

A new twist on RNA helicases: DExH/D box proteins as RNPases

Beate Schwer

DExH/D box proteins are required for the major transactions of RNA, including mRNA synthesis, pre-mRNA splicing, ribosome biogenesis, translation and RNA decay. In the popular imagination, DExH/D box proteins have become synonymous with 'RNA helicases', which are enzymes that unwind duplex RNAs in concert with the hydrolysis of nucleoside triphosphates (NTPs). But all DExH/D box proteins may not be RNA helicases and the energy of NTP hydrolysis by DExH/D box proteins may be harnessed for other purposes. Cellular RNAs are associated with proteins, often in large ribonucleoprotein (RNP) complexes. This review focuses on recent progress suggesting a role for DExH/D box proteins as 'RNPases' that use chemical energy to remodel the interactions of RNA and proteins.

DExH/D box proteins are defined by six conserved peptide motifs arrayed in a colinear fashion, of which the eponymous DExH/D box comprises motif II¹⁻³. Most DExH/D box proteins can hydrolyze NTPs to NDPs in a reaction that is either stimulated by or entirely dependent on a nucleic acid cofactor. Some DExH/D box proteins also possess intrinsic RNA or DNA helicase activity. Mutational studies, in conjunction with the crystal structures of several helicases, implicate conserved motifs I (GxGKT), II (DExH/D) and VI (H/QRxGRxGR) in NTP hydrolysis whereas motif III (S/TAT) couples the chemical reaction to the mechanical steps of duplex unwinding⁴⁻¹². Given that RNAs are often bound to proteins in the cell, the question arises whether DExH/D proteins are capable of disrupting protein-RNA interactions and what the biological ribonucleoprotein targets for DExH/D NTPases may be.

A DExH helicase can displace an RNA-bound protein

The exemplary DExH-box RNA helicase is vaccinia virus NPH-II. NPH-II is essential for virus replication and plays a role in mRNA transcription by enzymes encapsidated in the virion^{9,13}. NPH-II can processively unwind long stretches of duplex RNA in a 3' to 5' direction with a kinetic step size of about one-half helical turn^{14,15}. NPH-II is thought to unwind by translocating along the 'template' RNA strand to which it initially binds and displacing in its wake either DNA or RNA strands annealed to the RNA. In a recent report, Jankowsky *et al.*¹⁶ ask whether NPH-II is also capable of dislodging proteins from RNA. They designed a substrate that contains a high-affinity binding site for a well characterized RNA-binding protein, the U1A protein, embedded within a duplex region (Fig. 1). NPH-II unwound the RNA whether or not U1A was present, showing that the helicase was not impeded by RNA loops (caused by the inclusion of the U1A binding site) or by the bound U1A. Was displacement of U1A an active process or simply a reflection of passive dissociation of U1A from the RNA? The dissociation rate for U1A was increased by several orders of magnitude when NPH-II plus ATP were present. Detailed kinetic analyses showed that although NPH-II appears to 'stumble' when it encounters the U1A protein, it maintains its processivity.

Even though the experimental scenario is not physiological (that is, NPH-II is unlikely to encounter U1A as a target *in vivo*), this study clearly demonstrates that a DExD/H box RNA helicase

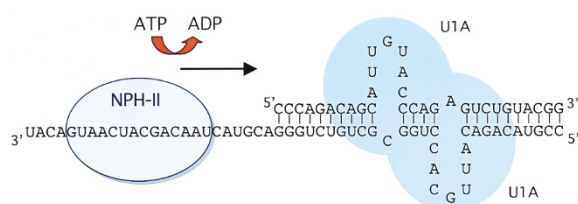


Fig. 1 Schematics of a synthetic RNP substrate¹⁶. The RNA binding protein is U1A, which is a component of the U1 small ribonucleoprotein particle (U1 snRNP) and it also binds its own pre-mRNA and inhibits polyadenylation of its own pre-mRNA. A high affinity binding site for the U1A protein, from the 3' untranslated region of U1A pre-mRNA, is embedded within duplex regions¹⁶. U1A protein (indicated in blue) binds as a dimer to the two asymmetric loops⁵⁵. A 3' single-stranded overhang serves as a binding site for the RNA helicase NPH-II (indicated in purple). NPH-II binds to the 3' ss tail and translocates on the RNA in a 3' to 5' direction, powered by ATP hydrolysis.

can function to displace proteins from RNA. Although often speculated upon^{17,18}, such an 'RNPase' function (a term coined by Jankowsky *et al.*¹⁶) had never been demonstrated for any DExH/D box protein. The RNPase activity of NPH-II provides clues to how other DExH/D box proteins may act upon complex ribonucleoprotein assemblies such as the spliceosome.

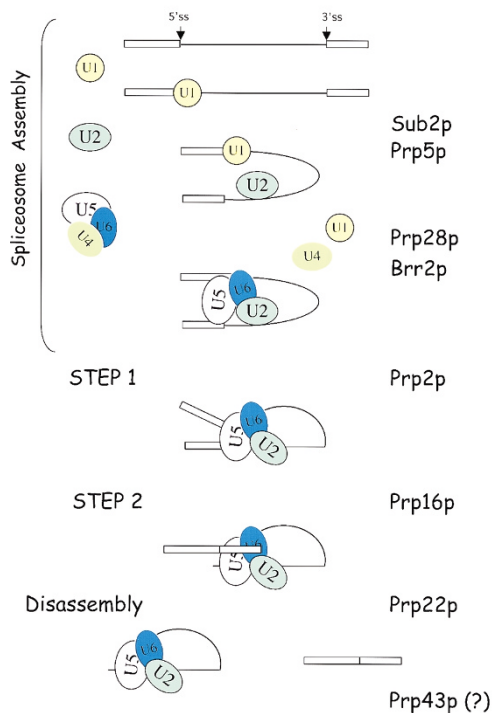
DExH/D box proteins and the spliceosome

The spliceosome is formed by the ordered assembly of U1, U2, and U4/U6/U5 snRNPs and various non-snRNP proteins onto the pre-mRNA (Fig. 2). Intron removal occurs by two sequential transesterification reactions. In step 1, the 5' splice site is cleaved and a branched lariat intermediate is formed. In step 2, the 3' splice site is cleaved and the two exons are joined¹⁹. Whereas the hypothesis that the chemical steps are catalyzed by snRNAs remains plausible, it is clear that proteins are required to assemble, modulate and disassemble the catalytic core²⁰⁻²². Phylogenetic, genetic and biochemical studies illuminate a dynamic network of RNA-RNA and RNA-protein interactions necessary for splice site recognition and accurate positioning of the reactive nucleotides for catalysis^{18,23,24}. The challenge now is to determine in molecular detail how these RNA-RNA and RNA-protein contacts are crafted and remodeled.

Department of Microbiology & Immunology, Weill Medical College of Cornell University, New York, New York 10021, USA.

Correspondence should be addressed to B.S. *email: bschwer@mail.med.cornell.edu*

review



Eight DEXD/H box proteins are important for pre-mRNA splicing in yeast or mammalian systems^{19,20}: Prp5p, Brr2p, Prp28p, UAP56/Sub2p, Prp2p, Prp16p, Prp22p and Prp43p. The DEXH NTPases Prp2p, Prp16p and Prp22p act sequentially at distinct steps in the splicing pathway (Fig. 2). ATP hydrolysis by Prp2p propels the first transesterification reaction^{25,26}. NTP hydrolysis by Prp16p is essential for the second transesterification step²⁷. The ATP-dependent helicase activity of Prp22p is crucial for the release of mature mRNA from the spliceosome¹⁰. Conformational changes in the spliceosome accompany these steps. For example, Prp16p effects a rearrangement at the 3' splice site (3' ss) and Prp22p activity leads to the disassembly of the spliceosome²⁸⁻³⁰. However, the immediate molecular targets of Prp2p, Prp16p and Prp22p action within the spliceosome are not defined.

Four recent reports shed light on the functions of two DEXD box proteins, Prp28p and Sub2p, in early steps of spliceosome assembly³¹⁻³⁴. They demonstrate that the essential functions of Prp28p and Sub2p can be bypassed *in vivo* by mutations in the U1C protein (or the U1 snRNA) or a deletion of the Mud2 protein, respectively, thereby implicating RNA-bound proteins as key components of the targets upon which Prp28p and Sub2p act^{31,32}. **Change of pairing partners at the 5' splice site.** In a genetic screen for suppressors of the cold sensitive (*cs*) phenotype of the *prp28-102* strain³⁵, Chen *et al.*³¹ identified dominant mutations in the gene encoding the essential U1C protein. U1C contains an N-terminal zinc finger motif that is important for its function^{36,37}. Each of three suppressing mutations mapped to a single leucine residue (Leu 13) in the zinc finger. Changing Leu 13 to all other 19 natural amino acids showed that all but six variants could suppress the *cs* phenotype of *prp28-102*. Remarkably, the suppressor strains readily lost *prp28-102*, arguing that the U1C[L13] mutants could bypass the essential function of the PRP28 gene product. U1C protein is important for the stable association of the U1snRNP with the 5' splice site (5' ss) sequence^{36,38}. The U1C[L13] suppressors likely affect this stabilizing function because the requirement for Prp28p can also be bypassed by certain mutations in U1 snRNA that destabilize the

Fig. 2 The splicing pathway. Stages of spliceosome assembly, the two transesterification steps and release of mRNA (disassembly) are shown. Small nuclear ribonucleoprotein particles (U1, U2, U4, U6, U5 snRNPs) assemble onto the pre-mRNA in an ordered manner. U1 binds to the 5' splice site (5' ss), U2 to the branchpoint sequence element. U4/U6/U5 enter the spliceosome as a tri-snRNP. U1 and U4 snRNPs are destabilized prior to step 1 transesterification. Please note that non-snRNP proteins, which associate with the pre-mRNA are omitted for simplicity (see refs 19 and 20 for more details). The DEXH/D proteins that act at the various ATP-dependent stages, are indicated on the right. Prp43p is thought to play a role in the disassembly after mRNA is released⁵⁶, but it is not clear whether ATP is required for this reaction.

U1/5' ss duplex. The disruption of the U1/5' ss helix allows for contacts of the 5' ss with U6 snRNA, an interaction that is essential for accurate 5' cleavage site selection²³ (Fig. 3).

Previous studies had implicated Prp28p in this switch of pairing partners with the 5' ss³⁹. However, because the switch from U1/5' ss to U6/5' ss is closely linked with unwinding of the extensively base-paired U4/U6 snRNAs and the necessity for another DEXH protein, Brr2p, it had not been feasible to define the point of action of Prp28p exactly (Fig. 3). The study by Chen *et al.*³¹ suggests a direct role for Prp28p in disrupting the U1/5' ss helix. But, how is Prp28p positioned for action? Does it interact with U1C, with RNA or both?

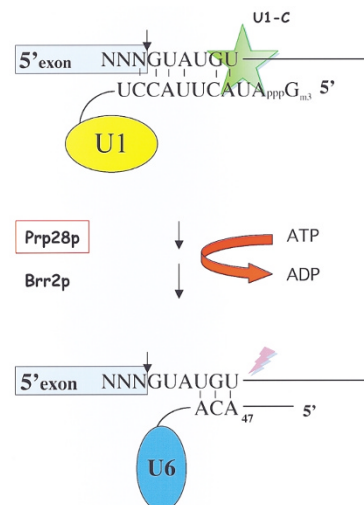
The human homolog hPrp28p is an integral component of the U5 and U4/U6/U5 snRNPs and thus likely to enter the spliceosome together with the tri-snRNP complex⁴⁰. Ismaili *et al.*⁴¹ have used site-specific crosslinking to identify hPrp28p as a protein that binds to intron sequences at the 5' ss. Kinetic analyses indicated that hPrp28p remains bound to the pre-mRNA throughout the splicing pathway. The site of the crosslink in the protein was mapped near motif III (TAT). In other DEXH/D box helicases, including Prp22p, NPH-II, hepatitis C virus NS3 and mammalian eIF-4A, motif III has been shown to couple the energy of ATP hydrolysis to duplex unwinding^{4,8-10}. Yeast Prp28p can hydrolyze ATP in an RNA-stimulated fashion⁴² and it will be interesting to determine if and how Prp28p uses ATP hydrolysis during the splicing reaction.

Handing off the branchpoint sequence to U2 snRNP. The 5' splice site is recognized at least twice — initially by U1 snRNP and then U6 snRNA — prior to the first catalytic step. Similarly, the branchpoint sequence (BPS) and 3' splice site (3' ss) region are contacted initially by proteins, and then the BPS interacts with U2 snRNA (Fig. 4). The branchpoint binding protein BBP/SF1 binds to the BPS in cooperation with U2AF65 or its yeast homolog Mud2p, which bind to a pyrimidine-rich element between the BPS and the 3' ss^{20,43-45}. The bound proteins are replaced in the pre-spliceosome by U2 snRNA. The switch of pairing partners at the BPS requires essential DEXH/D box proteins.

The yeast ATPase Prp5p is thought to modulate RNA structure in the U2 snRNP to make it accessible for interaction with the BPS^{46,47}. The ATP requirement for U2 association (but not the requirement for Prp5p *per se*) can be bypassed by deletion of the non-essential U2 snRNP protein CUS2. Perriman and Ares⁴⁸ suggest that ATP stimulates the rate of U2 snRNP recruitment to the spliceosome, in a manner that is controlled by CUS2 and the U2 snRNA structure. A mammalian counterpart for Prp5p has been identified (C. Query, pers. comm.), and additionally, mammalian UAP56, which was identified through its physical interaction with U2AF65, is essential for stable U2 snRNP association⁴⁹.

Three groups³²⁻³⁴ have now identified the DEXD box protein Sub2p as the yeast homolog of UAP56. Strong evidence that UAP56 and Sub2p are functional homologs is provided by the finding that UAP56 can support growth of a lethal *sub2Δ* mutant³⁴. Kistler and Guthrie³² postulated that if Sub2p is involved

Fig. 3 An RNA switch at the 5' splice site. The consensus sequences are those from yeast¹⁹. The cleavage site is indicated by an arrow at the exon/intron border. Yeast U1 snRNA forms base-pair interactions with the 5' ss. U1-C protein can be crosslinked to the pre-mRNA and the green star shows its relative position⁵⁷. The U1/5' ss interaction is disrupted at the expense of a mutually exclusive interaction between U6 snRNA and the 5' ss²³. ATP hydrolysis is necessary, and the two DEXH/D box proteins Prp28p and Brr2p act in this switch. The relative position in the intron (+7/+8 with respect to the site of cleavage) to which hPrp28p can be crosslinked⁴¹ is indicated (pink flash).



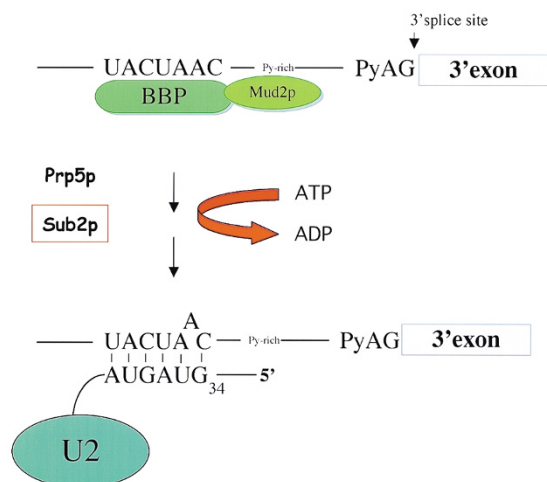
in the change of pairing partners at the BPS, its essential function might be to antagonize Mud2p and/or BBP, which must be displaced before U2 snRNP can gain access (Fig. 4). They found that deletion of the non-essential *MUD2* gene rescued the lethality of a *sub2Δ* strain. Given that Sub2p is a member of the DEXH box family, one can speculate that Sub2p hydrolyzes ATP to dislodge Mud2p. Zhang and Green³⁴ find that mutations of Sub2p at positions corresponding to amino acids essential for ATP hydrolysis in other DEXH/D box proteins inactivate Sub2p *in vivo*. However, it remains to be demonstrated that Sub2p has NTPase activity and, if so, whether Sub2p requires its ATPase activity to displace Mud2p (and possibly BBP) or to effect any other conformational changes during spliceosome assembly. *In vitro* studies using extracts from different *sub2* mutants suggest a role for the protein at several stages during spliceosome formation^{32–34}.

Prp28p function is not rate-limiting *in vivo* when the U1/5' ss interaction is weakened and Sub2p is not essential when the Mud2 protein is absent. However, the bypass strains fail to grow at low temperatures^{30,31}. A detailed analysis of pre-mRNA splicing in the bypass strains may uncover intermediate steps that may otherwise not be apparent, much in the way in which conditional mutants have been instrumental to define the splicing pathway at its current level of resolution^{18,19}. These strains also offer an opportunity to separate the ATP requirements of Prp28p and Sub2p from those of other DEXH/D box proteins that act at nearby steps. For example, Prp5p functions temporally near Sub2p (Fig. 4) and Brr2p acts close to Prp28p (Fig. 3).

Thus, the recent findings by Chen *et al.*³¹ and Kistler and Guthrie³² provide the means to dissect the splicing reaction in greater detail and they clearly define specific ribonucleoproteins within the spliceosome as targets for DEXH/D ATPases.

'Non-helicase' DEXH box ATPases

Exemplary DEXH ATPases that lack intrinsic helicase function but act nonetheless to remodel protein nucleic acid complexes include the chromatin remodeling factor SWI2/SNF2 and vaccinia virus



transcription termination factor NPH-I. NPH-I acts on the RNA polymerase ternary complex to effect the release of nascent mRNA chains in response to an upstream termination signal in the RNA. The ATPase activity of NPH-I, which is absolutely dependent on single-stranded DNA, is essential for termination^{50,51}. It is proposed that ATP hydrolysis is triggered by interaction of NPH-I with single stranded DNA in the transcription bubble.

SWI2/SNF2 enzymes, often as part of a large multiprotein complex, elicit structural alterations in the DNA–histone complex to make the DNA more accessible to transcription factors and the DNA repair machinery⁵². The DNA-stimulated ATPase activity of SWI2/SNF2 is crucial to its function and conserved residues in motifs that distinguish this DEXH family are important for *in vivo* and *in vitro* activity^{53,54}.

Common themes?

RNPase activity can now be added to the functional repertoire of DEXH/D box proteins along with NTP hydrolysis (the common denominator) and, in some cases, RNA unwinding. Is there a unifying mechanistic theme to all DEXH/D box proteins or are there fundamental differences in how energy is used? The question can be reduced to the issue of whether all DEXH/D box NTPases can translocate along RNA, powered by NTP hydrolysis, and effectively act as molecular cowcatchers (as on a locomotive) to sweep proteins and/or RNA duplexes out of their way. Some DEXH/D proteins may translocate, yet fail to score in a conventional *in vitro* RNA helicase assay, which typically demands unwinding of RNA duplexes of 15–30 base pairs. A translocation mechanism could accommodate the emerging models that implicate DEXH/D box proteins as catalysts of conformational changes in the spliceosome^{18,31,32}.

The NPH-II model of RNPase activity establishes proof-of-principle, but it does not resolve a fundamental question of whether protein displacement is necessarily coupled to RNA unwinding, because the U1A binding site is embedded within an RNA duplex¹⁶. The challenge for future studies is to devise substrates and assay systems to measure protein translocation along an RNA molecule independent of duplex unwinding.

Fig. 4 Switch of pairing partners at the branchpoint/3' splice site region. Shown are the yeast branchpoint and 3' ss consensus sequences¹⁹. The BPS/3' ss region in the intron is recognized by BBP and Mud2p (SF1/mBBP and U2AF65, respectively in the mammalian system²⁰). Interaction of the U2 snRNA with the BPS requires the DEAD ATPase Prp5p and the DEXH protein Sub2p.



An alternative model that invokes fundamental mechanistic differences among various DExH/D box proteins could entail direct binding of the DExH/D box NTPase to an RNA-binding protein (or next to it on RNA), leading directly to its displacement without translocation.

A biochemical characterization of each DExH/D box family member with respect to NTP hydrolysis and nucleic acid unwinding parameters (directionality, processivity, and so forth) is necessary to draw inferences about *in vivo* mechanisms. Valuable insights have been gleaned from the crystal structures of a helicase domain of the hepatitis C virus NS3, a DExH box protein^{11,12}. Structural analyses of other members of the family may help resolve unanswered questions regarding cofactor and nucleotide specificity and directionality of unwinding/translocation.

In summary, functions other than NTP hydrolysis cannot be attributed to DExH/D box proteins based solely on sequence conservation. Even highly related proteins, for example Prp22p and Prp2p or NPH-II and NPH-I, can differ in their biochemical properties (helicase *versus* non-helicase)^{6,25,29,30,50}. The ongoing identification of natural targets and cofactors for DExH/D box proteins will be critical to understand their biological functions.

Acknowledgments

I wish to thank C. Query for sharing his findings and for critical commentary on the manuscript. I am grateful to my colleagues for communicating results prior to publication.

Received 21 December, 2000; accepted 4 January, 2001.

- Gorbalenya, A.E. & Koonin, E.V. Helicases: amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol.* **3**, 419-429 (1993).
- de la Cruz, J., Kressler, D. & Linder, P. Unwinding RNA in *Saccharomyces cerevisiae*: DEAD-box proteins and related families. *Trends Biochem. Sci.* **24**, 192-198 (1999).
- Hall, M.C. & Matson, S.W. Helicase motifs: the engine that powers DNA unwinding. *Mol. Microbiol.* **34**, 867-877 (1999).
- Pause, A. & Sonenberg, N. Mutational analysis of a DEAD box RNA helicase: the mammalian translation initiation factor eIF-4A. *EMBO J.* **11**, 2643-2654 (1992).
- Martins, A., Gross, C.H. & Shuman, S. Mutational analysis of vaccinia virus nucleoside triphosphate phosphohydrolase I, a DNA-dependent ATPase of the DExH box family. *J. Virol.* **73**, 1302-1308 (1999).
- Gross, C.H. & Shuman, S. Mutational analysis of vaccinia virus nucleoside triphosphate phosphohydrolase II, a DExH box RNA helicase. *J. Virol.* **69**, 4727-4736 (1995).
- Gross, C.H. & Shuman, S. The QRxGRxGR'G motif of the vaccinia virus DExH box RNA helicase NPH-II is required for ATP hydrolysis and RNA unwinding but not for RNA binding. *J. Virol.* **70**, 1706-1713 (1996).
- Heilek, G.M. & Peterson, M.G. A point mutation abolishes the helicase but not the nucleoside triphosphatase activity of hepatitis C virus NS3 protein. *J. Virol.* **71**, 6264-6266 (1997).
- Gross, C.H. & Shuman, S. The nucleoside triphosphatase and helicase activities of vaccinia virus NPH-II are essential for virus replication. *J. Virol.* **72**, 4729-4736 (1998).
- Schwer, B. & Meszaros, T. RNA helicase dynamics in pre-mRNA splicing. *EMBO J.* **19**, 6582-6591 (2000).
- Yao, N. *et al.* Structure of the hepatitis C virus RNA helicase domain. *Nature Struct. Biol.* **4**, 463-467 (1997).
- Kim, J.L. *et al.* Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding. *Structure* **6**, 89-100 (1998).
- Gross, C.H. & Shuman, S. Vaccinia virions lacking the RNA helicase nucleoside triphosphate phosphohydrolase II are defective in early transcription. *J. Virol.* **70**, 8549-8557 (1996).
- Jankowsky, E., Gross, C.H., Shuman, S. & Pyle, A.M. The DExH protein NPH-II is a processive and directional motor for unwinding RNA. *Nature* **403**, 447-451 (2000).
- Gross, C.H. & Shuman, S. Vaccinia virus RNA helicase: nucleic acid specificity in duplex unwinding. *J. Virol.* **70**, 2615-2619 (1996).
- Jankowsky, E., Gross, C.H., Shuman, S. & Pyle, A.M. Active disruption of an RNA-protein interaction by a DExH/D RNA helicase. *Science* **291**, 121-125 (2001).
- Lorsch, J.R. & Herschlag, D. The DEAD box protein eIF4A. 1. A minimal kinetic and thermodynamic framework reveals coupled binding of RNA and nucleotide. *Biochemistry* **37**, 2180-2193 (1998).
- Staley, J.P. & Guthrie, C. Mechanic devices of the spliceosome: motors, clocks, springs, and things. *Cell* **92**, 315-326 (1998).
- Burge, C.B., Tuschl, T.H. & Sharp, P.A. In *RNA world II* (eds. Gesteland, R.F., Cech, T.R. & Atkins, J.F.) 525-560 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; 1999).
- Kramer, A. The structure and function of proteins involved in mammalian pre-mRNA splicing. *Annu. Rev. Biochem.* **65**, 367-409 (1996).
- Collins, C.A. & Guthrie, C. The question remains: Is the spliceosome a ribozyme? *Nature Struct. Biol.* **7**, 850-854 (2000).
- Ye, S.L., Wuenschell, G., Termini, J. & Lin, R.J. Metal-ion coordination by U6 small nuclear RNA contributes to catalysis in the spliceosome. *Nature* **408**, 881-884 (2000).
- Madhani, H.D. & Guthrie, C. Dynamic RNA-RNA interactions in the spliceosome. *Annu. Rev. Genet.* **28**, 1026 (1994).
- Nilsen, T.W. RNA-RNA interactions in the spliceosome: unraveling the ties that bind. *Cell* **78**, 1-4 (1994).
- Kim, S.H., Smith, J., Claude, A. & Lin, R.J. The purified yeast pre-mRNA splicing factor PRP2 is an RNA-dependent NTPase. *EMBO J.* **11**, 2319-2326 (1992).
- Kim, S.H. & Lin, R.J. Pre-mRNA splicing within an assembled yeast spliceosome requires an RNA-dependent ATPase and ATP hydrolysis. *Proc. Natl. Acad. Sci. USA* **90**, 888-892 (1993).
- Schwer, B. & Guthrie, C. PRP16 is an RNA-dependent ATPase that interacts transiently with the spliceosome. *Nature* **349**, 494-499 (1991).
- Schwer, B. & Guthrie, C. A conformational rearrangement in the spliceosome is dependent on PRP16 and ATP hydrolysis. *EMBO J.* **11**, 5033-5039 (1992).
- Schwer, B. & Gross, C.H. Prp22, a DExH-box RNA helicase, plays two distinct roles in yeast pre-mRNA splicing. *EMBO J.* **17**, 2086-2094 (1998).
- Wagner, J.D., Jankowsky, E., Pyle, A.M. & Abelson, J.N. The DEAD-box protein PRP22 is an ATPase that mediates ATP-dependent mRNA release from the spliceosome and unwinds RNA duplexes. *EMBO J.* **17**, 2926-2937 (1998).
- Chen, J.Y.F., Stands, L., Staley, J.P., Jackups, Jr., R.R., Latus, L.J. & Chang, T.H. Specific alterations of U1-C protein or U1 small nuclear RNA can eliminate the requirement of Prp28p, an essential DEAD-box splicing factor. *Mol. Cell in the press* (2001).
- Kistler, A.L. & Guthrie, C. Deletion of MUD2, the yeast homologue of U2AF65, can bypass the requirement for Sub2, an essential spliceosomal ATPase. *Genes Dev.* **15**, 42-49 (2001).
- Libri, D., Graziani, N., Saguez, C., & Boulay, J. Multiple roles for the yeast SUB2/UAP56 gene in splicing. *Genes Dev.* **15**, 36-41 (2001).
- Zhang, M. & Green, M.R. Identification and characterization of yUAP/Sub2p, a yeast homologue of the essential human pre-mRNA splicing factor hUAP56. *Genes Dev.* **15**, 30-35 (2001).
- Chang, T.H., Latus, L.J., Liu, Z. & Abbott, J.M. Genetic interactions of conserved regions in the DEAD-box protein Prp28p. *Nucleic Acids Res.* **25**, 5033-5040 (1997).
- Will, C.L., Rümpler, S., Gunnewiek, J.K., van Venrooij, W.J. & Lührmann, R. In vitro reconstitution of mammalian U1 snRNPs active in splicing: the U1-C protein enhances the formation of early (E) spliceosomal complexes. *Nucleic Acids Res.* **24**, 4614-4623 (1996).
- Tang, J., Abovich, N., Fleming, M., Séraphin, B. & Rosbash, M. Identification and characterization of a yeast homologue of U1 snRNP-specific protein C. *EMBO J.* **16**, 4082-4091 (1997).
- Heinrichs, V., Bach, M., Winkelmann, G. & Lührmann, R. U1-specific protein C needed for efficient complex formation of U1 snRNP with a 5' splice site. *Science* **247**, 69-71 (1990).
- Staley, J.P. & Guthrie, C. An RNA switch at the 5' splice site requires ATP and the DEAD box protein Prp28p. *Mol. Cell* **3**, 55-64 (1999).
- Teigelkamp, S., Mundt, C., Achsel, T., Will, C.L. & Lührmann, R. The human U5 snRNP-specific 100-kD protein is an RS domain-containing, putative RNA helicase with significant homology to the yeast splicing factor Prp28p. *RNA* **3**, 1313-1326 (1997).
- Ismail, N., Sha, M., Gustafson, H. & Konarska, M.M. The 100 kD U5 snRNP protein (hPrp28p) contacts the 5' splice site through its ATPase site. *RNA in the press* (2001).
- Strauss, E.J. & Guthrie, C. PRP28, a 'DEAD-box' protein, is required for the first step of pre-mRNA splicing in vitro. *Nucleic Acids Res.* **22**, 3187-3193 (1994).
- Abovich, N., Liao, X.C. & Rosbash, M. The MUD2 protein: an interaction with PRP11 defines a bridge between commitment complexes and U2 snRNP addition. *Genes Dev.* **8**, 843-854 (1994).
- Berglund, J.A., Chua, K., Abovich, N., Reed, R. & Rosbash, M. The splicing factor BBP interacts specifically with the pre-mRNA branchpoint sequence UACUAAAC. *Cell* **89**, 781-787 (1997).
- Berglund, J.A., Abovich, N. & Rosbash, M. A cooperative interaction between U2AF65 and mBPP/SF1 facilitates branchpoint region recognition. *Genes Dev.* **12**, 858-867 (1998).
- Ruby, S.W., Chang, T.H. & Abelson, J. Four yeast spliceosomal proteins (PRP5, PRP9, PRP11, and PRP21) interact to promote U2 snRNP binding to pre-mRNA. *Genes Dev.* **7**, 1909-1925 (1993).
- O'Day, C.L., Dalbadie-McFarland, G. & Abelson, J. The *Saccharomyces cerevisiae* Prp5 protein has RNA-dependent ATPase activity with specificity for U2 small nuclear RNA. *J. Biol. Chem.* **271**, 33261-33267 (1996).
- Perriman, R. & Ares, Jr., M. ATP can be dispensable for prespliceosome formation in yeast. *Genes Dev.* **14**, 97-107 (2000).
- Fleckner, J., Zhang, M., Valcárcel, J. & Green, M.R. U2AF65 recruits a novel human DEAD box protein required for the U2 snRNP-branchpoint interaction. *Genes Dev.* **11**, 1864-1872 (1997).
- Deng, L. & Shuman, S. Vaccinia NPH-I, a DExH-box ATPase, is the energy coupling factor for mRNA transcription termination. *Genes Dev.* **12**, 538-546 (1998).
- Christen, L.M., Sanders, M., Wiler, C. & Niles, E.G. Vaccinia virus nucleoside triphosphate phosphohydrolase I is an essential viral early gene transcription termination factor. *Virology* **245**, 360-371 (1998).
- Travers, A. An engine for nucleosome remodeling. *Cell* **96**, 311-314 (1999).
- Laurent, B.C., Treich, I. & Carlsson, M. The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. *Genes Dev.* **7**, 583-591 (1993).
- Richmond, E. & Peterson, C.L. Functional analysis of the DNA-stimulated ATPase domain of yeast SWI2/SNF2. *Nucleic Acids Res.* **24**, 3685-3692 (1996).
- Varani, L. *et al.* The NMR structure of the 38 kDa U1A protein-PIE RNA complex reveals the basis for cooperativity in regulation of polyadenylation by human U1A protein. *Nature Struct. Biol.* **7**, 329-335 (2000).
- Arenas, J. & Abelson, J. Prp43: an RNA helicase-like factor involved in spliceosome disassembly. *Proc. Natl. Acad. Sci. USA* **94**, 11798-11802 (1997).
- Zhang, D. & Rosbash, M. Identification of eight proteins that cross-link to pre-mRNA in the yeast commitment complex. *Genes Dev.* **13**, 581-592 (1999).