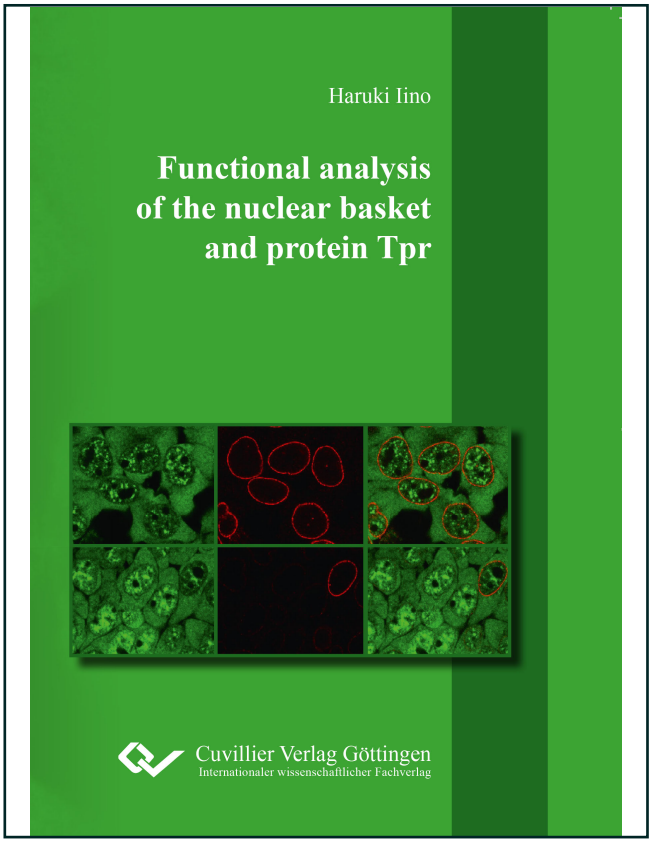
Haruki Iino (Autor)

Functional analysis of the nuclear basket and protein Tpr



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2. Abstract

A large coiled-coil protein, Tpr (Translocated promoter region) is located at the nuclear side of the nuclear pore complex (NPC), and plays an important role in the architecture of the nuclear basket (NB). Although its contribution to the NB structure has been characterized, little is known about the function of Tpr. In this work, we investigated whether and how Tpr contributes to the surveillance of mRNA export, especially, to the quality control (QC) of certain un-spliced mRNAs.

To answer these questions, we established a series of different reporter cell lines, allowing us to monitor the occurrence of un-spliced and spliced reporter transcripts. In the analysis of most of these reporter cell lines, the depletion of Tpr did not cause any obvious leakage of un-spliced reporter mRNAs. However, when the coding sequence of the HIV (Human immunodeficiency virus) Gag protein was used as a readout, the cytoplasmic levels of the un-spliced HIV-*gag* mRNAs were highly enhanced in the absence of Tpr. Such phenotype was only observed when a viral RNA export enhancer element, the CTE (the constitutive transport element), that recruits the general mRNA export receptor, NXF1/TAP, was part of the HIV-*gag* reporter gene. Intriguingly, a slight reduction in the cellular Tpr levels already caused the breakdown of a retention mechanism that normally keeps these HIV-*gag* transcripts in the nucleus. Results obtained with this and other transcripts showed that Tpr can indeed play a role in keeping certain transcripts within the nucleus in an export-pathway-dependent manner. However, they also reveal that this Tpr-dependent retention mechanism does not monitor intron-containing transcripts in general. In fact, at least in the case of the HIV-*gag* transcript, such retention did not depend on the presence of splice sites, the branch point sequence (BPS), or the poly-pyrimidine track (PPT). How such retention of certain transcripts along a distinct export pathway might be mechanistically explainable is being discussed.

3. Introduction

3.1 Gene expression in eukaryotes

In eukaryotes, the genetic material is surrounded by a double lipid bilayer, called the nuclear envelope (NE), and translation is physically separated from the transcription process. This compartmentalization is a specialty of eukaryotes and provides additional regulatory options in their gene expression. Molecule exchange between these two compartments is mediated by the nuclear pore complexes (NPCs) that are embedded in the NE (See 3.6).

Gene expression in eukaryotic cells begins with the synthesis of pre-mRNAs at the transcription site in the nucleus, and is organized by RNA polymerase II (Pol II). The carboxyl-terminal domain (CTD) of the Pol II large subunit is evolutionally conserved, and serves as a flexible platform for transcription-mediating proteins. The recruitment of those proteins is coordinated by the phosphorylation state of the CTD (Dahmus et al, 1996; Komarnitsky et al. 2000), and RNA chains are efficiently elongated. After the synthesis of the first ~20-24 nucleotides (nts), the terminal nucleotide of the 5' end is protected with a 7-methyl-guanylate (m^7G) (Capping), which in turn allows to recruit the cap binding complex (CBC). Consequently, the newly synthesized nascent RNAs are stabilized (See 3.4, Figure 3-4) (McCracken et al. 1997; Cho et al. 1997). Most of splicing occurs co-transcriptionally (Girard et al. 2012), and the introns of pre-mRNAs are spliced before the release from the transcription site (see 3.2.1). The exon junction complexes (EJCs) are assembled upstream of the exon-junctions at the late stage of splicing (Le Hir et al. 2001), and become a hallmark of spliced transcripts. In the termination step, cleavage and polyadenylation specificity factor (CPSF) recognizes polyadenylation signals (PAS: AAUAAA or AUUAAA) on the transcript, and cleavage stimulation factor (CstF) defines the cleavage site (poly(A) site: CA) by binding downstream of the PAS. Poly(A) polymerase (PAP) is then recruited to the poly(A) site, and cleaves the transcript. The cleavage site is further polyadenylated, and ~250-300 adenosine monophosphates (in human cells) (Brawerman et al. 1981; Wahle et al, 1995) are added to the 3' end of the transcript. This elongation process is achieved by PAP as well as additional poly(A)-binding proteins.

During the transcription, the RNA binding proteins bind to the synthesized RNAs with their associated proteins, and form mRNPs (messenger ribonucleoproteins). Only

mature mRNPs attract a group of export-mediating proteins. Such mRNPs are then selectively exported into the cytoplasm through the permeability barrier of the NPC which represents a hydrogel-like meshwork formed by the natively unfolded phenylalanine-glycine (FG) repeat domains of several different NPC proteins (nucleoporins) (See 3.6).

3.2 mRNA processing

3.2.1 Sequential splicing reactions

As stated above, the introns in pre-mRNAs are normally co-transcriptionally removed, and the splicing reactions are achieved in a stepwise manner by the spliceosome that consists of small nuclear RNAs (snRNAs) and numerous associated proteins (Figure 3-1) (e.g., reviewed in Wahl et al. 2009; Will & Lührmann 2011).

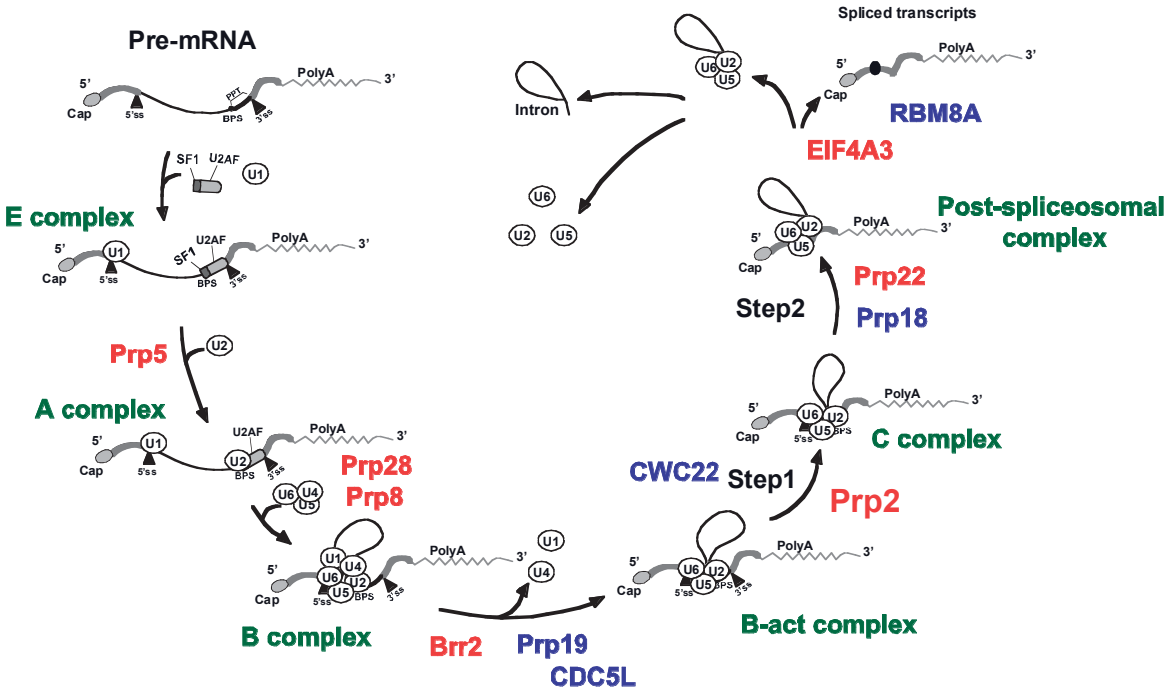

Figure 3-1. The sequential splicing events and the complex assembly
The depiction illustrates the assembly and disassembly of spliceosomal factors in splicing. The name of the complex of each stage is indicated in green. The helicases involved at distinct steps of the splicing process are shown in red and some further characteristic proteins in blue. The residence of the spliceosomal proteins on the spliceosome is not shown in the illustration, but is summarized in the Table 3-1. The Prp19 complex is recruited onto U6 snRNP-containing spliceosome. B^{act} complex (including CWC22) is loaded onto the Prp19 complex. The C complex second step II factors (including Prp18) associate with the B^{act} Complex. The step1 and step2 indicate the first and second transesterification reactions respectively. The depiction is based on Wahl et al., 2009, Will and Lührmann 2011, and Agafonov et al. 2011.

Name	Alias	Residence					Function
		E	A	B	B*	C	
SNRP70	U1-70K	**	**				U1snRNPs
SNRPA	U1A						
SNRPC	U1C						
SF1		**					U2snRNPs
U2AF2	U2AF65		**	*			
DDX46	Prp5	**	*				U2 recruitment
DDX23	Prp28			**	*	*	U5 associated protein/The B complex assembly/ the replacement of U5 at the exon of 5'ss
SNRNP220	Prp8						U5 associated protein/Coordination of Prp28 and Brr2 helicase activities
SNRNP200	Brr2			**	**	**	Annealing of U6 to U2 and releasing of U4 and U1
Prpf19	Prp19						After the B-complex before the C-complex step02
CDC5L					*	**	Required for the dissociation of U5 snRNPs from splicesome
Snip1				*	**	*	RES complex
DHX16	Prp2			*	**	**	C complex step I factor/ after the B activated complex
CWC22						**	EJC assembly component
EIF4A3						*	EJC component
Prpf18	Prp18						C complex step II factor
DHX8	Prp22					**	C complex step II factor
RBM8A	Y14						EJC component/ Heterodimer with MAGOH/ RNA binding motif

Table 3-1 The residence, phosphorylation state, and function of the spliceosomal proteins
The helicases required for the conformational change of the spliceosome are written in red. The green boxes indicate the presence of the protein at the stage of the spliceosome complex (E, A, B, B*,C). The degree of the green represents the abundance of the protein at the stage. The phosphorylation state of the protein is shown as the number of the asterisks (*). The described information is the summary from Agfonov et al. 2011 and Will and Lührmann 2011.

As a first step, the 5’ splice site (ss) (or Donor site (D)) of pre-mRNAs are initially recognized by U1 snRNA associated with the core U1 snRNA binding proteins (U1-70k, U1-A, and U1C). The branch point sequence (BPS) and ~20-40 nts of the poly-pyrimidine-track (PPT) upstream of the 3’ ss (or Acceptor site (A)) are attracted by splicing factor 1 (SF1) and the large subunit of U2 snRNA associated factor (U2AF65), while the U2AF2 small subunit (U2AF1) recognizes the AG of the 3’ss. The complex at this stage is called the spliceosomal E complex.

The ATP-dependent helicase activities of Prp5 and UAP56 are required for the further displacement of SF1 to U2 snRNPs at the BPS, and the A complex is assembled as a result. The tri-snRNPs of U4/U6.U5 are then recruited to the A complex together with the Prp19/CDC5L complex (the B complex). This conformational change is achieved by the U5 associated helicases, Prp28 and Prp8. This B complex is catalytically inactive, but is activated by the release of U1 and U4 snRNPs with the help of Brr2 (the B activated complex), and then, the spliceosome is rearranged by Prp2 to a catalytic form (the C complex).

In this stage, the 2’ OH group of the adenosine in the BPS attacks the guanosine of the 5’ ss, resulting in the cleavage between the 3’ OH group of the 5’ exon and the 3’ phosphate group of the intron. This free phosphate group subsequently binds to the 2’ OH group of the adenosine, and forms a lariat intermediate (Figure 3-2). This reaction is the first transesterification. Additionally, the 3’ OH group of the 5’ exon attacks the 3’ ss, and the 5’ exon and 3’ exon are jointed (Figure 3-2). This is the second transesterification. The intron lariat is consequently released from the 3’ exon. These reactions are facilitated by the C complex step II factors (e.g., Prp18, Prp16 (Ohrt et al.)).

The exon junction complexes (EJCs) are assembled ~20 nts upstream of the exon-exon junction on the transcripts. This assembly starts with the recruitment of CWC22 at the B-act complex, which then serves as a platform for eIF4A3, an EJC component (Steckelberg et al.). Subsequently, additional EJC components (MAGOH and RBM8A (Y14)) are recruited. Finally, Prp22 promotes the disassembly of the spliceosomal proteins (Schwer 2008; Fourmann et al. 2013) and spliced mRNA.