Mini Review

DEAD-box helicases: Posttranslational regulation and function

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1. Introduction

Helicases are enzymes that can separate duplex oligonucleotides in a NTP-dependent fashion and are essential in all aspects of DNA and RNA metabolism. Amino acid sequence analysis identified several conserved sequence motifs in DNA and RNA helicases allowing their classification into five major groups (Super families SF1–SF5) [1]. DExD/H helicases share eight conserved sequence motifs, whereas the DEAD-box helicase subgroup has an additional ninth conserved sequence motif [2]. These sequence motifs encompass an approximately 300–400 amino acid core region involved in ATP-binding/hydrolysis and RNA binding (Part 2: Fig. 1). Structural analyses of several DEAD-box proteins show this core region forms two RecA-like globular domains [2].

Work in a variety of eukaryotes has identified the biological functions of many DEAD-box helicases. The genome of the yeast Saccharomyces cerevisiae encodes 25 DEAD-box proteins. Counterparts for each of these, along with 11 additional DEAD-box genes, are found in the human genome. Although some of the shared DEAD-box genes have similar functions in both humans and yeast, it is clear that several human DEAD-box proteins have acquired additional functions [3,4]. How these functions are regulated within cellular or developmental contexts is less understood. The N-terminal and C-terminal sequences flanking the DEAD-box core regions are considerably more divergent among DEAD-box proteins and are thought to interact with RNA substrates or cofactors. Such interactions can thereby target and regulate their helicase activity or perform completely independent functions [3]. Although the structures of these divergent flanking sequences are largely unknown, a growing body of evidence suggests they are regulatory hot-spots for posttranslational modifications and protein–protein interactions (Fig. 1). Despite DEAD-box helicase conservation throughout the animal kingdom, the most comprehensive data on their posttranslational regulation comes from the human DEAD-box helicase family (DDX proteins). For the purposes of this review, we shall focus primarily on the data concerning human DDX proteins and look at our current understanding on how posttranslational modifications and protein–protein interactions regulate DEAD-box protein functions.

2. DDX1

DDX1 was originally identified as an overexpressed gene in retinoblastoma and neuroblastoma tumors and cell lines [5]. In its normal context, DDX1 is a nuclear protein expressed in many cell types early in development that later becomes restricted, indicative of a specific role in differentiated cells. While much of DDX1 function remains unknown, a number of studies suggest that interaction with other proteins directs its function as a cofactor in various biological processes. It is also the only human DEAD-box gene with a SPRY domain, which may facilitate some of its binding properties [5,6]. The DDX1 interaction with the RelA (p65) subunit of NF-κB recruits it to NF-κB-binding promoter sequences. A mammalian-two-hybrid binding assay suggests that amino acids in both the conserved DEAD-box core region and the N-terminal amino acids are important for this interaction [7]. A yeast two-hybrid screen identified DDX1 residues located in the DEAD-box conserved core region (amino acids 189–333) that bound HIV Regulator of virion (Rev) protein, which is required for nuclear export of viral transcripts [8]. Further in vitro binding analysis and in vivo co-immunoprecipitation in HEK293 cell extracts confirmed the interaction between Rev and DDX1 [9]. Overexpression of DDX1 in HIV infected cells corresponded to increased viral production, whereas siRNA knock-down of DDX1 altered Rev localization suggesting that DDX1 is an important cofactor for Rev function [9]. Following ionizing radiation, DDX1 protein colocalizes with ATM (ataxia telangiectasia mutated) to multiple foci within the nucleus. DDX1 is phosphorylated by ATM both in vitro and in vivo and phosphorylation of endogenous DDX1 is enhanced by ionizing radiation, supporting a role for DDX1 in the repair of double-strand DNA breaks within transcriptionally active regions of the genome [10]. Therefore, it is clear that posttranslational regulation plays an important role in DDX1 function.

3. DDX2 (eIF4A)

DDX2 (eIF4A) is an essential and universally conserved RNA helicase that plays a key role in initiation of translation by relieving secondary mRNA structure and allowing ribosome scanning [11]. The interaction of eIF4A with several factors is essential for its canonical role in the translation initiation complex. The ATPase activity of eIF4A is greatly enhanced when it is part of a multiprotein complex with initiation factors eIF4G, eIF4E, eIF4B and eIF4H compared to the ATPase activity of free eIF4A [3]. The helicase activity of eIF4A is stimulated by the presence of either eIF4B or
eIF4H, presumably through a direct interaction. Binding of programmed cell death factor 4 (Pdcd4) to eIF4A inactivates its helicase activity thereby inhibiting cap-dependent translation and competes for eIF4A binding with a C-terminal portion of eIF4G [3]. Mammalian two-hybrid and in vitro binding assays of eIF4A point mutants suggest Pdcd4 and eIF4G bind to partially overlapping sites within both N-terminal and C-terminal globular core domains of eIF4A [12]. Modification of eIF4A with the lipid molecule 15-deoxy-delta-12,14-prostaglandin J2 (15d-PGJ2) blocks the interaction between eIF4A and eIF4G thereby inhibiting translation. This is consistent with the signaling activity of 15d-PGJ2 that directs inhibition of cell proliferation [13]. Recent work in Drosophila demonstrates that eIF4A plays a role in germline stem cell maintenance by directly interacting and antagonizing the function of Bag of marbles (BAM). BAM preferentially interacts with an N-terminal portion of eIF4A in a yeast two-hybrid binding assay and also co-immunoprecipitates with eIF4A in Drosophila S2 cell extracts. The small marine natural product pateamine A (PatA) inhibits eukaryotic translation initiation by directly targeting eIF4AI and II. In vitro binding analyses demonstrate the N-terminal amino acids 1–220 of eIF4A are sufficient for its PatA interaction [14].

4. DDX3 (pl10)

DDX3 is a nucleocytoplasmic shuttling protein with several reported functions including mRNA translocation, RNA splicing, innate immunity, as well as its cooption in viral pathology [15,16]. The activity of DDX3 is modulated by several protein–protein interactions and posttranslational modifications. DDX3 amino...
acids Thr204 and Thr323, in the DEAD-box core region, are phospho- 
ylated in vitro by cyclinB/cdc2 [17]. Both in vitro and in vivo 
binding analyses suggest the X chromosomal isoform of DDX3 
(DDX3X) is a substrate for Tank binding kinase 1 (TBK1). DDX3 is 
also phosphorylated at 11 sites that are scattered throughout the 
DEAD-box core region. These TBK1-dependent phosphorylations 
are required for DDX3X to stimulate the type-I interferon (IFN)-β 
production through a transcriptional activation mechanism [18]. 
DDX3 interacts with elf4E through a conserved elf4E consensus 
binding sequence in its N-terminal residues 38–44 and this in- 
teraction represses translation [19]. Biochemical and structural anal- 
ysis show DDX3 also interacts with the vaccinia virus K7 protein 
through N-terminal amino acids 61–90 [20]. DDX3 C-terminal ami- 
ocids 553–622 directly and specifically interact with the hepa- 
titis C core protein. The vaccinia virus K7 protein and hepatitis C 
are required for DDX3 to induce interferon β (IFN-β) transcription [21].

5. DDX4 (Vasa)

DDX4 is the human Vasa ortholog, which is a highly conserved 
DEAD-box helicase involved in germ line formation and fertility. 
Nine conserved sequence motifs typify all DEAD-box genes [3]. 
Biochemical analyses show how these motifs, in Vasa and other 
DEAD-box proteins, confer its ATP-dependent RNA helicase cata-
lytic activity. Structural data also suggests that Vasa unwinds du-
plex RNA in a non-processive manner [23].

The mouse Vasa homolog is required for spermatogenesis and 
male fertility. Several studies indicate a functional relationship be-
tween Vasa and both the small interfering RNA and micro-RNA 
interactions [2,3]. Vasa protein binds to MIWI and MILI, which are mouse PIWI 
and Vasa homologs. In vitro binding data suggest these interactions occur 
through the N-terminal portion of Vasa protein. MILI and Vasa 
knockout mice have similar phenotypes and defects in spermato-
genesis indicative of cooperative molecular functions [23]. In MILI 
knockout mice, Vasa protein does not localize to the nuage struc-
tures [23]. However, it is still unknown whether MIWI is required 
for nuage and ultrastructural studies in MIWI knockout mice are 
needed. Exactly how these specific interactions influence Vasa, 
MIWI or MILI function is unclear.

Recent work has identified Maelstrom as a nuage component that 
interacts with both mouse Vasa and MIWI, is required for spermatogenesis 
and also is involved in silencing transposable elements [24]. In Drosophila, 
Maelstrom protein localizes to nuage in a Vasa-dependent manner. In maelstrom 
mutant oocytes, a higher molecular weight Vasa protein species is evident indi-
cating that Maelstrom is required for proper Vasa modification 
or processing.

Mouse Vasa also interacts with RanBPM. The N-terminal por-
tion of Vasa is sufficient for this interaction and both proteins colo-
ralize to nuage in maturing spermatocytes [25]. RanBPM is 
believed to be involved in recruiting Ran–GTP to microtubule 
assembly sites. These results suggest a functional relationship be-
tween Vasa and microtubule nucleation during meiosis. Much like 
another Vasa-interacting protein, Gustavus, RanBPM has contains a 
SPRY domain. It is unclear, however, whether the Vasa–RanBPM 
interaction occurs through its SPRY domain.

6. DDX5 (p68) and DDX17 (p72)

The human DDX5 (p68) and DDX17 (p72) are very similar RNA 
helicases required for splicing that also play roles in transcriptional regulation [26]. p68 is important for normal cell growth, differenti-
ation and proliferation [27,28]. Various types of posttranslational 
regulation, including covalent modifications and protein–protein 
interactions, appear to coordinate and control these activities. Colo-
rectal tumors are associated with increased expression and polyub-
iquitination of p68. However, it is unknown which portions of the 
p68 protein are ubiquitylated. Addition of the small ubiquitin-like 
modifier (SUMO) to proteins is also known to alter their localization, 
binding capabilities and function [29]. PIAS1-mediated SUMO mod-
ification of p68 on single site Lys553 modulates its transcriptional 
activity and promotes its interaction with HDAC1 [30]. Platelet-de- 
erived growth factor stimulates c-Abl kinase phosphorylation of 
p68 on Tyr593 in the nucleus. This phosphorylation is detected in se-
ven different cancer cell lines. Phosphorylated p68 interacts with 
nuclear β-catenin which then promotes cell proliferation by activat-
ing transcription of cyclin D1 and c-Myc genes and also promotes an 
epithelial–mesenchymal transition [31,32]. Several studies sug-
gest that the localization and function of p68 are regulated by inter-
actions with various proteins. For instance, fibrillarin and p68 
interact in HeLa cell extracts and colocalize in nascent nucleoli dur-
ing late telophase. This interaction requires p68 amino acids 67–483, 
suggesting that both N-terminal, C-terminal and conserved core 
regions are involved. During interphase, p68 can localize to the 
nuclear matrix by binding A-kinase-anchoring protein (AKAP95). The 
transcriptional coactivator CREB-binding protein (CBP/p300) and 
RNA polymerase II bind p68 in vivo, suggesting p68 mediates this 
multiprotein complex. In vitro binding analyses suggests the CBP/ 
p300 interaction occurs through p68 amino acids 176–388 in the 
DEAD-box core region and RNA polymerase II interacts with amino 
acids 1–80 on the p68 N-terminus. p68 and p72 preferentially form 
hetero-dimers [33]. This interaction regulates their activities as 
transcriptional coactivators.

7. DDX6 (RCK/p54)

The DDX6-like p54 genes are implicated in several biological pro-
cesses including translational regulation and function in various 
cytoplasmic bodies such as Caenorhabditis elegans P-bodies, Dro-
sophila sponge bodies and Xenopus Balbiani bodies [34]. Human RCK/p54 interacts with AG01 and AG02 in vitro and in vivo and facil-
itates P-body formation [35]. Much of the regulatory data comes from 
its Xenopus homolog Xp54. Potential nuclear export sequence 
and protein kinase CK2 sites were identified in N-terminal region 
and a C-terminal 44 amino acid segment respectively in Xenopus 
p54. Xenopus p54 interacts with cytoplasmic polyadenylation ele-
ment-binding protein (CPEB) [36,37]. A C-terminal portion of p54 
interacts with 4E-T and embryonic poly(A)-binding protein (ePAB) 
and is important for its P-body localization [38]. It is still unclear, 
however, if similar posttranslational regulatory events occur in 
humans.

8. DDX20 (dp103)

Functional analyses of DP103 knockout mice show that it is 
required for embryonic development as well as ovarian develop-
ment and function [39]. The DP103 C-terminus interacts with several 
proteins that target its function. DP103 represses transcrip-
tion by interacting with SUMOylated steroidogenic factor 1 (SF1), 
but it is unknown whether DP103 itself is SUMOylated [40]. This 
interaction was mapped to amino acids 721–825 within the 
non-conserved C-terminal region of DP103. This same region
interacts with the Ets repressor METS to form a transcription corepressor complex. A yeast two-hybrid screen for early growth response 2 (Egr2) interacting protein also identified an interacting clone containing the C-terminal amino acids 612–825 [40]. The C-terminal region of DP103 containing amino acids 666–824 interacts with Epstein–Barr virus nuclear antigens EBNA2 and EBNA3C. Together, these data suggest that the transcriptional corepressor activity of DP103 is important for embryonic and ovarian development.

9. DDX21 (RHII/Gu)

DDX21 functions in ribosomal RNA processing and is normally localized in the nucleolus. DDX21 interacts with c-Jun through its C-terminal 749–801 amino acids and functions as a transcriptional coactivator. Recent work shows that c-Jun also regulates DDX21 function. Depletion of c-Jun inhibits 18S and 28S rRNA accumulation and results in a mislocalization of DDX21 out of the nucleolus and into the nucleoplasm [41].

10. DDX23 (Prp28)

DDX23 is a spliceosome component required for splicing of nuclear pre-mRNA. The human DDX23 contains an N-terminal RS domain and can be phosphorylated in vitro by the CLK/Sty and the U1 snRNP-associated kinase, which both are known to phosphorylate RS domains. Consistent with this, DDX23 phosphorylation is required for its spliceosomal B complex association [42].

11. DDX25 (gonadotropin-regulated testicular helicase)

DDX25 (GRTH) is a gonadotropin-dependent testis-specific RNA helicase in Leydig and germinal cells essential for spermatogenesis. A leucine-rich sequence in the N-terminal amino acids 61–74 functions as a nuclear export sequence and phosphorylated GRTH displays a cytoplasmic localization, whereas unphosphorylated GRTH interacts with CRM1 [43].

<table>
<thead>
<tr>
<th>Protein</th>
<th>N-terminal</th>
<th>DEAD-box helicase core</th>
<th>C-terminal</th>
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<tbody>
<tr>
<td>DDX1 (DBP-RB)</td>
<td>–</td>
<td>T80, T83</td>
<td>Y628</td>
</tr>
<tr>
<td>DDX2 (eIF4A1)</td>
<td>–</td>
<td>Y126, T158, Y197</td>
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<td>DDX3 (PL10)</td>
<td>Y52, S60, S61, Y68, S63, S75, S77, S81, S91, Y103, S124, S130, Y162, K117 and K129</td>
<td>T203, Y242, Y282, Y300, T322, Y432, Y461 and Y465</td>
<td>S590, S593, S611 and S613</td>
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<tr>
<td>DDX4 (Vasa)</td>
<td>S200 and S202</td>
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<td>–</td>
</tr>
<tr>
<td>DDX5 (Rho80)</td>
<td>K32 and K33</td>
<td>Y202, Y244, Y297</td>
<td>–</td>
</tr>
<tr>
<td>DDX6 (Rck/p54)</td>
<td>T36 and T73</td>
<td>Y131 and Y131</td>
<td>Y473</td>
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<tr>
<td>DDX10</td>
<td>T4, S7</td>
<td>T255</td>
<td>S539, S569, T577 and S581</td>
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<td>Y281, Y323</td>
<td>T525, T574, Y584, S603, S605, S675, S676, S680, R688</td>
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<tr>
<td>DDX18 (MrDb)</td>
<td>S74, T130, S140</td>
<td>T225, S227, Y440 and Y442</td>
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<td>DDX19B (DBP5')</td>
<td>S86, Y89 and S90</td>
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<td>DDX20 (DP103)</td>
<td>S47 and S48</td>
<td>S187</td>
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<td>DDX21 (RHII/Gu)</td>
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<td>S287, S295, T302</td>
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<td>DDX41 (Abstract)</td>
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<td>S424, K448, K451</td>
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<td>T163, Y202</td>
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<td>Y304</td>
<td>S579, T582, S602</td>
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<td>DDX51</td>
<td>S83, S103</td>
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<td>Y35, S39, S99</td>
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<td>K517, K519, K523, S544, S594</td>
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<tr>
<td>DDX56</td>
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<td>DDX59</td>
<td>S64, S76, S160</td>
<td>S246</td>
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</table>

Y, S and T indicate phosphorylation on tyrosine, serine and threonine residues respectively. K indicates acetylation on lysine residues. R indicates methylation on arginine residues.

In vivo protein phosphorylation, methylation and acetylation sites were identified using the phosphorylated protein database http://www.phosphosite.org.

Y, S and T indicate phosphorylation on tyrosine, serine and threonine residues respectively. K indicates acetylation on lysine residues. R indicates methylation on arginine residues.

12. DDX39

DDX39 is a growth-associated RNA helicase involved in pre-mRNA processing and export. DDX39 is also upregulated in lung squamous cell carcinoma [44]. DDX39 interacts with cytokine induced protein 29 (CIP29), is polyubiquitylated when expressed in 293 cells and is degraded by the ubiquitin proteasome pathway. The DDX39 ubiquitylation occurs somewhere on its N-terminal portion [45]. Aly is a splicing factor that links pre-mRNA splicing to nuclear export and interacts with DDX39 but not the C-terminal truncated splice variant DDX39-S, implying that Aly interacts with the C-terminal portion of DDX39 [45]. These data suggest that the posttranslational regulation controlling proper levels of DDX39 protein are important for normal cell physiology.

13. DDX42

DDX42 is a component of 17S U2 snRNP splicing complex and also interacts with the Japanese Encephalitis Virus (JEV) NS4A
protein. A functional analysis suggests that the interaction between JEV NS4A and DDX42 impedes an interferon-α/β-mediated innate immune response to JEV infection [46]. The C-terminal 685–938 amino acids interacted with apoptosis-stimulating protein of p53 2 (ASPP2) in a yeast two-hybrid screen. A DDX42 protein consisting of amino acids 1–737 failed to coprecipitate with ASPP2, confirming that the C-terminal portion was required for its ASPP2 binding ability. DDX42 interaction with ASPP2 interferes with the apoptosis-stimulating properties of ASPP2 [47].

14. DDX54 (dp97)

DP97 is thought to function in transcriptional regulation, but its other roles are unclear. DP97 interacts with a 14-3-3 protein, in a ligand-dependent manner with estrogen receptors and with other nuclear receptors to repress their transcriptional activity [48]. This interaction was mapped to amino acids 657–865 in the C-terminal region. The transcriptional repression by DP97 maps to amino acids 413–865 in the C-terminal region.

15. Other DDX proteins

Functional and regulatory data are presently lacking for 23 of the 36 DEAD-box genes in the human genome. However, several recent proteomic analyses have shed light on in vivo posttranslational modification dynamics, phosphorylation in particular, during the cell cycle and in response to a number of different stimuli [49–59]. PhosphoSite is a curated, sequence-oriented protein database dedicated to in vivo phosphorylation sites that culls all the proteomic data into a searchable platform (http://www.phosphosite.org/). Using this database to search for posttranslational modifications detected on all human DDX proteins, we cataloged their location within either the N-terminal region, DEAD-box helicase conserved core region and C-terminal region (Table 1). While modifications were detected in all regions of the DDX proteins, the substantial majority occurred on their N-terminal and C-terminal regions (Fig. 1).

The human DEAD-box RNA helicases represent a large family of enzymes important for most, if not all, aspects of RNA function and regulation. Despite a similar and well-conserved catalytic core region, DDX proteins have remarkably different and specialized cellular, tissue and developmental functions. We posit that the divergent flanking N-terminal and C-terminal sequences serve as regions essential for proper DDX target recognition, localization and stability. The human DDX N-terminal and C-terminal sequences vary immensely in their sequence lengths and composition and generally contain no predicted structural motifs. The only notable exceptions are the C-terminal GUC domains in DDX21 and DDX50, as well as the C-terminal CHCZn-knuckle motif in DDX41. The GUC and CHCZn-knuckle domains are predicted to facilitate interactions with RNA. Since all DEAD-box proteins are already thought to bind and unwind RNA, these offer little functional insight. The data reviewed here suggests that, while all parts of a DDX protein are subject to posttranslational modification, the N-terminal and C-terminal portions are substantially preferred sites for such regulation through both posttranslational modifications and protein–protein interactions. Targeted analyses of these divergent regions using yeast two-hybrid, immunoprecipitation or affinity chromatography approaches will hasten our understanding of how these proteins function and how such functions are regulated.

References


