Cycling splicing factors

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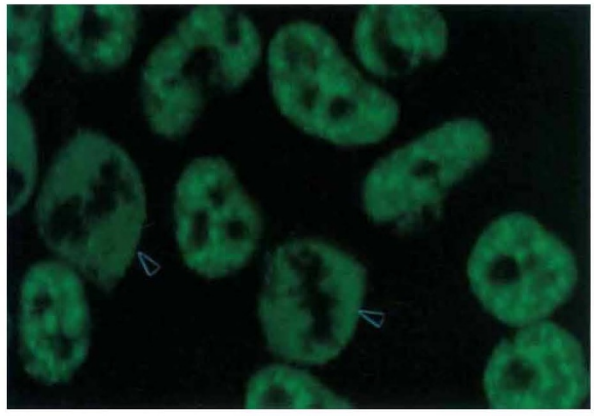
IT is 25 years since it was shown biochemically that small nuclear ribonucleic acids (snRNAs) redistribute to daughter cells at each cell division[1]. On page 678 of this issue[2], Gui *et al.* describe the identification of a kinase that may be responsible for the redistribution of splicing factors, including snRNPs which contain snRNAs, as cells enter mitosis. The same kinase inhibits splicing *in vitro*, so it may also participate in modulating pre-messenger RNA splicing activity.

During interphase, snRNPs (small nuclear ribonucleoprotein particles) and other pre-mRNA splicing factors are organized in a speckled pattern in the nucleus of mammalian cells. This pattern is composed of both perichromatin fibrils and interchromatin granule clusters (see ref. 3 for review). It has been proposed that the perichromatin fibrils represent nascent transcripts and the interchromatin granule clusters are storage or reassembly sites (or both) for splicing factors (see ref. 4 for review). Recent results have led to the suggestion that splicing factors are recruited from the storage/reassembly sites to the sites of active transcription of intron-containing genes[5]. On entry into mitosis, the speckled pattern breaks up as the splicing factors become redistributed throughout the cytoplasm. Late in mitosis the pattern begins to reform, and by the G1 phase of the cell cycle each daughter cell once again exhibits a speckled distribution of splicing factors (see ref. 3).



Pre-mRNA splicing factors are organized in a speckled pattern in interphase nuclei. Upon entry into mitosis (arrowheads) these factors are redistributed diffusely throughout the cytoplasm. A kinase, SRPK1, may be responsible for this redistribution. (Photo by David L. Spector.)

Although several studies have provided a glimpse of many of the players in this orchestra, the conductor has remained elusive. Enter Gui *et al.* — while examining the possibility that $p34^{cdc2}$ is the conductor, they have come up with a new virtuoso, SRPK1, a kinase whose activity is cell-cycle regulated. This kinase phosphorylates a family of proteins, referred to as SR proteins (ref. 6), as well as other splicing factors that contain a domain rich in arginine/serine (RS) repeats. The SR proteins are highly conserved from plants to humans (see ref. 7 for review), their RS domain being responsible for targeting them to nuclear speckles[8], and for protein–protein interactions among family members[9,10]. SR proteins can restore splicing activity to S100 extracts, and they are central to the process of constitutive or alternative pre-mRNA splicing (see ref. 11 for review).

Gui *et al.* have fractionated extracts from mitotic cells and find that the activity of SRPK1 is distinct from that of $p34^{cdc2}$ kinase. The newly identified kinase induces a redistribution of SR proteins and snRNPs when added in purified form to permeabilized cells, but has no apparent effect on several other nuclear constituents examined. It is not yet known if the action of the kinase results in the specific release of SR proteins and snRNPs from the interchromatin granule clusters, or if it causes a complete dissociation of these clusters. Also, more experiments will be required to determine if the kinase has additional substrates, and if the phosphorylation state of a particular substrate is directly responsible for the observed redistribution. Because SRPK1 activity was also evident in extracts from unsynchronized cells, Gui *et al.* raise the possibility that it may also be involved in the regulation of the activity of splicing factors during interphase. It is tantalizing to suggest that changes in the phosphorylation state of these factors may be the trigger for the observed recruitment of splicing factors between storage/reassembly sites and sites of active transcription[5].

There is evidence that phosphorylation and dephosphorylation have a part in the splicing reaction. For example, last year Woppmann *et al.*[12] identified a kinase activity that phosphorylates serines in the RS domains of the U1 70K protein and SF2/ASF, a member of the SR family. The substrate specificity of SRPK1 raises the possibility that it is the kinase responsible for this activity. Phosphatases have been implicated in both catalytic steps of pre-mRNA splicing *in vitro*, but not in spliceosome assembly[13,14]. The study of Gui *et al.* is the first to implicate phosphorylation in the cell-cycle regulation and organization of splicing factors. Because kinases usually work in concert with protein phosphatases, it is likely that one or more phosphatases will also be involved in this process.

In addition to its role in reorganizing splicing factors, Gui *et al.* found that addition of SRPK1 to a splicing reaction *in vitro* resulted in a dose-dependent inhibition of pre-mRNA splicing. It will be interesting to determine if the constitutive and alternative splicing activities of SR proteins differ when they are purified from interphase or mitotic cells.

Sequence analysis has revealed that SRPK1 is related to both the fission yeast kinase Dsk1 (ref. 15) and a hypothetical kinase in *Caenorhabditis elegans*. Although the *dsk1* gene affects chromosome segregation during mitosis, it is not essential for viability; overexpression, however, results in a delay in progression from G2 to mitosis. Although Dsk1 and SRPK1 share homology, they may have different roles in the cell — SR proteins have not yet been identified in yeast and, furthermore, it is unclear if all splicing factors are organized in a speckled pattern in yeast nuclei (see ref. 3 for review). The sequence of SRPK1 includes two stretches of basic amino acids, which may function as nuclear targeting signals, but it will be essential to determine the sub-localization of the kinase in the nucleus (is it in the speckles?) and whether or not its distribution is regulated by the cell cycle.

The findings of Gui *et al.* bring to the forefront the problems of how factors may be maintained in particular subnuclear domains, and how their redistribution is orchestrated. The whole story is not yet in. But the prologue is very thought-provoking, and we can expect exciting developments over the next few years. □

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