The post transcriptional processing of pre-mRNAs in the nucleus is an important step in regulating the expression of eukaryotic genes. Processing events include 5'-end capping, 3'-end processing, splicing, and editing. We developed a procedure whereby naturally assembled mammalian nuclear RNP complexes could be isolated in an undegraded form. Intact RNP particles, that sediment at 200S in sucrose gradients, have thus been isolated from a number of mammalian nuclei. Electron microscopy (EM) revealed large compact particles, 50 nm in diameter, that are composed of substructures. These particles were therefore termed large nuclear RNP (lnRNP) particles. The lnRNP particles package pre-mRNA transcripts that differ largely in their length and number of introns, in complexes of a unique size and hydrodynamic properties, indicating their universal nature.

Biochemical composition

The composition of the lnRNP particles is very similar to that of the in vitro assembled spliceosome. All of the uridine-rich small nuclear RNP particles (U snRNPs), which are required for pre-mRNA splicing, are present within them, as well as several protein splicing factors that include U2AF and the SR protein family. SR proteins are of special interest because they are proposed to play a role in alternative (regulated) splicing. Moreover, they are required for the association of the U snRNPs with pre-mRNA and thus for spliceosome assembly. Since this assembly is regulated by their phosphorylation, it is significant that we have found all of the phosphorylated nucleoplasmic SR proteins associated with lnRNP.

We have also demonstrated the presence of 5'-end capping and 3'-end cleavage and polyadenylation components within the lnRNP particles. Another RNA processing event is RNA editing through the conversion of specific adenosines to inosines by specific deaminases (ADAR proteins). The amino acid changes introduced by this A-to-I RNA editing result in significant alterations in the physiological properties of many gene products. Consistent with the notion that editing precedes pre-mRNA splicing, we have demonstrated the presence of both ADAR proteins and A-to-I RNA editing activity as integral components of the lnRNP particles.

Structural studies

Three-dimensional image reconstruction of isolated lnRNP particles by automated electron tomography was performed at an optimal resolution of 2 nm. The reconstructions revealed a quadrangular compact structure whose dimensions are 50 X 50 X 35 nm. The reconstructed model is composed of four major subunits of similar dimensions, which are connected to each other. An additional domain is sometimes observed towards the center of the particle. The automatic electron tomography studies were complemented by mass measurements of the lnRNP particles using scanning transmission electron microscopy (STEM). These studies showed that the mass of the tetrameric lnRNP particle is 21±1.6 mDa, and that the mass of each of its individual subunits is 4.8±0.5 mDa.
The estimated mass of the 60S in vitro spliceosome is 4.9 mDa, which is almost identical to the measured mass of a monomeric subunit of the lnRNP particle. Since the composition of the lnRNP particle and the spliceosome are similar, we have proposed that the lnRNP particle is primarily composed of four repeating subunits, presumably 60S spliceosomes, thus making a supraspliceosome complex associated with a single pre-mRNA molecule. In this configuration (Fig. 1), the lnRNP particle serves as a mold onto which the pre-mRNA is folded to juxtapose exons that should be spliced, while introns are looped around and out of each of the respective subunit. Current studies by cryo-electron tomography of unstained lnRNP particles in the frozen hydrated state that faithfully represent their native structure, are expected to provide structural information of higher resolution.

Our model of the supraspliceosome is also supported by the fact that strands and loops of RNA were often seen emanating from positively stained lnRNP particles. However, localization by EM of RNA within biological complexes, is not yet a straightforward undertaking. We are therefore developing a methodology for the covalent tagging of RNA molecules with gold clusters, thereby enabling their direct visualization by microscopy. Our strategy involves transcription in vitro of RNAs that carry free thiol groups, using thiolated ribonucleoside triphosphate analogs. This synthesis is followed by coupling of gold clusters to the thiolated transcript through a maleimido group. Visualization of such gold-tagged RNAs by EM showed spots of gold clusters, with a diameter of 1-2 nm, arranged at nearly regular distances on an imaginary curve that presumably corresponds to the RNA chain. This assignment was corroborated by atomic force microscopy (AFM) that exhibited images of RNA chains in which knobby-like structures, whose height corresponds to the diameter of the gold clusters, were clearly seen (Figure 2). This study demonstrates the potential use of nucleic acids that are covalently labeled with gold clusters for the structural characterization of protein-RNA complexes.

**Publications**


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