

# Functional expression of rat cytochrome *c* in *Saccharomyces cerevisiae*

(heterologous gene expression/respiratory chain/evolution/mitochondrial function)

RICHARD C. SCARPULLA AND STEVEN H. NYE

Department of Molecular Biology, Northwestern University Medical School, Chicago, IL 60611

Communicated by David Shemin, May 13, 1986

**ABSTRACT** To determine whether a mammalian cytochrome *c* could efficiently replace iso-1-cytochrome *c*, which is encoded by the yeast *CYC1* gene, the coding sequence of *RC9* (a nondefective processed gene from rat) was cloned in both single- and multiple-copy expression vectors under the direction of the yeast alcohol dehydrogenase 1 (*ADCI*) promoter. Upon transformation of a *CYC1* deletion strain, the multiple-copy construct restored a wild-type growth rate on lactate medium; such growth normally requires derepressed amounts of iso-1-cytochrome *c*. These transformants expressed a level of hybrid *ADCI/RC9* mRNA approximately 5- to 10-fold greater than the amount of message from the endogenous *ADCI* gene and produced a steady-state level of rat cytochrome *c* equivalent to that of the wild-type yeast protein. A requirement for the vector was evidenced by its absence in all transformants that lost the lactate growth phenotype after propagation in nonselective medium. In contrast, the level of vector-specific message in single copy was equivalent to that of the endogenous *ADCI* mRNA, but transformants exhibited no significant growth on lactate. Constructions having a small deletion or a mammalian intron within the rat cytochrome *c* coding region failed to support lactate-dependent growth, indicating that complementation depends upon proper translation of the correct rat coding sequence. Therefore, the rat polypeptide, when expressed at normal physiological levels, is recognized by the yeast machinery involved in the multiple steps required for the processing and transport of an active cytochrome *c* as well as its functional interaction with the respiratory apparatus.

Mammalian genomes contain families of about 30 different sequences that have a high degree of homology with DNA encoding the somatic form of cytochrome *c* (1, 2). Although it has not been directly established, considerable structural evidence supports the hypothesis that in the rat nearly all of these originate from the reverse transcription and genomic integration of the multiple mRNA products of *RC4*, a single intron-containing active gene (3). A second nonallelic clone, *RC9*, is a processed gene of very recent evolutionary origin and has a nondefective cytochrome *c* coding region. All of the other family members of known sequence have mutational defects in their coding regions, including replacements in codons for essential residues (3). Several of these changes are in highly invariant regions of the molecule to which no structural or functional significance has yet been assigned. The numerous processed pseudogenes in this family therefore represent a collection of mutated rat cytochrome *c* coding regions of defined structure.

To directly assay the biological function of the variant mammalian cytochromes encoded by these genes or by recombinant derivatives constructed *in vitro*, it would be useful to express them in a facultative anaerobe, where they

are required for growth only under defined conditions. Yeast is an ideal organism to serve as a recipient. Its growth on lactate as a carbon source depends upon normal levels of iso-1-cytochrome *c* (4), deletion mutants of the entire *CYC1* gene encoding this cytochrome have been isolated (4), and a sophisticated transformation system and expression vectors are well established for the organism (5, 6).

The production of a functional cytochrome *c* requires several steps following the cytoplasmic translation of the apocytochrome (7). These include the recognition of the apocytochrome by cytosolic factors and a membrane receptor that facilitates its mitochondrial uptake, the enzymatic attachment of heme as it traverses the mitochondrial membrane into the intermembrane space, and finally the cytochrome's specific interaction with the respiratory apparatus (8). Failure of the rat polypeptide to be efficiently recognized by components of the yeast machinery mediating any of these steps would result in defective cytochrome *c* function. Although cytochrome *c* is an ancient protein whose structure and activity is conserved for all major taxonomic groups, only about 60% homology in primary structure is retained between fungi and mammals, and other notable physicochemical differences exist as well (for reviews see refs. 8, 9, and 10). Therefore, as a first step toward evaluating yeast as a facultative anaerobe for the expression and analysis of variant mammalian cytochromes it is essential to establish whether rat cytochrome *c* is functionally equivalent to yeast iso-1-cytochrome *c* *in vivo*. In this work, the nondefective coding region from *RC9*, a rare rat processed gene of very recent evolutionary origin, is expressed in a *CYC1* deletion mutant under the direction of yeast regulatory sequences.

## MATERIALS AND METHODS

**Yeast Strains.** A host strain to serve as a recipient for the expression of rat cytochrome *c* was constructed by crossing F45 (*MAT $\alpha$* , *leu2-3*, *leu2-112*, *his4-34*, *ura3-52*), a derivative of AH22 (5) containing both a *leu2* double mutation and an insertional disruption of *ura3*, with D234-10A (*MAT $\alpha$* , *cycl-1*, *adel*, *trp1*), a derivative of *CYC1* deletion strain D234 (11). Diploids were allowed to sporulate and the haploid spores were released from asci by Glusulase treatment and sonication (12). After growth on YEPD (yeast extract/peptone/dextrose) rich medium, progeny were tested for the desired markers by replica plating. One of the mating products, designated N1B (*MAT $\alpha$* , *cycl-1*, *leu2-3*, *leu2-112*, *his4-34*, *ura3-52*), was used as the host strain for transformations with both multiple-copy and single-copy vectors, utilizing *LEU2* and *URA3* as selectable markers. Transformation of this strain with expression vectors was carried out by an established procedure (5).

Abbreviation: kb, kilobases.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

**Vectors.** Multiple-copy vectors for the expression of rat apocytochrome *c* were constructed by introducing the rat cytochrome *c* coding sequences into the unique *Hind*III cloning site of pAAH5 (13). This site is flanked by promoter and terminator regions of the alcohol dehydrogenase 1 (*ADC1* gene) transcriptional unit (14). The 5' *Msp* I and 3' *Acc* I ends of the *RC9* coding region restriction fragment were converted to *Hind*III by the addition of synthetic linkers after *S*1 nuclease treatment (3) to generate blunt ends. In *RC4* the *Msp* I site is displaced upstream because of the presence of a large intron (3). Therefore, to maintain an identical 5' end in both *RC4* and *RC9* constructs, the intron-containing *Nco* I-*Acc* I fragment from *RC4* was substituted for the analogous *RC9* fragment after conversion of the *Acc* I site to a *Hind*III site. A single-copy expression vector, designated YCpAD1, was constructed by inserting the *ADC1 Bam*HI fragment from pAAH5 into YCp50, a vector containing a functional centromere (32). Constructions containing the *RC4* or *RC9* coding regions were designated YE<sub>p</sub>RC4 or YE<sub>p</sub>RC9 in multiple-copy and YCpRC4 or YCpRC9 in single-copy expression vectors. Roman numerals denote the sense (I) and antisense (II) transcriptional orientations of the rat coding regions. A small deletion of nine nucleotides introduced at the *Nco* I site of *RC9* by digestion with *S*1 nuclease followed by ligation of the resulting blunt ends is indicated by a  $\Delta$ .

**Cell Growth Analysis.** Transformants were selected for their ability to restore N1B to either leucine or uracil prototrophy, depending upon the vector introduced. Individual isolates containing the various constructions were then tested for growth on DL-lactate as a carbon source (11) at 30°C and compared to the wild-type control strain expressing normal levels of yeast cytochrome *c*. For a quantitative comparison of cell growth, cells from cultures grown at 30°C with glucose as a carbon source were washed and inoculated into lactate medium ( $10^6$  cells per ml), and cell growth was monitored with a Klett-Summerson colorimeter using a no. 66 red filter.

**RNA Analysis.** Yeast cells were harvested from logarithmic-phase cultures grown in either glucose or lactate medium. Total cellular RNA was prepared by an established protocol (15) except that the breaking buffer contained 1.0% sodium dodecyl sulfate and diethoxydiformate was omitted. The polyadenylated RNA fraction was isolated as previously described (16) but with the addition of 0.05% sodium dodecyl sulfate to both the binding and elution buffers. Polyadenylated RNAs were resolved by electrophoresis on 1% agarose gels in the presence of formaldehyde, transferred to nitrocellulose filters (17), and detected by hybridization to radioactive restriction fragments prepared and  $^{32}$ P-labeled as before (18). Levels of the various mRNAs were quantified by densitometry of the autoradiograph.

**Immunological Methods.** Antiserum against total yeast cytochrome *c* (Sigma) was raised in New Zealand White rabbits. The antigen was mixed in an oil-in-water adjuvant system supplied by Ribl ImmunoChem Research and prepared according to the supplier's instructions except for the omission of cell wall skeleton. Immunization was carried out with 200  $\mu$ g of cytochrome *c* administered by a combination of both intramuscular and subcutaneous injections. Rabbit antiserum to mouse cytochrome *c* was a gift from Erwin Goldberg of Northwestern University.

Total protein extracts for use in immunoblotting were prepared from logarithmic-phase cultures of transformant and control cells by an established protocol (19). The extract supernatant was dialyzed against 0.025 M Tris-HCl, pH 7.4/0.1% sodium dodecyl sulfate, lyophilized, and dissolved in water. Protein concentrations were determined as described by Lowry *et al.* (20). Total cellular proteins were separated electrophoretically under denaturing conditions (21) on a 9% polyacrylamide slab gel (22). Proteins were

transferred and bound to nitrocellulose (0.2  $\mu$ m BA-83, Schleicher & Schuell) (23) by diffusion and solvent flow. Immunodetection was performed with 4-chloro-1-naphthol as a substrate as described previously (24) except that biotinylated goat anti-rabbit IgG and the streptavidin conjugate of horseradish peroxidase (Sigma) were used for secondary incubation.

## RESULTS

**Complementation of a *CYC1* Deletion by Expression of Rat Cytochrome *c* in Yeast.** To determine whether rat apocytochrome *c* can be functionally expressed in yeast, the coding sequences from both genes *RC4* and *RC9* were cloned in single- and multiple-copy expression vectors as summarized in Fig. 1. Transformants of the *CYC1* deletion strain N1B with each of the constructs were selected for restoration of either leucine or uracil prototrophy, depending upon the expression vector used, and then monitored for growth on DL-lactate as a carbon source. Fig. 2 demonstrates that growth on lactate is detected only in multiple-copy transformants of N1B in which the *RC9* coding region is intact and in the proper transcriptional orientation relative to the *ADC1* promoter and terminator. A wild-type growth rate of about 5–7 hr per generation is achieved by cells transformed with multicopy vector YE<sub>p</sub>RC9I. The only difference in growth between wild-type and YE<sub>p</sub>RC9I transformants is a longer lag phase in the latter, which was the same regardless of whether inoculum cells were grown on glucose or lactate

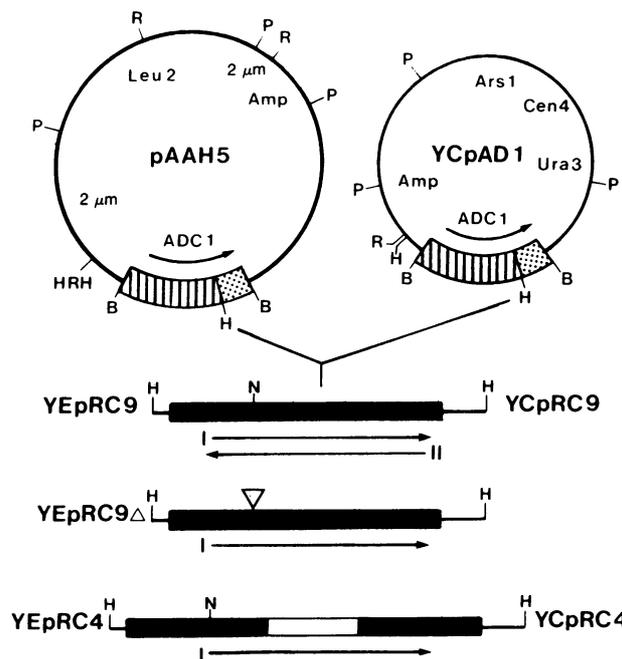


FIG. 1. Summary of vector constructions used for the expression of rat cytochrome *c* in yeast. Single-copy expression vector YCpAD1 was constructed by cloning the 1.9-kilobase (kb) *ADC1 Bam*HI restriction fragment from multiple-copy expression vector pAAH5 in YCp50. Promoter (hatched box) and terminator (stippled box) regions from the yeast *ADC1* gene are in the transcriptional orientation indicated by the arrow. Rat cytochrome *c* coding sequences from *RC4* or *RC9* shown below were introduced into the *Hind*III cloning site as described in *Materials and Methods*. Names of the resulting constructs are shown to the left (multiple-copy) and right (single-copy) of each map. The deletion at the *Nco* I site of *RC9* is indicated by  $\nabla$  and the sense (I) and antisense (II) transcriptional orientations, by roman numerals. Restriction enzyme sites are labeled as follows: R, *Eco*RI; B, *Bam*HI; H, *Hind*III; N, *Nco* I; and P, *Pst* I.

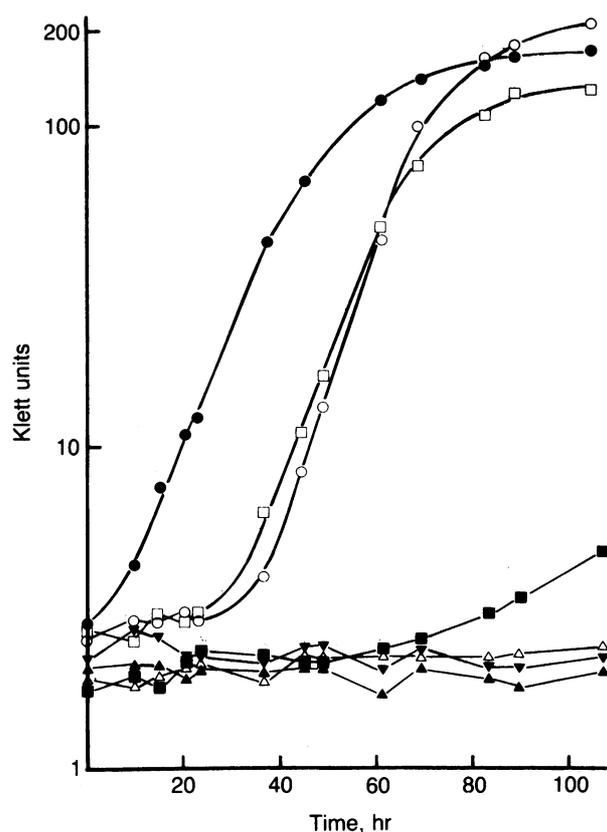


FIG. 2. Cytochrome *c*-dependent growth of transformants and control strains on lactate. Growth at 30°C of various transformants containing rat cytochrome *c* coding sequences under the direction of yeast *ADC1* regulatory regions is compared to that of the *CYC*<sup>+</sup> wild type (●) and the *CYC1* deletion strain N1B (▲). Curves depict the kinetics of cell growth of two independent isolates of N1B transformed with YEpRC9I (○, □), and with YCpRC9I (■), YEpRC9ΔI (△), or YCpRC4I (▼).

medium. The single-copy vector YCpRC9I confers no significant growth on lactate as a carbon source. The vector sequences alone had no effect on the growth of wild-type cells or the *CYC1* deletion strain N1B.

Growth is not detected when the rat cytochrome *c* sequences are cloned in the antisense transcriptional orientation (YEpRC9II) or when a small deletion is introduced at the unique *Nco* I site present in the coding region (YEpRC9ΔI). The intron-containing coding region of *RC4* was not stably

maintained at high copy, but no growth was observed with *RC4* at low copy, and mRNA analysis demonstrates that the yeast cells were unable to excise the mammalian intron (see Fig. 3). When the YEpRC9I transformants were plated on nonselective rich medium and replicas were made for phenotypic testing, all colonies that failed to grow on selective media lost both leucine and lactate growth phenotypes. In addition, no rearrangements of the vector sequences are detected by restriction mapping after their propagation in yeast. Taken together, these results support the conclusion that complementation of the *CYC1* deletion in these transformants requires the presence of the vector and occurs through expression of an intact rat apocytochrome *c*.

**Analysis of Rat Cytochrome *c* Gene Products Expressed in Yeast.** Because a wild-type growth phenotype is observed only in high-copy transformants, it is essential to distinguish whether complementation takes place through the overproduction of an inefficiently utilized rat apocytochrome or whether some step in the expression itself is inefficient. To this end both mRNA and protein levels were estimated and compared to those of wild-type cells.

When the polyadenylated RNA from each of the transformants is hybridized to the yeast iso-1-cytochrome *c* gene as probe (Fig. 3A), the endogenous cytochrome *c* mRNA of approximately 650 nucleotides (25) is detected only in the wild-type parent strain (lane 5). Iso-2-cytochrome *c* shares nearly 80% DNA sequence homology with the coding region of iso-1-cytochrome *c* (26), and a significant elevation in the expression of the *CYC7* gene, which encodes iso-2-cytochrome *c*, would be detected under the conditions of hybridization. The mRNA for this isoform yields only the very weak signal expected from the minute fraction of total yeast cytochrome *c* normally contributed by the *CYC7* gene (10). In contrast, with the rat cytochrome *c* gene as probe, a substantial level of mRNA expression is revealed in all transformants (Fig. 3B) and no detectable signal is seen in either the parental wild-type (lane 5) or the N1B deletion strain (lane 6) controls. The specific mRNA levels are higher in the multiple-copy transformants (lanes 1–4) compared to single-copy (lanes 8 and 9) and remain the same in cells transformed with YEpRC9I and grown with either glucose or lactate as a carbon source (lanes 1 and 2). In addition, the size of the major mRNA species of approximately 700 nucleotides corresponds to that expected from the size of the rat coding fragment (380 nucleotides) and the 5' and 3' ends of the *ADC1* mRNA, which map to 14–20 and 215 nucleotides, respectively, from the *Hind*III site (13, 14). Cells transformed with YCpRC4I express a larger mRNA (lane 8), whose size is consistent with the failure of the yeast splicing machinery to

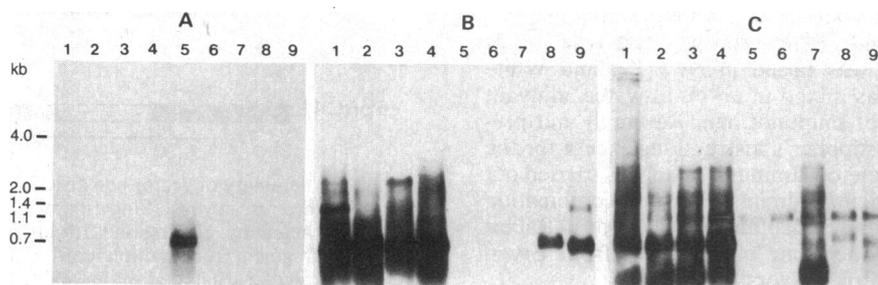


FIG. 3. Hybridization analysis of polyadenylated RNA from rat cytochrome *c* transformants and control strains. Hybridization was carried out at 40°C in 0.97 M Na<sup>+</sup> (0.75 M NaCl/0.075 M trisodium citrate) and 50% (vol/vol) formamide. A, B, and C show the autoradiographs from identical filters hybridized to <sup>32</sup>P-labeled probes made from the iso-1-cytochrome *c* coding region from pYC11 (1) the rat cytochrome *c* coding region from pRC9 (3), and the 3' noncoding and flanking region from the yeast *ADC1* gene, respectively (13, 14). The hybridization pattern from poly(A)<sup>+</sup> RNA (10 μg per lane) derived from cells transformed with YEpRC9I (lanes 1 and 2), YEpRC9II (lane 3), YEpRC9ΔI (lane 4), pAAH5 (lane 7), YCpRC4I (lane 8), and YCpRC9I (lane 9) is compared to that from the *CYC*<sup>+</sup> wild type (lane 5) and the *cyc1-1* host strain N1B (lane 6). RNA samples used in lanes 1 and 5 were isolated from lactate-grown cultures, while all others were prepared from cells grown in selective medium with glucose as a carbon source. Size markers include the 18S and 28S ribosomal RNAs and 1.4-, 1.1-, and 0.7-kb cytochrome *c* mRNAs from rat liver (18).

remove the 105-nucleotide mammalian intron. It is also notable that steady-state levels of *ADC1/RC* mRNAs are not significantly affected by the presence of the rat cytochrome sequences in the antisense orientation (lane 3) or by a small coding region deletion (lane 4), both of which fail to support lactate-dependent growth.

When the *ADC1* terminator region is used as a probe (Fig. 3C), the same size mRNA species in the same relative amounts are detected in the various transformants, reaffirming that the rat-specific mRNAs originate from the hybrid *ADC1/RC* transcriptional unit on the vectors. The multiple-copy vector without rat cytochrome sequences directs the synthesis of a major mRNA whose size of 300 to 400 nucleotides is consistent with the absence of the coding sequences (lane 7). The level of expression from the vector can be estimated by comparing the major vector-specific mRNA to the 1200-nucleotide mRNA from the chromosomal *ADC1* gene (14). Single-copy transformants express a level of *ADC1/RC* hybrid mRNA equivalent to the endogenous level of *ADC1* mRNA (lanes 8 and 9), while the level of the specific vector-directed mRNA is about 10-fold higher with the multiple-copy vector (lanes 1–4). However, a severalfold decrease in the level of endogenous *ADC1* mRNA is observed in lactate-grown cells, in keeping with the observation that its synthesis is repressed by a nonfermentable carbon source (27, 28). This repression does not appear to significantly alter the levels of the hybrid *ADC1/RC9* mRNA (Fig. 3B and C, lanes 1 and 2). Nevertheless, the differences in mRNA expression between single- and multiple-copy vectors are consistent with a 10-fold difference in vector copy number detected between single- and multiple-copy transformants (data not shown). Therefore a 5- to 10-fold overproduction of the *ADC1/RC9* mRNA encoded by the vector YEpRC9I supports normal wild-type growth on DL-lactate in the absence of the *CYC1* gene.

To determine whether YEpRC9I transformants also express a high steady-state level of rat cytochrome *c* protein, specific antibodies were utilized to directly compare cytochrome *c* levels in wild-type and transformant strains. Antisera raised against total yeast or mouse cytochrome *c* [rat and mouse cytochromes *c* are identical (29)] display no cross-reactivity as measured by a quantitative immunoblotting assay (Fig. 4, lanes 1 and 2). As expected, no rat or yeast cytochrome *c* is detected in N1B (lanes 3) and only yeast cytochrome *c* is found in the wild-type (lanes 4). The YEpRC9I transformant (Fig. 4B, lane 5) expresses a level of rat cytochrome *c* polypeptide (0.6  $\mu$ g per 100  $\mu$ g of total protein) comparable to but generally estimated by densitometry of the immunoblot to be about 50% higher than the derepressed level of yeast cytochrome *c* detected in the wild-type control (Fig. 4A, lane 4). A somewhat lower steady-state level of rat cytochrome polypeptide is detected in cells transformed with YEpRC9 $\Delta$ I (lane 8). The 9-nucleotide deletion in this construct is expected to result in the synthesis of a defective cytochrome devoid of amino acid residues Leu-32, His-33, and Gly-34, and as shown above this construct fails to support growth on lactate. In contrast, only approximately 1/5th as much rat cytochrome *c* could be detected in the YCpRC9I single-copy transformant (Fig. 4B, lane 6) after a growth lag of several days (Fig. 2). The low cytochrome *c* level in these cultures is most likely the result of spontaneous revertants that arise in the population during the long growth lag. These revertants, which were isolated by selection for normal growth on lactate, had approximately 50% of the normal derepressed level of cytochrome *c* observed in wild-type yeast (Fig. 4B, lane 7) and had an increased vector copy number that could account for the increased cytochrome *c* (data not shown). A very low level of protein is also found by using the yeast-specific antisera (Fig. 4A, lane 5). This most likely represents the derepressed

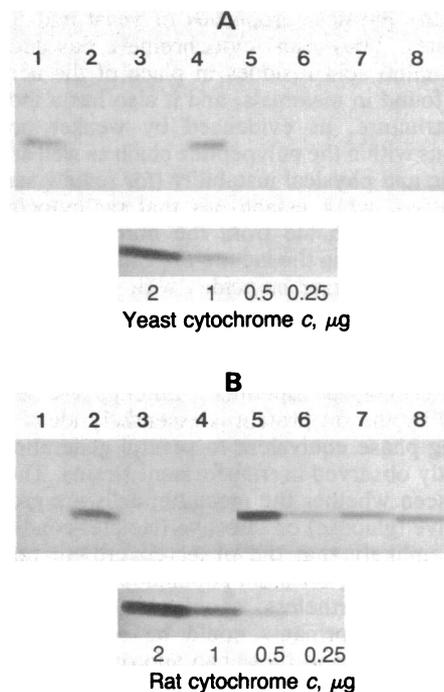


FIG. 4. Detection of rat and yeast cytochromes *c* in transformant and control strains by immunoblotting. (A) Immunodetection of cytochrome *c* after gel electrophoresis and transfer of total cellular proteins to a nitrocellulose membrane and visualization as described in *Materials and Methods* using a specific antiserum raised against total yeast cytochrome *c*. (B) Identical to A except that a specific antiserum raised against mouse cytochrome *c* is used for immunodetection. Each gel lane contained 350  $\mu$ g of reduced and denatured total cellular protein. Lanes 1 and 2 depict the colorimetric signal obtained with 1  $\mu$ g of yeast and rat cytochrome *c*, respectively, mixed with 350  $\mu$ g of total protein from the *CYC1* deletion strain N1B. The signal from N1B alone is shown in lane 3 and that from the *CYC1*<sup>+</sup> wild-type strain in lane 4. Immunodetection of cytochrome *c* in the total proteins of cells transformed with rat cytochrome *c* expression vectors is shown as follows: YEpRC9I (lane 5), YCpRC9I (lane 6), and YEpRC9 $\Delta$ I (lane 8). Lane 7 depicts the signal from a revertant of the YCpRC9I transformant selected for normal growth on lactate. Below each gel is a dilution series of authentic yeast (A) or rat (B) cytochrome *c* for approximate quantitation.

synthesis of iso-2-cytochrome *c*, which at its normal level of expression (5% of the total cytochrome *c*) is not sufficient to support significant growth on lactate (10). Therefore, the normal growth of multiple-copy transformants on lactate requires the expression of a steady-state level of structurally intact rat cytochrome *c* in amounts equivalent to the normal expression of yeast cytochrome *c* in the wild-type control.

## DISCUSSION

To evaluate yeast as a facultative anaerobe for the analysis of variant mammalian cytochromes *c*, it is essential to first establish whether the yeast and mammalian proteins have equivalent biological activities *in vivo*. Although a number of biochemical studies show striking similarities in the oxidative properties of cytochromes *c* from widely divergent organisms (for reviews see refs. 8 and 10) the formation of a fully functional molecule *in vivo* depends upon a complex and unique pathway for processing and transport that involves specific interactions of the apocytochrome with other cellular components (7). For example, heterologous polypeptides from yeast and mammals have 1/5th to 1/10th the affinity of the homologous apoprotein for binding to specific receptors associated with *Neurospora crassa* mitochondria (30). Other potential functional disparities are also suggested by differ-

ences in the physical properties of yeast and mammalian cytochromes. Yeast iso-1-cytochrome *c* has additional N-terminal amino acid residues in place of the acetylated N terminus found in mammals, and it also has a more loosely packed structure, as evidenced by weaker noncovalent interactions within the polypeptide chain as well as enhanced proteolytic and physical instability (for review see ref. 10).

The present work establishes that rat cytochrome *c* is nearly indistinguishable from the normal wild-type yeast protein in supporting the lactate-dependent growth of yeast. A normal growth rate coincides with the expression of a steady-state level of rat cytochrome equivalent to the normal derepressed level of iso-1-cytochrome *c*, indicating that the rat protein is efficiently utilized in yeast when expressed in proper physiological amounts. Although the growth rate supported by the rat protein is essentially identical to wild type, a lag phase equivalent to several generation times is consistently observed in transformant strains. The same lag phase is seen whether the inoculum cells are grown under nonselective (glucose) or selective (lactate) conditions. It is therefore unlikely that the observed growth results from selection operating on a subpopulation of cells during the growth lag. Nevertheless, spontaneous revertants of the single-copy transformants could be selected for normal growth on lactate, and these had approximately 50% of the normal derepressed level of cytochrome *c* observed in wild-type yeast. Thus, rat cytochrome *c* is not limiting for growth on lactate until its steady-state expression is below this level, suggesting a relatively high efficiency in its biological function.

In the host-vector system used in these experiments, the production of normal amounts of protein requires overproduction of the *ADC1/RC9* mRNA. This observation might be explained either by a decreased translational efficiency of the hybrid mRNA directing the synthesis of rat cytochrome or by a difference in polypeptide turnover that is compensated for by increased mRNA levels. It is of interest in this context that a generally higher degree of potential secondary structure is detected in the *ADC1/RC9* mRNA sequence than in the normal iso-1-cytochrome *c* message by using a computer modeling program (31). The discrepancy between protein and mRNA levels may be accounted for by differences in codon usage or by selective constraints on the coding sequence that operate at the level of mRNA structure and function. Alternatively, the differences may simply be an artifactual property of the particular hybrid constructions used in these experiments. More detailed studies of polypeptide turnover and alternative expression vehicles should further delineate parameters for efficient cytochrome *c* synthesis and function.

In conclusion, the experiments establish that yeast can serve as a host organism for the efficient functional expression of a mammalian cytochrome *c*. Thus, the structural features that mediate the subcellular localization of the molecule, the covalent posttranslational addition of a heme prosthetic group, and the catalytic interaction with components of the respiratory apparatus must all be functionally conserved between yeast and mammals. The heterologous system should provide a useful experimental tool for the production of variant mammalian cytochromes for use in biochemical studies as well as for the analysis of their biological function *in vivo*.

We are indebted to Dr. T. Donahue for instructing us in the basic techniques of yeast genetics and for his valuable advice and criti-

cisms throughout the course of this work. We thank Dr. S. Liebman for *CYC1* deletion strain D234-10A, Dr. E. Goldberg for antiserum raised against mouse cytochrome *c*, Dr. B. Anderson for help with immunological techniques, and S. Thomas for technical assistance. This work was supported by Grant GM32525 from the National Institutes of Health.

- Scarpulla, R. C., Agne, K. M. & Wu, R. (1981) *J. Biol. Chem.* **256**, 6480-6486.
- Scarpulla, R. C., Agne, K. M. & Wu, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 739-743.
- Scarpulla, R. C. (1984) *Mol. Cell. Biol.* **4**, 2279-2288.
- Sherman, F., Taber, H. & Cambell, W. (1965) *J. Mol. Biol.* **13**, 21-39.
- Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929-1933.
- Struhl, K. (1983) *Nature (London)* **305**, 391-396.
- Hennig, B., Kohler, H. & Neupert, W. (1983) in *Mitochondria 1983*, eds. Schweyen, R. J., Wolf, K. & Kaudewitz, F. (de Gruyter, New York), pp. 551-562.
- Margoliash, E. (1980) in *The Evolution of Protein Structure and Function*, eds. Sigman, D. S. & Brazier, M. A. B. (Academic, New York), pp. 299-321.
- Nolan, C. & Margoliash, E. (1968) *Annu. Rev. Biochem.* **37**, 727-790.
- Sherman, F. & Stewart, J. W. (1971) *Annu. Rev. Genet.* **5**, 257-296.
- Sherman, F., Stewart, J. W., Parker, J. H., Inhaber, E., Shipman, N. A., Putterman, G. J., Gardisky, R. L. & Margoliash, E. (1968) *J. Biol. Chem.* **243**, 5446-5456.
- Sherman, F., Fink, G. R. & Hicks, J. B. (1979) in *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 17-20.
- Ammerer, G. (1983) *Methods Enzymol.* **101**, 192-201.
- Bennetzen, J. L. & Hall, B. D. (1982) *J. Biol. Chem.* **257**, 3018-3025.
- Carlson, M. & Botstein, D. (1982) *Cell* **28**, 145-154.
- Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408-1412.
- Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201-5205.
- Scarpulla, R. C. & Wu, R. (1983) *Cell* **32**, 473-482.
- Ohashi, A., Gibson, J., Gregor, I. & Schatz, G. (1982) *J. Biol. Chem.* **257**, 13042-13047.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
- Baum, S. G., Horwitz, M. S. & Maizel, J. V. (1972) *J. Virol.* **10**, 211-219.
- Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350-4354.
- Hawkes, R., Niday, E. & Gordon, J. (1981) *Anal. Biochem.* **119**, 142-147.
- Boss, J. B., Gillam, S., Zitomer, R. S. & Smith, M. (1981) *J. Biol. Chem.* **256**, 12958-12961.
- Montgomery, D. L., Leung, D. W., Smith, M., Shalit, D., Faye, G. & Hall, B. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 541-545.
- Lutstorff, U. & Megnet, R. (1968) *Arch. Biochem. Biophys.* **126**, 933-944.
- Denis, C. L., Ferguson, J. & Young, E. T. (1983) *J. Biol. Chem.* **258**, 1165-1171.
- Carlson, S. S., Mross, G. A., Wilson, A. C., Mead, R. T., Wolin, L. D., Bowers, S. F., Foley, N. T., Muijsers, A. O. & Margoliash, E. (1977) *Biochemistry* **16**, 1437-1442.
- Hennig, B., Koehler, H. & Neupert, W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4963-4967.
- Zuker, M. & Stiegler, P. (1981) *Nucleic Acids Res.* **9**, 133-148.
- Johnston, M. & Davis, R. W. (1984) *Mol. Cell. Biol.* **4**, 1440-1448.