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Conformational Change in the Protein Complex SF3b upon Integration into the Spliceosomal U11/U12 di-snRNP as revealed by Electron Cryomicroscopy

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Splicing describes an essential process needed to precisely excise pre-mRNA intron sequences prior to translation of the mature mRNA into protein [1]. This removal of introns is catalysed by a large macromolecular complex, termed the spliceosome, that is assembled by the stepwise integration of snRNPs (small nuclear ribonucleoprotein) on pre-mRNA. Most intron sequences are removed by the so called major spliceosome. However, in some eukaryotes, a minor class of introns is removed by the minor (U12-dependent) spliceosome, which contains the snRNP heterodimer U11/U12. The U11/U12 di-snRNP is involved in pre-mRNA binding and interacts simultaneously with the 5' splice site and the branch site of the intron. Its components thus form a molecular bridge that functionally pairs the intron ends of the pre-mRNA in the early spliceosomal assembly phase of initial splice site selection.

We have determined the three-dimensional structure of the human U11/U12 di-snRNP by cryo-negative stain single-particle electron microscopy using angular reconstitution (~12 Å resolution) and random conical tilt techniques. SF3b [2], a heteromeric protein complex functionally important for branch-site recognition, was located in the U11/U12 di-snRNP complex by antibody labeling, and additionally, based on the identification of the tandem helical HEAT motif of the SF3b155 protein and the RRMs of proteins SF3b49 and p14 (Fig. 1). The conformation of SF3b bound to the U11/U12 di-snRNP differs strongly from that of isolated SF3b. Upon integration of SF3b into U11/U12 di-snRNP, the SF3b complex thus rearranges into a more open form which positions the protein p14 at the surface of the U11/U12 di-snRNP complex [3].

The way SF3b is integrated in the U11/U12 di-snRNP has important implications for the understanding of branch site recognition. A groove on the surface of the U11/U12 di-snRNP structure (Fig. 2) is lined up by a number of proteins that can directly be cross-linked in case of U2 snRNP and all assembly states of the major spliceosome to different pre-mRNA positions. In analogy, we interpret this groove in our current model as the potential U11/U12 di-snRNP binding site for the pre-mRNA which would allow the pre-mRNA to bind to U11/U12 di-snRNP complex in a straight line (Fig. 2).

References

[1] C.B. Burge et al., The RNA World, Cold Spring Harbor Laboratory Press (1999).
[2] M.M. Golas et al., Science (2003) 300.
[3] M.M. Golas et al., Mol Cell (2004) *in press*.
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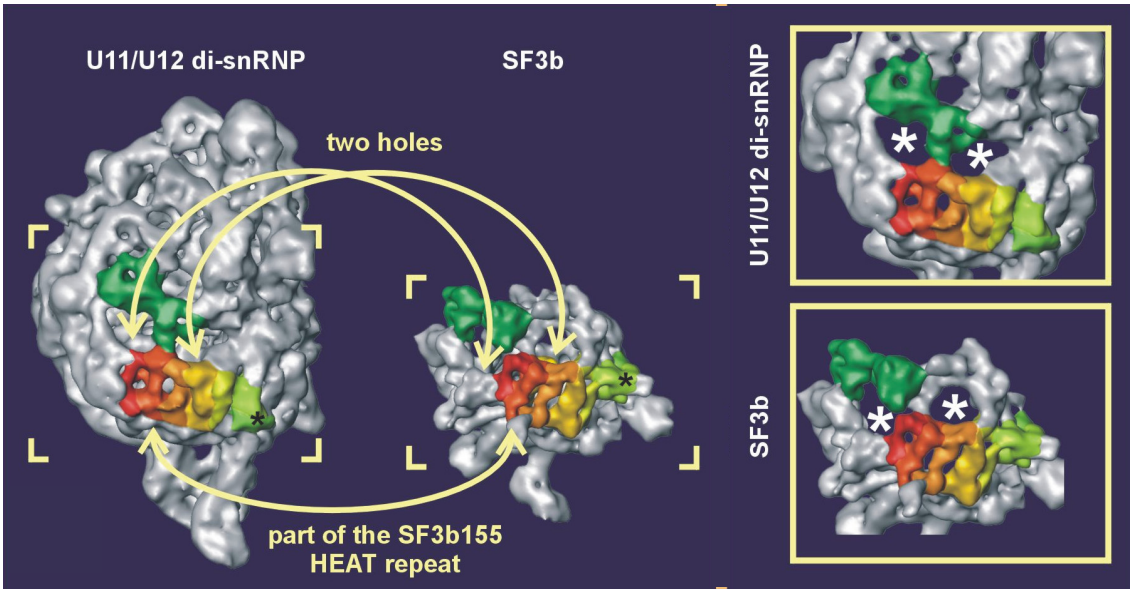

Fig.1 Similar structural elements of SF3b and U11/U12 di-snRNP used for fitting. The HEAT repeats of the SF3b155 protein are colored in rainbow colors and protein SF3b49 is shown in green. The second part of the SF3b complex (not shown here) had to be rotated as a rigid body to fit well into U11/U12 di-snRNP.

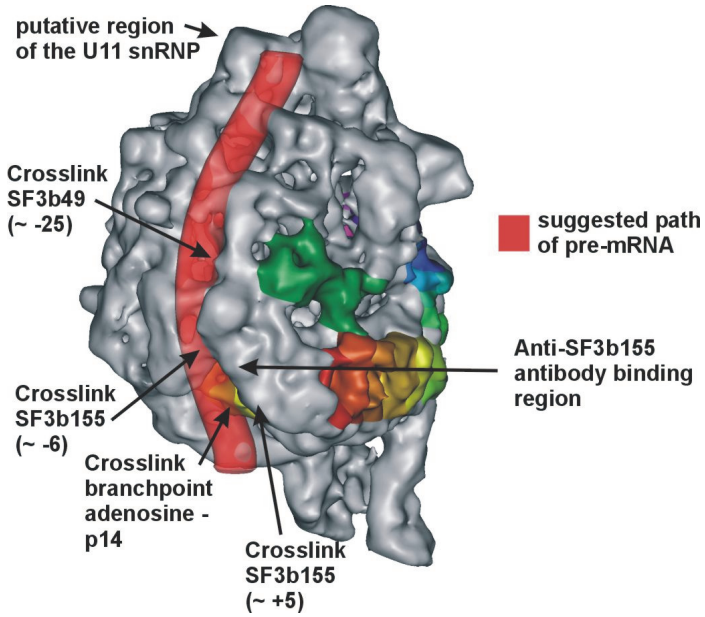

Fig. 2 Tentative model of the pre-mRNA (red) bound to a groove in the U11/U12 di-snRNP. The model agrees well with the available cross-linking data of pre-mRNA with SF3b proteins. The rearrangement of SF3b repositions p14 from the interior to the surface of U11/U12 di-snRNP and makes it accessible for the interaction with the pre-mRNA.

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