Are DEAD-box proteins becoming respectable helicases?

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The vaccinia NPH-II RNA helicase, a member of the DEAD/DExH-box protein family, has been shown to be a processive, unidirectional RNA helicase with a step size of about one half turn of a helix. This finding demonstrates that RNA helicases can function as molecular motors.

RNA helicases are found in all cellular organisms and in many viral genomes. They perform essential functions in RNA metabolism and are most likely to be involved in all processes involving RNA, as shown by extensive genetic analyses in the yeast *Saccharomyces cerevisiae*. Nevertheless, despite the wide interest in the study of viral and cellular RNA helicases, little is known about their biochemical characteristics. Moreover, their designation as RNA helicases has been questioned, since unlike DNA helicases, RNA helicases may not be required for unwinding long stretches of double stranded RNA, but could be involved in disrupting secondary structures or short RNA–RNA interactions of a few base pairs. To address the mode of action of RNA helicases, Jankowsky *et al.*[1] have now analyzed in detail the vaccinia nucleoside-triphosphate phosphohydrolase-II (NPH-II) protein. They show that NPH-II that is a highly processive 3'->5' RNA helicase, provided the supply of ATP is plentiful. In fact, this is the first demonstration of a processive RNA helicase. They found the step size to be ~6 base pairs (bp), which is similar to the step size of a processive DNA helicase, UvrD[2]. Thus, similarities between RNA and DNA helicases are becoming apparent, suggesting that differences between RNA and DNA helicases may essentially reside in the other proteins with which they interact and their particular substrates.

The RNA helicase families are characterized according to eight conserved sequence motifs[3–5]. Two of the motifs (I and II) are characteristic of NTP hydrolyzing proteins, whereas others have been shown to be involved in the coupling of ATP hydrolysis and unwinding (III) or are required for the nucleic acid dependent NTP hydrolysis (VI) (Fig. 1). The remaining motifs await an analysis to understand their roles in the function of these proteins. Motif variations allow the RNA helicases to be grouped into distinct families, such as DEAD, DEAH or DExH families, named according to the conserved motif II. In addition to the conserved motifs representing the core domain of RNA helicases, these proteins possess N- and C-terminal extensions that are required for cellular localization and/or interactions with RNAs or other proteins.

The best overview of cellular processes involving RNA helicases can be obtained from the study of DEAD-box and related proteins in the yeast *Saccharomyces cerevisiae*[6]. Genetic analyses have shown that RNA helicases are involved in transcription, pre-mRNA processing, ribosome biogenesis, RNA export, translational initiation, mitochondrial gene expression and RNA degradation (Fig. 2). Although the translation initiation factor eIF4A has served as the prototype for the DEAD-box proteins, its *in vivo* function is not known.

The first demonstration of an essential biological role for RNA helicases came from the pre-mRNA splicing field. The spliceosome is a transient and highly dynamic structure that assembles in a highly ordered and stepwise manner and undergoes several conformational changes. The spliceosome is made up of five small nuclear RNAs (snRNAs: U1, U2, U4, U5 and U6) and many different polypeptides. Several of the assembly steps that lead to the formation of a catalytically active spliceosome involve well-described changes in RNA–RNA interactions[7,8]. Indeed, the two transesterification steps in the splicing reaction do not require energy *per se*, but the splicing process is dependent on ATP hydrolysis. It is generally believed that the ATP-dependent RNA helicases are primarily responsible for the energy requiring rearrangements within the splicing process to allow the formation of new, sometimes mutually exclusive interactions to occur. Several of these RNA rearrangements have been attributed to specific RNA helicases, such as the unwinding of duplexes formed between U4 and U6 snRNAs by Brr2p (ref. 9).

Similarly, other studies show that a majority of the DEAD-box proteins are involved in ribosome biogenesis. This can be explained by the dynamic process of ribosome formation which involves numerous sequential steps of pre-ribosomal RNA (pre-rRNA) maturation and assembly with ribosomal proteins. Nevertheless, in the case of ribosome biogenesis, a correlation between *in vivo* requirements and defined biochemical steps, as in the case of the spliceosome rearrangements, are largely missing due to the absence of a reconstituted *in vitro* system.

Finally, studies on putative RNA helicases without clear homologs in *S. cerevisiae* have been reported, suggesting that these proteins could be involved in other processes not present in this lower eukaryote[6,10].

RNA helicases are expected to melt double stranded RNA molecules. This activity

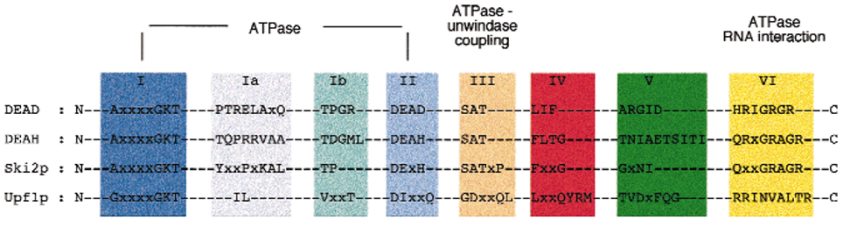

Fig. 1 Sequence comparison groups the putative RNA helicases in the DEAD, DEAH, DExH, and Upf1p families. Conserved residues are given, but variations within the family exist. Therefore, a clear assignment to a particular family can be made in most cases, but a few proteins show similarities to more than one family. While many members of these families have been analyzed, it is not clear whether members of different families use the same mode of action. The N- and C-terminal extensions, as well as the spacing between the motifs, vary and are only schematically represented here. Proteins of the Snf2p family involved in chromatin remodeling and transcription are not included since they are most likely not *bona fide* helicases[28].

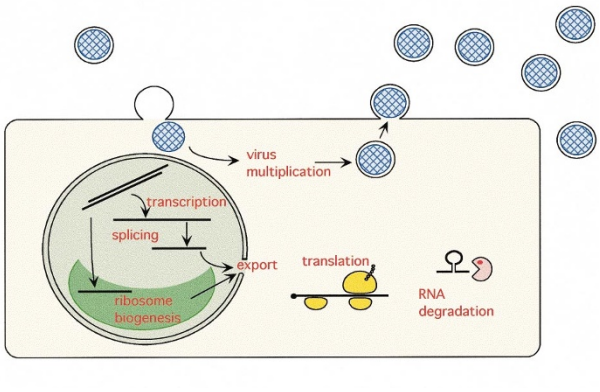

Fig. 2 RNA helicases play important roles in all processes involving RNA. Although RNA helicases from different organisms and viruses[29] have been biochemically and genetically analyzed, the most detailed picture has evolved from the use of molecular genetic methods in the yeast *Saccharomyces cerevisiae*.

is typically assayed by using double stranded RNAs (dsRNAs) with single stranded overhangs and by electrophoretic separation of the duplex and monomer products. The polarity of the single stranded tails of the duplex substrate, required for all characterized helicases to load onto the nucleic acid, allows them to be classified as either 5'->3' or 3'->5' helicases[11]. Some RNA helicases from cellular and viral origins have a demonstrable *in vitro* helicase activity[12–15], validating to some extent the notion of an RNA helicase family, although such an activity has not been demonstrated for most of these proteins. As expected for an active unwinding process, helicase activity requires ATP hydrolysis and indeed all *bona fide* RNA helicases and many other members of the families have been shown to possess ATPase activity. This activity is often stimulated by or largely dependent on the presence of nucleic acids. In some cases, this stimulation is most pronounced with particular RNAs, suggesting a substrate specificity of these helicases[16]. In addition to these catalytic activities, RNA helicases also show more or less pronounced RNA binding activities. The binding affinities for single stranded *versus* double stranded RNAs may be modulated according to the bound nucleotide (ADP *versus* ATP)[12,17].

So far all cellular processes requiring RNA helicases seem to involve dynamic RNA–RNA interactions and may require denaturation of short RNA duplexes. For example, essential but transient interactions occur between small nucleolar RNAs (snoRNAs) and pre-rRNA sequences and between small nuclear RNAs (snRNAs) and pre-mRNA sequences during ribosome biogenesis and pre-mRNA splicing, respectively. Likewise, secondary structures within mRNAs have been shown to inhibit the scanning process during translation initiation and the degradation of RNA by exonucleases in RNA decay pathways[18,19].

Unwinding of such short duplexes could take place in a passive or active manner (Fig. 3). First, it could occur by a simple strand displacement mechanism in which the RNA binding protein binds to or traps one of the two strands of RNA generated by the fraying of duplex ends. In this passive mode of unwinding, ATP hydrolysis is required for translocation of the protein along the single stranded nucleic acid. Alternatively, unwinding could occur by active duplex unwinding in which ATP hydrolysis is used to pull apart the two strands of nucleic acid. Whether ATP hydrolysis is also used for translocation in active duplex unwinding is not clear.

Proteins of the DEAD-box and related families that actively unwind duplexes in a nonprocessive manner have also been designated unwindases to contrast with the processive activity of DNA helicases[7]. Indeed, DNA helicases can unwind up to 30 kb of DNA under optimal conditions, at 1,000 bp s^{-1}, without dissociating from their substrate[11].

Because of these differences in the activities of RNA and DNA helicases and the fact that the sequence similarity between them is restricted to a few residues within some conserved motifs, it was gratifying that the three-dimensional structures of DNA and RNA helicases showed some similarities. Specifically, the structures of two DNA helicases (PcrA[20] and Rep[21]) and two RNA helicases (HCV[22] and eIF4A[23,24]) reveal a similar structural arrangement of all helicase motifs (I to VI), forming a nucleotide binding pocket. The structural similarities would suggest that the coupling of ATP hydrolysis and unwinding is highly conserved between DNA and RNA helicases. The differences between them, if they exist, therefore must reside in additional elements, which may be located within or outside the core domain. In fact, N- and C-terminal extensions may be required for their association with other proteins, which contribute to their *in vivo* function and permit their assembly into larger complexes[25].

To elucidate the mechanisms of RNA unwinding in more detail, Jankowsky *et al.*[1] have used the NPH-II protein from vaccinia virus. The NPH-II protein encoded by gene I8R of the dsDNA vaccinia genome is most closely related to cellular DEAH-box proteins and has previously been shown to have 3'->5' RNA unwinding activity[26]. Moreover, mutational analyses of this helicase have given insights into the function of some of its motifs. In an elegant *in vivo* rescue system, it has been shown that NPH-II is indeed required for propagation of viral particles, although its precise role is not yet known[27]. To gain insight into how this helicase functions, Jankowsky and coworkers addressed the following questions: (i) does NPH-II unwind RNA in sequential steps and, if so, what is the step size? (ii) does the helicase remain bound to the substrate between the steps (that is, is it a processive enzyme) or does it dissociate after each unwinding step?

Under normal assay conditions the activity of NPH-II was monitored by the final release of the monomeric products. Since helicase translocation is not rate limiting, it was necessary to search for suboptimal reaction conditions in order to follow the unwinding reaction. By using Co^{++} instead of Mg^{++} as cofactor for the ATPase, the lag phase of the unwinding reaction increased with increasing duplex length. This allowed the authors to estimate the average step size of the reaction to be ~6 bp, which is similar to the step size of the UvrD DNA helicase[2]. This step size corresponds approximately to a half turn of the dsRNA.

To test for processivity, a large excess of nonspecific RNA was added to trap the enzymes that dissociate from their substrates. In such an experiment the unwinding rates remained similar to the control in the absence of trap RNA, indicating that the translocation is not affected under these conditions. Although the amount of duplex substrate in presence of the trap RNA decreased, the fact that a large portion of the substrate was unwound at the same rate in presence of trap RNA, shows that the enzyme stays on the substrate and unwinds duplex RNA without being trapped by the nonspecific RNA. As expected, the possibility of dissociation from the substrate increased with increasing duplex length. Other experiments showed that the processivity is, in fact, dependent on the ATP concentration. At limiting ATP concentrations, the NPH-II proteins have a tendency to release the substrate, whereas in the presence of high ATP concentrations the enzyme continues to the end. Thus, ATP availability could be used for regulating the overall RNA helicase activity.

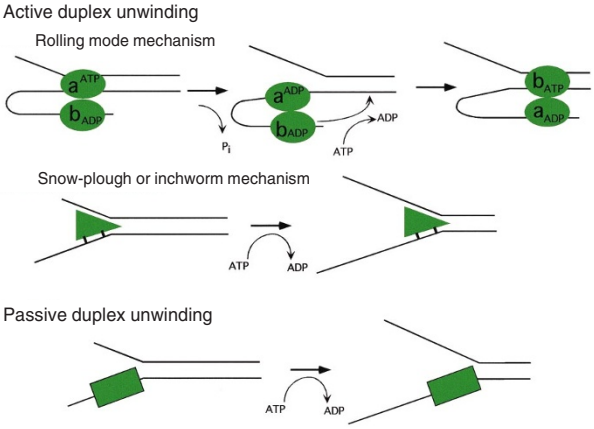

Fig. 3 Proposed models for the action of helicases. In the rolling model, an oligomeric helicase binds alternating to single and double stranded nucleic acid, where the ATP-bound proteins have a high affinity for double stranded nucleic acid. In the snow-plough model, the RNA helicase is moving along the fork and uses energy from NTP hydrolysis to melt hydrogen bonding between the two nucleic acid strands. By contrast, in the passive unwinding model, the local denaturation by thermal fluctuation could be fixed by a single stranded RNA (ssRNA) binding protein. The movement of the ssRNA binding protein along the single stranded nucleic acid would be an ATP-dependent reaction.

In conclusion, genetic experiments have been able to assign individual RNA helicases to specific cellular processes. Biochemical experiments have shown that in the presence of ATP and Mg^{++} some of these proteins are able to dissociate short RNA duplexes. With the report by Jankowsky *et al.*[1] our view of RNA helicases has expanded. RNA helicase have now been shown to migrate in a directional fashion, in distinct steps along the substrate RNA without dissociating from it.

Despite this important step forward, we remain at the beginning of our understanding of RNA helicase function. Indeed, we do not know how helicases unwind duplex molecules. Do they act as an active snow-plough, as a rolling oligomer tearing the substrate apart or by following in an ATPase-dependent manner the spontaneous denaturation of the duplex (Fig. 3)? In the case of active DNA helicases it has been shown that they exist in oligomeric complexes. So far there is little information on this concerning RNA helicases, and even in the case of 'monomeric' RNA helicases it cannot be excluded that oligomerization is induced by contact with the RNA.

Thus, it may turn out — depending on the molecular environment or their tertiary structure — that some RNA helicases unwind duplex substrates in a processive fashion, whereas others do not. Moreover, the RNA helicases are highly specific and cannot be freely interchanged. Thus, they most certainly possess specificity determinants and/or interact with other components that let them work in a controlled manner on the right substrate and at the right time.

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The servant with the scissors

In 1978, Werner Arber (Biozentrum der Universität, Basel, Switzerland), Dan Nathans and Hamilton Smith (both at Johns Hopkins University School of Medicine, Baltimore, Maryland, USA) were awarded the Nobel Prize in Physiology or Medicine for the discovery of "restriction enzymes and their application to problems of molecular genetics". Almost immediately, the application of these enzymes to genetics led to "new and far reaching results". In fact, it is hard to imagine what the biological sciences would look like today without restriction maps, cloning and the ability to alter genes at will, to name just a few everyday tools of the trade. But how did this crucial discovery come about?

While studying a phenomenon known as 'host controlled restriction of bacteriophages', Arber and Dussoix[1–3] found that it provided bacteria with a defense mechanism against invading foreign DNA, such as viral DNA. This process, which was shown to be a property of the recipient bacteria, could be divided into two parts: restriction and modification. Restriction involved the endonucleolytic cleavage of DNA at specific DNA sequences. Because this would restrict viral growth, these enzymes came to be known as restriction enzymes. Modification involved nucleotide methylation at these same specific DNA sequences in the genome. In this way, the bacteria's own DNA was protected from cleavage because it was methylated while the inappropriately or unmodified