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pre-mRNA splicing, small nuclear ribonucleoproteins

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Research topics

RNA molecules are not just messengers acting between DNA and proteins but rather required factors that play an active role in the expression of genes encoded in our genome. An RNA processing step called splicing can dramatically increase the diversity of proteins in human cells and tissues. RNA splicing is catalysed by a large macromolecular complex, the spliceosome, which is formed from several RNA-protein complexes called snRNPs. In our group we are interested in spliceosome assembly and the organization of RNA splicing in the cell nucleus. Using advanced microscopy techniques (e.g. live cell imaging, FRET, FCS) we explore where and when the spliceosome assembles in the cell nucleus. Experimental data are then used for modelling spliceosome assembly in the 3D space of the nuclear landscape. We identified the conserved nuclear compartment, the Cajal body, as the site of snRNP assembly and recycling, and we proposed a model stating that the presence of Cajal bodies increases the efficiency of snRNP formation. We also aim to determine how mutations in splicing factors can cause *retinitis pigmentosa*, a human genetic disease characterized by photoreceptor cell degeneration.

Current grant support

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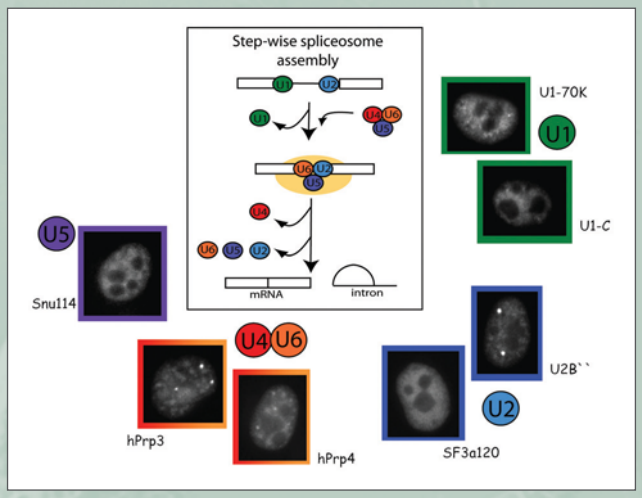
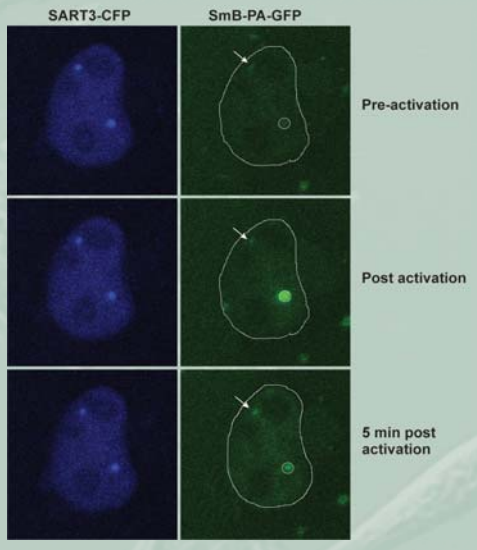
Selected recent papers

1. Klingauf M, Staněk D, Neugebauer KM. Enhancement of U4/U6 snRNP association in Cajal bodies predicted by mathematical modeling. **Mol Biol Cell.** 2006;17:4972-4981.
2. Staněk D, Neugebauer KM. Cajal bodies: a meeting place for snRNP in the nuclear maze. **Chromosoma.** 2006;115:343-354.
3. Staněk D, Přidalová-Hnilicová J, Novotný I, Huranová J, Blažíková M, Wen X, Sapra, A.K., Neugebauer, K.M. Spliceosomal snRNPs repeatedly cycle through Cajal bodies. **Mol Biol Cell.** 2008;19:2534-2543.
4. Cvačková Z, Albring KF, Koberna K, Ligasová A, Huber O, Raška I, Staněk D. Pontin is localized in nucleolar fibrillar centers. **Chromosoma**. 2008;117:487-497.



Assembly of the spliceosome in vivo. Currently, it is unknown how splicing machinery assembles in the cell nucleus. To analyse this problem we created a battery of splicing-specific proteins tagged with fluorescent proteins. These constructs are used for FRET, FRAP and FCS to measure interactions and dynamics of individual proteins directly in living cells.



Cycling of spliceosomal snRNPs between Cajal bodies. Specific marker of snRNPs - the SmB protein - was tagged with photoctivatable GFP, expressed in the cell and specifically activated in one Cajal body (circle). SART3-CFP serves as a marker of Cajal bodies. This and other experiments helped us to reveal the role of Cajal bodies in snRNP recycling.