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SURVEY AND SUMMARY

DExD/H box RNA helicases: multifunctional proteins with important roles in transcriptional regulation

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ABSTRACT

The DExD/H box family of proteins includes a large number of proteins that play important roles in RNA metabolism. Members of this family have been shown to act as RNA helicases or unwindases, using the energy from ATP hydrolysis to unwind RNA structures or dissociate RNA–protein complexes in cellular processes that require modulation of RNA structures. However, it is clear that several members of this family are multifunctional and, in addition to acting as RNA helicases in processes such as pre-mRNA processing, play important roles in transcriptional regulation. In this review I shall concentrate on RNA helicase A (Dhx9), DP103 (Ddx20), p68 (Ddx5) and p72 (Ddx17), proteins for which there is a strong body of evidence showing that they play important roles in transcription, often as coactivators or corepressors through their interaction with key components of the transcriptional machinery, such as CREB-binding protein, p300, RNA polymerase II and histone deacetylases.

INTRODUCTION

The DExD/H box family of proteins is distinguished by the presence of several conserved motifs, which include the characteristic 'DExD/H' sequence (where x can be any amino acid) and are highly conserved in proteins from viruses and bacteria to humans (1–3). Based on homology with the well-characterized DNA helicases (4) and the observation that some prototypic members exhibit RNA helicase activity *in vitro*, it has been proposed that DExD/H proteins act principally as ATP-dependent RNA helicases. However, it is now thought that rather than being processive RNA helicases, several DExD/H box proteins may be acting as RNA 'chaperones', promoting the formation of optimal RNA structures through local RNA unwinding, or as RNPases by mediating RNA–protein association/dissociation (5–8).

These proteins are now of major interest because they are known to play important roles in virtually all aspects of RNA synthesis and function, including pre-mRNA processing, ribosome biogenesis, RNA turnover, RNA export and translation, processes that involve multi-step association/dissociation of large RNP complexes as well as the modulation of complex RNA structures. Historically, most DExD/H box proteins have been associated with such cellular processes; indeed many members of this family were initially identified through investigations of mutants that led to specific defects in processes such as RNA processing, ribosome biogenesis and translation, and it is clear that the RNA unwinding activity of these proteins is essential for their function in such processes (9). However, there is now an emerging body of evidence indicating that several members of the DExD/H family of proteins have multiple functions in the cell, some of which may not require their RNA helicase activity. Although DExD/H box proteins show considerable sequence and structural homology within their conserved 'helicase' core, their flanking N- and C-terminal domains are highly divergent and are thought to provide specificity of function through interaction with specific RNA substrates or other interacting factors. A schematic figure showing the alignment of the conserved cores of the DExD/H proteins considered in this review and highlighting their different N- and C-terminal extensions is included (Figure 1). These N- and C-terminal domains through interaction with different partners would also provide the capability for their involvement in multiple processes. Of particular interest are the reports showing that some of these proteins, through interaction with components of the transcriptional machinery, play important roles in transcriptional regulation.

In this article I shall review current evidence demonstrating that some members of the DExD/H family have, in addition to their established roles as RNA helicases or unwindases, clear roles as transcriptional regulators which, in several cases, appear to be independent of their RNA helicase activity. For this purpose, I shall concentrate predominantly on those proteins for which most data are available in the literature: these include a member of the DExH subclass RNA helicase A (also known as Dhx9 and nuclear DNA helicase

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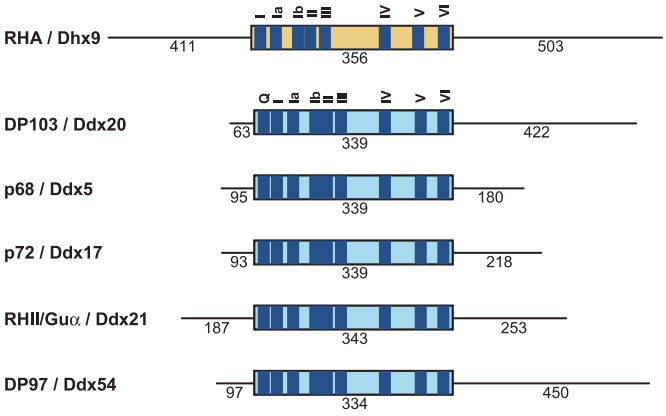

Figure 1. A schematic alignment of the human homologues of the DExD/H box proteins considered in this review, highlighting the different lengths of the divergent N- and C-terminal domains. The conserved helicase cores [in yellow for DExH and light blue for DEAD (DEVD in the case of Ddx21)] are shown, with the conserved motifs within them numbered (I–VI) and highlighted in dark blue. The number of amino acids in the helicase cores and the N- and C-terminal domains are indicated for each protein. The functions of the conserved motifs are as follows: Motifs Q, I, II and VI are required for ATP binding and hydrolysis; motifs Ia, Ib, III, IV and V are thought to be involved in RNA binding. These are described more fully in the contribution by Linder (2,3). The accession numbers for these proteins are Dhx9- Q08211, Ddx20- Q9UH16, Ddx5- P17844, Ddx17- Q92841, Ddx21- Q9NR30 and Ddx54- Q8TDD1.

II), as well as the DEAD subclass proteins DP103 (also known as Ddx20 and Gemin3) and the highly related p68/p72 proteins (Ddx5/Ddx17). The functions of these proteins are summarized in Table 1. I shall also briefly review evidence that highlights roles for DExD/H box proteins in regulating transcription of genes important in cell proliferation, cell cycle arrest or apoptosis. Finally, I shall summarize some common perspectives and explore current ideas about possible mechanisms by which DExD/H box proteins may be functioning in transcription, through acting as adaptor or bridging factors to recruit transcription factors or to stabilize transcriptional initiation complexes, as well as the roles they may play in linking or coupling the different processes in which they function.

RNA HELICASE A (Dhx9)

The first suggestion that a member of the DExD/H family was involved in the regulation of transcription came from the finding of Kuroda *et al*. (10) that the *Drosophila maleless* DEIH protein Mle is associated with hundreds of discrete sites along the X chromosome in males but not in females. They suggested that Mle plays a role in X chromosome dosage compensation by coordinately regulating expression of genes located on the X chromosome so that X-linked gene expression in males (XY) is equivalent to that in females (XX). Interestingly the association of Mle with the X chromosome appears to be RNA-dependent (11). Subsequently, two mammalian proteins with known helicase activity were shown to be homologues for Mle; these included an abundant nuclear protein from HeLa cells, called RNA helicase A (RHA) (12), which had been shown previously to unwind double-stranded RNA (13), and the bovine nuclear DNA helicase II (NDHII) (14), which also had been shown to have RNA and DNA helicase activities (15). Similar activities were then attributed to Mle (16).

Concrete evidence that RHA (Dhx9) played an active role in transcription came from the findings of Nakajima *et al*. (17), who showed that RHA acts as a bridging factor between the CREB-binding protein (CBP) and RNA polymerase II (Pol II), thus cooperating with CBP to activate transcription. The N-terminal domain of RHA, outside the conserved helicase core (Figure 1), was shown to interact with CBP while a region overlapping the N-terminal domain and the helicase domain was shown to interact with Pol II. Interestingly, RNA helicase-defective mutants of RHA showed a decreased ability to coactivate CBP-dependent transcription raising the possibility that, apart from being a bridging factor, RHA may enhance engagement of the transcriptional machinery at responsive promoters by inducing changes in chromatin structure through local DNA unwinding. This idea is consistent with the finding that RHA interacts with double-stranded DNA and with topoisomerase IIα (18). In a subsequent study, Aratani *et al*. (19) identified a minimal transactivation domain in RHA that is N-terminal to the conserved helicase core, and is the domain that actually interacts with Pol II. Through the use of a series of mutations in this activation domain and in the conserved ATP-binding motif they showed that Pol II recruitment was independent of ATPase activity. However, mutants in both the transactivation domain and the ATP-binding motif exhibited reduced CREB-dependent transcriptional activity when co-transfected with plasmids encoding CBP and a CRE-luciferase reporter (19). These observations and the findings of Nakajima *et al*. (17) led to the suggestion that RHA can independently regulate CREB-dependent transcription either through ATP-dependent mechanisms or through the recruitment of RNA Pol II.

RHA has been found to fulfill a similar bridging role between the breast cancer-specific tumour suppressor BRCA1 and the Pol II holoenzyme, interacting with the transcriptionally active C-terminal domain BRCA1 (20–22). Although BRCA1 is not itself a sequence-specific DNA-binding transcription factor, it has been shown to be involved in the transcriptional regulation of several genes involved in DNA repair, cell cycle and apoptosis (23–25), presumably as a coregulator of transcription through its interactions with factors in the transcriptional machinery. Interestingly, the interacting region for BRCA1 on RHA is separate from that for CBP implying that RHA also may interact specifically with other factors involved in transcription from specific promoters in response to particular signals. RHA was also found to interact with the p65 subunit of NF-κB and to enhance NF-κB-dependent transcription (26), while the *Drosophila* homologue was shown to associate with, and stimulate the transcriptional activity of, a region upstream of the *roX2* gene, which is critical for dosage compensation (27). Additionally, RHA has been shown to bind preferentially to the promoter sequence of the $p16^{INK4a}$ tumour suppressor gene in cells with transcriptionally active $p16^{INK4a}$ (28) and to be recruited to the promoter of the multidrug resistance gene (*MDR1*), upregulating transcription of the *MDR1* gene (29). Thus RHA may possess a more general transcriptional activity through its association with CBP/RNA Pol II and also a more specific activity through its interaction with specific transcription factors and/or recruitment to specific promoters.

Table 1. Summary of the commonly used names and reported functions for RHA, DP103, p68 and p72

Ddx/Dhx nomenclature	Other names	Functions in transcription	Other functions
Dhx9	RNA Helicase A (RHA) Nuclear DNA Helicase II (NDHII) *Drosophila* maleless (MLE)	Transcriptional coactivator-acting as bridging factor between transcription factors/co-factors (17,20) Binding to promoters (29)	snRNP assembly (34) Expression/nuclear export of retroviral RNAs (30–32) Translation (33) RNA editing (35)
Ddx20	DP103 Gemin3	Transcriptional repressor/corepressor-interacts with repressors (40,41,44,45)	snRNP assembly (38)
Ddx5	p68 Dbp2 (yeast homologue for p68/p72) RM62/Dmp68 (*Drosophila* homologue)	Transcriptional coactivator/repressor; interacts with transcription factors/cofactors (64–67,69–71) Transcriptional deactivation (72)	Pre-mRNA and pre-rRNA processing (56,58) Alternative splicing (59) mRNA decay (58) RNAi (63)
Ddx17	p72 (Dbp2-yeast homologue)	Transcriptional coactivator/repressor (65,71)	Pre-mRNA processing (57) Alternative splicing (60,61)

The findings discussed above highlight a very clear role for RHA in transcriptional regulation. However, there is also a strong body of evidence showing that RHA is involved in the expression/nuclear export of retroviral RNAs and in pre-mRNA processing. RHA was found to interact both with the constitutive transport element (CTE) of simian type D retrovirus, being important for CTE-mediated nuclear export (30,31), and with the Rev response element (RRE) of HIV, stimulating both CTE- and Rev-dependent gene expression (32). A recent report has also indicated a role for RHA in translation of selected mRNAs through interaction with their 5′-untranslated region (33). Additionally, RHA has been found to be associated with the survival motor neuron protein (SMN), in Gems (nuclear bodies that are often associated with Cajal bodies) and in cytoplasmic spliceosomal small nuclear ribonucleoprotein complexes (snRNPs) suggesting a possible involvement for RHA in the assembly of spliceosomes (34). Interestingly, studies on the *Drosophila* homologue, Mle, showed that a specific mutation (*mle*^{napts}) is not involved in dosage compensation but, instead, appears to result in aberrant splicing/exon skipping in a region of the *para* Na^+ channel that is involved in RNA editing; these findings suggest that it may be required to resolve RNA secondary structures in this region (35).

Thus RHA and its *Drosophila* homologue Mle function in several different cellular processes, including transcription, RNA processing and export, through their interaction with different components of the machineries involved in these processes, via their unique N- and C-terminal domains which lie outside the conserved helicase core [reviewed in (36)]. A diagram, highlighting the proteins that RHA/Mle interact with and the processes they are involved in, is shown in Figure 2A.

DP103 (Ddx20)

DP103 (Ddx20) was originally isolated as a DEAD box protein that associated with the Epstein–Barr virus nuclear proteins EBNA2 and EBNA3C, which play a role in the regulation of transcription of both latent viral and cellular genes (37). This protein was also independently isolated as Gemin3, a protein that interacts with SMN and is a component of the SMN complex in nuclear Gems (38), suggesting a role for Gemin3 in snRNP assembly. As with RHA the unique C-terminus of Gemin3 was required for interaction with SMN. A similar interaction with SMN was later confirmed for the murine homologue of DP103 (39). Thus, at an early stage, DP103 was suggested to be involved in both transcriptional regulation and RNA processing; this is highlighted in Figure 2B, which also indicates the proteins that have been shown to interact with DP103.

In recent years, however, most of the reports on DP103 have concentrated on its role in transcription and have provided more direct evidence for its role as a transcriptional regulator. The murine DP103 homologue was identified in a yeast two-hybrid screen as a protein that interacted with the proximal transcriptional repression domain within the nuclear steroidogenic factor SF-1, an orphan nuclear receptor that regulates expression of genes essential for reproductive and endocrine development (40). The interaction with SF-1 again was shown to occur through the C-terminal domain of DP103, outside the conserved helicase core (Figure 1). Moreover this domain was found to possess an autonomous intrinsic transcriptional repression activity and to repress the activation function of SF-1 (41). However, not all physiological target promoters of SF-1 tested were repressed, suggesting a measure of promoter specificity in repression by DP103 (41). Interestingly it has been suggested recently that DP103 mediates repression of SF-1 transcriptional activity by promoting sumoylation of SF-1 via the PIAS E3 SUMO ligases, which in turn results in subnuclear relocalization of SF-1 to discrete nuclear foci (42).

The C-terminal domain of DP103 was also isolated as an interacting protein for two other transcription factors from yeast two-hybrid screens. Firstly, it was found to interact with the N-terminal repression domain of the mitogenic Ets repressor METS [a member of the ETS-domain transcription factor family (43)], which is induced during macrophage differentiation and represses transcription of Ets target genes involved in Ras-dependent proliferation (44). The C-terminal domain of DP103 was again demonstrated to have intrinsic transcriptional repression activity and to be required for the METS-dependent block in cell proliferation. Moreover

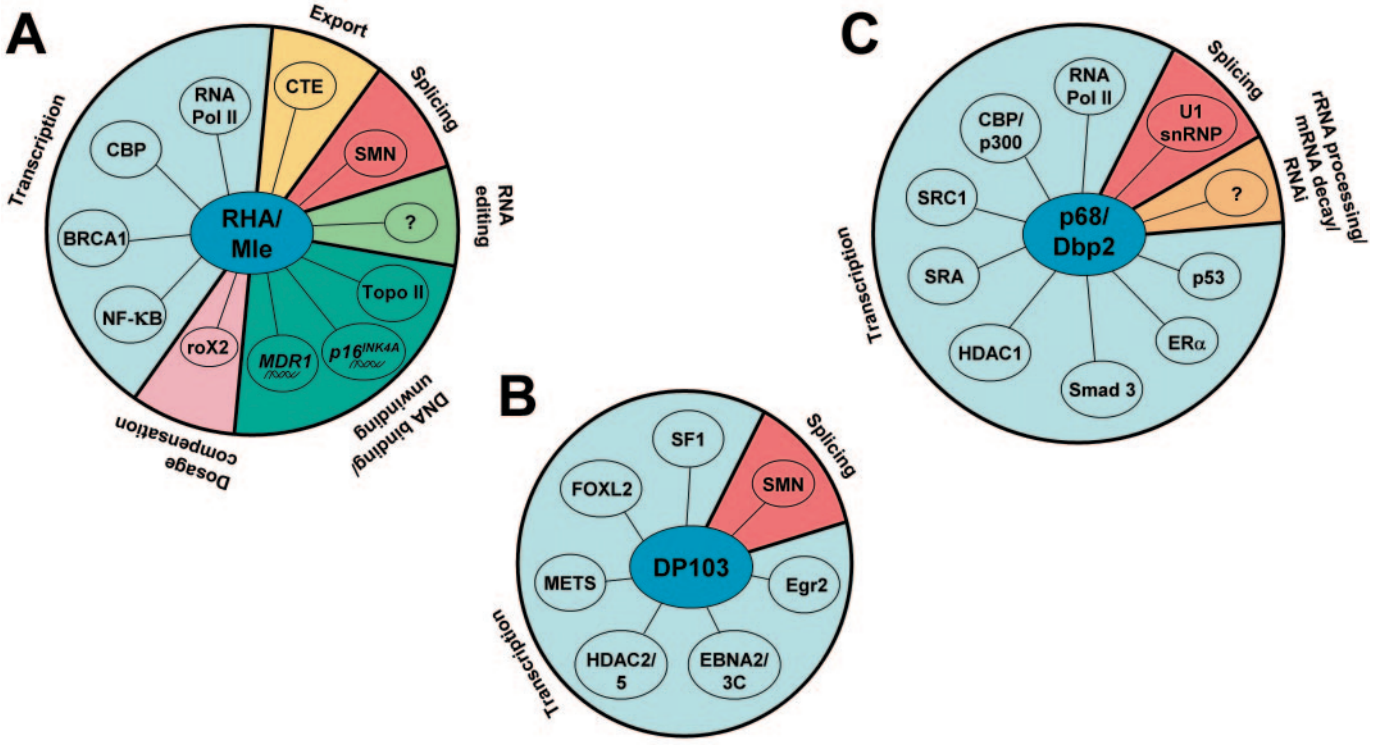

Figure 2. Proteins interacting with (**A**) RHA/Mle, (**B**) DP103 and (**C**) p68/Dbp2, and the cellular processes in which they are involved, as examples to illustrate the multifunctional properties of the DExD/H box proteins discussed in this review. RHA has also been shown to interact with the promoters of the *MDR1* and $p16^{INK4A}$ genes; therefore these are also indicated.

DP103 was shown to co-immunoprecipitate with the histone deacetylases HDAC2 and HDAC5 implying that DP103 may repress transcription through the recruitment of HDACs (44). Secondly, DP103 was found to interact with the early growth response 2 (Egr2/Krox-20) transcription factor, which is important for myelination of the peripheral nervous system and for establishing segmentation in the developing vertebrate hindbrain (45). Again DP103 was shown to repress Egr2-mediated transcriptional activation; however, this was not observed for all Egr2-responsive promoters, suggesting that this repression activity depended on the promoter context. Since DP103 had been shown to interact with HDAC2 and HDAC5, Gillian and Svaren (45) tested the effect of the HDAC inhibitor Trichostatin A (TSA) on the ability of DP103 to repress transcription. Using a range of reporter constructs they showed that, while TSA alleviated the transcriptional repression of promoters of the Egr2 target genes *Eph4A* and *FGF2* when DP103 was co-transfected with Egr2, it had no effect on the constitutively active GAL4-thymidine kinase and GAL4-Adenovirus major late promoters when DP103 was tethered to a GAL4 DNA-binding domain. Thus DP103 repression of Egr2-mediated transcriptional activation exhibits promoter specificity and is at least in part dependent on HDAC recruitment. On the other hand the intrinsic transcriptional repression function of DP103 appears to be HDAC-independent, suggesting that there is more than one mechanism for transcriptional repression by DP103.

The idea that DP103 may, at least partially, repress transcription through HDAC recruitment is particularly exciting, especially since DP103 was shown to repress SF-1 activity through sumoylation [see above (42)]. Sumoylation often correlates with transcriptional repression, which, at least in some cases, is mediated through HDAC recruitment (46).

p68 (Ddx5) and p72 (Ddx17)

p68 (Ddx5) is one of the prototypic members of the DEAD box family of proteins (47) and was one of the first proteins to be shown to exhibit RNA helicase activity *in vitro* (48). p72 (Ddx17) was fortuitously isolated from an antibody screen of a HeLa expression library and was found to have remarkable similarity to p68, sharing 90% amino acid sequence identity over the conserved core (49), although the N- and C-termini are significantly different. p72 and its alternative translation initiation product p82 have themselves been shown to have RNA helicase activity *in vitro* (50,51) and p68 and p72 were subsequently shown to exist as heterodimers in cells (52). However the function of these proteins remained elusive, although the expression of p68 was shown to be growth and developmentally regulated and to correlate with organ maturation/differentiation (53), p72 appeared to be down-regulated during development (54). The finding that p68 was one of the proteins co-purifying with spliceosomes in a mass spectrometric analysis of spliceosomal complexes (55) suggested a role for this protein in RNA splicing. This was subsequently confirmed when it was shown that p68 is an essential splicing protein which acts at the U1 snRNA-5′ splice site duplex (56). Interestingly p72 was also found to co-purify with U1 snRNP (57). Moreover the yeast p68/p72 homologue Dbp2 was shown to be

required for rRNA processing (58) and the human p68 and p72 proteins have been shown to be important for alternative splicing of the c-H-*ras* and CD44 genes, respectively (59–61). Roles for DEAD (and DExH) box proteins in pre-mRNA and pre-rRNA processing have been well documented (2,62). Interestingly, a study investigating proteins associating with the *Drosophila* fragile X protein, FMR1, has shown that the *Drosophila* p68 homologue (Dmp68/RM62) co-purifies with FMR1 and Argonaute 2 (a component of the RNA-induced silencing complex) and is required for RNAi (63), suggesting another potential function for p68.

Perhaps more surprising was the finding that p68 interacts with and acts as a transcriptional coactivator for the nuclear receptor estrogen receptor alpha (ERα) (64). This activity was subsequently found to be shared by p72 (65). Activation of gene expression by ERα is mediated by two autonomous transcription activation domains, AF1 and AF2. AF1 functions in a ligand-independent manner while the activity of AF2 is regulated by estrogen binding, which results in a conformational change in this domain and thus facilitates coregulator recruitment. Since p68/p72 stimulates the estrogen-induced activity of ERα and also interacts with steroid receptor coactivator, SRC1, and the RNA coactivator, SRA, it was suggested that p68 might coactivate ERα by mediating interactions between ERα, AF1 and the AF2 coactivator complex by direct binding to these coactivators (65). This implies that p68 and p72, like RNA helicase A (17,20), may act as a bridge between transcription factors/coactivators, an idea that has been reinforced by other recent reports. First, p68 was shown to interact with the transcriptional coactivators CBP/p300 and RNA Pol II, stimulating transcriptional activation mediated by CBP/p300 (66). Second, it was found to interact with Smad3 [a member of the Smad family of transcriptional activators that act as intracellular effectors of TGF-β (67)] and to form part of a ternary complex with Smad3 and CBP (68). Moreover, p68 was shown to be recruited to the promoter of the natural ERα target gene, pS2, in the presence of estrogen (69), suggesting an involvement for p68 in transcriptional initiation.

A recent report has demonstrated that p68 acts as a potent coactivator for the tumour suppressor p53, a latent transcription factor that is induced and activated in response to stresses, such as DNA damage, and induces transcription of genes involved in cell cycle arrest and apoptosis. Co-expression of p68 potently stimulated transcription from p53-responsive promoters. Furthermore RNAi suppression of p68 inhibited the expression of p53 target genes in response to DNA damage and p53-dependent apoptosis, suggesting a requirement for p68 in the p53 response (70). This activity is specific for p68 since RNAi suppression of p72 had no effect on the induction of p53 transcriptional activity by DNA damage. p68 was also found to be recruited to p53-responsive promoters in response to DNA damage (70) echoing the finding for p68/ERα in response to estrogen (69) and suggesting that p68 may play a role in transcriptional initiation, perhaps through facilitating the recruitment of other transcription factors or coactivators through its interaction with factors such as CBP, p300, SRC1 and RNA Pol II (64–66). In addition to their role as transcriptional coactivators, p68 and p72 have been shown to have other functions as transcriptional repressors: both proteins have been shown to interact with HDAC1 and to exhibit intrinsic promoter-specific transcriptional repressor activity when tethered to DNA using the GAL4 system (71). Interestingly, p68 and p72 showed subtle differences in their ability to repress transcription whereas p72 exhibited both HDAC-dependent and HDAC-independent transcriptional repression activity. This is reminiscent of the findings for DP103 (45). Another recent study, this time with the *Drosophila* homologue, Dmp68, has suggested a novel role for p68 in transcript clearance from transcription sites and in transcriptional deactivation (72).

An interesting point from the studies of p68/p72 as transcriptional coactivators/repressors was the finding that, in most cases, RNA helicase activity was not required for their function in transcriptional regulation. For ERα, although the interacting region between p68/p72 and ERα included part of the conserved helicase core and RNA binding was important for ERα coactivation, p68 helicase activity *per se* was not required (64,65). It is not yet clear whether the same applies for p72. In the case of p53, a helicase inactive mutant of p68 was able to stimulate p53 transcriptional activity as efficiently as the wild-type protein (70). Similarly, RNA helicase activity did not appear to be required for p68 and p72 to function as transcriptional repressors (71). These findings thus suggest that the activity of p68/72 in transcriptional regulation is distinct from their activity in RNA processing and supports the idea that they are truly multifunctional proteins with roles in several cellular processes. This is indicated in Figure 2C for p68 and its yeast homologue Dbp2, which was shown to be important in rRNA processing and nonsense mediated mRNA decay (58).

RHII/Guα (Ddx21) and DP97 (Ddx54)

Other members of the DExD/H family shown to act as transcriptional regulators include the DEVD subfamily protein RHII/Guα (Ddx21) and the DEAD subfamily protein DP97 (Ddx54).

RHII/Guα was first isolated as a nucleolar protein recognized by an autoantibody from a patient with watermelon stomach disease (73). Little was known about its function until it was identified as an interacting partner for c-Jun from a Tandem affinity purification/mass spectrometric analysis (74). This study went on to show that the C-terminal domain of RHII/Guα interacts with the N-terminal transactivation domain of c-Jun, a transcription factor which plays a key role in the response of cells to stress, apoptotic and differentiation signals. The interaction between RHII/Guα and c-Jun was enhanced by anisomycin treatment, which activates the c-Jun signalling pathway and results in a relocalization of RHII/Guα from the nucleolus to the nucleoplasm. Moreover, while overexpression of wild-type RHII/Guα had no effect on c-Jun-dependent transcription, overexpression of ATPase/helicase defective mutants or microinjection of anti-RHII/Guα antibody inhibited c-Jun transcription activity *in vitro*, suggesting that RHII/Guα is important for c-Jun transcription activation and that RHII/Guα helicase activity is involved in this function. Subsequently, RHII/Guα was found to be required for the processing of 20S rRNA to 18S, indicating an additional role for this helicase in rRNA processing (75–77).

DP97 was identified as a protein that interacts with the AF2 domain of ERα and of other nuclear receptors (78). Again the interaction was shown to occur through the C-terminal domain of DP97 but, in this case, was found to result in transcriptional repression of ERα-regulated genes.

A role for DExD/H proteins in cell growth/cell cycle regulation and cancer

For several years, reports demonstrating overexpression of some DExD/H box proteins in tumour cell lines and tumour tissues (79–83) have implied possible roles for these proteins in cancer development, although it has not been clear whether their association with cancer is causal or consequential. Therefore, the demonstration that several DExD/H proteins interact with proteins that are known to be involved in the transcriptional regulation of genes involved in DNA repair and cell growth/cell cycle control have highlighted potentially important roles for DExD/H proteins in the development of cancer. Some examples are discussed below.

RHA is the most extensively documented DExD/H protein in terms of possible links with tumour development. Through its interaction with BRCA1, RHA may play an important role in the function of BRCA1 in the transcriptional regulation of genes involved in DNA repair/genome maintenance, cell cycle regulation and apoptosis (23–25). Moreover, the finding that overexpression of a fragment of RHA that is known to bind to BRCA1 gives a dominant negative mutant and causes defects in ploidy and cytokinesis in mammary epithelial cells suggests a possible co-suppressor function for RHA (84). Thus RHA could be important in breast cancer development. Several other reports have also suggested roles for RHA in cell cycle or growth regulation and cancer through its function in transcription. Examples include (i) the finding that RHA is part of the MEF1 transcription factor complex which is recruited to the MDR1 promoter and enhances transcription of this gene in cancer cells, implying an involvement of RHA in the development of resistance to chemotherapeutic drugs (29), and (ii) the observation that RHA interacts with the p65 subunit of NF-κB and is important in mediating NF-κB transcriptional activity (26), suggesting that RHA could play a role in the known functions of NF-kB as a promoter of cell growth and survival or, as shown in some contexts, tumour suppression (85). Other possible connections for RHA with cancer development are suggested by the findings that RHA (i) stimulates the exonuclease activity of the Werner's syndrome protein, which is important in DNA repair and genome maintenance (86,87); (ii) preferentially interacts with the promoter of the $p16^{INK4a}$ tumour suppressor gene in cells in which this gene is transcriptionally active, suggesting another role for RHA in cell cycle control (28,88); and (iii) is overexpressed in human lung cancers (89) and maps to the prostate cancer susceptibility locus on chromosome 1q25 (90).

Several lines of evidence have suggested that p68 may also play an important role in cancer development. The finding that p68 coactivated ERα function (64) not only demonstrated for the first time a role for p68 in transcriptional regulation but also highlighted a potential link with tumour development. This was reinforced by reports showing that p68 is overexpressed and abnormally modified in colorectal tumours (91), and that there are changes in p68 phosphorylation associated with transformation and abnormal cell proliferation (92). Moreover, the finding that p68 stimulates the transcriptional activity of the p53 tumour suppressor and that RNAi depletion of p68 inhibits the induction of p53 target genes in response to DNA damage and p53-dependent apoptosis (70) suggests that p68 may act as a tumour co-suppressor for p53. Taken together these different lines of evidence suggest that p68 may be important in cancer development.

Other examples of DExD/H proteins having possible implications for cell growth regulation, as a result of their function in transcriptional regulation, include DP103 and RHII/Guα. DP103, through its interaction with the Ets repressor METS, is important in blocking Ras-dependent cell proliferation (44). Moreover, it has been suggested that the interaction of DP103 with the Epstein–Barr virus proteins EBNA2 and EBNA3C (37) may interfere with the METS/DP103 interaction and may contribute to transformation by this virus (44). DP103 has also been shown to interact with the forkhead transcription factor FOXL2 and to modulate its ability to induce apoptosis (93). Similarly RHII/Guα, through its coactivation of c-Jun transcriptional activity and promotion of c-Jun-mediated neuronal differentiation (74), could play a role in c-Jun mediated cell cycle regulation or apoptosis (94,95).

CONCLUSION/PERSPECTIVES

DExD/H proteins as nucleation/bridging factors in transcription complexes

DExD/H proteins have long been known to function as part of large RNP complexes in processes such as pre-mRNA processing, translation initiation and ribosome assembly (2). Many of the studies that have demonstrated roles for these proteins in transcription have shown that they function through interaction with transcriptional coactivators/corepressors. These findings have led to the suggestion that DExD/H proteins may regulate transcription either by acting as nucleating factors to recruit transcription factors and other coregulators to the transcriptional initiation complex or by stabilizing the initiation complex through interactions with multiple factors in the complex (Figure 2). Importantly, as discussed above for RHA (17,20), DP103 (44) and p68/p72 (65), these studies demonstrate, or at the very least suggest, that they function through acting as adaptors or bridging factors between coactivators/corepressors and factors in the transcriptional machinery. The idea that DExD/H proteins are acting as bridging factors to regulate transcription is particularly interesting when one considers that, in many cases, the interacting regions on the DExD/H proteins lie outside the conserved core, and could account for the high diversity and specificity of interactions exhibited by these proteins. Such diversity/specificity could be very important in the cell's response to different signals or stresses and the consequent responses. A model suggesting how RHA or p68/p72, by acting as adaptors between different components of the transcription machinery, could assist in recruitment of other factors or stabilization of the transcription initiation complex at promoters is shown in Figure 3.

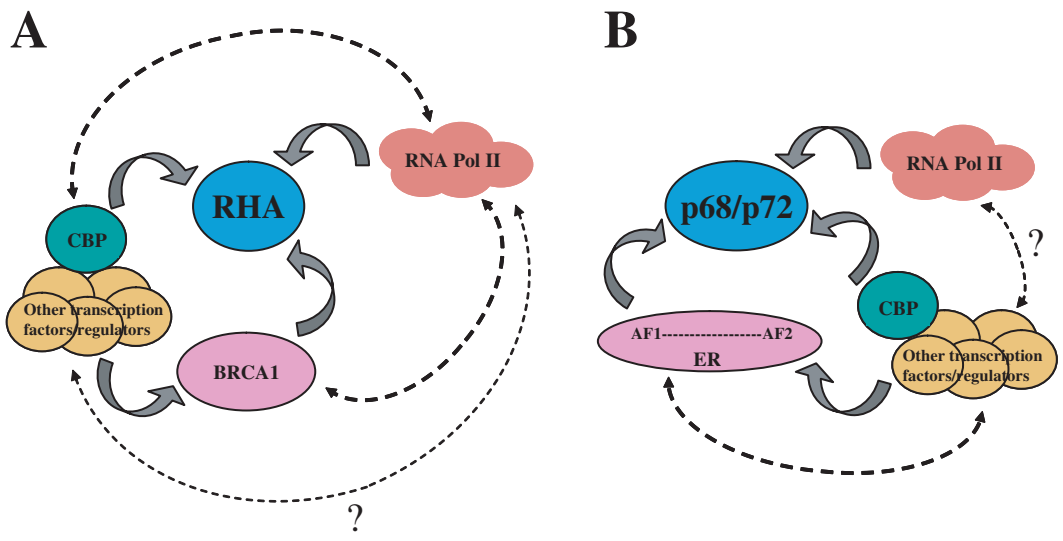

Figure 3. Models for mechanisms by which DExD/H box proteins, such as RHA and p68/p72, could recruit components of the transcription machinery to promoters or stabilize transcription initiation by acting as adaptor molecules or bridging factors, as suggested by the literature. Solid arrows indicate interactions documented in the literature; heavy dashed arrows are bridging roles suggested from the known interactions; faint dashed arrows with query marks are implied recruiting or bridging roles. (**A**) RHA through its interaction with CBP, RNA Pol II and BRCA1 has been suggested to act as a bridge between CBP and RNA Pol II and between BRCA1 and RNA Pol II. Since BRCA1 also interacts with other transcriptional regulators its interaction with RHA could facilitate in recruitment of RNA Pol II and other factors to the initiation complex or stabilize the initiation complex. (**B**) p68/p72 is known to interact with the AF1 domain of ERα and p68 has been shown to interact with CBP and RNA Pol II, leading to the suggestion that p68/p72 may act as bridges between the ERα AF1 domain and transcription factors interacting with the AF2 domain of ERα. p68/p72 could thus also enhance transcriptional activity by recruiting RNA Pol II to the initiation complex or by stabilizing the complex.

DExD/H RNA helicases as multifunctional coupling factors

The function of DExD/H box proteins in transcriptional regulation is likely to be dependent on interacting transcription factors, the signals they respond to, the promoter context and the cellular environment. Importantly, for most of the proteins discussed above, the regions interacting with transcription factors and involved in transcriptional regulation lie in the N- or C-terminal domains outside the conserved 'helicase' core. Moreover, in some cases at least, ATPase/RNA helicase activity does not appear to be required for their function in transcription. This strongly suggests that their role in transcription is distinct from their role in other processes, where they may act as bona fide helicases or unwindases, and indicates that these proteins have multiple functions within the cell, some of which may be biochemically independent of their role as bona fide helicases/unwindases. For many years it has been suggested that, while the conserved helicase core acts as a 'motor', the unique N- and C-terminal domains impart specificity to the interaction of these proteins with partner proteins, allowing them to unwind specific substrates. However, the finding that some DExD/H proteins have additional, possibly helicase-independent, functions encoded by these N- and C-terminal extensions strongly suggests that they are truly multifunctional.

Another very interesting idea that emerges from these findings is that DExD/H proteins may be acting to 'couple' processes such as transcription and RNA processing. In fact such a suggestion was made by Auboeuf *et al.* (96) recently for some coregulators of nuclear receptors, and by Bates *et al.* for p68 (70). Since several of these proteins have been shown to interact with factors from the transcriptional and RNA processing machineries, one of their functions may be to facilitate the recruitment of such factors. This is particularly relevant since it is clear that mRNA processing events (cap formation, splicing and 3′ end formation/polyadenylation) occur co-transcriptionally [reviewed in (97,98)]. Therefore, the idea that RNA helicases, which might be required to unwind RNA in later stages of gene expression (e.g. splicing), are also functioning as part of the transcription machinery (perhaps in an RNA helicase-independent manner), to facilitate mature mRNA production, is very exciting. It is tempting to speculate that some DExD/H proteins might perform such 'coupling' functions partly through their action as adaptor molecules. After transcriptional initiation, they may also interact with the nascent RNA molecule and with splicing factors and, possibly through conformational changes, may be then released from the transcription machinery to function in RNA processing. For example, as discussed above, RHA has been shown to associate both with components of the transcriptional machinery and with the SMN complex in snRNPs (17,34), although it is unclear whether the 'pools' of RHA molecules associating with transcription and spliceosome complexes are independent, or whether there is a transition from transcription- to spliceosome-associated RHA. A particularly interesting example of how a DExD/H protein might act to couple transcription and pre-mRNA processing lies with p68. This protein has been shown to be associated both with the transcriptional machinery (64,66) and with spliceosomes (55), and to specifically interact with the U1 snRNA at the 5′ splice site (56,99); moreover, it has been demonstrated to function in transcriptional initiation and

pre-mRNA splicing (56,70). Interestingly, U1 snRNA has been found to associate with the general transcription factor TFIIH and to regulate transcriptional initiation (100); this association has been suggested to provide a potential link between transcriptional initiation and mRNA processing (101). Furthermore, it has been known for a long time that the positioning of the first intron and its 5′ splice site have a profound effect on transcription efficiency in transgenic mouse models (102,103). More recent studies, using a retroviral minigene model, demonstrated that the 5′ splice site of the first intron and its recognition by the U1 snRNA are important for achieving efficient transcriptional activation (104), while U1 snRNP was shown to co-immunoprecipitate with RNA Pol II (105). It is therefore tempting to speculate that p68, by virtue of its interactions with components of the transcriptional machinery (64,66) and with U1 snRNA at the 5′ splice site (99), could act as a coupling factor between transcription and pre-mRNA processing. Additionally, the recently suggested functions for the p68 *Drosophila* homologue in transcript clearance and export (72) could imply a further function for p68 in ensuring that the synthesized mRNA is efficiently exported away from promoter sites. Although this study examined the role of p68 in transcript clearance for gene deactivation, it is also possible that p68 may play such a role to ensure efficient promoter clearance to increase efficiency of transcription. Moreover such functions, as is the case for the known role for p68 in splicing (56), may require a helicase-active protein, in contrast to its role in transcriptional initiation (64,70).

The picture now emerging on the function of many DExD/H proteins is considerably more complicated than their acting simply as motors to unwind RNA or displace proteins from RNA. The fact that several members of this family appear to be multifunctional and to have, in addition to their RNA unwinding functions, other helicase-independent functions suggests that the biological roles of these proteins may be more complex than previously imagined. Additionally, the idea that these proteins may act as adaptor molecules to facilitate the formation, or improve the stability, of specific protein complexes involved in transcription regulation would allow them to have different functions in the cell, depending on other factors present or the signals to which the cell responds. This would be consistent with several of these proteins being involved in cell growth and cell cycle regulation. Moreover, the ability of DExD/H proteins to act as adaptor molecules would also provide a means for them to act as coupling factors between different processes in the cell; this would impart a clear advantage in that it would help to ensure both efficient transcriptional activation and correct processing to yield mature mRNAs. Studies to examine the roles of DExD/H box proteins in the formation and modulation of transcription and splicing complexes should help to elucidate possible mechanisms by which these proteins could couple these different processes. Clearly there is much work to be done and the next few years in this area of DExD/H protein research should be very exciting.

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