19. N. Z. Scoville *et al.*, *ibid.* **289**, 129 (1985).
20. K. C. Freeman and G. de Vaucouleurs, *ibid.* **194**, 569 (1974).
21. M. Joy *et al.*, *ibid.* **330**, L29 (1988).
22. H. Arp, *Atlas of Peculiar Galaxies* (California Institute of Technology, Pasadena, 1966).
23. G. Neugebauer *et al.*, *Science* **224**, 14 (1984).
24. G. H. Rieke *et al.*, *ibid.* **231**, 807 (1986).
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Research Articles

# RNA Processing Generates the Mature 3′ End of Yeast *CYC1* Messenger RNA in Vitro

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**In whole cell extracts of *Saccharomyces cerevisiae*, incubation of precursor mRNA transcripts encoding the sequences essential in vivo for forming the 3′ end of the iso-1-cytochrome c mRNA (*CYC1*) revealed an endonuclease activity with the characteristics required for producing the mature mRNA 3′ end. The observed cleavage in vitro is (i) accurate, occurring at or near the polyadenylation site of *CYC1* RNA, (ii) 30 to 50 percent efficient, (iii) adenosine triphosphate dependent, (iv) specific for the 3′ ends of at least two yeast pre-mRNA's, and (v) absent with related pre-mRNA's carrying mutations that abolish correct 3′ end formation in vivo. In addition, a second activity in the extract polyadenylates the product under appropriate conditions. Thus, the mature 3′ ends of yeast mRNA's may be generated by endonucleolytic cleavage and polyadenylation rather than by transcription termination.**

In eukaryotic cells, messenger RNA's require post-transcriptional modification before export from the nucleus to the cytoplasm. These modifications include capping of the 5′ terminus, methylation of some internal adenosines, and RNA splicing to remove internal noncoding introns and then join the separated exons into a contiguous mRNA. In higher eukaryotes, the formation of the mature 3′ end of mRNA's also requires RNA processing which entails (except for most of the histone transcripts) endonucleolytic cleavage and polyadenylation of a primary transcript, or pre-mRNA, that extends some distance beyond the mature 3′ end of the message (*1*). The extent of gene expression depends on (i) efficient polyadenylation of mRNA's to enhance the stability of the message (*2*) and (ii) selection of the correct polyadenylation site to produce the desired protein product. For example, some human thalassemias result from mutations in the conserved AAUAAA polyadenylation signal that abolish correct 3′ end formation and thus reduce globin gene expression in the affected individuals (*3, 4*).

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Where alternative polyadenylation signals exist, selection among them provides a mechanism to control gene expression. In developing B cells, the shift from one polyadenylation signal to another causes a change in the production of immunoglobulin M heavy chain from the membrane bound to the secreted form (*5*), and selection of polyadenylation sites affects the relative amounts of adenovirus mRNA's during infection (*6, 7*). These examples reveal the widespread involvement of mechanisms determining mRNA 3′ end formation in the regulation of gene expression.

In higher eukaryotes, cleavage and polyadenylation require the nucleotide signal AAUAAA, which is about 20 nucleotides 5′ to the cleavage site (*8, 9*), as well as some less conserved GU- or U-rich sequences just distal to the polyadenylation site (*10–13*). In *Saccharomyces cerevisiae*, however, all known RNA polymerase II transcripts (including the histone mRNA's as well as aberrant mRNA's) become polyadenylated after synthesis, yet known sequences of yeast mRNA's do not share an AAUAAA signal or any other highly conserved sequence identified as responsible for 3′ end formation. Zaret and Sherman discovered that a mutation (*cyc1-512*) deleting 38 base pairs 3′ to the coding sequence of the iso-1-cytochrome *c* gene (*CYC1*) causes a 90 percent decrease in the amount of *CYC1* mRNA (*14*). The residual mRNA's produced are all longer than normal and they are all polyadenylated, suggesting that some crucial elements of the normal 3′ end formation signal are deleted. Comparison of sequences from the *cyc1-512* deletion with those from other DNA fragments that apparently direct mRNA 3′ end formation in yeast (*15–18*) shows similarities, but none of the proposed consensus sequences fit all of the known 3′ end regions. This lack of strong sequence similarity and the fact that all RNA polymerase II transcripts are polyadenylated led to the suggestion that polyadenylation might be coupled to transcription termination (*14*) and that a protein factor analogous to *Escherichia coli* transcription termination factor rho might be involved in mRNA 3′ end formation in yeast (*16*).

These considerations and the recent discovery that *E. coli* rho factor catalyzes the unwinding of RNA-DNA helices (*19*) led us to search for a rho-type helicase activity in yeast whole cell extracts. In the course of this search we discovered that *CYC1* pre-mRNA sequences are accurately cleaved and polyadenylated in vitro. The formation of 3′ ends of yeast mRNA's may thus be independent of transcription termination, and our results suggest that yeast process

the 3′ ends of their RNA polymerase II transcripts in a manner analogous to that in higher eukaryotes.

**Endonucleolytic cleavage at the *CYC1* polyadenylation site in vitro.** We incubated a helicase substrate, analogous to that of Brennan *et al.* (*19*), in a yeast whole cell extract and observed extensive hydrolysis of the duplexed portion by ribonuclease (RNase) H. We also detected small amounts of two RNA products whose lengths indicated cleavage of the RNA substrate by an endonuclease (Fig. 1A). With improved reaction conditions (*20*) we increased the extent of cleavage to 30 to 50 percent (Fig. 1B). Incubation of 5′ capped, $^{32}$P-labeled pre-mRNA spanning the 3′ end formation signal of *CYC1* (Fig. 1A) results in time-dependent cleavage of the RNA into two fragments whose lengths are consistent with cleavage close to the *CYC1* polyadenylation site. The larger fragment (**I**), which we show below is the 5′ portion, accumulates for up to 60 minutes and remains relatively stable in the extract for at least 3 hours. The smaller RNA fragment (**II**), however, reaches maximal levels by 15 minutes and appears unstable thereafter. This pattern of endonucleolytic cleavage and the relative stabilities of the products agree with similar results from mammalian systems in which the 5′ cleaved fragment accumulates and the 3′ fragment disappears as a result of exonucleolytic hydrolysis (*21, 22*).

We isolated the two endonuclease cleavage products and analyzed them by one-dimensional T1 RNase fingerprinting. The existence of the normal *CYC1* polyadenylation site within a T1 RNase product of 33 nucleotides (nt) (Fig. 2A) simplifies this analysis because endonucleolytic cleavage at this site should eliminate the 33-nt fragment from the T1 RNase products of the two RNA fragments, while yielding new products whose lengths depend on the precise point of the cleavage. As predicted, the 33-nt fragment does not appear among the T1 RNase products of either of the endonuclease cleavage products (Fig. 2B, lanes 3 and 4). The 200-nt RNA (**I**) yields T1 RNase oligonucleotides 5′ to the 33-nt fragment (that is, the 12-nt fragment, 17-nt fragment, and 21-nt fragment) while the 150-nt RNA (**II**) yields products 3′ to the cleavage site (that is, the 13-nt fragment and 11-nt fragment). Thus, the pattern of T1 RNase digestion products is consistent with cleavage of the *CYC1* pre-mRNA at a site within the 33-nt fragment to give a 5′ product (200-nt RNA) and a 3′ product (150-nt RNA). The precise point of

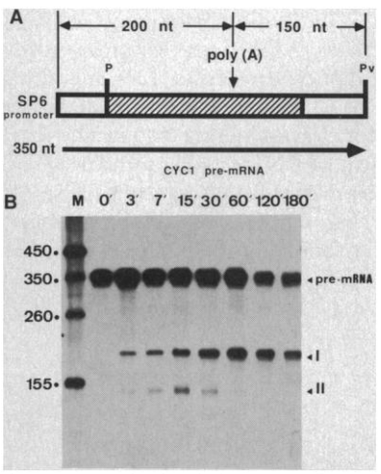


**Fig. 1.** Endonucleolytic cleavage of a *CYC1* pre-mRNA in vitro. (**A**) Map of pGYC1, a pGEM1 derivative, from the SP6 RNA polymerase initiation site to the Pvu II (Pv) site; P indicates the Pst I site. The shaded portion of the rectangle represents the 237-bp Taq I fragment derived from the untranslated 3′ end of *CYC1* [positions 351 to 588, UAA stop codon is at position 330 (*35*)] with the site of polyadenylation [positions 503 to 505 (*23*)] in vivo indicated by the arrow above. The *CYC1* pre-mRNA thus contains 149 nt of *CYC1* sequence 5′ to and 88 nt 3′ to the *CYC1* polyadenylation site. (**B**) Synthetic, capped precursor *CYC1* RNA synthesized from Pvu II restricted pGYC1 labeled with [α-$^{32}$P]GTP was incubated in a whole cell yeast extract prepared as described by Lin *et al.* (*26*) at 30°C for the indicated amounts of time. The final concentrations of the reaction components (including those contributed by the extract) in the 10-μl reactions were 4.6 m*M* Hepes (*p*H 7.0), 0.05 m*M* EDTA, 2 m*M* ATP, 1 m*M* magnesium acetate, 11 m*M* potassium chloride, 75 m*M* potassium acetate, 2 percent polyethylene glycol (PEG) 8000, 0.1 m*M* dithiothreitol (DTT), 4 percent glycerol, 23 percent extract (v/v), and 10 n*M* RNA (28,000 cpm/μl). The reactions were stopped by the addition of 2 μl of proteinase K (2 mg/ml), 130 m*M* EDTA, 2.5 percent SDS, with incubation at 37°C for 15 minutes. The reaction mixtures were then extracted twice with an equal volume of a mixture of phenol, chloroform, isoamyl alcohol (50:49:1) and precipitated with ethanol. The precipitated RNA's were dried and resuspended in water, and one-tenth of the total was suspended in 80 percent formamide, 0.1 percent xylene cyanole, and 0.1 percent bromophenol blue and heated at 90°C for 3 minutes. The precipitated material was then separated by electrophoresis (4 percent polyacrylamide, 0.08 percent bisacrylamide, 8*M* urea gel in 50 m*M* tris-borate (*p*H 8.3), 1 m*M* EDTA). The products were visualized by autoradiography (Kodak X Omat RP film). Lane M shows RNA molecular size markers whose lengths are given in nucleotides and whose positions are marked by the filled circles. The remaining lanes are designated according to the incubation time in minutes. The 5′ and 3′ cleavage products were about 200 nt and 150 nt and are indicated by I and II, respectively.

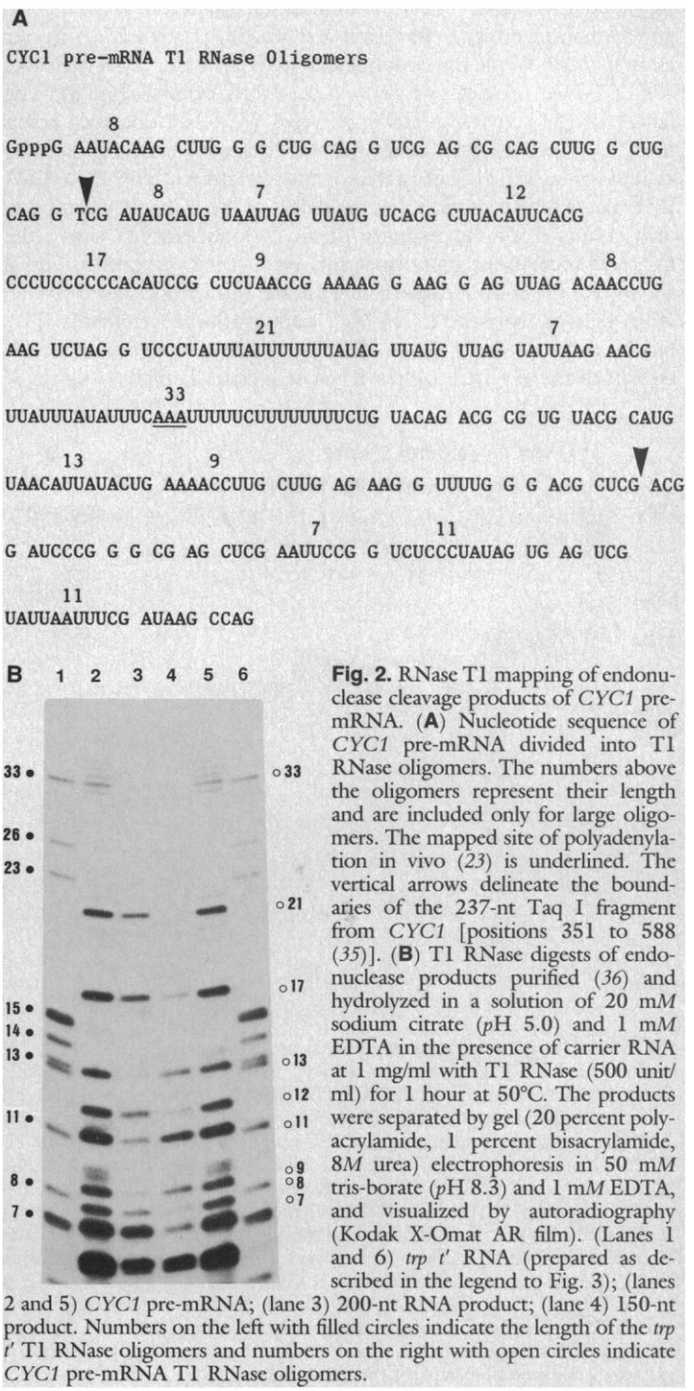


**Fig. 2.** RNase T1 mapping of endonuclease cleavage products of *CYC1* pre-mRNA. (**A**) Nucleotide sequence of *CYC1* pre-mRNA divided into T1 RNase oligomers. The numbers above the oligomers represent their length and are included only for large oligomers. The mapped site of polyadenylation in vivo (*23*) is underlined. The vertical arrows delineate the boundaries of the 237-nt Taq I fragment from *CYC1* [positions 351 to 588 (*35*)]. (**B**) T1 RNase digests of endonuclease products purified (*36*) and hydrolyzed in a solution of 20 m*M* sodium citrate (*p*H 5.0) and 1 m*M* EDTA in the presence of carrier RNA at 1 mg/ml with T1 RNase (500 unit/ml) for 1 hour at 50°C. The products were separated by gel (20 percent polyacrylamide, 1 percent bisacrylamide, 8*M* urea) electrophoresis in 50 m*M* tris-borate (*p*H 8.3) and 1 m*M* EDTA, and visualized by autoradiography (Kodak X-Omat AR film). (Lanes 1 and 6) *trp t′* RNA (prepared as described in the legend to Fig. 3); (lanes 2 and 5) *CYC1* pre-mRNA; (lane 3) 200-nt RNA product; (lane 4) 150-nt product. Numbers on the left with filled circles indicate the length of the *trp t′* T1 RNase oligomers and numbers on the right with open circles indicate *CYC1* pre-mRNA T1 RNase oligomers.

cleavage should, in principle, be discernible from the length of the new 17-nt fragment (Fig. 2B, lane 4) running with the 3′ T1 RNase products. Production of this oligonucleotide by T1 RNase means that its 3′ end coincides with the 3′G of the 33-nt fragment, thus putting the endonuclease cleavage site at the third of the three A's (Fig. 2A) at the in vivo polyadenylation site (*23*). The fact that the 3′ endonuclease product is unstable (Fig. 1B) and that we observe faint T1 RNase products at 18, 19, and 20 nt (Fig. 2B, lane 4) is consistent with cleavage slightly earlier with subsequent degradation by a 5′,3′-exonuclease (*22, 24*). In any case, the above results indicate that cleavage in vitro occurs at, or very close to, the site used in vivo.

**Substrate specificity and nucleotide cofactor requirement for endonucleolytic cleavage.** Most of the *CYC1* mRNA's in a *cyc1-512* mutant are longer than normal (up to 1000 nt) and all are polyadenylated (*14*). Since this indicates some defect in mRNA 3′ end formation in vivo, we tested a synthetic *cyc1-512* RNA in our assay in vitro. While the endonuclease activity in the extracts cleaves *CYC1* RNA, no cleavage of *cyc1-512* RNA occurs (Fig. 3). The failure of the activity to cleave the *cyc1-512* RNA does not result from the shorter length of the RNA because we observe the same result with a *cyc1-512* substrate equal in length to the *CYC1* RNA (*25*). These results suggest that the defect of the *cyc1-512* mutation in vivo results from the inability of an endonuclease to cleave the mRNA precursor at its normal site. As a negative control, Fig. 3 shows that the endonuclease activity in the extract does not cleave an RNA derived from the *E. coli trp t′* transcriptional terminator. This result, together with those described above, suggests that the endonuclease activity requires RNA sequences known to direct 3′ end formation in yeast.

The *cyc1-512* mutation also causes a defect in 3′ end formation of the adjacent, convergently transcribed *UTR* (*u*nidentified *tr*anscript) gene (*14*). We incubated a precursor RNA derived from the wild-type *UTR* gene in the extract and found cleavage of the RNA into two fragments of 80 nt and 230 nt (Fig. 3). Cleavage occurs with a lower efficiency with an RNA (*utr-512*) derived from a template carrying the *cyc1-512* deletion (Fig. 3). The partial cleavage of *utr-512* suggests that the *cyc1-512* mutation deletes some, although not all, of the information necessary for efficient processing of *UTR* mRNA and supports previous indications that the signals for 3′ end processing of *CYC1* and *UTR* mRNA's overlap (*14*).

Since RNA processing reactions often require adenosine triphosphate (ATP) for maximum efficiency (*21, 26*), we tested the nucleotide requirements of the endonuclease activity by assaying for cleavage in the presence or absence of various nucleotide cofactors. Efficient cleavage requires a nucleoside triphosphate, and ATP works best (Fig. 4). It appears that cytidine triphosphate (CTP) substitutes equally well for ATP but, as we show below, partial polyadenylation of the cleaved substrate (possibly only in the presence of ATP) results in an underestimate of the amount of cleavage in the ATP lanes. The nonhydrolyzable ATP analog ADP($CH_2$)P does not substitute for ATP, suggesting a requirement for hydrolysis of the β-γ bond of ATP for endonucleolytic cleavage. The nucleoside triphosphates guanosine triphosphate (GTP), uridine 5′-triphosphate (UTP), and deoxyadenosine triphosphate (dATP) work about half as well as ATP and 3′dATP does not work at all. Surprisingly, adenosine 5′-*O*-(3-thio)triphosphate (ATPγS) also works about half as well as ATP. Some enzymes slowly hydrolyze ATPγS to adenosine diphosphate (ADP) (*27*), and such a process coupled to conversion of ADP to ATP in the crude extract might provide enough energy for cleavage during long incubations.

We also tested the requirement of RNA capping for cleavage by assaying uncapped molecular size marker RNA's, one of which (350 nt) is the *CYC1* pre-mRNA (Fig. 3). The loss of the uncapped RNA's, in contrast to the stability of the capped RNA's in the other

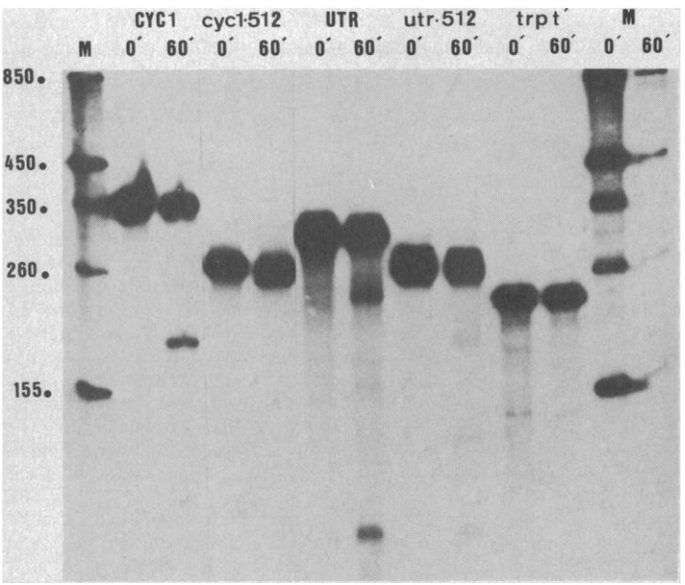


**Fig. 3.** Specificity of endonucleolytic cleavage in vitro. The *CYC1* and *UTR* precursor mRNA's were synthesized from Pvu–II restricted pGYC1 with SP6 RNA polymerase for *CYC1* RNA or Hind III–restricted pGYC1 with T7 RNA polymerase for UTR RNA. The *cyc1-512* and *utr-512* precursor RNA's were synthesized from Hind III–restricted pG200R (*36*) with T7 RNA polymerase for *cyc1-512* or Eco RI–restricted pG200R with SP6 RNA polymerase for *utr-512* RNA. The *trp t′* RNA was synthesized from Bam HI–restricted p65t′ (*19*) with SP6 RNA polymerase. The RNA's were capped and labeled with [α-$^{32}$P]GTP during synthesis. Each RNA (200 cpm/μl) was incubated for 0 to 60 minutes and processed as described in the legend to Fig. 1. The final concentration of each RNA was: *CYC1*, 1 n*M*; *cyc1-512*, 4 n*M*; *UTR*, 4 n*M*; *utr-512*, 1 n*M*; *trp t′*, 4 n*M*. At these concentrations, the rate of cleavage is proportional to the amount of added RNA (*38*). The last two lanes show the effect of incubation (from none to 60 minutes) in the extract of uncapped molecular size marker RNA's.

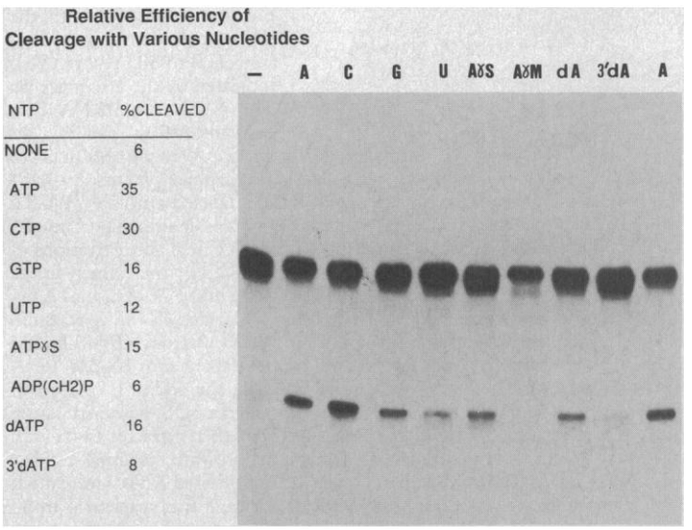


**Fig. 4.** Nucleotide requirement for endonucleolytic cleavage in vitro. *CYC1* precursor RNA (2000 cpm/μl) was incubated (as described in the legend to Fig. 1) at a final concentration of 1 n*M* for 60 minutes with the indicated nucleotide (2 m*M*) substituted for ATP. After incubation, the products were analyzed as described in the legend to Fig. 1. The percentage of cleaved product was determined by excising the precursor and product bands and then determining the amount of material in each by liquid scintillation counting. The abbreviations above the figure are, A, ATP; C, CTP; G, GTP; U, UTP; AγS, ATPγS; AγM, ADP($CH_2$)P; dA, dATP; and 3′dA, 3′dATP.

lanes, suggests that optimal stability requires capping of the RNA's. The specific disappearance of the 350-nt *CYC1* RNA results from cleavage and instability (determined by overexposure of the autoradiogram). These results show that, although capping increases RNA stability in the extracts, it is not required for endonucleolytic cleavage.

**Polyadenylation follows endonucleolytic cleavage of *CYC1* RNA.** If endonucleolytic cleavage of *CYC1* RNA is indeed a step in the formation of the mature 3′ end of the RNA, then we expect subsequent polyadenylation of the product in vitro. The time course of cleavage (Fig. 1) shows the accumulation, beginning at about 7 minutes, of small amounts of RNA with a length of about 280 nt. Chromatography of some of these cleavage products on poly(U)-Sephadex separates the RNA into unbound, poly(A)$^-$ RNA, and bound, poly(A)$^+$ RNA whose approximate size (280 nt) indicates the addition of about 80 adenosine residues to the 5′ cleaved fragment (Fig. 5A) in agreement with previous estimates of the length of poly(A) tails in yeast (*28*). The polyadenylated species we observe is not the result of polyadenylation of the 3′ cleaved product because decreasing the length of the substrate RNA at its 3′ end does not change the length of the polyadenylated product (*29*). The nucleotide 3′ dATP (cordycepin) inhibits polyadenylation in vitro (*21*), and therefore we included it in cleavage reactions and monitored the profiles of total and poly(A)$^+$ RNA (Fig. 5B). The profile of total RNA shows that 3′ dATP, in the presence of ATP, causes an increase in the amount of the 5′ cleavage product and a corresponding loss of the diffuse band of RNA at 280 nt. Since 3′ dATP does not act as a cofactor for cleavage (Fig. 4), the increased accumulation of the 5′ cleaved product results most likely from inhibition of its polyadenylation. The poly(A)$^+$ profile (Fig. 5B) shows that the diffuse 280-nt band of RNA is retained on a poly(U)-Sephadex column and that its production is ATP dependent and inhibited by 3′ dATP. These results support the conclusion that the 5′ cleavage product becomes preferentially polyadenylated after cleavage.

We have also observed, under conditions of high $Mg^{2+}$ (>2 m*M*) or low RNA (1 n*M*) concentrations, apparent nonspecific polyadenylation of the substrate RNA as well as RNA's that are not cleaved in the reaction (*29*). Since the rate of cleavage is low under such conditions relative to those used in Fig. 5 (1 m*M* $Mg^{2+}$, 10 n*M* RNA) we suspect that the activity that polyadenylates RNA in the extract is limiting, and therefore only efficient production of the 5′ cleaved product results in its preferential polyadenylation. We estimate, by comparing lanes 2 and 5 in Fig. 5B, that at least 10 percent of the cleaved RNA is polyadenylated in these experiments; efforts to improve the yield have failed thus far. This low efficiency of polyadenylation may result from loss or inactivation of the poly(A) polymerase during preparation of the extract, or 3′ end processing of endogenous pre-mRNA's may compete for polyadenylation with added substrate. In any case, the specificity of endonuclease cleavage and the preferential polyadenylation of the 5′ cleaved fragment, in the presence of equal or greater amounts of uncleaved substrate, provide strong support for our conclusion that this system correctly carries out mRNA 3′ end processing.

**Messenger RNA 3′ end formation in yeast.** Our results show that endonucleolytic cleavage and polyadenylation produce the 3′ end of *CYC1* pre-mRNA in vitro. Cleavage also occurs with the second yeast RNA (*UTR*) whose signals for 3′ end formation appear to partially overlap those of *CYC1*. This suggests that endonucleolytic cleavage and polyadenylation may form the mature 3′ ends of all yeast mRNA's as it appears to do in other eukaryotes (with the exception of most histone transcripts). Alternatively, the system processing *CYC1* and *UTR* mRNA's could be confined to certain classes of genes; whether other mRNA's are cleaved in this system remains to be determined.

In other eukaryotes, the highly conserved AAUAAA signal, along with downstream sequences, form the signal for mRNA 3′ end processing (*8, 9, 30*). Similar signals have not been identified for yeast mRNA's; in one case, 3′ end formation occurs in yeast on a *Drosophila* mRNA deleted for its AAUAAA signal (*31*), and the tripartite sequence (*14*) is found in many but not all yeast mRNA 3′ ends (*15–18*). In the absence of a highly conserved signal sequence we must entertain the possibility that RNA structure (or lack thereof), formed by disparate sequence elements, specifies the site of mRNA 3′ end formation in yeast, or that factors specific to certain classes of mRNA's, may direct cleavage and polyadenylation of those messages.

If our results showing endonucleolytic cleavage and polyadenylation in vitro reflect the mechanism used in vivo, then transcription by RNA polymerase II must extend beyond the mature mRNA 3′ end. However, in yeast, unlike higher eukaryotes, there is no evidence that transcription goes beyond the polyadenylation site. Indeed, recent experiments indicate that transcription stops fairly soon as a consequence of passing through the *CYC1* 3′ region (*32*) or the *CAT1* and *CAT2* 3′ regions (*33*). Proof that RNA polymerase transcribes past the mature 3′ end of a gene requires detection of such RNA products in vitro or in vivo. Experiments addressing this point have not been reported for yeast; detection of RNA products downstream of the polyadenylation site may be technically difficult if the polymerase continues only a short distance or the 3′ endonuclease cleavage product is unstable, as suggested by our experiments in vitro (Fig. 1B). We propose a simple model to reconcile these observations, in which RNA polymerase terminates transcription either in direct response to the mRNA processing reaction, or by the action of trans-acting factors such as an RNA:DNA helicase (*19*), or a 5′-3′ exonuclease (*34*), which might first interact with the 3′ cleavage product and then move along the RNA until it catches the

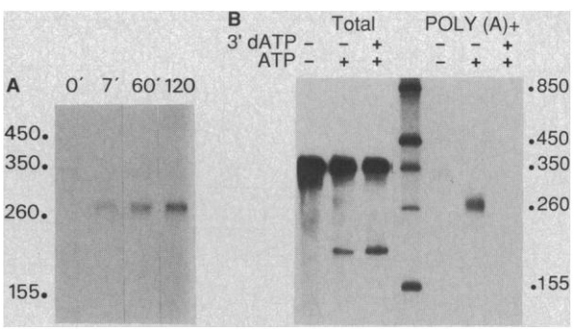


**Fig. 5.** Isolation of poly(A)$^+$ RNA by poly(U)-Sephadex chromatography. (**A**) RNA prepared as described in the legend to Fig. 1 was resuspended in 20 μl of 10 m*M* tris · HCl (*p*H 7.5), 300 m*M* NaCl, 1 m*M* EDTA, and 0.2 percent SDS and mixed with 300 μl of a slurry of poly(U)-Sephadex (Bethesda Research Labs) equilibrated in the same buffer. The mixture was heated at 65°C for 10 minutes and cooled to 25°C (10 minutes), before being transferred to a 1-ml syringe plugged with silanized glass wool. The resin was washed with ten volumes of 10 m*M* tris · HCl (*p*H 7.5), 1 m*M* EDTA, 0.2 percent SDS, 10 percent formamide (25°C) and the bound poly(A)$^+$ RNA was eluted with 300 μl of 10 m*M* tris · HCl (*p*H 7.5), 1 m*M* EDTA, 0.2 percent SDS, and 90 percent formamide. The eluted RNA was adjusted to 0.1*M* potassium acetate, 20 μg of carrier RNA was then added, and the RNA was precipitated with two volumes of ethanol. After being dried, the RNA was separated by electrophoresis as described in the legend to Fig. 1. The length of RNA molecular size markers in nucleotides is indicated at left and their position is indicated by the closed circles. The incubation time in the extract in minutes is indicated above. (**B**) Cleavage reactions were carried out for 60 minutes as described in Fig. 1. 3′dATP was included at 2 m*M* where indicated. The products were precipitated with ethanol and one-tenth of the total was separated by electrophoresis as described in Fig. 1; the remainder was separated by poly(U)-Sephadex chromatography and analyzed as described above.

polymerase and causes termination.

We have presented evidence that components in a yeast whole cell extract process a pre-mRNA spanning the 3′ end of the yeast *CYC1* gene to produce a polyadenylated, mature 3′ end similar to that seen in vivo. Our results imply that RNA processing, rather than transcription termination, forms the 3′ ends of mRNA's in *Saccharomyces cerevisiae*. Moreover, this discovery verifies that trans-acting factors must participate in mRNA 3′ end formation, and complements our recent development of a genetic selection for such factors (*23*). These combined approaches should lead to the identification of molecular components responsible for mRNA 3′ end formation in yeast and provide important insights into this process in all eukaryotes.


REFERENCES AND NOTES

1. M. L. Birnstiel, M. Busslinger, K. Strub, *Cell* **41**, 349 (1985).
2. D. R. Drummond, J. Armstrong, A. Coleman, *Virology* **13**, 7375 (1985).
3. D. R. Higgs *et al.*, *Nature* **306**, 398 (1983).
4. S. H. Orkin, T-C. Cheng, S. E. Antonarkis, H. H. Kazazian, Jr., *EMBO J.* **4**, 453 (1985).
5. G. Galli, J. Guise, P. Tucker, J. R. Nevins, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2439 (1988).
6. J. R. Nevins and J. E. Darnell, *Cell* **15**, 1477 (1978).
7. ———, *Nature* **290**, 113 (1981).
8. M. Fitzgerald and T. Shenk, *Cell* **24**, 251 (1981).
9. M. Wickens and P. Stephenson, *Science* **226**, 1045 (1984).
10. T. L. Green and R. P. Hart, *Mol. Cell. Biol.* **8**, 1839 (1988).
11. A. Gil and N. J. Proudfoot, *Cell* **49**, 399 (1987).
12. R. P. Hart, M. A. McDevitt, J. R. Nevins, *ibid.* **43**, 677 (1985).
13. M. A. McDevitt, M. J. Imperiale, H. Ali, J. R. Nevins, *ibid.* **37**, 993 (1984).
14. K. S. Zaret and F. Sherman, *ibid.* **28**, 563 (1982).
15. J. G. Yarger, G. Armilei, M. C. Gorman, *Mol. Cell. Biol.* **6**, 1095 (1986).
16. S. Henikoff and E. H. Cohen, *ibid.* **4**, 1515 (1984).
17. A. Sutton and J. R. Broach, *ibid.* **5**, 2770 (1985).
18. J. L. Bennetzen and B. D. Hall, *J. Biol. Chem.* **257**, 3018 (1982).
19. C. Brennan, A. J. Dombroski, T. Platt, *Cell* **48**, 945 (1987).
20. Cleavage occurs in the absence of added magnesium and in the presence of 4 m*M* EDTA, but it is enhanced about tenfold by the addition of 1 m*M* magnesium acetate, which works significantly better than magnesium chloride.
21. C. L. Moore and P. A. Sharp, *Cell* **41**, 845 (1985).
22. D. Zarkower, P. Stephenson, M. Sheets, M. Wickens, *Mol. Cell. Biol.* **6**, 2317 (1986).
23. H. Ruohola, S. M. Baker, R. Parker, T. Platt, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5041 (1988).
24. A. Stevens, *J. Biol. Chem.* **256**, 3080 (1980).
25. J. S. Butler, unpublished results.
26. R-J. Lin, A. J. Newman, S-C. Cheng, J. Abelson, *J. Biol. Chem.* **260**, 14780 (1985).
27. B. L. Stitt and M. R. Webb, *ibid.* **261**, 15906 (1986).
28. C. S. McLaughlin, J. R. Warner, M. Edmonds, H. Nakazato, M. Vaughan, *ibid.* **248**, 1466 (1973).
29. J. S. Butler, unpublished results.
30. H. Skolnik-David, C. L. Moore, P. A. Sharp, *Genes Dev.* **1**, 672 (1987).
31. S. Henikoff, J. D. Kelly, E. H. Cohen, *Cell* **33**, 607 (1983).
32. B. I. Osborne and L. Guarente, *Genes Dev.* **2**, 766 (1988).
33. M. Snyder, R. J. Sapolsky, R. W. Davis, *Mol. Cell. Biol.* **8**, 2184 (1988).
34. S. Connelly and J. L. Manley, *Genes Dev.* **2**, 440 (1988).
35. M. Smith *et al.*, *Cell* **16**, 753 (1979).
36. D. A. Peattie, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1760 (1979).
37. pG200R, a pGEM1 derivative, contains a 199-bp Taq I insert analogous to that in pGYC1 but with the *cyc1-512* 38-bp deletion and cloned in the opposite orientation.
38. J. S. Butler, unpublished results.


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