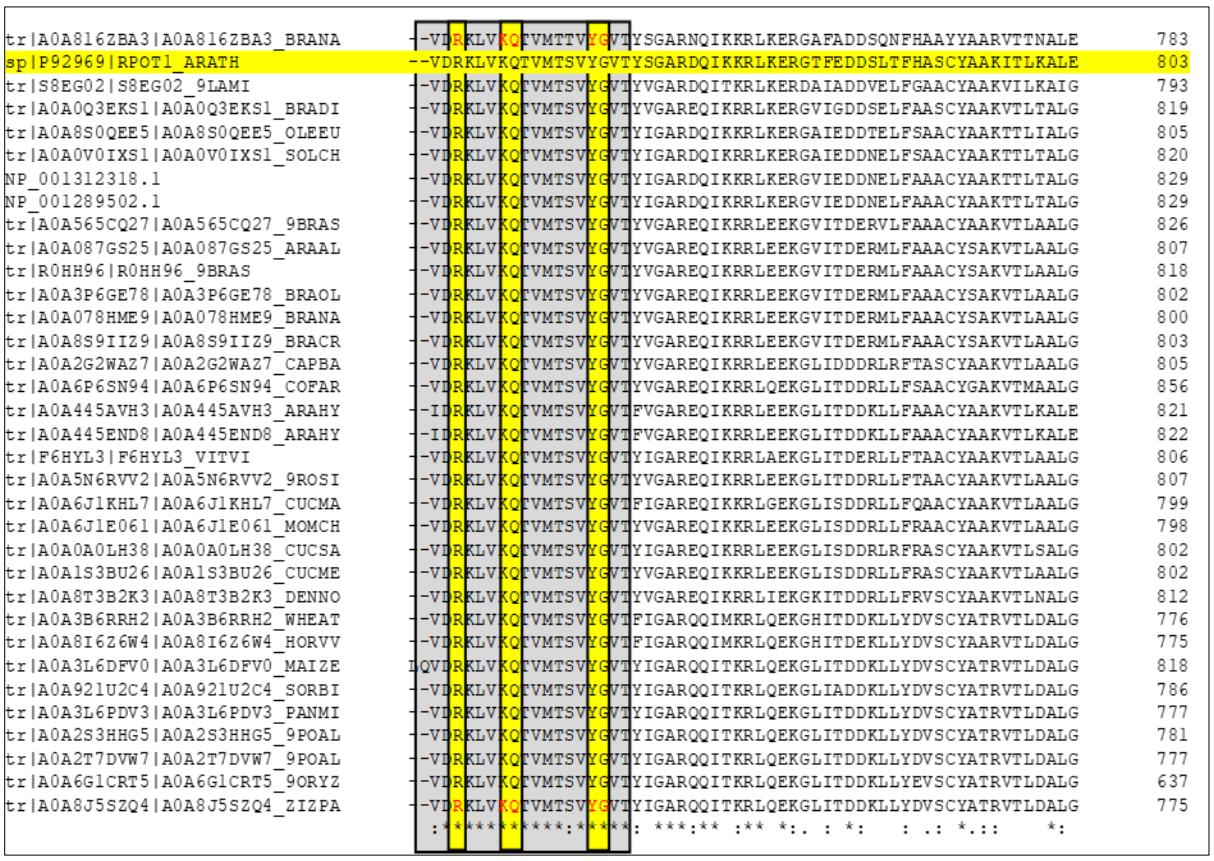
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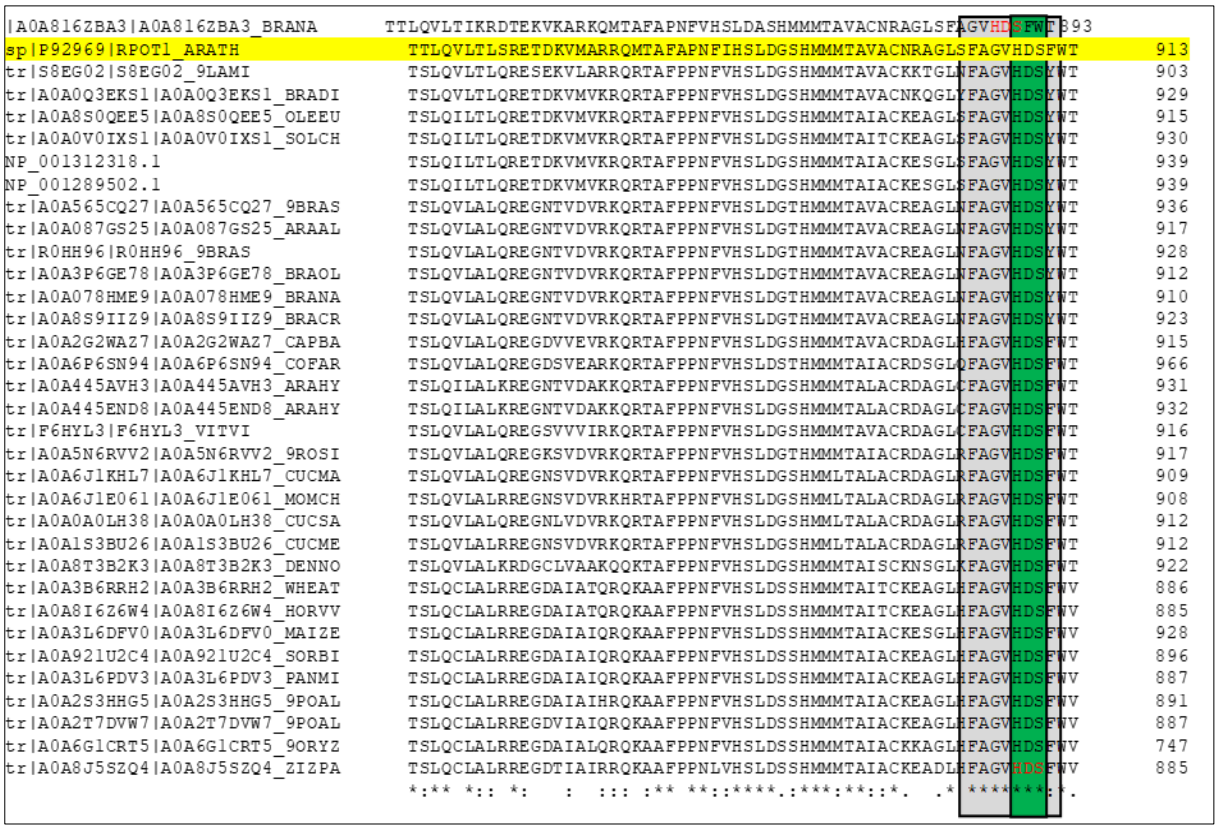
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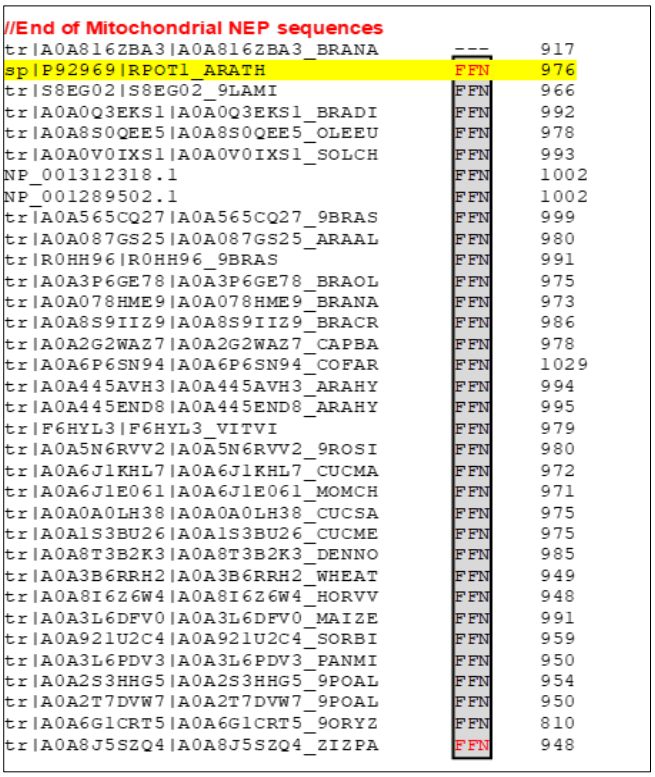

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A0A445END8_ARAHY *Arachis hypogaea*
A0A5N6RVV2_9ROSI *Carpinus fangiana*
A0A6J1E061_MOMCH *Momordica charantia*
A0A1S3BU26_CUCME *Cucumis melo*
A0A3B6RRH2_WHEAT *Triticum aestivum*
A0A3L6DFV0_MAIZE *Zea mays*
A0A3L6PDV3_PANMI *Panicum miliaceum*
A0A2T7DVW7_9POAL *Panicum hallii*
A0A8J5SZQ4_ZIZPA *Zizania palustris*

Figure 1 MSA of mitochondrial RNAPs (NEP) from various plant sources

3.2 'Mix and Match' MSA analysis of Plant Mitochondrial and Chloroplast NEPs

Figure 2 shows the 'mix and match' MSA of the plant mitochondrial and chloroplast NEPs. The N-terminal regions do not show much conservation, up to ~150 amino acids. Even though both show many consecutive Ss in the N-terminal regions, but the chloroplast N-terminal region shows a large number of consecutive Ss up to 9 of them and in addition to that, it also has a –DxD- type metal-binding motif (highlighted in green). BLASTp analysis of both the NEPs of *Arabidopsis thaliana* was performed. The mitochondrial NEP of *Arabidopsis thaliana* shows 59.05% identity to the chloroplast NEP, suggesting the NEPs of mitochondria and chloroplasts are distinctly different, but showed complete conservation of their catalytic regions (Fig. 2). Even though these NEPs are classified as T3/T7 RNA polymerase type, but they showed very little identity (the mitochondrial NEP of *Arabidopsis thaliana* showed only 28.24% identity to the T7 RNAP) suggesting a drastic divergence during evolution, but maintaining the catalytic cores still intact. The decapeptides in the NTD regions are highly conserved in both NEPs and highlighted. It is interesting to note that the metal-binding motifs, -HQ**D**-, -H**D**S- and –**D**v**D**-, and the catalytic core regions, viz. -QVD**R**KLV**KQ**TVMTSV**YG**VTY- are completely conserved in both the NEPs and highlighted. The CTD contains an invariant tetrad, –**YFFN**- motif (marked in red) in both the NEPs suggesting a possible common role.

3.1.2 CLUSTAL O (1.2.4) 'Mix and Match' MSA analysis of mitochondrial and chloroplast NEPs

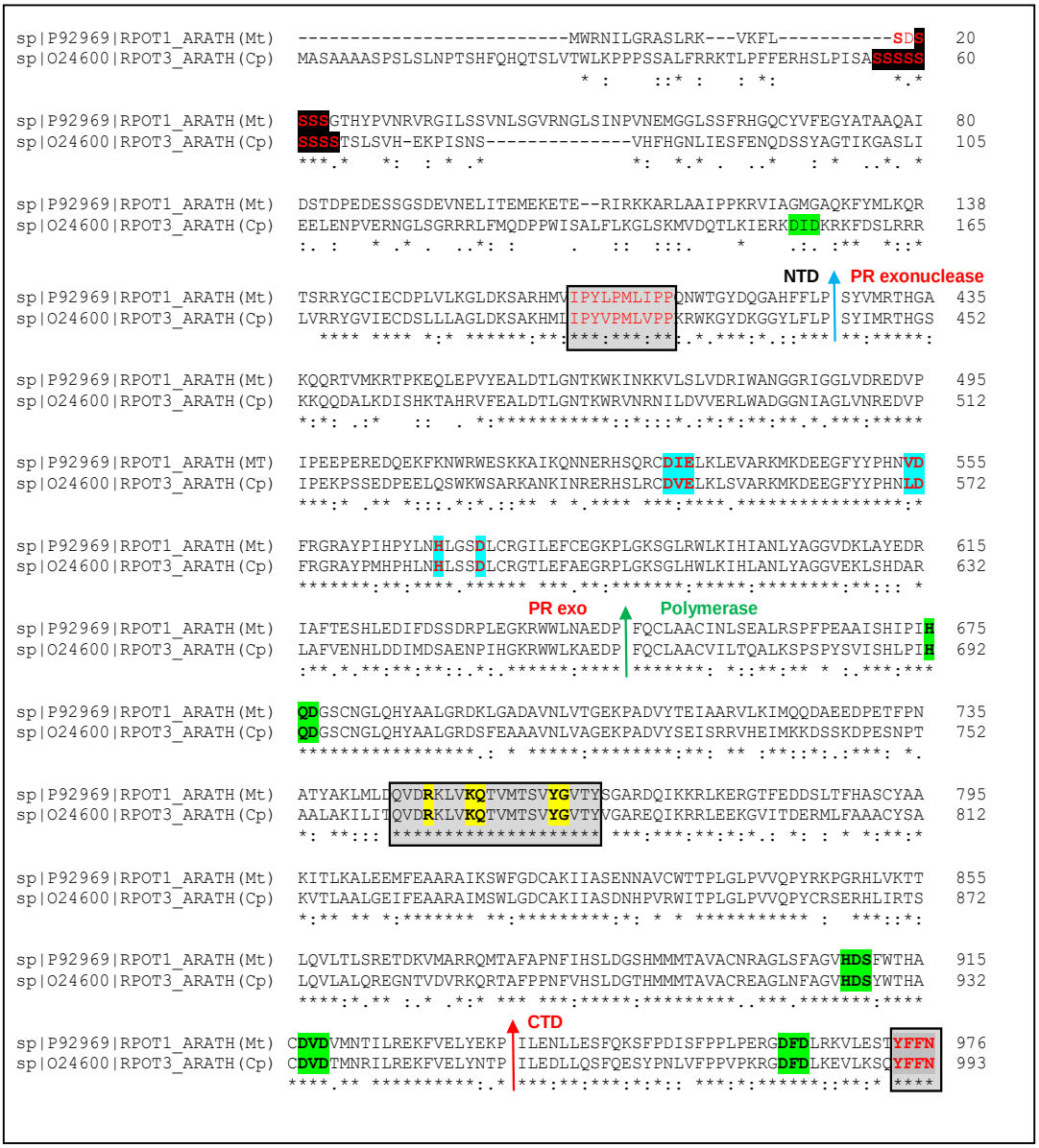


Figure 2 'Mix and Match' MSA analysis of the NEPs of mitochondria and chloroplasts from various plant sources

Figure 3 shows the organization of the NEPs of the mitochondria and chloroplast of *A. thaliana*. The NTDs are not conserved in both the cases, whereas the other three domains are highly conserved and organized in the same order.

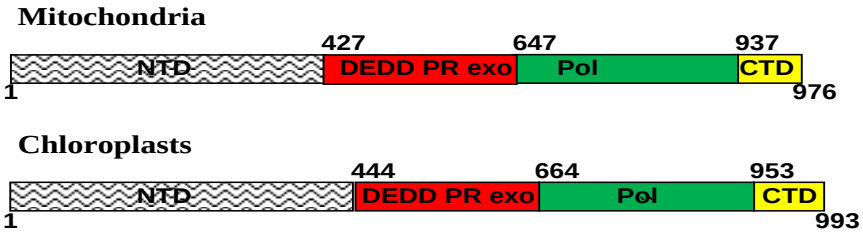


Figure 3 Domain-organization of the NEPs of mitochondria and chloroplasts (Numberings from the *A. thaliana* sequence)

3.3 Analysis of Active Site Amino Acids of the NEP from Plant Mitochondria

3.1.3 Active Site Amino Acids of the RNA Polymerase Catalytic Core

The catalytic core region essentially contains three components in DNA/RNA polymerases, viz. a template-binding pair –YG-, a basic catalytic amino acid -K/R- and a nucleotide discriminating amino acid –R-, placed at -4 to -5 from the catalytic K/R. These three highly conserved components of the catalytic core are found in the NEPs of the plant mitochondria also. Interestingly, the plant mitochondrial NEP's catalytic core, -^{748}D**R**$^{-4}$KLV**K**^{753}**Q**^{1}TVMTSV**Y**^{8}**G**V-, is identical to the catalytic core of the chloroplast NEP of *A. thaliana,* suggesting their common origin. Furthermore, the mitochondrial NEP's catalytic core regions are in close agreement with those already reported from other DNA/RNA polymerases (Table 2).

Table 2 Catalytic core regions of various RNA and DNA polymerases

Polymerase type	Catalytic core
SSU RNA/DNA pols	
Viral T7 SSU RNA pol	-^{620}WLAY^{-8}GVTR^{-4}SVTKR^{1}SVMTLAY^{8}GS-
Viral SP6 SSU RNA Pol	-^{612}WDSI^{-8}GITR^{-4}SLTKK^{1}PVMTLPY^{8}GS-
Mitochondrial SSU RNA pol (*Sc*)	-^{1009}TR^{-4}KVVKQ^{1}TVMTNVY^{8}GV-
Mitochondrial SSU RNA pol (*H. sapiens*)	-^{986}TR^{-4}KVVKQ^{1}TVMTVVY^{8}GV-
E. coli DNA pol I (SSU)	-^{753}QR^{-4}RSAK^{758}A^{1}INFGLIY^{8}GM-
Chloroplast SSU DNA pol IA (*ARATH*)	-^{873}ER^{-4}RKAK^{878}M^{1}LNFSIAY^{8}GK-
Chloroplast SSU DNA pol IB (*ARATH*)	-^{857}ER^{-4}RKAK^{862}M^{1}LNFSIAY^{8}GK-
Chloroplast SSU RNA pol (NEP) (*ARATH*)	-^{765}DR^{-4}KLVK^{770}Q^{1}TVMTSVY^{8}GV-
Mitochondrial SSU RNA pol (NEP) (*ARATH*)	-^{748}DR^{-4}KLVK^{753}Q^{1}TVMTSVY^{8}GV-

Adapted from Palanivelu [5]; *Sc, Saccharomyces cerevisiae; ARATH, Arabidopsis thaliana;* The active site amino acids, highlighted in dark blue, are confirmed by SDM; and other techniques.

3.1.4 Polymerase Metal-Binding Sites

The second important site for the polymerase function is the catalytic metal-binding site which involves in the addition of the incoming nucleotides to the 3'-OH of the growing primer. The catalytic metal-binding amino acids of the mitochondrial NEP are arrived at sequence similarity. The two **Ds** in the –HQ**D**^{677}- and –H**D**^{909}S- motifs (highlighted in dark green in Figs. 1 and 2) are proposed as the catalytic metal-binding amino acids and they could bind a Mg^{2+} during catalysis. Interestingly, these two motifs are completely conserved in the NEPs of both mitochondria and chloroplasts from various plant sources (Fig. 2). The –H**D**^{812}S- motif is also found in T7 RNAP and its involvement was confirmed by an SDM experiment. It was found that the –**D**^{812}→N in the –H**D**S- motif exhibited no detectable activity [17]. The 2Ds, proposed in the metal-binding sites are also reported to be highly conserved among most of the SSU RNA polymerases. Usually the D in –Q**D**- and the D in H**D**S are found to be involved in Mg^{2+}-binding and 'NTP charge shielding'. They are widely reported in the SSU RNA polymerases like T3/T7 phage RNA polymerases and SSU RNA polymerases of plant and fungal mitochondria and plant chloroplasts [7]. Based on these data, Fig. 4 shows the proposed active site amino acids of the polymerase domain of the plant mitochondrial NEP. For a detailed description of the polymerase's mechanism, see Palanivelu [19].

3.1.5 PR Exonuclease Active Site

The PR exonuclease active site amino acids of NEPs from various plant mitochondria are arrived at from MSA analysis and their sequence similarities to other well-established DEDD-exonuclease superfamilies. The NEPs from various plant mitochondria belong to DEDD(H) subfamily as discussed below. The DEDD-superfamily of PR exonucleases uses either a Y or a H as the catalytic proton acceptor. An invariant H is found in the active site of mitochondrial NEPs (Table 3), and thus, this one belongs to the DEDD(H)-subfamily. Fijalkowska and Schaaper [20] have found DEDD(H)-subfamily of

PR exonuclease in the ε-subunits of the bacterial replicase multienzyme complexes (DNA pols III) which belongs to the DnaQ-H-family with the four active site carboxylates (D^{12}, E^{14}, D^{103}, and D^{167}) with the invariant **H^{162}**, which acts as the general base in catalysis. They also found that modification of the two conserved amino acid residues, viz. **D^{12}**→Ala and **E^{14}**→Ala, in the ε-subunit by SDM experiments, resulted in the loss of the exonuclease function. These observations were further corroborated by X–ray crystallographic analysis of the ε-subunit, ε-186, by Hamdan et al. [21]. An interesting DEDD-superfamily of PR 3'→5' exonuclease was found not in polymerases, but in the tRNA processing enzyme, RNase T. At least five amino acid residues, viz. D^{23}, E^{25}, D^{125}, **H^{181}** and D^{186} were found on the active site of *E. coli* RNase T. The crystal structures of RNase T from *Pseudomonas aeruginosa* and *E. coli* have been reported by Zuo et al. [22] and they found it also belongs to DEDD(H)-subfamily.

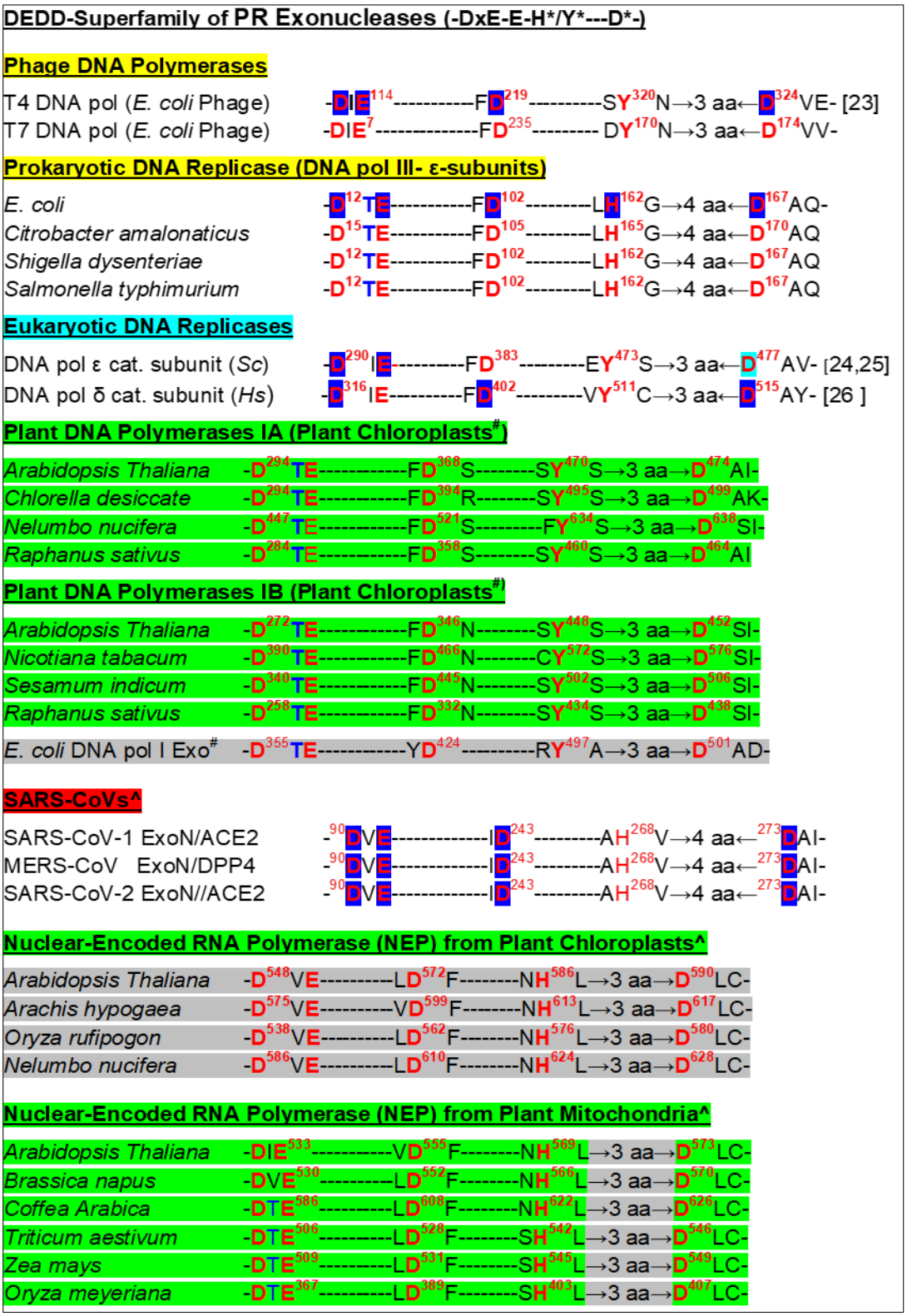
Tables 3 and 4 show the summary of the identified/confirmed DEDD(Y/H)-exonuclease active site amino acids in DNA/RNA polymerases from different viral, bacterial, fungal, plant and animal sources.

Table 3 DEDD-superfamily of PR exonuclease active site amino acids from viral, bacterial, fungal, plant and animal sources

Exo-Family	Consensus A-site Pattern	Proton Acceptor	Catalytic Metal ion*	ZBSs
DEDD(Y/H)-superfamily				
Prokaryotic PR Enzymes				
T4 DNA pol (*E. coli* Phage)	-D^{112}xE^{114}-D^{219}-Y^{320}-D^{324}-	Tyr	Zn^{2+}	1
DNA pol I (*E. coli*)	-D^{355}xE^{357}-D^{424}-Y^{497}-D^{501}-	Tyr	Zn^{2+}	1
DNA pol II (*E. coli*)	-D^{156}xE^{158}-D^{229}-Y^{331}-D^{335}-	Tyr	Zn^{2+}	1
RNase D (*E. coli*)	-D^{28}xE^{30}---D^{85}--Y^{151}-D^{155}-	Tyr	Zn^{2+}	1
DNA pol III, ε-subunit (*E. coli*)	-D^{12}xE^{14}-D^{103}-H^{162}-D^{167}-	His	Zn^{2+}	1
RNase T (*E. coli*)	-D^{23}xE^{25}-D^{125}-H^{181}-D^{186}-	His	Zn^{2+}	1
PR Enzymes in Eukaryotic DNA Replicases				
DNA pol ε cat. subunit (*Sc*)	-D^{290}VE^{292}-D^{383}-Y^{473}-D^{477}-	His	Zn^{2+}	1
DNA pol δ cat. subunit (*Hs*)	-D^{316}IE^{318}--D^{402}-Y^{511}-D^{515}-	His	Zn^{2+}	1
PR Enzymes in DNA Polymerases 1A and 1B from Plant Chloroplasts				
DNA polymerase 1A (*ARATH*)	-DTE^{296}--------D^{368}-----Y^{470}—D^{474}-	Tyr	Zn^{2+}	1
DNA polymerase 1B (*ARATH*)	-DTE^{272}--------D^{346}-----Y^{448}—D^{452}-	Tyr	Zn^{2+}	1
PR Enzymes in RNA Polymerase, NEP from Plant Chloroplasts				
Arabidopsis Thaliana	-DVE^{550}--------D^{572}-----H^{586}—D^{590}-	His	Zn^{2+}	1
Arachis hypogaea	-DVE----------D---------H------D-	His	Zn^{2+}	1
Oryza rufipogon	-DVE----------D---------H------D-	His	Zn^{2+}	1
Nelumbo nucifera	-DIE----------D---------H------D-	His	Zn^{2+}	1
Nicotiana tabacum	-DTE----------D---------H------D-	His	Zn^{2+}	1
Capsicum annuum	-DTE----------D---------H------D-	His	Zn^{2+}	1
Carpinus fangiana	-DTE----------D---------H------D-	His	Zn^{2+}	1
PR Enzymes in RNA Polymerase, NEP from Plant Mitochondria				
Arabidopsis Thaliana	-DIE^{533}--------D^{555}-----H^{569}—D^{573}-	His	Zn^{2+}	1
Brassica napus	-DVE----------D---------H-------D-	His	Zn^{2+}	1
Coffea Arabica	-DTE----------D---------H-------D-	His	Zn^{2+}	1
Triticum aestivum	-DTE----------D---------H-------D-	His	Zn^{2+}	1
Zea mays	-DTE----------D---------H-------D-	His	Zn^{2+}	1
Oryza meyeriana	-DTE----------D---------H-------D-	His	Zn^{2+}	1

Adapted from Palanivelu [5]. A-site, Active site; *Water-bound Zn^{2+}; ZBSs, Number of zinc-binding sites; Active site amino acids, confirmed by SDM are highlighted in dark blue and X-ray crystallography, in light blue. *Sc*, *Saccharomyces cerevisiae*; *Hs, Homo sapiens*; ARATH, *Arabidopsis thaliana.*

Table 4 DEDD(Y/H)-superfamily exonuclease active site amino acids and their distance conservation



Adapted from Palanivelu [5]. *Sc, Saccharomyces cerevisiae*; *Hs, Homo sapiens* *The distance between the proton acceptor (H/Y) and the last D are 3 to 4 amino acids Active site amino acids confirmed by SDM analysis are highlighted in dark blue and by X-ray are highlighted in light blue #Similar active site amino acids are found in *E. coli* DNA pol I ^Interestingly, similar active site amino acids are found in SARS-CoVs and other human CoVs ACE2, Angiotensin-Converting Enzyme 2; DPP4, Dipeptidyl peptidase 4.

Figure 4 shows the proposed polymerase and PR exonuclease active sites. In the NEP of mitochondria, the active site H^{569} accepts the proton from the metal-bound water molecule to initiate catalysis, which is followed by the formation of a highly reactive Zn-hydroxyl free radical, resulting in the removal of a misincorporated nucleotide. Thus, the 3'→5' exonuclease↔polymerase activities switch between excision and incorporation modes without dissociation of the enzyme-substrate complex [27]. For a detailed mechanism of polymerase, see Palanivelu [19].

Mitochondrial NEP (*ARATH*) (PR exo) –RC**D**^{531}I**E**^{533}------NV**D**^{555}F-----N**H**^{569}L—S**D**^{573}LC-

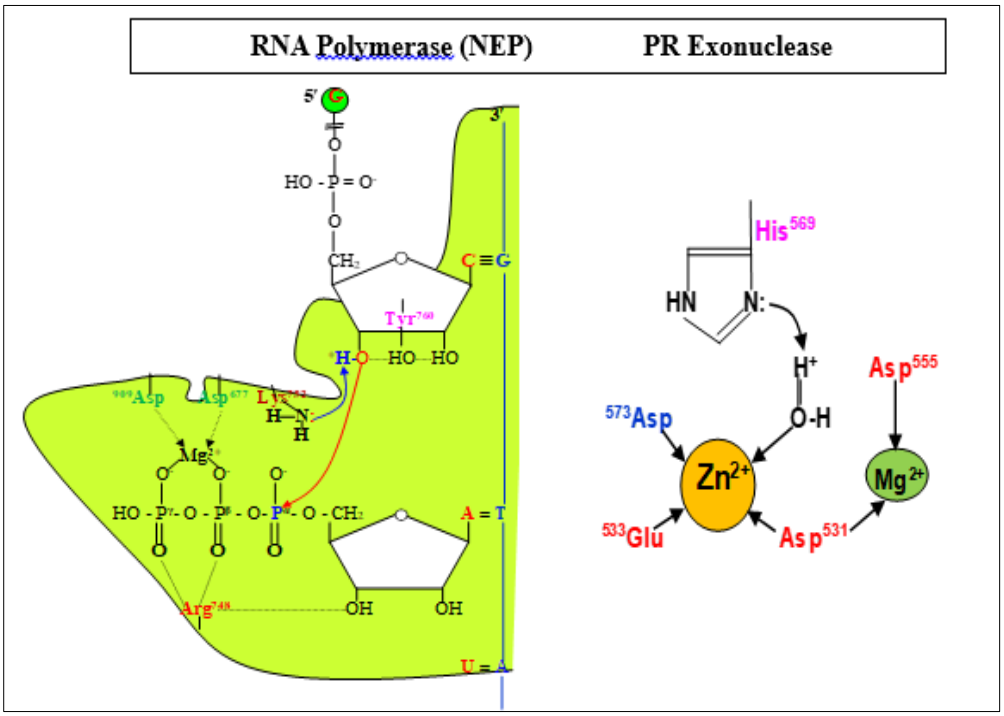


Figure 4 Proposed amino acids at the active sites of the polymerase and PR exonuclease of the NEP of plant mitochondria (Numberings from the *A. thaliana*'s sequence).

4 Conclusion

Mitochondria are semi-autonomous organelles and are found in all eukaryotic cells. They are partly controlled by their own genome and mostly by the nuclear imports. The nuclear-encoded RNA polymerase (NEP) is imported from the nucleus to the mitochondria and is involved in the transcription of all mitochondrial genes. The mitochondrial NEP showed only 59.05% identity to the NEP of chloroplasts from *Arabidopsis thaliana*, suggesting that the NEPs of mitochondria and chloroplasts are distinctly different. However, in both the NEPs, the polymerase catalytic core and PR exonuclease domains are completely conserved. The PR exonuclease of the mitochondrial NEP belongs to the DEDD-superfamily.

Compliance with ethical standards

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