Short Communication

An ATP-Dependent Step Is Required for the Translocation of Microinjected Precursor mRNA into Nuclear Speckles

(ATP-dependent step / HeLa cell nucleus / microinjected splicing-competent RNA / RNA translocation into nuclear speckles)

V. KOPSKÝ[1,2,3,+], J. VEČEŘOVÁ[1,2,+], I. MELČÁK[1,2,4], A. PLISS[1,2], J. ŠTULÍK[2], K. KOBERNA[1,2], L. TOMÁŠKOVÁ[1,2], I. RAŠKA[1,2]

[1]Department of Cell Biology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic

[2]Laboratory of Gene Expression, 1st Medical Faculty, Charles University, Prague, Czech Republic

Abstract. Nuclear speckles (speckles) represent a distinct nuclear compartment within the interchromatin space and are enriched in splicing factors. In a previous study (Melčák et al., 2001), it has been shown that the pre-spliceosomal assembly on microinjected splicing-competent precursor mRNA takes place in the speckles, and it has been suggested that the targeting of RNA into speckles consists of two interdependent steps, namely the diffusion process, followed by the energy-dependent translocation of RNA into the speckles. In the present study, we confirm the existence of these two steps and show that this latter translocation is ATP dependent.

Most primary transcripts of mammalian protein-coding genes contain introns and have to be spliced before being transported to the cytoplasm. Precursor mRNA (pre-mRNA) splicing takes place in a large ribonucleoprotein complex termed spliceosome. The spliceosomes are generated by the constitutive assembly of U1, U2, U5, U4/U6 small nuclear ribonucleoprotein particles (snRNPs) and various non-snRNP factors on pre-mRNAs in a cascade of sequence-specific steps (Steitz et al., 1988; Lamm and Lamond, 1993; Moore et al., 1993; Newman, 1994; Krämer, 1996). The formation of the functional spliceosome is thus preceded by the formation of a number of pre-spliceosomal complexes which contain, together with unspliced pre-mRNA, defined combinations of snRNP particles and non-snRNP factors (e.g. Steitz et al., 1988; Lamm and Lamond, 1993; Moore et al., 1993; Newman, 1994; Krämer, 1996).

When RNA polymerase II transcriptional and splicing components are mapped within the cell nucleus, they exhibit frequent sites of high local accumulation in the form of microclusters, which likely represent the sites of active transcription and co-transcriptional splicing (Neugebauer and Roth, 1997; Misteli and Spector, 1998). On the other hand, beside these microclusters, an accumulation of factors of the splicing apparatus is typically mapped to spatially distinct and large domains termed „speckles", „SC35 domains", the antibody to splicing factor SC35 (Fu and Maniatis, 1990) being used regularly for the visualization of speckles, or „splicing factor compartments" (reviewed in Spector, 1990; Fakan, 1994; Misteli, 2000). Even though there is a consensus that speckles play a role in RNA metabolism, their exact function is presently unknown. These structures are not usually correlated with RNA polymerase II transcription, but it has been shown at the level of activation of some specific unique genes that speckles serve as pools of splicing factors, which are redistributed to the transcription/splicing sites (Misteli et al., 1997).

The splicing of pre-mRNAs may be a co-transcriptional event (Beyer and Osheim, 1988; Neugebauer and Roth, 1997; Custodio et al., 1999), and it has been shown that transcription and splicing are coupled through interactions of certain factors participating in both these processes (Corden and Patturajan, 1997; McCracken et al., 1997; Steinmetz,

Received January 11, 2002. Accepted January 28, 2002.

This work was supported by grants from the Grant Agency of the Czech Republic 304/00/1481 and 304/01/0729, from the Academy of Sciences of the Czech Republic IAA5039103 and AV0Z5039906, and from the Ministry of Education, Youth and Sports MSM: 111100003.

Corresponding author: Ivan Raška, Department of Cell Biology, Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Albertov 4, 128 00 Prague, Czech Republic, Tel: +420 (2) 24910315, Fax: +420 (2) 24917418, e-mail: iraska@lf1.cuni.cz.

[3]Present address: Department of Animal Physiology and Developmental Biology, Faculty of Science, Charles University, Viničná 7, Prague 2, 128 44, Czech Republic

[4]Present address: Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399, USA

[+] V. K and J. V. were equally contributing authors

Abbreviations: pre-mRNA – precursor messenger RNA, snRNP – small nuclear ribonucleoprotein.

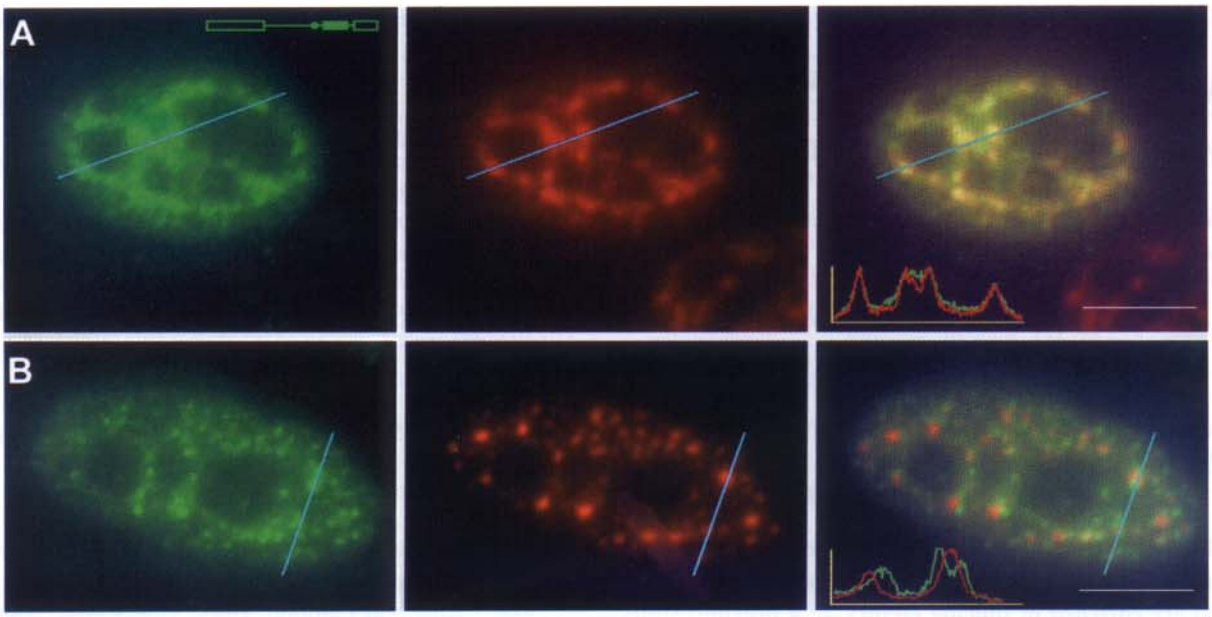

Fig. 1. (A, B) After the consecutive microinjection of apyrase (B) or buffer (A), and of the Ad1 RNA labelled with fluorochrome (in green), the cells were incubated at 37°C for 15 min. The cells were then labelled for the SC35 splicing factor (in red). The form of the Ad1 RNA, consisting of two exons and one intron, is shown in A. Note a part of a non-microinjected nucleus in A. The two left columns correspond to individual colour channels, the right column is the overlay. In A, RNA was accumulated within the speckles. In B, the ATP depletion resulted in the accumulation of RNA in dots adjacent to the speckles. In the quantitative evaluation (inserts in A and B), fluorescence intensities along the blue lines shown in A and B were scaled to the minimum and maximum values. The identical position of peaks A (insert) in the two colour channels testifies to the accumulation of RNAs in the speckles. Note the differences in the position of peaks in B (insert). The bar in A and B corresponds to 4 μm.

1997). However, not all pre-mRNA sequences are processed co-transcriptionally and post-transcriptional splicing does occur (Zachar et al., 1993; Baurén and Wieslander, 1994; Wuarin and Schibler, 1994). Importantly, isolated pre-mRNAs from mammalian cells may contain both introns and poly(A) tails. Splicing may then be a post-transcriptional event, at least in some cases (McCracken et al., 1997; Minvielle-Sebastia and Keller, 1999), and it has been suggested that speckles are involved in post-transcriptional splicing (Melčák and Raška, 1996). With regard to the spatial relationship of pre-mRNA accumulations relative to speckle domains of splicing factor accumulations, primary transcripts of certain genes as well as the spliced RNAs have been mapped at sites of active transcription and/or outside the speckles (Zhang et al., 1994; Smith et al., 1999). However, pre-mRNAs from some other genes have been shown to be associated, or overlapped, with nuclear speckles (Xing et al., 1993, 1995; Huang and Spector, 1996; Dirks et al., 1997; Ishov et al., 1997; Jolly et al., 1999; Smith et al., 1999; Snaar et al., 1999; Johnson et al, 2000; Melčák et al., 2000).

The suggested function of speckles in splicing has been probed using a completely different approach in which the behavior of microinjected (and fluorochrome-labelled) pre-mRNA into the nuclei of Hela cells was investigated (Wang et al., 1991; Melčák et al., 2001). The rationale for this approach is based on the findings of Graessmann and Graessmann (1982) that microinjected intron-containing RNA is processed into functional mRNA within the cell nucleus. These exogenous RNAs skip the transcription context and behave similarly to endogenous unspliced RNAs released from the sites of transcription. Using model RNAs with a single intron, it has been shown that these RNAs rapidly accumulate in the speckles after microinjection in a process that is dependent on the intron (Wang et al., 1991; Melčák et al., 2001) and that the pre-spliceosome assembly on RNAs takes place within the speckles (Wang et al., 1991; Melčák et al., 2001). RNA targeting to and accumulation within the speckles is the result of the cumulative loading of splicing factors to the pre-mRNA.

In this study, we expand the results with microinjected adenovirus pre-mRNAs and demonstrate that the targeting of microinjected pre-mRNA, together with formed pre-spliceosomal complexes, to the speckles consists of two interdependent steps, namely the movement of pre-mRNA towards the speckles and its translocation into the speckles, this second step requiring ATP.

Material and Methods

Synthesis of the fluorochrome-labelled and splicing-competent adenovirus Ad1 RNA, containing a single