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RNA-Degrading Exosome Complexes: Molecular Mechanisms and Structural Insights

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RNA exosome, nuclease, helicase, RNA processing, RNA degradation

Abstract

The RNA exosome is a conserved multiprotein complex essential for 3′-to-5′ RNA degradation in eukaryotic cells. In the cytoplasm, the exosome participates in messenger RNA surveillance and decay, while in the nucleus and nucleolus it performs a broader range of functions, from fully degrading cryptic RNAs generated by faulty or pervasive transcription to precisely trimming structured RNAs. An extended network of obligate cofactors and transient RNA helicase complexes has evolved to handle the large variety of substrates in each subcellular compartment. This network organizes in layers around the exosome core and regulates the irreversible 3′-to-5′ degradative action in synergy with the features of the substrates. In this review, we discuss findings derived from genetic, cellular, biochemical, and structural analyses of nuclear and cytoplasmic exosome complexes, and integrate them into molecular movies that illustrate the mechanistic principles of this versatile and dynamic machine in RNA processing and RNA decay.

Contents

1. INTRODUCTION

The lifespans of RNAs are determined by the balance between their rates of synthesis and degradation. Degradation is crucial not only for the turnover of normal, functional transcripts, but also for eliminating the many spurious or faulty transcripts that eukaryotic cells must contend with (**Figure 1**). In the cytoplasm, surveillance pathways linked to translating ribosomes monitor aberrant messenger RNAs (mRNAs), such as those with premature or missing stop codons, and direct their degradation, thus preventing the accumulation of malfunctioning proteins (Labno et al. 2016, Monaghan et al. 2023). The nucleus contains an even more active environment for RNA surveillance. Here, the error-prone nature of biogenesis processes for both coding and stable noncoding RNAs often results in improperly processed transcripts, and the pervasive nature of RNA polymerase II (Pol II) transcription leads to the abundant production of intergenic or antisense transcripts (Bresson & Tollervey 2018, Garland & Jensen 2024, Rambout & Maquat 2024). Yet, in opposition to these destructive roles, degradation can also serve a constructive function in the processing of RNA precursors into mature transcripts. An essential macromolecular machine, the RNA exosome, astoundingly operates across all these pathways, orchestrating precise partial trimming in RNA processing and total decay in RNA turnover and surveillance (Lingaraju et al. 2019b, Puno et al. 2019).

The RNA exosome was discovered in the late 1990s by David Tollervey's group while investigating the nuclear biogenesis of the 60S large ribosomal subunit in *Saccharomyces cerevisiae* (Mitchell et al. 1997). The name exosome reflected the 3′-to-5′ exoribonuclease activity responsible for the maturation of the 5.8S ribosomal RNA (rRNA) component. The five proteins originally identified as part of the exosome complex were named with the prefix Rrp to indicate their ribosomal-RNA-processing function (Mitchell et al. 1996). At the time, it was already evident that exosome subunits shared sequence similarities with bacterial 3′-to-5′ exoribonucleases (Mitchell et al. 1997). Human orthologs of yeast exosome subunits had already been identified as well. However, they were initially described as autoantigens from patients with scleroderma and polymyositis, indicating the first links to disease (Allmang et al. 1999b, Briggs et al. 1998, Brouwer et al. 2001). A quarter of a century later, the RNA exosome is now established as a juggernaut of nuclear and cytoplasmic RNA degradation across all eukaryotes studied to date—the equivalent of the proteasome to protein degradation (Chlebowski et al. 2013,

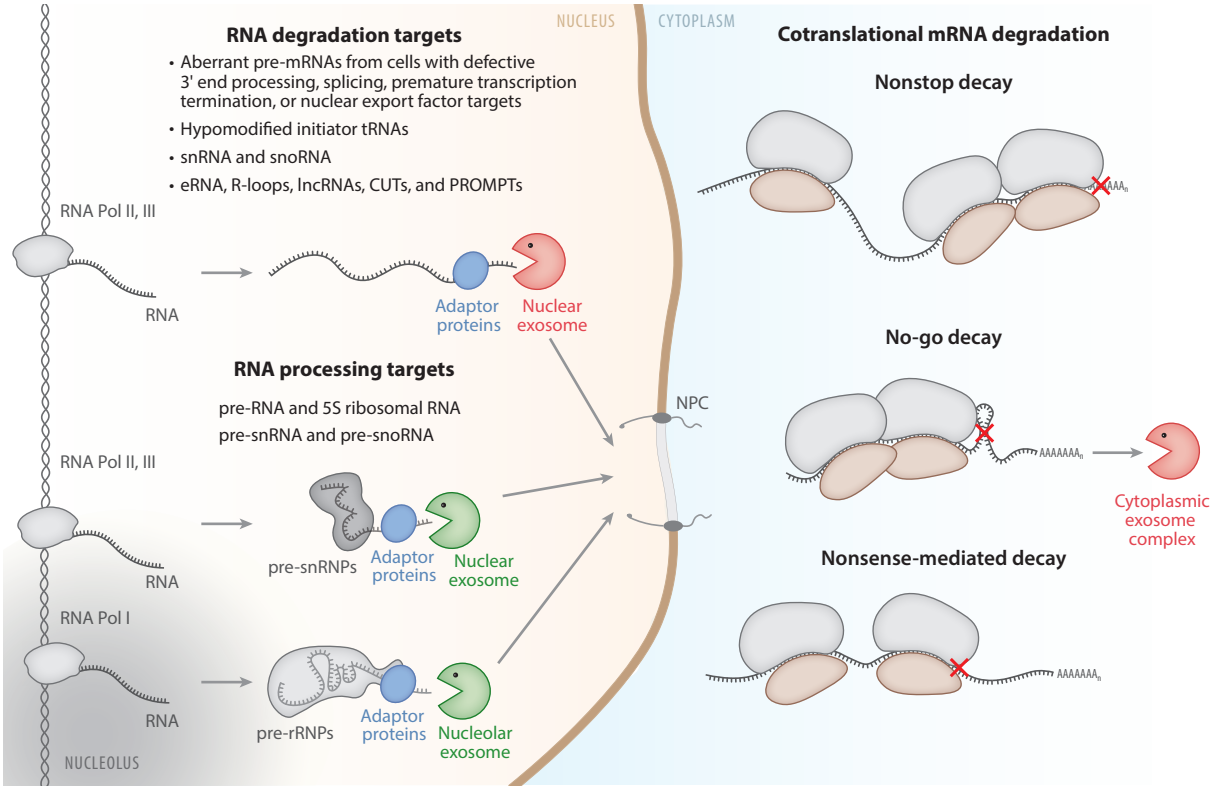


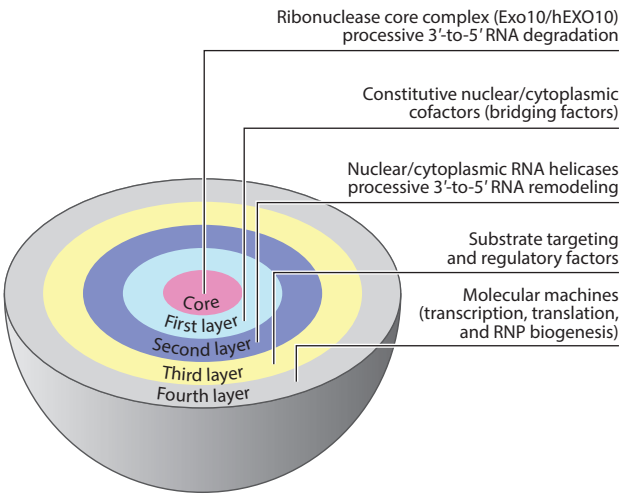
Figure 1

Schematic overview recapitulating the major RNA degradation pathways of RNA exosome complexes in the nucleus, nucleolus, and cytoplasm of eukaryotic cells. Different RNA degradation pathways lead to destructive decay functions (*red*) or constructive processing functions (*green*) of RNA exosome complexes. In the nucleus and the nucleolus (*left*), different RNA transcripts are recognized by a variety of adaptor proteins, whereas a single adaptor complex operates in the cytoplasm (*right*) to recognize the predominate substrate, mRNA. These adaptors (*blue*) are essential for substrate selection and the recruitment of the exosome. In the case of RNA degradation, the substrate is recognized and completely destroyed in a processive manner from the 3′ toward the 5′ end. In the case of RNA processing, the substrate is compacted in an RNP, which prevents its complete degradation and results in trimming of the respective RNA 3′ end. The exosome is released from the RNP after this processing step. Mature mRNAs are transported as mRNPs through the NPC to the cytoplasm. Here, they are engaged by the translation machinery (*gray* and *brown*) and translated into proteins. During this process, multiple factors (not shown for simplicity) monitor translation to identify aberrant mRNAs and target them to the cytoplasmic exosome complex for degradation. Abbreviations: CUTs, cryptic unstable transcripts; eRNA, enhancer RNA; lncRNA, long noncoding RNA; mRNA, messenger RNA; mRNP, messenger ribonucleoprotein; NPC, nuclear pore complex; Pol, polymerase; PROMPTs, promoter upstream transcripts; rRNP, ribosomal ribonucleoprotein; RNP, ribonucleoprotein; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; snoRNA, small nucleolar RNA; tRNA, transfer RNA.

Makino et al. 2013b, Zinder & Lima 2017). Many disease-associated mutations have been uncovered, particularly in the context of neuronal and autoimmune disorders as well as cancer (Bourgeois et al. 2018, Fasken et al. 2020, Ohguchi & Ohguchi 2023). In this review, we primarily focus on the budding yeast and human exosome complexes, as they have served as the main model systems for mechanistic investigations and exemplify both evolutionary conservation and species adaptation. Various genetically tractable model organisms, however, have been key for identifying exosome-interacting factors and functions, and we highlight selected contributions alongside comparisons with prokaryotic systems. Importantly, the exosome should not

be viewed as a single static complex but rather as a dynamic assembly existing in multiple forms. Each one is defined by cofactors and adaptors that associate with the exoribonuclease core in different subcellular compartments to enable the recognition of different substrates. These associated factors are centered on the recruitment and regulation of 3′-to-5′ RNA helicases and provide stringent regulation of substrate access—a crucial aspect given that removal of a ribonucleotide is irreversible. Building on insights from genetics, biochemistry, and structural biology, we discuss the molecular mechanisms governing nuclear and cytoplasmic exosome holocomplexes, proceeding from the inner ribonuclease (RNase) core to the outer regulatory layers (**Figure 2*a***).

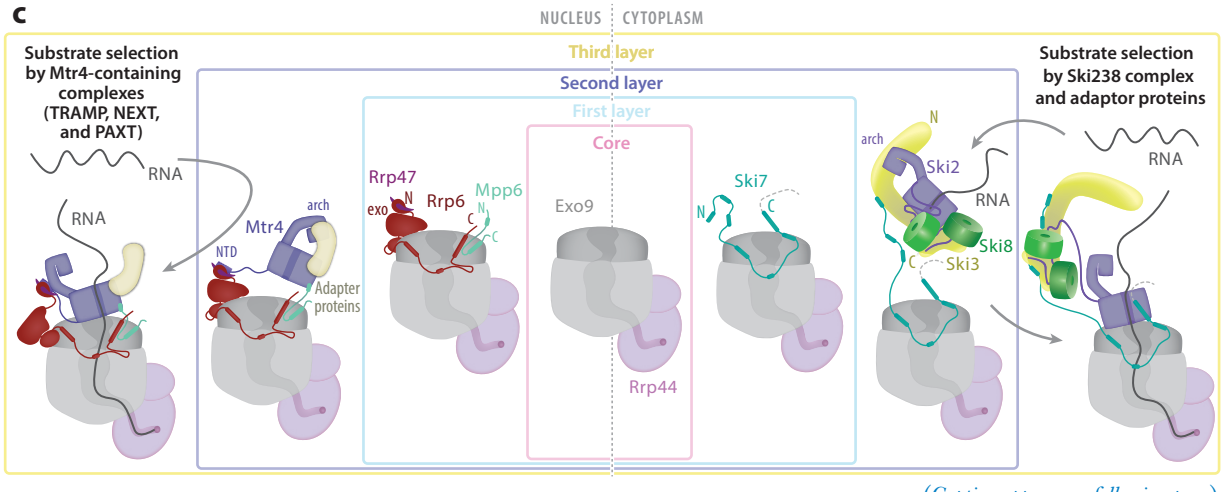
a



b

		S. cerevisiae	*H. sapiens*
Core	**Exo9 cap** S1/KH domain proteins	Csl4 and Ski4 Rrp4 Rrp40	EXOSC1 and hCSL4 EXOSC2 and hRRP4 EXOSC3 and hRRP40
	Exo9 ring/barrel S1/KH domain proteins	Rrp41 and Ski6 Rrp46 Mtr3 Rrp42 Rrp43 Rrp45	EXOSC4 and hRRP41 EXOSC5 and hRRP46 EXOSC6 and hMTR3 EXOSC7 and hRRP42 EXOSC8 and hRRP43 EXOSC9 and hRRP45
	Nuclease	Rrp44	hDIS3 and hDIS3L
First layer	**Nuclear**	Rrp6 Rrp47 and Lrp1 Mpp6	EXOSC10 and hRRP6 C1D hMPP6
	Cytoplasmic	Ski7	HBS1L3 and hSKI7
Second layer	**Nuclear** **Cytoplasmic**	Mtr4 Ski2	hMtr4, SKIV2L2, and MTREX hSKI2 and SKI2VL
Third layer	**Nuclear**	PAP2 and Trf4/5 Air1/2 Nop53 Utp18	PAPD5 and TENT4B ZCCHC7 NOP53 UTP18 NVL NRDE2 RBM7 ZCCHC8 ZFC3H1 RBM26/27 ZC3H3 PABPN1
	Cytoplasmic	Ski3 Ski8 Ska1	hSKI3 and TTC37 hSKI8 and WDR61q AVEN FOCAD

c



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Versatile compositions of nuclear and cytoplasmic exosome holo-complexes. (*a*) The exosome core complex interacts with different layers of cofactors and adaptors that lend their specific functions. The fourth layer is more general and does not involve a specific function, but rather a connection to major metabolic processes. (*b*) List of proteins belonging to the exosome core and the different layers from *Saccharomyces cerevisiae* and *Homo sapiens*. In the case of *H. sapiens*, the exonuclease hDIS3 is present in the nucleus and hDIS3L in the cytoplasm. Proteins grouped into the first, second, and third layers are further divided by localization to the nucleus or cytoplasm. While there is notable evolutionary conservation between the two species, a multitude of additional adaptors are present in *H. sapiens* (listed in the third layer). (*c*) Schematic overview of the nuclear and cytoplasmic exosome complexes in association with the compartment-specific adaptor proteins. All illustrations are based on current structural models from *S. cerevisiae*. The core consists of Exo9 (*dark gray* and *light gray*) in association with the exoribonuclease Rrp44 (*light purple*). The first layer contains the nuclear and cytoplasmic constitutive cofactors that serve to bridge the exosome to helicases (second layer) and adaptor proteins (third layer). Nuclear constitutive cofactors are Rrp6 (*red*), Rrp47 (*purple*), and Mpp6 (*turquoise*); and the cytoplasmic constitutive cofactor is Ski7 (*cyan*). Rrp6 contains an additional exoribonuclease domain (labeled exo), and the Rrp6 N terminus is intertwined with Rrp47. Ski7 has an additional C-terminal domain involved in the cotranslational surveillance pathway nonstop decay (*dotted line*). The second layer contains the nuclear and cytoplasmic helicases Mtr4 (*dark purple*) and Ski2 (*dark purple*), respectively. The large variety of nuclear adaptor proteins and complexes, which revolve around the Mtr4 helicase and are simplified by a single shape (*light yellow*), are part of different Mtr4-containing complexes. These adaptors can regulate the activity of Mtr4 by binding to the arch domain, blocking the Exo10-binding site, or blocking the RNA exit site. The cytoplasmic proteins Ski3 (*yellow*) and Ski8 (*green*) form a stable complex with the Ski2 helicase (termed the Ski complex). During substrate binding and upon ATP-dependent activation, the complex undergoes large conformational rearrangements involving the detachment of the Ski2 helicase module from the gatekeeping module (composed of the rest of the complex). Abbreviations: C, C terminus; N, N terminus; RNP, ribonucleoprotein. The drawing of the exosome and the Ski238 complex used in panel *c* was adapted from Kögel et al. (2024) (CC BY 4.0).

2. THE EXORIBONUCLEASE CORE COMPLEX

2.1. Exo9/hEXO9: The Catalytically Inactive RNA-Binding Cage

Exosome complexes in the nuclear, nucleolar, and cytoplasmic compartments share a structural framework known in yeast as Exo9. It includes nine evolutionarily conserved subunits: three so-called cap proteins with S1/KH domains (Rrp4, Csl4, and Rrp40) and three heterodimers formed from six RNase PH-like proteins (Rrp41-Rrp45, Rrp42-Mtr3, and Rrp43-Rrp46) (Liu et al. 2006, Lorentzen et al. 2005). The corresponding human orthologs are referred to either by their equivalent name in all capital letters prefixed with an "h" or as exosome components (EXOSC) (**Figure 2*b***). Depletion of any of these proteins leads to similar phenotypes and defects in the biogenesis of rRNA and small nuclear/nucleolar RNAs (sn/snoRNAs), but deletion of the corresponding genes is lethal in yeast (Allmang et al. 1999a,b). In humans, missense mutations in genes encoding hEXO9 proteins are linked to autosomal recessive disorders that typically result in abnormal neuronal development and/or degeneration, particularly of the cerebellum (Fasken et al. 2020). Structural modeling of amino acid substitutions suggests that disease mutations generally impair the stability or interactions of the exosome structural framework, while functional studies suggest that defects in ribosomal biogenesis and protein synthesis may underlie these diseases (Fasken et al. 2020).

The structural framework of Exo9/hEXO9 consists of three heterotrimers formed by the specific interactions between a cap protein and the corresponding RNase PH-like heterodimer (Liu et al. 2006). The three heterotrimers pack closely together with side-by-side interactions between the cap proteins and between the RNase PH-like proteins. This creates two stacked ring layers that collectively adopt an overall barrel-like shape (**Supplemental Video 1**). The yeast and human Exo9/hEXO9 structures are remarkably similar, the major difference being that hRRP45 contains a prominent C-terminal extension that wraps around the circumference of the barrel (Bonneau et al. 2009, Liu et al. 2006). In both species, the barrel has a rather narrow opening at the top of the S1/KH ring. The internal channel first extends vertically to reach the RNase PH-like ring and then bends horizontally within the Rrp41-Rrp45/hRRP41-hRRP45 heterodimer.

Supplemental Material >

This L-shaped internal channel is lined by evolutionarily conserved positively charged residues and functions to bind, encage, and direct RNA to the degradation site (Bonneau et al. 2009). The evolutionary conservation of the Exo9/hEXO9 barrel itself and its RNA-binding channel is also observed in prokaryotic exosome-like complexes, although the activities have diverged. Archaeal exosomes and bacterial PNPase have a simpler subunit composition and degrade RNA via a phosphorolytic mechanism (mediated by the corresponding Rrp41 subunit) (Buttner et al. 2005, Lorentzen & Conti 2005, Symmons et al. 2000). Although it was initially surprising, it is now well-established that in yeast, humans, and most other eukaryotes, all exosome RNase PH-like subunits have lost the ability to perform the phosphorolytic reaction due to subtle changes in their corresponding active sites (Dziembowski et al. 2007; Liu et al. 2006, 2007; Lorentzen et al. 2005). To acquire RNase activity, the Exo9/hEXO9 complex associates with a tenth subunit, the hydrolytic Rrp44/hDIS3/hDIS3L exoribonuclease (Exo10/hEXO10) (Bonneau et al. 2009, Dziembowski et al. 2007, Liu et al. 2006).

2.2. Rrp44/hDIS3/hDIS3L: The Processive 3′-to-5′ Exoribonuclease

S. cerevisiae Rrp44 interacts with Exo9 in both the nuclear and cytoplasmic compartments but is particularly enriched in the nucleolus (Okuda et al. 2020) (**Figure 2*b*,*c***). Rrp44 belongs to the RNB family of 3′-to-5′ exoribonucleases, similar to bacterial RNase II/R (Frazao et al. 2006). Its C-terminal exoribonuclease module contains the catalytic RNB domain and three OB-fold nucleic acid–binding domains (S1, CSD1, and CSD2). Although located at opposite ends in the sequence, the OB folds are positioned on the same side of the RNB domain in the three-dimensional structure. The CSD1 and RNB domains guide the RNA substrate toward the entrance of the catalytic chamber (Lorentzen et al. 2008). Rrp44 has an additional N-terminal region that tethers the RNase to the lower part of the Exo9 RNase-PH-like ring (Bonneau et al. 2009, Makino et al. 2013a). More specifically, the Rrp44 N-terminal region contains an enlarged PIN domain and binds Rrp41-Rrp42 near the exit of the Exo9 channel (Bonneau et al. 2009, Schaeffer et al. 2012). When bound to RNA, the Rrp44 RNase module repositions around the RNA exit site of Exo9, forming a continuous single RNA-binding channel. This pathway spans more than 160 Å and accommodates an RNA of approximately 25–30 ribonucleotides in length (Makino et al. 2013a). A corresponding RNA-binding footprint was verified with in vitro biochemical data from RNase protection assays (Bonneau et al. 2009). In support, a 5.8S rRNA maturation defect was identified by in vivo studies showing that the last 30-nucleotide region of its precursor rRNA remains unprocessed in the absence of nuclear exosome cofactors (Briggs et al. 1998). The Rrp44 PIN domain features a characteristic endoribonuclease active site, albeit exhibiting rather modest activity (Lebreton et al. 2008, Schaeffer et al. 2009, Schneider et al. 2009). In vivo disruption of the Rrp44 endoribonuclease site exacerbates the severe phenotype resulting from impairing the Rrp44 exoribonuclease site but has minor effects on its own (Lebreton et al. 2008, Schaeffer et al. 2009, Schneider et al. 2009). Overall, the current data suggest that the activity of the Rrp44 PIN domain mostly functions as a backup to the exoribonuclease.

Humans encode three distinct Rrp44 paralogs: hDIS3, hDIS3L, and hDIS3L2. The human hDIS3 paralog is localized in the nucleus and is the closest ortholog to yeast Rrp44 (Staals et al. 2010, Tomecki et al. 2010). *DIS3* is one of the most frequently mutated genes in multiple myeloma, with pathogenic mutations distributed throughout the protein (Ohguchi & Ohguchi 2023). At the molecular level, the mechanism by which the N-terminal region of hDIS3 binds hEXO9 is evolutionarily conserved (Gerlach et al. 2018, Weick et al. 2018). Yet, the interaction is weaker and exhibits fewer contacts compared to yeast (Gerlach et al. 2018), rationalizing why this association was difficult to detect by mass spectrometry (Staals et al. 2010, Tomecki et al. 2010, Weick et al.

2018). Another difference from yeast is that the exoribonuclease module does not reposition in the RNA-bound form. This arrangement guides the RNA to the catalytic chamber via a longer path between the CSD1 and S1 domains (Gerlach et al. 2018, Weick et al. 2018). This thus explains the longer RNA-binding footprints observed in vitro and the longer intermediate in the 5.8S rRNA maturation defect in human cells (Lubas et al. 2011, Tafforeau et al. 2013). Unlike yeast, hDIS3 is excluded from the nucleolus, though it remains unclear whether this exclusion is due to weak association properties with hEXO9 or a more direct mechanism (Staals et al. 2010, Tomecki et al. 2010).

The hDIS3L paralog is localized in the cytoplasm (Staals et al. 2010, Tomecki et al. 2010). hDIS3L also adopts an RNA-bound open conformation in the cytoplasmic exosome, but it has distinct features compared to the nuclear paralog (Kögel et al. 2024). First, hDIS3L is 20 times less abundant than hDIS3, perhaps reflecting the smaller pool of exosome substrates in the cytoplasmic transcriptome. Second, hDIS3L has lost the endonuclease site in the PIN domain. Third, hDIS3L contains an additional N-terminal extension that interacts with hEXO9 and contacts the C-terminal extension of human hRRP45. This in turn forms the binding platform for recruiting the hSKI7 cytoplasmic cofactor discussed in Section 3.1.2 (Kögel et al. 2024). The hDIS3L2 paralog is also restricted to the cytoplasm but lacks an N-terminal PIN domain and accordingly does not associate with the exosome, instead functioning in other pathways (Chang et al. 2013, Malecki et al. 2013).

2.3. RNA Degradation Mechanism: Processivity and Irreversibility

Rrp44/hDIS3/hDIS3L endows the exosome core with 3′-to-5′ exoribonuclease activity relying on a two-metal ion (magnesium) mechanism (Steitz & Steitz 1993). Although RNA-bound structures have generally been determined using mutations that disrupt exoribonuclease activity, wild-type Rrp44 from *Trypanosoma brucei* was recently reported in an active state after a cleavage reaction and before substrate release (Cesaro et al. 2023). Overall, the structural snapshots reveal how one of the magnesium ions helps position and activate the attacking water molecule, leading to the hydrolytic cleavage of the terminal 3′ nucleotide. As the cleaved nucleotide exits via a side channel, the newly formed 3′ end can translocate to the active site for the next cleavage event. The processive nature of the reaction, defined by sequential cleavages without releasing the substrate in between, is connected to the topology of the enclosed RNA-binding channel. This drives highly efficient degradation until the substrate is released once it is reduced to three to four nucleotides (Dziembowski et al. 2007, Lorentzen et al. 2008). The hydrolytic nature of the eukaryotic exosome reaction has one important consequence: It is irreversible due to the very high concentration of the reactant (i.e., water) in the cellular environment. This is a key difference from the phosphorolytic reactions in prokaryotic exosome-like complexes, which can reverse (i.e., add ribonucleotides to the 3′ end) when the phosphate concentration is lower than that of nucleotide diphosphates (Portnoy et al. 2005). Interestingly, plants, and possibly some protists, have retained RRP41 phosphorolytic activity, as shown by in vitro biochemical studies on *Arabidopsis thaliana* EXO9 (Lange & Gagliardi 2022, Sikorska et al. 2017). However, it remains unclear whether this activity serves a functional role in vivo or merely reflects a snapshot of evolutionary history, as protists/plants are a bridge between bacteria/archaea and fungi/animals in the evolutionary tree.

3. EQUIPPING THE CORE COMPLEX FOR SELECTIVITY

3.1. First Layer: The Constitutive Exosome Cofactors

The 10-subunit processive exoribonuclease core associates with a layer of obligate cofactors specific to distinct subcellular compartments.

3.1.1. The nuclear compartment: Rrp6, Rrp47, and Mpp6. Three evolutionarily conserved cofactors associate with the *S. cerevisiae* nuclear exosome at both the functional and physical levels: Rrp6, Rrp47 (also known as Lrp1), and Mpp6 (**Figure 2*b*,*c***) (Allmang et al. 1999b, Burkard & Butler 2000, Synowsky et al. 2009). Yeast strains simultaneously lacking Mpp6 and either Rrp47 or Rrp6 are nonviable, while individual deletions confer a slow growth phenotype and lead to defects in 3′ end maturation of 5.8S rRNA and sn/snoRNAs (Allmang et al. 1999a; Briggs et al. 1998; Milligan et al. 2008; Mitchell et al. 2003; van Hoof et al. 2000a,b). In addition, these factors participate in the degradation of noncoding RNAs produced by premature transcription termination of protein-coding genes or pervasive transcription from intergenic spacer regions across the yeast genome, referred to as cryptic unstable transcripts (CUTs) (Davis & Ares 2006, Marquardt et al. 2011, Milligan et al. 2008, Wyers et al. 2005). The corresponding orthologs in human cells, hRRP6 (also known as EXOSC10 or PM-Scl 100), hRRP47 (C1D), and hMPP6, are likewise involved in 5.8S rRNA biogenesis and RNA surveillance (Davidson et al. 2019; Fujiwara et al. 2022; Schilders et al. 2005, 2007a,b). These three nuclear exosome cofactors form a stable assembly with the exosome processive core complex (Exo13n/hEXO13n), as shown by biochemical and structural studies with both the yeast and human proteins.

Rrp6, the largest of the nuclear cofactors, has a conserved multidomain organization. Part of the unstructured C-terminal domain (CTD) interacts with the RNase PH-like proteins Mtr3 and Rrp43 and the S1/KH protein Csl4 with high affinity (with dissociation constants in the low nanomolar range in vitro) (Kowalinski et al. 2016, Makino et al. 2013a, Wasmuth et al. 2014). The central region of Rrp6 features the 3′-to-5′ exoribonuclease module, which is composed of the catalytic DEDD domain (which belongs to the RNase D enzyme family) and the regulatory HRDC domain (which contributes to RNA binding) (Makino et al. 2015, Midtgaard et al. 2006, Wasmuth et al. 2014, Zinder et al. 2016). Rrp6-mediated RNA cleavage uses a two-metal ion mechanism, but its RNase activity is distributive instead of processive (Briggs et al. 1998, Burkard & Butler 2000, Liu et al. 2006). The distributive nature of the reaction, characterized by substrate dissociation and rebinding after each cleavage, relates to the shallow and solvent-exposed active site of Rrp6 and is likely connected to its RNA trimming properties (Makino et al. 2015, Midtgaard et al. 2006). The Rrp6 N-terminal domain ($Rrp6_N$) interacts with Rrp47 to form an intertwined structural unit, rationalizing their mutual stabilization in the cellular environment (Feigenbutz et al. 2013, Schuch et al. 2014, Stead et al. 2007, Stuparevic et al. 2013). In the structure of a yeast Exo10-Rrp6-Rrp47 complex, the Rrp6 exoribonuclease module is observed in an engaged conformation, meaning it rests on top of the S1/KH ring by engaging primarily with the Rrp4 subunit (Makino et al. 2015) (**Supplemental Video 1**). In turn, the exoribonuclease module is surmounted by the $Rrp6_N$-Rrp47 module (Makino et al. 2015), which forms a recruiting platform for Mtr4 (Schuch et al. 2014).

Supplemental Material >

Rrp6 has more functional features in its C-terminal unstructured region: the so-called lasso that contributes to RNA degradation and nuclear localization signals (NLSs), which mediate import into the nucleus (Callahan & Butler 2008, Gonzales-Zubiate et al. 2017, Wasmuth & Lima 2017). The unstructured C-terminal region of Rrp47 also possesses positively charged stretches of amino acids that may act redundantly in increasing RNA binding (Costello et al. 2011). Similar biochemical and structural features have been either directly demonstrated or predicted for the human orthologs (Gerlach et al. 2018, Januszyk & Lima 2011). A significant difference in humans is that hRRP6 is thought to be the only exosome exoribonuclease in the nucleolus, as hDIS3 is excluded from this nuclear compartment (Staals et al. 2010, Tomecki et al. 2010). The nucleolar localization of hRRP6 is mediated by the ribosome biogenesis factor UTP3 (Bao et al. 2024). Yet under stressful conditions like oxygen deficiency, hRRP6 redistributes to the nucleoplasm in a

process dependent on the SUMOylation of its unstructured C-terminal region (Chen et al. 2023, Filippopoulou et al. 2024).

The third nuclear cofactor, Mpp6/hMPP6, is a small, unstructured and highly positively charged protein. As the acronym for M-phase phosphoprotein 6 implies, hMPP6 is a phosphoprotein present throughout the mitotic cell cycle (Matsumoto-Taniura et al. 1996). In both yeast and human, this cofactor uses the central region of the protein to bind two of the S1/KH subunits, Csl4 (EXOSC1/hCSL4) and Rrp40 (EXOSC3/hRRP40). The surface of the latter interaction is found to be mutated in human patients with pontocerebellar hypoplasia (Falk et al. 2017a, Wasmuth et al. 2017). Importantly, the N-terminal region of Mpp6/hMPP6 serves as another recruitment point for Mtr4/hMTR4, as it latches onto the helicase core with a mechanism conserved from yeast to human (Gerlach et al. 2018, Schuller et al. 2018, Weick et al. 2018). The joint recruitment of the essential helicase by Rrp6/Rrp47 and Mpp6, elucidated in biochemical and structural studies, explains their partial functional redundancy and why their simultaneous loss is synthetic lethal in yeast (Milligan et al. 2008). This functional redundancy is also observed with the human orthologs (Fujiwara et al. 2022). In this context, it is plausible that Mpp6 harbors an additional NLS in its positively charged unstructured regions, which likely contributes to the enhanced exoribonuclease activities of the exosome observed in vitro (Wasmuth et al. 2017).

3.1.2. The cytoplasmic compartment: Ski7/HBS1L3. A single cofactor, Ski7, associates with the *S. cerevisiae* exosome in the cytoplasm (**Figure 2*b*,*c***). The *SKI7* gene was discovered together with *SKI2*, *SKI3*, and *SKI8* from a genetic screen of superkiller mutants, which are characterized by the increased production of a viral killer toxin (Toh et al. 1978). The Ski7 protein was later recognized as the bridge between the exosome and the Ski2-Ski3-Ski8 complex in cytoplasmic mRNA decay pathways (Araki et al. 2001, van Hoof et al. 2000c). The unstructured N-terminal region of Ski7 ($Ski7_N$) is required and sufficient for exosome-mediated mRNA turnover (Araki et al. 2001). The central portion of $Ski7_N$ associates with Exo10 with high affinity by interacting with the RNase PH-like proteins Mtr3 and Rrp43 and the S1/KH protein Csl4 (Kowalinski et al. 2016) (**Supplemental Video 2**). Although cytoplasmic Ski7 and nuclear Rrp6 share little to no sequence similarity, with only a short motif in common, they are recognized by the same surfaces of Exo9, notably in a mutually exclusive manner (Kalisiak et al. 2017, Kowalinski et al. 2016), rationalizing how cells prevent the cytoplasmic exosome complex (Exo11c) from being imported into the nucleus. The terminal portion of $Ski7_N$ extends outward from the S1/KH ring and latches onto the helicase core of Ski2 during active RNA degradation by the cytoplasmic exosome holocomplex (Keidel et al. 2023). This interaction, however, is highly regulated, as discussed below.

Supplemental Material >

The human ortholog of yeast Ski7, HBS1L3/hSKI7, has an evolutionarily conserved hEXO9-binding region and also latches onto the hSKI2 helicase with a similar motif and regulation as in yeast (Kögel et al. 2024). Interestingly, previous studies on the yeast *Lachancea kluyveri* identified an alternatively spliced isoform of *HBS1* that exhibited similar function to *S. cerevisiae* Ski7 (Marshall et al. 2013). It is indeed this homology that enabled identification of hSKI7 from an alternatively spliced isoform of the *HBS1* gene (Kalisiak et al. 2017, Kowalinski et al. 2016). In retrospect, *S. cerevisiae* may be more of an exception than the norm in encoding the Ski7 and Hbs1 proteins by different genes, likely due to the ancient whole-genome duplication event in this species. Yeast Ski7 and Hbs1 also have an intriguing functional relationship as each contains a translational GTPase-like domain involved in mRNA surveillance pathways monitoring stalled ribosomes, like the nonstop decay (NSD) and no-go decay (NGD) pathways (D'Orazio & Green 2021). The GTP-binding domain in the C-terminal region of Ski7 ($Ski7_C$), which is dedicated to the NSD pathway (van Hoof et al. 2002), adopts a constitutively active conformation and lacks the essential residues required for GTPase activity (Kowalinski et al. 2015). In contrast, the Hbs1 GTPase is

activated by Dom34, which binds to the empty A site of stalled ribosomes in the NGD pathway, although it also contributes to NSD (Tsuboi et al. 2012). In humans, hSKI7 lacks a translational GTPase-like domain altogether (Kalisiak et al. 2017, Kowalinski et al. 2016), and HBS1-DOM34 (alternatively referred to as PELOTA) also functions in NSD (Saito et al. 2013).

3.2. Second Layer: The Transient RNA Helicases

The two RNA helicases, Mtr4 and Ski2, that are recruited to the exosome in the nuclear and cytoplasmic compartments, respectively, belong to the DExH family of RNA-dependent ATPases (Ozgur et al. 2015, Weick & Lima 2021). In yeast, Mtr4 (also known as Dob1) is encoded by an essential gene (de la Cruz et al. 1998). Yeast Mtr4 and its human ortholog hMTR4 (also known as MTREX and SKIV2L2) are involved in essentially all nuclear exosome-mediated pathways (**Figure 2*b***). In yeast, Ski2 and its interacting factors function redundantly with the 5′-3′ mRNA degradation pathway, as indicated by the synthetic lethality with the exoribonuclease Xrn1 (Anderson & Parker 1998, Johnson & Kolodner 1995). In human cells, hSKI2 (also known as SKIV2L) is found on mRNAs at ribosome-occupied regions and functions primarily in mRNA quality rather than in mRNA turnover (Tuck et al. 2020, Zinoviev et al. 2020). At the physiological level, hSKI2 is crucial for early B cell development and for limiting the accumulation of endogenous cytoplasmic RNAs that would otherwise activate an interferon response (Eckard et al. 2014). Missense mutations in hSKI2 as well as its interacting partner hSKI3 (TTC37) lead to a severe congenital disease, trichohepatoenteric syndrome (Bourgeois et al. 2018).

Structural studies on the helicase module of yeast Mtr4 and Ski2 first revealed a conserved architectural organization with a DExH catalytic core and a regulatory arch domain (Halbach et al. 2012, Jackson et al. 2010, Weir et al. 2010). The DExH core is composed of the typical RecA1 and RecA2 domains juxtaposed to a helical domain. In general, helicases of the DExH family translocate RNA in a processive, directional manner via a stepwise, inchworm mechanism: moving one nucleotide per ATP hydrolysis cycle in the single-stranded channel of the helicase (Gilman et al. 2017). The 3′-to-5′ directionality depends on consecutive conformational changes of the RecA domains. Additional bookend features are believed to prevent backsliding (the so-called ratchet helix and the RecA2 β-hairpin, which also wedges into an incoming duplex, likely aiding in strand separation) (Buttner et al. 2007, Gilman et al. 2017, Taylor et al. 2014, Weick et al. 2018). Importantly, a free single-stranded 3′ end is required to initiate RNA unwinding and translocation.

The arch domain is an addition to the DExH core and protrudes with a helical stalk and a globular Kyprides, Ouzounis, Woese (KOW) fold (Halbach et al. 2012, Jackson et al. 2010, Weir et al. 2010). The arch domains of Mtr4 and Ski2 helicases can adopt different conformations, generally hovering with the KOW domain above the entry of the DExH RNA-binding/unwinding channel. The KOW domain has RNA-binding properties, as shown both in biochemical assays and in structural studies, and it also facilitates substrate unwinding (Halbach et al. 2012, Jackson et al. 2010, Taylor et al. 2014, Weick et al. 2018, Weir et al. 2010). Furthermore, the Mtr4 KOW domain provides a major protein-protein interaction site, as discussed in the next paragraphs. Lastly, both Mtr4 and Ski2 have an N-terminal unstructured region. In the case of Mtr4, it binds Rrp6-Rrp47 (Schuch et al. 2014) and may also contain an NLS, while in the case of Ski2 it forms an intertwined interaction with Ski3 (Halbach et al. 2013, Kögel et al. 2022).

Structural studies have visualized both Mtr4/hMTR4 (Gerlach et al. 2022, Schuller et al. 2018, Weick & Lima 2021) and Ski2/hSKI2 (Keidel et al. 2023, Kögel et al. 2024) as they interact with yeast/human exosome complexes. These revealed how both helicases interact with their DExH core on the same surface of the S1/KH subunit Rrp4/hRRP4 and channel the 3′ end of

the unwound RNA substrate into the RNase core (**Figure 2*c***; **Supplemental Videos 1 and 2**). The helicase-exosome interaction is tightly regulated. For the nuclear exosome, the Rrp6 exoribonuclease switches to a disengaged conformation as it is displaced from the S1/KH ring to allow the Mtr4-exosome interaction (Schuller et al. 2018). For the cytoplasmic exosome, complex conformational changes take place to disengage the helicase from its interacting proteins to allow the Ski2-exosome interaction (Keidel et al. 2023, Kögel et al. 2024).

Supplemental Material >

3.3. Third Layer: Substrate Adaptors and Regulators of Helicases

The Mtr4 and Ski2 helicases interact with adaptor proteins to regulate RNA delivery to the exosome exoribonuclease core. These adaptors serve as a hub that tunes helicase activity and substrate targeting for exosome-mediated RNA degradation (**Figure 2*b***). Mtr4 associates with a variety of adaptors, reflecting the diverse types of exosome substrates in the nucleus [e.g., rRNA, transfer RNAs (tRNAs), precursor mRNAs (pre-mRNAs), and noncoding RNAs]. In contrast, Ski2 is embedded in a single complex in the cytoplasm, Ski2-Ski3-SKI8, reflecting the presence of a predominant exosome substrate in this subcellular compartment (i.e., mRNA).

3.3.1. Nuclear Mtr4 adaptor complexes. The simplest types of Mtr4-binding adaptors are those containing short arch-interacting motifs (AIMs) within intrinsically unstructured regions. AIMs were originally identified in the ribosome biogenesis factors Nop53 and Utp18, which were shown to be required for 5.8S rRNA processing and 5′ external transcribed spacer degradation, respectively (Thoms et al. 2015). The canonical Nop53 AIM binds at a surface of the Mtr4 KOW domain adjacent to the RNA-binding surface (Falk et al. 2017b, Schuller et al. 2018). Rather than a strict conservation, divergent sequences can function as AIMs and are recognized on the same surface of the KOW domain, as seen, for example, with the NVL ribosomal biogenesis factor (Lingaraju et al. 2019a).

Adaptors can also bind the DExH core of Mtr4, as is the case for the *S. cerevisiae* TRAMP complex (Trf4-Air2-Mtr4). The TRAMP complex was discovered through genetic studies in budding yeast focusing on the degradation of an aberrant tRNA species, namely a hypomodified initiator methionine tRNA ($tRNA_i^{Met}$) lacking the m^1A58 modification (Kadaba et al. 2004, LaCava et al. 2005, Vanacova et al. 2005, Wyers et al. 2005). The TRAMP-exosome pathway is now known to also target CUTs and other noncoding RNAs from intergenic regions (Houseley et al. 2007, Wyers et al. 2005) and defective forms of rRNAs from nonproductive ribosome assembly intermediates (Dez et al. 2006, Kadaba et al. 2006, LaCava et al. 2005). TRAMP is also involved in trimming snoRNA 3′ extensions (Callahan & Butler 2010, Carneiro et al. 2007, Dez et al. 2006). For both CUTs and snoRNAs, the TRAMP-exosome complex is recruited to these Pol II transcripts by the noncanonical transcription termination complex NNS (Nrd1-Nab3-Sen1) (Grzechnik & Kufel 2008, Tudek et al. 2014). Impairment of exosome recruitment results in the accumulation of 3′ oligoadenylated substrates that are generated by Trf4, a noncanonical polyA polymerase with distributive activity (Hamill et al. 2010, LaCava et al. 2005). Air2 is an RNA-binding protein containing five zinc knuckles (ZnK), whereby ZnK4 and ZnK5 bind the polyA-polymerase domain of Trf4, while ZnK1–3 likely mediate interactions with RNA substrates together with the Mtr4 KOW domain (Denson et al. 2024, Hamill et al. 2010, Holub et al. 2012). Trf4 and Air2 also contain unstructured regions that are responsible for the interaction with the DExH core of Mtr4 and potentially the KOW domain (Falk et al. 2014). Based on current structural information (Denson et al. 2024, Falk et al. 2014, Hamill et al. 2010), the most plausible mechanism for TRAMP function is that a substrate 3′ end will first undergo polyadenylation before being threaded into the helicase. The transition from unwinding to degradation involves the two nuclear exosome exoribonucleases (Das et al. 2021), with the specific degradation mechanism

possibly determined by the substrate. For snoRNA 3′ end processing, TRAMP function depends on Rrp6 activity, likely requiring a disengaged conformation of this exoribonuclease since the Rrp6 CTD is dispensable for TRAMP function in vivo (Callahan & Butler 2008, Chaudhuri et al. 2024).

In *S. cerevisiae*, different TRAMP complexes exist with different combinations of Trf4 (or its paralog Trf5) and Air2 (or its paralog Air1) (Delan-Forino et al. 2020, Schmidt et al. 2012). In human cells, the orthologous TRAMP complex (hMTR4-PAPD5-ZCCHC7) is exclusively localized in the nucleolus and involved in rRNA processing under normal cellular conditions (Fasken et al. 2011, Lubas et al. 2011). Infection by cytoplasmic RNA viruses, however, drives the nuclear export of hMTR4 and ZCCHC7, resulting in a cytoplasmic complex that specifically recognizes and induces degradation of viral mRNAs (Molleston et al. 2016). TRAMP complexes are broadly conserved across eukaryotes. Polyadenylation-dependent degradation is even more ancient, as it is a general RNA decay mechanism in bacteria (Mohanty & Kushner 2022).

In human cells, additional hMTR4 adaptor complexes have been identified and exhibit an even greater level of complexity, with large multidomain scaffold proteins that serve as platforms for diverse interactions. Among them, the NEXT (nuclear exosome targeting) complex is a key nuclear exosome adaptor that mediates the degradation of nonpolyadenylated (pA-) RNAs from spurious transcription (Lubas et al. 2011), of snoRNA precursors (Hrossova et al. 2015, Lubas et al. 2015), and of telomerase RNA during maturation (Gable et al. 2019, Tseng et al. 2015). Mutations in the NEXT subunits ZCCHC8 and RBM7 have been linked to human diseases, ranging from telomere and neuronal syndromes to cancer (Gable et al. 2019, Puno & Lima 2022). In terms of molecular mechanisms, ZCCHC8 is a multidomain protein with both scaffolding and regulatory functions. Its scaffolding roles include binding the DExH core and KOW domains of hMTR4 and the RNA recognition motif (RRM) domain of RBM7 as well as forming a tight homodimer (Falk et al. 2016; Gerlach et al. 2022; Lingaraju et al. 2019a; Puno & Lima 2018, 2022). Its regulatory roles include modulation of the RNA-dependent ATPase activity of hMTR4 and the binding ability to the exosome in an interdependent manner. Indeed, the ZCCHC8 CTD binds the bottom of the hMTR4 DExH domain in the absence of RNA substrates, blocking the interaction with the exosome, but disengages from the helicase core upon RNA-dependent ATP hydrolysis, allowing it to dock onto the exosome (Gerlach et al. 2022; Puno & Lima 2018, 2022). In addition, the ZnKs of ZCCHC8 contribute to the RNA-binding path of NEXT, connecting the RNA-binding sites of RBM7 to the hMTR4 helicase channel (Hrossova et al. 2015, Lubas et al. 2015, Puno & Lima 2022). RBM7 confers specificity to substrate recognition: Although it is loaded promiscuously on newly synthesized RNA, it preferentially accumulates at U-rich intron 3′ ends and potentially connects to splicing factors (Cordiner et al. 2023, Falk et al. 2016, Hrossova et al. 2015, Lubas et al. 2015). ZCCHC8 connects to the nuclear cap-binding complex (CBC)-ARS2 complex at the 5′ cap of Pol II transcripts via interactions with either ZC3H18 or ZC3H4 (Andersen et al. 2013, Dubiez et al. 2024, Rouviere et al. 2023), rationalizing the nexus for NEXT-exosome recruitment to short, capped transcripts (Garland & Jensen 2024). ZCCHC8 also connects to the human silencing hub complex, leading to NEXT-exosome-mediated degradation of transposable element transcripts (Garland et al. 2022).

The NEXT complex functions in synergy with PAXT (pA-tail-exosome-targeting), which preferentially directs longer and polyadenylated (pA+) Pol II transcripts to the exosome for degradation, thereby preventing RNAs arising from cryptic transcription events from reaching the cytoplasm (Meola et al. 2016, Ogami et al. 2017, Silla et al. 2018). This adaptor complex was originally discovered in *Schizosaccharomyces pombe*, the so-called NURS (nuclear RNA silencing) or MTREC (Mtl1-Red1 core) complex, and shown to promote degradation of unspliced pre-mRNAs, noncoding RNAs, and meiotic transcripts (Egan et al. 2014, Lee et al. 2013, Zhou et al. 2015). The core module is centered at the Mtl1-Red1 interaction (corresponding to the

hMTR4-ZFC3H1 interaction) (Lee et al. 2013, Meola et al. 2016). Red1 is a multidomain protein that uses a zinc finger domain to bind the KOW fold of the Mtl1 helicase (Dobrev et al. 2021) and a C-terminal region to form a homodimer (Foucher et al. 2022). In addition, *S. pombe* Red1 directly interacts with the CBC-binding protein Pir2 (Egan et al. 2014, Foucher et al. 2022, Zhou et al. 2015) and with the canonical poly(A) polymerase Pla1 (Soni et al. 2023, Zhou et al. 2015). In humans, ZFC3H1 binds CBC-ARS2, thereby competing with the NEXT complex for ARS2 binding (Meola et al. 2016, Ogami et al. 2017, Polak et al. 2023), and recruits the poly(A) polymerase gamma (PAPOLG) (Contreras et al. 2023). The MTREC/NURS complex includes the nuclear poly(A)-binding protein Pab2, the zinc-finger protein Red5, and the RRM-containing protein Rmn1 (Egan et al. 2014, Zhou et al. 2015). Analogous accessory factors are present in human PAXT (PABPN1, ZC3H3, and RBM26/27) (Meola et al. 2016, Silla et al. 2018). The link between PABPN1 and ZFC3H1 is likely indirect (hence the original name PAXT connection rather than PAXT complex) but establishes a functional connection between the nuclear poly(A) tail ribonucleoprotein (RNP) and the exosome (Meola et al. 2016). Given that the poly(A) RNP is also a feature of mature messenger (m)RNP particles that is important for nuclear export, the question arises as to how the same feature in labile mRNPs can be a signal for degradation. In this context, it has recently been shown that the concomitant presence of two distinct features, a 5′ splice site and a poly(A) junction, promotes the PAXT-dependent degradation of aberrant mRNAs arising from intronic polyadenylation (Soles et al. 2024).

Studies in fission yeast initially demonstrated that the Mtr4 ortholog Mtl1 forms a distinct complex with the proteins Nrl1 and Ctr1 (Lee et al. 2013, Zhou et al. 2015), which also have conserved human orthologs, NRDE2 and CCDC174. NRDE2 is enriched in nuclear speckles in human cells and has been linked to splicing factors (Flemr et al. 2023, Wang et al. 2019). NRDE2 also interacts with hMTR4 (Ogami et al. 2017), but it remains unclear whether this interaction inhibits or allows exosome association to hMTR4 (Flemr et al. 2023, Ogami et al. 2017). Structural studies have shown that NRDE2 binds both the KOW domain and the helicase core, with an interaction mechanism that confines the helicase conformation and appears to restrict RNA binding (Wang et al. 2019). The interaction sites for NRDE2 on hMTR4 overlap with those for the TRAMP complex (Falk et al. 2014) and the NEXT complex (Gerlach et al. 2022, Puno & Lima 2022), supporting the presence of multiple mutually exclusive hMTR4 adaptor complexes.

3.3.2. Cytoplasmic cofactor complex: Ski2-Ski3-Ski8 (Ski/hSKI). In *S. cerevisiae*, Ski2 associates with Ski3 and Ski8 to form a stable complex (Brown et al. 2000), exhibiting an unusual 1:1:2 stoichiometry (Halbach et al. 2013, Synowsky & Heck 2008). Ski3 is a helical repeat protein comprising over 30 tetratricopeptide repeats (TPRs) arranged into N-terminal and C-terminal supercoiled arms (Halbach et al. 2013). The Ski3 C-terminal arm spirals around the N-terminal unstructured region of Ski2 ($Ski2_N$), forming the scaffolding unit for binding two molecules of the β-propeller protein Ski8 at inner and outer surfaces of the superhelix ($Ski8_{IN}$ and $Ski8_{OUT}$) (Halbach et al. 2013). This portion of the complex serves a regulatory function and is currently referred to as the gatekeeping module. In the resting state of the Ski complex, the gatekeeping module interacts with the Ski2 helicase module by surrounding the DExH core and inserting an intricate loop structure of $Ski2_N$ (the so-called wedge) into the exit gate of the RNA unwinding channel (Halbach et al. 2013, Keidel et al. 2023). Structural analyses have shown how this closed conformation inhibits Ski2 at several levels (Keidel et al. 2023). First, the gatekeeping module restrains the RNA 3′ end from exiting the helicase, explaining the decreased ATPase activity of Ski2 in the complex. Second, it prevents the Ski2 helicase core from docking onto the exosome, reminiscent of the hMTR4-ZCCHC8 CTD interaction discussed in a previous paragraph. Third, it limits the binding of the Ski7 adaptor. The active state of the complex is an

open conformation whereby the helicase module disengages from the gatekeeping module while remaining covalently linked via the $SKI2_N$ region (Keidel et al. 2023). In this open conformation, the gatekeeping module can better accommodate the N-terminal portion of $Ski7_N$ between its TPR repeats. Meanwhile, the Ski2 DExH core can dock onto the S1/KH ring of the exosome and channel RNA to the Rrp44 exoribonuclease (**Figure 2*c***; **Supplemental Video 2**) (Keidel et al. 2023). Both the structure and conformational regulation between the inactive and active states of the Ski complex are conserved in the complex of human hSKI2, hSKI3 (TTC37), and hSKI8 (WDR61) (Kögel et al. 2022, 2024). Remarkably, these molecular mechanisms are conserved even though the hSKI3-binding domain in hSKI7 shares no sequence homology or structural similarity with that in *S. cerevisiae* (Kögel et al. 2024).

SKI complexes from different organisms also exhibit distinct characteristics. In fungal species, Ski8 relocalizes to the nucleus and associates with chromosomes during meiosis as part of the Spo11 complex, which initiates double-strand breaks during meiotic recombination (Arora et al. 2004). Strikingly, Spo11 and Ski3 have evolved the same Ski8-recognition motifs (Claeys Bouuaert et al. 2021, Halbach et al. 2013). It is currently unclear whether the link between mRNA decay and meiosis is coincidental or functional; detaching one β-propeller subunit ($Ski8_{OUT}$) from Ski3 compromises the biochemical properties but not the structural integrity of the Ski complex (Halbach et al. 2013). A subpopulation of the Ski complex in budding yeast interacts with Ska1 (YKL023W), which is thought to mediate the decay of specific classes of mRNAs independent of direct ribosome association (Zhang et al. 2019). The hSKI complex generally functions within cotranslational mRNA quality control pathways, alongside two additional factors, FOCAD and AVEN, which act in the context of stalled ribosomes (Tuck et al. 2020). FOCAD contributes to the stability of the SKI complex in human cells and is important for normal physiology, whereas its loss results in a pediatric syndrome (Moreno Traspas et al. 2022). FOCAD was originally identified as the human ortholog of RST1 (RESURRECTION 1), a factor required for the function of the cytoplasmic exosome in *A. thaliana* together with its interacting protein RIPR (Lange et al. 2019). Both RST1 and RIPR are involved in the mRNA surveillance pathways NGD and NSD in the plant lineage (Auth et al. 2021). It is currently unknown if human AVEN and plant RIPR share similarities at the molecular level.

4. EXOSOME-RIBOSOME SUPERCOMPLEXES: PROCESSING VERSUS DECAY

To date, the most complex substrate interactions of the exosome that have been elucidated involve ribosomal RNPs (Du et al. 2020; Kögel et al. 2022, 2024; Lau et al. 2021; Schmidt et al. 2016; Schuller et al. 2018). These studies also provide the first mechanistic insights into two different facets of exosome-mediated RNA degradation: the constructive role of the nuclear exosome in ribosome biogenesis and the destructive role of the cytoplasmic exosome in eliminating ribosome-bound mRNAs (**Figure 3**).

Studies with budding yeast proteins have captured a nuclear exosome supercomplex (Exo13n) associated with a precursor large ribosomal subunit (pre-60S) during 7S-to-5.8S rRNA processing. This structural snapshot was captured by biochemically halting the reaction at the 5.8S+30 intermediate state (Schuller et al. 2018) at which point Mtr4 and the Exo13n have already remodeled most of the ITS2 (internal transcribed spacer 2) and its interacting ribosomal biogenesis factors, which are released from the complex during this process. The structural data revealed how Mtr4 recognizes the preribosome substrate by docking to specific features of the 25S rRNA through its arch (KOW) domain. This initial recruitment enables the helicase Mtr4 to bind the 3′ extension of the 5.8S rRNA and channel it through the Exo10 toward the nuclease active site.

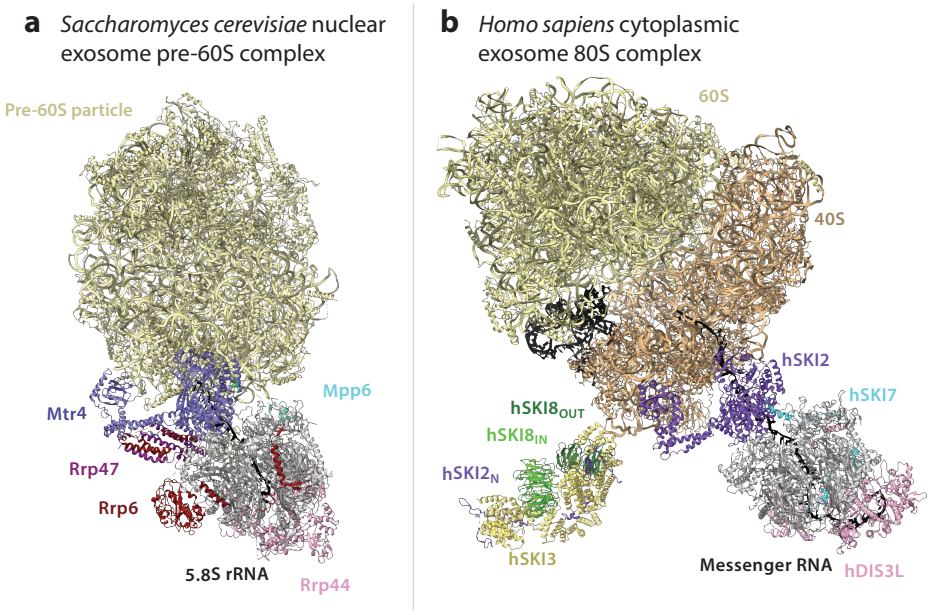


Figure 3

Atomic models of nuclear and cytoplasmic exosome supercomplexes in the context of ribosome-associated substrates. (*a*) Structure of a yeast nuclear exosome supercomplex captured during biogenesis of a pre-60S ribosome [Protein Data Bank (PDB): 6FT6 and 6FSZ]. The pre-60S particle (*beige*) contains the pre-5.8S rRNA (*black*). The 5.8S rRNA precursor is coated by different proteins, of which Nop53 (pre-60S foot) is required for the recruitment of the nuclear exosome and results in constructive 3′ end trimming. (*b*) Structure of a human cytoplasmic exosome captured as it acts on an 80S-bound RNA (PDB: 9G8M and 9G8O). The 80S ribosome consists of the large ribosomal subunit (60S) (*beige*) and the small ribosomal subunit (40S) (*brown*). The RNA substrate (*black*) is preassembled on a ribosome and has a 3′ overhang to mimic a substrate that is recognizable by the hSKI2-hSKI3-hSKI8 complex. In the structure, the RNA threads through the hSKI2 helicase and extends to the DIS3L exonuclease. In this active state, the helicase is detached from the gatekeeping module ($hSKI2_N$-hSKI3-hSKI8), which associates laterally with the 40S subunit. In their active states, the nuclear and cytoplasmic helicases Mtr4 and hSKI2, respectively, bridge the ribosome and exosome in conjunction with conformational changes of cofactors (i.e., the displacement of the nuclear Rrp6-Rrp47 exoribonuclease and of the cytoplasmic $hSKI2_N$-hSKI3-hSKI8 gatekeeping module). Colors are analogous to those used in **Figure 2*c***.

In this complex, the DExH helicase core of Mtr4 is positioned on the S1/KH ring of Exo9 in an edge-on conformation and forms a continuous RNA channel between Mtr4 and the Exo10 (**Figure 3*a***; **Supplemental Video 3**). This also requires Rrp6-Rrp47 to reposition on the side of the Exo10. Integrating the structure with years of biochemical and functional data allows us to propose a comprehensive model for how the substrate switches from the Rrp44 processive site to the Rrp6 distributive site for final trimming (Lingaraju et al. 2019b) (**Supplemental Video 3**). The exosome stops degrading this rRNA when it physically clashes against the surface of the pre-60S particle, as it cannot further extract and disentangle the rRNA embedded inside. Albeit resolved at lower resolution, the exosome has also been detected in cryo-electron microscopy analysis of the maturing 90S preribosome, indicating how Mtr4 acts in these early ribosome biogenesis stages (Du et al. 2020, Lau et al. 2021).

Supplemental Material >

Studies with human proteins have captured a cytoplasmic exosome complex (hEXO11c) and its hSKI helicase complex associated with an 80S-bound RNA substrate that mimics an mRNA that

has undergone endonucleolytic cleavage during cotranslation surveillance pathways (**Figure 3*b***) (Kögel et al. 2024). The structure reveals how hSKI2 bridges the 40S subunit of the cytoplasmic ribosome and hEXO11c. Specifically, hSKI2 engages the arch domain (KOW) to bind specific features of the 40S subunit (the 18S rRNA and its neighboring proteins uS3 and uS10) (Kögel et al. 2022, 2024), as also observed with a yeast 80S-Ski2-Ski3-Ski8 complex (Schmidt et al. 2016). hSKI2 captures the 3′ end of the mRNA-like substrate and channels it into hEXO10 by positioning the DExH helicase core on the S1/KH ring, threading it to hDIS3L (**Supplemental Video 4**). The structure thus explains how the mRNAs can be extracted and degraded, consistent with biochemical data (Zinoviev et al. 2020). In the complex, the $hSKI2_N$-hSKI3-hSKI8 gatekeeping module has disengaged from the helicase module and instead binds to an adjacent surface of the 40S (Kögel et al. 2024). The interaction between the $hSKI2_N$-hSKI3-hSKI8 gatekeeping module and the 40S occurs at a site that is often used by factors recognizing colliding ribosomes, a common feature of cotranslational mRNA surveillance pathways (Monaghan et al. 2023). While the precise sequence of events in mRNA surveillance remains to be clarified, the current structural information provides insights into how mRNAs identified in these pathways are extracted and completely degraded by the cytoplasmic exosome.

When viewing the structures of these exosome-ribosome supercomplexes (**Figure 3**), it is astounding how similar the helicase-exoribonuclease assemblies are, particularly when considering that they are from different species (yeast and human), include different nuclear and cytoplasmic cofactors, and engage different substrates (rRNA and mRNA), leading to different processing or decay outcomes. Conceptually, rather than rationalizing how RNA-degrading exosome complexes determine whether to process or degrade a substrate, it appears that it is the substrates themselves that play a decisive role in determining how RNA exosomes operate on them.

5. OUTLOOK

The emerging view is that cells maintain strict spatiotemporal control over the RNA-degradative activity of exosome complexes by modulating access to their exoribonuclease channel. Exosome-associated helicases serve as major regulatory hubs, employing conformational mechanisms to restrict or grant the delivery of RNA substrates to the exosome exoribonucleases. These conformational changes, engaging or disengaging gatekeeping features, are made possible by a combination of high-affinity anchoring interactions and weaker, flexible contacts. In the next years, we expect a more comprehensive understanding of these helicase-adaptor complexes, specifically, how they coordinate with other gene expression machineries (such as transcription termination and splicing complexes in the nucleus and collided ribosomes in the cytoplasm) and in the context of other physiological substrates (e.g., snoRNPs or telomerase RNPs). As mechanistic studies progress toward these larger assemblies, technological developments in cryo-electron tomography hold the potential to structurally visualize exosome complexes in their native cellular environment. A broader biological perspective is also warranted, for example, regarding potential feedback loops between pathways, which appears plausible given the dual roles of the same genes (e.g., yeast *SKI7* and human *HBS1L*). Furthermore, mass spectrometry analyses suggest that much remains to be discovered about new substrate adaptors and the impact of posttranscriptional and posttranslational modifications in different organisms. As more exosome-related pathologies are identified in humans, understanding how disease mutations affect function could provide a foundation for translating molecular insights into clinical applications. Perhaps more imaginatively, by extending the analogy with the proteasome, one can envision new technologies that target RNA for exosome degradation in a manner similar to how proteolysis targeting chimeras leverage the proteasome system for protein degradation (Samarasinghe & Crews 2021). The expansion of exosome

studies has far-reaching implications for understanding posttranscriptional gene regulation in the development of innovative technologies and therapies.

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Supplemental Material >

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