NEWS AND VIEWS

Mechanisms of Nuclear Transport in the cell: RNA exosome in *Saccharomyces cerevisiae*

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Abstract: Ribonucleases (RNases) functions in the cell include precise maturation of non- coding RNAs and degradation of specific RNA transcripts that are no longer necessary. RNAses are present in the cell as single units or assembled as multimeric complexes; one of these complexes is the RNA exosome, a highly conserved complex essential for RNA processing and degradation. In the yeast *Saccharomyces cerevisiae*, the RNA exosome comprises eleven subunits, two with catalytic activity: Rrp6 and Rrp44, where the Rrp6 subunit is exclusively nuclear. Despite the RNA exosome has been intensively investigated since its discovery in 1997, only a few studies were accomplished concerning its nuclear transport. This review describes recent research about cellular localization and transport of this essential complex.

Key words: Ribonucleases, exosome, nuclear transport, Rrp6, Rrp44, RNA processing, RNA degradation.

Introduction

RNA exosome is a multiprotein RNase involved in the modification of all RNAs in the cell; its primary function includes exonucleolytic and endonucleolytic cleavage of pre-ribosomal RNA (pre-rRNA), messenger RNA (mRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA)[1]. Other RNA exosome substrates were described and included a significant group of long noncoding RNAs (lncRNAs): cryptic unstable transcripts (CUT) in yeast and upstream promoter transcripts (PROMPT) in humans, prematurely terminated RNA (ptRNA), enhancer RNA (eRNA) and long intergenic RNA (lincRNA)[2] (Figure 1). Highly conserved throughout evolution, exosome complex can be found in several species such as *Archaea*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Arabidopsis thaliana*, *Mus musculus*, and humans, to mention some. In *Saccharomyces cerevisiae*, the exosome is present in both nucleus and cytoplasm, fulfilling different functions depending on its substrates. Two structures compose the exosome core: a barrel-like with six different subunits, each containing an inactive RNase PH domain (Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, Mtr3); and a cap, capping this barrel with three RNA binding subunits (Rrp4, Rrp40, Csl4). The catalytic subunits bind to opposite sides of the core: Rrp44 with endo- and exonucleolytic activities is present in both nuclear and cytoplasmic exosome[3-5]; Rrp6 with a 3'-5' exonucleolytic activity present only in the nuclear exosome[6] (Figure 2).

The nuclear exosome[7,8] processes degradation of incorrectly processed RNAs and maturation of ribosomal RNAs, small nuclear RNAs and small nucleolar RNAs. Furthermore, cytoplasmic exosome plays a vital role in regulating mRNA biogenesis's gene expression by mRNA degradation[9,10].

Nuclear transport of exosome

Nuclear transport is primarily mediated by specific interactions between a karyopherin, a protein transporter, and signal sequences (NLS; Nuclear Localization Signal) present in the cargo proteins[11,12] (Figure 3). In *Saccharomyces cerevisiae*, there is one karyopherin: Srp1. Besides, there are 14 karyopherins β: ten of which are involved in transport to the nucleus (importins - Kap95, Kap104, Sxm1/Kap108, Mtr10, Kap114, Nmd5, Kap120/Lph2, Pse1/Kap121, Kap122, Kap123), three are involved in transport to the cytoplasm (exportins - Cse1, Crm1/Xpo1, Los1), and one karyopherin is involved in transport in both directions, Msn5[13]. Interaction of karyopherins Srp1 and Kap95 with Rrp6 were observed initially in protein purification experiments[14-17] and co-immunoprecipitation experiments using Rrp6 as bait showing a surprising number of karyopherins: Kap95, Srp1, Kap114, Kap123, Sxm1, and Cse1[18].

Presence of NLSs in exosome subunits

In-silico and experimental analysis showed three subunits containing NLSs in their sequence: Rrp6, Rrp43, and Rrp44. Subunit Rrp43 contains a potential NLS between the residues 203 and 213: $_{203}$LKMKRKWSYVL$_{213}$ identified using the software NLS Mapper and NLStradamus[19,20]. No experiments are available to test if this motif could serve as a nuclear localization signal.

Subunit Rrp6 showed the presence of a three different NLSs in its sequence

NLS1 sequence $_{104}$NSKSRGSDLQYLGEFSGKNFSPTKRVEKP$_{132}$ identified by NLS Mapper and NLStradamus[19,20]. NLS2: $_{153}$KEKPNALKPLSESLRLVDDDENNPSHYPHPY$_{183}$, contains a multipartite proline–tyrosine nuclear localization signal called PY-NLS with a C- terminal R/H/K-X2–5-P-Y motif within a positively charged region of approximately 30 amino acids[18,21]. They could be recognized by importins Kap104, Sxm1/Kap108, Kap121, Kap114, Nmd5/Kap119, and Kap95[22-24].

NLS3: $_{697}$RQQKKRRFDPSSSDSNGPRAAKKRRPA$_{723}$, a classical NLS at the C-terminal region of Rrp6 that could be recognized by importin α Srp1[25]. It was observed that lack of any of the mentioned NLSs in the Rrp6 sequence lead to a partial mislocalization in the cell[18].

Subunit Rrp44 showed to possess three NLSs

NLS1: $_{172}$RAIRKTCQWYSEHLKPY$_{188}$, similar to Rrp6-NLS2 with a nuclear localization signal PY-NLS, NLS1 is located PinC domain of Rrp44[26].

NLS2 is located in the cold shock domain 2 CSD2[26] be-

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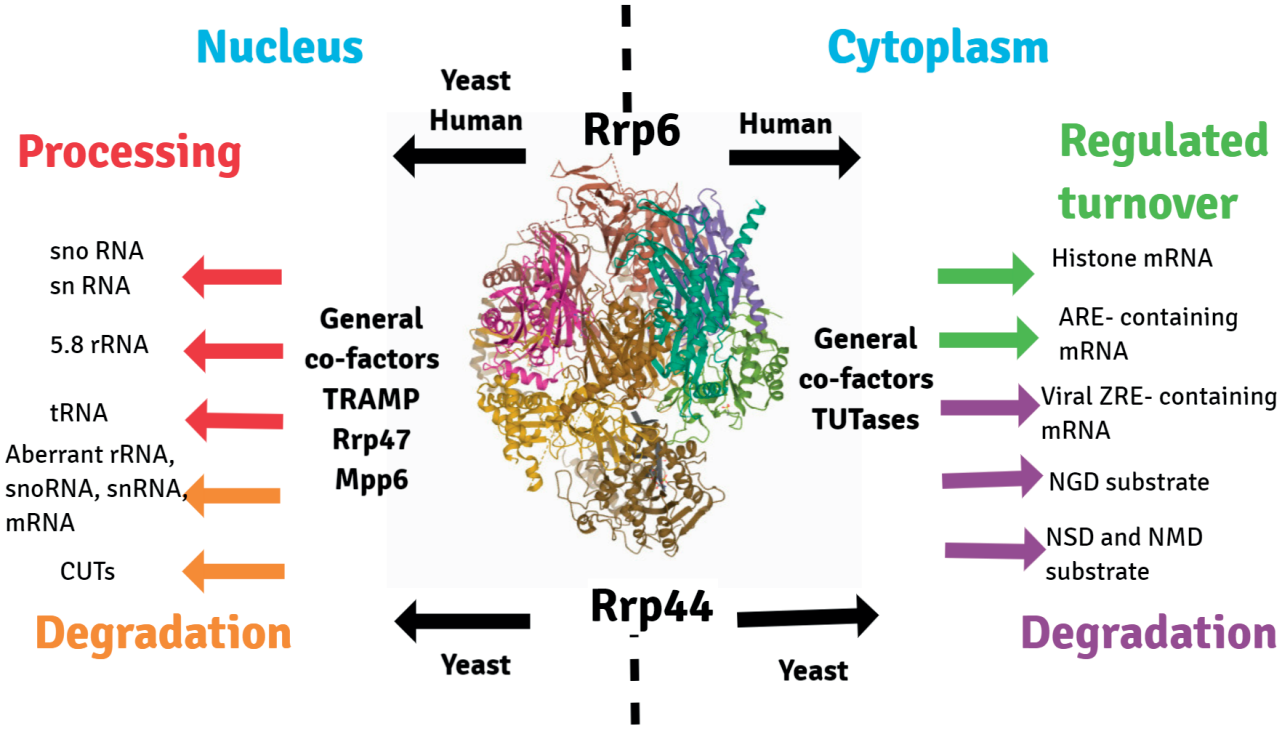

Figure 1. RNA quality control by the exosome for aberrant mRNA, tRNA, rRNA, and other noncoding RNA (ncRNA) species in every cell compartment. Arrows indicate the localization of active nucleases Rrp6 and Rrp44. TRAMP (complex consists of helicase Mtr4, polyA polymerase Trf4 or Trf5, and RNA-binding proteins Air1 or Air2), TUTases (terminal RNA uridylyltransferases). CUTs (Cryptic unstable transcripts), upstream promoter transcripts (PROMPTs), and upstream noncoding transcripts (UNTs). ARE (AU-rich instability element). Aberrant mRNAs with translation defects: NMD (Nonsense-mediated decay), NSD (non-stop decay), and NGD (no-go decay).

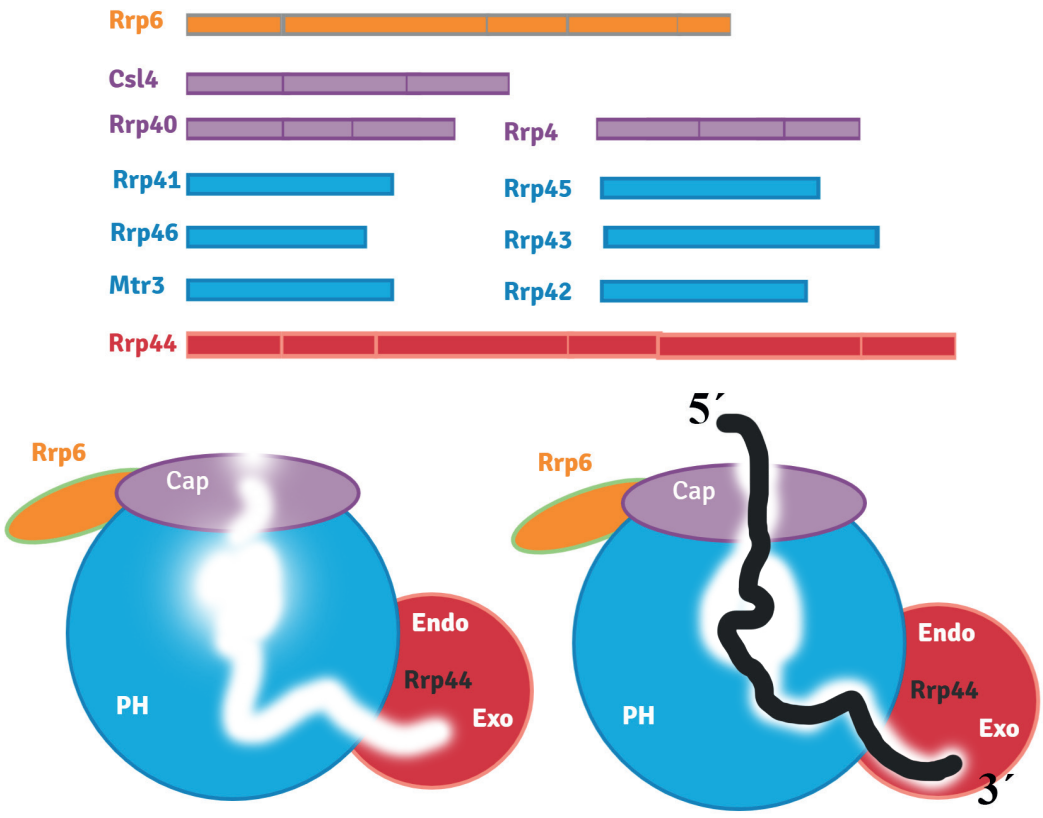

Figure 2. Exosome structure in yeast. A. Cap and barrel-like structures of exosome in purple and blue, respectively. Associated nucleases are in red (Rrp44) and orange (Rrp6). B. RNA-free form of the exosome. C. RNA going through the exosome channel to be processed.revistabionatura.com/2020.05.04.25.html

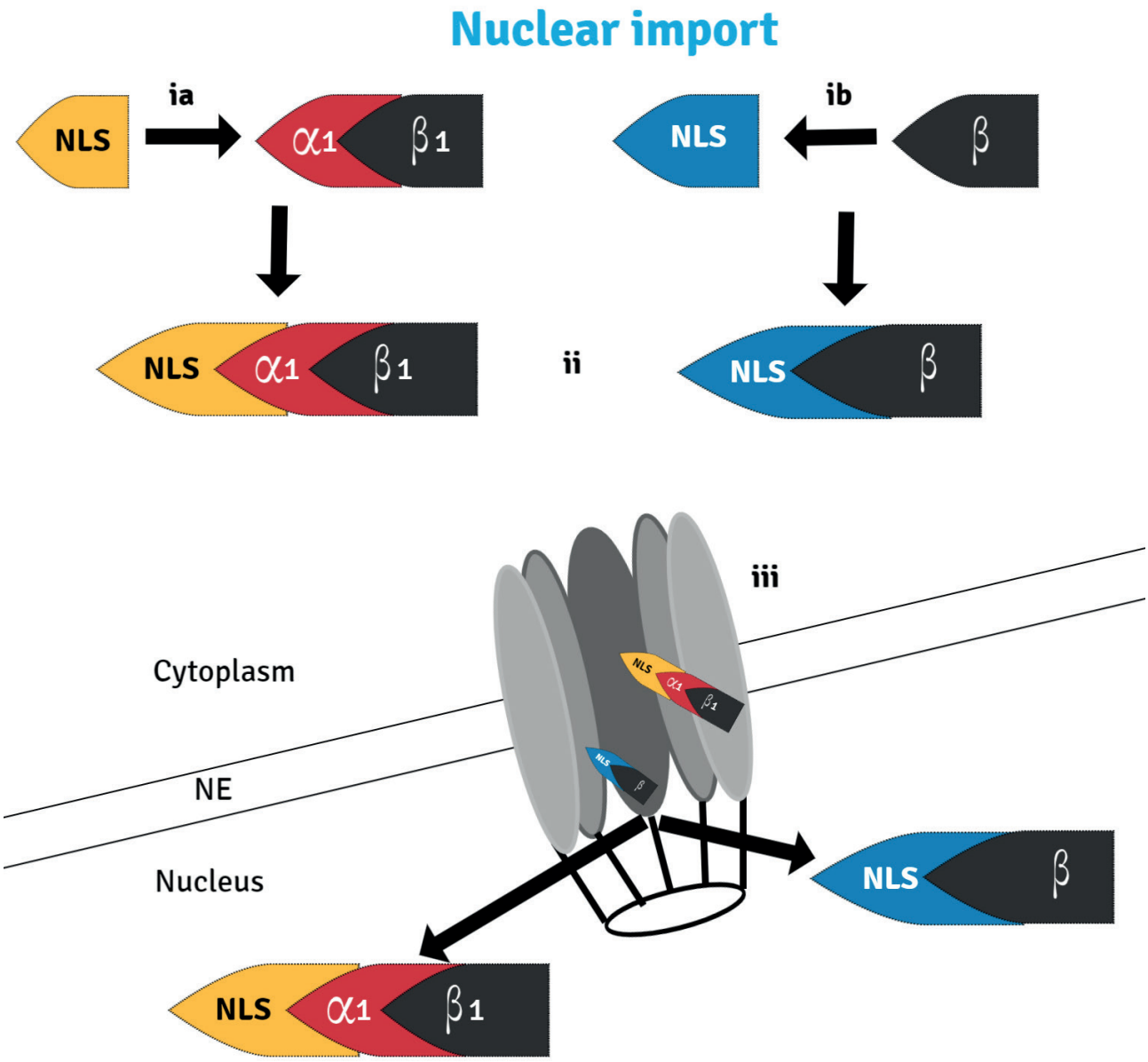

Figure 3. Nuclear transport through the Nuclear Pore Complex requires the recognition and binding of NLS-containing cargo proteins by either the importins α/β1 heterodimer (ia) or importin β alone (ib) on the cytoplasm. Once bound (ii), the transport complex moves through the NPC (iii) via a series of transient interactions between importin β and nucleoporins in the NPC.

tween the residues 370 and 401 of Rrp44 $_{370}$RRLLAKDAMIAQRSKKIQPTAKVVYIQRRSWR$_{401}$ and contains the consensus NLS R/K-X2-L-XnV/Y-X2-V/I-X-K/R-X3-K/R that can be recognized by the importins Kap114, Kap95, Kap123, Pse1, and Kap104[27].

NLS3 is located at the C-terminus of Rrp44: $_{988}$DPITSKRKAELLLK$_{1001}$. This NLS is similar to the NLS in Drosophila Rrp44 (dDis3), which is recognized by importin-3[28,29]. From the three NLSs presents in Rrp44 sequence, NLS1 is the most critical signal for Rrp44 nuclear import. NLS1, together with either NLS2 or NLS3 is sufficient for Rrp44 nuclear localization[18].

The presence of three nuclear localization signals in both Rrp6 and Rrp44 and more of one karyopherin involved in their nuclear transport confirm overlapping and redundancy in the import pathways.

Depletion of karyopherins affect the localization of exosome subunits

Depletion of essential karyopherin Srp1 in the cell after 14 hours showed Rrp6 visualized in the cytoplasm, although Rrp6 remained concentrated in the nucleus; levels of Rrp6 decreased upon inhibition of Srp1 expression, it could suggest when not efficiently transported to the nucleus, Rrp6 may be destabilized[18]. Inhibition of karyopherin Kap95 expression for 14 hours strongly affected Rrp6 localization; Rrp6 was observed in the cytoplasm and concentrated in the nucleus. Surprisingly, the deletion of the karyopherin Sxm1/Kap108 gene affected the localization of Rrp6 partially Sxm1 is not essential for growth but Rrp6 was detected in the cytoplasm, and its mislocalization was more robust at 37°C. These results showed that Rrp6 could associate with different importins for its transport to the nucleus[18].

Localization of Rrp44 in wild-type cells is mainly in the nucleus with a weak signal in the cytoplasm. The depletion of Srp1 or Kap95 levels in the cell leads to Rrp44 partial mislocalization, evidenced by a strong signal in the cytoplasm. Unlike Rrp6, the depletion of Sxm1 did not show any effect on Rrp44 localization[30].

Conclusions

The RNA exosome is localized in the cytoplasm and the nucleus (specifically in the nucleolus). Although its predominantly nuclear localization, only three exosome subunits contain NLSs in their sequence, and from these three, two have shown to be transported by karyopherins: Rrp6 and Rrp44. It is already known that Srp1 and Kap95 are the karyopherins involved in the nuclear transport of Rrp44 and Rrp6, with the extra help of karyopherin Sxm1 for Rrp6 transport. However, it is uncertain how the rest of the subunits are transported to the nucleus or transported independently or as a complex. Only further studies will help to elucidate these questions and better understand the transport mechanisms of this complex.

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