HYPOTHESIS

The exozyme model: A continuum of functionally distinct complexes

DANIEL L. KISS^{1,2} and ERIK D. ANDRULIS^{1,2}

¹Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106-4960, USA

²Program in Cell Biology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106-4934, USA

ABSTRACT

Exosome complexes are composed of 10 to 11 subunits and are involved in multiple facets of 3' → 5' RNA processing and turnover. The current paradigm stipulates that a uniform, stoichiometric core exosome, composed of single copies of each subunit, carries out all RNA metabolic functions in vivo. While core composition is well established in vitro, available genetic, cell biological, proteomic, and transcriptomic data raise questions about whether individual subunits contribute to RNA metabolic functions exclusively within the complex. Here, we recount the current understanding of the core exosome model and show predictions of the core model that are not satisfied by the available evidence. To resolve this discrepancy, we propose the exozyme hypothesis, a novel model stipulating that while exosome subunits can and do carry out certain functions within the core, subsets of exosome subunits and cofactors also assemble into a continuum of compositionally distinct complexes—exozymes—with different RNA specificities. The exozyme model is consistent with all published data and provides a new framework for understanding the general mechanisms and regulation of RNA processing and turnover.

Keywords: exosome; exozyme; RNA processing; RNA turnover; RNA surveillance; ribonuclease; Rrp6; Dis3; exosome subunit

INTRODUCTION

The RNA processing exosome complex was initially described as a five-polypeptide complex required for the 3' → 5' processing and degradation of rRNA precursors in *Saccharomyces cerevisiae* by David Tollervey and co-workers (Mitchell et al. 1997; Houseley et al. 2006; Schmid and Jensen 2008). Earlier and later works identified additional subunits that comprise the complex thereby rendering the complex a protean entity (Supplemental Fig. S1, left column; Allmang et al. 1999b). This larger complex, also called the exosome, degrades or processes many types of RNAs including mRNA, rRNA, snRNA, snoRNA, and tRNA precursors plus small RNAs such as cryptic unstable transcripts (CUTs), upstream non-coding transcripts (UNTs), and promoter upstream transcripts (PROMPTs) in both the nucleus and the cytoplasm (Supplemental Fig. S1, middle column; Houseley et al. 2006; Vanacova and Stefl 2007). An exosome-like complex is evolutionarily conserved in all domains of life, with a bacterial analog (PNPase), nine-subunit

forms in some archaea, and nine- to 11-subunit complexes found in all eukaryotes studied to date (Table 1; Koonin et al. 2001; Evguenieva-Hackenberg et al. 2003; Yehudai-Resheff et al. 2003; Schmid and Jensen 2008). Our understanding of exosome complexes is derived from a compilation of data from in vitro and in silico approaches coupled with genetic and biochemical evidence from several model organisms including *Saccharomyces cerevisiae* (Sc), *Sulfolobus solfataricus* (Ss), *Archaeoglobus fulgidus* (Afu), *Schizosaccharomyces pombe* (Sp), *Trypanosoma brucei* (Tb), *Arabidopsis thaliana* (At), *Oryza sativa* (Os), *Caenorhabditis elegans* (Ce), *Drosophila melanogaster* (Dm), and *Homo sapiens* (Hs) tissue culture cells (Mitchell et al. 1997; Briggs et al. 1998; Allmang et al. 1999a,b, 2000; Brouwer et al. 2001; Chen et al. 2001; Estevez et al. 2001, 2003; Andrulis et al. 2002; Brouwer et al. 2002; Butler 2002; Evguenieva-Hackenburg et al. 2003; Forler et al. 2003; Buttner et al. 2005; Vanacova and Stefl 2007; Yang et al. 2010).

Despite their established roles within the exosome complex at present, all but three of the subunits (Rrp42, Rrp43, and Rrp46) were historically identified as functionally independent of the exosome complex (Supplemental Fig. S1, left column). In this study, we present our best understanding of the core exosome model, outline its predictions, and test those predictions with previously published data. These tests affirm the utility and importance of the core

Reprint requests to: Erik D. Andrulis, Department of Molecular Biology and Microbiology and Program in Cell Biology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, USA; e-mail: exa32@case.edu; fax: (216) 368-3055.

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TABLE 1. Exosome subunits

Subunit	Domain(s)	Archaea	Yeast	Trypanosome	Plants	Fly	Human
Mtr3	RNase PH	n/a	Mtr3	EAP4	Mtr3	Mtr3	Mtr3/EXOSC6
Rrp41	RNase PH	Rrp41	Ski6/Rrp41	Rrp41A	Rrp41	Ski6	Rrp41/EXOSC4
Rrp42	RNase PH	Rrp42	Rrp42	EAP1	Rrp42	Rrp42	Rrp42/EXOSC7
Rrp43	RNase PH	n/a	Rrp43	EAP2	Rrp43	n/a	Rrp43/Oip2/EXOSC8
Rrp45	RNase PH	n/a	Rrp45	Rrp45	Rrp45B/cer7	Rrp45 ^a	Rrp45/PM-Scl-75/EXOSC9
Rrp46	RNase PH	n/a	Rrp46	Rrp41B	Rrp46	Rrp46	Rrp46/CML28/EXOSC5
Rrp40	S1/KH	n/a	Rrp40	Rrp40	Rrp40A	Rrp40	Rrp40/EXOSC3
Rrp4	S1/KH	Rrp4	Rrp4	Rrp4	Rrp4	Rrp4	Rrp4/EXOSC2
Csl4	S1/KH	Csl4	Csl4/Ski4	Csl4	Csl4	Csl4	Csl4/EXOSC1
Dis3	RNase R + PIN	n/a	Dis3/Rrp44/Mtr17	Rrp44	no	Dis3	Dis3 ^b /Dis3L1
Rrp6	RNase D + HRDC	n/a	Rrp6	Rrp6	no	Rrp6	Rrp6/PM-Scl-100/EXOSC10

^aThis subunit is present in cytoplasmic complex, but missing from nuclear complex.

^bConflicting data on whether the protein does/does not associate with the exosome complex.

(n/a) The organism lacks that gene.

exosome model for certain substrates; however, they reveal several anomalies that cannot be explained by the core model. In the final section of this review, we propose an overarching model—subsuming and expanding upon the core exosome—that accounts for all the available data from *in vivo* systems.

Predictions of the core exosome model

Although numerous publications have used the “exosome” or “core exosome” terminology, a formal promulgation of the “core exosome model” has not occurred. Based on our reading of the literature and the consensus that has evolved, we define the core exosome model as follows:

The core exosome is a stoichiometric complex containing single copies of each exosome subunit, all of which are essential for the structural integrity and catalytic activity of the complex in all RNA metabolic pathways.

We have compiled five predictions that emerge from the model articulated above:

1. Since all RNA metabolic functions of exosome subunits have been attributed to their roles within the core, exosome subunits are not expected to have RNA metabolic functions outside the core complex.
2. Since exosome subunit co-depletion has been interpreted as resulting from complex disassembly, then depletion or mutation of exosome subunits should cause complex disassembly. Therefore, individual subunit depletion should result in inactive complexes and yield similar effects on RNA processing and turnover.
3. Since core subunits function solely within the core and supernumerary subunits are actively degraded, exosome subunits are predicted to co-localize and have similar copy numbers in cells.
4. In the core model, exosome complex architecture (subunit–subunit interactions) should be conserved. Complex

purifications are predicted to yield identical complexes with similar non-exosome co-factors.

5. Since the core is the only functional complex, incomplete complexes should lack activity.

We have evaluated these predictions using all of the published data (about 301 papers) available to date. The results of most *in vitro* studies are consistent with the predictions of the core exosome model outlined above. Indeed, the data support the existence of 9–11-subunit core complexes responsible for certain RNA processing and turnover events. However, in many cases, these predictions, and therefore the exosome model, are contradicted by genetic, cell biological, proteomic, and biochemical results from different *in vivo* studies (Supplemental Fig. S1, right column; see below). Our analysis, summarized below, demonstrates that all five predictions of the core exosome paradigm are unfulfilled *in vivo*. This suggests that the core exosome model requires amendment or replacement with a more comprehensive overarching model.

Exosome complex functions *in vivo*

The complex is evolutionarily conserved from archaea to humans and consists of 9–11 subunits (Table 1), depending on the organism and subcellular compartment (Houseley et al. 2006; Evguenieva-Hackenberg and Klug 2009). *In vivo*, its functions can be divided into two broad categories, the 3′-end processing of stable RNAs and the surveillance and turnover of different RNA species (Mitchell et al. 1997; Tollervey and Caceres 2000; Perumal and Reddy 2002; Houseley et al. 2006; Vanacova and Stefl 2007; Schmid and Jensen 2008). Several reviews that describe the characterization and functions of exosome subunits as a complex are available (Houseley et al. 2006; Vanacova and Stefl 2007; Evguenieva-Hackenberg and Klug 2009). The focus of this study is to build a model for understanding the contributions

of individual subunits to many RNA metabolic pathways. Toward this end, we compiled a color-coded chart detailing which subunits have been tested for each function attributed to the exosome complex (Fig. 1). An expanded chart and function-by-function reference section, compiling all the references used in Figure 1, is included as supplementary information (Supplemental Tables S1, S2).

An examination of Figure 1 reveals that the collected knowledge of exosome subunit contributions to most RNA

processing or surveillance functions is incomplete (gray boxes). Functions attributed to every subunit of the complex are the processing (trimming of 3' extensions) of 5.8S rRNA and the turnover of the 5' external transcribed spacer (ETS) (Fig. 1; Mitchell et al. 1996, 1997; Allmang et al. 1999a,b). All exosome subunits were also shown to be required for the processing of the 7S rRNA precursor to its mature size (Allmang et al. 1999a,b, 2000). There are some discrepancies between studies for these substrates (Fig. 1, orange boxes;

Supplemental Table S1). Most notably, and contradictory to predictions 1 and 2 above, several distinct 5.8S rRNA processing and 5' ETS degradation intermediates, and different ratios of those intermediates, accumulate when different exosome subunits are mutated and/or depleted (Allmang et al. 1999a,b, 2000). Aside from the early works with those two substrates, all subsequent analyses have focused on examining a subset of the subunits (Fig. 1). Several other rRNA processing functions, such as the trimming and/or the complete turnover of other rRNA precursors (Fig. 1), were then attributed to the entire exosome complex and its cofactors (Allmang et al. 1999a,b, 2000; Suzuki et al. 2001; Houseley and Tollervey 2006; Chekanova et al. 2007). In addition to processing the 3' ends of rRNAs, several exosome subunits have been shown to be required for the proper 3'-end processing of other stable RNAs including small nucleolar RNAs (snoRNAs) and small nuclear RNAs (snRNAs) (Fig. 1; Allmang et al. 1999a; van Hoof et al. 2000a,c). Collectively, the thoroughly characterized functions attributed to all subunits of the exosome complex represent a minority of the available studies (Fig. 1).

The 3'-end processing data show that cells with mutant, deleted, or depleted exosome subunits accumulate different RNAs and/or distinct processing intermediates of the same RNA depending on which subunit was targeted (see Fig. 1; Supplemental Tables S1, S2). These results do not fulfill predictions 1 and 2 of the core exosome paradigm. In these studies, multiple (and different proportions of) distinct 3' extended processing and degradation intermediates accumulate in different exosome subunit mutant or GAL depletion strains (Briggs et al. 1998; Allmang et al. 1999a,b, 2000;

	RNase PH domain						S1/KH domain			II/R	D
	Mtr3	Rrp41	Rrp42	Rrp43	Rrp45	Rrp46	Rrp40	Rrp4	Csl4		
rRNA Processing											
5' ETS turnover											
3' ETS turnover											
trimming of 7S pre-rRNA											
trimming of 5.8S +27 pre-rRNA											
trimming of 27S pre-rRNA to 25S											
trimming of 35S pre-rRNA to 32S											
21S and 23S pre-rRNA turnover											
snoRNA maturation											
snRNA maturation											
tRNA degradation										*	
non-coding RNAs											
cryptic RNA turnover											
PROMPTs / UNTs											
SUTs											
microRNA											
antisense RNA turnover											
nuclear mRNA surveillance											
general mRNA turnover											
DRN											
co-transcriptional surveillance											
mis-spliced mRNA surveillance											
mRNA 3' end surveillance											
mRNA export											
histone mRNA turnover											
chromosome condensation											
heterochromatic gene silencing											
novel transcript											
sRNA											
Cytoplasmic mRNA surveillance											
general 3' to 5' mRNA turnover											
ARE mRNA decay											
intron lariat degradation											
non-stop decay											
no-go decay											
degradation of siRNA substrates											
degradation of NMD substrates											
RNA binding											
	Mtr3	Rrp41	Rrp42	Rrp43	Rrp45	Rrp46	Rrp40	Rrp4	Csl4	Dis3	Rrp6

FIGURE 1. The functions of the exosome complexes. The functions attributed to the complex are listed in the *leftmost* column. Exosome subunits were tested and found to be required (yellow boxes) or not required (red boxes). Orange boxes indicate conflicting data in separate studies; asterisks indicate that different degradation and/or processing intermediates accumulated in those subunits. Two asterisks indicate that the subunit alone was sufficient to perform the function in vitro. A complete expanded function table and citation index is included in Supplemental Tables S1 and S2.

Burkard and Butler 2000; Chekanova et al. 2000, 2002; Estevez et al. 2001). The most striking difference occurs in *rrp6Δ* yeast strains, which accumulate a specific 5.8S + 27 precursor (Allmang et al. 1999a,b; Burkard and Butler 2000). Depletions of other subunits such as Rrp41, Rrp42, and Rrp43 show several subunit-restricted 3' extended 5.8S rRNA species (Allmang et al. 1999a,b; Burkard and Butler 2000). Detailed examination of 5' ETS turnover in yeast also yields a similar profile of distinct intermediates (Allmang and Tollervey 1998; Allmang et al. 1999a,b, 2000). Other stable RNAs such as snoRNAs (U14, U18, and U24) and snRNAs (U4 and U5) also accumulate different processing intermediates in mutant or GAL depleted yeast strains (Allmang et al. 1999a,b, 2000). Furthermore, these processing intermediates were independently reproduced by multiple subsequent studies in many labs (Fig. 1; Supplemental Tables S1, S2). These results are all inconsistent with the predictions of the core exosome model, thus suggesting the need for a more comprehensive model.

Exosome subunits also have roles in targeted RNA turnover. The Anderson lab was the first to show a tRNA surveillance system involving Dis3 (Kadaba et al. 2004). Contradicting predictions 1, 2, and 5, subsequent work has shown that Dis3 alone was sufficient for the surveillance and turnover of hypomodified tRNA^{Met} in vitro (Fig. 1; Schneider et al. 2007). Many, but not all, exosome subunits have been implicated in the turnover of intergenic non-coding RNAs (ncRNAs) (Wyers et al. 2005). This diverse group of RNAs includes both short-lived transcripts such as the poly(A)⁺ cryptic unstable transcripts (CUTs) in yeast, upstream noncoding transcripts (UNTs) in plants, and promoter upstream transcripts (PROMPTs) in human cells (Wyers et al. 2005; Chekanova et al. 2007; Preker et al. 2008). Inconsistent with prediction 1, depletion of individual subunits yields different effects on these non-coding RNAs (Chekanova et al. 2007; Preker et al. 2008). In addition, certain subunits (Rrp4, Rrp40, and Rrp41) are required for the degradation of certain microRNA precursors, but others (Csl4 or Rrp6) are not (Camblong et al. 2007; Chekanova et al. 2007; Preker et al. 2008; Neil et al. 2009; Xu et al. 2009). As mentioned earlier, the dispensability of one or more exosome subunits in these RNA metabolic processes challenges the generality and applicability of the core exosome model.

Multiple exosome subunits, both singly and in combination, have also been implicated in specialized forms of mRNA turnover including co- and post-transcriptional mRNA surveillance (Das et al. 2003; Vasudevan and Peltz 2003; Vinciguerra and Stutz 2004; Houseley et al. 2006; Houseley and Tollervey 2008; Schmid and Jensen 2008). Many *Dm* exosome subunits have been shown to co-localize and co-purify with elongating RNA polymerase (RNAP) II on actively transcribing heat-shock genes (Andrulis et al. 2002; Saunders et al. 2003). Multiple exosome subunits are required for the surveillance and turnover of mis-spliced

and/or export-deficient mRNAs in addition to those with malformed 3' ends in yeast (some data conflict in different studies) (Fig. 1, orange boxes; Burkard and Butler 2000; Libri et al. 2002; Torchet et al. 2002; Zenklusen et al. 2002). Degradation of RNA in the nucleus (DRN), a general nuclear poly(A)⁺ RNA turnover mechanism, was also shown to require certain exosome subunits (Das et al. 2003, 2006; Kuai et al. 2004, 2005). Other functions including histone mRNA turnover, aspects of mitotic chromosome condensation, and heterochromatic gene silencing (Fig. 1) have been attributed to the entire complex, but may be specific to particular polypeptides (Buhler et al. 2007; Camblong et al. 2007; Canavan and Bond 2007; Murakami et al. 2007; Reis and Campbell 2007; Vasiljeva et al. 2008). As many subunits are not required for certain functions, these findings do not affirm predictions 1 or 5 of the core exosome model.

The exosome core has also been linked to the general turnover of mRNAs in the cytoplasm (Fig. 1; Anderson and Parker 1998; van Hoof et al. 2000b; Araki et al. 2001; van Dijk et al. 2007). These general turnover mechanisms apply to whole mRNAs, especially those with specialized elements, such as AREs, in their 3'-untranslated regions (UTRs) (Chen et al. 2001; Gherzi et al. 2004, 2006). RNAs targeted by small interfering RNAs (siRNAs) are also degraded in an exosome subunit-dependent manner (Orban and Izaurralde 2005; Ibrahim et al. 2006). In addition to the degradation of normal cytoplasmic mRNAs, certain, but not all, exosome subunits are needed for the turnover of multiple different types of aberrant mRNAs. Aberrant mRNAs, such as those with stalled ribosomes (no-go decay, NGD) or those that lack termination codons (non-stop decay, NSD), and certain intron lariats are also degraded with the aid of certain cytoplasmic exosome cofactors (van Hoof et al. 2002; Hilleren and Parker 2003; Doma and Parker 2006; Meaux and Van Hoof 2006). The nonsense-mediated decay (NMD) pathway is a translation-dependent mRNA surveillance pathway that detects mRNAs with premature termination codons (PTCs) and targets them for degradation (Behm-Ansmant et al. 2007). The turnover of NMD targeted transcripts has been well characterized in yeast and metazoan (human and fly) cells and occurs by different mechanisms depending on the organism studied (Mitchell and Tollervey 2003; Gatfield and Izaurralde 2004). Several subunits have been linked to this mRNA surveillance pathway, but conflicting data obscure our understanding of their specific contributions within or without the core (Fig. 1; Neu-Yilik et al. 2001; Neu-Yilik and Kulozik 2008; Rebbapragada and Lykke-Andersen 2009; Kiss and Andrulis 2010).

Although a great deal of study has been directed at the functions of exosome subunits in RNA turnover pathways, no data have been reported for many subunits (Fig. 1, gray boxes). For example, the exosome was suggested to be responsible for the turnover of intron lariats and NGD targeted transcripts, yet no bona fide exosome subunits, only exosome cofactors, were examined in those studies

(Fig. 1; van Hoof et al. 2002; Hilleren and Parker 2003; Doma and Parker 2006). The dispensability of some exosome subunits (Fig. 1, red boxes) for certain functions is also inconsistent with several predictions of the core exosome model. Furthermore, conflicting results in several published works raise questions about the veracity of those data (Fig. 1, orange boxes). To summarize, regardless of the approach used or RNA species tested and contrary to predictions 1 and 2 of the core exosome model, multiple non-identical RNA processing intermediates accumulate in yeast strains with depleted or mutated exosome subunits (Fig. 1; Supplemental Tables S1, S2).

Microarray studies show exosome subunits survey distinct RNAs in vivo

Data from several transcriptomic studies support those obtained via the targeted approaches above and contradict predictions 1 and 2 of the core exosome model. Based on the core exosome model, one would predict that microarrays would detect a conserved set of RNAs when individual subunits are mutated or depleted. This perspective is derived from the similar transcriptomic profiles observed when the components of other RNA processing or surveillance machineries, such as the NMD, nuclear export, and splicing machineries, were mutated or depleted (Herold et al. 2003; Rehwinkel et al. 2005, 2006; Pleiss et al. 2007). In contrast to those precedents, microarray studies in yeast, plants, and flies show that depletion or mutation of individual exosome subunits affects distinct sets of RNAs (Houalla et al. 2006; Chekanova et al. 2007; Kiss and Andrulis 2010).

The first microarray study examined RNA harvested from *rrp41-1*, *rrp6Δ*, and *rrp47Δ* *Sc* strains using gene expression arrays (Houalla et al. 2006). These arrays showed that the affected mRNAs were predominantly increased but often not shared between the different subunits (Houalla et al. 2006). *rrp6Δ* yeast strains also show distinct effects on global mRNA levels (Houalla et al. 2006). Similar results were obtained with whole-genome tiling arrays in *At* using RNA harvested from *csf4-2* mutants or plants depleted of Rrp4 or Rrp41 using RNAi (Chekanova et al. 2007). As with the *Sc* arrays, exosome subunit depletion or mutation in both plants and flies affected different RNAs (Chekanova et al. 2007; Kiss and Andrulis 2010). Different populations of mRNAs were also stabilized in *Dm* S2 cells when eight exosome subunits were depleted (Kiss and Andrulis 2010). Importantly, this trend was not limited to mRNAs and held true for rRNAs, miRNAs, tRNAs, snRNAs, snoRNAs, 7SL RNA, and UNTs in plants (Chekanova et al. 2007). In human cells, PROMPTs were also differentially affected by exosome subunit depletion (Preker et al. 2008). Collectively, the microarray studies show that mutation or depletion of different exosome subunits does not reveal a core set of exosome subunit-surveyed RNAs. The heterogeneous

nature of these data sets contradicts predictions 1 and 2 of the core exosome model.

Individual exosome subunits have complex-independent functions in vivo

Recent work from the Butler lab showed that *Sc* Rrp6 performs certain rRNA processing functions independent of the core (Callahan and Butler 2008, 2010). *Dm* Rrp6 also functions in cell cycle checkpoints, and these roles are independent of at least one other exosome subunit (Rrp40) (Graham et al. 2009b). *Dm* Rrp6 also appears to have a specialized role in the surveillance of NMD targeted transcripts (Kiss and Andrulis 2010). Contradicting prediction 2, these observations support the idea that Rrp6 has core exosome-independent functions in yeast and flies (Houalla et al. 2006; Callahan and Butler 2008; Kiss and Andrulis 2010).

Core exosome complex-independent RNA surveillance functions are not restricted to Rrp6. For example, recombinant *Sc* Dis3 and TRAMP complexes were sufficient for the surveillance and turnover of hypomodified tRNA^{Met} in vitro, a function that was initially attributed to the entire exosome complex (Kadaba et al. 2004, 2006; LaCava et al. 2005; Vanacova et al. 2005; Schneider et al. 2007). Human Dis3 has also been shown to have 28S rRNA processing functions that are distinct from three other exosome subunits (Slomovic et al. 2010). Finally, Rrp46 homodimers have been shown to bind and degrade RNA in vitro (Yang et al. 2010). In sum, certain RNA processing and surveillance functions attributed to the entire exosome complex could be formed independently by individual exosome subunits or subsets of exosome subunits.

Exosome subunits have different localization profiles and copy numbers

Co-depletion and overexpression results from several different systems have been interpreted to mean that the levels of exosome subunits are precisely balanced (Estevez et al. 2003; Graham et al. 2006; van Dijk et al. 2007; Kiss and Andrulis 2010). The current viewpoint is that surplus copies of subunits—superstoichiometric to the core—are actively degraded in a proteasome-dependent manner. This proteolysis occurs upon either overexpression of exosome subunits as in fly cells and trypanosomes, or by subunit knockdown as in trypanosomes, fly, and human systems. The third prediction of the core exosome model—that exosome subunits should have similar copy numbers—is derived from these observations and interpretations. We evaluated this prediction directly by mining yeast proteomic data. One study used GFP-tagged proteins expressed from their native promoters to estimate the copy number of the yeast proteome, and, contrary to prediction 3, the copy numbers of exosome subunits varied greatly (Ghaemmamghami et al. 2003). Dis3

was the least common subunit with approximately 600 copies/*Sc* cell, while Rrp46 had approximately 11,000 copies/cell. The copy numbers of the remaining exosome subunits also showed a broad range (from approximately 2100 to approximately 7400 copies per cell).

Because the core model stipulates that exosome subunits should be within an exosome core, all subunits should also co-localize. Several broad and targeted studies have tested this prediction. Differing localization patterns were observed via studies both with GFP-tagged and tandem affinity purification (TAP) tagged proteins in *Sc* (Huh et al. 2003; Gavin et al. 2006). Indirect immunofluorescence studies also demonstrated that both tagged and endogenous exosome subunits have non-overlapping localization profiles in *Dm* S2 cells (Graham et al. 2006).

Distinct complexes are purified from yeast and fly cells

The fourth prediction of the core exosome model states that similar complexes and complex-associated proteins should be purified, regardless of which exosome subunit was recovered. In proteomic studies from two groups, *Sc* exosome subunits were expressed from their endogenous promoters and TAP-tagged at their 3' ends, the resulting complexes purified, and their composition was determined by mass spectrometry using similar automated procedures (Gavin et al. 2002, 2006; Krogan et al. 2004, 2006). Contradicting prediction 4, very different complexes were recovered depending on which subunit was tagged (Fig. 2; Supplemental Table S3). For example, in the studies from Gavin and co-workers, between 8 and 11 exosome subunits were recovered regardless of which subunit was tagged (Gavin et al. 2002, 2006). Interestingly, Rrp46, an RNase PH domain subunit that should be required to form an intact hexameric ring, was the subunit most commonly absent from these purifications (Gavin et al. 2006; Liu et al. 2006). The second study used the same methodology and obtained complexes with greater compositional variability (Krogan et al. 2006). Notably, both groups purified several exosome cofactors as well as many proteins with no known role in exosome-mediated processes (Supplemental Table S3; Gavin et al. 2002, 2006; Krogan et al. 2004, 2006). These novel exosome subunit-interacting proteins may have functional roles in RNA processing and/or turnover; however, the core exosome model does not account for the presence or function(s) of these additional proteins.

FIGURE 2. TAP profiles of exosome subunits in *Sc*. The grid shows the subunits co-purifying with each TAP tagged exosome subunit. Yellow boxes indicate the subunit was recovered in the Gavin studies (Gavin et al. 2002, 2006); blue boxes indicate the subunit was recovered in the Krogan studies (Krogan et al. 2004, 2006); green boxes indicate the subunit was recovered in both studies; and red boxes indicate the subunit was not recovered in either study. All co-purifying proteins are listed in Supplemental Table S3.

Biochemical approaches to purify exosome complexes specifically from the nucleus or cytoplasm have shown that certain exosome subunits (Csl4, Rrp4, Rrp45, Rrp46, and Mtr3) were more prevalent in cytoplasmic complexes, and others (Rrp41, Rrp43, Dis3, and Rrp6) were over-represented in nuclear complexes (Synowsky et al. 2009). Only Rrp40 and Rrp42 were approximately evenly distributed between complexes purified from nuclear and cytoplasmic sources (Synowsky et al. 2009). As Rrp6 is nuclear-restricted in *Sc*, the complete absence of Rrp6 from the cytoplasmic complex is expected; however, the remaining results are inconsistent with prediction 3 of the core exosome model, which predicts a 1:1 ratio of subunits regardless of the compartment. Contradicting prediction 4, exosome complex purification experiments using Flag-tagged exosome subunits in *Dm* S2 tissue culture cells yielded heterogeneous complexes (Graham et al. 2006). One possible interpretation is that the poorly recovered exosome subunits are present in only a subset of the complexes, but this possibility is unaccounted for by the core exosome model.

Distinct exosome subunit arrangements are common

The fourth prediction of the core exosome model is that the composition (subunits) and structure (subunit-subunit interactions) of the complexes would be unchanged from study to study and organism to organism. Contradicting this prediction, many different complexes containing exosome subunits, most called exosomes, have been purified (Fig. 3; references in Supplemental Table S3). For example, two distinct *Afu* exosome complexes (Fig. 3) were reconstructed in vitro and crystallized (Buttner et al. 2005). The two complexes differed by the composition of their S1/KH domain subunit caps, which were either homo-trimers of

FIGURE 3. Known exosome subunit assemblages. A grid and schematic showing the composition of known exozymes. (Ss) *Sulfolobus solfataricus*; (Afu) *Archaeoglobus fulgidus*; (Sc) *Saccharomyces cerevisiae*; (Tb) *Trypanosoma brucei*; (At) *Arabidopsis thaliana*; (Os) *Oryza sativa*; (Ce) *Caenorhabditis elegans*; (Dm) *Drosophila melanogaster*; (Hs) *Homo sapiens*. References are listed in Supplemental Table S4. (*) Those subunits were absent from complexes harvested by different methods; (**) the subunits were added singly or in combination to the remaining subunits; (†) indicates that hDis3 has been replaced by Dis3L1 or Dis3L2; (‡) individual subunits had RNase activity on short, unstructured RNAs. “Other” proteins are (a) dnaG, (b) Ssa1p, (c) Ski7p, (d) TRAMP complex, (e) MPP6 and TRAMP complex, (f) *Ce* degradosome, (g) Spt6, (h) Importin α -3. The in vitro activity abbreviations are (R) RNase (random RNA or RNA oligonucleotide); (A) poly-adenylation; (n/d) not determined; (t) tRNA degradation; (m) mRNA degradation; (D) DNase activity.

Rrp4 or Csl4 and were also observed in Ss (Evgenieva-Hackenberg et al. 2008; Roppelt et al. 2010). While only two cap structures were resolved, the trimeric caps of archaeal exosomes could be comprised of all possible combinations of Rrp4 and/or Csl4 (Fig. 3), including caps with 1:2 ratios (Buttner et al. 2005). Indeed, the viability of *csl4-2* plants, a verified *Csl4* null allele in *At*, suggests that the subunits of plant S1/KH domain caps may behave similarly (Chekanova et al. 2007). The existence of structurally diverse exosome complexes in archaea suggests that other systems may possess structurally distinct exosome complexes.

Contrary to prediction 4 above, and in agreement with the archaeal data, certain eukaryotic model systems also have exosome complex variants that lack one or more core exosome subunits (Fig. 3). For example, both *At* and *Tb* exosome complexes lack Dis3 (Fig. 3), which is thought to be the sole catalytic component of the yeast complex (Estevez et al. 2003; Chekanova et al. 2007). Furthermore, complexes purified from *At* also lack Rrp6, and the viability of *csl4-2* plants shows that “missing” subunits also include structurally indispensable subunits. Importantly, *At* Rrp4 and *At* Rrp41 subunits appear to have retained their RNase activities over the course of their evolution (Chekanova

et al. 2000, 2002, 2007). Moreover, several other exosome subunit assemblages, which lack one or more subunits, have also been identified in yeast using macromolecular mass spectrometry (Synowsky et al. 2006, 2009). Diverging with the observations from plants, many of these complexes differ by the inclusion or exclusion of Csl4; however, other core subunits are also missing in certain cases (Synowsky et al. 2009).

Indeed, there are other examples in which functional exosome complexes lack one or more structurally critical subunits (Fig. 3; Andrulis et al. 2002; Forler et al. 2003; Graham et al. 2006). Two distinct exosome complexes, both lacking the RNase PH domain hexameric ring component Rrp43, have been purified from *Dm* tissue culture cells (Fig. 3; Andrulis et al. 2002; Forler et al. 2003). The Rrp43- and Rrp45-less *Dm* complex retained RNase PH activity in vitro, even though one-third of the core's RNase PH ring was missing (Andrulis et al. 2002). The presence of multiple, structurally distinct yet functional exosome complexes is inconsistent with the fourth prediction of the core exosome model.

Some exosome subunits form subcomplexes in vivo and in vitro

In addition to the larger exosome subunit assemblages mentioned above, several smaller, yet stable, subcomplexes have been identified in vivo or reconstructed in vitro (Fig. 3). First, our group has obtained biochemical results showing that Dis3 exists in a complex with Rrp6 and Importin- α 3, and these subcomplexes are independent of other exosome subunits (Graham et al. 2009a; Mamolen et al. 2010). The precise function of this tripartite complex has yet to be elucidated, but it may be involved in the transport of other exosome subunits (Graham et al. 2009a). In agreement with these observations, Importin α/β dimers have been shown to associate with other exosome components in yeast (Synowsky et al. 2009). Although this complex may be an assembly intermediate of the core, both evolutionary and experimental evidence suggest that Dis3- and Rrp6-like enzymes emerged functionally independent of the core.

Second, a recent study shows that the Rrp41, Rrp45, and Dis3 trimer forms a stable exosome subunit subcomplex in vitro (Bonneau et al. 2009). Third, new work has shown that *Ce*, *Os*, and *Hs* Rrp46 can form stable homodimers with species-specific RNase and DNase activities (Fig. 3; Yang et al. 2010). These findings expand upon previous observations showing a role for a subset of exosome subunits (Rrp6 and Rrp46) in the apoptotic degradosome of *Ce* (Parrish and Xue 2003, 2006). Furthermore, the new work offers an intriguing explanation for the link between Rrp46, also called CML28, and certain human cancers (Yang et al. 2002). Collectively, the existence of these subcomplexes contradict predictions 4 and 5 of the core

exosome model and imply that compartment- and substrate-specific exosome subcomplexes may exist in vivo. Despite the inconsistencies with the exosome model listed above, no alternative model has been proposed.

Exozymes

Given the incompatibility of the data with the core exosome model, the field requires a new integrated synthesis that can encapsulate all the data. On multiple occasions, our group has proposed that the aggregate data are consistent with a model in which individual exosome subunits assemble into multiple distinct complexes in vivo (Graham et al. 2006, 2009a,b; Kiss and Andrulis 2010; Mamolen et al. 2010). Prior to defining the exozyme model, we review the major evidence responsible for its construction. First, functional non-stoichiometric exosome complexes are prevalent in Archaea (Fig. 3; Buttner et al. 2005; Evgueniev-Hackenberg et al. 2008; Lorentzen and Conti 2008; Roppelt et al. 2010). Second, compositionally distinct exosome complexes are purified from several different organisms (Figs. 2, 3; Supplemental Table S3; Allmang et al. 1999b; Buttner et al. 2005; Gavin et al. 2006; Krogan et al. 2006; Synowsky et al. 2006, 2009; Graham et al. 2009a). Third, subsets of eukaryotic exosome subunits can independently perform RNA metabolic functions in vivo and in vitro (Schneider et al. 2007; Callahan and Butler 2008, 2010). Fourth, exosome subunits do not always co-localize in vivo (Huh et al. 2003; Gavin et al. 2006; Graham et al. 2006). Finally, mutation or depletion of different exosome subunits shows that they survey different RNAs and produce distinct RNA processing intermediates (Fig. 1; Supplemental Table S1). These observations are true for all available model systems including Archaea, yeast, trypanosomes, plants, and both fly and human tissue culture cells.

The exozyme model stipulates that, in addition to functioning in the core, exosome subunits assemble into and function as independent complexes termed exozymes. There are several axioms and predictions of this model:

1. Exozymes are composed of exosome subunits either alone or as a mélange of exosome subunits, exosome cofactors, and non-exosome polypeptides.
2. Exozymes range in size from one component to many components with the traditional core exosome and associated cofactors representing the largest exozymes.
3. Smaller exozymes may be dissociated from the core exosome, or they may maintain dynamic relationships with the core and other exosome subunits.
4. Exozymes should be subcellularly compartmentalized and have metabolic pathway-restricted substrates.
5. Exozyme complexes are predicted to function independently of other exozymes via different substrate specificities, or they may share RNA substrates by participating in the same or different steps of RNA processing.

6. Exozymes are predicted to have some non-RNA metabolic functions.

The exozyme model not only addresses the anomalies that the current paradigm has difficulty tolerating, but also affords a solution to the largest quandary remaining in the field of exosome biology: How do complexes achieve substrate specificity? We have known that certain exosome subunits can recognize RNAs directly; for example, Dis3 can recognize and survey tRNA, while Rrp41, Rrp43, and Rrp45 can preferentially bind ARE-like sequences *in vitro* (Anderson et al. 2006; Schneider et al. 2007). Under the core exosome model, the functional implications of these direct exosome subunit–RNA interactions, especially for Rrp41, Rrp43, and Rrp45, were difficult to explain. However, with the exozyme model, direct RNA–Rrp41/Rrp43/Rrp45 interactions occur through a specialized exozyme. In a general case, direct exozyme:RNA interactions could coordinate RNAs for specific processing and/or turnover events and hence afford substrate specificity to the exozyme complexes. New work showing the different substrate specificities of Rrp4-capped and Csl4-capped archaeal exosome complexes strongly supports this idea (Roppelt et al. 2010).

Other putative exozyme components (i.e., known exosome-associated proteins and/or cofactors) could serve as “specificity modules” for different exozymes. Such proteins would directly interact with RNAs, other RNA binding proteins, or with specific exosome subunits and serve as bridges between exozymes and RNAs to allow their degradation and/or processing. TTP and KSRP, which serve as binding adapters to ARE-containing mRNAs, are examples of exosome-interacting proteins that are known to fill these roles (Chen et al. 2001; Gherzi et al. 2004; Chou et al. 2006). The zinc-finger antiviral protein (ZAP) is another adapter candidate as it has also been shown to destabilize viral mRNAs via direct interactions with Rrp46 (Guo et al. 2007). There are also dozens of additional exosome subunit–interacting proteins (Supplemental Table S3), most of which have unknown relationships with exosome subunits, that have been identified through different proteomics approaches (Gavin et al. 2002, 2006; Krogan et al. 2004, 2006). These exosome subunit–interacting proteins include helicases (Prp43), RNA binding proteins (Pab1), and known nucleases (Mpp6), suggesting that other novel helicases and/or nucleases can be part of different exozyme complexes (Gavin et al. 2006; Krogan et al. 2006). Post-translational modifications of exosome subunits, such as the phosphorylation of Rrp4, Rrp6, Rrp40, and Csl4, could potentially act as switches for exozyme assembly/disassembly or exozyme–RNA binding and/or turnover activities (Synowsky et al. 2006, 2009).

According to the exozyme framework, some functions that have been attributed to the entire core may, in actuality, be performed by individual subunits—the smallest

exozymes. Dis3 is a good example, as recombinant Dis3 has robust endo- and exo-nuclease activities and was sufficient to carry out tRNA surveillance *in vitro* (Alexandrov et al. 2006; Schneider et al. 2007; Mamolen and Andrulis 2009; Schaeffer et al. 2009; Mamolen et al. 2010). Rrp6 and the exosome cofactor Rrp47/C1D/Lrp1 could typify another exozyme as their deletions yield the same 5.8S rRNA processing intermediate (Allmang et al. 1999a,b, 2000). Rrp6 could also be a single-component exozyme, since some of its RNA processing functions can be performed while unable to associate with the remainder of the exosome complex (although Rrp47 association was not tested) (Callahan and Butler 2008; Callahan and Butler 2010). Finally, exosome subunits could form smaller exozymes that carry out the RNA processing and turnover functions of the complex. One such exozyme, already mentioned, is the Rrp46 homodimer (Yang et al. 2010).

Several predictions of the exozyme model are borne out by published data. First, different RNA substrates would likely be surveyed by distinct exozymes. For example, complex substrates like large rRNA precursors would require exozymes with helicases that could resolve complex RNA secondary structures, while unstructured RNAs would be surveyed by non-helicase-containing exozymes. Indeed, a subunit-specific association of known and novel exosome subunit–interacting helicases is evident upon further analysis of the proteomic data (Gavin et al. 2006; Krogan et al. 2006). Second, several small (one to four subunit) exozymes have been characterized. These putative exozymes include Rrp6 alone, the Dis3-TRAMP complexes involved in tRNA surveillance, and the Rrp6–Dis3–Importin α -3 trimeric complex (Schneider et al. 2007; Callahan and Butler 2008, 2010; Graham et al. 2009a). Third, the composition of cytoplasmic and nuclear exozymes appears to differ in yeast and flies. Indeed, the different localization patterns and the uneven distribution of subunits observed by macromolecular mass spectrometry of the *Sc* exosome subunits suggest that this is the case (Ghaemmaghami et al. 2003; Huh et al. 2003; Graham et al. 2006; Synowsky et al. 2009). Fourth, microarray studies in *Dm* show that the proteins encoded by exosome subunit surveyed transcripts participate in distinct metabolic processes (Kiss and Andrulis 2010). Fifth, exozymes could achieve substrate specificity by targeting RNAs based on specific secondary structures or sequences via direct RNA–protein interactions. The direct ARE-specific RNA binding activities of Rrp41, Rrp43, and Rrp45 show that sequence-specific exozymes are possible (Anderson et al. 2006). New evidence showing substrate preferences of *Ss* Csl4 and Rrp4 complexes also offers a preliminary validation of this prediction (Roppelt et al. 2010). Indirect exosome subunit–RNA interactions via additional proteins such as KSRP and TTP are also postulated by the exozyme model (Chen et al. 2001; Gherzi et al. 2004; Chou et al. 2006). Finally, Rrp46 homodimers with DNA binding and DNase activity show that exozymes have RNA-independent functions

(Yang et al. 2010). As the exozyme model is both testable and consistent with all the previously published data, it should be examined and rigorously tested to determine if it is a viable explanation of exosome subunit function.

SUMMARY

In this review, we provide multiple lines of evidence that strongly contradict a rigid interpretation of the core exosome paradigm *in vivo*. We propose the exozyme model as a way to encapsulate all the available data while maintaining many aspects of the core exosome model, including a role(s) for the core in certain RNA processing and turnover events. The largest obstacles to critically evaluating the exozyme model are the incompleteness of the data (Fig. 1, gray boxes) and the conflicting results (Fig. 1, orange boxes) published to date. To test the exozyme model fully, a systematic *in vivo* analysis determining the relationship(s) of each exosome subunit to specific RNA metabolic functions is required. Many of these questions can be addressed simultaneously using genetic or RNAi approaches coupled to deep sequencing methodologies. Once these data have been reported, the relationship between the exosome core and exozymes will come into focus.

SUPPLEMENTAL MATERIAL

Supplemental material can be found at <http://www.rnajournal.org>.

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