Role of the 5'-cap in the biogenesis of spliceosomal snRNPs

Achim Dickmanns and Ralf Ficner

Abstract

The biogenesis of spliceosomal UsnRNPs in higher eukaryotes involves a nucleocytoplasmic shuttling cycle. After transcription and processing in the nucleus, the m^7G-cap-dependent export of the snRNAs U1, U2, U4, and U5 to the cytoplasm occurs. In the cytoplasm, these UsnRNAs specifically associate with seven Sm-proteins and form a doughnut-shaped snRNP core structure. This assembly, mediated by the SMN complex, is a prerequisite for the hypermethylation of the m^7G-cap to the 2,2,7-trimethylguanosine (m_3G)-cap. Snurportin1 (SPN1), specifically, recognises the m_3G-cap and facilitates the nuclear import of UsnRNPs. The recently determined crystal structure of human SPN1 reveals a significantly different binding mode for the cap structure in comparison to that of the m^7G-binding proteins CBC, eIF4E and VP39.

1 Introduction

In eukaryotic cells, mRNAs are generally transcribed as pre-mRNAs. One hallmark of the eukaryotic genome is the separation of genes into coding regions of variable length, by intervening non-coding regions. Thus, the newly synthesized pre-mRNAs also carry coding sequences, exons, and non-coding regions, introns. Before export to and subsequent translation in the cytoplasm, the introns are removed by the spliceosome (see also Chapter 7), a highly dynamic large ribonucleoprotein complex. Two forms of spliceosomes have been identified, the major spliceosome, which is responsible for the majority of the pre-mRNA splicing events, namely splicing of the so called U2 type introns, and the minor spliceosome processes a rare class of pre-mRNA introns, the U12 type (Burge et al. 1999).

Essential components of the major spliceosome are the five uridyl-rich small nuclear ribonucleoprotein particles (UsnRNPs) U1, U2, U4, U5, and U6 and several non-snRNP proteins (Hartmuth et al. 2002; Makarov et al. 2002). Each UsnRNP is composed of a UsnRNA and a set of seven proteins common, the Sm proteins, for U1, U2, U4, U5, or highly homologous proteins to those seven, the Lsm proteins, for U6. Additionally each U snRNP acquires a subset of particle specific proteins (reviewed in Will and Lührmann 2001).

Topics in Current Genetics, Vol. 12
H. Grosjean (Ed.): Fine-Tuning of RNA Functions by Modification and Editing
DOI 10.1007/b106799 / Published online: 27 January 2005

2 snRNP biogenesis

During U1, U2, U4, and U5snRNP biogenesis in metazoans from snRNA into fully assembled particles, they undergo one round of shuttling between the nuclear and cytoplasmic compartment and return into, and localize in subnuclear compartments within the nucleus. After transcription, addition of an m^7G-cap to the 5'-end, and 3'-end modifications, the RNA is exported into the cytoplasm where the Sm proteins assemble to it. Interestingly, before reimport into the nucleus a second round of RNA modification takes place at both ends. The 3' end is trimmed to the final length and the 5' cap is hypermodified into a trimethyl-2,2,7-G-cap. After reimport, the UsnRNA is modified a third time, in order to allow assembly of additional, particle-specific proteins, before the mature UsnRNP is formed. During both transport processes the structures of the RNA caps are important determinants mediating the transfer. In the following, we will describe in more detail the biogenesis of mammalian UsnRNPs especially focusing on the transport processes and structural aspects of recognition.

2.1 Transcription of snRNAs

In eukaryotes, the site for RNA transcription and maturation lies within the nucleus. Three RNA polymerases (RNA Pols) have been identified and their responsibilities assigned. RNA Pol II exclusively transcribes all RNAs to be translated, whereas the untranslated RNAs are transcribed by all three RNA Pols. RNA Pol I transcribes 5.8S, 8S and 28S rRNA in the nucleolus and RNA Pol III is responsible for transcription of tRNAs and 5S rRNA (for survey, see Paule and White 2000).

Like mRNAs, the U1, U2, U4, and U5 snRNAs are transcribed by RNAPol II, but the fate of the transcribed RNA is already determined by their promoters due to a simple structure comprising proximal and distal sequence elements (for a more detailed review, see Hernandez 2001).

The primary transcripts are modified at both, the 5' and 3' end (for review, see Cougot et al. 2004). The snRNAs acquire a guanosine to the first nucleotide at the 5' end of the RNA via a 5'-5'triphosphate linkage forming a GpppG/A-cap structure (Shatkin 1976; Coppola et al. 1983), catalyzed by a triphosphatase and a guanylyltransferase. Subsequently the cap guanosine is methylated at N7 by a methyltransferase (see also the chapter by Bokar in this volume for more details; for survey, see Shuman 2002). The three enzyme activities are part of the cellular capping apparatus. The addition and methylation of the cap occurs cotranscriptionally (Salditt-Georgieff et al. 1980) to all RNA Pol II transcripts. The m^7G-cap structure protects the RNA to 5' exonuclease activity (Furuichi et al. 1977; Shimotohno et al. 1977; Murthy et al. 1991) and is the important determinant for UsnRNA export (Shatkin 1976).

The m^7G-cap of UsnRNAs as well as mRNAs (see Chapter 5) is specifically recognized within the nucleus by the cap binding complex (CBC; Ohno et al. 1990; Jarmolowski et al. 1994). CBC is a heterodimer of the CBP20 and CBP80