BIOLOGY REPORTS

Published: 24 May 2010

Structure of spliceosomal ribonucleoproteins

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F1000 Biology Reports 2010, **2**:39 (doi:10.3410/B2-39)

Abstract

Splicing of the precursors of eukaryotic mRNA and some non-coding RNAs is catalyzed by the 'spliceosome', which comprises five RNA-protein complexes (small nuclear ribonucleoproteins, or snRNPs) that assemble in an ordered manner onto precursor-mRNAs. Much progress has been made in determining the gross morphology of spliceosomal assembly intermediates. Recently, the first crystal structure of a spliceosomal snRNP has provided significant insight into assembly and architecture of spliceosomal snRNPs in general and the structure-function relationship of human U1 snRNP in particular.

Introduction and context

Spliceosomes catalyze excision of introns and ligation of exons from precursor-mRNAs (pre-mRNAs) in a two-step *trans*-esterification reaction (Figure 1a). They are dynamic and comprise approximately 170 components during a round of splicing [1]. Integral to the spliceosome are the U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins (snRNPs). Together with non-snRNP factors, snRNPs assemble in an ordered manner onto pre-mRNAs, recognizing (a) the junction between 5′ exon and intron (5′ splice-site), (b) a conserved sequence within the intron (branch-site), and (c) the junction between the intron and 3′ exon (3′ splice-site). Assembly intermediates are designated E, A, B, B*, and C complexes. Initially, U1 snRNP recognizes the 5′ splice-site and non-snRNP proteins bind the branch-site and 3′ splice-site (E complex), and then U2 snRNP recognizes the branch-site (A complex). The U4/U6-U5 tri-snRNP joins to form B complex. Following structural/compositional rearrangements, U1 and U4 snRNPs are destabilized, with U1 displaced by U6, and an activated B complex (B*, containing U2, U5, and U6 snRNPs) catalyzes the first reaction. After significant further remodeling, C complex catalyzes the second reaction to yield spliced mRNA.

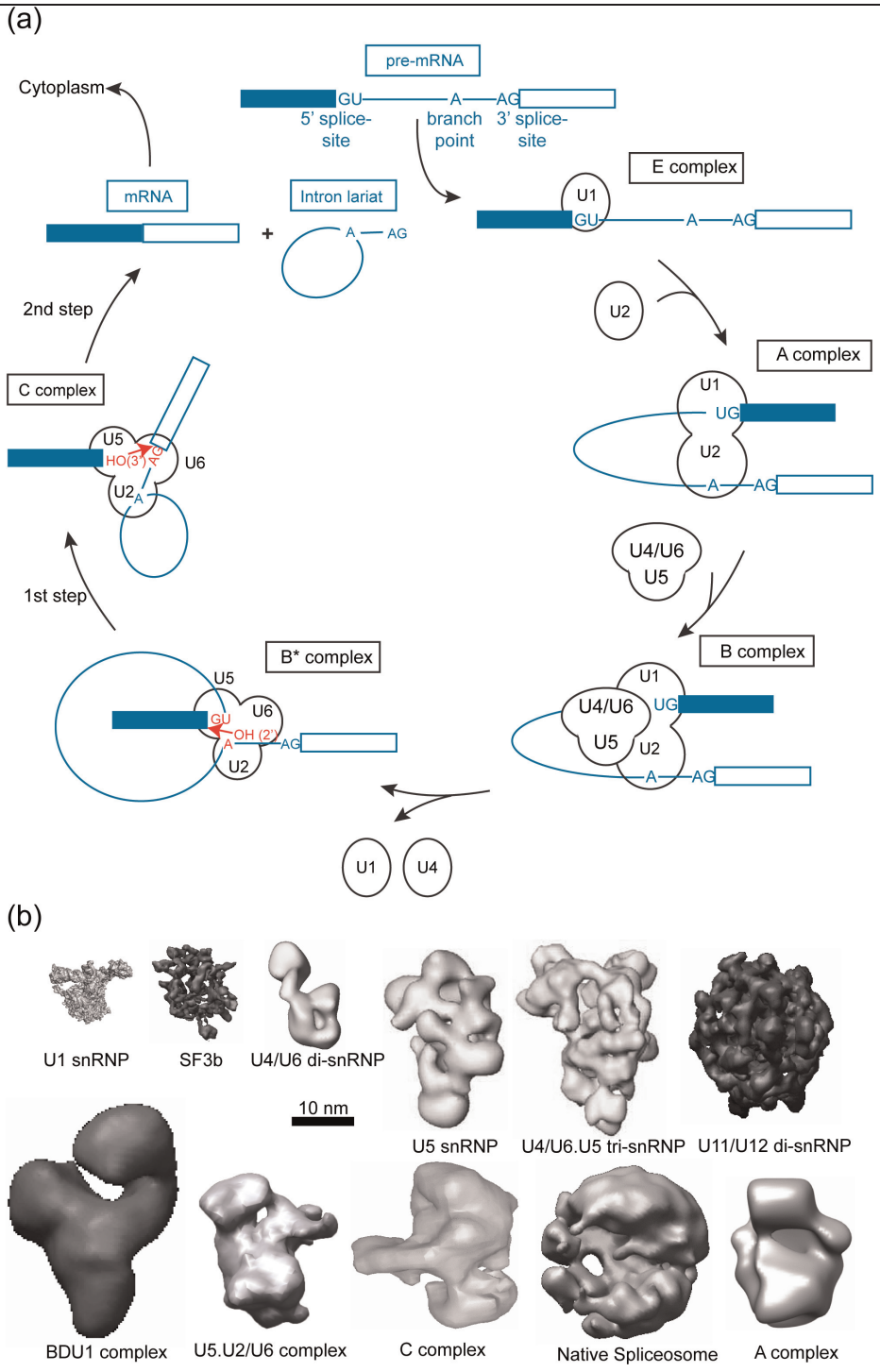
Major recent advances

I. Structural determination of snRNPs and their subcomplexes by electron microscopy

Spliceosomal snRNPs and their subcomplexes have been visualized by cryo-negative electron microscopy (CNEM) or single-particle cryo-electron microscopy (cryo-EM) (or both). Human U1 snRNP assumes a doughnut-shaped central structure from which emanate two protuberances, features observed in early negative-stained images and by cryo-EM [2,3].

The structure of SF3b (~450 kDa), a major component of U2 snRNP, was determined by CNEM at a resolution of approximately 10 Å [4]. It resembles a 'bivalve shell' with a stalk-like protuberance (Figure 1b). Three of seven proteins of SF3b have well-characterized protein folds: SF3b155 has 22 HEAT repeats; p14 (SF3b14a) and SF3b49 have one and two RNA recognition motifs (RRMs), respectively. The authors of the paper attribute three 'distinctly shaped blobs' to the RRMs and a ladder-like arrangement of density to SF3b155 HEAT repeats. The position of p14 is significant because it cross-links to the branch-point adenosine when U2 snRNP is incorporated into the spliceosome [5]. U11 and U12 snRNPs are equivalent to U1 and U2 snRNPs, but for minor intron

Figure 1. Spliceosomal assembly cycle and structures of spliceosomal complexes in the cycle, as determined by electron microscopy



(a) U small nuclear ribonucleoproteins (snRNPs) assemble onto a precursor-mRNA (pre-mRNA) transcript and catalyze two *trans*-esterification reactions, which result in the splicing of two exons and the release of an intron in the form of a lariat-like structure. **(b)** Electron microscopy structures of spliceosomal assembly intermediates and U snRNPs: SF3b [4], U4/U6 di-snRNP, U5 snRNP and U4/U6.U5 tri-snRNP [8], U11/U12 di-snRNP [6], BΔU1 complex [12], fission yeast U5.U2/U6 complex [19], C complex [17], native spliceosome [22], and the A complex [7]. Electron microscopy envelopes were obtained from the Electron Microscopy Data Bank [29]. A surface representation of the human U1 snRNP crystal structure with modeled SL2 and U1-A RRM1 (RNA recognition motif 1) [23] is included for comparison.

species, they function as a pre-formed U11/U12 di-snRNP (1.3 MDa). CNEM reveals an ellipsoidal structure with multiple protuberances [6] (Figure 1b). SF3b, also located within the di-snRNP, appears to adopt a more open conformation exposing p14 for interaction with the branch-point. A path for pre-mRNA across the di-snRNP is proposed on the basis of cross-links with identified protein components. The 1.8-MDa U4/U6-U5 tri-snRNP, determined at 21 Å by both cryo-EM and CNEM [7], shows an elongate tetrahedron, approximately 30.5 nm in length (Figure 1b). U5 snRNP and U4/U6 di-snRNP CNEM structures are also presented in this paper [7]. Comparison of particles showed that U5 snRNP, di-snRNP, and tri-snRNP all have rigid domains separated by flexible regions. By means of rigid-body fitting, U5 snRNP and U4/U6 di-snRNP structures were placed in the tri-snRNP. In this and a subsequent study [8], proteins were located in the tri-snRNP: U4/U6 di-snRNP proteins in an 'arm' domain, which can move relative to the rest of the particle; U5 proteins in a more static 'body' and 'head'; and tri-snRNP-specific proteins were found in a 'linker' region. Snu114, an elongation factor-G-like GTPase that regulates Brr2, is located near the hinge of the tri-snRNP, which allows the 'arm' to move. This raises the intriguing possibility that a conformational change of Snu114, driven by hydrolysis of GTP, powers the movement of the arm and brings the U4/U6 assembly into the proximity of Brr2, which can then unwind the base-paired region of the U4/U6 small nuclear ribonucleic acids (snRNAs).

II. Structural determination of spliceosomal assembly intermediates by electron microscopy

Splicing assembly intermediates have been prepared using pre-mRNA substrates that stall splicing at specific steps. Their structures have subsequently been investigated by Cryo-EM or CNEM (or both).

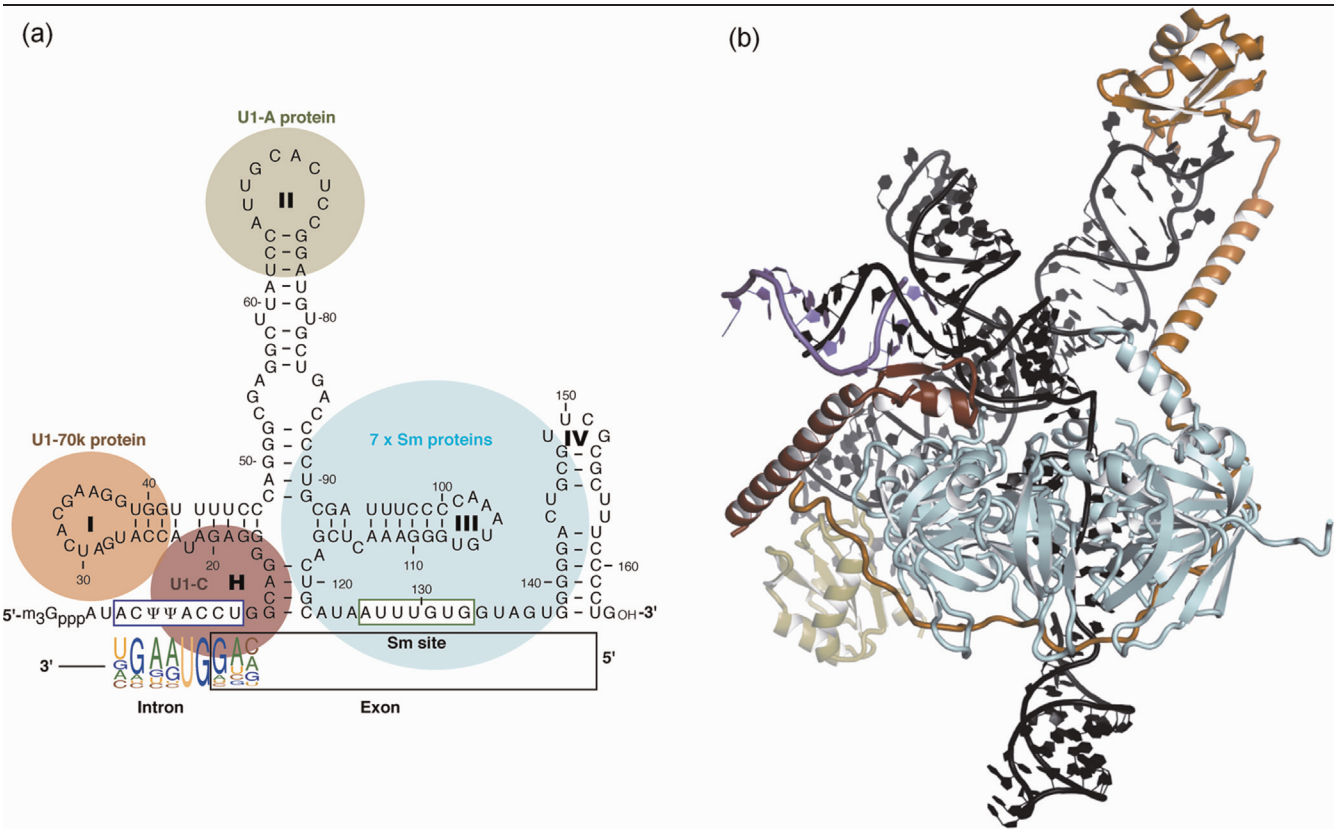
Human A complex contains all of the expected U1 and U2 snRNP proteins together with approximately 50 non-snRNP proteins. The CNEM structure reveals an asymmetrical particle consisting of a globular body, a head-like domain, and feet-like protrusions [9]. The A complex associates with the U4/U6-U5 tri-snRNP to form B complex. Around 100 proteins are associated with the B complex, which assumes a rhomboid shape, approximately 42 nm in its longest dimension [10]. The intron and both exons as well as U2 protein SF3b155 are located in its head domain [11]. U1 snRNP dissociates prior to formation of the activated B* complex. A CNEM structure of B complex lacking U1 snRNP (BΔU1) was determined at a resolution of approximately 40 Å [12]. The structure features a mobile head domain linked to a roughly triangular body, similar in size and shape to the U4/U6-U5 tri-snRNP. B complex activation requires major remodeling of RNA: base-pairing between U4/U6 snRNAs is destabilized before formation of a U2/U6 snRNA structure. DExD/H-box helicases orchestrate these rearrangements [13,14], leading to U4 snRNP dissociation and formation of activated B* complex, which catalyzes the first *trans*-esterification reaction. Over 50 proteins are exchanged [15], and the structure of the megadalton U5 snRNP is dramatically altered during the transition from B to B* complex [16]. A cryo-EM structure of the human C complex at approximately 30 Å shows a particle consisting of three separate lobes in an open conformation [17]. A non-spliceosomal RNA-binding protein, with a shape that is readily identified by EM, yielded insights into how complex C binds partially processed transcripts [18].

An approximately 29 Å cryo-EM structure of a 37S spliceosomal complex from *Schizosaccharomyces pombe* resembles the human C complex (U5.U2/U6). This structure, with dimensions of approximately 30 × 20 × 18 nm, is composed of distinct domains that contact each other at a central point [19]. Under certain low-salt conditions, complexes containing all five snRNPs together ('penta-snRNPs') have been isolated from yeast and human extracts [20,21] but their functional significance is unclear. An approximately 20 Å cryo-EM structure of a 'native' 200S spliceosome, purified from human cell nuclei, has an elongate structure made up of two globular bodies connected by a tunnel-like mesh. This 21-MDa particle is approximately 28 nm in its longest dimension [22]. The larger of the two bodies could accommodate a 'penta-snRNP'. The tunnel-like structure between the bodies is proposed to accommodate pre-mRNA.

III. Crystal structure of a U snRNP

Each spliceosomal snRNP is composed of a single RNA molecule (U snRNA), a set of seven Sm or LSm proteins, and snRNP-specific proteins. Human U1 snRNP (~245 kDa, 11 subunits) contains three U1-specific proteins: U1-70k, U1-A, and U1-C (Figure 2a). The crystal structure of a fully recombinant, 10-subunit human U1 snRNP at a resolution of 5.5 Å [23] revealed a hierarchical network of interactions between U1 snRNP subunits. Seven Sm proteins form a ring around the single-stranded Sm site, which fans out through its center (Figure 2b). Strikingly, the N-terminus of U1-70k extends approximately 180 Å from its RRM, which recognizes loop I of U1 snRNA, wrapping 'underneath' the entire Sm ring to finally contact U1-C, which is poised to recognize the 5′ splice-site. The circuitous path of U1-70k was unambiguously determined by a 'selenium-walk' through the electron density map [24]. The conserved, single-stranded 5′ end of U1 snRNA base-pairs with the 5′ splice-site, initiating

Figure 2. Structure of human U1 small nuclear ribonucleoprotein (snRNP) [23]



(a) Secondary structure of U1 small nuclear ribonucleic acid (snRNA) (black) and general location of the seven Sm proteins (cyan), U1-70k (peachy brown), U1-A (green), and U1-C (maroon). A consensus 5′ splice-site is indicated, base-pairing to the single-stranded 5′ end of U1 snRNA. **(b)** Crystal structure of U1 snRNP with SL2 and U1-A RRM1 (RNA recognition motif 1) modeled. The structure is color-coded as in (a), with the 5′ splice-site mimic RNA colored blue.

pre-mRNA recognition. In U1 snRNP crystals, the 5′ end of one U1 snRNA interacts with the 5′ end from an adjacent particle such that they mutually mimic the 5′ splice-site. U1-C from each particle interacts with the resulting RNA duplex. The EM structure of U1 snRNP shows a ring-shaped core domain with two large protuberances [3]. The ring-shaped core domain was interpreted as the Sm ring; it has a funnel-shaped hole passing through it. In the crystal structure, the four-helix junction lies directly over the center of the Sm ring and no such hole is observed; therefore, the interpretation of the EM envelope is not consistent with the crystal structure.

Future directions

Significant progress has been made toward determining the overall architecture of the spliceosome and its constituent snRNPs, particularly U1. In the future, improvements in EM structural resolution are expected, possibly facilitated by cross-linking [25], but ultimately the preparation of compositionally and structurally homogeneous samples is pivotal. Better purification strategies to identify stable core structures should yield better samples [26]. Improvements in labeling to localize pre-mRNA/proteins and dock higher-resolution structures of proteins or larger complexes will also be critical to provide a clearer picture of the spliceosome in its entirety. Further understanding of a 'native' complex structure, how post-translational modifications may alter structure [27], and the dynamics of assembly [28] will further enhance our understanding of this huge, dynamic machine: the spliceosome.

Abbreviations

CNEM, cryo-negative electron microscopy; cryo-EM, cryo-electron microscopy; EM, electron microscopy; RRM, RNA recognition motif; snRNA, small nuclear ribonucleic acid; snRNP, small nuclear ribonucleoprotein.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We thank Andy Newman for critical reading of the manuscript and Melanie Ohi for sending us the fission yeast U5.U2/U6 EM map. The U1 snRNP project was funded by the Medical Research Council of the UK and the Human Frontier Science Program (HFSP). DAPK was a recipient of an HFSP long-term fellowship.

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