# mRNA helicases: the tacticians of translational control

*Armen Parsyan*, Yuri Svitkin*¶, David Shahbazian‡¶, Christos Gkogkas*, Paul Lasko§, William C. Merrick|| and Nahum Sonenberg**

Abstract | The translation initiation step in eukaryotes is highly regulated and rate-limiting. During this process, the 40S ribosomal subunit is usually recruited to the 5′ terminus of the mRNA. It then migrates towards the initiation codon, where it is joined by the 60S ribosomal subunit to form the 80S initiation complex. Secondary structures in the 5′ untranslated region (UTR) can impede binding and movement of the 40S ribosome. The canonical eukaryotic translation initiation factor eIF4A (also known as DDX2), together with its accessory proteins eIF4B and eIF4H, is thought to act as a helicase that unwinds secondary structures in the mRNA 5′ UTR. Growing evidence suggests that other helicases are also important for translation initiation and may promote the scanning processivity of the 40S subunit, synergize with eIF4A to 'melt' secondary structures or facilitate translation of a subset of mRNAs.

*Department of Biochemistry and Goodman Cancer Research Centre, Faculty of Medicine, McGill University, Montreal, Quebec H3A 1A3, Canada.
‡Department of Pharmacology, School of Medicine, Yale University, New Haven, Connecticut 06511, USA.
§Department of Biology and Goodman Cancer Research Centre, McGill University, Montreal, Quebec H3A 1B1, Canada.
||Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106-4935, USA.
¶These authors contributed equally to this work.
Correspondence to N.S.
e-mail: nahum.sonenberg@mcgill.ca
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Initiation of translation is a highly regulated rate-limiting step of protein synthesis in eukaryotes[1]. Whereas in bacteria the ribosome is directly recruited to the vicinity of the initiation codon through the Shine–Dalgarno sequence of the mRNA[2,3], eukaryotes use a more intricate mechanism that is conserved from yeast to mammals. Initially, the small 40S ribosomal subunit is recruited to the 5′ untranslated region (UTR) of an mRNA through the coordinated action of several translation initiation factors at the 5′ cap structure on the mRNA[4]. The ribosome then scans along the mRNA in a 5′ to 3′ direction until an initiation codon is encountered (for a recent review, see REF. 5). To achieve this, ribosomes must often navigate through regions of the mRNA that contain extensive secondary structures or bound proteins, and energy must be provided to overcome these barriers. In most cases, this requires the action of helicases: enzymes that couple ATP hydrolysis to the unwinding of DNA or RNA duplex substrates.

The best-characterized helicase required for translation initiation is eukaryotic translation initiation factor 4A (eIF4A; also known as DDX2), which is thought to have a key role in unwinding secondary structures in the 5′ UTR of the mRNA. However, there is increasing evidence that other helicases, such as Ded1, DEAH-box 29 (DHX29), Vasa (VAS), RNA helicase A (RHA; also known as DHX9) and perhaps RCK (also known as p54 and DDX6) and DEAD-box 25 (DDX25), might have non-redundant roles in translation initiation. These helicases belong to the DEAD- and DEAH-box family of helicases that contain Asp-Glu-Ala-Asp (DEAD) or Asp-Glu-Ala-His (DEAH) motifs[6]. So far, it seems that none of these helicases alone has the high level of processivity required to 'melt' extensive mRNA 5′ UTR secondary structures and to allow the ribosome to 'glide' towards the initiation codon[7]. This may account for the need for several helicases to act in concert or to use modulatory proteins and binding partners that augment the unwinding activity and processivity of the helicase[7]. This requirement may vary depending on the length and composition of the 5′ UTR and the distance between the 5′ cap region, where eIF4A is recruited, and the initiation codon.

In this Review, we outline the functions of different helicases in the initiation of translation, and their mechanisms of action. We first focus on the function of eIF4A. We discuss how unwinding of mRNA by eIF4A may be augmented through its interaction with other translation initiation factors and how the major signalling cascades converge to regulate eIF4A function. We then describe the Ded1 helicase that maintains the scanning competence of the ribosomal complex on long 5′ UTRs. We also outline the proposed unique mechanism of action for DHX29 that is thought to enhance the ability of the 40S subunit to overcome 5′ UTR secondary structures. Although eIF4A, Ded1 and DHX29 are not thought to be target-specific, two helicases, RHA and VAS, regulate translation initiation of a specific subset of mRNAs. We discuss how RHA may be particularly important for a subset of mRNAs that possess a highly

structured 5′ UTR element, whereas VAS is believed to activate translation through a mechanism that is unique among translation initiation helicases: the recruitment of eIF5B to mRNAs and facilitation of 60S subunit recruitment and its joining to the 40S subunit. We also discuss the possible roles of other helicases, such as RCK and DDX25, that have been suggested to function in translation initiation but for which the exact participation in this process requires further studies.

## Stepwise control of translation initiation

Under most circumstances, the eukaryotic small 40S ribosomal subunit is recruited to the 5′ end of the mRNA in a process that is strongly facilitated by a 5′-terminal m$^7$G[5′]ppp[5′]N-cap structure, where N can be any nucleotide[4]. Translation of some eukaryotic mRNAs can be initiated independently of the m$^7$G-cap structure, in which case the 40S ribosomal subunit binds to an internal site usually located upstream of an initiation codon — an internal ribosome entry site (IRES)[8].

m$^7$G-cap-dependent translation initiation in eukaryotes requires at least 13 translation initiation factors (for a review, see REF. 5). The interaction between ribosome-bound translation initiation factors and the mRNA cap-bound eIF4F complex facilitates the recruitment of the 40S subunit (FIG. 1). eIF4F is a heterotrimeric protein complex, consisting of a large scaffold (eIF4G), an RNA helicase (eIF4A)[9,10], and eIF4E. eIF4E, as part of the complex, binds directly to the mRNA m$^7$G-cap structure. Several translation initiation factors, including the large multisubunit protein eIF3 (REF. 11), eIF1, eIF1A, eIF2–GTP and the initiator tRNA (Met-tRNA$^{Met}_i$)[12–15], bind to the 40S ribosomal subunit to form the 43S ribosomal pre-initiation complex (FIG. 1a). The interaction between eIF4G and eIF3 is thought to have a primary role in bringing the small ribosomal subunit to mRNA in mammals, but not in yeast[16], and this interaction is probably enhanced by other initiation factors[17–20].

When bound, the 43S pre-initiation complex scans the mRNA in the 5′ to 3′ direction until it encounters the initiation codon (FIG. 1b–e). However, the mRNA scanning mechanism is not universal, as it might not be used when translation is initiated via an IRES within an mRNA transcript. Following base pairing between the 40S P site-bound Met-tRNA$^{Met}_i$ and the initiation codon, the 48S initiation complex is formed (FIG. 1e). Subsequently, eIF5 promotes the hydrolysis of eIF2-bound GTP, the eIF5B–GTP complex assists in recruiting the 60S subunit to the 48S initiation complex and the translation initiation factors (except for eIF5B–GTP and eIF1A) are displaced (FIG. 1e,f). eIF5B then hydrolyses GTP and, together with eIF1A, is released from the 80S ribosomal complex that is now competent for translational elongation (FIG. 1g).

It has also been reported that mammalian 80S ribosomes can be recruited to the mRNA and direct polypeptide synthesis in the absence of translation initiation factors, on mRNAs without 5′ leader sequences[21]. In addition, in the presence of eIF1, the 43S ribosomal complex locates the AUG codon in an unstructured mRNA without requiring ATP hydrolysis[22]. By contrast, movement of the 43S pre-initiation complex on 5′ UTRs that contain even weak secondary structures requires ATP and RNA helicase activity[22]. More than half of human mRNA transcripts can be classified as having moderate or strong secondary structures in their 5′ UTR[23]. Thus, translation initiation of most mRNAs would require the scanning 43S complex to associate with helicase activity to overcome these secondary structures.

Figure 1 | **Stepwise process of translation initiation.** ► **a** | Before translation initiation commences, two separate macromolecular complexes are formed. Eukaryotic translation initiation factor 4F (eIF4F) is a heterotrimeric protein complex, consisting of a large subunit, eIF4G, an RNA helicase, eIF4A (also known as DDX2), and eIF4E. eIF3, eIF1, eIF1A, eIF2–GTP-initiator tRNA (Met-tRNA$^{Met}_i$) and probably eIF5 bind to the 40S ribosomal subunit to form the 43S ribosomal pre-initiation complex. **b** | eIF4E, within the eIF4F complex, binds to the mRNA m$^7$G-cap structure. The interaction between eIF4G and eIF3 brings the 43S ribosomal complex and mRNA together. eIF4B and eIF4H share a common binding site on eIF4A and their interactions are mutually exclusive[64]. Not shown is the circularization of the mRNA through interaction of the poly(A)-tail-bound poly(A)-binding protein and eIF4G. **c** | The 43S complex scans in a 5′ to 3′ direction until it encounters a secondary structure. It is unclear whether eIF4E stays associated with the m$^7$G-cap structure during scanning or dissociates. **d** | eIF4A, together with other helicases and accessory proteins not depicted here, unwinds the secondary structure in the mRNA. eIF4B and eIF4H enhance the activity of eIF4A. After the mRNA secondary structure is unwound, the 43S complex resumes scanning towards the initiation codon (AUG). **e** | Recognition of the initiation codon by the 43S complex leads to the formation of the 48S initiation complex and eIF5-mediated hydrolysis of eIF2–GTP. eIF5B–GTP assists in recruitment of the 60S subunit to the 48S initiation complex, to form the 80S complex, and concomitant displacement of eIF2–GDP, eIF1, eIF3, eIF4A, eIF4B, eIF4G, eIF4H and eIF5. **f** | eIF5B hydrolyses GTP and, together with eIF1A, is released from the 80S ribosomal complex. **g** | The translation elongation-competent 80S ribosomal complex is now formed. Images are modified, with permission, from REF. 127 © (2009) Elsevier, and REF. 5 © (2010) Macmillan Publishers Ltd. 

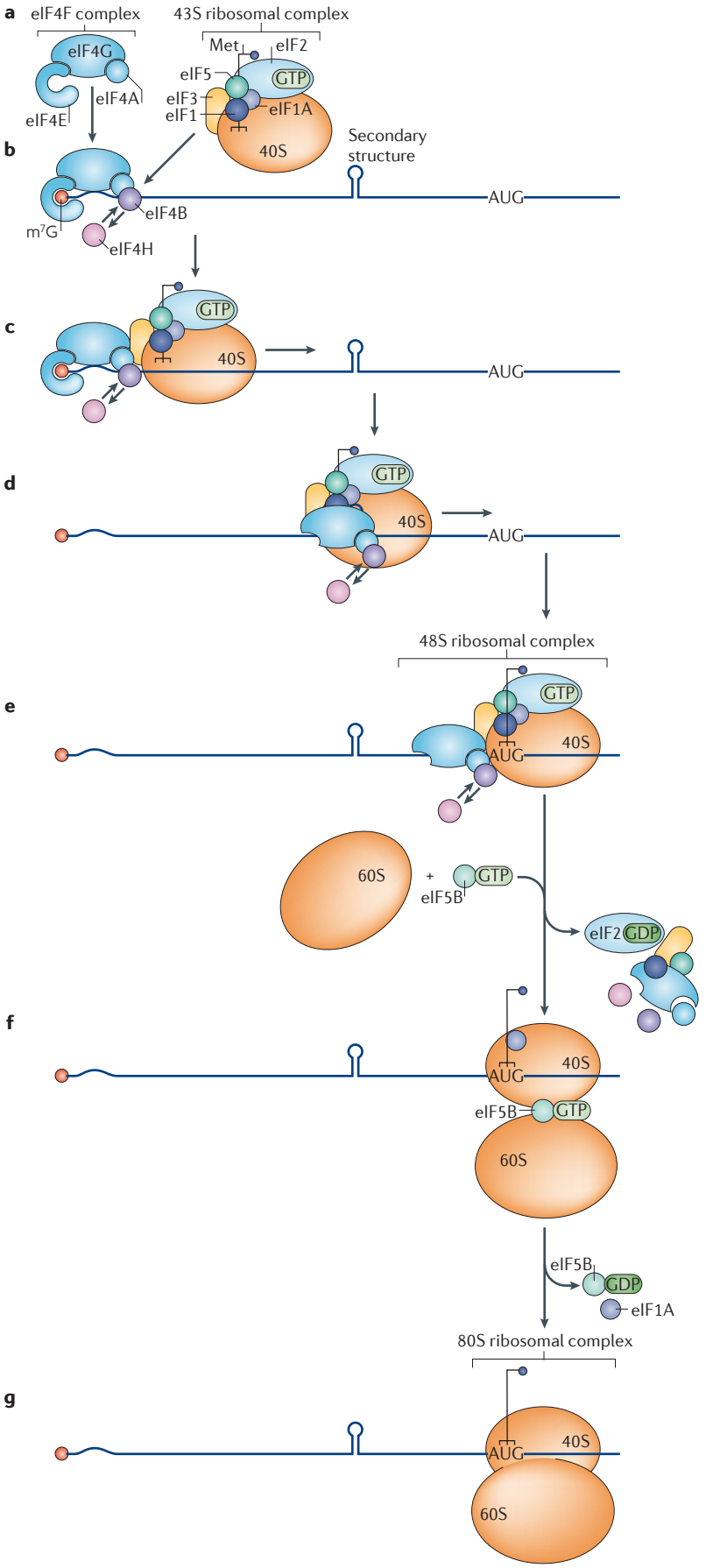
## Diverse families of helicases

Helicases function to unwind DNA or RNA in a wide range of key biochemical processes, including ribosome biogenesis, transcription, pre-mRNA splicing, mRNA export, RNA maturation and degradation, translation and ribonucleoprotein particle (RNP) remodelling[6,24–26]. The canonical helicases are thought to processively unwind the double-stranded region of the nucleic acid polymer by binding to, and then translocating along, the nucleic acid strand with multiple rounds of ATP hydrolysis (for a review, see REF. 27). In the early 1990s, helicases were grouped into several superfamilies based on their primary sequence alignments[28]. RNA helicases are predominantly members of superfamily II (SFII), which is the largest[6]. Within SFII, helicases are further

Shine–Dalgarno sequence
A ribosomal binding site of approximately eight nucleotides in the mRNA of bacteria, located upstream of the initiation codon. Helps to recruit the small ribosomal subunit to the mRNA to initiate protein synthesis.

Initiator tRNA
(Met-tRNA$^{Met}_i$). A tRNA, linked with Met through an aminoacyl group, that is used to initiate protein synthesis. Its anticodon is complementary to the AUG initiation codon. Forms a highly specific ternary complex with eukaryotic translation initiation factor 2 and GTP, and binds to the ribosomal P site.

P site
The site on the 40S ribosomal subunit that accommodates Met-tRNA$^{Met}_i$ or the tRNA that is linked to the growing peptide chain.




sub-classified into families that are defined by a series of conserved motifs. Although this classification provides an important framework, it is becoming clear that it should be revised to include current data on helicase function and from structural analyses. For example, some putative helicases do not unwind nucleic acids but instead couple nucleoside 5′-triphosphate (NTP) hydrolysis to directional motion or translocation along polynucleotides, and thus should be classified as translocases (for a review, see REF. 27). Other helicases do not translocate but only locally unwind or modulate double-stranded RNA structures[29].

eIF4A was the first helicase that exhibits a role in eukaryotic translation initiation to be identified. On the basis of the homology that many proteins show to eIF4A and their function in nucleic-acid unwinding, a family of helicases called DEAD-box proteins was identified within the SFII superfamily and named for the eponymous Asp-Glu-Ala-Asp (DEAD) motif[6] (FIG. 2). A related family of proteins is made up of the DEAH-box helicases, which contain the conserved sequence Asp-Glu-Ala-His (DEAH). Both DEAD- and DEAH-box proteins require ATP to unwind RNA helices by separating the strands from each other in a non-directional fashion[30–34]. The model that most closely describes the complex functions of these proteins views DEAD-box proteins not as poorly processive helicases but as ATP-dependent RNA-binding proteins[35]. It posits that DEAD-box proteins unwind duplexes using ATP-driven affinity changes of the enzyme and that RNA unwinding per se does not require energy provided by ATP hydrolysis[29,35]. Moreover, strand separation by DEAD-box proteins requires only the presence, not the hydrolysis, of ATP, and only a single ATP molecule is needed to separate an RNA duplex[36]. ATP hydrolysis seems to be required for enzyme dissociation from the RNA and subsequent enzyme recycling[35]. DEAD-box proteins are thought to 'open' the RNA duplex and reduce the number of base pairs within the duplex, leading to the dissociation of the remaining base pairs without further input from the helicase. Thus, the unwinding rate of DEAD-box helicases would decrease with increasing duplex length and stability[37]. This mechanism might explain why RNA helicases cannot readily unwind RNA duplexes that contain more than two helical turns[33,34].

### eIF4A as a canonical helicase

The eIF4A subunit of the eIF4F complex is thought to be responsible for unwinding of mRNA 5′ UTR secondary structures[38–40] (FIG. 3a). eIF4A is a canonical DEAD-box helicase that exhibits RNA-dependent ATPase and ATP-dependent bidirectional helicase activities[37,38,41–43]. It lacks RNA-binding domains and binds weakly to single-stranded RNA (with a dissociation constant ($K_d$) of ~100 μM) in a process that is independent of nucleotide sequence[39,43–47].

In mammals, there are three eIF4A isoforms, which are encoded by different genes. eIF4AI (also known as DDX2A) and eIF4AII (also known as DDX2B) share 91% amino-acid sequence identity and are functionally indistinguishable[48]; thus, they are cumulatively

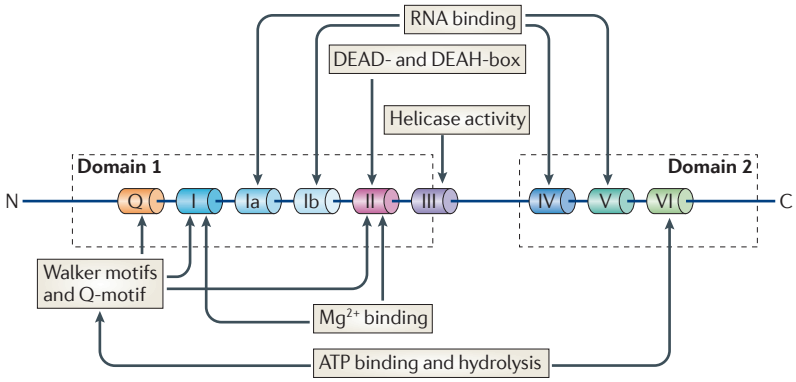



Figure 2 | **Motif structure of DEAD and DEAH helicases.** DEAD- and DEAH-box helicases are characterized by the presence of an Asp-Glu-Ala-Asp or an Asp-Glu-Ala-His motif, respectively (shown as motif II here). Both families of helicases contain amino-terminal and carboxy-terminal domains (domain 1 and domain 2, respectively) and nine characteristic motifs within these (depicted in the figure as boxes Q and I–VI). The known or proposed functions of each motif are shown. Motif II forms interactions with the β-phosphate and γ-phosphate of ATP through $Mg^{2+}$ and is required for ATP hydrolysis. Figure is modified, with permission, from REF. 24 © (1999) Elsevier, and REF. 25 © (2004) Macmillan Publishers Ltd. All rights reserved.

referred to as eIF4A. A third member of the eIF4A family, eIF4AIII (also known as DDX48), exhibits only 65% amino-acid sequence identity to eIF4AI and is crucial for the assembly of the exon junction complex after splicing[49–51]. eIF4AIII failed to substitute for eIF4AI in translation and thus is not likely to be involved in the control of initiation[52,53].

The recently solved crystal structure of yeast eIF4A has provided new insights into its function[54]. eIF4A has a distended 'dumbbell' structure, with two globular domains that are connected by an extended 11-residue linker. The dumbbell structure is in equilibrium with a compact form, in which the two domains interact with each other. ATP and RNA bind to the compact form and shift the equilibrium towards this conformation[55]. Within the eIF4F complex, eIF4A is bound to eIF4G through HEAT (huntingtin, EF3, PP2A and TOR1) domains in the middle domain (and also, in mammals, at the carboxyl terminus) of eIF4G[18,56–58]. The main site of interaction between eIF4A and the middle domain of eIF4G occurs on the surface of the C-terminal recombinase A (RecA)-like domain in eIF4A. Through additional interactions with the eIF4A amino-terminal domain, eIF4G acts as a soft clamp to stabilize a closed RNA-binding conformation of eIF4A[59]. A kinetic model has been proposed, in which eIF4A undergoes a cycle of ATP-induced conformational changes during translation initiation[43,60].

eIF4A has weak intrinsic helicase and ATPase activities and acts in a non-processive manner[37]. The initial rate of duplex unwinding by eIF4A decreases with increasing stability of the duplex[37]. The finding that the translation of mRNAs containing a strong secondary structure in their 5′ UTR is more susceptible to inhibition by the eIF4A dominant-negative mutant than the translation of mRNAs with relaxed structures provides indirect evidence that eIF4A melts secondary structures in the mRNA 5′ UTR[61]. This supports the model that the requirement for eIF4A in translation is determined by the degree of mRNA 5′ secondary structure. The helicase and ATPase activities of eIF4A are strongly stimulated when eIF4A is part of the eIF4F complex or is associated with eIF4B and eIF4H[38,39,42,62,63]. Furthermore, inhibition of translation by the eIF4A dominant-negative mutants is relieved much more efficiently by the eIF4F complex than by eIF4A alone[39]. Together, these results indicate that the eIF4G–eIF4E subcomplex acts to deliver the helicase activity of eIF4A to the 5′ end of mRNA and also to increase its activity.

***eIF4A modulation by eIF4B and eIF4H.*** eIF4B is homologous to eIF4H throughout the entire amino-acid sequence of eIF4H, but eIF4B contains additional N- and C-terminal domains. By stimulating the helicase activity of eIF4A, eIF4B and eIF4H allow eIF4A to unwind longer, more stable duplexes with an increased initial rate[37,38,41,62]. eIF4B and eIF4H modulate the affinity of eIF4A for ATP or ADP[10]. It was recently reported that eIF4B and eIF4H share a common binding site on eIF4A and their interactions with eIF4A are mutually exclusive[64]. These proteins may also increase the affinity of eIF4A for RNA[45,65], although further studies are required to confirm this.

Several additional mechanisms of action have been suggested for eIF4B and eIF4H. Both eIF4B and eIF4H also interact with RNA through their RNA-recognition motif (RRM) domains[63,66,67], and these interactions might be important for modulating local mRNA structures. For example, it is possible that eIF4B or eIF4H stabilize a single-stranded region of 5′ UTR where 40S initially binds to the mRNA[68]. It was also postulated that eIF4B and eIF4H prevent mRNA re-annealing, thereby promoting processive 5′ to 3′ directional eIF4A movement[10] and preventing ribosomes from sliding in the opposite (3′ to 5′) direction. A 'Brownian ratchet model' of 43S ribosomal complex movement along the mRNA has also been proposed[69], in which the one-dimensional diffusion of the 43S complex along the mRNA is rendered unidirectional (5′ to 3′) by the eIF4F complex using a 'ratchet-and-pawl' mechanism. In this model, ATP binding and its eIF4A-induced hydrolysis changes the affinity of eIF4A for eIF4B and for mRNA[69]. This results in the restriction of 3′ to 5′ backward sliding of the 43S complex along the mRNA, while allowing stochastic 5′ to 3′ movements.

Thus, eIF4A modulatory proteins might exert their function by several means: first, by stimulating eIF4A helicase activity and increasing its processivity; second, by stabilizing single-stranded RNA and preventing refolding of the 5′ UTR of the template mRNA; and third, by rendering 43S ribosome movement on the mRNA unidirectional.

***Modulating eIF4A activity through signalling cascades.*** eIF4B is a downstream effector of major signalling pathways that control translation initiation (FIG. 4). eIF4B phosphorylation at Ser422 is important for its interaction with eIF3 (REFS 70–72). It is possible that this interaction facilitates 40S ribosome recruitment through the

Translocase
An enzyme that couples ATP hydrolysis to directional movement on single- or double-stranded nucleic acids.

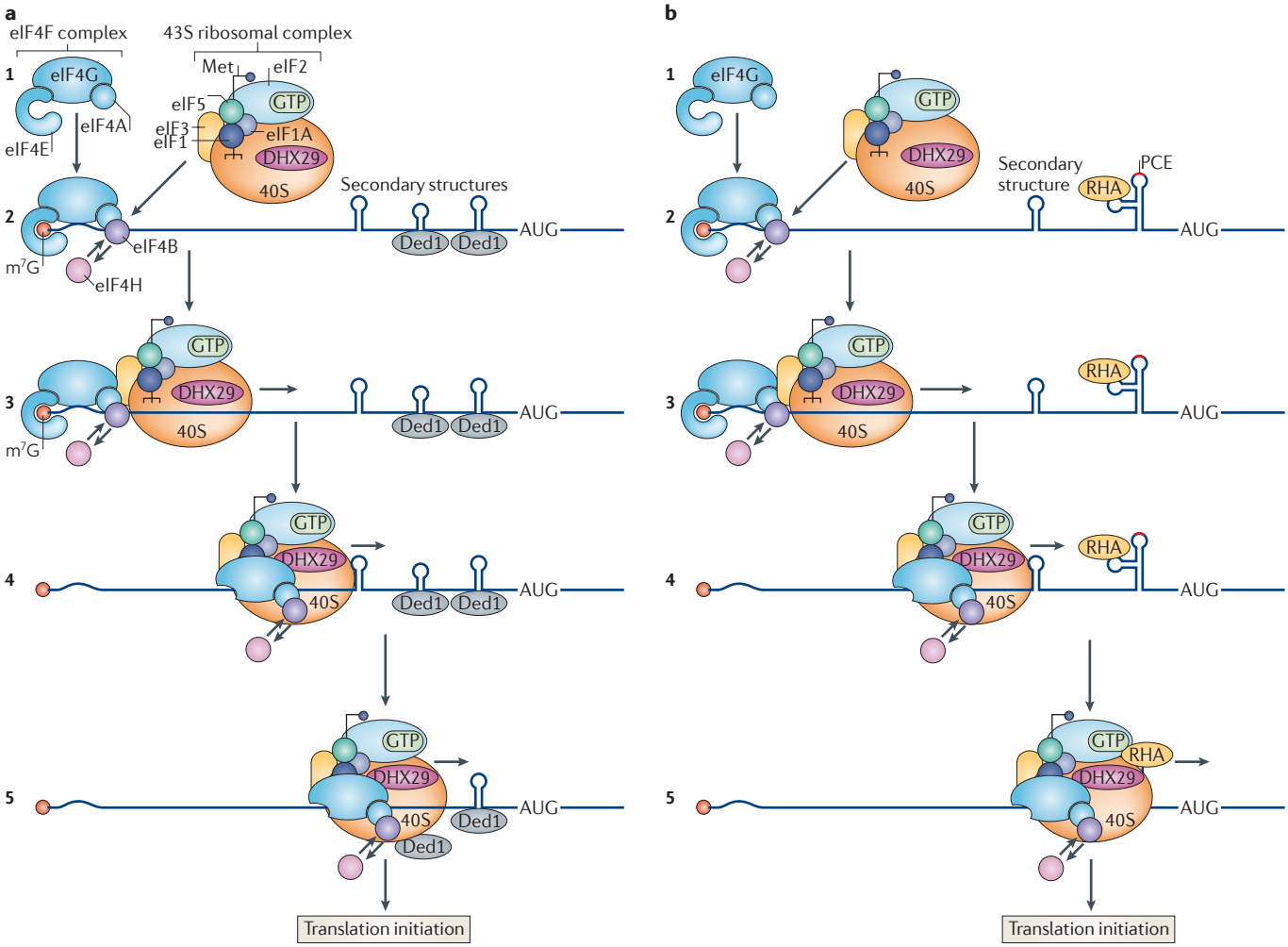



Figure 3 | **Translational control by helicases that unwind mRNA secondary structures or modulate the 40S subunit function. a** | The helicase activity of eukaryotic translation initiation factor 4A (eIF4A; also known as DDX2) and its processivity are promoted by its modulatory proteins eIF4B and eIF4H; together, these three proteins unwind secondary structures in the mRNA. The interactions of eIF4B and eIF4H with eIF4A may be mutually exclusive. The DEAH-box 29 (DHX29) helicase also remodels the 40S subunit in a manner that facilitates the ability of the ribosome to overcome 5′ untranslated region (UTR) secondary structures[89]. It probably does so at the entry of the mRNA into the 40S subunit. It is possible that DHX29 can also unwind secondary structures on the mRNA[92]. The combined action of eIF4A and DHX29 facilitates the scanning process and movement of the 40S subunit on structured mRNAs[92]. In yeast, Ded1 helicase has also been proposed to be required for translation initiation on mRNAs with long 5′ UTRs. The precise role of the mammalian Ded1 homologues, DEAD-box 3 (DDX3) and PL10 (also known as D1PAS1), is unknown. **b** | The interaction between RNA helicase A (RHA; also known as DHX9) and the post-transcriptional control element (PCE) seems to occur early after transcription[97]. The presence of RHA possibly leads to RNA rearrangement or unwinding that facilitates 40S scanning and/or eIF4A unwinding activity. The RHA–PCE interaction has also been proposed to stimulate ribosome recycling and mRNA circularization via interaction with poly(A)-binding protein[97] (not depicted here).

generation of a bridge between the eIF4F complex and ribosome-bound eIF3. Upon Ser422 phosphorylation, more ATPase activity is associated with immunoprecipitated eIF4B in interferon-stimulated cells[72]. On the basis of these data, it was proposed that upon phosphorylation, eIF4B interacts more efficiently with eIF4A[72]. The kinase responsible for Ser422 phosphorylation was identified as S6 kinase 1 (S6K1) and S6K2, which is activated by the phosphoinositide 3-kinase (PI3K)–mammalian target of rapamycin (mTOR) signalling pathway[70] (FIG. 4). It has been shown that the mitogen-activated protein kinase (MAPK)-induced kinase ribosomal S6 kinase (RSK) also phosphorylates Ser422 (REF. 70), as does AKT (also known as PKB)[73]. An additional mitogen-regulated phosphorylation site Ser406 on eIF4B has also been identified and shown to depend on both MAPK/ERK kinase (MEK) and mTOR activity[73]. Both Ser406 and Ser422 promote the translational activity of eIF4B[73]. Hence, major signalling cascades regulating mitogenesis and survival converge to phosphorylate eIF4B, which underlines the importance of this factor in the regulation of eIF4A activity.

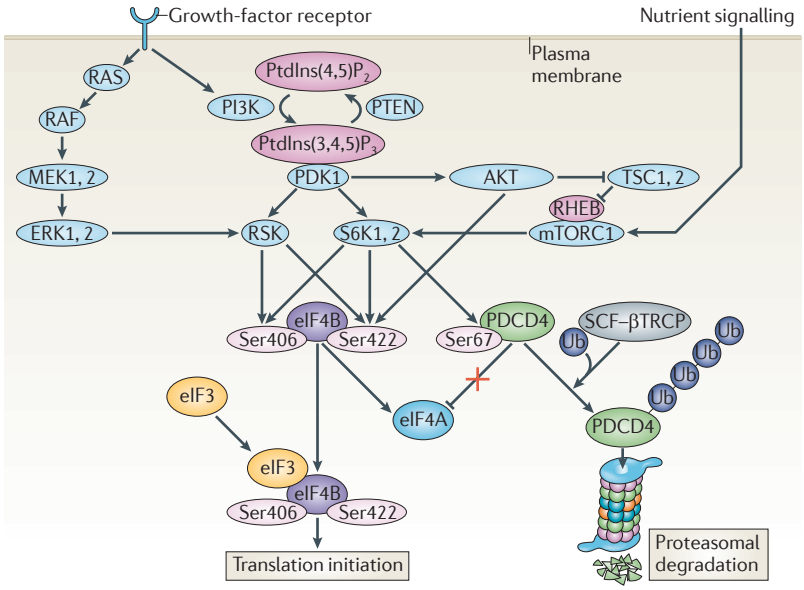



Figure 4 | **Control of eIF4A activity by signalling cascades.** Both the mitogen-activated protein kinase (MAPK) ribosomal S6 kinase (RSK) and the phosphoinositide 3-kinase (PI3K)–mammalian target of rapamycin (mTOR) complex 1 (mTORC1)–S6 kinase (S6K) signalling modules, which respond to changes in growth factor signalling, control the phosphorylation state and activation of eukaryotic translation initiation factor 4B (eIF4B)[70,72,73,77]. MAPK/ERK kinase 1 (MEK1), MEK2 and mTOR promote phosphorylation of eIF4B at Ser406 and RSK, AKT (also known as PKB), S6K1 and S6K2 phosphorylate Ser422 (REFS 70,73,128). Tuberous sclerosis 1 (TSC1; also known as hamartin) and TSC2 (also known as tuberin), through inhibition of mTORC1 activity, lead to reduced eIF4B phosphorylation at Ser406 and Ser422. Phosphorylation of eIF4B leads to stimulation of eIF4A (also known as DDX2) activity[72]. The negative regulator of eIF4A, programmed cell death 4 (PDCD4), is phosphorylated by S6K1 on Ser67 and is consequently targeted for ubiquitylation by the E3 ligase Skp–cullin–F-box (SCF)–β-transducin repeat-containing protein (βTRCP) and proteasomal degradation[75]. ERK, extracellular signal-regulated kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; PtdIns(4,5)$P_2$, phosphatidylinositol-4,5-bisphosphate; PtdIns(3,4,5)$P_3$, phosphatidylinositol-3,4,5-triphosphate; PTEN, phosphatase and tensin homologue; RHEB, Ras homologue enriched in brain; Ub, ubiquitin.

Another regulator of eIF4A activity is the translation inhibitor programmed cell death 4 (PDCD4), which is a tumour suppressor gene product[74]. In response to mitogens, PDCD4 is rapidly phosphorylated on Ser67 by S6K1 (FIG. 4) and subsequently degraded via ubiquitylation by the ubiquitin ligase Skp–cullin–F-box (SCF)–β-transducin repeat-containing protein (βTRCP)[75] and targeting to the proteasome. Hence, the PI3K–mTOR–S6K signalling module stimulates eIF4A via both eIF4B activation and PDCD4 degradation, providing a regulatory link from extracellular cues to translation initiation.

## Ded1 and scanning on long 5′ UTRs

Yeast Ded1 is an RNA-dependent ATPase and an ATP-dependent RNA helicase that is essential for viability[76,77]. As measured by sucrose density-gradient centrifugation analysis, conditional *ded1* mutants exhibit decreased translation, disruption of polysomes and accumulation of 80S ribosomes, suggesting that Ded1 is required for translation initiation[78,79]. Ded1 is proposed to act as an oligomer, so it could interact with single- and double-stranded RNA either through equivalent domains of distinct monomers or through different domains of a single monomer[33]. It has been suggested that Ded1 may be a more potent helicase than eIF4A and that this assists in scanning long 5′ UTRs[78–81] (FIG. 3a). Ded1 and the yeast eIF4A homologues, transcription intermediary factor 1 (Tif1) and Tif2, seem to be non-redundant, and the capacity to maintain scanning competence on a long 5′ UTR depends more on Ded1 than on eIF4A[81]. *Saccharomyces cerevisiae* also has a close homologue of Ded1 called Dbp1, which has been proposed to have a role in translation initiation that is similar to that of Ded1 (REFS 81,82). However, the role of Dbp1 in translation requires further characterization.

Mammalian homologues of Ded1 (DEAD-box 3 (DDX3) and PL10 (also known as D1PAS1)) are implicated in translation initiation, but their precise roles are unclear. Humans have two related proteins that share 91% similarity: DDX3Y (Y-chromosome-linked and translated only in the testes) and DDX3X (X-chromosome-linked and ubiquitously expressed)[83]. In addition to DDX3X and DDX3Y, rodents express an autosomally encoded PL10 protein[84], which can substitute for DDX3Y function during spermatogenesis[85]. Despite the fact that mouse PL10 shares only 53% amino-acid similarity with Ded1, it can functionally replace Ded1, suggesting that PL10 may also regulate translation[78].

There are contradictory views as to what the role of DDX3 in translation is. Depletion of DDX3 by small interfering RNA (siRNA) in HeLa cells inhibits translation from a β-globin reporter[86]. By contrast, another study reported that DDX3 does not significantly affect general translation but, similarly to the proposed role of Ded1 in yeast, promotes translation of mRNAs containing a long or structured 5′ UTR[87]. Both studies support the conclusion that DDX3 positively stimulates translation initiation. It has also been observed that DDX3 inhibits cap-dependent translation through a direct interaction with eIF4E that disrupts the integrity of the eIF4F complex; also, siRNA-mediated silencing of DDX3 increased cap-dependent translation from a capped bicistronic luciferase RNA and enhanced green fluorescent protein reporter[88]. The basis for this discrepancy is unclear and may be attributed to either the reporter used or cell-type-specific differences. Although the combined evidence from various studies in yeast and mammals favours a role of DDX3 in promoting translation initiation of mRNA with strongly structured and/or long 5′ UTRs, the actual function of DDX3 remains ambiguous and requires further characterization.

## DHX29 and 40S remodelling

DHX29 is a recently described translation initiation factor[89] that is required for cancer cell proliferation[23]. It is a DEAH-box protein that escaped identification as a translation initiation factor in early studies using *in vitro* reconstituted initiation systems[12,13,22,89,90]. *S. cerevisiae YLR419w*, which is a nonessential gene with unknown function, was proposed to be a potential orthologue of *DHX29*[91]. However, yeast YLR419w is unlikely to be the functional DHX29 orthologue as it is more closely related

Polysome
A cluster of mRNA-bound, elongating 80S ribosomal complexes. Also known as a polyribosome.

to mammalian DHX57 and DHX36 than to DHX29 (REF. 92). DHX29 is enriched in fractions containing the 40S ribosomal subunit but not in polysomes[23], indicating that, in common with other translation initiation factors, DHX29 dissociates from the 40S subunit upon completion of translation initiation. Both *in vitro*[89] and *in vivo*[23] studies have concluded that the main role of DHX29 is to promote translation initiation of mRNAs with moderate to strong 5′ UTR secondary structures. Evidence suggests that DHX29 remodels the 40S subunit, rendering it more processive[89]. This evidence mainly comes from distinct effects of DHX29 on 48S complex formation on different IRES elements. DHX29 significantly increased 48S complex formation on an IRES of *Encephalomyocarditis virus* (EMCV) but reduced binding of 40S subunits to the IRES of cricket paralysis virus (CrPV)[89]. The disparate effects of DHX29 on 48S complex formation at different IRESs favours the hypothesis that DHX29 causes conformational changes in 40S subunits. Most recent data suggest that DHX29 monitors mRNA 'entry' into the 43S complex[92]. The most likely mechanism is that DHX29 cycles between NTP- and nucleoside 5′-diphosphate (NDP)-bound states, engendering the corresponding opening and closing of the 40S ribosome entry channel, although direct unwinding of mRNA secondary structures by DHX29 in addition to this ribosomal remodelling cannot be excluded[92]. Determination of the structure of the 43S complex with NTP- and NDP-bound DHX29 would help to better understand the mechanism by which DHX29 influences translation initiation.

## RHA acts on a subset of mRNAs

RHA is a highly conserved protein that participates in transcription, splicing and nuclear export[93–96]. RHA is also thought to unwind secondary structures in the 5′ UTR of mRNA[97]. The major difference between RHA and the helicases described above is that it is target-specific. Target-sequence specificity is generally conferred to DEAD- and DEAH-box helicases by motifs outside the region of DEAD- and DEAH-box protein similarity (for an example, see reference REF. 98). RHA is essential for translation initiation of a selected set of mRNAs through binding to a complex 5′ post-transcriptional control element (PCE)[97]. This element is present in the RNA of retroviruses (such as HIV-1 and human T-lymphotrophic type 1 viruses) and is used by RHA to facilitate translation of the viral RNA, as well as to enhance the infectivity of progeny virions[99]. PCE is also present in the mRNA that encodes cellular JUND growth-control protein, which also contains a highly structured 5′ UTR and initiates translation by an m$^7$G-cap-dependent mechanism[100]. A model of RHA function in translation initiation has been proposed[97,101], in which RHA stimulates ribosome scanning, possibly through its helicase activity and control of RNA–protein rearrangements, and thus facilitates translation initiation (FIG. 3b). RHA is reported to bind to its target mRNA through its N-terminal domain and then to rearrange the complex 5′ UTR[102]. Thus, RHA relieves the barrier that is imposed by the complex 5′ UTR of PCE-containing RNAs.

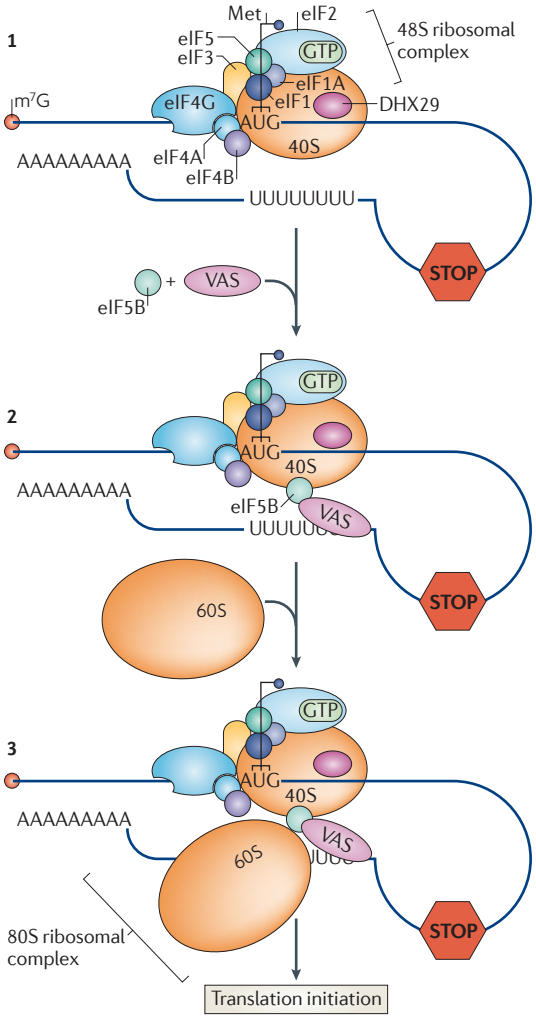



Figure 5 | **Translational control by VAS recruitment of eIF5B and the 60S subunit.** The Vasa (VAS) protein facilitates translation of a subset of mRNAs through binding to the U-rich element in the 3′ untranslated region. It then assists in the recruitment of eukaryotic translation initiation factor 5B (eIF5B) to the 40S subunit. This protein subsequently facilitates joining of the 40S and 60S ribosomal subunits to form the 80S complex[98]. DHX29, DEAH-box 29.

## VAS and 60S recruitment

Another target-specific helicase is VAS, which binds its targets through sequences outside of its core DEAD-box helicase domain[29,98]. VAS is a DEAD-box RNA-binding protein required in *Drosophila melanogaster* at several steps of oogenesis. It exhibits ATP-dependent RNA helicase activity[103] and associates with eIF5B[104,105]. The VAS–eIF5B interaction has been implicated genetically in the translation of *gurken* mRNA, which is expressed in the oocyte and is critical for embryonic patterning[106,107], and *mei-P26* mRNA, which regulates micro RNAs (miRNAs) and cell growth in the *D. melanogaster* ovarian stem cell lineage[98,108]. VAS binds specifically to a U-rich element in the *mei-P26* 3′ UTR and is believed to activate translation by recruiting eIF5B to the mRNA, which in turn allows the 60S subunit to be recruited and to join the 40S subunit[104,109] (FIG. 5). It remains unclear as to why, in

Micro RNA
A small RNA of ~21 nucleotides that binds to argonaute proteins and regulates the expression of a large number of mRNAs, with which it is partially complementary in sequence.

Chromatoid body
A large perinuclear ribonucleoprotein particle of germ cells that might organize and control RNA processing.

Piwi-interacting RNA
A small RNA of ~30 nucleotides that interacts with argonaute-related piwi proteins. Has been linked to transcriptional gene silencing of retrotransposons and other genetic elements in germ line cells, particularly those involved in spermatogenesis.

Processing body
A distinct cytoplasmic focus in the eukaryotic cell that contains many enzymes involved in mRNA turnover.

the absence of VAS, eIF5B cannot be efficiently recruited to the *mei-P26* mRNA. Precedents also exist in mammalian systems for regulation of translation at this late stage of initiation[110–112]. Mutational analysis of *D. melanogaster* VAS indicates that the interactions between its N- and C-terminal domains couple ATP hydrolysis to RNA unwinding through a mechanism that differs from those of other helicases[29]. Little is known about the role of the mammalian homologues of VAS in translation, except that mouse DDX4 (also known as MVH) is required for proliferation and differentiation of mouse male germ cells and associates in chromatoid bodies with proteins involved in the miRNA pathway[113,114]. *D. melanogaster* VAS also associates *in vivo* with components of the piwi-interacting RNA (piRNA) pathway in germ line structures that are analogous to chromatoid bodies[115]. Thus, VAS is unique in that it targets a subset of mRNAs by binding to the 3′ UTR and facilitates translation initiation by recruiting eIF5B.

## Other translation helicases

Growing evidence suggests that several RNA helicases have an important role in translation. Several helicases, in addition to eIF4A, are now known to function in translation initiation. Eukaryotic genomes typically encode 100 or more putative RNA helicases. Many of these remain uncharacterized and it is conceivable that some of those will be discovered to affect translational control. Although it appears that most helicases that do participate in translation affect the initiation step, RNA helicases might also be involved in other stages of translation, such as elongation and termination. For example, the yeast DEAD-box helicase Dbp5 (a DDX19 homologue) is proposed to function in translation termination[116,117].

Among the other helicases that have been linked to translation, yeast Dhh1 (which is homologous to mammalian RCK) has been proposed to repress translation[118]. This protein is thought to dissociate eIF4E from the m$^7$G-cap (and thereby promote decapping, which targets mRNA for degradation) by facilitating the access of mRNA-decapping enzyme 1 (Dcp1) to the cap[119] and also augmenting processing body (P-body) formation[118,120,121]. However, the mechanism of Dhh1 action in translation might be more complex, as one study has indicated that it is equally capable of repressing the translation of both capped and uncapped mRNA[118]. It inhibits the assembly of the 48S complex on the mRNA, thus affecting the process of translation initiation and not merely the stability of mRNAs[118].

DDX25, which is required for spermatogenesis, was reported to control translation of specific mRNAs in germ cells[122–125]. Although the role of DDX25 in translation is poorly characterized, it is thought that the protein is required for translation at early stages as it acts as a component of RNP particles[123,124]. Furthermore, a phosphorylated cytoplasmic form of DDX25 is associated with actively translating polysomes[125], which is consistent with this protein having a role in translation.

## Summary and outlook

RNA helicases are crucial for translation initiation in eukaryotes and are essential for many fundamental biological functions of the cell[23,98,126]. The functions, target specificities and sites at which helicases act during translation initiation seem to be diverse (TABLE 1). The canonical helicase eIF4A is required for unwinding secondary structures during 40S ribosomal subunit scanning at the mRNA 5′ UTR (FIG. 3a). Ded1 might be required to unwind long and highly structured 5′ UTRs (FIG. 3a) but the precise

Table 1 | **Diverse tactics of helicases in translation initiation**

| Helicase | Family | Proposed site of action | Mechanism of action in translation initiation | Comments |
|---|---|---|---|---|
| eIF4A, eIF4AI and eIF4AII (also known as DDX2, DDX2A and DDX2B, respectively) | DEAD | 5′ UTR of the mRNA | • Unwind mRNA 5′ UTR secondary structures<br>• Together with eIF4B and eIF4H, anchor the 40S complex to 5′ UTR to restrict its 3′ to 5′ diffusional sliding (within the 'ratchet-and-pawl' model)[69] | Can be positioned simultaneously in front of and behind the scanning edge of the 40S subunit[10]; helicase activity is significantly increased when it is a subunit of eIF4F, and by eIF4B and eIF4H proteins[38,39,42,62,63] |
| Ded1 | DEAD | 5′ UTR of the mRNA | • Considered to be a more potent helicase than eIF4A and proposed to assist in translation of mRNAs with long 5′ UTRs[78–80,92] | Role of mammalian homologue DDX3 is controversial; mouse Ded1 homologue, PL10 (also known as D1PAS1) can functionally replace Ded1, suggesting a role of PL10 in translation[78] |
| DHX29 | DEAH | 40S ribosome (possibly near the mRNA entry channel of the 40S subunit[92]) | • Remodels the 43S complex to facilitate its ability to overcome secondary structures<br>• Possibly unwinds mRNA secondary structures | Binds the 40S subunit[23,89]; no other known binding partners |
| RHA (also known as DHX9) | DEAH | 5′ UTR of mRNAs that contain a complex PCE[97] | • Unwinds the mRNA secondary structure of PCE[102] | Increases ribosome recycling through interaction with PABP |
| VAS | DEAD | *Drosophila melanogaster* VAS binds a URE in the 3′ UTR of mRNAs | • Assists ribosomal subunit joining through recruitment of eIF5B[98]<br>• Proposed to modulate local mRNA structure[29] | Interacts with eIF5B[98] |

DDX, DEAD-box; DHX29, DEAH-box 29; eIF, eukaryotic translation initiation factor; PCE, post-transcriptional control element; PABP, poly(A)-binding protein; RHA, RNA helicase A; URE, U-rich element; UTR, untranslated region; VAS, Vasa.

function of its homologues DDX3 and PL10 in mammalian translation initiation is still to be resolved. DHX29 seems to remodel the 40S subunit to assist it in overcoming secondary structures (FIG. 3a). Whereas eIF4A, Ded1 and DHX29 do not have target specificity, RHA and VAS helicases exert their functions on specific sets of mRNAs (FIGS 3b,5). Thus, the translation initiation machinery potentially uses various tactics to overcome mRNA secondary structures, ranging from melting those structures to modulating the processivity of the 40S complex. The decision as to which tactics to use most likely depends on the strength of a particular secondary structure, its context and its position at the 5′ UTR.

Given the fundamental role for helicases in translation, it is not surprising that their activity is highly regulated. The control of helicase activity is evident from studies of the eIF4A modulatory protein eIF4B, the phosphorylation of which is controlled by major signalling pathways that are important for mitogenesis and survival (FIG. 4). It is likely that the other helicases are also downstream effectors of such signalling cascades and are regulated either directly or through binding partners and modulatory proteins. The fundamental roles of helicases in translation and their associations with signalling pathways make them attractive candidates for pharmacological targeting of diseases.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**Competing interests statement**

The authors declare no competing financial interests.

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