Review Article

Translation initiation factor 4A: a prototype member of dead-box protein family

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ABSTRACT

DEAD-box proteins are characterized by nine conserved helicase motifs. Several different DEAD-box proteins are found in eukaryotes, whereas prokaryotes have small number of these proteins. They play important roles in almost all kinds of RNA metabolism including roles in remodeling ribonuclear protein complexes. These proteins are usually very specific and cannot mutually be replaced. Many of these DEAD-box proteins (but not all) have been shown to have RNA-dependent ATPase and ATP-dependent RNA helicase activities. Many of them have also been shown to contain DNA unwinding activities. Translation initiation factor 4A is the prototype of the DEAD-box family of proteins. Actually, the DEAD-box protein family was discovered on the basis of conserved sequence motifs of eIF4A. Motif II (or Walker B motif) has the amino acids D-E-A-D (Asp-Glu-Ala-Asp), which gave the name to the family. In general, the eIF4A is considered as a helicase that locally melts the secondary structures and makes the RNA accessible to nucleases. It is part of the cap-binding complex eIF4F but is also found to be present in a free form. The biochemical activities of eIF4A are reported to be upregulated by eIF4B, eIF4H and eIF4G. It has been proposed that eIF4A helps to unwind secondary structures in the 5'-untranslated region, which are inhibitory for protein synthesis. In plants, it has been shown to play a unique role in abiotic stress tolerance, which suggests a new pathway to engineer to increase the crop production under the stress conditions. **[Physiol. Mol. Biol. Plants 2008; 14(1&2) : 101-107]** *E-mail : narendra@icgeb.res.in*

Key words : *ATPase; DEAD-box helicases; helicase-conserved motifs; molecular motor; translation initiation; unwinding enzymes*

Abbreviations : *DEAD, helicase motif II (Asp-Glu-Ala-Asp); eIF, eukaryotic translation initiation factor; EJC, exon junction complex; NMD, nonsense mediated decay; RRM, RNA recognition motif; SF, super family of helicases; UTR, untranslated reagion*

DEAD-box proteins have been shown to be essential for translation initiation. The eukaryotic translation initiation factor 4A (eIF4A) is the best characterized member of the RNA helicase family that plays a key role in the first step of translation initiation together with the two other translation initiation factors eIF4B and eIF4F. eIF4A is a 46 kDA polypeptide (in mammals and plants) exhibiting RNA dependent ATPase and bidirectional RNA helicase actvities in cap dependent translation initiation. eIF4A is a prototype of a protein family termed as DEAD-box family named after one of the motif (motif II) shared by all family members. The related family members share the motif DEAH or DEXH (Sonenberg, 1993; Tuteja and Tuteja, 2004; Bleichert and Baserga, 2007). All family members have been shown to possess NTPase activity, but only a few members in addition to eIF4A were shown to possess double stranded RNA unwinding activity. The function and regulation of eIF4A activity are the best characterized of the RNA helicases, and provide a map for DExD/H-box family study. So far, eIF4A genes from following plants such as tobacco (Owttrim *et al*., 1991), *Arabidopsis* (Metz *et al*., 1992), wheat (Metz *et al*., 1993), rice (Nishi *et al*., 1993) and pea (Pham *et al*., 2000; Vashisht *et al*., 2005) have been isolated. In tobacco at least ten expressed genes of eIF4A have been identified (Owttrim *et al*., 1994). At least 6 eIF4As have been found in Arabidopsis plants. Two RNA helicases, eIF4A (Rozen *et al*., 1990) and Ded1 (Chuang *et al*., 1997; de la Cruz *et al*., 1997), have been identified to be important in cap-dependent translation initiation. Proteins related to eIF4A sequence can be found in all eukaryotic cells and in most eubacteria and archaebacteria.

DEAD-box family of helicases

DEAD-box family of helicases are one of the smallest motors of biological system, which harness the chemical

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free energy of ATP hydrolysis to catalyze the opening of energetically stable duplex nucleic acids and thereby are involved in almost all aspect of nucleic acid metabolism including replication, repair, recombination, transcription, translation, and ribosome biogenesis. The genome of the yeast *Saccharomyces cerevisiae* encodes at least 25 DEAD-box proteins and *Plasmodium falciparum* genome contains at least 22 full-length DEAD-box proteins (Linder, 2006; Tuteja and Pradhan, 2006). Based on their primary sequences, DNA helicases can be classified into families and superfamilies. Helicases constitute a large family of enzymes with diverse biological functions. A computer-assisted amino acid sequence analysis of helicases from many different organisms has revealed nine short conserved motifs, called 'helicase core motifs' (Gorbalenya *et al*., 1988; Linder *et al*., 1989; Tuteja and Tuteja, 2004; Bleichert and Baserga, 2007). With this discovery, the helicases have been classified into three superfamilies (SF) namely SF1, SF2, and SF3 based on the extent of similarity and the organization of these conserved motifs. High sequence conservation has been maintained in this large group of helicases, suggesting that the motifs containing helicases genes evolved from a common ancestor. Hence, these helicase motifs can be used efficiently for the detection and the prediction of new helicases in the genome databases. SF1 and SF2 are the largest and related superfamilies, which contain nine conserved motifs (Q, I, Ia, Ib, II to VI) (Fig. 1), while SF3 has just three motifs (A, B and C) (Hall and Matson, 1999). Motif III differs between SF1 and SF2 family proteins.

In general the SF1 family members (Rep, UvrD, PcrA-*Bacillus*, T4-Dda, and HSV-UL5) are considered to be ssDNA translocases while SF2 (eIF4A, RecQ, UvrB, RecG, PriA-*E. coli*, and HCV-NS3, UL9 and NPH-II) are dsDNA translocases (Hall and Matson, 1999). This property may be the distinction between the SF1 and SF2 family. Eukaryotic eIF4A is a prototype member of SF2. Within SF2, an Snf2 like family of eukaryotic proteins defines a variety of proteins with similarities to yeast protein Snf2 (also called Swi2) (Laurent *et al*., 1991). Because of the variations in motif II, the SF2 family of helicases are further subgrouped as DEAD, DEAH, DEXH box proteins. The examples of SF3 family of helicases are RuvB, MCM and some viral encoded proteins (Hall and Matson, 1999). Another group called family 4 (F4) contains five motifs (I, Ia, 2 to 4) (Hall and Matson, 1999). The Rho helicase is a RNA-DNA helicase and belong to family 5 (F5). The motifs of SF1 and SF2 are usually clustered in a region of 200-700 amino acids and can be called as a core-region. All the helicase motifs and their functions are shown in Figure 1. The DEAD-box motif together with motif I (or Walker A motif), the Q-motif and motif VI, is required for ATP binding and hydrolysis. Motif Ia and Ib, III, IV and V have not been characterized well but may be involved in interaction with RNA and in intramolecular rearrangements necessary for remodelling activity (Linder, 2006).

Role of eIF4A in translation initiation

eIF4A acts by unwinding secondary structure in the 5' UTR of the mRNA, enabling binding and scanning of the small ribosomal subunit for the start codon AUG (Svitkin *et al*., 2001). eIF4A participates in the eIF4F complex and interacts both genetically and biochemically with eIF4B. These factors are required for binding of the 43S complex, which contains the 40S subunit, eIF3, and eIF2/GTP/Met-tRNA, to the 5' end of the mRNA. Mutational analysis of

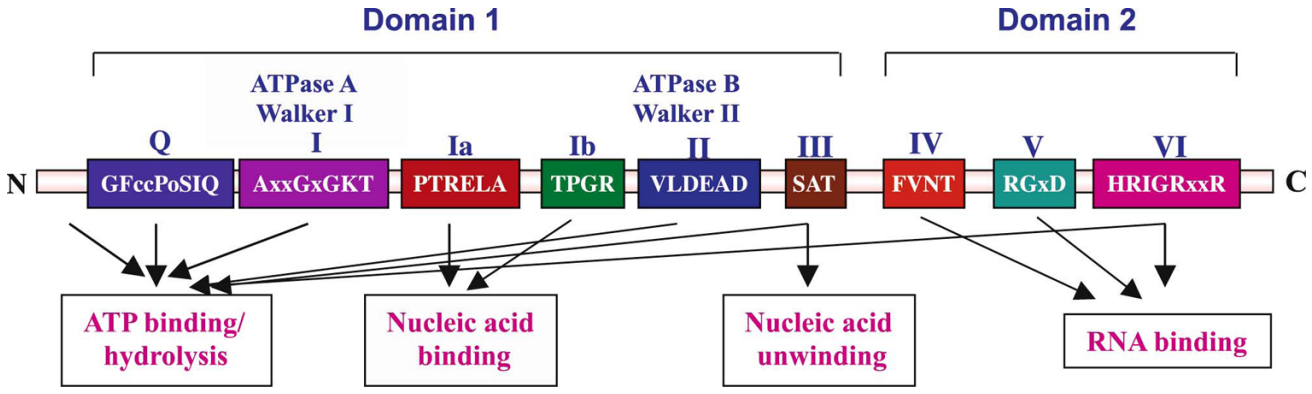

Fig. 1. Conserved Sequence Motifs of eIF4A/DEAD-box RNA Helicases of superfamily 2 and their functions. Motif II (Walker B) contains the amino acids DEAD in DEAD box RNA helicases. The Q motif is specific to the family of DEAD box proteins. eIF4As/RNA helicases share conserved sequence motifs that are located in two different domains: motifs I, Ia, Ib, II, and III in domain 1 and motifs IV, V, and VI in domain 2.

eIF4A identified conserved residues important for helicase activity (Pause and Sonenberg, 1992); similar mutations made in other helicase family members confer a dominant negative phenotype when overexpressed (Pause *et al*., 1994; Plumpton *et al*., 1994; Edwalds-Gilbert *et al*., 2000; Schneider *et al*., 2002). Evidence that the target of eIF4A is secondary structure in the 5' UTR comes from a study utilizing a dominant negative mammalian eIF4A with a series of substrates containing increasing amounts of 5' UTR secondary structure (Svitkin *et al*., 2001). The results showed that the degree of translation inhibition by mutant eIF4A correlates with the amount of 5' UTR secondary structure in the substrate. eIF4B and closely related cofactor eIF4H stimulate the otherwise low level of helicase activity of eIF4A by 20 fold (Rozen *et al*., 1990; Rogers *et al*., 2001a). Although there is no evidence for a direct physical interaction between eIF4A and eIF4B, eIF4B stimulates every known activity of eIF4A: RNA binding, RNA helicase, ATPase, ATP binding, and translation initiation (Rozen *et al*., 1990; Rogers *et al*., 2001a, 2001b). eIF4B binding to RNA promotes recruitment of the eIF4A containing complex, eIF4F, to the 5' UTR, suggesting both cooperative and bridging interactions between eIF4B and eIF4A. Within eIF4F, eIF4E recognizes the 5' cap on the mRNA and, when free from regulatory binding proteins, brings eIF4A and eIF4G to the 5'cap. eIF4B binds to the p170 subunit of eIF3 (Methot *et al*., 1996). eIF4G is thought to bind to eIF3, completing the bridge between eIF4B and eIF4A (Fig. 2).

In addition, multiple copies of eIF4B can suppress a temperature sensitive eIF4A mutation in yeast, demonstrating a genetic link between the two translation initiation factors (Coppolecchia *et al*., 1993). Yeast eIF4B also binds RNA very efficiently through specific residues in its basic C-terminus (Methot *et al*., 1994), and a RRM (RNA Recognition Motif) in the N-terminus, both of which are necessary to suppress eIF4A mutations (Coppolecchia *et al*., 1993). While the cofactor activity of eIF4B upon eIF4A is well characterized, complex formation between eIF4F, eIF3 and eIF4B is clearly important for inducing eIF4A activity upon the 5' UTR, and any protein that affects this formation would likely have the properties of a cofactor.

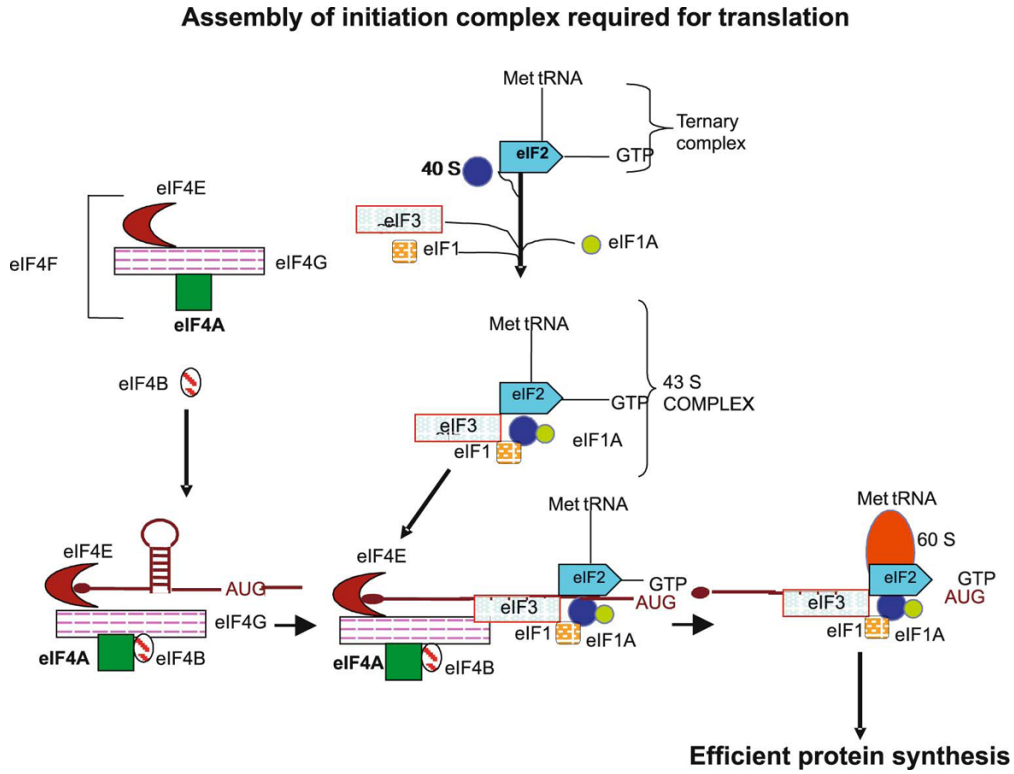

Fig. 2. The role of eIF4A in translation initiation. eIF4A was the first RNA helicase described and remains the best-characterized RNA helicase in eukaryotes. eIF4A is, together with eIF4G and eIF4E, a component of the eIF4F complex that is required for cap-dependent translation initiation The target of eIF4A is hypothesized to be secondary structure in 5' UTR of mRNA. eIF4B stimulates the eIF4A helicase activity. Upon ATP hydrolysis, inhibitory structure is relieved, and the mRNA can participate in the formation of initiation complex and further help in efficient protein synthesis.

On the basis of a large amount of experimental data, it can be assumed that eIF4A, which forms part of the cap-binding complex, unwinds or rearranges RNA-duplex structures at the 5' end of eukaryotic mRNA to prepare it for scanning by the small ribosomal subunit. Another possibility is that eIF4A removes proteins from mRNA, which is probably coated with many proteins after exit from the nucleus (Rogers *et al*., 2002, Svitkin *et al*., 1996). Whereas, several roles for eIF4A have been suggested, the function of another DEAD-box protein, Ded1, is not known, although its role in translation initiation has been indicated by several independent genetic and biochemical experiments (de La Cruz *et al*., 1997, Chuang *et al*., 1997, Noueiry *et al*., 2000, Grallert *et al*., 2000). The activities of eIF4A and Ded1 proteins might be required to various extents on different mRNA molecules (Svitkin *et al*., 2001). This implied that helicase activities of these proteins fulfill regulatory roles in gene expression.

In higher eukaryotes that undergo embryonic development, another DEAD-box protein, Vasa, is required for translational activation of germline-specific mRNAs in a spatially controlled fashion (Markussen *et al*., 1995). Although, helicase activity is essential for the function of Vasa, its precise role in activating these messages is not clear (Liang *et al*., 1994). There are three mammalian isoforms of eukaryotic translation initiation factor 4A; eIF4AI, eIF4AII, and eIF4AIII (Nielsen *et al*., 1988; Weinstein *et al*., 1997). Human eIF4AII is highly homologus to eIF4AI (89% identity) and is functionally equivalent (Yoder-Hill *et al*., 1993). eIF4AIII is functionally distinct from eIF4AI and eIF4AII. It has 65% identity at amino acid level with eIF4AI and eIF4AII and do not have any role in translation initiation (Li *et al*., 1999). In eukaryotes a surveillance mechanism known as nonsense-mediated decay (NMD) degrades the mRNA when premature termination (PTC) codon is present. NMD requires translation to read the frame of mRNA and detect the PTC. During pre mRNA splicing, the exon junction complex (EJC) is recruited to a region 20-24 nucleotide upstream of the exon junction on the mature RNA. The presence of PTC upstream the EJC elicits NMD. eIF4AIII is a novel component of exon junction complex (Chan *et al*., 2004). Ferraiuolo *et al*., (2004) showed that siRNA against eIF4AIII but not against eIF4AI/II, inhibit NMD. Moreover, eIF4AIII, but not eIF4AI, is specially recruited to the EJC during splicing. Thus eIF4AIII is functioning during NMD. Palacios *et al* (2004) identified eIF4AIII as component of a conserved protein complex that is essential for mRNA localization in flies and NMD in mammals.

The transformation suppressor Pdcd4 that inhibits tumour promoter induced neoplastic transformation has been shown as novel eukaryotic translation initiation factor 4A (eIF4A) binding protein that inhibits translation by inhibiting its helicase activity (Yang *et al*., 2003). Pdcd4 has a MA-3 (similar domain of eIF4G that helps in binding of eIF4A to it) domain by which it binds to eIF4A (Yang *et al*., 2004).

Abiotic stress regulated eIF4As from pea plant

Abiotic stress is an increasing threat in reducing agricultural productivity worldwide. Among abiotic stresses, the high salinity stress is most severe environmental stress, which impairs crop production on at least 20% of irrigated land worldwide (Mahajan and Tuteja, 2005). In saline soils, high levels of sodium ions lead to plant growth inhibition and even death. As salinity stress affects the cellular gene-expression machinery, it is evident that molecules involved in nucleic acid processing including helicases, are likely to be affected as well. In plants the role of eIF4A/helicases in abiotic stress is just begining to emerge. The two eIF4As from pea have been shown to play role in abiotic stress tolerance especially salinity and cold stress (Pham *et al*., 2000; Vashisht *et al*., 2005)

1. 45 kDa eIF4A from pea :

It contains both RNA and DNA unwinding activities and is also called as pea DNA helicase 45 (PDH45). Antibodies against the eIF4A/PDH45 inhibit *in vitro* translation, confirming its role in translation initiation (Pham *et al*., 2000). The enzyme is localized in the nucleus and cytosol and unwinds DNA in the 3' to 5' direction. The *eIF4A*/*PDH45* mRNA is induced in pea seedlings in response to high salinity and its over-expression in tobacco plants confers salinity tolerance, thus suggesting a new pathway for manipulating stress tolerance in crop plants. The T1 transgenics were able to grow to maturity and set normal viable seeds under continuous salinity stress, without any reduction in plant yield. Measurement of Na^+ in different parts of the plant showed higher accumulation in the old leaves and negligible in seeds of T1 transgenic lines as compared with the WT plants (Sanan-Misra *et al*., 2005). The induction of eIF4A/*PDH45* transcript was also observed to be induced by the phytohormone, ABA, which suggested that the stress effect might be mediated through ABA-mediated pathways. The exact mechanism of eIF4A-mediated tolerance of salinity stress is not understood. This protein may act at translational level or may associate with DNA multisubunit protein complex to alter gene expression. It differs from other stress regulated pea eIF4A in many properties, which are shown in Table 1.

2. 47 kDa eIF4A from pea

It also contains both the RNA and DNA helicase activities and is called as pea DNA helicase 47 (PDH47). It is a unique bipolar helicase that contains both the 3' to 5' and 5' to 3' directional helicase activities. The transcript of *PDH47* was induced under cold and salinity stress. ABA treatment did not alter its expression in shoot but induced its mRNA in root indicating the role of PDH47 in both the ABA-independent and ABA-dependent pathways in abiotic stress. This is also localized in the nucleus and cytosol (Vashisth *et al*., 2005). It differs from other stress regulated pea eIF4A/PDH45 in many properties, which are shown in Table 1.

CONCLUSIONS AND PERSPECTIVES

Many mRNAs have been shown to have extensive secondary structures within their coding sequences and even RNA of random sequence has been found to be ~50% base paired. This causes a potential thermodynamic and kinetic barrier to protein synthesis. Usually the translation machinery is highly efficient at unwinding helical structure in mRNA during the elongation phase, where the eIF4A play important role. Many proteins that are involved in cell signaling and growth control usually contain long 5'UTR that form inhibitory secondary structures, which are resolved by eIF4A and hence it also plays an important role in cell signaling and growth control. The role of eIF4A/helicase in protein synthesis and in RNA/DNA metabolisms makes them important molecules of the cell, and thereby has several implications. Despite the diversity of their functions and a large range of organisms in which these proteins have been identified, high sequence conservation has been maintained in the eIF4A, suggesting that all these eIF4A/helicase genes evolved from a common ancestor. With the completion of genome sequences of a number of organisms, it is interesting to note that each genome contains a large number of putative eIF4A/helicases. Although some eIF4A/helicases have been isolated from different sources, but the clear-cut role of only few has been reported. Still there is a need to find out the exact function of many eIF4As in stress signaling. Though the DEAD-box helicases/eIF4A contain common conserved sequences yet they differ mainly in N-and C-terminal sequences, which contain different targeting signals. Furthermore, many mechanisms of regulation both at the level of expression and at the post-transcriptional level explain the wide spectrum of functions involving DEAD-box RNA helicases.

In the near future, it will be important to identify and characterize specific substrates, interacting proteins, and cofactors of eIF4A/helicases to precisely elucidate their

Table 1. The differences between the properties of the eIF4A/PDH45 and eIF4A/PDH47

Properties	eIF4A/PDH45	eIF4A/PDH47
Sequence of motif II & motif IV:	DESD & SRT	DEAD & SAT
Optimum pH for unwinding activity:	pH: 7 – 9	pH: 5 – 10
Optimum concentrations of ATP/ Mg/ KCl for unwinding activity:	0.6/ 0.6/ 150 mM	2.0/ 2.0/ 75 mM
NTPs/dNTPs requirements:	All	Only ATP & dATP
ATPase activity stimulated by:	ssDNA>>>>dsDNA	ssDNA>dsDNA
Cofactors requirements:	Mg>Mn>>Ca	Mg=Mn>>>Zn>>Ca
Polarity of unwinding:	Unipolar: 3'-5'	Bipolar:3'-5' & 5'-3'
Transcript level:	root>shoot>flower	shoot>root>>flower
Transcript level induced by abiotic stress:	Mainly by high salt (NaCl), dehydration and ABA	Mainly by high salt, cold and not by dehydration and ABA
Stress tolerance via:	ABA-dependent pathway	ABA-independent pathway

specific functions in nucleic acid metabolism. Presently, low-resolution structural analysis, revealed by electron microscopy, is beginning to provide clues to some of the functions and mechanism of action of these remarkable proteins. A better understanding of these proteins in plant will still have to await the necessary breakthrough that hopefully is provided by the high-resolution structure of a helicase to be solved by X-ray crystallography. We still have to understand the molecular details of how other proteins modulate the activity of eIF4As. The crystal structures of SF1 and SF2 helicases have shown that the helicase motifs are clustered together in the tertiary structure, forming NTP-binding pocket and a portion of the nucleic acid binding site. According to Hall and Matson, (1999) the conserved helicase motifs can be envisioned as the engine of helicases generating energy by the consumption of fuel (NTPs) and using the energy to do work. The non-conserved portion of eIF4A/helicase structure may contain some specific domain such as protein-protein interaction domain, cellular localization signals, site-specific DNA recognition domains and oligomerization interfaces, which could be unique to individual helicases. Very interestingly, a new and important role of a eIF4A/helicase in the salinity and cold stress tolerance in plant suggests a novel pathway to engineer to maximize crop yield in sub-optimal conditions. This discovery should make an important contribution to our better understanding of protein synthesis and stress signaling in plants and will have a great biotechnological application of eIF4A/helicases in future.

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