Yeast cleavage factor Hrp1 is a novel guard protein that surveils pre-mRNA 3' processing

Dissertation

for the award of the degree

"Doctor of Philosophy" (Ph.D.)

Division of Mathematics and Natural Sciences

of the Georg-August-Universität Göttingen

within the doctoral program "Biology"

of the Georg-August University School of Science (GAUSS)

submitted by

Jing Li

From Shanxi, China

Göttingen, January 2022

Members of the Thesis Committee

Prof. Dr. Heike Krebber
Department of Molecular Genetics
Institute for Microbiology and Genetics

Dr. Oliver Valerius
Department of Molecular Microbiology and Genetics
Institute for Microbiology and Genetics

PD Dr. Wilfried Kramer
Department of Molecular Genetics
Institute for Microbiology and Genetics

Members of the Examination Board

Reviewer: Prof. Dr. Heike Krebber
Department of Molecular Genetics
Institute for Microbiology and Genetics

2nd Reviewer: Dr. Oliver Valerius
Department of Molecular Microbiology and Genetics
Institute for Microbiology and Genetics

Further members of the Examination Board:

PD Dr. Wilfried Kramer
Department of Molecular Genetics
Institute for Microbiology and Genetics

Prof. Dr. Stefani Pöggeler
Department of Genetics of Eukaryotic Microorganisms
Institute for Microbiology and Genetics

Prof. Dr. Jörg Stülke
Department of General Microbiology
Institute for Microbiology and Genetics

Prof. Dr. Kai Heimel
Department of Molecular Microbiology and Genetics
Institute of Microbiology and Genetics

Date of the oral examination: 22.03.2022

Affidavit

I hereby declare that I prepared this doctoral thesis titled “Yeast cleavage factor Hrp1 is a novel guard protein that surveils pre-mRNA 3’ processing” independently and with no other sources and aids than quoted.

Göttingen, January 2022

_____

Jing Li

Table of Contents

1. Abstract

Hrp1 is a component of the cleavage and polyadenylation complex (CPF-CF) for pre-mRNA cleavage and polyadenylation in *S. cerevisiae*. It specifically binds to a relatively conserved UA-rich domain called the efficiency-element (EE) upstream of the cleavage site to promote the accuracy and efficiency of pre-mRNA 3' processing. Moreover, it functions in nonsense-mediated decay (NMD) and therefore commutes between the nucleus and the cytoplasm. Interestingly, it shares similarities with guard proteins that surveil mRNA processing. It contains two RNA-binding motifs and a typical SR/RGG (serine-arginine/arginine-glycine-glycine) rich domain. We propose that Hrp1 might also be a guard protein that surveils pre-mRNA cleavage and polyadenylation and have carried out a series of experiments to support this idea. We found that Hrp1 physically and genetically interacts with the mRNA export machinery Mex67-Mtr2 and directly contacts a component of the nuclear pore complex (NPC) named Mlp1, which is important for surveillance of mRNA export. Similar to the other guard proteins, overexpression of *HRP1* is toxic to cells and retains mRNAs in the nucleus. Most strikingly, in comparison to the nuclear retention of faulty mRNAs in the exosome mutant *rrp6Δ* and the CPF-CF complex mutant *cft2-1*, these RNAs leak into the cytoplasm when functional Hrp1 is missing. In fact, we were able to show that 3'-elongated mRNAs reached the cytoplasm in the *hrp1-1 cft2-1* double mutant with cell fractionation experiments. Moreover, we discovered that Hrp1 binds more faulty mRNAs in *cft2-1* but recruits less Mex67, which is consistent with its function in nuclear retention. Interestingly, Hrp1 has lost physical interaction with its binding partner Rna14 in the CPF-CF complex in *cft2-1*. In this mutant, Rna14 is not incorporated into the CPF-CF complex anymore. Thus, we propose that Rna14 might be the trigger for Hrp1 mediated recruitment of Mex67 to mRNAs. In conclusion, our data reveal that Hrp1 is a novel guard protein that surveils the 3' processing of pre-mRNAs.

2. Introduction

2.1. mRNA biogenesis and export

2.1.1. mRNA transcription initiation and capping

mRNAs in all eukaryotic cells are transcribed from DNA templates in the genome by RNA Polymerase II (Pol II) in the nucleus. Upon gene activation, RNA Pol II transcription initiates at the promoter, which usually contains a transcription start site (TSS) and its upstream TATA box as the canonical core elements (Hampsey, 1998). A small portion of gene promoters can contain derivatives of the conserved TATA box for transcription initiation (Hampsey, 1998). The promoter core elements provide a platform for the preinitiation complex (PIC) assembly by recruiting RNA Pol II, general transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH), and the Srb/Mediator complex (Figure 1) (Lee and Young, 2000). Yeast RNA Pol II is comprised of 12 Rpb subunits, within which the two largest subunits, Rpb1 and Rpb2, form a conserved cleft as a core channel for RNA synthesis (Oh *et al.*, 2019; Lee and Young, 2000). The carboxy-terminal domain (CTD) of the yeast Rpb1 subunit contains 26 repeats of the YSPTSPS element and modulates transcription initiation in a state without phosphorylation (Harlen and Churchman, 2017). Upon DNA melting and the single-stranded template positioning at the active cleft of RNA Pol II, the PIC undergoes a dramatic conformational change and becomes an active open complex for transcription initiation (Figure 1) (Hahn, 2004). This step is an ATP-dependent process. Some abortive RNAs with a length of 3-10 bases are usually generated and rapidly degraded at the beginning of transcription, which is thought to be an integral process for RNA Pol II escaping from the core promoter region (Hahn, 2004; Hsu, 2009). Once a product of around 30 bases has been successfully produced, RNA Pol II moves to a downstream region of the TSS and switches to the elongation state (Figure 1) (Hahn, 2004). This switch is signaled by phosphorylation of the CTD and Ser5 phosphorylation enables recruitment of the mRNA capping enzymes (Figure 1 and 4) (Bharati *et al.*, 2016; Kim *et al.*, 2004a; Hsin and Manley, 2012).

The capping process is composed of three enzymatic reactions (Kim *et al.*, 2004a). First, the 5' triphosphate end of the newly synthesized mRNA is dephosphorylated to a diphosphate end via the 5' triphosphatase Cet1. Then the mRNA is capped by adding a guanosine to the 5'-5' triphosphate linkage via the RNA guanylyltransferase Ceg1. Finally, the cap is methylated at guanosine-7 by the RNA methyltransferase Abd1. An inverted 7-methyl-guanosine (m^7G) cap linked to the first nucleotide is essential for mRNA stability, protecting it from 5' to 3' exonuclease degradation (Ramanathan, Robb and Chan, 2016). It is also important for recruitment of the transcription elongation factors (Ramanathan, Robb and Chan, 2016). Once synthesized, the cap is immediately bound by the cap-binding complex (CBC), which helps to recruit the export adaptor protein Nlp3 for mRNA packaging and export into the cytoplasm (Figure 1) (Lewis and Izaurralde, 1997; Shen *et al.*, 2000; Lei, Krebber and Silver, 2001).

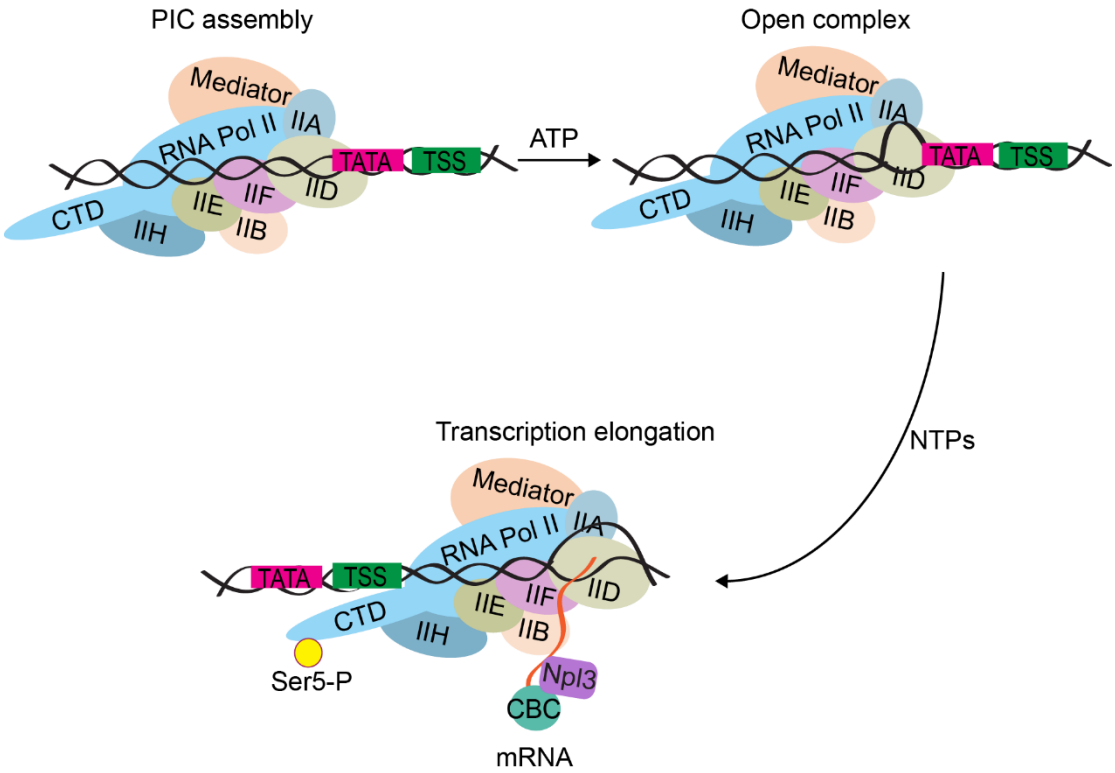


Figure 1: mRNA transcription initiation switches to elongation once around 30 nucleotides have been successfully synthesized.

Once the PIC is fully assembled, it transforms into an open complex by melting the DNA template and initiates mRNA transcription. In response to the CTD phosphorylation at Ser5, the

PIC escapes from the core promoter and elongates the transcript. The CBC immediately binds the m^7G-cap of the newly synthesized mRNA and recruits an export adaptor protein, Npl3, for early packaging. Adapted from (Hahn, 2004).

2.1.2. mRNA transcription elongation and splicing

With increased phosphorylation at the CTD, the proteins recruited by RNA Pol II change to facilitate elongation and later processing steps of pre-mRNAs (Lee and Young, 2000). Due to the highly compact structure of chromatin in eukaryotes, most elongation factors function to remodel the chromatin and nucleosome structures to remove the barriers that prevent RNA Pol II from moving further downstream (Svejstrup, 2002). Pob3/Spt16 (FACT), Swi/Snf, and Spt6 can bind histones directly and might act to remove histones from actively transcribed genes (Orphanides *et al.*, 1999; Bortvin and Winston, 1996; Schwabish and Struhl, 2007). The elongator complex was originally co-purified with the elongating RNA Pol II and is likely to promote transcription elongation with its histone acetyltransferase (HAT) activity (Otero *et al.*, 1999; Wittschieben *et al.*, 2000). In contrast, other elongation factors function in different ways to enhance the elongation rate. For example, according to Blythe *et al.*, Spt4/5 might facilitate transcription elongation via keeping the DNA template engaged in the elongation complex (Blythe *et al.*, 2016).

RNA Pol II also recruits splicing factors in a stepwise manner in response to the dynamic phosphorylation pattern of the CTD during transcription elongation (Hsin and Manley, 2012). Subsequently, the splicing factors define proper sites for splicing and promote assembly of the spliceosome. The spliceosome is a ribozyme that is made up of five small nuclear RNAs (snRNAs) known as U1, U2, U4, U5, and U6 with their binding proteins (Plaschka, Newman and Nagai, 2019). Spliceosome mediated splicing is a series of complicated catalyzing reactions that aim to remove the non-coding introns and connect the exons to form a continuous open reading frame (ORF) for protein expression. Gornemann *et al.* have found that CBC deletion entirely abolishes the spliceosome assembly, indicating that the 5'-capping and the CBC

recruitment are essential for functional spliceosome formation (Gornemann *et al.*, 2005). Due to significantly fewer intron-containing genes that exist in yeast as compared to humans, co-transcriptional splicing in *S. cerevisiae* is not as universal as in higher eukaryotes. However, splicing is still essential, mainly for generating functional mRNAs encoding ribosome proteins (Davis *et al.*, 2000; Spingola *et al.*, 1999). As shown in Figure 2, U1 snRNP is responsible for recognizing a conserved 5' splice site (5'SS) via U1 base pairing and then recruiting U2 snRNPs to the 3' splice site (3'SS) to form the A complex. Upon the association of U4/U6.U5 tri-snRNP with the A complex in an ATP-dependent manner, the spliceosome (B complex) is fully assembled (Will and Luhrmann, 2011). Subsequently, U1 and U4 leave the complex, and the B complex is activated to catalyze the processing reactions. Finally, the 5'SS and 3'SS are cleaved within several catalytic steps, and two exons are subjected to ligation, forming a continuous ORF (Will and Luhrmann, 2011; Plaschka, Newman and Nagai, 2019). After splicing, mRNAs are packaged with another two adaptor proteins, Gbp2 and Hrb1, and their covering export receptor, Mex67-Mtr2 (Figure 3 and 5) (Hackmann *et al.*, 2014).

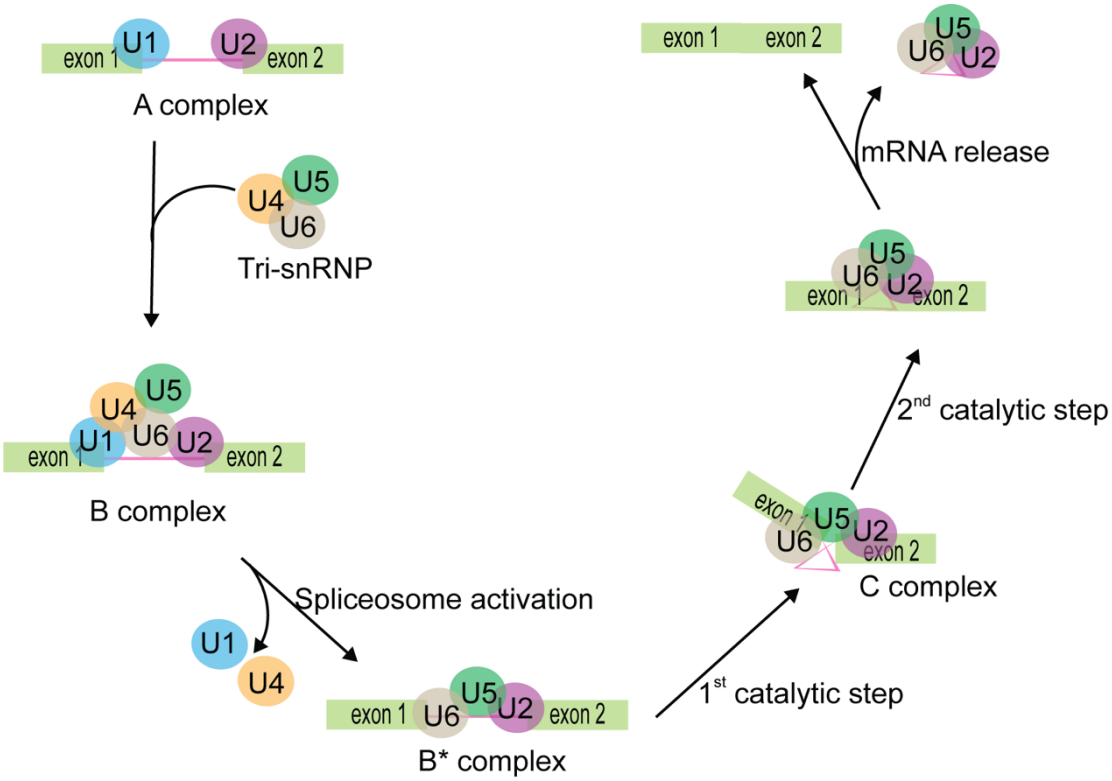


Figure 2: Splicing steps of the intron-containing mRNA.

Continuous recruitment of U1, U2, and U4/U6.U5 snRNPs to the target intron is required for the spliceosome assembly. The disassociation of U1 and U4 snRNP stimulates the spliceosome activity. U2, U5, and U6 snRNPs catalyze the splicing reactions and release properly spliced mRNAs. Adapted from (Will and Luhrmann, 2011)

2.1.3. mRNA 3'-end processing and transcription termination

The 3'-end processing and transcription termination are the last steps for mRNA maturation in the nucleus. For this purpose, a large group of proteins are co-transcriptionally incorporated into the cleavage and polyadenylation complex (CPF-CF) at the 3'-end of pre-mRNAs in response to increasing phosphorylation of the RNA Pol II CTD at Ser2 (Figure 3 and 4) (Proudfoot, 2004; Barilla, Lee and Proudfoot, 2001; Mapendano *et al.*, 2010). The CPF-CF complex plays in concert with multiple cis-elements to facilitate the 3'-end processing of pre-mRNAs (Figure 3) (Barilla, Lee and Proudfoot, 2001; Mandel, Bai and Tong, 2008). The CF IA subcomplex binds to the positioning element (PE) via its RNA-binding factor Rna15. CF IB (Hrp1) specifically binds to the upstream efficiency element (EE) to promote the efficiency and accuracy of the 3'-end processing of pre-mRNAs. Since single Rna15 seems to bind RNA without specificity, its specific contact with the PE is likely mediated by its physical interactions with Hrp1 and the bridge protein Rna14 in the CF IA subcomplex (Gross and Moore, 2001). The settled CF I subcomplex on the EE and the PE contributes to positioning of the CPF subcomplex through interactions among their components. RNA binding factors like Cft1, Fip1, and Yth1 in the CPF subcomplex directly interact with the flanking signal elements of the cleavage site to assist the endonuclease Ysh1 in cleaving pre-mRNAs at the cleavage site and the poly(A) polymerase Pap1 in subsequently adding adenosine nucleotides. Once the poly(A) tail is synthesized, Pab1 and Nab2 immediately bind to it to maintain its proper length (Soucek, Corbett and Fasken, 2012; Hector *et al.*, 2002; Amrani *et al.*, 1997; Beilharz and Preiss, 2007).

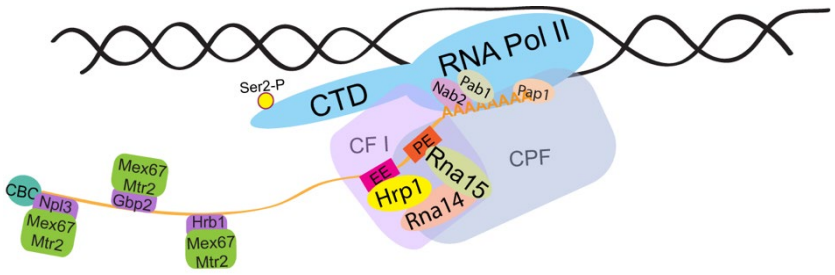


Figure 3: The CPF-CF complex is recruited to the mRNA 3'-end for cleavage and polyadenylation upon increasing CTD phosphorylation at Ser2.
The CF I components Hrp1 and Rna15 contribute to the positioning of the CPF complex at the cleavage site via anchoring to the EE and the PE. The poly(A) tail is generated by Pap1. Two poly(A) binding proteins, Pab1 and Nab2, associate with the 3' adenosines and monitor the length of the poly(A) tail.

The 3'-end cleavage and polyadenylation is highly coupled with transcription termination, which is likely supported by interactions between the CPF-CF components and the CTD of RNA Pol II (Proudfoot, 2004; Dichtl *et al.*, 2002; Barilla, Lee and Proudfoot, 2001; Meinhart and Cramer, 2004). Genes of the 3'-end processing machinery are usually essential, and many temperature-sensitive mutants are described to cause transcriptional readthrough of the canonical cleavage site of pre-mRNAs (Torchet *et al.*, 2002; Singh *et al.*, 2021; Mapendano *et al.*, 2010; Al-Husini *et al.*, 2017). Although the exact mechanism of transcription termination remains unclear, two potential models have been raised based on the achieved evidence (Rondon *et al.*, 2009; Buratowski, 2005). According to the torpedo model, rapid degradation of the 3' cleaved product disrupts the stable interaction of RNA Pol II with the DNA template and leads to its dissociation. However, the allosteric model argues that the release of RNA Pol II is attributed to its conformational change upon reaching a string of adenosines.

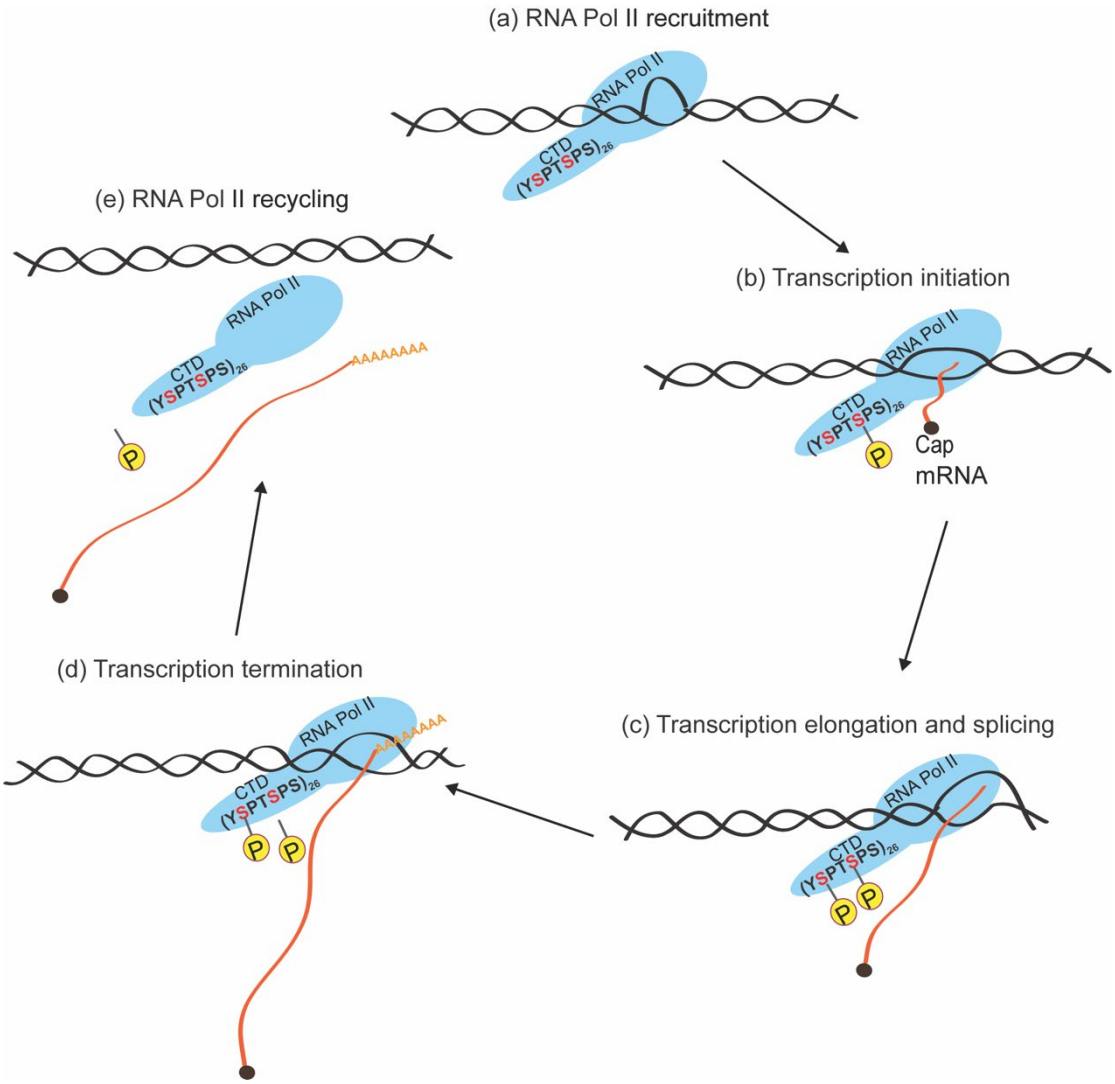


Figure 4: Dynamic Ser2 and Ser5 phosphorylation of RNA Pol II CTD are the main signals that orchestrate mRNA transcription.

(a) Unphosphorylated RNA Pol II is recruited onto an activated gene template and initiates transcription. (b) The CTD Ser5 phosphorylation signals the capping of a newly synthesized mRNA and promotes a shift of RNA Pol II for transcription elongation. (c) Transcription elongation and splicing are activated by concurrently increasing Ser2 phosphorylation. (d) Ser5 is gradually dephosphorylated and Ser2 phosphorylation indicates the 3'-end processing and transcription termination. (e) The RNA Pol II CTD is completely dephosphorylated after the 3'-end processing and released from the DNA template and the mature mRNA for recycling. Adapted from (Egloff and Murphy, 2008).

2.1.4. mRNA packaging and export

mRNA export from the nucleus to the cytoplasm relies on the export receptor composed of the heterodimer Mex67-Mtr2. Several molecules cover the mRNA and mediate its interaction with the gate keeper protein Mlp1 of the nuclear pore complex (NPC) (Figure 5) (Zander *et al.*, 2016; Strawn, Shen and Wente, 2001). Although Mex67-Mtr2 is capable of binding heat shock mRNAs directly and supporting their translocation in response to stress challenges, it is usually recruited onto the packaging mRNAs by its adaptor proteins in normal conditions (Figure 5) (Iglesias *et al.*, 2010; Zander and Krebber, 2017; Zander *et al.*, 2016). mRNA packaging is the process during which mRNA-binding proteins are gradually remodeled to a certain status that enables the mRNA to be transported through the NPC as a particle of messenger ribonucleoprotein (mRNP) assemblies. This process is co-transcriptionally coupled with the pre-mRNA maturation steps of capping, splicing, and 3'-end processing. As already mentioned in 2.1.1, the CBC is essential for recruitment of the export adaptor protein Npl3. Interestingly, the CBC also promotes targeting of another export factor, Yra1, to the newly synthesized transcripts (Sen *et al.*, 2019). With transcription elongation and splicing carrying on, an evolutionarily conserved heterotetramer of Tho2, Hpr1, Mft1, and Thp2 called the THO complex is recruited to the growing mRNA via the RNA Pol II CTD and splicing factors (Chanarat, Seizl and Strasser, 2011; Abruzzi, Lacadie and Rosbash, 2004; Meinel *et al.*, 2013). Association of the THO complex with mRNA is modulated by sumoylation of its component Hpr1 (Bretes *et al.*, 2014). The THO complex, together with Tex1, Sub2, and Yra1, contributes to the assembly of a transcription and export (TREX) complex, which incorporates more adaptor proteins onto mRNAs, including Gbp2, and Hrb1 for export (Abruzzi, Lacadie and Rosbash, 2004; Saguez *et al.*, 2013; Meinel *et al.*, 2013; Häcker and Krebber, 2004). Yeast cells that grew in the absence of TREX factors like Hpr1, Sub2, and Yra1 have been shown to result in the formation of macromolecular chromatin complexes and display a significant accumulation of poly(A) RNAs in the nucleus, implying their essential roles in regulating mRNA export (Jensen *et al.*, 2001; Zenklusen *et al.*, 2001;

Tutucci and Stutz, 2011). During mRNA 3'-end maturation, the two poly(A) binding proteins Nab2 and Pab1 are loaded, with Nab2 being able to function as an adaptor protein for Mex67-Mtr2 covering (Brune *et al.*, 2005; Iglesias *et al.*, 2010). Intriguingly, all the proteins that can recruit Mex67 are shuttling proteins except for Yra1, which is consistent with their special role as guard proteins for mRNA export (Brune *et al.*, 2005; Zander *et al.*, 2016). In contrast, Yra1 is subjected to the E3 ligase Tom3-mediated ubiquitination and is degraded shortly before mRNP export at the NPC (Iglesias *et al.*, 2010). Given that Yra1 binds to Sub2 and Mex67 in a mutually exclusive manner and can enhance Nab2-Mex67 interaction on mRNAs, Yra1 appears to act as a regulator for Mex67 recruitment (Iglesias *et al.*, 2010; Strasser *et al.*, 2002). On the cytoplasmic side of the NPC, mRNPs are remodeled by the DEAD-box protein Dbp5, releasing the CBC, Mex67, and Nab2 into the cytoplasm (Torvund-Jensen *et al.*, 2014; Adams and Wente, 2020; Ishigaki *et al.*, 2001; Windgassen *et al.*, 2004). Npl3, Gbp2, Hrb1, and Pab1 remain bound to mRNAs during translation initiation (Adams and Wente, 2020; Poornima *et al.*, 2021; Estrella, Wilkinson and Gonzalez, 2009; Windgassen *et al.*, 2004).

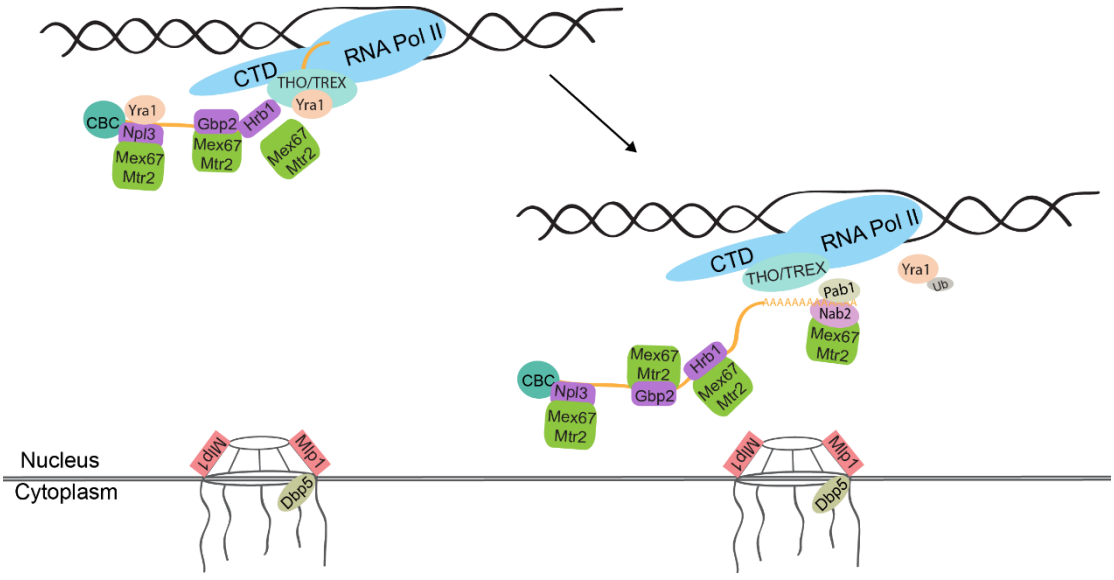


Figure 5: mRNAs are co-transcriptionally packaged into mRNP particles by recruiting adaptor proteins and the associated export receptor Mex67-Mtr2 for transport through the NPC.

Export adaptor protein Npl3 and export factor Yra1 are loaded onto the mRNA 5'-end once the cap is formed and bound to the CBC. Following transcription elongation, the THO/TREX complex facilitates extensive recruitment of another two adaptor proteins, Gbp2 and Hrb1, for export. Nab2 binds to the poly(A) tail and recruits Mex67-Mtr2 for export during the 3'-end processing. Export factor Yra1 is ubiquitinated and degraded before mRNA export. All adaptor proteins for Mex67-Mtr2 are covered by Mex67-Mtr2 to form an export competent mRNP particle.

2.2. Functions of Hrp1

HRP1 is an essential gene in *S. cerevisiae* and has originally been identified as a suppressor of *NPL3* (Henry *et al.*, 1996). Hrp1 localizes to the nucleus at a steady state. However, it frequently shuttles between the nucleus and the cytoplasm (Henry *et al.*, 1996; Kessler *et al.*, 1997). The C-terminal domain of Hrp1, which contains three repeats of arginine-glycine-glycine (RGG), is likely to be a target of the arginine methyltransferase Hmt1 for methylation (Shen *et al.*, 1998; Valentini, Weiss and Silver, 1999). Shen *et al.* have shown that Hrp1 methylation is important for its shuttling, since it fails to shuttle into the cytosol in the absence of Hmt1 (Shen *et al.*, 1998). Hrp1 shares similar RNA recognition motifs (RRMs) to the hnRNP A/B in vertebrates and specifically binds to the EE, which is a repeat motif of $(UA)_3$ that is upstream of the cleavage site (Guo and Sherman, 1996; Kessler *et al.*, 1997). According to Kessler and colleagues, Hrp1 is the only component of the CF IB subunit of the CPF-CF complex that mediates mRNA 3'-end processing (Kessler *et al.*, 1997). However, Minvielle-Sebastian and others argue that Hrp1 is not required for the cleavage reaction itself but rather controls the cleavage site selection (Minvielle-Sebastia *et al.*, 1998). Consistently, *HRP1* mutation leads to increased use of the distant poly(A) site of *SUA7* mRNA, and *HRP1* overexpression enhances the usage of the proximal termination site in yeast cells (Kim Guisbert, Li and Guthrie, 2007). Mutations within the RRMs of Hrp1 result in a temperature-sensitive phenotype, suggesting that recognition of the UA-rich element is crucial for mRNA maturation at the 3'-end. Subsequent studies have confirmed that UAUAUA is the canonical binding site of Hrp1, although some alternative 3'-end sequence elements could also be specific targets for

Hrp1, but with lower binding affinity (Kim Guisbert *et al.*, 2005; Chen and Hyman, 1998; Graber *et al.*, 1999; Perez-Canadillas, 2006; Graber, McAllister and Smith, 2002). Hrp1 directly interacts with Rna14 and Rna15 of the CF IA complex to facilitate the efficiency and accuracy of cleavage and polyadenylation (Leeper *et al.*, 2010; Kessler *et al.*, 1997; Barnwal *et al.*, 2012). A second important function of Hrp1 is in the cytoplasm, where it marks aberrant transcripts with a premature termination codon for nonsense-mediated decay (NMD), which releases the stalled ribosome and directs the faulty mRNA for degradation (Gonzalez *et al.*, 2000). Therefore, Hrp1 mediates the cytosolic surveillance of faulty mRNAs that contain a nonsense codon for translation termination. The reimport of Hrp1 into the nucleus from the cytoplasm has been shown to be facilitated via the importin Kap104 (Lange *et al.*, 2008).

2.3. Nuclear quality control of mRNA

2.3.1. General functions of SR/RGG proteins

Most human SR/RGG proteins are actively involved in modulating mRNA splicing, especially functioning as regulators of alternative splicing via binding to the exonic splicing enhancers or splicing silencers on pre-mRNAs (Howard and Sanford, 2015; Anko, 2014; Busch and Hertel, 2012; Zhu, Mayeda and Krainer, 2001). Emerging roles of mammalian SR/RGG proteins have also been described within the last two decades, including regulating transcription elongation (Lin *et al.*, 2008; Ji *et al.*, 2013; Lemieux *et al.*, 2015), facilitating mRNA export (Huang and Steitz, 2001; Swartz *et al.*, 2007; Mili *et al.*, 2001), modulating translation (Swartz *et al.*, 2007; Sanford *et al.*, 2004; Maslon *et al.*, 2014; Yao *et al.*, 2017; Torvund-Jensen *et al.*, 2014) and mediating mRNA decay (Reznik, Clement and Lykke-Andersen, 2014). As a result, mammalian SR/RGG proteins significantly and profoundly impact gene expression in multiple ways.

In contrast to mammals, fewer SR/RGG-containing mRNA-binding proteins have been identified in yeast cells of *S. cerevisiae*, which is likely due to their predominant roles

in mRNA splicing. In fact, only around 5% of genes in budding yeast contain introns, and they represent about 25% of mRNAs due to strong expression (Hackmann *et al.*, 2014; Davis *et al.*, 2000). The most studied SR/RGG protein in yeast is Npl3, which has two RRMs and a C-terminal SR/RGG domain with 15 RGG and 8 SR/RS repeats (Shen *et al.*, 1998; Bossie *et al.*, 1992). The shuttling protein Npl3 has been originally characterized as an mRNA carrier protein that is co-transcriptionally recruited for mRNA export (Singleton *et al.*, 1995; Lee, Henry and Silver, 1996; Lei, Krebber and Silver, 2001). Some *NPL3* mutants show a severe mRNA export defect at semi-permissive or nonpermissive temperatures. Later, Npl3 has been found to interact with the CBC, therefore, Npl3 seems to be recruited at an early time during mRNA transcription (Shen *et al.*, 2000). Combined data suggests that Npl3 might play a role in the quality control of the mRNA capping step in the nucleus, but the exact mechanism has not been shown yet (Zander *et al.*, 2016; Zander and Krebber, 2017). Npl3 has also been discovered to physically interact with the C-terminal domain of RNAP II, stimulating its activity for transcription elongation until Npl3 is phosphorylated, which results in transcription termination (Dermody *et al.*, 2008; Lei, Krebber and Silver, 2001). Active transcription elongation is supported by methylation of the SR/RGG domain of Npl3 by the arginine methyltransferase Hmt1, which represses termination (Wong *et al.*, 2010; McBride *et al.*, 2005). Intriguingly, transcription termination defects and considerably longer transcripts have been observed in the absence of Npl3, indicating that Npl3 promotes 3'-end processing of mRNAs (Holmes *et al.*, 2015). However, earlier results from Bucheli and Deka suggest an opposite function of Npl3 in transcription termination. They argue that Npl3 is competing with Rna15 for binding of the mRNA 3'-end (Bucheli and Buratowski, 2005; Bucheli *et al.*, 2007; Deka *et al.*, 2008).

As a canonical SR/RGG protein in yeast, Npl3 has also been implicated in promoting co-transcriptional recruitment of splicing factors to mRNAs (Kress, Krogan and Guthrie, 2008; Muddukrishna, Jackson and Yu, 2017). A recent study has shown that Npl3 is

essential for regulating splicing of mRNAs in the meiotic network and, as a consequence, might control a proper cell cycle program (Sandhu, Sinha and Montpetit, 2021). In addition, Npl3 has been reported to function in transcription initiation by mediating ribosomal subunit joining (Baierlein *et al.*, 2013) and as a translational repressor that promotes the accuracy of translation termination via mRNP remodeling in the cytoplasm (Estrella, Wilkinson and Gonzalez, 2009; Windgassen *et al.*, 2004). Reimport of Npl3 is supported by the SR-specific protein kinase Sky1 and mediated by the importin Mtr10 (Häcker and Krebber, 2004).

According to Häcker and others, Gbp2 and Hrb1 are two mRNA-binding proteins that both contain three C-terminal RRMs and an N-terminal SR/RGG domain (Häcker and Krebber, 2004; Hurt *et al.*, 2004; Windgassen and Krebber, 2003). They are recruited by the TREX complex onto mRNA transcripts and are, like Npl3, involved in mRNA export (Häcker and Krebber, 2004; Hurt *et al.*, 2004). In addition, recent structure analysis has shown that both the non-canonical RRM3 and the SR/RGG domain of Gbp2 and Hrb1 are essential for their interaction with the TREX complex (Hurt *et al.*, 2004; Martinez-Lumbreras *et al.*, 2016; Xie *et al.*, 2021). As first shown in the work of Windgassen *et al.*, Gbp2 and Hrb1 are transported together with mRNAs into the cytoplasm and are part of the translating mRNPs (Windgassen *et al.*, 2004). A recent study has found that Gbp2 functions to repress the translation of a *GFP* reporter mRNA in vivo, with the SR/RGG motif being important for this suppression (Poornima *et al.*, 2021). Strikingly, Gbp2 and Hrb1 have also been discovered to act as guard proteins both in the nucleus and in the cytoplasm (Hackmann *et al.*, 2014; Grosse *et al.*, 2021). With respect to splicing surveillance, Gbp2 and Hrb1 function to determine the fate of mRNAs for export or degradation in the nucleus, whereas in the cytoplasm, they contribute to recognizing premature codons in faulty mRNAs and targeting them for NMD. Similar to Npl3, the reimport of Gbp2 and Hrb1 is supported by the SR-kinase Sky1 and the import factor Mtr10 (Häcker and Krebber, 2004).

Nab2 and Hrp1 are two SR/RGG proteins that have been found to be important for mRNA 3'-end maturation in budding yeast (Kessler *et al.*, 1997; Fasken, Corbett and Stewart, 2019). As introduced in 2.2, Hrp1 is a shuttling SR/RGG protein. It functions as the CF IB for cleavage and polyadenylation in the nucleus and is also involved in NMD in the cytoplasm. Like the other SR/RGG proteins in yeast, Nab2 also commutes between the nucleus and the cytoplasm (Zander *et al.*, 2016). Interestingly, similar to Hrp1, Nab2 export is likely to be mediated via Hmt1 methylation at the SR/RGG box and its reimport relies on the importin Kap104 (Truant *et al.*, 1998; Green *et al.*, 2002; Marfatia *et al.*, 2003; Soniat *et al.*, 2013). Since deletion of the SR/RGG box or the N-terminal motif of Nab2 has been shown to cause nuclear accumulation of poly(A) mRNAs, Nab2 translocation is highly coupled with mRNA export (Marfatia *et al.*, 2003). Nab2 was originally identified as a nuclear ribonucleoprotein that intimately binds to poly(A) mRNAs via its zinc-binding motif (Anderson *et al.*, 1993). Subsequent functional and structural studies suggest that the evolutionally conserved C-terminal zinc finger motif of Nab2 is essential for recognizing the poly-adenosines of mRNA (Kelly *et al.*, 2007; Marfatia *et al.*, 2003; Martinez-Lumbreras *et al.*, 2013). This specific interaction allows Nab2 to facilitate mRNA packaging via self-dimerization and to monitor the length of the poly(A) tail (Soucek, Corbett and Fasken, 2012; Aibara *et al.*, 2017). Additionally, the capacity of Nab2 to bind the poly(A) tail is important for mRNA export and stability, implying a role for Nab2 in mRNA quality control in the nucleus. Soucek *et al.* suggest that Nab2 might also participate in combining mRNA splicing with the subsequent 3'-end processing via interaction with the spliceosome component (Soucek *et al.*, 2016). A recent study with a Nab2 Anchor-Away strain by Alpert and her colleagues has observed considerable chimeric transcripts with retained introns from upstream genes, indicating a role of Nab2 in transcription termination (Alpert *et al.*, 2020).

2.3.2. Shuttling SR/RGG proteins mediated nuclear quality control of mRNA

During evolution, cells have developed diverse surveillance systems to prevent the synthesis of malfunctioning proteins that are usually toxic to cells and lead to cell death or illnesses in higher eukaryotes (Wegener and Muller-McNicoll, 2018). Eukaryotic cells are perfectly compartmentalized and confine mRNA synthesis to the nucleus. Nuclear quality control is the first and most important defense layer, which sets numerous checkpoints to make sure that mRNAs are correctly processed during biogenesis. In line with this, several shuttling RNA-binding proteins surveil mRNA processing steps and target them either for retention in the nucleus or for export into the cytoplasm (Wegener and Muller-McNicoll, 2018; Tutucci and Stutz, 2011; Eberle and Visa, 2014). Yeast shuttling SR/RGG proteins Gbp2 and Hrb1 have been the first two export adaptors identified to function as guard proteins for pre-mRNA processing (Figure 6) (Hackmann *et al.*, 2014). In contrast to the nuclear retention of faulty mRNAs in the mutants of the TREX complex or the exosome, it has been shown that the absence of Gbp2 or Hrb1 results in significant leakage of unspliced mRNAs. Given that Gbp2 and Hrb1 physically interact with the export receptor Mex67-Mtr2 and the exosome co-factor TRAMP complex in a mutually exclusive manner, the two guard proteins are assumed to function as a switch for mRNA export and degradation in the nucleus (Hackmann *et al.*, 2014). In addition, the direct interaction of Gbp2 and Hrb1 with the NPC component Mlp1 further supports their surveillance role at the final checkpoint for export. In brief, Gbp2 and Hrb1 are loaded during splicing and recruit the TRAMP complex by default (Hackmann *et al.*, 2014). If the intron-containing pre-mRNAs are appropriately processed, the TRAMP complex is displaced by Mex67 recruitment to facilitate mRNP export. However, if the spliceosome fails to remove the inserted intron, Gbp2 and Hrb1 prefer to retain the TRAMP complex and subsequently recruit the exosome, eliminating unspliced faulty mRNAs in the nucleus (Hackmann *et al.*, 2014). Although a detailed mechanism is still missing, another shuttling SR/RGG protein, Npl3, has been implicated in monitoring mRNA capping (Zander and Krebber,

2017). Another SR/RGG protein, Nab2, has the capability of controlling mRNA poly(A) tail length and mRNA export, which indicates a role in quality control of nuclear polyadenylation (Green *et al.*, 2002; Iglesias *et al.*, 2010; Soucek, Corbett and Fasken, 2012). Moreover, subsequent functional and structural studies investigating the interaction between Nab2 and the NPC further support its similar surveillance mechanism for mRNA export to the other guard proteins (Adams and Wente, 2020; Green *et al.*, 2003; Grant *et al.*, 2008).

Although mRNA export is controlled by SR/RGG guard proteins in normal conditions, yeast cells behave differently in response to stress situations (Zander *et al.*, 2016). The study has shown that the export receptor Mex67-Mtr2 and its adaptor proteins Npl3, Gbp2, Hrb1, and Nab2 all dissociate from regular mRNAs to support the export of actively generated heat shock mRNAs. Interestingly, the rapid export of heat shock mRNAs does not even need adaptor proteins for Mex67-Mtr2 recruitment, indicating the nuclear quality control is bypassed for the stress-responsive transcripts. Strikingly, the distinct fates of regular mRNAs and stress-stimulated transcripts seem to be determined by their own promoters, illustrating how yeast cells escape regular mRNA surveillance to survive in extreme environments.

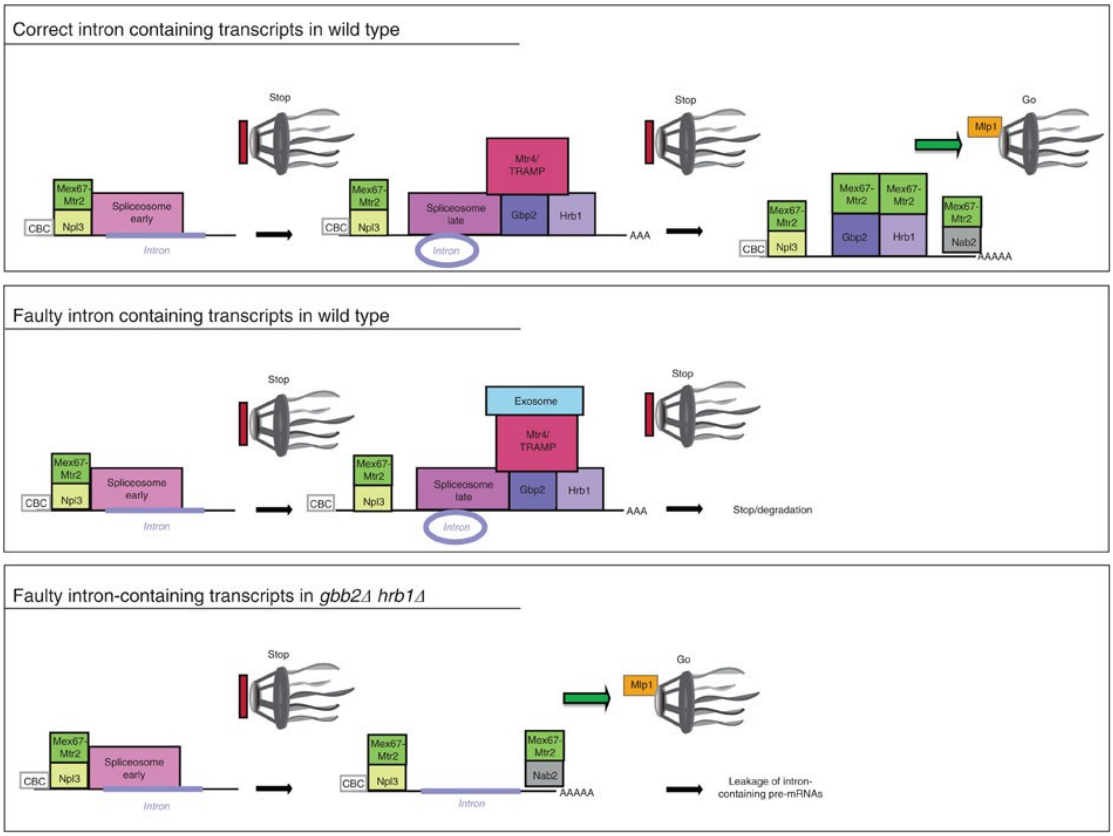


Figure 6: mRNA nuclear quality control in budding yeast is mediated by guard proteins. Npl3 is recruited to the newly synthesized mRNA via the CBC and is assumed to surveil mRNA capping. Its interaction with the early spliceosome retains mRNAs in the nucleus. Gbp2 and Hrb1 are shown to monitor mRNA splicing and they only recruit the export receptor Mex67-Mtr2 onto properly spliced mRNAs for export (top). Faulty mRNAs are subjected to nuclear retention and degradation via the TRAMP complex and the exosome (middle). If Gbp2 and Hrb1 are missing for splicing surveillance, aberrant intron-containing mRNAs leak into the cytoplasm (bottom). Nab2 is capable of recruiting Mex67-Mtr2 on a properly formed poly(A) tail. Mlp1 is the gatekeeper protein of the NPC. Taken from (Hackmann *et al.*, 2014).

2.3.3. mRNA surveillance and export through the NPC

The conserved nuclear gatekeeper NPC is a large doughnut-shaped nucleoporin complex that is embedded in the double-layer nuclear envelope (NE) and facilitates molecule translocation between the nucleus and the cytoplasm (Aitchison and Rout, 2012). The octagonal core channel interspersed within the NE is highly symmetrical and emanates peripheral filaments to both sides of the NPC to establish direct contact with the nuclear and cytosolic contents. The filaments on the nucleoplasmic side of the

NPC form a basket structure that provides nuclear docking sites for mRNP export through the NPC (Xie and Ren, 2019; Green *et al.*, 2003; Niepel *et al.*, 2013; Vinciguerra *et al.*, 2005). Although small molecules can passively diffuse through the NPC, large particles like mRNPs are subjected to selective transport (Fernandez-Martinez and Rout, 2009). The selective control of mRNP export relies on the interaction of the covering export receptor Mex67-Mtr2 with the permeability barrier that is primarily constructed by the NPC anchored phenylalanine-glycine rich nucleoporins (FG-Nups) (Li, Goryaynov and Yang, 2016). Among all the nucleoporins making up the NPC, about one third contain FG-rich domains (Li, Goryaynov and Yang, 2016). FG repeats are distributed in certain patches and are separated by linker sequences (Rout and Wente, 1994). Instead of folding into any typical secondary or tertiary structures, FG domains are intrinsically disordered and flexible, forming highly net charged and low-level hydrophobic clouds that mainly localize in the interior of the core channel and extend to the nuclear basket and cytosolic filaments of the NPC (Lemke, 2016; Li, Goryaynov and Yang, 2016). Although bulk mRNA export is independent of the nuclear transport factor 2 (Ntf2) mediated Ran-GTP gradient, the mRNA export receptor Mex67-Mtr2 contains at least two FG-binding sites and one of them is structurally similar to Ntf2 (Li, Goryaynov and Yang, 2016; Terry and Wente, 2009). Given the disordered configuration of FG-repeats, Mex67-Mtr2 interacts with them transiently and guides mRNPs rapidly passing through the NPC (Xie *et al.*, 2021; Li, Goryaynov and Yang, 2016). Once moving to the cytoplasmic side of the NPC, the mRNP docks at the FG-Nups and is rapidly disassembled by Dbp5-mediated remodeling, which prohibits it from moving back into the nucleus (Fasken and Corbett, 2009).

Intriguingly, aberrant mRNAs that target the NPC basket in normal circumstances are not permitted to move through, and they are preferentially retained in the nucleus for rapid degradation (Soheilypour and Mofrad, 2018; Fasken and Corbett, 2009; Hackmann *et al.*, 2014). Accordingly, the NPC basket has been implicated as the last

checkpoint for mRNA quality control before export (Fasken and Corbett, 2009). Saroufim and his colleagues have shown that mRNAs seem to scan the nuclear periphery before export through tracking the moving path of a single mRNA from the nucleus into the cytoplasm via live cell microscopy (Saroufim *et al.*, 2015). The NPC basket component Mlp1, its close relative Mlp2, and another three associated proteins Pml39, Esc1, and Nup60 are thought to work together to trap faulty mRNAs in the nucleus (Fasken, Stewart and Corbett, 2008; Palancade *et al.*, 2005; Hackmann *et al.*, 2014; Galy *et al.*, 2004; Lewis, Felberbaum and Hochstrasser, 2007). Among them, Pml39, Esc1, and Nup60 seem to function upstream to position the executive factor Mlp1 and Mlp2 onto the NPC basket to perform quality control (Galy *et al.*, 2004; Palancade *et al.*, 2005; Lewis, Felberbaum and Hochstrasser, 2007). Mlp1 is the most studied surveillance factor of the NPC basket and has been shown to retain the intron-containing mRNPs via recognizing the naked guard proteins Gbp2 and Hrb1 without the export receptor Mex67-Mtr2 coverage (Hackmann *et al.*, 2014; Galy *et al.*, 2004). Deletion of *MLP1* or *GBP2* and *HRB1* prevents the nuclear retention of unspliced mRNAs and leads to their cytoplasmic leakage (Galy *et al.*, 2004; Soheilypour and Mofrad, 2018; Hackmann *et al.*, 2014; Zander *et al.*, 2016). The physical interactions of Mlp1 with Npl3 and Nab2 are also important for targeting mRNP particles to the NPC for export (Fasken, Stewart and Corbett, 2008; Häcker and Krebber, 2004; Green *et al.*, 2003). In addition to retaining faulty mRNAs that are signaled by uncovered guard proteins, Mlp1 and Mlp2 might also recognize properly processed mRNAs and concentrate them at the NPC via direct interactions with mRNA export factors like Yra1 or Mex67 (Vinciguerra *et al.*, 2005; Soheilypour and Mofrad, 2018; Niepel *et al.*, 2013). Therefore, the NPC quality control system might combine the functions of retaining aberrant mRNAs and selecting correctly processed mRNAs to ensure proper export of mRNPs.

2.3.4. Nuclear quality control related mRNA degradation

2.3.4.1. Rat1/Rai1-mediated decapping and degradation of mRNAs

Newly synthesized mRNAs are supposed to be protected by an inverted 7-methyl-guanosine (m^7G) cap that is bound to the CBC from 5' degradation in the nucleus (Lewis and Izaurralde, 1997; Ramanathan, Robb and Chan, 2016; Sen *et al.*, 2019). Proper capping is surveilled and failure in this step leads to rapid degradation of faulty mRNAs by the 5' to 3'-end exoribonuclease Rat1 with its activator Rai1 in the nucleus (Xiang *et al.*, 2009; Jiao *et al.*, 2010). In contrast to the canonical decapping enzyme Dcp2, Rai1 functions as a decapping endonuclease that specifically targets mRNAs with an unmethylated cap or 5'-triphosphates end (Jiao *et al.*, 2010). Deletion of *RAI1* results in significant accumulation of aberrant mRNAs upon nutritional stress (Jiao *et al.*, 2010). Rat1 has been shown to stimulate the hydrolysis activity of Rai1, and the resultant product with a stable secondary structure appears to be degraded more easily by Rat1 (Jiao *et al.*, 2010; Xiang *et al.*, 2009). The Rat1/Rai1 complex is also essential for transcription termination and responsible for eliminating the 3' cleaved product (Houseley and Tollervey, 2009).

2.3.4.2. The TRAMP complex and the exosome mediated mRNA degradation

Faulty mRNAs that are not properly spliced or inappropriately processed at the 3'-end are subjected to nuclear retention and subsequent rapid degradation via the 3' nuclear degradation machinery called the exosome (Torchet *et al.*, 2002; Hackmann *et al.*, 2014; Bousquet-Antonelli, Presutti and Tollervey, 2000; Singh *et al.*, 2021). Nuclear elimination of aberrant mRNA that are defective in packaging and export also relies on the exosome (Houseley and Tollervey, 2009; Singh *et al.*, 2021). Accordingly, the exosome mediated nuclear decay is the major pathway for pre-mRNA turnover.

An evolutionarily conserved exosome is composed of a group of protein factors that construct a hexameric core channel and a trimeric cap on top (Schneider and Tollervey, 2013; Liu, Greimann and Lima, 2006). Dis3/Rrp44 is an additional core channel

associated protein in yeast cells that enables the 3'-5' exonuclease activity inside of the complex (Liu, Greimann and Lima, 2006). The Dis3/Rrp44 included exo-10 complex exists in both the nucleus and the cytoplasm. However, another exonuclease protein, Rrp6, is exclusively localized in the nucleus, associating with the exo-10 complex to form the nuclear holoenzyme exo-11 (Schneider and Tollervey, 2013). Interestingly, an unexpected endonuclease activity was identified at the N-terminus of Dis3/Rrp44, which is also likely to contribute to substrate digestion (Schneider and Tollervey, 2013). Since the core channel is only wide enough for a single-stranded RNA, the substrate is assumed to be unwound and threaded through the core channel to the catalytic site at the bottom (Schneider and Tollervey, 2013; Liu, Greimann and Lima, 2006).

Except for targeting pre-mRNAs for degradation, the exosome also actively participates in eliminating rRNA, tRNA and other kinds of RNA substrates (Allmang *et al.*, 2000; Kadaba *et al.*, 2004; van Hoof, Lennertz and Parker, 2000; Wlotzka *et al.*, 2011). Extensive studies have shown that the exosome is likely to facilitate the 3'-end maturation of a growing list of RNA transcripts (Briggs, Burkard and Butler, 1998; van Hoof, Lennertz and Parker, 2000; Ciais, Bohnsack and Tollervey, 2008; Allmang *et al.*, 1999; Lemay *et al.*, 2010). Given the significant diversity in substrates and functions, the exosome is highly modulated by its cofactors, which guide the machinery towards specific targets for efficient degradation or 3'-end trimming (Schmidt and Butler, 2013). With respect to pre-mRNA surveillance in the nucleus, the Trf4/5-Air1/2-Mtr4 polyadenylation (TRAMP) complex is recruited via the guard proteins and plays in concert with the exosome to degrade the aberrant transcripts (Hackmann *et al.*, 2014; Bousquet-Antonelli, Presutti and Tollervey, 2000; Hilleren *et al.*, 2001; Torchet *et al.*, 2002). In brief, together with the zinc-finger mRNA-binding subunits Air1/2, the RNA helicase factor Mtr4 unwinds the mRNA substrates and therefore facilitates polymerase components Trf4/5 to add few adenosines following the 3'-end (Houseley and Tollervey, 2009). The single-stranded poly-adenosines establish a landing

platform for the exosome that promotes its 3'-5' exonuclease activity (Houseley and Tollervey, 2009). Intriguingly, the length of poly(A) tails added by the TRAMP complex is usually much shorter than the canonical ones synthesized via Pab1 for normal polyadenylation. In comparison with 60-80 adenosines for normal mRNA poly(A) tails, Schmidt and Butler have shown that the distribution of the short poly(A) tails peaks at 4-5 nucleotides, making the aberrant mRNAs easily distinguishable for degradation rather than export (Schmidt and Butler, 2013).

2.3.4.3. The Nrd1-Nab3-Sen1(NNS) complex mediated fail-safe transcription termination

Although the CPF-CF complex mediated cleavage and polyadenylation predominates in the transcription termination of protein-coding genes, the majority of noncoding RNAs are transcriptionally terminated in a cleavage-independent manner via the NNS complex (Lemay and Bachand, 2015; Thiebaut *et al.*, 2006). Nrd1 and Nab3 are two RNA-binding proteins that specifically recognize the essential cis-elements GUAA/G and UCUU for transcription termination via their single RRM, respectively (Carroll *et al.*, 2004; Carroll *et al.*, 2007). Sen1, a putative RNA-DNA helicase, is likely to be recruited by Nab3 and relies on ATP hydrolysis to proceed with its movement forward (Franco-Echevarria *et al.*, 2017). The NNS complex is co-transcriptionally recruited to noncoding RNAs by the properly phosphorylated RNA Pol II CTD at Ser5. Upon catching RNA Pol II at the 3' end, the NNS complex facilitates termination via displacement of RNA Pol II (Lemay and Bachand, 2015). Based on the physical interaction of Nrd1 with Trf4, the NNS complex is usually coupled with the TRAMP-exosome complex to target noncoding RNAs for 3'-end maturation or 3'-5' degradation (Grzechnik and Kufel, 2008; Vasiljeva and Buratowski, 2006). Interestingly, the binding site of Nrd1 for the RNA Pol II CTD and Trf4 is mutually exclusive, indicating that the NNS complex organizes RNA transcription termination and processing/degradation through shifting to alternative binding partners (Tudek *et al.*, 2014).

Surprisingly, the NNS complex has also been identified in terminating mRNA transcription, which rescues the readthrough RNA Pol II in the *rat1-1* mutant and targets the 3'-end extended transcripts for degradation (Rondon *et al.*, 2009). The role of the NNS complex in in the fail-safe termination has been further confirmed by a recent study, which has shown that malfunctioning Nrd1 stabilizes the readthrough mRNAs in the mutant *rna14-1* for 3'-end processing (Singh *et al.*, 2021). Additionally, it has been revealed that the RNA Pol II CTD binding domain (CID) of Nrd1 is essential for its co-transcriptionally recruitment to unterminated mRNAs. Since lacking the CID domain abolishes the physical interaction of Nrd1 with the TRAMP-exosome complex, it is also the key motif for recruiting the degradation machinery (Heo *et al.*, 2013). The NNS complex-mediated fail-safe termination of mRNA transcription (Figure 7) effectively prevents perturbation of downstream gene expression and contributes to the maintenance of genome integrity.

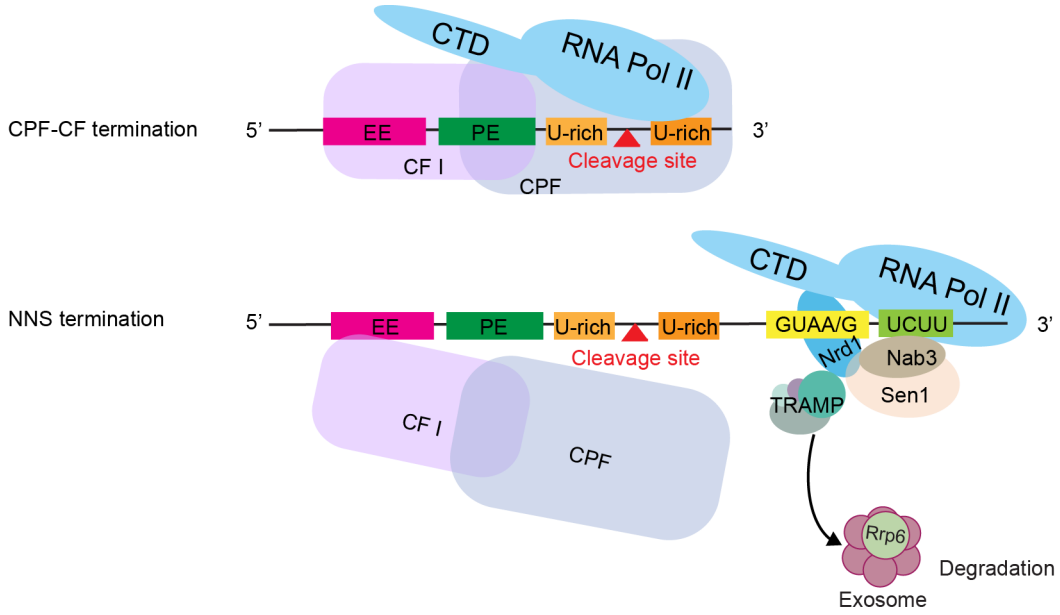


Figure 7: The NNS complex mediates termination and degradation of readthrough mRNAs.

In the upper figure, the CPF-CF complex is properly incorporated at the 3'-end of the pre-mRNA and facilitates transcription termination at the regular cleavage site. However, if the CPF-CF termination fails, as shown in the lower figure, the NNS termination pathway rescues the

readthrough mRNA and targets the faulty mRNA for degradation via the TRAMP-exosome complex.

2.4. Polyadenylation signals

In contrast to the conserved AAUAAA sequence for the mRNA 3'-end processing in human cells, the current understanding of polyadenylation signals in budding yeast is based on five cis-elements at the 3'-end of pre-mRNAs (Figure 7 and 8) (Tian and Graber, 2012). The UA-rich EE usually resides 35-60 nucleotides upstream of the poly(A) site and functions in facilitating the efficiency and accuracy of the 3'-end processing (Graber, McAllister and Smith, 2002). However, in some rare cases, it might be more distant (Tian and Graber, 2012). Although alternative sequences exist, UAUAUA is the most canonical and frequently used EE that provides the strongest signal for mRNA 3'-end formation (Chen and Hyman, 1998; Kim Guisbert, Li and Guthrie, 2007; Kim Guisbert *et al.*, 2005; Tian and Graber, 2012). Among the nucleotides in the EE, the first and fifth uridines have been shown to be the most important nucleotides for its function (Irniger and Braus, 1994). The PE is typically positioned 10-30 nucleotides upstream of the poly(A) site, which is likely not conserved and only enriched in adenosines in most cases (Graber, McAllister and Smith, 2002). Since mutation or deletion of the PE alters the poly(A) site location, it might function to direct the cleavage and polyadenylation to the downstream poly(A) site (Russo *et al.*, 1991; Wahle and Ruegsegger, 1999). The poly(A) site in budding yeast often appears as a cluster of adenosines following a pyrimidine downstream of the PE. Computer analysis has also identified two U-rich elements that sit closely up and downstream of the poly(A) site (Graber *et al.*, 1999; van Helden, del Olmo and Perez-Ortin, 2000). Extensive research implies that the U-rich elements probably function to make the poly(A) site more accessible to the 3'-end processing complex (Barabino, Ohnacker and Keller, 2000). Intriguingly, the cis-elements for the 3'-end processing in budding yeast are quite degenerated and redundant, which means mutations or deletions of one or two elements might only slightly reduce the cleavage activity (Dichtl and Keller,

2001; Mandel, Bai and Tong, 2008; Guo *et al.*, 1995; Zhao, Hyman and Moore, 1999). Therefore, yeast poly(A) signals are unexpectedly complicated.

2.5. The cleavage and polyadenylation complex

The polyadenylation signals are recognized by the CPF-CF complex, and accordingly, they play in coordination to manage the 3'-end cleavage and polyadenylation of pre-mRNAs (Figure 8). Around 20 protein factors are incorporated into the huge processing machinery and they can be further organized into the cleavage factor (CF) complex and the cleavage and polyadenylation factor (CPF) complex (Mandel, Bai and Tong, 2008).

For the CF complex, four subunits of Rna14, Rna15, Pcf11, and Clp1 constitute the CF IA, and a single component, Hrp1, represents the CF IB (Mandel, Bai and Tong, 2008). *In vitro* reconstitution with bacterial expressed proteins revealed that the molar ratio of the CF IA components Rna14, Rna15, Pcf11, and Clp1 is 2:2:1:1 (Gordon *et al.*, 2011). Rna14 is a central scaffold protein and physically interacts with other factors of the CF subcomplex. As mentioned in 2.1.3, Hrp1 specifically recognizes the EE and tethers the CF IA to the PE through interaction with Rna14 (Gross and Moore, 2001). Extensive studies indicated that Rna14 contacts the RRM of Hrp1 and the middle region of Rna15 to bridge the two RNA binding proteins (Moreno-Morcillo *et al.*, 2011; Barnwal *et al.*, 2012). The positioning of Rna15 at the PE is essential to the 3'-end processing *in vitro*, and reduced affinity of Rna15 to the A-rich element is lethal *in vivo* (Gross and Moore, 2001).

The CPF subcomplex has initially been separated into the cleavage factor II (CF II) and polyadenylation factor I (PF I) subcomplexes according to the contributions of the components to the *in vitro* cleavage and polyadenylation reactions (Chen and Moore, 1992). Interestingly, together with the CF I complex, the CF II complex has been shown to be sufficient to proceed with cleavage of *GAL7* and *CYC1* precursors. In contrast,

the essential complex for polyadenylation of pre-cleaved *GAL7* mRNA includes the CF I complex, PF I complex, and Pap1. Given the complicated catalytic activities and dynamic interactions within the compex, a recent study has organised the CPF components into three enzyme modules via computational analysis (Casanal *et al.*, 2017). The nuclease module for cleavage is centered around the endonuclease Ysh1/Brr5 and contains additional Cft2 and Mpe1, whereas the polymerase module for polyadenylation includes the poly(A) polymerase Pap1 and four other factors, which are Cft1, Pfs2, Fip1, and Yth1. For coupling transcription termination and 3'-end processing, two core phosphatases, Ssu72 and Glc7, and five other components, including Ref2, Swd2, Pta1, Pti1, and Syc1, constitute the phosphorylation module to regulate the phosphorylation status of the RNA Pol II CTD. Interestingly, the phosphorylation module is consistent with a group of the CPF factors identified via the tagged-Syc1 purification analysis, indicating that they are more likely to function as a subcomplex (Nedea *et al.*, 2003). In addition, Syc1 is highly homologous to the endunuclease Ysh1/Brr5 and might negatively regulate the 3'-end processing via competing with Ysh1/Brr5 for its mutually exclusive binding site on Pta1.

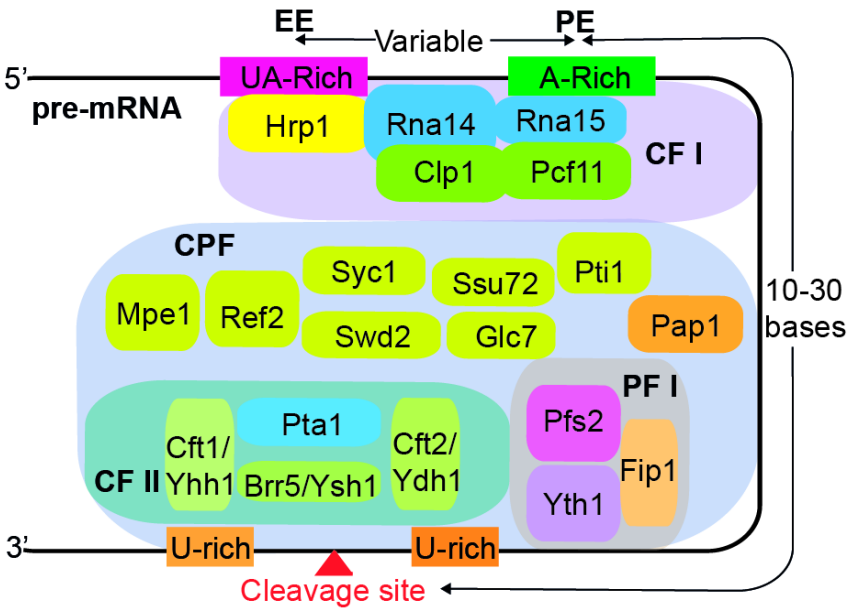


Figure 8: The CPF-CF complex fully assembles upon poly(A) signal recognition.

A total of twenty protein components are recruited onto the pre-mRNA 3'-end to form the complete CFP-CF complex. The CF IA subcomplex targets the A-rich PE via Rna15, whereas the CF IB is anchored to the UA-rich EE. The CPF complex can be further separated into the CF II and PF I subcomplexes. The endonuclease Brr5/Ydh1 is supposed to cleave the pre-mRNA at the cleavage site. The RNA polymerase Pap1 subsequently adds a poly(A) tail to the 3' cleaved end. Adapted from (Mandel, Bai and Tong, 2008).

2.6. Aim of the study

As shown in Figure 6, mRNA nuclear quality control in budding yeast relies on the shuttling guard proteins, including Npl3, Gbp2, Hrb1, and Nab2 (Hackmann *et al.*, 2014). On the one hand, the co-transcriptionally recruited surveillance factors function as export adaptors to recruit the export receptor Mex67-Mtr2 for mRNA export through the NPC. On the other hand, they act as checkpoints to monitor crucial mRNA processing steps and direct faulty mRNAs for rapid degradation in the nucleus. Npl3 seems to surveil mRNA capping, while Gbp2 and Hrb1 have been shown to monitor the splicing of intron-containing mRNAs. Although Nab2 appears to be a quality control factor for polyadenylation, the guard protein for cleavage remains unknown. Malfunctioning cleavage factors usually lead to production of 3'-extended mRNAs in budding yeast (Torchet *et al.*, 2002; Singh *et al.*, 2021; Mapendano *et al.*, 2010; Turner *et al.*, 2021; Al-Husini *et al.*, 2017). Brodsky and others have shown that mRNAs in the CPF-CF mutants are retained in the nucleus, indicating that the readthrough mRNAs are surveilled in the nucleus (Brodsky and Silver, 2000; Carneiro *et al.*, 2008; Hammell *et al.*, 2002). All of the mRNA nuclear quality control factors identified in yeast contain a SR/RGG-rich domain, which is the target of post-translational modification and is important for their export or import (Häcker and Krebber, 2004; Green *et al.*, 2002; Marfatia *et al.*, 2003). As has long been described, yeast cleavage factor Hrp1 is also a SR/RGG protein and commutes between the nucleus and the cytoplasm (Zhao, Hyman and Moore, 1999; Kessler *et al.*, 1997). Given the significant similarities of Hrp1 with the other guard proteins, it appears very likely that Hrp1 might participate in the nuclear quality control of the 3'-end processing. In this study, we aim to identify the

function of Hrp1 in mRNA nuclear quality control and try to reveal the hidden mechanism of this function.

3. Materials and Methods

3.1. Chemicals and Consumables

Table 1 - List of consumable materials

Materials	Company / Source
Agar	Carl Roth
Agarose NEEO Ultra	Carl Roth
Amersham™ protran® Western blotting membrane, nitrocellulose, pore size 0.45 µm	GE Healthcare
Clycogen	Carl Roth
Complete EDTA-free protease inhibotor	Roche
Cy3-oligo-d(T)$_{50}$ probe 1:400	Biospring
DAPI	Merck
dNTPs	Thermo Fisher Scientific
DTT	Carl Roth
Formaldehyde 37%	Sigma-Aldrich
GeneRuler™ 1 kb DNA Ladder	Thermo Fisher Scientific
GFP-trap beads	Chromotek
Glass beads	Carl Roth
GlycoBlue Coprecipitant	Thermo Fisher Scientific
HDGreen Plus DNA Stain	Intas Science Imaging
Lambda DNA / EcoRI plus HindIII marker	Thermo Fisher Scientific
MF-Millipore™ Membrane Filter, 0.025 µm pore size	Merck
Myc-trap beads	Chromotek
Oligos	Sigma-Aldrich
PageRuler™ Prestained Protein Ladder, 10 to 180 kDa	Thermo Fisher Scientific
Poly-L-lysine solution	Sigma-Aldrich

qPCRBIO SyGreen Mix Lo-ROX	Nippon Genetics
Random Hexamer Primers	Thermo Fisher Scientific
Ribolock RNAse inhibitor	Thermo Fisher Scientific
RiboLock RNase Inhibitor	Thermo Fisher Scientific
Rotiphorese Gel 30 (37.5:1) acrylamide	Carl Roth
Salmon sperm DNA	Sigma-Aldrich
TritonX100	Carl Roth
TRIzol™ Reagent	Thermo Fisher Scientific
tRNAs	Sigma-Aldrich
Tween 20	Carl Roth
WesternBright Quantum HRP substrate	Advansta
Whatman blotting Paper	Hahnemühle
Enzymes	
DreamTaq DNA Polymerase	Thermo Fisher Scientific
Phusion® High-Fidelity DNA polymerase	New England Biolabs
Q5® High-Fidelity DNA polymerase	New England Biolabs
restriction endonuclease	Thermo Fisher Scientific
restriction endonuclease	New England Biolabs
RNase A	Qiagen
RNase-Free DNase	Quiagen
T4 DNA Ligase	Thermo Fisher Scientific
Zymolyase 20T	Zymo Research
Antibodies	
Anti-Hem15 (rabbit) 1:5,000	Courtesy of Prof. Ulrich Mühlenhoff
Anti-myc (A-14) (rabbit) 1:1,000	Santa Cruz
Anti-Nop1 (mouse) 1:1,000	Santa Cruz
Anti-Mex67 (rabbit) 1:1,000	Davids Biotechnology
Anti-GFP (GF28R) (mouse) 1:4,000	Thermo Fisher Scientific

Anti-GFP (rabbit) 1:4,000	Chromotek
Anti-rabbit IgG-HRP (goat) 1:10,000	Dianova
Anti-mouse IgG-HRP (goat) 1:10,000	Dianova
Anti-Tdh1 1:4000 (mouse) 1:50000	Thermo Fisher Scientific
Anti-Zwf1 (rabbit) 1: 20,000	Courtesy of Prof. Ulrich Mühlenhoff
Kits	
Dynabeads™ mRNA Purification Kit (for mRNA purification from total RNA preps)	Invitrogen
FastGene® Scriptase II cDNA Kit	NIPPON Genetics
NucleoBond® Xtra Midi	MACHEREY-NAGEL
NucleoSpin® Gel and PCR Clean-up	MACHEREY-NAGEL
NucleoSpin® Plasmid	MACHEREY-NAGEL
NucleoSpin® RNA	MACHEREY-NAGEL

Table 2 - List of equipment

Equipment	**Company / Source**
AF6000 microscope with Leica DFC360 FX camera	Leica
Bio Photometer	Eppendorf
CFX Connect 96FX2 qPCR cycler	Bio-Rad Laboratories
Eclipse E400 tetrad microscope	Nikon
Electro Blotter PerfectBlue Semi-Dry, Sedec M	Peqlab
FastPrep-24® Cell homogenizer	MP Biomedicals
Fusion-SL-3500.WL	Vilber Lourmat
Gene Pulser Xcell™ Electroporation System	Bio-Rad Laboratories
Heraeus™ Fresco™ 21	Thermo Fisher Scientific
Heraeus™ Multifuge™ X3 with TX-750 or F15-8x50cy rotor	Thermo Fisher Scientific
Heraeus™ Pico™ 21	Thermo Fisher Scientific

Improved Neubauer counting chamber	Carl Roth
INTAS UV gel detection system	INTAS
Milli-Q® Water purification system	Millipore
My Cycler 1.065	Bio-Rad Laboratories
Nano Drop 2000 spectrophotometer	Peqlab
Primo Star light microscope	Zeiss
T100™ Thermal Cycler	Bio-Rad Laboratories

Table 3 - List of Software

Software	Developer
CFX manager 3.1	Bio Rad
Filemaker	Filemaker, Inc.
Fusion .Capt Software	Vilber
Illustrator CS5	Adobe
Image J	https://imagej.nih.gov/ij/download.html developed by National Institutes of Health
Leica AF 2.7.3.9723	Leica
Office® 2011/2019	Microsoft
Photoshop CS5	Adobe
Primer-BLAST	NCBI
Prism 7	GraphPad
Snapgene viewer	GSL Biotech

Table 4 - List of *E. coli* media

Type of medium	Component	Amount
LB	Tryptone	1% (w/v)
	Yeast extract	0.5% (w/v)
	Agar	1.5% (w/v)

	NaCl	85 mM
	Ampicillin	150 µg/ml
SOC	Yeast extract	0.5% (w/v)
	NaCl	10 mM
	Peptone	2% (w/v)
	KCl	2.5 mM
	$MgCl_2$	10 mM
	$MgSO_4$	10 mM
	Glucose	20 mM

(Sambrook, 1989)

Table 5 - List of *Saccharomyces cerevisiae* media

Type of medium	Component	Amount
YPD (plates)	Yeast extract	1% (w/v)
	Peptone	2% (w/v)
	Glucose	2% (w/v)
	(Agar*)	1.8% (w/v)
YPGal (plates)	Yeast extract	1% (w/v)
	Peptone	2% (w/v)
	Galactose**	2% (w/v)
	(Agar*)	1.8% (w/v)
Selective media / (plates)	Yeast drop out mix	0.2% (w/v)
	Yeast nitrogen base	0.17% (w/v)
	Ammonium sulfate	0.5% (w/v)
	Glucose* / Galactose**	2% (w/v)
	(Agar*)	1.8% (w/v)
	Yeast extract	0.25% (w/v)
	Potassium acetate	150 mM

Super-Spo medium	Glucose**	0.05% (w/v)
	Uracil**	40 mg/l
	Adenine**	40 mg/l
	Tyrosine**	40 mg/l
	Histidine**	20 mg/l
	Leucine**	20 mg/l
	Lysine**	20 mg/l
	Tryptophan**	20 mg/l
	Methionine**	20 mg/l
	Arginine**	20 mg/l
	Phenylalanine**	100 mg/l
	Threonine**	350 mg/l
B plates	Nitrogen base	0.17% (w/v)
	Ammonium sulphate	3 mM
	Glucose*	2%
	Agar*	3% (w/v)
FOA plates	Yeast drop out mix	0.2% (w/v)
	Yeast nitrogen base	0.17% (w/v)
	Ammonium sulfate	0.5% (w/v)
	Glucose/ Galactose*	2% (w/v)
	Agar*	1.8% (w/v)
	FOA**	0.1% (w/v)

The drop out mix consists of all amino acids except the ones for marker gene selection.

* Autoclaved separately

** Sterile filtered

(Sherman, 2002; Sprague, 1991)

Table 6 – List of yeast strains used in this study

Number	Genotype full	Source	Parental strains
HKY314	*his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Euroscarf	
HKY644	*mex67::HIS3; ade2Δ0; his3Δ1; leu2Δ0; trp1Δ0; ura3Δ0; +pUN100-mex67-5 LEU2 CEN*	(Segref *et al.*, 1997)	
HKY1001	*MLP1-TAP:HISMX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Open Biosystems	
HKY1028	*rrp6::kanMX4; his3Δ1; leu2D0; lys2Δ0; ura3Δ0*	Euroscarf	
HKY1060	*mlp1::kanMX4 leu2Δ0; met15Δ0; ura3Δ0; his3Δ1*	Open Biosystems	
HKY1861	*HRP1-GFP:HIS3MX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Invitrogen	
HKY1882	*HRP1/hrp1::kanMX4; ura3Δ0/ura3Δ0; leu2Δ0/leu2Δ0; his3Δ1/his3Δ1; met15Δ0/MET15; LYS2/lys2Δ0*	Euroscarf	
HKY2035	*cft2-1:kanMX; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0*	Euroscarf	
HKY2059	*cft2-1:kanMX; rrp6::kanMX4; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0*	This study	HKY1028 x HKY2035
HKY2120	*hrp1::kanMX4; his3Δ1; leu2Δ0; lys2Δ0; +phrp1-1-MYC URA CEN*	This study	HKY1882
HKY2123	*hrp1::kanMX4; his3Δ1; leu2Δ0; lys2Δ0; +phrp1-1-MYC URA CEN*	This study	HKY1882
HKY2124	*hrp1::kanMX4; his3Δ1; leu2Δ0; lys2Δ0; +phrp1-1-MYC URA CEN*	This study	HKY1882

HKY2126	*hrp1::kanMX4; rrp6::kanMX4; his3Δ1; leu2D0; lys2Δ0; +phrp1-1-MYC URA CEN*	This study	HKY2123 x HKY1028
HKY2128	*hrp1::kanMX4; cft2-1:kanMX4; his3Δ1; lys2Δ0; + phrp1-1-MYC URA CEN*	This study	HKY2124 x HKY2035
HKY2138	*hrp1::kanMX4; his3Δ1; leu2Δ0; lys2Δ0; +phrp1-1-GFP URA CEN*	This study	HKY1882
HKY2139	*hrp1::kanMX4; his3Δ1; leu2Δ0; lys2Δ0; +pHRP1-GFP URA CEN*	This study	HKY1882
HKY2257	*hrp1::KanMX4; cft2-1:KanMX; rrp6::KanMx4; his3Δ1; leu2Δ0; lys2Δ0; + phrp1-1-MYC URA CEN*	This study	HKY2059 x HKY2120
HKY2273	*CFT1-GFP:HIS3MX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Invitrogen	
HKY2274	*PFS2-GFP:HIS3MX6; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	Invitrogen	
HKY2306	*hrp1::kanMX4; mex67::HIS3; ade2Δ0; +phrp1-1-MYC URA CEN; +pUN100-mex67-5 LEU2 CEN*	This study	HKY644 x HKY2120
HKY2381	*hrp1::kanMX4; mlp1::kanMX4; his3Δ1; leu2Δ0; +phrp1-1-MYC URA CEN*	This study	HKY2124 x HKY1060

Table 7 – List of plasmids used in this study.

Number	Genotype	Source
pHK87	*LEU2; CEN; AMP^R*	(Sikorski and Hieter, 1989)
pHK88	*URA3; CEN; AMP^R*	(Sikorski and Hieter, 1989)
pHK240	*$_{GAL1}$GFP-HRP1; URA3; 2μ; AMP^R*	Krebber lab
pHK750	*PADH:NLS-NES-MYC-MYC-MYC CEN, URA3*	Krebber lab

pHK778	*9x MYC-NPL3; LEU2; CEN; AMP^R*	Krebber lab
pHK1664	*HRP1-MYC; URA3; CEN; AMP^R*	This study (origin pHK750)
pHK1682	*pFRE5-GFP; LEU2; CEN; AMP^R*	Krebber lab
pHK1695	*hrp1-1-MYC; URA3; CEN; AMP^R*	This study (origin pHK1664)
pHK1702	*hrp1-1-GFP URA3; CEN; AMP^R*	This study (origin pHK1695)
pHK1703	*HRP1-GFP; URA3; CEN; AMP^R*	This study (origin pHK1702)
pHK1728	*RNA14-GFP; LEU2; CEN; AMP^R*	This study (origin pHK1682)
pHK1816	*RNA14-3x MYC; LEU2; CEN; AMP^R*	This study (origin pHK1728)
pHK1817	*RNA14-9x MYC; LEU2; CEN; AMP^R*	This study (origin pHK1728)

Table 8 – List of primers used for qPCR in this study

Number	Sequence	Name
HK1867	5'-CAAACGGTGAGAGATTTCTGTGC-3'	*ITS1* Forward (rRNA)
HK1868	5'-GCCCCGATTGCTCGAATG-3'	*ITS1* Reverse (rRNA)
HK1879	5'-ATGCGAAAGCAGTTGAAGACAAG-3'	*ETS1* Forward (rRNA)
HK1880	5'-CTAGGCAGATCTGACGATCACC-3'	*ETS1* Reverse (rRNA)
HK3089	5'-AGTTACGCTAGGGATAACAGGG-3'	*21s* Forward (mitochondrial)
HK3090	5'-TGACGAACAGTCAAACCCTTC-3'	*21s* Reverse (mitochondrial)
HK3222	5'-TGTGTTTTGTCTCTCCCTTTTCT-3'	*ACT1* Forward (cleavage site)
HK3223	5'-GATGATCATATGATACACGGTCCA-3'	*ACT1* Reverse (cleavage site)
HK3226	5'-GTCAAATCGTTGGTAGATACGTTGT-3'	*ADH1* Forward (cleavage site)
HK3227	5'-GCTATACCTGAGAAAGCAACCTGA-3'	*ADH1* Reverse (cleavage site)

HK3362	5'-AGGGTAATTTGCCAGGTGT-3'	*MRP2* Forward (cleavage site)
HK3363	5'-AGCTACTTGTTTTTCTCCCAGT-3'	*MRP2* Reverse (cleavage site)
HK3366	5'-AGCCGCACAGTATCACAAAA-3'	*MEX67* Forward (cleavage site)
HK3367	5'-ACACCAAGGAAAGGGAAAAAGGAA-3'	*MEX67* Reverse (cleavage site)

Table 9 – List of primers used for cloning in this study (mismatched overhangs are marked in red)

Construct	Number	Sequence	Name
pHK1664	HK3373	5'-AGGGCGAATTGGAGCTCC ACCGCGGTGGCGGCCGCAT ACTAAGAAATATAGGTTGCTG CAAGGAAG-3'	*HRP1* Forward (Gibson assembly)
	HK3374	5'-AAATCAACTTTTGTTCACC TCTAGAGGAATTCTGCCTATT ATATGGATGGTAGCCATTATT ACGTC-3'	*HRP1* reverse (Gibson assembly)
pHK1695	HK3618	5'-TATAGGGCGAATTGGAGC TCCACCGCGGTGGCGGCCG CATACTAAGAAATATAGGTTG CTGCAAGGAAGAtG-3'	*HRP1* Forward (Gibson assembly)
	HK3619	5'-TCAGAAATCAACTTTTGTT CACCTCTAGAGGAATTCTGC CTATTATATGGATGGTAGCCA TTATTACGTCTATTG-3'	*HRP1* reverse (Gibson assembly)
pHK1702	HK3743	5'-ACGTAATAATGGCTACCAT	*GFP* Forward

		CCATATAATAGGCAGAATTCT ATGGCTAGCAAAGGAGAAGA ACTCTTCACTG-3'	(Gibson assembly)
	HK3744	5'-GAAGGGGGAGTAAAAATA AGTATACCGAAGCTT-3'	*NUF2* 3' UTR reverse (Gibson assembly)
pHK1703	HK3793	5'-TCCGCCACTGTAATTAAAA ACAAAGGATTGAAC-3'	*HRP1* Forward (Gibson assembly)
	HK3794	5'-CAGTGAAGAGTTCTTCTCC TTTGCTAGCCATAGAATTCTG CCTATTATATGGATGGTAGCC ATTATTACGTCTATTG-3'	*HRP1* reverse (Gibson assembly)
pHK1728	HK4120	5'-ACCCTCACTAAAGGGAAC AAAAGCTGGAGCTCACTTTCT ACAAATTCTTCATCATCCTCA AG-3'	*RNA14* Forward (Gibson assembly)
	HK4121	5'-AGTTCTTCTCCTTTGCTAG CCATTCCTGCAGGACCTGAC TTGGTGCTCTCAAC-3'	*RNA14* Reverse (Gibson assembly)
pHK1816	HK4358	5'-GATTCCAACAGTTGAGAG CACCAAGTCAGGTCAGAATT CCTCTAGAGGTGAACAAAAG TTG-3'	*MYC* Forward (Restriction free cloning)
	HK4359	5'-GTGTTTCAAGATTATGTTC TCGTGATTGACAACTTACGAC AGGTACCTTCACATGTTCCG CAGATTTTG-3'	*NUF2* 3' UTR (Restriction free cloning)
pHK1817	HK4367	5'-GATTCCAACAGTTGAGAG CACCAAGTCAGGTACCATAA GGCCTATGGGTGAAC-3'	*MYC* Frame Forward (Restriction free cloning)

	HK4368	5'-GATATCGAATTCCTGCAGC TTCGAAGAATGCTTTATTCAG ACATAGGCCTTCCGTTCAAG-3'	*MYC* Frame Reverse (Restriction free cloning)

Table 10 – List of primers used for analytical PCR in this study

Number	Sequence	Name
HK205	5'-GTGCCCATTAACATCACC-3'	GFP Reverse
HK423	5'-AGGCATAAATTCCGTCAGCC-3'	*KanMX4* Reverse
HK690	5'-GGAATTCCATATGTCCGTTCAAGTC TTCTTCTG-3'	*MYC* Reverse
HK1153	5'-CGAGATGAGCTTGAGAACTCC-3'	*RRP6* 5' UTR Forward
HK1157	5'-CCACATATCGCAGAAAGC-3'	*MLP1* 5' UTR Forward
HK1264	5'-CCTTCCTTTTCGGTTAGAGC-3'	*CYC1* 3' UTR Reverse
HK3088	5'-ATGAGCTCTGACGAAGAAGA-3'	*HRP1* Forward
HK3093	5‘-GCCACCTAATGCAATGAC-3'	*HRP1* Forward
HK3094	5‘-AATCCGCTTTCGAACGTTC-3'	*HRP1* Reverse
HK3181	5'-TGAAAAAGCGTGCATAATAC-3'	*HRP1* 5' UTR Forward
HK3182	5'-AACGTTCGAAAGCGGATTTGTC-3'	*HRP1* Forward
HK3183	5'-AGCTTGTGATTATACATTCTAGC-3'	*HRP1* 3' UTR Reverse
HK3487	5'-TAAGGAGAGCCTACCGCAAG-3'	*CFT2* Forward
HK3496	5'-ATGTTGAATGCCAAGCACTTC-3'	*MEX67* 5' UTR Forward
HK4078	5'-ATTTCCCACTTCGTTTAAAATG-3'	*RNA14* 5' UTR Forward
HK4079	5'-ATGTGGAATAGATACACTCAATG-3'	*RNA14* Forward
HK4122	5'-ACCATAAGTGAAAGTAGTGACAAG-3'	*GFP* Reverse
HK4133	5'-ATTTGGTAAATGTCGTAAACTG-3'	*RNA14* Forward
HK4145	5'-AGCTTCCAACAGAGGTTC-3'	*RNA14* Forward
HK4150	5'-AGCGTATGACTCTTGAGTTTC-3'	*RNA14* Reverse
HK4246	5'-TGGAAAGCATGCGCCTTTTATAC-3'	*HRP1* 5' UTR Reverse

HK4247	5'-ATCCCAATGGCATTTTTTAGCTAC-3'	*NUF2* 3' UTR Forward
HK4248	5'-TCACACAGGAAACAGCTATGAC-3'	M13 Reverse
HK4360	5'-TCTATGAGGCCTATGCTTG-3'	*CFT1* Forward
HK4361	5'-ATCAATAACGACATCAATGCTG-3'	*PFS2* Forward
HK4369	5'-TGTAAGCGTGACATAACTAATTAC-3'	*CYC1* 3' UTR Reverse
HK4905	5'-AGTGCCATCTTGCTTACTAGAAG-3'	*HRP1* Reverse

3.2. DNA isolation and cloning

3.2.1. Purification of genomic DNA from yeast cells

The method of genomic DNA (gDNA) extraction from yeast cells is modified from (Rose, 1991). Yeast cells (10 ml, grown to saturation in YPD) were collected by centrifugation (4000 rpm for 5 min) and transferred to a new screw-top eppi with 500 µl H_2O. After washing, the cells were spun down and the supernatant was removed. Then 500 µl detergent lysis buffer, 500 µl phenol and 300 µl glass beads were added to lyse the cells with FastPrep at 6 m/s for 2x 20 seconds. Since the DNA molecules are hydrophilic, the aquatic phase (upper phase) was transferred into a fresh tube after the sample was centrifuged at 13000 rpm for 5 min. The same amount of phenol was then added, the tube was vigorously shaken, and the new upper phase was separated again via centrifugation.

To further purify the gDNA, the last step was repeated with the same amount of phenol/chloroform/isoamylalcohol (25:24:1) and later with chloroform/isoamyl alcohol (24:1) in the same way. Following that, the final aquatic phase was mixed with 1/10 volume of 3 M sodium acetate (pH=5.2) and 3x volumes of precooled ethanol (100%). After incubation in the freezer (-20 °C) for 60 min, DNA molecules were precipitated and spun down at 13000 rpm (4 °C) for 30 min. 70% ethanol was used for washing the pellets. The gDNA pellet was then airdried and resuspended in 50 µl DEPC H_2O.

For usage as templates in PCR, gDNA was digested with one or two restriction enzymes without cutting the target genes. 1 µl gDNA was diluted in a 20 µl digestion system, and 1 µl digested gDNA was used for PCR.

3.2.2. DNA digestion with restriction enzymes

For DNA digestion with restriction endonuclease, a general digestion system is shown in Table 11. For double digestion, a recommended combination of enzymes and buffer was found with the ‘‘DoubleDigest Calculator—Thermo Scientific’’. The amount of water in the reaction was adjusted according to the volume of the other elements. The reaction was inactivated by incubation at 60 °C or 80 °C, depending on the enzyme that was used.

Table 11 - General digestion system

Add	Amount (µl)
Nuclease-free water	16
10x digestion buffer	2
DNA (0.5-1 µg/µl)	1
Restriction enzyme	0.5-2
Mix gently and spin down	
Incubate at 37 °C for 1 – 16 hours	

3.2.3. Polymerase chain reaction (PCR)

Dream *Taq* polymerase or proofreading polymerases (e.g., Phusion, Q5, VELOCITY) were used to amplify DNA fragments for analytical or cloning purposes, respectively (reaction mix see Table 12, reaction cycle see Table 13).

Table 12 - Standard 25 µl reaction mix for PCR

	Dream *Taq*	**Phusion**	**Q5**	**VELOCITY**
Template	5-100 ng plasmid DNA or 1 µl digested gDNA			
Reaction buffer	2.5 µl	5 µl		
dNTPs	200 µM each	200 µM each	200 µM each	250 µM each
primers	0.2 µM each	0.5 µM each	0.5 µM each	0.2-0.6 µM each
polymerase	0.025 U/µl	0.02 U/µl	0.02 U/µl	0.02-0.04 U/µl
DEPC H_2O	Add to 25 µl			

Table 13 - PCR reaction cycles

	Dream *Taq*	**Phusion**	**Q5**	**VELOCITY**	**Repeat**
Initial denaturation	95 °C 3 min	98 °C 30 s	98 °C 30 s	98 °C 2 min	1
Denaturation	95 °C 30 s	98 °C 10 s	98 °C 10 s	98 °C 30 s	30-35
Annealing	45-60 °C (depends on the annealing temperatures of the primers); 30 s				
Extension	72 °C 1 min/kb	72 °C 30 s/kb			
Final extension	72 °C; 5-10 min				1

3.2.4. Agarose gel electrophoresis and gel extraction

PCR products or plasmid digestion were analyzed with agarose gel electrophoresis. DNA samples were mixed with 6x loading dye (10 mM Tris pH=7.5, 60% (v/v) glycerol, 0.03% (w/v) Bromophenol blue, 60 mM EDTA) and loaded on the agarose gel (1%-2% agarose and 5 µl/100 ml Intas HDGreen™ Plus DNA dye in TAE buffer (40 mM Tris base, 0.1% Acetic acid, 1 mM EDTA, millipore water)). According to their sizes, DNA molecules were separated during electrophoresis for 30-50 min at a voltage of 120 V.

The bands of DNA molecules were visualized with a UV-transilluminator and the sizes of bands were shown with a DNA marker. For DNA purification on a gel, the kit NucleoSpin® Gel and PCR Clean-up (MACHEREY-NAGEL) was used with their protocol. The concentration of DNA yield was measured by light absorbance at 260 nm with a Nanodrop spectrophotometer.

3.2.5. Gibson assembly

The Gibson assembly used for plasmid construction is based on (Gibson *et al.*, 2009). Several overlapping DNA fragments with varying lengths and compatibility were combined in one isothermal process. The backbone was obtained by linearizing an existing plasmid with restriction enzymes. The DNA insert fragment was amplified via PCR with a proofreading polymerase and a pair of long primers (around 60 bp for each) containing around 30 bp overhangs. The sequences of overhangs were identical to their neighboring segment on the backbone, respectively. Both the DNA backbone and the insert were purified with the kit NucleoSpin® Gel and PCR Clean-up (MACHEREY-NAGEL). The concentration of the yields was measured with a Nanodrop Spectrophotometer. NEBioCalculator was used for calculating the amount of DNA insert that should be used for cloning. The backbone and insert fragments were joined in one Gibson assembly reaction (see Table14) with T5 exonuclease (creates single strand DNA overhangs for the annealing of the complementary DNA fragments), Phusion DNA polymerase (fills up the gaps in the annealed fragments) and *Taq* DNA ligase (ligates fragments), processing at 50 °C for 1 h.

Table 14 - Gibson assembly reaction

Add	amount
Gibson assembly master mix (see Table 15)	10 µl
Backbone (linearized plasmid)	100 ng
DNA insert fragment	2-5 folds molar excess of backbone
DEPC H_2O	To 20 µl

Table 15 - Gibson assembly master mix

Add	amount
T5 exonuclease	0.04 U
Phusion DNA polymerase	0.25 U
Taq DNA ligase	40 U
PEG 8000	5% (v/v)
Tris/HCl pH=7.5	100 mM
MgCl2	10 mM
DTT	10 mM
dNTPs	200 µM
NAD	1 mM

3.2.6. Restriction free cloning

Restriction free cloning was also used to generate new plasmids and was based on two rounds of PCR (Bond and Naus, 2012). For the first round, the DNA insert fragment was amplified with flanking sequences complementary to the target plasmid. Then the insert was used as a mega primer to replicate the destination plasmid. The methylated plasmid was degraded using the DpnI restriction enzyme (overnight) and the resulting products were used for transformation of *E. coli*.

3.2.7. Transformation of *E. coli* with electroporation and subsequent colony PCR

Benefiting from the high efficiency of DNA molecule introduction into the cell and rapid growth, *E. coli* was preferred as a host for gene cloning. Electro competent cells were prepared as described in the work of Dower and others and stored at -80 °C (Dower, Miller and Ragsdale, 1988). To make an efficient transformation, Gibson assembly reaction mix or daughter products from restriction-free cloning were dialyzed by dropping them onto a nitrocellulose membrane (0.025 µm pore size) floating in

deionized water in a Petri dish for 30-60 min. After removing inhibitory substances via drop dialysis, DNA samples were used for *E. coli* transformation. One aliquot of 50 µl competent cells was thawed on ice and mixed with dialyzed DNA products. Then the mixture was transferred into a precooled electroporation cuvette and pulsed (exponential decay, 1.5 kV, 50 µF, 150 Ω) with an electroporator. 1 ml of SOC medium was immediately added and the cells were transferred into a fresh tube. After incubation at 37 °C for 1 h, cells were harvested via centrifugation and a suspension in 100 µl sterile H_2O was plated on a LB-Agar plate with ampicillin. Following a 12-15 h incubation at 37 °C, colonies on the plate were taken for colony PCR to screen cells for the correct plasmid. 1-2 µl suspension of a single colony in 20-30 µl sterile H_2O was applied as template for PCR.

3.2.8. Plasmid isolation from *E. coli* and DNA sequencing

E. coli cells from each positive colony suspension were grown in 10 ml LB media with ampicillin at 37 °C for 12-15 h and collected via centrifugation (4000 rpm, 10 min). Plasmids were purified with a NucleoSpin Plasmid purification kit (MACHEREY-NAGEL) and sequenced by LGC Genomics via Sanger sequencing. The NucleoBond Xtra midi kit (MACHEREY-NAGEL) was used for larger scale purification.

3.3. *S. cerevisiae* cell cultivation

3.3.1. Growth and storage condition of yeast cells

Yeast cells with a metabolic auxotrophy of certain amino acids (L-Adenine, L-Histidine, L-Leucine, L-Lysine, L-Tryptophan, or Uracil) created through knockout of respective genes were normally grown in full medium YPD. Plasmid containing yeast cells with the marker gene which grants prototrophy for auxotrophic growth were grown in selective media. For the purpose of gene overexpression under a *GAL1* promoter, cells were grown in media with sucrose and subsequently induced with galactose for 2-3 h. Yeast cells were generally grown at 25 °C, but if temperature-sensitive mutants were

used, all the strains in one experiment were shifted to a nonpermissive temperature for an appropriate time. Cultures in the same volume for one experiment were harvested at the logarithmic growth phase (1-3x 10^7 cells/ml). The density of cultures was either determined by counting the cells with a Neubauer counting chamber, or by measuring the value at the wavelength of 600 nm with a standard photometer.

Yeast cells were mixed with 50% glycerin and frozen at -80 °C for long-term storage. For a short time (around 2 weeks), strains were streaked out on plates and kept in the fridge at 4 °C. A FOA plate was used to select cells that had lost their plasmids with the *URA3* gene.

3.3.2 Crossing and sporulation of yeast strains

Yeast strains with new genotypes were generated via crossing. Haploid strains used in this study were either *MATa* or *MATα* in their mating type. Two haploid strains with the opposite mating types and different marker genes were mixed on YPD plates to form diploids. The diploid strain and parental haploid strains were further streaked out on a double selective plate. Only diploids with two marker genes could grow on the selective plate. The super-spo medium was used for diploid sporulation due to its low nutrient level. One diploid cell can form a tetrad with four spores after meiosis. Sufficient tetrads were usually obtained after 3-7 days of sporulation and were identified with a light microscope. 100 µl sporulation culture was centrifuged and cells were suspended in 50 µl P-solution (0.1 M phosphate buffer pH=6.5, 1.2 M sorbitol). 2.5 µl zymolyase (20 mg/ml) was used for digesting the tetrads for 5.5 min, which enabled tetrad dissection under a tetrad microscope. After about 3-4 days, spores on a YPD plate were restreaked on another YPD plate to get sufficient cell materials and then were stored in a 96-well plate with 200 µl glycerin in each well at -80 °C.

The selection makers were detected by stamping the spores on selective plates. The mating type of the spores was determined by crossing them with both the *MATa* and

MATα reference strains, which were valine and isoleucine auxotrophic. Only diploids could grow on B-plates. Therefore, the mating types of the spores were opposite to those of the reference strains, with which they could form diploids. Further genotypes were determined by colony PCR. Yeast cells were suspended in 50 µl of PBS (2.7 mM KCl, 137 mM NaCl, 2 mM Na_2HPO_4, 10 mM KH_2PO_4) and digested with 10 µl of zymolyase (20 mg/ml) at 37 °C for 1 h, followed by 10 min at 95 °C. After centrifugation (13000 rpm, 1 min), 2 µl of supernatant containing gDNA was used as a template for PCR.

3.3.3. Transformation of yeast cells with plasmids

Yeast cells were transformed with plasmids via the lithium acetate method (Gietz *et al.*, 1992). A preculture (5 ml) was prepared and grown overnight. Cells were counted and inoculated in another tube of medium (5 ml) from 1x 10^7 cells/ml. At the logarithmic phase, cells were collected and washed with 1 ml of sterile H_2O and with 1 ml of TE lithium acetate buffer (100 mM lithium acetate, 1 mM EDTA, 10 mM Tris, pH=7). After resuspension in 50 µl of the TE lithium acetate buffer, 1 µg of the plasmid, 5-10 µl ssDNA (preheated at 95 °C for 5 min and cooled on ice for 2 min before it was used), and 300 µl of PEG TE lithium acetate buffer (100 mM lithium acetate, 10 mM Tris pH=7.5, 1 mM EDTA, 40% (v/v) polyethylene glycol 4000) were added into the tube. The tube was incubated at 25 °C for 30 min on a rotator and heated at 42 °C for 15 min. Afterwards, cells were harvested and plated on a selective plate.

3.4. Cell biology methods

3.4.1. Growth test

Yeast strains were precultured overnight and were diluted and counted on the next day. Afterwards, cells for each strain were diluted into 10^7, 10^6, 10^5, 10^4, and 10^3 cells/ml and dropped on agar plates. After 2-3 days of incubation at different

temperatures (16 °C, 25 °C, 30 °C, 35 °C, and 37 °C), plates were scanned and yeast growth was analyzed by comparing the intensity and size of the colonies.

3.4.2. Fluorescence *in situ* hybridization (FISH)

The experiment was performed as described in an earlier work (Hackmann *et al.*, 2014). A Cy3-labled oligo-d(T)$_{50}$ probe was used to target mRNAs with a poly(A) tail in yeast cells. 10 ml of yeast culture for each strain was grown at 25 °C and shifted to 37 °C for 3 h to obtain the defects in mutant strains. Cells were fixed with 37% formaldehyde (1 ml for each) for 45 min on the rotator and harvested on ice. After washing three times with 1 ml of P-solution for each, cells were treated with DTT (10 mM) in 100 µl of P-solution for 10 min. Then zymolyase digestion (5 µl 10 mg/ml, 10-30 min, monitored under a light microscope) was performed to get intact spheroplasts. Once 70% of cells appeared dark, the spheroplasts were collected via centrifugation (2000 rpm, 2 min, 4 °C) and washed with 1ml of P-solution. 20-30 µl of resuspended cells (in P-solution) for each strain were applied to the wells of a poly-L-lysine coated slide and incubated for 30-60 min at room temperature. 0.5% (v/v) triton-X100/P-solution was used to permeabilize the nuclear envelope that enables probes to penetrate into the nucleus. To block unspecific hybridization, 20 µl of pre-hybridization buffer including Hybmix (see table 16), tRNA (1/20 v/v, 10 mg/ml) and ssDNA (1/20 v/v, preheated at 95 °C for 10 min and cooled on ice for 5 min) was applied to each well at 37 °C for 30-60 min in a humidified chamber. The Cy3-labled oligo-d(T)$_{50}$ probe was diluted (1:200) into fresh pre-hybridization buffer and 20 µl for each well was added for hybridization at 37 °C overnight. One well of the wild type cells on the slide without adding probes served as a negative control. The day after that, cells on the slide were washed with 2x SSC (0.3 mM NaCl, 30 mM sodium citrate, pH=7) for 1 h and 1x SSC for 1 h at 25 °C. Then 0.5x SSC was used for two washing steps, once at 37 °C and once at 25 °C. The nucleus was stained with DAPI (1:10000 diluted in PBS) for 5 min at 25 °C. PBS was used for washing 3-5 times, each for 5 min. The slide was dried and coated with mounting medium (2% n-propyl gallate, 80% glycerol, 20% PBS pH=8). A glass slide on top was

used to cover the wells and nail polish was used to seal the slide. The slide was stored at -20 °C for several months.

Cell fluorescence was detected with the camera DFC360 FX of the Leica DMI6000B fluorescence microscope. Pictures were taken on the platform of the LAS AF1.6.2 software.

Table 16 - Hybmix (40 ml, stored at -20 °C)

Add	Amount
deionized formamide	20 ml
20x SSC DEPC	10 ml
500 mM EDTA; pH=8	400 µl
10% Tween-20	400 µl
50x Denhardt's (see table 17)	800 µl
Heparin, 10 mg/ml	400 µl
DEPC-Water	7,2 ml

Table 17- 50x Denhardt's (5 ml)

Add	Amount
Ficoll	0.05 g
Polyvinylpyrrolidone	0.05 g
BSA (Pentax fraction V)	0.05 g
DEPC H_2O	to 5 ml

3.5. Biochemical methods

3.5.1 Protein co-Immunoprecipitation

The protein co-immunoprecipitation experiment was performed as described in an earlier work (Zander *et al.*, 2016). Cells in a 400 ml or 800 ml culture for each strain

were harvested and frozen at -20 °C. To lyse the cells with a FastPrep Cell homogenizer (3x 30 s, 5 min on ice in between), the same amount of PBSKMT buffer (see table 18) and glass beads were added. Clear supernatants were transferred to fresh tubes after centrifugation (13000 rpm, 10-20 min) and 30 µl from each was used as an input control. For immunoprecipitation, GFP-trap beads were used to pull down GFP-tagged proteins. To reduce unspecific precipitation, GFP beads were washed 3 times with PBSKMT (2000 rpm, 2 min) and blocked for 30-60 min on a rotator at 25 °C with BSA (50-100 mg/ml) and glycogen (10 µl/reaction, 20 mg/ml). Then the beads were washed again for 3 times and split in equal volumes for each reaction. The same amount of lysate from each strain was applied to GFP bead aliquots and incubated at 4 °C on a rotator for 2-3 h. After 3-5 times of washing steps with PBSKMT, both beads and lysates were mixed with 30 µl of 2xSDS sample buffer (2% (w/v) SDS, 125 mM Tris/HCl pH=6.8, 25% (v/v) Glycerol, Bromophenol blue, 10% (v/v) ß-mercaptoethanol (freshly added)) and denatured at 95 °C for 6-8 min. All the samples were centrifuged (13000 rpm, 30 s) and afterwards loaded onto a SDS gel.

Table 18 - PBSKMT buffer

Add	Amount
1x PBS pH=7.5	137 mM
KCl	3 mM
$MgCl_2$	2.5 mM
Triton-X-100 (freshly added)	0.5% (v/v)
protease inhibitor	5 µl/100 ml pellet

3.5.2. SDS-PAGE and western blot

SDS-PAGE (sodium dodecyl sulfate polyacrylamide gelelectrophoresis) was used to separate proteins of different sizes (Garfin, 2009). Protein samples and the marker were loaded into the wells. Overnight electrophoresis was running with a current of 6 mA.

Table 19- SDS gel

	Stacking gel (top)	Resolving gel (bottom)
Rotiphorese Gel 30 acrylamide mix	16.7% (v/v)	33.3% (v/v)
ddH_2O	40% (v/v)	68% (v/v)
Tris/HCl pH=8.0	/	375 mM
Tris/HCl pH=6.8	125 mM	/
SDS	0.1% (w/v)	0.1% (w/v)
APS	0.1% (w/v)	0.1% (w/v)
TEMED	0.1% (w/v)	0.1% (w/v)

With a semi-dry blotting system, proteins were transferred (1.5 mA/cm^2, 2 h) from a SDS gel to a nitrocellulose membrane for analyzing targeting proteins with specific antibodies (Alegria-Schaffer, 2014). Ponceau S (0.2% (w/v) Ponceau S, 5% (v/v) acetic acid) staining was applied to visualize all the proteins on the membrane. Depending on the size of target proteins, the membrane was cut into pieces. Followed by a washing step with water (5-10 min), 5% (w/v) milk powder in TBST buffer (150 mM NaCL, 50 mM Tris base, 0.1% (v/v) Tween 20) was added and the blot was further incubated for 30-60 min to block unspecific binding. Then the specific primary antibody in TBST/2% (w/v) milk powder was added to each membrane piece with the target protein on it. The incubation was performed on a shaker at 4 °C overnight. On the next day, the membrane pieces were washed with TBST for 3 times (each for 5-10 min) and incubated with the corresponding secondary antibody in TBST for 2-3 h. For protein detection, the membrane pieces were firstly washed with TBST (3x, 5-10 min for each) and secondly, covered with ECL substrate solution.

After incubation for 10-30 s, the signals of proteins were detected with a chemiluminescence imaging system of Fusion-SL-3500.WL (Vilber Lourmat). Different

exposure times were applied depending on the intensity of protein bands on the membrane.

3.5.3. RNA co-Immunoprecipitation

RNA co-immunoprecipitation was used to analyze whether RNAs would bind to target proteins (Zander *et al.*, 2016). GFP-tagged proteins were pulled down in a similar way that was done in protein co-immunoprecipitation (see 3.5.1), but with RIP buffer (see table 20) instead of PBSKMT buffer. In the last washing step after incubation at 4 °C, each sample was split into 300 µl for protein analysis and 700 µl for RNA purification. Protein detection was performed with SDS-PAGE and western blot. RNAs from both lysate and eluate were isolated with Trizol and chloroform after incubated with DNase (30-60 min) to eliminate DNAs in samples. Glycogen and glycoblue in isopropanol were used to precipitate RNAs from aqueous layer in lysate and eluate, respectively. On the next day, RNAs were pelleted with centrifugation at a high speed (13000 rpm, 30 min) and washed two times with precooled 70% ethanol. Then the pellet was dried at 65 °C for 10 min and dissolved in DEPC H_2O (100 µL for lysate samples and 20 µl for eluate samples) at 65 °C for 10 min. The concentration of each sample was measured with the NanoDrop2000 spectrophotometer (Peqlab).

Table 20 – RIP buffer

Add	Amount
Tris/HCl	25 mM
$MgCl_2$	2 mM
NaCl	150 mM
DTT (freshly added)	0.5 mM
Triton-X-100 (freshly added)	0.2% (v/v)
PMSF (freshly added)	0.2 mM
Protease inhibitor (freshly added)	5 µl/100 ml pellet
RiboLock (freshly added)	0.02 U/ml

3.5.4. cDNA synthesis and Quantitative Realtime-PCR

The FastGene Scriptase II Kit (NIPPON Genetics) was used to synthesize cDNA from purified RNA. The same amount of purified RNA was taken from each sample and reverse transcribed into complementary cDNAs with random hexamer primers. The cDNA synthesis mix was then diluted to 1 µg/µl with DEPC H_2O for qPCR analysis. In case there was significant DNA contamination in the RNA samples, negative controls of RNA dilution (1 µg/µl) in DEPC H_2O were also prepared for qPCR analysis.

The CFX Connect 96FX2 qPCR cycler (Bio-Rad Laboratories) was used to perform qPCR. Expression of a specific gene was quantified by measuring the Cq value when there was detectable SYBR Green fluorescence. For each qPCR, 5 µl qPCRBIO SyGreen Mix, 0.48 µl primers (0.24 µl for each, 10 mM), 2 µl cDNA template and 2.52 µl DEPC H_2O were mixed and added into one well of a 96-well plate. A master mix without templates was prepared to minimize pipetting errors. Three replicates were performed for each qPCR with cDNA templates. Negative controls with RNA templates were performed once for each.

3.5.5. Cell fractionation

A cell fractionation experiment was performed to analyze the leakage of faulty mRNAs in the cytoplasm (Shukla and Parker, 2014). Yeast cells (logarithmic) from 400 ml culture for each strain were harvested with centrifugation (2000 rpm, 5 min), washed once with H_2O and once with 1 ml of YPD/1 M sorbitol/2 mM DTT. Then spheroplasts were obtained by zymolyase digestion in 1 ml of YPD/1 M sorbitol/1 mM DTT for 10-30 min at 25 °C. Cells were collected and resuspended in 50 ml of YPD/1 m sorbitol for 30 min on a rotator at 25 °C. For accumulation of the defects in temperature sensitive mutants, cells were then shifted to 37 °C for 3 h. Afterwards, cells for each strain were split into 20 ml (for protein and RNA control in the total lysate) and 30 ml (for cytosolic protein control and cytosolic RNA isolation), collected via centrifugation (2000 rpm, 5 min) respectively. Cells collected for total lysate controls were washed

with 1 ml H_2O and spilt into 700 µl (for protein control, in a screw-top tube) and 300 µl (for total RNA isolation). To obtain the cytosolic fraction, cells were resuspended in 800 µl of lysis buffer (18% Ficoll 400, 10 mM HEPES pH=6.0) and treated with 1.6 ml of buffer A (50 mM NaCl, 1 mM $MgCl_2$, 10 mM HEPES pH=6.0). The mixture was vortexed for 10 s and centrifuged (4000 rpm) for 15 min at 4 °C. The supernatant was taken as the cytosolic fraction. 100 µl was frozen for protein detection in the cytoplasm and the rest was stored for RNA isolation.

For protein detection, total lysate was obtained via cell lysis (see 3.5.1) and the cytosolic fraction was directly used for preparing the protein sample with 2x SDS sample buffer. SDS-PAGE and western blot were performed for the detection. Nop1 and Zwf1 were detected as positive controls for nuclear protein and cytosolic protein, respectively.

If there was detectable Zwf1 signal for each strain in the cytosolic fraction, but not for Nop1, the cell fractionation was successful and RNA isolation was then performed with a NucleoSpin RNA kit (MACHEREY-NAGEL). cDNAs were synthesized and analyzed via qPCR (see 3.5.4.).

3.5.6. mRNA isolation

mRNA isolation from total RNAs was performed using the Dynabeads™ mRNA Purification Kit (Invitrogen) according to the manufacturer's instructions as written in the manual. Total RNAs were extracted with the NucleoSpin RNA Kit (MACHEREY-NAGEL).

3.6. Statistical analysis

At least three independent repeats were performed for all the experiments in this study. The error bars reflect the standard deviation. The p values of two unpaired arrays were

determined with a t-test (two tails, heteroscedastic type). Significance was shown with stars according to the p value (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

4. Results

4.1. Hrp1 might be a potential quality control factor for mRNAs in the nucleus

Npl3, Gbp2, Hrb1, and Nab2 are SR like proteins that shuttle between the nucleus and the cytoplasm (Zander and Krebber, 2017; Zander *et al.*, 2016; Kim Guisbert *et al.*, 2005; Flach *et al.*, 1994). They have been later identified as guard proteins that co-transcriptionally bind mRNAs and surveil mRNA processing during its biogenesis in the nucleus (Hackmann *et al.*, 2014; Zander and Krebber, 2017). Afterwards, all these guard proteins are part of an export competent mRNP particle that is transported into the cytoplasm for translation (Hackmann *et al.*, 2014; Grosse *et al.*, 2021; Windgassen *et al.*, 2004). Since Hrp1 has also been reported as a shuttling SR protein and it is essential for efficient and accurate cleavage and polyadenylation of mRNAs in the nucleus (Häcker and Krebber, 2004; Kessler *et al.*, 1997; Zhao, Hyman and Moore, 1999; Kim Guisbert *et al.*, 2005; Minvielle-Sebastia *et al.*, 1998), it might be a good candidate for mRNA surveillance at the 3'-end processing in the nucleus. We compared Hrp1's amino acid sequence and functional domains to those of the other guard proteins and show that Hrp1 is highly homologues and shares RRM and SR/RGG domains with Gbp2, Hrb1, and Npl3 (Figure 9). Among those SR proteins, Npl3 has a more canonical SR/RGG domain which is rich in SR and RGG repeats at the C terminus. Gbp2 and Hrb1 both include an N-terminal SR/RGG domain which contains several SR repeats. Similarly, Nab2 has a SR/RGG domain that includes four RGG repeats. Instead of RRMs for mRNA binding, the C-terminal zinc finger domain is critical for Nab2 to recognize poly-adenosines in mRNAs. The N-terminal domain of Nab2 has been shown to be essential for mRNA export (Marfatia *et al.*, 2003). In respect to Hrp1, although there are fewer SR or RGG repeats, the SR/RGG domain is rich in single serine and arginine amino acids. Hrp1 exhibits an overall identity of about 26-27% with Gbp2, Hrb1, and Npl3 (Figure 9). Nab2 has no significant sequence identity with the other shuttling SR/RGG proteins due to a lack of a canonical RRM.

The overall similarities in functional domain organization might suggest that Hrp1 could be another shuttling SR like protein and might also behave similarly.

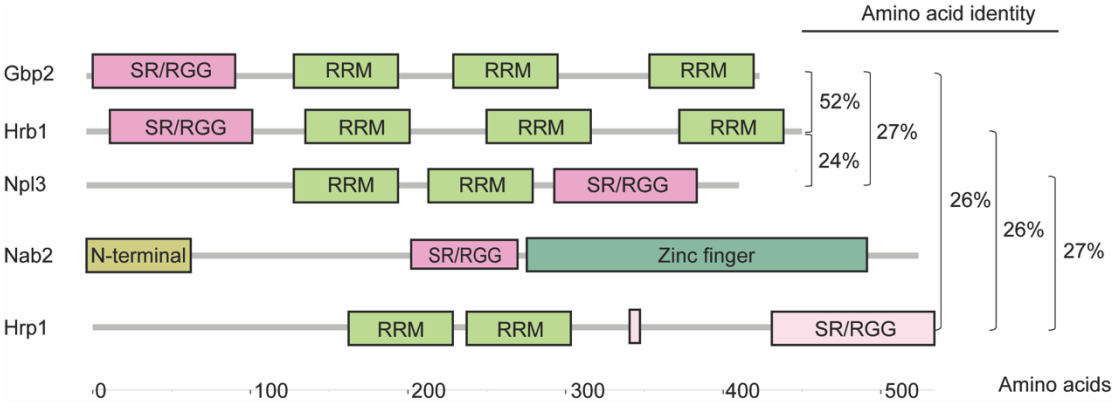


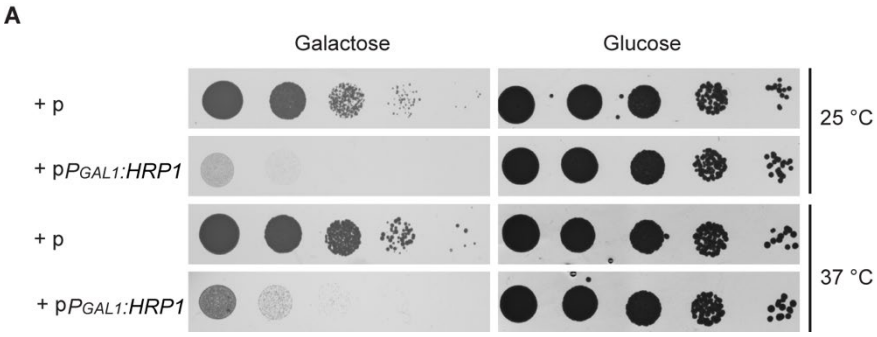
Figure 9: The shuttling protein Hrp1 shares significant sequence similarities with Gbp2, Hrb1, and Npl3.

The amino acid sequences of the proteins were obtained from the SGD (Saccharomyces GENOME DATABASE). An online website (http://smart.embl-heidelberg.de/) was used to explore the domain architectures of each protein. The identities of the amino acid sequences of proteins were determined with NCBI blastp.

4.2. Hrp1 behaves like a nuclear retention factor

Overexpression of *NPL3* or *GBP2* generates an excessive amount of guard proteins in the nucleus, which is toxic to cells and results in nuclear retention of poly(A)-mRNAs (Häcker and Krebber, 2004; Baierlein *et al.*, 2013; Windgassen and Krebber, 2003). *HRB1* overexpression is not toxic for yeast cell growth, which is likely due to the changed localization of Hrb1 from the nucleus in the normal condition to the cytoplasm during overexpression (Häcker and Krebber, 2004). The harmful interfering effects of excessive mRNA guard proteins in the nucleus might be attributable to the depletion of the mRNA export receptor and other binding proteins. Given the sequence and structure similarities of shuttling Hrp1 to the other guard proteins (Figure 9), we expected a similar behavioral pattern. First, the effect of *HRP1* overexpression on yeast cell growth was tested. For this purpose, the wild type cells were transformed with either a plasmid containing *HRP1* under a *GAL1* promoter (pP_{GAL1}:*HRP1 URA3*),

or an empty plasmid with only a marker gene of *URA3*. *HRP1* was overexpressed with galactose induction, driven by the strong *GAL1* promoter. In contrast, the *GAL1* promoter was suppressed when cells were grown on a plate with glucose. Interestingly, a severe growth defect of the cells grown on plates with galactose was observed at both 25 °C and 37 °C (Figure 10A) in growth analysis, but cells on plates with glucose showed normal growth. This indicates that *HRP1* overexpression is toxic to yeast cells, which is consistent with the growth defects due to overexpression of *NPL3* and *GBP2* that encode the other two guard proteins. Afterwards, to detect whether the distribution of the mRNA was affected by the overexpression of *HRP1*, we performed a FISH experiment with a Cy3-labeled oligo $d(T)_{50}$ probe. Strikingly, cells with *HRP1* under a *GAL1* promoter that were grown on galactose containing plates displayed strong nuclear accumulation of mRNAs at both temperatures (Figure 10B), indicating that Hrp1 might act as a nuclear retention factor for mRNA export. This, in turn, explained the toxicity of *HRP1* overexpression, which was likely due to the disrupted export of mRNAs caused by its overexpression.



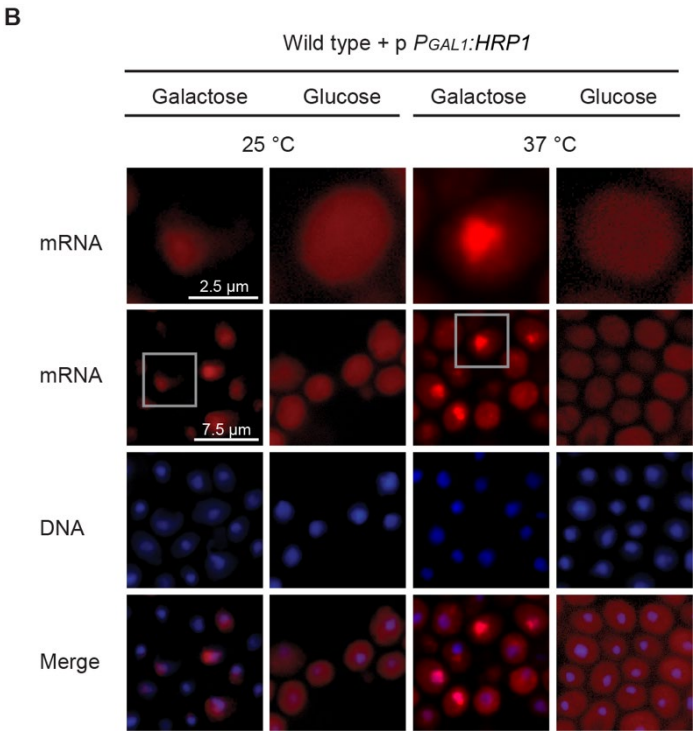


Figure 10: *HRP1* overexpression is toxic to yeast cells and retains mRNAs in the nucleus. (A) A growth analysis shows the toxicity of *HRP1* overexpression. The wild type cells transformed with either pP_{GAL1}:*HRP1 URA3* or p*URA3* were spotted in 10-fold serial dilutions onto -ura agar plates with galactose or glucose. Plates were incubated at 25 °C or 37 °C for 2-3 days. Cells that were transformed with empty plasmids and grown on plates with glucose served as a negative control. (B) The FISH experiment reveals the nuclear retention of mRNAs caused by *HRP1* overexpression. For hybridization, a Cy3-labeled oligo d$(T)_{50}$ probe targeting mRNAs with a poly(A) tail was used. The nucleus was stained with DAPI. For a negative control, the wild type cells with pP_{GAL1}:*HRP1 URA3* were cultured in glucose-containing medium.

4.3. Hrp1 functions as an adaptor protein for mRNA export

Hrp1 shares sequence similarities with the other guard proteins (Figure 9) and may function as a nuclear retention factor for mRNAs in the nucleus (Figure 10). This might suggest that Hrp1 may have more common features with the other guard proteins. For mRNA surveillance in the nucleus, the capability of recruitment of the export machinery is especially important. The Mex67-Mtr2 heterodimer, known as the main export receptor for all kinds of RNAs in budding yeast (Faza *et al.*, 2012; Chatterjee *et al.*,

2017; Tuck and Tollervey, 2013; Erkmann and Kutay, 2004), is recruited by the other nuclear guard proteins for mRNA export (Hackmann *et al.*, 2014; Zander *et al.*, 2016). To test the hypothesis that Hrp1 might also be an adaptor protein for mRNA export, we studied if Hrp1 directly interacts with Mex67. The physical interaction was examined via a co-immunoprecipitation experiment. As shown in Figure 11B, GFP-tagged Hrp1 was pulled down with GFP-trap beads and co-immunoprecipitated Mex67 was detected in the eluates. RNase treatment weakened, but did not abolish the physical interaction (Figure 11B), indicating that mRNA is important for stable binding of the two proteins. The genetic interaction of *HRP1* and *MEX67* was tested with a double mutant strain of *hrp1-1 mex67-5*, created via crossing of the *hrp1Δ+phrp1-1* and *mex67Δ+pmex67-5* single mutants. As illustrated in the growth analysis (Figure 11A), the combination of defects in *hrp1-1* and *mex67-5* exhibited synthetic lethality at 35 °C, indicating that the function of Hrp1 *and* Mex67 are coupled for mRNA export. The genetic and physical interactions of Hrp1 and Mex67 suggest that Hrp1 might function as an adaptor protein for the recruitment of the export machinery.

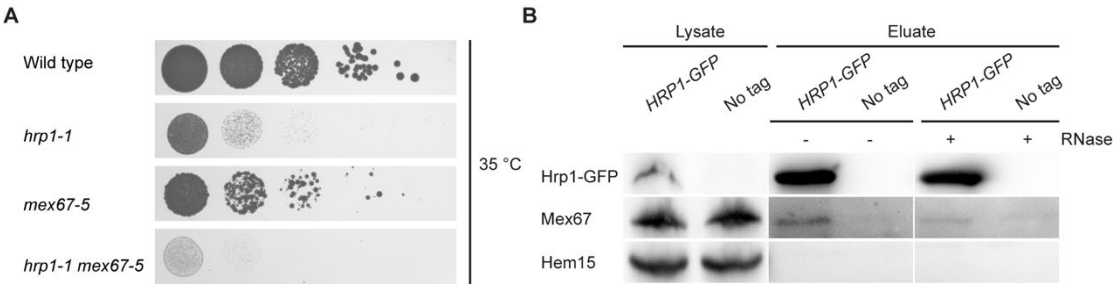


Figure 11: Hrp1 genetically and physically interacts with Mex67.

(A) Drop dilution analysis displays synthetic lethality of *hrp1-1 mex67-5* at 35 °C. 10-fold serial dilutions of the indicated strains were spotted on the -ura -leu agar plate and incubated at 35 °C for 2-3 days. This figure was obtained together with Luisa Querl. (B) The western blot of a co-immunoprecipitation experiment shows the physical interaction of Hrp1 with Mex67. The wild type strain containing endogenous *HRP1* without a *GFP* tag was used as a negative control for pull down. The mitochondrial protein Hem15 was detected as a negative control indicating sufficient washing steps following the precipitation. The samples treated with RNase were processed in parallel to the ones without this treatment. Once RNase A was added, all samples were incubated for another 30 min at 4 °C before washing.

4.4. Hrp1 might mediate mRNA surveillance via Mlp1 at the nuclear pore complex

mRNA export through the nuclear pore complex is facilitated by the interaction of the Mex67 covered adaptor proteins with the nuclear pore complex (Soheilypour and Mofrad, 2018; Soheilypour and Mofrad, 2016). Mlp1 and Mlp2, two large gate keeper proteins on the nucleoplasmic side of the nuclear pore complex, have been shown to dock and monitor the mRNP candidates for cytoplasmic export (Soheilypour and Mofrad, 2018; Fasken, Stewart and Corbett, 2008). All of the identified guard proteins, including Npl3, Gbp2, Hrb1, and Nab2 for mRNA quality control in the nucleus, have been reported to physically interact with Mlp1 (Hackmann *et al.*, 2014; Soheilypour and Mofrad, 2016). Therefore, a *TAP*-tagged *MLP1* strain transformed with a plasmid containing *HRP1-GFP* was used in co-immunoprecipitation experiments to investigate whether Hrp1 might also interact with Mlp1. As demonstrated in Figure 12B, Hrp1 co-precipitated Mlp1 in an RNase A sensitive manner, implying that Hrp1 may act in the same way as the other guard proteins to mediate mRNA inspection at the nuclear pore complex for export. The growth test in Figure 12A also shows the genetic interaction between Hrp1 and Mlp1, which further supports their coupled functions in mRNA export and surveillance at the NPC.

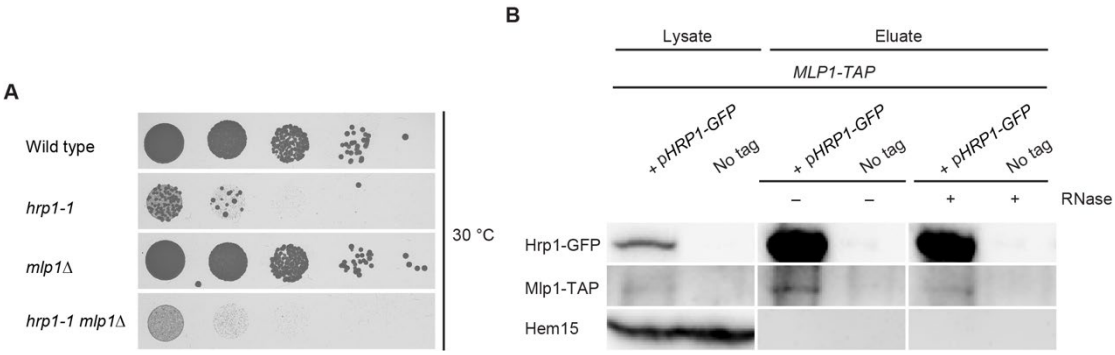


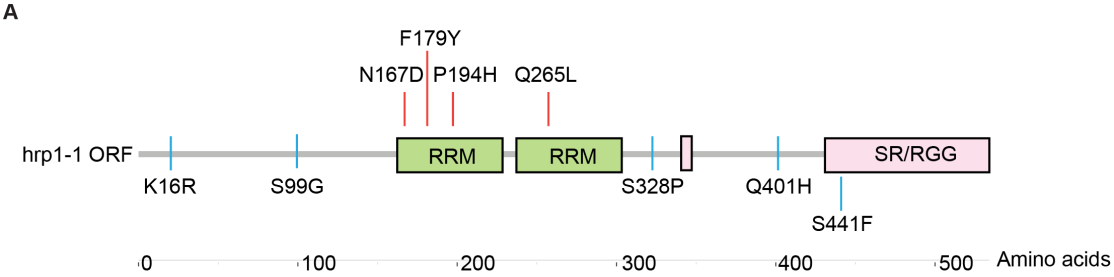
Figure 12: Hrp1 genetically and physically interacts with Mlp1.

(A) Drop dilution analysis exhibits a more severe growth defect in *hrp1-1 mlp1Δ* at 30 °C. 10-fold serial dilutions of the indicated strains were dropped on the -ura agar plate and incubated at 30 °C for 2-3 days. This figure was obtained together with Luisa Querl. (B) The western blot shows co-precipitation of Mlp1 by Hrp1. For a no tag control, the *MLP1-TAP* strain was

transformed with an empty plasmid. Hem15 was used as a negative control for unspecific binding. The cell lysate for each strain was split equally with the same volume of GFP-trap beads for two sets of immunoprecipitation reactions. All samples were treated in the same manner for precipitation and washing, except the RNase treatment, which was included only for one set of precipitation for 30 min.

4.5. *hrp1-1* exhibits no mRNA export defect, and the mutant protein is stable at the restrictive temperature

Given the interaction of Hrp1 with the export receptor Mex67 and the NPC surveillance factor Mlp1 (Figure 11 and 12), and its function in the nuclear retention of mRNAs (Figure 10), we propose a novel role for Hrp1 in the nuclear quality control of mRNAs. For this purpose, a knockout strain of *HRP1* would be perfect for further research. However, since *HRP1* is an essential gene and its deletion leads to cell death of yeast, a temperature sensitive mutant strain of *hrp1-1* from Euroscarf was used in the following experiments. To identify the mutations in this strain, a PCR product of the open reading frame (ORF) of *hrp1-1* was sequenced and mutations were analyzed for amino acid substitutions. As shown in Figure 13A, hrp1-1 contains 9 amino acid alterations that are widely spread throughout the ORF, and four of them are within the two RRMs. To characterize this mutant, a FISH experiment was performed to test if there is an export defect in *hrp1-1*. Unlike *mex67-5,* which displayed a severe mRNA export defect, *hrp1-1* did not show any export deficiency, indicating that Hrp1 is not essential for mRNA transport (Figure 13B). To investigate the mutant protein further, we analyzed its expression status upon a temperature shift to 37 °C from 0.5 h to 3 h. As illustrated in Figure 13C, hrp1-1 was quite stable at the nonpermissive temperatures.



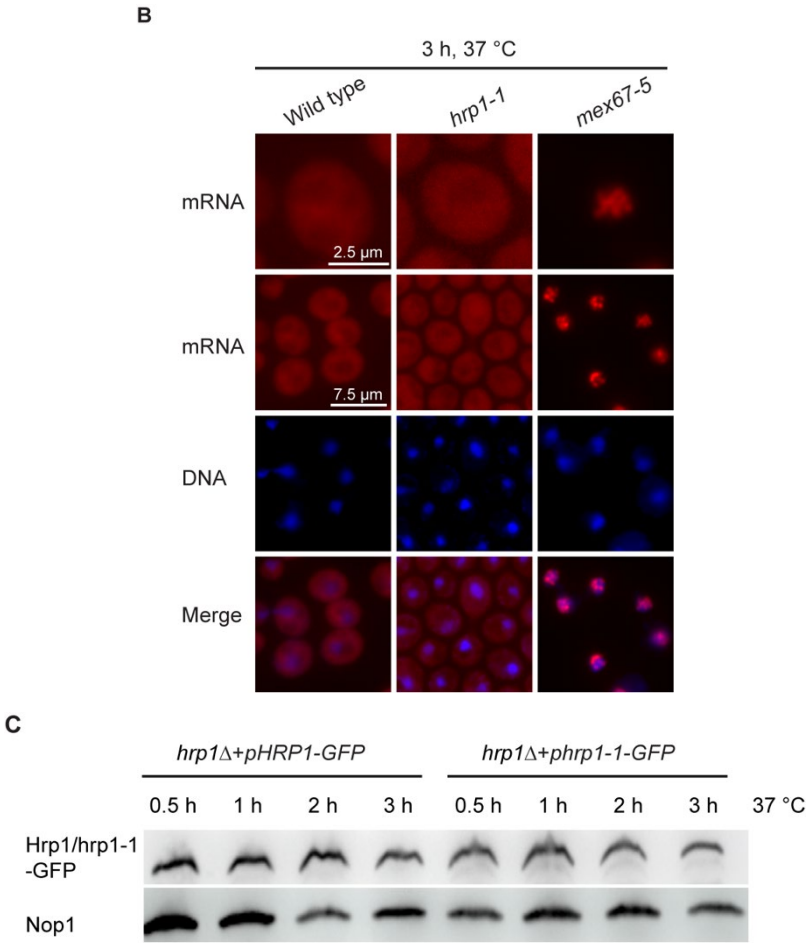


Figure 13: hrp1-1 has 9 point mutations, and no mRNA export defect was observed in *hrp1-1.*

(A) The schematic diagram of amino acid substitutions in hrp1-1. The red bars indicate the mutations in the RRMs and the blue ones show other alterations within the ORF. (B) The FISH experiment with a Cy3-labeled oligo $d(T)_{50}$ probe that targets mRNAs containing a poly(A) tail reveals that *hrp1-1* has no mRNA export defect. Cells for each strain were shifted to 37 °C for 3 h. *mex67-5* was used as a positive control to show mRNA export defects. DAPI was used to stain the nucleus. (C) Western blot reveals that hrp1-1 is stably expressed after being shifted to 37 °C for up to 3 h. Equal volumes of the logarithmic yeast cultures were harvested after being shifted to 37 °C for 0.5 h, 1 h, 2 h, and 3 h, respectively. All samples were treated in the same way to obtain cell lysate that was loaded onto the SDS-gel for western blot analysis. The nucleolar protein Nop1 was detected as a loading control.

4.6. The specific binding of hrp1-1 to the efficiency element of mRNAs is abolished

To identify the defect in hrp1-1, we analyzed its binding capability to mRNAs that contain a specific efficiency element after shifting cells to 37 °C for 3 h. GFP-tagged Hrp1 and hrp1-1 were pulled down (Figure 14A) with GFP-trap beads, and the associated RNAs of both proteins were purified and then analyzed via qPCR following cDNA synthesis. *ACT1*, *ADH1*, *MRP2*, and *MEX67* were chosen as specific mRNA targets of Hrp1. *ACT1*, *MRP2*, and *MEX67* have one or more copies of the canonical binding motif (UAUAUA) of Hrp1. In comparison, *ADH1* does not have a typical $(UA)_3$ but might have a different binding site for Hrp1. As *ADH1* can compete with the $(UA)_3$ containing *ADH2* for Hrp1 binding and crosslink with Hrp1, it is also a specific target of Hrp1, although with a lower affinity (Chen and Hyman, 1998). As shown in Figure 14C, in comparison with the wild type Hrp1, there was a significantly decreased binding of hrp1-1 to mRNA targets containing the canonical efficiency element (*ACT1*, *MRP2*, and *MEX67)*. For *ADH1*, the decreased binding of hrp1-1 to it was not as significant as for other targets (Figure 14C), which is consistent with its lower binding efficiency for Hrp1. When measuring the concentration of purified RNAs with a nanodrop spectrophotometer, we discovered that there were much more total RNAs associated with hrp1-1 (Figure 14B), suggesting that hrp1-1 might have lost its binding specificity and is depleted by unspecific RNAs. Since rRNA is the most abundant RNA species in cells, we randomly analyzed two regions of the 35s rRNA, the internal spacer region1 (*ITS1*) and the external spacer region1 (*ETS1*) via qRCRs. Indeed, hrp1-1 was bound to them with a marked increase (Figure 14C). Thus, the results of the RNA co-immunoprecipitation experiment suggested that the interaction of hrp1-1 with its specific mRNA targets was disrupted at the non-permissive temperature, probably due to its depletion through unspecific RNAs such as rRNAs. These findings indicate that *hrp1-1* could be used for further study. The protein is depleted from specific mRNAs, which is a prerequisite for studying its quality control function for its usual target RNA with the efficiency elements.

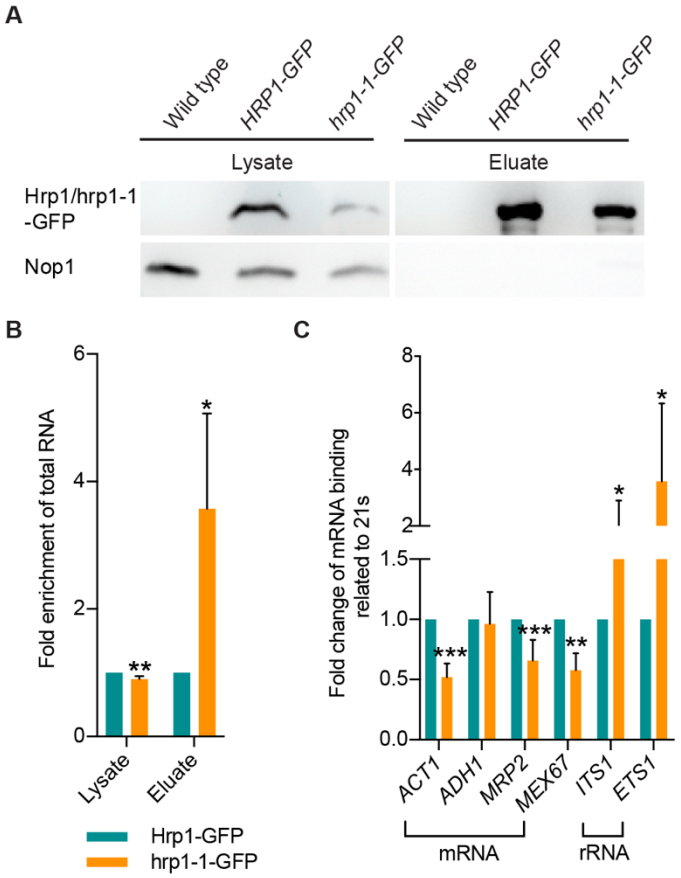


Figure 14: RNA co-immunoprecipitation reveals defects in the binding of hrp1-1 to the efficiency element containing mRNA targets.

(A) The western blot shows that Hrp1-GFP and hrp1-1-GFP were pulled down. Nop1 was detected as a negative control for unspecific binding. (B) hrp1-1-GFP precipitated approximately 3.5 folds more total RNAs than Hrp1-GFP. The concentration of total RNAs isolated in the lysate and the eluate for each strain was measured with a nanodrop spectrophotometer at OD=260 nm after RNA purification. The value of RNA concentration in the eluate for each sample was related to the amount of protein that was pulled down. The signal intensity of each pull down shown on the western blot was measured via image J. The amount of the RNA in the eluates for tagged proteins was related to that of the no tag control. The final fold enrichment of RNAs precipitated by hrp1-1-GFP was obtained via relating it to that of Hrp1-GFP. (C) About half of the amount of hrp1-1 was depleted from most mRNA targets by interacting with unspecific rRNAs after a temperature shift to 37 °C for 3 h. The binding of different targets was identified through relation to the no tag control and to Hrp1-GFP, respectively.

4.7. Hrp1 plays a role as an mRNA quality control factor in the nucleus

Since hrp1-1 shows a reduced contact to its target mRNAs (Figure 14C), we investigated if there is leakage of faulty mRNAs into the cytosol via a FISH experiment. The Cy3-labeled oligo $d(T)_{50}$ probe was applied to target mRNAs with a poly(A) tail. The double mutant of *hrp1-1 rrp6Δ* was generated via crossing of the two single mutants *hrp1-1* and *rrp6Δ*. Rrp6 is a component of the nuclear exosome, which is involved in eliminating faulty mRNAs generated in the nucleus with its 3'-5' exonuclease activity. As shown in Figure 15B with the knockout strain of *RRP6*, the aberrant mRNAs accumulated in the nucleus. Strikingly, when combined with *hrp1-1*, the strong signal of nuclear accumulation in *rrp6Δ* disappeared and the double mutant of *hrp1-1 rrp6Δ* showed significant leakage of the faulty mRNAs into the cytoplasm (Figure 15B). Moreover, the leakage of faulty mRNAs in the double mutant was reversed when the strain was transformed with a plasmid that contains a copy of the wild type *HRP1* (Figure 15B), indicating that Hrp1 is a nuclear quality control factor for mRNA export. This was further supported by the genetic interaction of *HRP1* with *RRP6* shown in Figure 15A, which demonstrated that the double mutant of *hrp1-1 rrp6Δ* grew much slower than the single mutants, probably due to the severe toxicity of the many faulty mRNAs that leak into the cytoplasm. Thus, wild typic *HRP1* expression suppressed the *hrp1-1* mutant (Figure 15A).

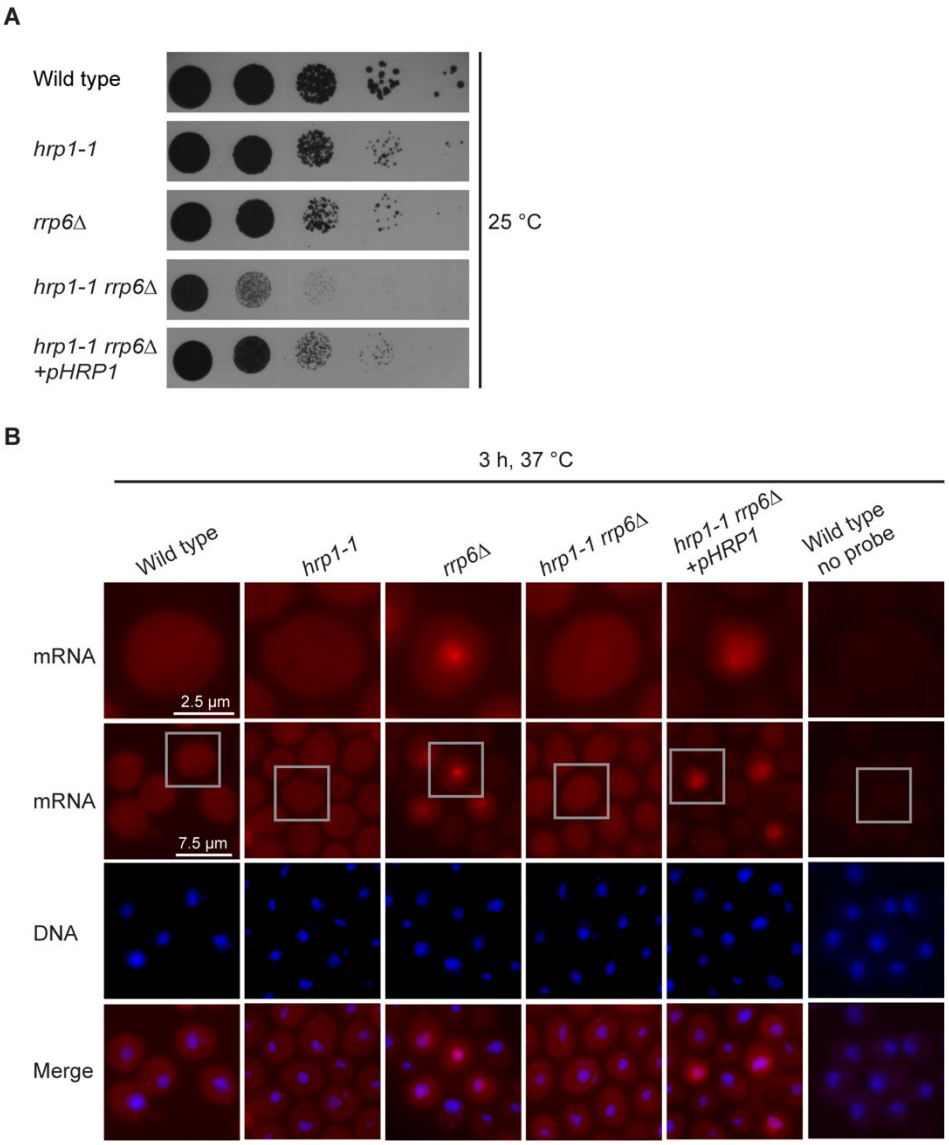


Figure 15: The faulty mRNAs that accumulate in *rrp6Δ* leak into the cytoplasm in *hrp1-1 rrp6Δ*.

(A) *HRP1* genetically interacts with *RRP6*. The indicated strains were spotted onto a -ura agar plate in 10-fold serial dilutions. The plate was incubated at 25 °C for 2-3 days. (B) A FISH assay reveals that *hrp1-1* leads to the leakage of faulty mRNAs into the cytosol. A Cy3-labeled oligo $d(T)_{50}$ probe was used for mRNA detection. Cells of the indicated strains were grown to a log phase before they were shifted to 37 °C for 3 h. The wild type cells with no probe served as a negative control for unspecific fluorescence background. DAPI was used to mark the nucleus.

4.8. Hrp1 surveils the 3'-end processing of mRNAs in the nucleus

Considering the fact that Hrp1 might act as an mRNA quality control factor in the nucleus (Figure 15), and its role as the single component of CF IB in the cleavage and polyadenylation complex (Zhao, Hyman and Moore, 1999; Kessler *et al.*, 1997; Kim Guisbert, Li and Guthrie, 2007), we assumed that it might operate as a surveillance factor for the 3'-end processing of mRNAs. To test this speculation, we performed another FISH experiment with a mutant strain of *cft2-1*. Cft2 is a subunit of the cleavage and polyadenylation complex in yeast, which is essential for pre-mRNA cleavage and polyadenylation (Kyburz *et al.*, 2003). It has been shown that the 3'-end processing of mRNAs in the mutant of *cft2-1* is defective, resulting in the readthrough of the CPF-CF site and leading to usage of the distant cleavage site downstream of the canonical CPF-CF site (Kyburz *et al.*, 2003). As we expected, those faulty mRNAs in *cft2-1* were retained in the nucleus during the temperature shift at 37 °C for 3 h (Figure 16B). Since we assumed that Hrp1 was the surveillance factor for the 3'-end processing of mRNAs, a double mutant of *hrp1-1 cft2-1* was created via crossing and used to detect leakage of faulty mRNAs via FISH. As shown in Figure 16B, the 3'-extended mRNAs that were retained in *cft2-1* were released into the cytoplasm in *hrp1-1 cft2-1*, indicating that Hrp1 is a quality control factor for proper cleavage and polyadenylation of mRNAs. It is known that mRNAs in *cft2-1* are undergoing a rapid degradation at the restrictive temperature (Kyburz *et al.*, 2003), and therefore the signals were relatively weak (Figure 16B). To confirm the leakage of aberrant mRNAs for cleavage and polyadenylation in the absence of Hrp1 quality control, *rrp6Δ* was then combined with *cft2-1* or *cft2-1 hrp1-1* to create a double mutant of *cft2-1 rrp6Δ* and a triple mutant of *hrp1-1 cft2-1 rrp6Δ*, respectively. As displayed in Figure 16B, there was significantly increased intensity of mRNA nuclear retention in *cft2-1 rrp6Δ* and the leakage of abnormal mRNAs was also observed within the triple mutant of *hrp1-1 cft2-1 rrp6Δ*. These findings demonstrate that Hrp1 monitors the 3'-end processing of mRNAs in the nucleus. The decreased proliferation of the double mutant of *hrp1-1 cft2-1* revealed a

genetic interaction of *HRP1* with *CFT2* (Figure 16A), which additionally substantiated that the deficiency in Hrp1 surveillance for the 3'-end processing was harmful to yeast cells.

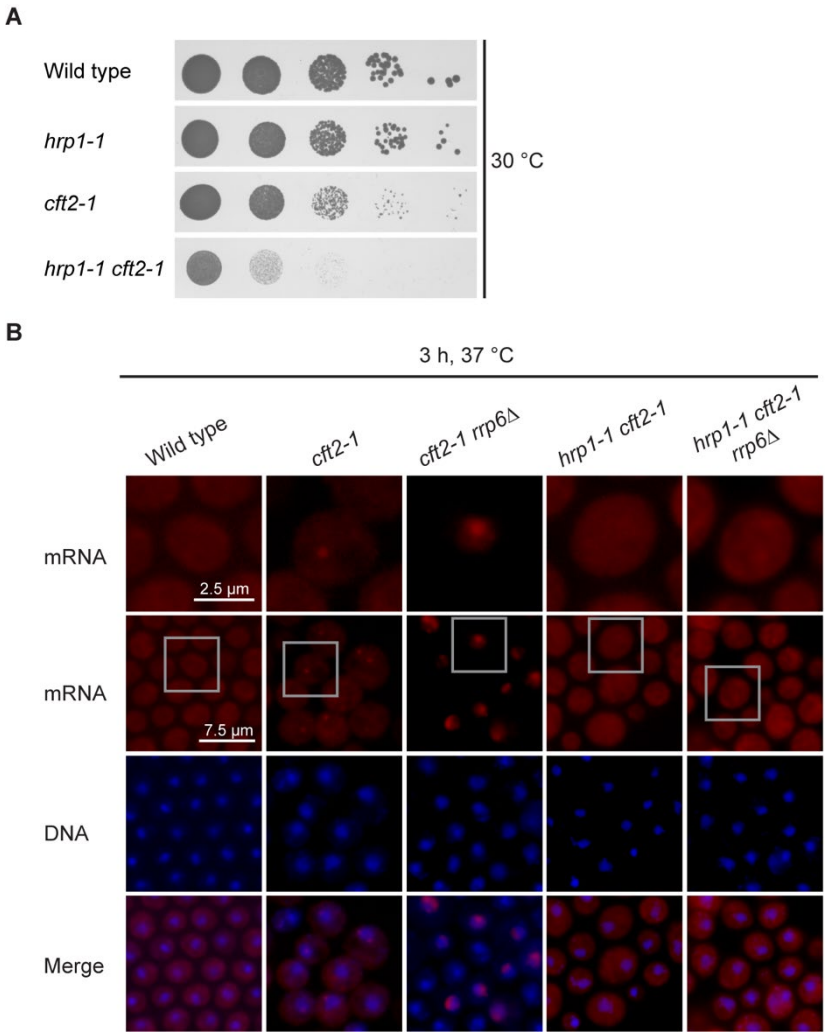


Figure 16: Lacking Hrp1 3'-end processing quality control prevents nuclear retention of faulty mRNAs which are toxic to cells.

(A) Growth analysis shows the genetic interaction of *HRP1* with *CFT2*. 10-fold serial dilutions of the relevant strains were dropped onto a -ura agar plate and incubated at 25 °C for 2-3 days. (B) Readthrough mRNAs generated in *cft2-1* leak into the cytoplasm without Hrp1 surveillance. FISH experiment is shown with a Cy3-labeled oligo $d(T)_{50}$ probe against mRNAs with a poly(A) tail. All strains were shifted to 37 °C for 3 h to enhance the corresponding defects in the mutants. The nucleus was stained with DAPI.

4.9. The cytosolic fraction of *hrp1-1 cft2-1* contains significantly more of the readthrough mRNAs

To confirm the leakage of the 3'-extended mRNAs in *cft2-1 hrp1-1*, a cell fractionation experiment was performed and the amount of the readthrough mRNAs in the cytoplasm was analyzed via qPCR. The faulty mRNAs were detected by a pair of primers flanking the cleavage site (Figure 17A). All the indicated strains were grown to the logarithmic phase before they were digested with zymolyase to obtain spheroplasts. Afterwards, the spheroplasts were shifted to 37 °C for 3 h and thereafter subjected to cell fractionation. As shown in Figure 17B, the cytosolic fraction for each strain was successfully separated from the corresponding cell lysate since there was no visible signal of the nucleolar protein Nop1 in the cytoplasmic fraction. The readthrough mRNAs in the cytosol and the lysate were detected separately via qPCR and the ratio was calculated to show leakage of the faulty mRNAs. qPCR analysis demonstrated that the 3'-extended mRNAs were significantly released into the cytoplasm in the double mutant of *cft2-1 hrp1-1* (Figure 17C), which further supported the leakage of faulty mRNAs in the 3'-end processing via FISH (Figure 16B).

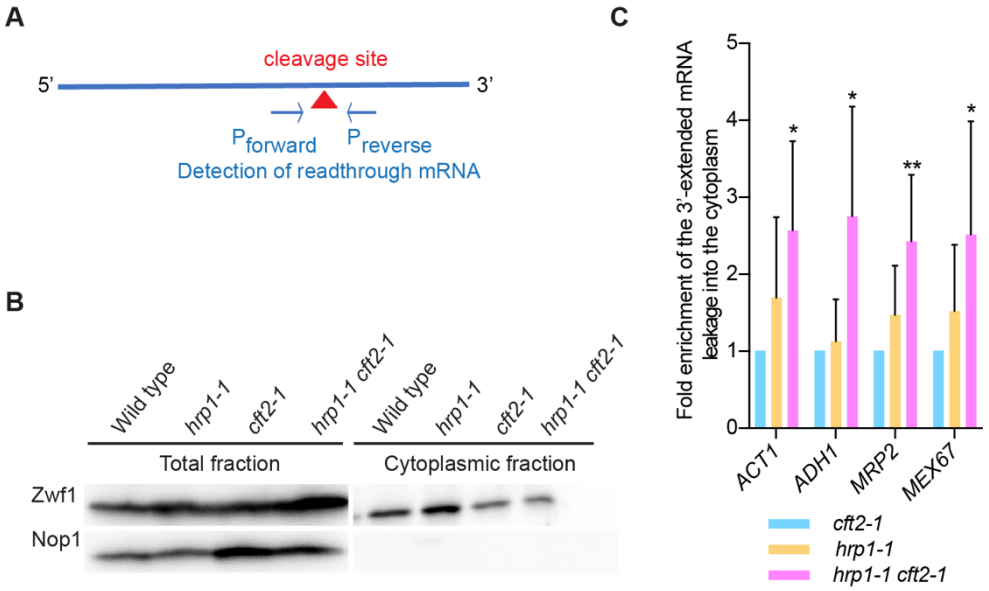


Figure 17: Cell fractionation reveals that the 3'-extended mRNAs containing efficiency elements leak into the cytosol in the absence of functional Hrp1.

(A) Primers flanking the cleavage site were designed for each specific mRNA target of Hrp1 to detect the 3'-extended faulty mRNAs via qPCR. (B) The western blot shows that the cytosolic fraction was successfully separated via cell fractionation. The glycolytic enzyme Zwf1 was used as a positive control for cytoplasmic proteins. The nucleolar protein Nop1 was detected as a nuclear marker. (C) qPCR analysis exhibits leakage of the 3'-extended faulty mRNAs into the cytoplasm. Leakage in the mutant strains was all related to that in the wild type and, subsequently, to *cft2-1*.

4.10. Hrp1 retains the 3'-extended mRNAs in the nucleus without recruiting the export receptor

As revealed in the above results, Hrp1 could act as an adaptor protein to recruit Mex67-Mtr2 for mRNA export when the mRNAs were properly processed at the 3'-end (Figure 11B). As Hrp1 retains faulty transcripts in the nucleus (Figure 15B, 16B, and 17C), one possible mechanism that would lead to retention would be that it would not recruit Mex67 anymore. But how Hrp1 determines the retention was not known. One simple postulation was that Hrp1 still binds to those faulty mRNAs, but is unable to recruit the export receptor on 3'-extended mRNAs. To analyze this, we first conducted a co-immunoprecipitation assay to detect the physical interaction between Hrp1 and Mex67-Mtr2 in *cft2-1*. As shown in Figure 18A, a similar amount of Mtr2-GFP was pulled down in either the wild type or the *cft2-1* strain with GFP-trap beads. Consistent with the formation of the heterodimer Mex67-Mtr2 for mRNA export (Senay *et al.*, 2003; Aibara *et al.*, 2015), Mex67 was precipitated in approximately the same amount in both strains (Figure 18A). But remarkably, Hrp1 precipitation was significantly reduced in *cft2-1* (Figure 18A), indicating that the export heterodimer of Mex67-Mtr2 might not be appropriately recruited onto faulty mRNAs by Hrp1. In support of this observation, we also performed an RNA co-immunoprecipitation experiment to confirm the binding of Hrp1 with its mRNA targets. Surprisingly, there was even more Hrp1 that was loaded on the abnormal mRNAs with deficiencies in 3'-end processing in *cft2-1* (Figure 18C), implying that a stronger retention was mediated by Hrp1 surveillance. Since it has been

shown that mRNAs are rapidly degraded in *cft2-1* at a non-permissive temperature (37 °C) and there is still readthrough during transcription termination at 30 °C (Kyburz *et al.*, 2003), all strains that were indicated for the protein or RNA co-immunoprecipitation experiments were grown at 30 °C until the logarithmic phase for harvesting. In order to show that mRNAs in *cft2-1* were relatively stable at 30 °C, we extracted the total RNAs from an equivalent volume of cell pellets of the wild type and *cft2-1* and purified mRNAs from the same amount of total RNA samples. As revealed in Figure 18B, a similar amount of total RNAs was isolated from *cft2-1* compared to the wild type, and of those, almost equal amounts of mRNAs were contained in both strains. These findings suggest that Hrp1 retains the aberrant mRNAs that fail the 3'-end processing in the nucleus, but does not recruit the export machinery Mex67-Mtr2.

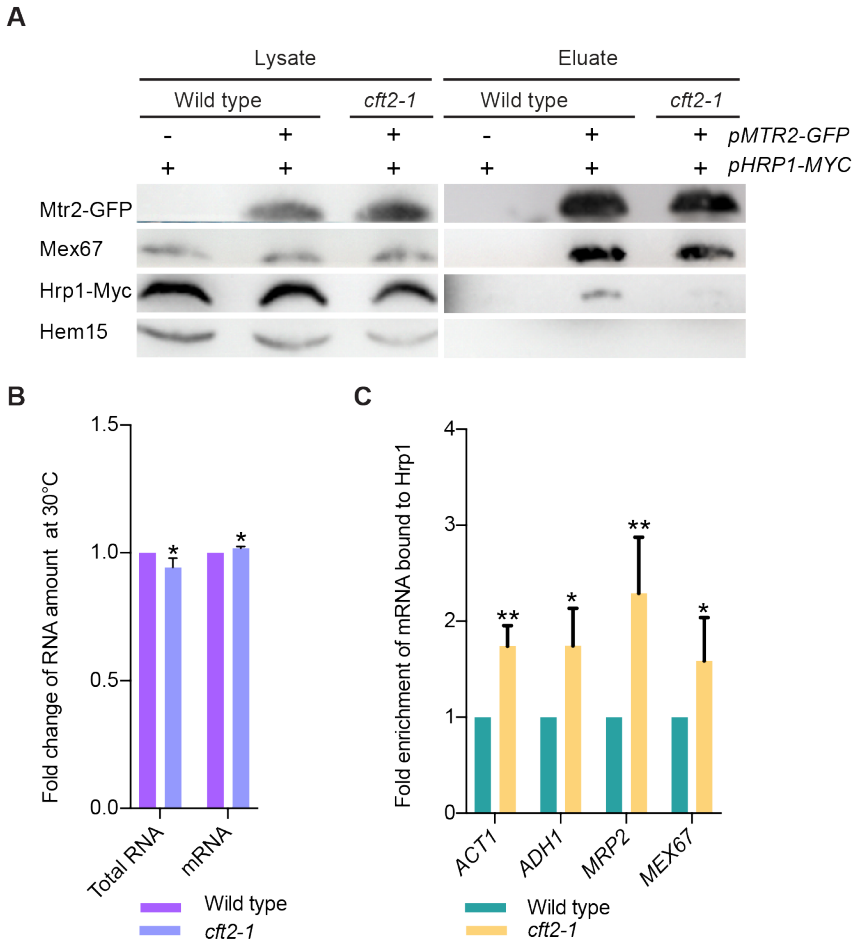


Figure 18: Hrp1 does not recruit the export machinery to the readthrough mRNAs in *cft2-1* but retains them in the nucleus instead.

(A) The physical interaction of Hrp1 with Mex67-Mtr2 is reduced in *cft2-1*. A western blot is shown of the indicated strains that were cultivated at 30 °C to the log phase. Subsequently, GFP-tagged Mtr2 was pulled down with GFP-trap beads. Hem15 was detected as a control for unspecific binding. (B) mRNAs were not degraded in *cft2-1* at 30 °C. An equal volume of cell cultures of the wild type and *cft2-1* was harvested. Total RNAs were isolated from an equal volume of cell pellet of each strain with the NucleoSpin RNA Kit (MACHEREY-NAGEL). The same amounts of total RNAs extracted from the wild type and *cft2-1* were taken for mRNA purification with the Dynabeads™ mRNA Purification Kit (Invitrogen). The concentration of RNAs in each sample was measured for light absorbance at 260 nm via a nanodrop spectrophotometer. The amount of total RNAs and mRNAs of *cft2-1* was related to that of the wild type. (C) The RNA co-immunoprecipitation experiment shows an increased binding of Hrp1 to faulty mRNAs in *cft2-1* at 30 °C. The wild type and *cft2-1* strains were transformed with a plasmid containing *HRP1-GFP*. A no-tag control with an empty plasmid in the wildtype was added. GFP-tagged Hrp1 was pulled down from the cell lysate of each indicated strain above and the associated RNAs were purified for cDNA synthesis and qPCR analysis. *ACT1*, *ADH1*, *MRP2*, and *MEX67* were detected as the specific mRNA targets of Hrp1. The amount of each specific target bound to Hrp1 was related to that of the no tag control and subsequently to that of Hrp1 in the wild type. The RNA co-immunoprecipitation experiments for Figure 18C were performed by Luisa Querl.

4.11. Rna14 might be the trigger for the recruitment of the export receptor to mRNAs by Hrp1

Hrp1 is unable to recruit Mex67-Mtr2 to faulty mRNAs with an extended 3'-end in the mutant of *cft2-1* and thus retains those readthrough mRNAs in the nucleus (Figure 18). However, it remains unclear how Hrp1 decides whether or not to recruit the export machinery to mRNA targets. Since Rna14 and Rna15 are the only two components of the CPF-CF complex that have been shown to physically interact with Hrp1, it is reasonable to propose that these interactions might be altered on an aberrant mRNA with deficiencies in cleavage and polyadenylation, such as in *cft2-1*. Therefore, a co-immunoprecipitation experiment was performed to test the physical interaction of Hrp1 with Rna14 in *cft2-1*. Intriguingly, as shown in Figure 19, a similar amount of GFP-

tagged Rna14 was pulled down for the wild type and *cft2-1*, but significantly declined for the co-precipitated Hrp1 and Mex67 in *cft2-1*. These findings suggest that the binding of Rna14 to Hrp1 is altered and might be necessary for the recruitment of Mex67.

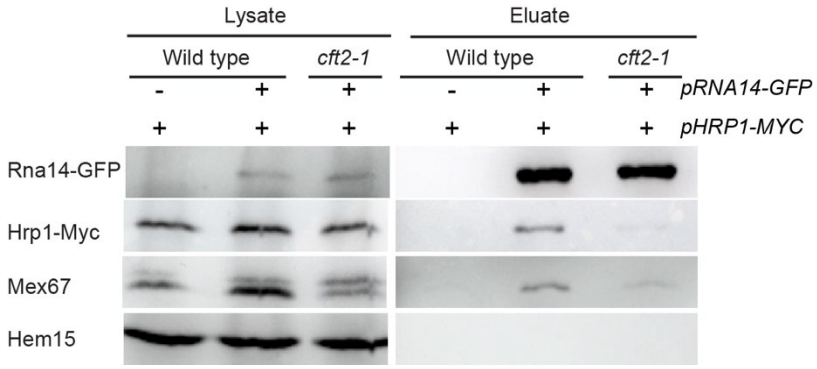


Figure 19: Co-immunoprecipitation illustrates the reduced binding of Rna14 to Hrp1 on the faulty mRNAs in *cft2-1*.

Cells of the corresponding strains in this experiment were grown at 30 °C and collected during the logarithmic phase. The wild type strain, which was only transformed with a plasmid of p*HRP1-MYC*, was added as a no tag control for pull down. Hem15 was used as a negative control for unspecific binding.

4.12. Rna14 might not be loaded on the faulty mRNAs that are retained by Hrp1

In support of the previous finding showing that Rna14 might be the trigger for Hrp1 to recruit Mex67 (Figure 19), we also conducted an RNA co-immunoprecipitation assay to confirm the absence of Rna14 on the 3'-extended mRNAs in *cft2-1*. The wild type and *cft2-1* strains were transformed with a plasmid that includes *RNA14-GFP* under its own promoter. For a no tag control, the wild type cells were transformed with an empty plasmid. As shown in Figure 20, Rna14-GFP co-precipitated much less mRNA targets in *cft2-1* compared to that in the wild type strain, implying that Rna14 is less loaded on the readthrough mRNAs in *cft2-1*.

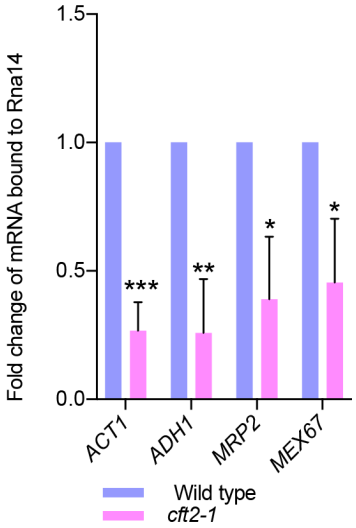


Figure 20: Rna14 co-immunoprecipitated less faulty mRNAs in *cft2-1*.
The GFP-tagged Rna14 was pulled down from the cell lysate of the indicated strains that were grown at 30 °C. The RNAs associated with Rna14-GFP were isolated for cDNA synthesis and qPCR analysis. *ACT1*, *ADH1*, *MRP2*, and *MEX67* were detected as specific targets of Hrp1 and Rna14. The amount of each specific target that was co-precipitated with Rna14-GFP was related to the no tag control first and afterwards to that of Rna14-GFP in the wild type.

4.13. Rna14 might not be incorporated into the cleavage and polyadenylation complex on 3'-extended mRNAs

To further support the results revealing that Hrp1 is not recruiting the export receptor of Mex67-Mtr2 due to a lack of Rna14 contact (Figure 19 and 20), two co-immunoprecipitation experiments were performed to test the physical interaction of Rna14 with the CPF subcomplex. Cft1 and Pfs2 are two components of the CF II and PF I subcomplex, respectively (Stumpf and Domdey, 1996; Dichtl *et al.*, 2002). Cft1 and Pfs2 have been found to be essential for cleavage and polyadenylation and they also physically interact with Rna14 (Dichtl *et al.*, 2002; Ohnacker *et al.*, 2000; Ghazy *et al.*, 2012; Casanal *et al.*, 2017). Therefore, Cft1 and Pfs2 are perfect candidates for determining the incorporation of Rna14 in the cleavage and polyadenylation complex. Interestingly, as shown in Figure 21A and B, GFP tagged Cft1 or Pfs2 were pulled down with the same amount of GFP-trap beads, but a significant decrease of co-

precipitated Rna14-Myc was observed in *cft2-1* for both experiments, implying that Rna14 is not part of the complex for the 3'-end processing in *cft2-1*. Together with the former results (Figure 19 and 20), our data suggest that Rna14 might be the trigger for Hrp1 to recruit Mex67-Mtr2 to mRNAs for export.

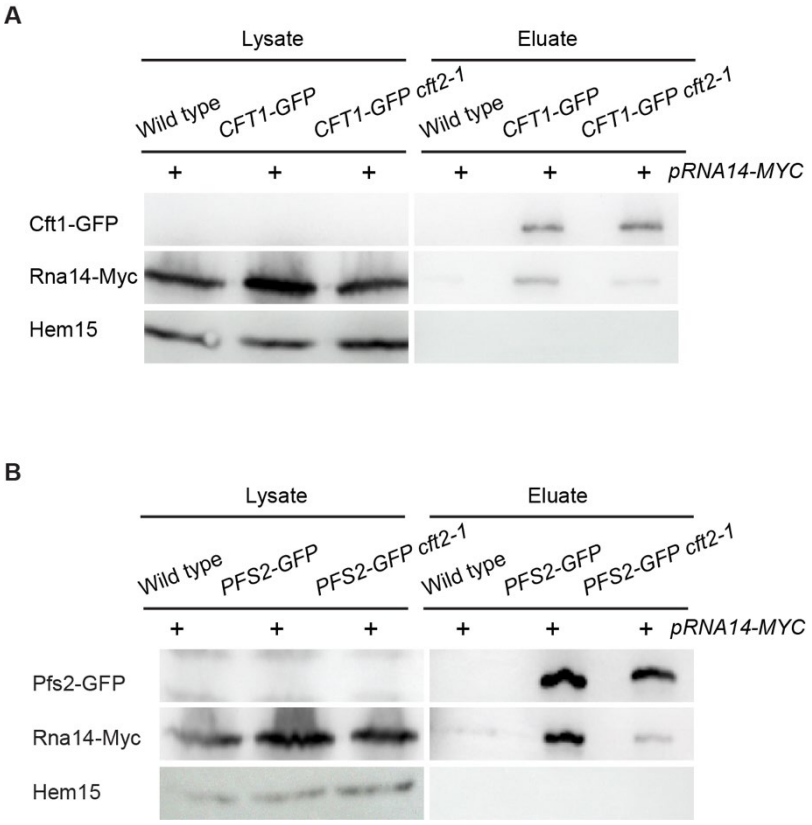


Figure 21: Rna14 loses physical contact with the CPF-CF complex on aberrant mRNAs in *cft2-1*.

(A/B) The western blots of the co-immunoprecipitation assays reveal that Cft1 and Pfs2 co-precipitate less Rna14 in *cft2-1*. All the relevant strains were inoculated in the same volume of cultures and grown at 30 °C. The cells for each strain were harvested at the logarithmic phase. Hem15 was detected as the negative control for unspecific binding. Figure 21B was obtained together with Luisa Querl.

5. Discussion

5.1. Hrp1 shares several features with typical mRNA nuclear guard proteins and surveils pre-mRNA 3'-end processing

Based on the fact that Hrp1 is a shuttling SR/RGG protein, we further explored its functions in the nucleus and the idea that it might be involved in the nuclear mRNA quality control. First, *HRP1* overexpression is toxic and retains mRNAs in the nucleus, indicating that Hrp1 functions as a retention factor for mRNA export (Figure 10). Second, Hrp1 acts as an mRNA export adaptor protein based on its physical interaction with the export receptor Mex67 (Figure 11). Third, Hrp1 contacts the NPC surveillance component Mlp1, mediating the crosstalk between mRNPs and the NPC (Figure 12). Fourth, hrp1-1 that is defective for the targeted mRNA binding leads to significant leakage of faulty mRNAs into the cytoplasm (Figure 15B). Interestingly, the nuclear retained faulty mRNAs in the exosome mutant *rrp6*Δ seem to leak completely into the cytoplasm in the double mutant of *hrp1-1 rrp6*Δ, which implies that Hrp1 might be a dominant quality control factor that retains faulty transcripts for nuclear degradation by the exosome.

More specifically, given the known function of Hrp1 as a cleavage factor, we further showed that aberrant 3'-extended mRNAs in a mutant of the cleavage factor Cft2 were released into the cytosol when combined with *hrp1-1* as shown via FISH and cell fractionation experiments (Figure 16B and 17C). Considering the nuclear accumulation of poly(A) mRNAs in other mutants of the CPF-CF complex, such as *rna15-58*, *pcf11-1*, *rna14-49*, and *rna14-1 rrp6*Δ (Carneiro *et al.*, 2008; Hammell *et al.*, 2002), it is very likely that the retention is due to Hrp1 3' quality control and a lack of Hrp1 surveillance will also result in leakage of readthrough mRNAs in these mutants. Retention of a single mRNA reporter using a probe targeting the *GFP* tag has been shown earlier (Brodsky and Silver, 2000; Zander *et al.*, 2016). Therefore, it would be more supportive

if we could also show single mRNA leakage in the absence of Hrp1 quality control. Although it was difficult to detect the fluorescence signal of a strongly expressed *GFP* mRNA reporter with multiple Cy3-labeled probes in our system, remarkable leakage of 3'-extended single mRNA targets was clearly shown in the double mutant of *hrp1-1 cft2-1* in a cell fractionation assay (Figure 17C). Unexpectedly, only slight leakage of readthrough mRNAs was observed in the single mutant of *hrp1-1* compared to *cft2-1* (Figure 17C). Since the pre-mRNA of *HRP1* also has a canonical EE and is a specific target of the CPF-CF termination complex, it is likely that *HRP1* expression is reduced in *cft2-1* during the temperature shift to 37 °C and therefore Hrp1 mediated nuclear surveillance is weaker than in the wild type. Consistently, the raw data showed certain leakage of 3'-extended mRNAs in *cft2-1* compared to the wild type (Figure 23). In fact, it might also be the case that the nuclear retention of faulty mRNAs in *cft2-1* overwhelms the cell, which inevitably causes some leakage and through this elevates the baseline of faulty mRNA leakage. Another explanation might be that Hrp1 functions as a 3'-end surveillance factor rather than being required for the cleavage reaction. In this way, fewer 3'-extended mRNAs are generated in *hrp1-1* than in the *cft2-1* mutant. The randomly produced readthrough mRNAs in *hrp1-1* escape from concurrent nuclear degradation via the NNS pathway due to rapid leakage into the cytoplasm. This explanation could be supported by Minvielle's work, as they have shown that CF IA and CF II were sufficient to cleave mRNA precursors (Minvielle-Sebastia *et al.*, 1998). Nevertheless, in contrast to *cft2-1*, the significant enrichment of readthrough mRNAs in the cytoplasm of the double mutant *hrp1-1 cft2-1* is strong evidence of leakage caused by a lack of Hrp1 surveillance (Figure 17C).

5.2. Transcriptional readthrough mRNAs are supposed to be terminated via the NNS pathway

Usage of the proximal or distant poly(A) sites of a pre-mRNA raises the topic of alternative cleavage and polyadenylation (APA), which is helpful to explore the relationship between CPF-CF and NNS attributed transcription termination. Although

in mammalian cells, APA is crucial for generating protein isoforms that feature diversity in cell types, cell status, and tissues, the physiological effects of APA in yeast remain largely unknown (Neve *et al.*, 2017). The species specificity of APA profiles in different kinds of yeast cells indicates that alternative poly(A) sites might be biological noises (Neve *et al.*, 2017; Moqtaderi *et al.*, 2013).

In budding yeast, it has been estimated that around 70% of genes undergo APA, which is mediated via usage of weak poly(A) sites downstream of the main termination site (Ozsolak *et al.*, 2010). The canonical poly(A) site that is regularly used in budding yeast is usually upstream of weak signals, which is consistent with the fact that the EE is mainly enriched for the first cleavage site (Liu *et al.*, 2017). Oppositely, in fission yeast, the UA-rich and A-rich motifs are more enriched towards the last poly(A) site, which enables efficient expression of longer mRNA isoforms (Liu *et al.*, 2017). If RNA Pol II fails to terminate mRNA transcription at the canonical poly(A) site, elongated mRNAs will be generated due to transcription readthrough. Interestingly, a significant increase in the amount of mRNA products terminated at the last cleavage site were frequently observed in the mutants that are defective for mRNA 3'-end maturation (Kim Guisbert, Li and Guthrie, 2007; Kyburz *et al.*, 2003; Mandart and Parker, 1995; Vo *et al.*, 2001). In line with the role of the NNS complex in fail-safe transcription termination, the most distant poly(A) site in budding yeast usually appears to be surrounded by putative NNS binding sites (Liu *et al.*, 2017). Minor utilization of the middle cryptic poly(A) sites in both wild type and mutant cells probably implies that they are rather weak in competition among all the termination sites. Since widespread 3'-extended mRNAs were observed in cells grown without Npl3, a role in transcription termination for Npl3 has been suggested (Holmes *et al.*, 2015). Strikingly, a transcriptome-wide RNA-binding analysis of the NNS component Nab3 in their study revealed a dramatic shift from its processing targets to the surveillance substrates of readthrough mRNAs in *npl3Δ,* which suggests a general role of the NNS complex in fail-safe termination. Consistently, readthrough mRNAs are further transcribed and stabilized if the NNS

complex is malfunctioning or the binding sites for the complex are mutated, which indicates that the NNS pathway appears to be a powerful fail-safe termination mechanism (Rondon *et al.*, 2009; Singh *et al.*, 2021). The NNS termination pathway can be further enhanced by chromatin obstacles established by the nucleosomes and DNA-binding proteins such as intergenic Reb1 (Colin *et al.*, 2014; Roy *et al.*, 2016). Together, they efficiently contribute to the fail-safe termination of mRNA transcription that protects downstream genes from perturbation.

The NNS fail-safe termination complex is assumed to direct readthrough mRNAs for the TRAMP-exosome complex mediated nuclear degradation (Rondon *et al.*, 2009; Singh *et al.*, 2021), which explains why we were not able to detect direct interaction between Hrp1 and the degradation machinery (Figure 24). Since the NNS complex is recruited by the RNA Pol II CTD and activated upon binding to its specific RNA elements, Hrp1 must not necessarily bind the NNS complex to facilitate its guarding function (Figure 25) (Lemay and Bachand, 2015; Rondon *et al.*, 2009). It rather functions to monitor and mark 3'-extended mRNAs and retain those faulty mRNAs in the nucleus.

5.3. Rna14 depletion from readthrough mRNAs might prevent Hrp1 from recruiting the export receptor Mex67-Mtr2

Only fully processed mRNAs that are properly packaged by adaptor proteins and the export receptor Mex67-Mtr2 are competent for transport into the cytoplasm (Hackmann *et al.*, 2014). The spliceosome that is stuck on intron-containing mRNAs might lead to higher affinity of Gbp2 and Hrb1 for the TRAMP-exosome complex, thereby restricting the recruitment of Mex67-Mtr2 for export (Hackmann *et al.*, 2014). In line with this, we discovered that the physical interaction between Hrp1 and Mex67-Mtr2 is abolished in *cft2-1*, implying that a lack of Mex67-Mtr2 recruitment is a common consequence of

surveillance by nuclear guard proteins. Uncovered quality control factors are subsequently detected by the NPC monitoring factor Mlp1 and therefore mediate nuclear retention of aberrant pre-mRNAs.

Interestingly, we further found that Rna14 is absent from readthrough mRNAs and the CPF-CF complex in *cft2-1* (Figure 20 and 21). Although Rna15 was also shown to contact Hrp1 in structural analysis, the bridging protein Rna14 might be the main factor that connects Hrp1 with the rest of the CPF-CF complex (Leeper *et al.*, 2010; Barnwal *et al.*, 2012). Therefore, one plausible mechanism could be that arrival of Rna14 is sensed by Hrp1 and this triggers the recruitment of Mex67-Mtr2 onto mRNAs. The binding domain of Hrp1 to Rna14 has been revealed (Barnwal *et al.*, 2012), however, the interaction domain of Hrp1 that contacts Mex67 remains unclear. It would be interesting to further analyze how Hrp1 manages to regulate the interactions with Rna14 and Mex67 to facilitate its role in monitoring pre-mRNA 3' processing. Since Hrp1 fails to exit the nucleus without being methylated by Hmt1, the methylation status of Hrp1 might be one possible explanation. In addition, Rna15 seems to be depleted from the CPF-CF complex if the interaction with Rna14 is impaired (Moreno-Morcillo *et al.*, 2011; Kim *et al.*, 2004b). Therefore, Rna14 and Rna15 might not be incorporated into the 3'-end processing machinery in *cft2-1*, and conceivably, the whole CPF-CF complex might be inappropriately assembled in this mutant.

5.4. Hrp1 might cooperate with Nab2 to coordinate pre-mRNA 3' processing and surveillance

Hrp1 and Nab2 are two essential mRNA-binding factors for pre-mRNA 3'-end maturation in budding yeast. Methylation at the SR/RGG domain of both proteins via Hmt1 plays an important role in coupling their transport with mRNA export (Green *et al.*, 2002). They are both cargos for the import receptor Kap104 and contain the conserved PY-NLS-like sequences, a domain which is essential for Hrp1 reimport (Lange *et al.*, 2008). Nab2 is proposed as a nuclear surveillance factor that controls

mRNA poly(A) tail length and links 3' maturation with the export of mRNAs (Hector *et al.*, 2002; Soucek, Corbett and Fasken, 2012; Turtola *et al.*, 2021; Iglesias *et al.*, 2010). Our finding of the novel role of Hrp1 in the 3'-end processing surveillance fills the gap of cleavage quality control. Given the prevalence of pre-mRNA 3'-end processing, it is conceivable that Hrp1 and Nab2 cooperate to quality control the 3'-end cleavage and polyadenylation reaction.

5.5. Npl3 may regulate Hrp1 surveillance for pre-mRNA 3'-end processing

The genetic suppression of *npl3-1* by *HRP1* and *RNA15* mutants of the CF I complex has been the initial hint that implicates a role of Npl3 in the 3'-end processing of pre-mRNAs (Henry *et al.*, 1996). Early research has shown that Npl3 competes with the CF IA component Rna15 for mRNA binding and mutations within *NPL3* result in enhanced transcription termination (Bucheli and Buratowski, 2005). Consistently, extensive studies have further demonstrated that Npl3 might suppress utilization of weak poly(A) sites via active competition with 3'-end processing factors of the CF I complex (Bucheli *et al.*, 2007). Interestingly, the competition might be regulated by Npl3 methylation and phosphorylation via the methyltransferase Hmt1 and casein kinase 2 in the SR/RGG domain, respectively (Dermody *et al.*, 2008; Wong *et al.*, 2010). Different from the cytoplasmic SR protein kinase Sky1 that is important for Npl3 reimport, casein kinase 2 likely phosphorylates the SR/RGG domain of Npl3 in the nucleus (Gilbert, Siebel and Guthrie, 2001; Häcker and Krebber, 2004; Dermody *et al.*, 2008). Npl3 methylation promotes transcription elongation, whereas Npl3 phosphorylation has been shown to facilitate its dissociation from mRNAs and contribute to Rna15 recruitment. Consistently, a mutant of Npl3 defective for Ser411 phosphorylation results in transcription readthrough (Dermody *et al.*, 2008). Accordingly, Npl3 likely functions to mask the weak poly(A) signals and regulate assembly of the CPF-CF complex onto a proper poly(A) site. However, a later study by Holmes and colleagues suggests the opposite role of Npl3 in mRNA transcription

termination (Holmes *et al.*, 2015). They have discovered 3'-end readthrough mRNAs across the genome in the absence of Npl3, indicating that Npl3 is not an antagonist of the CPF-CF complex. Intriguingly, mRNAs with the canonical EE for Hrp1 binding at the 3' untranslated region exhibited nearly no readthrough, which might suggest that the high efficiency of the cleavage and polyadenylation attributed to Hrp1 is sufficient for transcription termination without Npl3. Therefore, Npl3 appears to cooperate with Hrp1 for the 3'-end processing of mRNA with an uncanonical poly (A) site. The controversial conclusions from different studies might result from the targets that have been tested. It is possible that the specific *GAL* reporter used in the earlier studies is limited to show a general role of Npl3 for pre-mRNA 3' processing. Nevertheless, all the information confirms the crucial function of Npl3 in mRNA 3' maturation and its coupled transcription termination. Combined with the physical interaction between Npl3 and Hrp1 (Figure 26), one expectation would be that Npl3 might regulate the role of Hrp1 in the surveillance of mRNA 3'-end processing.

5.6 Hrp1 appears to cooperate with the other guard proteins to maintain mRNA quality throughout the cell

Shuttling SR/RGG proteins Gbp2 and Hrb1 are quality control factors that surveil mRNA splicing in the nucleus (Hackmann *et al.*, 2014). Interestingly, mRNA quality control in the nucleus and in the cytoplasm are very likely linked by shuttling guard proteins. As shown in earlier works of our colleagues, Gbp2 and Hrb1 are still bound to mRNAs during the first round of translation, targeting aberrant mRNAs with a premature stop codon for NMD in the cytoplasm (Grosse *et al.*, 2021; Windgassen *et al.*, 2004). The continuous quality control function of SR/RGG proteins appears to be highly conserved. In metazoan cells, the exon-junction complex (EJC) including auxiliary SR proteins acts as a long-lasting guardian of mRNAs (Lu and Krebber, 2021). It monitors and marks mRNA splicing in the nucleus and further stimulates NMD in the cytoplasm only if a nonsense codon occurs upstream of the complex. Due to a lack of such an EJC complex in budding yeast, Gbp2 and Hrb1 are assumed to be a prototype

of the EJC (Lu and Krebber, 2021; Grosse *et al.*, 2021). Since only about 25% of mRNAs that are transcribed from around 5% of genes undergo splicing in budding yeast (Hackmann *et al.*, 2014), most mRNAs without introns are supposed to be surveilled by other guard proteins for NMD. Indeed, Hrp1 has been reported to participate in NMD in the cytosol to eliminate faulty mRNAs (Gonzalez *et al.*, 2000). Therefore, it is conceivable that Hrp1 continues its role as an mRNA surveillance factor from the nucleus to the cytoplasm. However, according to our findings that indicate that Hrp1 surveils the 3' processing of the EE-containing mRNAs in the nucleus, it should be a general quality control factor for such mRNAs no matter if they contain introns or not. In this way, it would be interesting to figure out how Hrp1, together with Gbp2 and Hrb1, is arranged to direct faulty intron-containing mRNAs for NMD in the cytosol in general.

5.7. Model for Hrp1 and the other guard proteins in the nuclear quality control of mRNA

In this study, we identified a novel role of yeast cleavage factor Hrp1 in monitoring pre-mRNA 3'-end processing in the nucleus. We showed general features of Hrp1 as a nuclear guard protein and leakage of 3'-extended mRNAs into the cytoplasm in the absence of Hrp1 quality control. Moreover, we revealed that the nuclear retention of readthrough mRNAs is due to defects in recruiting the export receptor Mex67-Mtr2, which is likely sensed by the NPC gatekeeper protein Mlp1. Finally, a possible mechanism that suggests that the interaction of Hrp1 with Rna14 determines Mex67-Mtr2 recruitment was proposed. Therefore, we confirmed the common role of SR/RGG shuttling mRNA-binding proteins in concurrent quality control of co-transcriptional mRNA processing and subsequent export, and were able to add a new guard protein to the list of nuclear mRNA surveillance factors: Hrp1.

Based on the findings of this study and the given knowledge of mRNA nuclear surveillance, we propose the following model for Hrp1 and the other guard proteins in

the nuclear quality control of mRNAs (Figure 22). Under normal conditions, mRNAs are transcribed by RNA Pol II and correctly processed at each maturation step. Proper maturation is detected by SR/RGG guard proteins including Hrp1, which recruits the export receptor Mex67-Mtr2 for efficient transport through the NPC. However, if RNA Pol II transcription reads through the canonical poly(A) site due to defective assembly of the CPF-CF complex as present in *cft2-1*, Hrp1 cannot bind Rna14 as it is missing in the CPF-CF complex. This in turn prevents the attachment of Mex67 to Hrp1, which is sensed by the NPC surveillance factor Mlp1 and leads to mRNA retention. The aberrant 3'-extended mRNAs are recognized by the NNS complex and, subsequently, degraded by the TRAMP-exosome complex. Mutant hrp1-1 is depleted from the EE-containing mRNAs through binding to unspecific targets, leading to a lack of Hrp1 quality control on 3'-elongated EE- containing mRNAs, which results in the escape of faulty mRNAs from the nucleus and leakage into the cytoplasm.

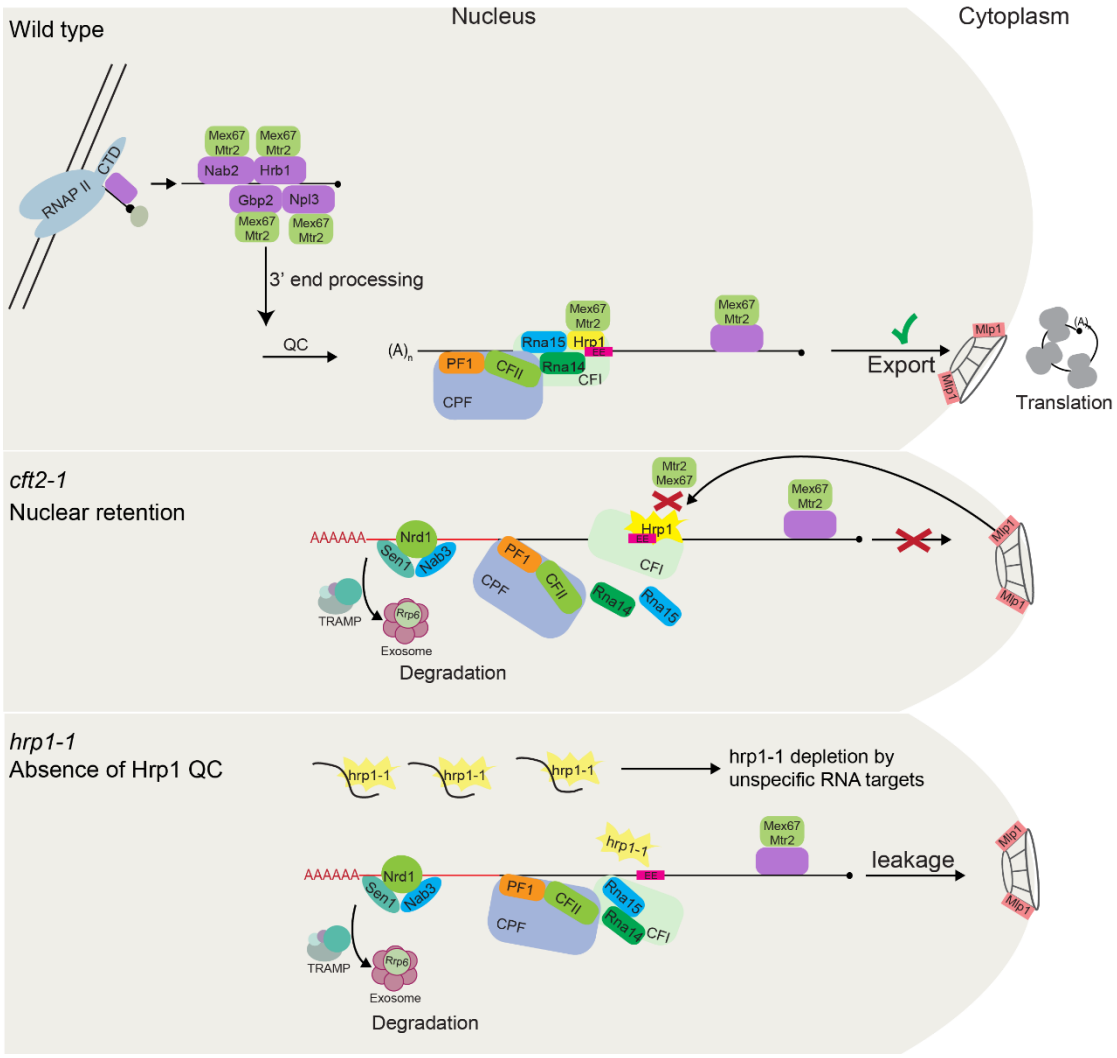


Figure 22: Model for Hrp1 and the other guard proteins in nuclear quality control of mRNAs.

In the wild type cells, shuttling proteins such as Npl3, Gbp2, Hrb1, and Nab2 act as guard proteins that surveil mRNA capping, splicing and poly(A) tail synthesis. Hrp1 specifically binds to the newly produced EE-containing pre-mRNAs and inspects if the 3'-end processing is correct or not. If the mRNA is properly cleaved and polyadenylated and thus bound to Rna14, Hrp1 recruits the export receptor Mex67-Mtr2 for mRNA export. If the 3'-end processing fails, for example in *cft2-1*, Hrp1 will not recruit Mex67-Mtr2, probably due to a lack of Rna14 in the CPF-CF complex. Therefore, the elongated mRNA will be captured by the NNS complex, marked with a short poly(A) tail via the TRAMP complex, and degraded by the exosome in the nucleus. In the *hrp1-1* mutant, the absence of Hrp1 for the 3' processing surveillance due to hrp1-1 depletion leads to the leakage of readthrough mRNAs into the cytoplasm.

6. Appendix

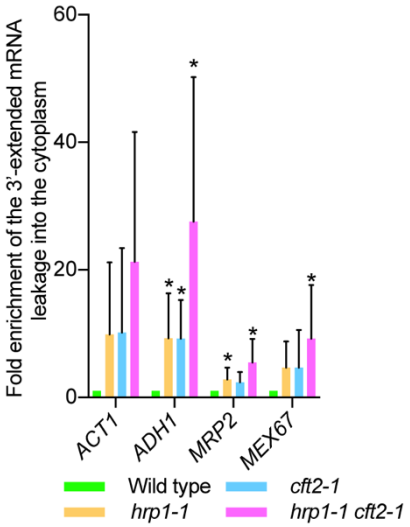


Figure 23: Cell fractionation shows leakage of the 3'-extended mRNAs into the cytoplasm.
qPCR analysis shows fold enrichment of 3'-elongated mRNAs that leaked into the cytoplasm. Leakage in the mutant strains was all related to that in the wild type.

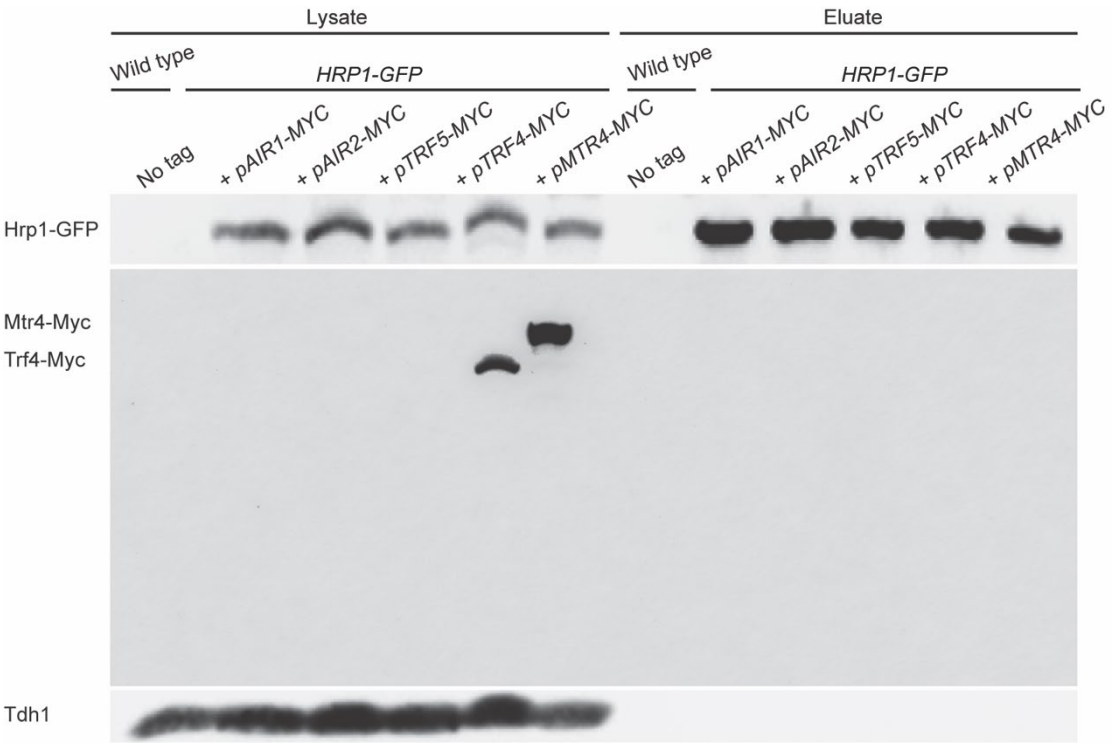


Figure 24: Hrp1 does not physically interact with the TRAMP complex.
The western blot shows no direct interaction between Hrp1 and the TRAMP complex. The *HRP1-GFP* strain was transformed with *pAIR1-MYC*, *pAIR2-MYC*, *pTRF4-MYC*, *pTRF5-MYC*, and *pMTR4-MYC* for immunoprecipitation experiments, respectively. The GFP-tagged Hrp1

was pulled down with the GFP-trap beads. No signal was detected for Myc-tagged components of the TRAMP complex on the membrane. The glyceraldehyde-3-phosphate dehydrogenase Tdh1 was used as a negative control.

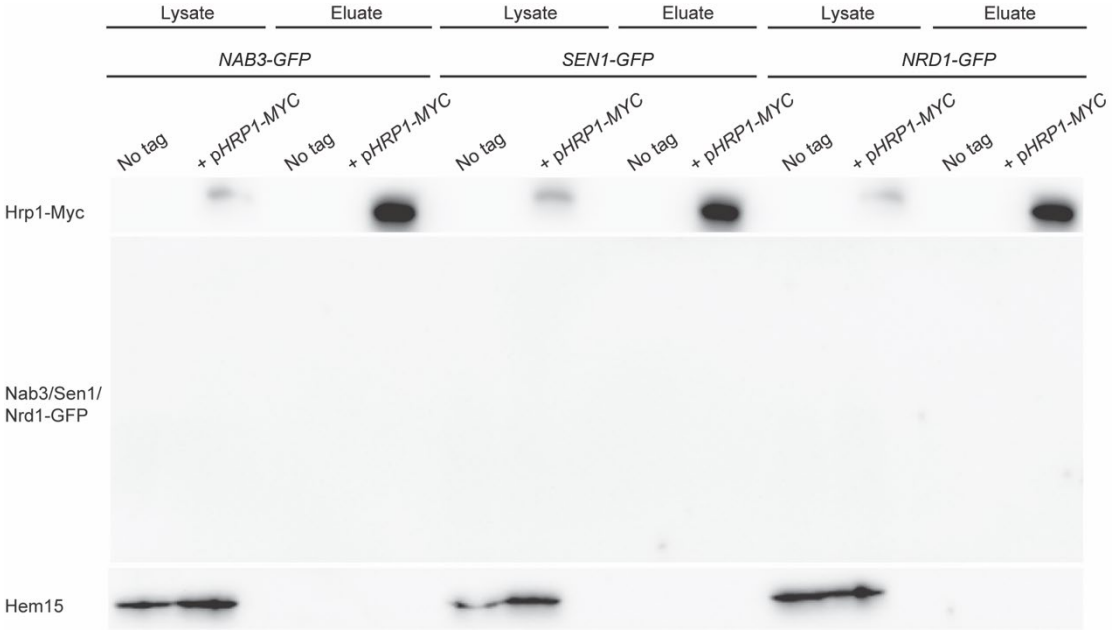


Figure 25: Hrp1 does not physically interact with the NNS complex.
The Myc-tagged Hrp1 was pulled down with the Myc-trap beads. The western blot exhibits no co-immunoprecipitation signals of the NNS complex. Hem15 served as a negative control.

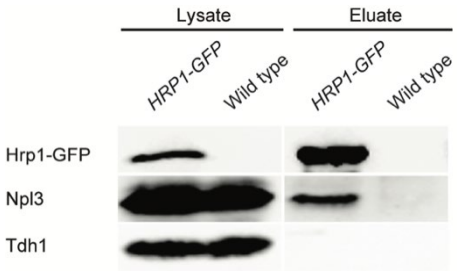


Figure 26: The western blot of a co-immunoprecipitation assay shows the physical interaction between Hrp1 and Npl3.
Hrp1-GFP was pulled down with the GFP-trap beads. The wild type was used as a no-tag control for pull down. Tdh1 was detected as a negative control for co-immunoprecipitation.

7. References

Abruzzi, K. C., Lacadie, S. and Rosbash, M. (2004) 'Biochemical analysis of TREX complex recruitment to intronless and intron-containing yeast genes', *EMBO J,* 23(13), pp. 2620-31.

Adams, R. L. and Wente, S. R. (2020) 'Dbp5 associates with RNA-bound Mex67 and Nab2 and its localization at the nuclear pore complex is sufficient for mRNP export and cell viability', *PLoS Genet,* 16(10), pp. e1009033.

Aibara, S., Gordon, J. M., Riesterer, A. S., McLaughlin, S. H. and Stewart, M. (2017) 'Structural basis for the dimerization of Nab2 generated by RNA binding provides insight into its contribution to both poly(A) tail length determination and transcript compaction in Saccharomyces cerevisiae', *Nucleic Acids Res,* 45(3), pp. 1529-1538.

Aibara, S., Valkov, E., Lamers, M. and Stewart, M. (2015) 'Domain organization within the nuclear export factor Mex67:Mtr2 generates an extended mRNA binding surface', *Nucleic Acids Res,* 43(3), pp. 1927-36.

Aitchison, J. D. and Rout, M. P. (2012) 'The yeast nuclear pore complex and transport through it', *Genetics,* 190(3), pp. 855-83.

Al-Husini, N., Sharifi, A., Mousavi, S. A., Chitsaz, H. and Ansari, A. (2017) 'Genomewide Analysis of Clp1 Function in Transcription in Budding Yeast', *Sci Rep,* 7(1), pp. 6894.

Alegria-Schaffer, A. (2014) 'Western blotting using chemiluminescent substrates', *Methods Enzymol,* 541, pp. 251-9.

Allmang, C., Kufel, J., Chanfreau, G., Mitchell, P., Petfalski, E. and Tollervey, D. (1999) 'Functions of the exosome in rRNA, snoRNA and snRNA synthesis', *EMBO J,* 18(19), pp. 5399-410.

Allmang, C., Mitchell, P., Petfalski, E. and Tollervey, D. (2000) 'Degradation of ribosomal RNA precursors by the exosome', *Nucleic Acids Res,* 28(8), pp. 1684-91.

Alpert, T., Straube, K., Oesterreich, F. C., Herzel, L. and Neugebauer, K. M. (2020) 'Widespread Transcriptional Readthrough Caused by Nab2 Depletion Leads to Chimeric Transcripts with Retained Introns', *Cell Rep,* 33(13), pp. 108496.

Amrani, N., Minet, M., Le Gouar, M., Lacroute, F. and Wyers, F. (1997) 'Yeast Pab1 interacts with Rna15 and participates in the control of the poly(A) tail length in vitro', *Mol Cell Biol,* 17(7), pp. 3694-701.

Anderson, J. T., Wilson, S. M., Datar, K. V. and Swanson, M. S. (1993) 'NAB2: a yeast nuclear polyadenylated RNA-binding protein essential for cell viability', *Mol Cell Biol,* 13(5), pp. 2730-41.

Anko, M. L. (2014) 'Regulation of gene expression programmes by serine-arginine rich splicing factors', *Semin Cell Dev Biol,* 32, pp. 11-21.

Baierlein, C., Hackmann, A., Gross, T., Henker, L., Hinz, F. and Krebber, H. (2013) 'Monosome formation during translation initiation requires the serine/arginine-rich protein Npl3', *Mol Cell Biol,* 33(24), pp. 4811-23.

Barabino, S. M., Ohnacker, M. and Keller, W. (2000) 'Distinct roles of two Yth1p domains in 3'-end cleavage and polyadenylation of yeast pre-mRNAs', *EMBO J,* 19(14), pp. 3778-87.

Barilla, D., Lee, B. A. and Proudfoot, N. J. (2001) 'Cleavage/polyadenylation factor IA associates with the carboxyl-terminal domain of RNA polymerase II in Saccharomyces cerevisiae', *Proc Natl Acad Sci U S A,* 98(2), pp. 445-50.

Barnwal, R. P., Lee, S. D., Moore, C. and Varani, G. (2012) 'Structural and biochemical analysis of the assembly and function of the yeast pre-mRNA 3' end processing complex CF I', *Proc Natl Acad Sci U S A,* 109(52), pp. 21342-7.

Beilharz, T. H. and Preiss, T. (2007) 'Widespread use of poly(A) tail length control to accentuate expression of the yeast transcriptome', *RNA,* 13(7), pp. 982-97.

Bharati, A. P., Singh, N., Kumar, V., Kashif, M., Singh, A. K., Singh, P., Singh, S. K., Siddiqi, M. I., Tripathi, T. and Akhtar, M. S. (2016) 'The mRNA capping enzyme of Saccharomyces cerevisiae has dual specificity to interact with CTD of RNA Polymerase II', *Sci Rep,* 6, pp. 31294.

Blythe, A. J., Yazar-Klosinski, B., Webster, M. W., Chen, E., Vandevenne, M., Bendak, K., Mackay, J. P., Hartzog, G. A. and Vrielink, A. (2016) 'The yeast transcription elongation factor Spt4/5 is a sequence-specific RNA binding protein', *Protein Sci,* 25(9), pp. 1710-21.

Bond, S. R. and Naus, C. C. (2012) 'RF-Cloning.org: an online tool for the design of restriction-free cloning projects', *Nucleic Acids Res,* 40(Web Server issue), pp. W209-13.

Bortvin, A. and Winston, F. (1996) 'Evidence that Spt6p controls chromatin structure by a direct interaction with histones', *Science,* 272(5267), pp. 1473-6.

Bossie, M. A., DeHoratius, C., Barcelo, G. and Silver, P. (1992) 'A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast', *Mol Biol Cell,* 3(8), pp. 875-93.

Bousquet-Antonelli, C., Presutti, C. and Tollervey, D. (2000) 'Identification of a regulated pathway for nuclear pre-mRNA turnover', *Cell,* 102(6), pp. 765-75.

Bretes, H., Rouviere, J. O., Leger, T., Oeffinger, M., Devaux, F., Doye, V. and Palancade, B. (2014) 'Sumoylation of the THO complex regulates the biogenesis of a subset of mRNPs', *Nucleic Acids Res,* 42(8), pp. 5043-58.

Briggs, M. W., Burkard, K. T. and Butler, J. S. (1998) 'Rrp6p, the yeast homologue of the human PM-Scl 100-kDa autoantigen, is essential for efficient 5.8 S rRNA 3' end formation', *J Biol Chem,* 273(21), pp. 13255-63.

Brodsky, A. S. and Silver, P. A. (2000) 'Pre-mRNA processing factors are required for nuclear export', *RNA,* 6(12), pp. 1737-49.

Brune, C., Munchel, S. E., Fischer, N., Podtelejnikov, A. V. and Weis, K. (2005) 'Yeast poly(A)-binding protein Pab1 shuttles between the nucleus and the cytoplasm and functions in mRNA export', *RNA,* 11(4), pp. 517-31.

Bucheli, M. E. and Buratowski, S. (2005) 'Npl3 is an antagonist of mRNA 3' end formation by RNA polymerase II', *EMBO J,* 24(12), pp. 2150-60.

Bucheli, M. E., He, X., Kaplan, C. D., Moore, C. L. and Buratowski, S. (2007) 'Polyadenylation site choice in yeast is affected by competition between Npl3 and polyadenylation factor CFI', *RNA,* 13(10), pp. 1756-64.

Buratowski, S. (2005) 'Connections between mRNA 3' end processing and transcription termination', *Curr Opin Cell Biol,* 17(3), pp. 257-61.

Busch, A. and Hertel, K. J. (2012) 'Evolution of SR protein and hnRNP splicing regulatory factors', *Wiley Interdiscip Rev RNA,* 3(1), pp. 1-12.

Carneiro, T., Carvalho, C., Braga, J., Rino, J., Milligan, L., Tollervey, D. and Carmo-Fonseca, M. (2008) 'Inactivation of cleavage factor I components Rna14p and Rna15p induces sequestration of small nucleolar ribonucleoproteins at discrete sites in the nucleus', *Mol Biol Cell,* 19(4), pp. 1499-508.

Carroll, K. L., Ghirlando, R., Ames, J. M. and Corden, J. L. (2007) 'Interaction of yeast RNA-binding proteins Nrd1 and Nab3 with RNA polymerase II terminator elements', *RNA,* 13(3), pp. 361-73.

Carroll, K. L., Pradhan, D. A., Granek, J. A., Clarke, N. D. and Corden, J. L. (2004) 'Identification of cis elements directing termination of yeast nonpolyadenylated snoRNA transcripts', *Mol Cell Biol,* 24(14), pp. 6241-52.

Casanal, A., Kumar, A., Hill, C. H., Easter, A. D., Emsley, P., Degliesposti, G., Gordiyenko, Y., Santhanam, B., Wolf, J., Wiederhold, K., Dornan, G. L., Skehel, M., Robinson, C. V. and Passmore, L. A. (2017) 'Architecture of eukaryotic mRNA 3'-end processing machinery', *Science,* 358(6366), pp. 1056-1059.

Chanarat, S., Seizl, M. and Strasser, K. (2011) 'The Prp19 complex is a novel transcription elongation factor required for TREX occupancy at transcribed genes', *Genes Dev,* 25(11), pp. 1147-58.

Chatterjee, K., Majumder, S., Wan, Y., Shah, V., Wu, J., Huang, H. Y. and Hopper, A. K. (2017) 'Sharing the load: Mex67-Mtr2 cofunctions with Los1 in primary tRNA nuclear export', *Genes Dev,* 31(21), pp. 2186-2198.

Chen, J. and Moore, C. (1992) 'Separation of factors required for cleavage and polyadenylation of yeast pre-mRNA', *Mol Cell Biol,* 12(8), pp. 3470-81.

Chen, S. and Hyman, L. E. (1998) 'A specific RNA-protein interaction at yeast polyadenylation efficiency elements', *Nucleic Acids Res,* 26(21), pp. 4965-74.

Ciais, D., Bohnsack, M. T. and Tollervey, D. (2008) 'The mRNA encoding the yeast ARE-binding protein Cth2 is generated by a novel 3' processing pathway', *Nucleic Acids Res,* 36(9), pp. 3075-84.

Colin, J., Candelli, T., Porrua, O., Boulay, J., Zhu, C., Lacroute, F., Steinmetz, L. M. and Libri, D. (2014) 'Roadblock termination by reb1p restricts cryptic and readthrough transcription', *Mol Cell,* 56(5), pp. 667-80.

Davis, C. A., Grate, L., Spingola, M. and Ares, M., Jr. (2000) 'Test of intron predictions reveals novel splice sites, alternatively spliced mRNAs and new introns in meiotically regulated genes of yeast', *Nucleic Acids Res,* 28(8), pp. 1700-6.

Deka, P., Bucheli, M. E., Moore, C., Buratowski, S. and Varani, G. (2008) 'Structure of the yeast SR protein Npl3 and Interaction with mRNA 3'-end processing signals', *J Mol Biol,* 375(1), pp. 136-50.

Dermody, J. L., Dreyfuss, J. M., Villen, J., Ogundipe, B., Gygi, S. P., Park, P. J., Ponticelli, A. S., Moore, C. L., Buratowski, S. and Bucheli, M. E. (2008) 'Unphosphorylated SR-like protein Npl3 stimulates RNA polymerase II elongation', *PLoS One,* 3(9), pp. e3273.

Dichtl, B., Blank, D., Sadowski, M., Hubner, W., Weiser, S. and Keller, W. (2002) 'Yhh1p/Cft1p directly links poly(A) site recognition and RNA polymerase II transcription termination', *EMBO J,* 21(15), pp. 4125-35.

Dichtl, B. and Keller, W. (2001) 'Recognition of polyadenylation sites in yeast pre-mRNAs by cleavage and polyadenylation factor', *EMBO J,* 20(12), pp. 3197-209.

Dower, W. J., Miller, J. F. and Ragsdale, C. W. (1988) 'High efficiency transformation of E. coli by high voltage electroporation', *Nucleic Acids Res,* 16(13), pp. 6127-45.

Eberle, A. B. and Visa, N. (2014) 'Quality control of mRNP biogenesis: networking at the transcription site', *Semin Cell Dev Biol,* 32, pp. 37-46.

Egloff, S. and Murphy, S. (2008) 'Cracking the RNA polymerase II CTD code', *Trends Genet,* 24(6), pp. 280-8.

Erkmann, J. A. and Kutay, U. (2004) 'Nuclear export of mRNA: from the site of transcription to the cytoplasm', *Exp Cell Res,* 296(1), pp. 12-20.

Estrella, L. A., Wilkinson, M. F. and Gonzalez, C. I. (2009) 'The shuttling protein Npl3 promotes translation termination accuracy in Saccharomyces cerevisiae', *J Mol Biol,* 394(3), pp. 410-22.

Fasken, M. B. and Corbett, A. H. (2009) 'Mechanisms of nuclear mRNA quality control', *RNA Biol,* 6(3), pp. 237-41.

Fasken, M. B., Corbett, A. H. and Stewart, M. (2019) 'Structure-function relationships in the Nab2 polyadenosine-RNA binding Zn finger protein family', *Protein Sci,* 28(3), pp. 513-523.

Fasken, M. B., Stewart, M. and Corbett, A. H. (2008) 'Functional significance of the interaction between the mRNA-binding protein, Nab2, and the nuclear pore-associated protein, Mlp1, in mRNA export', *J Biol Chem,* 283(40), pp. 27130-43.

Faza, M. B., Chang, Y., Occhipinti, L., Kemmler, S. and Panse, V. G. (2012) 'Role of Mex67-Mtr2 in the nuclear export of 40S pre-ribosomes', *PLoS Genet,* 8(8), pp. e1002915.

Fernandez-Martinez, J. and Rout, M. P. (2009) 'Nuclear pore complex biogenesis', *Curr Opin Cell Biol,* 21(4), pp. 603-12.

Flach, J., Bossie, M., Vogel, J., Corbett, A., Jinks, T., Willins, D. A. and Silver, P. A. (1994) 'A yeast RNA-binding protein shuttles between the nucleus and the cytoplasm', *Mol Cell Biol,* 14(12), pp. 8399-407.

Franco-Echevarria, E., Gonzalez-Polo, N., Zorrilla, S., Martinez-Lumbreras, S., Santiveri, C. M., Campos-Olivas, R., Sanchez, M., Calvo, O., Gonzalez, B. and Perez-Canadillas, J. M. (2017) 'The structure of transcription termination factor Nrd1 reveals an original mode for GUAA recognition', *Nucleic Acids Res,* 45(17), pp. 10293-10305.

Galy, V., Gadal, O., Fromont-Racine, M., Romano, A., Jacquier, A. and Nehrbass, U. (2004) 'Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1', *Cell,* 116(1), pp. 63-73.

Garfin, D. E. (2009) 'One-dimensional gel electrophoresis', *Methods Enzymol,* 463, pp. 497-513.

Ghazy, M. A., Gordon, J. M., Lee, S. D., Singh, B. N., Bohm, A., Hampsey, M. and Moore, C. (2012) 'The interaction of Pcf11 and Clp1 is needed for mRNA 3'-end formation and is modulated by amino acids in the ATP-binding site', *Nucleic Acids Res,* 40(3), pp. 1214-25.

Gibson, D. G., Young, L., Chuang, R. Y., Venter, J. C., Hutchison, C. A., 3rd and Smith, H. O. (2009) 'Enzymatic assembly of DNA molecules up to several hundred kilobases', *Nat Methods,* 6(5), pp. 343-5.

Gietz, D., St Jean, A., Woods, R. A. and Schiestl, R. H. (1992) 'Improved method for high efficiency transformation of intact yeast cells', *Nucleic Acids Res,* 20(6), pp. 1425.

Gilbert, W., Siebel, C. W. and Guthrie, C. (2001) 'Phosphorylation by Sky1p promotes Npl3p shuttling and mRNA dissociation', *RNA,* 7(2), pp. 302-13.

Gonzalez, C. I., Ruiz-Echevarria, M. J., Vasudevan, S., Henry, M. F. and Peltz, S. W. (2000) 'The yeast hnRNP-like protein Hrp1/Nab4 marks a transcript for nonsense-mediated mRNA decay', *Mol Cell,* 5(3), pp. 489-99.

Gordon, J. M., Shikov, S., Kuehner, J. N., Liriano, M., Lee, E., Stafford, W., Poulsen, M. B., Harrison, C., Moore, C. and Bohm, A. (2011) 'Reconstitution of CF IA from overexpressed subunits reveals stoichiometry and provides insights into molecular topology', *Biochemistry,* 50(47), pp. 10203-14.

Gornemann, J., Kotovic, K. M., Hujer, K. and Neugebauer, K. M. (2005) 'Cotranscriptional spliceosome assembly occurs in a stepwise fashion and requires the cap binding complex', *Mol Cell,* 19(1), pp. 53-63.

Graber, J. H., Cantor, C. R., Mohr, S. C. and Smith, T. F. (1999) 'Genomic detection of new yeast pre-mRNA 3'-end-processing signals', *Nucleic Acids Res,* 27(3), pp. 888-94.

Graber, J. H., McAllister, G. D. and Smith, T. F. (2002) 'Probabilistic prediction of Saccharomyces cerevisiae mRNA 3'-processing sites', *Nucleic Acids Res,* 30(8), pp. 1851-8.

Grant, R. P., Marshall, N. J., Yang, J. C., Fasken, M. B., Kelly, S. M., Harreman, M. T., Neuhaus, D., Corbett, A. H. and Stewart, M. (2008) 'Structure of the N-terminal Mlp1-binding domain of the Saccharomyces cerevisiae mRNA-binding protein, Nab2', *J Mol Biol,* 376(4), pp. 1048-59.

Green, D. M., Johnson, C. P., Hagan, H. and Corbett, A. H. (2003) 'The C-terminal domain of myosin-like protein 1 (Mlp1p) is a docking site for heterogeneous nuclear ribonucleoproteins that are required for mRNA export', *Proc Natl Acad Sci U S A,* 100(3), pp. 1010-5.

Green, D. M., Marfatia, K. A., Crafton, E. B., Zhang, X., Cheng, X. and Corbett, A. H. (2002) 'Nab2p is

required for poly(A) RNA export in Saccharomyces cerevisiae and is regulated by arginine methylation via Hmt1p', *J Biol Chem,* 277(10), pp. 7752-60.
Gross, S. and Moore, C. L. (2001) 'Rna15 interaction with the A-rich yeast polyadenylation signal is an essential step in mRNA 3'-end formation', *Mol Cell Biol,* 21(23), pp. 8045-55.
Grosse, S., Lu, Y. Y., Coban, I., Neumann, B. and Krebber, H. (2021) 'Nuclear SR-protein mediated mRNA quality control is continued in cytoplasmic nonsense-mediated decay', *RNA Biol,* 18(10), pp. 1390-1407.
Grzechnik, P. and Kufel, J. (2008) 'Polyadenylation linked to transcription termination directs the processing of snoRNA precursors in yeast', *Mol Cell,* 32(2), pp. 247-58.
Guo, Z., Russo, P., Yun, D. F., Butler, J. S. and Sherman, F. (1995) 'Redundant 3' end-forming signals for the yeast CYC1 mRNA', *Proc Natl Acad Sci U S A,* 92(10), pp. 4211-4.
Guo, Z. and Sherman, F. (1996) '3'-end-forming signals of yeast mRNA', *Trends Biochem Sci,* 21(12), pp. 477-81.
Häcker, S. and Krebber, H. (2004) 'Differential export requirements for shuttling serine/arginine-type mRNA-binding proteins', *J Biol Chem,* 279(7), pp. 5049-52.
Hackmann, A., Wu, H., Schneider, U. M., Meyer, K., Jung, K. and Krebber, H. (2014) 'Quality control of spliced mRNAs requires the shuttling SR proteins Gbp2 and Hrb1', *Nat Commun,* 5, pp. 3123.
Hahn, S. (2004) 'Structure and mechanism of the RNA polymerase II transcription machinery', *Nat Struct Mol Biol,* 11(5), pp. 394-403.
Hammell, C. M., Gross, S., Zenklusen, D., Heath, C. V., Stutz, F., Moore, C. and Cole, C. N. (2002) 'Coupling of termination, 3' processing, and mRNA export', *Mol Cell Biol,* 22(18), pp. 6441-57.
Hampsey, M. (1998) 'Molecular genetics of the RNA polymerase II general transcriptional machinery', *Microbiol Mol Biol Rev,* 62(2), pp. 465-503.
Harlen, K. M. and Churchman, L. S. (2017) 'The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain', *Nat Rev Mol Cell Biol,* 18(4), pp. 263-273.
Hector, R. E., Nykamp, K. R., Dheur, S., Anderson, J. T., Non, P. J., Urbinati, C. R., Wilson, S. M., Minvielle-Sebastia, L. and Swanson, M. S. (2002) 'Dual requirement for yeast hnRNP Nab2p in mRNA poly(A) tail length control and nuclear export', *EMBO J,* 21(7), pp. 1800-10.
Henry, M., Borland, C. Z., Bossie, M. and Silver, P. A. (1996) 'Potential RNA binding proteins in Saccharomyces cerevisiae identified as suppressors of temperature-sensitive mutations in NPL3', *Genetics,* 142(1), pp. 103-15.
Heo, D. H., Yoo, I., Kong, J., Lidschreiber, M., Mayer, A., Choi, B. Y., Hahn, Y., Cramer, P., Buratowski, S. and Kim, M. (2013) 'The RNA polymerase II C-terminal domain-interacting domain of yeast Nrd1 contributes to the choice of termination pathway and couples to RNA processing by the nuclear exosome', *J Biol Chem,* 288(51), pp. 36676-90.
Hilleren, P., McCarthy, T., Rosbash, M., Parker, R. and Jensen, T. H. (2001) 'Quality control of mRNA 3'-end processing is linked to the nuclear exosome', *Nature,* 413(6855), pp. 538-42.
Holmes, R. K., Tuck, A. C., Zhu, C., Dunn-Davies, H. R., Kudla, G., Clauder-Munster, S., Granneman, S., Steinmetz, L. M., Guthrie, C. and Tollervey, D. (2015) 'Loss of the Yeast SR Protein Npl3 Alters Gene Expression Due to Transcription Readthrough', *PLoS Genet,* 11(12), pp. e1005735.
Houseley, J. and Tollervey, D. (2009) 'The many pathways of RNA degradation', *Cell,* 136(4), pp. 763-76.
Howard, J. M. and Sanford, J. R. (2015) 'The RNAissance family: SR proteins as multifaceted regulators of gene expression', *Wiley Interdiscip Rev RNA,* 6(1), pp. 93-110.
Hsin, J. P. and Manley, J. L. (2012) 'The RNA polymerase II CTD coordinates transcription and RNA processing', *Genes Dev,* 26(19), pp. 2119-37.

Hsu, L. M. (2009) 'Monitoring abortive initiation', *Methods,* 47(1), pp. 25-36.

Huang, Y. and Steitz, J. A. (2001) 'Splicing factors SRp20 and 9G8 promote the nucleocytoplasmic export of mRNA', *Mol Cell,* 7(4), pp. 899-905.

Hurt, E., Luo, M. J., Rother, S., Reed, R. and Strasser, K. (2004) 'Cotranscriptional recruitment of the serine-arginine-rich (SR)-like proteins Gbp2 and Hrb1 to nascent mRNA via the TREX complex', *Proc Natl Acad Sci U S A,* 101(7), pp. 1858-62.

Iglesias, N., Tutucci, E., Gwizdek, C., Vinciguerra, P., Von Dach, E., Corbett, A. H., Dargemont, C. and Stutz, F. (2010) 'Ubiquitin-mediated mRNP dynamics and surveillance prior to budding yeast mRNA export', *Genes Dev,* 24(17), pp. 1927-38.

Irniger, S. and Braus, G. H. (1994) 'Saturation mutagenesis of a polyadenylation signal reveals a hexanucleotide element essential for mRNA 3' end formation in Saccharomyces cerevisiae', *Proc Natl Acad Sci U S A,* 91(1), pp. 257-61.

Ishigaki, Y., Li, X., Serin, G. and Maquat, L. E. (2001) 'Evidence for a pioneer round of mRNA translation: mRNAs subject to nonsense-mediated decay in mammalian cells are bound by CBP80 and CBP20', *Cell,* 106(5), pp. 607-17.

Jensen, T. H., Patricio, K., McCarthy, T. and Rosbash, M. (2001) 'A block to mRNA nuclear export in S. cerevisiae leads to hyperadenylation of transcripts that accumulate at the site of transcription', *Mol Cell,* 7(4), pp. 887-98.

Ji, X., Zhou, Y., Pandit, S., Huang, J., Li, H., Lin, C. Y., Xiao, R., Burge, C. B. and Fu, X. D. (2013) 'SR proteins collaborate with 7SK and promoter-associated nascent RNA to release paused polymerase', *Cell,* 153(4), pp. 855-68.

Jiao, X., Xiang, S., Oh, C., Martin, C. E., Tong, L. and Kiledjian, M. (2010) 'Identification of a quality-control mechanism for mRNA 5'-end capping', *Nature,* 467(7315), pp. 608-11.

Kadaba, S., Krueger, A., Trice, T., Krecic, A. M., Hinnebusch, A. G. and Anderson, J. (2004) 'Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae', *Genes Dev,* 18(11), pp. 1227-40.

Kelly, S. M., Pabit, S. A., Kitchen, C. M., Guo, P., Marfatia, K. A., Murphy, T. J., Corbett, A. H. and Berland, K. M. (2007) 'Recognition of polyadenosine RNA by zinc finger proteins', *Proc Natl Acad Sci U S A,* 104(30), pp. 12306-11.

Kessler, M. M., Henry, M. F., Shen, E., Zhao, J., Gross, S., Silver, P. A. and Moore, C. L. (1997) 'Hrp1, a sequence-specific RNA-binding protein that shuttles between the nucleus and the cytoplasm, is required for mRNA 3'-end formation in yeast', *Genes Dev,* 11(19), pp. 2545-56.

Kim Guisbert, K., Duncan, K., Li, H. and Guthrie, C. (2005) 'Functional specificity of shuttling hnRNPs revealed by genome-wide analysis of their RNA binding profiles', *RNA,* 11(4), pp. 383-93.

Kim Guisbert, K. S., Li, H. and Guthrie, C. (2007) 'Alternative 3' pre-mRNA processing in Saccharomyces cerevisiae is modulated by Nab4/Hrp1 in vivo', *PLoS Biol,* 5(1), pp. e6.

Kim, H. J., Jeong, S. H., Heo, J. H., Jeong, S. J., Kim, S. T., Youn, H. D., Han, J. W., Lee, H. W. and Cho, E. J. (2004a) 'mRNA capping enzyme activity is coupled to an early transcription elongation', *Mol Cell Biol,* 24(14), pp. 6184-93.

Kim, M., Ahn, S. H., Krogan, N. J., Greenblatt, J. F. and Buratowski, S. (2004b) 'Transitions in RNA polymerase II elongation complexes at the 3' ends of genes', *EMBO J,* 23(2), pp. 354-64.

Kress, T. L., Krogan, N. J. and Guthrie, C. (2008) 'A single SR-like protein, Npl3, promotes pre-mRNA splicing in budding yeast', *Mol Cell,* 32(5), pp. 727-34.

Kyburz, A., Sadowski, M., Dichtl, B. and Keller, W. (2003) 'The role of the yeast cleavage and

polyadenylation factor subunit Ydh1p/Cft2p in pre-mRNA 3'-end formation', *Nucleic Acids Res,* 31(14), pp. 3936-45.

Lange, A., Mills, R. E., Devine, S. E. and Corbett, A. H. (2008) 'A PY-NLS nuclear targeting signal is required for nuclear localization and function of the Saccharomyces cerevisiae mRNA-binding protein Hrp1', *J Biol Chem,* 283(19), pp. 12926-34.

Lee, M. S., Henry, M. and Silver, P. A. (1996) 'A protein that shuttles between the nucleus and the cytoplasm is an important mediator of RNA export', *Genes Dev,* 10(10), pp. 1233-46.

Lee, T. I. and Young, R. A. (2000) 'Transcription of eukaryotic protein-coding genes', *Annu Rev Genet,* 34, pp. 77-137.

Leeper, T. C., Qu, X., Lu, C., Moore, C. and Varani, G. (2010) 'Novel protein-protein contacts facilitate mRNA 3'-processing signal recognition by Rna15 and Hrp1', *J Mol Biol,* 401(3), pp. 334-49.

Lei, E. P., Krebber, H. and Silver, P. A. (2001) 'Messenger RNAs are recruited for nuclear export during transcription', *Genes Dev,* 15(14), pp. 1771-82.

Lemay, J. F. and Bachand, F. (2015) 'Fail-safe transcription termination: Because one is never enough', *RNA Biol,* 12(9), pp. 927-32.

Lemay, J. F., D'Amours, A., Lemieux, C., Lackner, D. H., St-Sauveur, V. G., Bahler, J. and Bachand, F. (2010) 'The nuclear poly(A)-binding protein interacts with the exosome to promote synthesis of noncoding small nucleolar RNAs', *Mol Cell,* 37(1), pp. 34-45.

Lemieux, B., Blanchette, M., Monette, A., Mouland, A. J., Wellinger, R. J. and Chabot, B. (2015) 'A Function for the hnRNP A1/A2 Proteins in Transcription Elongation', *PLoS One,* 10(5), pp. e0126654.

Lemke, E. A. (2016) 'The Multiple Faces of Disordered Nucleoporins', *J Mol Biol,* 428(10 Pt A), pp. 2011-24.

Lewis, A., Felberbaum, R. and Hochstrasser, M. (2007) 'A nuclear envelope protein linking nuclear pore basket assembly, SUMO protease regulation, and mRNA surveillance', *J Cell Biol,* 178(5), pp. 813-27.

Lewis, J. D. and Izaurralde, E. (1997) 'The role of the cap structure in RNA processing and nuclear export', *Eur J Biochem,* 247(2), pp. 461-9.

Li, C., Goryaynov, A. and Yang, W. (2016) 'The selective permeability barrier in the nuclear pore complex', *Nucleus,* 7(5), pp. 430-446.

Lin, S., Coutinho-Mansfield, G., Wang, D., Pandit, S. and Fu, X. D. (2008) 'The splicing factor SC35 has an active role in transcriptional elongation', *Nat Struct Mol Biol,* 15(8), pp. 819-26.

Liu, Q., Greimann, J. C. and Lima, C. D. (2006) 'Reconstitution, activities, and structure of the eukaryotic RNA exosome', *Cell,* 127(6), pp. 1223-37.

Liu, X., Hoque, M., Larochelle, M., Lemay, J. F., Yurko, N., Manley, J. L., Bachand, F. and Tian, B. (2017) 'Comparative analysis of alternative polyadenylation in S. cerevisiae and S. pombe', *Genome Res,* 27(10), pp. 1685-1695.

Lu, Y. Y. and Krebber, H. (2021) 'Nuclear mRNA Quality Control and Cytoplasmic NMD Are Linked by the Guard Proteins Gbp2 and Hrb1', *Int J Mol Sci,* 22(20).

Mandart, E. and Parker, R. (1995) 'Effects of mutations in the Saccharomyces cerevisiae RNA14, RNA15, and PAP1 genes on polyadenylation in vivo', *Mol Cell Biol,* 15(12), pp. 6979-86.

Mandel, C. R., Bai, Y. and Tong, L. (2008) 'Protein factors in pre-mRNA 3'-end processing', *Cell Mol Life Sci,* 65(7-8), pp. 1099-122.

Mapendano, C. K., Lykke-Andersen, S., Kjems, J., Bertrand, E. and Jensen, T. H. (2010) 'Crosstalk between mRNA 3' end processing and transcription initiation', *Mol Cell,* 40(3), pp. 410-22.

Marfatia, K. A., Crafton, E. B., Green, D. M. and Corbett, A. H. (2003) 'Domain analysis of the

Saccharomyces cerevisiae heterogeneous nuclear ribonucleoprotein, Nab2p. Dissecting the requirements for Nab2p-facilitated poly(A) RNA export', *J Biol Chem,* 278(9), pp. 6731-40.
Martinez-Lumbreras, S., Santiveri, C. M., Mirassou, Y., Zorrilla, S. and Perez-Canadillas, J. M. (2013) 'Two singular types of CCCH tandem zinc finger in Nab2p contribute to polyadenosine RNA recognition', *Structure,* 21(10), pp. 1800-11.
Martinez-Lumbreras, S., Taverniti, V., Zorrilla, S., Seraphin, B. and Perez-Canadillas, J. M. (2016) 'Gbp2 interacts with THO/TREX through a novel type of RRM domain', *Nucleic Acids Res,* 44(1), pp. 437-48.
Maslon, M. M., Heras, S. R., Bellora, N., Eyras, E. and Caceres, J. F. (2014) 'The translational landscape of the splicing factor SRSF1 and its role in mitosis', *Elife*, pp. e02028.
McBride, A. E., Cook, J. T., Stemmler, E. A., Rutledge, K. L., McGrath, K. A. and Rubens, J. A. (2005) 'Arginine methylation of yeast mRNA-binding protein Npl3 directly affects its function, nuclear export, and intranuclear protein interactions', *J Biol Chem,* 280(35), pp. 30888-98.
Meinel, D. M., Burkert-Kautzsch, C., Kieser, A., O'Duibhir, E., Siebert, M., Mayer, A., Cramer, P., Soding, J., Holstege, F. C. and Strasser, K. (2013) 'Recruitment of TREX to the transcription machinery by its direct binding to the phospho-CTD of RNA polymerase II', *PLoS Genet,* 9(11), pp. e1003914.
Meinhart, A. and Cramer, P. (2004) 'Recognition of RNA polymerase II carboxy-terminal domain by 3'-RNA-processing factors', *Nature,* 430(6996), pp. 223-6.
Mili, S., Shu, H. J., Zhao, Y. and Pinol-Roma, S. (2001) 'Distinct RNP complexes of shuttling hnRNP proteins with pre-mRNA and mRNA: candidate intermediates in formation and export of mRNA', *Mol Cell Biol,* 21(21), pp. 7307-19.
Minvielle-Sebastia, L., Beyer, K., Krecic, A. M., Hector, R. E., Swanson, M. S. and Keller, W. (1998) 'Control of cleavage site selection during mRNA 3' end formation by a yeast hnRNP', *EMBO J,* 17(24), pp. 7454-68.
Moqtaderi, Z., Geisberg, J. V., Jin, Y., Fan, X. and Struhl, K. (2013) 'Species-specific factors mediate extensive heterogeneity of mRNA 3' ends in yeasts', *Proc Natl Acad Sci U S A,* 110(27), pp. 11073-8.
Moreno-Morcillo, M., Minvielle-Sebastia, L., Fribourg, S. and Mackereth, C. D. (2011) 'Locked tether formation by cooperative folding of Rna14p monkeytail and Rna15p hinge domains in the yeast CF IA complex', *Structure,* 19(4), pp. 534-45.
Muddukrishna, B., Jackson, C. A. and Yu, M. C. (2017) 'Protein arginine methylation of Npl3 promotes splicing of the SUS1 intron harboring non-consensus 5' splice site and branch site', *Biochim Biophys Acta Gene Regul Mech,* 1860(6), pp. 730-739.
Nedea, E., He, X., Kim, M., Pootoolal, J., Zhong, G., Canadien, V., Hughes, T., Buratowski, S., Moore, C. L. and Greenblatt, J. (2003) 'Organization and function of APT, a subcomplex of the yeast cleavage and polyadenylation factor involved in the formation of mRNA and small nucleolar RNA 3'-ends', *J Biol Chem,* 278(35), pp. 33000-10.
Neve, J., Patel, R., Wang, Z., Louey, A. and Furger, A. M. (2017) 'Cleavage and polyadenylation: Ending the message expands gene regulation', *RNA Biol,* 14(7), pp. 865-890.
Niepel, M., Molloy, K. R., Williams, R., Farr, J. C., Meinema, A. C., Vecchietti, N., Cristea, I. M., Chait, B. T., Rout, M. P. and Strambio-De-Castillia, C. (2013) 'The nuclear basket proteins Mlp1p and Mlp2p are part of a dynamic interactome including Esc1p and the proteasome', *Mol Biol Cell,* 24(24), pp. 3920-38.
Oh, J., Xu, J., Chong, J. and Wang, D. (2019) 'Structural and biochemical analysis of DNA lesion-induced RNA polymerase II arrest', *Methods,* 159-160, pp. 29-34.
Ohnacker, M., Barabino, S. M., Preker, P. J. and Keller, W. (2000) 'The WD-repeat protein pfs2p bridges two essential factors within the yeast pre-mRNA 3'-end-processing complex', *EMBO J,* 19(1), pp. 37-47.

Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M. and Reinberg, D. (1999) 'The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins', *Nature,* 400(6741), pp. 284-8.

Otero, G., Fellows, J., Li, Y., de Bizemont, T., Dirac, A. M., Gustafsson, C. M., Erdjument-Bromage, H., Tempst, P. and Svejstrup, J. Q. (1999) 'Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation', *Mol Cell,* 3(1), pp. 109-18.

Ozsolak, F., Kapranov, P., Foissac, S., Kim, S. W., Fishilevich, E., Monaghan, A. P., John, B. and Milos, P. M. (2010) 'Comprehensive polyadenylation site maps in yeast and human reveal pervasive alternative polyadenylation', *Cell,* 143(6), pp. 1018-29.

Palancade, B., Zuccolo, M., Loeillet, S., Nicolas, A. and Doye, V. (2005) 'Pml39, a novel protein of the nuclear periphery required for nuclear retention of improper messenger ribonucleoparticles', *Mol Biol Cell,* 16(11), pp. 5258-68.

Perez-Canadillas, J. M. (2006) 'Grabbing the message: structural basis of mRNA 3'UTR recognition by Hrp1', *EMBO J,* 25(13), pp. 3167-78.

Plaschka, C., Newman, A. J. and Nagai, K. (2019) 'Structural Basis of Nuclear pre-mRNA Splicing: Lessons from Yeast', *Cold Spring Harb Perspect Biol,* 11(5).

Poornima, G., Srivastava, G., Roy, B., Kuttanda, I. A., Kurbah, I. and Rajyaguru, P. I. (2021) 'RGG-motif containing mRNA export factor Gbp2 acts as a translation repressor', *RNA Biol*, pp. 1-12.

Proudfoot, N. (2004) 'New perspectives on connecting messenger RNA 3' end formation to transcription', *Curr Opin Cell Biol,* 16(3), pp. 272-8.

Ramanathan, A., Robb, G. B. and Chan, S. H. (2016) 'mRNA capping: biological functions and applications', *Nucleic Acids Res,* 44(16), pp. 7511-26.

Reznik, B., Clement, S. L. and Lykke-Andersen, J. (2014) 'hnRNP F complexes with tristetraprolin and stimulates ARE-mRNA decay', *PLoS One,* 9(6), pp. e100992.

Rondon, A. G., Mischo, H. E., Kawauchi, J. and Proudfoot, N. J. (2009) 'Fail-safe transcriptional termination for protein-coding genes in S. cerevisiae', *Mol Cell,* 36(1), pp. 88-98.

Rose, M., Winston, F., and Hieter, P. (1991) *Methods in Yeast Genetics — A Laboratory Course Manual.* Biochem. Educ. *19*, 101–102.

Rout, M. P. and Wente, S. R. (1994) 'Pores for thought: nuclear pore complex proteins', *Trends Cell Biol,* 4(10), pp. 357-65.

Roy, K., Gabunilas, J., Gillespie, A., Ngo, D. and Chanfreau, G. F. (2016) 'Common genomic elements promote transcriptional and DNA replication roadblocks', *Genome Res,* 26(10), pp. 1363-1375.

Russo, P., Li, W. Z., Hampsey, D. M., Zaret, K. S. and Sherman, F. (1991) 'Distinct cis-acting signals enhance 3' endpoint formation of CYC1 mRNA in the yeast Saccharomyces cerevisiae', *EMBO J,* 10(3), pp. 563-71.

Saguez, C., Gonzales, F. A., Schmid, M., Boggild, A., Latrick, C. M., Malagon, F., Putnam, A., Sanderson, L., Jankowsky, E., Brodersen, D. E. and Jensen, T. H. (2013) 'Mutational analysis of the yeast RNA helicase Sub2p reveals conserved domains required for growth, mRNA export, and genomic stability', *RNA,* 19(10), pp. 1363-71.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual. Cold Spring Harbor laboratory press.*

Sandhu, R., Sinha, A. and Montpetit, B. (2021) 'The SR-protein Npl3 is an essential component of the meiotic splicing regulatory network in Saccharomyces cerevisiae', *Nucleic Acids Res,* 49(5), pp. 2552-2568.

Sanford, J. R., Gray, N. K., Beckmann, K. and Caceres, J. F. (2004) 'A novel role for shuttling SR proteins in mRNA translation', *Genes Dev,* 18(7), pp. 755-68.

Saroufim, M. A., Bensidoun, P., Raymond, P., Rahman, S., Krause, M. R., Oeffinger, M. and Zenklusen, D. (2015) 'The nuclear basket mediates perinuclear mRNA scanning in budding yeast', *J Cell Biol,* 211(6), pp. 1131-40.

Schmidt, K. and Butler, J. S. (2013) 'Nuclear RNA surveillance: role of TRAMP in controlling exosome specificity', *Wiley Interdiscip Rev RNA,* 4(2), pp. 217-31.

Schneider, C. and Tollervey, D. (2013) 'Threading the barrel of the RNA exosome', *Trends Biochem Sci,* 38(10), pp. 485-93.

Schwabish, M. A. and Struhl, K. (2007) 'The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo', *Mol Cell Biol,* 27(20), pp. 6987-95.

Segref, A., Sharma, K., Doye, V., Hellwig, A., Huber, J., Luhrmann, R. and Hurt, E. (1997) 'Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)+ RNA and nuclear pores', *EMBO J,* 16(11), pp. 3256-71.

Sen, R., Barman, P., Kaja, A., Ferdoush, J., Lahudkar, S., Roy, A. and Bhaumik, S. R. (2019) 'Distinct Functions of the Cap-Binding Complex in Stimulation of Nuclear mRNA Export', *Mol Cell Biol,* 39(8).

Senay, C., Ferrari, P., Rocher, C., Rieger, K. J., Winter, J., Platel, D. and Bourne, Y. (2003) 'The Mtr2-Mex67 NTF2-like domain complex. Structural insights into a dual role of Mtr2 for yeast nuclear export', *J Biol Chem,* 278(48), pp. 48395-403.

Shen, E. C., Henry, M. F., Weiss, V. H., Valentini, S. R., Silver, P. A. and Lee, M. S. (1998) 'Arginine methylation facilitates the nuclear export of hnRNP proteins', *Genes Dev,* 12(5), pp. 679-91.

Shen, E. C., Stage-Zimmermann, T., Chui, P. and Silver, P. A. (2000) '7The yeast mRNA-binding protein Npl3p interacts with the cap-binding complex', *J Biol Chem,* 275(31), pp. 23718-24.

Sherman, F. (2002) 'Getting started with yeast', *Methods Enzymol,* 350, pp. 3-41.

Shukla, S. and Parker, R. (2014) 'Quality control of assembly-defective U1 snRNAs by decapping and 5'-to-3' exonucleolytic digestion', *Proc Natl Acad Sci U S A,* 111(32), pp. E3277-86.

Sikorski, R. S. and Hieter, P. (1989) 'A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae', *Genetics,* 122(1), pp. 19-27.

Singh, P., Chaudhuri, A., Banerjea, M., Marathe, N. and Das, B. (2021) 'Nrd1p identifies aberrant and natural exosomal target messages during the nuclear mRNA surveillance in Saccharomyces cerevisiae', *Nucleic Acids Res,* 49(20), pp. 11512-11536.

Singleton, D. R., Chen, S., Hitomi, M., Kumagai, C. and Tartakoff, A. M. (1995) 'A yeast protein that bidirectionally affects nucleocytoplasmic transport', *J Cell Sci,* 108 (Pt 1), pp. 265-72.

Soheilypour, M. and Mofrad, M. R. (2016) 'Regulation of RNA-binding proteins affinity to export receptors enables the nuclear basket proteins to distinguish and retain aberrant mRNAs', *Sci Rep,* 6, pp. 35380.

Soheilypour, M. and Mofrad, M. R. K. (2018) 'Quality control of mRNAs at the entry of the nuclear pore: Cooperation in a complex molecular system', *Nucleus,* 9(1), pp. 202-211.

Soniat, M., Sampathkumar, P., Collett, G., Gizzi, A. S., Banu, R. N., Bhosle, R. C., Chamala, S., Chowdhury, S., Fiser, A., Glenn, A. S., Hammonds, J., Hillerich, B., Khafizov, K., Love, J. D., Matikainen, B., Seidel, R. D., Toro, R., Rajesh Kumar, P., Bonanno, J. B., Chook, Y. M. and Almo, S. C. (2013) 'Crystal structure of human Karyopherin beta2 bound to the PY-NLS of Saccharomyces cerevisiae Nab2', *J Struct Funct Genomics,* 14(2), pp. 31-5.

Soucek, S., Corbett, A. H. and Fasken, M. B. (2012) 'The long and the short of it: the role of the zinc finger

polyadenosine RNA binding protein, Nab2, in control of poly(A) tail length', *Biochim Biophys Acta,* 1819(6), pp. 546-54.

Soucek, S., Zeng, Y., Bellur, D. L., Bergkessel, M., Morris, K. J., Deng, Q., Duong, D., Seyfried, N. T., Guthrie, C., Staley, J. P., Fasken, M. B. and Corbett, A. H. (2016) 'The Evolutionarily-conserved Polyadenosine RNA Binding Protein, Nab2, Cooperates with Splicing Machinery to Regulate the Fate of pre-mRNA', *Mol Cell Biol,* 36(21), pp. 2697-2714.

Spingola, M., Grate, L., Haussler, D. and Ares, M., Jr. (1999) 'Genome-wide bioinformatic and molecular analysis of introns in Saccharomyces cerevisiae', *RNA,* 5(2), pp. 221-34.

Sprague, G. F., Jr. (1991) 'Assay of yeast mating reaction', *Methods Enzymol,* 194, pp. 77-93.

Strasser, K., Masuda, S., Mason, P., Pfannstiel, J., Oppizzi, M., Rodriguez-Navarro, S., Rondon, A. G., Aguilera, A., Struhl, K., Reed, R. and Hurt, E. (2002) 'TREX is a conserved complex coupling transcription with messenger RNA export', *Nature,* 417(6886), pp. 304-8.

Strawn, L. A., Shen, T. and Wente, S. R. (2001) 'The GLFG regions of Nup116p and Nup100p serve as binding sites for both Kap95p and Mex67p at the nuclear pore complex', *J Biol Chem,* 276(9), pp. 6445-52.

Stumpf, G. and Domdey, H. (1996) 'Dependence of yeast pre-mRNA 3'-end processing on CFT1: a sequence homolog of the mammalian AAUAAA binding factor', *Science,* 274(5292), pp. 1517-20.

Svejstrup, J. Q. (2002) 'Chromatin elongation factors', *Curr Opin Genet Dev,* 12(2), pp. 156-61.

Swartz, J. E., Bor, Y. C., Misawa, Y., Rekosh, D. and Hammarskjold, M. L. (2007) 'The shuttling SR protein 9G8 plays a role in translation of unspliced mRNA containing a constitutive transport element', *J Biol Chem,* 282(27), pp. 19844-53.

Terry, L. J. and Wente, S. R. (2009) 'Flexible gates: dynamic topologies and functions for FG nucleoporins in nucleocytoplasmic transport', *Eukaryot Cell,* 8(12), pp. 1814-27.

Thiebaut, M., Kisseleva-Romanova, E., Rougemaille, M., Boulay, J. and Libri, D. (2006) 'Transcription termination and nuclear degradation of cryptic unstable transcripts: a role for the nrd1-nab3 pathway in genome surveillance', *Mol Cell,* 23(6), pp. 853-64.

Tian, B. and Graber, J. H. (2012) 'Signals for pre-mRNA cleavage and polyadenylation', *Wiley Interdiscip Rev RNA,* 3(3), pp. 385-96.

Torchet, C., Bousquet-Antonelli, C., Milligan, L., Thompson, E., Kufel, J. and Tollervey, D. (2002) 'Processing of 3'-extended read-through transcripts by the exosome can generate functional mRNAs', *Mol Cell,* 9(6), pp. 1285-96.

Torvund-Jensen, J., Steengaard, J., Reimer, L., Fihl, L. B. and Laursen, L. S. (2014) 'Transport and translation of MBP mRNA is regulated differently by distinct hnRNP proteins', *J Cell Sci,* 127(Pt 7), pp. 1550-64.

Truant, R., Fridell, R. A., Benson, R. E., Bogerd, H. and Cullen, B. R. (1998) 'Identification and functional characterization of a novel nuclear localization signal present in the yeast Nab2 poly(A)+ RNA binding protein', *Mol Cell Biol,* 18(3), pp. 1449-58.

Tuck, A. C. and Tollervey, D. (2013) 'A transcriptome-wide atlas of RNP composition reveals diverse classes of mRNAs and lncRNAs', *Cell,* 154(5), pp. 996-1009.

Tudek, A., Porrua, O., Kabzinski, T., Lidschreiber, M., Kubicek, K., Fortova, A., Lacroute, F., Vanacova, S., Cramer, P., Stefl, R. and Libri, D. (2014) 'Molecular basis for coordinating transcription termination with noncoding RNA degradation', *Mol Cell,* 55(3), pp. 467-81.

Turner, R. E., Harrison, P. F., Swaminathan, A., Kraupner-Taylor, C. A., Goldie, B. J., See, M., Peterson, A. L., Schittenhelm, R. B., Powell, D. R., Creek, D. J., Dichtl, B. and Beilharz, T. H. (2021) 'Genetic and

pharmacological evidence for kinetic competition between alternative poly(A) sites in yeast', *Elife,* 10.
Turtola, M., Manav, M. C., Kumar, A., Tudek, A., Mroczek, S., Krawczyk, P. S., Dziembowski, A., Schmid, M., Passmore, L. A., Casanal, A. and Jensen, T. H. (2021) 'Three-layered control of mRNA poly(A) tail synthesis in Saccharomyces cerevisiae', *Genes Dev,* 35(17-18), pp. 1290-1303.
Tutucci, E. and Stutz, F. (2011) 'Keeping mRNPs in check during assembly and nuclear export', *Nat Rev Mol Cell Biol,* 12(6), pp. 377-84.
Valentini, S. R., Weiss, V. H. and Silver, P. A. (1999) 'Arginine methylation and binding of Hrp1p to the efficiency element for mRNA 3'-end formation', *RNA,* 5(2), pp. 272-80.
van Helden, J., del Olmo, M. and Perez-Ortin, J. E. (2000) 'Statistical analysis of yeast genomic downstream sequences reveals putative polyadenylation signals', *Nucleic Acids Res,* 28(4), pp. 1000-10.
van Hoof, A., Lennertz, P. and Parker, R. (2000) 'Yeast exosome mutants accumulate 3'-extended polyadenylated forms of U4 small nuclear RNA and small nucleolar RNAs', *Mol Cell Biol,* 20(2), pp. 441-52.
Vasiljeva, L. and Buratowski, S. (2006) 'Nrd1 interacts with the nuclear exosome for 3' processing of RNA polymerase II transcripts', *Mol Cell,* 21(2), pp. 239-48.
Vinciguerra, P., Iglesias, N., Camblong, J., Zenklusen, D. and Stutz, F. (2005) 'Perinuclear Mlp proteins downregulate gene expression in response to a defect in mRNA export', *EMBO J,* 24(4), pp. 813-23.
Vo, L. T., Minet, M., Schmitter, J. M., Lacroute, F. and Wyers, F. (2001) 'Mpe1, a zinc knuckle protein, is an essential component of yeast cleavage and polyadenylation factor required for the cleavage and polyadenylation of mRNA', *Mol Cell Biol,* 21(24), pp. 8346-56.
Wahle, E. and Ruegsegger, U. (1999) '3'-End processing of pre-mRNA in eukaryotes', *FEMS Microbiol Rev,* 23(3), pp. 277-95.
Wegener, M. and Muller-McNicoll, M. (2018) 'Nuclear retention of mRNAs - quality control, gene regulation and human disease', *Semin Cell Dev Biol,* 79, pp. 131-142.
Will, C. L. and Luhrmann, R. (2011) 'Spliceosome structure and function', *Cold Spring Harb Perspect Biol,* 3(7).
Windgassen, M. and Krebber, H. (2003) 'Identification of Gbp2 as a novel poly(A)+ RNA-binding protein involved in the cytoplasmic delivery of messenger RNAs in yeast', *EMBO Rep,* 4(3), pp. 278-83.
Windgassen, M., Sturm, D., Cajigas, I. J., Gonzalez, C. I., Seedorf, M., Bastians, H. and Krebber, H. (2004) 'Yeast shuttling SR proteins Npl3p, Gbp2p, and Hrb1p are part of the translating mRNPs, and Npl3p can function as a translational repressor', *Mol Cell Biol,* 24(23), pp. 10479-91.
Wittschieben, B. O., Fellows, J., Du, W., Stillman, D. J. and Svejstrup, J. Q. (2000) 'Overlapping roles for the histone acetyltransferase activities of SAGA and elongator in vivo', *EMBO J,* 19(12), pp. 3060-8.
Wlotzka, W., Kudla, G., Granneman, S. and Tollervey, D. (2011) 'The nuclear RNA polymerase II surveillance system targets polymerase III transcripts', *EMBO J,* 30(9), pp. 1790-803.
Wong, C. M., Tang, H. M., Kong, K. Y., Wong, G. W., Qiu, H., Jin, D. Y. and Hinnebusch, A. G. (2010) 'Yeast arginine methyltransferase Hmt1p regulates transcription elongation and termination by methylating Npl3p', *Nucleic Acids Res,* 38(7), pp. 2217-28.
Xiang, S., Cooper-Morgan, A., Jiao, X., Kiledjian, M., Manley, J. L. and Tong, L. (2009) 'Structure and function of the 5'-->3' exoribonuclease Rat1 and its activating partner Rai1', *Nature,* 458(7239), pp. 784-8.
Xie, Y., Clarke, B. P., Kim, Y. J., Ivey, A. L., Hill, P. S., Shi, Y. and Ren, Y. (2021) 'Cryo-EM structure of the yeast TREX complex and coordination with the SR-like protein Gbp2', *Elife,* 10.
Xie, Y. and Ren, Y. (2019) 'Mechanisms of nuclear mRNA export: A structural perspective', *Traffic,* 20(11),

pp. 829-840.
Yao, P., Wu, J., Lindner, D. and Fox, P. L. (2017) 'Interplay between miR-574-3p and hnRNP L regulates VEGFA mRNA translation and tumorigenesis', *Nucleic Acids Res,* 45(13), pp. 7950-7964.
Zander, G., Hackmann, A., Bender, L., Becker, D., Lingner, T., Salinas, G. and Krebber, H. (2016) 'mRNA quality control is bypassed for immediate export of stress-responsive transcripts', *Nature,* 540(7634), pp. 593-596.
Zander, G. and Krebber, H. (2017) 'Quick or quality? How mRNA escapes nuclear quality control during stress', *RNA Biol,* 14(12), pp. 1642-1648.
Zenklusen, D., Vinciguerra, P., Strahm, Y. and Stutz, F. (2001) 'The yeast hnRNP-Like proteins Yra1p and Yra2p participate in mRNA export through interaction with Mex67p', *Mol Cell Biol,* 21(13), pp. 4219-32.
Zhao, J., Hyman, L. and Moore, C. (1999) 'Formation of mRNA 3' ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis', *Microbiol Mol Biol Rev,* 63(2), pp. 405-45.
Zhu, J., Mayeda, A. and Krainer, A. R. (2001) 'Exon identity established through differential antagonism between exonic splicing silencer-bound hnRNP A1 and enhancer-bound SR proteins', *Mol Cell,* 8(6), pp. 1351-61.

8. Acknowledgement

At this point, I would like to express my gratitude to everyone who helped in the achievement of this work. A special thanks to the China Scholarship Council for offering me a great opportunity to experience PhD study in Germany.

I would like to thank my supervisor, Prof. Dr. Heike Krebber, and express my deepest appreciation. Thank you for offering me the opportunity to work on the interesting topic of mRNA quality control and supervising me during my PhD study.

Sincere acknowledgements to Dr. Oliver Valerius and to PD Dr. Wilfried Kramer. Thank you for your valuable advice on each TAC meeting. I particularly thank Dr. Oliver Valerius for reviewing my PhD thesis.

I feel deeply grateful to all of my colleagues. Thank you all for your academic advice and all your support. I especially thank Luisa Querl for joining this project at a later time and doing a great collaboration with me. Extensive thanks to Markus Röder. Thank you for helping me overcome difficulties in cloning. Special thanks to Dr. Yen-yun Lu. I was highly inspired to organize all the thoughts in the discussion.

Last but not least, many thanks to my parents, my brother, and all of my friends. Thank you for your support and motivation.