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MOLECULAR CHARACTERIZATION OF THREE *his4* DELETION MUTANTS IN SACCHAROMYCES CEREVISIAE

by

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DNA was isolated from haploid strains of Saccharomyces cerevisiae carrying three different deletions in the *his4* gene, *his4–15*, *his4–24* and *his4–29*, as well as a *his4* point mutant and a strain carrying the *HIS4* wild type gene. Samples of the DNA preparations were treated with restriction endonucleases BamHI, EcoRI, HindIII, PstI and SalI and the DNA fragments were separated according to size by agarose gel electrophoresis. A bacterial plasmid containing the yeast DNA sequences of a region including the *HIS4* gene allowed the detection by molecular hybridization of the fragments in the electropherograms that contained these sequences. From the sizes of these fragments the mutations *his4–15* and *his4–24* were both determined to be deletions of about 0.5 kb, while *his4–29* was found to be a deletion of approximately 0.9 kb. The data allowed an extension of our previously constructed cleavage map of the region as well as an approximate location of the deletions on the map. Comparisons with available data on the *his4* gene and its product allowed an approximate positioning of the three functional regions of the *his4* gene on the map and indicated that no large intervening sequences are present within the *his4AB* region.

1. INTRODUCTION

The *HIS4* gene in yeast encodes the enzymatic activities catalyzing the 3rd (*HIS4A*), 2nd (*HIS4B*) and the 10th (*HIS4C*) steps in the pathway of histidine biosynthesis (2). A great number of point mutants and several deletion mutants have been isolated and used for genetic mapping of the region (3,4). Genetic (7) and biochemical studies (1) employing various nonsense, missense and frameshift mutations have shown that the *HIS4* gene codes for a single polypeptide (molecular weight 95,000) which contains three domains coded for by *HIS4A*, *HIS4B* and *HIS4C* respectively. Each domain

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has one of the three enzymatic activities and the direction of translation of the *HIS4* messenger RNA is from *HIS4A* to *HIS4C*.

In the accompanying paper (5) we have reported the construction of several plasmids which contain a 9.4 kb yeast chromosomal DNA sequence including the *HIS4* gene. In one of these plasmids, pC502, the 9.4 kb segment is carried on the bacterial plasmid pBR325. Using molecular hybridization with radioactive pC502 DNA, we have in the present study determined the sizes of different restriction endonuclease fragments originating from the *HIS4* region. By comparison of the data for three different *his4* deletion mutants with the wild type pattern the approximate physical positions and sizes of the deletions were determined.

2. MATERIALS AND METHODS

The following haploid Saccharomyces cerevisiae strains were used as sources of DNA: S288C (*HIS4*), IV-1 (*his4–260*), K5–5A (*his4–15*), C75–M23 (*his4–24*) and C75–M26 (*his4–29*). Strain S288C is a prototrophic reference strain from which the *his4* mutations were derived (3,4). The complete genotypes of the other strains have been given (6).

The preparation of total yeast DNA has been described (6). The DNA was further purified by adding 1.24 g CsCl per ml of DNA solution followed by centrifugation at 40,000 rpm in a Beckman 50 Ti rotor at 15 °C for 48 hours. Fractions containing DNA were selected according to viscosity, pooled, precipitated with ethanol and dissolved in 10 mM-Tris–HCl, 0.1 mM-EDTA, pH 7.5.

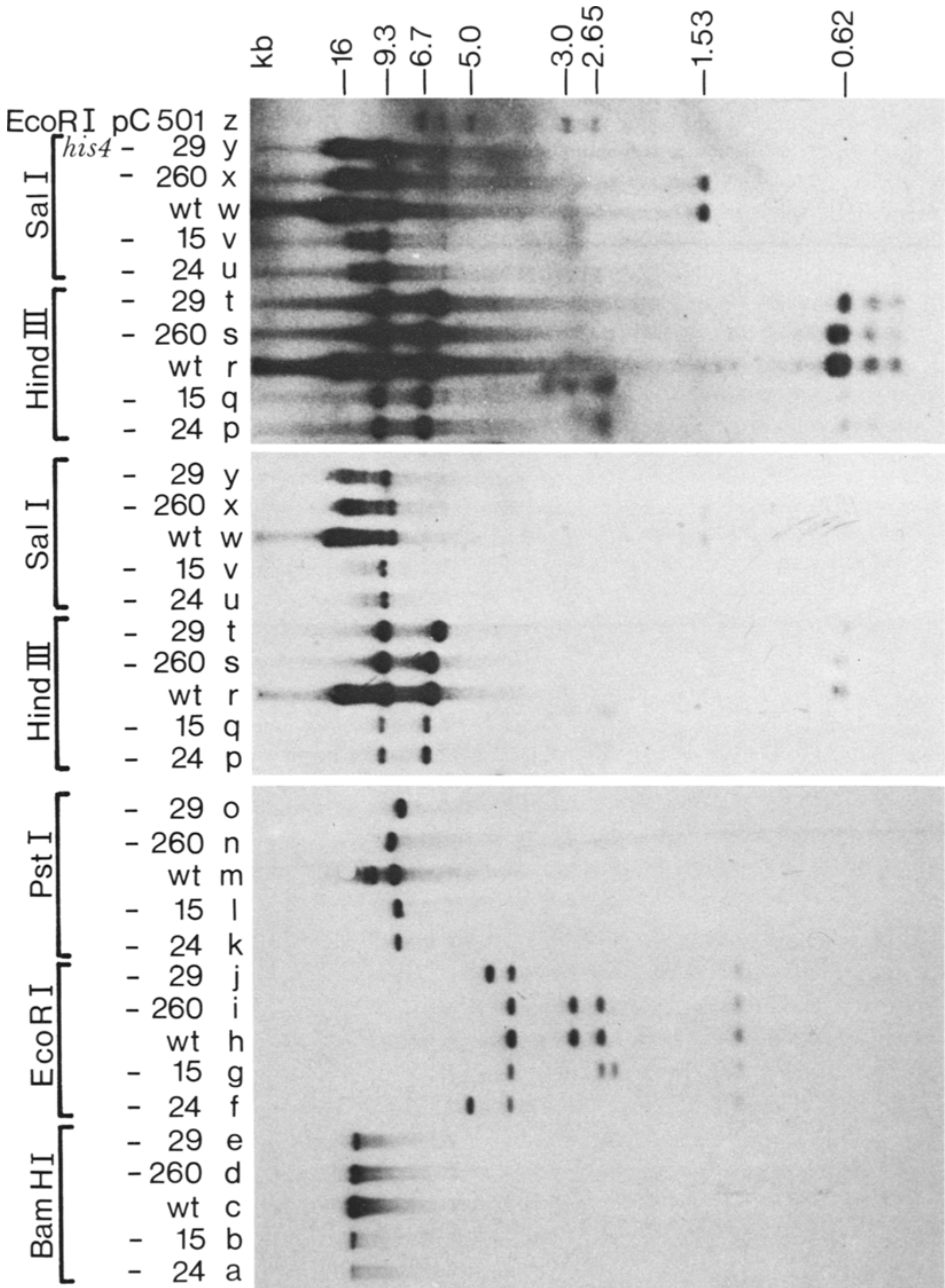
Autoradiographic exposure was for 2 to 7 days with or without Kodak X-Omatic regular intensifying screens. All other methods used in this study have been described (5).

3. RESULTS

DNA was isolated from yeast strains carrying the deletions *his4–15, his4–24* and *his4–29*. For comparison, DNA was also prepared from a strain carrying the *his4–260* point mutation and a histidine prototrophic strain (*HIS4*). Samples of each of the 5 DNA preparations were digested with the restriction endonucleases PstI, EcoRI, BamHI, SalI and HindIII and subjected to electrophoresis in 0.7% agarose. After electrophoresis the DNA in the gel slab was transferred to a sheet of cellulose nitrate filter as described by SOUTHERN (8), with the modification that the DNA was slightly depurinated by exposing the gel to pH 2.7 (5) for 4 hours prior to the denaturing conditions in alkali. This step was introduced to induce hydrolytic cleavage at depurinated sites by the alkali which decreases the molecular weight of large restriction fragments. It resulted in total transfer to the filters, also of the larger restriction fragments, as judged by staining the gels with ethidium bromide after the transfer. The DNA bound to the filters was then hybridized with denatured ^{32}P-labelled pC502 (*HIS4*) DNA for 48 hours. Autoradiograms of the filters are shown in Figure 1. When DNA from a *HIS4* yeast strain is digested with EcoRI, electrophoresed and probed with ^{32}P-labelled pC502 DNA, 4 bands of 4.1, 3.0, 2.65 and 1.4 kb are obtained (lane h). *his4–260* DNA has an identical pattern (lane i). All 3 deletion strains show an altered hybridization pattern when compared to the wild type (*HIS4*) or to each other. DNA from *his4–29* (lane j) shows only 3 bands. It has retained the upper and lower of the wild type bands (4.1 and 1.4 kb) and has a new band at 4.8 kb instead of the two middle wild type bands. From our physical mapping of the 9.4 kb PstI fragment (5) we know that the 3.0 and 2.65 kb fragments are adjacent in the chromosome. The pattern of the *his4–29* strain therefore shows that the deletion covers the EcoRI site between these two fragments and has

Figure 1. Restriction endonuclease fragments from 4 different *his4* mutants.

Samples consisting of about 1 µg of DNA digested with the following restriction endonucleases were separated by electrophoresis: BamHI (lanes a-e), EcoRI (lanes f-j), PstI (lanes k-o), HindIII (lanes p-t) and SalI (lanes u-y). Fragments which contain sequences in and around the *HIS4* gene were detected by molecular hybridization with $1.5 \cdot 10^7$ cpm of ^{32}P-labelled pC502 (5). The DNA was extracted from yeast strains carrying the following *his4* alleles: *his4–29, his4–260, HIS4, his4–15 and his4–24*. Lane z shows EcoRI digested pC501 (5). For better visualization of faint bands after HindIII and SalI digestion a longer exposure is shown to the right.


kb
16
9.3
6.7
5.0
3.0
2.65
1.53
0.62
EcoR I pC 501 z
his4 - 29 y
- 260 x
wt w
- 15 v
- 24 u
- 29 t
- 260 s
wt r
- 15 q
- 24 p
Sal I
Hind III
- 29 y
- 260 x
wt w
- 15 v
- 24 u
- 29 t
- 260 s
wt r
- 15 q
- 24 p
Sal I
Hind III
- 29 o
- 260 n
wt m
- 15 l
- 24 k
- 29 j
- 260 i
wt h
- 15 g
- 24 f
- 29 e
- 260 d
wt c
- 15 b
- 24 a
Pst I
EcoR I
Bam HI

the approximate size of 0.9 kb. The deletion *his4–24* (lane f) shows the same type of change, but here the new band has a mobility corresponding to 5.2 kb indicating that the *his4–24* deletion covers 0.45 kb. The *his4–15* deletion shows 4 bands (lane g), 3 of which are identical to wild type bands. The *HIS4* 3.0 kb fragment is missing and a 2.5 kb fragment appears, showing that the *his4–15* deletion is situated within the 3.0 kb EcoRI fragment and has a size of 0.5 kb.

The hybridization pattern after cleavage of DNA from the *HIS4* strain with HindIII (lane r) reveals 6 fragments of 9.5 (H1), 6.4 (H2), 0.69 (H3), 0.62 (H4), 0.46 (H5) and 0.39 kb (H6). As in the case of the EcoRI digestion, the HindIII pattern of the point mutation (lane s) is identical to that of the wild type (lane r). All 3 deletions show the same type of change when compared to *HIS4:* The H3 fragment is missing and the H2 fragment has changed slightly in size. This shows that H2 and H3 are adjacent and that all 3 deletions cover the HindIII site between these two fragments. The deletions *his4–15* and *his4–24* are both slightly smaller than the H3 fragment, since they cause a minor increase in the size of H2 (lanes p and q). The *his4–29* deletion must be larger than the H3 fragment as the H2 fragment decreases in size (lane t).

Although the digestion of the 5 DNA preparations with SalI is incomplete (lanes u-y), 3 bands in the wild type pattern appear to correspond to total digestion: S1 of about 16 kb, S2 of 9.3 kb and S3 of 1.55 kb. As for the other restriction enzymes the pattern of *HIS4* and *his4–260* are indistinguishable. The SalI digestion of the DNA from the deletion strains (lanes u, v and y) shows a pattern analogous to that of the HindIII digestion: The S3 fragment is absent and the S2 fragment changes in size indicating that all 3 deletions cover the SalI recognition site between these two fragments. In all 3 cases the S2 fragment becomes larger, showing that the deletions are smaller than the S3 fragment. The S2 fragment is smaller in *his4–29* than in the 2 other deletions, again revealing that this deletion is the largest.

Cleavage of the DNA from all 5 strains with either PstI (lanes k-o) or BamHI (lanes a-e) reveals only one band which hybridizes to *HIS4* DNA (except for the incompletely digested DNA of lane m). The BamHI fragments are bigger than the PstI fragments. This is consistent with the facts that the *HIS4* carrying 9.4 kb fragment in pC501 (5) originally was isolated after digestion of total yeast DNA with PstI (6) and that restriction mapping of this PstI fragment

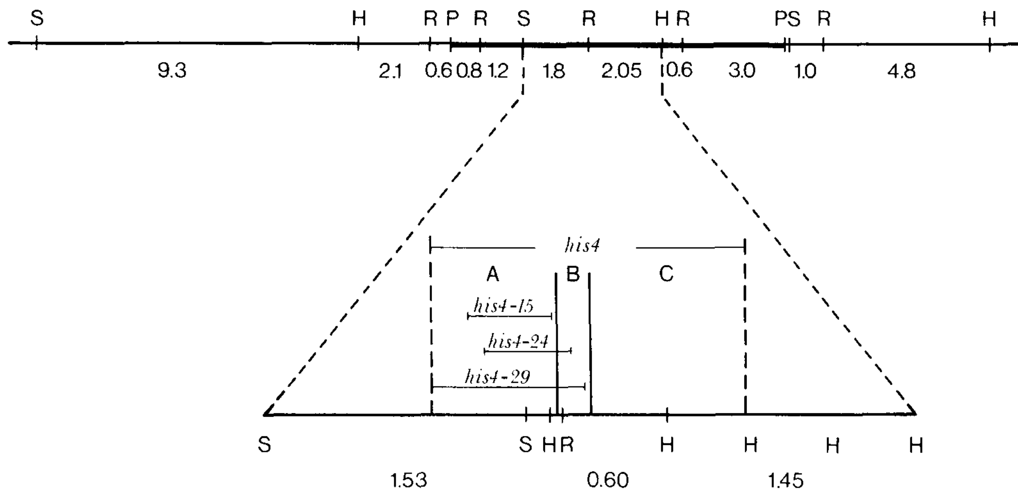


Figure 2. Restriction endonuclease map of the *HIS4* region of chromosome III in strain S288C.

H, P, R and S designate recognition sites for the restriction endonucleases HindIII, PstI, EcoRI and SalI, respectively. A, B and C designate the *his4A*, *his4B* and *his4C* functional segments. The map is complete only between the two PstI sites.

does not reveal any BamHI recognition sites (5). In this fragment size range the deletions do not cause large and reproducible changes in mobility with the possible exception of *his4–29* (lanes e and o).

Combining the information in Figure 1 with our mapping data (5) we have constructed a restriction endonuclease map of the region on chromosome III containing the *HIS4* gene (Figure 2). The EcoRI sites outside the 9.4 kb PstI fragment can be positioned as follows: Since the distance from the right end of the cloned DNA to the nearest EcoRI site in this DNA is 3.0 kb, the 1.4 kb EcoRI fragment observed in chromosomal DNA (Figure 1, lane h) must cover the left end of the cloned DNA. The positions of the 2 SalI sites outside the cloned DNA could be determined, since the deletions extend into the 9.3 kb SalI fragment. Similarly, the 6.4 kb wild type HindIII fragment could be positioned, since it contains part of the deletions. It was not possible to determine the positions of the two BamHI recognition sites. The map is complete for the 5 restriction endonucleases only within the cloned 9.4 kb region, since the method employed does not allow detection of more than two cleavage sites outside this region for any of the enzymes.

In summary, we have determined the sizes of deletions *his4–15* and *his4–24* to be about 0.5 kb and that of *his4–29* to be about 0.9 kb. Their approximate positions have been derived from the restriction enzyme cleavage sites which they cover (Figure 2).

4. DISCUSSION

The deletions in the *his4* region were isolated and characterized by FINK and STYLES (4) by the following two criteria: They did not revert and did not recombine with two or more *his4* mutants which recombined with each other. We have shown that three of these mutants are in fact lacking DNA sequences present in the wild type. The genetical positions of the deletions within the *his4* locus (4) allow us to place the *his4A*, *his4B* and *his4C* regions on the restriction endonuclease map presented in Figure 2. Deletion *his4–15* covers the part of *his4A* which is proximal to *his4B*. *his4–24* covers less of *his4A* but extends into *his4B*. Since the EcoRI site deleted by *his4–24* and *his4–29* is not deleted by *his4–15*, this EcoRI site must be at the border between *his4A* and *his4B* or in the part of *his4B* deleted by *his4–24*. This places *his4B* to the right of *his4–15* and thereby to the right of *his4A* on the restriction map in Figure 2. SHAFFER et al. (7) reported that the nonsense mutation *his4–864* which maps in the very beginning of the C-region instead of the 95,000 molecular weight wild type polypeptide has a polypeptide of approx. 45,000 with *HIS4A* and *HIS4B* activities. Also, the *HIS4C* activity in *his4–29* sediments more slowly in a sucrose gradient than the normal complex (4). Assuming that the *his4–29* deletion is extending to or beyond the beginning of the *his4A* coding region, we can therefore conclude that the region covered by the deletion codes for a little less than 45,000 of molecular weight of the wild type *his4* protein. With an average molecular weight of an amino acid residue of 110 this corresponds to 400 amino acids of 1.2 kb of coding region. Since *his4–29* has been found to be of about the same size (0.9 kb) we suggest that there are no large intervening sequences in the *his4A* or *his4B* regions.

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