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The Chromatin Structure at the Promoter of a Glyceraldehyde Phosphate Dehydrogenase Gene from *Saccharomyces cerevisiae* Reflects Its Functional State

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The chromatin structure of *TDH3*, one of three genes encoding glyceraldehyde phosphate dehydrogenases in *Saccharomyces cerevisiae*, was analyzed by nuclease digestion. A large hypersensitive region was found at the *TDH3* promoter extending from the RNA initiation site at position −40 to position −560. This hypersensitive domain is nucleosome free and includes all putative *cis*-acting regulatory DNA elements. It is equally present in cells grown on fermentable as well as nonfermentable carbon sources. In a mutant which lacks the *trans*-activating protein GCR1 and which as a consequence expresses *TDH3* at less than 5% of the wild-type level, the chromatin structure is different. Hypersensitivity between −40 and −370 is lost, due to the deposition of nucleosomes on a stretch that is nucleosome free in wild-type cells. Hypersensitivity is retained, however, further upstream (from −370 to −560). A similarly altered chromatin structure, as in a gcr1 mutant, is found in wild-type cells when they approach stationary phase. This is the first evidence for a growth-dependent regulation of the *TDH3* promoter.

Saccharomyces cerevisiae promoters are composed of multiple sequence elements (for reviews, see references 10–12, 32). The TATA box and the RNA initiation site determine the accuracy of transcription initiation, but for transcription to actually take place, upstream elements are also required. These upstream elements are called upstream activating sequences. They seem to be functionally equivalent to enhancers in higher eucaryotes (12). Through the interaction with regulatory proteins, the upstream elements are also responsible for the regulatory properties of a specific promoter.

For some time, our interest has been in the role that the chromatin structure plays in gene regulation. Eucaryotic DNA is normally packaged in chromatin, with the nucleosome being a basic subunit (for reviews, see references 9, 9a, 28, 33, 37). It is composed of 146 base pairs (bp) of DNA wrapped tightly around a histone octamer consisting of two copies each of the histones H2A, H2B, H3, and H4. Functionally important DNA elements have, however, been found to reside in many cases in nucleosome-free regions, which makes these sequences highly susceptible to nucleases used to probe the structure of chromatin. For this reason these regions are usually called hypersensitive sites.

We have previously shown that an upstream activating sequence element from the promoter of *PHO5*, a strongly regulated acid phosphatase gene from *S. cerevisiae*, is contained within a short hypersensitive region under conditions of *PHO5* repression (1). The remaining part of the *PHO5* promoter is organized in positioned nucleosomes under these conditions. Upon *PHO5* activation, these nucleosomes are removed and the entire promoter turns hypersensitive (2).

In an effort to address the question of whether a chromatin transition at a promoter is the cause or the result of gene activation, we extended our analyses to another strong promoter in *S. cerevisiae*. We chose *TDH3*, a heavily transcribed gene for a glyceraldehyde phosphate dehydrogenase (GAPDH), for that purpose. Yeast has three GAPDH structural genes, *TDH1*, *TDH2*, and *TDH3* (TDH stands for triosephosphate dehydrogenase) that contribute about 10, 30, and 60% of the total GAPDH activity in wild-type cells (14, 15, 17, 25). There is only a small effect of the carbon source in the medium on GAPDH activity (25). However, for the high-level expression of *TDH1*, *TDH2*, *TDH3*, and a number of other genes for glycolytic enzymes, the activity of GCR1, a *trans*-activating protein, is required (7, 8). The corresponding gene has recently been cloned and sequenced (4, 18). Transcription of *TDH3* drops to a level of less than 5% of the usual value if the GCR1 gene is disrupted (18), but the molecular basis of the pleiotropic action of GCR1 is not understood.

For *TDH3*, it is known that a region encompassing 680 bp upstream of the structural gene contains all *cis*-acting DNA elements necessary for promoter function (5). Our results show that special features of the chromatin structure are indeed found in precisely that region. Moreover, the chromatin structure looks different when the GCR1 protein is absent, indicating that GCR1 has a direct influence on both *TDH3* transcription and the chromatin organization of the *TDH3* promoter.

MATERIALS AND METHODS

Strains and growth conditions. The two GCR1 strains used were IH2 (a *his4-519 trp1 leu2-3 leu2-112 ura3-251 ura3-328 ura3-373 ade2*) (30) and S173-6B (α *leu2-3 leu2-112 his3-1 trp1-289 ura3-52*) (18). They gave identical results in our analyses. A gcr1 deletion mutant was constructed from S173-6B by M. Holland, University of California, Davis (8) and was kindly provided together with the parent strain. The strains were grown in YPA medium (2% Bacto-Peptone [Difco Laboratories], 1% yeast extract [Difco], and 100 mg of adenine per liter) or minimal medium containing 0.67% yeast nitrogen base without amino acids (Difco) supplemented with the necessary amino acids, and uracil and

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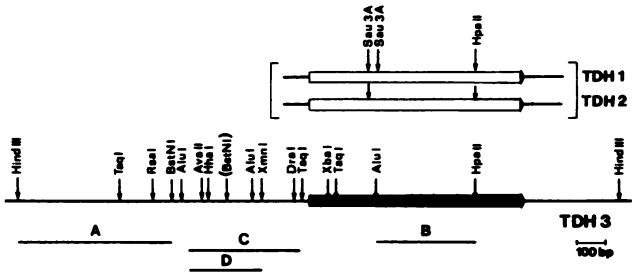

FIG. 1. Restriction map of the *TDH3* locus based on the data of Holland and Holland (14) and Bitter and Egan (5). The *Bst*N1 site shown in parentheses is missing in strain S173-6B and derivatives thereof. Subcloned DNA fragments (see Materials and Methods) used as hybridization probes are indicated (A to D). The gene loci for *TDH1* (18) and *TDH2* (16) with pertinent restriction sites (see text) are shown at the top.

adenine. Carbon sources were 2% glucose, 2% glycerol plus 2% lactate, or 2% ethanol.

Isolation of nuclei, nuclease digestion, gel electrophoresis, hybridization, and DNA probes. Nuclei were isolated by a modification of the procedure of Wintersberger et al. (34) as described previously (1). Nuclease digestion, gel electrophoresis, and hybridization were performed as described previously (1). Nylon membranes (GeneScreen Plus, Dupont NEN Research Products) were used for Southern blot transfer throughout. The DNA probes used were pBR322 subclones derived from the 2.1-kilobase (kb) *Hin*dIII fragment containing the *TDH3* gene (14, 17) kindly provided by A. Hinnen, CIBA-GEIGY AG, Basel, Switzerland. The positions and sizes of restriction fragments used for the subcloning (see Fig. 1) are as follows: A, *Hin*dIII (ca. −1040)-*Bst*NI (−491), 550 bp; B, *Alu*I (+240)-*Hpa*II (+602), 362 bp; C, *Hin*fI (−431)-*Taq*I (−27), 404 bp; D, *Hin*fI (−432)-*Xmn*I (−171), 261 bp. The restriction sites are derived from the sequence data of Bitter and Egan (5) and Holland and Holland (14) and are listed relative to the *TDH3* initiation codon.

RESULTS

Large hypersensitive region at the *TDH3* promoter. The chromatin structure of the *TDH3* promoter region was investigated by digestion of yeast nuclei with different nucleases. Accessibility to nucleases was monitored by the indirect end-labeling procedure (27, 36). The strategy employed is shown in Fig. 1, which shows a restriction map of the *TDH3* gene locus based on the data of Holland and Holland (14) and Bitter and Egan (5). To get a view of the promoter from upstream, we first digested nuclei (e.g., with DNase I), isolated DNA, restricted it with *Hin*dIII, and used a subclone called probe A in Fig. 1 for hybridization. Similarly, probe B was used for a view from downstream after restriction of the isolated DNA with *Hpa*II. To overcome the problem that probe B crosshybridizes with the two other TDH genes (15, 16), we included *Sau*3A in the secondary digestion. As indicated in Fig. 1, this procedure generated two short fragments from the other TDH genes, which made it possible to assign any fragments larger than those unambiguously to *TDH3*.

Nuclei were isolated from exponentially dividing cells that had been grown with glucose as the carbon source. In the chromatin digestion patterns, cleavage by DNase I was not uniformly distributed but was confined to a specific region within the promoter (Fig. 2 and 3). The DNA further upstream and the transcribed region itself were much more resistant to the nuclease. A highly accessible region of the kind we observed is usually referred to as a hypersensitive site and is one of the hallmarks of active genes. That preferential cleavage by DNase I is truly a property of the chromatin structure was demonstrated by control experiments with free DNA (shown on the right in Fig. 2 and 3), which showed fairly uniform DNase I cleavage across the entire DNA that was being analyzed. The slight banding pattern in the free DNA digests was due to a weak DNA sequence preference of the DNase. This sequence preference can also explain the bands seen within the hypersensitive region in the chromatin digests, since DNA within that region behaved more or less like free DNA.

The boundaries of the hypersensitive region can be accurately mapped relative to known restriction sites by including appropriate restriction digests of either genomic DNA or of the cloned *TDH3* fragment in the gel analyses. From coelectrophoresis with such fragments, we have found that the hypersensitive region starts around an *Rsa*I site at position −563 and extends down to a *Dra*I site at position −50, which is where transcription of the *TDH3* gene is initiated (16, 26). The gene region itself is not particularly sensitive to DNase I. It should be remembered, however, that elevated DNase I sensitivity, as was reported in many cases for transcribed regions (28, 33, 37), would not be detected by the approach we took.

Digestion of nuclei with restriction nucleases constitutes an alternative approach that can be used to map sensitive regions in chromatin, with the added benefit that accessibility can be quantitated. If a particular restriction site was not accessible in the nucleus, a 2.1-kb fragment was generated after secondary digestion with *Hin*dIII (Fig. 4), while a shorter fragment was present if the restriction nuclease actually did cleave the sequence. Therefore, in each case the intensity of the small fragment compared with that of the large fragment is a direct measure of the accessibility of that site. The results presented in Fig. 4 confirmed our expectation that restriction sites contained within the hypersensitive region are accessible, while sites further upstream (e.g., *Taq*I) or downstream (*Xba*I) are barely cleaved. Digestion with *Taq*I was particularly informative in this respect. Of all the *Taq*I sites within the 2.1-kb *Hin*dIII fragment (six within the sequenced part), only one, at position −27, was cleaved to an appreciable extent (30 to 40%). This site is at the very end of the hypersensitive region, which might explain why it is not fully susceptible. The results presented in Fig. 4 and those obtained in the same way with a number of additional restriction nucleases are summarized in Fig. 5 together with the data from DNase I digestion.

It has been reported that there is some influence by the carbon source on the level of GAPDH activity, although the differences appeared to be rather small (25). Therefore, we investigated whether there was any effect of the carbon source on the chromatin configuration of the *TDH3* promoter and analyzed nuclei from cells grown in gluconeogenic media with ethanol or glycerol-lactate. DNase I patterns obtained with these conditions were identical to those obtained with glucose-containing media, and similarly, growing cells in minimal medium rather than rich medium had no effect on the chromatin structure of the *TDH3* promoter.

The chromatin structure at the *TDH3* promoter changes when cells approach stationary phase. An unexpected result was obtained when the chromatin configuration of the *TDH3* promoter was analyzed in cells that were approaching sta-

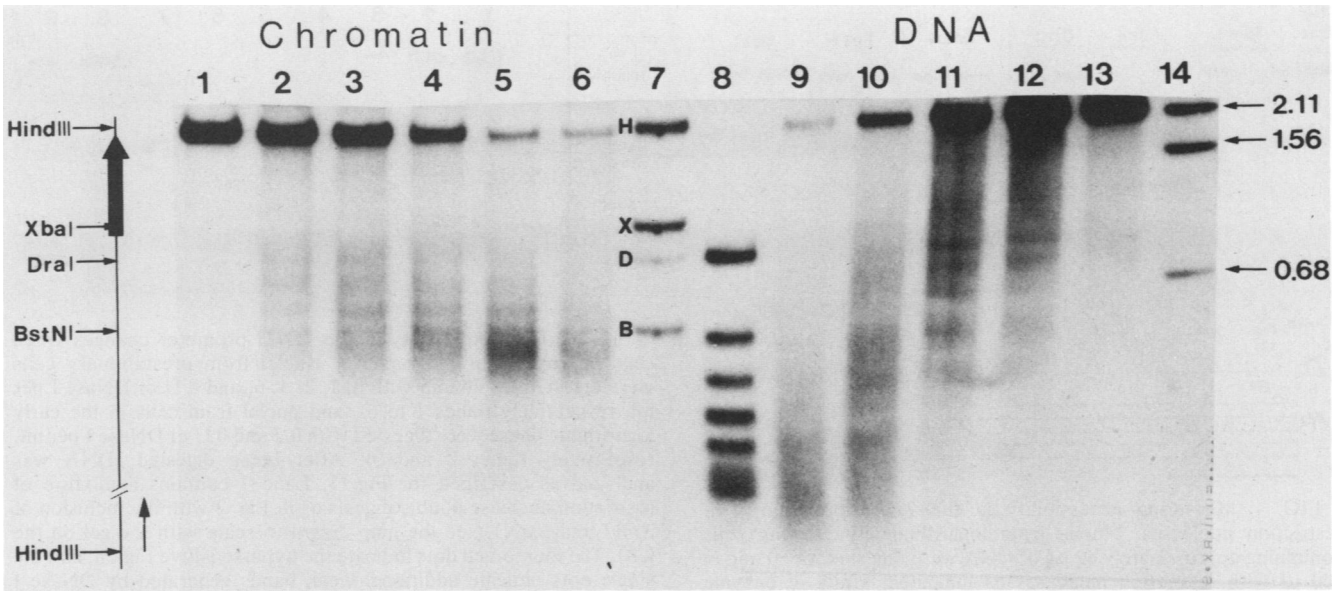

FIG. 2. Upstream view of the hypersensitive region at the *TDH3* promoter. Nuclei from logarithmically growing cells or the isolated 2.1-kb *Hin*dIII fragment were digested for 20 min with 0, 1, 2, 4, 6, and 8 U of DNase I per ml, respectively (lanes 1 to 6), or with 0.16, 0.08, 0.04, 0.02, and 0 U of DNase I per ml, respectively (lanes 9 to 13). DNA was isolated, cleaved with *Hin*dIII, separated in a 1.5% agarose gel, blotted, and hybridized with probe A (see Fig. 1). Lane 7 contains a mixture of restriction nuclease digests of the isolated 2.1-kb *Hin*dIII fragment (H) with either *Xba*I, *Dra*I, or *Bst*N1 (X, D, and B, respectively). The locations of these sites relative to the *TDH3* gene are shown on the left in a map drawn to scale with the gel. The arrow denotes the probe. A pBR322 subclone digested with *Hpa*II serves as a molecular weight reference in lane 8. Sizes of the five largest fragments are 914, 527, 404, 305, and 242 bp. An *Rsa*I digest of pBR322 DNA is shown in lane 14, with sizes given on the right in kilobases.

tionary phase. The accessibility to DNase I was restricted to a much shorter stretch (marked with four dots in Fig. 6). The hypersensitive region started at about the same far-upstream position as in logarithmically growing cultures, but it spanned only about 200 bp and ended around a *Hha*I site at position −370. The promoter region proximal to the gene was no longer hypersensitive; instead, two weak bands spaced about 150 bp apart (single dots in Fig. 6) were generated by DNase I. Identical patterns were obtained when cells grown in gluconeogenic media approached stationary phase, indicating that this transition in the chromatin structure was not simply due to the exhaustion of glucose.

It is not clear what actually triggers the transition in the chromatin structure that we observed when the cells stopped dividing exponentially. It might be depletion of GAPDH substrates, and since GAPDH is involved in both (the

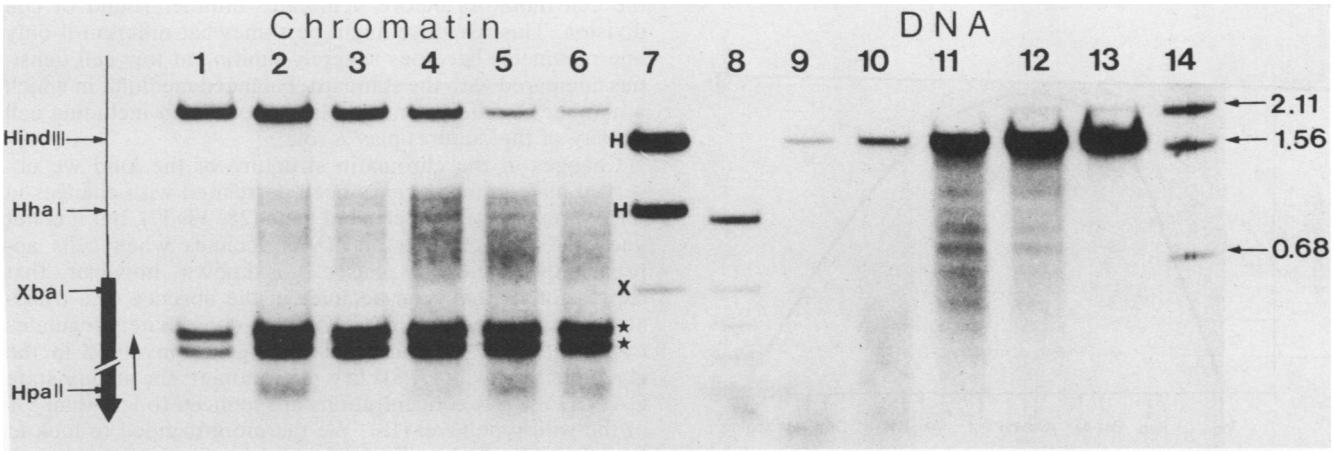

FIG. 3. View from downstream of the hypersensitive region at the *TDH3* promoter. The DNase I digests analyzed in Fig. 2 were cleaved with *Hpa*II and *Sau*3A (chromatin) or only *Hpa*II (DNA) instead of *Hin*dIII. The DNA samples were separated in a 1.5% agarose gel, blotted, and hybridized with probe B (see Fig. 1). The stars mark 334- and 389-bp fragments derived from *TDH1* and *TDH2*, respectively, which crosshybridize with probe B (see Fig. 1). Lane 7 contains a mixture of restriction nuclease digests of the isolated 2.1-kb *Hin*dIII fragment digested with *Hpa*II to give an *Hpa*II-*Hin*dIII fragment (upper H), with *Hpa*II plus *Hha*I (lower H), or with HpaII plus XbaI (X). The locations of these sites relative to the *TDH3* gene are shown on the left in a map drawn to scale with the gel. The arrow denotes the probe. Lanes 8 and 14 contain the molecular weight references shown in Fig. 2.

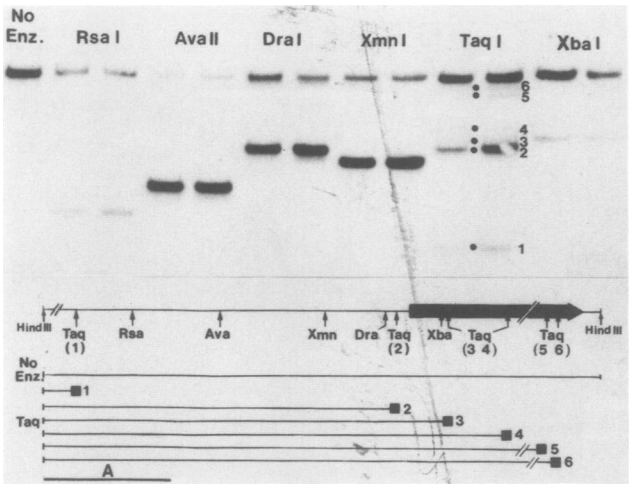

FIG. 4. Measuring accessibility at the *TDH3* promoter with restriction nucleases. Nuclei from logarithmically growing cells containing approximately 40 μg of DNA were digested for 60 min in 100 μl with restriction nucleases as indicated. Units of enzyme added were as follows (from left to right): 60, 120 (*Rsa*I); 20, 40 (*Ava*II); 50, 100 (*Dra*I); 40, 80 (*Xmn*I); 60, 120 (*Taq*I); 80, 160 (*Xba*I). A control incubation without enzyme is shown on the left. After being digested, DNA was isolated and analyzed as in Fig. 2. A map of the *TDH3* locus with the restriction sites used is shown. The principle of the method is illustrated for *Taq*I below. All possible *Taq*I fragments that would be detected with probe A are numbered from 1 to 6 and indicated in the autoradiogram.

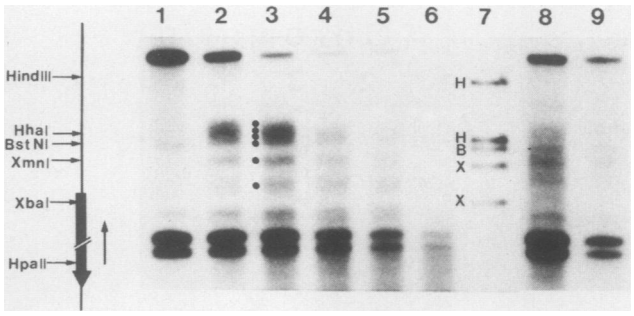

FIG. 6. Hypersensitivity at the *TDH3* promoter changes when cells approach stationary phase. Nuclei from prestationary cells were digested for 20 min with 0, 1, 2, 4, 6, and 8 U of DNase I per ml, respectively (lanes 1 to 6), and nuclei from cells in the early logarithmic phase were digested with 0.2 and 0 U of DNase I per ml, respectively (lanes 8 and 9). After being digested, DNA was analyzed as described for Fig. 3. Lane 7 contains a mixture of restriction nuclease double digests as in Fig. 3 with the inclusion of *Xmn*I and *Bst*N1 (see the map drawn to scale with the gel on the left). The four joined dots indicate the hypersensitive region, and the single dots indicate additional weak bands generated by DNase I (see text for details).

glycolytic and the gluconeogenic pathway), depletion of either carbon source might give the effect. Alternatively, it might be part of a more general growth control effect. In that case, starvation of the cells for any of a number of different components might trigger the response. In a first attempt to differentiate between these possibilities, we grew the cells in minimal medium with a limiting amount of added leucine. They become stationary at a lower cell density under these conditions, with excess glucose still being present in the medium, since the strain is auxotrophic for leucine. When we analyzed chromatin at the *TDH3* promoter of cells approaching stationary phase under these conditions, patterns were observed that resembled the late logarithmic growth phase patterns (late patterns) except that hypersensitivity in the downstream region was not completely lost. The same kind of intermediate patterns were obtained with cells becoming stationary in YPA medium with less glucose (0.25%) added. Again, growth of the cells leveled off at lower cell densities.

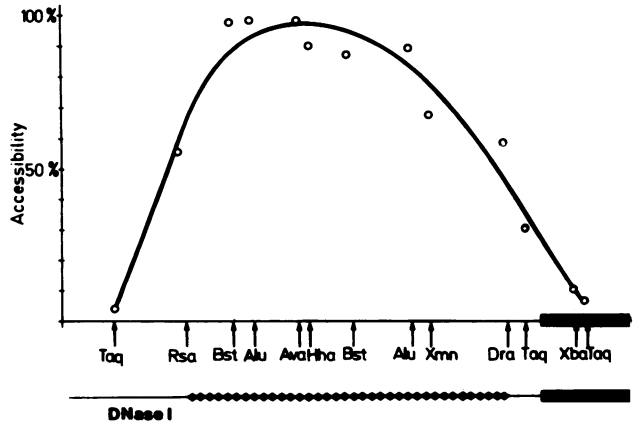

FIG. 5. Accessibility of the *TDH3* promoter to restriction nucleases. Extent of cleavage at different restriction sites in nuclei was measured as shown in Fig. 4 by determining the ratios of the band intensities in the autoradiograms. The region hypersensitive to DNase I is shown for comparison below (◆).

The cells do have to go through a process of starvation, however, for the pattern to change. When logarithmically growing cells were washed free of leucine or glucose and suspended in leucine- or glucose-free medium, the cells retained the pattern typical of logarithmically growing cultures. Under these conditions, there was no further cell division upon transfer of the cells to the depleted medium.

We interpret these results to mean that the late pattern reflects a complex response to different parameters which the cell monitors before it initiates another round of cell division. This response might be somewhat different if only one parameter becomes severely limiting at low cell densities compared with the standard, balanced medium, in which a number of different metabolic factors (also including cell density of the culture) play a role.

Changes in the chromatin structure of the kind we observed have in many cases been correlated with changes in the expression of the pertinent gene (28, 33, 37), but it is not known if transcription of *TDH3* declines when cells approach the stationary phase. It is known, however, that expression of this gene declines in the absence of a *trans*-acting regulatory protein, GCR1, that coordinately regulates transcription of a large number of genes involved in the glycolytic pathway (7, 8). In a gcr1 mutant, the steady-state GAPDH mRNA concentrations are reduced to less than 5% of the wild-type level (18). We therefore decided to look at the effect of a gcr1 null mutation on the chromatin structure of the *TDH3* promoter.

The GCR1 protein affects the chromatin structure of the *TDH3* promoter. Analysis of the *TDH3* promoter in a gcr1 null mutant that was kindly made available to us by M. Holland gave a clear result. Hypersensitivity was again confined to the far upstream region between positions −560

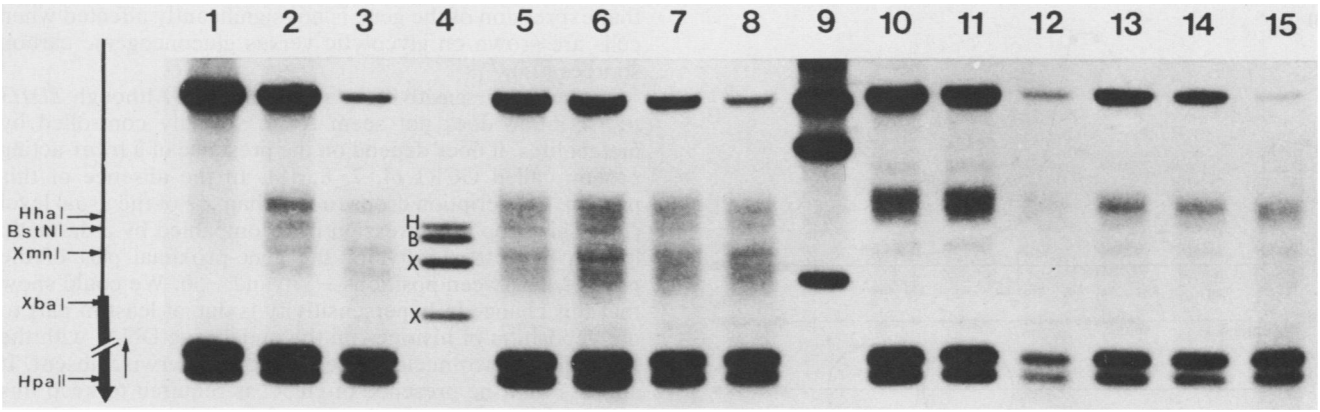

FIG. 7. Hypersensitivity at the *TDH3* promoter is affected by the growth state of the cells and the GCR1 protein. Shown are DNase I hypersensitivity measurements of the *TDH3* promoter under different conditions. Analysis was as described for Fig. 3. The strains and the growth conditions, including the carbon sources, were as follows: lanes 1 to 3, early logarithmic growth phase wild-type nuclei (carbon source, glucose); lanes 5 to 8, early logarithmic growth phase wild-type nuclei (carbon source, glycerol-lactate); lanes 10 to 12, late logarithmic growth phase wild-type nuclei (carbon source, glucose); lanes 13 to 15, early logarithmic growth phase gcr1 nuclei (carbon source, glycerol-lactate). DNase I concentrations were 0, 0.4, and 0.8 U/ml, respectively (lanes 1 to 3); 0.4, 0.8, 1.2, and 1.6 U/ml, respectively (lanes 5 to 8); and 1.5, 2.0, 3.0, 0.2, 0.4, and 0.6 U/ml, respectively (lanes 10 to 15). The map on the left is drawn to scale with the gel.

and −370 (Fig. 7, lanes 13 to 15). The rest of the promoter lost its hypersensitivity in precisely the same way that wild-type cells do in the late logarithmic growth phase (Fig. 7, cf. lanes 10 and 11). Significantly, however, in the mutant this pattern was always obtained regardless of the growth state of the cells, and there was no further reduction in hypersensitivity when the cells approached stationary phase.

It was important to show that this change in the chromatin structure was really due to the lack of GCR1 and not simply a strain-specific variation. Fortunately, the gcr1 phenotype in the mutant had been generated by gene disruption (18), and the parent wild-type strain was also available to us. When we analyzed the chromatin structure at the *TDH3* promoter in that strain, we found no differences between it and our own wild-type strain as far as medium and growth state dependence were concerned. The pattern that was typical of the mutant was indistinguishable from the late pattern of the parent wild-type strain and very different from the pattern seen in the early logarithmic growth phase (early pattern) of GCR1 cells (see Fig. 7). It is therefore reasonable to correlate the late pattern with the absence of GCR1 and, as a consequence, inactivation of the promoter, and to equate the early pattern with an active promoter.

Loss of hypersensitivity at the inactive *TDH3* promoter is due to the presence of nucleosomes. The loss of hypersensitivity at the *TDH3* promoter was accompanied by the appearance of two narrow, weakly sensitive regions spaced at 150-bp intervals (Fig. 6 and 7). This is suggestive of the presence of positioned nucleosomes located in the immediate vicinity of the part of the hypersensitive region that persists in the inactive pattern. In order to investigate this possibility, we digested nuclei extensively with micrococcal nuclease, thus converting most of the chromatin to mononucleosomes and short oligonucleosomes. These extensive digests were then hybridized without secondary restriction against a probe derived from the region of interest (Fig. 8). Hybridization signals typical of nucleosomes were barely detectable when the active promoter, which yields the early pattern, was analyzed. In contrast, there was clear evidence of nucleosomes present on this stretch of DNA in the gcr1 mutant and in late logarithmic growth phase wild-type cells. We interpret these results to mean that a highly accessible chromatin region in the active *TDH3* promoter becomes associated with nucleosomes under conditions in which the promoter is no longer active or that nucleosomes are removed upon activation of the promoter. This interpretation is also in accord with data on the accessibility of various restriction sites in chromatin from gcr1 cells. Our results for the late chromatin pattern are summarized in Fig. 9, and the

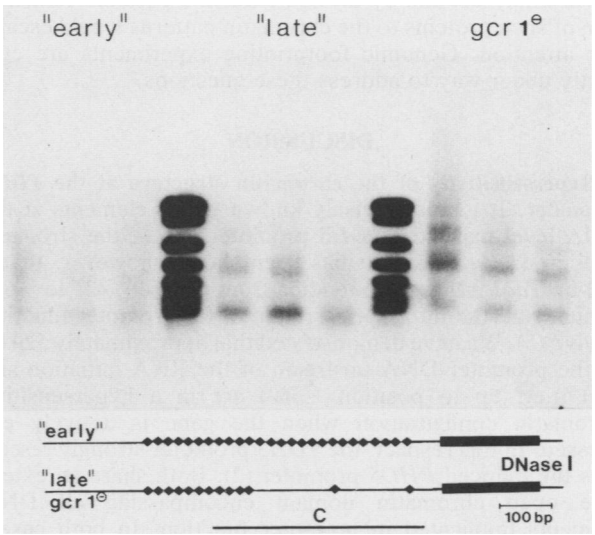

FIG. 8. Nucleosome substructure at the *TDH3* promoter. Nuclei from either early logarithmic growth phase wild-type cells, late logarithmic growth phase wild-type cells, or early logarithmic growth phase gcr1 cells (all grown with glycerol-lactate as the carbon source) were digested for 20 min with 8, 12, 20, 35, 40, 60, 9, 18, and 36 U of micrococcal nuclease per ml, respectively, from left to right. DNA was isolated and hybridized against probe C without prior restriction. The most rapidly migrating band corresponds to core particle DNA of approximately 150 bp. pBR322 DNA digested with *Hpa*II was used as a molecular weight reference (lanes 4 and 8). The position of probe C relative to the DNase I-hypersensitive regions (◆) in the different nuclei (Fig. 7) is indicated at the bottom.

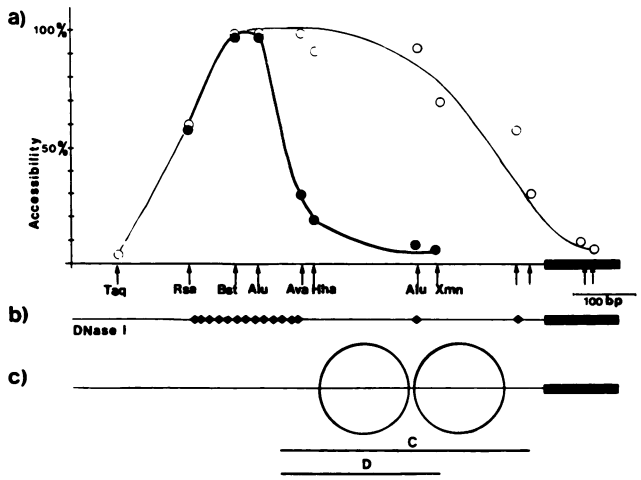

FIG. 9. Effect of the GCR1 protein on the chromatin structure at the *TDH3* promoter. (a) Accessibility of restriction sites in nuclei from gcr1 cells (●) was determined as described for Fig. 4. These values are compared with the corresponding ones from early logarithmic growth phase wild-type cells (○). (b) DNase I hypersensitivity (◆) at the *TDH3* promoter in gcr1 cells. (c) Nucleosome substructure at the *TDH3* promoter in the gcr1 mutant. Our conception of the *TDH3* promoter in the gcr1 mutant with two positioned nucleosomes (circles) detected by probe C or D (see Fig. 8) is shown. In logarithmically growing wild-type cells, this region is largely nucleosome free.

two additional nucleosomes that distinguish the inactive promoter from the active one are indicated. It is important to realize, however, that at our current level of resolution the presence of nonhistone proteins would tend to go undetected in our analyses, and by the same token a possible contribution of such proteins to the chromatin patterns would escape our attention. Genomic footprinting experiments are currently under way to address these questions.

DISCUSSION

Hypersensitivity of the chromatin structure at the *TDH3* promoter. It is not precisely known which elements at the DNA level make the *TDH3* promoter one of the strongest ones in *S. cerevisiae*. It has been shown, however, that a 652-bp *Taq*I fragment extending from position −27 to −678 contains all the information required for promoter function in vivo (5). We have demonstrated that approximately 520 bp of the promoter DNA upstream of the RNA initiation site (16) (i.e., up to position −560) are in a hypersensitive chromatin configuration when the gene is actively expressed. In this respect, the *TDH3* promoter strongly resembles the induced *PHO5* promoter (2). Both share an extensive open chromatin domain encompassing all DNA elements implicated in promoter function. In both cases, accessibility of the promoter is due to the absence of nucleosomes, as has been proposed in many other cases in which hypersensitive sites have been examined (for reviews, see references 9, 9a, 28, 37). It should be noted, however, that we cannot distinguish between total absence of nucleosomes and a persistence of nucleosomes with drastically altered properties, such as no longer being able to protect DNA against restriction nucleases and micrococcal nuclease.

For *TDH3*, this open domain is present regardless of the carbon source available. This is consistent with the finding that expression of the gene is not significantly affected when cells are grown on glycolytic versus gluconeogenic carbon sources (25).

Loss of hypersensitivity in a gcr1 mutant. Although *TDH3* transcription does not seem to be strongly controlled by metabolites, it does depend on the presence of a *trans*-acting protein called GCR1 (4, 7, 8, 18). In the absence of this protein, transcription drops to less than 5% of the usual level (18). This drop in expression is accompanied by a dramatic loss in hypersensitivity for the gene-proximal part of the promoter between positions −370 and −50. We could show that this change in hypersensitivity is due at least in part to the deposition of histones on the underlying DNA, with the formation of two nucleosomes that are otherwise absent. It appears that the presence of GCR1 is required to keep this part of the promoter nucleosome free and thereby active.

DNA from about −560 to −370 remains hypersensitive, however, even in a gcr1 null mutant. Hypersensitivity at this region further upstream might point to a functional role of the underlying DNA in the reversible opening and closing of the adjacent promoter in response to GCR1 (see below). Consistent with this hypothesis is the finding of *cis*-acting DNA elements between −540 and −440 involved in *TDH3* regulation (M. Holland, personal communication), i.e., precisely within this upstream hypersensitive region.

Regulation of *TDH3* expression during transition to stationary phase. When wild-type yeast cells cease to grow exponentially, a striking change in the chromatin structure at the *TDH3* promoter is observed that precisely mimics the situation that is found with the gcr1 mutant, except that in the latter it is not restricted to the late growth phase but is always encountered. This similarity strongly suggests that the *TDH3* promoter is at least partially shut down under these conditions. It is not known whether this chromatin transition towards the end of exponential growth is brought about by GCR1 or by other *trans*-acting factors interacting with the *TDH3* promoter. If it were due to GCR1, it might be the result of a modification of the protein, e.g., by a phosphorylation-dephosphorylation event. This mechanism has been shown to affect a number of *trans*-activating proteins in mammalian cells, e.g., AP-1 (3) and AP-2 (19). It is interesting in this context that a cAMP-dependent protein kinase is involved in growth control in yeast (24).

It is known that yeast cells undergo a distinct set of metabolic changes when they switch from exponential growth to the stationary phase (13). TDH mRNA levels per cell are indeed severalfold lower in stationary-phase cells compared with vegetative cells (20), but are clearly above background; this is consistent with the finding that GAPDH continues to be synthesized in stationary phase (6). From these studies it cannot be determined, however, if this residual activity is due to all three TDH genes or only a subset. In addition, since the stability of TDH mRNA in cells approaching stationary phase is not known, it is unclear at what stage TDH transcription had actually occurred.

Chromatin structure and gene regulation. Hypersensitivity at promoter regions seems to be a universal property of genes that are expressed or poised for expression (9, 9a, 28, 37). In the case of *PHO5*, we showed that an upstream activating sequence element recognized by the regulatory protein PHO4 (K. Vogel, personal communication) is precisely contained within a short hypersensitive site with adjacent positioned nucleosomes under conditions of *PHO5* repression (1). This region is responsible for the PHO4-dependent removal of adjacent nucleosomes upon induction of *PHO5* (C. Straka and W. Hörz, manuscript in prepara-

tion), and as a consequence, essentially all of the promoter becomes nucleosome free and thereby accessible (2).

For *TDH3*, a similar situation is encountered. In the absence of GCR1, hypersensitivity is confined to a short stretch, located further upstream of the gene, which contains *cis*-acting regulatory DNA elements (M. Holland, personal communication). The GCR1 protein, possibly in combination with these DNA elements and other regulatory proteins (18), seems to be responsible for opening up the more downstream part of the promoter, including the TATA box, by the removal of two nucleosomes. This would be consistent with the view that the chromatin structure of the promoter directly affects its state of activity and that at least one function of regulatory proteins is to modify the chromatin configuration of a promoter (e.g., by removing histones), as activating proteins would be well equipped to do given their acidic domain (12). Support for this notion comes from constitutive yeast promoters that depend on oligo(dA · dT) stretches serving as upstream elements (31). Nucleosomes do not form on poly(dA · dT) in vitro (22, 29). This has led to the proposal that an open chromatin window in the absence of *trans*-activating proteins can be sufficient for promoter activity (32).

That nucleosomes can render a promoter inactive has been demonstrated by in vitro experiments (21, 23). In one study, it was even shown that a particular transcription factor, TFIID, can overcome this repression by nucleosomes if it is allowed to bind prior to chromatin assembly (35). By a rather different approach, Han et al. (12a) showed that the *S. cerevisiae PHO5* promoter can be activated in vivo if histone H4 synthesis is shut off and the chromatin structure becomes severely disturbed. The sophisticated molecular genetics available makes *S. cerevisiae* the organism which is most likely to provide a definitive answer to the intriguing question of what role nucleosomes play in gene regulation.

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