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Detection of dsRNA-binding domains in RNA helicase A and *Drosophila* maleless: implications for monomeric RNA helicases

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ABSTRACT

Searches with dsRNA-binding domain profiles detected two copies of the domain in each of RNA helicase A, *Drosophila* maleless and *C.elegans* ORF T20G5_11 (of unknown function). RNA helicase A is unusual in being one of the few characterised DEAD/DExH helicases that are active as monomers. Other monomeric DEAD/DExH RNA helicases (p68, NPH-II) have domains that match another RNA-binding motif, the RGG repeat. The DEAD/DExH domain appears to be insufficient on its own to promote helicase activity and additional RNA-binding capacity must be supplied either as domains adjacent to the DEAD/DExH-box or by bound partners as in the eIF-4AB dimer. The presence or absence of extra RNA-binding domains should allow classification of DEAD/DExH proteins as monomeric or multimeric helicases.

INTRODUCTION

Helicases are enzymes that unwind double-stranded nucleic acid by translocation along one strand. They are now thought to be involved in numerous areas of nucleic acid biology, including gene transcription, transcript splicing, protein synthesis and DNA repair. A helicase requires two features, an ATP-driven conformational rearrangement that can generate force, and a minimum of two nucleic acid binding sites between which this force can act to promote a translocation step. Known DNA helicases are either homodimers or more complex multimeric forms, allowing models to be proposed in which the DNA-binding sites are distributed among the subunits (reviewed in 1). Such a model may also apply to an RNA helicase, potyvirus CI protein, which purifies as a large complex (2). However, two recently described human nuclear RNA helicases, p68 and RNA helicase A, and one from vaccinia, NPH-II are unusual in that they are active in the monomeric form (3–5).

These four RNA helicases share sequence homology and belong in the rapidly growing DEAD/DExH group of putative nucleic acid helicases, which is a subset of the superfamily II helicases (listed and reviewed in 6–8). Notwithstanding general expectation, a number of DEAD/DExH proteins have failed to demonstrate helicase activity *in vitro* although they have shown nucleic acid-dependent NTPase activity (e.g. 9–12). The prototype DEAD/DExH family member eIF-4A, has RNA-dependent NTPase activity as a monomer but functions as a helicase when dimerised with eIF-4B, which binds RNA through an RNP RNA-binding domain (13). It is currently thought that most DEAD/DExH proteins either require accessory proteins to function or else recognise a specific sequence in an unknown RNA substrate.

The sequence of RNA helicase A revealed it to be the human orthologue of the gene product of the *Drosophila* gene *maleless* (*MLE*) with which it shares 49% identity (14). MLE protein is found at hundreds of sites on the male X chromosome where it is essential for gene dosage compensation (15). Lesions in *MLE* that result in failure to upregulate X-linked gene expression are lethal to male progeny. Although RNA helicase A cannot have this precise function, both proteins may play a role in a more general process modulating levels of gene transcripts (14).

A growing number of protein domain classes that function in RNA recognition have now been identified in proteins that interact with RNA. Different classes may bind to single or double-stranded RNA, while some are base-specific and others non-specific (reviewed in 16,17). One such domain, termed the double-stranded RNA-binding domain (dsRBD), was first identified as ~70 residue repeated motifs in three proteins: dsRNA-dependent (DAI) protein kinase; *Xenopus* RNA-binding protein xlrbpa; *Drosophila* maternal effect protein staufen (18,19). Database searches with motif-based patterns then revealed dsRBDs in several other proteins such as TAR-binding protein (TRBP) and *E.coli* RNAse III (19). Non-specific dsRNA-binding activity has been demonstrated for domains from DAI, xlrbpa and staufen (18,19). While it is premature to conclude that the domain always binds non-specifically, direct sequence recognition need not be postulated for any of the known dsRBD-containing proteins.

We now report that database searches with dsRBD profiles found homologous domains in the MLE/helicase A sequences. The presence of additional RNA-binding sites outside the DExH

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domain suggests an explanation as to why helicase A can function as a monomer, unlike many other DEAD/DExH proteins.

MATERIALS AND METHODS

Preparation and use of dsRBD profiles

Previously identified dsRBDs were extracted and aligned by hand. Additional RNAse III homologues were isolated by keyword search with SRS (20) and by database searches. A profile (21) was prepared from the alignment using the program PROFILEWEIGHT (22). The profile was calculated with the BLOSUM 62 residue substitution matrix (23), weighting for sequence divergence, and excision of INDEL columns if gaps were present in greater than 20% of the sequences (22,24). At INDEL sites, gap opening penalties were set to 1/10th the penalty for other columns. A second profile was also prepared which included the 8 newly detected dsRBD domains.

Searches of protein databases were conducted with the GCG program PROFILESEARCH (21, 25) while TPROFILE-SEARCH (24) was used to search 6-frame translations of the DNA-databases. Default normalisations for amino acid compositions and sequence length were turned off. For the reported scores, penalties were 1.00 for gap opening and 0.05 for gap extension.

Dotplots, comparing profiles against sequences (as in Figure 1), were produced with PROPLOT (22).

Database searches with single sequences

These were conducted against SWISS-PROT v. 27 (26) using the EMBL BLITZ network service (27). BLITZ compares sequences using MPsrch (J. Collins and S. Sturrock, Edinburgh) which conducts a full Smith-Waterman (28) local similarity search. A query with a dsRBD sequence (~70 residues) takes under 30 seconds. The BLOSUM 62 matrix with gap penalty set to 8 were used in all searches.

RESULTS AND DISCUSSION

Detection of candidate dsRNA-binding domains by profile searches

Database searches were undertaken with profiles (21) prepared from an alignment of 23 previously identified dsRBD motifs, using BLOSUM residue substitution matrices (23) and weighting according to sequence divergence (22,24). Table I records the high scoring entries. Five sequences not already in the profile had scores comparable to entries known to contain the dsRBD. *Xenopus* 4F.1, scoring near the top of the score range, is a recently published dsRNA-binding sequence with two copies of the dsRBD (29). A newly deposited RNAse III from the rickettsia *Coxiella burnetti* scored 7.30. The remaining 3 sequences scored just lower than the 6.90 for Pac1 which is the worst scoring sequence contributing to the profile. *C.elegans* cosmid T20G5, reading frame 11, scored 6.86, human RNA Helicase A scored 6.85 and Drosophila MLE 6.70. These values are much closer to the sequences contributing to the profile than is the next highest score of 5.38 (Table 1).

A dotplot, comparing the helicase A sequence against the dsRBD profile, shows two high scoring regions, residues 3–69 and 180–250, preceding the DExH helicase domain (Figure 1A). Similarly, using dotplots and PROFILEGAP scores (21), two high scoring regions were also found to be present in MLE and T20G5_11.

Table 1. Top scoring entries detected by the dsRBD profiles in SWISS-PROT and EMBL databases

			Original dsRBD Profile			Revised dsRBD Profile		
Sequence Name	Entry[1]	Accession No.	SW Rank[3]	Search Score	Entry is in Profile	SW Rank[3]	Search Score[4]	Entry is in Profile
Tar-BP	em:Hstrbp	M60801		13.90	*		-13.00	*
RBPA	em:Xlrnabipr	U08998		13.17	*		-12.68	*
P68/DAI kinase	Kp68_Human	P19525	1	12.74	*	1	+12.96	*
4F.1	em:Xl07155	U07155		12.13			+13.04	*
Staufen	Stau_Drome	P25129	2	11.97	*	3	-11.44	*
RNAse III	Rnc_Ecoli	P05797	3	11.94	*	2	+12.12	*
RNAse III	em:Crpcs20	X58242		11.29	*		+12.20	*
P68/TIK	em:Mmp68kin	M93567		11.17	*		+11.36	*
E3L	Ve03_Varv	P33863	4	10.98	*	4	-10.44	*
E3L	Ve03_Vaccc	P21081	5	10.98	*	5	-10.44	*
E3L	Ve03_Vaccv	P21605	6	10.97	*	6	-10.34	*
Son-A	em:Hssona	X63753		9.38	*		+9.54	*
NS34	Vn34_Rotbs	P34717	7	9.13	*	8	-8.24	*
K12H4_8	Ym68_Caeel	P34529	8	8.57	*	9	-7.36	*
NS34	Vn34_Rotpc	P27586	9	7.83	*		-6.74	*
RNAse III	em:Cbrer	L27436		7.30			+9.26	*
Pac1/RNAse III	Pac1_Schpo	P22192	10	6.90	*	10	+7.30	*
T20G5_11	em:Cet20g5	Z30423		6.86			+7.62	*
Helicase A	em:Hsrnahela	L13848		6.85			+9.52	*
Maleless	Mle_Drome	P24785	11	6.70		7	+9.30	*
Notch	Notc_Drome[2]	P07207	12	5.38		39	-4.14	
NGG1	Ngg1_Yeast[2]	P32494	13	5.33		12	-4.98	
Histone 1B	H1B_Strpu[2]	P15869	25	4.85		13	-4.68	

[1]SWISS-PROT unless prefixed by em:
[2]Highest scoring entries in SWISS-PROT that lack a dsRBD
[3]Position in SWISS-PROT search
[4]'+' if score is higher score with respect to first profile, '–' if lower

The nine high scoring regions from the five newly detected proteins were aligned to the known sequences by a combination of profile alignment (21) and manual correction of the most conserved positions and allowed gaps. The alignment of the matching regions with the other dsRBD domains (Figure 1C) reveals that key conserved residues (especially the positive and the small hydrophobic columns in block 4) are present in al the newly added sequences. In addition, at positions in the blocks where residue conservation is weak, the amphipathic nature of the original dsRBD alignment is maintained by the new sequences (Figure 1C).

Table I also records the results of database searches with a new profile prepared using the 32 aligned dsRBD sequences. The newly added sequences now scored substantially higher, which is inevitable and of no significance. The two previously highest scoring entries scored lower, which is also guaranteed to occur whenever distant sequences are added. For the remainder of the dsRBD containing entries, scores were a mixture of both higher and lower scores. By contrast the highest scoring false positives uniformly scored lower, and the gap between the lowest real score and highest false positive widened from 1.32 to 2.60. Overall, this behaviour is consistent with an unambiguous increase in discriminatory power of the new profile, suggesting that the newly incorporated sequences are dsRBD homologues.

SWISS-PROT searches with dsRBD sequences

Table II records the ranks and scores for searches of SWISS-PROT with individual dsRBD sequences using the fast and sensitive BLITZ server (27). Using a dsRBD sequence from the DAI kinase, which scores highly against the dsRBD profile, Mle_Drome is ranked 9th—above several existing dsRBD entries. A search with a dsRBD sequence which scores poorly against the profile, Pac1, fails to rank five of the ten dsRBD entries in the top 100 scores. Searching with the MLE/1 sequence also fails to find 5 entries, while the detected entries are ranked more highly than for Pac1 with the first false positive at position

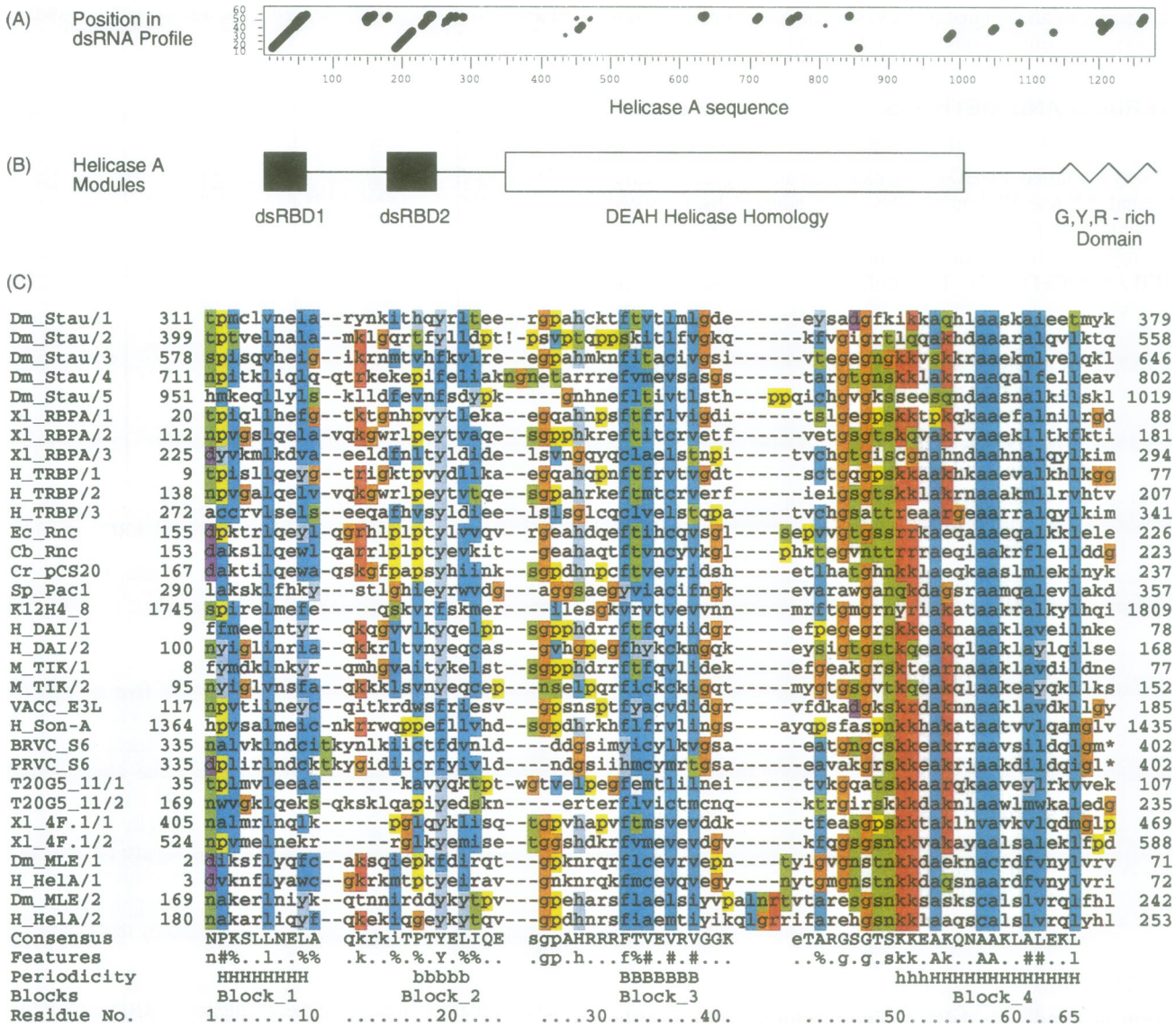

Figure 1. (**A**) Dotplot of dsRBD profile against the helicase A sequence. Values were taken from the profile matrix according to residues in the sequence and summed over a window of 21 residues. Large dots were plotted for the top 0.05% of the score range, smaller ones for scores in the top 0.1%. Two strong diagonals are visible. (For method, see 22). (**B**) Deduced multidomain structure of helicase A and MLE. Globular domains are shown as rectangles, in black for the dsRBD domains. (**C**) Alignment of known dsRBD sequences, colour coded to highlight similarities. An ! marks a 92 residue insertion in staufen repeat 2. Also shown are a consensus representing the most frequent residue at each position, the conserved features and the four blocks showing amphipathic periodicities consistent with either α-helix or b-strand secondary structures. Feature symbols: # strongly conserved hydrophobicity; % semi-conserved hydrophobicity. All G (orange) and P (yellow) residues are coloured. Other colouring is by conserved property in more than 40% of a column: uncoloured residues lack a sufficiently conserved property. Blue, hydrophobic; light blue, partially hydrophobic; red, positive; purple, negative; green, hydrophilic. Numbers either side of the sequences show the position of each domain in the protein: note that the pCS20 RNAse III is in an unannotated frame so is numbered from the start of the clone. Database entries and accession numbers are listed in Table I. The figure was prepared with the GDE alignment editor (S. Smith, Harvard University) and COLORMASK (J. Thompson, EMBL).

6. The remaining motifs in MLE and T20G5__11 can all rank dsRBD sequences in the top 100, but perform worse than Pac1. Importantly, the queries always detect either KP68__human or Stau__Drome, SWISS-PROT entries that are not only highest scoring against the profile, but together contain eight repeats of the dsRBD domain. Conversely, these queries do not detect the entries that score poorest against the profile, Pac1__Schpo and Vn34__Rotpc.

In general the searches with single dsRBD sequences perform much less well than the profile searches, with many false positives when searching with even the best matching sequences. Both short domain length and high sequence divergence contribute to a poor signal to noise. However, the results show that the new sequences behave as expected for divergent dsRBD sequences, with MLE/1 reciprocal detection well within the range of existing dsRBD sequences.

New dsRBD-containing sequences

Three protein sequences are reported here which each appear to have two copies of a domain whose only known function is to bind double-stranded nucleic acid. The sequence T20G5__11 is a 386 amino acid predicted spliced ORF, with no previous

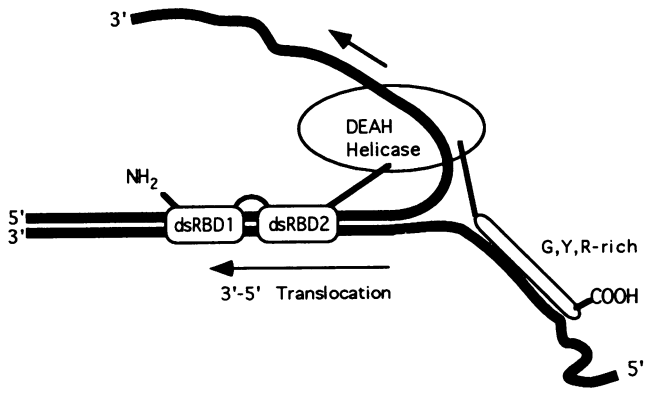

Figure 2. Hypothetical scheme showing how the various modules of helicase A may fit to a forked substrate RNA. The helicase domain is shown binding to the 3′ single-stranded region, which it pulls away from the other strand, and along which it translocates. Models in which the C-terminal domain binds to the 3′ strand instead of the 5′ strand are also plausible.

clues to function, in a cosmid sequenced by the *C.elegans* sequencing project (30). A role in RNA metabolism now seems likely.

The remaining two proteins are the orthologs MLE and helicase A that are already known to be associated with RNA. In addition to the DExH domain, MLE/helicase A also has a C-terminal extension containing numerous degenerate repeats of the motif GGYRG. It is anticipated that this region will bind RNA (14) since it strongly resembles the RGGF motifs in nucleolin that bind to and unstack ssRNA (31,32). The modular structure of MLE/helicase A is summarised in Figure 1B.

The *C.elegans* sequencing project (30) has yielded a second sequence with a dsRBD, the predicted 1,882 residue protein K12H4_8, which was used in the original profile due to reported similarity to RNAse III. This unusual sequence consists of at least 3 regions, an N-terminal DExH domain and a C-terminal RNAse III, separated by ~900 residues with no known similarity. Although this provides another example of a putative helicase linked with a dsRBD, the following discussion is probably not directly relevant as the dsRBD belongs to the RNAse III portion of the molecule and is presumably involved in dsRNA degradation rather than translocation.

Monomeric RNA helicases have two or more discrete RNA-binding domains

The finding that the RNA helicase A sequence possesses several discrete RNA-binding domains as well as a DExH helicase domain fits with the monomeric helicase activity demonstrated *in vitro* (14). It has been supposed that RNA helicases should recognise dsRNA (33) although dsRNA-specific regions have not previously been established. As shown in the schematic in Figure 2, the protein may have sufficient capacity to bind both the single strands as well as the double strand in a forked RNA template. Only one of the ssRNA-binding sites need have strand-specificity to account for the 3′−5′ translocation polarity of helicase A.

Such a model presupposes that the DEAD/DExH domain has too few RNA-binding sites to function as an active helicase on its own, consistent with the observations that several family members lack helicase activity *in vitro*: The enzymatic domain presumably possesses just one RNA-binding site (that which promotes ATPase activity), constructed around the conserved Arg-rich motifs (6). In the case of eIF-4A, recent mutagenesis and cross-linking studies imply that there is a single RNA-binding site and that the conserved HRIGRxxR motif is essential for RNA-binding activity (34). Therefore, the helicase domain must be supplied with additional RNA-binding domains which, in many cases, will be provided by accessory proteins. This model implies that p68 and NPH-II, the other DEAD/DExH helicases known to function alone *in vitro*, should also have additional RNA-binding domains. The p68 sequence (35) has N- and C-terminal extensions bracketing the helicase domain which could function as (presumably non-specific) RNA-binding domains: residues 1−100 are 23% positively charged (Arg, Lys, His) while residues 430−530 are 22% positively charged, and RGG repeats have been noted previously (16). The NPH-II sequence (5) has an analogous N-terminal extension in which residues 1−90 are 21% positively charged. It is not yet reliably known which translocation polarities are possessed by p68 or NPH-II but, given the different modules with respect to helicase A and the eIF-4AB dimer (a bidirectional helicase), it will be of considerable interest to see how they compare for both directionality and processivity.

Table II. Scores and ranking positions resulting from searching the SWISS-PROT database with individual dsRBD sequences[1]

	DAI/1		PAC1		MLE/1		MLE/2		T20G5_11/1		T20G5_11/2	
Entry	Pos.	Score	Pos.	Score	Pos.	Score	Pos.	Score	Pos.	Score	Pos.	Score
Kp68_Human	1	352	4	54	26	49	7	54	-	-	6	55
Stau_Drome	5	82	-	-	5	57	-	-	77	46	8	54
Rnc_Ecoli	6	72	-	-	-	-	-	-	-	-	-	-
Ve03_Varv	2=	99	9=	52	2=	63	-	-	-	-	-	-
Ve03_Vaccc	2=	99	9=	52	2=	63	-	-	-	-	-	-
Ve03_Vaccv	2=	99	9=	52	2=	63	-	-	-	-	-	-
Vn34_Rotbs	-	-	-	-	-	-	-	-	-	-	-	-
Vn34_Rotpc	17	52	-	-	-	-	-	-	-	-	-	-
Ym68_Caeel	48	47	-	-	-	-	26	51	-	-	19	49
Pac1_Schpo	10	54	1	344	-	-	-	-	-	-	-	-
Mle_Drome	9	54	-	-	1	364	1	364	-	-	-	-
Top false hit	7	55	2	58	6	57	2	62	1	64	1	59

[1]Positions and scores are not recorded for entries scoring below the top 100.

Implications for putative RNA helicases

The realisation that monomeric RNA helicases are multidomain proteins with several RNA-binding sites should be helpful to experimentalists working on the biochemistry of the large related group of putative RNA helicases. Inspection of DEAD/DExH family sequences should reveal whether or not they have additional domains that may have RNA-binding capability. For example the *Drosophila* DEAD-box protein Vasa (36,37), which is expected to be a functional helicase (8), also has a probable RNA-binding domain: the N-terminal 200 residues are highly positively charged with many degenerate copies of the RGG motif.

An *E.coli* DEAD protein, DbpA, has an unusual 70 residue C-terminal domain with 25% positive charge and 29% Gly/Ala (small residues). DbpA has been shown to possess two separable RNA-binding capabilities: one which is specific for 23s ribosomal RNA and induces ATPase activity, and a second nonspecific binding capability that does not induce ATP hydrolysis (12). Since 23s-specific binding induces ATPase activity, it is likely located on the DEAD domain. If non-specific binding is located in the C-terminal domain, there is a strong prospect that DbpA is a 23s RNA-specific helicase.

In contrast to these multidomain proteins that are likely to be helicases in their own right, the DEAD/DExH proteins clearly lacking additional RNA-binding domains are predicted to acquire helicase function only within complexes.

The modular nature of monomeric RNA helicases can be exploited by domain swap and deletion experiments to address a number of questions. Can other DEAD/DExH sequences function as helicases when they are linked to RNA-binding domains? How many and what classes of RNA-binding domains are needed for 5′−3′, 3′−5′ or bidirectional activity? Is the RNA-binding site on the DEAD/DExH domain polarity-specific? If so, is it always the same polarity? Finally, as regards the many DEAD/DExH family helicases lacking more than one RNA-binding site, their biology will only be understood after their RNA-binding partners have been identified.

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