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Review

Looking back on the birth of DEAD-box RNA helicases ☆

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ABSTRACT

DEAD-box proteins represent the largest family of RNA helicases, present in all three kingdoms of life. They are involved in a variety of processes involving RNA metabolism and in some instances also in processes that use guide RNAs. Since their first descriptions in the late 1980s, the perception of their molecular activities has dramatically changed. At the time when only eight proteins with 9 conserved motifs constituted the DEAD-box protein family, it was the biochemical characterization of mammalian eIF4A that first suggested a local unwinding activity. This was confirmed *in vitro* using partially double stranded RNA substrates with the unexpected result of a bidirectional unwinding activity. A real change of paradigm from the classical helicase activity to localized RNA unwinding occurred with the publication of the vasa•RNA structure with a bend in the RNA substrate and the insightful work from several laboratories demonstrating local unwinding without translocation. Finally, elegant work on the exon-junction complex revealed how DEAD-box proteins can bind to RNA to serve as clamps to function as nucleation centers to form RNP complexes. This article is part of a Special Issue entitled: The Biology of RNA helicases — Modulation for life.

1. Introduction

Helicases unwind double stranded nucleic acid helices in an energy-dependent manner. The substrates can be DNA or RNA and in most cases require a single stranded extension for loading of the helicase prior to translocation and unwinding. RNA helicases can be classified into 6 superfamilies, which are composed of families/subfamilies according to their sequence information [1].

DEAD-box RNA helicases belong to superfamily 2 and represent the largest family of helicases. They are characterized by 12 conserved motifs that, like a barcode, unambiguously identify their members (Fig. 1). These motifs are involved in ATP binding and hydrolysis, RNA binding, and intramolecular interactions. DEAD-box proteins are present in almost all living organisms and many of them are essential in eukaryotic cells. In eukaryotes they are involved in all processes involving RNA from transcription to decay, i.e., from birth to "DEAD." In prokaryotes they are involved in ribosome biogenesis, translation initiation and RNA decay, making them crucial players in adaptations to environmental changes and stress response.

In the following paragraphs we will highlight a few important milestones in the analysis of DEAD-box proteins (Fig. 2). Needless to say, many of these remarkable publications were made possible by a multitude of findings that cannot all be described and cited here.

2. The translation initiation factor eIF4A

The "godfather" of the DEAD-box proteins [2] is without any doubt the eukaryotic translation initiation factor 4A, eIF4A [3–5]. Together with other translation initiation factors it helps the recruitment of the small ribosomal subunit to the 5′ cap structure, to allow subsequent scanning for the initiator AUG. This initiation factor is very abundant and is present as part of the cap-binding complex, eIF4F, and in a free form. The translation initiation factor eIF4A was found to possess RNA dependent ATPase activity that was stimulated by eIF4B and eIF4F, the cap-binding complex [6], and later was shown to be also stimulated by eIF4H [7]. An important step towards the idea of an unwinding activity was obtained through the work by the Merrick lab that showed ATP-dependent structural changes of reovirus mRNA induced by eIF4A [8]. In these experiments reovirus RNA was probed using RNases after having been exposed to eIF4A in presence and absence of ATP. The results clearly showed an eIF4A-dependent structural change and the authors proposed an unwinding activity by eIF4A.

Although the report by Ray and collaborators [8] strongly influenced the nascent field of DEAD-box proteins, it was the *in vitro* unwinding of short duplexes by p68 [9] and by eIF4A that showed convincingly a local unwinding activity [10]. In the work by the Sonenberg lab [10] 10 base pair G•C duplexes with either 3′ or 5′ extensions were used as substrates for eIF4A unwinding activity. The double strand dissociation

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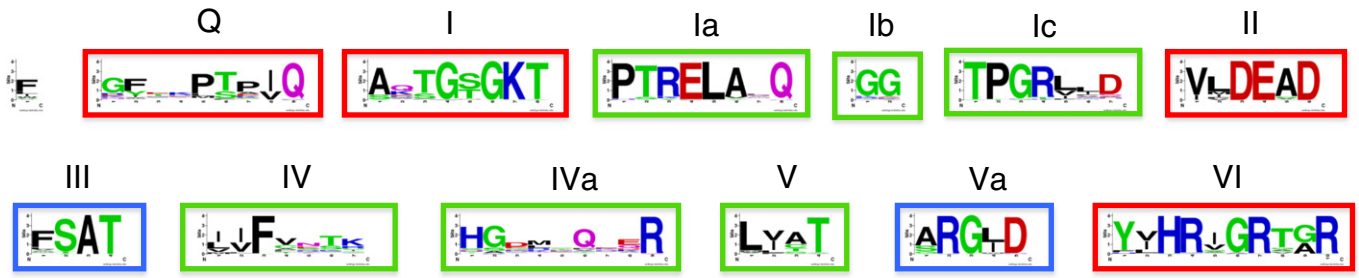

Fig. 1. Twelve highly conserved sequence motifs allow unambiguous identification of DEAD-box proteins and distinction from other DNA and RNA helicases. The conserved motifs present within the helicase core were defined using 25 proteins from the yeast *Saccharomyces cerevisiae*, 36 proteins from *Homo sapiens*, 5 proteins from *Escherichia coli* and 4 proteins from *Bacillus subtilis*. The list of proteins from yeast and humans are from Ref. [81]. Motifs interacting with ATP are boxed in red, motifs interacting with RNA are boxed in green, and motifs involved in intramolecular interactions are boxed in blue.

activity of eIF4A and eIF4F was stimulated by eIF4B and was dependent on ATP or dATP. Moreover, ATP analogs or other nucleotides, that were previously shown not to interact with eIF4A [11], were unable to stimulate dissociation supporting the idea of ATP-hydrolysis dependent unwinding of the substrate. This was consistent with the previous demonstration that mutation of motif I (AxxGxGKT) abrogated nucleotide binding when the lysine was replaced by an alanine [12]. Intriguingly, the unwinding reaction was possible with both 3′ and 5′ single stranded extensions. Whereas in this paper, a blunt-ended substrate with ($\Delta G = -35$ kcal/mol) was not unwound, such an activity was reported later [13] ($\Delta G = -21.1$ kcal/mol) suggesting binding to dsRNA, albeit less efficiently than to ssRNA. Interestingly, a cap-analog reduced eIF4F-dependent unwinding of the 5′ single-strand extended substrate, but not the 3′ single stranded substrate. This was in accordance with previous results that showed inhibition of the ATPase activity by a cap-proximal double stranded region [14]. Although these experiments were carried out with an excess of protein, they clearly demonstrated that 1) the *in vitro* dissociation of a dsRNA in an ATP dependent manner; 2) the unexpected bidirectional activity; and 3) the stimulation of the helicase activity by eIF4F and eIF4B.

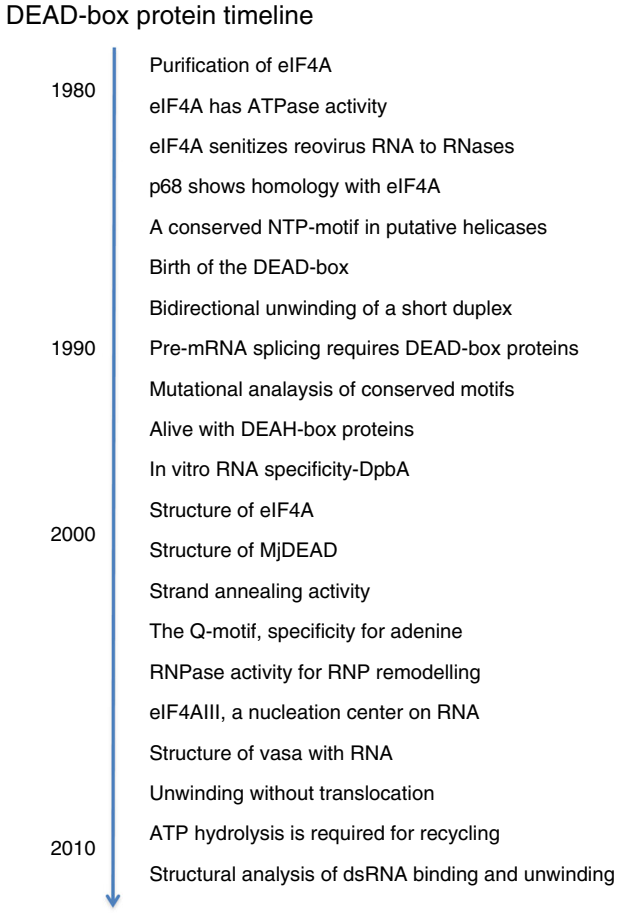

Fig. 2. Timeline of the DEAD-box protein family. Although an enormous amount of work contributed to the evolution of our understanding of DEAD-box proteins, several events could be considered as landmarks. In this non-exhaustive list we find purification of eIF4A [82], eIF4A has ATPase activity [6], eIF4A sensitizes reovirus RNA to RNases [8], p68 shows homology with eIF4A [27], a conserved NTP-motif in putative helicases [26], birth of the DEAD-box [28], bidirectional unwinding of a short duplex [9,10], pre-mRNA splicing requires DEAD-box proteins [83], mutational analysis of conserved motifs [22], alive with DEAH-box proteins [84,85], *in vitro* rRNA specificity of DpbA [51], structure of eIF4A [59,60,62], structure of MjDEAD [61], strand annealing activity [86], the Q-motif — specificity for adenine [87], RNPase activity for RNP remodeling [73], eIF4AIII — a nucleation center on RNA [77], unwinding without translocation [47,63], structure of vasa with RNA [39], ATP hydrolysis is required for recycling [64,65], structural analysis of RNA-duplex binding and unwinding [45].

Kinetic analyses by the Herschlag [15,16], the Merrick [13,17,18] and other laboratories showed that RNA affinity is influenced by the nucleotide and that eIF4A is a non-processive helicase, with decreasing strand-separation activity as the stability of the duplex increases. Importantly, competition experiments showed that ssRNA actively competed the unwinding of a blunt-ended substrate, whereas dsRNA did so to a lesser extent. This work also suggested a dsRNA binding activity allowing blunt-ended substrate unwinding [13], although such an activity could not be measured previously [16].

These activities are consistent with the proposed activity of eIF4A as an RNA helicase, unwinding secondary structures at the 5′ untranslated region (5′ UTR) of mRNAs to allow binding of the eIF4F complex and the small ribosomal subunit, and thereby permitting scanning for the initiator AUG codon. Translation initiation of mRNAs is reduced in the presence of a dominant negative eIF4A mutant protein and this effect is exacerbated with increasingly stable secondary structures in the 5′ UTR [19]. Intriguingly, a study with increasing length of the 5′UTR did not show dependence on eIF4A, but on Ded1, another DEAD-box protein involved in translation initiation [20]. It is therefore not clear whether the increased requirement for eIF4A in the presence of secondary structure is a direct effect of a necessity to unwind such scanning barriers, or whether it is a secondary consequence of a generally weakened translation initiation efficiency. Moreover, pioneering mutational analyses of eIF4A [21,22] together with the use of an *in vitro* translation system indicated a general effect on mRNA translation, indicating that eIF4A is not simply required for unwinding 5′UTR secondary structures in many mRNAs [23]. On the other hand, not all mRNAs seem to require eIF4A for translation [24]. Moreover, it is worth mentioning that other RNA helicases were found to be involved in translation initiation (see Ref. [25] this issue).

Although eIF4A has served as paradigm of DEAD-box protein function and many contributions on the function of eIF4A have significantly influenced the field of DEAD-box RNA helicases, elucidation of the precise role(s) of eIF4A in translation initiation remains a challenge for the future.

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