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Uncoupling of mRNA 3′ Cleavage and Polyadenylation by Expression of a Hammerhead Ribozyme in Yeast*

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An artificial messenger RNA containing a derivative of a tobacco ringspot virus ribozyme was expressed in the bakers' yeast *Saccharomyces cerevisiae*. This mRNA was able to cleave itself efficiently *in vivo*. Using this system, the two steps of mRNA 3′ processing, *i.e.* cleavage and the addition of a poly(A) tail, can be separated in yeast *in vivo*. The ribozyme-cleaved transcript was shown to be polyadenylated. The poly(A) tail length was similar to the poly(A) tail length of an endogenous yeast mRNA. Therefore, cleavage of the precursor RNA at the polyadenylation site and the addition of adenosine residues to the 5′ product require independent cellular machineries in yeast and can be separately analyzed. This is in contrast to higher eukaryotes where both processes are coupled.

The yeast *Saccharomyces cerevisiae* represents a feasable model system to study eukaryotic transcription because many aspects of this process are conserved from yeast to man. From *in vitro* systems in mammals and in yeast, it can be concluded that the 3′-ends are formed by endonucleolytic cleavage of precursor transcripts followed by the addition of A residues at the polyadenylation site (Moore and Sharp, 1984, 1985; Butler *et al.*, 1990). An additional step is the actual termination of transcription, which seems to require a pausing signal for the RNA polymerase II and which takes place in various distances downstream of the processing site. Beside these common aspects, there are differencies within the eukaryotes concerning these processes. The length of the poly(A) tail varies from about 60 A residues in yeast (Groner *et al.*, 1975) to approximately 200 residues in higher eukaryotes (for reviews, see Humphrey and Proudfoot (1988), Manley (1988), and Wickens (1990)). Furthermore, whereas in higher eukaryotes histone mRNAs do not contain a poly(A) tail, all mRNAs seem to be polyadenylated in yeast (Fahrner *et al.*, 1980).

Higher eukaryotes require the highly conserved hexanucleotide AAUAAA sequence on the nascent RNA as a signal for 3′-end formation. This canonical element is not functional in yeast and shows no homology to the yeast signal sequences that have been proposed so far (Hyman *et al.*, 1991; Proudfoot, 1991; Irniger *et al.*, 1993). A decade ago, the degenerate tripartite sequence motif TAG...TA(T)GT(A)...TTT had been suggested as polyadenylation signal because a deletion of this sequence had resulted in a *CYC1* mutant defective in mRNA 3′-end formation (Zaret and Sherman, 1982). Further analysis of intragenic *CYC1* revertants that had restored proper mRNA 3′-end formation revealed that two of six revertants had acquired the bipartite motif TAG...TATGTA and that four revertants had created the modified sequences TATATA or TACATA (Russo *et al.*, 1991). Irniger *et al.* (1992) found that the nonanucleotide sequence TAGTATGTA, similar to the bipartite *CYC1* sequence motif was required for function of the cauliflower mosaic virus polyadenylation site in *S. cerevisiae*. Mutational analysis of the yeast *GAL7* polyadenylation signal demonstrated that a sequence element of 26 bp[1] containing an alternating TA stretch was essential for 3′-end formation (Abe *et al.*, 1990). A similar sequence element, *i.e.* the octanucleotide TTTTTATA, has also been suggested as a signal sequence (Henikoff and Cohen, 1983). However, the absence of a unique processing and polyadenylation signal suggested that there is no single mechanism for 3′-end formation in *S. cerevisiae* (Yu and Elder, 1989; Irniger *et al.*, 1991).

Cleavage and polyadenylation are performed by similar multicomponent complexes that are partially equivalent in mammals and in yeast. In higher eukaryotes, there are five known factors in addition to the poly(A) polymerase that are required for this process. The poly(A) polymerase (Raabe *et al.*, 1991; Wahle *et al.* 1991) and two protein factors, *i.e.* the cleavage and polyadenylation specifity factor and the poly(A) binding protein II, are required for specific polyadenylation (Christofori and Keller, 1988; Takagaki *et al.*, 1988, 1989; Gilmartin and Nevins, 1989; Bienroth *et al.*, 1993). Three factors involved in endonucleolytic cleavage, designated cleavage factors I and II and cleavage-stimulation factor have been separated from HeLa nuclear extracts (for review, see Wahle and Keller (1992)).

In *S. cerevisiae*, the gene for the poly(A) polymerase has been cloned by Lingner *et al.* (1991a). In addition, there are at least three trans-acting factors that are involved in 3′-end formation. Chen and Moore (1992) showed that *in vitro* cleavage of the precursor transcript occurred upon the combination of fractions containing cleavage factor I and cleavage factor II without requiring the poly(A) polymerase or the polyadenylation factor I. These two factors in addition to the cleavage factor I were required for the specific polyadenylation reaction of a precleaved *GAL7* RNA. Both processes, the cleavage and the polyadenylation, were dependent on the UA repeat within the poly(A) signal (Chen and Moore, 1992).

While in mammals, cleavage and polyadenylation of the precursor transcript are coupled *in vivo* (for review, see Wahle and Keller (1992)) the situation is unclear in yeast (Butler and Platt, 1988; Zaret and Sherman, 1982). Cleavage and polyadenylation can be uncoupled *in vitro* using yeast extracts (Chen and Moore, 1992; Sadhale and Platt, 1992).

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[1] The abbreviations used are: bp, base pair(s); PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); RACE, rapid amplification of cDNA ends; MCS, multiple cloning site.

This situation prompted us to ask whether it is possible to uncouple cleavage and polyadenylation in yeast *in vivo*. We present here the first *in vivo* data showing that, in yeast as a simple eukaryotic model system, cleavage and polyadenylation can be separated into two processes. Separation of the processes was shown by expressing a heterologous ribozyme sequence under the control of a RNA polymerase II promoter.

EXPERIMENTAL PROCEDURES

Yeast Strains, Media for Yeast Strain Cultivation, and Methods—The *S. cerevisiae* strain used for these studies was RH 1416 (MATa *ura3–52*), a derivative of the *S. cerevisiae* laboratory standard strain X2180–1A (*MATa gal2 SUC2 mal CUP1*). Yeast strains were cultivated in YEPD complete medium (2% bactopeptone, 1% yeast extract; 2% glucose) or MV minimal medium (0.145% yeast nitrogen base, 0.525% ammonium sulfate, 2% glucose, 1% succinic acid) (Miozzari *et al.*, 1978). Yeast transformation (Ito *et al.*, 1983), DNA isolation (Braus *et al.*, 1985), and Southern analysis (Southern, 1975) were previously described.

Site-directed Mutagenesis—Point mutations were created using oligonucleotides as primers that contained the desired mutations and the polymerase chain reactions according to Giebel and Spritz (1990). The ribozyme sequence of the obtained test constructs including the flanking regions of the *ACT1* promoter and the *ADH1* terminator were sequenced using the dideoxy method (Sanger *et al.*, 1977), thereby ruling out possible second site mutations.

Construction of Yeast Strains Carrying the Various Test Constructs—All yeast strains carrying the test constructs were obtained by integration at the chromosomal yeast *URA3* locus. Therefore the yeast strain RH1416 (*MATa ura3–52*) was transformed with the modified integrative plasmid pSP64. The plasmid contained the 1.1-kilobase *Hin*dIII-*URA3* gene fragment, which was cloned blunt ended into the *Hin*dIII site. In addition, the 1041-bp *Bam*HI-fragment with the test cassette for 3'-end formation was cloned into the *Bam*HI site (Irniger *et al.*, 1992). The test cassette was modified by inserting a polylinker (Asp-718/*Cla*I/*Bgl*II) into the *Xho*I site of the test cassette. The test cassette was restricted with *Cla*I and *Bgl*II to obtain the various constructs. The self-cleaving 84-bp *Cla*I/*Bam*HI ribozyme fragment (Eckner *et al.*, 1991) was cloned into the polylinker of the test cassette in positive orientation and resulted in construct 1. Test construct 2 was obtained by cloning the 84-bp *Cla*I/*Bam*HI ribozyme fragment into a test cassette with a negatively inserted polylinker. Test construct 3 was generated like construct 1 with the difference that the sequence CCGTCA within the ribozyme was mutated to CCCGGG. In test construct 4, the nucleotides TCTGATGAGTCCGTGAGGACGAAACGGTG were deleted. This was achieved by amplification of the ribozyme fragment with the oligonucleotides GGGCCAGATCTCGCGTAAACACAACGCGTGACGG with an overhanging *Bgl*II restriction site and with GCGCTTGCACCATCC that binds to a sequence within the *ACT1* promoter. After restriction with *Cla*I and *Bgl*II, the shortened fragment could be inserted into the polylinker of the test cassette.

Isolation of RNA and Northern Hybridization—Total RNA was isolated according to the method of Zitomer and Hall (1976) using glass beads to disrupt the yeast cells. Poly(A) RNA was obtained by enrichment over an oligo(dT) cellulose column (Aviv and Leder, 1972). For Northern hybridization experiments, 30 μg of total RNA or 5 μg of poly(A)-enriched RNA were separated on a denaturing formaldehyde gel according to Rave *et al.* (1979). After transferring to Hybond™ nylon membranes (Amersham, UK), the bound RNA was hybridized at 42 °C with the random labeled 440-bp *Mlu*I/*Xho*I DNA element of the *ACT1* 5' region (Feinberg and Vogelstein, 1984). The RNA was visualized by autoradiography. Band intensities from autoradiographs were quantified by scanning analysis.

Nuclease S1 Mapping of 3'-mRNA Termini—For the mapping of 3'-ends of transcripts, DNA fragments were restricted at the *Ava*II site in the *ACT1* promoter and at the *Sph*I site in the *ADH1* terminator and labeled with [α-^{32}P]dATP using avian myeloblastosis virus reverse transcriptase. The fragments with the 84-bp *Cla*I/*Bam*HI ribozyme fragment between the *ACT1* promoter and the *ADH1* terminator had a size of 448 bp. After radiolabeling, the fragments were annealed to 50 mg of total or 10 μg of poly(A) RNA in 30 μl of 80% formamide, 0.04 M PIPES (pH 6.4), 0.4 M NaCl, 1 mM EDTA by denaturing them for 15 min at 85 °C followed by hybridization at 42 °C (Favoloro *et al.*, 1980). A total of 300 ml of ice-cold S1-buffer (0.28 M NaCl, 0.05 M sodium acetate (pH 4.6), 4.5 mM $ZnSO_4$, 20 mg of denatured sonicated calf thymus DNA/ml) and 200 units of nuclease S1 were also added and incubated for 30 min at 37 °C. The reaction was stopped by the addition of 75 μl of 2.5 M ammonium sulfate, 50 mM EDTA. The nucleic acids were then ethanol precipitated and fractionated on denaturing polyacrylamide gels containing 7 M urea.

PCR Mapping of 3'-mRNA Termini (RACE)—Mapping of the 3'-ends was carried out by a procedure modified by Frohman *et al.* (1988) used and illustrated by Russo *et al.* (1991). We used the oligonucleotide with the sequence CCGAAGCTCGAGCTGCGGATCC$(T)_{17}$ for the reverse transcription reaction. For the specific amplification of the cDNA, the two used oligonucleotides were GGGAATTCGGTCAATCTTTGTTAAAGAATAGG, with a 5'-overhanging *Eco*RI restricition site and binding in the *ACT1* promoter, and the oligonucleotide CCGAAGCTCGAGCTGCGGATC binding the flanking sequence of the oligonucleotide used for the production of the cDNA. The amplified fragments were isolated over an 0.8% low melting agarose gel, restricted with the enzymes *Eco*RI and *Bam*HI, cloned into pGEM7® Zf(+), restricted with the same enzymes, and sequenced using the dideoxy method (Sanger *et al.*, 1977).

RESULTS

Construction and Expression of an Artificial Yeast Test Gene Containing a Hammerhead Ribozyme—An artificial mRNA was constructed that contained the potential of its own cleavage. A yeast test cassette for 3' processing consisting of the strong *ACT1* promoter for RNA polymerase II, a multiple cloning site, and the *ADH1* polyadenylation signal was used as a basis. Putative 3' processing elements cloned into the multiple cloning site (*MCS*) of the test cassette resulted in short truncated (*T*) transcripts in case of functionality and in long readthrough transcripts (*RT*) in case of no functionality (Fig. 1). A modified plant ribozyme sequence was introduced into the multiple cloning site of the test cassette. The used ribozyme sequence was designed by Eckner *et al.* (1991) and was based on the results of Haseloff and Gerlach (1988). It is a derivative of satellite RNAs of tobacco ringspot virus, which is of the hammerhead type and which directs intramolecular RNA self-cleavage in plants. For proper function the cleavage site (*cs*) including the sequence GUC and the catalytic domain (*cd*) are necessary (Haseloff and Gerlach, 1988; Fig. 1). The ribozyme was cloned into the multiple cloning site of the test cassette resulting in construct 1. The various constructs were integrated at the *URA3* locus to avoid multicopy effects and were expressed in yeast.

The Hammerhead Ribozyme Is Active in the Heterologous Host S. cerevisiae—The part of the ribozyme sequence of the artificial mRNA was 84 nucleotides in length. The test construct containing this element was expected to result in potential readthrough transcripts of a length of 550 bp and in potential truncated transcripts caused by ribozyme cleavage of a length of 266 bp. Northern hybridization revealed that the RNA from construct 1 was exclusively of the short truncated type with a length of approximately 260 bp, indicating that the transcript was cleaved within the ribozyme sequence (Fig. 2, *lane 2*). No readthrough transcript of 550 bp could be detected, indicating that the ribozyme was highly efficient in the yeast cells. The finding of the full activity *in vivo* reflected the *in vitro* situation where the ribozyme-containing transcript, transcribed by the T7 RNA polymerase, was entirely cleaved (data not shown). In case of the test cassette without any DNA cloned into the MCS (TC), the truncated transcript was not present, but a readthrough transcript of 470 bp appeared on the Northern blot (Fig. 2, *lane 1*). This indicated that in construct 1 the cleavage was directed by the ribozyme sequence. Quantification of five different Northern blots revealed that the amount of the truncated transcript was between 30 and 35% in comparison with the readthrough transcript. The control construct 2 with the ribozyme sequence in the negative orientation was created to further confirm this finding. RNA from this control construct 2 was exclusively of the readthrough type with 550 bp in length (Fig. 2, *lane 2*). These results demonstrate (i) that the

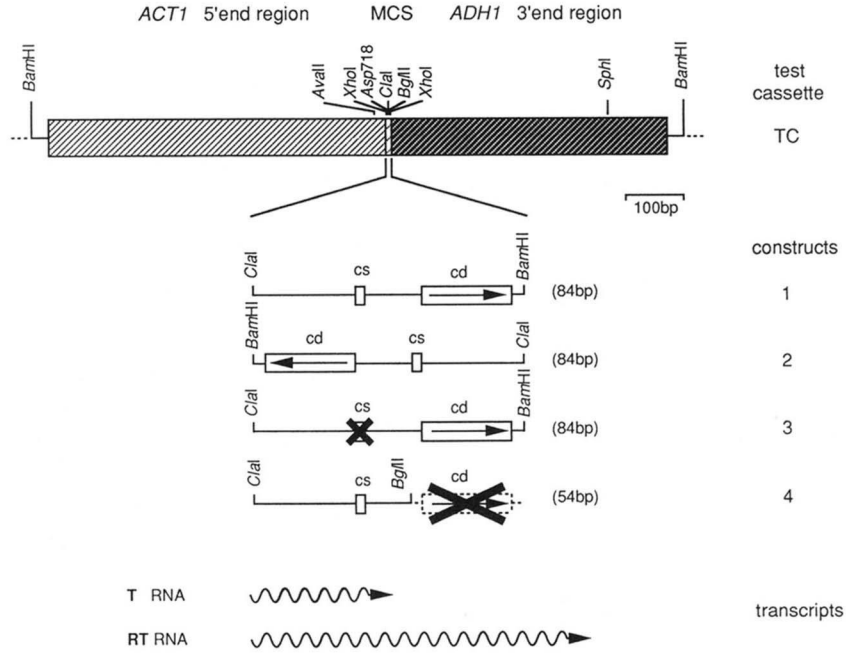

FIG. 1. **Test system for 3′-end formation.** The test cassette (*TC*) consisted of the *ACT1* promoter, a multiple cloning site (*MCS*) and the *ADH1* terminator. 3′ Processing sites cloned into the MCS resulted in short truncated transcripts (*T*) in case of functionality. Nonfunctional sites resulted in long readthrough transcripts (*RT*). The ribozyme containing both the cleavage site (*cs*) and the catalytic domain (*cd*) necessary for the cleavage activity was cloned into the MCS (*construct 1*). Three control constructs were used: the ribozyme fragment cloned in the negative orientation (*construct 2*), point mutations of the cleavage site substituting the sequence CCGTCA for CCCGGG (*construct 3*), and deletion of 30 nucleotides from the 3′-end of the ribozyme fragment including the catalytic domain (*construct 4*).

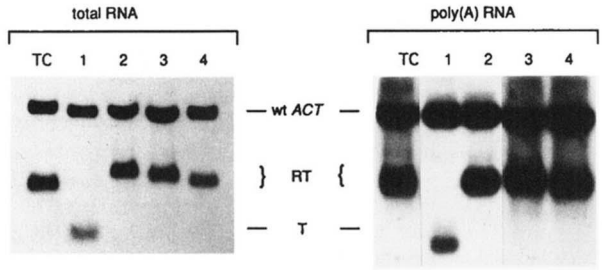

FIG. 2. **Analysis of the effect of ribozyme processing on mRNA 3′-end formation.** Either total or poly(A)-enriched RNA isolated from *S. cerevisiae* strains carrying the different constructs integrated into the chromosomal *URA3* locus were assayed. Hybridization to the chromosomally encoded *ACT1* transcript was used as a control for RNA quantification. For nomenclature of the products, see Fig. 1.

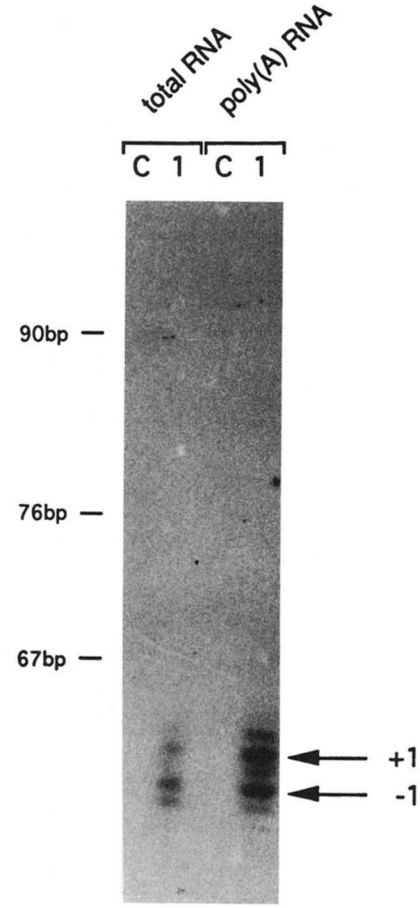

FIG. 3. **S1 nuclease mapping of the 3′-ends of the ribozyme-cleaved transcripts of the test construct 1.** Either total or poly(A) RNA was used for this analysis. Two signals of 61 and 63 bp corresponded to the active site of the ribozyme at the location −1 and +1 (see Fig. 4). As negative control (*C*), Torula yeast RNA was used.

ribozyme causes a specific truncated transcript *in vivo*, and (ii) that no readthrough transcript could be detected, suggesting that the ribozyme cleaves with an efficiency of close to 100%.

The Hammerhead Ribozyme Cleaves Specifically Its Own Transcript in Yeast Cells—Two elements, the cleavage site with the conserved nucleotides GUC and the catalytic domain, are required for ribozyme activity. Two additional constructs were generated to test whether the truncated transcript was the result of the ribozyme self-cleavage activity or caused by either a cryptic 3′ processing signal or by cleavage of an endogenous yeast nuclease. In construct 3, the cleavage site was destroyed by point mutations in all four nucleotides from position −2 to +1. In construct 4, the catalytic domain was deleted (Fig. 1). Both alterations resulted in complete loss of the cleavage activity (Fig. 2, *lanes 3* and *4*). Two additional methods were applied to further confirm the ribozyme activity and to exclude the presence of cryptic yeast polyadenylation signals. The 3′-end of the cleaved transcript was analyzed either by nuclease S1 mapping (Fig. 3) or by sequencing of cDNA clones obtained by RACE experiments (Fig. 4). Both methods revealed that the 3′-end of the truncated transcript was located within the ribozyme cleavage site, indicating that the cleavage reaction was ribozyme-dependent. The polyadenylation sites and the transcripts were named as −1 for transcript A and +1 for transcript B.

The Hammerhead Ribozyme-cleaved Transcripts Are Polyadenylated—Two independent methods showed that the short self-cleaved transcript from construct 1 was polyadenylated. Firstly, the transcript was still present on the Northern blot after oligo(dT) affinity chromatography (Fig. 2, *lane 2*). Ten different Northern blots with total RNA and poly(A) RNA were compared by scanning analysis. The relative amounts of the truncated transcript were the same on the Northern gel with

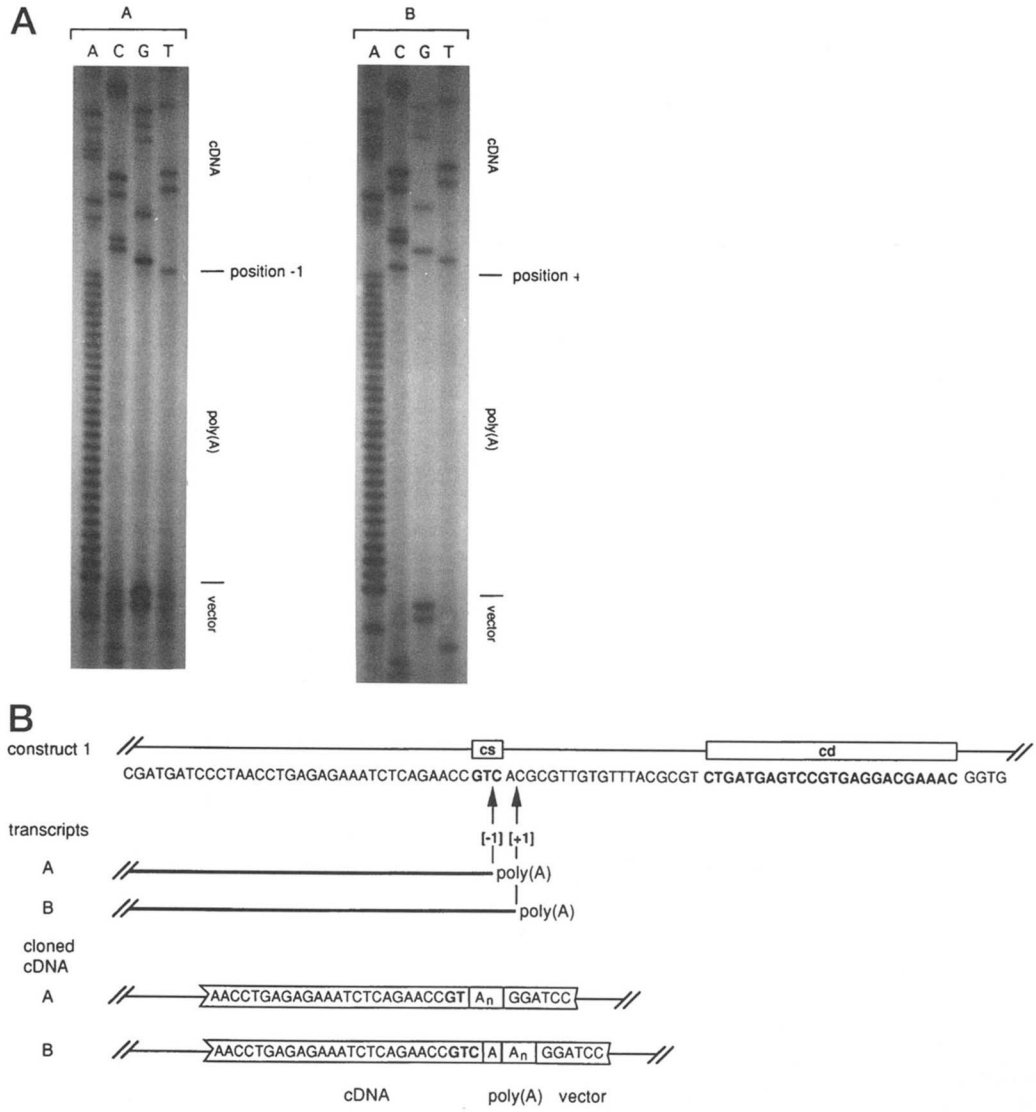

FIG. 4. **Sequence analysis of the cloned cDNA of construct 1.** RACE analysis exhibited two different 3′-ends of ribozyme-cleaved transcripts which contain poly(A) tails of comparable length to endogenously generated transcripts. *Panel A*, the cDNAs of the ribozyme-cleaved transcripts were amplified, cloned into an *Eco*RI/*Bam*HI-cleaved pGEM7® 7Zf(+) vector and sequenced. Nine cDNAs were analyzed, two corresponded to *transcript A*, seven to *transcript B*. The transcripts were amplified using the RACE method (Russo *et al.*, 1991). For the cDNA of both transcripts, one example is given with a corresponding poly(A) tail of 27 (*A*) and 28 A residues (*B*), respectively. *Panel B*, the corresponding ribozyme sequence and the transcripts 3′-ends. The nucleotides from the cleavage site (*cs*) and from the catalytic domain (*cd*) are written in bold. The 3′-ends of the transcripts are assigned as *[–1]* and *[+1]* in agreement to the earlier described cleavage site (Haseloff and Gerlach, 1988).

total and with poly(A) RNA, demonstrating that the cleaved transcript was polyadenylated. Secondly, we used the RACE method and produced cDNA of the truncated transcripts using an internal oligonucleotide and an additional (dT_{17}) oligonucleotide. Afterwards the cDNA was amplified and cloned. Sequencing of nine independent ribozyme-induced RACE products gave an average poly(A) tail length of 21 A residues. This finding indicated that the transcripts were polyadenylated. The endogenous yeast mRNA of the *GCN4* gene was simultanously investigated by using the same procedure. Interestingly the poly(A) tail length of the *GCN4* RACE product was with 24 A residues in the same range as the measured poly(A) tail length of the ribozyme-induced RACE product.

DISCUSSION

Although the process of 3′-end formation is much better understood in higher than in lower eukaryotes, most information in this field suggests that the principal mechanisms involved are conserved in all eukaryotes. We show here one of the differencies in 3′-end formation between yeast and mammals. While the two steps of 3′ processing, *i.e.* cleavage of the precursor transcript and addition of a poly(A) tail, seem to be strongly coupled in higher eukaryotes (for review, see Wahle and Keller (1992)), we show that it is possible to separate the two reaction steps *in vivo* in the yeast *S. cerevisiae*.

The rationale of our assay was to separate the polyadenylation from the cleavage reaction *in vivo* by using a self-cleaving ribozyme. Thus, the cellular 3′ processing machinery was not involved in the cleavage reaction. The ribozyme element contained all of the required sequence elements to form a hammerhead structure with the active cleavage site. When expressed in yeast, the ribozyme was surprisingly fully active, which is rare for hammerhead ribozymes in heterologous systems. The cleavage site of the ribozyme in the heterologous yeast system was shown to be located at the sequence GUC, which is a peculiarity for this type of hammerhead ribozymes. By sequencing cDNA clones and by nuclease S1 mapping, two polyadenylation sites differing in two nucleotides were identified. It is most likely that ribozyme cleavage happens at the cleavage site and that the two different 3′-ends are generated

after the cleavage reaction by cellular activities. The first polyadenylation site (−1) was located one nucleotide upstream of the cleavage site and is thought to be generated by an endonucleolytic activity. The second polyadenylation site (+1) was located just downstream of an A residue. Therefore this A residue can be either encoded by the DNA template or it can be the first nucleotide of the poly(A) tail added by poly(A) polymerase. This two-band pattern was observed earlier in the work of Eckner *et al.* (1991) and seems to be a feature of this special ribozyme. The specificity of this cleavage reaction was further confirmed by destroying the cleavage site by point mutagenesis in the cleavage site and by deletion of the catalytic domain. Each of these modifications turned this sequence into a completely inactive element. By these results, the accidental generation of a cryptic yeast cleavage site could be excluded.

The ribozyme activity cleaved the precursor transcript of the test gene, which resulted in a 5′- and a 3′-cleavage product. The 5′-cleavage product corresponds to the truncated short transcript detectable on the autoradiographs in Fig. 2. This transcript should be capped and was shown to be polyadenylated. The amount of the ribozyme-cleaved transcript was lower than that of the corresponding readthrough transcript. This might be due to an instability of ribozyme-cleaved transcripts or to decreased polyadenylation efficiency. The 3′-cleavage product, which was uncapped, was undetectable by Northern hybridization. These results indicated that the 3′-product was rapidly degraded, presumably due to the lack of the Cap structure. This is in agreement with earlier findings that a Cap structure is crucial for mRNA stability (Gerstel *et al.*, 1992).

The length of the poly(A) tail was estimated from the sequence of the RACE products. Since the (dT) oligonucleotide was able to bind at any position within the poly(A) tail, this method does only allow to determine the relative length of the poly(A) tails. The poly(A) tail lengths of all sequenced RACE products of the test construct 1 and of all *GCN4*-derived transcripts used as control were in the same range varying from 17 to 28 A residues. These data suggest that the poly(A) tail of the truncated transcript was of a comparable length as the poly(A) tail of the *GCN4* mRNA.

The most interesting result of this report is that the yeast poly(A) polymerase is able to recognize the 3′-ends of a ribozyme precleaved transcript and to add a regular poly(A) tail *in vivo* in a yeast cell. The exact chemical mechanism of the addition of the first A residue is difficult to explain. The poly(A) polymerase requires a free 3′ hydroxyl end, whereas the ribozyme cleavage should result in a 2′,3′-cyclic phosphate. Therefore we cannot exclude the possibility that in addition to the cleavage and the polyadenylation activity, a third cellular activity is required. The polyadenylation reaction does not require and is not coupled to the regular cleavage reaction, which normally takes place in the cell. We cannot discriminate whether the poly(A) polymerase requires only a free 3′-end and is otherwise unspecific or whether it is able to recognize additional specific features on a pre-mRNA. Several data support the idea that there is an unspecific polyadenylation activity in the yeast cell. Zaret and Sherman (1982) have found that the *CYC1–512* deletion in the 3′ region of the gene resulted in aberrant longer transcripts that all seemed to be polyadenylated. In contrast to other eukaryotes, the yeast histone genes encode for transcripts that are polyadenylated (Fahrner *et al.*, 1980). There are also *in vitro* data with purified enzyme and unspecific RNA including *Escherichia coli* tRNA or rRNA (Lingner *et al.*, 1991b) which suggest that the yeast poly(A) polymerase is an enzyme that recognizes unspecifically the 3′-end of a RNA molecule. However, there are also reports that suggest that there is a certain specificity in the polyadenylation reaction in yeast. Butler and co-workers (Butler *et al.*, 1990) have found that an ammonium sulfate fraction of a yeast extract did not polyadenylate unprocessed precursor RNAs, whereas the 5′ cleavage products were efficiently polyadenylated. Even in mammals, an unspecific and a specific polyadenylation function can be found *in vitro*. In the presence of Mg^{2+} ions, the mammalian enzyme has an unspecific polyadenylation activity. However, the cleavage and polyadenylation specificity factor that is dependent on the AAUAAA signal sequence is required for the specific addition of the poly(A) tail and the poly(A) binding protein II for its elongation (Christofori and Keller, 1988; Takagaki *et al.*, 1988; 1989; Gilmartin and Nevins, 1989; Bienroth *et al.*, 1993).

It might be possible that the difference between yeast and mammals is a more stable and tight complex of the poly(A) polymerase, specificity factors, stimulating factors, and cleavage factors in mammalian cells in comparison to yeast. In mammals, the poly(A) polymerase seems to be part of the cleavage complex of the precursor RNA. In yeast, the poly(A) polymerase does not seem to be required for cleavage. In yeast it might therefore be possible that the poly(A) polymerase can easily dissociate from the other factors and can provide a higher unspecific polyadenylation activity in the yeast cell.

In summary, we have expressed a heterologous ribozyme in yeast and have found that this ribozyme is highly active in yeast. The ribozyme-cleaved transcript was stable and polyadenylated and is therefore the first transcript where it was possible to separate the cleavage and the polyadenylation of 3′-end processing of a mRNA *in vivo*. This system might be useful to study polyadenylation and RNA cleavage independently from each other and might facilitate the understanding of mRNA 3′-end formation in eukaryotes.

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