Perspective

Spliceosomes and Snurposomes

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THE RNAs THAT DIRECT PROTEIN SYNTHESIS IN ANIMAL and plant cells are synthesized in the nucleus as large precursors (pre-mRNAs). The protein coding sequences in pre-mRNA molecules are arranged in discontinuous segments—exons—interspersed with noncoding sequences—introns. The production of mature mRNAs requires the precise removal of introns and the joining of exons by RNA splicing (*1*). Splicing reactions can occur in vitro when short RNA molecules that contain one or a few introns are incubated with appropriate nuclear extracts. Thus it is possible to follow the different steps in splicing and to define the major components necessary for the reactions. Among the most thoroughly studied of these components are the highly conserved small nuclear RNAs (snRNAs) designated U1, U2, U4, U5, and U6 (*2*). In cell extracts, the snRNAs exist associated with proteins as small nuclear ribonucleoproteins (snRNPs, pronounced "snurps"). In an in vitro splicing reaction, the snRNPs, other essential protein factors (*3*), and the pre-mRNA form a macromolecular complex known as a spliceosome (*4*). Spliceosomes can be isolated from the reaction in relatively pure form for biochemical studies (*5*); in the electron microscope they appear as 40- to 60-nm particles with a distinctive morphology (*6*). Much of our knowledge about splicing comes from in vitro biochemical experiments.

In living cells the synthesis of snRNAs and the assembly of snRNPs has been extensively studied, but relatively little is known about splicing itself (*7*). It is not clear how and where spliceosomes are formed or, indeed, whether structures comparable to in vitro spliceosomes occur in the nucleus.

Insight into these questions can be gained by examining the distribution of splicing components in the nucleus. About 10 years ago Steitz and her colleagues showed that sera from some patients with autoimmune diseases precipitate snRNPs from cell extracts (*8*). When used for immunofluorescent staining of cultured cells, these sera highlight 20 to 50 bright spots or "speckles" in the nucleus, set against a diffusely stained background (*9*). Because snRNPs and other splicing factors (*3*) are concentrated in the speckles, it has been suggested that splicing occurs in these regions. However, it is equally possible that the speckles are sites for assembly or storage of splicing components. Much depends on where RNA transcription takes place and whether splicing occurs while transcription is still in progress. The first of these questions was addressed by Spector, who mapped the regions of RNA synthesis within individual interphase nuclei; he concluded that the speckles and the regions of RNA synthesis are not identical (*10*). The interpretation of this observation hinges on whether RNA synthesis and splicing are concurrent events. If they are, splicing may occur in the diffusely stained regions, not in the speckles.

The crucial question of the temporal relation between splicing and transcription has been studied in giant chromosomes by examining the regions of active RNA synthesis—the puffs on polytene chromosomes of flies (*11*) and the loops on lampbrush chromosomes (Fig. 1) from amphibian oocytes (*12*). snRNPs have been detected in both types of chromosome, demonstrating that essential splicing components occur on the nascent RNA. However, these observations do not prove that splicing begins before the completion of transcription. The best evidence for the latter possibility comes from the electron micrographs (EMs) of Beyer and her colleagues (*13*), which show that active *Drosophila* chorion genes have bowed regions on individual nascent transcripts that correspond in size and position to known introns. Furthermore, transcripts at the end of transcription units are shorter than those near the middle, indicating that there is a loss of sequences during transcription. Taken together, the EM and immunofluorescence data suggest that the splicing machinery is in place and can function during transcription.

The same immunofluorescence observations that demonstrated snRNPs on the actively transcribing loops of lampbrush chromosomes revealed thousands of brightly staining granules in the nucleoplasm surrounding the chromosomes (*12*). These granules were stained by two antibodies, one that detected the Sm antigen common to several snRNPs and one that recognized a specific feature of snRNAs, the trimethylguanosine cap at the 5′ end of each molecule. Recently, we have used in situ nucleic acid hybridization to localize specific snRNAs in the granules and have extended the immunofluorescence observations with additional antibodies (*14*). It is now clear that snRNPs occur in at least three morphologically and biochemically distinct granules in the amphibian oocyte nucleus. We refer to these granules as A, B, and C *snurposomes*. The term snurposome emphasizes the composition of the granules without implying specific functions.

The A snurposomes are the simplest. They contain only U1 snRNA and U1-associated proteins. They range in size from less than 1 μm to about 4 μm, and they often look like miniature red blood cells (*14*). The B snurposomes (Figs. 1 and 2) are homogenous granules about the same size as As, but much more complex in composition. They contain all five splicing snRNAs in roughly equivalent amounts, as demonstrated by in situ hybridization with ^{3}H-labeled probes. Immunofluorescent staining reveals the presence of U1- and U2-specific proteins, the trimethylguanosine cap, the Sm antigen, the non-snRNP splicing factor SC35 (*3*), and several proteins commonly associated with pre-mRNAs. EM sections through B snurposomes show that they consist of thousands to millions of particles 20 to 30 nm in diameter, about the size of ribosomes (*15*). All components so far identified in B snurposomes also occur on the loops of the lampbrush chromosomes.

The C snurposomes range in size from less than 1 μm to giant structures 20 μm in diameter and usually have one to many B snurposomes embedded in their surface (Fig. 2). Because of their remarkably spherical shape, large Cs with their accompanying Bs have long been known in the cytological literature as spheres or sphere organelles (*16*). Most spheres occur free in the nucleoplasm, but some are attached to specific chromosome loci, the sphere organizers. C snurposomes stain with antibodies to the Sm antigen and the trimethylguanosine cap and thus contain snRNPs of some

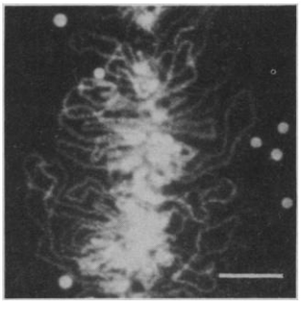

Fig. 1. Short segment of a newt lampbrush chromosome showing some of the many loops on which RNA synthesis takes place. Immunofluorescent staining was with monoclonal antibody Y12, which reacts with several snRNP proteins. A few B snurposomes are also present in this field. Bar = 10 μm.

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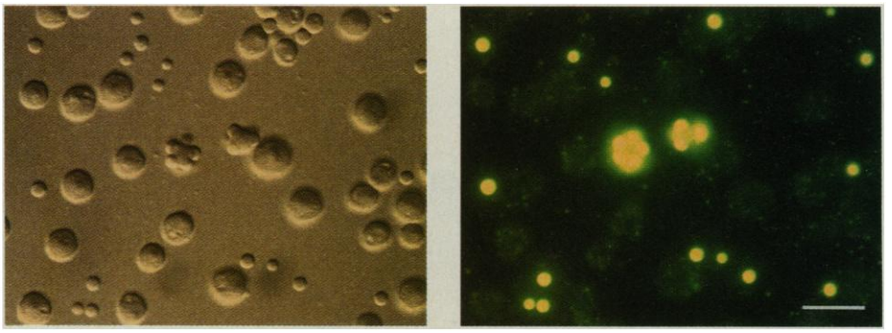

Fig. 2. Nucleoli and snurposomes from an oocyte nucleus of the toad *Xenopus*. (**Left**) The larger structures are nucleoli, of which there are about 1000 in an oocyte nucleus. (**Right**) The same field after immunofluorescent staining with monoclonal antibody αSC35, which detects an essential splicing factor (*3*). About 15 B snurposomes are brightly stained. Near the center of the field are clusters of B snurposomes on the surface of two C snurposomes. Bar = 10 μm.

type. However, none of the splicing snRNAs have been detected in small Cs (large Cs present complications because of the Bs on their surface). Consequently the composition of C snurposomes remains unclear.

The functions of snurposomes are at present a matter of speculation, but some suggestions are possible from what is known about their molecular components. The simple composition of A snurposomes implies an equally simple function, such as storage of U1 snRNPs. A storage function is in keeping with observations made several years ago that a *Xenopus* oocyte nucleus contains enough stored U1 (and U2) snRNPs to supply all of the embryonic nuclei up to the mid-blastula stage (*17*). In fact, storage (and perhaps recycling) of snRNPs is a possible function for B and C snurposomes as well, because all three snurposomes remain abundant in mature oocytes, when transcription of pre-mRNAs, and hence splicing, shuts down.

An additional and more important possible function for B snurposomes is suggested by the fact that they share with the chromosome loops at least a dozen RNA packaging and splicing components. As many as 50 such components—snRNPs, non-snRNP splicing factors, and other proteins—associate with pre-mRNAs in the form of heterogeneous nuclear ribonucleoprotein particles (hnRNPs), each of which probably contains dozens of individual molecules (*18*). On kinetic grounds alone, it seems unlikely that such complex hnRNP-snRNP particles are assembled on each new segment of RNA as it is transcribed. We suggest, instead, that these particles, or subunits of them, are preassembled in the B snurposomes and then travel to the sites of transcription on the chromosome loops. The 20- to 30-nm particles that compose B snurposomes could be these preassembled complexes. An analogy can be drawn to ribosome assembly. The 70 or so ribosomal proteins first come together in the nucleolus, along with ribosomal RNA and several snRNAs, to form the large and small ribosomal subunits. These assembled subunits travel from the nucleolus to the cytoplasm where they function in protein synthesis.

If all or part of the RNA processing machinery is preassembled in B snurposomes in the oocyte, something comparable is probably true for somatic nuclei. The speckles are possible candidates for somatic homologs of the B snurposomes. Because of their small size, it will be difficult to determine if individual speckles are as complex as B snurposomes. Nevertheless, snRNAs have been demonstrated in the speckles and in three to five more intensely stained foci by fluorescent in situ hybridization (*19*). The foci are also stained by antibodies to peptides from the splicing factor U2AF (*20*). The relations between foci, speckles, and the three types of snurposomes remain to be elucidated.

The giant oocyte nucleus, with its wealth of structural detail, has long been a source of important information about chromosome structure, transcription, and ribosome biosynthesis. With the discovery of snurposomes, it also promises new insight into the cell biology of RNA processing.

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