Polymerase chain reaction mapping of yeast *GAL7* mRNA polyadenylation sites demonstrates that 3' end processing *in vitro* faithfully reproduces the 3' ends observed *in vivo*

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ABSTRACT

In general, synthetic RNA transcripts corresponding to the 3' ends of *Saccharomyces cerevisiae* genes appear to be accurately cleaved and polyadenylated *in vitro* under appropriate conditions in yeast cell extracts. Initially, however, the endpoints observed *in vitro* for the *GAL7* gene failed to correlate adequately with those reported *in vivo* as derived from traditional S1 nuclease protection analyses. This led us to apply an independent method for analyzing mRNA 3' ends, using the polymerase chain reaction, with a first strand primer that incorporated a BamHI restriction site sequence near its 5' end, followed by (dT)₁₇. This proved to be a sensitive and accurate means for determining precisely the major and minor polyadenylation sites of the *GAL7* mRNA. Moreover, there was complete agreement between the sites identified with this technique when applied to cellular RNA and those generated *in vitro* by our 3' end mRNA processing reaction. This provides further support for the likelihood that processing *in vitro* faithfully reflects the endonucleolytic cleavage and polyadenylation events that occur within the living cell.

INTRODUCTION

The 3' ends of messenger RNA molecules can be generated by a variety of different mechanisms. In bacteria, termination of transcription by RNA polymerase appears to play the primary role, though processing events may also participate (1). In contrast, higher eukaryotic organisms rely mainly on complex RNA processing mechanisms that involve accurate endonucleolytic cleavage and subsequent polyadenylation. In lower eukaryotes, such as the budding yeast *S. cerevisiae*, the mechanism of 3' end maturation was unclear until fairly recently. In 1988, Butler and Platt (2) used a synthetic precursor mRNA encompassing the polyadenylation site of the *S. cerevisiae* *CYC1* gene to demonstrate correct processing and polyadenylation in a whole cell extract *in vitro*. Butler et al. (3) then showed that

synthetic pre-mRNAs corresponding to a variety of genes can be cleaved and polyadenylated when incubated in the same extract, although with varying efficiencies. This suggested that processing is a general method of mRNA 3' end formation in *S. cerevisiae*. Although for all of the transcripts examined thus far, the processing sites *in vitro* lie in the regions encompassing the respective 3' ends *in vivo*, the accuracy of processing has been confirmed only for the *CYC1* transcript (2).

In our initial studies on the genes involved in galactose metabolism, we encountered a disturbing discrepancy between the processing site observed for the *GAL7* pre-mRNA *in vitro* and the reported position of the poly(A) site *in vivo*, about 100 nt farther upstream (4). This brought into question the fidelity of processing for pre-mRNAs *in vitro*. The situation was further complicated by our discovery of a site of unusual S1 nuclease sensitivity within the *GAL7* 3' untranslated region. To help determine which endpoints were the real ones, we turned to the alternative technology offered by the polymerase chain reaction (PCR) for analyzing poly(A) sites (5) applied to the 3' processing sites in yeast mRNA. Here we report evidence derived from PCR amplification of polyadenylated RNA transcripts, to compare the *bona fide* sites of 3' end formation for *GAL7* mRNA *in vivo* and *in vitro*. Our findings show that a *GAL7* pre-mRNA is processed *in vitro* at precisely the same polyadenylation sites as are utilized *in vivo*, confirming the fidelity of 3' end processing *in vitro* and providing a simple method for analyzing polyadenylation sites in other systems.

MATERIALS AND METHODS

Plasmids and strains

The 246 nt long *SspI* fragment encompassing the 3' end of the *GAL7* gene (Fig. 1) was cloned into the *HincII* site in pGEM3Z (Promega corp) to create plasmid pSB28. The orientation of the insert is such that the direction of both *GAL7* and SP6 transcription is the same. pNN452 was created by end filling the 739 nt long *HindIII-XbaI* fragment of pNN77 (6) with the Klenow fragment of DNA polymerase I (Klenow) and cloning this

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fragment into the *Sma*I site of pNN451 (a pUC18 derivative with the *Sph*I site destroyed by T4 polymerase and the *Hind*III site filled with Klenow). The orientation of the insert in pNN452 is such that the *Kpn*I site of the polylinker is proximal to the filled *Xba*I site. The yeast strain EP148 was used to make whole cell extracts. The yeast strains YNN318 or T481 were used to prepare total cellular RNA.

RNA substrates and processing of synthetic pre-mRNAs in vitro

Transcriptions in vitro with SP6 polymerase were carried out as described by Butler and Platt (2). The capped ^{32} P labelled synthetic RNA was separated from rest of the reaction components by electrophoresis on 5% polyacrylamide gel containing 8M urea. The RNA was eluted from the gel and subjected to processing in yeast cell extracts as described previously (3).

cDNA synthesis and subsequent specific amplification by polymerase chain reaction

RNA was precipitated from the processing reactions and resuspended in 10 μ l water, of which 1 μ l was used in a coupled reverse transcriptase and PCR reaction (7). First strand synthesis was carried out with 200 units of MMuLV reverse transcriptase (BRL) in a final volume of 20 μ l 1 $\times$ PCR buffer (50 mM KCl, 20 mM Tris-Cl pH 8.4 and 2.5 mM MgCl₂) with 1mM each of the four dNTPs, 20 units of RNasin, 100 pmoles of oligo-dT primer (5'-T₃GGATCCT₁₇-3'). The reaction was initiated at room temperature for 10 minutes, incubated at 30°C for 30 minutes, and boiled for 5 minutes to inactivate the reverse transcriptase. After cooling on ice, the reaction was supplemented with 8 μ l of 10 $\times$ PCR buffer, 100 pmoles of the upstream primer (5'-GAATACTCAAGCTTG-3') and 1 unit of Taq polymerase (BRL). The reaction mix was overlaid with mineral oil and run through 30 cycles with the following parameters: denaturation for 1 minute at 94°C, annealing for 2 minutes at 30°C and extension for 2 minutes at 72°C. A small portion (10%) of the reaction mixture was run on 3% agarose gel in TBE to analyse the products of the reaction. Remaining DNA was purified by GeneClean (BIO 101; used according to manufacturer's suggestions) and cloned in the *Sma*I site of pGEM3Z. The insert was sequenced by Sanger Dideoxy chain termination method using the Sequenase kit (USB).

To determine the 3' ends of mRNA in vivo, the RNA was isolated from yeast grown in presence of galactose by the glass bead method (8). 1 μ g of total RNA was used in 20 μ l reverse transcriptase reaction and then the cDNA was diluted up to 1000 fold and 1 μ l of it was used in 100 μ l PCR reactions as described above.

Isolation of RNA and S1 nuclease mapping analysis

YNN318 was grown to log phase in YP medium supplemented with 2% glucose or galactose. The cells were pelleted and directly frozen at -70°C. The frozen pellets were resuspended in 0.1 M NaCl, 10 mM Tris pH 7.5, 1 mM EDTA and RNA was prepared by a modified hot phenol extraction procedure as described by Sachs and Davis (9). S1 mapping was performed by a modification of the procedure by Maniatis et al. (10). Hybridization was performed in a 15 μ l reaction volume and digestion with S1 was performed by addition of 0.2 ml ice-cold [0.28 M NaCl, 0.3 M Na-acetate pH 4.5, 4.5 mM ZnCl₂], 0.1 mg/ml carrier DNA, containing 50 to 500 units of S1 nuclease

Fig. 1. Processing in vitro of a synthetic *GAL7* transcript. (a) Map of pSB28 in linear form. The plasmid pSB28 contains the 246 bp *Ssp*I fragment downstream of the open reading frame of the *GAL7* gene (Fig. 4a). The *Eco*RI linearized pSB28 gives a 306 nt long transcript that spans the 3' end of *GAL7* mRNA. Relevant restriction sites are indicated and the vertical arrow indicates the position of the polyadenylation site reported in this paper; * indicates the position of the 3' end mapped by Tajima et al. (4). (b) The in vitro reactions were carried out as previously described. The products of reactions stopped immediately (lane 1) or incubated for 30 min (lanes 2 and 3) were separated on a 5% acrylamide gel. The reaction in lane 3 also contained 2mM 3'dATP, which prevents polyadenylation of the cleavage product. The pre-mRNA (pre-), 5' cleavage product (5'-), polyadenylated cleavage product (pA-), and 3' cleavage product (3'-) are indicated.

(Sigma). The reaction was stopped with 6 μ l of 0.25 M EGTA, followed by direct precipitation with 2.5 volumes ethanol. The specific conditions in the experiments are described below in Results.

RESULTS

Processing of a synthetic *GAL7* pre-mRNA

The 1.2 kb *GAL7* mRNA encodes a 1095 nt open reading frame. To examine processing in vitro, we utilized a 306 nt SP6 runoff RNA transcribed from *Eco*RI linearized pSB28 (Fig. 1a), that encompasses the 3' end region of the normal transcript. Incubation of this *GAL7* pre-mRNA with the 40% ammonium sulfate fraction of whole cell yeast extracts prepared according to Butler and Platt (2) reveals three new species that were not seen in the input RNA (Fig. 1b, lanes 1 and 2). The shortest species is approximately 140 nt long, the middle one is approximately 160 nt long while the upper one is between 210–220 nts. Since specific polyadenylation activity is coupled to cleavage of the *CYC1* pre-mRNA under similar conditions (2), we tested whether the longer species was actually generated by polyadenylation of one of the shorter ones. Incubation of the *GAL7* pre-mRNA in the presence of cordycepin triphosphate (3'dATP), which inhibits polyadenylation but not cleavage (11), inhibits production of the ca. 220 nt long RNA, and increases

Fig. 2. (a) The sequence of the GAL7-28 transcript. The nucleotide sequence of this synthetic pre-mRNA was deduced from the published sequence of the *GAL7* gene and pGEM3Z vector sequence (4). The two vertical arrows bracket the *GAL7* sequence. The previously published 3' end is indicated by the encircled lowercase letter, t. The positions mapped to be the 3' ends in this report are indicated by the boxed As. Since it is not possible to determine which of the As among the cluster in each case is the site for the addition of poly(A), all the As are boxed. Abe et al. (10) identified the 3' end as within the CTG sequence just before this A-cluster. The positions of the two oligonucleotides priming PCR amplification are shown in boldface. (b) The products of PCR amplification. The RNAs isolated from two of the reactions shown in Fig. 1 were used for PCR amplification. Lane 1 shows the PCR product (A) corresponding to the pre-mRNA alone while lane 2 shows an additional product (B) corresponding to the polyadenylated cleavage product. The position of unreacted primers in the reactions is shown.

accumulation of the unpolyadenylated 160 nt RNA (Fig. 1b, lane 3). This suggested that the 160 nt species is the 5' cleavage product that gets polyadenylated in presence of ATP. The shortest species is the 3' cleavage product, which lacks a 5' cap structure and is prone to rapid degradation. This is analogous to *CYC1* pre-mRNA for which the 3' cleavage product was only transiently visible, and rapidly degraded, presumably by 5'→3' exonucleases (2).

Mapping of the 3' end of the cleavage product by PCR amplification

The size of the 5' cleavage product of the *GAL7* pre-mRNA places the cleavage site about 60 nt distal to the *GAL7* 3' end and originally reported by Tajima et al. (4), but roughly agrees with the more recent results of Abe et al. (12). Since interpretation of these findings became further clouded by our discovery of a site of unusual S1 nuclease sensitivity in this same region (see below), an independent method for determining the 3' end locations in vitro and in vivo seemed desirable. With a strategy based on PCR technology, we constructed a 5' primer that corresponds to the first 16 nts of the pre-mRNA (encoded by the transcription vector pGEM3Z) and a 3' primer comprised of oligo-dT with a *Bam*HI site incorporated near its 5' end. The latter is a slightly simpler version of the poly(A) specific primer used by Frohman et al. (5). The oligo-dT will hybridize to both the A-rich portion of the pre-mRNA at position 210–232 from the 5' end and the processed product at the poly(A) stretch. The amplification of sequence between the positions of these two primers on the pre-mRNA will generate a fragment of ca. 240 bp. The amplification of the sequence after polyadenylation at the site detected in vitro should generate a fragment about 200 bp long. The RNAs used for PCR amplification were the same as those in the reactions shown in Fig. 1b. Fig. 2b shows the result of PCR on pre-mRNA before (lane 1) and after (lane 2) processing in vitro in the presence of ATP. Only one product arises from the pre-mRNA and since about 50% of the pre-mRNA remains after 30 minutes of processing, the same band appears in lane 2, although with reduced intensity. The lower band in lane 2 is specific to the processing reaction and is derived

Fig. 3. The sequence of the PCR amplified products. The PCR products were sequenced using the Sequenase kit and the upstream primer used for PCR amplifications. The dideoxynucleotide sequencing reactions are labelled A, C, G and T at the top of each lane. The nucleotide sequence deduced from each of these sequencing reactions is shown along the sides of sequencing reactions. pre and pA above each set indicates the PCR product used in the reaction as template.

from the polyadenylated cleavage product (Fig. 1b, lane 2). The RNA from processing reactions in the presence of 3'dATP does not yield the lower product after PCR amplification (data not shown). Each of the two bands was gel purified and cloned in the *Sma*I site of the vector pGEM3Z and sequenced. The comparison of panels A and B (Fig. 3) with the known *GAL7* sequence shows that the polyadenylation site in vitro is at the cluster of three A's located 70 nt downstream of the unique *Sall* site.

S1 protection analysis reveals a site of unusual nuclease sensitivity

A further technique-dependent complication of 3' end determination in vivo was revealed by careful mapping of the 3' end of *GAL7* mRNA from galactose-induced cells, when both the enzyme concentration and temperature were varied in an S1

Fig. 4. (a) DNA probes used for S1 mapping of in vivo *GAL7* mRNA. The upper bar shows the *GAL7* gene and the downstream region to the *EcoRI* site. The lower bar shows the restriction map of the *GAL7* 3' end on an enlarged scale. *M*, major 3' end; *m*, minor 3' end; *a*, nuclease S1 sensitive region. Below the lower map are the S1 mapping probe DNAs used. pNN77 was cut with *HindIII* and *EcoRI* to provide the 1.86 kb probe DNA. pNN452 was cut with *Sau3AI* and *KpnI* to provide the 543 bp probe DNA. pNN452 was cut with *DdeI* and *KpnI* to provide the 453 bp probe DNA. (b) S1 mapping *GAL7* mRNA. 20 μ g total RNA isolated from cells grown on glucose (Glc) or galactose (Gal) were hybridized for 3 hr at 37°C with the 453 bp ^{32} P-dATP end-labelled *DdeI*-*KpnI* probe DNA (labelled at the *DdeI* site). Digestion was performed at 18°C for 45 min with 100 units of nuclease S1, and the reaction products were run on an 8% polyacrylamide, 8 M urea gel. Molecular weight markers were prepared by digesting the probe DNA with *Sall* (124), *MaeIII* (150), *HinfI* (partial digest, 200), *MaeI* (215), *Avall* (232) and *AluI* (283). (c) S1 mapping *GAL7* mRNA as a function of S1 concentration. 20 μ g total RNA isolated from cells grown on galactose was hybridized for 6 hr at 37°C with the 1.86 kb ^{32} P-dCTP end labelled *HindIII*-*EcoRI* probe DNA (labelled at the *HindIII* site). Digestion was performed at 11°C for 45 min with the indicated units of nuclease S1, and the reaction products were run on an 8% polyacrylamide, 8 M urea gel. Molecular weight markers were prepared by digesting the probe DNA with *HinfI* (314), *SspI* (partial digest, 349), *Sall* (415), *MaeIII* (441), *MaeI* (506) and *XbaI* (739). (d) S1 mapping *GAL7* mRNA as a function of reaction temperature. 25 μ g total RNA isolated from cells grown on galactose were hybridized for 4 hr at 37°C with the 543 bp ^{32} P-dCTP end labelled *Sau3AI*-*KpnI* probe DNA (labelled at the *Sau3AI* site). Digestion was performed at the indicated temperatures for 45 min with 100 units of nuclease S1, and the reaction products were run on an 6% polyacrylamide, 8 M urea gel. Molecular weight markers were prepared by digesting the probe DNA with *Sall* (214), *MaeIII* (240), *MaeI* (305), *Avall* (322) and *SspI* (partial digest, 394).

protection assay. The probes used were double stranded 3' end labeled DNA from pNN77 or pNN452 as shown in Fig. 4a. Figure 4b shows that two galactose-induced transcripts protect the *DdeI* end-labelled probe DNA. The 3' ends of these transcripts map 70 nt (band *M*) and 155 nt (band *m*) downstream of the unique *Sall* site. Another small protected species (band *a*, Fig. 4c and d) roughly corresponds to the previously published 3' end (4), but its endpoint is in fact approximately 25 nt upstream of the *Sall* site, correlating with the position of the T-rich tract, T₈A₃T₆, as determined by a chemical sequencing ladder (datanot shown). When the S1 concentration used in the reaction is lowered, the intensity of this band decreases relative to the S1 protected bands mapping 70 nt and 155 nt downstream of the *Sall* site (bands *M* and *m*, Fig. 4c). When the temperature of the S1 reaction is decreased, the intensity of the protected bands corresponding to the galactose-induced transcripts increases (bands *M* and *m*, Fig. 4d). These results indicate that band *a* is artifactual and due to the unusual S1 nuclease sensitivity of a T-rich tract. They also support the interpretation that the major *GAL7* 3' end (corresponding to band *M*) lies about 70 nt

downstream of the *Sall* site as suggested by the data of Butler et al (3) and supported more recently by Abe et al (12).

Use of PCR to determine 3' ends of polyadenylated mRNA in vivo

Having identified a false signal and confirmed by these S1 nuclease titrations that the major 3' end of *GAL7* mRNA in vivo is at the same location seen in vitro, we decided to test the applicability of the PCR technique to the determination of 3' ends of yeast mRNAs in vivo. Total cellular RNA was isolated from yeast cells grown in presence of either 2% glucose or 2% galactose. The cDNA was made as described in the methods section using the oligo-dT primer. The PCR reaction was carried out essentially in similar fashion except with a 5' primer (5'-G-GCTCCTTTGAATGCCACT-3') complementary to the *GAL7* cDNA about 230 nts upstream from the *Sall* site. The amplification of the major cDNA specific for *GAL7* gene with this primer would generate a product of about 350 bp. To increase the stringency of the PCR reaction, the cDNA was diluted about 1000 fold before amplification, the annealing temperature was

Fig. 5. (a) The PCR products from *GAL7* mRNA in vivo. The cDNA corresponding to the *GAL7* mRNA was amplified by PCR as described in the results section. Lane 1 shows 10% of the reaction products obtained from amplification of 1:1000 dilution of cDNA reverse transcribed from 1 μ g of total RNA from yeast grown in presence of galactose. (b, c) Sequencing of the in vivo PCR products. The PCR products shown in Fig. 5a were cloned in *Sma*I site of pGEM3Z. The clones were sequenced with the universal primer. The ddNTPs in each reaction are indicated above each lane. Fig. 5b represents the major and 5c the minor product in the PCR reaction. The relevant portion of the sequence deduced is shown alongside the respective gel.

raised to 45°C and duration of extension was reduced to 30 seconds. Fig. 5a shows the PCR products obtained from the amplification of cDNA specific for *GAL7* mRNA. Although there are no other major products seen on this gel, possibly other products might have resulted from amplification of cDNAs representing minor 3' ends (as revealed by S1 mapping in this report and by Abe et al (12)). To test this possibility total DNA from the PCR reaction was cloned into the *Sma*I site of pGEM3Z. The DNA was sequenced by using the M13(-40) sequencing primer that hybridizes the pGEM vector also about 40 bp upstream from the polylinker. This allowed sequencing of all clones irrespective of the sequence of the insert. We discovered that four out of five of the clones with inserts from 3' end of the *GAL7* gene represented the major 3' end about 70 nts downstream of the *Sall* site (Fig. 5b) while one had an insert corresponding to a polyadenylation site about 30 nts upstream of the *Sall* site (Fig. 3 and 5c). This latter site corresponds to that reported by Abe et al as a minor 3' end in vivo (12). We have confirmed this site to be a weak processing site by in vitro analysis of a longer substrate (data not shown). We have not detected a processing product in vitro corresponding to the protected species *m* in our S1 analysis nor have we found a clone representing a PCR product corresponding to a polyadenylation site in that region. The absence of any product that would correspond to priming at the long A-tract (see Fig. 2a) suggests that few if any transcripts of that length or longer survive intact.

DISCUSSION

The yeast *S. cerevisiae* has been used extensively as a model eukaryotic cell in the study of various phenomena, including the study of mRNA 3' end formation, using both genetic and biochemical approaches (2,3,12–18). Since one of our goals is to use processing in extracts to analyze various mutants defective in 3' end formation, we felt it was essential to determine the fidelity of processing in vitro. The substrate used to test in vitro processing of *GAL7* pre-mRNA revealed a major 3' processing product much larger than expected based on the previously reported 3' end (4). In an attempt to resolve this discrepancy,

we mapped the polyadenylation site in vitro as described above. To compare these in vitro results with in vivo polyadenylation sites we then carried out S1 nuclease protection analysis of the RNA from cells grown in presence of galactose. These experiments established that there are in fact at least two 3' ends of *GAL7* mRNA and that the major 3' end lies about 70 nt downstream of the unique *Sall* site while the minor one lies about 30 nts upstream of it. The results of S1 mapping have been supported by in vitro processing and sequencing of amplified products corresponding to each of the polyadenylated products both in vivo as well as in vitro. Our inability to find a clone corresponding to the S1 protected band *m* (Fig. 4), may reflect the fact that its representation among the PCR products is small and too few clones have been examined. On the other hand the only evidence we have for this site as a probable 3' end is the S1 mapping analysis where the change in intensity of both bands *M* and *m* is similar with the increase in the concentration of S1. The sequencing of PCR amplified DNA corresponding to the polyadenylated products showed that the major site of polyadenylation in vitro is indeed the same as the major site in vivo. Although we observe the minor protected species *a* showing a different pattern indicating that it is an artifact due to inherent S1 sensitivity, we do not detect a protected species corresponding to the true minor polyadenylation site found by PCR analysis. This might reflect on the difference in the sensitivities of the two techniques.

Abe et al. (12) recently published an elegant analysis of signals required for 3' end formation of *GAL7* mRNA, and determined the *GAL7* 3' end by RNase protection analysis. Based on size mapping of their protected bands with relation to DNA molecular weight markers, they identified the 3' end as within the CUG sequence 3–4 nts upstream of the position determined by our present work using both S1 and PCR mapping techniques. Although this discrepancy is not serious, it highlights the potential pitfalls of mapping by size rather than sequence. Comparison of regions flanking our mapped major and the minor sites, aligned at the sequence AAT, reveals considerable similarity (underlined) between the two sequences: ATgACATCATtaTCTGTAAAT and ATtcATATCATattttTCTATTAAAT. We postulate that the vast

difference in efficiencies between the two may be due to the differences in these adjacent regions and this can now be specifically tested.

Our results suggest that a simple in vitro pre-mRNA processing assay (2, 3) accurately reproduces the *bona fide* 3' endpoints of mRNA in *S. cerevisiae*. The 3' ends of the polyadenylated mRNAs can be determined by sequence analysis of PCR products derived by amplification of the RNA generated by processing in vitro, as shown in this report. Since the length of average poly(A) tail of yeast mRNAs is about four fold greater than that of the oligo-dT primer, specific amplification products might be expected to be heterogenous in length. Although this will be true in early cycles, over the course of 30 repetitions, an average length attenuation of less than 2 A residues per cycle will reduce a population with poly(A) tracts of 50–70 to a homogeneous length corresponding to the that of the primer itself. While our work was in progress, we learned that Russo et al. (19) were using a similar technique to analyze variant *CYC1* mRNA 3' ends in vivo, but with a (dT)₂₀ primer degenerate at the 3' nucleotide to minimize mismatching across the poly(A) site. Since we have not encountered any clones having an oligo dT stretch longer than 17 nts (length of the dT stretch in our oligo-dT primer) based on sequencing data, the Russo et al. (19) modifications of the oligo-dT primer to achieve size homogeneity appear unnecessary. Frohman et al. (5) also found that their (dT)₁₇ primer was sufficient to reduce the much longer poly(A) tracts of higher eukaryotes to near homogeneity. A minor drawback of these variations is that it is not possible to map the polyadenylation site to the nucleotide if it falls in a stretch of A residues as seen at the *GAL7* (and normal *CYC1*) polyadenylation sites.

In conjunction with our demonstration that 3' end processing of pre-mRNAs corresponding to a variety of genes in *S. cerevisiae* (a budding yeast) can be observed in vitro (3), we have recently shown that processing is functionally interchangeable with *S. pombe* (a fission yeast) both in vivo and in vitro (20). Our results imply that the in vitro system accurately reflects the processing events that occur in vivo, and that examining the biochemical details of the processing mechanism will illuminate how the mature 3' ends of RNA polymerase II transcripts are generated not only in *S. cerevisiae*, but in other eukaryotic microorganisms as well.

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