Orientation-dependent function of a short *CYC1* DNA fragment in directing mRNA 3' end formation in yeast

(polyadenylation/RNA polymerase II/RNA processing/transcription termination)

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ABSTRACT We have cloned an 82-base-pair region spanning the site of normal 3' end formation of *Saccharomyces cerevisiae* *CYC1* mRNA into an integrative vector carrying the 5' end of the actin gene (including its intron) fused in frame to *HIS4ABC* sequences. This vector can confer the ability to grow on histidinol if *HIS4C* (encoding histidinol dehydrogenase) is sufficiently expressed. With the *CYC1* fragment cloned in its wild-type (forward) orientation within the actin intron, transformants cannot grow on histidinol, whereas cells transformed with the vector carrying the reverse orientation of this fragment are able to grow well. RNA transfers demonstrate that transformants containing the forward orientation accumulate <40% of the control level of full-length mRNA and reveal the presence of a short, stable (≈ 300 nucleotides) poly(A) RNA that represents 60–70% of the transcripts originating from the same promoter. The reverse orientation of the insert allows near-normal levels of full-length mRNA. Mapping of the 3' end of the truncated RNA indicates that poly(A) addition is variable in length but occurs at the same location as in the normal *CYC1* transcript. Dominant and recessive suppressor mutations permit growth on histidinol despite the inserted fragment. Genetic analyses indicate that most of the dominant mutants are cis-acting and that the recessive mutants define a minimum of three complementation groups, indicating that defects in several different genes can restore higher levels of *HIS4C* expression.

An understanding of the molecular mechanisms involved in mRNA 3' end formation depends on identifying and deciphering signals residing within the nucleic acid sequence and structure. It also depends on determining how RNA polymerase responds, either alone or in conjunction with additional factors, to affect the events required for subsequent termination or RNA 3' end processing. The formation of the mRNA 3' ends in prokaryotes probably results from transcription termination by RNA polymerase, in direct response either to signals in the RNA or to the action of accessory proteins. In higher eukaryotes, 3' end formation generally occurs by RNA processing after RNA polymerase has transcribed as much as hundreds of nucleotides farther downstream (for reviews, see refs. 1 and 2). In yeast, mRNA processing appears similar to other eukaryotes in that transcripts are capped and many are spliced. All yeast mRNAs examined thus far (including histone transcripts) are polyadenylated. However, 3' processing signals similar to those conserved in genes of higher eukaryotes have not been identified in yeast nor does yeast possess the transcription termination signals characteristic of bacterial systems.

A region of DNA apparently specifying transcription termination was identified in the *CYC1* gene (encoding iso-1-cytochrome *c*) of *Saccharomyces cerevisiae* by Zaret and

Sherman (3) when they found that the *cyc1-512* mutation [a 38-base-pair (bp) deletion spanning the wild-type *CYC1* mRNA 3' end point] resulted in a 90% reduction in the overall accumulation of transcripts encoding the *CYC1* product; many of the mRNAs were longer, and all were polyadenylated. Several other yeast genes share limited homology to a subset of the sequences deleted by the *cyc1-512* mutation. Local sequence changes in these regions can restore partial function (4–6); however, no definitive sequence has yet been proved to specify 3' end formation. Analysis of *cyc1-512* revertants demonstrated that mutations in trans-acting factors can also act to restore the efficiency of 3' end formation and accumulation of stable mRNAs (4, 7).

In this report we describe a combined genetic and biochemical approach to understanding 3' end formation and polyadenylation of yeast mRNA. We have used an in-frame fusion of the actin and *HIS4* genes (8) to show that an 82-bp sequence, which spans the normal site of the *CYC1* mRNA 3' end point, acts in an orientation-dependent manner to reduce expression of the distal *HIS4* gene by decreasing the yield of full-length transcript. We confirm that this short sequence can direct specific 3' end formation and polyadenylation in yeast with at least 60–70% efficiency. A positive selection for growth on histidinol reveals that cis- and trans-acting mutations suppress the ability of the 82-bp fragment to reduce expression of the distal *HIS4C* activity.

MATERIALS AND METHODS

Strains and Materials. Yeast strains were derived from those of Parker and Guthrie (8); genetic markers include *ura3-52* for selection of *URA3* transformants, *his4-401* [a 2-kilobase (kb) deletion encompassing the promoter and most of the coding sequences], and the dominant *HOL1* mutation to permit uptake of the histidine precursor, histidinol. In addition, either *ade2* or *ade5* markers were introduced into the strains to permit selection of diploids used in complementation tests. Yeast were transformed after spheroplasting (9), and other standard genetic techniques and growth media were as described by Sherman *et al.* (10). In SC + Hol medium, 0.064% histidinol was included in standard synthetic complete medium lacking histidine (8). Histidinol (Hol) phenotypes were determined by examining the extent of growth occurring after incubation at 30°C for 3, 4, and 6 days. Restriction enzymes were purchased from New England Biolabs, [α -³²P]dCTP and [α -³²]GTP were purchased from Amersham, and other reagents were from Sigma.

DNA and RNA Analysis. Transformants were analyzed for integration of single copies of pYAH-12, pYAH-82F, and

Abbreviation: nt, nucleotide(s).

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pYAH-82R plasmids (Fig. 1) by separating genomic DNA digested with *Pst* I on agarose gels and hybridizing the transferred DNA with nick-translated pBR322 as described (11). Total RNA was prepared by the glass bead lysis technique (12). Poly(A) RNA, purified according to Domdey *et al.* (13), was electrophoresed in either a 10% acrylamide/urea gel with transfer to diazobenzoyloxymethyl-paper or a 1.5% agarose/formaldehyde gel with transfer to nitrocellulose or GeneScreenPlus. RNA was probed with an α -³²P-labeled 3.3-kb *Bam*HI-*Xba* I fragment from pYAH-12 (carrying the actin-*HIS4* fusion product) or with α -³²P-labeled antisense RNA probe synthesized with SP6 polymerase (Promega Biotec, Madison, WI) from a linearized template encoding the 632-nucleotide (nt) *Bam*HI-*Sal* I region of pYAH-82F that includes actin and *CYC1* segments. Hybridization signals were quantitated by excising the radioactive bands from the membrane and assaying them by liquid scintillation spectrometry.

Mapping the RNA 3' End. A linearized SP6 template with the 82-bp *CYC1* sequence in reverse orientation was used to generate a 114-nt antisense probe that has 10 extra nt at its 5' end (corresponding to added *Xho* I linkers) and 22 nt of polylinker sequence at the 3' side. Hybridization reaction mixtures contained 5–10 × 10⁵ cpm of gel-purified probe (≈5 × 10⁶ cpm/pmol) and 25–150 μg of total RNA in 20 μl of hybridization buffer (75% formamide/40 mM Pipes, pH 6.7/400 mM NaCl/1 mM EDTA). After heating for 5 min at 80°C, hybridizations were continued 8–16 hr at 38°C. Single-stranded RNA was digested with RNase A (80 μg/ml) and RNase T1 (2000 units/ml) at 25°C for 30–45 min. Samples were treated with proteinase K (Beckman), extracted with phenol, and electrophoresed on 10% acrylamide/8 M urea gels.

RESULTS

Hol Phenotype Depends upon the Orientation of *CYC1* 3' Sequences. In the pYAH-12 construction correct splicing of the 309-bp actin intron as well as undisrupted read-through of the fusion gene are required for production of functional *HIS4* gene product. We inserted a *Xho* I-linked 82-bp *Ava* II-*Fnu*DII fragment that spans the region corresponding to the 3' end of the *Saccharomyces* *CYC1* mRNA [located 55–58 bp downstream of the *Ava* II site (3)] into the unique *Xho* I site present in the actin gene intron carried by pYAH-12 (Fig. 1). Linear pYAH-12 and the derivatives carrying the *CYC1* fragments, each cut with *Xba* I, were transformed into a *ura3-52*, *his4-401*, *HOL1* haploid host strain. Ura⁺ transformants were picked and screened by Southern analysis to identify those with a single copy of the vector integrated at the correct chromosomal site just 3' to the *his4-401* deletion in the host (data not shown). Transformants with a single copy of pYAH-12, pYAH-82F, and pYAH-82R are designated YP-0, YP-82F, and YP-82R, respectively, referring to either the absence, forward orientation, or reverse orientation of the 82-bp *CYC1* fragment. Representative transformants obtained from each of the three plasmids were tested for the ability to grow on selective medium lacking histidine and uracil but containing histidinol. All six of the YP-0 and YP-82R transformants tested grew on histidinol, whereas none of the YP-82F transformants could do so. The three different transformants were crossed with *ura3-52* tester strains containing either the *his4-401* or the *HIS4* wild-type allele, and the resulting diploids were sporulated. In all tetrads examined (an average of 15 for each diploid) the characteristic Hol phenotype of the transformed strain cosegregated with the *URA3* plasmid marker and the *his4* chromosomal marker.

***CYC1* Fragment Directs Efficient 3' End Formation of a Short, Stable RNA.** The single-copy transformants were examined by RNA transfer analysis to determine if the YP-82F

FIG. 1. Plasmid pYAH-12 is a *URA3* derivative of YIP5 that carries the 5' region of the yeast actin gene including its intron, fused in frame shortly thereafter to *HIS4* sequences (8). Translation of the spliced mRNA yields a fusion polypeptide with histidinol dehydrogenase activity (encoded by *HIS4*C). The ability of cells to grow on histidinol in place of histidine provides a phenotypic assay for the amount of functional *HIS4*C gene product since reduction in the expression of the fusion gene by >50% results in a mutant Hol[−] phenotype (8). The 82-bp fragment spanning the normal *CYC1* site of 3' end formation with added *Xho* I sites at each end (gift of L. Guarente, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA) was cloned in both orientations into the unique *Xho* I site of pYAH-12 in the actin gene intron as indicated. The resulting vectors, pYAH-82F (*CYC1* forward orientation) and pYAH-82R (reverse orientation), were cleaved at the unique *Xba* I site in the 3' flanking sequence of *HIS4* for targeted integration into the *his4-401* deletion strain. Sites of cleavage of various restriction enzymes are designated as follows: B, *Bam*HI; S, *Sal* I; X, *Xba* I; and Z, *Xho* I. Dark shaded blocks represent actin sequences surrounding the intron (open block) fused to the *HIS4* coding sequence (light shaded block). The thin line in the bottom portion represents the region of *HIS4* deleted in the host chromosome. AMP^r, ampicillin resistant.

Hol[−] phenotype either was a consequence of defective splicing of the fusion transcript or was due to 3' end formation directed by the *CYC1* insert. Total RNA from the host and the transformed strains was resolved on a formaldehyde gel, transferred, and hybridized to a 632-nt antisense RNA probe that spans the actin promoter and part of the intron (see *Materials and Methods*). This probe detects the endogenous 1.2-kb actin transcript and the 2.8-kb actin-*HIS4* fusion transcript produced in each of the transformed strains (Fig. 2). RNA from YP-82F contains a reduced level of the 2.8-kb fusion transcript with the corresponding appearance of a stable RNA of 250–350 nt. The results obtained from two independent experiments and four separate measurements show that after normalization to the signal obtained from hybridization to the actin mRNA (see *Materials and Methods*), the relative amount of the truncated transcript detected (Fig. 2, lane 3) represents 60–70% of the full-length fusion transcript levels present in the YP-0 control (Fig. 2, lane 1), whereas the relative accumulation of the full-length transcript detected in YP-82F is only about 30%. The inverse orientation of the *CYC1* insert present in YP-82R also directs formation of a small RNA, but at less than about 5% efficiency (Fig. 2, lane 2; see *Discussion*).

Short, Stable mRNA 3' End Is Polyadenylated at the Normal *CYC1* Site. Poly(A)⁺ RNA was isolated from the transformed strains and resolved on an acrylamide/urea gel. After transfer to diazobenzoyloxymethyl-paper the RNA was hybridized with α -³²P-labeled 3.3-kb *Bam*HI-*Xba* I fragment obtained from pYAH-12 (Fig. 1). The probe hybridizes to a broad band of poly(A) RNA from the YP-82F transformant that ranges from 250 to 350 nt in length (data not shown), just as was seen with total RNA (Fig. 2, lane 3).

FIG. 2. RNA transfer analysis of the transformed strains. RNA isolated from YP-0 (lane 1), YP-82R (lane 2), or YP-82F (lane 3) was hybridized to an antisense RNA probe derived from the 5' region of the actin gene. The endogenous 1.2-kb actin transcript was used to normalize the levels of the other RNAs. The relative abundance of the 2.8-kb actin-*HIS4* fusion transcript present in the YP-82F transformant is <40% of the levels seen in either other strain. The short RNA of about 0.3 kb represent at least 60% of the level of the 2.8-kb fusion transcript in the YP-0 control. A slightly longer transcript is also detected in the YP-82R transformant at very low levels.

The 3' end of the truncated mRNA was mapped to determine if multiple sites of 3' end formation contributed to the size heterogeneity of the truncated transcript. The results show that the majority of the 3' ends of the truncated YP-82F

transcripts occur in the same region as the *CYC1* transcript. RNA from a wild-type *CYC1*⁺ strain protects a 58- and 59-nt region of the probe (Fig. 3 *Upper*, lane 2), whereas the truncated transcript from YP-82F protects 67, 69, and 70 nt (Fig. 3 *Upper*, lane 3). Although several minor RNAs are also protected by the RNA from YP-82F, these represent only a small fraction of the total RNA and occur in the same pattern obtained from the less abundant *CYC1* mRNA when the autoradiogram is overexposed. We do not yet know if the additional strong band with YP-82F is of any significance.

Selection and Analysis of Revertants to *HOL*⁺. In control experiments, complementary mating types of YP-82F, YP-82R, and the *his4-401* deletion host were used in pair-wise crosses to determine the *Hol* phenotype of the resulting diploid strains that were either homozygous or heterozygous for the integrated plasmids. All of the diploids containing at least one copy of pYAH-82R (from YP-82R) were *Hol*⁺, whereas the homozygous YP-82F diploid, the YP-82F/*his4-401* diploid, and the *his4-401/his4-401* diploid control were *Hol*⁻. These *Hol*⁻ phenotypes permit classical complementation analysis of the recessive *Hol*⁺ revertants, which were isolated by plating the YP-82F transformant onto selective medium containing histidinol. After 7 days at 30°C, ≈ 1 in 10^5 cells plated gave rise to spontaneous *Hol*⁺ colonies. Over 200 *Hol*⁺ revertants were initially characterized and tentatively classified as dominant, weakly dominant, or recessive, as judged by the *Hol* phenotype of diploids created by crossing the different *Hol*⁺ revertants either to the YP-82F (*Hol*⁻)

FIG. 3. Polyadenylation of the short mRNA occurs at the normal site. (*Upper*) Total RNA from YP-82R (75 μ g, lane 1), an untransformed *CYC1*⁺ strain (150 μ g, lane 2), and YP-82F (75 μ g, lane 3) was hybridized to an antisense RNA probe corresponding to the 82-bp region from the 3' end of *CYC1* and digested with RNase to determine the location of the 3' end of the 260- to 350-nt transcript. As the antisense RNA probe used for mapping (derived from the 82-bp *CYC1* fragment present in pYAH-82F) contains an additional 10 nt of linker sequences (italicized in *Lower*) homologous to YP-82F (82F) but not to the wild-type (WT) *CYC1* gene, the protected RNA from YP-82F (lane 3) is longer than that from a wild-type *CYC1* strain (lane 2). The native chromosomal *CYC1* transcript is not detected in either the YP-82R or the YP-82F transformant (lanes 1 and 3) because the parental strain itself is *cyc1*⁻ and has at least 10 times less *CYC1* mRNA than the *CYC1*⁺ control as determined by RNA transfer analysis (results not shown). (*Lower*) Nucleotide sequence of cloned fragment: 5' linker sequences are italicized, the normal site of polyadenylation is underlined, and the overbar delimits the extent of the *cyc1-512* deletion (3). Numbering is from the *CYC1* UAA termination codon.

parental strain or to the *his4-401* deletion strain. About 70% of the mutants gave rise to Hol⁺ diploids when crossed to either tester strain and were classified as phenotypical Hol⁺ dominant.

To determine if the dominant Hol phenotype was linked to the Ura⁺ plasmid marker the diploids created from six independent dominant mutants and the *his4-401* tester strain were sporulated and the resulting Ura⁺ progeny were tested for their Hol phenotype. In all six crosses about 95% of the Ura⁺ haploid progeny were also Hol⁺, indicating that the majority of the dominant Hol⁺ mutations are cis-acting. Of the remaining 62 putative recessive mutants, 16 were tentatively classified as weakly dominant because they grew marginally on histidinol when crossed with YP-82F but were unable to grow when crossed with the *his4-401* deletion strain. When the remaining 46 mutants were crossed to either tester strain, the resultant diploids were all Hol⁻. Unlike the dominant mutants, the recessive Hol⁺ (haploid) mutants displayed a wide spectrum of growth patterns on histidinol, ranging from confluent growth after 2–3 days to marginal growth after 5–6 days.

The recessive Hol⁺ revertants were further characterized by crossing them with each other and testing the diploids for their Hol phenotype; initially we examined only those mutants exhibiting the clearest complementation pattern, and the results of these crosses (Table 1) define a minimum of three complementation groups. To determine the cis- or trans-acting nature of the recessive Hol⁺ mutants, representatives of the different complementation groups were crossed with the *his4-401*, *ura3-52* tester strain and, after the resulting diploids were sporulated, the Hol phenotype of the Ura⁺ haploid progeny was scored. In each of seven mutants examined in this manner, roughly half of the Ura⁺ haploid progeny were also Hol⁺, indicating that the recessive mutations are trans-acting and are not tightly linked to the plasmid *URA3* marker. Relative to the amount of full-length transcript present in YP-82F, all nine of the recessive Hol⁺ mutants examined by RNA transfer analysis exhibit levels well above the 50% threshold required for growth on histidinol (data not shown). Since, in all cases, the Hol⁺ character reflects increased *HIS4C* expression, we have tentatively designated these as *tef* mutations, causing defects in 3' end formation that result in greater read-through transcription. Preliminary characterization of other mutants reveals a complicated complementation pattern that may reflect either epistatic or allele-specific interactions (data not shown), but future work should clarify the nature of these interactions.

Table 1. Spontaneous Hol⁺ mutants obtained from *MATa ade2* and *MATa ade5* derivatives of YP-82F

<i>MATa/MATa</i>	TEF	II		III	
		<i>tef2-3</i>	<i>tef2-4</i>	<i>tef3-1</i>	<i>tef3-2</i>
TEF	—	—	—	—	—
I					
<i>tef1-1</i>	—	—	—	—	—
<i>tef1-2</i>	—	—	—	—	—
<i>tef1-3</i>	—	—	—	—	—
II					
<i>tef2-1</i>	—	+	+	—	—
<i>tef2-2</i>	—	+	+	—	—

Stable mutants that consistently exhibited a recessive phenotype in crosses to *his4-401* and YP-82F (*TEF*⁺) strains were then crossed with each other and replica plated onto SC – Ade, – Ura medium (to control for mating efficiency) and Hol medium (SC + Hol, – Ade, – His, – Ura) (8, 10). In these tests it is important to note that the negative Hol⁻ phenotype is expected if the two mutants complement each other, whereas a Hol⁺ phenotype suggests the mutations occur in the same gene. TEF, 3' end formation.

DISCUSSION

We have shown here that a surprisingly small (82 bp) *CYC1* fragment carries sufficient information to direct 3' end formation of a short, stable RNA to 60–70% efficiency, with nearly the same specificity and consequent polyadenylation as occurs when it is in its native location adjacent to the *CYC1* coding region. We cannot rule out that flanking sequences in the actin intron partially contribute to 3' end formation, but the minimal effect of the reverse orientation of the 82-bp fragment belies this interpretation, and the intron does not contain sequences obviously homologous to those thought to be involved in 3' end formation in yeast (3, 5). In addition, previous studies suggest that part of the signal is contained within the even smaller 38-bp sequence deleted in the *cyc1-512* mutation (3). Although this deletion alters function bidirectionally (causing abnormally long transcripts for *CYC1* and the convergently transcribed *UTR1* gene), revertants in the DNA sequence restored partial function in one direction but not the other; hence, Zaret and Sherman (4) suggested that there were two distinct but partially overlapping signals. Our finding that the 82-bp fragment is 70% efficient in pYAH-82F, but <5% efficient in pYAH-82R, supports the inference that the *UTR1* signal requires a substantial contribution from outside the 82-bp fragment, whereas *CYC1* does not.

We have entertained four possibilities for explaining why the 82-bp fragment appears to be only 60–70% efficient in its forward orientation. (i) Increased transcription from the actin promoter (relative to that occurring from *CYC1*) may amplify the extent of read-through transcription, and we cannot yet rule this out. (ii) Our observed efficiency may reflect that of *CYC1* itself, if all transcripts resulting from a residual 30–40% read-through are extremely unstable [as is indeed the case for read-through transcription with the *cyc1-512* mutation (4)]. (iii) Some portion of the signal for 3' end formation may reside outside the 82-bp segment, and our results reflect only partial function. If such signals are nearby, a larger fragment encompassing the *CYC1* 3' region should direct more efficient formation of the short RNA. (iv) We cannot exclude the possibility that splicing or spliceosome formation directly competes with and reduces the efficiency of 3' end formation, and attempts are necessary to determine the level of truncated and full-length transcripts produced in derivatives of pYAH-82F containing mutations that prevent splicing (8).

Klinz and Gallwitz (14) have shown that insertion of a 173-bp DNA fragment into the *Xho* I site of the actin intron does not interfere with splicing, and the orientation-dependent response we observe for the smaller 82-bp *CYC1* insert confirms that the size itself has little effect. However, the introduction of a signal capable of directing mRNA 3' end formation within a relatively small intron could cause interference with the formation of complexes required either for splicing or for 3' end formation. It is unlikely that the *CYC1* insert depresses *HIS4C* expression by affecting splicing, since longer exposures of RNA transfers similar to that shown in Fig. 2 do not reveal accumulation of unspliced pre-mRNA or other identifiable splicing intermediates. Because the total amount of YP-82F transcription (30–40% full-length plus 60–70% truncated RNA) is near normal, transcript instability must be negligible for the short RNA. Moreover, when the *CYC1* signal is recognized in this system, the steps required for 3' end formation must occur before splicing or otherwise preclude it. This appears similar to the situation in the adenovirus E3 gene where polyadenylation usually competes with, or precedes, splicing of the transcript (15).

In higher eukaryotes, part of the transcription termination signal may occur within a few hundred nucleotides downstream of the 3' processing signals (16–18), and in some

instances an intact poly(A) signal is also required for normal termination to occur (19–21). Previous studies have shown that a variety of sequences may promote 3' end formation and polyadenylation in yeast (4, 5, 22), though the canonical AAUAAA signal of higher eukaryotes is usually not present. It is not known whether correct 3' end formation of mRNA in yeast requires transcription termination and/or processing of a longer transcript, but if specific polyadenylation or processing signals are used, they must lie within our 82-bp insert [it is notable that a similarly sized region of 80 bp of the mouse histone H4 gene contains signals controlling cell-cycle-dependent RNA 3' processing (23)]. Alternatively, all yeast mRNA may become polyadenylated by mechanistic or temporal coupling to termination of transcription by polymerase II, as suggested by Zaret and Sherman (4). Conceivably, termination and processing may both operate, as seen with *trp* operon mRNA of *Escherichia coli*, where 25% of the mature 3' ends appears to be the consequence of transcription termination and 75% appears to be the result of RNA processing events (24).

One approach to understanding 3' end formation has utilized selections for revertants of *cyc1-512* strains to obtain cis-acting mutations (chromosomal rearrangements and local mutations in 3' flanking sequences) as well as recessive trans-acting *sut* mutants that restore *CYC1* mRNA levels 2- to 4-fold relative to the *cyc1-512 SUT⁺* parent (4, 7). It is not known whether this is due to more efficient polyadenylation, to termination, or to longer mRNA half-life, but in all cases *sut⁻* mutations generally restore *CYC1* expression without restoring termination at the normal site.

The system we describe, in contrast, provides an ideal selection for mutants that allow elevated read-through expression to *HIS4C* and are thus candidates that may have defects in 3' end formation (*tef*). Selection for *Hol⁺* revertants of YP-82F ensures that splicing is not impaired, but some mutations that result in efficient read-through transcription may be lethal due to a major defect in correct 3' end formation. Viable revertants may therefore be only partially defective in 3' end formation but can be identified because the *Hol⁺* phenotype requires only a small increase in full-length mRNA (read-through in the *Hol⁻* parent is 30–40%, and cells with 50% expression become *Hol⁺*).

In summary, we have shown that a small 82-bp region, overlapping the site of *CYC1* mRNA 3' end formation, directs efficient and accurate 3' end formation and polyadenylation when present within the actin intron. Since the orientation-dependent signal reduces read-through transcription below the level required for growth on histidinol, our system is ideally suited for identifying relatively small regions of DNA that specify signals involved in mRNA 3' end formation in yeast and for the selection of mutations that are defective in this function. Preliminary complementation analysis of *Hol⁺* revertants that increase *HIS4C* expression defines a minimum of three different genes, which we have designated *TEF1*, -2, and -3, that may influence the efficiency of 3' end formation. Further genetic analysis should permit identification of some of the major components involved in 3' end formation and polyadenylation, which may include RNA

polymerase II subunits, RNA processing enzymes, and even small ribonucleoprotein particles.

Note Added in Proof. After this paper was accepted, we learned that Osborne and Guarente (25) reported that the same *CYC1* fragment used here can direct 3' end formation in both orientations when inserted at some, but not all, other locations; it remains to be seen whether these differences reflect contextual effects or are related to distance from the promoter. They correctly state that the identity with *CYC1* also covers 83 bp, not 82 bp, as can be verified in Fig. 3 Lower.

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