Minireview

Lifting the veil on the transcriptome

Kevin P Callahan* and J Scott Butler*†

Addresses: *Departments of Biochemistry and Biophysics, and †Microbiology and Immunology, University of Rochester Medical Center, Elmwood Avenue, Rochester, NY 14642, USA.

Correspondence: J Scott Butler. Email: btlr@mail.rochester.edu

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Abstract

Inhibition of the cellular RNA surveillance system in *Arabidopsis thaliana* results in the accumulation of thousands of transcripts arising from annotated and unannotated regions of the genome. This normally hidden transcriptome is replete with noncoding RNAs with the potential to regulate wide-ranging physiological activities.

The eukaryotic genome was once thought to be collinear, with defined regulatory regions controlling the initiation of transcription of their respective downstream protein-coding regions. Recent genome-wide analyses demand a revision of this view by revealing a genomic architecture now best described as interleaved and modular [1]. Tiling microarray analyses have identified thousands of RNAs arising from annotated and unannotated regions of eukaryotic genomes, with transcription often occurring on both strands of the same region of DNA. Some of the RNAs produced from these noncoding regions appear to play a physiological role in the cell, but the details remain obscure. In a recent paper in *Cell*, Belostotsky and colleagues (Chekanova *et al.* [2]) now reveal an additional layer of complexity to the transcriptome of *Arabidopsis thaliana* that appears upon inhibition of the exosome, a component of the RNA surveillance system (Figure 1). They have found novel species of RNA whose regulated expression may control critical physiological processes in eukaryotes. In addition, they show that inhibition of RNA surveillance causes developmental abnormalities that provide clues to the physiological roles of some of these noncoding RNAs.

RNA surveillance by the exosome

The exosome, a highly conserved RNA-processing protein complex, appears to provide the major 3'-5' exoribonucleolytic activity in eukaryotic cells [3]. Present in both the nucleus and the cytoplasm, exosomes degrade aberrant noncoding and coding RNAs and catalyze the accurate 3'-end formation of ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). Remarkably, recent experiments in the budding yeast *Saccharomyces cerevisiae* showed that these noncoding RNA intermediates carry poly(A) tails synthesized by the TRAMP complex [4], a protein complex containing the poly(A) polymerases Trf4p or Trf5p, the zinc-knuckle RNA-binding proteins Air1p or Air2p and the RNA helicase Mtr4p (Figure 1). Polyadenylation of an RNA by the TRAMP complex facilitates its degradation by the exosome; thus, inactivation of the exosome results in the accumulation of thousands of poly(A)$^+$ noncoding RNAs.

Structure and function studies on the exosome from yeast and humans showed that its structural integrity requires all nine subunits, and that its catalytic activity resides in the exoribonucleases Rrp44p and Rrp6p [5,6]. This differs from the situation in *A. thaliana* where the exosome component RRP41 possesses exoribonuclease activity, and a component of the core exosome, CSL4, is dispensable for growth. The *Arabidopsis* homolog of Rrp44p - RRP44 - does not co-purify with the plant exosome. Chekanova *et al.* [2] exploited these differences to evaluate the genome-wide consequences of the absence of CSL4, or of the depletion of the essential RRP41or RRP4 in *A. thaliana*. Their results illuminate an unappreciated functional plasticity of the exosome, and uncover hidden layers of the transcriptome that are under

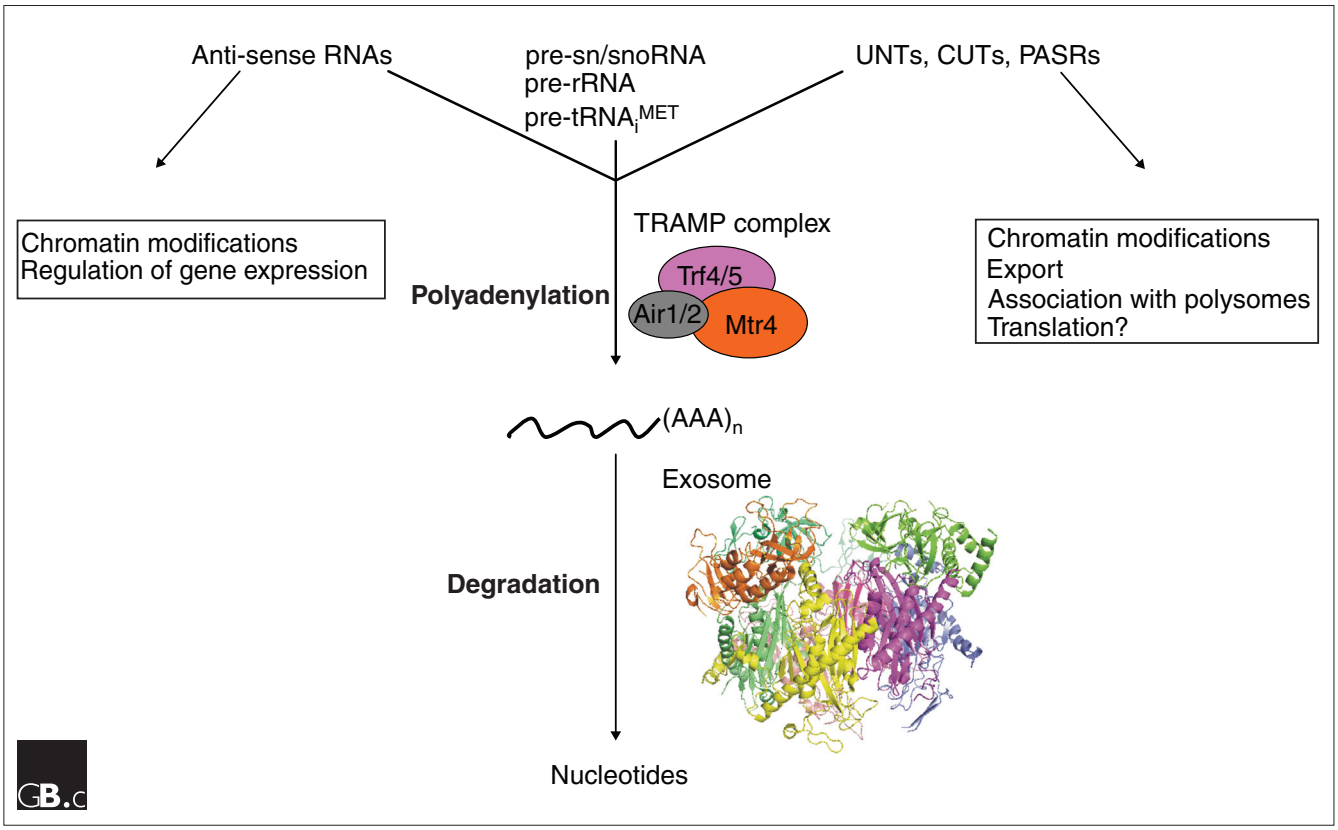

Figure 1
Surveillance of noncoding RNA production by the exosome. A variety of small noncoding RNAs are transcribed from the genome. These RNAs are polyadenylated by the TRAMP complex, which facilitates their degradation by the exosome. Some of these transcripts appear to participate in regulatory events before their degradation. The molecular model of the human exosome was generated using PyMOL from PDB coordinates 2NN6 deposited by Liu *et al.* [6]. CUTs, cryptic untranslated transcripts; PASRs, promoter-associated short RNAs; UNTs, upstream noncoding transcripts.

the control of widespread oligoadenylation, reminiscent of prokaryotic RNA surveillance [7].

Following depletion of RRP41 and RRP4 by estradiol-induced RNA interference (RNAi), Chekanova *et al.* [2] generated transcriptional profiles by interrogating tiling arrays with oligo(dT)-primed cDNA probes. The results showed upregulation of more than 1,500 transcripts, many presumed to be direct targets of the exosome. The RNAs arose from transcription by RNA polymerases I, II and III, and encompassed all known RNAs as well as some novel species. Interestingly, many of the RNAs exhibited an exosome subunit-specific response, suggesting that unique exosome subcomplexes carry out specific functions in the cell. Consistent with this conclusion, analysis of T-DNA insertion mutations in CSL4, RRP41 and RRP4 revealed distinct phenotypes in homozygotes. Loss of function of CSL4 caused no significant defects in plant development. In contrast, mutation of RRP41 blocked the formation of female gametophytes and loss of RRP4 function arrested plant development at early embryonic stages.

Antisense RNA and other small noncoding RNAs

One of the most striking discoveries by Chekanova *et al.* [2] was the role of the exosome in controlling the levels of antisense transcripts from rRNA loci. After RNAi-mediated suppression of RRP41 or RRP4, increases and decreases in the level of antisense transcripts occurred in chromosomal regions that form boundaries to the mature rRNAs, suggesting the possibility of a new layer of regulation for these highly transcribed genes. It seems likely that the antisense transcripts play a direct role in controlling rRNA levels, as their abundance correlates inversely with the levels of the adjacent mature rRNA. Perhaps antisense transcription affects the local chromatin environment and thus the accessibility of the respective rRNA promoter. Indeed, a recent report suggests that exosome control of the level of cryptic transcripts from the rDNA locus plays an important role in heterochromatic gene silencing [8]. Chekanova *et al.* [2] also detected polyadenylated antisense transcripts that accumulated near mRNA promoter regions. Recently, Camblong and colleagues [9] showed that antisense RNA production near the *PHO84* promoter in *S. cerevisiae*

results in the recruitment of the histone deacetylase Hda1 and subsequent repression of *PHO84* transcription. Interestingly, the stabilization of an antisense transcript near a gene from the same family, *PHO5*, results in increased production of the mature mRNA [10]. The increased transcription correlates with changes in the chromatin environment; in this case, however, the antisense RNA probably enhances chromatin plasticity, leading to activation of the gene traversed by the antisense sequence. The regulatory potential of antisense transcripts is highlighted by recent work showing that transcription of murine *Xist* RNA, which triggers X-chromosome inactivation, is negatively regulated *in cis* by an antisense gene [11].

Not all transcription from protein-coding genes results in full-length mRNA. Chekanova *et al*. [2] observed collinear transcription from the 5' ends of annotated genes, producing what they refer to as upstream noncoding transcripts (UNTs). This transcription appears distinct from that of the 'main' transcription units by RNA polymerase II, and the UNTs accumulate to higher levels than the mature mRNA. Similar transcripts, called promoter-associated short RNAs (PASRs) accumulate in human cells, where their expression correlates strongly with gene transcription [12]. Human PASR expression also correlates with that from syntenic regions in mouse, suggesting a conserved function for these small RNAs [12]. Although no experimental evidence for a role for PASRs exists, a clue comes from the study of small RNAs in budding yeast termed cryptic untranslated transcripts (CUTs) [13,14]. A CUT sequence called *SRG1* overlaps the promoter of the *SER3* gene and negatively regulates its expression by promoter occlusion [15]. This glimpse of function and the widespread conservation of small RNAs overlapping the 5' end of genes suggest that further work on these transcripts will uncover novel roles in gene regulation.

The widespread, and apparently regulated, production of UNTs and CUTs suggests that these transcripts have physiological roles in the cell. In this view, the cell may only require appreciable levels of these transcripts at specific times or upon receiving specific stimuli. The cell could then upregulate production of these 'poised' transcripts by increasing recruitment of the transcriptional machinery or possibly by local inhibition of the exosome surveillance pathway. Indeed, in *Schizosaccharomyces pombe*, meiosis-specific mRNAs accumulate upon loss of Rrp44 and of Cid14, a homolog of Trf4p, suggesting that the exosome constantly degrades those RNAs to prevent ectopic meiosis [16]. The absence of accumulation of UNTs, PASRs and CUTs in wild-type cells implies a very limited or tightly regulated role *in vivo*. Alternatively, some of this transcription may not result in functional RNA, but instead represents a vestige of nonspecific transcription that provides an organism with the ability to evolve new, functional transcription units [17].

The work of Chekanova *et al*. [2] also points to an apparent functional specialization among subunits of the exosome and suggests the existence of unique exosome subcomplexes performing specific functions throughout the cell. Is it possible that a 'degradation exosome' or a 'processing exosome' exists, or that location in the cell dictates an exosomes's function? Evidence for the latter case exists in *Drosophila*, where individual exosome subunits have distinct localizations *in vivo* [18]. The inability of the *Arabidopsis* RRP44 protein to co-purify with the plant exosome also points to the existence of exosome subcomplexes. The unique transcriptome profile of the *Arabidopsis csl4* mutant and the sub-stoichiometric amounts of the protein in affinity-purified exosomes also suggest the existence of functional exosome subcomplexes. Indeed, in humans a trimeric complex containing Rrp6, the RNA helicase Mtr4 and the RNA-binding protein MPP6 seems to participate in the correct processing of 5.8S rRNA [19]. The plasticity may not stop there, as the poly(A) polymerases of the TRAMP complex also exhibit differences in specificity [20]. It is conceivable that such dynamic behavior of the surveillance components evolved to regulate the large number of small RNAs produced by the transcriptome.

The importance of the exosome-dependent surveillance pathway is highlighted by the fact that cells deficient in this pathway accumulate chromosomal abnormalities similar to those observed in cancer patients [16]. In addition, exosome-deficient yeast cells exhibit a growth defect in the presence of the chemotherapeutic drug 5-fluorouracil (5FU), and exposure of wild-type cells to 5FU results in exosome-enhanced accumulation of polyadenylated noncoding RNAs [21]. The ubiquitous nature of the exosome makes it an ideal tool for identifying and understanding new classes of RNA. Importantly, the depletion of the individual exosome subunits in *A. thaliana* leads to unique RNA profiles and developmental phenotypes [2]. These new findings suggest a previously unrecognized role for the exosome in regulating the levels of noncoding RNAs that may play critical roles in gene regulation and organismal development.

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