Structural biology

A clue to the catalytic activation of splicing

Soo-Chen Cheng

The processing of messenger RNA during splicing requires the activity of a complex of RNAs and proteins termed the spliceosome. Structural data shed light on previously mysterious aspects of splicing in humans. See p.842

Most genes in eukaryotic organisms (those such as plants, animals and fungi, with cells that have a nucleus) are interrupted by non-coding sequences called introns, which need to be removed from the corresponding RNA molecule by a process called splicing. On page 842, Schmitzová *et al.*[1] provide insights into an important step needed for splicing to occur.

The processing of unspliced precursor messenger RNA (pre-mRNA) is catalysed by a complex of RNAs and proteins called the spliceosome. Splicing generates a mature version of the mRNA, from which the intron has been excised in a loop (lariat) form (Fig. 1). The spliceosome is a highly complex structure, comprising five RNAs called small nuclear RNAs (snRNAs), termed U1, U2, U4/U6 (these two RNAs form a complex together) and U5, and numerous protein factors[2]. The protein-bound snRNAs are called snRNPs. The spliceosome is assembled by the stepwise association and dissociation of these components to the pre-mRNA to form an RNA-based, catalytically poised structure, termed the B^{act} complex.

The breakdown (hydrolysis) of the molecule ATP, catalysed by proteins belonging to a family of enzymes called DExD/H-box RNA helicases, provides the energy that is required for most of the steps involving release of components of the spliceosome[3]. In yeast (*Saccharomyces cerevisiae*), eight such DExD/H-box proteins are required for splicing, but the human spliceosome contains an extra helicase called Aquarius, which is incorporated into the spliceosome during spliceosome activation, and which remains associated with the spliceosome during the subsequent steps[4]. Yet the function of Aquarius in splicing was previously unknown.

Schmitzová *et al.* report the structure, obtained using cryo-electron microscopy (cryo-EM), of a splicing complex that has stalled at the step before the catalytic reaction occurs. The structure sheds light on the role of Aquarius and provides key insights into the mechanism of the catalytic activation of splicing.

The spliceosome is activated on formation of what is called the RNA catalytic centre. However, such a spliceosome still cannot catalyse a splicing reaction, because a key region of RNA involved in splicing — known as the branch site of the pre-mRNA (Fig. 1) — is sequestered 50 ångströms away from the catalytic centre[5] by the SF3B protein complex, which contains a large subunit called SF3B1 that binds to U2 snRNP. The helicase PRP2, which belongs to the DEAH subfamily of DExD/H proteins, promotes the release of the proteins SF3A/B (these are subcomplexes of U2 snRNP) to enable catalytic activation. The pre-mRNA retention and splicing (RES) complex is also removed on PRP2 action, although it does not prevent the interaction of the branch site with the catalytic centre.

In yeast, Prp2 (the yeast version of PRP2) is recruited to the spliceosome on interacting with the protein Brr2, and Prp2 is then transferred to the pre-mRNA at a site 23–33 nucleotides downstream of the binding site[6]. It has been proposed that Prp2 moves along the pre-mRNA in the direction from the 3′ end to the 5′ end of the RNA to displace the SF3A/B and RES complexes. A similar mechanism has also been proposed for how another DEAH-box protein, Prp22, releases mRNA on completion of splicing[7]. Nevertheless, neither protein has been captured in action.

Schmitzová and colleagues used a mutant version of Aquarius that was defective in its ability to hydrolyse ATP as a way to block splicing. The authors then isolated the stalled spliceosome, which they named B^{AQR}, for cryo-EM study. Their structure reveals B^{AQR}

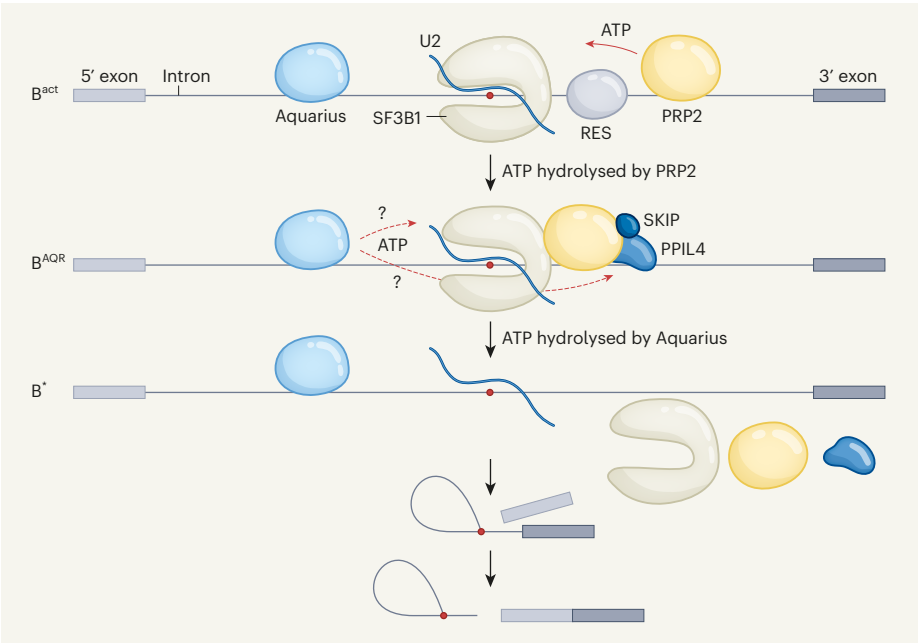

Figure 1 | The processing of human messenger RNA. Non-coding sequences (introns) are removed from between mRNA segments called exons during an event called splicing. Part of the intron, called the branch site (red circle), binds to an RNA called U2 and forms an RNA helix termed the branch helix, which is enclosed by a protein called SF3B1. For splicing to occur, SF3B1 must be released to expose the branch site and enable it to interact with the 5′ end of the intron. The B^{act} complex, which is poised for splicing, forms when the RNA-based catalytic centre (not shown) forms. PRP2 (a type of enzyme called an RNA helicase) then binds to the intron downstream of the branch site and moves towards the branch site on PRP2's breakdown (hydrolysis) of the molecule ATP. This movement destabilizes a complex called RES. Schmitzová *et al.*[1] present a structure termed B^{AQR}, which reveals the next step towards splicing. PRP2 movement halts when it interacts with the proteins PPIL4 and SKIP. The helicase Aquarius hydrolyses ATP as it completes the liberation of the branch site — this might occur by releasing PPIL4 to allow PRP2 movement, or movement of Aquarius might promote SF3B1 release. The catalytically activated structure is termed B*, from which the intron can then be removed in the form of a loop.

halfway through catalytic activation. Compared with the B^{act} complex, several components, such as the RES complex and SF3B6 (a protein subunit of the SF3B complex), are destabilized (positioned at different locations and more loosely associated with the pre-mRNA than before), and the protein PPIL4 is a newly associated component. Several other components were also repositioned.

Strikingly, in B^{AQR}, PRP2 occupies a region 7–13 nucleotides downstream of the branch site, rather than 26–32 nucleotides downstream, as in the B^{act} complex; this reflects the movement of PRP2 along the intron by 19 nucleotides towards the spliceosome core. Moreover, PRP2 is rotated approximately 70° anticlockwise, with certain domains of the protein (the RecA-like domains) adopting the open conformation characteristic of that found after ATP hydrolysis.

A region of PRP2 (the amino-terminal domain) that is not observed in the B^{act} complex exhibits an extended conformation in B^{AQR}, and is organized into three distinct modules. These are referred to as the hook, the clip and the pin. PRP2 interacts with PPIL4 and the protein SKIP through the clip, and together they form a stable 3D assembly that binds to the intron at nucleotides 17–20 downstream of the PRP2 binding site. Moreover, a region of SKIP inserts like a wedge between the two RecA and the HB domains of PRP2, locking PRP2 into an open conformation. Thus, it is proposed that PPIL4 and SKIP form a brake-like structure to stop the movement (translocation) of PRP2.

Translocation of PRP2 leads to a structural change in SF3B1. The HEAT domain of SF3B1, which encloses the branch helix in a closed conformation in the B^{act} complex, exhibits a loose conformation in the B^{AQR} complex, disrupting approximately 60% of SF3B1's interface with the RNA. This alteration to the SF3B1 structure weakens the interaction between the SF3B complex and the protein PRP8 — probably assisting the subsequent release of the SF3A/B complex, which would occur only when Aquarius can catalyse ATP hydrolysis.

An interesting question arises as to how Aquarius coordinates with PRP2 to remodel the spliceosome. PPIL4 and SKIP bind to the intron immediately downstream of PRP2 in B^{AQR}. If PPIL4 and SKIP indeed act as a molecular brake to PRP2, then Aquarius might destabilize the PPIL4 and SKIP bound to RNA, thereby permitting PRP2 movement and thus release of SF3A/B. Alternatively, PRP2 and Aquarius might act sequentially to destabilize the RNA binding of components found downstream and upstream of the branch site, respectively, ultimately liberating the branch site.

In both scenarios, PPIL4 and SKIP need to interact with the intron and PRP2 in a timely manner — only after PRP2 has moved near to the branch site but before it proceeds farther. How the action of PPIL4 and SKIP is temporally regulated is thus a fascinating question.

Saccharomyces cerevisiae lacks Aquarius or PPIL4; instead, its version of PRP2 is responsible for the entire process. So it would seem that PPIL4 and Aquarius co-evolved. Given that PPIL4 is observed only in the B^{AQR} complex, it might not be required for other steps of the pathway. Conceivably, depleting PPIL4 might bypass the need to have Aquarius for splicing. The evolution of PPIL4 and Aquarius in eukaryotes such as humans indicates that another layer of splicing regulation might exist, compared with the situation in yeast. Whether similar regulation also applies to other DEAH-box helicases remains to be studied.

It is a matter of debate whether DEAH-box helicases pull their RNA substrate towards them from a distance or act by stepwise movement (translocation) along the RNA to destabilize their targets during remodelling of the spliceosome[6–9]. The B^{AQR} complex structure provides key evidence supporting the translocation mechanism for PRP2.

A yeast study of Prp16, using the technique of crosslinking, also supports the translocation mechanism[10]. Prp16 was found to crosslink to a region downstream of the branch site when the second splicing reaction was blocked, whereas an ATPase-defective Prp16 mutant crosslinked farther downstream of the branch site — indicating translocation of Prp16 from its anchoring site towards the spliceosome core during the reaction[10]. Further study will be needed to determine whether this represents a shared mechanism for all splicing that depends on DEAH-box helicases.

Soo-Chen Cheng is at the Institute of Molecular Biology, Academia Sinica, Taipei City 115, Taiwan.
e-mail: mbscc@ccvax.sinica.edu.tw

1. Schmitzová, J., Cretu, C., Dienemann, C., Urlaub, H. & Pena, V. *Nature* **617**, 842–850 (2023).
2. Wilkinson, M. E., Charenton, C. & Nagai, K. *Annu. Rev. Biochem.* **89**, 359–388 (2020).
3. Liu, Y.-C. & Cheng, S.-C. *J. Biomed. Sci.* **22**, 54 (2015).
4. De, I. *et al. Nature Struct. Mol. Biol.* **22**, 138–144 (2015).
5. Yan, C., Wan, R., Bai, R. & Shi, Y. *Science* **353**, 904–911 (2016).
6. Liu, H.-L. & Cheng, S.-C. *Mol. Cell. Biol.* **32**, 5056–5066 (2012).
7. Schwer, B. *Mol. Cell* **30**, 743–754 (2008).
8. Semlow, D. R., Blanco, M. R., Walter, N. G. & Staley, J. P. *Cell* **164**, 985–998 (2016).
9. Bai, R. *et al. Science* **371**, eabe8863 (2021).
10. Chung, C.-S. *et al. Nucleic Acids Res.* **47**, 899–910 (2019).

The author declares no competing interests.
This article was published online on 10 May 2023.

Optical imaging

Cocktails of tags enhance microscopy resolution

Alistair Curd

A limit on the resolution of optical-microscopy techniques has been broken by using a mixture of tags to label copies of target molecules in a sample, opening the way to better views of molecular organization in cells. **See p.711**

On page 711, Reinhardt *et al.*[1] report an optical-microscopy technique that can distinguish between tiny objects that are less than one nanometre apart, equivalent to a separation of just several atoms. They use this approach to visualize adjacent points along a DNA double helix and individual proteins found in pairs in cells — accomplishments that other optical-imaging methods would struggle to achieve. The technique could lead to biologists probing the organization of complex arrangements of the molecules of life in greater detail than before, in the native environment of the cell.

For more than 150 years, the builders of optical microscopes have known that there is a limit to the detail that can be seen in an image using conventional techniques, no matter what the magnification. This is because of the way in which light waves spread out after they are confined — by the aperture of a lens, for instance. The resolution limit long prevented scientists from seeing the detailed arrangements of cellular machinery, which works at a scale well beyond that limit. However, over the past couple of decades, researchers have invented ways of working around this limit.

One important group of techniques involves labelling all the copies of a molecule of interest with a tag, and then causing these tags to 'blink', or light up with fluorescence, a few at a time. The positions of the resulting blurred image spots can be calculated much more precisely than when all the molecules are visible at the same time. Over many repetitions of this process, the positions of many of