RNA Processing In Vitro Produces Mature 3' Ends of a Variety of Saccharomyces cerevisiae mRNAs

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Ammonium sulfate fractionation of a Saccharomyces cerevisiae whole-cell extract yielded a preparation which carried out correct and efficient endonucleolytic cleavage and polyadenylation of yeast precursor mRNAs substrates corresponding to a variety of yeast genes. These included CYC1 (iso-1-cytochrome c), HIS4 (histidine biosynthesis), GAL7 (galactose-1-phosphate uridylyltransferase), H2B2 (histone H2B2), PRT2 (a protein of unknown function), and CBP1 (cytochrome b mRNA processing). The reaction processed these pre-mRNAs with varying efficiencies, with cleavage and polyadenylation exceeding 70% in some cases. In each case, the poly(A) tail corresponded to the addition of approximately 60 adenosine residues, which agrees with the usual length of poly(A) tails formed in vivo. Addition of cordycepin triphosphate or substitution of CTP for ATP in these reactions inhibited polyadenylation but not endonucleolytic cleavage and resulted in accumulation of the cleaved RNA product. Although this system readily generated yeast mRNA 3' ends, no processing occurred on a human α-globin pre-mRNA containing the highly conserved AAUAAA polyadenylation signal of higher eucaryotes. This sequence and adjacent signals used in mammalian systems are thus not sufficient to direct mRNA 3' end formation in yeast. Despite the lack of a highly conserved nucleotide sequence signal, the same purified fraction processed the 3' ends of a variety of unrelated yeast pre-mRNAs, suggesting that endonuclease cleavage and polyadenylation may produce the mature 3' ends of all mRNAs in S. cerevisiae.

In eucaryotic cells, mRNAs undergo posttranscriptional modification before export from the nucleus to the cytoplasm. Of these processing events, the addition of a poly(A) tail to the 3' end of mRNA precursors results in one of the distinguishing features of mature mRNAs (for a review, see reference 14). In mammalian cell-free systems, a specific endonucleolytic cleavage precedes polyadenylation of the mRNA, rationalizing earlier observations that RNA polymerase II transcribes past the polyadenylation sites of eucaryotic mRNAs (16). In mammalian cells, the highly conserved AAUAAA found 20 to 30 nucleotides (nt) upstream of the polyadenylation site together with some less conserved downstream sequences function as signals for this mRNA 3' end processing event (4, 7, 8, 22, 28). Cleavage and polyadenylation occur on relatively large complexes (12, 17, 22, 23, 33) and appear to require factors in addition to an endonuclease and a poly(A) polymerase (3, 26, 27).

The ubiquitous nature of the AAUAAA 3' end formation signal in higher cells and the apparent lack of such a highly conserved signal in the yeast Saccharomyces cerevisiae initially suggested differences between the mechanisms of mRNA 3' end formation in yeast and mammalian cells. Identification of possible sequence elements involved in yeast mRNA 3' end processing came from studies of a 38-base-pair deletion (cyc1-312) found in the 3' noncoding region of the yeast iso-1-cytochrome c gene (CYC1) (31). This mutation resulted in a 90% reduction in the steady-state levels of CYC1 mRNAs, the remainder of which are longer (up to 1,000 nt) than normal and all of which are polyadenylated. Comparison of the sequence deleted with known sequences near the polyadenylation sites of other yeast genes and with cis-acting revertants of cyc1-312 that restore normal mRNA levels (32) shows similarities proposed as a tripartite signal for transcription termination (31, 32). Analysis of steady-state mRNA 3' ends of a yeast 2μm circle plasmid (24) and of the Ty element long terminal repeat (30) provided additional evidence for the involvement of this tripartite signal. However, linker substitution mutagenesis of the region containing the tripartite sequence in CYC1 decreased its function less than twofold (18). The inability of anything less than a large deletion to affect function (10, 29) and the lack of strong sequence similarity among the many polyadenylation sites examined are reminiscent of rho-factor-dependent termination in procaryotes (20). This and the fact that all mRNAs (including the replication-dependent histones) in yeast are polyadenylated led to the suggestion that yeast mRNA 3' ends might be formed directly by transcription termination rather than by the processing of a precursor mRNA (31, 32).

We recently described a cell-free system from yeast capable of endonucleolytic cleavage of yeast CYC1 pre-mRNA at its polyadenylation site (1). This reaction requires the presence of a nucleoside triphosphate for maximum efficiency and fails with pre-mRNA substrates carrying mutations that disrupt mRNA 3' end formation in vivo. Although this system showed a high degree of accuracy and specificity for cleavage, the efficiency of polyadenylation, though specific for the cleaved substrate, was only about 10% (1). The system also failed to process several pre-mRNAs derived from the 3' ends of different yeast genes. We show here that modification of our original procedure resulted in a preparation that accurately and efficiently polyadenylates a variety of yeast pre-mRNAs. These results suggest that despite the apparent lack of a strong consensus signal for mRNA 3' end formation, endonucleolytic cleavage and subsequent polyadenylation may produce all yeast mRNA 3' ends.

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MATERIALS AND METHODS

Reagents. Nucleotides were purchased from Pharmacia, Inc. Restriction enzymes and bacteriophage RNA polymerases were purchased from Bethesda Research Laboratories, Inc., or Boehringer Mannheim Biochemicals. [α-32P]GTP (400 mCi/mmol) was purchased from Amersham Corp. Zymolyase 60000 was purchased from Seikagaku Kogyo-Japan. RNasin was purchased from Promega Corp.

Transcription templates and synthesis of radiolabeled precursor mRNAs. The following linearized plasmid DNA templates carrying the appropriate bacteriophage RNA polymerase promoters were used to prepare pre-mRNAs. For CYC1 and cyc1-312 pre-mRNAs, PvuII hydrolyzed pGYC1 and HindIII hydrolyzed pG200R by using SP6 and T7 RNA polymerases, respectively (1). For CBI1, HindIII hydrolyzed pBS1-5 (15) by using T7 RNA polymerase. For GAL7, EcoRI hydrolyzed pS28 by using SP6 RNA polymerase. pS28 was derived by insertion of an SstI fragment containing the 3' end of GAL7 (positions 1123 to 1371 [25]) into the HincII site of pGEM1. For HIS4, BstEII hydrolyzed pHTE14 by using T7 RNA polymerase. pHTE14 was derived by insertion of the Hpal-BamHI fragment from pYAH12 (19) containing the 3' end of HIS4 (positions 1894 to 3420 [6]) into the HincII-BamHI site of pGEM4Z. For H2B2, BglII hydrolyzed pHG9D by using T7 RNA polymerase. pHG9D was derived by insertion of the 1.2-kilobase HindIII fragment containing the 3' ends of the H2B2 and PRT2 genes (fragment G' in reference 11) into the HindIII site of pGEM1. For PRT2, BglII hydrolyzed pHG9D by using SP6 RNA polymerase. For human α-globin, EcoRI hydrolyzed pSP6αM (kindly provided by N. Proudfoot) by using SP6 polymerase.

RNAs were synthesized from restriction enzyme-linearized plasmid DNAs with SP6 or T7 bacteriophage RNA polymerase in 50-μl reaction volumes. For T7 RNA polymerase, reactions contained 40 mM Tris hydrochloride (pH 8.0), 25 mM NaCl, 8 mM MgCl2, 2 mM spermidine, 5 mM dithiothreitol (DTT), 0.4 mM ATP, 0.4 mM CTP, 0.4 mM UTP, 0.02 mM GTP, 0.2 mM diguanosinetriphosphate (GpG), 40 U of RNasin, 1 µg of plasmid DNA, 20 µCi of [α-32P]GTP, and 50 U of T7 RNA polymerase. For SP6 RNA polymerase, reactions contained 40 mM Tris hydrochloride (pH 7.9), 6 mM MgCl2, 2 mM spermidine, 1 mM DTT, 0.4 mM ATP, 0.4 mM CTP, 0.4 mM UTP, 0.02 mM GTP, 0.2 mM GpG, 40 U of RNasin, 1 µg of plasmid DNA, 20 µCi of [α-32P]GTP, and 15 U of SP6 RNA polymerase. Uncapped CYC1 pre-mRNA was synthesized as described above with the omission of GpG from the SP6 RNA polymerase reaction mixture. RNA synthesis in each case was carried out for 60 min at 37°C and terminated by ethanol precipitation. The RNAs were separated from unincorporated 32P-labeled nucleotides on polyacrylamide-urea gels followed by excision and elution of the desired product from the gel (1).

Preparation of mRNA 3'-end-processing extracts. The procedure to prepare mRNA 3'-end-processing extracts was modified from a protocol designed by Lin et al. for the preparation of yeast-splicing extracts (13). We generally used the strain EJ101 (MATα his+ pep4-3 prb1 prc1), but we have prepared active extracts from a variety of unrelated strains. Yeast cells were grown in 1 liter of YEPD (1% yeast extract, 2% Bacto-Peptone, 2% dextrose) to an A600 between 4 and 6. Cultures grown to higher densities usually gave preparations with low activities. Cultures grown to lower densities gave active preparations only when the volume of cells used was increased proportionately. The cells were collected by centrifugation at 1,000 × g for 5 min at room temperature and suspended in 15 ml of buffer S (50 mM Tris hydrochloride [pH 7.8], 1 M sorbitol, 10 mM MgCl2, 30 mM DTT) and incubated at room temperature without shaking for 15 min. The cells were collected by centrifugation at 1,000 × g for 5 min at room temperature and resuspended in 15 ml of buffer S. To this suspension of cells, 0.09 ml of Zymolyase 60000 (20 mg/ml) was added, and the mixture was incubated with gentle shaking at 30°C for 40 min. The spheroplasts were collected by centrifugation (4°C) at 1,000 × g for 5 min and were suspended in 8 ml of buffer A [10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-KOH (pH 7.0), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT; 4°C]. The spheroplasts were lysed with five strokes of a Dounce homogenizer (Wheaton Industries glass homogenizer, pestle A). The suspension was brought to 0.2 M KCl and stirred gently on ice for 30 min. The supernatant was collected by centrifugation at 22,000 × g, and the collected supernatant was centrifuged (4°C) further at 145,000 × g for 60 min. The supernatant was collected and brought to 40% saturation by the addition of solid (NH4)2SO4 (2.2 g/ml of starting solution) and gentle stirring (4°C) until the salt dissolved. The solution was allowed to sit without stirring for 20 min at 4°C, and precipitated protein was collected by centrifugation at 15,000 × g for 20 min. The pellet was suspended in 1 to 2 ml of buffer B (20 mM HEPES-KOH [pH 7.0], 0.2 mM EDTA, 50 mM KCl, 20% [vol/vol] glycerol, 0.5 mM DTT) and dialyzed against 1,000 volumes of the same buffer for 3 h at 4°C. The extract was frozen in 50- to 100-μl portions and stored at -70°C. Under these conditions, the mRNA 3'-end-processing activities were stable for at least 9 months, and the extracts could be thawed and refrozen several times without apparent loss of activity. We found that an extract with good activity had an A280 above 4/ml and an A260/A280 ratio of about 1.

mRNA 3' end processing in vitro. Processing reactions were carried out in 10-μl volumes at 30°C and terminated as described previously (1). Reaction mixtures were assembled on ice, and the last two components added to the tube were extract and RNA. The products were precipitated by the addition of 50 µl of 1 mM EDTA, 0.3 M potassium acetate (pH 5), 0.5 mg of carrier RNA per ml, and 120 µl of ethanol and analyzed by electrophoresis as previously described (1).

RESULTS

Partially purified yeast extracts correctly and efficiently process yeast mRNA 3’ ends. Despite the accuracy and specificity of CYC1 pre-mRNA processing in extracts prepared as previously described (1), we found that polyadenylation was inefficient and that the extracts did not process pre-mRNAs carrying the polyadenylation sites from several other yeast mRNAs. Upon fractionation of these extracts, we found that dialysis of S-100 fractions resulted in a substantial loss of poly(A) polymerase activity without inactivation of the endonuclease. However, ammonium sulfate fractionation of the S-100 prior to dialysis yielded a preparation (0 to 40% saturation) containing stable cleavage and polyadenylation activities (Fig. 1); apparently ammonium sulfate fractionation removes some activity which inactivates or destabilizes the poly(A) polymerase. Significantly, incubation of a CYC1 pre-mRNA in this fraction resulted in rapid, low efficient cleavage and polyadenylation of the pre-mRNA (Fig. 1 and 2). Incubation at 30°C for 30 min converted about 70% of the input pre-mRNA (as determined...
by scintillation counting of the gel bands) into correctly polyadenylated RNA and two residual species 200 and 150 nt long, corresponding, respectively, to the 5' (upstream of the polyadenylation site) and 3' (downstream of the polyadenylation site) cleavage products. The identities of these two cleavage products were established previously by RNA fingerprint analysis (1).

Three lines of evidence support the identity of the polyadenylated product, which appeared as a broad band at approximately 260 nt (Fig. 1 and 2). First, its length corresponded to the addition to the 200-nt 5' cleavage product of approximately 60 adenosines (Fig. 2, lane 30'), which is the average length of poly(A) tails in yeast (9). The apparent poly(A) tail shortening evident in Fig. 2 is discussed below. Second, this species alone is retained on poly(U)-Sephadex columns (1). Third, addition of the polyadenylation inhibitor cordycepin triphosphate inhibited the appearance of this product and resulted in the accumulation of the 5' cleavage product (Fig. 3).

High-efficiency processing retains specificity and exclusivity. We tested whether this improved processing preparation retains the specificity observed in cruder fractions by incubating a pre-mRNA encompassing the cys1-512 deletion under conditions which efficiently process a CYC1 pre-mRNA. The result shows that the mutation also disrupts normal 3' end processing in vitro (Fig. 3). Close inspection of the autoradiogram in Fig. 3 reveals a faint band of RNA running at approximately 300 nt in lane 5 whose appearance is inhibited by the addition of cordycepin triphosphate in place of ATP (compare lanes 5 and 6). This finding suggests that this is a polyadenylated species derived either from low-level polyadenylation of the precursor or from inefficient cleavage and polyadenylation near the end of the precursor. The fact that the reaction did not process the cys1-512 pre-mRNA under conditions in which it efficiently processed the CYC1 pre-mRNA demonstrates that cleavage and polyadenylation in vitro retain the specificity observed in vivo.

Processing does not require a 5' cap. We also tested the
requirement of the cleavage and polyadenylation reaction for a 5' terminal cap on the pre-mRNA. Comparison of the reaction kinetics for capped and uncapped substrates showed similar rates of processing for the two substrates, suggesting that prior capping of the pre-mRNA does not directly affect cleavage and polyadenylation in vitro (Fig. 4). It appears, however, that the cap enhances the stability of the pre-mRNA because all of the RNA species added to and generated by the reaction with a capped pre-mRNA remained stable in the extract for at least the first 30 min, with the exception of the 3' cleavage product (Fig. 1 to 4). This 3' fragment was the only uncapped RNA species present, hence its lability suggests hydrolysis by a 5'-3' exonuclease.

Since all of the RNA species in the uncapped reaction disappeared with kinetics similar to that of the 3' cleavage product, we believe that they suffered the same fate because of the lack of a 5' cap. These results indicate indirectly that uncapped pre-mRNAs added to our reactions did not become capped during the course of the incubation and, therefore, that mRNA 3' processing of yeast mRNAs in vitro does not require a 5'-terminal cap.

A time course of the reaction showed a decrease in the average length of the poly(A)+ product between 30 and 60 min, with the average length stabilizing between 60 and 120 min (Fig. 2). This may have resulted from random hydrolysis of the product during the reaction, but the facts that the pre-mRNA carried a 5' cap and that the average length of the polyadenylated product stabilized with time suggest that the decrease may have resulted from shortening of the poly(A) tail. Interestingly, the decrease (ca. 15 nt) in the average length of the poly(A)+ RNA observed here corresponds to the amount of poly(A) tail shortening observed in vivo in yeast (21).

RNA processing generates the mature 3' ends of a variety of yeast mRNAs. We tested several different yeast pre-mRNAs for 3' end processing in our purified extracts. A number of these (CYC1, GAL7, CBP1, and HIS4) have 3' endpoints mapped in vivo, and therefore, one can predict the cleavage and polyadenylation pattern expected in vitro in each case. For each pre-mRNA, we carried out processing reactions in the presence of either ATP or CTP; the latter acts as a control for polyadenylation since CTP functions as a cofactor for cleavage but not for polyadenylation (Fig. 5; 1). Evidence for this effect of CTP comes from the comparison of the incubation of CYC1 with CTP (Fig. 5, lane 3) and reactions which included the polyadenylation inhibitor cordycepin triphosphate (Fig. 4, lane 3). Both nucleotides inhibited the appearance of the broad band of polyadeny-
lated product produced in the ATP reaction leading to accumulation of the polyadenylated 5' cleavage product. These two nucleotides had different influences on the yield of 3' cleavage product, probably because of the effects on the activities degrading this product. Incubation of other yeast pre-mRNAs under these conditions resulted in a similar pattern of product formation in each case. Significantly, in cases where we know the polyadenylation site in vivo (CYC1, GAL7, CBP1, and HIS4), the lengths of the 5' and 3' cleavage products suggest that cleavage in vitro takes place at the site used in vivo (Fig. 5). For example, a CBP1 pre-mRNA yielded, in the presence of CTP, two products, the sum of whose lengths (approximately 300 and 600 nt) equaled that of the pre-mRNA (ca. 900 nt), indicating that they arose from endonucleolytic cleavage of the pre-mRNA (Fig. 5, lane 9). The lengths of these cleavage products also suggest that this cleavage occurs at the polyadenylation site used in vivo (15), which lies 305 nt from the 5' end of the pre-mRNA (15). Polyadenylation of the 305-nl product in the presence of ATP yielded a broad band of RNA about 370 nt in length, corresponding to the addition of a normal 60-residue poly(A) tail (Fig. 5, lane 8).

A GAL7 pre-mRNA also yielded two cleavage products (ca. 160 and 140 nt) and a poly(A) + product (ca. 220 nt) from the 300-nt pre-mRNA (Fig. 5, lanes 5 and 6). The presence of CTP prevented formation of the poly(A) + product at 220 nt, resulting in accumulation of the 160-nt product, indicating that the reaction normally polyadenylates this RNA fragment (Fig. 5, lane 6). The site of cleavage and polyadenyla-
tion indicated by these results in vitro differs from the published determination of the 3' end of GAL7 mRNA in vivo (25). However, we have recently mapped the sites used in vitro and in vivo and have found that the published site in vivo corresponds to a region of S1 nuclease hypersensitivity and that the genuine polyadenylation site in vivo corre-
sponds to the one used in vitro (P. Sadhale, R. Sапolsky, J. S. Butler, R. Davis, and T. Platt, unpublished data).

Analysis of the processing of HIS4 pre-mRNA is complicated by the fact that mapping of the 3' end shows major and minor polyadenylation sites in vivo (6). For processing in vitro, two aspects of the pattern of cleavage and polyadenyla-
tion suggest multiple sites of processing (Fig. 5, lanes 10 to 12; note that for HIS4, cleavage produced a relatively small 3' fragment [ca. 130 nt] which has migrated out of the gel). First, the band of polyadenylated RNA extended over about 70 nt and appeared to contain a major and a minor species of about 710 and 750 nt, respectively. Second, blocking polyaden-
ylation with CTP resulted in the accumulation of a major product of about 650 nt and a minor product of about 690 nt, each lacking the 60 or so adenosines of the polyadenylated products. The lengths of these major and minor products suggest that their 3' ends correspond closely to the major and minor 3' endpoints reported for HIS4 in vivo (6).

For H2B2 and PR2, whose polyadenylation sites in vivo remain unmapped, the lengths of major products generated in vitro indicate that cleavage and polyadenylation occurred downstream of the coding sequence as expected (Fig. 5). In each case, the polyadenylated product (ca. 400 to 460 nt for H2B2 and 230 nt for PR2), identified by its inhibition by CTP, corresponds to the addition of approximately 60 nt to the 5' cleavage products (ca. 370 and 390 nt for H2B2 and 170 nt for PR2). This analysis suggests that H2B2 has two strong polyadenylation sites within 20 nt of each other. The 3' cleavage products of H2B2 are probably obscured by the poly(A) + band in the ATP lane and lost in the CTP lane (Fig. 5).

FIG. 6. Inability of yeast to process a pre-mRNA with mammalian 3' end formation signals; autoradiogram of gel electrophoretic separation of the products of processing reactions incubated for the times (in minutes) indicated at the top. Lanes M are RNA molecular weight markers whose lengths (from top to bottom) are 450, 350, 260, and 155 nt for the left set and 850, 450, and 350 nt for the right set. A + indicates poly(A) + RNA, and 5' indicates the 5' cleavage products. The products of the HIS4 pre-mRNA processing reaction as well as adjacent molecular weight markers were separated by electrophoresis prior to and during the separation of the other RNAs on the same gel.

The formation of polyadenylated mRNA 3' ends that we observed in vitro for yeast pre-mRNAs corresponds mechanistically to the process observed in vitro in mammalian cell systems in that a specific endonucleolytic cut precedes the addition of a poly(A) tail (16). The highly conserved mammalian AAUAAA 3' end formation signal lies within the 3' end of some yeast mRNAs, and variations of it exist in many other 3' regions. To test whether the AAUAAA sequence signals 3' end processing in yeast, we incubated in yeast extracts a synthetic pre-mRNA carrying the 3' end formation signals of the human α-globin gene (Fig. 6). Cleavage and polyadenylation of this pre-mRNA at its natural site should generate a product approximately 270 nt long, while cleavage alone should produce a 5' cleavage product about 210 nt long. We observed no processing of the α-globin pre-mRNA under conditions in which efficient processing of CYC1, GAL7, and HIS4 pre-mRNAs occurred (Fig. 6). This result suggests that the AAUAAA and associated mammalian processing signals cannot, by themselves, signal mRNA 3' processing in yeast.

DISCUSSION

We have demonstrated the efficient, accurate, and specific generation of the mature 3' ends of a variety of yeast mRNAs in vitro. The time course of processing of CYC1 exemplifies the efficiency of this system in that the reaction converted a significant fraction of the precursor mRNA into polyadenylated product in only 30 min (Fig. 2). Several other pre-mRNAs, notably CBP1, HIS4, and H2B2, exhibited even higher processing efficiencies than CYC1 did (Fig. 5). These differences in the relative rates of processing may reflect intrinsic differences in the rate of 3' end processing in vivo, or they may result from loss or depletion, during preparation of the extract, of some additional factor(s) required for efficient processing of particular mRNAs. On the other hand, we have employed synthetic pre-mRNAs carrying only the 3' portion of each mRNA, and so they may not carry some nucleotide sequence elements affecting the
efficiency of 3' end processing. Interestingly, the most efficient pre-mRNAs (CBP1, HIS4, and H2B2) all carry some portion of their coding sequence and therefore all of the information between the termination codon and the polyadenylation site, while CYC1 and GAL7 do not (Fig. 5; it is not known if PRT2 carries its coding sequence). This could simply reflect a length dependence of the processing reaction, but mammalian mRNAs carry sequence elements upstream of the AAUAAA signal which sometimes have important effects on the efficiency of cleavage and polyadenylation (2, 5).

The 3'-end-processing system described here displays an accuracy and exclusivity consistent with sites of polyadenylation in vivo. Support for this conclusion comes from the fact that for the cases where the polyadenylation site in vivo is known (CYC1, GAL7, CBP1, and HIS4), processing in vitro efficiently generated only those products expected from endonucleolytic cleavage and polyadenylation of the pre-mRNAs at their authentic polyadenylation sites (Fig. 5). Polyadenylation also exhibited significant specificity in that only the expected cleavage product underwent polyadenylation in vitro (Fig. 5). This was especially striking early in the reaction with CYC1, where unprocessed pre-mRNA was the majority of the RNA in the reaction, yet only the 5' cleavage product became polyadenylated (Fig. 2). This implies that polyadenylation requires prior endonucleolytic cleavage of the mRNA and argues against models for mRNA 3' end formation in yeast which feature polyadenylation at any free mRNA 3' end.

The requirement of the polyadenylation reaction for prior endonucleolytic cleavage suggests that the cleavage reaction determines the specificity of mRNA 3' end formation in yeast. A deletion (cyc1-S12) of sequences required for accurate and efficient mRNA 3' end formation of CYC1 in vivo destroyed the ability of this mRNA to function as a substrate for mRNA 3' end formation in vitro (Fig. 3). In this case, no cleavage and therefore no polyadenylation occurred presumably because the poly(A) polymerase cannot efficiently recognize the 3' end of the cyc1-S12 pre-mRNA as a correct substrate. The determinants of the specificity of endonucleolytic cleavage and polyadenylation in yeast remain mysterious. Clearly, sequence elements crucial for processing of CYC1 must reside in the 38 base-pair deleted in cyc1-S12, yet extensive deletion-substitution mutagenesis failed to reveal smaller sequence elements, such as AAUAAA, necessary for 3' end formation (18). The 38-base-pair region resembles, in this respect, the processing signals downstream of the polyadenylation sites in mammalian mRNAs in that it appears large or composed of several shorter, redundant elements and does not contain a highly conserved nucleotide sequence (34). The processing system in vitro described here provides a means to test variants of yeast mRNA 3' end formation signals for function in the absence of effects on transcription or mRNA stability. It should therefore aid the determination of sequence and higher-order structural elements involved in 3' end processing in yeast. One such experiment showed that the signals used in mammalian cells, including AAUAAA, do not function for cleavage and polyadenylation in S. cerevisiae in vitro (Fig. 6).

The mechanism of mRNA 3' end processing in yeast appears similar to that in higher eucaryotes in spite of the dissimilarities in the sequence determinants. Both systems use a pre-mRNA extending past the mature 3' end as a substrate and process this RNA with (at minimum) an endonuclease and a poly(A) polymerase. This suggests similarity among the factors necessary for mRNA 3' end forma-

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