

CUTting genetic noise by polyadenylation-induced RNA degradation

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Silencing of genomic regions in eukaryotes is thought to be the result of transcriptional repression. Recent results show that nuclear RNA degradation plays a major role in discarding RNA molecules with no obvious roles that are produced by cryptic RNA polymerase II transcription throughout the *Saccharomyces cerevisiae* genome. These cryptic transcripts are polyadenylated at their 3'-end by a poly(A) polymerase complex distinct from that used by the mRNA factory, which serves to tag these aberrant transcripts for nuclear degradation.

Introduction

Discriminating between expressing useful genetic information and repressing the expression of genetic 'junk' in eukaryotes has long been thought to rely on exquisite transcriptional control. In classical models, promoter elements and positive regulatory sequences, in the context of an accessible chromatin environment, promote the assembly of productive transcription initiation complexes that lead to the production of RNA transcripts. According to this view, transcription is absent in genetically silent regions either because of the absence of active promoters or because the chromatin is inaccessible (heterochromatin). A recent study by Wyers *et al.* [1] provides a complementary mechanism by which the expression of a fraction of the *Saccharomyces cerevisiae* genome is muted. In this paper, the authors show that several loci thought to be genetically silent are indeed actively transcribed, but the RNAs produced from these loci are rapidly degraded within the nucleus. This mechanism seems widespread throughout the genome and is estimated to affect as much as 10% of the intergenic regions of the yeast genome. The authors named the RNAs produced from these cryptic transcription units 'CUTs' – for 'cryptic unstable transcripts'. CUTs had previously been detected by serial analysis of gene expression (SAGE) of the yeast transcriptome [2], but their significance remained unclear. Like mRNAs, CUTs show discrete 5'-ends, with cap structures, but they display variable 3'-polyadenylated ends. CUTs exhibit no apparent genetic information as they lack extended open-reading frames (ORFs). Wyers *et al.* show that CUTs are normally degraded in the nucleus by the exosome, a complex of 3'-5' exoribonucleases [3], but they can be detected in a yeast strain lacking the nuclear exosome component Rrp6p. The detection of these

transcripts in cells lacking Rrp6p is not due to aberrant transcription of these regions in the absence of Rrp6p, as the density of RNA polymerases in these regions is similar in wild-type cells and in cells lacking Rrp6p. Thus, CUTs are transcribed actively in normal cells, but they are rapidly degraded by the nuclear exosome.

An alternative polyadenylation complex tags CUTs and other transcripts for degradation

A second surprise from this study came from the fact that the poly(A) tails found at the 3'-ends of CUTs were added not by the classical poly(A) polymerase (PAP) Pap1p, but rather by the TRAMP complex containing the non-canonical PAP Trf4p, the RNA-binding proteins Air1p and Air2p and the putative helicase Mtr4p [4] (Figure 1). Polyadenylation of CUTs by the TRAMP complex directs these RNAs for degradation (Figure 1) as CUTs are further stabilized when both Rrp6p and Trf4p activities are absent. In contrast to typical mRNAs, CUTs show heterogeneous 3'-ends, raising the question of the mechanism of formation of their 3'-ends. From the data presented by Wyers *et al.*, it seems likely that CUTs detected in cells lacking Rrp6p correspond to intermediate species of the degradation of larger transcripts. While it is clear that Trf4p is responsible for adding the poly(A) tails at the 3'-end of CUTs detected in cells lacking Rrp6p, longer and more discrete-sized poly(A)-containing species accumulate in the absence of both Rrp6p and Trf4p. In this circumstance, the polyadenylation of these species is dependent on the classical PAP, Pap1p. Whether or not these larger transcripts correspond to primary CUT transcripts is questionable as they are not observed in normal conditions and they might reflect a default pathway that these transcripts undergo when the degradative activities of Trf4p and Rrp6p are absent. Thus, a major unanswered question is to understand the precise 3'-processing and degradative pathways that give rise to CUTs and to clarify the precursor-product relationships that might exist between the longer species polyadenylated by Pap1 and the shorter CUTs polyadenylated by Trf4p. Whatever the precise mechanism of 3'-end formation of CUTs, these results show that Trf4p-mediated polyadenylation is required for their degradation (Figure 1).

A role for Trf4p-mediated polyadenylation in RNA degradation was not completely unexpected. In a pioneering study from the Anderson laboratory, Trf4p-mediated polyadenylation had been implicated in tagging a tRNA

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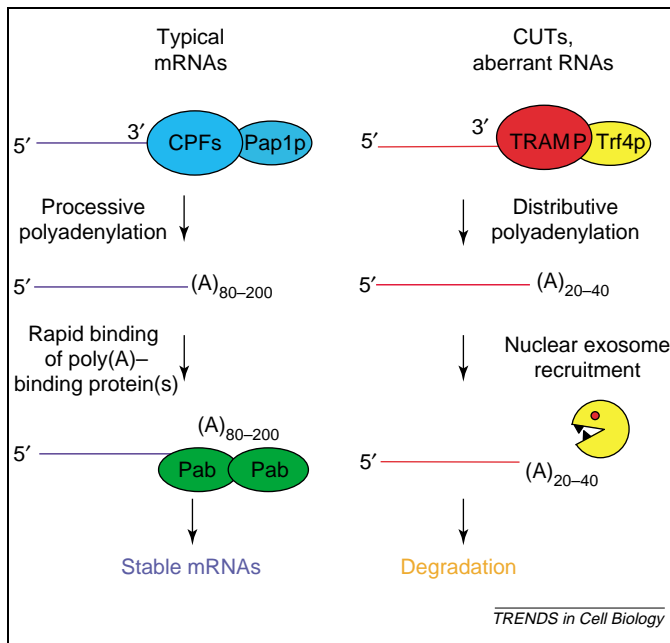


Figure 1. Models of polyadenylation mechanisms and functional consequences for typical eukaryotic mRNAs or cryptic unstable transcripts (CUTs) and aberrant RNAs. Abbreviations: CPFs, cleavage and polyadenylation factors of mRNAs; TRAMP, Trf4p/Air2p/Mtr4p polyadenylation complex. Although part of the CPFs or TRAMP complexes, respectively, Pap1p and Trf4p are shown as separate subunits. The lengths of the poly(A) tails added by each of the poly(A) polymerases are approximate.

lacking nucleotide modifications for degradation [5]. Subsequently, *in vitro* studies by Vanacova *et al.* showed that Trf4p PAP activity focuses on misfolded or structurally unstable tRNAs that result from the absence of some modifications [6]. This study and work by LaCava *et al.* also demonstrated that Trf4p-mediated polyadenylation can stimulate exosome activity *in vitro* [4,6], suggesting that the poly(A) tails added by Trf4p can tag RNA molecules for degradation by the exosome. This mechanism operates on misfolded tRNAs [5,6], as well as on several noncoding RNAs [1,4,6]. More generally, Wyers *et al.* have shown that Trf4p-mediated polyadenylation plays a widespread role in directing cryptic polymerase II transcripts to the RNA degradation pathway [1].

Taken together, these studies challenge the classical dichotomous model in which 3'-polyadenylation acts as an RNA destabilization element in bacteria, but acts as a stabilization signal in eukaryotes [7]. A more refined eukaryotic model suggests that Pap1-induced polyadenylation stabilizes eukaryotic mRNAs, whereas Trf4p-mediated polyadenylation triggers nuclear degradation by the exosome (Figure 1). Differences in the functional consequences of poly(A) tail addition by each PAP complex might be linked to their distinct kinetic properties [4,6]: Pap1p fast and processive activity might promote rapid binding of poly(A)-binding proteins or other factors that would protect the 3'-end of the RNA from exosome activity and/or promote RNA export [8], whereas slow and distributive poly(A) polymerization by Trf4p might allow access of the 3'-end to the exosome, triggering degradation (Figure 1).

What distinguishes CUT transcription units from 'normal' transcription units?

The most intriguing question that arises from this study is how the cellular machinery discriminates transcription units that generate CUTs from regular mRNA transcription units. It is clear that promoter identity is not responsible – as swapping a promoter that gives rise to CUTs to a heterologous promoter does not stabilize these transcripts [1]. One possibility is that, as CUTs lack extended ORFs, they might be distinguished from regular transcripts by a nuclear mechanism analogous to nonsense-mediated decay [9] that would recognize the absence of ORFs and trigger degradation. In addition, it is known that co-transcriptional loading of mRNA export factors is linked to 3'-end formation in yeast [8]. Thus, it is reasonable to speculate that mRNA export factors might be loaded less efficiently on CUTs, because their 3'-end formation mechanism is distinct from that of 'normal' mRNAs. This would result in a slower rate of export and in nuclear sequestration. In the absence of export, they might be degraded by default in the nucleus. It is also possible that CUTs represent a novel class of noncoding RNAs and that they might not be as 'useless' as generally presented. Although they lack any apparent genetic information and are degraded under standard growth conditions, they might bear some function as noncoding RNAs in nonstandard laboratory conditions (e.g. stationary phase or sporulation), where their degradation would be repressed.

How conserved is polyadenylation-induced RNA degradation among eukaryotes?

Wyers *et al.* propose that this mechanism might be used in higher eukaryotes to complement transcriptional repression. Tiled microarray studies in human cells have shown an abundance of noncharacterized transcripts, some of which are polyadenylated and found exclusively in the nucleus, as would be expected for CUTs [10]. This observation would favor the existence of conserved pathways. An alternative possibility is that degradation of the RNAs generated from cryptic promoters is specific to compact genomes, such as that of *S. cerevisiae*. Degradation of CUTs might avoid having to establish regions of chromatin inaccessibility when actively transcribed regions are located nearby, while preserving the possibility of evolving functional loci from these cryptic transcription units, as has been suggested [1]. Although the *trans*-acting factors involved in CUT degradation are conserved in higher eukaryotes, it is tempting to speculate that this way of muting genetic noise has evolved with the compactness of the *S. cerevisiae* genome. In organisms with larger genomes, transcriptional repression by heterochromatin formation might be more prominent, but possibly still involving the same *trans*-acting factors. Indeed, Cid12, a protein similar to Trf4p, was recently shown involved in heterochromatin formation in fission yeast [11], suggesting an independent function for polyadenylation in transcriptional silencing in higher eukaryotes.

Concluding remarks

Whatever the functional conservation of these degradative pathways, this study provides a landmark in our understanding of gene repression. It shows that a significant fraction of the yeast genome is repressed by degrading RNA transcripts that occur from cryptic transcription events in intergenic regions. The use of a variant poly(A) polymerase and the difference in the mechanism of 3'-end formation of these transcripts are likely to distinguish these aberrant RNAs from productive ones, allowing the cells to eliminate them using a novel quality-control mechanism.

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The actin cytoskeleton, RAS–cAMP signaling and mitochondrial ROS in yeast apoptosis

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The release of reactive oxygen species (ROS) by mitochondria instigates the pathways of programmed cell death in eukaryotic cells. Gourlay and Ayscough present intriguing experimental evidence that mutations in the genes encoding the regulatory proteins End3p and Sla1p, which influence actin dynamics in budding yeast, lead to a loss of mitochondrial membrane potential, resulting in ROS production and apoptosis. This effect can be suppressed by down-regulation of the RAS–cAMP signaling pathway, thus establishing the existence of a new and complex regulatory network.

Introduction

Recent evidence indicates that crosstalk between the dynamics of the actin cytoskeleton, the release of reactive oxygen species (ROS) by mitochondria (induced by either hypo- or hyper-polarization of the mitochondrial membrane) and RAS signaling mediates pathways controlling programmed cell death in eukaryotic cells. The current

work from Gourlay and Ayscough [1,2] now provides wide-reaching insights into the potential connection between ageing and apoptosis and highlights the role of the actin cytoskeleton in preventing the release of high ROS concentrations into the cytosol.

ROS production and actin cytoskeleton dynamics

Several proteins are known to regulate actin dynamics in the budding yeast *Saccharomyces cerevisiae*. Formation of one or more multiprotein complex(es) containing Sla1p, End3p, Pan1p, Las17p, Abp1p (proteins that have been shown to be involved in the maintenance of actin polymerization and dynamics in yeast) and possibly also CAP, a yeast protein interacting with adenylate cyclase, ultimately stimulates Arp2/3-mediated polymerization of actin filaments. Sla1p, an adaptor protein containing multiple Src-homology 3 (SH3) domains, is thought to play a pivotal role in assembling components at the sites of actin polymerization in yeast actin patches. The interaction between Sla1p and Sla2p regulates a substantial fraction of actin dynamics in yeast cells during endocytosis [3,4].

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