

Transcription of the *his3* Gene Region in *Saccharomyces cerevisiae*

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In exponentially growing cells of the yeast *Saccharomyces cerevisiae*, approximately 85% of a 10.1×10^3 base region of DNA that includes the *his3* gene is transcribed into discrete messenger RNA species. These species map at five or six non-overlapping regions on the cloned DNA, and presumably represent the transcriptional products of five or six genes. The spacing between the RNA coding regions ranges between 300 and 600 base-pairs. The steady-state levels of these RNAs average about one to three molecules per cell. Under a condition in which a yeast strain carrying the *rna1-ts1* mutation accumulates transfer RNA precursors and fails to transport RNA from the nucleus to the cytoplasm, the mRNA species homologous to this 10.1×10^3 base region appear unaltered in size.

The smallest mRNA species (0.7×10^3 bases in length) maps to the same location as the *his3* gene determined by complementation patterns in yeast and in *Escherichia coli* of physically defined derivatives of the cloned *his3* gene. The 5'-end of this transcript maps within 50 base-pairs of the sequence that is recognized as a promoter necessary for *his3* expression in *E. coli*. *S*₁ nuclease mapping of this transcript strongly suggests that the *his3* gene does not contain intervening sequences. The steady-state level of the *his3* mRNA is about ten times higher in cells starved of histidine; the levels of the other mRNA species are unaffected by this physiological condition.

1. Introduction

We have chosen the *Saccharomyces cerevisiae* (bakers' yeast) as a model system for eukaryotic gene expression. The ability to transform yeast cells with cloned DNA (Hinnen *et al.*, 1978) and the development of vectors useful for a wide variety of molecular genetic manipulations (Struhl *et al.*, 1979) allows a detailed study of gene expression *in vivo*. A physical and genetic map of the *his3* region has been obtained with the use of deletion mutants of a *His3* hybrid phage (Struhl & Davis, 1980). The cloned *his3* gene functions both in *Escherichia coli* and yeast cells (Struhl *et al.*, 1976, 1979; Hinnen *et al.*, 1978). Thus, it is of interest to compare expression of this gene in a prokaryotic organism to expression in its native eukaryotic host. Previous genetic and physiological experiments have concerned the expression of *his3* in *E. coli* (Struhl *et al.*, 1980; Struhl & Davis, 1980; Brennan & Struhl, 1980).

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In this paper, the original cloned *his3*-containing DNA fragment and derivatives of it are used as nucleic acid hybridization probes for transcriptional products homologous to this region. The location and orientation of the *his3* and adjacent transcripts are determined by using a variety of techniques. In addition, we examine *his3* regulation by measuring the steady-state level of the *his3* messenger RNA under different physiological conditions.

2. Materials and Methods

(a) Phage, bacterial and yeast strains and their propagation

Methods for the growth of phage and bacterial strains used in this work have been described (Struhl & Davis, 1980). The wild-type yeast strain, S288C, was grown at 30°C either in YPD medium (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose) or in standard minimal medium (0.67% (w/v) Difco yeast nitrogen base without amino acids, 2% glucose). When aminotriazole was added to the medium, the final concentration was 10 mM. The mutant yeast strain M304 (ax *rna1-1/rna1-1 GAL⁺*) (Bossinger & Cooper, 1976) was obtained from John Abelson. This strain was grown at 23°C because the *rna1* allele prevents growth at 37°C (herein defined as the restrictive temperature).

(b) DNAs

The co-ordinate scale and restriction endonuclease cleavage map for the 10.1 kb† *his3*-containing *EcoRI* DNA fragment has been defined (Struhl & Davis, 1980). Plasmid and λ hybrid DNA molecules containing various sub-regions of this original fragment were characterized and prepared as before (Struhl & Davis, 1980).

DNA fragments are purified from agarose gels in the following manner. The gel slice is dissolved at 42°C in 5 vol. of a filtered solution containing 6 M-sodium perchlorate, 30 mM-sodium phosphate (pH 7.0). This mixture is loaded on a column of hydroxylapatite (Bio-Rad; HTP) equilibrated with 6 M-sodium perchlorate. Very little resin is needed (50 to 100 µg is generally sufficient) and it is important that the particles be coarse, otherwise the column will flow very slowly. After the sample is loaded, the column is washed with 20 to 30 column volumes of 30 mM-sodium phosphate (pH 7.0). The DNA is eluted with 5 to 10 column volumes of 0.5 M-sodium phosphate (pH 7.0), dialyzed extensively against 10 mM-Tris (pH 7.5), 1 mM-EDTA, 0.3 M-NaCl, and concentrated by precipitation with ethanol. DNA (as large as 30 kb) is recovered in at least 50% yield and is of high enough quality to permit essentially any enzyme reaction.

(c) RNA isolation

Wild-type yeast cells were grown until the absorbance at 600 nm was 2.0 (exponential phase). The cells were concentrated 10-fold by centrifugation for 1 min at room temperature and resuspended in 50 mM-potassium phosphate (pH 7.5), 0.95 M-sorbitol, 14 mM-2-mercaptoethanol, and 3 units lyticase/original ml of culture (Scott & Schekman, 1979). After 2 to 3 min at 30°C, sodium dodecyl sulfate and diethylpyrocarbonate were added to 1%. An equal volume of phenol/chloroform/isoamyl alcohol (24:24:1, by vol.) was immediately added and the resulting mixture mixed vigorously. Total nucleic acid was prepared by 2 phenol/chloroform extractions, 2 ether extractions, and a precipitation with ethanol. For relatively large-scale RNA preparations (greater than 100 ml), the procedures were performed in 50 ml screw cap plastic tubes (Corning). For small preparations (1 to 5 ml), the steps were performed in 1.5 ml Eppendorf microfuge tubes. This procedure minimizes the time from growing culture to cellular lysis and prevents significant RNA degradation. Typically 20 mg of total nucleic acid is obtained from 1 l of yeast; 99% of this nucleic acid is RNA.

† Abbreviations used: kb, 10³ bases or base-pairs, as appropriate; cDNA, complementary DNA.

Poly(A)-containing RNA was isolated by loading the total nucleic acid (in 0.7 M-NaCl, 10 mM-EDTA, 1% sodium dodecyl sulfate) on a column of poly(U)-Sephacrose (Pharmacia). The column was washed exhaustively with 0.5 M-NaCl, 10 mM-EDTA, and the poly(A)-containing RNA was eluted in water. The poly(A)⁺ fraction represents 1 to 2% of the total nucleic acid and is 50 to 80% pure with respect to ribosomal RNA contaminants.

For experiments using M304 (carrying the *rna1-tsl* mutation), the RNA isolation procedure was somewhat different. Osmotically stabilized spheroplasts capable of normal RNA metabolism were prepared from cells grown at 23°C in 1% yeast extract, 2% peptone, 2% galactose (Hutchinson & Hartwell, 1967). The culture of spheroplasts was split: half was shifted to 37°C (the restrictive temperature) and half remained at 23°C. At various times after the split, total nucleic acid was prepared as described above.

(d) Hybridization procedures

³²P-labeled hybridization probes were prepared as follows. DNA complementary to yeast poly(A)-containing RNA (cDNA) was synthesized using reverse transcriptase. A 20 µl reaction mixture containing 1 µg RNA, 0.2 µg oligonucleotides about 6 to 12 bases in length (prepared by DNAase I digestion of calf thymus DNA and obtained from M. Wolfner), 100 µg actinomycin D/ml, 40 µCi ³²P-labeled deoxy-GTP (about 400 Ci/mmol), and 100 µM-unlabeled deoxy-ATP, TTP, and CTP in standard buffer conditions was incubated with an empirically determined quantity of enzyme for 40 min at 37°C. The reaction was terminated by addition to 0.2 M-NaOH, 40 mM-EDTA, 1% sodium dodecyl sulfate containing 50 µg of salmon sperm DNA. The cDNA probe was separated from unincorporated triphosphate precursors by using a Sephadex G50 column. Nick-translated DNA probes were prepared as described (Struhl & Davis, 1980).

Hybridization of labeled probes to nitrocellulose paper containing electrophoretically separated DNA was performed as described (Struhl & Davis, 1980). Hybridization to electrophoretically separated RNA was performed by using the following modifications to the procedure of Alwine *et al.* (1977). RNA was incubated at 50°C in 50% dimethyl sulphoxide, 1 M-glyoxal, and 10 mM-sodium phosphate (pH 7.0) (McMaster & Carmichael, 1977). RNA was separated in a 1.7% (w/v) agarose gel buffered with 10 mM-sodium phosphate (pH 7). The transfer of this RNA to diazotized paper was performed as described except that the washing solution was 0.1 M-sodium acetate (pH 4). Hybridization procedures were performed as described above.

All the experiments in this paper involving poly(A)-containing RNA from strain S288C (wild-type) used the identical preparation of RNA. The RNA-bound strips of diazotized paper are re-usable. ³²P-labeled hybridized nucleic acid can be removed by washing the strips for 4 h at 65°C in 99% (v/v) formamide and 1% sodium dodecyl sulfate (several changes). Remaining ³²P label on the paper, if detectable, will eventually decay. The strips are stable for at least 1 year.

(e) *S*₁ nuclease mapping

A 50-µl reaction containing 20 µg of poly(A) RNA and 1 µg of *Bam*HI-cleaved *lgt4-Sc2601* DNA were incubated for 5 min at 70°C (to denature the DNA) and then for 4 h at 54°C in 70% formamide (recrystallized), 0.3 M-NaCl, 0.1 M-PIPES (pH 7.2), 1 mM-EDTA. Then 9 vol. *S*₁ nuclease buffer containing 15 µg of boiled *Myxococcus xanthus* DNA and an empirically determined quantity of *S*₁ nuclease were added and the *S*₁-resistant nucleic acid was isolated after 30 min of treatment as described (Struhl & Davis, 1980). This nucleic acid was denatured in alkali, electrophoretically separated in a 1.5% alkaline agarose gel (30 mM-NaOH, 2 mM-EDTA), transferred to nitrocellulose, and challenged for hybridization with a ³²P-labeled probe of pBR322-Sc2676 DNA.

3. Results

(a) *Most of a 10.1 kb his3-containing DNA fragment is transcribed in yeast cells*

The *his3* gene was originally isolated as a 10.1 kb DNA fragment generated by *EcoRI* endonuclease cleavage (Struhl *et al.*, 1976). Using restriction endonuclease cleavage sites as markers, a physical map of Sc2601 DNA was obtained (Struhl & Davis, 1980). In addition, this region was physically fractionated by subcloning various segments of Sc2601 DNA and by isolating deletion mutants of λ gt4-Sc2601. The mapping positions of the subcloned fragments and of the deletion endpoints were determined with respect to the restriction endonuclease cleavage sites (Struhl & Davis, 1980).

To determine which regions of Sc2601 DNA are transcribed, a large number of DNA fragments representing different regions of Sc2601 were individually challenged for hybridization with a ^{32}P -labeled probe representing sequences complementary to poly(A)-containing RNA isolated from an exponentially growing culture of yeast cells. The DNA fragments were generated by cleavage of pMB9-Sc2601 DNA and some of its derivatives with a variety of restriction endonucleases. These DNA fragments were separated by agarose gel electrophoresis to produce the restriction spectra shown in Figure 1. Following denaturation in alkali, these DNA fragments were transferred *in situ* to a sheet of cellulose nitrate paper by a modification of the technique of Southern (1975). The hybridization probe was synthesized *in vitro* as DNA complementary to total poly(A)-containing RNA by using reverse transcriptase. The RNA was primed for this complementary DNA synthesis by random oligonucleotides (6 to 10 nucleotides in length). cDNA made in this manner should provide an accurate representation of the sequences present in the poly(A)-containing RNA (St. John & Davis, 1979). Though sequences homologous to Sc2601 DNA represent only a small percentage of the total cDNA synthesized (roughly 0.1%), they are detectable by hybridization to the fragments of denatured Sc2601 DNA. The Sc2601 DNA fragments immobilized on the filter are unique in the yeast genome (Struhl & Davis, 1977) and are present in a 10^6 -fold molar excess over the cDNA in the hybridization probe. Therefore, the relative abundance of any particular transcribed sequence should be directly proportional to the relative hybridization intensity. However, fragments with more nucleotides of yeast DNA may potentially hybridize to more of the cDNA probe. Therefore, the observed intensity of hybridization must be normalized to the number of yeast nucleotides present on the particular DNA fragment.

An autoradiograph from this experiment is shown in Figure 1 and the results are indicated in Figure 2. Essentially every segment of Sc2601 DNA but no segment derived solely from pMB9 DNA hybridizes to the cDNA probe. The only exception is a small region located between co-ordinates 7.7 to 8.0. When normalized for sequence complexity, the regions centered between co-ordinates 4.5 and 6.0, and between 9.5 and 10.1 appear to code for RNA which is two to four times more abundant than RNA coded by other regions of Sc2601 DNA. These results indicate that most of Sc2601 DNA is transcribed in yeast cells into poly(A)-containing RNA.

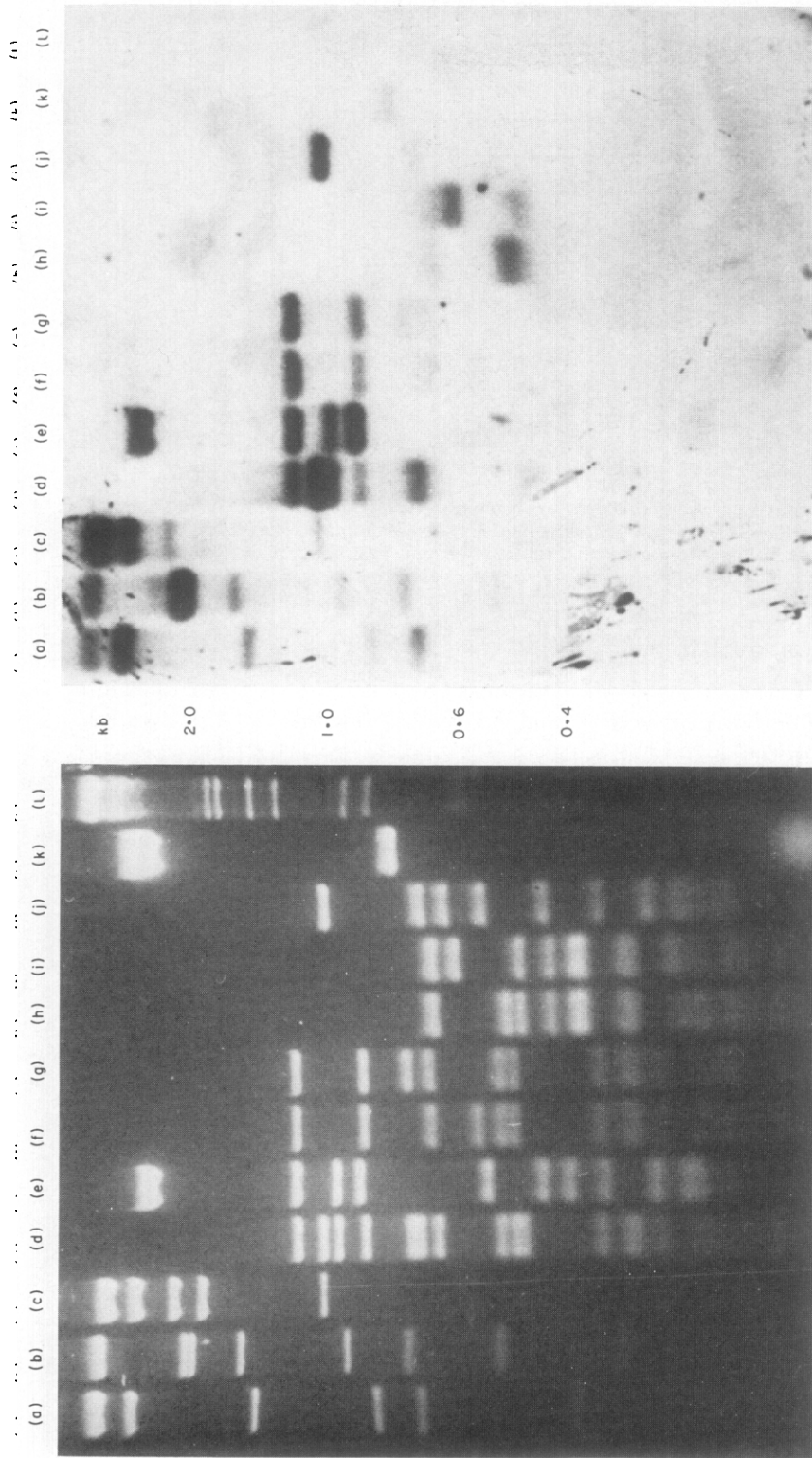


FIG. 1. cDNA hybridization to fragments of Sc2601 DNA. 1 to 2 μ g of various DNAs were cleaved with various restriction endonucleases and the fragments electrophoretically separated in a 1.5% agarose gel. The left panel shows the following restriction spectra: (a) *Hind*III-cleaved pMB9-Sc2601; (b) *Bam*HI and *Sal*I-cleaved pMB9-Sc2601; (c) *Hpa*I and *Sal*I-cleaved pMB9-Sc2601; (d) *Hha*I and *Sal*I-cleaved pGT2-Sc2605; (e) *Hpa*II and *Sal*I-cleaved pGT2-Sc2605; (f) *Hha*I and *Bam*HI-cleaved pGT1-Sc2602; (g) *Hha*I-cleaved pGT1-Sc2602; (h) *Hha*I and *Bam*HI-cleaved pBR322-Sc2676; (i) *Hha*I-cleaved pBR322-Sc2676; (j) *Hpa*II and *Bam*HI-cleaved pBR322-Sc2676; (k) *Bam*HI and *Hind*III-cleaved pBR322-Sc2678; (l) *Hind*III and *Eco*RI-cleaved λ 1857 S7. The right panel shows an autoradiograph resulting from hybridization of cDNA (synthesized by reverse transcription of poly(A)⁺-containing RNA primed with random hexanucleotides) to a sheet of nitrocellulose containing denatured DNA fragments corresponding in position to the left panel.

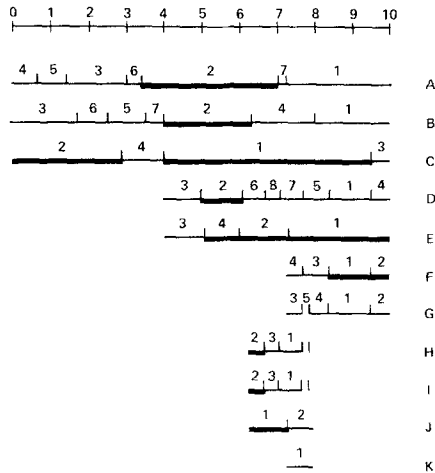


FIG. 2. Transcribed regions of Sc2601 DNA. The co-ordinate system of Sc2601 DNA (in kb units) is defined by Struhl & Davis (1980). Each horizontal line below the co-ordinate scale (ordered as in Fig. 1) indicates the DNA fragments from a particular restriction endonuclease cleavage that hybridize cDNA. Thick lines indicate a relatively high band intensity (not normalized for fragment length); the absence of a line indicates no hybridization.

(b) *Mapping the discrete mRNA transcripts on Sc2601 DNA*

The previous section indicates which regions of Sc2601 DNA are transcribed into poly(A)-containing RNA. In this section, it is shown that this RNA is organized into discrete transcripts.

The identical preparation of poly(A)-containing RNA used in the previous section was denatured by treatment with glyoxal and subjected to gel electrophoresis in an agarose gel (McMaster & Carmichael, 1977). The RNA species, fractionated by molecular weight, were transferred *in situ* to a sheet of diazotized paper by a modification of the procedure of Alwine *et al.* (1977). After cutting this RNA-paper into five strips, the discrete poly(A)-containing RNA species homologous to specific regions of Sc2601 DNA were identified by separate hybridizations using different ^{32}P -labeled probes (Fig. 3). This experiment yields information complementary to that obtained in the previous section.

There are at least six discrete poly(A)-containing RNA species homologous to Sc2601 DNA (Fig. 4, lane (1)). Though it is not formally proven, we shall assume that these discrete species represent messenger RNAs. The molecular weights of these mRNAs are inversely and exponentially proportional to their relative mobilities from the origin of electrophoresis (McMaster & Carmichael, 1977). Their actual molecular weights were determined using those of the yeast ribosomal RNA species (1.71 and 3.36 kb) as size standards (Philippsen *et al.*, 1978). The mRNA species encoded by Sc2601 DNA are 2.3, 1.9, 1.55, 1.45, 1.2 and 0.7 kb in length. The bands are of roughly equal intensity with the exception of the 2.3 kb RNA which is about two to four times more intense. It is possible that a given hybridization band may represent more than one discrete mRNA species.

The mapping positions of these mRNA species are determined by determining which particular region of Sc2601 DNA is homologous to a given sized RNA. The

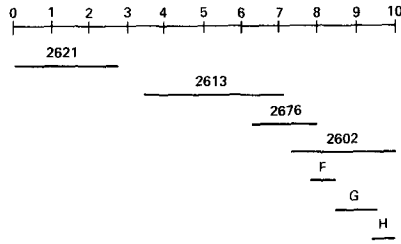


FIG. 3. Hybridization probes used for mRNA mapping. Each horizontal line below the co-ordinate scale indicates the regions of Sc2601 DNA used as probes for the experiment in Fig. 4 (see Struhl & Davis, 1980). The ^{32}P -labeled probes were produced by nick-translation of intact hybrid DNA molecules (numbered) or of fragments (lettered) following agarose gel electrophoresis (see Materials and Methods) of *Hha*I-cleaved pMB9-Sc2601 DNA.

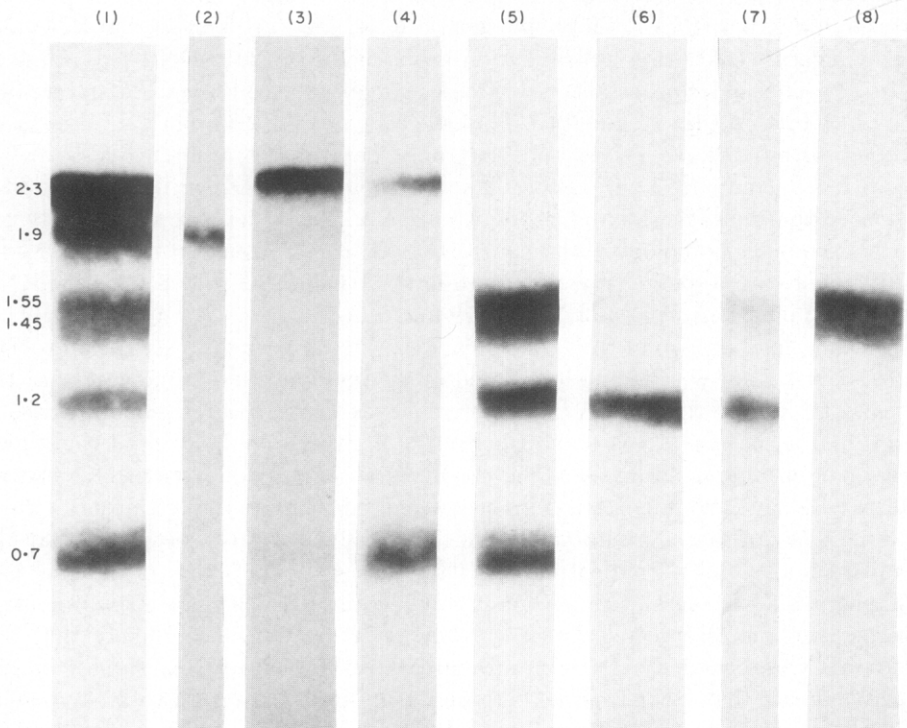


FIG. 4. Mapping the discrete transcripts on Sc2601 DNA. 40 μg of poly(A)-containing RNA was denatured in glyoxal, separated in an agarose gel (1.7%), and transferred to diazotized paper. The resulting paper was cut into 5 identical strips and hybridized with the following nick-translated DNA probes: (1) pMB9-Sc2601; (2) λgt -Sc2621; (3) λ554 -Sc2613; (4) pBR322-Sc2676; (5) pGT1-Sc2602; (6) Sc2605 *Hha*I.F; (7) Sc2605 *Hha*I.G; and (8) Sc2605 *Hha*I.H. RNA sizes (in kb) are indicated at the left of the Figure.

relative intensity of a particular hybridization response depends upon the abundance of the transcript and upon the degree to which the mRNA sequences overlap with the specific probe. By comparing ratios of band intensities obtained with a given probe to the same ratios obtained with the entire Sc2601 DNA fragment, it is possible to estimate the amount of overlap between a particular mRNA species and a particular region of DNA. The ^{32}P -labeled hybridization probes were all obtained by nick-translating (Rigby *et al.*, 1977) various hybrid DNA molecules containing defined regions of Sc2601 DNA. The locations of these regions are indicated in Figure 3. A collection of autoradiographs is shown in Figure 4.

The approximate map location of a given poly(A)-containing RNA species is based on its molecular weight, the intensities of its hybridization responses to various probes, and the assumption that it contains no or very small intervening sequences. The validity of this assumption is explored in the Discussion. The analysis reported below begins from the leftmost region of Sc2601 DNA and proceeds rightward.

$\lambda\text{gt-Sc2621}$ DNA (co-ordinates 0 to 2.7) hybridizes only to the band corresponding to mRNA(s) 1.9 kb in length (Fig. 4, lane (2)). This sized mRNA also responds very weakly to a hybridization probe of $\lambda\text{554-Sc2613}$ DNA (co-ordinates 3.4 to 7.1). This latter probe also hybridizes strongly to a 2.3 kb mRNA and weakly to a 0.7 kb mRNA (Fig. 4, lane (3)). This suggests that the 2.3 kb mRNA is entirely encoded within Sc2613 DNA, and that only part of the other mRNAs are so encoded. By comparing the ratios of band intensities as described above, it is estimated that both the 1.9 and the 0.7 kb mRNA species share approximately 200 bases of sequence homology with Sc2613 DNA. This indicates that there is a 1.9 kb mRNA species mapping between co-ordinates 1.7 and 3.6 and a 0.7 kb mRNA species mapping between co-ordinates 6.9 and 7.6.

The mapping position of the 1.9 kb mRNA accounts for its hybridization to $\lambda\text{gt-Sc2621}$ DNA. However, results presented in the previous section indicate that the leftmost 1.4 kb of Sc2601 DNA (defined by cleavage with *Hind*III endonuclease as shown in Fig. 1, lane (a)) is transcribed at roughly the same level as the region between co-ordinates 1.4 and 4.0 (Fig. 2). Because the only discrete mRNA species detected by $\lambda\text{gt-Sc2621}$ DNA is 1.9 kb, it seems likely that the leftmost 1.4 kb of Sc2601 DNA encodes a 1.9 kb transcript. In other words, it is proposed that the band with a mobility indicating a 1.9 kb transcript actually represents two different mRNA species. Though many eukaryotic mRNAs map in two or more dispersed regions (Berget *et al.*, 1977; Chow *et al.*, 1977), it is unlikely that one 1.9 kb mRNA is encoded both by co-ordinates 0 to 1.4 and 3.1 to 3.6. Figures 1 and 2 clearly indicate that DNA fragments representing co-ordinates 1.7 to 2.5 and 1.6 to 3.0 hybridize equivalent amounts of cDNA. From the data presented, it is impossible to determine the endpoints of this proposed leftmost mRNA species. However, the cDNA hybridization results suggest that most or all of the leftmost 1.4 kb of Sc2601 DNA encodes this mRNA. If this 1.9 kb mRNA is entirely encoded by Sc2601 DNA, it would share sequences with the other 1.9 kb mRNA; however, such overlap could be avoided easily by postulating that some of the leftmost mRNA is not encoded by Sc2601 DNA. Another way to account for the observed

results is to propose that RNA homologous to the region between co-ordinates 0 and 1.4 does not exist as a discrete species. This could result from random transcription through the region or by extremely rapid processing. We consider this possibility unlikely especially considering that the *leu2*, *arg4*, *ura3*, *trp5* and *trp1* genes all code for discrete sized mRNA transcripts (data not shown).

Moving further to the right, lane (4) of Figure 4 indicates that pBR322-Sc2676 DNA (co-ordinates 6.3 to 8.0) hybridizes normally to the 0.7 kb species, relatively weakly to the 2.3 kb species and faintly to the 1.2 kb species. From the band intensities, about 300 bases of the 2.3 kb mRNA and 100 nucleotides of the 1.2 kb mRNA overlaps Sc2676 DNA. Therefore, the mapping co-ordinates of the 2.3 kb mRNA are 4.3 to 6.6 and those of the 1.2 kb species are 7.9 to 9.1. That the 0.7 kb RNA is entirely encoded by Sc2676 DNA is expected from the mapping position determined above. These results are consistent with those obtained by cDNA hybridization, which indicated that the region between co-ordinates 7.7 and 8.0 is not transcribed. Though roughly 100 base-pairs of homology are predicted, it is probably not detectable under the experimental conditions.

The hybridization pattern of pGT1-Sc2602 DNA indicates that the rightmost 2.9 kb of Sc2601 DNA (co-ordinates 7.2 to 10.1) encodes four discrete mRNA species (Fig. 4, lane (5)). Hybridization to the 0.7 and the 1.2 kb species was expected from other results. In addition, there is a doublet centered around 1.5 kb. The intensity of hybridization to the 0.7 kb mRNA suggests that about half of its sequences are homologous to pGT1-Sc2602 DNA; this confirms the mapping position established previously. Mapping in this region was performed in greater detail by the use of small non-overlapping probes of Sc2602 DNA (Fig. 4, lanes (6) to (8)). As expected, the 0.7 kb mRNA does not hybridize to DNA fragments from co-ordinates 7.7 to 10.1, while the 1.2 kb species hybridizes to fragments representing both 7.7 to 8.3 and 8.3 to 9.5, but not 9.5 to 10.1. The two mRNA species centered around 1.5 kb in length show identical hybridization patterns. They hybridize normally to the region defined by 9.5 to 10.1, weakly to 8.3 to 9.5, and undetectably to 7.7 to 8.3. Both these mRNA species have one endpoint at co-ordinate 9.4; the other probably occurs 0.8 kb past the *EcoRI* site on the cloned Sc2601 DNA fragment.

These results corroborate those obtained by cDNA hybridization. A total of 85% of the original cloned Sc2601 DNA fragment is transcribed by exponentially growing yeast cells. All the mRNA species are transcribed at roughly the same level except for the 2.3 kb transcript which is about three times more abundant. The mRNAs (with the possible exception of the adjacent 1.9 kb RNAs) are all encoded by discrete, non-overlapping regions of Sc2601 DNA. The average spacing between transcripts is about 300 to 400 base-pairs. These results are summarized pictorially in Figure 6.

(c) *Direction of mRNA transcription*

To determine the direction of transcription for each of the mRNA species, the identical strips of RNA-bound paper used in the previous section, were challenged for hybridization with ³²P-labeled probes representing the individual DNA strands of Sc2601 DNA. Such probes were prepared in the following manner. λ gt4-Sc2601'

DNA (with the *his3*-containing fragment in the inverted orientation with respect to Fig. 3) was denatured in alkali, and the resulting single strands separated physically by centrifugation to equilibrium in a CsCl density gradient in the presence of poly(U, G) RNA. Under these conditions, the strand with the highest apparent density (the *r* strand) is complementary to RNA species representing rightward 5'-3' transcription on bacteriophage λ (Szybalski *et al.*, 1971); the less dense strand (*l* strand) is complementary to leftward transcripts. Using a random collection of oligonucleotides to prime each separated strand, a ^{32}P -labeled probe was synthesized *in vitro* with DNA polymerase I. The directions of transcription can be assigned by taking into account the facts that (1) only the complementary DNA strand of a given separated strand is copied and thereby ^{32}P -labeled, and (2) the Sc2601 DNA fragment is inserted into $\lambda\text{gt}4$ in the opposite orientation from that defining the co-ordinate scale.

The results from this experiment are shown in Figure 5 and included in the map shown in Figure 6. Each discrete transcript hybridizes preferentially (by a factor of 5 to 10) to one of the separated strand DNA probes; that each mRNA species will respond to both probes probably happens because the separated strands are only about 80 to 90% pure. The 2.3 and the 0.7 kb mRNAs are transcribed leftward with respect to Sc2601 DNA while the double at 1.5 kb and the species at 1.2 kb are

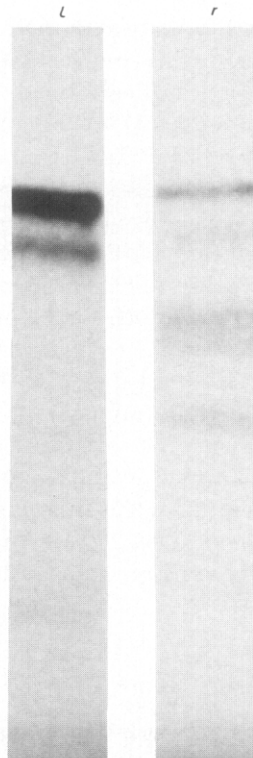


FIG. 5. Direction of transcription. ^{32}P -labeled separated strand probes were hybridized to the strips containing poly(A) RNA described in the legend to Fig. 4. *l*, indicates leftward transcripts (defined on the normal co-ordinate system) while *r* indicates rightward transcripts.

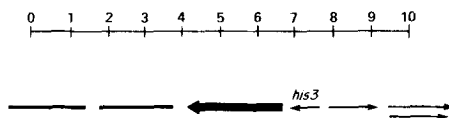


FIG. 6. mRNA map of Sc2601 DNA. The locations, directions of transcription, and relative abundance (thickness of line) for mRNA species encoded by Sc2601 DNA are indicated. See the text for caveats concerning this map.

transcribed rightward. Though the hybridization pattern to the band at 1.9 kb indicates leftward transcription, the situation is complicated by the fact that this band probably represents two different species. Though it seems likely that both transcripts proceed leftward, it is conceivable that the weak hybridization signal indicating rightward transcription could result from one of these 1.9 kb mRNAs. Consequently, the transcriptional directions for both of these species are not indicated in Figure 6.

(d) *The rna1-ts1 mutation has no detectable effect on structures of Sc2601-encoded transcripts*

The *rna1-ts1* mutation prevents yeast cells from growing at 37°C but not at 23°C. Under the restrictive condition, the bulk of mRNA does not appear to be transported to the cytoplasm (Hutchinson *et al.*, 1969), and transfer RNA genes with intervening sequences accumulate precursors that are not spliced to the mature form (Hopper *et al.*, 1978; Knapp *et al.*, 1978). To determine the effect of the *rna1-ts1* mutation on mRNA transcription, a culture of metabolizing spheroplasts incubating at 23°C was split such that half was shifted to 37°C and half remained at 23°C. At various times after the shift, total nucleic acid was extracted and examined for the presence of discrete transcripts homologous to Sc2601 DNA. The results shown in Figure 7 indicate no detectable qualitative or quantitative difference in the hybridization pattern produced by RNA extracted at various times at either temperature. In addition, the hybridization patterns produced from these total RNA preparations are not distinguishable from those from poly(A)-containing RNA preparations. The banding pattern is observed even after spheroplasts have been incubated at the restrictive temperature for one hour; this indicates that the mRNA species are relatively stable. When the identical RNA-bound paper was probed for *gal1,7,10* (St. John & Davis, 1979) or Ty1 (Cameron *et al.*, 1979) sequences, larger transcripts were easily detected after 30 minutes at the restrictive temperature (T. St. John & R. W. Davis, unpublished results).

(e) *The 0.7 kb mRNA maps indistinguishably from the his3 complementation unit determined by expression in E. coli*

The *his3* gene was initially located on Sc2601 DNA by determining which of a set of physically defined deletion mutants of a bacteriophage λ *his3* hybrid expressed this yeast gene in *E. coli* (Struhl *et al.*, 1980; Struhl & Davis, 1980). Such a complementation unit was located between co-ordinates 6.9 and 7.6. By

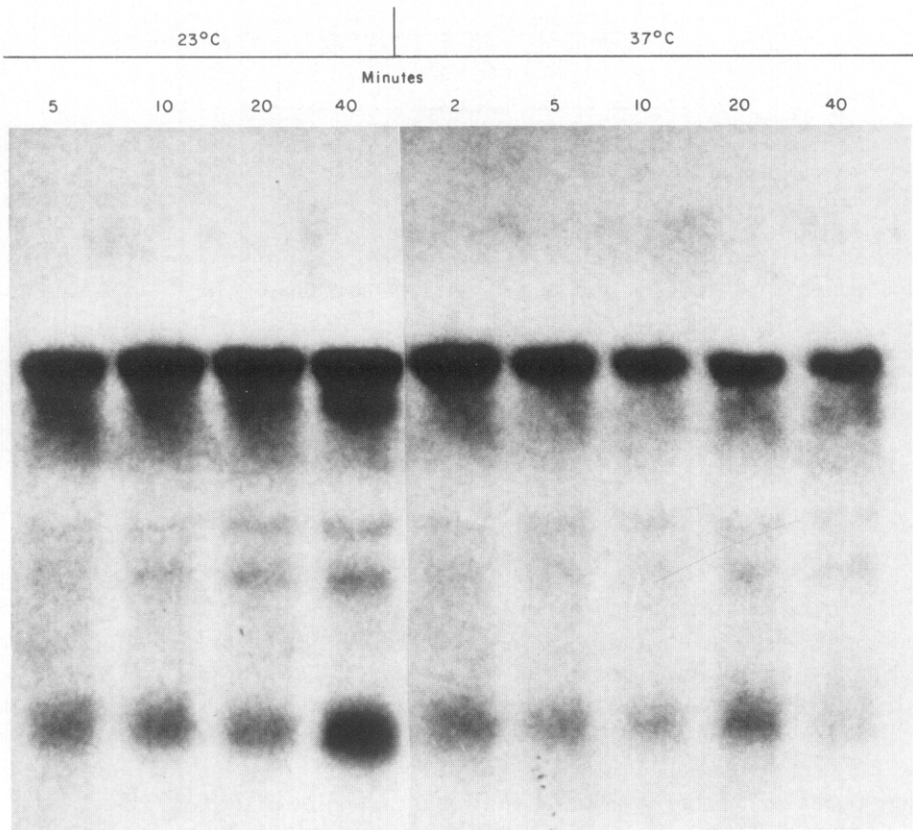


FIG. 7. Effect of *rna1-tsl1*. A culture of metabolizing spheroplasts from log growth phase cells of strain M304 (Knapp *et al.*, 1978) growing at 23°C in broth containing 2% galactose as a source of carbon was split in half. One part was shifted to 37°C, the other remaining at 23°C. Total nucleic acid was isolated at the indicated times after the shift and examined for mRNA species homologous to pMB9-Sc2601 DNA. Each lane has roughly 30 µg nucleic acid.

transformation of yeast cells with many of the same *λhis3* deletion alleles, the *his3* complementation unit determined in yeast cells maps to this same region (Struhl & Davis, 1981). This position corresponds exactly to that of the 0.7 kb mRNA, thus identifying the 0.7 kb transcript as the *his3* mRNA. Furthermore, the direction of transcription of this RNA agrees with that predicted from genetic results (Struhl & Davis, 1980).

(f) *The his3 gene is unlikely to contain intervening sequences*

Many eukaryotic mRNAs map in two or more discontinuous blocks of the encoding DNA (Berget *et al.*, 1977; Chow *et al.*, 1977). To determine whether the yeast *his3* gene contains intervening sequences, the experiment outlined in Figure 8 was performed. *Bam*HI endonuclease-cleaved *λgt4*-Sc2601 DNA was denatured in alkali and incubated with poly(A)-containing RNA under conditions specific for formation of RNA-DNA duplex molecules (Casey & Davidson, 1977). After hybridization, the reaction mixtures were treated with S_1 nuclease to remove all

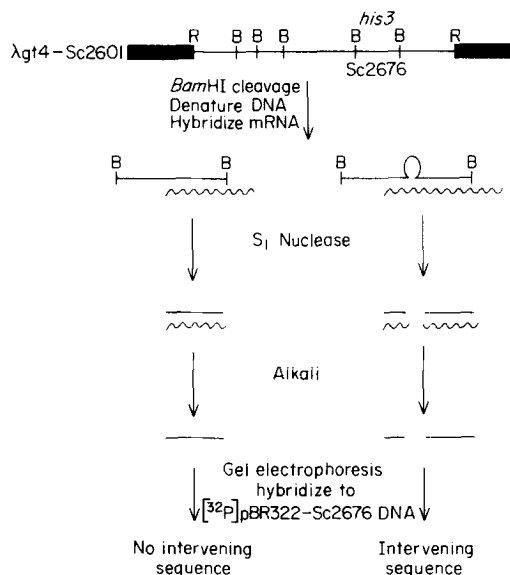


FIG. 8. S₁ nuclease mapping of *his3* DNA-*his3* mRNA duplexes. 10 μ g of poly(A)-containing RNA and 2 μ g of BamHI-cleaved, denatured λ gt4-Sc2601 DNA were incubated at 54°C in 70% formamide, 0.4 M-NaCl, 40 mM-PIPES (pH 7.3), 1 mM-EDTA for 4 h. The mixture was diluted into buffer containing S₁ nuclease and treated as described by Struhl & Davis (1980). The products were electrophoretically separated in an alkaline agarose gel (1.5%) (McDonnell & Studier, 1977), transferred to nitrocellulose, and probed with ³²P-labeled nick-translated pBR322-Sc2676 DNA. The left and right halves of the Figure indicate, respectively, the results expected if *his3* does or does not contain an intervening sequence.

single-stranded nucleic acid. The RNA-DNA duplex molecules resistant to S₁ nuclease digestion were treated with alkali to destroy the RNA components. The resulting single-stranded DNA molecules were electrophoretically separated in an alkaline agarose gel, transferred *in situ* to a strip of nitrocellulose, and challenged for hybridization with ³²P-labeled pBR322-Sc2676 DNA (representing co-ordinates 6.3 to 8.0). As indicated in Figure 8, S₁ nuclease treatment of RNA-DNA duplexes formed by genes with intervening sequences and the homologous mRNA should generate at least two bands because S₁ nuclease can cleave the looped-out DNA region from such genes. By contrast, RNAs colinear with the encoding DNA should protect one DNA fragment of identical size to that of the RNA.

Because λ gt4-Sc2601 DNA was cleaved with BamHI endonuclease and the probe was specific for the *his3*-containing BamHI DNA fragment (Sc2676), the experiment is formally analogous to hybridization of yeast poly(A)-containing RNA to Sc2676. From the mRNA mapping positions determined in the previous section, this DNA fragment codes for the entire *his3* mRNA, roughly 300 base-pairs of the 2.3 kb mRNA, and about 100 nucleotides of the 1.2 kb RNA. Therefore, if the transcribed regions homologous to Sc2676 DNA do not contain intervening sequences, the procedure described above should produce protected DNA fragments of roughly 700, 300 and 100 nucleotides. From the autoradiograph from such an experiment (Fig. 9), bands representing 675 and 275 nucleotide species are clearly evident. These bands do not appear when poly(A)-containing RNA is

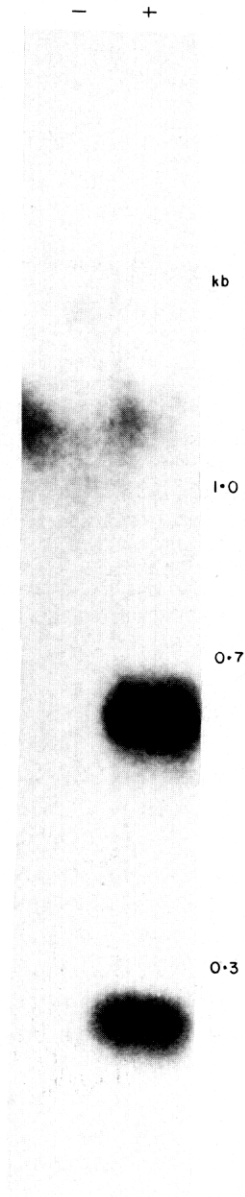


FIG. 9. Results of S₁ nuclease mapping. Autoradiograph of the experiment diagrammed in Fig. 8. - Indicates no mRNA added to the initial reaction mixture; + indicates mRNA added. The observed band pattern is explained in the text.

omitted from the hybridization mixture. The expected band corresponding to the predicted 100 nucleotide species is not observed because its mobility precluded its presence in the gel. The size difference between the 700 base mRNA and the 675 base protected DNA fragment is within experimental error; in addition, it could be accounted for by the presumed stretch of poly(A) residues at the 3'-end of the RNA, which is probably not encoded by the *his3* gene. These results indicate that the *his3* mRNA is colinear with the gene. If there is an intervening sequence in *his3*, it must be very small (less than 50 base-pairs) and located at the extreme 5' or 3'-end of the transcript.

(g) *Histidine-starved yeast cells accumulate five to ten times the normal amount of his3 mRNA*

Aminotriazole is a potent competitive inhibitor of yeast imidazoleglycerol-phosphate dehydratase, the *his3* gene product (Klopotoski & Wiater, 1965). Addition of aminotriazole to the medium results in temporary cessation of growth due to histidine starvation (Wolfner *et al.*, 1975). This growth inhibition is overcome within a few hours, presumably by increased imidazoleglycerolphosphate dehydratase activity. The levels of many amino acid biosynthetic enzymes are

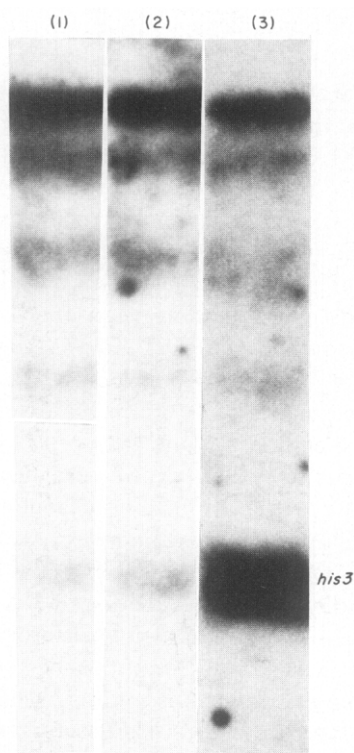


FIG. 10. Induction of *his3* mRNA. 25 μ g of total nucleic acid prepared from strain S288C growing in glucose minimal medium containing either (1) no supplements, (2) 100 μ g histidine/ml, or (3) 10 mM-aminotriazole was examined for mRNA species homologous to pMB9-Sc2601 DNA.

increased when cells resume growth in the presence of aminotriazole (Wolfner *et al.*, 1975).

Because this physiological condition is likely to reflect directly the activity level of the *his3* gene product, it was of interest to determine the steady-state level of the *his3* mRNA as a function of histidine starvation. Total RNA was extracted from yeast cells growing under three different physiological conditions and the transcripts homologous to Sc2601 DNA examined by the methods used in the previous sections. From Figure 10, it is apparent that the transcription pattern of yeast cells growing in glucose minimal medium in the presence or in the absence of histidine is identical to that seen when they are grown in rich medium (lanes (1) and (2)). However, addition of aminotriazole to minimal medium results in accumulation of ten times the normal quantity of *his3* mRNA, but normal amounts of the other mRNA species encoded by Sc2601 DNA (Fig. 10, lane (3)).

4. Discussion

Understanding the molecular mechanisms by which a eukaryotic organism expresses and regulates its genes depends upon the characterization of transcriptional products. In this work, we characterize the RNA transcripts encoded by a 10.1 kb DNA fragment containing the yeast *his3* gene that are synthesized in growing yeast cells.

Essentially the entire Sc2601 region is transcribed in normally growing yeast cells. This is rather higher than previous estimates of the percentage of the yeast genome that is transcribed; these ranged from 20 to 40% (Hereford & Rosbash, 1977; Kaback *et al.*, 1979). Transcription from the *his3* gene region is arranged into at least five and probably six non-overlapping regions on Sc2601 DNA. Thus, it is likely that Sc2601 contains coding sequences for five or six different genes. The ambiguity in the number of genes exists because hybridization to a single RNA size class could result from homology to more than one transcript. Though the most likely explanation for the data is that DNA represented by co-ordinates 0 to 4.0 codes for two mRNA species each 1.9 kb in length, it is not conclusive. The RNA species represented by the doublet at 1.5 kb in Figure 4 map identically on Sc2601 DNA, though most of the coding regions are probably homologous to the DNA fragment normally adjacent to Sc2601 in the yeast genome. Probably, these two mRNAs are transcriptional products of the same gene. Which, if either, of these is translated is unknown. The sequence difference between these RNAs probably occurs past the most 3'-proximal region coded by Sc2601 DNA; this could be either at the 3'-end or in the middle of the RNA. Different RNA species that share almost all of their sequences in common have also been observed in the yeast *gal1,7,10* cluster (T. P. St. John & R. W. Davis, unpublished results).

All the mRNAs homologous to Sc2601 are expressed at roughly the same level with the exception of the 2.3 kb species. Considering that (1) haploid yeast cells contain approximately equal amounts of DNA and poly(A)-containing RNA, (2) a large fraction of the genome is transcribed, and (3) some mRNA species are more abundant than the majority (Hereford & Rosbash, 1977), it is crudely estimated

that yeast contains an average of about one to two molecules of a typical mRNA species (such as *his3*) per cell.

Map positions of the mRNA species were made by assuming that the encoding genes contained small or no intervening sequences. The experiment shown in Figure 9 indicates that this is the case for the *his3* gene. This is also suggested by correlation of mRNA and polypeptide sizes for the *gal1*, *gal7*, *gal10* and *his4* genes (Hopper & Rowe, 1978; St. John & Davis, 1979; G. R. Fink, personal communication). DNA and protein sequences of the yeast iso-1-cytochrome *c* (*cyc1*) gene directly indicate the absence of intervening sequences within the structural gene (Smith *et al.*, 1979). A general search for yeast genes with intervening sequences greater than 100 base-pairs was unsuccessful (Kaback *et al.*, 1979). Therefore, it is likely that most chromosomal yeast genes do not contain intervening sequences. In this regard, it is interesting that the *rna1*-*ts1* lesion does not affect the sizes of the mRNAs homologous to Sc2601 DNA. At the restrictive temperature, this lesion results in the accumulation of tRNA precursors containing intervening sequences (Hopper *et al.*, 1978; Knapp *et al.*, 1978) and the inhibition of mRNA transport from the nucleus to the cytoplasm (Hutchinson *et al.*, 1969). This latter phenomenon is not due to rapid cytoplasmic degradation because discrete mRNA species are visible 60 minutes after the temperature shift.

The *his3* mRNA maps to the identical location of the His⁺ complementation unit determined by *his3* expression in *E. coli*. In particular, the 5'-end of this mRNA maps less than 50 base-pairs from the sequence which, in *E. coli*, acts as a promoter to initiate *his3* transcription. This adds support to the idea that *his3* transcriptional initiation may share similar features in yeast and in *E. coli* (Struhl & Davis, 1980).

The experiment shown in Figure 10 indicates that the level of *his3* mRNA found in yeast cells varies with the physiological condition. By analogy with a wide variety of examples representing many different organisms, this strongly intimates, but does not prove, that *his3* is regulated at the transcriptional level. For example, the difference in mRNA levels found for the yeast *ura3* and the *cyc1* genes can be accounted for by altered rates of RNA synthesis (Bach *et al.*, 1979; Zitomer *et al.*, 1979). Conditions causing the increased level of *his3* mRNA also result in the increased activity levels of many amino acid biosynthetic enzymes (Wolfner *et al.*, 1975); therefore, it seems reasonable that *his3* is under the same general control system as these other related genes. Mutations in at least six genes alter this general control mechanism (G. R. Fink, personal communication). Four of these are incapable of elevated levels of enzyme activity; they are unable to grow in the presence of aminotriazole. The other two synthesize constitutively high levels of these enzymes and also affect the yeast cell cycle (Wolfner *et al.*, 1975). The likelihood that *his3* is under transcriptional regulation suggests that the altered enzyme levels are due to increases in the relative levels of the encoding mRNAs. Figure 10 indicates that the levels of the five presumed genes linked to *his3* are unchanged by this physiological condition. In addition, the fact that the 2.3 kb mRNA is unchanged in expression even though it is downstream and transcribed in the same direction as *his3* strongly suggests that the *his3* gene is not part of an operon.

The physical and physiological characterization of mRNA species encoded by

his3 and five other linked genes provide the basis for further studies concerning the expression and regulation of the *his3* gene in yeast cells. In particular, it will be important to incorporate these findings with respect to the phenotypes and DNA structures of *his3* mutations that alter the expression and/or the regulation of this gene.

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