Chapter 4
The Nuclear RNA Exosome and Its Cofactors

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Abstract The RNA exosome is a highly conserved ribonuclease endowed with 3′–5′ exonuclease and endonuclease activities. The multisubunit complex resides in both the nucleus and the cytoplasm, with varying compositions and activities between the two compartments. While the cytoplasmic exosome functions mostly in mRNA quality control pathways, the nuclear RNA exosome partakes in the 3′-end processing and complete decay of a wide variety of substrates, including virtually all types of noncoding (nc) RNAs. To handle these diverse tasks, the nuclear exosome engages with dedicated cofactors, some of which serve as activators by stimulating decay through oligoA addition and/or RNA helicase activities or, as adaptors, by recruiting RNA substrates through their RNA-binding capacities. Most nuclear exosome cofactors contain the essential RNA helicase Mtr4 (MTR4 in humans). However, apart from Mtr4, nuclear exosome cofactors have undergone significant evolutionary divergence. Here, we summarize biochemical and functional knowledge about the nuclear exosome and exemplify its cofactor variety by discussing the best understood model organisms—the budding yeast *Saccharomyces cerevisiae*, the fission yeast *Schizosaccharomyces pombe*, and human cells.

Keywords RNA exosome · Nuclear RNA decay · Exosome cofactors · Polyadenylation · TRAMP · NEXT · PAXT

4.1 The RNA Exosome

4.1.1 The Core

The central core of the RNA exosome is barrel-shaped and composed of six RNase PH-like proteins that form a ring. This ring associates with 3 S1/KH RNA-binding

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M. Oeffinger, D. Zenklusen (eds.), *The Biology of mRNA: Structure and Function*,
Advances in Experimental Medicine and Biology 1203,
https://doi.org/10.1007/978-3-030-31434-7_4

domain containing proteins positioned on one end of the barrel, typically pictured as the "top" (Januszyk and Lima 2014; Liu et al. 2006; Lorentzen et al. 2007). The structure of the resulting 9 protein subunit core complex, termed Exo9, is very similar to eubacterial exonucleases RNase PH and PNPase, and the archaeal RNA exosome, which all have active phosphorolytic exonuclease sites positioned in the central cavity of a characteristic barrel-shaped structure (Januszyk and Lima 2014). In contrast, most eukaryotic Exo9 homologs have altered active site residues, which results in a catalytically inactive exosome core (Dziembowski et al. 2007; Liu et al. 2006). Notable exceptions are plants and some early-branching nonplant eukaryotes, where one of the RNase PH domains has retained phosphorolytic activity (Sikorska et al. 2017).

4.1.2 Catalytic Subunits

To compensate for the widespread loss of activity within their cores, eukaryotic exosomes assemble with the processive 3′–5′ exonuclease and endonuclease Dis3 (often also referred to as Rrp44; DIS3 in humans) and the distributive 3′–5′ exonuclease Rrp6 (EXOSC10 in humans), which bind to the bottom and the top of the core exosome, respectively (Dziembowski et al. 2007; Makino et al. 2013; Mitchell et al. 1997; Wasmuth et al. 2014; Zinder et al. 2016). Exosome complexes comprising Dis3 or Dis3 plus Rrp6 are commonly referred to as Exo10 and Exo11, respectively. Dis3 receives RNAs that are threaded down through the central channel of the exosome core, whereas Rrp6 accesses RNA from the exosome top without threading through the core structure (Kowalinski et al. 2016; Liu et al. 2016; Makino et al. 2013; Wasmuth et al. 2014; Zinder et al. 2016). Even so, Rrp6 functions are intimately linked with the exosome core, and its position close to the entry site of the central channel is consistent with data, suggesting that Rrp6 may control RNA threading to Dis3 (Makino et al. 2015; Wasmuth et al. 2014). Conversely, core KH domain proteins contribute to the binding of RNAs processed by Rrp6 (Zinder et al. 2016). In addition, Rrp6 and its partner Rrp47 provide critical binding surfaces for exosome cofactors, such as Mtr4 (Fig. 4.1) (Falk et al. 2017a; Schuch et al. 2014).

Dis3 and Rrp6 association with the exosome core varies to some extent between organisms and subcellular compartments. Both budding and fission yeasts possess a single Dis3 and Rrp6 paralog, with Rrp6 being exclusively nuclear, while Dis3 is present on both nuclear and cytoplasmic exosomes (Allmang et al. 1999; Mitchell et al. 1997). The situation is more complex for higher eukaryotes; the human genome, for example, encodes three different Dis3 paralogs: DIS3, "DIS3 like" (DIS3L), and DIS3L2. While DIS3 and DIS3L inhabit nuclear and cytoplasmic exosomes, respectively (Staals et al. 2010; Tomecki et al. 2010), DIS3L2 exercises cytoplasmic 3′–5′ exonucleolytic activities independent of the core exosome (Chang et al. 2013; Lubas et al. 2013; Malecki et al. 2013). Moreover, even though the single human Rrp6 paralog EXOSC10 is primarily nuclear, some cytoplasmic presence has also been reported (Brouwer et al. 2001; Lejeune et al. 2003; Tomecki et al. 2010).

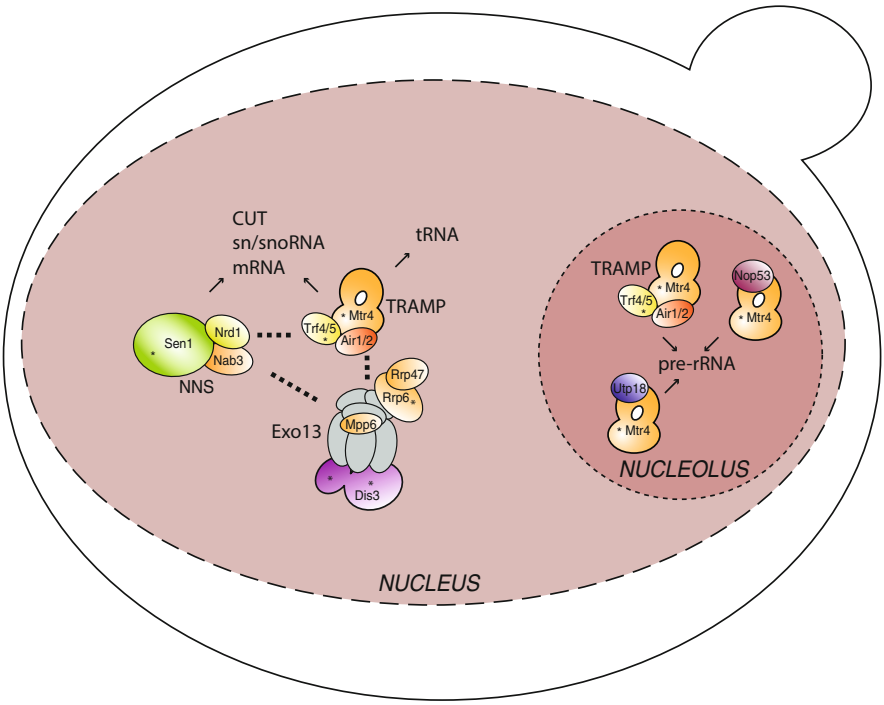

Fig. 4.1 The exosome and its cofactors in the *S. cerevisiae* nucleus. The major exosome cofactor in *S. cerevisiae* nuclei is the TRAMP complex (Mtr4, Air1/Air2, and Trf4/Trf5), which acts in the nucleoplasm and nucleolus. In the nucleoplasm, the NNS adaptor complex (Nrd1, Nab3, Sen1) is important for exosome targeting of all major RNAPII transcript classes, such as CUTs, sn/snoRNAs, and mRNAs. In nucleoli, TRAMP and the Mtr4-interacting proteins Nop53 and Utp18 recruit the exosome for the processing of rRNA precursors and the decay of processing by-products. TRAMP also facilitates decay of hypomodified tRNA. Asterisks denote enzymatic activities. See text for more detail

The mechanisms determining the subcellular fractioning of exosomes and which nucleases they carry still remain to be elucidated.

4.1.3 Lrp1 and Mpp6

In addition to the core and catalytic components, the Lrp1 (often also referred to as Rrp47, C1D in humans) and Mpp6 (MPP6 in humans) proteins are considered constituents of the nuclear exosome, yielding Exo13 (Makino et al. 2015; Milligan et al. 2008; Mitchell et al. 2003; Schilders et al. 2005; Schuch et al. 2014; Wasmuth et al. 2017). Both Lrp1 and Mpp6 are nuclear restricted and were originally proposed to act as exosome adaptors by facilitating exosome access to specific substrates either by direct RNA binding or by contacting specific RNP components (Milligan

et al. 2008; Schilders et al. 2005). More recent structural studies position both proteins on top of the exosome core, in close contact with Mtr4, suggesting that they contribute to exosome core function by aiding the Mtr4–exosome interaction and its RNA threading activity (Fig. 4.1) (Makino et al. 2015; Schuch et al. 2014; Wasmuth et al. 2017; Falk et al. 2017a). Lrp1 binds to, and stabilizes, Rrp6, wherefore its in vivo functions are largely overlapping those of Rrp6 (Mitchell et al. 2003). Mpp6, on the other hand, contacts other exosome core subunits, but it is curiously enough only associated in substoichiometric amounts, suggesting a more specialized function (Schilders et al. 2005; Shi et al. 2015). Recently, budding yeast Mpp6 was suggested to promote RNA threading to Dis3, whereas Mpp6 absence would result in the threading-independent decay by Rrp6 (Kim et al. 2016). Whether such an Mpp6-mediated switch in decay mechanism is general and conserved in other organisms remains to be determined.

4.2 RNA Helicase Activities Central to Exosome Function: Mtr4/Ski2

While the various RNA exosome assemblies outlined above in principle can bind and degrade RNA, efficient activity and substrate recognition depend on additional protein complexes, with RNA helicases of the Mtr4/Ski2 (MTR4 (SKIV2L2)/SKIV2L in humans) family playing central roles (Johnson and Jackson 2013; Zinder and Lima 2017). Binding to the top of the exosome, these proteins hand RNA substrates to the exosome core, possibly using the RNA helicase activity to inject the substrate for threading down to Dis3 or for presenting the RNA to Rrp6 (Falk et al. 2017a; Halbach et al. 2013; Zinder et al. 2016). Critically, Mtr4/Ski2 are also part of other complexes, containing so-called adaptor proteins, which serve to directly recognize exosome substrates (see below). Despite some commonalities, these complexes have diverged considerably within and between different eukaryotic species. Ski2 homologs are generally cytoplasmic, while Mtr4 homologs are nuclear (Zinder and Lima 2017). As this chapter focuses on nuclear exosome biology, the next sections will describe the different Mtr4-containing complexes in the three model organisms of choice.

4.3 *S. cerevisiae*

4.3.1 The TRAMP and NNS Complexes

The *S. cerevisiae* Trf4–Air2–Mtr4 polyadenylation (TRAMP) and Nrd1–Nab3–Sen1 (NNS) complexes were among the first nuclear exosome cofactors to be discovered (Fig. 4.1) (LaCava et al. 2005; Vanacova et al. 2005; Wyers et al.

2005). TRAMP consists of Mtr4, the poly(A) polymerase Trf4, and the RNA-binding protein Air2. Trf4 and Air2 can be replaced by their paralogs Trf5 and Air1, yielding different possible TRAMP compositions (LaCava et al. 2005; Vanacova et al. 2005; Wyers et al. 2005). Current evidence suggests that at least a subset of these different TRAMP complexes are present in vivo and serve partially nonoverlapping functions (San Paolo et al. 2009; Schmidt et al. 2012). The molecular contribution of TRAMP complexes to exosome activity is believed to involve the addition of short A-tails to RNA 3′ ends by the Trf4/5 enzymes (Schmidt and Butler 2013; Zinder and Lima 2017). Consistently, in wild-type cells, TRAMP targets can be found carrying short (~4 nt) oligo(A) tails, which are lost in Trf4/5 mutants but accumulate upon exosome inactivation (Jia et al. 2011; Tuck and Tollervey 2013; Wlotzka et al. 2011). These short unstructured tails are then suggested to facilitate the loading of RNA 3′ ends by Mtr4 to promote the exosomal threading of otherwise structured RNAs. The Zn-finger containing Air1/2 proteins may provide RNA-binding capacity to the TRAMP complex, while also promoting overall complex stability through cooperative binding with Trf4/5 to Mtr4 (Falk et al. 2014; Hamill et al. 2010).

In budding yeast, TRAMP engages in a wide variety of nuclear exosome functions, including the decay of aberrant tRNA, processing of rRNA precursors, and decay of processing by-products (Schmidt and Butler 2013). In these cases, the combined adenylation and helicase activities of TRAMP may allow for the decay of otherwise highly structured substrates resilient to exosomal attack. Moreover, TRAMP also targets RNA polymerase II (RNAPII) products, e.g., facilitating decay of the so-called cryptic unstable transcripts (CUTs; see below) and the 3′ trimming of snRNA and snoRNA precursors (Schmidt and Butler 2013). These latter exosome substrates are unlikely to be highly structured, reflecting that TRAMP might also serve as an RNA-binding adaptor, in addition to its role as enzymatic activator.

How does TRAMP get in contact with RNA? At least some targeting capacity is likely to be mediated by direct RNA contacts via the Air proteins (Holub et al. 2012; Schmidt and Butler 2013; Schmidt et al. 2012). However, in the case of RNAPII-produced substrates, target recognition is often mediated by the NNS complex through the sequence-specific RNA-binding domains of the Nrd1 and Nab3 proteins (Tudek et al. 2014; Vasiljeva and Buratowski 2006; Wlotzka et al. 2011). Nrd1 further contains a so-called C-terminal domain (CTD) interaction domain (CID), which specifically binds the Ser5P phosphorylated CTD of the largest subunit of RNAPII, while also directly binding to the TRAMP complex component Trf4 (Gudipati et al. 2008; Tudek et al. 2014; Vasiljeva et al. 2008). Hence, the NNS complex associates with both, early elongating RNAPII, and the TRAMP and exosome complexes (Fig. 4.1). In doing so, it serves two functions: (1) promoting transcription termination of RNAPII from short transcription units (TUs), and (2) channeling 3′ ends derived from such termination events for TRAMP/exosome-mediated trimming or decay (Arigo et al. 2006; Gudipati et al. 2008; Schulz et al. 2013; Steinmetz et al. 2006; Thiebaut et al. 2006; Tudek et al. 2014). The contemporary view suggests that NNS function relies on the binding of Nrd1

and Nab3 to their respective RNA recognition sites during early transcription (Porrua and Libri 2015). This likely involves the interaction of Nrd1 with RNAPII, since the CTD Ser5-P modification is most prominent at TU 5′ ends. Since the helicase activity of Sen1 can promote the disassembly of RNAPII transcription complexes in vitro (Porrua and Libri 2013), this explains the termination function of the NNS complex. After transcription termination, NNS supposedly "hands" the resulting transcript to the RNA exosome via the Nrd1–Trf4 interaction (Tudek et al. 2014). However, disruption of this interaction causes only a moderate stabilization of NNS targets (Tudek et al. 2014). Moreover, Nrd1/Nab3 can directly contact the exosome components Mpp6 and Rrp6 independent of TRAMP (Fasken et al. 2015; Kim et al. 2016). Thus, TRAMP appears to not be strictly required for exosome association with NNS targets but may rather serve to promote degradation of transcripts that are not directly amenable to exosomal decay.

Exosome removal of NNS-targeted transcripts is highly efficient, and typically, these RNAs are only revealed in NNS-, TRAMP-, or exosome-depleted cells, hence their nomenclature as CUTs (Neil et al. 2009; Wyers et al. 2005; Xu et al. 2009). The RNA sequence motifs recognized by Nrd1 and Nab3 are short and abundantly present in the *S. cerevisiae* genome but conspicuously absent from the coding strand of protein-coding genes (Cakiroglu et al. 2016; Schulz et al. 2013). This explains how the NNS complex discriminates the numerous RNAs produced by spurious transcription, either bidirectionally from gene promoters or antisense to mRNAs, from protein-coding transcripts. At sn/snoRNA TUs, NNS activity facilitates the production of short stable RNAs. This is presumably due to the highly structured and protein-bound nature of mature sn/snoRNAs, which stops RNA exosome progress after its initial removal (processing) of the unstructured 3′ extensions (Coy et al. 2013). CUTs do not assemble stable structures and are thus completely decayed.

Although the NNS and TRAMP complexes were long believed to target only ncRNAs, recent data revealed NNS and Mtr4 interaction with a host of mRNAs whose expression changes in response to glucose depletion (Bresson et al. 2017). This suggests the interesting possibility that mRNAs can be targeted for nuclear decay and that this can be regulated in a stimulus-specific manner. Such potential re-purposing of the NNS complex from ncRNA to mRNA targeting is consistent with an earlier study, showing that Nrd1 is dephosphorylated during nutrient depletion and that this influences nutrient-dependent protein-coding gene expression (Darby et al. 2012). However, whether Nrd1/Mtr4 mRNA targeting elicits decay and if so, how such regulation may occur remains to be determined.

4.3.2 Nucleolar Exosome Cofactors

In addition to its role in TRAMP, *S. cerevisiae* Mtr4 also interacts directly with the nucleolar proteins Nop53 and Utp18 (Fig. 4.1, "NUCLEOLUS"). This occurs through the so-called arch domain of Mtr4, which binds a conserved short sequence motif, the arch interaction motif (AIM) (Falk et al. 2017b; Thoms et al. 2015).

Nop53 is a component of nuclear ribosomal pre-60S particles, which contain 5.8S rRNA precursors, and its interaction with Mtr4 is required for the exosomal trimming of 3′ extensions of 5.8S pre-rRNAs. Utp18, instead, is part of ribosomal pre-90S particles and takes part in the release, and Mtr4-dependent decay, of the nonfunctional 5′ external transcribed spacer (5′ETS) (Thoms et al. 2015). Interestingly, TRAMP is also implicated in 5′ETS removal (Houseley and Tollervey 2006), but the functional relationship between Mtr4's action in the context of TRAMP and together with Utp18 has not been disentangled. Assembly of the TRAMP complex does not depend on the Mtr4 arch domain, and it is therefore possible that Utp18 recruits Mtr4 as part of the TRAMP complex for 5′ETS decay (Falk et al. 2017b; Thoms et al. 2015). At the same time, there are nonessential direct contacts between the Mtr4 arch domain and Air2 within TRAMP (Falk et al. 2017b), suggesting that Air2 and Utp18 interactions with Mtr4p influence each other to control 5′ETS decay.

4.4 *S. pombe*

4.4.1 TRAMP

The composition of the fission yeast TRAMP complex is overall similar to its budding yeast paralog with subunits Cid14 (homologous to Trf4/5), Air1, and Mtr4 (Fig. 4.2, "NUCLEOLUS") (Keller et al. 2010). Compared to *S. cerevisiae*, *S. pombe* TRAMP appears to be a more specialized exosome cofactor, still implicated in the processing or decay of nucleolar substrates but with a less general role in the nucleoplasm (Larochelle et al. 2012; Win et al. 2006). Consistently, a functional analog of the *S. cerevisiae* NNS complex has not been identified (Lemay et al. 2016; Wittmann et al. 2017). Instead, Cid14 and the exosome subunit Rrp6 were shown to be involved in RNAi-independent heterochromatin formation processes, pointing toward a still ill-defined link between decay of heterochromatin-derived transcripts and the deposition of chromatin marks (Buhler et al. 2007; Keller et al. 2010; Reyes-Turcu et al. 2011; Wang et al. 2008).

4.4.2 MTREC

To engage in nuclear activities outside of nucleoli, the fission-yeast specific nucleoplasmic-residing Mtr4 paralog, called Mtr4-like 1 (Mtl1), forms a tight complex with the Zn-finger protein Red1 (Fig. 4.2). This dimer then interacts with numerous other proteins to form higher-order complexes termed MTREC (Mtl1–Red1 core) or NURS (nuclear RNA silencing) (Egan et al. 2014; Lee et al. 2013; Zhou et al. 2015). Red1 is required for MTREC's association with the *S. pombe* exosome, supposedly compensating for Mtl1's loss of a specific N-terminal domain required for Mtr4:Rrp6/Lsd1 interaction (Schuch et al. 2014; Zhou et al. 2015). The

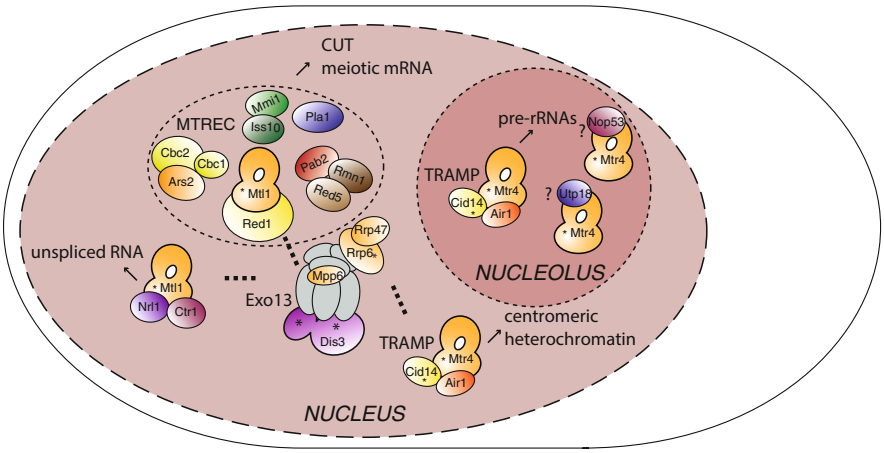

Fig. 4.2 The exosome and its cofactors in the *S. pombe* nucleus. Mtl1 and Red1 form the core of the major exosome cofactors in the *S. pombe* nucleoplasm. MTREC comprises Mtl1–Red1 and a number of other protein complexes, including nCBC (Cbc1, Cbc2, Ars2); a subcomplex comprising Mmi1 and Iss10; a subcomplex of Pab2, Rmn1, and Red5; and the poly(A) polymerase Pla1. MTREC targets include meiosis-specific RNAs during vegetative growth but also other RNAPII-derived transcripts, such as CUTs. In addition to MTREC, Mtl1 is part of a complex including Ctr1 and Nrl1, which also binds the exosome and is involved in decay of unspliced RNAs. The *S. pombe* TRAMP complex functions in the nucleoplasm where it targets centromeric heterochromatin and in the nucleolus where it is involved in rRNA processing. Utp18 and Nop53 homologs contain AIM domains and are conserved, and therefore also likely to act as exosome cofactors via Mtr4. Asterisks denote enzymatic activities. Question marks are used to symbolize that roles of Utp18 and Nop53 have not been demonstrated in *S. pombe*. See text for more detail

MTREC complex comprises a number of other proteins, including Red5, Iss10, Mmi1, poly(A)-binding protein Pab2, poly(A) polymerase Pla1, and the nuclear mRNA 5′ cap-binding complex (nCBC) proteins (Egan et al. 2014; Lee et al. 2013; Zhou et al. 2015) (Fig. 4.2). The presence of Pla1 and Pab2 in MTREC might provide a means to add and recognize A-tails of MTREC targets, independent of TRAMP. At the same time, Pla1 (and probably Pab2) are also required for the production of regular mRNAs that normally need to avoid nuclear decay. How this distinction is achieved is presently under intense investigation (see below).

MTREC should not be seen as a single well-defined complex, but rather comprises a number of functionally distinct subcomplexes associating around the Mtl1–Red1 core (Fig. 4.2). Mtl1 also engages in a Red1-independent complex with the Nrl1 and Ctr1 proteins, which interact with the splicing machinery and seem to be specifically involved in exosomal decay of unspliced transcripts, including those containing so-called cryptic introns (Lee et al. 2013; Zhou et al. 2015).

Interestingly, homologs of many MTREC components are also cofactors of the human RNA exosome (Table 4.1) and several harbor RNA-binding domains, which might contribute to target recognition. An example is the sequence-specific RNA-binding protein Mmi1, which serves a highly specific role in the targeting of

Table 4.1 Exosome components and cofactors

Complex	*S. cerevisiae*	*S. pombe*	Human	Domains
Exo13	Csl4	Csl4	EXOSC1	S1
	Rrp4	Rrp4	EXOSC2	S1/KH
	Rrp40	Rrp40	EXOSC3	S1/KH
	Rrp41	Rrp41	EXOSC4	RNase PH
	Rrp46	Rrp46	EXOSC5	RNase PH
	Mtr3	Mtr3	EXOSC6	RNase PH
	Rrp42	Rrp42	EXOSC7	RNase PH
	Rrp43	Rrp43	EXOSC8	RNase PH
	Rrp45	Rrp45	EXOSC9	RNase PH
	Rrp6	Rrp6	EXOSC10	3′–5′ exonuclease (RNase D)
	Dis3 (Rrp44)	Dis3	DIS3, DIS3L	3′–5′ exonuclease (RNase II), PIN endonuclease domain
	Lrp1 (Rrp47)	Cti1	C1D (LRP1)	C1D
	Mpp6	Mpp6	MPP6	–
Mtr4	Mtr4	Mtr4 Mtl1	MTREX (MTR4, SKIV2L2)	ATP-dependent RNA helicase
TRAMP	Trf4, Trf5	Cid14	PAPD5 (TRF4-2)	poly(A) polymerase
	Air1, Air2	Air1	ZCCHC7 (AIR1)	Zn-knuckle
NNS	Nrd1	Seb1[#]	? SCAF4, SCAF8	RRM, CID
	Nab3	Nab3[#]	? RALY	RRM
	Sen1	Sen1[#]	SETX (ALS4, AOA2)[#]	ATP-dependent RNA helicase
NEXT			RBM7	RRM
			ZCCHC8	Zn-finger
nCBC	? Cbc2 (Cbp20)	Cbc2	NCBP2 (CBP20)	RRM
	? Sto1 (Cbp80)	Cbc1	NCBP1 (CBP80)	–
		Pir2	SRRT (ARS2)	Zn-finger
			ZC3H18	Zn-finger
MTREC/ PAXT		Red1	ZFC3H1	Zn-finger
		Red5	? ZC3H3	Zn-finger
		Mmi1	? YTHDF1/2/3	YTH domain
		Iss10	ZFC3H1 N terminus	–
		Rmn1	? RBM26, RBM27	RRM
	Pap1	Pla1	? PAPOLA, PAPOLG	Poly(A) polymerase
		Pab2	PABPN1	RRM (poly(A) binding)
–		Ctr1	? CCDC174	–
		Nrl1	? NRDE2	–

(continued)

Table 4.1 (continued)

Complex	*S. cerevisiae*	*S. pombe*	Human	Domains
–	Utp18	? Utp18	? UTP18 (WDR50)	WDR40, AIM
	Nop53	? Rrp16	? NOP53 (GLTSCR2)	AIM
–	? Rix7	? Rix7	NVL (NVL2)	AAA ATPase

List of exosome components and cofactors from *S. cerevisiae*, *S. pombe*, and human cells. Listed are standard gene names from the *S. cerevisiae* and *S. pombe* genome databases (www.yeastgenome.org and www.pombase.org) as well as approved symbols for human genes from the "HUGO" Gene Nomenclature Committee (www.genenames.org). Alternative, commonly used names are in parenthesis. Sequence homologs which are not proposed to have a functional connection with the nuclear RNA exosome are marked with "#," and sequence homologs where a functional connection to the exosome is possible but not yet demonstrated are marked with "?"

meiosis-specific mRNAs during *S. pombe* vegetative growth (Harigaya et al. 2006). This silencing is partly achieved by the posttranscriptional decay of these transcripts mediated by Mmi1–MTREC binding to cognate sites in the target RNAs and their subsequent handover to the nuclear exosome (Chen et al. 2011; Harigaya et al. 2006; Yamashita et al. 2012). In addition to posttranscriptional decay, silencing of meiosis-specific genes involves the formation of heterochromatic islands around affected loci, and the MTREC complex is involved in the deposition of repressive chromatin marks (Egan et al. 2014; Lee et al. 2013). This activity is independent on Cid14 and therefore provides a unique link between RNA decay and heterochromatin formation in *S. pombe* that has not been reported in other organisms.

4.5 Human

4.5.1 TRAMP

A TRAMP-like complex, although still poorly characterized, also exists in human cells and is composed of MTR4, the poly(A) polymerase PAPD5, and the Zn-finger protein ZCCHC7 (Lubas et al. 2011). This complex, hTRAMP, localizes to nucleoli, and its depletion mainly results in phenotypes affecting nucleolar substrates (Lubas et al. 2011), which is consistent with the presence of distinct adaptor complexes serving exosome functions in the nucleoplasm. This yields a conceptually similar setup as for *S. pombe* (Fig. 4.3). MTR4 also interacts with other nucleolar proteins, such as NVL, which promotes pre-rRNA processing, and the human homologs of *S. cerevisiae* Nop53 and Utp18, suggesting that these proteins are also exosome cofactors in human rRNA metabolism (Lubas et al. 2011; Yoshikatsu et al. 2015).

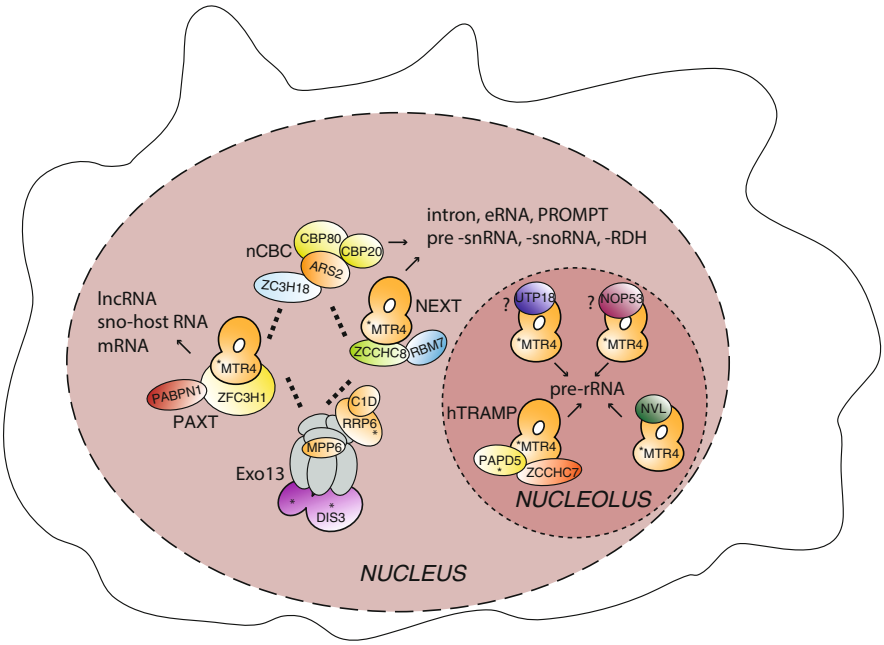

Fig. 4.3 The exosome and its cofactors in the human cell nucleus. Human exosome cofactors NEXT (MTR4, ZCCHC8, and RBM7) and PAXT (MTR4, ZFC3H1, and PABPN1) are present in the nucleoplasm. PAXT targets poly(A)$^+$ lncRNAs, including the subclass hosting intronic snoRNAs ("sno-host RNA"), and mRNAs. NEXT facilitates decay of numerous unstable transcripts (i.e., some PROMPTs, eRNAs, and spliced-out introns) and the 3′ processing of pre-snRNAs, pre-snoRNAs, and replication-dependent histone (RDH)-encoding mRNAs. Both PAXT and NEXT bind to the CBCA complex (CBC80, CBC20, ARS2) via the protein ZC3H18, but a functional role of CBCA in exosomal decay has primarily been shown for NEXT targets. Cofactors in human nucleoli include hTRAMP (MTR4, ZCCHC7, PAPD5) and the MTR4-interacting protein NVL. In addition, human MTR4 associates with NOP53 and UTP18, suggesting a conserved role of these proteins, even though the AIM domain is only conserved in NOP53. Nucleolar cofactors facilitate pre-rRNA processing and decay of processing by-products. Asterisks denote enzymatic activities. Question marks are used to symbolize that roles of UTP18 and NOP53 have not been demonstrated in humans. See text for more detail

4.5.2 NEXT

The nuclear exosome targeting (NEXT) complex is presently the best-characterized human exosome adaptor complex. It consists of the RNA-binding protein RBM7, linked to MTR4 by the Zn-finger protein ZCCHC8 (Lubas et al. 2011) (Fig. 4.3). NEXT facilitates the exosomal decay of many promoter-upstream transcripts (PROMPTs, also called upstream antisense (ua)RNAs) and other labile ncRNAs, like enhancer RNAs (eRNAs) (Lubas et al. 2011, 2015; Meola et al. 2016). Moreover, it mediates the exosomal trimming of 3′-end extensions of snRNAs, snoRNAs, and histone-encoding mRNAs (Lubas et al. 2011, 2015).

Human snRNAs and histone-encoding mRNAs are produced from autonomous TUs using specialized transcription termination mechanisms based on the Integrator and CPSF complexes, respectively (Guiro and Murphy 2017; Marzluff and Koreski 2017). In contrast, most human snoRNAs are hosted within the introns of pre-mRNAs and pre-ncRNAs, where from they are produced by the trimming of excised intron 5′ ends by the exonuclease XRN2 and 3′ ends by the exosome (Valen et al. 2011). RBM7 Individual-nucleotide resolution Cross-Linking and Immuno-Precipitation (iCLIP) experiments demonstrated that the protein promiscuously contacts RNAs in a manner unlikely to involve sequence-specific target recognition (Lubas et al. 2015). Thus, RBM7 binding appears to only be consequential in combination with the presence of an unprotected 3′-end. At the same time, RBM7 also interacts with the splicing factor SF3B2 (also termed SAP145), which likely underlies the enriched binding of RBM7 to intronic 3′ ends and explains how NEXT facilitates the exosomal decay of intronic regions (Falk et al. 2016).

Interestingly, RBM7 gets phosphorylated upon cellular UV damage, which debilitates the ability of the protein to bind RNA without otherwise affecting NEXT complex integrity (Blasius et al. 2014; Tiedje et al. 2015). This provides a first characterization of a posttranslational modification of an RNA exosome cofactor, and a further delineation of its physiological consequence(s) and mechanistic background will be revealing for how nuclear RNA decay might be regulated in response to external stimuli.

4.5.3 PAXT

A third human MTR4-containing complex assembles around the stable MTR4–ZFC3H1 dimer (Meola et al. 2016). ZFC3H1 and MTR4 depletions both lead to the accumulation of the mature products of some snoRNA host genes as well as numerous other nuclear transcripts (Meola et al. 2016; Ogami et al. 2017). Many ZFC3H1-specific targets are also stabilized upon depletion of the nuclear poly(A)-binding protein PABPN1, and exosomal decay depends on their polyadenylation by the canonical poly(A) polymerase PAP prompting the idea of a so-called PAP-mediated RNA decay (PPD) pathway (Beaulieu et al. 2012; Bresson and Conrad 2013; Bresson et al. 2015; Meola et al. 2016). The PPD pathway was originally suggested to primarily affect ncRNAs, but transcriptome-wide analysis of ZFC3H1 and PABPN1 inactivation indicated that mRNAs are also frequently targeted (Meola et al. 2016; Silla et al. 2018). Taken all evidence together, the emerging picture suggests that PABPN1 binding to RNA poly(A) tails will lead to recruitment of the exosome via MTR–ZFC3H1 unless the RNA manages to escape the nucleus (Meola and Jensen 2017). Consistently, PABPN1 associates with MTR4 in a ZFC3H1-dependent manner (Meola et al. 2016), yet, this interaction is less robust than that of the core MTR4–ZFC3H1 dimer, inspiring the proposition of a "poly(A) exosome targeting" (PAXT) connection, comprising MTR4, ZFC3H1, and PABPN1 (Meola et al. 2016) (Fig. 4.3). The term "connection" recognizes that this

is not a stable complex and the suboptimal binding of PABPN1 may indeed help explain how stable polyadenylated transcripts evade decay (see below).

ZFC3H1 and PABPN1 are the human homologs of the *S. pombe* Red1 and Pab2 MTREC components, suggesting an overall conserved function between PAXT and MTREC in the decay of polyadenylated nuclear RNA, including mRNA.

4.5.4 The Nuclear RNA Cap-Binding Complex

Both NEXT and PAXT components can be physically bridged to the nuclear 5′ cap-binding complex (nCBC) and nCBC proteins also co-IP the nuclear exosome (Andersen et al. 2013; Lubas et al. 2011; Meola et al. 2016). The link between NEXT/PAXT and the nCBC is mediated by the ZC3H18 protein, which further binds to the nCBC proteins Cbp20 and Cbp80 (also termed NCBP1 and NCBP2) via the protein ARS2 (also termed SRRT) (Giacometti et al. 2017) (Fig. 4.3). Individual depletion of all of these proteins leads to the stabilization of some nuclear exosome substrates, suggesting that nCBC in some instances contribute to exosome recruitment (Andersen et al. 2013; Iasillo et al. 2017). In addition, nCBC and ARS2, but neither ZC3H18 nor NEXT, are required for the efficient termination of RNAPII transcription at PROMPT, snRNA, and histone mRNA TUs, which suggests an active coupling between transcription termination and decay (Andersen et al. 2013; Iasillo et al. 2017). This is reminiscent of budding yeast NNS activity, which also promotes transcription termination before offering substrates to the exosome for decay. nCBC components are also part of *S. pombe* MTREC (Egan et al. 2014; Lee et al. 2013; Zhou et al. 2015), suggesting an omnipresent role of the RNA 5′ cap in facilitating nuclear 3′–5′ decay. While this at first sight seems counterproductive due to the unwanted targeting of capped RNAs with functional roles in the cell, it may indeed provide an important connection, enabling the quality control of capped transcripts.

4.6 Nuclear Decay vs. RNA Export

An emerging concept in RNA biology suggests that nuclear RNA decay, as described above, is in competition with RNA nuclear export, to prevent the cytoplasmic appearance of too many nonfunctional molecules. In line with this notion, there are clear indications that nuclear exosome cofactors impact RNA export. This is perhaps best exemplified by the nCBC and ARS2 (forming the CBCA complex), which also actively facilitates RNA export by interacting with the “phosphorylated adaptor for RNA export” (PHAX) protein (Giacometti et al. 2017; Hallais et al. 2013). Interestingly, this interaction is mutually exclusive with the binding of ZC3H18 to the CBC, which would otherwise bridge the CBCA complex to the exosome adaptors NEXT and PAXT (Giacometti et al. 2017). Moreover, the nuclear

mRNA export factor ALYREF binds at transcript 5′- and 3′-ends via interactions with the nCBC and PABPN1, respectively (Fan et al. 2017; Shi et al. 2017). As described above, both nCBC and PABPN1 also interact with PAXT and/or NEXT, indicating that RNA association with exosome cofactors is generally mutually exclusive with binding of export factors. Consistent with this idea, the PAXT component ZFC3H1 appears to be capable of retaining RNA exosome substrates in the nucleus, as upon depletion of ZFC3H1, numerous PAXT targets are now found in the cytoplasm where they may even engage in translation (Ogami et al. 2017; Silla et al. 2018). This ability of ZFC3H1 to counter untimely RNA export appears to reach beyond simply preventing the binding of export factors as exosome substrates accumulating in exosome-depleted cells concentrate in ZFC3H1-dependent subnuclear aggregates (Silla et al. 2018). ZFC3H1 contains long low complexity regions, suggesting a direct role of ZFC3H1 in forming such foci, which probably reflect RNP complexes formed to prevent their unsolicited export from the nucleus.

But what then decides how RNAs are chosen for decay or export? Targeting of polyadenylated RNAs for exosomal decay mediated by PAXT or MTREC involves PABP recruitment, which occurs not only on exosome targets but also on to stable mRNAs. This conundrum has inspired the so-called nuclear timer model, where PABPs serve to initially protect poly(A) tailed RNA only later to elicit decay of transcripts that remain nuclear (Libri 2010; Meola and Jensen 2017). Mechanistically, this could be achieved through transient interactions of PABPN1/Pab2 with MTR4–ZFC3H1/Mtl1–Red and the exosome, leading to the slow assembly of a decay-promoting complex and avoiding decay of timely exported mRNAs (Meola et al. 2016).

4.7 Concluding Remarks

The nuclear RNA exosome partakes in the processing and/or decay of virtually all types of transcripts. Being able to handle such diverse tasks depends on exosome interaction with a number of adapter proteins as described above. This places the RNA exosome as a central player in cellular RNA metabolism. It may therefore come as no surprise that the RNA exosome and some of its cofactors have been linked to various disease states. For example, the exosome subunit DIS3 is recurrently found mutated in multiple myeloma, and mutations in several exosome core subunits are linked to inherited neurodegenerative diseases (Morton et al. 2017; Robinson et al. 2015). In addition, the RNA exosome and its cofactors also figure in the arms races occurring between viruses and their hosts. This is exemplified by influenza viruses, which, on one hand, have been shown to actively hijack the nuclear RNA exosome to produce RNA fragments required for priming transcription of their genomes (Rialdi et al. 2017), while, on the other hand, the cellular defense against other types of RNA viruses involves export of hTRAMP proteins to the cytoplasm to aid in the decay of viral RNA (Molleston et al. 2016). Although still

immature, these examples provide a glimpse of the central role of the nuclear RNA exosome in cell biology. While the composition and function of basic exosome machinery is now reasonably understood, much still remains to be learned about the regulation and cellular function of the various exosome cofactors and their relation to cell physiology in different systems.

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