Meeting report

Structure and dynamics of the post-transcriptional mRNA-processing machinery

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A report on the 'RNA processsing' and 'Nuclear Import and Export' minisymposia at the first meeting of the European Life Scientist Organization (ELSO), Geneva, 2-6 September, 2000.

Eukaryotic gene expression depends on the synthesis, processing, export and translation of mRNA. These minisymposia tackled multiple aspects of the post-transcriptional pathway of mRNAs including the structure and dynamics of proteins implicated in splicing, polyadenylation and nuclear export.

Dynamics of the cell nucleus

It has been known for some time that the mammalian cell nucleus contains numerous subcompartments, or bodies, which have been implicated in essential processes such as transcription and RNA processing. Cytological studies in which researchers observed the localization of various nuclear proteins have led to the definition of distinct nuclear subcompartments: the nucleolus, the nuclear speckles and several small nuclear bodies including the Cajal bodies (also called coiled bodies). An emerging theme of the meeting was the realization that these nuclear subcompartments are extremely dynamic and that proteins that are usually localized within these compartments are in fact moving rapidly throughout the entire nucleus. Highlights of these minisymposia included reports on the dynamics of proteins localized in these nuclear compartments from Tom Misteli (National Cancer Institute, National Institutes of Health, Bethesda, USA) and the biochemical purification of nucleoli and Cajal bodies from Angus Lamond (University of Dundee, UK).

Using photobleaching techniques, Misteli and co-workers have shown that the vast majority of proteins move within the nucleoplasm by a passive, energy-independent mechanism. Photobleaching experiments have been used for many years to measure the mobility of proteins within lipid bilayers. Misteli and co-workers applied the method to analyze the mobility of proteins in the nucleus of living cells. In these experiments, a small area in the nucleus of a living cell that expresses a protein fused to green fluorescent protein (GFP) is bleached with a short, targeted laser pulse (Figure 1). The recovery of the fluorescent signal in the bleached area as the

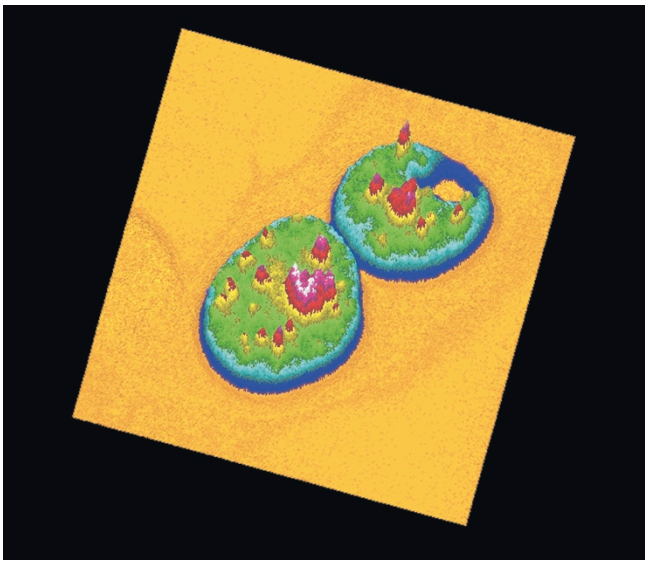

Figure 1
The principle of FRAP. FRAP experiments have been used to analyze the mobility of proteins in the nuclei of living cells. Color-coded fluorescence intensity in the nuclei of two living cells expressing the pre-mRNA splicing factor SF2/ASF fused to GFP is shown. The red peaks represent nuclear speckles, where GFP-SF2/ASF is concentrated *in vivo*. The blue 'holes' in the cell background represent the bleached area. The recovery of fluorescence as a consequence of protein mobility is followed over time. Images courtesy of Tom Misteli.

consequence of movement of the labeled protein is recorded at different times. The kinetics of recovery are a measure of the mobility of the labeled protein. Using this approach, known as fluorescence recovery after photobleaching (FRAP), Misteli analyzed the mobility of the nucleolar protein fibrillarin, several DNA-binding proteins, including HMG-14 and HMG-17, and the pre-mRNA splicing factor SF2/ASF, which is concentrated in speckles but is also present within the nucleoplasm. The astonishing conclusion of these studies is that nuclear proteins that reside in these nuclear structures are nevertheless highly mobile and are rapidly exchanged between these compartments and the nucleoplasm. The high mobility of proteins suggests that most nuclear proteins may rapidly roam the nucleus to find appropriate binding partners and substrates.

This report was complemented by Lamond's work on the biochemical purification and dynamics of Cajal bodies and nuclei. Strikingly, Lamond's studies revealed that the nucleolus, the most extensively studied nuclear subcompartment, is largely composed of 'unknown' proteins. Further information on the sequence, structural domains and function of these novel proteins is eagerly awaited by many.

Nuclear export of messenger RNA

Fully processed mRNAs are exported to the cytoplasm to direct protein synthesis. A general feature of mRNA export is that it is an active, receptor-mediated process. The mRNA-export receptors are thought to recognize and bind either directly or indirectly (via protein-protein interactions) to the mRNA-export substrate and facilitate its translocation across the central channel of the nuclear pore complex (NPC). On the cytoplasmic side of the NPC, the exported mRNA is released and the receptor returns to the nucleoplasm, without the cargo, to initiate additional rounds of export. Published work from the laboratory of Ed Hurt (University of Heidelberg, Germany) and from my group indicate that the yeast protein Mex67p and its human homolog TAP (also called NXF1) are likely to fulfill the function of an mRNA-export receptor. Both groups have now identified a family of evolutionarily conserved RNA-binding proteins that may mediate the interaction between TAP or Mex67p and the mRNA. These proteins are known as Yra1p in yeast and REFs or Aly in higher eukaryotes. Interestingly, some vertebrate REFs localize to nuclear speckles, suggesting that these proteins also interact with components of the splicing machinery and may provide a link between splicing and export (Figure 2).

Both in the nucleus and in the cytoplasm, mRNAs form complexes (messenger ribonucleoproteins or mRNPs) with multiple RNA-binding proteins that affect RNA processing, transport, translation, stability and localization. An excellent example of this is the Balbiani ring mRNPs from the insect *Chironomus tentans*, which are composed of an RNA molecule of

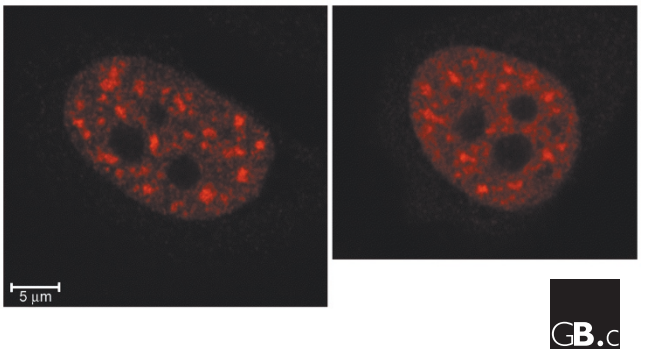

Figure 2
Subcellular localization of the Ref protein REF2-II in HeLa cells. REF2-II is distributed at lower levels throughout the nucleoplasm and is concentrated in 20-40 distinct domains, called the nuclear speckles, which are nuclear subcompartments enriched in splicing factors. Two identical cells are shown.

35-40 kilobases associated with about 500 protein molecules. Published work from the laboratory of Bertil Daneholt (Karolinska Institute, Stockholm, Sweden), describes the identification of many of the proteins associated with this mRNA, including the splicing factor Hrp45 (a homolog of SF2/ASF) and several RNA-binding proteins. Daneholt reported at this meeting that monomeric actin is also associated with the Balbiani ring mRNP in the nucleoplasm. Although the role of actin in this context is unknown, the data raise the intriguing possibility that actin may be involved in some aspects of mRNA export, perhaps facilitating the movement of large mRNPs in the nucleoplasm or across the NPC.

From function to structure

Removal of introns from pre-mRNA is mediated by the spliceosome, a large complex consisting of four small ribonucleoprotein particles (snRNPs) and numerous proteins. Although the determination of the structure of the spliceosome is lagging behind that of the recently reported structures of ribosomal subunits, remarkable progress has been made in determining the structure of some of its individual components. The determination of the structure of the U1 snRNP using cryoelectron microscopy was reported by Holger Stark (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany).

Two other structures of processing or export factors were reported at the meeting. Walter Keller (Biozentrum, Basel, Switzerland) presented the structure of poly(A) polymerase (PAP) at 2.6 Å resolution, and I presented the structure of the amino-terminal domain of human TAP at 2.4 Å resolution. A common theme of these presentations was the realization that proteins with no sequence similarities can

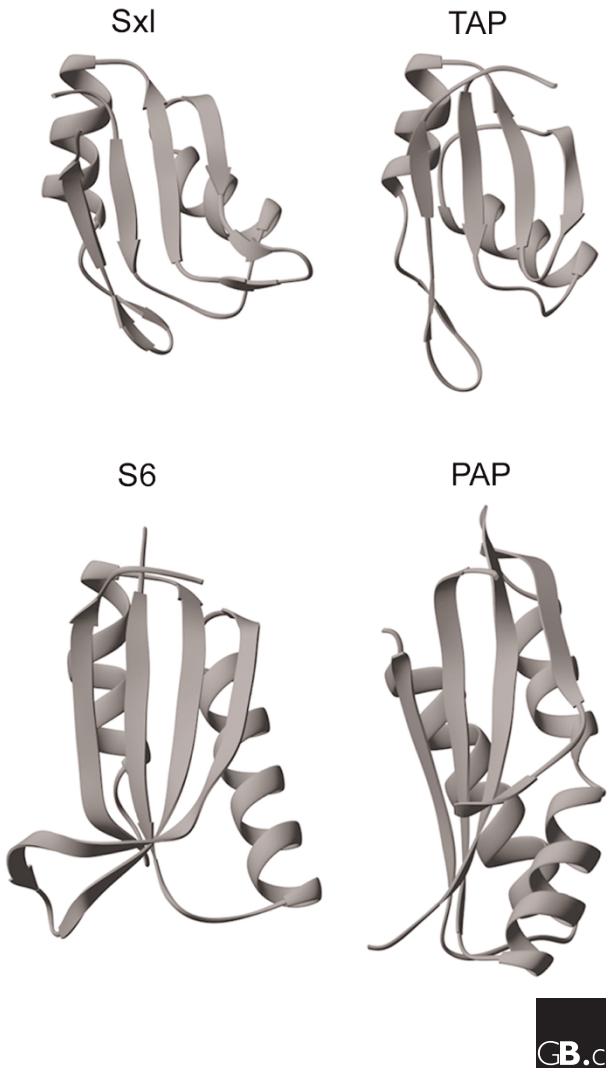

Figure 3
Ribbon diagrams of the structures of the RNA-binding domains of TAP and PAP compared with the known structures of Sex lethal and ribosomal protein S6. The structures are shown in a similar orientation after optimal superposition. Note that, despite the similar folding, none of these proteins exhibits significant sequence similarities. Images courtesy of Elena Conti (EMBL, Heidelberg, Germany).

nevertheless have a very similar three-dimensional folding pattern. PAP has an RNA-binding domain that is folded similarly to the RNA-binding domain of ribosomal protein S6, whereas the RNA-binding domain of TAP has a very similar folding to that of a *Drosophila* protein Sex lethal, which is implicated in splicing regulation (Figure 3).

The RNA processing and export field are undoubtedly benefiting from the use of structural biology, proteomics and imaging techniques, which has allowed important contributions to our understanding of gene expression events *in vivo*.