A New Type of Fusion Analysis Applicable to Many Organisms: Protein Fusions to the **URA3** Gene of Yeast

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**ABSTRACT**

We have made constructs that join the promoter sequences and a portion of the coding region of the *Saccharomyces cerevisiae* **HIS4** and **GAL1** genes and the *E. coli lacZ* gene to the sixth codon of the *S. cerevisiae** **URA3** gene (encodes orotidine-5'-phosphate (OMP) decarboxylase) to form three in frame protein fusions. In each case the fusion protein has OMP decarboxylase activity as assayed by complementation tests and this activity is properly regulated. A convenient cassette consisting of the **URA3** segment plus some immediately proximal amino acids of **HIS4C** is available for making **URA3** fusions to other proteins of interest. **URA3** fusions offer several advantages over other systems for gene fusion analysis: the **URA3** specified protein is small and cytosolic; genetic selections exist to identify mutants with either increased or decreased **URA3** function in both yeast (*S. cerevisiae* and *Schizosaccharomyces pombe*) and bacteria (*Escherichia coli* and *Salmonella typhimurium*); and a sensitive OMP decarboxylase enzyme assay is available. Also, OMP decarboxylase activity is present in mammals, Drosophila and plants, so **URA3** fusions may eventually be applicable in these other organisms as well.

Protein fusions between a gene of interest and a gene whose activity can be monitored genetically in vivo and assayed biochemically in vitro have been used to investigate a wide range of biological problems. Fusions bearing some protein of interest at the amino terminus and β-galactosidase at the carboxy terminus have been successfully used to characterize regulatory elements, to identify intragenic signal export elements and to distinguish transcriptional from post-transcriptional control (Silhavy and Beckwith 1985; Guarente and Ptashne 1981; Rose, Casadaban and Botstein 1984). A number of other genes have been identified which retain functional activity in protein fusions; these include galK, cat, npt, phoA, dhfr and uidA (Silhavy and Beckwith 1985; Stueber et al. 1984; Gorman, Moffat and Howard 1982; Jefferson, Burgess and Hirsh 1986).

In this paper we describe protein fusions that contain the **GAL1**, **HIS4**, and **lacZ** gene products at the amino terminus and the *Saccharomyces cerevisiae** **URA3** gene product, orotidine-5'-phosphate (OMP) decarboxylase at the carboxy terminus. In each case we find that OMP decarboxylase is functional and that OMP decarboxylase expression is dependent on the regulatory elements of the gene to whose product it is fused. The yeast **URA3** gene product has a number of features that makes it particularly attractive for fusion analysis: (A) There are direct genetic selections both for and against **URA3** function (Boeke, Lacroute and Fink 1984). (B) An extremely sensitive assay for OMP decarboxylase activity exists (Lieberman, Kornberg and Simmons 1955; Rose, Grisafi and Botstein 1984). (C) OMP decarboxylase is small (monomer 25 kD) and active as a dimer, and may therefore be more tractable for some purposes than β-galactosidase, which is large (monomer 116 kD) and functions only as a tetramer (Rose and Botstein 1983; Brody and Westheimer 1979; Contaxis and Reithel 1971). (D) The same enzymatic activity is found in *Escherichia coli*, Drosophila, plants and higher animal cells, and nucleotide analogs have been used in some of these organisms to select against OMP decarboxylase function (Jones 1980). Therefore, the possibility exists that the **URA3** fusion cassette described here can be used for genetic analysis in a wide range of organisms.

**MATERIALS AND METHODS**

**Bacterial strains:** MM294 (F-, endoA, hsdR, supE44, thiA) was the standard strain used for plasmid amplification and DNA manipulation (Guarente *et al.* 1980), KC-8 (rx1486, M*, K12, leuB600, trpC9830, pyrF::Tn5, hisB463, ΔlacX74, strA, galU, galK) was provided by K. Struhl. KC-8 transformed with pNK627 (relevant genotype tet, lacI, N. Kleckner laboratory collection) was used to assay the **lacZ-URA3** gene fusions. **lacZ-URA3** gene fusion products were identified in NK5830 (F' lacI, lacI-ΔL8, Ara-, Mer-, NalR, Rif', srl::Tn10, recA56, argEam, supE, ΔalcproN8) (Roberts *et al.* 1985).

**Yeast strains:** DBY745 (MATα, adel-100, leu2-3, leu2-112, ura3-52) was obtained from D. Botstein. NKY280 (MATα, adel-100, leu2-3, leu2-112, ura3-52, trp1::hisG) bears a 1.1-kb insertion of Salmonella hisG DNA in the **TRP1** gene of DBY745. The construction of this strain is
described elsewhere (Alani, cao and KLECKNER 1987). TD50 (MAA \( \text{his}^{s-619}, \text{ura}^{3-52} \)) was kindly provided by Tom DONAHUE ( DONAHUE, FARABAUGH and FINK 1982).

**Media:** E. coli were grown in LB broth or on LB agar. E. coli used for p790: Tn10 complementation experiments were grown in M9 salts supplemented with 0.2% glucose and 0.004% each of histidine, tryptophan and leucine. Ampicillin and tetracycline were supplemented at 100 \( \mu \text{g} \)/ml and 15 \( \mu \text{g} \)/ml, respectively. Yeast were grown in either YPD or minimal selective media. Selective media contained 0.7% yeast nitrogen base (Difco), 2% agar and 2% glucose. Addition of 0.004% leucine, tryptophan, adenine and uracil was made according to strain requirements. In the galactose induction study, yeast were grown in media containing 2% of each of the following carbon sources: galactose plus sucrose, galactose plus glucose and galactose. 5-Fluoroorotic acid (5-FOA) was purchased from SCM Specialty Chemicals, Gainesville, Florida. 5-FOA plates were prepared as described previously (Borke, Lacroix and Fink 1984).

**Yeast transformation:** Yeast lithium acetate transformation were performed by standard methods (Ito et al. 1983). Integration of the HIS4-URA3 fusion into NKY280 was performed as follows: pNKY54 was digested with SpH1 and the 4.5-kb fragment bearing the HIS4-URA3 fusion and the TRP1 gene was introduced into NKY280 by homologous recombination between the 5' and 3' ends of the fragment and the chromosomal HIS4 locus. Sixteen Trp+ transformants were tested for uracil and histidine prototrophy. Eleven of these candidates were Ura+, sensitive to 5-FOA and 1.1-kb fragment bearing the URA3 fusion cassette was isolated and inserted into the SpH1 site of pYEP24 with a partial HindIII digest. A 4.0 kb SpH1 fragment encoding HIS4 (isolated from a B54 plasmid molecule whose SstI site had been converted into an SpH1 site) was inserted into the SpH1 site of pNKY2003 to form pNKY2005. A 2.5-kb BglII fragment bearing LEU2 was isolated from YEP13 and inserted into the BamHI site of pNKY2005 to form pNKY2010. Finally, a 1.5-kb BglII-BamHI fragment encoding the URA3 gene was isolated from a YEP24 derivative containing a BglII linker at the EcoRI site closest to the 5' end of URA3 and inserted into the BglII site of pNKY2009 to form pNKY2024 (Figure 1).

**B184:** B115, a pBR322 derived plasmid bearing the distal region of HIS4 was digested with BglII and integrated into the HIS4C locus of TD50 (his4-619). Chromosomal DNA of this integrant was made, cleaved with BamHI, ligated and transformed into MM294. Plasmids bearing the entire HIS4 locus (12 kb) were isolated by restriction mapping.

**pNKY2028:** pNKY2028 was made by replacing the 1.4-kb SpH1-XhoI fragment which spans the HIS4 initiation codon in pNKY2024 with the corresponding fragment from B118.

**pNKY54:** pNKY2024 was digested with Smal and BstE2 and a BglII 8-bp linker was inserted to form pNKY2029. After a BglII linker was inserted into the EcoRI site of YRP7 closest to the 5' end of the TRP1, an 850-bp BamHI-BglII fragment containing an intact TRP1 gene was isolated and inserted into the BglII site of pNKY2029. An SpH1 fragment from pNKY2029 which encodes the HIS4-URA3 fusion and the TRP1 gene was isolated and inserted into the SpH1 site of pBR322 to form pNKY54.

**pNKY48:** In order to sequence the HIS4-URA3 fusion junction, a HindIII fragment from pNKY2029, which encodes 93 amino acids of HIS4 fused to 267 amino acids of URA3, was isolated and inserted into the HindIII site of pYK47. The sequencing primer in pNKY48 is 290 bp from the HIS4-URA3 fusion junction. The backbone for the construct, pNKY47, is a derivative of pGCl. To make pNKY47, the existing HindIII site in pGCl was destroyed by a T4 polymerase filling in reaction and a new HindIII site was created in the poly linker by inserting an 8-bp HindIII linker into the BamHI site (filled in with T4 DNA polymerase, BamHI site regenerated). pNKY59,60: pNKY48 was digested with HindIII and the 1.1-kb fragment bearing the URA3 fusion cassette was isolated and inserted into the HindIII site of pUR290. The in-frame construct is pNKY59 and the opposite orientation construct is pNKY60.

**pNKY1069:** An EcoRI octamer linker was inserted into the HindIII site of pNKY48 closest to the primer to form pNKY70. pNKY70 was then digested with EcoRI and the 1.1-kb HIS4-URA3 EcoRI fragment was inserted into the EcoRI site of pRY116. The fusion construct (pNKY71) was selected and a 1.9-kb BamHI-SaiII fragment from pNKY71 (bearing the GAL1-URA3 fusion) was inserted into the Sall and BamHI sites of YRP7 to form pNKY1069.

**Protein gels:** A 1.0 M culture of NK5830 transformed with the lacZ-URA3 protein fusion plasmid (pNKY59) was grown to exponential phase, treated with 1 mM isopropyl \( \beta \)-D-thiogalactopyranoside (IPTG) and plated on 2% agar with 0.2 M EDTA, 0.5 M Tris, 5% Triton X-100, pH 8.0; and treated with 10 \( \mu \text{g} \) of lysozyme for 5 min on ice. After an equal volume of 0.1% SDS loading buffer was added, the samples were boiled for 1.5 min and then applied to a 5% Laemmli gel. Gel was prepared and run according to standard procedures and stained with Coomassie brilliant blue R250 (Laemmli 1970).

**Nucleic acid techniques:** All restriction enzymes, BAL31, T4 DNA ligase and T4 DNA polymerase were purchased from New England Biolabs and used according to manufacturer's specifications. Plasmid DNA was isolated by a cleared lysate protocol and DNA manipulations were described previously (Maniwa, Frisch and Sambrook 1982). DNA sequencing was described previously (Messing 1983).

**Yeast lithium acetate transformation:** Yeast transformation was performed by standard methods (Ito et al. 1983). Integration of the HIS4-URA3 fusion into NKY280 was performed as follows: pNKY54 was digested with SpH1 and the 4.5-kb fragment bearing the HIS4-URA3 fusion and the TRP1 gene was introduced into NKY280 by homologous recombination between the 5' and 3' ends of the fragment and the chromosomal HIS4 locus. Sixteen Trp+ transformants were tested for uracil and histidine prototrophy. Eleven of these candidates were Ura+, sensitive to 5-FOA and 1.1-kb fragment bearing the URA3 fusion cassette was isolated and inserted into the SpH1 site of pYEP24 with a partial HindIII digest. A 4.0 kb SpH1 fragment encoding HIS4 (isolated from a B54 plasmid molecule whose SstI site had been converted into an SpH1 site) was inserted into the SpH1 site of pNKY2003 to form pNKY2005. A 2.5-kb BglII fragment bearing LEU2 was isolated from YEP13 and inserted into the BamHI site of pNKY2005 to form pNKY2010. Finally, a 1.5-kb BglII-BamHI fragment encoding the URA3 gene was isolated from a YEP24 derivative containing a BglII linker at the EcoRI site closest to the 5' end of URA3 and inserted into the BglII site of pNKY2010 to form pNKY2009. BAL31 deletions were performed at the BglII site of pNKY2009 to form the protein fusion vector pNKY2024 (Figure 1).

**Integration of the HIS4-URA3 fusion into NKY280 was made, cleaved with BamHI, ligated and transformed into MM294. Plasmids bearing the entire HIS4 locus (12 kb) were identified by restriction mapping.**

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**pNKY1069:** An EcoRI octamer linker was inserted into the HindIII site of pNKY48 closest to the primer to form pNKY70. pNKY70 was then digested with EcoRI and the 1.1-kb HIS4-URA3 EcoRI fragment was inserted into the EcoRI site of pRY116. The fusion construct (pNKY71) was selected and a 1.9-kb BamHI-SaiII fragment from pNKY71 (bearing the GAL1-URA3 fusion) was inserted into the Sall and BamHI sites of YRP7 to form pNKY1069.
**RESULTS**

**Construction of HIS4-URA3 gene fusion:** Our goal in this study was to first make URA3 gene fusions that retained OMP decarboxylase activity and then to construct a URA3 cassette that could be inserted into any gene of interest to create a fusion protein that expresses OMP decarboxylase. The *URA3* gene, which encodes a 267 amino acid protein, has been cloned and sequenced on an 1100-bp DNA segment (Rose, Grisafi and Botstein 1984). Our strategy was to make a collection of random deletions at the 5' end of *URA3* and fuse these deletions to the 3' end of a well-characterized gene and then use this fusion as a cassette to make other *URA3* fusions.

As an initial step toward constructing a *URA3* fusion cassette, we chose to make fusions of *URA3* to the yeast *HIS4* gene. The *HIS4* gene encodes a single translation product with A, B and C domains. Each domain is responsible for a separate biosynthetic step in histidine biosynthesis (Donahue, Farabaugh and Fink 1982). *HIS4-URA3* fusions which express OMP decarboxylase were built in two steps (Figure 1). An intact *URA3* gene was inserted into a plasmid bearing the C domain of *HIS4* and an initial BAL31 treatment was performed to eliminate the *URA3* start signals without deleting into the coding sequence. A second BAL31 treatment was performed on one appropriate deletion variant in such a way as to simultaneously delete the *URA3* start codon and fuse the remaining codons of *URA3* to *HIS4*.

The characterization of *HIS4-URA3* fusion constructs described involved a 2μ plasmid which is present in high copy. To assess more sensitively the OMP decarboxylase activity of the fusion protein, we examined the *URA3* expression of a single copy *HIS4-URA3* construct that had been integrated into the yeast genome at the *HIS4* locus. We found that the single copy *HIS4-URA3* fusion still complements the *ura3-52* mutation, and furthermore, cells bearing this single copy fusion are still sensitive to 5-FOA. These results suggest that the *HIS4-URA3* fusion protein probably retains a reasonably high specific activity.

**URA3 protein fusions to other genes:** The above results demonstrate that *HIS4-URA3* protein fusions can be made which express *URA3* function. To show that the OMP decarboxylase is active in a *URA3* fusion...
that bears some gene product of interest at the amino terminus, we made lacZ-URA3 and GALI-URA3 gene fusions (Figure 2) using a cassette derived from the HIS4-URA3 fusion (Figure 3). Both URA3 fusions contain a 93 codon bridge of HIS4 sequence proximal to the URA3 cassette because a restriction site is not present at the exact fusion junction between HIS4C and URA3.

It has been previously shown that the yeast URA3 gene product can complement E. coli strains that are deficient in OMP decarboxylase (pyrF::Tn5) (ROSE, GRISAFI and BOTSTEIN 1984). We constructed lacZ-URA3 protein fusions that express OMP decarboxylase in E. coli in response to IPTG induction. When induced, this fusion complements E. coli pyrF mutants. Also, an intact fusion protein having the size predicted of a continuous lacZ-URA3 encoded polypeptide was detected on Laemmli gels.

We inserted the URA3 fusion cassette in both orientations at the 3' end (polylinker site) of the lacZ gene of pUR290. pUR290 bears the lacZ gene under lac promoter and operator control and this vector is designed to generate fusions to the carboxy terminus of lacZ. The URA3 cassette inserted into pUR290 in the correct orientation is predicted to specify a 150-kd polypeptide (pNKY59). The cassette insertion in the opposite orientation serves as a negative control (pNKY60). Both constructs were introduced into the E. coli strain KC-8 bearing pNK627 (relevant genotype pyrF::Tn5, ΔlacX74, lacIq). As shown in Table 1, only background growth was observed for cells grown without IPTG. We believe that this growth is due to leakiness of the pyrF::Tn5 insertion. At levels of IPTG greater than 0.1 mM, the pyrF::Tn5; cells bearing the in-frame fusions were able to grow on M9 media lacking uracil. The opposite orientation construct failed to complement the pyrF mutant in all cases.

Thus the above results suggest that URA3 expression is dependent on the lac promoter. Also, the lacZ-URA3 fusion transformants of pNKY59 grew as bright blue colonies on XG (5-bromo-4-chloro-3-indolyl-ß-D-galactoside) plates with and without IPTG, suggesting that the fusion also expresses ß-galactosidase activity. The OMP decarboxylase and ß-galactosidase activities in fusion proteins derived from pNKY59 are present in a single physical fusion polypeptide. The lacZ-URA3 fusion construct was introduced into NK5830 (relevant genotype lacIq). Exponentially growing cells were induced with IPTG for two hours and total cell protein was examined on a 5% Laemmlı gel. As shown in Figure 4, a 150-kD protein which matches the size predicted by the lacZ-URA3 fusion protein was specifically induced by IPTG.

As a second example to assess the general applications of the URA3 fusion cassette, we constructed a GALI-URA3 gene fusion that encodes a 385 amino acid polypeptide (Figure 2) consisting of 28 amino acids derived from the GALI gene product and the remaining 357 amino acids from the URA3 fusion cassette. This fusion was inserted into a high copy ARS vector and transformed into the yeast strain NKY280 (relevant genotype ura3-52, trp1Δ). Transcription of the wild-type GAL1 gene is activated by galactose and repressed to undetectable lev-
Yeast URA3 Protein Fusions

FIGURE 3. — URA3 fusion cassette. Construction of pNKY48 and pNKY70 are described in MATERIALS AND METHODS. H = HindIII, B = BamHI, Bg = BglII, N = NcoI, E = EcoRI, C = ClaI, Xb = XbaI, X = XhoI, S = SalI.

TABLE 1
Average colony diameter (mm) of E. coli strain KC-8 (pyrF) bearing NK627 (laeig) and lacPOZ-URA3 fusion plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Minimal media</th>
<th>Minimal media + uracil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IPTC concentration (mM):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10^{-2}</td>
</tr>
<tr>
<td>pUR290 (lacPOZ)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>YEP24 (URA3)</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>pNKY59 (lacPOZ-URA3)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>pNKY60 (lacPOZ-ura3)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Fresh single colony transformants of E. coli KC-8 were diluted in 0.85% NaCl and plated onto petri dishes containing M9 media, plus various concentrations of IPTG. Approximately 30 colonies were plated onto each dish and the efficiency of plating was 1.0. Colony diameter was determined after 3 days growth at 37°.

DISCUSSION

We have constructed hybrid proteins that contain a bacterial or yeast gene product at the amino terminus and the URA3 gene of S. cerevisiae at the carboxy terminus. A convenient URA3 cassette has been constructed for use in generating URA3 fusions to other genes of interest. Since all three of the hybrid proteins described here show URA3 specified OMP decarboxylase activity, it seems likely that most fusions of URA3 product to other gene products will behave in a similar fashion.

Advantages and applications of URA3 fusion analysis: The yeast URA3 gene product has a number of features which make it particularly attractive for fusion analysis. First and most important, there are direct genetic selections both for and against URA3 function. Increase in function is selected by requiring growth of an OMP decarboxylase deficient mutant on defined medium in the absence of uracil. Decrease or absence of function is selected by requiring growth of such a mutant in the presence of both uracil and 5-FOA. Second, there exists a sensitive assay for OMP decarboxylase activity: release of $^{14}$CO$_2$ from [14C]orotidine 5'-phosphate (LIEBERMAN, KORNBERG and SIMMONS 1955; ROSE, GRISAFI and BOTSTEIN 1984). Third, the native enzyme has several features that help make derived fusion proteins especially tractable: the monomer polypeptide is small (27 kD) and the active form of the enzyme is a simple dimer and is cytosolic (BRODY and WESTHEIMER 1979; JONES 1980).

Gene fusion analysis is frequently applied to analysis of gene regulation. The URA3 fusions are clearly suitable for this application, since expression of OMP
**TABLE 2**

Average colony diameter (mm) of yeast strain NKY280 (ura3-52, trp1) bearing GALI-URA3 fusion plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Minimal media</th>
<th>Minimal media + uracil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GLU</td>
<td>GAL</td>
</tr>
<tr>
<td>YRP7 (TRP1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pNKY1069 (GAL1-URA3, TRP1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fresh single colony transformants of *S. cerevisiae* NKY280 were diluted in water and plated onto yeast minimal media supplemented with the carbon sources shown above. Approximately 30 colonies were plated onto each dish and the efficiency of plating was 1.0 in all cases where colonies were visible. Colony diameter was measured after 5 days growth at 30°C.

![FIGURE 4](image_url)

**FIGURE 4.**—IPTG dependent induction of a 150-kD protein in an *E. coli* strain bearing *lacZ-URA3* fusion plasmids. NK5830 (*laci*) transformed with either pUR290 (*laciPOZ*) or pNKY59 (*laciPOZ-URA3*) were grown to exponential phase and induced with 1 mM IPTG (see MATERIALS AND METHODS). Total cell protein was run on a 5% Laemmli gel and stained with Coomassie brilliant blue R250. The induced 116-kD polypeptide observed in NK5830 bearing pUR290 corresponds to the *β*-galactosidase protein in the molecular weight standard mix (S). + = IPTG induction, − = no IPTG induction.

decarboxylase activity is properly controlled by upstream regulatory regions in all three of the fusions described above. Enzymatic assays of cells containing a protein fusion can be used to quantitate changes in the level of a gene product, and with some assumptions about the specific activity of the fusion protein, to obtain a rough idea about the absolute level of the product present in the cell. The dimeric nature of the active enzyme make *URA3* fusions more attractive than the popular *lacZ* fusion constructs for this type of analysis. In yeast especially, genes of interest are often expressed at such low levels that protein fusions to *β*-galactosidase do not express a detectable level of enzyme in standard ONPG assays (for example, HUISMAN et al. 1987). Also, in both yeast and in bacteria, measured *β*-galactosidase activity is artificially low in whole cell assays if cells are producing an average of fewer than four (active) monomers per cell per generation (for example, RAILEIGH and KLECKNER 1986). Only a minority of cells which by chance contain at least four monomers contribute to the measured enzymatic activity, a problem which can lead to underestimation of expression levels and to overestimation of induction ratios when gene expression is regulated. This problem should be less severe for *URA3* fusions, where only two monomers per cell are required for an active (dimeric) molecule (BRODY and WESTHEIMER 1979).

Most important for gene expression analysis, the positive and negative genetic selections can be used to search for linked and unlinked regulatory mutations and for structural gene mutations that increase or decrease expression under conditions of interest. The one significant disadvantage of the *URA3* fusion system is the absence of any colorimetric colony assay for OMP decarboxylase function.

Gene fusion analysis has also been applied effectively to the analysis of protein localization and secretion, both in identifying and following the locations of particular proteins, and for selecting mutants that are altered in their localization or secretion behavior. *URA3* fusions should be readily applicable to this type of analysis as well. It should be straightforward to raise antibodies to yeast OMP decarboxylase because the enzyme can be purified to homogeneity in only two chromatographic steps (BRODY and WESTHEIMER 1979). The small size of the *URA3* fusion polypeptide may make it particularly advantageous for secretion and localization studies, which can sometimes be hampered by the failure of a large molecule such as *β*-galactosidase to be properly handled.

Finally, gene fusions are often used as a tool for both purifying proteins and generating antibodies to polypeptide sequences of interest. *URA3* fusions can be used for this purpose. A highly specific affinity column based on a competitive inhibitor of OMP decarboxylase (6-azauridine 5′-phosphate) has been used to purify the native protein and should be equally applicable to the purification of fusion proteins (BRODY and WESTHEIMER 1979).

**General applicability of *URA3* fusion analysis to**
many organisms: We have described above the analysis of URA3 protein fusions in two organisms, the bacterium *E. coli* and the yeast *S. cerevisiae*. The analysis described above can be applied essentially without modification to other bacteria; (Salmonella typhimurium) and another yeast (Schizosaccharomyces pombe). In both of these organisms mutants deficient in OMP decarboxylase have been identified and such mutants can be positively selected with 5-FOA (Boeke, Lacroute and Fink 1984; G. Fink, personal communication).

It seems likely that URA3 fusion analysis can eventually be applied to many other types of organisms. OMP decarboxylase activity is universal; it has been identified in Drosophila, mammals and plants (Jones 1980; G. Fink, personal communication). Also, mutations which either enhance or decrease OMP decarboxylase activity have been identified in many of these organisms. In mouse tissue culture, for example, fluorouracil has been used to select mutants which have reduced levels of OMP decarboxylase activity (Levinson, Ullman and Martin 1979). The URA3 gene should be equivalent to other reporter genes such as *cat*, *dhfr*, and luciferase for direct analysis of transcript formation (Stueber et al. 1984; Gorman, Moffatt and Howard 1982; Ow et al. 1986). In organisms where suitable mutants lacking endogenous OMP decarboxylase activity can be isolated, URA3 should also be as useful as other systems for assaying transient expression of the gene product itself. The most unique potential advantage of the URA3 system would be its applicability to genetic analysis for the selection of mutations that either increase or decrease the expression of OMP decarboxylase.

We are grateful to David Botstein for pointing out many of the advantages of URA3 fusion analysis and for encouraging us to develop the protein fusion cassette, to Patrick Errada, Paul McDonald, Richard Lynn and Jim Lilley for advice on DNA sequencing and fusion protein detection, and to Anna Ferris for manuscript preparation. E.A. was funded by a National Institutes of Health Genetics Training Grant awarded to the Department of Biochemistry and Molecular Biology.

**LITERATURE CITED**


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