The EMBO Journal vol.12 no.1 pp.177-186, 1993

CDC39, an essential nuclear protein that negatively regulates transcription and differentially affects the constitutive and inducible *HIS3* promoters

Martine A.Collart and Kevin Struhl

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

Communicated by R.Treisman

The yeast *HIS3* promoter region contains two functionally distinct TATA elements, T_C and T_R, that are responsible respectively for initiation from the +1 and +13 sites. Both T_C and T_R support basal *HIS3* transcription and require the TATA binding protein TFIID, but only T_R responds to transcriptional activation by GCN4 and GAL4. By selecting for yeast strains that increase transcription by a GCN4 derivative with a defective activation domain, we have isolated a temperature-sensitive mutation in *CDC39*, a previously defined gene implicated in cell-cycle control and the pheromone response. This *cdc39*-2 mutation causes increased basal transcription of many, but not all genes, as well as increased transcriptional activation by GCN4 and GAL4. Surprisingly, basal *HIS3* transcription from the +1 initiation site is strongly increased, while initiation from the +13 site is barely affected. Thus, unlike acidic activator proteins that function through T_R, *CDC39* preferentially affects transcription mediated by T_C. *CDC39* is an essential gene that encodes a very large nuclear protein (2108 amino acids) containing two glutamine-rich regions. These observations suggest that *CDC39* negatively regulates transcription either by affecting the general RNA polymerase II machinery or by altering chromatin structure.

Key words: eukaryotic promoters/GCN4 activation/*Saccharomyces cerevisiae*/TATA element/transcriptional repression

Introduction

Constitutive and GCN4-inducible expression of the yeast *HIS3* gene involves two TATA elements, T_C and T_R, that promote transcription by different molecular mechanisms (Struhl, 1986; Chen and Struhl, 1988; Harbury and Struhl, 1989; Mahadevan and Struhl, 1990; Ponticelli and Struhl, 1990). T_C is responsible for transcription initiated from the +1 site, whereas T_R-dependent transcription is initiated almost exclusively from the +13 site. In the absence of GCN4, *HIS3* transcription is initiated with equal efficiency from +1 and +13 because both T_C and T_R act in conjunction with an upstream poly(dA)·poly(dT) sequence (Struhl, 1985). However, during GCN4-activated transcription, initiation from the +13 site is increased 5-fold, whereas initiation from +1 remains essentially unaffected. Preferential initiation at +13 is also observed when GAL4 activates transcription from hybrid promoters in which the *GAL* enhancer is fused at various positions upstream of the *HIS3* TATA region (Struhl, 1984). These transcriptional initiation patterns arise because T_R can respond to transcriptional stimulation by GCN4 and GAL4, whereas T_C cannot.

Besides their distinct responses to acidic activator proteins, T_R and T_C differ in other ways. The T_R element is defined primarily by the canonical TATA sequence, TATAAA (Chen and Struhl, 1988; Harbury and Struhl, 1989), and its functional activity strongly correlates with its ability to bind TFIID (Strubin and Struhl, 1992) and support TFIID-dependent transcription *in vitro* (Ponticelli and Struhl, 1990; Wobbe and Struhl, 1990). In contrast, T_C does not contain a sequence that fits the TATAAA consensus, is surprisingly tolerant of mutations (Mahadevan and Struhl, 1990), does not support transcription in yeast nuclear extracts (Ponticelli and Struhl, 1990), and does not yield DNase I footprints with TFIID (A.S.Ponticelli and K.Struhl, unpublished results). Nevertheless, T_C-dependent transcription requires TFIID because temperature or proteolytic inactivation of TFIID *in vivo* results in an equally rapid decline in the +1 and +13 transcripts (Cormack and Struhl, 1992). Finally, micrococcal nuclease sensitivity in the *his3* TATA region is associated with T_C- but not T_R-dependent transcription (Oettinger and Struhl, 1985; Struhl, 1986).

We have begun a genetic approach to investigate the mechanism of GCN4 activation and the distinction between T_R- and T_C-mediated transcription. Starting with a strain containing a GCN4 derivative with a partially defective acidic activation domain, we have isolated unlinked suppressor mutations that increase *his3* transcription. In this study, we describe a mutation in *CDC39* that causes the novel phenotype of increasing *his3* transcription from the +1 site while hardly affecting initiation from the +13 site. *CDC39* was originally identified by a temperature-sensitive mutation that arrests cells in G_1 and inappropriately activates the pheromone response pathway (Reed, 1980; de Barros Lopes *et al.*, 1990). However, molecular and genetic analyses presented here suggest that *CDC39* is not directly involved in cell-cycle control, but rather is a general negative regulator of transcription that differentially affects the constitutive and inducible *his3* promoters.

Results

Isolation of a temperature-sensitive suppressor mutation

To obtain suppressor mutations that increase activation by GCN4 derivatives with a partially defective activation domain, we utilized a genetic approach based on the fact that the degree of GCN4 function is correlated with cell growth in medium containing aminotriazole (AT), a competitive inhibitor of the *his3* gene product (Hope and Struhl, 1986). The starting strain, KY1600, contains a chromosomal deletion of *GCN4*, a plasmid expressing the gcn4-C163 derivative (amino acids 119-281) from the

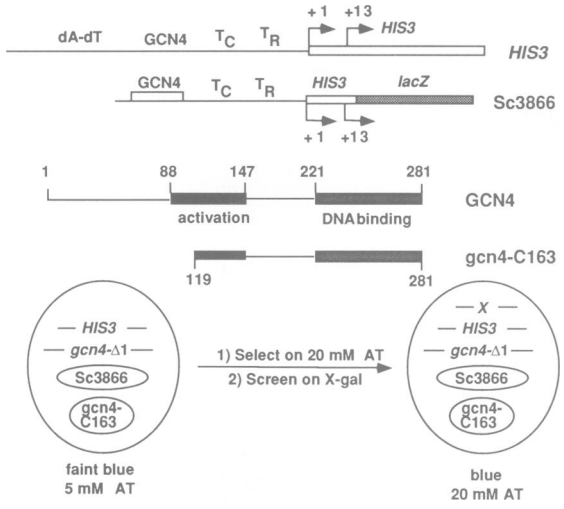


Fig. 1. Genetic selection. The structures of the *HIS3* and *his3−lacZ* reporter genes (promoter elements and transcriptional initiation sites indicated) and the GCN4 and gcn4-C163 proteins (transcriptional activation and DNA binding domains indicated) are shown. The parental strain (relevant chromosomal alleles indicated as lines and plasmid alleles indicated as circles) was subjected to a two-step genetic selection and screen to obtain unlinked suppressor mutations (X).

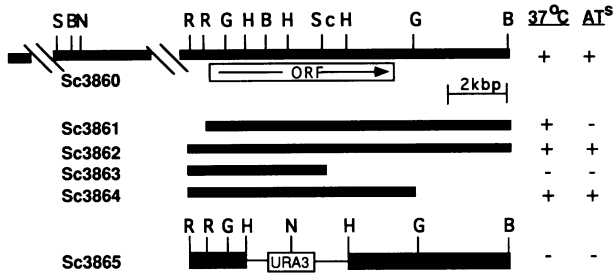


Fig. 2. The *CDC39* locus. The structures and phenotypes (determined by complementation) of the indicated DNA fragments are shown below the restriction map containing the *CDC39* gene region (broken lines shown for Sc3860 indicate uncertainty in the lengths). The location and transcriptional direction of *CDC39* are indicated by the open box and arrow. S, *Sal*I; B, *Bam*HI; N, *Nco*I; R, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; Sc, *Sac*I.

constitutive *DED1* promoter (Hope and Struhl, 1986), and a reporter plasmid in which a *his3−lacZ* fusion is controlled by a promoter with one optimal GCN4 binding site (Figure 1). KY1600 grows on 5 mM AT and is faintly blue on X-gal indicator plates. By selecting for derivatives of KY1600 that grow on 20 mM AT and are darker blue, we isolated 13 independent suppressor mutations.

Genetic analysis indicated that the suppressor mutations are recessive and define three complementation groups. One of these complementation groups is defined by a single mutation that also prevents growth at 37°C on rich medium. Diploids between the mutant and wild-type strains grow at 37°C, indicating that the temperature-sensitive growth phenotype is also recessive. The temperature-sensitive and AT-resistant phenotypes cosegregate 2:2 in tetrads, indicating that a single suppressor mutation confers both phenotypes. The suppressor mutation does not seem to confer any phenotype in the absence of GCN4, as the mutant and parental strains grow indistinguishably on 2 mM AT.

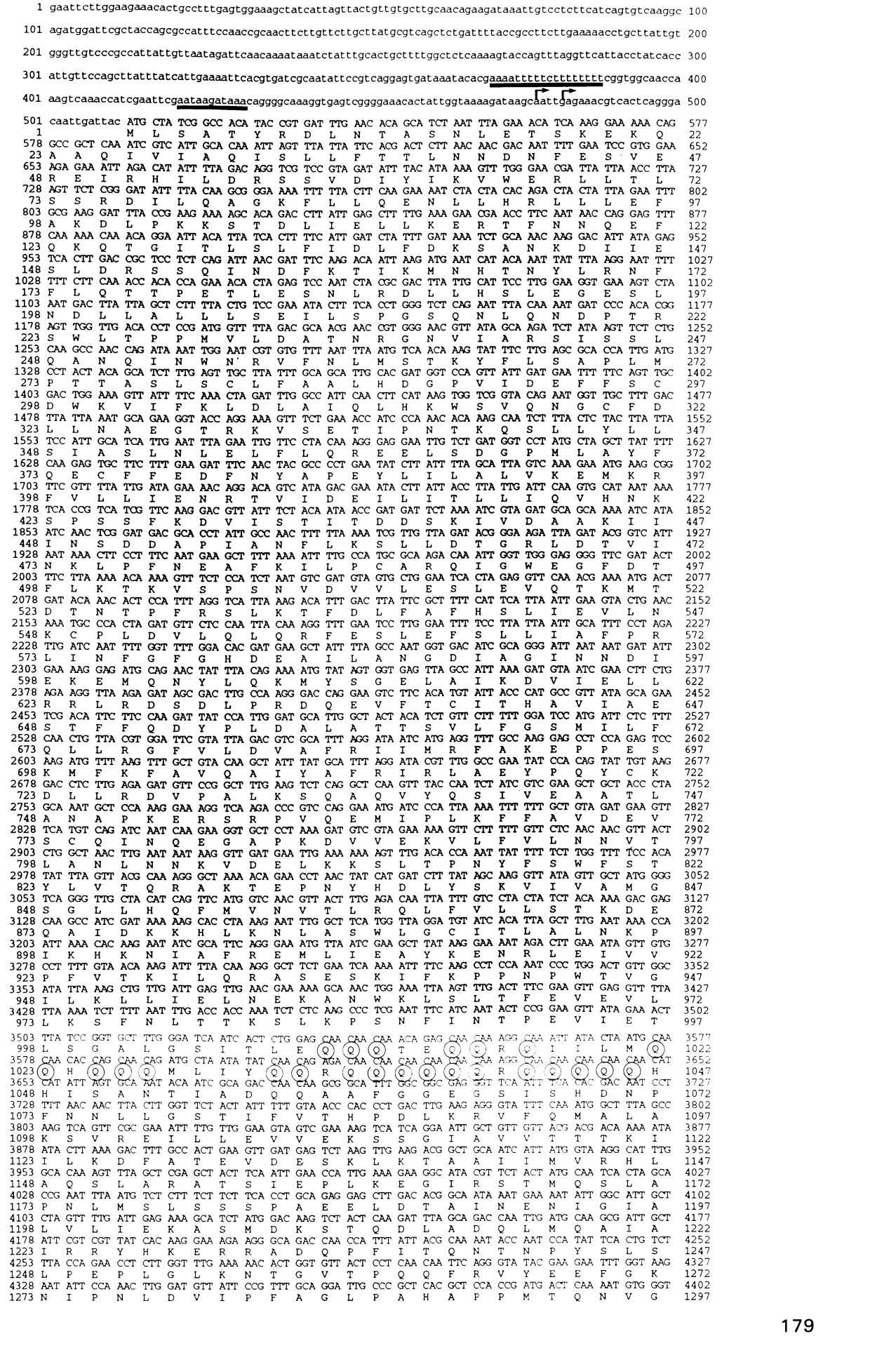
Cloning and molecular analysis of CDC39

The gene corresponding to the suppressor mutation was cloned by complementation of the temperature-sensitive and AT-resistant phenotypes. A plasmid containing 20 kb of yeast DNA (Sc3860) was recovered from one such transformant and shown to complement both phenotypes conferred by the suppressor mutation. Genomic integration and genetic mapping indicate that Sc3860 is tightly linked to the suppressor mutation. By phenotypically testing restriction fragments, the complementing gene was delimited to a 7.4 kb region (Figure 2).

The nucleotide sequence of the *Eco*RI−*Bgl*II fragment (Sc3864) encompassing the suppressor gene reveals a single significant open reading frame of 2108 amino acids (Figure 3). Replacement of 3276 bp of coding sequence by the *URA3* gene (Figure 2) eliminates complementation of both phenotypes, indicating the functional importance of the putative protein. Despite its very large size, the putative protein shows no significant similarities to proteins in the NBRF database. However, the sequence in the regions between amino acids 1009−1046 and 1300−1329 respectively contain 63 and 52% glutamine residues.

Genomic hybridization indicates that the suppressor gene is present in single copy and that patterns observed for the wild-type and suppressor strains are indistinguishable (Figure 4A). Hybridization of this same probe to a collection of λ bacteriophages covering most of the yeast genome (Olson *et al.*, 1986) reveals that the suppressor gene maps close to the genetically defined *CDC39* locus on the right arm of chromosome III (L.Riles, personal communication). *CDC39* was initially identified by a temperature-sensitive mutation (*cdc39*-1) that causes cell-cycle arrest in G_1, pachytene arrest and other effects in meiosis, and constitutively activates the pheromone response pathway (Reed, 1980; Shuster and Byers, 1989; de Barros Lopes *et al.*, 1990). Sequence comparisons show that our suppressor gene and *CDC39* are identical (S.I.Reed, personal communication) and correspond to YCR93W of chromosome III (Oliver, 1992). Moreover, *cdc39*-1/*cdc39*-2 heterozygotes do not grow at high temperature indicating that the alleles fail to complement. Thus, we now refer to our suppressor mutation as *cdc39*-2 and the corresponding gene as *CDC39*.

In parental and *cdc39*-2 strains, *CDC39* is expressed at equivalent levels as a single 7 kb RNA (Figure 4B). This transcript is long enough to encompass the putative *CDC39* protein of 2108 residues. The 5′ end of this RNA was mapped by primer extension analysis to two initiation sites, a major one 30 and a minor one 34 nucleotides upstream of the first ATG of the putative *CDC39* protein (Figure 4C). An AT-rich region containing the sequence GATAAA is centered 51 bp upstream of the RNA start sites and is likely to serve as the TATA element for *CDC39* transcription. About 50 bp upstream of this putative TATA element is a poly(dA)·poly(dT) stretch, such as is often found in yeast promoters and utilized as an upstream promoter element (Struhl, 1985). Interestingly, Sc3861 (Figure 2), a DNA fragment that contains the putative TATA element and entire protein coding region but lacks the poly(dA)·poly(dT) stretch and sequences further upstream, complements the temperature-sensitive but not the AT-resistant phenotype. This suggests that both elements are required for full *CDC39* expression and that lower levels of CDC39 are sufficient for viability but not for suppression.



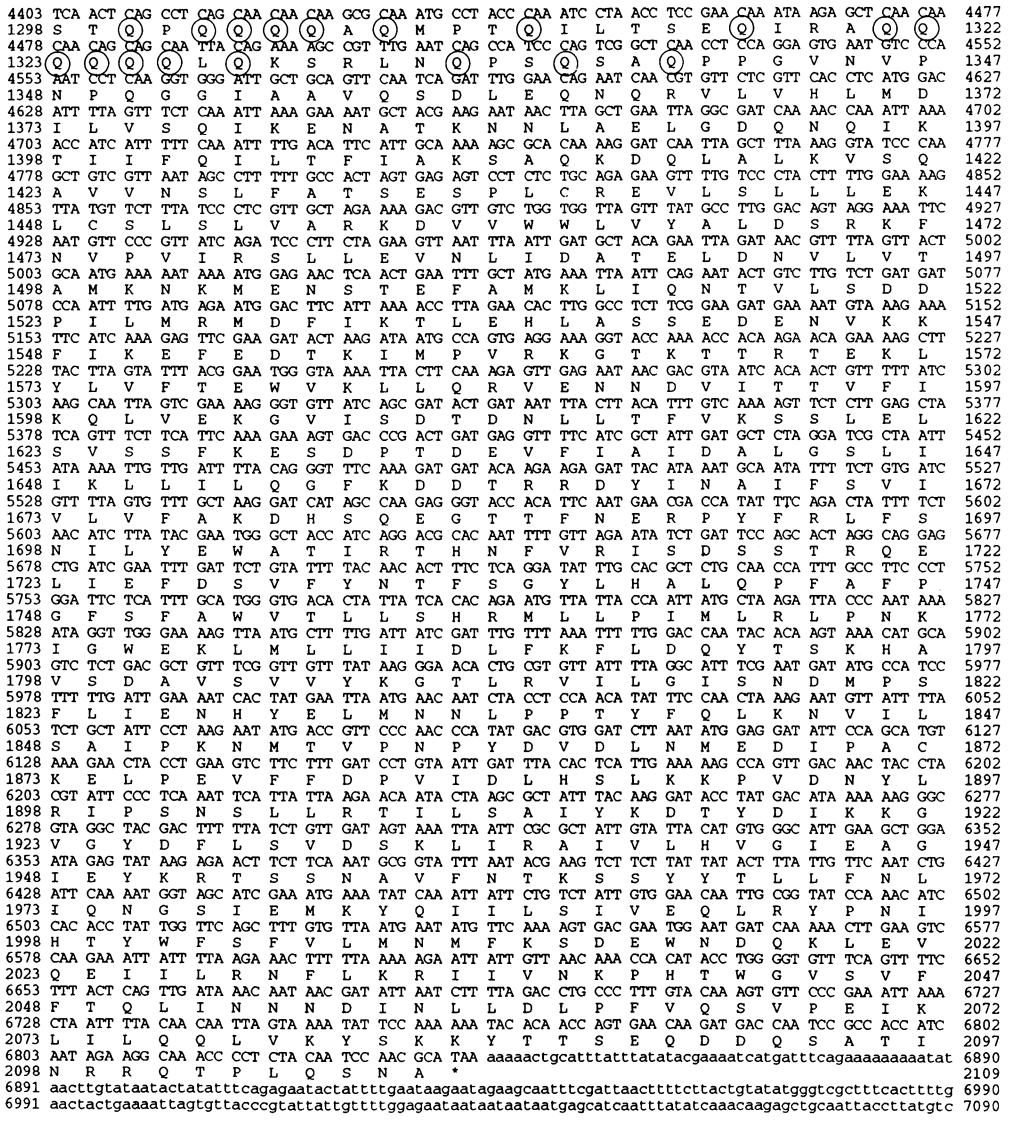


Fig. 3. *CDC39* sequence. The nucleotide sequence of Sc3864 and the putative amino acid sequence of CDC39 is shown with the two major RNA start sites (arrows), potential promoter elements (underlined) and glutamine-rich region (glutamine residues circled).

CDC39 is essential for viability

Strains containing either the original (*cdc39*-1) or present (*cdc39*-2) allele are unable to grow at 37°C, suggesting that *CDC39* is an essential gene. To determine the phenotype of a *cdc39* null mutation, we disrupted the *CDC39* locus of a diploid strain (KY805) with the *cdc39*::*URA3* substitutution allele described above. Genomic hybridization confirms that the resulting strain (KY1640) is heterozygous for the wild-type and disrupted *CDC39* alleles (Figure 4A). RNA analysis of the heterozygous diploid shows that the disrupted copy of *CDC39* is expressed as two small transcripts ending in the *URA3* inserted gene (Figure 4B). Sporulation and dissection of KY1640 resulted in tetrads with two viable and two inviable progeny. All viable progeny were Ura$^-$ indicating the presence of the *CDC39* allele and the absence of the *cdc39*::*URA3* allele. Microscopic examination indicated that the inviable spores had germinated and yielded microcolonies consisting of 10−20 normal looking cells. Thus, *CDC39* encodes an essential gene required for cell growth.

Suppression is independent of the mating pheromone response pathway

The initial *cdc39*-1 allele constitutively activates the mating pheromone response pathway by acting at the level of the transducing G protein (de Barros Lopes *et al.*, 1990). It was deduced that the cell-cycle defect was a consequence rather than a cause of the pheromone pathway induction. Although our *cdc39*-2 mutant strain does not exhibit a G_1 arrest phenotype at the restrictive temperature but arrests asynchronously, it remained possible that the *cdc39*-2 mutation affected the pheromone response pathway and indirectly caused the suppression phenotype.

To address this issue, we constructed *ste4*Δ::*URA3* and *ste12*Δ::*URA3* derivatives of KY1603 that lack a functional pheromone response pathway. These strains do not mate because *STE4*, the β subunit of the transducing G protein (Whiteway *et al.*, 1989) and *STE12*, a pheromone-responsive transcription factor (Dolan *et al.*, 1989; Errede and Ammerer, 1989), are essential components of the pheromone response pathway. However, these strains grow as well on

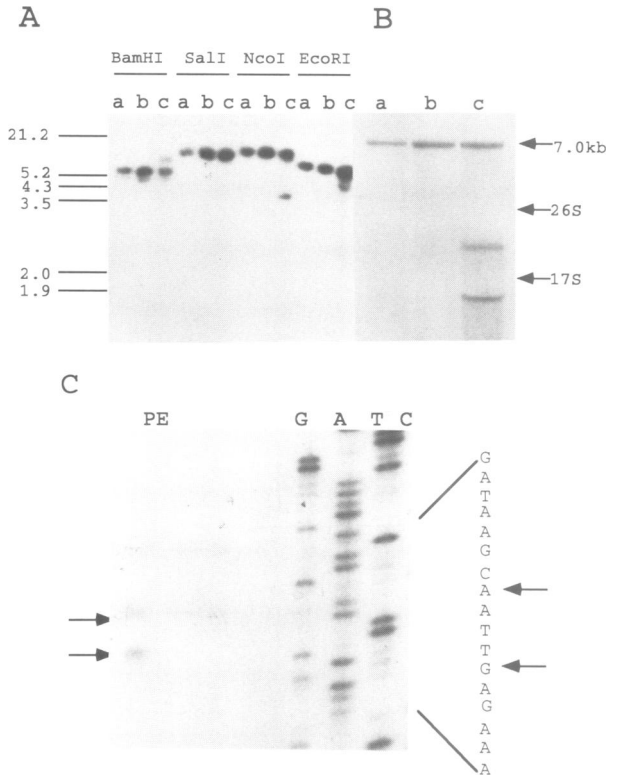


Fig. 4. Genomic and transcriptional analysis of *CDC39*, *cdc39*-2 and *CDC39/cdc39*::*URA3* strains. (**A**) Genomic DNAs from the wild-type KY803 (a), mutant KY1617 (b), and heterozygous disruption KY1640 (c) strains were hybridized to a radiolabeled *CDC39* probe. The positions of molecular weight markers are shown on the left. The *CDC39* and *cdc39*-2 alleles should have restriction fragments of the following sizes: *Bam*HI, 6 kb; *Sal*I, 20 kb; *Nco*I, 20 kb; *Eco*RI, 10 kb. The fragment sizes of the *cdc39*::*URA3* allele should be as follows: *Bam*HI, 11 kb because a site is lost; *Sal*I, 18 kb; *Nco*I, 4 kb and 17 kb because of a site in *URA3*; *Eco*RI, 8 kb. (**B**) Total RNAs from the same strains were electrophoretically separated, and hybridized to the *CDC39* probe. The positions of the 26S, 17S rRNAs and 7 kb CDC39 RNA are indicated on the right. (**C**) Primer extension product (PE), generated by extending 5′-end-labeled *CDC39* oligonucleotide primer along RNA from KY803, compared with products of DNA sequencing using the same primer. Arrows indicate the 5′ end of the CDC39 RNA.

40 mM AT as KY1603, indicating that *cdc39*-2 suppresses the gcn4-C163 activation mutant independently of the pheromone response pathway. Conversely, the parental *CDC39* strain and its *ste12*Δ::*URA3* or *ste4*Δ::*URA3* derivatives have indistinguishable AT-resistance phenotypes indicating that elimination of the mating response pathway does not lead to suppression.

Effect of cdc39-2 on transcriptional activation by various GCN4 derivatives

In order to test whether suppression by *cdc39*-2 was specific to the gcn4-C163 derivative used for its isolation, we introduced a number of different GCN4 derivatives into wild-type and *cdc39*-2 strains and tested AT resistance of the resulting transformants. As shown in Table I, *cdc39*-2 increases AT resistance in strains containing gcn4-Δ34 and gcn4-Δ44, partially functional derivatives with different portions of the activation domain. In contrast, *cdc39*-2 does not appear to increase transcription in the strain containing gcn4-Δ52, a derivative that lacks a functional transcriptional activation region.

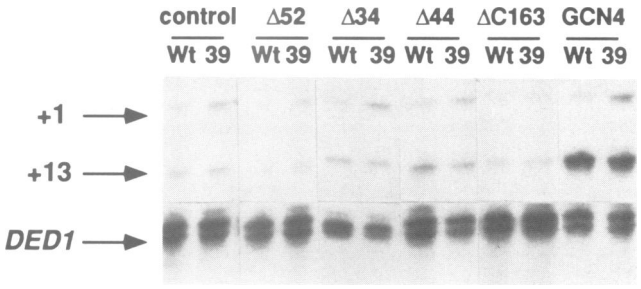


Fig. 5. Transcriptional activation by the indicated GCN4 derivatives in wild-type and *cdc39*-2 strains grown at the permissive temperature. Total RNA samples (25 μg) were hybridized to completion with an excess of *HIS3* and *DED1* oligonucleotide probes and digested with S1 nuclease. The positions of the *DED1* and the *HIS3* +1 and +13 transcripts are indicated. As described previously (Hope and Struhl, 1986; Hope *et al.*, 1988), gcn4-52 contains residues 107–121 fused to 179–281, gcn4-Δ34 contains residues 1–95 fused to 144–281, gcn4-Δ44 contains residues 107–137 fused to 179–183, and gcn4-C163 residues 119–281. Hybridization reactions were carried out in parallel, and all products were analyzed on the same gel; however, the autoradiographic exposure times for detecting the various transcripts were not equivalent.

Table I. Effect of *cdc39*-2 on AT resistance and *HIS3* transcript levels in strains containing various GCN4 derivatives

Genotype[a]		Growth[b]	*HIS3* transcription[c] +1	+13	ratio +1/+13
gcn4-Δ1	*CDC39*	<2	20	21	0.95
gcn4-Δ1	*cdc39*-2	<2	21	22	0.95
gcn4-Δ52[d]	*CDC39*	<2	22	23	0.95
gcn4-Δ52[d]	*cdc39*-2	<2	19	20	0.95
gcn4-Δ34[d]	*CDC39*	2	19	27	0.70
gcn4-Δ34[d]	*cdc39*-2	10	23	29	0.79
gcn4-Δ44[d]	*CDC39*	5	20	26	0.77
gcn4-Δ44[d]	*cdc39*-2	40	25	29	0.86
gcn4-C163	*CDC39*	5	19	28	0.68
gcn4-C163	*cdc39*-2	40	26	32	0.81
GCN4	*CDC39*	>120	19	60	0.32
GCN4	*cdc39*-2	>120	30	63	0.48

[a]Strains contain a chromosomal *gcn4*-Δ1 allele (lacks GCN4 coding sequences) and a plasmid expressing the indicated (or no) *gcn4* derivative.
[b]Maximal AT concentration (mM) that supports cell growth.
[c]Levels of +1 and +13 transcripts (arbitrary units) normalized to the level of the *DED1* internal control in the same hybridization reaction.
[d]Described by Hope *et al.* (1988).

To examine directly the effect of *cdc39*-2 on GCN4-activated *HIS3* transcription, the levels of the +1 and +13 transcripts were quantitated with respect to the internal *DED1* control (Figure 5; Table I). As expected, transcriptional activation by GCN4 and partially functional derivatives in wild-type strains increases transcription from the +13 site but not the +1 site. In accord with the growth phenotypes, *cdc39*-2 slightly increases overall *HIS3* transcription in strains containing GCN4 or derivatives capable of partial activation (Δ34, Δ44, C163), but not in strains containing the non-activating derivative gcn4-Δ52. Surprisingly, the

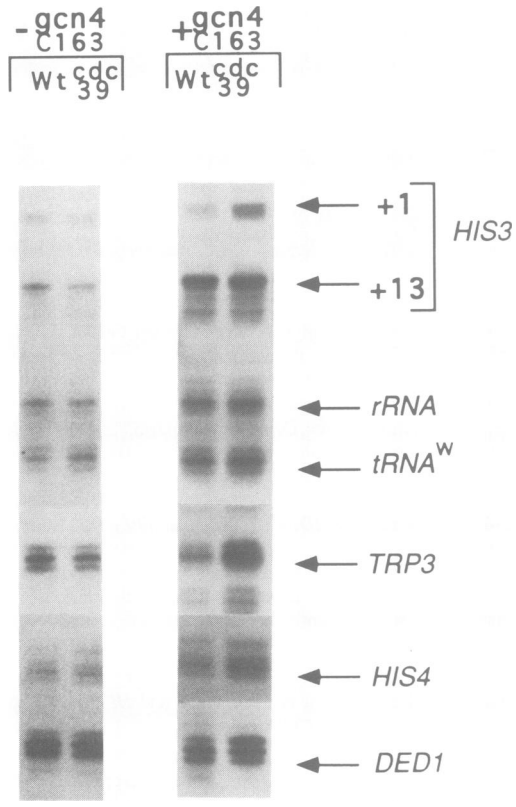


Fig. 6. Transcription of various genes in wild-type and *cdc39*-2 strains grown at the permissive temperature. Total RNA samples (25 μg) were hybridized to completion with an excess of the indicated probes and digested with S1 nuclease. Hybridization reactions were carried out in parallel, and all products were analyzed on the same gel; however, the autoradiographic exposure times for detecting the various transcripts were not equivalent.

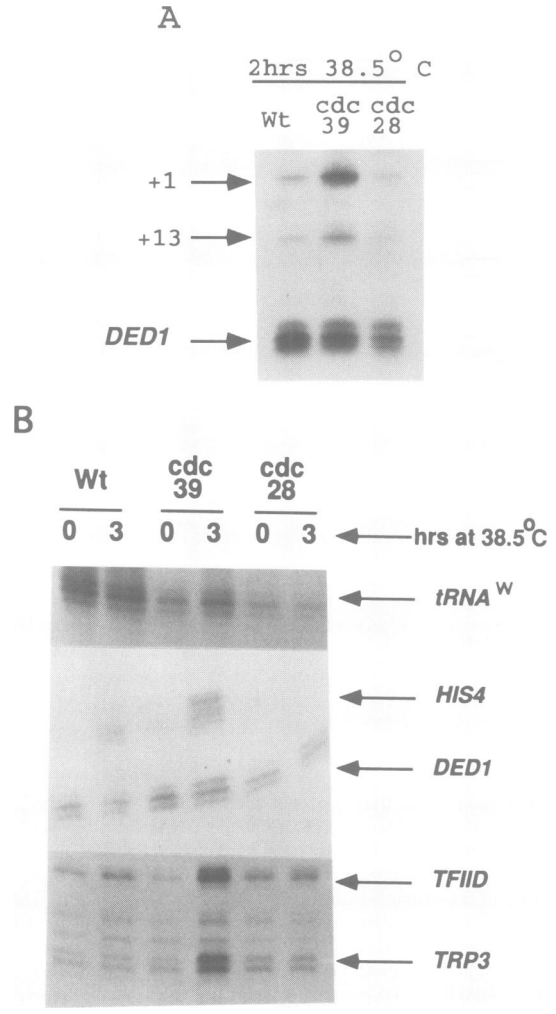


Fig. 7. Transcription of various genes in wild-type, *cdc39*-2 and *cdc28* strains after shifting to the restrictive temperature. Total RNA samples (25 μg) were hybridized to completion with an excess of the indicated probes and digested with S1 nuclease. Hybridization reactions were carried out in parallel, and all products were analyzed on the same gel; however, the autoradiographic exposure times for detecting the various transcripts were not equivalent. All strains are deleted for the chromosomal *gcn4* locus.

effect of *cdc39*-2 on GCN4-dependent activation is more pronounced on the +1 initiation site. Indeed, *cdc39*-2 causes stronger activators to produce more +1 transcripts, unlike the situation in wild-type strains where +1 transcription is unaffected by GCN4. Thus, suppression by *cdc39*-2 appears to require an acidic activation region, but is not specific for particular *GCN4* proteins.

Effect of cdc39-2 on transcription of other genes

The small effects of *cdc39*-2 on *his3* transcription are unlikely to account fully for the more significant differences in AT resistance. However, AT resistance is determined not only by *his3* enzyme levels but also by flux through the histidine biosynthetic pathway and by *ATR1* (Kanazawa *et al.*, 1988), suggesting that *cdc39*-2 might increase transcription of other GCN4-activated genes. We therefore examined RNA levels of several genes in *cdc39*-2 and wild-type strains containing a control or a gcn4-C163 expressing plasmid (Figure 6). In the presence of gcn4-C163, GCN4-regulated genes (*HIS3*, *TRP3* and probably *HIS4*) show increased transcription in the *cdc39*-2 background. In contrast, *cdc39*-2 does not affect the basal level of these genes, nor does it affect the transcription of unrelated genes such as *DED1*, ribosomal RNA and tryptophan tRNA. These results suggest that *cdc39*-2 increases activation by gcn4-C163 but does not affect basal transcription.

To determine whether *cdc39*-2 also increases transcription stimulated by different activator proteins, *lacZ* reporter plasmids under the control of the *GAL1* or *PHO5* promoter region were introduced into mutant and wild-type strains (Table II). Transcriptional induction of the *GAL1* promoter in galactose medium, which is mediated by the GAL4 activator, is increased ~3-fold in the *cdc39*-2 strain. In glycerol/ethanol medium, *cdc39*-2 appears to have a minimal effect although the very low activity of the *GAL1* promoter makes such a determination difficult. In contrast, *PHO5* transcription is unaffected by *cdc39*-2 either in normal conditions or in medium depleted for inorganic phosphate, which results in activation by PHO2 and PHO4. Taken together, these results suggest that *cdc39*-2 does not affect basal transcription, whereas it can increase transcriptional activation of some, but not all genes.

Transcription in cdc39-2 strains shifted to the restrictive temperature

The results presented so far describe transcriptional alterations in growing *cdc39*-2 cells, conditions where

Table II. Effect of *cdc39*-2 on expression of *GAL1*−*lacZ* and *PHO5*−*lacZ*[a]

	GAL1−*lacZ*[b]			*PHO5*−*lacZ*[c]	
Genotype	Glu	Gly+Eth	Gly+Eth+Gal	$+P_i$	$-P_i$
CDC39	<0.01	0.1	80	8	95
cdc39-2	<0.01	0.15	270	5	97

[a]β-galactosidase activities (Miller units).
[b]Cells grown in 2% of the indicated carbon sources (glucose, glycerol, ethanol, galactose).
[c]Cells grown in the phosphate-depleted medium in the presence or absence of inorganic phosphate.

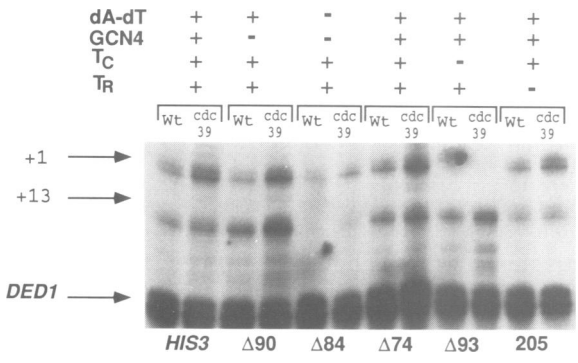


Fig. 8. Effect of loss of *CDC39* function on transcription from various *HIS3* promoters. Wild-type and *cdc39*-2 strains (deleted for *GCN4*) containing the indicated *his3* alleles were shifted for 2 h to the restrictive temperature, and total RNAs were hybridized to completion with an excess of *HIS3* and *DED1* probes and digested with S1 nuclease. As described previously (Struhl and Hill, 1987; Harbury and Struhl, 1989; Mahadevan and Struhl, 1990), *his3*-Δ90 deletes the GCN4 binding site (−109 to −80), *his3*-Δ84 deletes all sequences upstream of T_C (−447 to −94), *his3*-Δ−74 retains all known promoter elements but lacks the region between −447 and −156, *his3*-Δ93 deletes T_C (−83 to −46) and *his3*-205 has a point mutation (TGTAAA) in T_R.

CDC39 function has not been completely eliminated. We investigated the role of the wild-type *CDC39* more definitively by analyzing transcription of several genes after shifting *cdc39*-2 cells to the restrictive temperature (38.5°C). To distinguish direct effects of *CDC39* inactivation from indirect effects of growth arrest, we also analyzed a strain with a temperature-sensitive mutation in *CDC28*, a gene encoding the cyclin-associated protein kinase that mediates entry into the cell cycle. These temperature-shift experiments were carried out in strains deleted for GCN4, and hence measure basal (i.e. non-GCN4 activated) transcription.

A strong increase in *HIS3* transcription, predominantly from the +1 initiation site, is observed in the *cdc39*-2 strain but not in the wild-type or *cdc28* strain (Figure 7). This effect occurs within 30 min at 38.5°C (or within 1−2 h at 37°C) and is maintained for at least 4 h (not shown). Similarly, basal transcription of *HIS4*, *TRP3* and *TFIID* is increased specifically in the temperature-shifted *cdc39*-2 strain. In contrast, *DED1* transcript levels in the three strains are indistinguishable at all time points indicating that *CDC39* is not essential for transcription by RNA polymerase II. The levels of rRNA (not shown) and tryptophan tRNA are similar in the *cdc39* and *cdc28* strains; they are decreased relative to the wild-type strain, presumably because the cell growth is inhibited. These results confirm the preferential effects on initiation from the *HIS3* +1 site, and they demonstrate that *CDC39* negatively regulates basal transcription of *HIS3* and other genes. However, it appears that GCN4-activated transcription is more sensitive to *CDC39* function than basal transcription because it is preferentially affected when *cdc39*-2 cells are grown at the permissive temperature.

Effect of cdc39-2 on various his3 promoter derivatives

To determine which element(s) in the *HIS3* promoter region is responsible for negative regulation by *CDC39*, we assayed AT-resistance (not shown) and basal *HIS3* transcription in *cdc39*-2 and wild-type strains containing several *his3* promoter alleles (Figure 8). In all cases, AT resistance and *HIS3* transcription are higher in the *cdc39*-2 strain, although the magnitude of the increase varies depending on the allele. Deletion of the poly(dA)·poly(dT) sequence and/or GCN4 binding site does not significantly reduce the magnitude of the *cdc39*-2 effect, indicating that *HIS3* promoter sequences upstream of the TATA region are not required for negative regulation by *CDC39*. The *cdc39*-2 effect appears somewhat reduced in strains containing either a deletion of T_C or a point mutation in T_R (TGTAAA); as expected, the increase in +1 transcription depends on T_C whereas the increase in +13 transcription depends on T_R. This suggests that *CDC39* does not affect the inherent properties of the +1 and +13 initiator elements, but rather negatively regulates transcription mediated by both T_C and T_R. However, in the wild-type *HIS3* promoter, loss of *CDC39* function leads to a preferential increase in +1 transcription. This suggests that when both *HIS3* TATA elements are present, *CDC39* affects T_C-dependent transcription more strongly than T_R-dependent transcription.

CDC39 is a nuclear protein

To determine the cellular localization of CDC39 protein, we expressed CDC39, with or without an N-terminal tag (the flu epitope), from the constitutive *DED1* promoter. Expression of either protein complemented both the temperature-sensitive and AT-resistant phenotypes of the *cdc39*-2 mutation. Proteins from total cellular, cytoplasmic and nuclear extracts were electrophoretically separated and then probed with antibodies to the flu epitope (Figure 9). A protein migrating with an apparent molecular weight of ~200 kDa is detected in total and nuclear extracts of transformants containing the flu-tagged CDC39 only, indicating that CDC39 is a nuclear protein.

Discussion

CDC39 is a transcriptional regulatory protein

In this work, we isolated a temperature-sensitive mutation in *CDC39* by selecting for yeast strains that increase *HIS3* transcription by a GCN4 derivative with a defective activation domain. *CDC39* was originally identified by a temperature-sensitive mutation that causes cell-cycle arrest in G_1 and pachytene arrest in meiosis, and inappropriately activates the mating pheromone response (Reed, 1980; Shuster and Byers, 1989; de Barros Lopes *et al.*, 1990). Many of the phenotypes conferred by the original *cdc39*-1 mutation, including transcriptional induction of pheromone

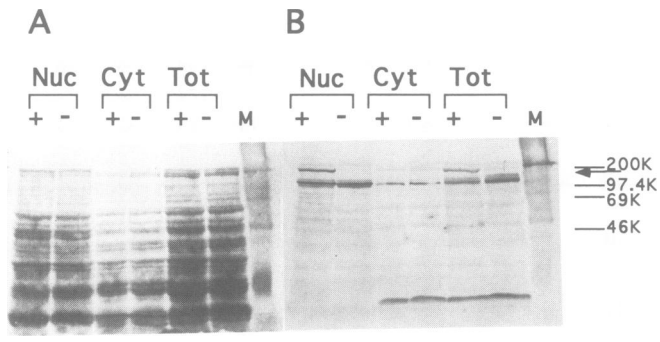


Fig. 9. Subcellular localization of CDC39. Protein (50 μg) from total (Tot), nuclear (Nuc) and cytoplasmic (Cyt) extracts prepared from strains expressing CDC39 with (+) or without (−) the flu epitope tag were analyzed by SDS−PAGE and assayed (**A**) for protein by Ponceau staining and (**B**) for epitope-tagged CDC39 by Western blotting using specific antibodies to the epitope. The positions corresponding to molecular weight markers and the tagged CDC39 protein (arrow) are indicated.

responsive genes (*FUS1*, *GPA1*, *STE4*), were interpreted as occurring as a consequence of activating the pheromone response (de Barros Lopes *et al.*, 1990). It was hypothesized that *CDC39*, in concert with other genes (*CDC36*, *CDC72*, *CDC73* and *SRM1*) that when mutated confer the same phenotypes (Reed *et al.*, 1988; Clark and Sprague, 1989), might regulate the activity of G proteins in the mating pheromone response and other signaling pathways (de Barros Lopes *et al.*, 1990).

Several lines of evidence presented here strongly argue that *CDC39* is primarily a transcriptional regulatory protein. First, the *cdc39*-2 mutation described here increases transcription of many unrelated genes that are not involved in the pheromone response pathway. Although the quantitative effects on individual genes are often modest, the phenotypic consequences, which depend on the activity of many genes, are marked. Second, *cdc39*-2 strains arrest asynchronously in the cell cycle, and their transcriptional effects (assayed by AT resistance) occur even when the pheromone response pathway is inactivated. In this regard, it was previously noted that *CDC39* function is essential even when the pheromone response pathway is inactivated by mutation or in **a**/α diploids (de Barros Lopes *et al.*, 1990). Third, loss of *CDC39* function affects T_C-dependent transcription more strongly than T_R-dependent transcription of the wild-type *HIS3* gene, thus leading to a preferential increase in initiation from the +1 site. Fourth, *CDC39* encodes a nuclear protein. For these reasons, we suggest that the cell-cycle and pheromone response phenotypes caused by the original *cdc39*-1 mutation arise indirectly as a consequence of increased transcription of genes responsible for these functions.

CDC39 negatively regulates basal and activated transcription of many genes

The basic observation that *cdc39*-2, a recessive and hence presumably loss-of-function mutation, causes increased RNA levels formally defines *CDC39* as a transcriptional inhibitory protein. At the restrictive temperature, *cdc39*-2 increases the basal (i.e. non-GCN4-activated) transcription of *HIS3*, *HIS4*, *TRP3* and *TFIID*. We presume, but have not tested, that GCN4-activated transcription of *HIS3*, *HIS4*, *TRP3* is also increased in the absence of *CDC39* function. However, *DED1* transcription appears unaffected, suggesting that negative regulation by *CDC39* is not universal to all genes transcribed by RNA polymerase II. In addition, synthesis of tryptophan tRNA by RNA polymerase III and rRNA by RNA polymerase I (not shown) are not significantly affected.

At the permissive temperature, *cdc39*-2 increases GCN4-activated, but not basal, transcription of *HIS3*, *HIS4* and *TRP3*. Moreover, *cdc39*-2 affects activation by GAL4 and by GCN4 derivatives with different portions of the acidic activation region; however, it does not increase PHO2/PHO4-mediated activation of *PHO5*. Under these conditions, it seems likely that the cdc39-2 protein retains sufficient function to repress basal transcription but not activation by GCN4 derivatives and GAL4. In this regard, Sc3861, a fragment that lacks sequences upstream of the putative TATA element and presumably expresses less CDC39 protein, permits growth at high temperature but does not negatively regulate GCN4-activated transcription (assayed by AT resistance). Although we cannot exclude the possibility that repression of basal and activated transcription involves distinct functions, the simplest interpretation of these results is that negative regulation of GCN4 (and GAL4) activation requires more of a common *CDC39* function than negative regulation of basal transcription.

Yeast cells contain a number of negative regulatory proteins that affect transcription of many genes such as the HMG1-like protein SIN1 (Kruger and Herskowitz, 1991), SIN3 (Wang *et al.*, 1990; Vidal *et al.*, 1991), the putative helicase MOT1 (Davis *et al.*, 1992) and the SSN6−TUP1 complex (Schultz *et al.*, 1990; Williams and Trumbly, 1990; Williams *et al.*, 1991). These proteins may function by distinct molecular mechanisms. It has been suggested that SIN1 functions through the C-terminal tail of the largest subunit of RNA polymerase II (Peterson *et al.*, 1991) and that the SSN6−TUP1 complex functions by directly interacting with specific DNA binding proteins (Keleher *et al.*, 1992). CDC39 may be functionally related to MOT1 in that both proteins are essential for cell growth and constitutively activate the pheromone response pathway. Given the similarities of G_1-arrest and mating response phenotypes in mutant strains, it is also possible that CDC36, CDC72, CDC73 and SRM1 (Reed *et al.*, 1988; Clark and Sprague, 1989; de Barros Lopes *et al.*, 1990) might also be negative regulators of transcription.

Potential molecular mechanisms

There are two general classes of model that can explain how *CDC39* negatively regulates the basal and acidic activator-mediated transcription of many unrelated genes. In one

model, *CDC39* is involved in maintaining the normal chromatin structure that is highly repressive for transcription. Indeed, mutations that reduce or eliminate the function of histones result in increased transcription of many genes (Clark-Adams *et al.*, 1988; Han and Grunstein, 1988; Han *et al.*, 1988; Durrin *et al.*, 1992). In the alternative model, *CDC39* inhibits the activity of a general component of the RNA polymerase II transcription machinery such as TFIID, TFIIB, TFIIA or the enzyme itself. Such inhibition might be mediated either by a direct interaction to such a component(s) or indirectly through intermediary proteins. In this regard, the human Dr, NC1 and NC2 factors interact with TFIID and repress basal and activated transcription *in vitro* (Meisterernst and Roeder, 1991; Inostroza *et al.*, 1992). Either of these two classes of explanation are compatible with non-equivalent effects of *CDC39* on different genes or with the distinction between basal and activated transcription observed at the permissive temperature.

The most striking and mechanistically informative observation about CDC39 is its preferential repression of T_C-mediated transcription from the wild-type *HIS3* promoter. The stronger increase in +1 over +13 transcription is novel, having never been observed in the wide variety of *his3* promoter derivatives analyzed over the past decade. The *CDC39* effect is clearly distinct from transcriptional induction by acidic activator proteins which is mediated almost exclusively through T_R and results in preferential initiation from +13 (Struhl, 1986); hence loss of *CDC39* function cannot lead to increased transcription by unmasking the potential of acidic activator proteins. Consistent with this view, GCN4 derivatives with increasingly strong activation regions stimulate increasingly more transcription from +13, whereas *cdc39*-2 strains show GCN4-dependent activation from the +1 site. Although T_C does not normally respond to transcriptional activators (Struhl, 1986; Ponticelli and Struhl, 1990), the present results suggest that this might not reflect an inherent defect of T_C but rather inhibition by proteins such as CDC39.

In considering the two classes of model for CDC39 function, we disfavor the view that CDC39 is involved in maintaining chromatin in its normally repressive state. Although T_R and T_C support comparable levels of basal *HIS3* TFIID-dependent transcription *in vivo* (Cormack and Struhl, 1992), only T_R efficiently binds TFIID and supports TFIID-dependent transcription *in vitro* (Ponticelli and Struhl, 1990). Thus, if loss of *CDC39* activity alleviated nucleosome repression, one might expect to observe transcriptional patterns resembling those obtained on naked DNA templates *in vitro*. Indeed, nucleosome loss *in vivo*, obtained by repressing histone H4 mRNA synthesis, leads to increased *HIS3* transcription that is almost exclusively mediated through T_R (Durrin *et al.*, 1992). In contrast, *CDC39* preferentially mediates its repressive effects through T_C. We therefore favor the idea that CDC39 directly or indirectly inhibits a component(s) of the general transcription machinery. TFIID is an attractive candidate for such a component because of the functional distinctions between T_R and T_C (Struhl, 1986; Mahadevan and Struhl, 1990; Ponticelli and Struhl, 1990) and because of the numerous proteins with which TFIID physically interacts (Dynlacht *et al.*, 1991; Lee *et al.*, 1991; Lieberman and Berk, 1991; Meisterernst and Roeder, 1991; Timmers and Sharp, 1991; Comai *et al.*, 1992; Eisenmann *et al.*, 1992; Inostroza *et al.*, 1992). However, CDC39 could affect other general transcription factors (e.g. TFIIA, TFIIB, RNA polymerase II) or influence chromatin structure in a manner more subtle than nucleosome disruption.

Materials and methods

Yeast strains

The starting strain used to isolate the suppressor mutations was KY1600, a derivative of KY803 (**a** *ura3*-52 *trp1*-Δ1 *leu2-PET56 gal2 gcn4*-Δ1; Hope and Struhl, 1986) that carries YCp88-Sc4363, a *URA3* centromeric vector that expresses the gcn4-C163 derivative from the constitutive *DED1* promoter (Hope and Struhl, 1986), and YCp87-Sc3866, a *LEU2* centromeric vector containing a *his3*−*lacZ* fusion protein under the control of a promoter containing an optimal AP-1 site fused upstream of the *HIS3* TATA region (position −86). KY1603, the initial strain containing *cdc39-2*, was generated as a spontaneous revertant of KY1600 that grew on glucose minimal plates containing 20 mM aminotriazole (AT) and was blue on X-gal indicator plates. To test the effect of *cdc39*-2 on various GCN4 proteins, the plasmids were cured from KY1603 to generate strain KY1617 which were then transformed with YCp88 plasmids expressing various GCN4 derivatives (Hope and Struhl, 1986; Hope *et al.*, 1988). To determine the phenotype of loss of *CDC39* function, the *cdc39*::*URA3* substitution allele (Sc3865) was introduced by one-step gene replacement (Rothstein, 1983) into KY805, an **a**/α diploid strain generated by *HO*-mediated diploidization of KY803 (Herskowitz and Jensen, 1991). To eliminate the mating pheromone response pathway, *ste4*::*URA3* (Whiteway *et al.*, 1989) or *ste12*::*URA3* (Errede and Ammerer, 1989) substitution alleles were introduced into KY803 and KY1617 by one-step gene replacement. The resulting strains were then transformed by pRS314-Sc4363 (a *TRP1* centromeric vector expressing gcn4-C163 from the *DED1* promoter). Isogenic derivatives of KY1600 and KY1603 containing various *his3* promoter alleles were obtained by two successive gene replacement events (Struhl, 1987).

DNA manipulations

YCp87-Sc3866 was generated by substituting the *Eco*RI−*Hin*dIII fragment of Sc4099 (Hill *et al.*, 1986) for the corresponding region of Sc3802 (Singer *et al.*, 1990) in the YCp87 vector (a *LEU2* derivative of YCp86 constructed by Joan Sellers). pRS314-Sc4363 contains the *Sma*I−*Eco*RI fragment of YCp88-Sc4363 (Hope and Struhl, 1986) cloned into pRS314 (Sikorski and Hieter, 1989).

CDC39 was cloned by complementation of the *cdc39*-2 allele. Specifically, a YCp50 library containing partial *Sau*3A fragments of yeast genomic DNA (obtained from Mark Rose) was introduced into KY1617 containing pRS314-Sc4363 and screened for AT-sensitive colonies. A plasmid containing 20 kb of yeast DNA (Sc3860) was recovered from one such transformant and shown to complement both phenotypes conferred by the suppressor mutation. YCp50-Sc3861 was generated by religating the largest *Eco*RI fragment of YCp50-Sc3860. YCp50-Sc3862 was obtained by partially digesting YCp50-Sc3860 with *Eco*RI digestion and religating. Sc3863 was generated by ligating a partial *Eco*RI−*Sac*I fragment of YCp50-Sc3862 into pRS316 (Sikorski and Hieter, 1989). Sc3864 was obtained by ligating a *Sac*I−*Bgl*II fragment of Sc3861 and a *Sal*I−*Sac*I fragment of Sc3863 into pRS316. For sequencing *CDC39*, deleted derivatives of pRS316-Sc3864 were generated either by subcloning restriction fragments or by digestion with exonuclease III and S1 nuclease. Sc3865 was obtained by replacing the *Hin*dIII fragments of Sc3862 with the 1.1 kb *Hin*dIII *URA3* fragment.

To construct the plasmid expressing the flu-tagged CDC39 derivative, GCCATCGATAATGCTATCGGCCACACATACCG and CGGGGTAC-CTTCTGCATTTAATAAGTCAAAGC were used as primers to amplify *CDC39* sequences between the ATG initiation codon and the proximal *Kpn*I site. The amplified DNA was digested with *Cla*I and *Kpn*I and cloned into pBS29 (constructed by Alice Vincent), a molecule containing an *Nde*I site upstream of the flu epitope. An *Nde*I blunted *Kpn*I fragment from the resulting molecule and a *Kpn*I−*Sac*I fragment from clone Sc3863 were cloned into *Hin*dIII blunted *Sac*I cleaved YCp92, a derivative of YCp88-GCN4 (Hope and Struhl, 1986) constructed by Brendan Cormack in which the coding region was replaced by an oligonucleotide containing a *Sac*I site and stop codons in all three frames. A *Sac*I−*Eco*RI fragment containing the 3′ region of *CDC39* was then substituted for the 3′ region of *GCN4* to complete the gene encoding the desired protein. A control construct without a flu tag at the N-terminus was made by cloning an *Eco*RI blunted *Sac*I fragment from Sc3861 and a *Sac*I−*Eco*RI fragment from *CDC39* derivative containing the flu tag into *Hin*dIII blunted *Eco*RI cleaved YCp92.

Phenotypic analyses

GCN4 function was assayed by the ability of cells to grow in glucose minimal medium containing various concentrations of AT as described previously (Hope and Struhl, 1986; Hope *et al.*, 1988). The degree of AT resistance is related to the ability of GCN4 to activate transcription of *HIS3* (which encodes the enzyme that is competitively inhibited by AT), other histidine biosynthetic genes (which affect the level of the substrate utilized by the *HIS3* enzyme), and *ATR1* (which encodes a membrane associated protein that lowers intracellular AT levels) (Kanazawa *et al.*, 1988). For strains containing any particular GCN4 derivative, the degree of AT resistance is directly related to *HIS3* RNA levels (Hill *et al.*, 1986; Chen and Struhl, 1988). To assay *GAL1* expression, strains were transformed with 131 (Ma and Ptashne, 1987), a centromeric plasmid containing a *GAL1–lacZ* fusion and grown in supplemented minimal medium containing 2% glucose or 2% each glycerol and ethanol ± 2% galactose. *PHO5* expression was assayed by transforming cells with pMH313 (Han and Grunstein, 1988), a high copy *PHO5–lacZ* fusion plasmid, and growing the resulting strains in supplemented glucose minimal medium that was or was not depleted for inorganic phosphate.

RNA analysis

For experiments carried out under permissive growth conditions, strains were grown overnight in glucose minimal medium containing 1% casamino acids but selective for plasmid maintenance, diluted to A_{600} = 0.2 in YPD, and grown for 4 h. Temperature-shift experiments were performed as described previously (Cormack and Struhl, 1992). Cells were grown in YPD at 30°C to A_{600} = 0.3, shifted to 37°C for 20 min, returned to 30°C for 1 h, and shifted again to the restrictive temperature (typically 38.5°C). Aliquots (20 ml) were taken before the first shift, before the second shift, and at various times after the second shift, and the cells were quickly pelleted and frozen at −80°C. This protocol avoids looking at the consequences of a general heat shock response, especially at the early time points (Nicolet and Craig, 1991).

Total RNA prepared from the various cultures was quantitated by absorbance at 260 nm and by ethidium bromide staining of 17S and 26S RNAs after electrophoretic separation. To quantitate RNA levels of individual genes, 30 mg of total RNA were hybridized to completion with an excess (2 ng) of the appropriate oligonucleotides, and the products were digested with S1 nuclease and electrophoretically separated (Chen *et al.*, 1987; Cormack and Struhl, 1992). The *HIS4* oligonucleotide, GGAGAATCTC-TTCATTACTCAGGCTCGAGCCATCCAAAAGTACCTGACCAAC-AAGTGCCTTAG, has not been described previously. All hybridization reactions contained multiple probes to ensure that the determinations were internally controlled. When appropriate, the levels of the +1 and +13 *HIS3* transcripts were quantitated with a phosphoimager using the constitutively expressed *DED1* RNA as an internal control. *CDC39* RNA was examined by Northern blotting using an *Eco*RI–*Bam*HI fragment (nucleotides 413–2509) as a hybridization probe. The 5′ ends of *CDC39* RNA were mapped by extending a radiolabeled primer, CACGGTATGTGGCCG-ATAGCATG, on total RNA and comparing the products with a sequencing ladder obtained with the same primer.

Western analysis

Total cellular and nuclear extracts were prepared as described previously (Ponticelli and Struhl, 1990), and cytoplasmic extracts were obtained as the supernatant after separation from the pelleted nuclei. Total protein from each extract (50 mg as quantitated by the Bradford assay) was separated on a 5% SDS–polyacrylamide gel, visualized by Ponceau staining, and electroblotted on to nitrocellulose. For tagged protein detection, HA1-specific monoclonal antibody (12CA5, ascites fluid, 14 mg/ml protein obtained from Scripps Institute) was used at a 1:1000 dilution followed by a 1:7500 dilution of goat anti-mouse IgG-specific antibody conjugated to alkaline phosphatase.

Acknowledgements

We thank Salvatore Oliviero and Hugo Giambarella for help in carrying out some of the experiments, Steve Reed for freely exchanging *CDC39* sequences prior to publication, Linda Riles for supplying the genomic mapping filters and interpreting the hybridization results, Ben Rich and John Marquardt for help with computer analysis, Brendan Cormack, Elaine Elion and Alice Vincent for yeast strains and DNAs, and Brendan Cormack, Elaine Elion, Michael Grunstein, Salvatore Oliviero and Steve Reed for fruitful discussions during the course of the experiments, and Dominique Belin for useful comments on the manuscript. This work was supported by a postdoctoral fellowship to M.A.C. from the Swiss National Fund and by grants from the National Institutes of Health (GM30186) and the Lucille Markey Foundation to K.S.

References

Chen,W. and Struhl,K. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 2691–2695.
Chen,W., Tabor,S. and Struhl,K. (1987) *Cell*, **50**, 1047–1055.
Clark,K.L. and Sprague,G.F.J. (1989) *Mol. Cell. Biol.*, **9**, 2682–2694.
Clark-Adams,C.D., Norris,D., Osley,M.A., Fassler,J.S. and Winston,F. (1988) *Genes Dev.*, **2**, 150–159.
Comai,L., Tanese,N. and Tjian,R. (1992) *Cell*, **68**, 965–976.
Cormack,B.P. and Struhl,K. (1992) *Cell*, **69**, 685–696.
Davis,J.L., Kunisawa,R. and Thorner,J. (1992) *Mol. Cell. Biol.*, **12**, 1879–1892.
de Barros Lopes,M., Ho,J.-Y. and Reed,S.I. (1990) *Mol. Cell. Biol.*, **10**, 2966–2972.
Dolan,J.W., Kirkman,C. and Fields,S. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 5703–5707.
Durrin,L.K., Mann,R.K. and Grunstein,M. (1992) *Mol. Cell. Biol.*, **12**, 1621–1629.
Dynlacht,B.D., Hoey,T. and Tjian,R. (1991) *Cell*, **66**, 563–576.
Eisenmann,D.M., Arndt,K.M., Ricupero,S.L., Rooney,J.W. and Winston,F. (1992) *Genes Dev.*, **6**, 1319–1331.
Errede,B. and Ammerer,G. (1989) *Genes Dev.*, **3**, 1349–1361.
Han,M. and Grunstein,M. (1988) *Cell*, **55**, 1137–1145.
Han,M., Kim,U.J., Kayne,P. and Grunstein,M. (1988) *EMBO J.*, **7**, 2221–2228.
Harbury,P.A.B. and Struhl,K. (1989) *Mol. Cell. Biol.*, **9**, 5298–5304.
Herskowitz,I. and Jensen,R.E. (1991) *Methods Enzymol.*, **194**, 132–146.
Hill,D.E., Hope,I.A., Macke,J.P. and Struhl,K. (1986) *Science*, **234**, 451–457.
Hope,I.A. and Struhl,K. (1986) *Cell*, **46**, 885–894.
Hope,I.A., Mahadevan,S. and Struhl,K. (1988) *Nature*, **333**, 635–640.
Inostroza,J.A., Mermelstein,F.H., Ha,I., Lane,W.S. and Reinberg,D. (1992) *Cell*, **70**, 477–489.
Kanazawa,S., Driscoll,M. and Struhl,K. (1988) *Mol. Cell. Biol.*, **8**, 664–673.
Keleher,C.A., Redd,M.J., Schultz,J., Carlson,M. and Johnson,A.D. (1992) *Cell*, **68**, 709–719.
Kruger,W. and Herskowitz,I. (1991) *Mol. Cell. Biol.*, **11**, 4135–4146.
Lee,W.S., Kao,C.C., Bryant,G.O., Liu,X. and Berk,A.J. (1991) *Cell*, **67**, 367–376.
Lieberman,P.M. and Berk,A.J. (1991) *Genes Dev.*, **5**, 2441–2454.
Ma,J. and Ptashne,M. (1987) *Cell*, **48**, 847–853.
Mahadevan,S. and Struhl,K. (1990) *Mol. Cell. Biol.*, **10**, 4447–4455.
Meisterernst,M. and Roeder,R.G. (1991) *Cell*, **67**, 557–567.
Nicolet,C.M. and Craig,E.A. (1991) *Methods Enzymol.*, **194**, 710–717.
Oettinger,M.A. and Struhl,K. (1985) *Mol. Cell. Biol.*, **5**, 1901–1909.
Oliver,S.G. (1992) *Nature*, **357**, 38–46.
Olson,M.V., Dutchik,J.E., Graham,M.Y., Brodeur,G.M., Helms,C., Frank,M., Maccollin,M., Scheinman,R. and Frank,T. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2444–2448.
Peterson,C.L., Kruger,W. and Herskowitz,I. (1991) *Cell*, **64**, 1135–1143.
Ponticelli,A.S. and Struhl,K. (1990) *Mol. Cell. Biol.*, **10**, 2832–2839.
Reed,S.I. (1980) *Genetics*, **95**, 561–577.
Reed,S.I., Ferguson,J. and Jahng,K.-Y. (1988) *Cold Spring Harbor Symp. Quant. Biol.*, **53**, 621–627.
Rothstein,R.J. (1983) *Methods Enzymol.*, **101**, 202–211.
Schultz,J., Marshall-Carlson,L. and Carlson,M. (1990) *Mol. Cell. Biol.*, **10**, 4744–4756.
Shuster,E.O. and Byers,B. (1989) *Genetics*, **123**, 29–43.
Sikorski,R.S. and Hieter,P. (1989) *Genetics*, **122**, 19–27.
Singer,V.L., Wobbe,C.R. and Struhl,K. (1990) *Genes Dev.*, **4**, 636–645.
Strubin,M. and Struhl,K. (1992) *Cell*, **68**, 721–730.
Struhl,K. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7865–7869.
Struhl,K. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 8419–8423.
Struhl,K. (1986) *Mol. Cell. Biol.*, **6**, 3847–3853.
Struhl,K. (1987) *Cell*, **50**, 841–846.
Struhl,K. and Hill,D.E. (1987) *Mol. Cell. Biol.*, **7**, 104–110.
Timmers,H.T.M. and Sharp,P.A. (1991) *Genes Dev.*, **5**, 1946–1956.
Vidal,M., Strich,R., Esposito,R.E. and Gaber,R.F. (1991) *Mol. Cell. Biol.*, **11**, 6306–6316.
Wang,H., Clark,I., Nicholson,P.R., Herskowitz,I. and Stillman,D.J. (1990) *Mol. Cell. Biol.*, **10**, 5927–5936.
Whiteway,M.L., Hougan,L., Dignard,D., Thomas,D.Y., Bell,L., Saari,G.C., Grant,F.J., O'Hara,P. and MacKay,V.L. (1989) *Cell*, **56**, 467–477.
Williams,F.E. and Trumbly,R.J. (1990) *Mol. Cell. Biol.*, **10**, 6500–6511.
Williams,F.E., Varanasi,U. and Trumbly,R.J. (1991) *Mol. Cell. Biol.*, **11**, 3307–3316.
Wobbe,C.R. and Struhl,K. (1990) *Mol. Cell. Biol.*, **10**, 3859–3867.

Received on September 7, 1992; revised on October 6, 1992