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Biochemistry

Production of a functional eukaryotic enzyme in *Escherichia coli*: Cloning and expression of the yeast structural gene for imidazoleglycerolphosphate dehydratase (*his*3)

(gene expression/cloned yeast genes/gene selection/yeast *his*3)

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ABSTRACT **A cloned segment of yeast DNA containing the structural gene for imidazoleglycerolphosphate dehydratase (D-*erythro*-imidazoleglycerolphosphate hydro-lase, EC 4.2.1.19) is transcribed and translated in *Escherichia coli* with sufficient fidelity to produce functional enzyme. This segment of yeast DNA was isolated as a viable molecular hybrid of bacteriophage λ (λgt-Sc2601) which complements a nonrevertible *his*B auxotroph of *E. coli* lacking dehydratase activity. The equivalent segments of DNA cloned from two independent *his*3 mutants of yeast lacking IGP dehydratase activity do not complement the *his*B auxotroph. The two nonfunctional *his*3 alleles cloned in bacteriophage λ can be recombined in *E. coli* to generate a hybrid phage which complements the *his*B auxotroph. The dehydratase activity produced in *E. coli* by the cloned segment of yeast DNA strongly resembles the activity found in yeast.**

A basic question is whether cloned eukaryotic DNA can be functionally expressed in *Escherichia coli*. That is, can eukaryotic DNA be transcribed and subsequently translated with sufficient fidelity to produce a functional eukaryotic protein? In a previous publication (1), we reported the isolation of a viable molecular hybrid of bacteriophage λ DNA containing a segment of DNA from the eukaryote *Saccharomyces cerevisiae* (baker's yeast) which complements a nonrevertible mutant of *E. coli* lacking imidazoleglycerolphosphate (IGP) dehydratase (D-*erythro*-imidazoleglycerolphosphate hydrolyase, EC 4.2.1.19) activity (*his*B463). When this hybrid is integrated into the chromosome of *his*B463, the resultant bacterium grows in the absence of histidine. The complementation dependent upon this segment of yeast DNA is specific to *E. coli* mutants lacking IGP dehydratase, and the *his*B463 lesion is unrevertible and unsuppressible by an *E. coli* mechanism even after various mutagenic treatments. For these reasons, we concluded that it was almost certain that there was production of a functional yeast IGP dehydratase in *E. coli*. However, the possibility of suppression of the *his*B463 lesion remained. Since then, Ratzkin and Carbon (2) have independently confirmed the complementation of *his*B463 and, in addition, have reported several other complementation events with cloned yeast DNA.

In this publication, we extend our previous characterization of the nature of functional genetic expression of yeast DNA in *E. coli*. Our analysis of the complementation of *his*B463 includes direct genetic and biochemical characterization of the product of functional genetic expression of the yeast DNA in *E. coli*. The genetic characterization involves the cloning of the equivalent DNA sequences from strains of yeast lacking IGP dehydratase activity. Such sequences should contain nonfunctional allelic genes and should not complement *his*B463. The biochemical characterization involves the determination of enzymatic parameters of IGP dehydratase activity that results from the complementation of *his*B463 by a segment of cloned yeast DNA. These experiments address the following two issues: (*i*) Does the cloned segment of yeast DNA contain the structural gene for IGP dehydratase (*his*3)? (*ii*) What is the fidelity of expression determined by enzymological characterization of the product encoded by the yeast DNA?

MATERIALS AND METHODS

Yeast, Bacterial, and Phage Strains. Isogenic yeast strains S288C, *his*3-38, and *his*3-532 were obtained from Gerry Fink (3). D585-11C was obtained from Ben Hall. Bacterial strain *his*461 (Δ*his*) was obtained from Phil Hartmann (4). The strain is deleted for the entire histidine operon as determined by complementation analysis. λgt6-*ara*6 contains the left end fragment of λgt4 (5), a fragment containing the arabinose (*ara*) operon of *E. coli* generated by cleavage of pLC 24–41 (6) with *Eco*RI* (7), and a derivative of the right end of λgt4 that has the b522 deletion (8). pMB9 DNA (9) was obtained from Rick Kramer. All other strains have been described (1, 10, 11). Growth of strains and preparation of DNA have also been described (10–12). The experiments were performed under P2,EK1 containment conditions as described by the National Institutes of Health "Guidelines for Recombinant DNA Research."

Cloning Methods. Hybrid DNA with λgt-*ara*6 or λgt6-*ara*6 DNA as the vector and total yeast DNA from strain S288C, *his*3-38, or *his*3-532 were constructed as described by Thomas *et al.* (10). After transfection, all viable phages were pooled (termed a "yeast hybrid pool") as described (11, 13). Hybrid pools made from the *his*3$^-$ yeast strains were screened by the plaque filter hybridization of Benton and Davis (14). The probe used was pMB9-Sc2601 [^{32}P]DNA synthesized by "nick translation" (15). From the hybrid pool made with *his*3-38 DNA, one phage (λgt-Sc2612) containing sequences homologous to Sc2601 was isolated out of the approximately 50,000 screened. This low frequency was due to a high background of uncut vector DNA and the fact that λgt-*ara*6 grows better than the λgt yeast hybrids (13). The frequency of a phage containing Sc2601 sequences from the hybrid pool made with *his*3-532 DNA (cloned in λgt6) was 5 in approximately 50,000.

IGP Dehydratase Assays. IGP was synthesized by a modification of the method of Ames (16). The concentration of IGP was estimated by a modification of the method used for determination of imidazoles (17). By using histidinol phosphate and histidine as standards, the empirical relationship used for calculation of the imidazole concentration was 1.0 unit of ab-

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Abbreviations: IGP, imidazoleglycerolphosphate; kb, kilobase pairs.

Table 1. IGP dehydratase and histidinol phosphate phosphatase activity levels

Bacterial strain	pMB9–Sc2601	IGP dehydratase	Histidinol *P* phosphatase
*his*461 (Δ*his*)	–	<0.1	<0.1
*his*461 (Δ*his*)	+	0.8	<0.1
*his*B463	–	<0.1	3.5
*his*B463	+	1.2	3.2
W3110 (*his*$^+$)	–	1.5	2.3

The parental strains (those without recombinant DNA) are compared to derivatives containing the hybrid pMB9-Sc2601. Entries in the table represent specific activities. Strains were grown in the presence of histidine.

sorbance at 520 nm equivalent to 50 nmol of imidazole. IGP dehydratase assays were performed with extracts exhaustively dialyzed at 4° against 20 mM triethanolamine pH 7.5. All assays were performed at pH 7.5 as described by Ames (16) except where otherwise stated. Each point was determined by the following method. From the mean A_{290} of duplicate tubes containing the total reaction mixture, the mean A_{290} of duplicate tubes without exogenously added IGP was subtracted. A factor representing the A_{290} of IGP was also subtracted. Duplicate points were accurate to ±1%. For a given extract under defined conditions, assay reproducibility was within 10%. There was, however, significant extract-to-extract variability (possibly as high as a factor of 2) with regard to the level of IGP dehydratase activity. We estimate that the measured kinetic parameters are accurate to within half an order of magnitude.

Histidinol phosphate phosphatase assays were performed as described (18). All enzyme levels were normalized to the protein concentration determined by the Lowry *et al.* (19) method.

RESULTS

IGP Dehydratase Activity Dependent upon a Cloned Segment of Yeast DNA Is Not the Result of Suppression of the *his*B463 Lesion. All strains of *E. coli* that grow in M9 minimal medium must synthesize functional IGP dehydratase. Therefore, merely assaying derivatives of *his*B463 that contain the complementing yeast DNA for IGP dehydratase yields little information on the nature of the complementation. The detection of IGP dehydratase activity in such cells does not distinguish between functional expression of a yeast structural gene in *E. coli* and suppression of the original mutation in *his*B463. A distinction can be made, however, if IGP dehydratase activity is found in cells containing the yeast sequence and in which the entire *his* operon is deleted from the *E. coli* chromosome. If activity is found in these cells, the suppression model can be ruled out.

Accordingly, the yeast DNA sequence cloned in pMB9 (pMB9-Sc2601) was introduced into a strain of *E. coli* deleted for the entire histidine operon (*his*461). Selection and maintenance of this strain, *his*461 (pMB9-Sc2601), relied upon the plasmid-coded trait for resistance to tetracycline because it would not grow without histidine. This strain had significant IGP dehydratase activity in contrast to the background levels of the parent, *his*461 (Table 1). Neither strain had detectable levels of histidinol phosphate phosphatase activity, as expected. The results with *his*B463 and the pMB9-Sc2601-containing derivative were similar, with the expected difference that both of these strains had wild-type levels of histidinol phosphate phosphatase. The experiments provide direct evidence that the IGP dehydratase activity dependent upon the cloned yeast DNA is measurable by an enzyme assay of a crude extract and that the activity and the complementation are not the result of suppression of the *E. coli hisB* locus.

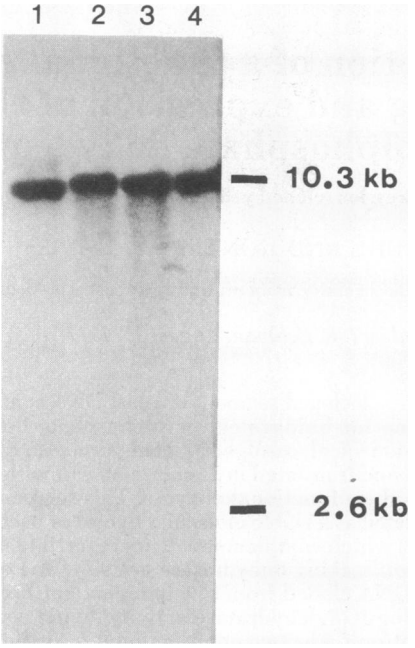

FIG. 1. Nucleic acid hybridization. Total yeast DNA (1 μg) from strains D585-11C (lane 1), S288C (lane 2), *his*3-38 (lane 3), and *his*3-532 (lane 4) were digested with *Eco*RI endonuclease, electrophoretically separated in 0.7% agarose, and transferred to a strip of nitrocellulose paper (1, 20). "Nick translated" [^{32}P]DNA of λgt-Sc2601 was hybridized to the strip. The faint bands are most likely due to *Eco*RI* cleavage of the yeast DNAs.

The Segment of Yeast DNA that Complements *his*B463 Contains the Structural Gene for IGP Dehydratase Defined by the *his*3 Locus. *his*3 mutants of yeast do not have IGP dehyratase activity (3). It is believed that the structural gene for IGP dehyratase in yeast is defined by the *his*3 locus (3). We adopted the following experimental strategy to determine whether the complementation of hisB463 is dependent upon a functional *his*3 gene in yeast. The original *his*$^+$ complementing phage, λgt-Sc2601, contained an *Eco*RI fragment 10.3 kilobase pairs (kb) long (Sc2601) derived from strain A364a XH79-20.3α (1). By a modification of the original procedure, the equivalent *Eco*RI fragment, Sc2630, has been cloned from a haploid strain of yeast, S288C, which also contains the wild-type allele for *his*3 (D. T. Stinchcomb and R. W. Davis, unpublished data). If complementation of the *his*B463 mutation depends upon a functional *his*3 locus, cloning of the equivalent *Eco*RI fragments from *his*3 mutants of yeast otherwise isogenic to strain S288C should result in hybrids unable to complement *his*B463.

Two wild-type (*his*3$^+$) and two *his*3$^-$ strains (*his*3-38 and *his*3-532) of yeast contain a 10.3-kb *Eco*RI fragment of DNA homologous to λgt-Sc2601. Fig. 1 shows an autoradiograph from an experiment in which DNA from λgt-Sc2601 labeled with ^{32}P was hybridized across *Eco*RI restriction spectra of total yeast DNA eluted from each of four agarose gels according to the method of Southern (20). In all cases, a single band with a mobility indistinguishable from 10.3 kb was observed, indicating that these four yeast strains contain a single *Eco*RI fragment of the same size as the cloned *his*$^+$ complementing fragment found in λgt-Sc2601.

Because strains *his*3-38 and *his*3-532 have a single *Eco*RI fragment homologous to λgt-Sc2601, it was possible to clone these "mutant" fragments by the *Eco*RI/DNA ligase technique

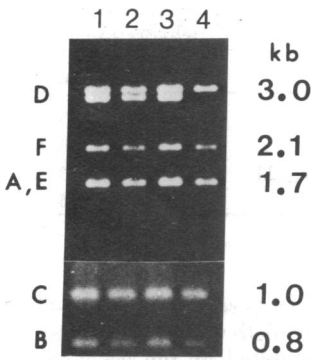

FIG. 2. Restriction spectra of hybrids containing *his*3 DNA sequences. DNA (1 μg) from the following sources were digested with a combination of *Bam*H-1 and *Eco*RI and electrophoretically separated in 0.7% agarose: Lane 1, λgt-Sc2601 (contains the "wild type" *his*3 gene); lane 2, λgt-Sc2612 (contains the "mutant" *his*3 gene from strain *his*3-38); lane 3, λgt-Sc2665 (*his*$^+$ revertant of λgt-Sc2612); and lane 4, pMB9-Sc2601. Fragment lengths were calibrated by using restriction enzyme fragments of λ DNA as standards. The bands at 2.9 kbases (kb) in lanes 1, 2, and 3 correspond to fragments that contain λ sequences. This figure is a composite of two different exposures of the same gel.

used in the construction of λgt-Sc2601. Accordingly, hybrid pools were constructed with a λgt vector and total yeast DNA from either *his*3$^-$ strain and screened for the presence of a phage containing Sc2601 DNA sequences by the plaque filter hybridization method described by Benton and Davis (14). From each pool, such a phage was isolated. These phages are called λgt-Sc2612 (from strain *his*3-38) and λgt6-Sc2679 (from strain *his*3-532).

Neither λgt-Sc2612 nor λgt6-Sc2679 complement *his*B463. By restriction enzyme analysis, the yeast DNA sequences of Sc2601, Sc2612, and Sc2679 are indistinguishable. These hybrids each contain a 10.3-kb *Eco*RI fragment of yeast DNA with indistinguishable *Bam*H-1 and *Sal* I restriction spectra (Fig. 2 and data not shown). Heteroduplex analysis also indicates, that these sequences are indistinguishable (Fig. 3). From both of

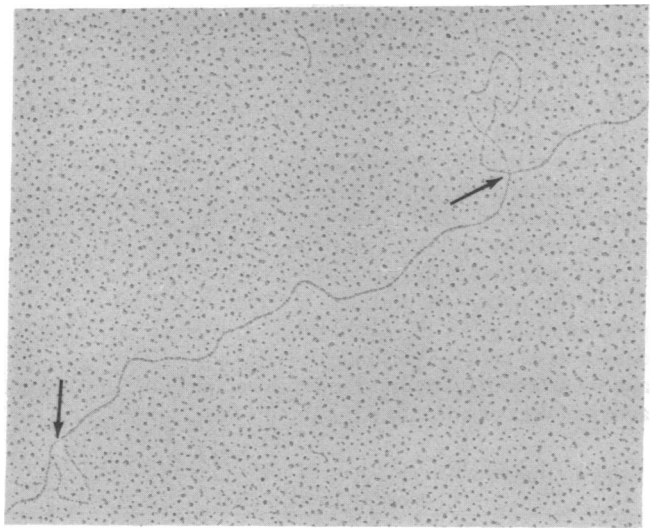

FIG. 3. Heteroduplex of λgt-Sc2601′ and λgt6-Sc2679′. This was performed as described (21) The arrows represent the end points of the sequence homology in the inserted 10.3-kbase *Eco*RI fragments. The left and right end points are defined by the regions of nonhomology that result from the fact that the λgt and λgt6 vectors have different structures (see Fig. 4). The small substitution loop on the left is due to the difference in the λ*Eco*RI A fragments of λgt and λgt6. The somewhat larger deletion loop on the right is due to a sequence of DNA that is present in λgt6 but not in λgt.

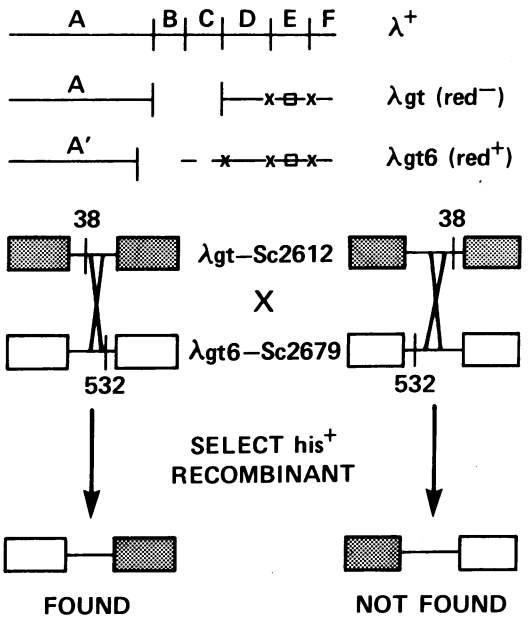

FIG. 4. Possible outcomes of a phage cross to determine the order of the cloned *his*3 lesions with respect to λ. The upper part of the figure illustrates the structures of wild-type λ, λgt, and λgt6. Vertical lines indicate *Eco*RI restriction sites. λgt-Sc2612 contains the mutant *his*3 gene from *his*3-38. λgt6-Sc2679 contains the mutant *his*3 gene from *his*3-532. The left and right *Eco*RI fragments of λgt and λgt6 are physically distinguishable; λgt6 derivatives are *red*$^+$ while λgt derivatives are *red*$^-$. To determine which of the two possible diagrammed orders of the *his*3$^-$ lesions was correct, λgt-Sc2612 and λgt6-Sc2679 were crossed and *his*$^+$ recombinants were selected. All five recombinants analyzed were *red*$^-$ and had physical structures consistent with the order: λ left end, *his*3-38, *his*3-532, λ right end. Physical structures were analyzed by cleavage of the recombinants with a combination of *Eco*RI and *Bam*H-1. *His*$^+$ recombinants were detected at a frequency of 10^{-3} to 10^{-4} of the total input phage.

these analyses, the *Eco*RI fragments Sc2612 and Sc2679 are inserted into the λ vector in the same orientation as λgt-Sc2601.

Three independent experiments indicated that the Sc2612 and Sc2679 fragments contain a cloned mutant *his*3 gene. First, it was possible to obtain spontaneous revertants of λgt-Sc2612, at a frequency of 5×10^{-8}, that were able to complement *his*B463. Analysis of one of these revertants, λgt-Sc2665, with *Eco*RI, *Bam*H-I, and *Sal* I indicates that the restriction spectra of the "revertant" are indistinguishable from the "mutant" and "wild type" (Fig. 2 and data not shown). Spontaneous revertants of λgt6-Sc2679 were not found ($<10^{-8}$). Second, it was possible to obtain *his*$^+$ recombinants from phage crosses between noncomplementing (*his*$^-$) deletion mutants of λgt4-Sc2601 and either λgt-Sc2612 or λgt6-Sc2679 (K. Struhl and R. W. Davis, unpublished data). Third, it was possible to obtain recombinants from a phage cross between λgt-Sc2612 and λgt6-Sc2679 that complemented *his*B463. Because the left and right *Eco*RI end fragments of λgt and λgt6 DNA have different restriction spectra, it was possible to order the *his*3$^-$ lesions of Sc2612 and Sc2679. The two possible outcomes of this three-factor cross are diagrammed in Fig. 4. Restriction enzyme analysis of a *his*$^+$ recombinant from such a cross indicated that the lesion in Sc2612 mapped to the left of the lesion in Sc2679. These genetic results indicate that the cloned mutant genes have sequences that are allelic to the cloned wild-type gene in λgt-Sc2601.

We conclude, therefore, that the complementation of *his*B463 by a segment of yeast DNA is dependent upon a functional *his*3 locus, because the equivalent *Eco*RI fragment

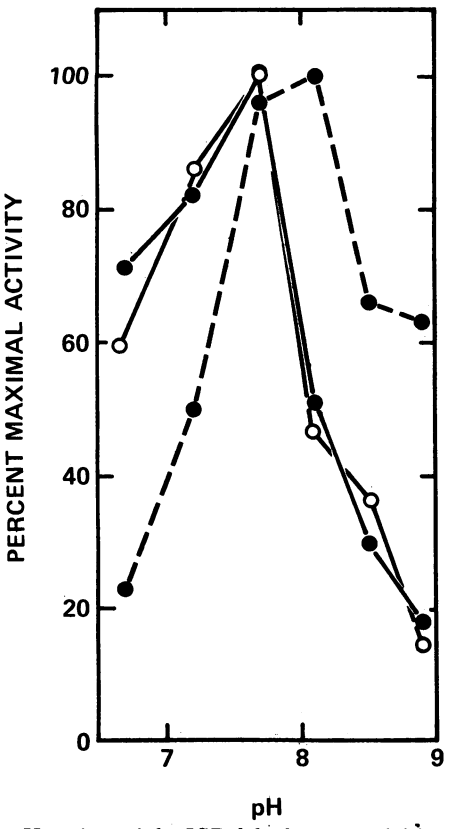

FIG. 5. pH optima of the IGP dehydratase activities. The IGP dehydratase activity in *his*461 (pMB9-Sc2601) is designated the "yeast in coli" activity. The solid lines represent the "yeast in coli" activity (open circles) and the wild-type yeast activity (closed circles). The dashed line represents the activity found in *E. coli* due to the *his*B protein. All points are the mean of three independent experiments.

from strains isogenic except for the *his*3 locus can be differentiated by virtue of functional genetic expression in *E. coli*. Furthermore, we conclude that we have cloned the structural gene for *his*3 because a functional *his*3 locus, whether in yeast or cloned as recombinant DNA in *E. coli*, results in the production of functional IGP dehydratase. Allelic forms of the *his*3 locus that are not functional in yeast are also not functional in *E. coli*.

IGP Dehydratase Made from Cloned Yeast DNA in *E. Coli* Is Enzymologically Similar to IGP Dehydratase in Wild-Type Yeast. The IGP dehydratase reaction is the Mn^{2+}-dependent dehydration of IGP to imidazole acetol phosphate (IAP). Enzymological parameters of the IGP dehydratase activity found in extracts prepared from *his*461 (Δ*his*) containing the yeast *his*3 genes cloned in pMB9 (designated the "yeast in coli" activity) were compared to the activity in extracts from wild-type strains of yeast. The wild-type yeast and wild-type *E. coli* IGP dehydratase activities were similar (data not shown). However, one clear difference was in the pH optima of the activities. Yeast IGP dehydratase activity had an optimum around pH 7.5 and was significantly less active at pH values above 8. The *E. coli* activity had a broader range with an optimum slightly above pH 8 and significant activity at pH 9. These findings were expected from previous results (22–24). Fig. 5 indicates that the "yeast in coli" activity closely resembles the activity found in wild-type yeast.

Table 2 summarizes the enzymological data comparing the "yeast in coli" activity with the wild-type yeast activity. The "yeast in coli" activity had a K_m of 300 μM for IGP and a binding constant of 20 μM for Mn^{2+}. These values were close to those of wild-type yeast dehydratase activity, 200 μM and 30 μM, respectively. Both wild-type yeast and the "yeast in coli" activities were inhibited by the herbicide aminotriazole or phosphate ion. The inhibitory constants (K_i) for these compounds were similar. The inhibition by aminotriazole was potentiated by the presence of phosphate ion (data not shown). This synergistic effect of aminotriazole and phosphate has been reported previously (24). IGP dehydratase from either extract was dependent upon Mn^{2+} as a divalent cation; Mg^{2+} and Ca^{2+} were completely ineffective as substitutes for this requirement (Table 3). High concentrations of Zn^{2+} (about 50-fold higher than the binding constant for Mn^{2+}) restored about 15% of full activity to the "yeast in coli" activity but was ineffective for the yeast wild-type activity.

Table 2. Enzymological parameters of "yeast in coli" and wild-type yeast IGP dehydratase activities

Parameter*	"Yeast in coli"	Yeast
K_m for IGP, μM	300	200
K for Mn^{2+}, μM	20	30
K_i for aminotriazole, μM	10	30
K_i for phosphate, mM	3	8

K_i, inhibitory constant.

From these experiments, we conclude that the "yeast in coli" IGP dehydratase is similar to the wild-type yeast activity. The activities may not appear to be identical. There is a small but differential effect of Zn^{2+} on the IGP dehydratase activities from these two sources and the "yeast in coli" activity may be slightly more sensitive to inhibition by aminotriazole and phosphate. However, because the assay is not very sensitive and because all determinations were made from crude extracts, these differences may not be significant. It is clear, however, that the similarities between the "yeast in coli" activity and the wild-type yeast activity are striking.

DISCUSSION

From the experiments in this paper and the preceding publication (1), we conclude that we have cloned the yeast structural gene for IGP dehydratase. This gene can be transcribed and translated by *E. coli* to produce an enzyme activity that is strikingly similar to the IGP dehydratase enzyme activity in wild-type yeast.

Evidence that the yeast DNA cloned in λgt-Sc2601 contains the structural gene for IGP dehydratase (*his*3) is both genetic and biochemical. *His*3 mutants of yeast have no IGP dehydratase activity and it is assumed (although not conclusively proved) that *his*3 is the structural gene for IGP dehydratase (3). In this paper, we correlate a physiological effect (complementation of an *E. coli* histidine auxotroph lacking IGP dehydratase) with the genetic parameter of the yeast *his*3 locus and the biochemical parameter of measurable IGP dehydratase activity. We consider the experiments dealing with these pa-

Table 3. Ion specificity of "yeast in coli" and wild-type yeast IGP dehydratase activities

Cation	"Yeast in coli"	Yeast
Mn^{2+}	100	100
None	<5	<5
Mg^{2+}	<5	<5
Ca^{2+}	<5	<5
Zn^{2+}	15	<5

Entries are expressed as percent of maximal activity.

rameters to answer different questions in regard to functional genetic expression of eukaryotic DNA in *E. coli*. Our definition of a structural gene has two aspects. First, a structural gene is defined by a polypeptide, in this case as assayed by enzyme activity. Second, a structural gene is defined by mutational lesions that produce altered forms of the defining polypeptide. The biochemical experiments clearly indicate that IGP dehydratase enzymatic activity is dependent upon the cloned segment of yeast DNA and is not the result of suppression of the *his*B lesion in *his*B463. This is strongly supported by the previous observations (1) that this segment of yeast DNA complements two different *E. coli* auxotrophs lacking IGP dehydratase but no other histidine auxotroph lacking one of the other histidine enzymes and that the *his*B463 lesion cannot be suppressed in *E. coli*. Therefore, λgt-Sc2601 contains the information for a structural gene having IGP dehydratase activity. The genetic experiments clearly indicate that the structural entity coded for by the yeast DNA in λgt-Sc2601 is determined by the *his*3 allele. When correlated with the observation that *his*3 mutants in yeast lack IGP dehydratase activity, the evidence that λgt-Sc2601 contains the functional structural gene for IGP dehydratase and that λgt-Sc2612 and λgt6-Sc2679 each contains a mutationally altered and functionally deficient version is compelling. This is supported by the isolation of revertants of λgt-Sc2612 that are able to complement *his*B463 at a frequency compatible with that of missense mutations and the ability to obtain *in vivo* recombinants from phage crosses between two noncomplementing derivatives that are able to complement *his*B463.

Support for the cloning of the structural gene for IGP dehydratase also comes from a physiological parameter of the expression of the gene in yeast and in *E. coli*. Growth of bacterial strains dependent upon the functional genetic expression of this yeast DNA is strongly inhibited by aminotriazole (K. Struhl and R. W. Davis, unpublished data). It is believed that aminotriazole is a specific inhibitor for IGP dehydratase in yeast (25).

The results presented here indicate that expression of a yeast gene in *E. coli* produces an enzymatic activity similar to that found in wild-type yeast. We do not consider this to be particularly surprising because our selection requires a functional enzyme. Because translational initiation and termination signals are universal for both prokaryotes and eukaryotes, it is likely that translation will proceed from an AUG codon. If the correct start codon is recognized at some efficiency, it is almost certain that termination will occur at the correct place. Because the "yeast in coli" protein is both functional and similar to the wild-type yeast protein, we consider it likely that the primary translation product of the *his*3 gene is the same in yeast and *E. coli*. Post-translational modifications may differ, however. We are somewhat surprised at the efficiency of the expression of yeast DNA in *E. coli*. When the Sc2601 fragment is cloned in pMB9, the level of enzyme activity approached that found in uninduced wild-type *E. coli* (Table 1). From the evidence to date, the nature of transcriptional fidelity is unclear. In *E. coli* the same functional protein can be produced even when transcription is initiated from different promoters (26, 27). Previously, we have argued that transcription necessary for the complementation is initiated in the fragment of yeast DNA (1). More support for this claim comes from the observations that the Sc2601 fragment complemented *his*B463 when cloned in three different plasmid vectors and two different λ vectors in either of the two possible orientations (28). We now have preliminary genetic evidence suggesting that this *E. coli* transcriptional initiation site on the yeast DNA maps no more than 100 base pairs from the start of the structural gene. This evidence is based upon the physical mapping of deletion mutants of λgt4-Sc2601 that have been genetically characterized by using the present knowledge of λ transcription.

In summary, we have cloned a segment of yeast DNA by selecting for its ability to complement a bacterial auxotroph lacking IGP dehydratase. The segment of yeast DNA contains the yeast structural gene for IGP dehydratase (*his*3). Expression of this DNA in *E. coli* occurs with sufficient fidelity so that the activity produced is enzymologically similar to the analogous activity in wild-type strains of yeast.

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1. Struhl, K., Cameron, J. R. & Davis, R. W. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1471–1475.
2. Ratzkin, B. & Carbon, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 487–491.
3. Fink, G. R. (1964) *Science* **146**, 525–527.
4. Garrick-Silversmith, L. & Hartmann, P. E. (1970) *J. Bacteriol.* **66**, 231–244.
5. Clarke, L. & Carbon, J. (1976) *Cell* **9**, 91.
6. Panasenko, S. M., Cameron, J. R., Davis, R. W. & Lehman, I. R. (1977) *Science* **196**, 188–189.
7. Polisky, B., Greene, P. J., Garfin, D. E., McCarthy, B. J., Goodman, H. M. & Boyer, H. W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3310–3314.
8. Davis, R. W. & Parkinson, J. S. (1971) *J. Mol. Biol.* **56**, 403–423.
9. Rodriguez, R. L., Bolivar, F., Goodman, H. M., Boyer, H. W. & Betlach, M. (1976) in *Molecular Mechanisms in the Control of Gene Expression* (Academic Press, New York), pp. 471–477.
10. Thomas, M., Cameron, J. R. & Davis, R. W. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4579–4583.
11. Cameron, J. R., Panasenko, S. M., Lehman, I. R. & Davis, R. W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3416–3420.
12. Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91**, 315–328.
13. Cameron, J. R. & Davis, R. W. (1977) *Science* **196**, 212–215.
14. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
15. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.*, **113**, 237–251.
16. Ames, B. N. (1957) *J. Biol. Chem.* **228**, 131.
17. Koessler, K. K. & Hanke, M. T. (1919) *J. Biol. Chem.* **39**, 497–519.
18. Martin, R. G., Berkerich, M. A., Ames, B. N., Davis, W. W., Goldberger, R. F. & Yourno, J. D. (1971) in *Methods of Enzymology*, eds. Tabor, H. & Tabor, C. W. (Academic Press, New York), Vol. 17B, pp. 3–44.
19. Lowry, O. H., Rosebrough, N. J, Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
20. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–517.
21. Davis, R. W., Simon, M. N. & Davidson, N. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21, pp. 413–438.
22. Hilton, J. L., Kearney, P. C. & Ames, B. N. (1965) *Arch. Biochem. Biophys.* **112**, 544–547.
23. Brady, D. R. & Houston, L. L. (1973) *J. Biol. Chem.* **248**, 2588–2592.
24. Klopotowski, T. & Wiater, A. (1965) *Arch. Biochem. Biophys.* **112**, 562–566.
25. Hilton, J. L. (1960) *Weeds* **8**, 392.
26. Franklin, N. C. (1971) in *The Bacteriophage* λ, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 621–645.
27. Miller, J. H., Reznikoff, W. S., Silverstone, A. E., Ippen, K., Signer, E. R. & Beckwith, J. R. (1970) *J. Bacteriol.* **104**, 1273–1279.
28. Struhl, K. & Davis, R. W. (1976) in *Molecular Mechanisms in the Control of Gene Expression* (Academic Press, New York), pp. 495–506.