reports provide strong evidence that the human Sm and Lsm proteins are structurally and functionally related to Hfq. This finding suggests that Sm and Sm-like proteins, including Hfq, evolved from an ancient mediator of RNA transactions that facilitated RNA-RNA interactions. The detailed mechanism by which Sm proteins stabilize RNA-RNA interactions remains to be elucidated. For example, mere simultaneous binding by the proteins to two RNA strands could enhance interaction between the strands. Alternatively or additionally, interactions between a particular RNA strand and the protein might prevent formation of competing secondary structures or other complexes, so that the RNA is available for interaction with its target and the protein facilitates the RNA-RNA association by acting as an RNA chaperone.

William F. Donahue and Kevin A. Jarrell
Department of Pharmacology and Experimental Therapeutics
Boston University Medical Center
80 East Concord Street
Boston, Massachusetts 02118

The Spliceosome: No Assembly Required?

In this issue of Molecular Cell, Stevens et al. purify a large particle from yeast extracts that contains all five of the U snRNPs required for pre-mRNA splicing. The existence of this "penta-snRNP" suggests the provocative possibility that spliceosome assembly does not depend upon a pre-mRNA substrate.

Nuclear pre-mRNA splicing is catalyzed by a massive ribonucleoprotein complex, the spliceosome, comprised of five U snRNPs (U1, U2, and U4/U6.U5) and more than 30 non-snRNP proteins. Current dogma holds that the spliceosome (unlike the ribosome) does not exist as a preformed entity. Rather, the spliceosome is thought to assemble in a stepwise and temporally ordered manner in the presence of intron-containing pre-mRNAs. In this view, the pre-mRNA serves as an obligate scaffold for spliceosome formation.

In its simplest form, the accretion model of spliceosome assembly states that U1 snRNP recognizes the substrate first via interaction with the 5' splice site; U1 snRNP then promotes the interaction of U2 snRNP with the branch point region; finally, U4/U6.U5 tri-snRNP joins (reviewed in Moore et al., 1993). This picture of ordered assembly is supported by extensive analyses (primarily electrophoresis and/or sedimentation) in a variety of systems, and it has been extremely valuable in interpreting a large body of data. Nevertheless, many disparate observations are difficult to reconcile with the canonical assembly pathway, particularly those that demonstrate "early" roles for snRNPs thought to enter the pathway "late" (see Das et al., 2000; Maroney et al., 2000; Johnson and Abelson, 2001; Hastings and Krainer, 2001; Will and Lührmann, 2001 for recent examples and discussion). Collectively, these outlying observations have suggested the possibility that snRNPs functionally interact prior to engaging the pre-mRNA.

Now, Stevens et al. (2002) provide solid evidence for a functionally relevant particle that contains all of the spliceosomal snRNPs. Their basic observation derives from sedimentation analyses at different salt concentrations; in very low salt (50 mM), a fast sedimenting species is specific. It will prove interesting to determine which (if any) of these proteins are required for formation and/or integrity of the penta-snRNP complex. Finally, a smattering of proteins not obviously related to splicing per se were also found. While there exist plausible reasons for the presence of these proteins, their relevance to the particle (or their stoichiometry) remains to be determined.

The protein and RNA data support the notion that the

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penta-snRNP is a specific particle and not an aggregate, but does the complex have functional relevance to splicing? Unfortunately, the particle itself has no demonstrable activity. However, two lines of experiment indicate that the penta-snRNP is functionally significant. First, extracts depleted of endogenous RNAs by nuclease digestion regain splicing activity when supplemented with penta-snRNP. This result might have been anticipated since the particle contains all of the RNPs known to be essential for splicing. More compelling are mixing experiments in intact extracts using tagged snRNAs which show that penta-snRNP functions as a preformed unit in splicing; i.e., its snRNA constituents do not exchange with endogenous snRNPs during spliceosome assembly.

In combination, the physical characterization and functional analyses of the penta-snRNP provide convincing evidence for a yeast spliceosome that is largely preformed. Importantly, a similar (but less well-characterized) entity was detected many years ago in mammalian extracts (Konarska and Sharp, 1988). On the surface, it may appear that “preassembly” is in direct conflict with the large body of evidence supporting stepwise assembly; however, this may not be the case. It is well-established that the spliceosome is dynamic; i.e., spliceosome maturation involves multiple conformational changes that accompany (or result from) dramatic RNA/RNA rearrangements that precede catalytic activation (reviewed in Staley and Guthrie, 1998; Nilsen, 1998). It seems likely that most (if not all) of these dynamic rearrangements progressively stabilize previously tenuous contacts between the spliceosomal apparatus and the pre-mRNA. Because spliceosome assembly is routinely assayed under fairly stringent conditions, only stable interactions are detected. Accordingly, the apparent stepwise assembly process (see above) might well reflect stepwise stabilization of interactions, not stepwise recruitment of components. This view has the advantage of accommodating both the large amount of data that supports the “traditional” model as well as the growing body of evidence that seems to contradict it.

The existence of a functional multi-snRNP complex certainly has implications beyond rationalizing heretofore puzzling observations. Just as one example, our view of substrate recognition may well need to be revised. As currently understood, the pre-mRNA is recognized in small bits (i.e., the 5' splice site, the branch point region, and the 3' splice site) by sequential engagement of factors; however, this scenario does not adequately explain the specificity or efficiency of intron recognition, especially in higher eukaryotes where splicing signals are not well conserved. An appealing possibility, made plausible by a preformed spliceosome, is that the entire intron is recognized by a multiplicity of concurrent weak interactions. Such a mechanism might explain some currently poorly understood phenomena (e.g., why the splicing apparatus ignores what appear to be perfectly good “splice sites”). In this regard, it will be of considerable interest to determine the complement of factors that are required for penta-snRNP to engage a pre-mRNA. Such a determination should be straightforward (e.g., by identifying those proteins that specifically associate with pre-mRNA in nuclease-treated extract).

Finally, regardless of its function in vivo, the existence of a purifiable multi-snRNP provides an extremely powerful tool for the biochemical analysis of splicing mechanism. As noted by Stevens et al. (2002), the particle contains the majority of proteins known to be involved in splicing. Noticeably absent are most of the RNA-dependent ATPases which are thought to drive the conformational changes required for spliceosome maturation and activation (Staley and Guthrie, 1998) as well as some proteins known to be important in substrate recognition. If penta-snRNP can be rendered active in the presence of this rather small subset of proteins, the stage will be set to determine both the definitive substrates of the ATPases and the details of the reactions they catalyze.

**Timothy W. Nilsen**
Center for RNA Molecular Biology
Case Western Reserve University School of Medicine
10900 Euclid Avenue
Cleveland, Ohio 44106

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