The NineTeen Complex (NTC) and NTC-associated proteins as targets for spliceosomal ATPase action during pre-mRNA splicing

Rogerio Alves de Almeida and Raymond T O’Keefe
Facility of Life Sciences; The University of Manchester; Manchester, UK

Introduction

Pre-mRNA splicing is an essential step in gene expression that removes intron sequences efficiently and accurately to produce a mature mRNA for translation. It is the large and dynamic RNA-protein complex called the spliceosome that catalyzes intron removal. To carry out splicing, the spliceosome not only needs to assemble correctly with the pre-mRNA but the spliceosome requires extensive remodelling of its RNA and protein components to execute the 2 steps of intron removal. Spliceosome remodelling is achieved through the action of ATPases that target both RNA and proteins to produce spliceosome conformations competent for each step of spliceosome activation, catalysis and disassembly. An increasing amount of research has pointed to the spliceosome associated NineTeen Complex (NTC) of proteins as targets for the action of a number of the spliceosomal ATPases during spliceosome remodelling. In this point-of-view article we present the latest findings on the changes in the NTC that occur following ATPase action that are required for spliceosome activation, catalysis and disassembly. We proposed that the NTC is one of the main targets of ATPase action during spliceosome remodelling required for pre-mRNA splicing.

Keywords: ATPase, Brr2, Cwc2, NineTeen Complex, PremRNA splicing, Prp19, Prp2, Prp16, Prp43, RNA helicase

© Rogerio Alves de Almeida and Raymond T O’Keefe
*Correspondence to: Raymond T O’Keefe; Email: rokeefe@manchester.ac.uk
Submitted: 10/31/2014
Revised: 12/04/2014
Accepted: 12/04/2014
http://dx.doi.org/10.1080/15476286.2015.1008926
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.
Figure 1. NTC protein remodelling by ATPases during the spliceosome assembly, activation and disassembly process. The pathway of complexes formed on the pre-mRNA during spliceosome assembly, activation and disassembly are indicated with arrows and the names of each complex are given below the complex. The ATPases are shown in red under the arrow for the step that each ATPase promotes. The NTC core complex (dark orange) and NTC-associated proteins (light orange) are shown below each complex that they associate with. Arrows from the NTC complex are used to indicate the proteins that leave following ATPase action. Question marks are used to indicate that experimental evidence for Yju2 removal from the NTC and Brr2 action during spliceosome disassembly is not in agreement.
the intron and join the 2 exons. The resulting splicing complex is called the post-splicing complex and this complex must be disassembled to release the mRNA, leaving the intron associated with the snRNPs as the intron-lariat spliceosome (ILS). Finally, the ILS is disassembled allowing recycling of the snRNPs for subsequent rounds of splicing. The rearrangements and conformational changes required for spliceosome assembly, activation and disassembly are catalyzed by the spliceosomal ATPases. In this "Point of View" we will describe an increasing amount of experimental evidence that identifies the NTC, and NTC-associated proteins, as targets for a number of the spliceosomal ATPases required for remodelling the spliceosome during premRNA splicing. We will concentrate on the yeast Saccharomyces cerevisiae system where there is the most evidence to date for this idea.

There are 8 ATPases involved in splicing (Table 1) and they share 8 conserved motifs divided into 2 domains, RecA1 and RecA2. The spliceosomal ATPases are used to modulate RNA-RNA, RNA-protein and protein-protein interactions during the splicing cycle. The ATPases promote correct conformations of the spliceosome for progression through the 2 steps of intron removal with accuracy and fidelity. It is the second role of some of the ATPases, that of proofreading certain steps of splicing, which provides the fidelity in the splicing process. Spliceosome conformations are monitored by the ATPases and can be rejected if a certain complex is not formed correctly or in a timely manner. The order of action of the ATPases during splicing is Sub2, Prp5, Prp28, Brt2, Prp2, Prp16, Prp22, Prp43 and Brt2 (Fig. 1). Of these ATPases, the action of Prp2, Prp16, Prp22, Prp43 and Brt2 appear to be related to changes in the interactions of the NTC and NTC-associated proteins with the spliceosome. The modulation of the NTC, therefore, is essential for the progression of the spliceosome through the splicing cycle.

The NTC and NTC-associated proteins are found within the B, B*, C, post-splicing complex and ILS (Fig. 1). The core NTC complex nucleated by Prp19, is found in all these complexes, it is composed of 8 proteins, but an additional 18 NTC-associated proteins interact with, or co-purify with, these core proteins and can be found in one or more of the splicing complexes. At the core of the NTC, Prp19 forms tetramers via its central coiled-coil domain which are bound by Cef1. Prp19 also contains WD domains with 2 molecules of Cwc2 interacting with these WD domains in the Prp19 tetramer. The NTC is linked to the spliceosome active site through Cwc2 which interacts directly with both the U6 snRNA and the pre-mRNA. Recently, the S. pombe homolog of Cef1, called Cdc5, has been shown to bind double stranded RNA in vitro suggesting that Cef1/Cdc5 may also link the NTC to the active site RNAs of the spliceosome. The NTC, and NTC-associated proteins, are involved in a number of the spliceosome rearrangements and conformational changes during the splicing cycle. Associations and interactions of certain NTC proteins change following the action of certain ATPases. We now present the latest findings on the changes in the NTC that occur following ATPase action that are required for spliceosome activation, catalysis and disassembly.

**Prp2 Remodelling of the Spliceosome for the First Step of Splicing Includes NTC Protein Remodelling**

During the transition of the spliceosome B complex to the B* complex, which is now competent for the first step of splicing, it is the ATPase Prp2 that makes the final rearrangement of the spliceosome. The remodelling by Prp2 involves a number of NTC proteins. Prp2 action releases the SF3a/b complex associated with the U2 snRNP. The removal of the SF3a/b complex creates high affinity binding sites for the NTC proteins Cwc25 and Yju2. Both Cwc25 and Yju2 are required to enable the catalytically-activated spliceosome to carry out the first step of splicing. The action of Prp2 requires the NTC-associated proteins Spp2 and Cwc22. Prp2 remodelling also involves dissociation of the NTC proteins Cwc24 and Cwc27. Therefore, it is clear that the action of Prp2 not only requires NTC proteins but Prp2 action results in the association and dissociation of NTC proteins with the spliceosome required for the transition through the first step of splicing.

**Prp16 Acts Through the NTC to Rearrange the Spliceosome for the Second Step of Splicing**

Prp16 is an ATPase that proofreads the first step of splicing and promotes rearrangement of the spliceosome during the second step of splicing. During splicing a series of RNA-RNA interactions occur, and one crucial interaction is between the U2 and U6 snRNAs which base-pair to form helix I. The U2/U6 helix I is formed following the release of the U1 and U4 snRNPs, with helix I required for the first step of splicing then the second step and exon joining. It has been suggested that sequences encompassing helix I (U6 AGC triad) may form

---

**Table 1. Yeast Spliceosomal ATPases**

| ATPase       | Family   | Function                                              |
|--------------|----------|-------------------------------------------------------|
| Sub2/UAP56   | DEAD-box | Association of U2 snRNA with pre-mRNA                 |
| Prp5         | DEAD-box | Proofreads U2-branchsite interaction                   |
| Prp28        | DEAD-box | Release of U1 by disrupting the base-pairing          |
| Prp2         | DEAH-box | Release of SF3a/SF3b                                   |
| Prp16        | DEAH-box | Release of Yju2 and Cwc25                              |
| Prp22        | DEAH-box | U2/U6 helix I remodelling                             |
| Prp43        | DEAH-box | Release of mature mRNA                                 |
| Brt2         | Ski2-like| Disassembly of the ILS                                 |
|              |          | Disrupt U4/U6 base-pairing                            |
|              |          | Disrupt U2/U6 base-pairing                            |
tertiary interactions with the U6 ACA-GAGA box and the U6 internal stem loop (ISL) to bind a metal ion, enabling the spliceosome to have an active site resembling that found in group II introns. Evidence over time has pointed to a role for Prp16 in modulating a conformational change in the spliceosome involving the U2/U6 helix I. Following the second step of splicing, it is known that Prp2, stabilizes U2/U6 helix I and appears to antagonize Prp16 action. The interactions of Cwc2 with the U6 snRNA and the pre-mRNA are influenced by Prp16 mutation. The prp16–302 allele stabilizes Cwc2 interactions with the U6 snRNA and destabilizes Cwc2 interactions with the pre-mRNA indicating that Cwc2 is one target for Prp16 action during splicing. Additionally, we have found that another NTC protein, Cwc2, interacts only transiently with the spliceosome, its influence on U2/U6 helix I must be applied through other spliceosome proteins. The first clue to how Prp16 could exert its action was found when deletion of the NTC protein Isy1 was shown to suppress the cold sensitive prp16–302 allele. This was the first link between Prp16 action and the NTC complex. Recently, we have found that another NTC protein, Cwc2, stabilizes U2/U6 helix I and appears to antagonize Prp16 action. The interactions of Cwc2 with the U6 snRNA and the pre-mRNA are influenced by Prp16 mutation. The prp16–302 allele stabilizes Cwc2 interactions with the U6 snRNA and destabilizes Cwc2 interactions with the pre-mRNA indicating that Cwc2 is one target for Prp16 action during splicing. Additionally, we have found that Cwc2 and Isy1 functionally cooperate during splicing. All together, these data point to the NTC proteins Isy1 and Cwc2, either directly or indirectly, as targets for Prp16 action in helix I remodelling during splicing.

In addition to modulating RNA-RNA and RNA-protein interactions between the 2 steps of splicing, there is evidence from both immunoprecipitation experiments and a purified yeast splicing system that Prp16 can also modulate the interactions of NTC proteins with the spliceosome prior to the second step of splicing. The binding of NTC proteins Cwc2 and Yju2 to the spliceosome, catalyzed by Prp2, is required to promote the first step of splicing. Following the first step of splicing Cwc25, and possibly Yju2, are removed to most likely allow new factors to bind to the spliceosome and promote the second step of splicing. It is the action of Prp16 that removes Cwc25, and potentially Yju2, after the first step of splicing. Using immunoprecipitation it was first shown that the action of Prp16 resulted in the release of Cwc25 and Yju2 from the spliceosome. However, recent work utilizing a purified yeast splicing system combined with mass spectrometry and dual-color fluorescence cross-correlation spectroscopy has found that Prp16 action causes a structural change in the spliceosome that reduces the binding affinity of Cwc25 allowing subsequent dissociation of Cwc25, but Prp16 action was not observed with this system to dissociate Yju2. Despite the conflicting data on Yju2 dissociation from the spliceosome, it is clear that the action of Prp16 influences the affinity of NTC proteins for the spliceosome to allow the second step of splicing.

### Prp22 Disassociates the NTC Proteins Cwc21 and Cwc22 During Spliceosome Disassembly Along with the RES Complex

Following the second step of splicing the post-splicing complex must be disassembled to release the mRNA and recycle the snRNPs. The first step of the disassembly process is carried out through the action of the ATPase Prp22. During splicing, the U5 snRNA interacts with the 5′ exon and 3′ exon sequences to align the 2 exons for joining during the second step of splicing. After the second step of splicing, the interactions of the U5 snRNA with the 2 exon sequences are disrupted by the ATPase activity of Prp22 promoting release of the mature mRNA. Recent use of the purified yeast splicing system combined with mass spectrometry to follow the spliceosome disassembly process has revealed how the protein composition of the post-splicing complex changes following Prp22 action. It was found that the NTC-associated proteins Cwc21 and Cwc22 are significantly reduced in the ILS produced by Prp22 action. In addition, the RES (RETention and Splicing) complex proteins were also found to be significantly less abundant, or absent, from the ILS following Prp22 action. The RES complex associates with the B complex along with the NTC and is required for enhancing the splicing of certain pre-mRNAs and retention of unspliced pre-mRNAs. Significantly, the RES complex protein Bud13/Cwc26 is an NTC protein found to associate with Cef1. Therefore, it appears that the action of Prp22 targets proteins of the NTC to induce spliceosome disassembly.

### Prp43 Disassociates the ILS to Allow Recycling of the snRNPs and the NTC for Further Rounds of Splicing

The second phase of spliceosome disassembly involves the removal of the snRNPs from the intron lariat RNA, but also dissociation of the snRNPs from each other, allowing the snRNPs to be recycled for subsequent rounds of splicing. The ATPase Prp43 is recruited for this disassembly step of the spliceosome. Prp43 associates with Ntr1 and Ntr2 (NTC-related proteins) and forms the NTR complex. It is Ntr1 that activates the ATPase activity of Prp43 to trigger release of the snRNPs from the intron lariat. The use of the purified yeast splicing system combined with mass spectrometry has also revealed how the protein composition of the snRNPs changes following Prp43 action to release the intron lariat and the snRNPs from each other. It has been found that the action of Prp43 completely dissociates the NTC protein Ntc20 from the snRNPs and intron-lariat. The other NTC proteins in the ILS appear to remain associated with the U2 and U5 snRNPs as well as the intron-lariat, but it is not clear how the NTC proteins are then further recycled from the released snRNPs and intron-lariat following Prp43 action. Nevertheless, it is apparent that the action of Prp43 influences NTC proteins during disassembly of the ILS.

### Brr2 is Linked to the ATPases that Remodel the NTC During Splicing

The ATPase Brr2 is an essential U5 snRNP protein involved in remodeling RNA-RNA interactions during spliceosomal activation and disassembly. Brr2 disrupts the base-pairing of the U4/U6
snRNAs to promote the release of U4, but once the catalytic steps of splicing are completed, Br2 again disrupts the base-pairing of U2/U6 marking the start of the spliceosome disassembly process.\textsuperscript{27-59} Br2 activity is regulated by the GT/Pase Snu114.\textsuperscript{60} Br2 contains 2 helicase casettes, with the N-terminal cassette able to hydrolyse ATP whereas the C-terminal cassette has evolved into a protein binding module.\textsuperscript{60} While it does not appear that Br2 ATPase action directly influences the NTC, Br2 is known to interact with a number of ATPases that do remodel the NTC during splicing. Br2 has been shown to interact with Prp2 by the 2-hybrid assay but also directly by pull-down assays.\textsuperscript{61,62} It has been proposed that Prp2 is recruited to the spliceosome by its interaction with Br2.\textsuperscript{61} Br2 has also been shown to interact with Prp16 which may be the way in which Prp16 associates with the spliceosome.\textsuperscript{62} Prp43 interacts with Ntr1 and Ntr2, with Prp43 being recruited to the spliceosome through Ntr2 interaction with Br2.\textsuperscript{63} Ntr2 binding to Br2 may be prevented by Prp16 and Snu7 binding to Br2 providing a mechanism by which Prp43 action is regulated.\textsuperscript{64} Overall, Br2 appears to be a binding platform for a number of the ATPases that modulate the NTC during splicing, indirectly linking Br2 to NTC dynamics during splicing.

Conclusions and Future Directions

It is clear that the action of the ATPases Prp2, Prp16, Prp22 and Prp43 are related to the modulation of the NTC and NTC-associated proteins with the spliceosome during the splicing cycle. These changes in the NTC brought about by ATPase action are essential for providing the spliceosome conformations required for the first and second steps of splicing. Additionally, ATPase action is also required to modulate NTC interactions during spliceosome disassembly. Once the NTC is assimilated into the spliceosome it may not operate as a discrete complex as it appears only certain NTC proteins are modulated by ATPase action. Alternatively, it may be that the whole NTC is modulated by ATPase action but evidence is now only available for a few of the NTC proteins. In many cases it is not known whether the ATPases act directly or indirectly on the NTC proteins. In future, it will be important to determine the interaction network within the spliceosome by which the actions of the ATPases are transmitted to the NTC. There is no evidence to date for the action of the ATPases Sub2, Prp5 and Prp28 influencing the NTC as they act before the association of the NTC with the spliceosome. In humans a number of other DExD box ATPases like DDX5 (p68) and DDX17 (p72) are associated with the spliceosome.\textsuperscript{65} It is conceivable that the action of other ATPases may induce conformations during spliceosome assembly that allows incorporation of the NTC and NTC-associated proteins with the spliceosome. Nevertheless, the NTC appears to be a major target for ATPase remodelling of the spliceosome and the NTC is therefore intimately associated with the essential remodelling steps required for pre-mRNA splicing.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Due to space limitations we apologize to authors whose relevant primary publications were not cited. We would like to thank an anonymous reviewer for excellent comments and suggestions for improving the manuscript.

Funding

The work was supported by the Biotechnology and Biological Sciences Research Council.

References

1. Singh RK, Cooper TA. Pre-mRNA splicing in disease and therapeutics. Trends Mol Med 2012; 18:472-82; PMID:22819011; http://dx.doi.org/10.1016/j.\ molmed.2012.06.006
2. Chen HC, Chia SC. Functional roles of protein splicing factors. Bioessays Rep 2012; 32:345-59; PMID:22762203; http://dx.doi.org/10.1002/bies.20120007
3. Wahl MC, Will CL, Lührmann R. The spliceosome: design principles of a dynamic RNP machine. Cell 2009; 136:701-18; PMID:19239890; http://dx.doi.\ org/10.1016/j.cell.2009.02.009
4. Chan SP, Kao DI, Tsai WY, Cheng SC. The Prp19p-associated complex in spliceosome activation. Science 2003; 302:279-82; PMID:12970570; http://dx.doi.org/10.1126/science.1086602
5. Hogg R, McGrai JC, O’Keefe RT. The function of the NineTern Complex (NTC) in regulating spliceosome conformations and fidelity during pre-mRNA splicing. Biochem Soc Trans 2010; 38:1115-8; PMID:20659013; http://dx.doi.org/10.1042/BST0381110
6. Saha D, Khandelia P, O’Keefe RT, Vajargahyan U. Saccharomyces cerevisiae NineTern complex (NTC)-associated factor Bud31/Tc68/3w assembles on precatalytic spliceosomes and improves first and second step pre-mRNA splicing efficiency. J Biol Chem 2012; 287:5390-9; PMID:22221566; http://dx.doi.org/10.1074/jbc.M111.298547
7. Ohto T, Prior M, Dannenberg J, Odenwalder P, Dykhov O, Rasche N, Schmitzova J, Gregor I, Fabrizio P, Enderlein J, et al. Prp2-mediated protein rearrangements at the catalytic core of the spliceosome as revealed by deCCs. RNA 2012; 18:1244-56; PMID:22535589; http://dx.doi.org/10.1261/rna.028516.112
8. Ohto T, Odenwalder P, Dannenberg J, Prior M, Warocki Z, Schmitzova J, Karaduman R, Gregor I, Enderlein J, Fabrizio P, et al. Molecular dissection of step 2 catalysis of yeast pre-mRNA splicing investigated in a purified system. RNA 2013; 19:902-15; PMID:23685439; http://dx.doi.org/10.1261/rna.039024.113
9. Cordin O, Begij JD. RNA helicases in splicing. RNA Biol 2010; 10:83-95; PMID:23229095; http://dx.doi.org/10.4161/rna.10.1.10511
10. Jankowsky E. RNA helicases at work: binding and rearranging. Trends Biochem Sci 2011; 36:19-29; PMID:20813532; http://dx.doi.org/10.1016/j.\ tibs.2010.07.008
11. Burgess SM, Gunthie A. A mechanism to enhance mRNA splicing fidelity: The RNA-dependent ATPase Prp16 governs usage of a discordant pathway for aberrant lariat intermediates. Cell 1993; 73:1377-91; PMID:8324826; http://dx.doi.org/10.1016/0092-8674\(1993\)00434-3
12. Koodarthingil P, Novak T, Picciollo JA, Staley JP. The DEAH box ATPases Prp16 and Prp43 cooperate to proofread 5′ splice site cleavage during pre-mRNA splicing. Mol Cell 2010; 39:385-95; PMID:20705241; http://dx.doi.org/10.4161/rna.22547
13. Koodarthingil P, Staley JP. Splicing fidelity: DEAH-box ATPases as molecular clocks. RNA Biol 2013; 10:1073-9; PMID:23770752; http://dx.doi.org/10.4161/rna.25245
14. Smith DJ, Query CC, Konarska MM. “Nought may endure but mutability”: Spliceosome dynamics and the regulation of splicing. Mol Cell 2008; 30:657-66; PMID:18570869; http://dx.doi.org/10.1016/j.r\na.2008.04.013
15. Whedever AM, Staley JP. The DExD/H-box ATPase Prp2p destabilizes and proofreads the catalytic RNA core of the spliceosome. RNA 2014; 20:282-94; PMID:24442613; http://dx.doi.org/10.1261/\rne.042598.113
16. Fabrizio P, Dannenberg J, Dube P, Kastner R, Stark H, Urlaub H, Lührmann R. The evolutionarily conserved core design of the catalytic activation step of the yeast spliceosome. Mol Cell 2009; 36:593-608; PMID:19941820; http://dx.doi.org/10.1016/j.molcel.2008.09.040
17. Fourmann JB, Schmitzova J, Christian H, Urlaub H, Ficner R, Boon KL, Fabrizio P, Lührmann R. Dissection of the factor requirements for spliceosome disassembly and the elucidation of its dissociation products using a purified splicing system. Genes Dev 2013; 27:413-28; PMID:23431055; http://dx.doi.org/10.1101/gad.207797.112
18. Ohi MD, Vander Kooi CW, Rosenberg JA, Ren L, Hirsch JP, Chazin WJ, Walé T, Gould KL. Structural and functional analysis of essential pre-mRNA splicing
33. Madhani HD, Guthrie C. A novel base-pairing interaction between U2 and U6 snRNAs suggests a mechanism for the catalytic activation of the spliceosome. Cell 2004; 117:493-503; PMID:15245631; http://dx.doi.org/10.1016/j.cell.2004.06.017.

34. Fica SM, Turtel N, Novak T, Li NS, Lu J, Koodathinugil P, Dai Q, Staley JP, Piccirilli J. RNA catalyses pre-mRNA splicing. Nature 2013; 503:229-34; PMID:24197618.

35. Keating K, Caccamo N, Perlman PS, Pyle AM. A structural analysis of the group II intron active site and implications for the spliceosome. RNA 2010; 16:19-61; PMID:19948765; http://dx.doi.org/10.1261/rna.197310.

36. Lee C, Jaladat Y, Mohammadi A, Sharif A, Geisler S, Vidal-Ferran R, Matutes S, Pablos E, Fuchs Z. A spliceosome maestro: prp43 promotes both catalytic steps of pre-mRNA splicing in vivo. RNA 2010; 16:2226-38; PMID:20826700; http://dx.doi.org/10.1016/j.ymgme.2011.07.0910.

37. Madhani HD, Guthrie C. Genetic interactions between the yeast RNA helicase homolog Prp16 and spliceosomal snRNAs identify candidate ligands for the Prp16 RNA-dependent ATPase. Genes Dev 1994; 15:677-87; PMID:8088513.

38. Villa T, Guthrie C. The Isp1p component of the NineTeen complex interacts with the ATPase Prp16p to regulate the fidelity of pre-mRNA splicing. Genes Dev 2005; 19:1894-904; PMID:15913217; http://dx.doi.org/10.1261/mbc.e04-11-0343.

39. Li W, Thompson XP, Yates JR, 3rd, Stevens SW. Release of Sf3 from the intron branchpoint activates the first step of pre-mRNA splicing. RNA 2010; 16:516-28; PMID:20088683; http://dx.doi.org/10.1261/rna.203930.104.

40. Hamada HD, Zaug AJ, Beese LS. The yeast RNA helicase Ddx4 interacts directly with the U6 snRNA to link the snRNA to the spliceosome. Genes Dev 2009; 23:338-50; PMID:19213236; http://dx.doi.org/10.1101/gad.1336305.

41. Schwer B, Gross CH. Prp22, a DExH-box RNA helicase, promotes the first catalytic step of pre-mRNA splicing. Mol Cell 2010; 39:487-97; PMID:20844807.

42. Schwer B, Guthrie C. Prp22, a DExH-box RNA helicase, plays two distinct roles in yeast pre-mRNA splicing. EMBO J 1998; 17:2086-94; PMID:9542120; http://dx.doi.org/10.1093/emboj/17.13.2086.

43. Schwer B, Guthrie C. Prp22, a DExH-box RNA helicase, promotes both catalytic steps of pre-mRNA splicing. Genes Dev 1998; 12:3813-22; PMID:9688660; http://dx.doi.org/10.1101/gad.82049.

44. Newnam AJ, Seavwright J, Rosen MA, Lewin A, Faber PS, Artemisin P, Wang X, Elshawi I, Xue L, Li J, Zhang W, Zheng X, Weng W, Kowalczykowski SC. Splicing factor Cwc22 is required for the function of the spliceosome. Mol Cell 2004; 24:10101-20; PMID:15109921; http://dx.doi.org/10.1016/j.molcel.2004.05.003.

45. Newnam AJ, Seavwright J, Rosen MA, Lewin A, Faber PS, Artemisin P, Wang X, Elshawi I, Xue L, Li J, Zhang W, Zheng X, Weng W, Kowalczykowski SC. Splicing factor Cwc22 is required for the function of the spliceosome. Mol Cell 2004; 24:10101-20; PMID:15109921; http://dx.doi.org/10.1016/j.molcel.2004.05.003.

46. Frank X, Li JS, Jiao HK, Deng H, Grabowski M, Zhou Z, de Boer YL, Vegter SW, van der Fels-Kraeling J, Xu J, Mei P, Enright AJ, Mayo MS, van der Meer P. Dynamic interactions of Ntr1 with Prp43 to mediate spliceosome disassembly. Mol Cell 2008; 30:743-54; PMID:18570877; http://dx.doi.org/10.1016/j.molcel.2008.05.003.

47. O'Keefe RT, Norman C, Newman AJ. The invariant U5 snRNA loop 1 in the second catalytic step of yeast pre-mRNA splicing. EMBO J 1998; 17:565-74; PMID:9430647; http://dx.doi.org/10.1093/emboj/17.2.565.

48. O'Keefe RT, Newman AJ, Schwer B. A conformational rearrangement in the spliceosome sets the stage for Prp22-dependent mRNA release. Mol Cell 2008; 30:743-54; PMID:18570877; http://dx.doi.org/10.1016/j.molcel.2008.05.003.

49. Dreumont M, Seraphin B. Rapid screening of yeast mutants with reporters identifies new splicing phenotypes. FEBS J 2013; 280:2712-26; PMID:23566877; http://dx.doi.org/10.1111/febs.12277.

50. Dziembowski A, Ventura AP, Rutz B, Caspary F, Faux P, Frey C, Czaplinski K, Martienssen R. HA-mediated intron retention and splicing. EMBO J 2004; 23:4847-56; PMID:15565172; http://dx.doi.org/10.1038/sj.emboj.7600482.

51. Scherrer FW, Jr., Spingola M. A subset of Mer1p-dependent introns requires Bfd1p for splicing activation and normal retention of the RNA. Mol Cell 2006; 12:1617-22; PMID:16738408; http://dx.doi.org/10.1016/j.molcel.22760806.

52. Ohi MD, Link AJ, Ren LP, Jennings JL, McDonald WH, Gould KL. Proteomics analysis reveals stable multiprotein complexes in both fusion and budding yeasts containing Myo1p. Mol Biol Cell 2004; 15:7691-700; PMID:15296370; http://dx.doi.org/10.1093/mbc/e15.11.7691.