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A Physiological Study of Functional Expression in *Escherichia coli* of the Cloned Yeast Imidazoleglycerolphosphate Dehydratase Gene

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Functional expression in *Escherichia coli* of bacteriophage λ and plasmid hybrids containing the *Saccharomyces cerevisiae* (yeast) gene for imidazoleglycerolphosphate (IGP) dehydratase (*his*3) has been characterized in growing *E. coli* cells lacking the bacterial IGP dehydratase activity and during lytic infection of these cells. *his*3 expression of an integrated bacteriophage λ*his*3 hybrid requires transcriptional initiation from a "promoter" in the yeast DNA. A deletion mutant lacking this promoter, but containing the *his*3 structural gene has been isolated. Fusion of a DNA segment containing this promoter to a segment containing the intact structural gene for tetracycline resistance (*tet*^{r}) but lacking an intact promoter results in expression of the *tet*^{r} gene.

Using four physiological criteria, the level of *his*3 expression in growing *E. coli* cells is affected by gene dosage. By these criteria, cells containing multiple copies of the *his*3 gene produce nearly wild-type *E. coli* activity levels of yeast IGP dehydratase. There is no evidence for regulation of *his*3 expression at the gene level as a function of histidine starvation.

Expression of the *his*3 gene during lytic infection by bacteriophage λ hybrids has been assessed by the ability of such hybrids to grow in histidine-starved *E. coli* cells lacking IGP dehydratase. Phage containing a functional *his*3 gene grow with a single burst of five under these conditions and also form "plaques without lawns" on the starved cells. Lytic expression depends on transcription from the λ promoter P_L; the level depends on the distance from P_L to the *his*3 gene.

The results indicate that expression in *E. coli* of a eukaryotic gene obeys prokaryotic rules of gene expression and that it can occur at a significantly high level. This suggests that the basic gene recognition signals of eukaryotes and prokaryotes may not be so different.

1. Introduction

The functional expression of eukaryotic genes in *Escherichia coli* has been demonstrated in a number of cases (Struhl *et al.*, 1976; Struhl & Davis, 1977; Ratzkin & Carbon, 1977; Vapnek *et al.*, 1977). In these cases, a bacteriophage λ or plasmid hybrid containing a eukaryotic DNA sequence was isolated by the complementation of a non-revertible *E. coli* auxotroph lacking a defined enzymatic activity. The usual

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assumption has been that a cloned eukaryotic DNA sequence which specifically complements mutations of a defined *E. coli* structural gene contains the analogous structural gene from the eukaryote. Further experiments have supported this assumption. Vapnek *et al.* (1977) have shown that a *Neurospora crassa* DNA sequence in *E. coli* directs the synthesis of a protein indistinguishable, by antigenic and physical criteria, from a protein found in the eukaryote. Hinnen *et al.* (1978) have shown that the yeast DNA sequence which complements *leuB* mutations of *E. coli* (Ratzkin & Carbon, 1977) transforms *leu2*$^-$ yeast cells prototrophy; therefore it contains the *leu*2 gene. Struhl & Davis (1977) have shown that the complementation by a yeast DNA sequence of a specific class of *E. coli his*B mutations (Struhl *et al.*, 1976) is dependent on a functional *his*3 allele of yeast. The enzyme activity produced by hybrids containing the *his*3 gene strongly resembles the activity found in wild-type yeast cells (Struhl & Davis, 1977).

The yeast *his*3 gene was originally isolated as a hybrid of bacteriophage λ (λgt-Sc2601) containing a 10·1 kb segment of yeast DNA (Struhl *et al.*, 1976). The fragment (Sc2601) has been recloned in pMB9 and pSC101 (Struhl & Davis, 1976) (see Fig. 1). Ratzkin & Carbon (1977) isolated a ColE1 hybrid containing the *his*3 gene that essentially contains a subset of the yeast DNA sequence of Sc2601 (Struhl & Davis, 1979). The following paper (Struhl & Davis, 1980) describes the isolation and the mapping of derivatives of λgt4-Sc2601 which delete sequences of the cloned yeast DNA.

In this paper, two modes of complementation of an *E. coli* auxotroph lacking imidazoleglycerolphosphate dehydratase activity (*hisB*463) by λ hybrids containing the yeast *his*3 gene are described. Lysogenic complementation occurs when *hisB*463

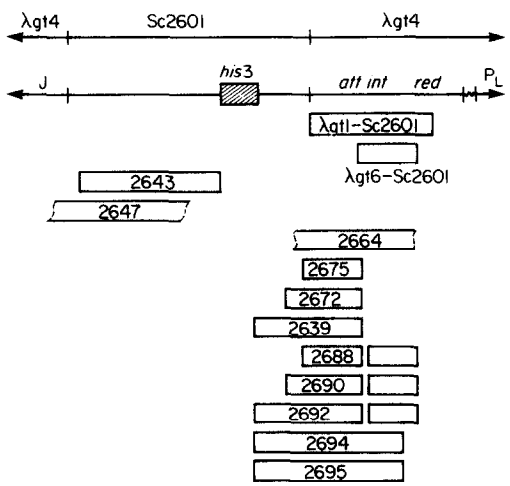

FIG. 1. Map of the *his*3 hybrids used.

The top line represents the λgt4 and the Sc2601 components of λgt4-Sc2601. The vertical lines are the *Eco*RI sites used for joining λ and yeast DNA fragments. The lower horizontal line indicates the genetic markers on this hybrid phage. The physical locations of the alterations in the λ*his*3 hybrids used in this study are indicated. Wavy lines at the end of the open bars indicate that the endpoints are not localized precisely. These data are taken from Struhl & Davis (1980).

cells, lysogenized by integrated λ*his*3 hybrids, grow in the absence of histidine. Lytic complementation is defined by the ability of λ*his*3 hybrids to produce a "plaque without a lawn" on *hisB*463 cells starved for histidine. Using these two complementation assays for yeast *his*3 expression in *E. coli*, we address ourselves to three issues: (1) initiation of transcription in *E. coli* of the yeast *his*3 gene; (2) the isolation of a deletion mutant which inactivates a promoter in the yeast DNA segment, but which contains the intact *his*3 structural gene; (3) the level of *his*3 expression and its effect on the physiology of *E. coli* cells.

2. Materials and Methods

(a) *Phage, bacteria and plasmids*

All strains used in this study have been described elsewhere, except for λgt4-Sc2601′, λgt6-Sc2601 and λgt6-Sc2601′ (Struhl *et al.*, 1976; Struhl & Davis, 1977,1980). These were isolated by cloning the *his*3-containing 10·1 kb† *Eco*RI DNA fragment in the phage vectors λgt4 and λgt6 (Panasenko *et al.*, 1977; Struhl & Davis, 1977). The absolute orientation of Sc2601 with respect to λ DNA or pMB9 DNA is defined in the following paper (Struhl & Davis, 1980). The notation Sc2601′ indicates that the *Eco*RI DNA fragment is inserted into a given vector in the opposite orientation from the original isolate.

(b) *Growth of bacterial strains*

The minimal medium used was M9 containing 0·5% (w/v) glucose. Histidine and adenine were added to 1 mM when necessary. Aminotriazole was added to the concentrations indicated in Table 4. Since, as is the case in *Salmonella typhimurium* (Hilton *et al.*, 1965), aminotriazole inhibits both IGP dehydratase and an enzyme necessary for adenine biosynthesis, adenine was always added to cultures containing aminotriazole.

Cells (25 ml) were grown in 250-ml Erlenmeyer flasks at 37°C. Periodically, 1 ml was removed and the optical density at 600 nm determined with a Zeiss spectrophotometer. Growth was monitored over 4 doubling times of exponential growth. For a given medium (Table 2), all strains, including a control of W3110 grown on minimal medium, were incubated at the same time and in the same incubator.

(c) *Enzyme assays*

Enzyme assays on dialyzed crude extracts were performed as described by Struhl & Davis (1977).

(d) *Kinetics of phage growth*

An exponentially growing culture of *hisB*463 cells in M9 medium containing 0·5% maltose and histidine was harvested, washed twice with an equal volume of 10 mM-$MgSO_4$, and resuspended in 0·4 vol. 10 mM-$MgSO_4$. A total of 10^7 phage in 0·1 ml of 10 mM-Tris (pH 7·5), 10 mM-$MgCl_2$ was adsorbed to 0·1 ml of cells for 10 min at 37°C (multiplicity of infection = 0·1). Then 0·8 ml of 10 mM-$MgSO_4$ was added and 20 μl of this mixture were inoculated into 20 ml of pre-warmed (37°C) medium and incubated at 37°C for up to 3 h. Portions of 1 ml were taken periodically. In general, these portions were treated with chloroform and placed on ice. At the end of the experiment, 0·1 ml of a 10-fold dilution was titered on C600 cells. The first sample was taken at the time of inoculation into the pre-warmed medium. The number of infecting phage was determined by immediately plating portions after adsorption without chloroform treatment.

(e) *Formation of double lysogens of hybrid phage*

This was performed by the integration helper method described by Struhl *et al.* (1976).

† Abbreviations used: kb, 10^3 base-pairs; IGP, imidazoleglycerolphosphate.

(f) *Lytic* his3 *plaque assay*

A modification of the Struhl & Davis (1976) procedure developed by Dan Stinchcomb was used. A fresh over night culture of *his*B463 cells grown on M9 medium containing maltose and histidine was spun down and resuspended in 0·4 vol. 10 mM-$MgSO_4$. Approximately 100 phage (in 0·1 ml) were adsorbed to 0·2 ml of cells at 37°C for 15 min. The mixture was plated on solid M9 medium containing glucose and lacking histidine. The soft agar (0·6%) overlay contained glucose minimal medium to which 1 μg histidine/ml and 75 μg/ethidium bromide/ml had been added. Plaques developed in 24 to 36 h. Ethidium bromide does not interfere with phage growth and is not essential for visualization of the "plaques without lawns". It is included for photographic purposes.

(g) *Phage crosses*

An exponentially growing culture of *his*B463 cells in T broth (0·1 M-NaCl, 1% Tryptone) containing 0·5% maltose was harvested and resuspended in 0·4 vol. 10 mM-$MgSO_4$. Phage to be crossed (in 0·1 ml) were adsorbed to 0·1 ml of cells for 10 min at 37°C (multiplicity of infection being roughly 5 for each phage). Then 0·8 ml of 10 mM-$MgSO_4$ was added and 10 μl of this mixture were inoculated into 1 ml of T broth containing 0·5% glucose. After incubation at 37°C for 60 min, the lysate was treated with chloroform.

3. Results

(a) his3 *gene expression during lytic infection by bacteriophage λ hybrids*

*his*B463 cells starved for histidine do not generally support λ phage growth. However, when such cells are infected by λgt-Sc2601, viable phage are produced (Fig. 2). Expression of the *his*3 gene is clearly necessary for this phenomenon. The single burst size of λgt-Sc2601 grown on *his*B463 cells in minimal medium is five phage per cell. The latent period (time after adsorption necessary for production of phage) is 90 minutes. These measurements indicate that phage growth dependent on *his*3 complementation of *his*B463 is both poor and slow. Growth of λgt-Sc2601 in cells

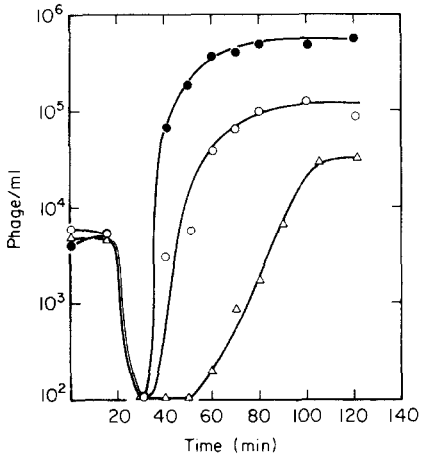

FIG. 2. Kinetics of lytic *his*3 expression.

The kinetics of phage infection of *his*B463 by λgt-Sc2601 was investigated under a variety of physiological conditions. (●) Growth in L broth. (○) Growth in M9 minimal medium containing glucose and histidine. (△) Growth in M9 minimal medium containing glucose.

of *his*B463 grown in minimal medium with a histidine supplement occurs with a burst size of 20 and a latent period of 60 minutes. When the cells are grown in rich broth, the burst size is 70 and the latent period is 40 minutes. As expected, *his*3 expression has no effect upon the burst size or the length of the latent period of phage grown in cells which are not starved for histidine. Phage growth dependent on lytic *his*3 expression in *his*B463 is influenced by the amount of residual histidine in the cells. Dependence on lytic *his*3 expression is most dramatic when *his*B463 cells are washed twice prior to infection. Under these conditions, λgt-Sc2601 has a burst size of five, while λgt4 has a burst size of less than 0·1. If the cells are washed three times, the burst size of λgt-Sc2601 is reduced to one phage per cell. However, if the cells are washed only once, λgt-Sc2601 grows with an increased burst size of nine, but λgt4 now grows with an observable burst of three.

By a modification of previously published techniques (Franklin, 1971; Struhl & Davis, 1976), we have shown that λgt-Sc2601 can form a plaque on a "negative lawn" of starved *his*B463 cells. Further studies in this paper generally use this lytic plaque assay for expression of the *his*3 gene. Phage that form a "plaque without a lawn" are termed lytically his$^+$.

(b) *Dependence of lytic* his3 *expression upon a* P_L*-initiated transcript*

Franklin (1971) established a mode of lytic expression of the *E. coli* tryptophan (*trp*) genes in λ*trp* transducing phage dependent on the *N* gene product of λ. In the presence of *N*, the transcript initiated at the λ promoter P_L "reads through" the normal termination site (T_L) and into the *trp* genes. Adhya *et al.* (1974) with λ*gal* transducing phage and Hopkins *et al.* (1976) with λ*trp* phage constructed *in vitro* as recombinant DNA have reported analogous findings. We report a similar situation with λ hybrids contqining the *his*3 gene from yeast.

λgt1-Sc2601 is lytically His$^+$, while λgt1-Sc2601′ (with an inverted *his*3 relative to λ) is lytically His$^-$. The dependence of lytic expression upon the orientation of the *his*3 gene with respect to λ is also observed when the *his*3 is closed in λgt4 and in λgt6. These results imply that lytic *his*3 expression depends on a promoter in λ DNA and not a promoter located in the yeast DNA. The only reasonable candidate for a λ promoter is P_L. Readthrough transcription from P_R is extremely weak (Hopkins *et al.*, 1976) and almost certainly would not be detected in the lytic plaque assay. Readthrough from the *int* promoter is precluded, since some of the lytically His$^+$ phages lack this promoter.

To demonstrate that P_L-initiated transcription is necessary for lytic *his*3 expression, a mutation in P_L (*sex*1) was crossed into several lytically His$^+$ phages. The *sex*1 mutation reduces leftward transcription by roughly a factor of ten (Franklin, 1971). λgt1-Sc2601 and λgt-Sc2671 were crossed with λ*imm*21c; appropriate recombinants were identified as plaques without lawns on *his*B463 (λ$^+$). The λ*his*3 *imm*21 phages were then crossed with λ*sex*1. Recombinants with λ immunity were screened for *his*3 sequences by the plaque filter hybridization method of Benton & Davis (1977). Derivatives of λgt1-Sc2601 and λgt-Sc2671 that carry the *sex*1 allele are incapable of lytic complementation of *his*B463. These phage contain an intact *his*3 structural gene because they lysogenically complement *his*B463 cells.

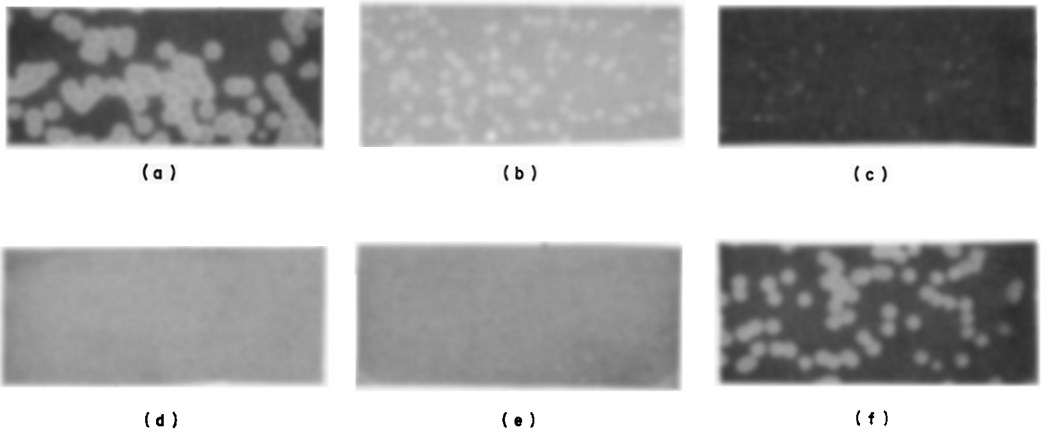

FIG. 3. Plaques without lawns.

Phages shown are as follows (P_L to *his*3 distance indicated): (a) λgt-Sc2639 (8 kb); (b) λgt-Sc2672 (9 kb); (c) λgt-Sc2675 (10 kb); (d) λgt4-Sc2601 (12 kb); (e) λgt-Sc2619 (*his*3$^-$); (f) λgt-Sc2601 (7 kb, *red*$^-$).

TABLE 1

Properties of λ his3 *hybrids*

Phage	His$^+$ lytic	His$^+$ lysogenic	Int	Att	P_L^- His3	Reference
λgt1-Sc2601	+++	+	—	—	6	A
λgt1-Sc2601′	—	+	—	—	—	A
λgt4-Sc2601	±	+	±	+	12	B
λgt4-Sc2601′	—	+	±	+	—	C
λgt6-Sc2601	±	+	—	POΔ	9	C
λgt6-Sc2601′	—	+	—	POΔ	—	C
λgt-Sc2612	pt	pt	—	—	—	D
λgt-Sc2619	Δ	Δ	+	ΔOP′	—	B
λgt-Sc2643	±	+	+	±	12	B
λgt-Sc2647	±	+	±	+	12	B
λgt-Sc2664	+++	+	—	—	6	B
λgt-Sc2675	+	+	+	ΔOP′	10	B
λgt-Sc2672	++	+	±	ΔOP′	9	B
λgt-Sc2639	+++	+	+	ΔOP′	8	B
λgt-Sc2688	++	+	—	ΔOΔ′	7	C
λgt-Sc2690	+++	+	—	ΔOΔ′	6	C
λgt-Sc2692	+++	+	—	ΔOΔ′	5	C
λgt-Sc2694	+++	—	—	—	5	C
λgt-Sc2695	+++	—	—	—	5	C
λgt1-Sc2601 *imm*21	+++	+	—	—	6	C
λgt-Sc2671 *imm*21	+++	+	+	ΔOP′	8	C
λgt1-Sc2601 *sex*1	—	+	—	—	6	C
λgt-Sc2671 *sex*1	—	+	+	ΔOP′	8	C

The level of lytic expression was determined by plaque size under defined conditions (see Fig. 3). λgt-Sc2688, -2690 and -2692 were made by crossing λgt6-*ara*6′ (Struhl & Davis, 1977) with λgt-Sc2675, -2672 and -2639, respectively. The genetic properties of these phage and the P_L to *his*3 distance (in kb) were determined in the following paper. References: A, Struhl *et al.*, 1976; B, Struhl & Davis, 1980; C, this work; D, Struhl & Davis, 1977.

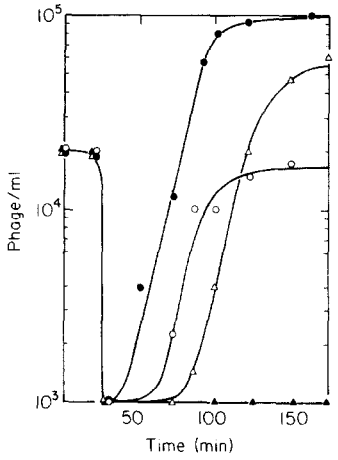

FIG. 4. Kinetics of lytic *his*3 expression.
The infection was performed in cells growing in M9 minimal medium containing glucose. (●) Infection by λgt-Sc2639. (○) Infection by λgt4-Sc2601. (△) Infection by λgt-Sc2675. (▲) Infection by λgt-Sc2619.

These results imply that the direction of transcription of the *his*3 gene is from right to left in λgt1-Sc2601. The direction of transcription of the *his*3 gene has been confirmed by hybridization of yeast messenger RNA to separated strands of a λ hybrid containing the *his*3 gene (K. Struhl & R. W. Davis, unpublished results).

(c) *Relationship of the level of lytic expression and the P_L to* his3 *distance*

Table 1 indicates the P_L to *his*3 distance for all the hybrids tested. The size of the "plaque without a lawn" is taken to be a measure of the level of lytic expression of the *his*3 gene. In Figure 3, the plaque sizes of various hybrids are visualized by staining with ethidium bromide (Struhl & Davis, 1976). These data are summarized in Table 1. The size of the plaque of a given phage is inversely related to the distance of P_L to the *his*3 gene. The effects of the *int* and *att* loci do not seem to be important. Hybrids with a P_L to *his*3 distance of less than 8 kb produce maximally large plaques. The distance effect is most clearly seen in the range of 8 to 10 kb (compare deletion mutants Sc2675, Sc2672 and Sc2639). The hybrids involved are identical except for the deletion endpoints within the yeast sequence, which differ by 1 kb intervals. Figure 3 seems to indicate that hybrids with a P_L to *his*3 distance of 12 kb are lytically *his*$^-$. This is not the case, however. λgt-Sc2643 and λgt-Sc2647 yielded a lytic response that was visualizable in two out of four experiments. This variability is probably a result of small variations in the amount of histidine in the medium (see Materials and Methods). Under standard conditions for the lytic plaque assay, we do not observe plaque formation by λgt4-Sc2601. However, such plaques are observed if the amount of histidine in the soft agar is 3 μg/ml. Under these less stringent conditions, a lytic *his*$^-$ phage does not make a plaque. The difference between λgt4-Sc2601 (an over-packaged phage) and λgt-Sc2643 or λgt-Sc2647 might be explained by instability or lower packaging efficiency of λgt4-Sc2601. Lytic expression of the *his*3 gene in λgt4-Sc2601 is also clearly demonstrated by the kinetic study shown in Figure 4. The burst size of one is probably too low to result in plaque formation under normal

conditions, but it is significant when compared to the burst size of λgt4. Figure 4 also indicates that a phage which makes large plaques (λgt-Sc2639) has a larger burst size and a shorter latent period than a phage that makes small plaques (λgt-Sc2675).

In separate experiments, the absolute plaque size of a given hybrid is somewhat variable. In each individual experiment, therefore, all hybrids were grown on the identical culture of cells. In all four separate experiments, the relative plaque sizes always followed the pattern shown in Table 1.

(d) *Lysogenic complementation depends on* his3 *transcription initiated from a promoter in yeast DNA*

Complementation of an *E. coli* auxotroph lacking IGP dehydratase activity (*hisB*463) by λ*his*3 hybrids requires a functional structural gene and a promoter that, in *E. coli*, initiates transcription of the gene. In the previous section it was shown that lytic complementation depends on the λ promoter P_L. In this section, strong evidence is presented that transcription necessary for lysogenic complementation is initiated from a promoter in the original yeast DNA segment (Sc2601). The results summarized below include those published previously (Struhl *et al.*, 1976).

Lysogenic complementation is defined by the ability of *hisB*463 cells, lysogenized by integrated λ*his*3 hybrids, to grow in the absence of histidine. It can occur even if all known λ promoters are repressed or deleted. For example, λgt-Sc2601 and λgt-Sc2664 delete the *b*2 region and the *int–xis* region. When integrated as prophages into *hisB*463 cells, the lytic promoters P_L, P_R and P'_R are repressed; the only known λ promoter functioning under these conditions (Prm) is very efficiently terminated long before the *his*3 gene. Lysogenic complementation is not dependent on the orientation of the *his*3 gene with respect to the λ vector. Sc2601, cloned in λgt1, λgt4 and λgt6, in either of the two possible orientations, complements the *hisB*463 mutation. Therefore, if the promoter necessary for *his*3 expression is not located in yeast DNA, two new λ promoters would be required. This latter possibility is clearly not compatible with observations that *E. coli* genes (*trp* and *lac*) inserted into this location in λ are not expressed as lysogens (Hopkins *et al.*, 1976; Casadaban, 1976).

The sequence in Sc2601 DNA that in *E. coli* acts like a promoter to read the yeast *his*3 gene is termed P_{his3}. It remains possible that more than one site in Sc2601 DNA could act as a promoter to initiate *his*3 transcription. It is also possible that certain derivatives of λ*his*3 hybrids may appear to be lysogenically His^+ because of a fortuitous promoter generated by the deletion event.

(e) *Isolation of a deletion mutant lacking the yeast promoter that in* E. coli *initiates transcription of the* his3 *gene*

The properties of the lytic and lysogenic complementation assays of *hisB*463 are listed in Table 2. Both complementation modes require an intact structural gene. However, lysogenic complementation depends on transcription from a promoter in the yeast DNA (P_{his3}), while lytic complementation requires transcription from P_L. Therefore, a deletion mutant lacking P_{his3} but containing the *his*3 structural gene should complement *hisB*463 lytically but not lysogenically. However, the frequency of such a deletion mutant would likely be quite low. In addition, lysogenic complementation by *int*$^-$ *att*$^-$ hybrids is somewhat inefficient (Struhl *et al.*, 1976). The

TABLE 2

Properties of lytic and lysogenic complementation of hisB*463*

Property	Lytic	Lysogenic
Functional *his*3 structural gene	Essential	Essential
Orientation dependence of *his*3	Yes	No
Level of response	Hybrid dependent	Hybrid independent
Frequency of response	1	Hybrid dependent (10^{-2} to 10^{-4})
Promoter necessary for expression	P_L	P_{his3}
Dependence of level of expression	P_L to *his*3 distance	Gene dosage

standard lysogenic complementation assay is too cumbersome for screening large numbers of prospective hybrids.

To circumvent these difficulties, we devised a single test that assays both lytic and lysogenic complementation of *his*B463. A sample of 1μl containing between 10^3 and 10^4 of a given hybrid phage is spotted on a lawn of *his*B463 cells on glucose minimal medium lacking histidine. A total of 25% of the bacterial cells were previously lysogenized by an integration helper which is cI^+ *att*$^+$ *int*$^+$. The remaining 75% of the cells are not lysogenized. A λ*his*3 hybrid containing the yeast promoter and the structural gene forms a plaque on the *his*B463 cells and *his*$^+$ colonies when integrated into the lysogenized derivatives. This simultaneous lytic and lysogenic complementation of *his*B463 is visualized as a "turbid plaque without a lawn" (Fig. 5). The degree of turbidity (number of *his*$^+$ colonies in a plaque) depends on the *int* and *att* alleles of the λ*his*3 hybrid. Phage without an intact *his*3 structural gene do not produce an observable response. A deletion mutant lacking P_{his3} but containing the *his*3 structural gene sould produce a plaque but not *his*$^+$ colonies; that is a "clear plaque without a lawn".

A total of 80 independently derived pools of deletion mutants of λgt4-Sc2601 was obtained as described in the following paper. Deletion mutants containing a functional *his*3 structural gene were isolated from each of these pools by lytic complementation of *his*B463. Out of 686 such deletion mutants tested by spotting onto the mixed indicator lawn, six (representing 2 independent isolates) produced a clear plaque without a lawn. The remaining 680 all produced turbid plaques without lawns. Upon retesting by more standard methods, these two isolates, λgt-Sc2694 and λgt-Sc2695, complement *his*B463 lytically but not lysogenically. The frequency of occurrence of these deletion mutants was between 10^{-9} and 10^{-10} (Table 3).

The isolation of λgt-Sc2694 and λgt-Sc2695 confirm directly the existence of a promoter in the yeast DNA segment which, in *E. coli*, initiates transcription of the *his*3 gene. In addition, it confirms the requirements necessary for lysogenic and lytic expression of the *his*3 gene in *E. coli* (Table 2).

(f) *Fusion of* P_{his3} *to the gene for tetracycline resistance*

The *Hin*dIII cleavage site of pMB9 DNA is located in the promoter sequence necessary for transcription of the plasmid-coded gene for tetracycline resistance,

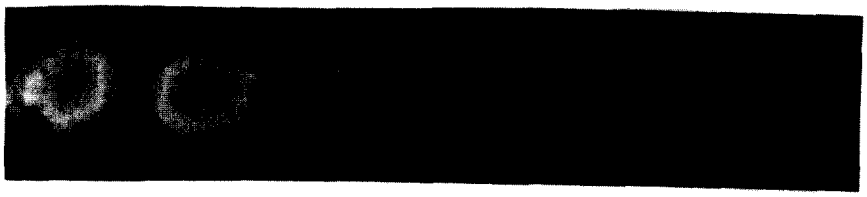

FIG. 5. Turbid and clear plaques without lawns.

Each spot indicates 10^3 to 10^4 of a given phage on a mixed indicator lawn (see the text). (a) λgt-Sc2639; (b) λgt-Sc2675; (c) λgt-Sc2601; (d) λgt-Sc2694; (e) λgt-Sc2695; (f) λgt-Sc2619. Phage and cells were incubated for 24 to 48 h at 37°C on minimal plates containing ethidium bromide.

TABLE 3

Isolation of deletion mutants lacking P_{his3}

Step	Frequency
(1) High titer stocks of λgt4-Sc2601	10^{-10}
(2) EDTA killing	10^{-7}
(3) Phage growth in EDTA	10^{-4}
(4) Selection for lytic *his*$^+$ phage	10^{-3}
(5) Selection for clear plaque without a lawn	1

tet^R (Backman *et al.*, 1976). Creation of a novel joint at this *Hin*dIII site (by insertion or deletion of DNA) generally inactivates this promoter; therefore, such plasmids do not confer tetracycline resistance on their host. However, fusion of a functional promoter to the intact structural gene results in a plasmid that does confer tetracycline resistance on the host (Backman *et al.*, 1976).

pGT1-Sc2602 was constructed by the deletion of all the *Hin*dIII fragments of pMB9-Sc2601 with the exception of the one fragment containing the plasmid replicon and the gene specifying colicin E1 resistance (Struhl & Davis, 1979). The evidence presented in the following paper indicates that the yeast DNA segment (Sc2602) contains P_{his3}. In pGT1-Sc2602, DNA, P_{his3} is located approximately 200 base-pairs from the 5′ end of the *tet* gene in the orientation conducive to transcription of the gene. HB101 (pGT1-Sc2602) forms colonies on plates containing tetracycline at concentrations up to 6 μg/ml. Derivatives of this strain resistant to 15 μg of tetracycline per ml are isolated at a frequency of 10^{-6}. In contrast, HB101 (pGT1-Sc2607) cells, which were selected in an analogous manner by resistance to colicin E1, do not form colonies in the presence of tetracycline at concentrations greater than 1 μg/ml. Sc2607 is the 0·2 kb *Eco*RI·*Hin*dIII fragment of yeast ribosomal DNA (Philippsen *et al.*, 1978).

(g) *Level of* his3 *gene expression in growing* E. coli *cells*

*his*B463 cells lack measurable IGP dehydratase activity and do not grow in the absence of histidine. The presence of a functional yeast *his*3 gene in these cells results in production of measurable IGP dehydratase activity and the ability to grow in

TABLE 4

Doubling times of E. coli *cells containing the yeast* his3 *gene*

Parent	Hybrid	Minimal medium with additions				
		None	His	Ade 2 mM-NA_3	Ade 8 mM-NA_3	Ade His 10 mM-NA_3
*his*B463	λgt-Sc2601	1·7	1·2	12	14	1·5
*his*B463	λgt-Sc2601′	1·7	1·2	12	13	1·2
*his*B463	pSC101-Sc2601	1·2	1·0	3	6	1·0
*his*B463	pMB9-Sc2601	1·2	1·1	4	6	1·1
*his*B463	ColE1-Sc2606	1·1	1·0	2	3	1·1
*his*B463	None	—	1·1	—	—	1·1
*his*461 (*Δhis*)	None	—	1·1	—	—	1·1
*his*461	pMB9-Sc2601	—	1·1	—	—	1·1
W3110 (*his*$^+$)	None	1·1	1·1	1·0	1·6	1·1

All strains were grown at 37°C in M9 minimal medium containing B1 and glucose. Histidine (1 mM), adenine (1 mM), and aminotriazole (NA_3) (concentrations indicated) were supplemented when desired. Entries in the Table are presented in hours. Strains containing λ*his*3h ybrids were also lysogenized with λ*cI*$^+$*int*$^+$*att*$^+$ in order to prevent cell lysis at 37°C.

the absence of histidine (Struhl & Davis, 1977). In this section of the Results, the *his*3 gene is stably present in the *hisB*463 cell as a hybrid of bacteriophage λ or a plasmid vector. A *hisB*463 cell, when lysogenized by a hybrid of bacteriophage λ, contains one copy of the *his*3 gene. A *hisB*463 cell containing the *his*3 gene as a hybrid of a plasmid vector (such as pSC101, pMB9 and ColE1) presumably has multiple copies of the *his*3 gene. By four independent methods, we measured the level of *his*3 gene expression in *his* B463 cells containing bacteriophage λ or plasmid hybrids.

(i) *Growth of cells dependent on* his*3 expression*

The doubling times of various strains is measured during growth in the absence of histidine (Table 4). Cells dependent on expression of a λ hybrid (one copy of the *his*3 gene) clearly grow more slowly than the wild-type control, and hence are limited in their growth rate by insufficient biosynthesis of histidine. In the presence of histidine, such cells grow at the wild-type rate. The doubling time does not depend on the orientation of the yeast DNA with respect to λ DNA. The doubling time depends only on the presence of the *his*3 gene and not the derivative containing the gene. The rate of colony formation by cells containing the λ hybrids listed in Table 1 on solid minimal medium lacking histidine is constant (data not shown). In contrast, cells with multiple copies of the *his*3 gene (as hybrids of any of the plasmids tested) grow at wild-type rates, even in the absence of histidine.

(ii) *Inhibition of* his*3-dependent growth by aminotriazole*

Aminotriazole is a competitive inhibitor of yeast and *E. coli* IGP dehydratases (Hilton *et al.*, 1965; Klopotowski & Wiater, 1965). Wild-type *E. coli* cells grown in medium containing aminotriazole derepress synthesis of the histidine biosynthetic enzymes. Consequently, the doubling time of wild-type cells in the presence of aminotriazole is only slightly slower than in the absence of the inhibitor (Table 4). Since it is likely that *his*3 gene expression is not regulated in *E. coli*, cells dependent on *his3* gene expression for IGP dehydratase production should be very sensitive to addition of aminotriazole to the medium. The data in Table 4 show this to be the case. Cells dependent on *his*3-containing λ hybrids for growth are significantly more sensitive to aminotriazole than cells dependent on plasmid hybrids. At two different concentrations of aminotriazole the growth rates of cells dependent on plasmid hybrids do not seem to be influenced significantly by the plasmid vector. This is unexpected in view of the fact that pSC101 is a stringently replicating plasmid (Cabello *et al.*, 1976) and that pMB9 and ColE1 are relaxed replicating plasmids (Rodriguez *et al.*, 1976; Clewell & Helinski, 1972).

(iii) *Level of IGP dehydratase activity in crude extracts*

The results of IGP dehydratase activity determinations are shown in Table 5. All values in the Table are expressed as specific activity (units) per doubling time (h). This number should be indicative of the approximate rate of synthesis of IGP dehydratase. The level of IGP dehydratase activity in a given strain is not significantly different in the presence or in the absence of histidine. Cells containing plasmid hybrids

TABLE 5

Enzyme levels in his*3-containing strains of* E. coli

Parent	Hybrid	−Histidine		+Histidine	
		Dehydratase	Phosphatase	Dehydratase	Phosphatase
*his*B463	λgt-Sc2601	0·2	26	NT	NT
*his*B463	λgt-Sc2601′	0·2	20	0·2	3
*his*B463	pSC101-Sc2601	1·1	9	0·9	3
*his*B463	pMB9-Sc2601	0·8	8	0·8	3
*his*B463	ColE1-Sc2606	1·1	8	0·7	3
*his*B463	None	—	—	<0·1	3
*his*461 (*Δhis*)	None	—	—	<0·1	<0·1
*his*461	pMB9-Sc2601	—	—	0·8	<0·1
W3110 (*his*$^+$)	None	1·5	2	1·3	3
W3110 (derepressed)	None	14	20	1·6	3

Cells were grown to the middle of exponential phase in M9 medium containing glucose in the presence or absence of histidine. Entries in the Table represent specific activities. NT, not tested. W3110 was derepressed by addition of 10 mM-aminotriazole to minimal medium.

and thus multiple gene copies produce roughly four times the enzyme activity as compared to cells containing a single gene copy as a λ hybrid prophage. The enzyme level in cells containing either of the plasmid hybrids is less than a factor of two lower than the level in repressed wild-type *E. coli*.

(iv) *Derepression of the* E. coli *histidine operon*

The state of the cells with respect to histidine starvation has also been monitored by assaying histidinolphosphate phosphatase activity. Starvation of *E. coli* for histidine causes an increase in the levels of all the histidine biosynthetic enzymes in the *his* operon (Ames *et al.*, 1961). Cells starved for histidine produce more histidinol-phosphate phosphatase, the quantity depending on the severity of starvation. Cells with an excess of endogenous histidine have a baseline level of this enzyme. The results in Table 5 indicate that cells containing λ hybrids are severely starved for histidine, while cells containing plasmid hybrids are merely hungry.

4. Discussion

Complementation of a bacterial auxotroph lacking IGP dehydratase activity by the yeast *his*3 gene requires both the structural gene sequences and other sequences that *E. coli* can recognize to express the gene. This physiological study functionally distinguishes structural from non-structural sequences. The distinction is made possible by defining the necessary conditions for two modes by which *E. coli* expresses the yeast *his*3 gene.

In the lysogenic mode, expression of the *his*3 gene in integrated λ hybrids appears to depend on a promoter in the yeast DNA. This is demonstrated directly by the isolation of deletion mutants that inactivate this promoter (λgt-Sc2694 and λgt-Sc2695). It is strongly supported by the fact that *hisB*463 cells lysogenized by any *his*3$^+$ phage grow at roughly the same rate in the absence of histidine, and by the previous observation that the complementation was independent of the orientation of the *his*3 DNA with respect to the λ vector (Struhl *et al.*, 1976). The promoter P_{his3} presumably functions in the plasmid hybrids that contain the same fragment of yeast DNA. Recently, J. A. Jaehning (unpublished results) has shown that *E. coli* RNA polymerase holoenzyme binds specifically to the P_{his3} region of Sc2601 DNA.

*his*3 expression during lytic infection by bacteriophage λ hybrids depends on readthrough transcription initiated at the λ promoter P_L. The lytic expression is orientation dependent, independent of the promoter in the yeast DNA (P_{his3}), independent of *int* and *att*, dependent on the P_L allele, and dependent on the P_L to *his*3 distance. The lytic expression depends on synergy between the host cell and the infecting phage. The cell must contain a residual amount of histidine to permit the initial events of phage infection. Once the *his*3 gene is expressed, the cell begins to grow and the phage can complete a normal infection process in the growing cell. The level of residual histidine is critical: too high, all phage grow; too low, no phage grow. Since P_{his3} is not sufficient to allow lytic *his*3 expression, it is likely that a stronger promoter is necessary. From the orientation dependence, the direction of *his*3 transcription must be from right to left in λgt1-Sc2601. This has been confirmed by nucleic acid hybridization experiments (K. Struhl & R. W. Davis, unpublished results).

In the literature there has been some question as to whether the level of read-through transcription depends on the distance of P_L to the gene. Adhya *et al.* (1974) has clearly shown a distance effect for the *gal* genes, but Franklin (1971; and personal communication) has not observed it for the *trp* genes. Our results indicate a distance effect for P_L to *his*3 lengths greater than 8 kb. The response becomes saturated at lengths smaller than 8 kb. All the *trp* phages studied by Franklin have P_L to *trp* distances of less than 8 kb. Three possible explanations for the P_L to *his*3 distance effect are: (1) random termination of the P_L initiated transcript; (2) weak transcriptional termination sequences located at a fairly large number of discrete sites (an average of 1 per 500 base-pairs for example); or (3) an increase in time after infection for expression of the *his*3 gene for derivatives with longer P_L to *his*3 distances, which disrupts the synergy necessary for lytic expression.

Isolation of λ hybrids that delete the yeast promoter P_{his3} was greatly facilitated by the ability to combine the lytic and lysogenic complementation assays into a single test. Hybrids containing P_{his3} and the *his*3 structural gene form "turbid plaques without lawns" on the mixed indicator plate (Fig. 5). Such hybrids, when spotted on the mixed "lawn", grow lytically on the starved *hisB*463 cells. Therefore, within the plaque there is a high local concentration of the hybrid phage and of histidine from the lysed cells. These two conditions result in lysogenic complementation (His^+ colonies) within a plaque. As expected, the number of colonies within the plaque depends on the frequency of integration of a hybrid phage into *hisB*463 cells (see Materials and Methods). Using this mixed complementation assay, it is possible to screen a large number of hybrids containing the *his*3 structural gene for the presence of absence of P_{his3}. Hybrids lacking P_{his3} (λgt-Sc2694 and λgt-Sc2695) produce "clear plaques without lawns", since they are unable to complement *hisB*463 lysogenically.

The fact that lytic *his*3 expression requires only a functional structural gene has great utility. To study the expression of the yeast *his*3 gene in yeast and in *E. coli* cells, it is useful to have derivatives that alter the expression of the gene while retaining the intact structural gene. In particular, it is now possible to assess the physiological effect in yeast cells of *his*3 derivatives that delete sequences near the 5′ end of the structural gene. When cloned in appropriate vectors, such derivatives may be introduced into yeast cells by DNA transformation (Hinnen *et al.*, 1978; Struhl *et al.*, 1979). The lytic complementation assay insures that any particular derivative contains a fully functional *his*3 structural gene.

In *E. coli*, the level of *his*3 expression is relatively high. By four independent criteria, it seems likely that the level of *his*3 gene expression is affected by gene dosage. Multiple copies of the gene in an *E. coli* cell correlate with increased expression as compared to a single copy. The physiological evidence indicates that plasmid hybrids produce about four times more IGP dehydratase activity than the single copy level. It is surprising that the level of expression from the pSC101 hybrid is indistinguishable from the level of the pMB9 or the ColE1 hybrid. pSC101 is usually present in five copies per cell (Cabello *et al.*, 1976), while pMB9 and colE1 are usually present in 20 copies per cell (Rodriguez *et al.*, 1976; Clewell & Helinski, 1972). This apparent anomaly may be explained by one or a combination of the following models. (1) A plasmid-coded promoter that reads the *his*3 gene. (2) Inhibition or *his*3 transcription in certain vectors by a variety of possible mechanisms. (3) The possibility

that the plasmid copy number in cells grown under these conditions might be different from the usually obtained values. We doubt that the *his*3 gene is regulated at the level of gene expression, because IGP dehydratase levels of strains grown in the presence or in the absence of histidine are not significantly different and because all hybrid containing cells are quite sensitive to aminotriazole. Inhibition by aminotriazole is sufficiently stringent to allow for the selection of strains resistant to its action. Some of these resistant strains overproduce yeast IGP dehydratase (Brennan & Struhl, 1980).

The *his*3 gene studied in *E. coli* is presumably identical to the native gene found in yeast. We think it extremely unlikely that isolation of the original *his*3-containing hybrid by gene expression selected for an altered form of the *his*3 capable of expression. The frequency of His^+ hybrid phage from a pool of hybrid phage (Struhl *et al.*, 1976) is indistinguishable from the frequency of phage that contain Sc2601 DNA sequences (Struhl & Davis, 1977). The frequency of reversion to His^+ of a cloned mutant (His^-) gene is compatible with the frequency of reversion of a simple point mutant. If the cloned *his*3 gene in Sc2601 is altered by mutation, the frequency of this alteration must be close to 100%. Therefore, a native yeast gene is expressed in *E. coli* with high fidelity (Struhl & Davis, 1977) and at a fairly high level. The number or eukaryotic genes selected by complementation of *E. coli* auxotrophs is significant (Struhl *et al.*, 1976; Ratzkin & Carbon, 1977; Vapnek *et al.*, 1977; Clarke & Carbon, 1978). These findings imply that the schism between prokaryotic and eukaryotic organisms with respect to gene expression may not be so wide.

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