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Functional genetic expression of eukaryotic DNA in *Escherichia coli*

(bacteriophage λgt-yeast hybrids/*E. coli his* B/gene selection)

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ABSTRACT **We have isolated a segment of DNA from the eukaryote *Saccharomyces cerevisiae* (baker's yeast) as a viable molecular hybrid of bacteriophage λ DNA which, when integrated into the chromosome of an *E. coli* histidine auxotroph, allows this bacterium to grow in the absence of histidine. The nonrevertable, histidine auxotroph lacks the enzymatic activity of imidazole glycerol phosphate (IGP) dehydratase (EC 4.2.1.19). From genetic experiments, we conclude that expression of the segment of yeast DNA results in the production of a diffusible substance and that transcription necessary for the complementation is most likely initiated from the segment of eukaryotic DNA.**

Techniques have been recently developed for the construction and cloning of viable molecular hybrids between bacteriophage λ and any foreign DNA (1, 2). Pools of molecular hybrids containing many different fragments of DNA from any given genome can be generated (3). Genetic selection systems which depend on the functional expression of the foreign DNA sequence have been used to isolate specific hybrids of interest. Hybrid phage containing specific bacterial genes were isolated by lytic growth in hosts having lesions in those genes. The gene for DNA ligase was isolated from *Escherichia coli* (3), and the genes for DNA polymerase I were isolated from *Klebsiella aerogenes* and *Klebsiella pneumoniae* (Struhl and Davis, in preparation). Since bacteriophage λ is capable of integrating into the bacterial chromosome to form stable lysogens, selections can also be performed without killing the host. Such selections were used to isolate hybrids containing the gene for 3-enolpyruvylshikimate 5-P synthetase (*aroA*) from *K. aerogenes* and *K. pneumoniae* and the gene for chorismate synthetase (*aroC*) from *E. coli* (Struhl and Davis, unpublished results).

In this paper, we have used a selection employing the lysogenic state of λ to obtain a specific hybrid which contains DNA from the yeast *Saccharomyces cerevisiae*. This selection is based upon complementation of a nonreverting bacterial mutant which has a single, defined, enzymatic lesion. Such a selection requires either the synthesis of a functional gene product from eukaryotic genetic information by *E. coli* or suppression of the specific lesion.

MATERIALS AND METHODS

Chemicals, Phage, Bacteria, DNA, and Enzymes. Bacterial strain his463 was obtained from Dr. P. Hartmann. Originally, this strain had been characterized as a *hisC* mutant (4). It was recharacterized as a *hisB* mutant which lacks the activity for imidazole glycerol phosphate (IGP) dehydratase (EC 4.2.1.19) but not the activity for histidinol phosphatase [L-histidinol-phosphate phosphohydrolase (EC 3.1.3.15)]. We constructed another *hisB* mutant of *E. coli* by mating of the *S. typhimurium* strain TR74 (F′ *hisB2404/ser821 arg501 his*Δ*712*) which harbors an episome containing the histidine operon from *E. coli* with his461 (Δ*his*) and selecting for growth on minimal plates supplemented with histidinol. The resulting strain produces an *E. coli his* B protein which lacks the activity for IGP dehydratase only (B. Cooper and J. Roth, personal communication). Other histidine auxotrophs were constructed in the same manner.

The λgt vector used in this paper contains all the essential genes necessary for phage growth but is too small to be packaged. An insertion of an *Eco*RI endonuclease generated fragment of yeast DNA between the λgt ends results in a viable molecular hybrid (1). λgti is a derivative of λgt-λC (1) which has a mutation in a recognition site for *Eco*RI endonuclease (RI-3). Other reagents have been described previously (1, 3, 5, 6).

Generation of λgt Hybrid Pools with Yeast DNA. DNA from the *Saccharomyces cerevisiae* strain A364a × H79-20.3α was supplied by Dr. B. Hall. We made hybrid DNA molecules using λgt-λB DNA (1) as the vector by the *Eco*RI endonuclease-DNA ligase method described previously (3, 6). The hybrid molecules were transfected into calcium treated *E. coli* SF8 cells (*C600 rK⁻ mK⁻ recBC⁻ lop 11 lig⁺*). Plaques representing individual viable hybrid phage were pooled by scraping the plates, and the phage pool was grown on C600 *rK⁻ mK⁺* to make a high titer stock. The viable hybrids are thereby K modified and can therefore infect any K12 strain of *E. coli* which is sensitive to infection by bacteriophage λ. These pools were more fully characterized by Cameron and Davis (in preparation).

Cultivation of Bacteria. The minimal medium used was standard M9 to which vitamin B1 was added at a concentration of 1 μg/ml, histidine at 100 μg/ml, and histidinol at 1 mM when required as supplements. Carbon sources were added to a final concentration of 0.4%.

Formation of Double Lysogens of HisB463. Since the vector used for hybrid pools was λgt-λB, all hybrid phage were deleted for the gene necessary for integration (*int*) and the phage attachment site (*att*) contained in the *Eco*RI-λC fragment. Hybrids were integrated into the bacterial chromosome as double lysogens by using an integration helper phage (λgti) which is *int⁺att⁺*. Double lysogens are formed by linkage of the prophages at the bacterial attachment site (7, 8). Because the hybrids have no phage attachment site, the double lysogens are not formed in the usual tandem linkage. Instead, the hybrids can be integrated into the chromosome by general bacterial recombination by homology with λgti. Recombination mediated formation of double lysogens has been shown to occur at a frequency of 1% (9). When induced, double lysogens formed in this manner yield equal numbers of the two prophages. HisB463 was coinfected with the integration helper and phage from the yeast hybrid pool (each at a multiplicity of in-

Abbreviations: IGP dehydratase, imidazole glycerol phosphate dehydratase; SSC, standard saline–citrate solution (0.15 M sodium chloride–0.015 M sodium citrate, pH 7); ×SSC means that the concentration of the solution used is times that of the standard saline–citrate solution.

fection of 1) in order to obtain a phage which complements this strain.

Curing of λ Prophages from Lysogenic Strains. All phage used have the *cI857* mutation, thus permitting temperature induction of the prophages. From 10^4 to 10^6 lysogens were inoculated into 1 ml of L broth and grown at 30° for 1–2 hr. The culture was shifted to 42° for 5 min to induce prophages and then returned to 30° for 2–4 hr. An aliquot was streaked on L plates at 42° and colonies were subsequently tested.

Induction of Double Lysogens and Purification of Phage. A culture of the double lysogen, growing exponentially at 30°, was induced for 15 min at 42° and then shifted to 37°. After lysis, a loopful of phage was streaked for single plaques. Since the λgti integration helper is *red*$^+$ and the hybrid phage is *red*$^-$, it is easy to distinguish between them by their plaque size. A small (*red*$^-$) plaque was picked and tested for the *red*$^-$ character by its inability to plate on either lig ts7 or H560 (*polA*$^-$).

Preparation of Complementary RNA. RNA was synthesized (10) with labeled [^{32}P]ATP and [^{32}P]GTP at 3 Ci/mmol and the appropriate phage DNA as a template. Incubation was for 2 hr at 37° with *E. coli* RNA polymerase holoenzyme. RNA was extracted twice with phenol and chromatographed on a Sephadex G-50 column.

Agarose Gel Electrophoresis, Hybridization, and Autoradiography. Agarose gel electrophoresis was performed as in Thomas and Davis (6). DNA was transferred from an 0.7% agarose cylindrical (0.6 × 14 cm) gel to strips of cellulose nitrate as in Southern (11). Complementary RNA (5×10^5 dpm in 0.6 ml) was hybridized to the strips for 18 hr at 43° in 50% formamide and 6 × SSC. Strips were washed with 1 M NaCl, 10 mM EDTA, and 100 mM sodium phosphate at pH 7.0 for 2 hr, soaked for 15 min in 2 × SSC, dried, and autoradiographed.

RESULTS

Complementation of the hisB463 lesion by a phage from the yeast hybrid pool

Two pools of viable molecular hybrids were constructed as described in *Materials and Methods* using the same preparation of yeast DNA. The pools, which were made 3 months apart, contained phage from about 10,000 and 14,000 independent plaques. These hybrids were stably integrated into the chromosomes of cells of the histidine auxotroph hisB463 by the integration helper method. After incubation for 60 hr on glucose minimal plates at 30°, colonies growing without histidine (*his*$^+$) were found at a frequency of approximately 10^{-8}. It was possible to detect complementation at this frequency by using either of the yeast hybrid pools. The colonies isolated in this manner are not revertants of hisB463. The strain reverts to *his*$^+$ with a frequency of less than 10^{-11}; in fact, we and previous workers have never seen a revertant. It is also impossible (less than 10^{-10}) to induce reversion of this strain by ultraviolet light, ethyl methane sulfonate, nitrosoguanidine, or ICR 191. Attempts to isolate this gene by this method from *E. coli*, *K. aerogenes*, *K. pneumoniae*, and *Dictyostelium discoideum* were unsuccessful, a result probably due to *Eco*RI endonuclease cleavage within the histidine operon.

Complementation of the hisB lesion depends on a λ prophage

The *his*$^+$ colonies presumably containing a prophage capable of complementing the *hisB* mutation, were purified on minimal glucose plates at 30°. Since all phage used in these experiments have a temperature sensitive repressor (*cI857*), stable lysogeny and cell viability should occur only at temperatures less than 35°. All 200 *His*$^+$ colonies were unable to grow on rich medium at 42°. If the complementation is in fact dependent upon a λ prophage, removal of the prophage (by curing) from the chromosome of the *his*$^+$ lysogen should result in the original hisB463 strain. Indeed, of 50 cured lysogens, all required histidine for growth at both 30 and 42°.

The *his*$^+$ colonies should contain two different prophages; the complementing prophage and the integration helper. Temperature induction of the *his*$^+$ colonies resulted in the production of approximately 20 phage per bacterium. Phage were isolated by equilibrium cesium chloride density gradient centrifugation. Two bands were clearly seen, confirming the fact that the *his*$^+$ colonies contained two different prophages. As expected equal numbers of the two phage were produced (see *Materials and Methods*).

Isolation and characterization of the phage capable of complementing hisB463

The prophage necessary for complementation of hisB463 was induced and a single plaque isolate was obtained as described in the *Materials and Methods*. Phage descendants from this plaque are designated by the strain name λgt-Sc2601. In this paper, they will be referred to by the more descriptive name λgt-Sc his. When hisB463 cells are coinfected with λgt-Sc his and the integration helper, *his*$^+$ double lysogens are found at the relatively low frequency of 10^{-4} after 3 days. Double lysogens produced by our methods are typically found at a frequency of 10^{-2}. Nevertheless, the frequency of 10^{-4} represents an enrichment of 10^4 from the original hybrid pool. The *his*$^+$ colonies obtained by using purified λgt-Sc his are indistinguishable from the *his*$^+$ colonies originally isolated with the yeast hybrid pool. They are temperature sensitive for growth, and curing of the prophages renders the strain his$^-$.

Single infection of hisB463 by λgt-Sc his, at 30°, resulted in stable *his*$^+$ colonies at the low frequency of 10^{-8}. These single lysogens are temperature sensitive and upon curing are *his*$^-$ at all temperatures. Phage can be induced from such a single lysogen although only 0.2 phage per bacterium are produced, an efficiency of only 1% compared to induction of the *his*$^+$ double lysogens. This poor efficiency is not due to a lowered burst, but rather to poor excision, since only 1% of the single lysogens induce any phage at all.

λgt-Sc his is also capable of complementing another hisB$^-$ strain (B2404) which synthesizes a *hisB* protein which lacks only the IGP dehydratase activity (see *Materials and Methods* for construction of this strain). When complementation was done in an analogous manner as with hisB463, *his*$^+$ double lysogens were also found at a frequency of 10^{-4}. λgt-Sc his does not complement any of the histidine auxotrophs tested which belong to other complementation groups (*hisA,C,D,E,F,G,I*) or a single site *hisB* mutant lacking both IGP dehydratase and histidinol phosphate phosphatase activities.

λgt-Sc his is a hybrid containing yeast DNA

DNA from λgt-Sc his was characterized by cleavage with *Eco*RI endonuclease and gel electrophoresis in 0.5% agarose (Fig. 1). The expected 4-band pattern for a λgt hybrid is seen. The band nearest the top of the gel represents a hydrogen bonded association of the two λgt end fragments. The two bands of intermediate mobility correspond to the individual λgt end fragments. The lowest band corresponds to the inserted segment of DNA. The length of the insert is about 10.3 kilobase pairs as determined by mobility in the agarose gel and length measurements in the electron microscope.

The facts that (1) λgt-Sc his could be obtained from two in-

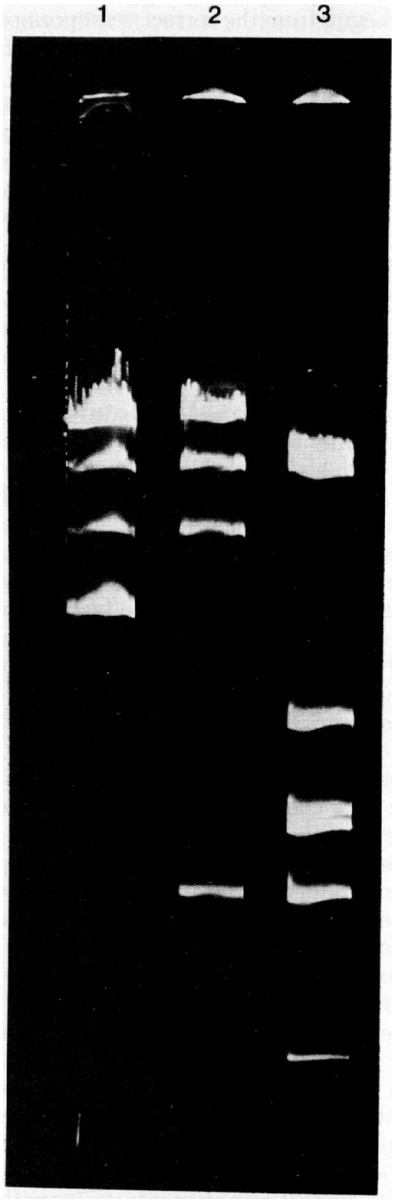

FIG. 1. Agarose gel electrophoresis. DNA was cleaved with *Eco*RI endonuclease and electrophoretically separated on a 0.5% agarose gel as in Thomas and Davis (1). Samples are (1) λgt-Sc his, (2) λgt-λB, and (3) λ*cI857* S_7. The upper band in gels 1 and 2 represents the right and left ends of λgt which are hydrogen bonded via the λ cohesive ends.

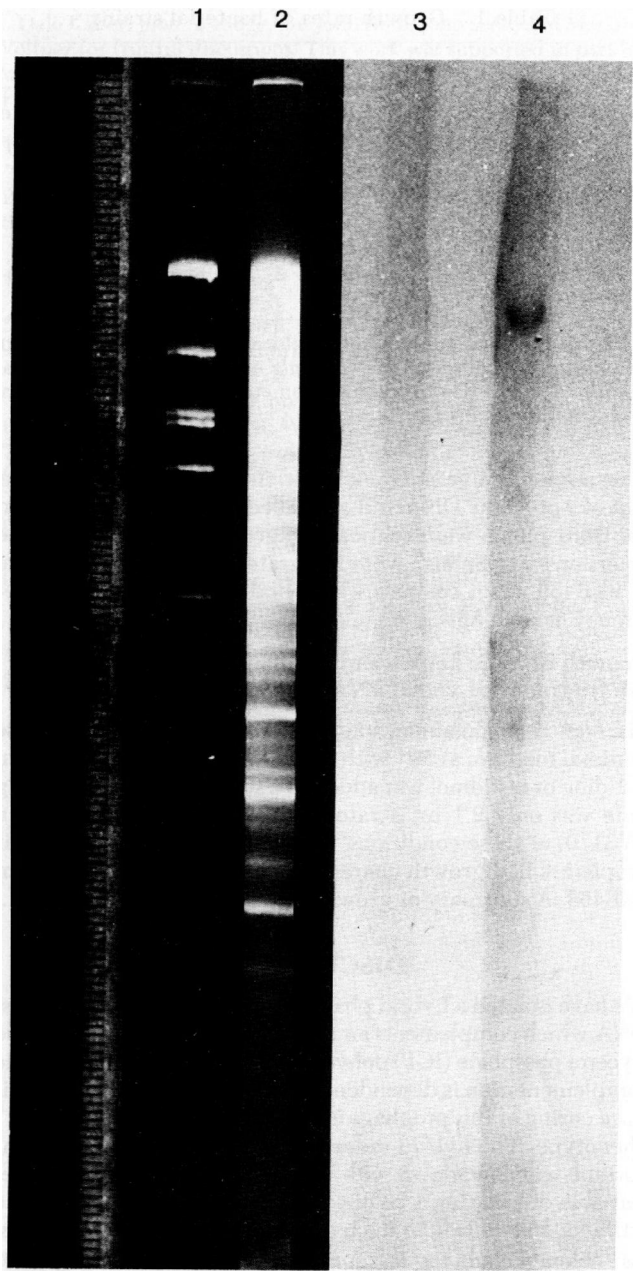

FIG. 2. Nucleic acid hybridization. This was performed as stated in the *text*. All DNA was cleaved with *Eco*RI endonuclease, and all gels were 0.7% agarose. (1) Gel of λ*cI857* S_7 DNA, (2) gel of total yeast DNA, (3) autoradiograph of cRNA to λgt-λB DNA hybridized to total *Eco*RI cleaved yeast DNA, (4) autoradiograph of cRNA to λgt-Sc his DNA hybridized to total *Eco*RI cleaved yeast DNA.

dependently prepared pools of yeast hybrids, (2) selections from all other hybrid pools including *E. coli* were unsuccessful, and (3) λgt-Sc his could not complement a *hisB* mutant lacking both activities made it unlikely that the inserted segment of DNA was a non-yeast contaminant. To avoid possible contamination in the preparation of yeast DNA, we made a new preparation of yeast DNA from another strain (D585-11C) as in Cameron and Davis (in preparation). This DNA was cleaved with *Eco*RI endonuclease, electrophoretically separated on a 0.7% agarose gel, denatured in alkali, and then transferred to a strip of cellulose nitrate paper by a technique developed by E. M. Southern which maintains the gel pattern on the strip (11). ^{32}P-labeled RNA complementary to λgt-Sc his DNA was synthesized *in vitro* with *E. coli* RNA polymerase and hybridized to the cellulose nitrate strip containing the yeast DNA. An autoradiograph from such an experiment is shown in Fig. 2, gel 4. One band corresponding to a length of DNA of 10-11 kilobases is evident, which is the same size as the inserted segment of DNA in λgt-Sc his. cRNA made to DNA from λgt-λB does not detectably hybridize to yeast DNA.

Complementation of hisB463 by λgt-Sc his does not depend upon orientation of the fragment of yeast DNA

λgt-Sc his DNA was cleaved with *Eco*RI endonuclease and then resealed with DNA ligase. Following transfection of these molecules into calcium treated cells, three plaques were picked and grown. The DNAs from these three phage were characterized by heteroduplex analysis with DNA from the original λgt-Sc his phage (12). Two of the three clearly contained the

Table 1. Growth rates of bacterial strains

Strain	Supplement*		
	None	Histidinol	Histidine
W3110	2.1	2.0	2.2
hisB463	No growth	2.1	2.1
His$^+$ double lysogen	2.7	2.2	2.2
Cured double lysogen	No growth	2.1	1.9

All cultures were grown at 29° in glucose minimal medium. Supplements were added as in *Materials and Methods*. Growth was monitored with a Klett spectrophotometer. Table entries represent doubling times in hours.

same segment of yeast DNA, but in the inverted orientation as that of λgt-Sc his DNA and are called λgt-Sc his′. DNA from the third phage was identical to λgt-Sc his DNA. When the inversions, λgt-Sc his′, were integrated into the chromosome of hisB463, *his*$^+$ colonies were obtained with the same frequency as with λgt-Sc his.

Growth of cells dependent upon complementation by the fragment of yeast DNA

The *his*$^+$ cells containing a λgt-Sc his prophage grow in glucose minimal medium at 29° with a doubling time of 2.7 hr. When histidine or histidinol was added as a supplement, the doubling time was only 2.1 hr, a rate comparable to the wild strain (W3110) at these conditions. Cells which were cured of their prophages had growth characteristics indistinguishable from hisB463. A summary of growth rates is presented in Table 1.

DISCUSSION

We have isolated a hybrid phage containing a segment of yeast DNA which complements an *E. coli* mutant lacking imidazole glycerol phosphate (IGP) dehydratase activity. The intergeneric complementation is dependent upon the presence of λgt-Sc his since curing of this prophage is accompanied by loss of the his$^+$ phenotype. The hybrid indeed contains yeast DNA. It is important when working with such potent selective pressures (capable of isolating 1 colony out of 10^{10}) to rule out possible artifacts. In addition to the trivial case of bacterial revertants, his$^+$ colonies could result from hybrids with inserted fragments which are DNA contaminants, recombinants, or rearrangements. The possibility of such an artifact is quite real considering that the hybrid phage are passaged on *E. coli* strains which are essentially wild type.

The nature of the complementation indicates that the segment of yeast DNA in hybrid λgt-Sc his codes for the synthesis of a diffusible yeast product which complements hisB463 *in vivo*. The complementation is not a site-specific effect on the *E. coli* histidine operon since the integration site for λ prophages does not map near this operon, inversion of the yeast DNA fragment with respect to λ (and the bacterial chromosome) has no effect, and because λgt-Sc his specifically complements hisB mutants lacking IGP dehydratase. The transcription for such a product is almost certainly initiated in the fragment of yeast DNA for two reasons. First, the expression occurs in a λ lysogen in which the λ promoters are either strongly repressed or have been deleted in λgt-Sc his. Second, since inversion of the yeast fragment has no effect, two as of yet unknown λ promoters would be required if the promoter did not reside in the yeast fragment itself. However, we have no evidence as to whether transcription begins from the correct yeast promoter or merely from a sequence fortuitously recognized by *E. coli* RNA polymerase.

The frequency of his$^+$ colonies formed after 3 days when purified λgt-Sc his is used is only 1% of the expected value. Typically, 1% of the lysogens formed by our methods are double lysogens. The actual frequency of phenotypically his$^+$ double lysogens (10^{-4}) may be explained by inefficient expression of the yeast DNA, inefficient functioning of the yeast product, instability of the yeast product, or by mutational changes in the sequence of the yeast fragment during lytic growth of the hybrid. The variability in his$^+$ colony size on selective medium and the observation that more his$^+$ colonies become visible after 3 days could be explained by any or a combination of the above. The single his$^+$ lysogens formed by the *int*$^-$ *att*$^-$ hybrid alone are almost certainly the result of abnormal integration. The frequency of such his$^+$ single lysogens is extremely low (10^{-8}) and the poor induction of viable phage indicates that abnormal excision is required.

The frequency of isolating a specific phage from the yeast hybrid pool capable of complementing hisB463 appears to be quite low. This is explained by three factors: the actual frequency of the specific yeast hybrid in the pool (about 10^{-4}), the frequency of forming double lysogens (10^{-2}), and the effectiveness of the complementation (10^{-2}). It should be possible to isolate other genes from yeast by similar selections in which different *E. coli* auxotrophs are used. However, selection of yeast and other eukaryotic genes by virtue of complementation is unlikely to be a completely general technique. Such complementation not only requires adequate genetic expression of the eukaryotic DNA in *E. coli*, but in addition requires activity of the protein product. For example, genes coding for eukaryotic enzymes which have significantly different enzymatic behavior from the analogous enzymes in *E. coli*, or enzymes which are part of an enzyme complex are less likely to be isolated by *in vivo* complementation.

Complementation of a single, defined, enzymatic lesion requires either the synthesis and functioning of an equivalent gene product or suppression of the original mutation. Although no direct evidence is presented in this paper, it seems unlikely that the complementation of hisB463 is the result of suppression of this specific lesion. The effect of the integrated yeast DNA is very specific, since it complements strains with a revertable or a nonrevertable mutation in hisB lacking IGP dehydratase activity, but not a strain with a revertable mutation in hisB lacking both IGP dehydratase and histidinol phosphate phosphatase activities. Complementation is not observed for any of the other genes in the histidine operon. A postulated suppressor would have to suppress two different specific mutations in hisB lacking IGP dehydratase activity and not suppress mutations in other his genes.

The mutant hisB463 used in this work is extremely stable. No growth is detected in the absence of histidine for at least 10 days. No revertants have ever been found spontaneously or after many different mutagenic treatments. The *his*B463 lesion is most probably a deletion which affects the IGP dehydratase activity but not the histidinol phosphatase activity of the hisB protein. It is difficult to conceive of how the product of a segment of yeast DNA could suppress a mutation which is unrevertable and unsuppressible by some *E. coli* mechanism even after a variety of mutagenic treatments. Nonsense and missense suppression of single base changes can occur via tRNAs but the fragment of yeast DNA does not detectably hybridize to yeast tRNA under conditions in which other λgt-yeast hybrids do. A frameshift suppressor is unlikely since hisB463 does not revert

after treatment with ICR 191. It is also unlikely that the yeast DNA codes for a product which stabilizes the mutant hisB463 protein. Besides the unusual nature of such a specific interaction which would be required for two different mutant hisB proteins, bacterial strains with mutations resulting in the production of such proteins would not be expected to have such a stringent requirement for histidine or histidinol.

Therefore, although we have not yet excluded the possibility of suppression, we believe it more likely that the yeast DNA in the hybrid phage codes for the structural gene for imidazole glycerol phosphate dehydratase (*his 3*) or some other gene with a similar activity, and that this DNA is transcribed and subsequently translated with enough fidelity by *E. coli* to produce a functional protein. The genes for IGP dehydratase and histidinol phosphatase are unlinked in *S. cerevisiae*. Therefore, a single 10 kilobase segment of its DNA should code for only one of these activities. This is consistent with λgt-Sc his complementing the *E. coli* mutants lacking only the IGP dehydratase activity. In any event, functional genetic expression of the fragment of yeast DNA in λgt-Sc his results in the specific complementation of *E. coli hisB* mutants which lack IGP dehydratase.

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1. Thomas, M., Cameron, J. R. & Davis, R. W. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4579–4583.
2. Murray, N. W. & Murray, K. (1974) *Nature* **251**, 476–481.
3. Cameron, J. R., Panasenko, S. M., Lehman, I. R. & Davis, R. W. (1974) *Proc. Natl. Acad. Sci. USA* **72**, 3416–3420.
4. Garrick-Silversmith, L. & Hartmann, P. E. (1970) *J. Bacteriol.* **66**, 231–244.
5. Martin, R. G., Berberich, M. A., Ames, B. N., Davis, W. W., Goldberger, R. F. & Yourno, J. D. (1971) in *Methods in Enzymology*, eds. Tabor, H. & Tabor, C. W. (Academic Press, New York), Vol. 17, Part. B, pp. 3–44.
6. Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91**, 315–328.
7. Campbell, A. (1965) *Virology* **27**, 340–348.
8. Gottesman, M. E. & Yarmolinsky, M. B. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 735–747.
9. Gottesman, M. E. & Yarmolinsky, M. B. (1968) *J. Mol. Biol.* **31**, 487–505.
10. Berg, D., Barrett, K. & Chamberlin, M. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21, part D, pp. 506–519.
11. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–517.
12. 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.