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Exosome complex and pervasive transcription in eukaryotic genomes

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Exosome complex is widely conserved, functionally versatile, and essential constituent of the machinery regulating gene expression in the nucleus as well as in the cytoplasm. While the most fundamental enzymatic property of exosome is ribonucleolytic activity, its *in vivo* functions are varied, highly specific, and tightly regulated, and include RNA degradation, processing, and quality control. Recent reports reveal that exosome also has a prominent role in gene silencing as well as in regulating the expression of a wide variety of noncoding RNAs. Taken together with the emerging notion of pervasive genomewide transcription, these findings indicate that 'policing the transcriptome' may well turn out to be the major role of exosome in eukaryotes.

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Introduction

Exosome was first described in 1997 as a stable complex of RNase-like and RNA binding proteins implicated in rRNA biogenesis [1]. Subsequent studies revealed that it has a large number of substrates and participates in first, 3′-end processing of stable structural RNAs from their extended precursors; second, RNA degradation, for example during homeostatic or regulated mRNA turnover; third, quality control and selective elimination of molecules that are not properly processed, folded, and/or assembled into RNP particles; fourth, attenuation-like regulation of alternative transcripts; fifth, post-transcriptional gene silencing; and sixth, regulation of noncoding RNA output of eukaryotic genomes. This article focuses on the recent findings in the last three areas (see [2–4] for recent reviews on exosome structure and function).

Nuclear and cytoplasmic forms of exosome share 10 common subunits. Six of these, RNase PH domain-containing proteins Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, and Mtr3 are organized into a hexameric ring, capped on one side by a trimer of subunits (Rrp40, Rrp4, and Csl4) that contain S1 and KH RNA binding domains. This nine-subunit architecture is structurally similar to the archaeal exosome complex that possesses three active sites [5]. However, RNase PH-like subunits in eukaryotes are catalytically inactive, owing to amino acid replacements that disable binding of RNA, phosphate ion, or catalysis [6,7] (one exception is plant exosome, possessing a catalytically active Rrp41 subunit [8]). Instead, active sites are contributed by Rrp44 (Dis3) as well as by substoichiometric, nuclear-specific subunit Rrp6. Exonucleolytic activity of Rrp44, often considered the 10th subunit of the exosome core, is essentially equivalent to that of the 10-subunit complex and important for optimal growth [6]. Unexpectedly, Rrp44 was recently found to also have a biologically significant endonucleolytic activity, which probably generates entry points that facilitate subsequent exonucleolytic digestion [9–11]. Rrp6 subunit, present only in the nuclear exosome, has several unique functions, such as during the final stages of the 5.8S rRNA processing as well as in mRNA quality control events in the vicinity of transcription sites and/or nuclear pores [12]. Moreover, Rrp6 has additional functions not associated with the exosome core [13]. Functional versatility of exosome is further facilitated by a number of auxiliary factors, most prominently TRAMP (for *Trf*4/5–*A*ir1/2–*M*tr4 *p*olyadenylation) complex, whose Trf4 (or Trf5) subunit oligoadenylates aberrant mRNA transcripts as well as a class of 200–500 nt long intergenic cryptic unstable transcripts (CUTs, below) and thereby marks them for degradation [2].

Key variables determining the susceptibility of a given transcript to exosomal decay in the nucleus include its transcriptional termination and polyadenylation pathways, as well as the presence of stabilizing secondary structures and protective RNA binding proteins [14]. For example, during mRNA processing the downstream fragment resulting from the pre-mRNA cleavage is degraded in the 5′–3′ direction, while the upstream fragment is polyadenylated by conventional poly(A) polymerase, followed by its rapid association with the poly(A) binding protein(s). On the other hand, in the case of CUTs, the recognition of specific RNA sequences by the RNAP-II-associated Nrd1/Nab3/Sen1 complex triggers a distinct mode of termination that is coupled to polyadenylation by

TRAMP. This is followed by a rapid degradation of CUT, facilitated by direct physical interaction of Nrd1 and exosome [15].

Exosome and gene regulation via alternative transcription start site choice

Transcriptional attenuation, widely used in bacteria, generates alternative transcripts with distinct functional fates. Recent reports suggest that exosome partakes in functionally parallel mechanisms in eukaryotic cells. One such case concerns the yeast *IMD2* gene encoding the key enzyme of GMP biosynthesis, whose expression is inversely correlated with cellular GTP levels [16,17]. In GTP-replete conditions, transcription from *IMD2* promoter produces CUT that starts with guanosine, terminates in Nrd1 complex-dependent manner and is rapidly degraded by exosome. By contrast, in low GTP, the same TATA box directs RNAP II to scan past the CUT transcriptional start site (TSS) and initiate with an A near the end of the CUT-encoding region, resulting in the functional, full-length *IMD2* mRNA. Similarly, in the case of yeast *URA2* gene, the same TATA box directs transcriptional machinery to different TSSs under activating and repressing conditions, resulting in the production of mRNA and CUT, respectively. Under repressing (high uracil) condition, a futile cycle of initiation, termination, and rapid exosome-mediated decay of the upstream CUT prevents productive expression of the downstream *URA2* mRNA [18]. Under activating conditions, *URA2* CUT does not diminish (as opposed to the case of *IMD2*), yet productive initiation at the *URA2* mRNA TSS somehow increases without changing the frequency of firing from the common upstream promoter. Hence, a constitutively negative effect of the *URA2* CUT is somehow negated upon activating conditions. One intriguing possibility is that the CUT RNA itself may help capture and redirect the RNAP II molecules to the *URA2* mRNA initiation site [18]. In this scenario, exosome would antagonize transcriptional site switch. How widespread such a mechanism might be is not yet clear, but numerous other transcripts encoding nucleotide biosynthetic genes have associated CUTs [18], and recent reports suggest that heterogeneous unstable RNAs may be associated with standard mRNA promoters genomewide (below).

Exosome and gene silencing

The most widely appreciated mechanisms of gene silencing in eukaryotes depend on small RNA-directed mRNA degradation and/or translational repression in the cytoplasm as well as chromatin-level effects in the nucleus (such as repressive histone modifications). It now appears that the exosome complex has joined the pantheon of gene silencing. In *S. pombe*, defects in Rrp6, Dis3/Rrp44, or Cid14/Trf4 derepress telomeric, silent mating type, and centromeric loci [19•,20]. This seems to be a direct consequence of the defect in the degradation of RNA transcribed from these loci, since heterochromatin formation remains unaffected [19•]. Whether Cid14 acts on or off chromatin remained debatable [20] but surprisingly, silencing of transcripts derived from the regions flanking centromeric repeats is mediated by cytoplasmic, rather than nuclear, form of the exosome, since these species are upregulated in *cid14*, *mtr4*, and *dis3* but not in *rrp6* mutant cells [21••]. Remarkably, loss of Cid14 also causes dramatic redistribution in the spectrum of Ago1-associated siRNAs, from mostly repeat-associated to those derived predominantly from rRNA and tRNA [21••]. This indicates that in the absence of exosome-mediated degradation, abundant aberrant RNA species can successfully compete for RNAi biogenesis machinery and thus interfere with heterochromatic silencing in *S. pombe*. This concept of competition for substrates between the TRAMP/exosome and RNAi machineries recalls other examples of cross-talk between RNA silencing and RNA quality control pathways, and therefore has far-reaching implications. For example, aberrant RNAs in plants tend to enter RNAi pathways unless they are degraded by exosome or 5′–3′ pathway [22–24].

Gene silencing also occurs in bakers yeast, which lacks RNAi machinery. For example, *S. cerevisiae* rDNA loci are silenced via both transcriptional (Sir2 deacetylase) and post-transcriptional (exosome) mechanisms [25,26]. Interestingly, a noncoding RNAP II transcript IGS1-R in the rDNA tandem array is downregulated by Trf4 and exosome in a manner that is independent of Trf4 polyadenylation activity. Perhaps Trf4 polyadenylation mainly aids in degrading highly structured RNAs, but is dispensable for less tightly folded molecules. Furthermore, it appears that the role of Trf4 at this locus is mostly to stimulate chromatin remodeling and/or promote DNA repair, which in turn contributes to stable maintenance of rDNA copy number [25]. Degradation of IGS1-R also requires Nrd1 complex, and active chromatin domain spreads beyond the IGS1-R region upon its loss [26], paralleling the observation that the loss of Rrp6 in *S. pombe* triggers a strong reduction in H3K9 methylation [27]. Hence, the exosome-dependent maintenance of heterochromatic marks may be a widespread mechanism. However, exosome can have an opposite effect at other loci, as recently shown for *PHO84* gene in aging *S. cerevisiae* cells [28••]. At this locus, an antisense CUT facilitates the recruitment of Hda1/2/3 deacetylase complex, while exosome downregulates CUT and hence prevents *PHO84* silencing. The recruitment of Rrp6 to *PHO84* (but not its activity) is reduced in aging cells, indicating a requirement for the nuclear exosome in *cis*. Likewise at *GAL10*, noncoding RNA transcription recruits methyltransferases and histone deacetylases in *cis*, while TRAMP and exosome antagonize this transcript [29]. Yet another effect of CUT is illustrated by the *PHO5* locus [30]. In this case, rapid chromatin remodeling and

Table 1

Major classes of noncoding transcripts demonstrably or potentially regulated by the exosome complex

Class	Organism	Detection	Main characteristics	Reference
CUTs	*S. cerevisiae*	In rrp6 and/or trf4-deficient cells	Capped, adenylated, heterogenous ends, 200–500 nt average size, Nrd1-dependent termination, associated with bona fide promoters, originate in shared 5′ or 3′ NFRs	[33,34,35••,36••]
UNTs	*A. thaliana*	Knockdown of core exosome subunits	Adenylated, 100–600 nt in size, apparently collinear with 5′-ends of bona fide mRNAs, often terminate in first intron	[42•]
PASRs	Human, mouse	Tiling arrays in human cell lines, deep sequencing	22–200 nt, capped RNAs, cluster at 5′ termini of annotated genes, syntenic in mouse, expression correlates with the expression level of overlapping genes. Capable of suppressing gene expression in *trans*	[43••,44••]
TASRs	Human, mouse	Tiling arrays in human cell lines	22–200 nt, cluster at 3′ termini of annotated genes, syntenic in mouse	[43••]
PALRs	Human, mouse	Tiling arrays in human cell lines	Hundreds of nt in length, adenylated, likely serve as precursors for PASRs	[43••]
TSSa RNAs	Mouse	Deep sequencing	Peaks between −300 and −100 and 0 to +50 relative to TSS, 20–90 nt in length, arise by bidirectional transcription	[45••]
Ripple effect	Mouse	Expression and tiling arrays	Arise owing to orientation and position (up or downstream)-independent induction of transcripts proximal to highly transcribed regions in the genome. Adenylated, likely unstable.	[46••]
PROMPTs	Human	Tiling arrays, exosome depletion	Peak at −1 kb upstream from annotated TSSs, both sense and antisense, associated with activity of nearby bona fide promoter, sequence-independent	[47••]

recruitment of RNAP II during activation is dependent on an antisense transcript synthesized under the repressing conditions. Taken together, these examples highlight the amazing versatility of this complex in impacting gene silencing and chromatin plasticity, and suggest that we have scratched only the surface of the rich repertoire of underlying mechanisms.

A guardian of the 'dark matter' in the transcriptome?

In 2005, the RIKEN team reported full-length sequences of 102 801 mouse transcripts, revealing a whole new universe of noncoding RNA in mammals [31]. This and subsequent studies, such as the one by the ENCODE consortium [32], led to estimates that as much as 90% of mammalian genomes is transcribed. Simultaneously, it was found that yeast genome transcribes an abundant class of 200–500 nt long CUTs corresponding to the intergenic regions represented on commercial expression arrays [33]. Previously, CUTs escaped detection because they are rapidly degraded by nuclear exosome and do not accumulate to appreciable levels in WT cells. Many mysteries surround this dark matter in the transcriptome. Do CUT-like transcripts exist in other species? How many noncoding transcripts are really there (e.g. compared to bona fide mRNAs)? What are their initiation and termination rules? Do they represent a uniform noise, or a hidden set of well-defined transcription units? What would be consequences of their misregulation by exosome? More generally, what is their functional role? Several recent studies (summarized below and in Table 1) shed new light on these questions.

Yeast

A PCR products-based tiling array study produced a far-reaching insight that CUTs may be broadly associated with yeast promoters and reflect a novel type of RNAP II activity that does not lead to functional mRNAs [34]. New reports vindicate this view and further illuminate the association of CUTs with inherent bidirectionality of promoters. Xu *et al.* [35••] used tiling arrays to globally define the boundaries of CUTs, stable unannotated transcripts (SUTs), and ORF-coding transcripts (ORF-Ts) in a variety of WT as well as RRP6-deficient strains, and correlated them with nucleosome-free regions (NFRs). Although all three classes of transcripts showed depletion of nucleosomes upstream of TSSs, most remarkable was the startling prevalence of bidirectional promoters, reflected in sharing of upstream NFR between neighboring divergent transcription units that are often coexpressed. Perhaps bidirectional transcription helps maintain open chromatin architecture at promoters, and/or facilitates local spreading of transcriptional regulatory signals together with the resulting noncoding RNAs.

Neil *et al.* [36••] enriched for CUTs using a dual strategy of eliminating nuclear exosome combined with a pull-down of capped nuclear transcripts, followed by deep

sequencing of long SAGE tags. One major finding from the resulting high-resolution comprehensive map of CUTs is their sheer number, which is comparable to the number of mRNAs. Significantly, yeast CUTs appear to be well-defined, discrete transcriptional units initiating from NFRs few hundred bases upstream from the annotated genes, that is they are associated with bona fide promoters and do not result from random transcriptional noise. One class of CUTs is transcribed in the same direction as the major transcription unit, for example the CUT associated with the *NRD1* gene. *NRD1* is negatively autoregulated via a premature transcriptional termination assisted by its encoded protein, Nrd1. Interestingly, in this case exosome not only degrades the CUT, but also helps direct this premature termination event [37•]. Moreover, *NRD1* CUT and *NRD1* mRNA seem to share the same TSS, reminiscent of the upstream noncoding transcripts in *Arabidopsis* (UNTs, below). On the other hand, the CUTs that originate upstream of ORF-coding genes attenuate their expression in a different way. For example, transcription of the *SRG1* CUT across the *SER3* promoter causes transcriptional interference via occlusion of activator binding sites [38]. However, most commonly the CUTs arise as a result of bidirectional transcription, such that the 'real' gene and its antisense CUT compete for the general transcription factors that land in the common intergenic region. Why does the 'real' gene usually win? One intriguing possibility is that the exosome-mediated downregulation of the antisense CUT has a direct role in the outcome of this competition. Notably, a protozoan parasite *Giardia lamblia* that apparently lacks nuclear exosome complex, produces an abundance of antisense transcripts originating bidirectionally from promoters [39].

Examination of the transcriptional landscape in *S. pombe* likewise indicates that >90% of its genome is transcribed, although only 36 of 427 novel noncoding RNAs were downregulated by exosome [40], perhaps indicating a more specialized function compared to *S. cerevisiae*. For example, *S. pombe* RRP6 has been specifically implicated in selective elimination of meiotic transcripts from vegetative cells [41].

Arabidopsis

A genomewide survey of exosome targets in *Arabidopsis* [42•] revealed a suite of known as well as novel substrates, including stable structural RNAs, select subset of mRNAs, byproducts of miRNA biogenesis as well as numerous transcripts derived from tandemly repeated heterochromatic loci. Since such loci tend to give raise to endogenous siRNAs, it will be interesting to study the effect of exosome on plant siRNA population, especially vis-à-vis the dramatic effect on siRNAs in exosome-deficient *S. pombe* cells [21••]. In addition, ~60 ORF-encoding loci showed accumulation of polyadenylated 200–500 nt long noncoding RNAs (UNTs) apparently colinear with 5′ parts of known ORF-coding transcription units. These species seem to originate from the transcription events distinct from those that give rise to the 'main' mRNAs. The apparent coincidence of the UNTs 5′-ends with those of the main RNAs distinguishes them from mammalian PROMPTs and PASRs (below), but is reminiscent of *NRD1*-associated CUT in yeast. Whether plant UNTs are terminated by Nrd1-like pathway remains to be determined.

Mammals

High-throughput studies in mammalian cells contributed greatly to the emerging concept of pervasive genomewide transcription. A flurry of recent reports further points to a widespread occurrence of promoter-associated noncoding transcripts and suggests that nuclear exosome may play a prominent role in controlling the output of this transcriptional activity (Table 1).

A tiling array-based survey of polyadenylated human nuclear and cytosolic long RNA (lRNA, >200 nt) as well as of total short RNA (sRNA, <200 nt) revealed a complex interleaved pattern of low-abundance transcripts associated with termini of known annotated transcription units, PASRs and TASRs (promoter-associated and terminator-associated small RNAs). Notably, as much as 41.8% of all transcribed sequences are never exported from the nucleus [43••], indicating a massive requirement for RNA quality control, processing, and degradation activities such as exosome. Many of these RNAs are syntenic in mouse. Moreover, overexpression of a synthetic PASR *in trans* can downregulate its cognate gene — the first direct indication of a functional relevance of ncRNAs of this type [44••].

Using deep sequencing, Seila *et al.* identified a novel class of transcription start site-associated (TSSa) RNAs of 20–90 nt [45••]. TSSa RNAs are associated with over half of all annotated mouse genes and can run in either direction, with peak counts centered between positions 0 to +50 downstream and −100 to −300 upstream of the TSS. How do TSSa RNAs arise and do they have a function? ChIP experiments indicate that RNAP II and H3K4 trimethylation are more significantly associated with genes that have TSSa RNAs compared to a random gene set. Moreover, while RNAP II peaks positioned precisely over the maxima of sense and antisense TSSa RNA signals, the H3K79me2 mark that is associated with productive elongation was only found downstream of TSSs. These data suggest that TSSa RNAs commonly arise as a result of divergent transcription over short distances at active promoters, and may help maintain a state poised for subsequent regulation. Apparently, RNAP II frequently pauses after initiating in either direction, but some undetermined mechanism prevents its escape into productive elongation in antisense direction while allowing elongation into the major transcription unit. The TSSa RNAs must be rapidly degraded (possibly by

exosome) because their abundance is very low (10^{-1}/cell), while the TSSa RNAP II ChIP signals are quite robust. These findings represent a striking parallel to those in yeast (above), and it will be interesting to test whether exosome helps determine the outcome of the competition between the antisense and sense transcripts.

Another study demonstrates that transient, intense transcription of immediate early genes (IEGs) can upregulate their neighboring loci in what was dubbed a 'ripple effect' [46••]. Because IEGs and their neighbors are not structurally or functionally related and their expression levels are not comparable, this effect cannot be explained by known mechanisms regulating clusters of coexpressed genes. Upregulation of neighbors is orientation and position-independent, occurs in a gradient of intensity centered at IEG, and is not confined to genes with annotated functions, that is some of the 'ripples' encoded noncoding RNAs. Conversely, growth factors responsive noncoding RNAs can trigger ripple effect in the neighboring ORF-encoding regions. Although the fate of the 'ripple RNA' remains to be determined, one might predict that such transcripts are removed by the quality control pathways, such as exosome. Indeed ripple effect does not perceptibly contribute to the cytoplasmic mRNA pool [46••].

PROMPTs (for *prom*oter u*p*stream *t*ranscripts), like TSSa RNAs, occur in both directions, but are found further upstream from TSSs (~1 kb) [47••]. Also, PROMPTs are more distal from TSSs than PASRs (which center at ~0.5 kb coordinate on either side of TSS) but more proximal than transcriptional ripples, and as opposed to both, detectable only upon the exosome knockdown. PROMPT regions contain RNAP II and marks of active chromatin (H3K9Ac and DNase I hypersensitivity) but are not associated with transcription initiation factors. Hence, it appears that they arise as 'beneficiaries' of the activity of nearby bona fide promoters rather than originate from PROMPT-dedicated ones. Moreover, a strong positive correlation exists between the transcriptional signal strength over the PROMPT region and the main transcription unit located downstream of it. Amazingly enough, PROMPTs can be generated even from completely heterologous DNA, as demonstrated by the transfection of an artificial construct linking a fragment of the lambda phage DNA with the CMV promoter-driven beta globin gene.

How and why are PROMPTs degraded, and why are they there in the first place? It appears that the exosome is uniquely specialized in their degradation, as neither deadenylase PARN nor 5′ decay pathway components Dcp2 and Xrn1 affect their abundance. Interestingly, RRP6 and RRP44, the two active ribonucleases in the human exosome, appear to act redundantly in degrading PROMPTs. It is likely that degradation takes place in the nucleus, although whether it occurs on or off chromatin is not known. Exosome-mediated removal of PROMPTs may facilitate disengagement of the bound RNAP II molecules, thus freeing them up for productive transcription ('a RNAP II warmup' model), and/or assist in chromatin remodeling around promoters so as to create a favorable environment for subsequent transcription. Finally, PROMPTs may affect DNA methylation in the CpG islands [47••]. In any event, widespread occurrence of PROMPTs indicates their functional potential as raw material for the evolution of gene regulatory mechanisms.

Conclusions

While some noncoding RNAs are associated with locus-specific gene regulatory functions, results of the highly parallel expression profiling indicate that eukaryotic genomes are pervasively transcribed. The sheer scale of genomewide transcription calls for a specialized machinery dedicated to processing and turnover of resulting RNA. Exosome complex, which may have evolved originally to regulate specific genes and pathways, is likely to have been co-opted for the purpose of dealing with the consequences of such pervasive transcription, raising an intriguing possibility that policing the noncoding transcriptome has become its major role.

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