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Review

Roles of helicases in translation initiation: A mechanistic view☆,☆☆

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ABSTRACT

The goal of this review is to summarize our current knowledge about the helicases involved in translation initiation and their roles in both general and mRNA-specific translation. The main topics covered are the mechanisms of helicase action, with emphasis on the roles of accessory domains and proteins; the functions performed by helicases in translation initiation; and the interplay between direct and indirect effects of helicases that also function in steps preceding translation initiation. Special attention is given to the dynamics of eIF4A binding and dissociation from eIF4F during mRNA unwinding. It is proposed that DHX29, as well as other helicases and translation initiation factors could also cycle on and off the translation initiation complexes, similar to eIF4A. The evidence in favor of this hypothesis and its possible implications for the mechanisms of translation initiation is discussed. This article is part of a Special Issue entitled: The biology of RNA helicases — Modulation for life.

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1. Introduction

Eukaryotic translation initiation is a multistep process involving over 10 eukaryotic initiation factors (eIFs), composed of more than 30 different polypeptides. The majority of mRNAs in the cell are capped and polyadenylated, and start codon recognition involves recruitment of the 43S pre-initiation complex (43S pre-IC) to the 5′-cap, followed by scanning. Therefore, for the purposes of this review, "general" eIFs are the proteins involved in translation initiation on capped, polyadenylated mRNAs. Certain types of mRNAs contain Internal Ribosome Entry Sites (IRESs) that require only a subset of these factors, with some only requiring the 40S subunit. In addition to the general translation factors acting on most mRNAs, there are many proteins that either stimulate or repress translation initiation on specific types of mRNAs (reviewed in [1–4]).

The 43S complex consists of the small, 40S ribosomal subunit, the eIF2•GTP•Met-tRNA$_i$ ternary complex (TC), as well as eIFs 1, 1A, 3 and 5. It is recruited to the mRNA 5′-cap by the eIF4E/4G/4A/4B complex. In higher eukaryotes, eIF4E, 4G and 4A form a stable complex called eIF4F. eIF4E is the cap-binding protein; eIF4G serves as a scaffold interacting with the other members of the complex, as well as with the poly-A binding protein (PABP) bound to the 3′-poly-A tail at the other end of the mRNA. eIF4A/DDX2 is a DEAD-box (DDX) helicase whose activity is stimulated by eIF4G and eIF4B (and also eIF4H in higher eukaryotes). 43S complex recruitment is mediated by eIF4G (possibly helped by eIF4B) and at least in higher eukaryotes involves binding to eIF3. The 43S complex then scans along the mRNA in the 3′ direction until it reaches the start codon. eIF4F remains bound to the scanning 43S complex and promotes unwinding of secondary structures on its path, while its association with the 5′-cap is likely lost allowing for a new 43S complex to be recruited while the first one is still scanning [5]. eIF1 and eIF1A are important for scanning and start codon selection. eIF1 discriminates against non-AUG codons, AUG codons located too close to the 5′-end, or AUG codons in poor sequence context that does not match the so-called Kozak consensus sequence. Basepairing between the start codon and the tRNA anticodon triggers major conformational changes in the 43S complex, leading to adoption of a "closed" scanning-incompetent conformation: the 48S IC. eIF5 is required to promote GTP hydrolysis by eIF2 in the 48S IC, followed by phosphate release and displacement of eIF1 from its binding site on the 40S subunit. GTP hydrolysis lowers the affinity of eIF2 for the Met-tRNA$_i$. eIF2•GDP dissociates and another G-protein eIF5B replaces it on the Met-tRNA$_i$. eIF5B together with eIF1A promotes the recruitment of the large 60S ribosomal subunit. Finally, ribosomal subunit joining promotes GTP hydrolysis by eIF5B, which dissociates together with eIF1A, leaving an 80S ribosome with a Met-tRNA$_i$ in the P-site, ready for translation elongation (reviewed in [1–4]).

For a long time, eIF4A was the only helicase eIF, although yeast Ded1 was also reported to play a role in general translation initiation. This changed in recent years with the discovery that the helicase DHX29

Abbreviations: eIF, eukaryotic translation initiation factor; IC, initiation complex; TC, ternary complex; EJC, Exon Junction Complex; PABP, poly-A binding protein; SF2, Superfamily 2; DDX, DEAD-box protein; DHX, DExH-box protein; RHA, RNA helicase A; UTR, untranslated region; PCE, post-transcriptional control element; IRES, Internal Ribosome Entry Site; ORF, open reading frame; uORF, upstream ORF; WT, wild type; mRNA, messenger RNA; mRNP, mRNA ribonucleoprotein; tRNA, transfer RNA; Met-tRNA$_i$, initiator methionyl-tRNA; ssRNA, single-stranded RNA; dsRNA, double-stranded RNA; ssDNA, single-stranded DNA; SSB, ssDNA-binding protein; OB, oligonucleotide/oligosaccharide binding fold domain; dsRBD, dsRNA-binding domain; WH, winged helix domain; RRM, RNA recognition motif domain; UBA, Ubiquitin-associated domain; miRNA, microRNA

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(DExH-box protein 29) is important for scanning 5′-untranslated regions (UTRs) containing stable hairpins [6–8].

In addition to eIF4A and DHX29, the helicases Ded1/DDX3, Vasa/DDX4, RNA helicase A (RHA/DHX9), and Dhh1/RCK/DDX6 also play roles related to translation initiation (reviewed in [9–11]). Dhh1/RCK is a translational repressor that acts at least in part at the level of translation initiation. However, this helicase will not be discussed here since its functions relate mainly to translational repression and mRNA decay [12–14]. Vasa/DDX4 [15–18] and RNA helicase A (RHA/DHX9) [19–22] have been reported to stimulate translation initiation of specific mRNAs, but the molecular mechanisms of their functions remain unclear. Yeast Ded1 and its mammalian homolog DDX3 have been reported by different groups to stimulate or to repress translation and to act as general or mRNA-specific factors [23–30]. These apparently conflicting data about Ded1/DDX3 illustrate the complexity of translation initiation and the current limits of our understanding of its molecular mechanisms. To further complicate matters, Ded1/DDX3 and RHA/DHX9, and maybe also Vasa/DDX4, all appear to play roles at steps preceding translation initiation, such as transcription, pre-mRNA splicing and transport [27,31–36], making it difficult to separate direct from indirect effects on translation initiation.

The goal of this review is to propose a framework for the analysis of the roles of helicases in translation initiation, with the hope of clarifying some apparent contradictions in the field or suggesting experimental approaches to address them. The main topics covered are: i) the mechanisms of helicase action, with emphasis on the roles of accessory domains and proteins; ii) the functions performed by helicases in translation initiation; iii) the interplay between direct and indirect effects of helicases that also function in steps preceding translation initiation; and iv) the dynamics of helicase binding and release from initiation complexes. In-depth descriptions of the structures and detailed enzymatic mechanisms of RNA helicases, which are beyond the scope of this article, can be found in a number of recent reviews [9–11,37,38].

2. Mechanism of action and domain structure of DEAD and DExH box helicases

2.1. Mechanism of helicase action

All helicases known to function in translation initiation are Superfamily 2 (SF2) helicases that belong to the DEAD-box or DExH-box families, named after the sequence of the helicase motif II [39,40]. These helicases contain two RecA domains, often surrounded by additional accessory or regulatory domains. While in the absence of ATP and RNA, the two RecA domains are not stably bound to each other ("open" conformation), both the ATP- and the RNA-binding sites are formed by the RecA domains coming in a specific orientation ("closed", catalytically active conformation). ATP is bound in a pocket between the two RecA domains while the RNA binds on the surface. The equilibrium between open and closed conformations is controlled by ATP and RNA binding, as well as by accessory domains and/or proteins (Fig. 1). The ADP-bound conformation (after ATP hydrolysis but before ADP release) is also "closed" but somewhat different from the ATP-bound state (reviewed in [9–11]).

2.2. Roles of accessory domains

Unlike replicative helicases, the DEAD and DExH box helicases involved in translation initiation cannot processively unwind long duplexes and appear to have a different mode of unwinding [41–44]. The helicase activity is mediated by binding to RNA in the presence of ATP, followed by cycles of ATP hydrolysis, release of the helicase from the RNA and re-binding, or partial release and sliding along the RNA. Strand separation is driven by the high affinity of the helicase for ssRNA. In crystal structures of DEAD/DExH box helicases in complex with ATP and RNA, the ssRNA is kinked, which may also promote strand separation [42]. A single cycle of ATP binding and hydrolysis is often sufficient to unwind a relatively short hairpin [45]. In some reports, ATP hydrolysis was not required for strand separation [46], whereas in others strand separation occurred after ATP hydrolysis, but before phosphate release [47]. The source for this discrepancy is not clear but it could be explained by the relative rates of ATP hydrolysis (fast), phosphate release (slow and rate-limiting), and strand separation, which can vary among different helicases. In principle, the mechanism of action of DEAD/DExH box helicases described above is consistent with the helicase binding to one RNA strand and displacing the second strand in a short duplex, or displacing a large enough portion of it to cause it to dissociate. In this scenario, ATP hydrolysis would not be required for the act of strand separation but for lowering the affinity of the helicase for RNA, leading to dissociation of the helicase and a new cycle of binding and release [10].

A helicase must only be active when bound to the right substrate at the right time, in order to perform its specific functions in the cell. This is accomplished by a combination of accessory domains and proteins that either stimulate or inhibit the helicase activity. The most common mechanisms of regulation of helicase activity are listed below and illustrated in Fig. 1A, followed by descriptions of the individual helicases involved in translation initiation.

1. *Promotion of ATP binding and hydrolysis by stabilizing the "closed", active conformation*. This is typically achieved through binding to both RecA domains in a "closed" conformation (activating domain in Fig. 1A). Since ATP and RNA bind cooperatively, this would also promote RNA binding. For example, the HEAT-1/MIF4G domain of eIF4G stimulates the activity of eIF4A by binding to both RecA domains. The interaction with the N-terminal domain of eIF4A (eIF4A-NTD) is much weaker, acting as a soft clamp, which allows stimulation of ATP binding without trapping the enzyme in the closed conformation (Fig. 1B) [48]. eIF4B- and eIF4H-mediated stimulation of ATP binding and hydrolysis by eIF4A [49–51] likely follow a similar mechanism, since stable binding to eIF4B and eIF4H requires an ATP/transition state analog and both eIF4A domains [52–54].
2. *Inhibition of ATP binding and hydrolysis by destabilizing the "closed" active conformation*. This can be achieved allosterically through binding to both RecA domains in an "open" conformation (inhibitory domain in Fig. 1A). Since ATP and RNA bind cooperatively, RNA binding would also be inhibited. For example, the tumor suppressor protein Pdcd4 inhibits eIF4A activity by binding to both RecA domains in an inactive conformation, as well as directly blocking the RNA-biding surface of eIF4A (see Mechanism #4, below) [55,56]. Binding at the interdomain interface, including the ATP-binding site would also lead to inhibition.
3. *Stabilization of RNA binding through additional protein–RNA interactions anchoring the helicase to its RNA substrate*. For example, in addition to directly stimulating eIF4A activity, eIF4B, eIF4H and eIF4G all have one or more RNA-binding regions that both increase the eIF4A affinity for RNA [9–11] and influence its substrate specificity [57–59]. The accessory RNA-binding domains/proteins may have preference for ssRNA or dsRNA (ssRBD and dsRBD in Fig. 1A). ssRNA binding domains could also promote helicase activity by binding to newly unwound regions of RNA (either the segment bound to the helicase RecA domains or the opposite RNA strand) and trapping them in single-stranded form [52]. Although this is yet to be experimentally demonstrated for proteins involved in translation initiation, ssDNA-binding proteins (SSBs) stabilize the newly-formed ssDNA behind replicative helicases and are integral part of every replication system [60].
Sequence- and structure-specific RNA-binding domains have additional functions in targeting the helicase to specific mRNAs or to the ribosome (see next section). For example, RHA is targeted to mRNAs containing the so-called post-transcriptional control element (PCE) [61,62], and DHX29 is recruited directly to the 40S ribosomal subunit [6,8,63]. While targeting itself is independent of any direct effects

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