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A Physical, Genetic and Transcriptional Map of the Cloned *his*3 Gene Region of *Saccharomyces cerevisiae*

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A cloned fragment of *Saccharomyces cerevisiae* (yeast) DNA containing the structural gene for imidazoleglycerolphosphate dehydratase (*his*3) has been mapped using a combination of physical techniques and classical bacteriophage λ genetics. A physical map has been constructed using subcloned restriction endonuclease fragments from the original yeast DNA fragment (Sc2601) and using deletion mutants of a bacteriophage λ hybrid containing Sc2601. The deletion endpoints within the yeast DNA segment have been mapped with respect to restriction endonuclease cleavage sites of Sc2601. The wild-type *his*3 gene, as defined by complementation of an *Escherichia coli* auxotroph lacking imidazoleglycerolphosphate dehydratase activity, is localized to a 700 base-pair region. The 5′ and 3′ endpoints of the gene are defined within limits of 50 base-pairs. The lesions in cloned mutant *his*3 genes that are non-functional in yeast and in *E. coli* have been mapped by phage recombination using deletion mutants of the *his*3 gene generated in *E. coli*. Transcription of the *his*3 gene in *E. coli* is initiated from a promoter located less than 100 base-pairs from the start of the structural gene.

1. Introduction

The understanding of the molecular mechanisms involved in the regulation of gene expression in prokaryotes such as *Escherichia coli* is quite advanced. The extensively studied regulatory systems are involved with such basic biological events as the adaptation to changes in the external environment (exemplified by the *E. coli* lactose operon) and the developmental cycle of an organism (such as bacteriophages λ and P22). Detailed studies of these and other systems have depended on both the availability of physiological variants that are amenable to genetic analysis, and the isolation of the relevant genes and gene products. With the recent technological ability to clone eukaryotic genes as hybrids of bacteriophages or plasmids that replicate in *E. coli*, it should be possible to probe the molecular mechanisms that govern their expression in significantly more detail.

We have chosen the *his*3 gene of *Saccharomyces cerevisiae* (yeast) as a model system for the study of eukaryotic gene expression. The *his*3 gene codes for the structural gene of imidazoleglycerolphosphate dehydratase (Fink, 1964). Many mutations of this locus are available (G. Fink, unpublished results), as are unlinked mutations,

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which presumably affect its expression (Wolfner *et al.*, 1975). A viable molecular hybrid of bacteriophage λ and yeast DNA has been isolated by complementation of an *E. coli* auxotroph lacking IGP† dehydratase (Struhl *et al.*, 1976). By genetic and biochemical criteria, this complementation was shown to result from the cloning of the structural gene for IGP dehydratase (*his*3) (Struhl & Davis, 1977). Cloned mutant *his*3 alleles do not function in *E. coli* as assayed by complementation. *E. coli* cells containing the yeast *his*3 gene produce an activity that strongly resembles the IGP dehydratase activity found in wild-type yeast cells.

Since λ is a temperate phage, it can be propagated in an *E. coli* lysogen (as an integrated prophage) or as a lytic virus. In the preceding paper, we physiologically characterize two modes of yeast *his*3 expression in *E. coli* of bacteriophage λ*his*3 hybrids. Lysogenic expression depends on transcriptional initiation from a promoter located in the yeast DNA. Lytic expression depends on "readthrough" transcription intiated from the λ promoter P_L.

A necessary prelude to studies concerning the expression of the yeast *his*3 gene in *E. coli* and in yeast is a physical fine structure map. In this paper, we describe such a map for a 10·1 kb fragment (Sc2601) of yeast DNA containing the *his*3 gene. Deletion mutants of a bacteriophage λ hybrid containing Sc2601, which were generated during lytic growth of the hybrid in *E. coli*, were employed in the construction of the map. Endpoints of the deletion mutants have been mapped relative to sites of cleavage by restriction endonucleases within Sc2601. Various fragments generated by restriction endonuclease cleavage have been subcloned.

We have used the physical mapping data to understand various aspects of the expression of the yeast *his*3 gene in *E. coli*. By determining which of the deletion mutants complement IGP dehydratase-deficient *E. coli* cells, the location of the *his*3 gene function in *E. coli* has been defined. The deletion mutants are also used for a unique form of eukaryotic fine structure genetic mapping. The location of *his*3 mutations of yeast are determined by classical deletion mapping in *E. coli* using bacteriophage λ hybrids containing cloned mutant *his*3 genes. The location of the promoter that initiates transcription of the *his*3 gene has been determined by comparing the lytic and lysogenic expression of many of the deletion mutants. These approaches therefore allow one to construct a functional and genetic map of a eukaryotic gene that is related directly to the physical structure of the gene.

2. Materials and Methods

(a) *Terminology*

All hybrid DNAs are defined in terms of a vector and of a yeast DNA component. The vector name is determined by the vector DNA component in the final hybrid molecule. A given *S. cerevisiae* (yeast) DNA sequence is numbered (e.g. Sc2601). Hybrid phage and hybrid DNA are named by both the vector and the yeast DNA component separated by a hyphen (e.g. λgt4-Sc2601). The term λgt is used generically for all vector components that are derived from a λgt vector, but which are not identical to any of the λgt vectors described previously. The yeast DNA isolation number is retained if the yeast sequence is simply transferred between vectors (e.g. λgt-Sc2601 and pMB9-Sc2601).

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

(b) *Chemicals and enzymes*

^{32}P-labelled deoxy GTP (approximately 350 Ci/mmol) was obtained from Amersham Searle. *Eco*RI endonuclease, *E. coli* DNA ligase and *E. coli* DNA polymerase I were gifts from Marjorie Thomas, Paul Modrich and Michael Goldberg, respectively. All other restriction endonucleases and T4 DNA ligase were purchased from New England BioLabs. DNase I was obtained from Worthington and S_1 nuclease was obtained from Miles.

(c) *Bacterial, phage and plasmid strains, and their preparation*

C600 *hsr*$^-$*hsm*$^+$ and *his*B463 have been described elsewhere (Struhl *et al.*, 1976). Phage strains not characterized in this paper were λgt-Sc2601, λgt-Sc2601′ (Struhl *et al.*, 1976), λgt-Sc2612, λgt-Sc2679 (Struhl & Davis, 1977), and λgt4 (Panasenko *et al.*, 1977). Plasmid strains used for cloning were pSC101 (Cohen *et al.* 1973), pMB9 (Rodriguez *et al.*, 1976), and pBR322 (Bolivar *et al.*, 1977).

E. coli cells with or without plasmids were grown in L broth or in M9 minimal medium with glucose (Struhl *et al.*, 1976). Tetracycline (10 μg/ml) and ampicillin (50 μg/ml) were added to media for drug selection. For colicin selections, an empirically determined quantity of colicin E1 (obtained from David Finnegan) was added to cells just prior to plating. High titer phage stocks were prepared by growth on C600 *hsr*$^-$*hsm*$^+$ on solid medium containing L broth.

(d) *Preparation of DNA*

λ DNA was prepared as described by Thomas & Davis (1975). Plasmid DNA was prepared by a modification of the procedure developed by Clewell & Helinski (1972). *E. coli* cells (100 ml) containing autonomously replicating plasmid DNA molecules were sedimented by centrifugation, washed once with 10 mM-Tris (pH 7·5), 1 mM-EDTA and resuspended in 1·5 ml of 0·8 M-sucrose, 50 mM-Tris (pH 8·3), 40 mM-EDTA. The sample was chilled on ice and treated with 0·6 ml of a solution containing 5 mg of egg-white lysozyme/ml. After 10 min, 2·7 ml of 10 mM-EDTA, 50 mM-Tris (pH 7·5) and 0·1% Triton X-100 were added. The cells were mixed gently until lysis was complete (approx. 10 min). Cellular debris and chromosomal DNA from the lysed cells were removed by centrifugation in a Beckman JA20 rotor for 20 min at 18,000 revs/min at 0°C. Then 4·2 ml of the cleared lysate and 0·2 ml of a 10 mg/ml solution of ethidium bromide were added to 3·9 g of CsCl (the density was 1·55 g/cm^3). The sample was transferred to a Beckman polyallomar ultracentrifuge tube (SW 50.1) and banded to equilibrium by centrifugation at 30,000 revs/min for 3 days. Plasmid DNA was isolated by puncturing the side of the centrifuge tube with a syringe needle. Ethidium bromide was removed by extraction with 1-butanol. The DNA was diluted with 3 vol. water, precipitated with ethanol, and resuspended in 10 mM-Tris (pH 7·5), 1 mM-EDTA. Though such DNA samples are useful for most applications, they were occasionally purified further by a second banding in CsCl in order to remove contaminating DNA and RNA. For rapid analysis of DNA structures, the procedures of Cameron *et al.* (1977) and of Rambach & Hogness (1977) were followed.

(e) *Enzyme reactions*

Typical restriction endonuclease reactions were performed in 0·4 ml plastic tubes (Sarstedt) and contained 0·2 to 1·0 μg of DNA in a final volume of 20 μl. Reactions were incubated at 37°C for approximately 2 h and were stopped by heating to 70°C for 5 min. All reactions were performed at pH 7·5 in 6 mM-β-mercaptoethanol and 100 μg of autoclaved gelatin/ml. Conditions for the restriction digests were as follows: *Eco*RI, *Pvu*I, *Sst*I, *Sal*I, *Xba*I in 50 mM-Tris, 100 mM-NaCl, 10 mM-$MgCl_2$; *Bam*H1, *Hin*dIII, *Hha*I, *Pst*I, *Xba*I in 10 mM-Tris, 50 mM-NaCl, 7 mM-$MgCl_2$; *Bgl*II, *Hpa*I, *Hpa*II, *Kpn*I in 10 mM-Tris, 10 mM-NaCl, 10 mM-$MgCl_2$. The amount of enzyme necessary for a total digest was determined empirically.

Ligations were typically performed in plastic tubes at 14°C in a reaction volume of 10 μl. The reaction conditions for *E. coli* DNA ligase were 30 mM-Tris (pH 8), 1 mM-EDTA, 4 mM-$MgCl_2$, 10 mM-$(NH_4)_2SO_4$, 50 μg of bovine serum albumin/ml and 50 μM-NAD^+.

The reaction conditions for T4 DNA ligase were 50 mM-Tris (pH 7·5), 100 mM-NaCl, 10 mM-$MgCl_2$, 10 mM-dithiothreitol, and 1 mM-ATP. The amount of enzyme necessary for ligation was determined empirically.

^{32}P-labelled DNA was prepared by a modification of the nick translation procedure of Rigby *et al.* (1977). A volume (20 μl) of a buffer containing 50 mM-Tris (pH 7·5), 10 mM-$MgCl_2$, 1 mM-dithiothreitol, 20 μM each of deoxy CTP, ATP and TTP, and 0·25 μg of DNA was added to a plastic tube containing 25 μCi of deoxy GTP that had been dried. Then 0·2 μl of a 10^{-5} dilution of a 1 mg/ml solution of DNase I and 0·2 μl of a 2 mg/ml solution of DNA polymerase I were added. The diluent for DNase I was 10 mM-Tris (pH 7·5), 10 mM-$MgCl_2$, 1 mg of bovine serum albumin/ml. The reaction was incubated for 3 to 4 h at 12 to 14°C.

(f) *Construction and selection of viable hybrids*

All hybrid DNAs were constructed in the following general way. DNA samples were combined and cleaved with the desired restriction endonuclease. Generally 0·3 to 0·6 μg of plasmid DNA and 1 to 2 μg of phage DNA were used per hybrid construction. The cloning of small DNA fragments was facilitated by high concentrations of DNA. The circularization of large prospective hybrid DNAs was favored by relatively low concentration of the input DNAs. Restriction endonuclease cleavage was stopped by heating the reaction mixture to 70°C for 5 min. For ligation with T4 DNA ligase, the reaction mixture (in 10 μl) was brought to the correct conditions by the addition of the appropriate amounts of concentrated stock solutions. For ligation with *E. coli* DNA ligase, the reaction mixture was brought to 25 mM-EDTA and 0·25 M-NaCl, and 2 vol. redistilled ethanol were added. The sample (total vol. 75 μl) was frozen at −70°C in a solid CO_2/ethanol bath for 15 min. DNA was precipitated using a Beckman Microfuge, washed with 100 μl of 70% ethanol, dried briefly, and resuspended in 9 μl of 10 mM-Tris (pH 7·5), 1 mM-EDTA. Then 1 μl of a 10-fold buffer solution necessary for ligation, followed by 0·1 μl of *E. coli* DNA ligase were added. Ligation with either *E. coli* or T4 DNA ligase was performed at 12 to 14°C for 6 to 24 h.

Hybrid DNA molecules were introduced into *E. coli* cells by a modification of the procedure of Mandel & Higa (1970). To recover viable hybrid phage, the transfection procedure of Thomas *et al.* (1974) was used. To recover *E. coli* strains containing hybrid plasmid DNAs, the cells to be transformed were harvested by centrifugation, resuspended in an equal volume of ice-cold 50 mM-$CaCl_2$, and incubated at 0°C for 20 min. Following centrifugation in a Beckman JA20 rotor (3000 revs/min for 5 min), the cells were resuspended with 0·05 the original volume of 50 mM-$CaCl_2$. A portion (0·1 ml) of this cell suspension was added to 0·1 ml of 10 mM-Tris (pH 7·5), 10 mM-$CaCl_2$, 10 mM-$MgCl_2$ containing 10 to 30 ng of vector DNA equivalent. Cells and DNA were incubated together for 20 min at 0°C followed by 3 min at 37°C: 1 ml of L broth was added and the cells were allowed to grow for 30 min at 37°C. For drug and colicin selections, 2·5 ml of soft agar (0·7%) containing L broth were added and the resulting mixture was plated on selective solid medium. For direct selection of the *his*$^+$ characteristic, cells were allowed to grow in L broth for an additional 60 min, pelleted by centrifugation, resuspended in 0·1 ml of 10 mM-$MgCl_2$, and spread on M9 minimal plates containing glucose. Since strain *hisB*463 is "K-restricting", it is essential that all DNAs used to transform this strain be "K-modified". When this was not the case, hybrid DNA molecules were transformed into C600 *hsr*$^-$*hsm*$^+$. Hybrid plasmid DNA isolated from this strain could then be tested for *his*$^+$.

pMB9-Sc2601 was constructed by *Eco*RI cleavage of pMB9 and λgt-Sc2601 DNAs followed by ligation. Upon transfection into *hisB*463 cells, tetracyline resistant (*tet*^{r}) colonies that grew in the absence of histidine (*his*$^+$) were selected. pSC101-Sc2601 was constructed analogously.

λgt4-Sc2601 was constructed by *Eco*RI cleavage of pMB9-Sc2601 and λgt4 DNAs followed by ligation. *his*$^+$ phage were selected and then tested for genetic characteristics (see below) and for physical structure of the DNA. As expected, this phage was slightly more sensitive than wild-type λ to EDTA and/or heat treatments (data not shown).

pGT1-Sc2602 was constructed by *Hin*dIII cleavage of pMB9-Sc2601 DNA followed by ligation. Colicin-resistant colonies were screened for yeast DNA sequences using the colony filter hybridization method of Grunstein & Hogness (1975). The probe was ^{32}P-labeled, nick-translated λgt-Sc2601 DNA. This method of construction deletes all the *Hin*dIII fragments of pMB9-Sc2601 DNA except for the fragment that contains the plasmid replicon and the gene for colicin resistance. This effectively clones the *Hin*dIII-*Eco*RI fragment of yeast DNA adjacent to the large *Hin*dIII-*Eco*RI fragment of pMB9 DNA (see Fig. 2).

pGT2-Sc2605 DNA was constructed in an analogous manner as pGT1-Sc2602, except that *Sal*I was employed instead of *Hin*dIII.

λ554-Sc2613 was constructed by *Hin*dIII cleavage and ligation of λ554 and pMB9-Sc2601 DNAs. Recombinant phage were screened by the plaque filter hybridization method of Benton & Davis (1977). λ554-Sc2613 was selected for hybridization to pGT2-Sc2605 DNA but not to pGT1-Sc2602 DNA.

pBR322-Sc2676 was constructed by cleavage of pGT2-Sc2605 and pBR322 DNAs with *Bam*HI followed by ligation. The colony of interest was selected by sensitivity to tetracycline and resistance to ampicillin.

pBR322-Sc2678 was constructed analogously to pBR322-Sc2676, except that the DNAs were cleaved by *Bam*HI and *Hin*dIII. Of 3 tetracycline sensitive, ampicillin resistant, hybrid-containing strains tested, all had structures identical to pBR322-Sc2678.

pBR322-Sc2710 was constructed by cleavage of pBR322 and pGT2-Sc2605 DNAs with *Pst*I followed by ligation. The colony of interest was selected by resistance to tetracycline and sensitivity to ampicillin.

pMB9-Sc2731 was constructed by *Kpn*I cleavage of pMB9-Sc2601 DNA followed by ligation. The colony of interest was tetracycline resistant.

In all hybrid constructions, the actual structure of the hybrid DNA was determined by restriction enzyme analysis. This analysis was facilitated significantly by the rapid isolation of plasmid and phage DNAs.

(g) *Isolation of deletion mutants of λgt4-Sc2601*

Independent plaques of λgt4-Sc2601 were picked and grown on solid medium into high titer phage stocks by infection of C600 *hsr*$^-$*hsm*$^+$. Phage stocks were harvested by overlaying the plates with 5 ml of 10 mM-Tris (pH 7·5), 1 mM-$MgCl_2$ for 12 h at 4°C. To assure independence of all deletion mutants, these stocks were always maintained separately throughout the selection procedure described below. Phage from each high titer stock were diluted at least 100-fold into 10 mM-Tris (pH 8·5), 10 mM-EDTA, and were heated to 37°C for 15 min. For effective killing by EDTA, it is essential that the concentration of Mg^{2+} be less than 0·1 mM, even in the presence of 10 mM-EDTA (data not shown). After 15 min at 37°C, the infectivity of λgt4-Sc2601 is reduced by a factor greater than 10^3. The EDTA killing was stopped by addition of $MgCl_2$ to a final concn of 10 mM. The surviving phage were immediately infected into a culture of C600 *hsr*$^-$*hsm*$^+$ growing exponentially in L broth. The infected culture was plated on solid medium containing 1% trypticase (BBL), 1 mM-EDTA (pH 8), 100 mM-NaCl. Wild-type length phage are unable to form plaques on this medium. Deletion mutants that have lost greater than 1·5 kb of DNA plate with an efficiency of 1 under these conditions (data not shown). Therefore, every plaque visible on these plates has arisen from a deletion mutant of the original phage. From each original independent plaque, the resulting deletion mutants were either picked individually or pooled together.

(h) *Genetic tests*

The number of *Eco*RI sites in a given phage DNA were determined by the efficiency of plating on a strain (C600 *rRI*$^+$*mRI*$^+$) that contained *Eco*RI endonuclease (Thomas *et al.*, 1974). The efficiency of plating depends on the number of *Eco*RI sites in the phage DNA.

For each *Eco*RI site, the phage is restricted by a factor of approximately 10. For convenience, 0·1 ml of a 10^{-2} dilution of a picked plaque was plated on C600 rRI^+mRI^+ and C600 hsr^-hsm^+.

The test for *red* was the ability to plate on H560 ($polA^-$). red^+ but not red^- phage plate on this strain (Zissler *et al.*, 1971).

The test for *int* and *xis* was the *red* plaque test of Enquist & Weisberg (1976).

We devised a test for *att* based on the fact that int^+att^+ phage can act as integration helpers for int^-att^- phage. int^+his3^- hybrids were co-infected with λgt-Sc2601′, at a multiplicity of about 5 phage/cell each, into *hisB*463 cells (Struhl *et al.*, 1976). Co-infection with int^+att^+ hybrids resulted in an almost confluent lawn of his^+ colonies; co-infection with $int^+attΔ0P'$ hybrids resulted in 100 to 1000 his^+ colonies/plate; co-infection with int^-att^- hybrids resulted in less than 10 colonies/plate. int^+his3^+ phage were tested by single infection into *hisB*463 cells. The frequency of his^+ colonies similarly depended on the *int* and *att* genotype. By virtue of the screening procedures used for selection of deletion mutants of λgt4-Sc2601, all int^- phage were also att^-.

Phage that contained specific sequences of DNA were screened by the plaque filter hybridization method of Benton & Davis (1977). The probes used were ^{32}P-labelled, nick translated, plasmid hybrid DNAs containing subcloned fragments of Sc2601 DNA.

Phage were screened for the ability to complement *hisB*463 by the double lysogen method (Struhl *et al.*, 1976) or by the lytic plaque assay described in the accompanying paper (Struhl & Davis, 1979).

(i) *Gel electrophoresis*

Agarose gel electrophoresis of DNA samples cleaved by restriction endonucleases was performed by a modification of the method of McDonnell *et al.* (1977). Typically, 200 ml of agarose in 90 mM-Tris base, 2·5 mM-EDTA, 90 mM-boric acid (final pH 8·3) were poured into a Lucite mold (13 cm × 21 cm × 0·5 cm): 2% gels were used to separate fragments from 0·2 to 1·5 kb: 0·7% gels were used for fragments ranging from 1 to 8 kb. To determine if the Sc2601 DNA sequence, when cleaved by any single restriction endonuclease, produced a fragment of DNA between 0·03 kb and 0·4 kb, samples were separated on an 8% acrylamide gel. No fragments below 150 base-pairs in length were detected. The size standards used in all cases were appropriate restriction digests of λ DNA. The length of λ DNA was taken to be 49·0 kb (Philippsen *et al.*, 1978).

(j) *Nucleic acid hybridization to cellulose nitrate filters*

Cellulose nitrate filters containing DNA to be radioactively probed were prepared in a variety of ways. For colony filter hybridization, the procedure of Grunstein & Hogness (1975) was used with one minor modification. The filter was treated with the appropriate solutions by successive placement on presoaked Whatman 3MM paper. For plaque filter hybridization, the procedure of Benton & Davis (1977) was followed. The procedure of Southern (1975) was employed with some modifications in order to transfer to nitrocellulose the DNA that had been electrophoretically separated in an agarose gel. Following electrophoresis, the gel was soaked in 0·5 M-NaOH, 2·5 M-NaCl for 15 to 45 min, followed by 1 M-Tris (pH 7·5), 3 M-NaCl for an additional 15 to 45 min. In general, low percentage gels were washed for shorter times than high percentage gels. The washed gel was placed on top of 8 sheets of Whatmann 3MM paper presoaked with 10 × PNE (PNE is 0·04 M-sodium phosphate (pH 7·0), 0·4 M-NaCl, 1 mM-EDTA). A sheet of nitrocellulose paper cut to the exact size of the gel was soaked in PNE and placed on the gel. Between 10 and 20 sheets of 3MM paper cut to the appropriate size were placed on top of the nitrocellulose strip, and a large stack of paper towels was placed on top of the 3MM paper. To insure efficient transfer of the DNA to the cellulose nitrate filter, it is essential that the bottom layer should always be soaked with 10 × PNE, all contact points between the various layers should be free from air bubbles, the dry paper should never contact wet paper except directly through the sandwich, and the entire sandwich should be weighted down

sufficiently to insure good contact between layers. Generally, the transfer process was allowed to proceed overnight. When the DNA fragments to be transferred were large (greater than 5 kb), the gel was irradiated with short-wave ultraviolet light (259 nm) for 2 to 10 min. After the transfer was complete, the strip was soaked in PNE to remove excess salt. In all cases, after the DNA was transferred, the nitrocellulose filter was baked *in vacuo* at 80°C for 90 min.

Prior to hybridization, the strips were pretreated for at least 4 h at 65°C in 2·5 × PNE, 0·3% sodium lauryl sulfate, 1 mg of denatured, sonicated, salmon sperm DNA/ml, 0·02% Ficoll (Sigma), 0·02% polyvinylpyrollidine, and 0·02% bovine serum albumin. Pre-treatment and subsequent hybridization were performed in heat-sealed plastic bags. Following pre-treatment, the solution was poured out, and the hybridization solution was added to the bag. The hybridization solution was identical to that used for pre-treatment, except that alkali-denatured, ^{32}P-labeled, nick translated probe was included. Enough hybridization solution was used such that it flowed freely and that there were few if any air bubbles. Hybridization proceeded for 18 to 48 h at 65°C. The extent of nucleic acid hybridization is limited kinetically. Therefore, hybridization signals were improved by longer hybridization times and by increasing the amount of radioactivity in the bag. Generally, for nick translated probes with approx. spec. act. = 10^8 cts/min per μg of DNA, 10^6 to 10^7 cts/min of probe was used for a filter with dimensions of 11 cm × 14 cm. It is possible to increase the radioactivity in the bag to 5×10^7 cts/min without significantly increasing the non-specific background. After hybridization, the filter was washed at the hybridization conditions for at least 2 h with at least 4 changes of buffer (lacking the salmon sperm DNA), twice in PNE, and dried in air. The filter was wrapped in Saranwrap, and autoradiographed using Kodak XR 5 film and a DuPont intensifying screen (Lighning Plus).

(k) *Heteroduplex-S_1 mapping*

A given deletion mutant and λgt-Sc2601 DNAs (0·25 μg each) were denatured in 45 μl of 0·1 M-NaOH, 10 mM Na_4EDTA, and 0·1 M-CsCl for 10 min at 25°C; then 5 μl of 2 M-Tris (pH 7·2) was added and the DNAs allowed to renature at 65°C for 30 min. The heteroduplexes were cooled quickly on ice. Then 5 μg of thermally denatured, sonicated, salmon sperm DNA followed by 0·45 ml of S_1 nuclease buffer (60 mM-sodium acetate (pH 4·6), 0·25 M-NaCl, 2 mM-$ZnCl_2$) containing an empirically determined quantity of S_1 nuclease were added. This reaction was incubated for 30 min at 37°C. *E. coli* tRNA (20 μg) was added and the reaction stopped immediately by the addition of 1 ml of re-distilled ethanol. The sample was frozen for 15 min at −70°C in a solid CO_2/ethanol bath. Following precipitation with ethanol using a Beckman Microfuge, and resuspension in 10 mM-Tris (pH 7·5), 1 mM-EDTA, the samples were subjected to gel electrophoresis. This entire series of steps was performed in a 1·5-ml capped plastic tube. Following electrophoresis, the DNA was transferred to nitrocellulose and challenged for hybridization with a ^{32}P-labeled, nick translated probe of pGT2-Sc2605 DNA.

3. Results

(a) *Characterization of hybrids containing subcloned fragments of Sc2601*

Sc2601 is a 10·1 kb *Eco*RI DNA fragment that codes for the wild-type *his*3 gene from strain A364a × H79-20·3 α (Struhl & Davis, 1977). It has been cloned as hybrids of derivatives of bacteriophage λ (Struhl *et al.* 1976) and as hybrids of the plasmids pSC101, pMB9 and ColE1 (Struhl, Stinchcomb & Davis, 1980). Various fragments generated by restriction endonuclease cleavage of Sc2601 DNA have been subcloned using λ or plasmid vectors as described in Materials and Methods. The hybrid DNAs each contain one subcloned restriction endonuclease fragment of Sc2601 DNA (Fig. 1).

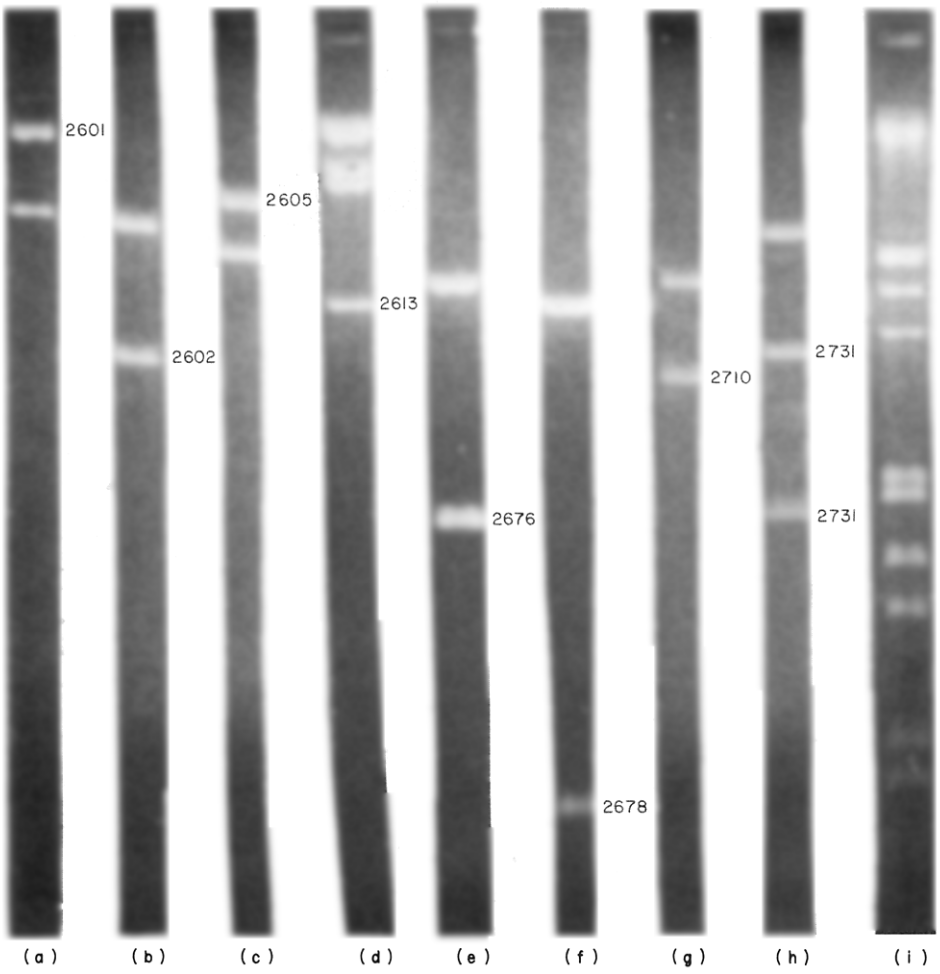


FIG. 1. Subcloned DNA fragments.

Each lane contains 0·2 μg of DNA cleaved by the appropriate restriction endonuclease(s). (a) *Eco*RI cleaved pMB9-Sc2601; (b) *Eco*RI-*Hin*dIII cleaved pGT1-Sc2602; (c) *Eco*RI-*Sal*I cleaved pGT2-Sc2605; (d) *Hin*dIII cleaved λ554-Sc2613; (e) *Bam*H1 cleaved pBR322-Sc2676; (f) *Bam*H1-*Hin*dIII cleaved pBR322-Sc2678; (g) *Pst*I cleaved pBR322-Sc2710; (h) *Kpn*I-*Eco*RI cleaved pMB9-Sc2731; (i) 0·5 μg of *Eco*RI-*Hin*dIII λcI857S7. DNAs were electrophoretically separated in 0·7% agarose.

Sc2602 is a 2·9 kb *Hin*dIII-*Eco*RI DNA fragment cloned in pGT1; Sc2605 is a 6·1 kb *Sal*I-*Eco*RI DNA fragment cloned in pGT2; Sc2613 is a 4·0 kb *Hin*dIII DNA fragment cloned in the λ554 vector of Murray & Murray (1975); Sc2676 is a 1·7 kb *Bam*H1 fragment cloned in the plasmid vector pBR322 of Bolivar *et al.* (1977); Sc2678 is a 780 base-pair *Bam*HI-*Hin*dIII DNA fragment cloned in pBR322; Sc2710 is a 2·8 kb *Pst*I DNA fragment cloned in pBR322; and Sc2731 is a deletion of Sc2601 that removes the internal *Kpn*I yeast DNA fragments (see Fig. 3).

Hybrids containing each of the subcloned fragments were tested for the ability

to complement an *E. coli* strain lacking IGP dehydratase (*hisB*463). Only Sc2605, Sc2676 and Sc2710 complement *hisB*463. Therefore, the *his*3 gene, as defined by this complementation, is located within the 1·2 kb region common to Sc2676 and Sc2710 (see Fig. 3).

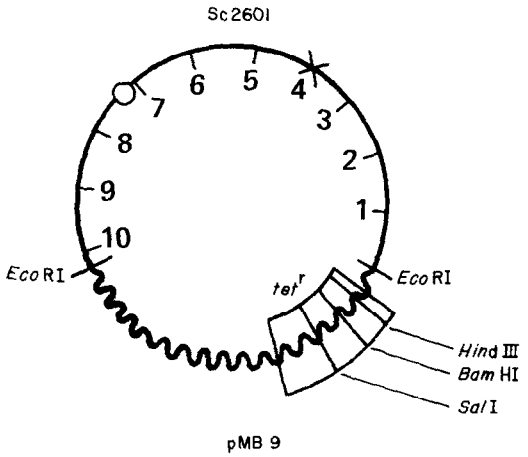
(b) *Restriction endonuclease cleavage map of Sc2601*

The physical map of Sc2601 is linearly defined in kb units (Fig. 2). The position defined as zero is the left endpoint of the 10·1 kb *Eco*RI DNA fragment cloned as λgt-Sc2601. Because λgt-Sc2601 was constructed by the *Eco*RI-DNA ligase technique (Struhl *et al.*, 1976), the zero point represents the junction between the left end *Eco*RI fragment of λgt and the inserted yeast DNA fragment. There is one *Sal*I recognition site in Sc2601 DNA because cleavage of λgt-Sc2601 DNA with both *Eco*RI and *Sal*I produced two fragments (4·0 and 6·1 kb in length) in addition to fragments due to recognition sites in the λgt vector. The location of this *Sal*I site with respect to the defined zero point was determined by *Sal*I cleavage of λgt-Sc2601 (Fig. 2). The site occurs at co-ordinate 4·0, since the *Sal*I fragment resulting from cleavage at this site and the site at 0·677 on the λ map (P. Philippsen, unpublished results) is 7·1 kb in length. This result is confirmed by the presence of a 5·0 kb *Sal*I fragment in λgt-Sc2601′, a hybrid that has the 10·1 kb *Eco*RI fragment cloned in the opposite orientation with respect to λgt from that of λgt-Sc2601. The orientation of Sc2601 with respect to pMB9 DNA has been established for the hybrid pMB9-Sc2601 in an analogous manner (Fig. 2).

The mapping of restriction endonuclease recognition sites in Sc2601 DNA is described below. The cloned 2·9 kb *Eco*RI-*Hin*dIII DNA fragment in pGT1-Sc2602 is localized at the right end of Sc2601 DNA map co-ordinates 7·2 to 10·1) by virtue of the construction of the hybrid (see Materials and Methods). The subcloned 6·1 kb *Eco*RI-*Sal*I DNA fragment in pGT2-Sc2605 must occur between co-ordinates 4·0 to 10·1 for analogous reasons. This also confirms the orientation of Sc2601 DNA with respect to pMB9 DNA. By appropriate restriction enzyme digests of pMB9-Sc2601, pGT1-Sc2602 and pGT2-Sc2605 DNAs, the *Hin*dIII, *Bam*H1, and *Hpa*I sites in Sc2601 DNA were mapped. Restriction endonuclease cleavage sites in the vector DNA were factored out by cleavage of pMB9 DNA with the appropriate enzymes. Using the knowledge from these results, a 1·7 kb *Bam*H1 fragment (Sc2676) and a 0·8 kb *Bam*HI-*Hin*dIII fragment (Sc2678) were also subcloned. These latter subcloned fragments were used with those described previously to map the *Hpa*II and *Hha*I sites in Sc2605. Restriction endonuclease recognition sites for *Pst*I, *Xho*I, *Kpn*I, *Xba*I, and *Bgl*II were determined by the appropriate digestion of pMB9-Sc2601 DNA followed by separate cleavages with *Eco*RI or *Sal*I. *Sma*I, *Sst*I and *Pvu*I do not cleave Sc2601 DNA. These data are summarized in Tables 1 and 2. A restriction map incorporating all these data is shown in Figure 3. The location of the subcloned DNA fragments with respect to Sc2601 DNA is indicated.

(c) *Isolation of deletion mutants of λgt4-Sc2601*

Wild-type λ phage are inactivated rapidly by chelating agents and/or heat (Parkinson & Huskey, 1971). Deletion mutants of λ, which occur spontaneously at a frequency of 10^{-6} per generation, are resistant to these treatments (Parkinson & Huskey, 1971).



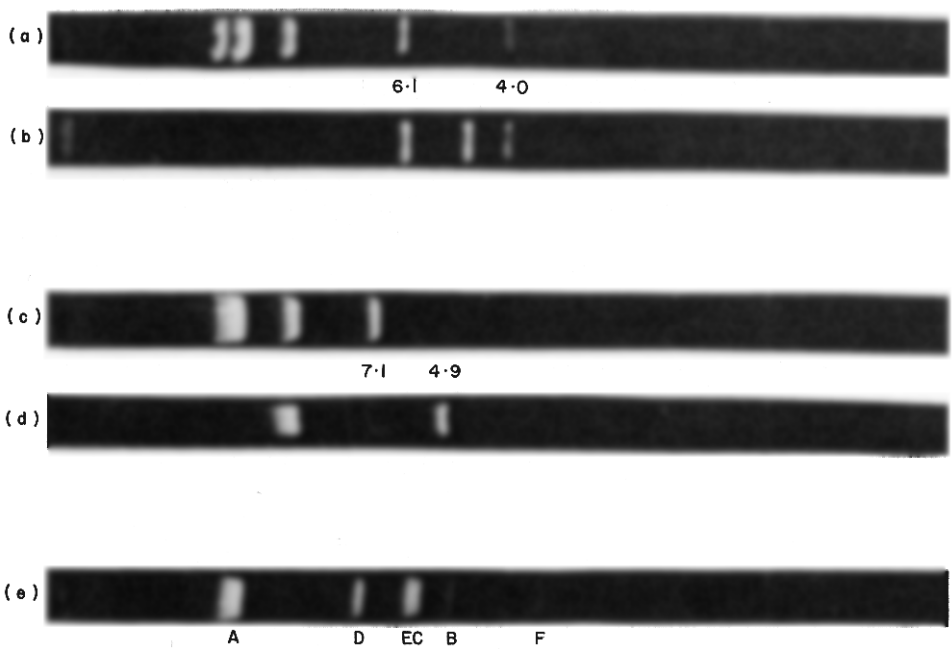


FIG. 2. Orientation of the physical map of Sc2601 DNA.

The linear molecule at the top of the Figure is λgt-Sc2601 DNA. λgt sequences are indicated by wavy lines; yeast (Sc2601) DNA sequences are indicated by a straight line. The junctions

TABLE 1

Restriction endonuclease fragments of Sc2601 DNA

Enzyme	Fragment lengths (kb)
*Eco*RI	A = 10·1
*Sal*I	A = 4·0; B = 6·1
*Hin*dIII	A = 0·6; B = 0·8; C = 1·6; D = 0·4; E = 3·7; F = 0·2; G = 2·9
*Bam*H1	A = 1·7; B = 0·8; C = 1·0; D = 2·8; E = 1·7; F = 2·1
*Hpa*I	A = 2·9; B = 6·5; C = 0·6
*Pst*I	A = 6·9; B = 2·8; C = 0·4
*Xho*I	A = 6·7; B = 3·4
*Kpn*I	A = 1·7; B = 1·7; C = 3·5; D = 3·2
*Xba*I	A = 2·1; B = 0·4; C = 3·2; D = 4·4
*Bgl*II	A = 2·6; B = 3·0; C = 0·7; D = 1·0; E = 2·8
*Hpa*II	A = 1·1; B = 0·9; C = 1·3; D = 2·8
*Hha*I	A = 1·0; B = 1·1; C = 0·6; D = 0·4; E = 0·6; F = 0·7; G = 1·1 H = 0·6

DNA fragments listed in the Table were generated by cleavage of Sc2601 DNA with *Eco*RI and a given endonuclease. The fragments are ordered (alphabetically) from left to right as defined in Fig. 2. A physical map of these fragments is shown in Fig. 3.

TABLE 2

Co-ordinates of restriction endonuclease cleavage sites of Sc2601 DNA

Enzyme	Co-ordinates
*Eco*RI	0; 10·1
*Sal*I	4·0
*Hin*dIII	0·6; 1·4; 3·0; 3·4; 7·0; 7·2
*Bam*H1	1·7; 2·5; 3·5; 6·3; 8·0
*Hpa*I	2·9; 9·4
*Pst*I	6·9; 9·7
*Xho*I	6·7
*Kpn*I	1·7; 3·4; 6·9
*Xba*I	2·1; 2·5; 5·7
*Bgl*II	2·6; 5·6; 6·3; 7·3
*Hpa*II	5·1; 6·0; 7·3
*Hha*I	5·0; 6·1; 6·7; 7·1; 7·7; 8·4; 9·5

The co-ordinates for cleavage of Sc2601 DNA by a given restriction endonuclease are indicated (to the nearest 100 base-pairs). A physical map of these sites is shown in Fig. 3.

between λgt and Sc2601 sequences are *Eco*RI sites. Sc2601 DNA is defined in kb units (0 to 10·1). The circular molecule at the bottom of the Figure is pMB9-Sc2601 DNA. Wavy lines represent pMB9 DNA. The *Hin*dIII, *Bam*H1, and *Sal*I sites in the gene for tetracycline resistance (*tet*) are indicated. The circle at co-ordinate 7·2 is a *Hin*dIII site; the × at co-ordinate 4·0 is the unique *Sal*I site. pGT1-Sc2602 and pGT2-Sc2605 were constructed by ligation of these ends to those produced by *Hin*dIII and *Sal*I, respectively, which are located in the *tet* gene. Each lane of the 0·3% agarose gel contains 0·2 μg DNA. Samples are: (a) *Sal*I-*Eco*RI cleaved λgt-Sc2601; (b) *Sal*I-*Eco*RI cleaved pMB9-Sc2601; (c) *Sal*I cleaved λgt-Sc2601; (d) *Sal*I cleaved pMB9-Sc2601; (e) *Eco*RI cleaved λcI857*S*7 (fragments indicated).

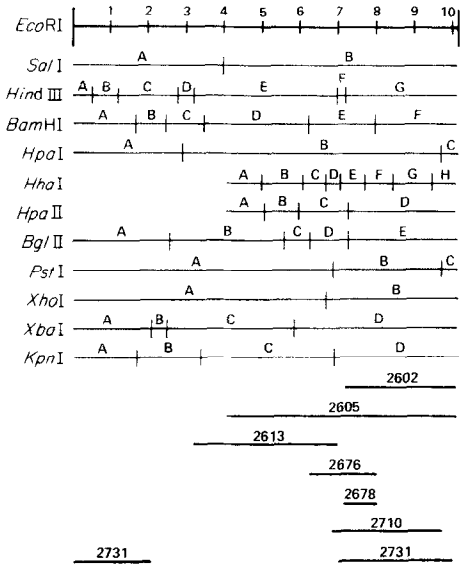


FIG. 3. Restriction endonuclease cleavage map of Sc2601 DNA.
Fragments generated by cleavage with *Eco*RI and the endonuclease listed in the left column are ordered alphabetically from left to right.

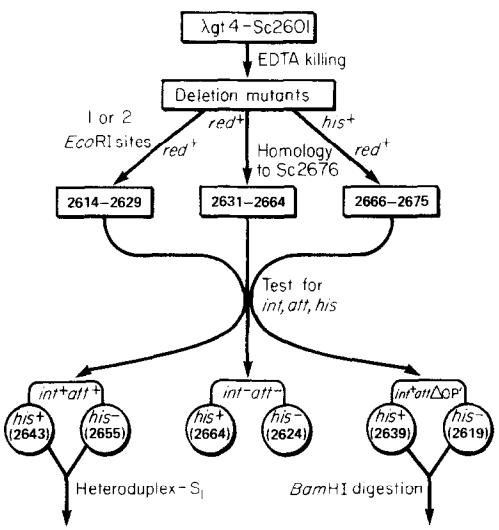


FIG. 4. Isolation of deletion mutants of λgt4-Sc2601.
Deletion mutants of λgt4-Sc2601 were isolated by resistance to EDTA killing. Some deletion mutants were preselected as indicated, and all were tested for *int*, *att*, and *his3*. Sample deletion mutants (of each class) are indicated in the circles at the bottom of the Figure. Properties of all the deletion mutants are summarized in Table 3.

All the deletion mutants used in this study were derived from λgt4-Sc2601. This phage, unlike deletion mutants derived from it, is extremely sensitive to chelating agents, since its DNA is of wild-type length. Over 200 independently derived deletion mutants of λgt4-Sc2601 have been physically and/or genetically characterized to some extent.

Characterization of the deletion mutants was facilitated greatly by the preliminary series of simple genetic tests listed below and shown diagramatically in Figure 4.

(1) Number of *Eco*RI recognition sites: λgt4-Sc2601 DNA has two *Eco*RI recognition sites. Deletion mutants have zero, one or two *Eco*RI recognition sites. The efficiency of plating of a given delction mutant on an *Eco*RI restricting host was used as a measure of the number of *Eco*RI sites (Thomas *et al.*, 1974). In general, deletion mutants containing only one *Eco*RI site were analyzed further. These deletions have one endpoint in the yeast DNA sequence and one endpoint in λ DNA. Some deletion mutants containing two *Eco*RI sites (deletions totally within the yeast DNA sequence) were also analyzed.

(2) *red:* The *red* (recombination) genes of bacteriophage λ map approximately 5 kb to the right of the yeast DNA sequence. All *red*$^-$ derivatives examined contained two *Eco*RI restriction sites and were not studied further, since they are not deleted for yeast DNA sequences.

(3) *int* and *attλ*: Approximately 70% of deletion mutants generated by vegetative growth of λ have a common endpoint. The endpoint lies at the λ attachment site (Parkinson & Huskey, 1971). All deletion mutants of this type (*int*$^+$*attΔ*OP′) were mapped physically.

(4) Hybridization to subcloned fragments: 34 deletion mutants (Sc2631 to Sc2664) were preselected by the plaque filter hybridization method of Benton & Davis (1977). Since deletions with endpoints in or near the *his*3 gene region were of primary interest, ^{32}P-labeled, nick translated probes were chosen using the information reported in the previous section. All these deletion mutants hybridized to a probe of pBR322-Sc2676 DNA, and some of these hybridized to a probe of pGT1-Sc2602 DNA.

(5) *his*$^+$ complementation: Deletion mutants Sc2666-Sc2675 were preselected by the lytic complementation of hisB463 (Struhl *et al.*, 1980). The degree of lytic complementation varied from pinpoint to large plaques without lawns (Struhl *et al.*, 1980). A summary of the properties of the deletion mutants is shown in Table 3.

(d) *Physical mapping of deletion mutants of λgt4-Sc2601*

The map co-ordinates of deletion mutants were determined with respect to the *Bam*HI restriction endonuclease cleavage sites in Sc2601 DNA. *Bam*HI cleaves λgt4-Sc2601 DNA at eight sites to generate six yeast DNA-containing fragments (A to F) shown diagramatically in Figure 5. Deletion mutants having a known endpoint between *int* and *attλ* (that is, *int*$^+$*attΔ*OP′) were simply mapped by *Bam*H1 cleavage of the hybrid DNAs. Four examples are shown in Figure 6. All have fragments A to D, lack E and F, and have a new fragment of length *Y*. Since the distance of the common end to the nearest *Bam*H1 site in λ DNA is known to be approximately 275 base-pairs (Schreier *et al.*, 1977), the endpoint within the *Bam*H1·E fragment is $Y-275$ base-pairs from the *Bam*H1 site that defines the D/E border. Since deletion

TABLE 3

Properties of deletion mutants of λgt4-Sc2601

Class (1) *his*$^-$*int*$^+$*attΔOP′*
2627 (A); 2658 (2·6); 2621 (2·7); 2662 (3·7); 2660 (4·0); 2620(4·5); 2649 (4·6); 2623 (5·0); 2638 (5·5); 2650 (5·6); 2618 (5·7); 2625 (5·8); 2644 (5·8); 2615 (5·9); 2640 (5·9); 2645 (6·0); 2634 (6·1); 2635 (6·1); 2640 (6·3); 2641 (6·3); 2629 (6·4); 2628 (6·5); 2622 (6·5); 2626 (6·6); 2631 (6·6); 2636 (6·7); 2648 (6·9); 2616 (7·3); 2619 (7·5)

Class (2) *his*$^+$*int*$^+$*attΔOP′*
2639 (7·6); 2666 (7·6); 2667 (7·6); 2669 (7·6); 2670 (7·6); 2671 (7·8); 2637 (8·1); 2774 (8·2); 2773 (8·5); 2772 (8·8); 2775 (9·7)

Class (3) *his*$^-$*int*$^-$*att*$^-$
2624 (D)

Class (4) *his*$^+$*int*$^-$*att*$^-$
2668 (E); 2664 (F)

Class (5) *his*$^-$*int*$^+$*att*$^+$
2617 (7·4); 2651 (7·4); 2655 (7·5); 2652 (7·5); 2654 (E); 2661 (E); 2656 (F); 2657 (F); 2663 (F)

Class (6) *his*$^+$*int*$^+$*att*$^+$
2647 (D); 2696; 2697; 2698; 2699; 2700; 2701; 2702

Class (7) *his*$^+$ internal deletions
2635 (A, D); 2643 (1·5, 6·7)

Class (8) *his*$^-$ internal deletions
2646 (2·7, 7·3); 2659 (3·4, 7·6); 2642

Class (9) Deletions of P_{his3} (*int*$^-$*att*$^-$)
2694 (7·6); 2695 (7·6)

The deletion mutants are divided into 9 phenotypic classes. Classes (1) and (2) are presumably *int* mediated (Parkinson & Huskey, 1971) and have a common endpoint at *att*. These deletions were mapped by *Bam*H1 cleavage. Classes (3) and (4) delete yeast DNA to the *right* of the endpoint 2624 and 2668 retain the *Bam*H1 cleavage site at 0·581 on the λ map (Philippsen, unpublished results). The endpoint in λ DNA of 2664 is undetermined. Classes (5) and (6) delete all the yeast DNA to the *left* of the endpoint (the λ DNA endpoint being localized between the *J* gene and co-ordinate 0·445). The endpoint in yeast DNA was precisely determined by the heteroduplex-S_1 method and crudely by *Bam*H1 cleavage. Classes (7) and (8) are deleted internally within Sc2601 DNA, since the derivatives each contain 2 *Eco*RI sites. These were mapped in a manner similar to that used for classes (5) and (6). Class (9) deletions were mapped by a combination of *Bam*H1 digestion and the heteroduplex-S_1 method. The co-ordinate(s) of the endpoint(s) within Sc2601 DNA of a given deletion mutants are indicated parenthetically. Endpoints designated by a letter indicate a location within a given *Bam*H1 fragment of Sc2601 DNA. Deletion mutants without indicated endpoints have not been mapped.

mutants of the *int*$^+$*attΔ*OP′ variety have one common endpoint, they define the map by a directional, linear series of DNA regions that are deleted successively. A physical map of these deletion mutants is shown in Figure 7.

Deletion mutants that do not have one defined endpoint are more difficult to map. *Bam*H1 cleavage of the DNAs from such deletion mutants locates one or both endpoints within a given fragment (A to F). Properties of deletions with one endpoint in fragment D or fragment F are summarized in Table 3. More precise localization of these endpoints has not been determined. Deletions with one endpoint in fragment E (the 1·7 kb *Bam*H1 fragment cloned as Sc2676 which complements *hisB*463) were mapped by the heteroduplex-S_1 method of Shenk *et al.* (1974). Heteroduplexes

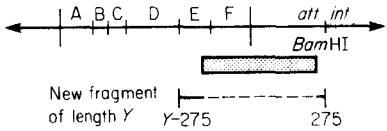


FIG. 5. Mapping deletion mutants of λgt4-Sc2601 by *Bam*H1 digestion.

Cleavage of λgt4-Sc2601 DNA (horizontal line) by *Bam*H1 generates the following fragments: B, C, D and E, which are internally within Sc2601 DNA; a 16 kb fragment containing Sc2601 fragment A and a large portion of the left end *Eco*RI fragment of λgt4; a 4·1 kb fragment containing Sc2601 fragment F, which is bounded on the right by the *Bam*H1 site in *int* indicated on the diagram; and 3 fragments of λgt4 (not shown). The location of a deletion of λgt4-Sc2601 DNA is indicated by the stippled box. The deletion is of the *int*$^+$*att*Δ*OP′* variety. The right endpoint of such a deletion is located in *att*; i.e. to the left of the *Bam*H1 site in *int*. The left endpoint of the deletion mutant occurs in fragment E. Therefore, *Bam*H1 cleavage of this deletion mutant DNA generates a new fragment (Y) in place of fragments E and F. Since the distance from the *Bam*H1 site in *int* to the common endpoint in *att* is 275 base-pairs (Schreier *et al.*, 1977), the co-ordinate of the left endpoint of the deletion is $6{\cdot}3 + (Y - 0{\cdot}275)$.

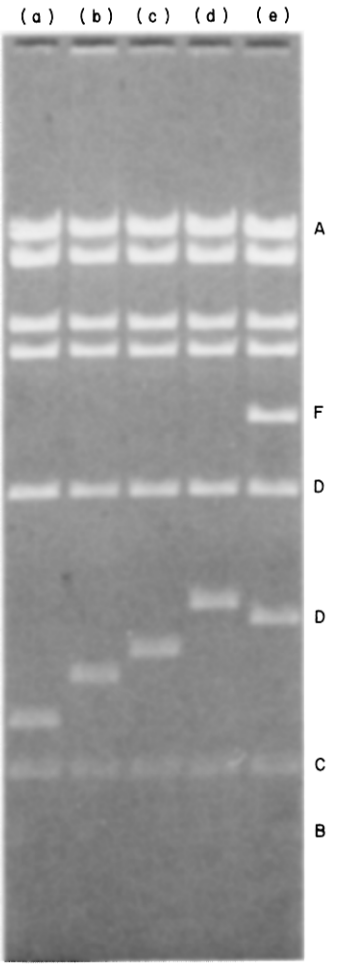


FIG. 6. *Bam*H1 cleavage of several deletion mutants of λgt4-Sc2601 DNA.

Each lane contains 0·4 μg of DNA cleaved with *Bam*H1. (a) λgt-Sc2616; (b) λgt-Sc2619; (c)λgt-Sc2639; (d) λgt-Sc2671; (e) λgt-Sc2601. DNAs were separated in 0·7% agarose.

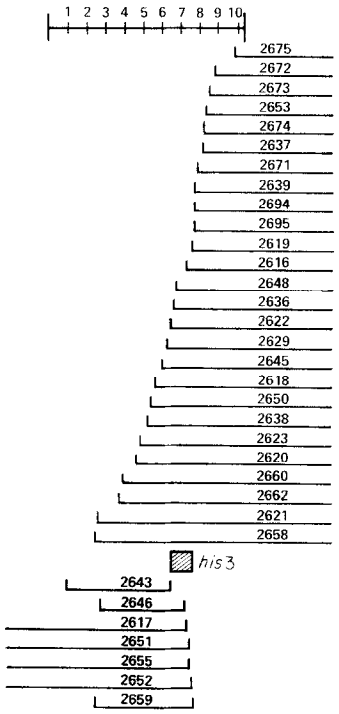


FIG. 7. Map of some of the deletion mutants of λgt4-Sc2601.

The *his*3 gene is localized by complementation analysis to the hatched box. Deletions above this box start at the common endpoint in *att* and delete DNA successively from the right side of Sc2601. Deletions below this box are missing DNA to the left of the *his*3 gene. Some (2617, 2651, 2655, 2652) delete all the yeast DNA to the left of the endpoint; others (2643, 2646, 2659) delete internally within Sc2601 DNA.

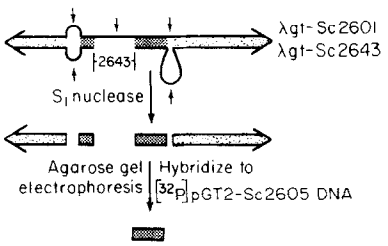


FIG. 8. Heteroduplex-S_1 nuclease mapping.

The upper molecule is a heteroduplex between λgt-Sc2601 and λgt-Sc2643 DNAs. The heavily stippled regions indicate homology between the yeast DNA sequences of these two phages. The lightly stippled regions indicate homology between λ sequences. Single-stranded regions are indicated by unbroken lines. The position of the 2643 deletion is indicated. Cleavage by S_1 nuclease (arrows) results in the products shown on the second line. Only 1 of these DNA fragments contains Sc2605 DNA sequences (bottom line).

between a given deletion mutant and λgt-Sc2601 DNAs were digested with S_1 nuclease. As shown in Figure 8, this method generates a DNA fragment of length X, which defines the co-ordinate of the deletion endpoint as $10{\cdot}1 - X$. The data for such an experiment are shown in Figure 9 and included in the map shown in Figure 7.

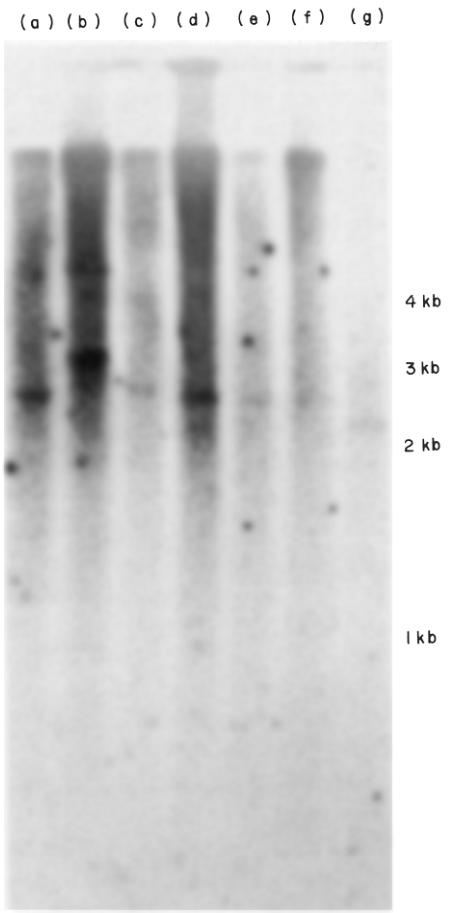


FIG. 9. Autoradiogram from heteroduplex-S_1 nuclease mapping.
The following deletion mutants were mapped as described in Materials and Methods and in Fig. 8: (a) λgt-Sc2617; (b) λgt-Sc2643; (c) λgt-Sc2646; (d) λgt-Sc2651; (e) λgt-Sc2652; (f) λgt-Sc2655; (g) λgt-Sc2659. The left endpoints of the internal deletions were determined by *Eco*RI cleavage of the DNAs. Such cleavage makes it possible to estimate the total length of the deleted DNA.

Deletion mutants λgt-Sc2694 and λgt-Sc2695 lack the yeast promoter necessary for *his*3 transcription in *E. coli*, but contain the intact structural gene (Struhl & Davis, 1980). *Bam*H1 digestion of these DNAs indicated that the deletions have one endpoint in the *Bam*H1·E fragment and one end point to the right of *att*. In order to determine these endpoints, the phage DNAs were mapped by a combination of

the previous methods (Fig. 10). Samples consiting of DNAs from a given deletion mutant and from pBR322-Sc2676 were cleaved with *Bam*H1, denatured in alkali, renatured, and treated with S_1 nuclease. The DNA was electrophoretically separated in an agarose gel, transferred to nitrocellulose paper, and challenged for hybridization with a probe of ^{32}P-labeled, nick translated pBR322-Sc2676 DNA. The results from such an experiment are shown in Figure 11. In addition to bands due to homoduplex renaturation of pBR322-Sc2676 DNA (at 1·7 and 4·3 kb), a new band corresponding to heteroduplex renaturation of pBR322-Sc2676 and the deletion mutant DNAs is observed. The length of this new band is indicative of the distance from the *Bam*H1 site at co-ordinate 6·3 to the deletion endpoint. By this method, the deletion endpoints of λgt-Sc2694 and λgt-Sc2695 occur at co-ordinate 7·6. This mapping procedure was also performed on deletion mutants λgt-Sc2616, λgt-Sc2619, λgt-Sc2639 and λgt-Sc2671. The results shown in Figure 11 confirm those shown in Figure 7.

(e) *Location of the* his*3 gene by complementation analysis*

The deletion mutants of λgt4-Sc2601 described in the previous section were analyzed for the ability to complement an *E. coli* auxotroph (*hisB*463) lacking IGP dehydratase activity. Complementation of a given deletion mutant was assayed during either lysogenic or lytic propagation of the hybrid in these cells (see Materials and Methods). A lysogenic *his*$^+$ phage, when integrated into the chromosome of *hisB*463, allows the cell to grow in the absence of histidine. A lytic *his*$^+$ phage can form a "plaque without a lawn" on starved *hisB*463 cells (Struhl *et al.*, 1980).

The complementation analysis of the deletion mutants of λgt4-Sc2601 is shown in Table 3. All deletion mutants tested, with the exception of λgt-Sc2694 and λgt-Sc2695, were either *his*$^+$ or *his*$^-$ by both lytic and lysogenic complementation. From this analysis the co-ordinates of the *his*3 gene are 6·9 to 7·6. The right endpoint occurs between the deletion endpoints of Sc2619 and Sc2639, a distance of approximately 100 base-pairs. The left endpoint occurs to the right of the *Pst*I cleavage site at co-ordinate 6·9 and to the left of the *Kpn*I cleavage site, a distance of less than 50 base-pairs. Therefore, the maximum length of the *his*3 gene as determined by complementation of an *E. coli* auxotroph is approximately 750 base-pairs. As expected, these 750 base-pairs are located totally within the 1·7 kb *Bam*H1 fragment (Sc2676) which complements *hisB*463. These results are shown diagramatically in Figure 12.

(f) *Deletion mapping in* E. coli *of yeast* his*3 mutations*

We have described the cloning of the *his*3 genes from two *his*3$^-$ mutants of yeast (*his*3-38 and *his*3-532) which lack IGP dehydratase activity (Struhl & Davis, 1977). These cloned mutant genes do not complement *hisB*463. The lesions in these cloned mutant genes have been mapped by phage recombination with some of the non-complementing (*his*$^-$) deletion mutants described in the previous section. The progeny of a given cross were scored for *his*$^+$ recombinants by the lytic complementation assay. The frequency of such recombinants, when they occurred, was between 10^{-3} and 10^{-4}. When recombinants were not observed, the frequency was less than 10^{-6}. Table 4 shows the results of this deletion mapping, which are included in the diagram of Figure 12. The lesion in *his*3-38 (cloned as λgt-Sc2612) maps between the

deletion endpoints of Sc2616 and Sc2648. The lesion in *his*3-532 (cloned as λgt6-Sc2679) maps between the deletion endpoints of Sc2616 and Sc2619. These results confirm the mapping order of the cloned *his*3 mutations determined by a three-factor cross between the two hybrid phages (Struhl & Davis, 1977).

(g) *Direction of transcription of the* his*3 gene*

Results presented in the preceding paper indicate that the *his*3 gene is transcribed from right to left as shown in Figure 10. These results depend on the observations that expression of the *his*3 gene during lytic infection of λ hybrids depends on transcription

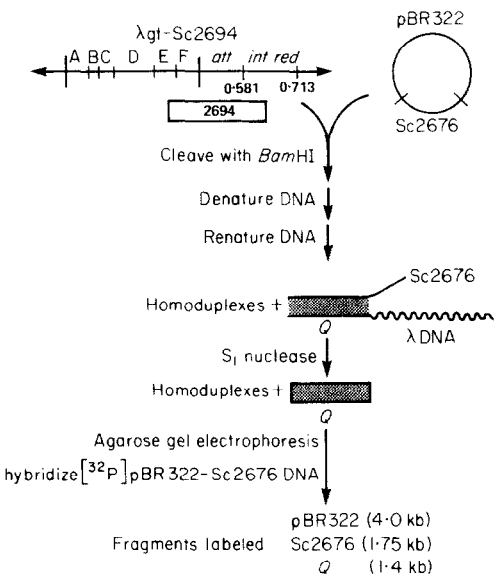


FIG. 10. *Bam*H1-heteroduplex-S_1 nuclease mapping.

The structures of λgt4-Sc2601 and pBR322-Sc2676 DNAs are drawn at the top of the Figure. The positions of the *Bam*H1 cleavage sites (perpendicular lines) and the 2694 deletion (open box) are indicated. *Bam*H1 cleavage followed by denaturation and renaturation of the fragments generates homoduplex molecules and the indicated heteroduplex molecule. The heteroduplex molecule consists of an homologous region of length *Q* (stippled region), a single-stranded region from the remainder of Sc2676 sequences (unbroken line), and a single-stranded region from λ sequences (wavy line). Treatment of the heteroduplex molecules with S_1 nuclease produces a double-stranded DNA fragment of length *Q*; homoduplexes are not affected by S_1 nuclease. The length of fragment *Q* is determined by agarose gel electrophoresis and hybridization analysis.

initiated from the λ promoter P_L. Transcripts initiated from P_L are known to read-through into adjacent genes (Franklin, 1971; Adhya *et al.*, 1974; Hopkins *et al.*, 1976). The 5′ to 3′ direction of such a transcript with respect to Sc2601 would be from right to left. When the 5′ to 3′ orientation of Sc2601 is inverted with respect to λ (as in λgt-Sc2601′), lytic *his*3 expression is not observed. The direction of transcription of the *his*3 gene has been confirmed by nucleic acid hybridization experiments using messenger RNA from yeast cells and ^{36}P-labeled separated strands of λgt-Sc2601 DNA (Struhl & Davis, unpublished results).

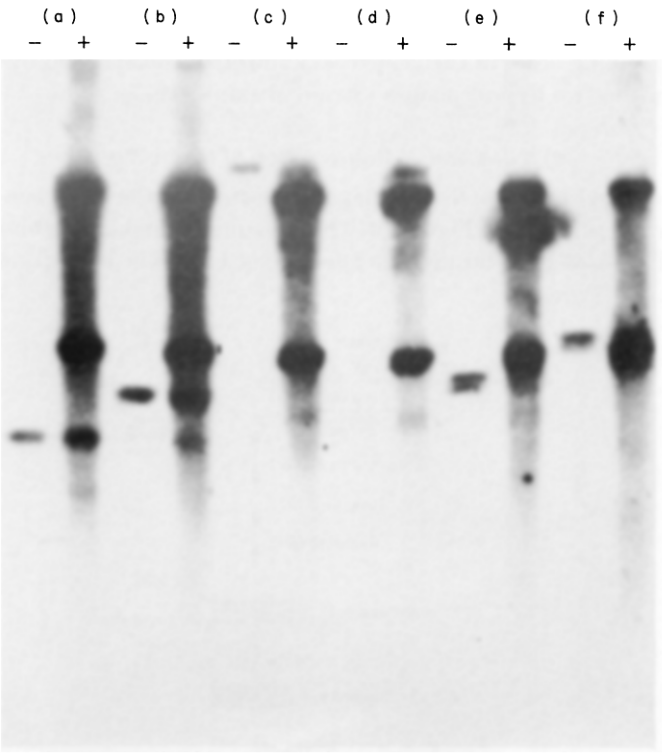


FIG. 11. Mapping of deletions with endpoints near P_{his3}.

The following deletion mutants were mapped by the *Bam*H1-heteroduplex-S_1 nuclease technique: (a) λgt-Sc2616; (b) λgt-Sc2619; (c) λgt-Sc2694; (d) λgt-Sc2695; (e) λgt-Sc2639; (f) λgt-Sc2671. Each lane contains 0·5 μg of a given deletion mutant DNA. Half of the lanes contain 0·3 μg of pBR322 DNA (+), and the other half do not (−). In all cases, DNAs were cleaved with *Bam*H1. These DNA fragments were treated as described in section (k) of Materials and Methods. DNA fragments were electrophoretically separated in 0·9% agarose and probed with ^{32}P-labelled, nick translated pBR322-Sc2676 DNA.

(h) *Localization of a yeast DNA sequence which, in* E. coli, *initiates transcription of the* his*3 gene*

The yeast DNA sequence, which behaves as a promoter in *E. coli*, has been localized utilizing results presented in the accompanying paper concerning the lytic and lysogenic complementation of the deletion mutants. The lysogenic complementation of *his*B463 by the yeast *his*3 gene depends on a promoter located in the yeast DNA as well as an intact structural gene (Struhl *et al.*, 1976; Struhl & Davis, 1977). Lytic complementation depends on the λ promoter P_L as well as an intact structural gene (Struhl *et al.*, 1980).

Deletion mutants Sc2619 and Sc2639 have deletion endpoints that are approximately 100 base-pairs apart. Otherwise they are identical, since both contain Sc2601 DNA sequences defined by co-ordinates 0 to 7·5, and both lack Sc2601 DNA sequences defined by co-ordinates 7·6 to 10·1 (Fig. 7). Sc2639 (and four other independent

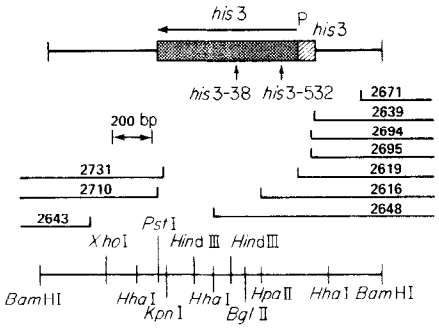


FIG. 12. Map of the *his*3 region.

A physical, genetic and transcriptional map of the 1·7 kb *BamH*1·E fragment (Sc2676) of Sc2601 DNA. The following items are shown: location of the *his*3 gene; location of the promoter that transcribes the *his*3 gene in *E. coli* (P_{his3}); the location of 2 cloned *his*3 lesions; the location of deletion mutants; the location of subcloned restriction endonuclease fragments; and restriction endonuclease sites within this DNA fragment. The complementation unit is defined by a 700 base-pair (bp) structural gene region and a small "promoter" region, which is immediately adjacent to it. The 3′ end of the gene is localized between the *Pst*I and the *Kpn*I site (a distance of less than 50 base-pairs). The 5′ end is localized between deletion end points of λgt-Sc2619 and λgt-Sc2639. Deletion mutants λgt-Sc2694 and λgt-Sc2695 inactive P_{his3} but not the *his*3 structural gene.

TABLE 4

Deletion mapping in E. coli *of yeast* his*3 lesions*

Cloned *his*3 point mutants	λgt4	*his*⁻ deletion mutants λgt-Sc2619	λgt-Sc2616	λgt-Sc2648
λgt-Sc2612 (*his*3-38)	—	+	+	—
λgt6-Sc2679 (*his*3-532)	—	+	—	—

Non-complementing deletion mutants were crossed with non-complementing point mutants. The point mutants were isolated by cloning the *his*3-containing fragment from *his*3 mutants of yeast (Struhl & Davis, 1977). A plus indicates that *his*⁺ recombinants occurred at a frequency of 10^{-3} to 10^{-4}. Recombinants were observed using the lytic complementation assay.

isolates, Sc2666, Sc2667, Sc2669 and Sc2670, which are physically indistinguishable from Sc2639) are lysogenically and lytically *his*⁺. Sc2619 is lysogenically and lytically *his*⁻. These data strongly imply that the promoter responsible for the lysogenic expression of the *his*3 gene is located between the deletion endpoints defined by Sc2619 and Sc2639 (Fig. 12). The location of this promoter is supported strongly by the physical mapping of λgt-Sc2694 and λgt-Sc2695. These deletion mutants lack P_{his3}, though they contain the *his*3 structural gene (Struhl *et al.*, 1980). The data illustrated in Figure 11 indicate that the deletion endpoints of these mutants map very close to and probably within the 100 base-pair region defined by the endpoints of Sc2619 and Sc2639. In the two previous sections we showed that the 5′ end of the structural gene (as defined by complementation) is located between these same two endpoints. Therefore, the promoter, which in *E. coli* initiates transcription of the *his*3 gene, is located less than 100 base-pairs from the start of the structural gene.

4. Discussion

This paper describes a physical, genetic and transcriptional map of the *his*3 gene region of *Saccharomyces cerevisiae*. The *his*3 gene has been defined by its ability to complement mutants of *E. coli* lacking IGP dehydratase activity (Struhl & Davis, 1977). Originally, the *his*3 gene was cloned in bacteriophage λ as a 10·1 kb *Eco*RI fragment of yeast DNA (Sc2601) (Struhl *et al.*, 1976). This fragment is relatively large and defined by only one parameter. Therefore, it was imperative to obtain a fine structure map of Sc2601 DNA in order to simplify the analysis of further experiments. Sc2601 DNA has been defined physically by restriction endonuclease cleavage sites and by the endpoints of deletion within Sc2601. A novel mapping method allowed most of the deletion mutants to be mapped rapidly with respect to the *Bam*H1 cleavage sites of Sc2601 DNA. In these ways, the 10·1 kb *Eco*RI fragment has been sub-divided into a continuous series of small, well-defined regions. Furthermore, Sc2601 DNA has been fractionated physically by the use of subcloned DNA fragments generated by restriction endonuclease cleavage and by the use of deletion mutants. In this paper we have used these physically altered derivatives of Sc2601 DNA to answer questions pertaining to the expression of the *his*3 gene in *E. coli*. Further studies concerning the expression of the *his*3 gene in yeast or in *E. coli* have and will continue to depend on the ability to use defined DNAs as genetic and physical probes.

We have shown that a region of less than 750 base-pairs located totally within the 1·7 kb *Bam*H1 DNA fragment (Sc2676) is necessary and sufficient for the complementation of an *E. coli* auxotroph lacking IGP dehydratase activity. Since the product of the *his*3 gene in *E. coli* strongly resembles the product of the *his*3 gene in yeast (Struhl & Davis, 1977), it is likely that this 750 base-pair region contains the structural information for yeast IGP dehydratase. Since all deletion mutants of λgt4-Sc2601 that are *his*$^+$ as lysogens of *hisB*463 are expressed equally well (Struhl *et al.*, 1980), it is unlikely that the protein coding region extends significantly, if at all, beyond the boundaries of the 750 base-pair region. Our preliminary evidence indicates that yeast cells synthesize a 0·65 kb poly(A)-containing RNA species that maps within the 750 base-pair region defined by complementation in *E. coli*. The DNA sequences necessary for *his*3 expression in yeast might be sufficient for expression in *E. coli*. This issue is best resolved by introducing the cloned *his*3 gene and its derivatives into yeast by the method of Hinnen *et al.* (1978).

*his*3 genes cloned from *his*3 mutants of yeast have been described (Struhl & Davis, 1977). The lesions in such mutant eukaryotic genes can be mapped genetically by bacterial genetic techniques in *E. coli* and thereby localized on the physical map. The locations of the *his*3 mutations lie within the confines of the 750 base-pair region.

Strong evidence is presented to support the contention that the promoter, which in *E. coli* can initiate transcription of the *his*3 gene, is located less than 100 base-pairs from the start of the structural gene. Deletion mutants of λgt4-Sc2601 that presumably inactivate this promoter, but which contain a functional structural gene, have been isolated. The deletion endpoints of λgt-Sc2694 and λgt-Sc2695 map within the predicted region of the promoter. These results provide direct evidence for the existence and location of a yeast DNA sequence that functions as an *E. coli* promoter. Based on the close proximity of this promoter to the start of the structural gene, we speculate that the yeast RNA polymerase may recognize a similar genetic signal as the

E. coli RNA polymerase. Physical mapping of the *his*3 messenger RNA in yeast indicates that its 5′ end is located close to if not within this promoter region.

This and the accompanying paper deal with the expression of a yeast gene in *E. coli*. A map has been constructed that correlates genetic tools (in *E. coli*) with physically defined and physically isolable DNA sequences. Future work will be directed toward questions involving the expression of this yeast gene in yeast. The physical and genetic map provides the groundwork for further studies on the structure, function, and control of the *his*3 gene in yeast and in *E. coli*.

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