A supraspliceosome model for large nuclear ribonucleoprotein particles based on mass determinations by scanning transmission electron microscopy

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Author(s) Müller, S; Wolpensinger, B; Angenitzki, M; Engel, A; Sperling, J; Sperling, R
Author(s) at UniBasel Engel, Andreas ;
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Pre-mRNA splicing is an important regulatory step in the expression of most eukaryotic genes. In vitro studies have shown splicing to occur within 50-60 S multi-component ribonucleoprotein (RNP) complexes termed spliceosomes. Studies of mammalian cell nuclei have revealed larger complexes that sediment at 200 S in sucrose gradients, termed large nuclear RNP (lnRNP) particles. These particles contain all factors required for pre-mRNA splicing, including the spliceosomal U snRNPs and protein splicing factors. Electron microscopy has shown them to consist of four apparently similar substructures. In this study, mass measurements by scanning transmission electron microscopy of freeze-dried mammalian lnRNP preparations, both confirm the similarity between the lnRNP particles and reveal the mass uniformity of their subunits. Thus, the tetrameric lnRNP particle has a mass of 21.1(+/-1.6) MDa, while each repeating subunit has a mass of 4.8(+/-0.5) MDa, which is close to the estimated mass of the fully assembled 60 S spliceosome. The 1.9 MDa discrepancy between the lnRNP particle's mass and the cumulative masses of its four subunits may be attributed to an additional domain frequently observed in the micrographs. Notably, strands and loops of RNA were often seen emanating from lnRNP particles positively stained with uranyl formate. Our results support the idea that the nuclear splicing machine is a supraspliceosome complex. For clarity, we define spliceosomes devoid of pre-mRNA as spliceosome cores, and propose that the supraspliceosome is constructed from one pre-mRNA, four spliceosome cores, each composed mainly of U snRNPs, and additional proteins. In this way a frame is provided to juxtapose exons about to be spliced.
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