Termination as a Factor in “Quality Control” during Ribosome Biogenesis*

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In eukaryotes, nascent rDNA and 5 S rRNA gene transcripts undergo 3’ end processing after termination. Mutations in which terminator sequences in these ribosomal RNA genes are deleted completely result in highly unstable transcripts, which are not properly processed and integrated into stable ribosome structure. Mutations that retard RNA processing by extending the 3’ external transcribed spacer or by introducing additional secondary structure in the spacers have a similar effect on stable transcript integration. The results indicate that proper termination coupled with efficient rRNA processing acts as a “quality control” process, which helps to ensure that only normal rRNA precursors are effectively processed and assembled into active ribosomes.

It is generally assumed that the termination of RNA transcription, even when genes are tandemly arranged, is largely an economic consideration, which leads to a conservation of cellular energy and allows for individual gene regulation. The wide distribution of often very large introns, which are rapidly discarded after transcription and RNA splicing, appears to make this measure less important, and it is surprising that with tandemly arranged highly repeated genes such as the rDNA, in which transcribed spacers actually can be longer than the non-transcribed regions, the termination signal is repeated (1, 2) and even fail safe copies are present (3, 4). Furthermore, in another tandemly arranged family, the genes encoding the 5 S rRNA, termination occurs only a few nucleotides after the 5 S rRNA sequence with a complex processing scheme essential for a mature RNA product (5–7). Both features clearly are not consistent with an economical use of cellular energy but still are widely conserved in eukaryotic organisms.

In recent studies on eukaryotic ribosome biosynthesis and rRNA processing, we have been expressing mutant genes in vivo in order to identify important structural features in the transcripts that contribute to rRNA function and ribosome assembly. Efficiently expressed “tagged” RNA systems have been developed for both the yeast 5 S rRNA gene (8, 9) and rDNA expression (10, 11). These can result in cellular RNA and ribosome populations that are 50–90% mutant with no adverse effects on cellular growth or function. In the course of these studies, we have been making changes in the 3’ external transcribed spacers (3’-ETS)† and termination regions to determine specific features that affect 3’-ETS processing (eg. Ref. 12). The changes have included controls in which the termination signals have been altered or removed entirely. Here we report that the yields of the mature RNAs are severely reduced when the termination signals are compromised, an observation which indicates that proper termination coupled with RNA processing can be an important component of a cell’s control on the quality of its RNA transcripts.

As previously reported (8, 11, 12) and illustrated by the example analyses in Fig. 1, when a yeast rDNA transcriptional unit or a gene encoding the 5 S rRNA is inserted in a high copy shuttle vector and expressed in yeast after cell transformation, an efficient and even preferential expression of plasmid-encoded RNA is observed with 50–90% of the RNA population being derived from plasmid-encoded transcripts. With direct 5 S rRNA quantification (Fig. 1A), the electrophoretic marker shows that 80–90% of the cellular 5 S RNA is mutant (lane b). Similarly, when 3’-end termini are mapped using S1 nuclease digestion (Fig. 1B) and a mutant RNA-specific probe (12), the termination sites, processing intermediates, and mature 25 S rRNA are all clearly evident (lane d). In striking contrast, however, when the termination signals are deleted using PCR-mediated targeted mutagenesis (9, 12), almost no plasmid-derived RNA is evident. As shown in Fig. 1, steady state analyses of the cellular 5 S RNA population (Fig. 1A) indicate that, without normal termination (lane c), the plasmid-derived transcripts are very unstable with no plasmid-derived RNA being observed after methylene blue staining. Similarly (Fig. 1B), only trace amounts or no plasmid-derived 25 S rRNA or processing intermediates are evident (lane c) when unterminated RNA transcripts are characterized by S1 nuclease digestion studies. Both results clearly demonstrate a striking instability in nascent transcripts that are not properly terminated, with no mature plasmid-derived RNA being detected.

As indicated in Fig. 2, the normal 5 S rRNA transcript (a, b) is extended by about 12 nucleotides, terminating in a polyuridylic acid cluster of 4–5 residues (5–7), this extension being rapidly removed during maturation. As illustrated with two examples in Fig. 3, this spacer sequence could be modestly altered, both with respect to the nucleotide sequence and length without an effect on the mature rRNA product. For example, when the spacer was abbreviated to only a poly(U) cluster (lane c), which constitutes the basic termination signal (5–7), or modestly lengthened to 24 nucleotides with poly(C) clusters (lane d), the plasmid-derived molecules continued to constitute 80–90% of the cellular 5 S rRNA population. In sharp contrast, however, when the poly(U) cluster was substantially displaced (lane e) with a long spacer (490 base pairs), a mature product was again absent as was observed earlier (Fig. 1) with a deleted termination signal.

Because the spacer sequence appeared not to be directly critical to RNA product stability and ribosomal integration, other features were examined, namely secondary structure and length. As also illustrated in Figs. 2 and 3, both of these proved to be important factors in RNA stability. When the sequence was altered to insert a small (5 base pairs) hairpin structure in

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† The abbreviations used are: ETS, external transcribed spacers; PCR, polymerase chain reaction; pol, polymerase.
Effect of transcript termination on rRNA stability in ribosome biogenesis. A, a truncated 5S rRNA gene without its 3'-end termination region was synthesized by PCR amplification (9) using a primer complementary to the 3'-end of the mature 5S rRNA sequence and from a plasmid DNA template (pYF5A99) containing a structurally marked (A99) 5S rRNA gene sequence (8). The mutant gene was cloned into pYF404, a high copy (30–40 copies/cell) yeast shuttle vector (22), and a plasmid DNA template (pYF5A99) containing a structurally marked (A99) 5S rRNA gene sequence (8). The position of the normal and plasmid-derived RNAs are indicated by arrows. B, a truncated rDNA 3'ETS region lacking the three "Salbox"-like termination signal repeats (12) was synthesized by PCR amplification (24) using a primer complementary to the sequence immediately upstream of the terminator repeats and a plasmid template (pFL20/Sp25NotI) containing Schizosaccharomyces pombe rDNA with a unique NotI cloning site in the 25S rRNA sequence (10, 12). The truncated 3'-end was substituted in pFL20/Sp25NotI using the unique NotI and PvuII sites (12), resulting in a rDNA construct (pFL20/Sp23T), which contained no known termination signal, and the sequence was confirmed by DNA sequencing. Whole cell RNA was prepared from exponentially growing pFL20Sp23T transformed S. pombe cells, strain Δ-leu1–32 ura 4-D18 (lane c), as well as untransformed (lane a) and pYF5A99-transformed cells (lane b), fractionated on 12%-non-denaturing polyacrylamide gels, and stained with methylene blue as described previously (8, 9). The positions of the normal and plasmid-derived RNAs are indicated by arrows. C, a truncated rDNA 3'ETS region lacking the three "Salbox"-like termination signal repeats (12) was synthesized by PCR amplification (24) using a primer complementary to the sequence immediately upstream of the terminator repeats and a plasmid template (pFL20/Sp25NotI) containing Schizosaccharomyces pombe rDNA with a unique NotI cloning site in the 25S rRNA sequence (10, 12). The truncated 3'-end was substituted in pFL20/Sp25NotI using the unique NotI and PvuII sites (12), resulting in a rDNA construct (pFL20/Sp23T), which contained no known termination signal, and the sequence was confirmed by DNA sequencing. Whole cell RNA was prepared from exponentially growing pFL20Sp23T transformed S. pombe cells, strain Δ-leu1–32 ura 4-D18 (lane c), as well as untransformed (lane a) and pYF5A99-transformed cells (lane b), fractionated on 12%-non-denaturing polyacrylamide gels, and stained with methylene blue as described previously (8, 9). The positions of the normal and plasmid-derived RNAs are indicated by arrows. D, a truncated rDNA 3'ETS region lacking the three "Salbox"-like termination signal repeats (12) was synthesized by PCR amplification (24) using a primer complementary to the sequence immediately upstream of the terminator repeats and a plasmid template (pFL20/Sp25NotI) containing Schizosaccharomyces pombe rDNA with a unique NotI cloning site in the 25S rRNA sequence (10, 12). The truncated 3'-end was substituted in pFL20/Sp25NotI using the unique NotI and PvuII sites (12), resulting in a rDNA construct (pFL20/Sp23T), which contained no known termination signal, and the sequence was confirmed by DNA sequencing. Whole cell RNA was prepared from exponentially growing pFL20Sp23T transformed S. pombe cells, strain Δ-leu1–32 ura 4-D18 (lane c), as well as untransformed (lane a) and pYF5A99-transformed cells (lane b), fractionated on 12%-non-denaturing polyacrylamide gels, and stained with methylene blue as described previously (8, 9). The positions of the normal and plasmid-derived RNAs are indicated by arrows.

Taken together, the results shown in Figs. 1 and 3 indicate that termination follows by efficient processing is essential for transcript stability. Presumably, with inefficient processing or no processing at all, the nascent transcript is not integrated into ribosomal structure and is susceptible to cellular "housekeeping" degradation, which may even be mediated, at least in part, by the processing enzymes. In a number of organisms (13–15), 5S rRNA processing has been shown to be dependent, at least in part, on exonuclease cleavage, and while the details are less clear, nucleolar RNA processing is also dependent, at least in part, on exonuclease trimming (see Ref. 16). It appears, therefore, that when the termination site is too distant from the mature RNA or encumbered with secondary structure, processing is sufficiently retarded or eliminated, resulting in transcript degradation without maturation.

Indeed, the significance of termination as a factor in quality control is not likely restricted to pol I or III transcripts. Although the termination signal has not been clearly defined with pol II transcripts (17), mRNA instability often has been linked to changes in the termination region. When many mRNA are not polyadenylated (see Refs. 18 and 19 for reviews), rapid turnover is frequently reported, and there is evidence that the function of intrinsic terminators is coupled to the functioning of the 3'-end maturation signals (20), indicating that defects which prevent cleavage of the nascent transcript also prevent the functioning of the downstream terminator. A conclusion cannot be drawn until pol II termination is further clarified. In the interim, the observations made here clearly show that, at least for pol I and III transcripts, proper termination appears to play a role in ensuring that only normal precursors are being assembled into ribosomes. Furthermore, when the present results are taken together with recent reports of relationships between enhancer and terminator sequences in rDNAs (see Ref. 21), they may even indicate that correct termination plays a role in coordinate regulation through the same degradation mechanism.
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