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Genetics

Selection of functional cDNAs by complementation in yeast

(gene expression/recombinant DNA/transformation)

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ABSTRACT **Yeast cDNA was prepared in a yeast expression plasmid to generate a cDNA plasmid pool composed of approximately 40,000 members. Several yeast mutants were transformed with the cDNA plasmid pool, and the cDNAs for *ADC1*, *HIS3*, *URA3*, and *ASP5* were isolated by functional complementation. Restriction enzyme analysis confirmed the genetic identity of the *ADC1*, *HIS3*, and *URA3* cDNAs and demonstrated that the *URA3* cDNA contains 5′ noncoding sequences. The relative abundance of the various cDNAs in the cDNA plasmid pool paralleled the abundance of the mRNAs in total poly(A)$^+$ RNA, which ranged from approximately 0.01% to 1%. The utility of this approach to isolate rare cDNAs from higher eukaryotes is discussed.**

The ability to transform yeast (1, 2) with replicating plasmids (2, 3) has allowed the rapid development of yeast as a host system for gene expression studies. By using plasmids containing fragments of genomic yeast DNA (4, 5), numerous yeast genes have been isolated on the basis of complementation of yeast mutants. However, the expression in yeast of genomic DNA sequences from higher eukaryotes has only been successful so far with genes that lack intervening sequences (6–10). One approach that circumvents the problem of intervening sequences in yeast is to insert full-length cDNA between promoter and terminator sequences in a yeast expression vector.

Preparing a functional cDNA pool requires the cDNA to be oriented properly with respect to the flanking transcriptional promoter and terminator sequences, and it should be joined to an ATG in the proper translational reading frame. Use of the cDNA cloning procedure of Okayama and Berg (11) is ideally suited for the efficient cloning of cDNA molecules in functional form in a suitable expression vector. In this procedure, an oligo(dT)-tailed vector serves as a primer for cDNA synthesis on a poly(A)$^+$ RNA template and, thus, allows the cDNA to be cloned in a defined orientation (11). The vector-cDNA·mRNA is hybridized to a vector fragment containing a homopolymer tail that is complementary to a homopolymer tail previously added to the vector-cDNA·mRNA (11); the molecule is subsequently cyclized by ligation. In this procedure the tailing and hybridizing steps act to select full-length cDNAs (11). The selection of full-length cDNAs allows the use of the translational initiation signals encoded in the cDNA and, thereby, ensures the use of the proper reading frame. However, the published procedure (11) generates cDNA containing an oligo(dG) sequence attached to the 5′ end of the mRNA strand. Because yeast genes that encode protein generally lack guanylate residues in the 5′ noncoding regions of their mRNA (12), we have modified the cDNA synthesis procedure by attaching an oligo(dC) tail to the 5′ end of the mRNA strand.

In the present work, we have created a pool of total yeast cDNA in an expression vector and have selected specific recombinant plasmids by their ability to complement specific yeast mutants. We describe here the construction of a yeast cDNA pool in an expression vector and the cloning of cDNAs derived from mRNA molecules present in yeast in medium abundance (*ADC1*) and in low abundance (*URA3*, *HIS3*, and *ASP5*). These procedures provide a powerful approach to the isolation of rare cDNAs from yeast and from various higher eukaryotes without any requirements for mRNA enrichment, specific oligonucleotide probes, or the availability of specific antibodies.

MATERIALS AND METHODS

Yeast, Bacteria, and Plasmid. The *Saccharomyces cerevisiae* strains used were X2180-1B (*MATα SUC2 CUP1 gal2*) obtained from L. Hartwell, 302-21 (*MATα trp1 adc1-11 adr2-43 adm his4*) obtained from E. T. Young, S150-2B (*MATa trp1-289 his3-Δ1 ura3-52 leu2-3,112*) obtained from S. Baim, and X1049-9C (*MATa trp1 ura3 asp5 his8 arg8*) obtained from F. Sherman. The *Escherichia coli* strain used was RR1. The yeast expression vector pMAC561 (Fig. 1), constructed by S. Bektesh, contains the *ADC1* promoter fused to the coding region and transcriptional terminator of *CYC1*, the 2-μm plasmid origin of replication, the yeast *TRP1*$^+$ gene, and most of pBR322.

Enzymes and Chemicals. Restriction endonucleases were obtained from Bethesda Research Laboratories and New England BioLabs. Terminal deoxynucleotidyl transferase, T4 DNA ligase, RNase H, and the Klenow fragment of *E. coli* DNA polymerase I were obtained from Bethesda Research Laboratories. All of the above enzymes were used in the conditions specified by the supplier except where otherwise noted. The avian myeloblastosis virus reverse transcriptase was obtained from Life Sciences. The oligo(dT)- and oligo(dA)-celluloses were obtained from Collaborative Research.

Preparation of Poly(A)$^+$ RNA. Total RNA was extracted (13) from strain X2180-1B grown in glucose medium to late logarithmic phase. Poly(A)$^+$ RNA was purified by two cycles of adsorption to and elution from oligo(dT)-cellulose (14).

Preparation of Tailed DNA Fragments. After digestion of pMAC561 DNA with *Kpn* I, homopolymer tails of approximately 100 deoxythymidylate residues were added to the 3′ termini by terminal deoxynucleotidyl transferase. The DNA was digested with *Eco*RI, and the 8.5-kilobase (kb) *Eco*RI–*Kpn* I fragment was purified by agarose gel electrophoresis. Oligo(dT)-tailed DNA was further purified by adsorption to and elution from oligo(dA)-cellulose (11). The oligo(dT)$^+$ DNA was resuspended in 50 μl of 10 mM Tris·HCl, pH 7.5/1 mM EDTA and stored at 4°C. The *Eco*RI–*Kpn* I oligo(dT)-tailed fragment functions as a vector primer (11) in the cDNA synthesis reaction. A second aliquot of pMAC561 DNA was digested with *Eco*RI, and the termini were blunt-ended by fill-in synthesis with the Klenow fragment of *E. coli* DNA polymerase I. Homopolymer

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Abbreviations: bp, base pairs; kb, kilobase pair(s); ADH, alcohol dehydrogenase.

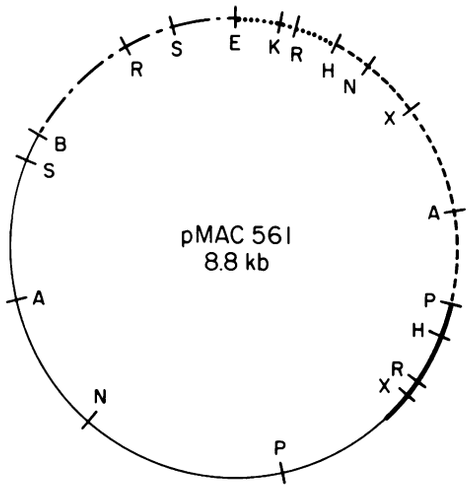

FIG. 1. The cDNA expression vector pMAC561. The *ADC1* promoter fragment (—·—), the fragment containing the *CYC1* coding region and transcriptional terminator (·····), the fragment containing the 2-μm plasmid origin of replication (– – –), the fragment containing the *TRP1*$^+$ gene (▬), and most of pBR322 (——) are shown. Location of restriction endonuclease sites: A, *Ava* I; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; N, *Nde* I; P, *Pst* I; R, *Eco*RV; S, *Sph* I; and X, *Xba* I. The *Eco*RI–*Kpn* I fragment of *CYC1* is ultimately replaced during the preparation of the cDNA plasmid pool with the properly oriented cDNAs containing oligo(dC) tails adjacent to the *Eco*RI site (see text for details).

tails of approximately 20 deoxycytidylate residues were added to the 3′ termini by terminal deoxynucleotidyl transferase, and the DNA was then digested with *Bam*HI. The 1.5-kb *Bam*HI–*Eco*RI fragment was purified by agarose gel electrophoresis, resuspended in 20 μl of 10 mM Tris·HCl, pH 7.5/1 mM EDTA, and stored at 4°C. Approximately 60% of the recovered fragment contained an oligo(dC) tail, as determined by the relative intensity of the *Sph* I–*Eco*RI doublet after *Sph* I digestion and agarose gel electrophoresis. The *Bam*HI–*Eco*RI oligo(dC)-tailed fragment functions as a linker fragment (11) in the recircularization of the cDNA-containing plasmids.

Preparation of the cDNA Plasmid Pool. The cDNA pool was prepared by following the general outline of the Okayama and Berg procedure (11). The 10-μl reaction volume contained 2.6 μg of oligo(dT)$^+$ DNA (0.5 pmol), 1.7 μg of poly(A)$^+$ RNA (6 pmol), and 11 units of reverse transcriptase in the reaction mixture previously described (11), except that the KCl concentration was 50 mM. The cDNA reaction was incubated for 30 min at 37°C and then stopped and treated as described (11). Homopolymer tails of approximately 20 deoxyguanylate residues were added to the 3′ termini of the cDNAs in the cDNA·mRNA hybrids by using 7 units of terminal deoxynucleotidyl transferase in a 25-μl reaction volume. The DNA was extracted with phenol, precipitated with ethanol, and digested with 5 units of *Bam*HI for 90 min in a 20-μl volume. The digestion was stopped by addition of 2 μl of 0.25 M EDTA and 1 μl of 10% sodium dodecyl sulfate and by extraction with phenol/chloroform/isoamyl alcohol, 24:24:1 (vol/vol). The DNA was precipitated with ethanol, resuspended in 10 μl of 10 mM Tris·HCl, pH 7.5/1 mM EDTA, and stored at 4°C. The oligo(dG)-tailed cDNA·mRNA plasmid DNA was hybridized with 0.75 pmol of the oligo(dC)-tailed DNA fragment in a 100-μl volume as described (11). The mixture was then adjusted to 1 ml of ligase buffer containing 25 units of T4 DNA ligase and incubated overnight at 12°C. The ligation reaction was then adjusted to 100 mM KCl, 17 units of RNase H were added, and the mixture was incubated at 23°C for 1 hr. Aliquots of 40 μl were used to transform competent (15) *E. coli* RR1, and transformants were selected on the basis of ampicillin resistance. Approximately 40,000 ampicillin-resistant colonies were pooled and amplified by growth, and plasmid DNA was isolated (16).

Yeast Transformation and Plasmid Recovery. The yeast-transformation protocol used was a modification of the procedure described by Beggs (2). Strain 302-21 was transformed with 2 μg of the cDNA plasmid pool and plated on synthetic medium lacking tryptophan. After 2 days of growth, the plate was overlayed with antimycin A to select for alcohol dehydrogenase (ADH) activity (17). Strains S150-2B and X1049-9C were each transformed with 17 μg of the cDNA plasmid pool and plated on synthetic medium lacking tryptophan. After approximately 1 week of growth, the Trp$^+$ cells were recovered from the agar medium and resuspended in 30 ml of water. Various dilutions of the Trp$^+$ cell suspension were plated on synthetic medium lacking tryptophan and histidine (strain S150-2B) or lacking tryptophan and uracil or aspartate (strain X1049-9C). A few colonies from each plate were subcloned, grown in nonselective medium, and replica-plated onto various media to test for mitotic instability and cosegregation of the Trp$^+$ and either His$^+$, Ura$^+$, or Asp$^+$ phenotypes. Plasmid DNAs were recovered from yeast, after spheroplast formation by treatment with Zymolyase, by using the procedure described by Birnboim and Doly (18). Subsequently, the DNA was used to transform competent (15) *E. coli* RR1, and plasmid DNA was isolated (18).

RESULTS

Selection of cDNAs by Complementation. We chose to isolate cDNAs for the genes *ADC1*, *HIS3*, and *URA3* because the abundance of the respective mRNA is known and because these genes have been isolated and characterized. Knowledge of the physical structure of these genes facilitated the characterization of their respective cDNAs.

Transformation of the Adh$^-$ yeast strain 302-21 with the cDNA plasmid pool yielded approximately 60 Adh$^+$ transformants per μg of plasmid DNA. The vector contains the *TRP1*$^+$ gene, which provides an internal standard for the comparison of transformation efficiencies. The Adh$^+$ transformants represented approximately 1% of the Trp$^+$ transformants, which indicates that approximately 1% of the cDNA inserts in the plasmid pool encode ADH activity. Plasmid DNA was recovered from two different Adh$^+$ transformants and designated pYC1 and pYC2, respectively.

Transformation of yeast strain S150-2B (*trp1 his3*) yielded His$^+$ cells at a frequency of 0.02% among the pooled Trp$^+$ cells. This result indicates that approximately 0.02% of the cDNA inserts complement the *his3* mutation. We analyzed two His$^+$ transformants and found that the His$^+$ phenotypes of both were mitotically unstable, even though the *TRP1*$^+$ plasmid markers were mitotically stable after growth in nonselective medium. One His$^+$ transformant was subcloned, and four of the colonies were analyzed for mitotic instability of the His$^+$ phenotype and cosegregation with the *TRP1*$^+$ plasmid marker. Each of the four subclones exhibited mitotic instability of the His$^+$ phenotype, and one showed cosegregation with the *TRP1*$^+$ plasmid marker. These results indicate that the *HIS3*$^+$ phenotype is plasmid-borne and linked to the *TRP1*$^+$ gene on the vector. Furthermore, these results suggest that the original His$^+$ transformants contained multiple plasmids, all of which carried the *TRP1*$^+$ plasmid marker but only one of which conferred the His$^+$ phenotype. Plasmid DNA recovered from the *HIS3*$^+$ *TRP1*$^+$ subclone was designated pYC3.

Transformation of yeast strain X1049-9C (*trp1 ura3 asp5*)

yielded Ura$^+$ and Asp$^+$ cells at a frequency of approximately 0.004% and 0.04% among the pooled Trp$^+$ cells, respectively. We analyzed two Ura$^+$ transformants and found the Ura$^+$ phenotypes of both to be mitotically unstable; the Ura$^+$ phenotype of one transformant cosegregated with the mitotically unstable *TRP1*$^+$ plasmid marker after growth in nonselective medium. Plasmid DNA recovered from this *URA3*$^+$ *TRP1*$^+$ transformant was designated pYC4. We analyzed two Asp$^+$ transformants and found the Asp$^+$ phenotype of one to be mitotically unstable and the Asp$^+$ phenotype of the other to be stable after growth in nonselective medium. Because the unstable Asp$^+$ phenotype did not cosegregate with the *TRP1*$^+$ plasmid marker, we subcloned the unstable Asp$^+$ transformant and tested four of the colonies. Each of the four subclones exhibited mitotic instability of the Asp$^+$ phenotype, and one showed cosegregation with the *TRP1*$^+$ plasmid marker. Plasmid DNA recovered from this *ASP5*$^+$ *TRP1*$^+$ subclone was designated pYC5.

Restriction Analysis of cDNA Inserts. We expected that the *Eco*RI site would be restored by the combined procedures of blunt-ending the *Eco*RI-generated termini by fill-in synthesis and (dC)-tailing of the blunt end. Yet, none of the plasmids pYC1–pYC5 contained an *Eco*RI site (data not shown). In contrast, the *Kpn* I site should not have been restored by (dT)-tailing of the *Kpn* I termini. In fact, only those plasmids expected to have a *Kpn* I site in the cDNA did so (see below). The loss of the *Eco*RI and *Kpn* I sites prevented the precise excision of the tailed-cDNA inserts. However, the inserts could be excised by digestion with *Eco*RV, whose sites flank the tailed cDNA by 703 and 106 base pairs (bp), or by digestion with *Xba* I and *Sph* I (see Fig. 1). The *Xba* I and *Sph* I sites in the vector DNA flank the cDNA insert by approximately 1 kb and 0.4 kb, respectively. The size of the cDNA inserts in pYC1 and pYC3–pYC5 was determined by digestion of the plasmids with *Xba* I and *Sph* I. The results (Fig. 2) indicate the cDNA inserts in pYC1, pYC3, pYC4, and pYC5 are 1.3, 1.05, 1.15, and 1.5 kb in length,

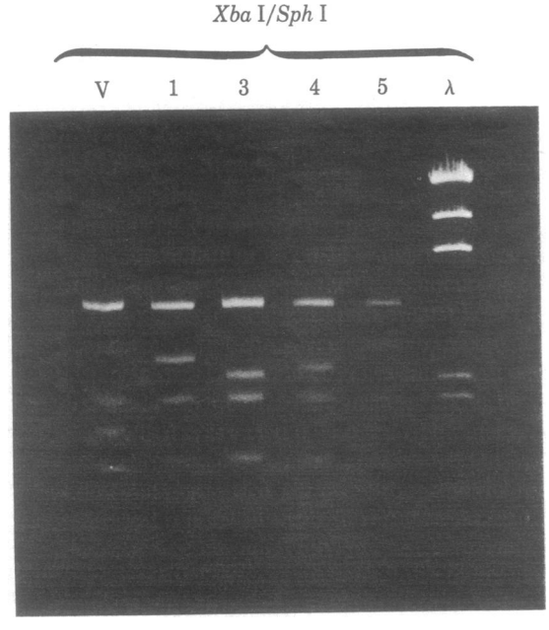

FIG. 2. Sizing of the cDNA inserts. The plasmid DNAs, including expression vector pMAC561 (lane V), pYC1 (lane 1), pYC3 (lane 3), pYC4 (lane 4), and pYC5 (lane 5), were digested with *Xba* I and *Sph* I, and the fragments were separated by electrophoresis in 1% agarose. The λ DNA (lane λ) was digested with *Hin*dIII, resulting in fragments of the following sizes: 23.1, 9.4, 6.6, 4.3, 2.3, and 2.0 kb. The size of the cDNA inserts was estimated by subtracting 1.4 kb from the size of the fragment unique to each plasmid (see text for details).

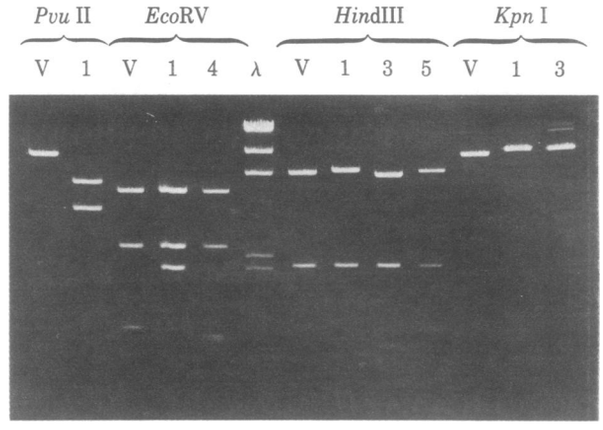

FIG. 3. Restriction endonuclease analysis of the cDNA inserts. The plasmid DNAs were digested with either *Pvu* II, *Eco*RV, *Hin*dIII, or *Kpn* I, and the fragments were separated by electrophoresis in 1% agarose. Lanes: V, expression vector pMAC561; 1, pYC1; 3, pYC3; 4, pYC4; 5, pYC5; λ, phage λ DNA. The phage λ DNA was digested with *Hin*dIII. The cDNA inserts contain the restriction endonuclease site when an extra fragment is present relative to the parent plasmid pMAC561. However, the cDNA inserts contain a *Kpn* I site if the plasmid is linearized because the *Kpn* I site in the vector was destroyed by tailing (see text for details).

respectively. The sizes of the inserts were confirmed by digestion of the plasmids with *Eco*RV (data not shown).

The genes *ADC1* and *ADR2* that encode the ADH isozymes can be distinguished by the presence of the unique sites *Pvu* II and *Eco*RV, respectively (19, 20). The cDNA insert in pYC1 contained a *Pvu* II site but not an *Eco*RV site, which indicates that pYC1 contains *ADC1* cDNA (Fig. 3). Further confirmation that pYC1 contains *ADC1* cDNA is seen in Fig. 3, where pYC1 is shown to contain the predicted sites for *Hin*dIII and *Kpn* I (19). The recovery of *ADC1* but not of *ADR2* from our cDNA pool made from cells grown in high-glucose medium was ex-

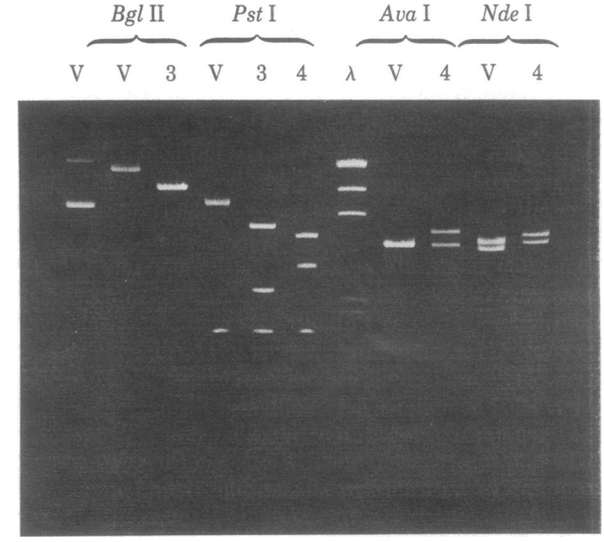

FIG. 4. Restriction endonuclease analysis of the cDNA inserts. The plasmid DNAs were digested with *Bgl* II, *Pst* I, *Ava* I, or *Nde* I, and the fragments were separated by electrophoresis in 1% agarose. Lanes: V, expression vector pMAC561; 3, pYC3; 4, pYC4; λ, phage λ DNA. The phage λ DNA was digested with *Hin*dIII. The cDNA inserts contain the restriction endonuclease site when an extra fragment is present relative to the parent plasmid pMAC561. Because digestion of pMAC561 with *Ava* I generates two fragments of virtually identical size, the cDNA insert lacks an *Ava* I site if a larger fragment is present relative to pMAC561.

pected because, in the presence of glucose, *ADC1* is expressed whereas *ADR2* is repressed (21). On the basis of size and the presence of restriction endonuclease sites expected for *ADC1*, the plasmids pYC1 and pYC2 appeared indistinguishable (data not shown).

The *HIS3* gene contains sites for *Hin*dIII, *Kpn* I, *Bgl* II, and *Pst* I (22). As expected, these sites are present in pYC3 (Figs. 3 and 4). Because the parent plasmid pMAC561 contains no *Bgl* II sites, an undigested sample of pMAC561 is included in Fig. 4 for comparison.

The *URA3* gene is known to contain sites for *Eco*RV, *Pst* I, *Nde* I, and *Ava* I (23). The *Eco*RV site is located in the coding region of *URA3* (23). The 5′ end of the *URA3* mRNA mapped between the *Pst* I and *Nde* I sites, which are located 22 and 89 bp, respectively, upstream of the ATG initiation codon in the 5′ leader region of *URA3* mRNA (23). The 3′ end of the *URA3* mRNA mapped upstream of the *Ava* I site, 76 bp distal to the TAA termination codon (23). As expected, the plasmid pYC4 contains *Eco*RV and *Pst* I sites but not *Nde* I or *Ava* I sites, as shown in Figs. 3 and 4. Because *Ava* I digestion of pMAC561 generated two fragments of virtually identical size, the presence of the larger *Ava* I fragment in pYC4 indicates the absence of an additional *Ava* I site (Fig. 4). These results are consistent with pYC4 containing *URA3* cDNA.

The *ASP5* gene has not been isolated previously to our knowledge. A *Hin*dIII site was found in pYC5 and is shown in Fig. 3. The *Hin*dIII site in the *ASP5* cDNA may be useful in the determination of the DNA sequence of *ASP5*.

DISCUSSION

We inserted yeast cDNA sequences in a yeast expression vector so that the mRNA 5′-proximal sequences are attached to the *ADC1* promoter (8) and the 3′-distal mRNA sequences are attached to the *CYC1* terminator (24). It is unknown whether the *CYC1* terminator is necessary for the expression of yeast cDNAs, but it is expected to enhance the expression of heterologous cDNAs (25). All necessary translation signals are provided by the cDNA insert. Using this approach, we genetically selected functional cDNAs for the yeast *ADC1*, *HIS3*, *URA3*, and *ASP5* genes by complementation in yeast. The genetic identity of the *ADC1*, *HIS3*, and *URA3* cDNAs was confirmed by restriction endonuclease analysis. The *ADC1* cDNA was shown to contain the expected sites for *Eco*RV, *Hin*dIII, and *Kpn* I, which are all located in the coding region (19). The mRNA for *ADC1* has been shown to be approximately 1.2 kb in length (21), which is similar to the 1.3-kb cDNA insert, including flanking tails, characterized here.

The *HIS3* cDNA was shown to contain the expected sites for *Hin*dIII, *Bgl* II, *Kpn* I, and *Pst* I. However, the mRNA for *HIS3* has been reported to be approximately 0.7 kb in length (26). In contrast to those results, the functional *HIS3* cDNA insert that we characterized is 1.05 kb, including flanking tails of approximately 20 deoxycytidylate and 100 deoxythymidylate residues.

The *URA3* cDNA was shown to contain the predicted sites for *Pst* I and *Eco*RV. The 5′ end of the mRNA strand in the *URA3* cDNA was found to map between the *Nde* I and *Pst* I sites, which are located 22 and 89 bp upstream of the ATG initiation codon, respectively (23). These results indicate the *URA3* cDNA contains 5′ noncoding sequences. The 3′ end of the mRNA strand in the *URA3* cDNA was found to map upstream of the *Ava* I site. The locations of the 5′ and 3′ ends of the *URA3* cDNA are consistent with the known locations of the 5′ and 3′ ends of the *URA3* mRNA (23).

The mRNA for *ADC1* comprises approximately 1% of the poly(A)$^+$ RNA in glucose-grown yeast cells (21). Because we recovered Adh$^+$ transformants at a frequency of approximately 1% of the Trp$^+$ transformants, the cDNAs present in the plasmid pool appear to be represented at the same frequency as in total poly(A)$^+$ RNA (Table 1). Similarly, the *HIS3* and *URA3* mRNAs each comprise approximately 0.01% of poly(A)$^+$ RNA, which corresponds to approximately one mRNA molecule per cell (26, 27). We recovered *HIS3* and *URA3* cDNAs at a frequency of approximately 0.02% and 0.004%, respectively, which closely parallels the abundance of these mRNAs in total poly(A)$^+$ RNA (see Table 1). These results indicate that the cDNA pool described here is representative of yeast poly(A)$^+$ RNA and contains rare cDNAs present at approximately 0.01%.

Table 1. Comparison of complementation frequencies and mRNA abundances

Plasmid	Gene	Complementation frequency	Known mRNA abundances
pYC1, pYC2	*ADC1*	1%	1%
pYC3	*HIS3*	0.018%	0.01%
pYC4	*URA3*	0.004%	0.01%
pYC5	*ASP5*	0.037%	—

The complementation frequency is the ratio of His$^+$ cells (e.g., to Trp$^+$ cells determined by plating various dilutions of the Trp$^+$ cell suspension on the respective selective media).

Most of the transformants we analyzed were found to be cotransformed with multiple plasmids. This suggests that the complementation frequency may be overestimated by 2- to 3-fold. Because competent yeast cells frequently become cotransformed with multiple plasmids (28) and because we transformed yeast with large amounts of DNA, the observed cotransformation frequency is not surprising. However, the desired plasmid can be readily purified by continued growth of the transformants on selective media.

We modified the cDNA synthesis procedure of Okayama and Berg (11) in several ways. First, we prepared cDNAs that contain an oligo(dC) tail instead of an oligo(dG) tail attached to the 5′ end of the mRNA strand. Second, we omitted the nick-translation repair of the cDNA·mRNA hybrid and transformed *E. coli* RR1 directly with the cDNA·mRNA hybrid plasmid. The use of commercially available preparations of *E. coli* DNA polymerase I in the nick-translation repair of the cDNA·mRNA hybrid dramatically lowered our recovery of *E. coli* transformants (unpublished data). For this reason we omitted the nick-translation repair step. Third, we used the Rec$^+$ *E. coli* strain RR1 instead of the *recA*$^-$ *E. coli* strain HB101. In addition, we used a yeast expression vector instead of a cloning vector containing simian virus 40 DNA. The success of the approach described here indicates that the presence of an oligo(dC) segment in the 5′ noncoding region of the resulting plasmid-encoded transcripts is permissive to translation. The efficiency of expression of the cDNAs has not been measured. However, only low levels of expression of the cDNAs are probably required for complementation of metabolic mutants.

The selection of functional cDNAs by complementation in yeast provides a promising approach for the isolation of rare cDNAs from a great variety of eukaryotic organisms, including mammals and plants. The approach is limited only by the availability of appropriate yeast mutants and the ability of a heterologous protein to provide function in yeast. The greatest utility of this approach is the isolation of cDNAs that encode metabolic functions and are expressed at low levels. For example, the biochemical functions for which certain yeast strains are mutant are those involved in many types of human genetic disease.

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