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Genetics

Yeast genes fused to β-galactosidase in *Escherichia coli* can be expressed normally in yeast

(deletion selection *in vivo*/*lacZ* gene fusions/*URA3* gene/*Saccharomyces cerevisiae*/regulation)

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ABSTRACT **A plasmid was constructed that allows the selection *in vivo* of gene fusions between the *Escherichia coli* β-galactosidase gene and the yeast (*Saccharomyces cerevisiae*) *URA3* gene. A large yeast DNA fragment containing the *URA3* gene was placed upstream of an amino-terminally deleted version of the *lacZ* gene. The plasmid vehicle contains sequences that allow selection and maintenance of the plasmid in both yeast and *E. coli*. Selection for Lac$^+$ in *E. coli* yielded numerous deletions that fused the *lacZ* gene to the *URA3* gene and flanking yeast sequences, to the bacterial tetracycline-resistance gene from the parent plasmid pBR322, and to the yeast 2-μm plasmid DNA. Some of these fusion plasmids produced β-galactosidase activity when introduced into yeast. One of the fusions to the *URA3* gene itself has been shown to place the expression of β-galactosidase activity under uracil regulation in yeast.**

Gene and operon fusions to the *Escherichia coli* gene specifying β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) (the *lacZ* gene) have become powerful and increasingly important tools in prokaryotic molecular genetics. Fusions have been particularly useful in studies of the signals that regulate expression of genes; the activity of these signals can easily be assayed (as β-galactosidase activity) without ambiguities introduced by the presence of variable amounts of the normal gene product(s) (1–5). In this paper, we describe a general way in which this powerful analytic tool can be applied to the study of the genes of the simple eukaryote *Saccharomyces cerevisiae* (baker's yeast).

The yeast gene that we chose as a target for gene fusions with the *lacZ* gene was the *URA3* gene, which encodes the enzyme orotidine-5′-monophosphate decarboxylase (orotidine-5′-phosphate carboxy-lyase, EC 4.1.1.23). The *URA3* gene is regulated in yeast (6–8) and, thus, could serve as a good test of whether one can obtain proper regulation of gene fusions in yeast. Further, the *URA3* gene has been cloned in *E. coli* and functions in this host to complement *pyrF* mutations (8).

The fusion system we used makes use of a previously described (9, 10) fragment of the *lacZ* gene that lacks the information encoding the amino terminus of the β-galactosidase protein. For this reason, the protein cannot be produced from this fragment. However, fusion of the truncated gene into the proper reading frame of another gene should result in production of β-galactosidase activity, because Beckwith and others have shown that substitution of up to about 30 amino-terminal residues of β-galactosidase with a variety of other protein sequences results in a hybrid protein with substantial β-galactosidase activity (11). Further, unpublished experiments suggested that certain random DNA sequences from yeast fused to *lacZ* result in expression of β-galactosidase in yeast.

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The *lacZ* fragment was put into a plasmid cloning vector that can be selected for and maintained in both *E. coli* and *S. cerevisiae*, and the *URA3* gene was placed in front. Fusions were selected *in vivo* in *E. coli* on the basis of their ability to produce β-galactosidase. Analysis of fusions by physical methods allowed the identification of several as authentic fusions between *URA3* and *lacZ*. When introduced into yeast, these produce β-galactosidase activity, which is regulated in a way essentially identical to the regulation of the intact *URA3* gene.

MATERIALS AND METHODS

Strains and Media. *E. coli* strain DB4572 [(*lacIPOZY*) ▽X74 *galU galK strA pyrF*74::Tn5] was made by P1 transduction of the *pyrF*74::Tn5 into strain M182 (from J. Beckwith). *S. cerevisiae* strain DBY689 (MAT*a* *ura3*-50 *leu2*-3 *leu2*-112) was constructed by crossing the transformable strain AH22 (which carries the two *leu2* mutations; ref. 12) with a derivative of strain FL100 (gift of F. Lacroute) in which the nonreverting *ura3*-50 mutation had been isolated. Yeast strain DBY 941 (MATα *ura1*-21 *leu2*-3 *ade2*-1) was constructed by crossing in the *ura1* mutation from a derivative of FL100 (from F. Lacroute) into strain 294 (MATα *leu2*-3 *ade2*-1 *his4*-712, gift of G. R. Fink). Published methods were employed to transform *E. coli* (13) and yeast (12) with plasmid DNA.

E. coli were grown in LB (complete) or M-9 (minimal) medium (14); ampicillin was added where appropriate to a final concentration of 100 μg/ml. Yeast were grown in YEP-glucose (complete) or SD (minimal) medium (15); conditions for crosses and sporulation were standard (15).

Plasmid DNA Isolation and Analysis with Restriction Endonucleases. A modification (16) of the method of Clewell and Helinski (17) was used to prepare plasmid DNA from *E. coli*. Such preparations were used for transformation of either yeast or *E. coli* and for analysis with restriction endonucleases (New England BioLabs).

Construction of Plasmid pRB45. The components of pRB45 (Fig. 1) consist of most of the well-known plasmid pBR322 (18, 19), the *Eco*RI A fragment from the 2-μm plasmid of yeast (20, 21), a *Bam*HI–*Bgl* II fragment of yeast chromosomal DNA carrying the *URA3* gene, a *Sal* I–*Xho* I fragment of yeast chromosomal DNA carrying the *LEU2* gene (12), and a *Bam*HI–*Sal* I fragment of *E. coli* chromosomal DNA carrying most of the *lac* operon but missing all of the promoter, operator, and 22 base pairs (7⅓ amino acid codons) of the amino-terminal coding sequence for β-galactosidase (*lacZ*). The construction and uses of the *lac* fragment have been published (9, 10).

Isolation and Characterization of Lac$^+$ Derivatives of pRB45. Many isolated clones of *E. coli* strain DB4572 containing pRB45 were grown to saturation in LB medium containing

Abbreviation: kb, kilobase.

ampicillin, washed twice with M-9 medium containing no sugar, and spread on plates with M-9 medium containing 0.2% lactose and 40 μg of uracil per ml, which were then incubated at 37°C. After 2 days of incubation, Lac^+ colonies appeared at a frequency of about 8×10^{-9}, increasing to about 10^{-7} after 5 or more days. Candidate colonies were purified on plates with LB medium containing ampicillin and were tested for growth in the absence of uracil on plates containing M-9 medium (0.2% glucose), for growth and color on MacConkey agar (Difco) containing 1% lactose, and again for growth on plates with M-9 minimal medium containing 0.2% lactose. In later experiments, Lac^+ colonies on the original selection plates were replica-plated directly to M-9 medium containing glucose (no uracil) to identify Pyr^- candidates. Lac^+ derivatives certain to be independent were cultured and their plasmid DNA was extracted and analyzed with restriction endonucleases as described above.

Assay for β-Galactosidase. *Plate assays.* Bacteria were spotted onto two kinds of plates. Control experiments showed that growth on M-9 minimal medium containing lactose required about 10 units of activity whereas a minimum of about 100 units was required to produce any red color on MacConkey-lactose medium; higher levels could be distinguished on the latter medium as well. Yeast were spotted on plates with SD minimal medium containing glucose and the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside; Bachem Fine Chemicals, Torrance, CA). Double-strength SD medium was buffered at pH 7 by addition of 0.1 M KH_2PO_4, titrated with KOH, and filter-sterilized, after which X-Gal in *N,N*-dimethylformamide was added to a final concentration of 40 μg/ml. An equal volume of molten agar was then added along with sterile glucose to a final concentration of 2% (wt/vol).

Enzyme assay. Bacterial assays were performed by the standard method (14); cells were grown in LB medium containing ampicillin, washed, and resuspended in M-9 medium. Yeast were assayed by growing cultures in minimal medium selecting Leu^+; saturated overnight, cultures were diluted and allowed to double before assay. Cells were chilled for 15 min, centrifuged, and resuspended in 0.1 or 0.05 vol of 0.1 M Tris·HCl, pH 8.0/20% (vol/vol) glycerol/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride (Sigma). Glass beads (0.45 mm, VWR Scientific) were added to the meniscus, and the mixture was frozen at −20°C. Cell extracts were made by vigorous shaking on a Vortex mixer (six times, 15 sec each) at 4°C. An equal volume of the above buffer was added, and the solution was clarified by centrifugation for 15 min in an Eppendorf centrifuge. β-Galactosidase activity was determined as for the bacterial assay, except that the activity was normalized to protein concentration in the extract. Protein was measured using the dye-binding method of Bradford (ref. 22; Bio-Rad) with bovine serum albumin as the standard. For starvation experiments, exponentially growing cells were washed and resuspended in prewarmed medium supplemented with either 1 or 50 μg of uracil/per ml. After incubation with shaking for about one doubling time (about 150 min; the starving cultures had stopped growing), extracts were prepared and assayed as above.

RESULTS

In order to obtain gene fusions between the yeast *URA3* gene and the *E. coli lacZ* gene that could be introduced into both *E. coli* and *S. cerevisiae*, we constructed the plasmid pRB45 (Fig. 1). The plasmid contains a bacterial replication system, a gene (amp^R) allowing selection in *E. coli*, a segment of yeast 2-μm DNA to allow autonomous replication in yeast, and a gene (*LEU2*) that can be selected in yeast.

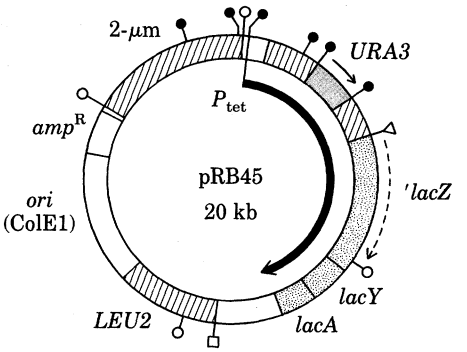

FIG. 1. Structure of pRB45. Yeast sequences, except for the *URA3* gene, are hatched. The stippled area is derived from the *E. coli* lac operon and was obtained from plasmid pMC874 (9, 10). Other areas are derived from pBR322 (18). The tetracycline-resistant gene promoter initiates transcription in a clockwise direction. The *URA3* gene and the *'lacZ* gene are both oriented so that clockwise transcription is from the sense strand. Restriction enzyme sites are as follows: ♀, *Eco*RI; ♦, *Hin*dIII; ♈, *Bam*HI; ♑, *Sal* I.

The coding information in the *'lacZ* fragment in pRB45 begins in the eighth residue from the amino terminus of the β-galactosidase protein and continues through the *lacY* and *lacA* genes. The fragment cannot be expressed unless fused because it lacks the signals for initiation of both translation and transcription. The *'lacZ* fragment has a *Bam*HI restriction target at its amino-terminal (with respect to β-galactosidase) end; this is the only *Bam*HI site in pRB45. The yeast *URA3* gene lies in a section of yeast DNA placed just in front of the *'lacZ* fragment; the coding region for orotidine-5'-monophosphate decarboxylase is completely contained within the central *Hin*dIII fragment, as shown in Fig. 1. About 1.9 kilobases (kb) of 5' flanking yeast DNA is present, making it very likely that all required regulatory sequences from yeast were retained. The source of the *URA3* gene is the yeast strain FL100, which we have shown contains no promoter capable of initiating transcription of the *URA3* gene in *E. coli* (unpublished data). Expression of the *URA3* gene in pRB45 is dependent upon a bacterial promoter (p_{tet}; from the tetracycline-resistance gene of the parent plasmid pBR322) which lies beyond the *URA3* gene segment. A control plasmid with an intact *lacZ* gene (but no promoter) was constructed (not shown); this plasmid makes β-galactosidase, indicating that there are no strong transcription termination sites in the yeast sequences between p_{tet} and *lacZ* in pRB45.

The structure of pRB45 suggested that the only reason for the failure to produce β-galactosidase was the lack of a functional initiation of translation. To apply selection for Lac^+, pRB45 was introduced by transformation into an *E. coli* host, DB4572, which carries a deletion of the *lac* operon and a Tn5 insertion mutation in the *pyrF* gene; the transformants are Lac^- but pyrimidine-independent in phenotype, because the *URA3* gene on the plasmid complements the *pyrF* mutation. Lac^+ derivatives were obtained after 5 days of incubation on plates with minimal medium containing lactose at frequencies up to 1×10^{-7}. About 20% of these mutants were now pyrimidine-requiring, indicating that part or all of the *URA3* gene had been deleted. Plasmids purified from about 50 Lac^+ derivatives were analyzed with restriction enzymes. Extents of deletions were estimated from the changes in sizes of fragments that wholly contain the deletion.

The map of the deletions resulting from selection in *E. coli* for expression of the *lacZ* gene is shown in Fig. 2. It is clear that there are many different classes of deletions that allow expression of β-galactosidase. Each of these must, at a minimum, bring

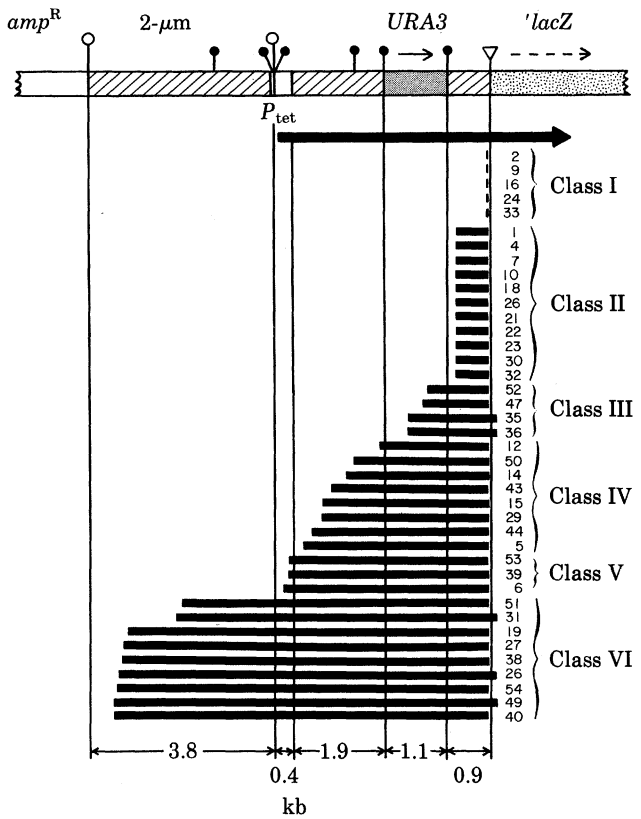

FIG. 2. Deletion map of fusion plasmids selected by Lac$^+$ phenotype in *E. coli*. The structure of the relevant portions of pRB45 are shown at the top. Left to right corresponds to clockwise progress in Fig. 1; the map is approximately drawn to scale. Deletion extents are shown with the right-hand end point assumed to lie close to the *Bam*HI site. Deletions 35, 36, 31, 26, and 49 all remove the *Bam*HI site and so presumably end in the *'lacZ* sequence; the remaining plasmids retain the *Bam*HI site. The various classes are defined in the test and in Table 1. ⚲, *Eco*RI; ⚲, *Hin*dIII; ▽, *Bam*HI.

an initiator codon in frame with the β-galactosidase nucleotide sequence with no intervening nonsense codons. The distribution of deletions shows two classes that account for a considerable fraction of the total. Class I consists of point mutations or small deletions that do not detectably alter the mobility of any restriction fragment. Class II consists of a set of indistinguishable deletions with endpoints in the segment of yeast DNA between the end of the *URA3* gene and the *'lacZ* fragment. These two classes represented "hotspots" of sufficient magnitude that the additional screening of plasmid phenotype for loss of ability to complement the *pyrF* mutation in the host was carried out. Among the Lac$^+$ Pyr$^-$ deletions, several were found that ended within the 1.1-kb *Hin*dIII segment known to contain the *URA3* gene (class III), several ended in yeast DNA segments beyond the *URA3* gene (class IV), a few deletions ended in what remains of the tetracycline-resistant gene of pBR322 (class V) and finally, surprisingly, many deletions ended in the 2-μm DNA segment beyond p_{tet} (class VI). The great variety of deletions that resulted in expression of β-galactosidase activity suggests that there are many sequences which meet the requirements of providing initiation of translation in *E. coli* and that the selection might be satisfied by virtually any initiator codon.

Our primary interest was to determine whether the hybrid proteins detected in *E. coli* could be detected in yeast and whether the β-galactosidase activity of these could be used to follow the regulation of expression of, for example, the *URA3* gene. In the case of fusions to sequences not known to be expressed in yeast, we wanted also to learn whether they would be expressed and, thereby, whether the β-galactosidase fusion system could be used to define transcription and translation start signals in yeast. To these ends, a representative set of the plasmids that conferred a Lac$^+$ phenotype in *E. coli* (i.e., at least one member of each of the classes defined in Fig. 3) was used to transform yeast, selecting for the *LEU2* marker. Expression of β-galactosidase in yeast was determined by observing the color of transformants on plates containing a chromogenic substrate (X-Gal) and by direct assay of β-galactosidase activity in extracts of the transformant yeast cells grown in the absence of leucine, thus selecting maintenance of the plasmid.

Table 1. β-Galactosidase specific activities in *E. coli* and yeast

Class	Point of fusion	Deletion no.	Extent of deletion, kb	β-Galactosidase activity: Yeast	β-Galactosidase activity: *E. coli*
I	At *Bam*HI site	2	<0.1	4.0	58
		9	<0.1	1.0	91
II	Downstream of *URA3*	1	0.7	303	390
		4	0.7	206	352
III	In *URA3* gene	52	1.3	450	663
		47	1.4	3.9	103
		35	1.8	402	626
		36	1.8	362	564
IV	Upstream of *URA3*	12	2.3	<1.0	16
		5	3.9	<1.0	135
V	In *tet*^{R} gene	6	4.1	<1.0	131
VI	In 2-μm DNA	31	6.2	24	770
		19	7.2	<1.0	91
—	pRB45	None	—	2.4	4

Extracts were prepared from transformed yeast cells grown in SD minimal medium with uracil (40 μg/ml), and the presence of the plasmid was selected for by omission of leucine. Extracts were prepared from *E. coli* transformants grown in LB broth containing ampicillin at 100 μg/ml. Activities are expressed as nmol of *o*-nitrophenol-β-D-galactosidase cleaved per min/mg of protein. In yeast, protein was measured directly; in *E. coli* the method of Miller (14) was used in which a culture of cells at a turbidity of 1.4 OD at 600 nm is assumed to contain 1×10^9 cells per ml or 150 μg of protein per ml. For all assays, $A_{420} = 0.0045$ represents 1 nmol of *o*-nitrophenol (14).

The X-Gal test showed that several of the plasmids do express β-galactosidase. Table 1 gives the results of quantitative assays of β-galactosidase activity measured both in *E. coli* and in yeast. Three of the four *URA3-lacZ* (class III) fusions produced about the same β-galactosidase activity in yeast and in *E. coli*. The activity in yeast for one of these was linear with time and with extract concentration (Fig. 3), showing that the activity is quite

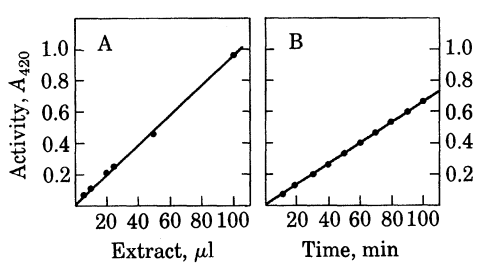

FIG. 3. Assays of β-galactosidase activity synthesized in yeast carrying the *URA3-lacZ* fusion plasmid 35. Extracts were assayed for β-galactosidase activity by using *o*-nitrophenyl-β-D-galactosidase cleavage as described by Miller (14). (*A*) Indicated amounts of extract were assayed for 15 min. (*B*) Extract (10 μl) was assayed for the indicated times. Assays were conducted for up to 24 hr with less than 20% loss of activity (data not shown).

stable in yeast extracts. The fourth *URA3-lacZ* fusion plasmid is exceptional in that it produces less activity in both organisms for reasons not yet understood.

Comparison of β-galactosidase activity in yeast and *E. coli* for the remaining classes of fusion plasmids (Table 1) showed that in addition to fusions to the *URA3* gene, only a few other fusions produced β-galactosidase activity in yeast. Most importantly, none of the fusions to sequences of bacterial origin (e.g., class V, fusions to the tetracycline gene of pBR322) or to yeast sequences upstream of the *URA3* gene (class IV) were expressed. This result implies that expression in yeast requires something additional to what is required in *E. coli*, most likely a source of transcription. In this light, the remaining classes of plasmids that are capable of expressing their fusion proteins [i.e., class II (fused to yeast sequences lying downstream of the *URA3* gene) and class VI (fused to yeast sequences derived from the 2-μm plasmid)] can be interpreted as evidence for normal yeast transcription of the yeast sequences to which *lacZ* was fused.

To demonstrate normal regulation of expression of the *URA3-lacZ* fusion proteins, we examined the response of this expression to conditions known to affect expression of the *URA3* gene. Lacroute and coworkers (6, 7) have discovered that the level of *URA3* gene expression is altered in various *ura*$^-$ mutants; specifically it is elevated (3- to 6-fold) in *ura1*$^-$ strains. Bach *et al.* (8) have shown that this regulation appears to occur at the level of transcription, and more recently (23) it has been shown that regulation is normal (although the absolute level is many-fold elevated) when the *URA3* gene is placed on the yeast 2-μm plasmid. A *URA3-lacZ* (class III) fusion plasmid was introduced into a *ura1*$^-$ genotype yeast strain by mating the haploid yeast strain (genotype *ura3*$^-$ *leu2*$^-$) bearing the plasmid with another haploid strain of opposite mating type carrying the *ura1*$^-$ as well as a *leu2*$^-$ mutation. Diploids with the plasmid were sporulated, and haploid spores retaining the plasmid (recognized by the Leu$^+$ phenotype) and having each of the four combinations of *ura* genotypes were identified. These were grown in the absence of leucine, and extracts were assayed for β-galactosidase activity with or without prior starvation for uracil. Because the yeast hosts that we crossed were not isogenic, several segregants of each type were assayed to minimize the effect of any differences in genetic background that might have segregated among the spores. The averages and standard deviations of β-galactosidase specific activity are shown in Table 2. Consistently higher levels of β-galactosidase activity were found in *ura1*$^-$ strains whether or not the cells had been starved for uracil. β-Galactosidase levels in *ura3*$^-$ strains were inducible. But the strongest result is that the strains with the *ura1*$^-$, *ura3*$^-$ double mutant genotype always showed the full derepressed level of expression. The maximal derepression ratio (about 6-fold) found here for β-galactosidase specific activity is comparable to that found previously for constitutive synthesis of orotidine-5′-monophosphate decarboxylase (the product of the *URA3* gene) both on the chromosome and on the 2-μm plasmid in yeast (8, 23). These data show that the *URA3-lacZ* fusion is under uracil control and strongly support the idea that transcription of the fusion protein begins at the correct yeast promoter.

Table 2. Regulation of β-galactosidase levels in yeast

Relevant genotype	Grown in uracil		Starved for uracil	
	Specific activity*	Ratio†	Specific activity*	Ratio†
URA$^+$	180 ± 89	(1)	241 ± 85	1.3
ura1	434 ± 17	2.4	651 ± 40	3.6
ura3	162 ± 62	0.9	585 ± 174	3.3
ura1, ura3	909 ± 452	5.0	1170 ± 482	6.5

Yeast strain DBY689 containing fusion plasmid 35 was crossed to strain DBY941, and several plasmid-containing spores of the indicated genotypes were obtained. Cells were grown and assayed before and after uracil starvation. Six *URA*$^+$ strains, three *ura1*$^-$ strains, three *ura3*$^-$ strains, and seven *ura1*$^-$, *ura3*$^-$ double mutant strains were assayed.

* Specific activities are expressed as nmol cleaved per min/mg of protein (see Table 1), and the results are ±SEM.

† Ratios are relative to level of wild type grown in uracil.

DISCUSSION

We have fused the *E. coli lacZ* gene to sequences derived from yeast (*S. cerevisiae*) using a method that should be generally applicable. Some of the fused genes produce β-galactosidase activity assayable in yeast. An amino-terminal deletion of the *lacZ* gene described previously (9, 10) was placed into a plasmid vehicle that can be selected and maintained in both yeast and *E. coli*. By selecting expression of β-galactosidase in *E. coli*, we obtained numerous spontaneous deletions that fuse the *lacZ* gene to sequences placed upstream. We recovered and characterized fusions to yeast sequences in and around the *URA3* gene and sequences from the yeast 2-μm plasmid and fusions to the tetracycline-resistant gene of the bacterial plasmid pBR322. Although all the fusions make detectable β-galactosidase activity in *E. coli*, only a subset of the fusions to yeast sequences expressed β-galactosidase activity in yeast. These included fusions to the *URA3* gene itself, a segment of yeast DNA immediately downstream of the *URA3* gene, and a portion of the 2-μm DNA. In yeast, the regulation of expression of β-galactosidase activity from a *URA3-lacZ* fusion was that expected for regulation of the *URA3* gene itself, suggesting that the fusion technique described here can be applied generally to the study of gene regulation in yeast.

The general system for obtaining fusions to yeast genes (or, in principle, genes from any source) depends on three important features of the plasmid pRB45, which we constructed for this purpose. First, as described before (9, 10), the terminally-deleted (′*lacZ*) β-galactosidase gene cannot be expressed without first being fused to a functional amino terminus. In *E. coli* this might be as simple as provision of an AUG codon upstream that is in frame with the β-galactosidase coding sequence and the absence of intervening in-frame nonsense codons. Second, transcription is provided by the bacterial promoter (p_{tet}) which normally transcribes the tetracycline-resistant gene of pBR322. Thus fusions can be detected in *E. coli* even when the target yeast gene contains no signals for initiation of transcription recognized by *E. coli* RNA polymerase. This indeed was a necessity in the case of the form of the *URA3* gene we used, which contains no functional *E. coli* promoter. The provision of transcription makes pRB45 suitable for selection in *E. coli* of fusions to any eukaryotic or prokaryotic sequence not containing a transcription-termination signal upstream. Third, the ability to select fusions simply by looking for spontaneous Lac$^+$ derivatives of *E. coli* containing the plasmid frees one from the necessity of knowing anything about the nucleotide sequence of the target genes. All fusions are necessarily in the β-galactosidase reading frame. Furthermore, the selection method allows the recovery of many fusions, deleting different amounts of the target sequence. In the particular case described above, the spectrum of fusions obtained suggests the presence of another previously undetected yeast gene downstream of the *URA3* gene itself and provides additional evidence for a transcribed and translated sequence in the yeast 2-μm plasmid (24, 25).

The usefulness for studies of gene regulation in yeast of fusions to the *E. coli* β-galactosidase gene is validated by our observation that the *URA3-lacZ* fusion produces β-galactosidase

activity under uracil regulation. Similar results were obtained for fusions to the yeast *CYC1* gene (see accompanying paper, ref. 26) and the yeast *LEU2* gene (unpublished data). A new result concerning *URA3* regulation is the observation that the *URA3-lacZ* fusion is weakly derepressible by starvation for uracil in yeast strains which are *ura3*$^-$. This may be a hint that there are elements of autoregulation in the control of the yeast uracil pathway. It is worthwhile noting that such a result can only be obtained easily by gene fusion (27).

Another use for the fusion system described here is the delineation of the minimum sequences required to retain normal regulation of the *URA3* gene in yeast. Because the fusions we report here are carboxy-terminal deletions of the *URA3* gene, our results indicate that none of the information required for proper regulation lies in the carboxy half of the gene. A similar conclusion is drawn concerning the *CYC1* gene in the accompanying paper (26). It should be noted also that such effects can be detected with the fusion system, even if the effect is at the level of translation. An extension of this kind of investigation is the use of properly regulated fused genes to recognize (possibly even select) regulatory mutants based upon their synthesis of β-galactosidase in yeast. Yeast cells containing functional β-galactosidase are unable to grow on lactose as the sole carbon source. This result is most likely due to the inability of lactose to enter the cell. Similar results have been obtained by Dickson, who placed the β-galactosidase gene of a related yeast, *Kluyveromyces lactis*, into *S. cerevisiae* (28).

One of the less obvious virtues of a system that selects fusion proteins in *E. coli* (as opposed to schemes that work directly in yeast) derives from the expectation (as yet not directly tested) that the same hybrid protein should be produced by a given fusion plasmid in both organisms. The ability to examine the fusion protein in both yeast and *E. coli* should be especially valuable in searches for regulatory mutants, because structural gene mutations should affect the protein in both organisms and many kinds of regulatory mutations might affect synthesis only in yeast.

We envision many additional uses for fusions in yeast (and other eukaryotes). Many interesting genes have been detected in yeast whose gene products have yet to be identified. These included the cell-division-cycle genes, some of which already have been cloned by complementation of mutant alleles (29, 30). The regulation of these genes can be studied nevertheless by the gene-fusion technique. Whole classes of genes that are similarly regulated could be identified and isolated based only on the similarity in their regulation; similar ideas have been successfully used in *E. coli* (31, 32). Finally, the production of hybrid proteins which might, through the raising of antibodies against determinants of the yeast protein moiety, allow the isolation of the natural protein products of such genes by affinity methods (33).

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