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DEAD-box Helicases: Posttranslational Regulation and Function

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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 5 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: Figure 1A). 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 (Figure 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.

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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.

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].

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 phosphorylated *in vitro* by cyclinB/cdc2 [17]. Both *in vitro* and *in vivo* binding analyses suggest the × 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 eIF4E through a conserved eIF4E consensus binding sequence in its N-terminal residues 38–44 and this interaction represses translation [19]. Biochemical and structural analysis show DDX3 also interacts with the vaccinia virus K7 protein through N-terminal amino acids 61–90 [20]. DDX3 C-terminal amino acids 553–622 directly and specifically interact with the hepatitis C core protein. The vaccinia virus K7 protein and hepatitis C virus core protein interactions evade the innate immunity response by preventing DDX3 from inducing IFN-β transcription [21].

DDX4 (Vasa)

DDX4 is the human Vasa ortholog, which is a highly conserved DEAD-box helicase involved in germline 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-dependant RNA helicase catalytic activity. Structural data also suggests that Vasa unwinds duplex RNA in a non-processive manner [2,3].

The mouse Vasa homolog is required for spermatogenesis and male fertility. Several studies indicate a functional relationship between Vasa and both the small interfering RNA and micro-RNA processing pathways. One essential component in both of these pathways is the RNase III endonuclease Dicer, which, in mice, colocalizes with Vasa in nuage [22]. Ectopically expressed Vasa and Dicer protein interact in COS cell lysate and this interaction requires the C-terminal portion of Vasa. The C-terminal RNaseIII region of Dicer is sufficient to interact with Vasa and the remaining N-terminal ATPase/helicase-PAZ domain region was unable to bind Vasa [22].

Vasa protein binds to MIWI and MILI, which are mouse PIWI 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 spermatogenesis indicative of cooperative molecular functions [23]. In MIWI knockout mice, Vasa protein does not localize to the nuage structures [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 indicating that Maelstrom is required for proper Vasa modification or processing.

Mouse Vasa also interacts with RanBPM The N-terminal portion of Vasa is sufficient for this interaction and both proteins colocalize 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 between 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.

DDX5 (p68), 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, differentiation and proliferation (Stevenson *et al*., 1998; Heinlein, 1998). Various types of posttranslational regulation, including covalent modifications and protein-protein interactions, appear to coordinate and control these activities. Colorectal tumors are associated with increased expression and polyubiquitylation 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 [27]. PIAS1-mediated SUMO modification of p68 on single site Lys53 modulates its transcriptional activity and promotes its interaction with HDAC1 [28]. Platelet-derived growth factor stimulates c-Abl kinase phosphorylation of p68 on Tyr593 in the nucleus. This phosphorylation is detected in seven different cancer cell lines. Phosphorylated p68 interacts with nuclear β-catenin which then promotes cell proliferation by activating transcription of cyclin D1 and c-Myc genes and also promotes an epithelial-mesenchymal transition [29,30]. Several studies suggest that the localization and function of p68 are regulated by interactions with various proteins. For instance, fibrillarin and p68 interact in HeLa cell extracts and colocalize in nascent nucleoli during 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 [31]. This interaction regulates their activities as transcriptional coactivators.

DDX6 (RCK/p54)

The DDX6-like p54 genes are implicated in several biological processes including translational regulation and function in various cytoplasmic bodies such as *C. elegans* P-bodies, *Drosophila* sponge bodies and *Xenopus* Balbiani bodies [32]. Human RCK/p54 interacts with AGO1 and AGO2 *in vitro* and *in vivo* and facilitates P-body formation [33]. 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 element-binding protein (CPEB) [34,35]. 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 [36]. It is still unclear, however, if similar posttranslational regulatory events occur in humans.

DDX20 (DP103)

Functional analyses of DP103 knock out mice show that it is required for embryonic development as well as ovarian development and function [37]. The DP103 C-terminus interacts with several proteins that target its function. DP103 represses transcription by interacting with SUMOylated steroidogenic factor 1 (SF1), but it is unknown whether DP103 itself is SUMOylated [38]. This interaction was mapped to amino acids 721 to 825 within the nonconserved 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 [38]. 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.

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 to 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 [39].

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 [40].

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 [41].

DDX39

DDX39 is a growth-associated RNA helicase involved in pre-mRNA processing and export. DDX39 is also upregulated in lung squamous cell carcinoma [42]. 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 [43]. 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 [43]. These data suggest that the posttranslational regulation controling proper levels of DDX39 protein are important for normal cell physiology.

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 [44]. 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 [45].

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 represses their transcriptional activity [46]. 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.

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 [47–57]. 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 catalogued 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 (Figure 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 GUCT domains in DDX21 and DDX50, as well as the C-terminal CCHC Zn-knuckle motif in DDX41. The GUCT and CCHC Zn-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 regulation, 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.

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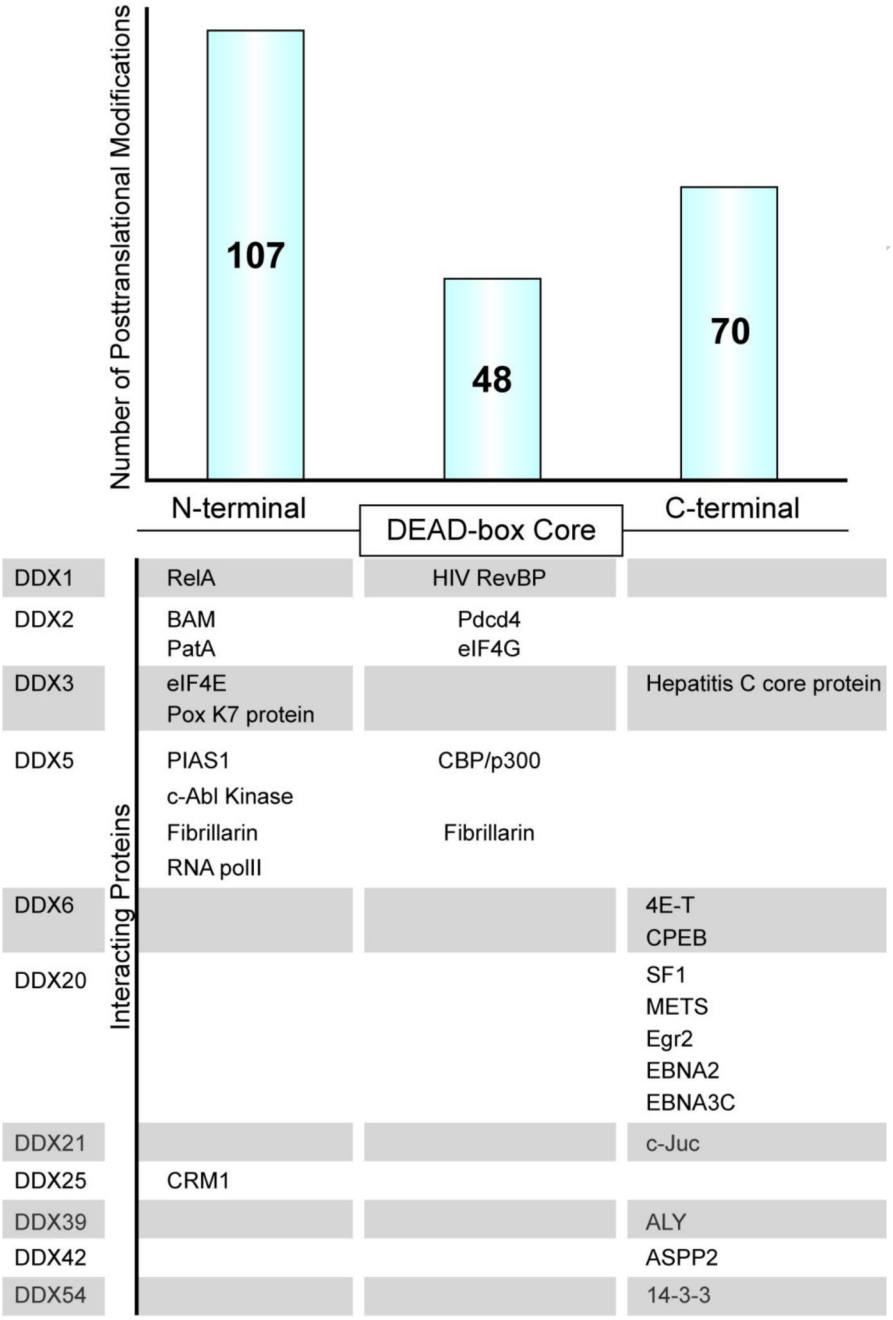

Figure 1. Human DEAD-box protein (DDX) posttranslational modifications and protein-protein interactions

A schematic representation of the DDX protein domain architecture features the conserved central DEAD-box core region along with the unique flanking N-terminal and C-terminal regions. Above shows the number of posttranslational documented in Table 1 with respect to their location within the DDX protein. Below lists known DDX-binding proteins, as well as where the interaction occurs on their respective DDX open reading frame.

Table 1

Posttranslational DDX modifications

Protein	N-terminal	DEAD-box helicase core	C-terminal
DDX1 (DBP-RB)	-	T80, T83	Y628
DDX2 (eIF4A1)	-	Y126, T158, Y197	-
DDX3 (PL10)	Y52, S60, S61, Y68, S63, S75, S77, S81 S91, Y103, S124, S130, Y162, K117 and K129	T203, Y242, Y282, Y300, T322, Y342, Y461 and Y465	S590, S593, S611 and S613
DDX4 (Vasa)	S200 and S202	-	-
DDX5 (p68)	K32 and K33	Y202, Y244, Y297	T438, S480, T507, Y518, S520, Y593 and Y595, R502
DDX6 (RCK/p54)	T36 and K73	Y312 and Y313	Y473
DDX10	T4, S7	T255	S539, T569, T577 and S831
DDX17 (p72)	Y77	Y281, Y323	T525, T574, Y584, S603, S605, S675, S676, S680, R688
DDX18 (MrDb)	S74, T130, S140	T225, S227, Y440 and Y442	-
DDX19B (DBP5)	S86, Y89 and S90	-	-
DDX20 (DP103)	S47 and S48	S187	S500, S532, T552, S649, S652, S654, S656, S672, S677, S678, T688, S703, T705, S714, Y756 and Y787
DDX21 (RHII/Gu)	S13, S65, S71 S89, S121, S164, S168, S171, S173	T296, T315	S567, S776
DDX23 (Prp28)	S14, S23, T25, S67, S107, S109	K642	-
DDX24	S82, S92, S93, S94, S166, S192	S287, S295, T302	-
DDX26 (DBI-1)	-	Y351	S804
DDX27	S54, S56, S79, S166, S171, T173, Y175, S176 and S177	-	S766
DDX28	S99	-	-
DDX29	S71, S192, S200	R647, Y811	-
DDX39 (URH49)	Y13, S37, Y38, S40	T171, K333	-
DDX41 (Abstrakt)	S21, S23, Y33	-	-
DDX42 (SF3b)	T10, K25, S96, T98, S104, S109, S11, Y160, Y183, S185, T193,	-	S744, S751, S754, T757, S758
DDX43 (HAGE)	-	Y449, S459, S460	T592
DDX46 (Prp5)	S40, S79, Y294, S295, S296, K271	-	K776, K779, S804
DDX47	-	-	S424, K448, K451
DDX48 (elF4A-III)	S12	T163, Y202	-
DDX50 (Gu-beta)	S82	-	S579, T582, S602
DDX51	S83, S103	Y304	-
DDX52 (ROK1-like)	Y35, S39, S99	-	-
DDX54 (DP97)	S34, S39, S41, S71, T74, S75	-	S644, S782, S788

Protein	N-terminal	DEAD-box helicase core	C-terminal
DDX55	-	S234, Y305	K517, K519, K523, S544, S594
DDX56	-	S126	-
DDX59	S64, S76 S160	S246	-

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 respectfully

K indicates acetylation on lysine residues

R indicates methylation on argentine residues