Shortcuts to the end

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Three recent reports identify the roles of specific ribonucleases necessary for efficient termination of transcription by RNA polymerase II. In one case, the primary cleavage event is carried out by a ribozyme encoded in the pre-mRNA itself.

Genes have informational borders but no physical boundaries. This poses restrictions to the ways RNA polymerases use to initiate and terminate transcription accurately. The simplest strategy is the recognition of specific DNA or nascent RNA sequences by the RNA polymerase, either directly or through interactions with other sequence-specific binding proteins. This mode of action prevails in prokaryotic and eukaryotic initiations and in prokaryotic termination. However, termination of eukaryotic protein-encoding genes by RNA polymerase II (Pol II) does not depend on universal terminator sequences, as it does in bacteria, and may occur at various positions located hundreds or even thousands of bases downstream of the cleavage and polyadenylation site, which marks the 3′ end of the last exon. Termination is important to recycle polymerase molecules for further transcription rounds and to prevent read-throughs that could interfere with transcription initiation of downstream genes.

Two alternative models have been proposed to explain Pol II termination, both consistent with the fact that the release of Pol II is dependent on transcription through a functional poly(A) signal[1,2] (the canonical AATAAA). An antitermination model, similar to the mode of action of the N protein of phage λ in *Escherichia coli*, assumes that elongation factors traveling with Pol II dissociate over the poly(A) signal, resulting in a termination-competent form of the enzyme. The addition of the poly(A) tail does not seem to be critical for this mechanism because yeast mutants defective in the polyadenylation enzyme but having a normal poly(A) signal terminate normally. Instead, findings that poly(A) site-cleavage factors also have activities in Pol II elongation and are able to interact with the C-terminal domain (CTD) of Pol II seem to explain why the poly(A) signal is required for termination[3]. The alternative 'torpedo' model[2,4] (**Fig. 1**) postulates that cleavage of the nascent mRNA at the poly(A) site provides an unprotected (uncapped) 5′ end that is degraded by 5′→3′ exonucleases (torpedoes), leading to the destabilization of the template–transcript–Pol II ternary complex and final dissociation of the enzyme. Although the two models are not necessarily mutually exclusive, three recent studies in *Nature*[5–7] highlight the importance of the torpedo mechanism by identifying and characterizing specific ribonucleases that are necessary for proper transcriptional termination in a human gene and in yeast.

The Proudfoot lab had previously shown that efficient transcriptional termination in the human β-globin gene not only requires a normal poly(A) signal but also depends on a novel cotranscriptional cleavage event (named CoTC) that takes place at a pre-mRNA segment encoded within the 3′ flanking region, between the poly(A) site and the termination region[8]. A study by Teixeira *et al.*[5] from the Akoulitchev and Proudfoot labs now elegantly demonstrates that the CoTC activity is not carried by a protein endonuclease acting *in trans* upon the β-globin transcript but involves a ribozyme activity encoded by the CoTC DNA region itself. Synthetic transcripts obtained from plasmid templates with the 800-nucleotide CoTC element in its original orientation become degraded *in vitro* in the presence of Mg^{2+} and GTP, under protein-free conditions, whereas control transcripts from plasmid templates with the CoTC sequence in opposite orientation are not degraded. The degradation of CoTC-containing transcripts exhibits first-order kinetics suggesting a single-molecule catalytic reaction as described for some self-cleaving ribozymes. The minimal region for sufficient autocatalytic cleavage was mapped to the most 5′ 200-nucleotide segment of CoTC, which adopts a stable secondary structure with an (A+U)-rich stem essential for activity. This CoTC core includes the cleavage site and is sufficient to promote termination *in vivo* when transplanted to the 3′ flanking region of a β-globin gene devoid of its full CoTC element.

How does CoTC promote termination? The second study shows that CoTC cleavage provides an entry site for Xrn2, the major nuclear 5′→3′ exonuclease found in human cells. West *et al.*[6] show that Xrn2 depletion by RNA interference inhibits termination of β-globin gene plasmids transfected in HeLa cells. The decrease in termination efficiency was demonstrated by nuclear run-on analysis and an RNase protection assay, which revealed increases in, respectively, nascent and steady-state RNA beyond the CoTC site. These observations were corroborated by chromatin immunoprecipitation (ChIP) using an antibody to Pol II, which showed a recovery of higher Pol II densities beyond the poly(A) site in Xrn2-depleted cells. CoTC generates 3′-OH and 5′-P ends[5]. Consistent with these results, Xrn2 requires a 5′ phosphate for activity as replacement of CoTC by a hammerhead ribozyme, whose cleavage generates 5′-OH ends, prevented Xrn2 degradation and termination. The RNA by-product generated from sequence between the poly(A) and CoTC sites is also degraded because it is not protected by a cap or a poly(A) tail. However, its levels are not affected by Xrn2

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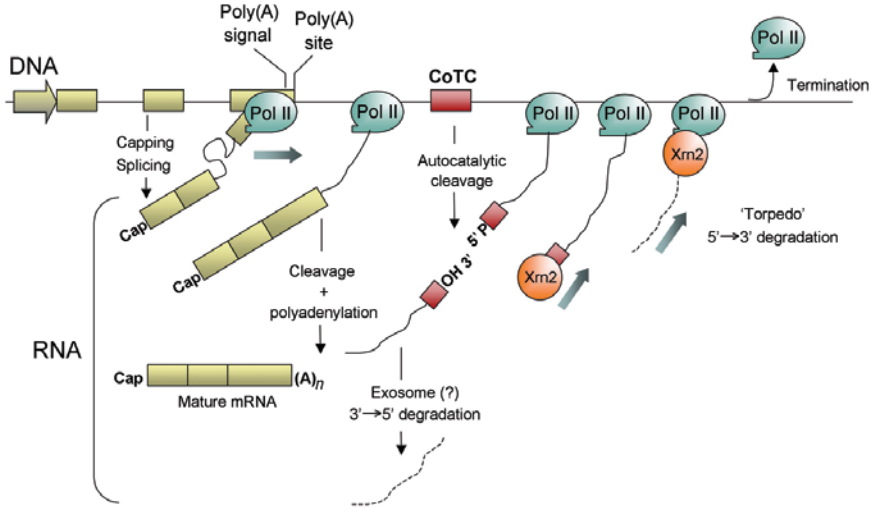

Figure 1 The torpedo model for transcription termination by RNA polymerase II in the human β-globin gene. β-globin pre-mRNA is subjected to a series of covalent modifications during its synthesis: capping, splicing, cleavage at the poly(A) site, polyadenylation, cleavage at the CoTC site, 5′→3′ degradation of downstream RNA and degradation of the RNA by-product between the poly(A) and CoTC sites. CoTC is a ribozyme, 5′→3′ degradation of downstream RNA is carried out by Xrn2 exonuclease and both processes are required for termination.

depletion, indicating that the 5′-P end generated at the poly(A) site is not a good substrate for Xrn2 degradation. This means that although a 5′-phosphate terminus generated cotranscriptionally is required, only a specific type of cleavage event is able to trigger Xrn2-dependent termination in the β-globin gene (**Fig. 1**). It is not known why CoTC activity is preferred to poly(A) cleavage in generating a substrate for Xrn2 degradation in the β-globin gene. One possibility is that both events have different kinetics and that in the context of Pol II's own kinetics, CoTC acts preferentially.

The CoTC-like ribozyme strategy seems to be restricted to the human β-globin gene and its orthologs in other primates[5], suggesting that other types of endonucleolytic events, including poly(A) cleavage, may be acting in torpedo terminations in other genes. Support for a more general version of the torpedo model is provided by the third study, which was carried out in yeast by the Buratowski and Greenblatt labs[7]. The CTD of the large subunit of Pol II consists of multiple heptapeptide repeats (26 in yeast and 52 in humans) with the consensus sequence YSPTSPS, where the serines at positions 2 and 5 are subjected to regulatory phosphorylations. Ser5 phosphorylation at early stages of transcription promotes promoter release and association with capping factors, whereas subsequent Ser2 phosphorylation promotes elongation, 3′-end formation and termination. Yeast polyadenylation factors, also required for termination, are cotranscriptionally recruited to Ser2 phospho-CTD (P-CTD). Looking for other proteins able to interact with Ser2 P-CTD peptides, Kim *et al.*[7] found Rtt103, a protein with an RPR domain (or CID, for CTD-interacting domain) present in other two yeast factors involved in mRNA processing (Pcf11 and Nrd1). Most interestingly, it was found that Rtt103 interacts with Rat1, the yeast homolog to human Xrn2. ChIP experiments confirmed that Rat1 is preferentially recruited at the 3′ end of three yeast genes, suggesting a role in 3′-end processing. The suspicion that Rat1 5′→3′ exoribonuclease activity was responsible for the degradation of RNA downstream of poly(A) cleavage was confirmed by the observation that downstream RNA is accumulated in a yeast mutant strain defective in Rat1. Hybridization of a tiled oligonucleotide microarray covering yeast chromosome III with immunoprecipitated chromatin with an antibody to Pol II (a powerful technique nicknamed "ChIP on chip") revealed that in Rat1-defective cells, Pol II remained attached to 3′ extensions of several genes, as compared with wild-type cells. Consistently, the exonuclease activity of Rat1, and not its mere presence, is needed for transcriptional termination because an active site Rat1 point mutant fails to complement, when expressed at normal levels, the Rat1-defective strain.

The above accounts call for a general role of Rat1 and Xrn2 5′→3′ exonucleases in transcriptional termination. It is conceivable that the endonucleolytic cleavage enabling these exonucleases to find their substrates and promote termination is carried out primarily and more generally at the poly(A) site. If so, CoTC, so far found only in the β-globin genes, may represent a particular strategy for a more generalized torpedo mechanism. Torpedo-like strategies may be ancient in evolution. Rat1 and Xrn2 use their substrates as leading threads to 'chase' the polymerase. This resembles Rho-dependent termination in bacteria. Rho loads onto mRNA cotranscriptionally at a C-rich sequence. Once bound, Rho acts as a 5′→3′ ATP-dependent helicase to translocate to the site of transcription and disengage the polymerase[9].

The new findings reported in the *Nature* papers[5–7] add important actors and activities to the mechanisms of cotranscriptional pre-mRNA processing. The realization that capping, splicing, 3′-end formation and termination, and mRNA export are coupled with transcription has swapped our static view of the basics of eukaryotic gene expression for a more dynamic and integrated one (recently reviewed in refs. 10–13). Because each process is extremely complex in itself, and involves hundreds of protein factors, RNA molecules, DNA sequences and possibly different chromatin configurations, we should be cautious in any attempts to generalize or simplify and bear in mind that certain molecular interactions or kinetic constraints might be relevant for a particular gene or set of genes but not for others.

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