**CDC68, a Yeast Gene That Affects Regulation of Cell Proliferation and Transcription, Encodes a Protein with a Highly Acidic Carboxyl Terminus**

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The cell cycle of the budding yeast *Saccharomyces cerevisiae* has been investigated through the study of conditional *cdc* mutations that specifically affect cell cycle performance. Cells bearing the *cdc68-1* mutation (J. A. Prendergast, L. E. Murray, A. Rowley, D. R. Carruthers, R. A. Singer, and G. C. Johnston, Genetics 124:81–90, 1990) are temperature sensitive for the performance of the G regulatory event, START. Here we describe the *CDC68* gene and present evidence that the *CDC68* gene product functions in transcription. *CDC68* encodes a 1,035-amino-acid protein with a highly acidic and serine-rich carboxyl terminus. The abundance of transcripts from several unrelated genes is decreased in *cdc68-1* mutant cells after transfer to the restrictive temperature, while at least one transcript, from the *HSP82* gene, persists in an aberrant fashion. Thus, the *cdc68-1* mutation has both positive and negative effects on gene expression. Our findings complement those of Malone et al. (E. A. Malone, C. D. Clark, A. Chiang, and F. Winston, Mol. Cell. Biol. 11:5710–5717, 1991), who have independently identified the *CDC68* gene (as *SPT16*) as a transcriptional suppressor of β-insertion mutations. Among transcripts that rapidly become depleted in *cdc68-1* mutant cells are those of the *G1* cyclin genes *CLN1, CLN2*, and *CLN3/WHI1/DAF1*, whose activity has been previously shown to be required for the performance of START. The decreased abundance of cyclin transcripts in *cdc68-1* mutant cells, coupled with the suppression of *cdc68-1*-mediated START arrest by the *CLN2-1* hyperactive allele of *CLN2*, shows that the *CDC68* gene affects START through cyclin gene expression.

Cell cycle regulation in the budding yeast *Saccharomyces cerevisiae* is exerted primarily at a step in *G1* termed START (19, 46). Components of the START regulatory machinery have been identified, in large part, as a result of investigations that exploit particular conditional mutations that affect the cell division cycle. One collection of cell division cycle (*cdc*) mutations defines the *CDC28* gene that encodes the *S. cerevisiae* homolog of the p34<sup>cdc2</sup> protein kinase, which has been shown to regulate cell cycle progression in a wide variety of eukaryotes (reviewed in references 29 and 42). In *S. cerevisiae* the performance of START depends on the periodic activation of the p34 kinase upon association with one or more members of a family of proteins known as *G1* cyclins (47, 66). In addition, the performance of START is responsive to the biosynthetic status of the cell and, in the case of haploid cells, to the presence of mating pheromones. In a previous study we employed a novel gradient selection procedure to identify additional genes that affect START (45). In particular, we sought to identify mutations that affect START without significant impairment of biosynthetic activity; such mutations should define additional genes that impinge upon the START machinery in a fairly direct manner. This genetic approach yielded conditional START mutations in three previously uncharacterized genes. Cells bearing one of these mutations, *cdc68-1*, are temperature sensitive for the performance of START; *cdc68-1* mutant cells proliferate normally at 23°C but arrest proliferation as unbudded cells within one cell cycle after transfer to the restrictive temperature of 37°C. Arrested *cdc68-1* mutant cells retain significant biosynthetic capacity and remain mating competent for several hours (45). Here we describe the molecular characterization of the *CDC68* gene and present molecular and genetic evidence that the *CDC68* gene product plays a general role in transcription.

The *CDC68* gene has been independently identified (as *SPT16*) by its ability to suppress, in multiple copy number, certain β-element insertion mutations that alter transcription of the *HIS4* and *LYS2* genes (32). The identification of the *CDC68* gene by this approach is consistent with a role for the Cdc68 protein in transcription.

**MATERIALS AND METHODS**

**Strains and media.** Yeast strains used in this study are listed in Table 1. Yeast cells were grown in YNB liquid defined medium (26) or in the complex rich medium YEPD as described elsewhere (18). Cell concentrations were determined with an electronic particle counter (Coulter Electronics, Inc.). Cell morphology was assessed by microscopic examination of at least 200 cells per sample. Nuclear morphology was assessed by staining with 4',6-diamidino-2-phenylindole (Sigma Chemical Co.).

**Recombinant DNA manipulations.** Yeast transformation was performed by the spheroplast method described by Hinnen et al. (23). Recombinant DNA manipulations were carried out essentially as described by Maniatis et al. (33). Plasmid pSC2-1 containing the *CDC68* gene has been previously described (45), as have vectors YEp24 (3) and YEp352 (22). Plasmid pUTX144, kindly provided by D. Finkelstein, is a pBR322-based plasmid containing the *S. cerevisiae* 2μm origin of replication, the *LEU2* gene to allow selection in *leu2* mutant cells, and sequence from −334 to +282 of the

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### TABLE 1. *S. cerevisiae* strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype or phenotype</th>
<th>Source*</th>
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<tr>
<td>21R</td>
<td>MATa ade1 leu3-112 ura3-52</td>
<td>J.E.H.</td>
</tr>
<tr>
<td>ART68-1†</td>
<td>MATa cdc68-1 ura3-52 leu3-112 ade3</td>
<td>This study</td>
</tr>
<tr>
<td>68507A‡</td>
<td>MATa cdc68-1 ura3-52 ade3</td>
<td>This study</td>
</tr>
<tr>
<td>L577</td>
<td>MATa his4-128 lys2-1288 ura3-52</td>
<td>E.A.M.</td>
</tr>
<tr>
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<td>MATa ura3-52::[HSP90-lacZYA pln1]</td>
<td>C.B.</td>
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<tr>
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<td>cdc68-1 ura3-52::[HSP90-lacZYA pln1]</td>
<td>This study</td>
</tr>
<tr>
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<td>This study</td>
</tr>
<tr>
<td>GCY26</td>
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<td>S.L.</td>
</tr>
<tr>
<td>CLD82α</td>
<td>MATa Can't Ade3 His+ Trp- Ura- HSP82::LEU2 HCSC2::LEU2</td>
<td>S.L.</td>
</tr>
</tbody>
</table>

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**HSP82 gene (12)** fused in frame to codon 8 of the *Escherichia coli* lacZ gene.

**Northern (RNA) analysis.** Total RNA was extracted from cells (2 × 10⁶ to 4 × 10⁷/ml) by the method of Penn et al. (44). Oligonucleotide probes were end labelled with T4 poly nucleotide kinase and [a-³²P]dATP as described by Sambrook et al. (51). Hybridization with restriction fragment probes was essentially as described by Thomas (61). Hybridization with oligonucleotide probes was performed as described by Wallace and Miyada (62). The following DNA restriction fragments were purified and labelled for use as probes. The *CLN1* and *CLN2* open reading frames were excised as 1.6-kbp *Nde1-BamHI* fragments from insertions in pRK171, kindly provided by C. Wittenberg. The *CLN3* probe was a 1.7-kbp *EcoRI-XhoI* restriction fragment from a plasmid containing the *CLN3/YWH1/DAF1* gene (a gift from F. Cross). The *ACT1* probe was a 1-kbp *HindIII-XhoI* restriction fragment from pRS208 (a gift from R. Storms). The *LEU2* probe was a 1.7-kbp *HpaI-AccI* fragment from YEp351 (provided by J. Hill). The *CDC68* probe was a 2.2-kbp *HpaI* fragment from the *CDC68* open reading frame, excised from pSCC-1. The *lacZ* probe was a 6.2-kbp *BamHI-Sall* fragment from pPUTX144 and contained *lacZ* gene sequences only. The *HSP82* probe was an oligonucleotide of sequence 5'-CAAGGCCATGATGTTCTACCC-3' complementary to nucleotides 2130 to 2149 of the *HSP82* coding sequence (12). The specificity of this probe was confirmed by hybridization to RNA extracted from strains PLD82α and CLD82α deleted for *HSP82* and *HCSC2*, respectively (kindly provided by S. Lindquist).

**Radiolabeling, protein extraction, and gel electrophoresis.** Pulse-labeling of cells (2 × 10⁶ to 4 × 10⁷/ml) with [³⁵S]methionine (NEN) and sample preparation were as previously described (36). Proteins from cell lysates containing equal amounts of radiolabelled trichloroacetic acid-precipitable proteins were resolved by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (27).

**DNA sequencing.** Overlapping restriction fragments from the cloned *CDC68* gene were subcloned into M13 um20 or um21 (International Biotechnologies Inc.), and nested sets of deletions were generated by the method of Henikoff (20). DNA sequencing (52) was performed with Sequenase version 2.0 (United States Biochemicals) and [³²P]dATP (United States Biochemicals). Both strands were completely sequenced. DNA sequence data were analyzed by using the Wisconsin Genetics Computer Group sequence analysis software (9).

**Nucleotide sequence accession number.** The GenBank accession number of the *CDC68* nucleotide sequence is M75333.

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**RESULTS**

**CDC68 nucleotide sequence.** The wild-type *CDC68* gene was cloned previously by complementation of the recessive *cdc68-1* temperature-sensitive mutation (45). To localize the *CDC68* gene we subcloned into the high-copy-number vector YEp53. A number of restriction fragments from the 1.8-kbp plasmid pSCC-2, the smallest complementing clone obtained in our earlier studies (45). The nucleotide sequence of a complementing fragment (a 5.2-kbp *BamHI* fragment; Fig. 1A) revealed a 3,105-bp open reading frame which we conclude specifies the *CDC68* gene product (Fig. 1B). The *CDC68* nucleotide sequence predicts a 1,035-amino-acid protein of 118,556 molecular weight. The predicted protein is highly charged, with 31.2% charged residues (14.2% basic and 17.0% acidic). Moreover, distribution of charged residues is different in different areas of the protein; the amino-terminal end of the *CDC68* gene product, from amino acid residues 1 to 451, is only moderately charged (26% overall, 12.2% acidic, and 13.8% basic), whereas the following region, encompassing amino acids 451 to 810, contains considerably more charged residues (37.2%, 15.8% acidic, and 21.4% basic). Particularly striking is the density of acidic amino acids at the carboxyl terminus; from residues 957 to 1021, 36 of 65 amino acids (55%) are either aspartic acid or glutamic acid, with a complete absence of basic amino acids (Fig. 1B). Of the remaining 29 residues within this acidic region, 12 (18%) are serine and 1 is threonine. Contained within this region are several consensus recognition sites for casein kinase II phosphorylation (34, 67), raising the possibility that the *CDC68* gene product is phosphorylated by casein kinase II in vivo.

**CDC68 and SPT16 are the same gene.** The *cdc68-1* mutation was previously mapped to chromosome VII, between *ADE5* and *KEX1* (45). Subsequent genetic mapping of a temperature-sensitive mutation in *SPT16*, a gene isolated as a high-copy-number suppressor of 8-element insertions in *his4-9126* and *lys2-1288*, suggested that this mutation, *spt16-197*, is an allele of the *CDC68* gene (32). Upon further analysis, *cdc68-1* and *spt16-197* temperature-sensitive mutations failed to complement when combined in a heterozygous diploid and displayed tight linkage in tetrad analysis when this diploid was subsequently sporulated (32). Furthermore, transformation of cells containing the *spt16-197* temperature-sensitive mutation (strain L577) with the *CDC68* subclones described above gave the same pattern of complementation as in *cdc68-1* mutant cells (Fig. 1A). These genetic and molecular criteria establish that the *cdc68-1* and *spt16-197* mutations define the same gene.
cdc68-1 and spy16-197 mutations confer the same mutant phenotype. S. cerevisiae cells containing the cdc68-1 mutation have been shown previously to be defective in performance of the cell cycle regulatory step, START (45). Cells containing the spy16-197 mutation (strain L577), when transferred from the permissive temperature (23°C) to the restrictive temperature (37°C), were also found to arrest proliferation promptly, within one cell cycle, with 80 to 90% un budded cells. (For S. cerevisiae, bud morphology provides an estimate of cell cycle position; cells within the G1 interval are unbudded [19]). Thus, like cdc68-1 mutant cells, cells harboring the spy16-197 mutation are conditionally defective in the G1 interval.

The SPT16 gene was identified by its ability, in multiple copy number, to suppress the eight insertions in his4-9128 and lys2-1288 (32). However, the his4-9128 and lys2-1288 mutations can also be suppressed by the spy16-197 temperature-sensitive mutation (32). We therefore assessed the ability of the cdc68-1 mutation to suppress his4-9128 and lys2-1288. Analysis of meiotic segregants from a cross between strains ART68-1 (cdc68-1) and FY56 (his4-9128 lys2-1288) demonstrated that the cdc68-1 mutation, like spy16-197, can suppress his4-9128 and lys2-1288. Thus, two independently isolated mutations in the CDC68/SPT16 gene exhibit the same cell cycle and 8-element suppression phenotypes.

Cyclin transcript abundance is decreased in cdc68-1 mutant cells. Recent investigations (8, 40, 48, 66) have revealed the involvement of a family of proteins, termed G1 cyclins, in the activation of the START machinery. Since cells harboring the cdc68-1 mutation are defective in the performance of START (45), we determined whether cdc68-1-mediated arrest involved altered expression of any of the three G1 cyclin genes (8, 17, 40, 48, 66). To address this question, transcript levels of the three G1 cyclin genes, CLN1, CLN2, and CLN3 (previously designated WHI1 [4, 40, 58] and DAF1 [7]), were examined by Northern analysis (Fig. 2A). After transfer of cdc68-1 mutant cells to the restrictive temperature, the levels of all three CLN transcripts rapidly and permanently decreased (Fig. 2A). A transient decrease in CLN2 transcript abundance in wild-type cells after transfer to 37°C is coincident with a previously described transient accumulation of the cells within the G1 interval (50, 53). The rapid decrease in CLN mRNA abundance in cdc68-1 mutant cells may be responsible for the START arrest phenotype exhibited by mutant cells at the restrictive temperature. Indeed, decreased cyclin expression has been previously shown to cause a first-cycle G1 arrest (8, 48).

The CLN2-1 mutation abrogates the G1 arrest of cdc68-1 mutant cells. To test the possibility that the START arrest caused by the cdc68-1 mutation is a consequence of decreased CLN gene expression, we examined the effect of CLN2-1, a hyperactive or hyperstable allele of the CLN2 gene (17). The CLN2-1 allele encodes a truncated Cln2 protein that is thought to exhibit increased stability due to the deletion of carboxyl-terminal PEST sequences implicated in protein degradation (49). The phenotype of cells containing the CLN2-1 mutation, including decreased cell size at bud emergence, a shortened G1 interval, moderate α-factor resistance, and failure to arrest at START in response to starvation, indicates that cyclin protein in these mutant cells is no longer rate limiting for cell cycle progression.

To assess the effect of increased cyclin stability on the START arrest caused by the cdc68-1 mutation, we constructed a diploid strain heterozygous for both the CLN2-1 and cdc68-1 mutations. Temperature sensitivity segregated 2:2 in meiotic segregants from this cross, indicating that the CLN2-1 mutation did not suppress the temperature sensitivity imposed by the cdc68-1 mutation. Both cdc68-1 and cdc68-1 CLN2-1 segregants exhibited an approximately two-fold increase in cell concentration after transfer to the restrictive temperature (Fig. 3A). However, the cell cycle behavior of cdc68-1 CLN2-1 double-mutant cells was altered. Whereas cdc68-1 segregants arrested proliferation as
FIG. 1—Continued.
unbudded cells unable to initiate a subsequent cell cycle, cdc68-1 CLN2-1 double-mutant segregants arrested proliferation primarily as budded cells that had initiated but failed to complete an additional cell cycle (Fig. 3B and C). The cdc68-1 CLN2-1 double-mutant cells arrested proliferation with nuclear morphologies typical of all stages of the cell cycle (data not shown). Thus, cdc68-1 CLN2-1 double-mutant cells perform START but do not complete the new cell cycle. The ability of the CLN2-1 mutation to suppress the START arrest of cdc68-1 mutant cells, combined with the cdc68-1-mediated decreases in CLN transcript levels, led us to conclude that START arrest of cdc68-1 mutant cells at the restrictive temperature is a consequence of cyclin limitation.

**Abundance of other transcripts is decreased in cdc68-1 mutant cells.** To further define the effects of the cdc68-1 mutation on gene expression, we examined the levels of other transcripts in cdc68-1 mutant cells. Transcripts from the ACT1 and LEU2 genes, like those from the three G1 cyclin genes, decreased in abundance after transfer of cdc68-1 mutant cells to the restrictive temperature (Fig. 2B). Even at the permissive temperature the LEU2 transcript was present at lower levels in cdc68-1 mutant cells (Fig. 2B).

The identification of CDC68 as an SPT gene suggests that the decreased transcript levels in cdc68-1 mutant cells are brought about by decreased transcription. The rapidity of change in CLN and LEU2 transcript abundance after transfer of cdc68-1 mutant cells to 37°C suggests that functional Cdc68 protein is rapidly depleted under these restrictive conditions. Even the more gradual effect on ACT1 transcript levels is consistent with an inhibition of transcription, since ACT1 mRNA has a relatively long half-life (approximately 30 min [21]) and is therefore expected to persist in the absence of further transcription.

**CDC68 transcript abundance is decreased in cdc68-1 mutant cells.**
Cells. A 3.2-kb transcript was detected in both wild-type and cdc68-1 mutant cells grown at 23°C by using a restriction fragment internal to the CDC68 open reading frame as a probe (Fig. 2C, lanes 1 and 6). This transcript size is consistent with the 3.105-kb CDC68 open reading frame shown in Fig. 1. Unexpectedly, the abundance of the 3.2-kb transcript decreased to barely detectable levels in cells containing the cdc68-1 mutation within 20 min of transfer to 37°C (Fig. 2C, lanes 6 to 10). This finding suggests that functional Cdc68 protein is required for transcription of the CDC68 gene itself.

Mutations in the CDC68 gene cause abnormal synthesis of heat shock proteins. Previous studies have demonstrated that significant levels of protein and RNA synthesis are maintained in cdc68-1 mutant cells at the restrictive temperature (45), suggesting that the cdc68-1 mutation is not deleterious to the expression of all genes. Therefore, we compared the patterns of proteins synthesized in wild-type and cdc68-1 mutant cells to identify other effects of cdc68 mutations. Total cellular proteins were extracted and resolved by one-dimensional SDS-PAGE following brief exposure of cells to [35S]methionine. The use of a short labelling period (10 min) to label proteins argues that any differences seen between wild-type and mutant cells reflect altered protein synthesis rather than altered protein stability. The pattern of proteins synthesized in wild-type cells before and after transfer to 37°C is shown in Fig. 4 (lanes 1 to 3). As expected, wild-type cells responded to increased temperature by transiently inducing the synthesis of heat shock proteins (reviewed in reference 30) while decreasing the synthesis of others. The pattern of newly synthesized proteins extracted either from cdc68-1 (Fig. 4, lanes 4 to 6) or from spt16-197 (data not shown) mutant cells showed a number of differences: the synthesis of many proteins decreased after transfer to 37°C but, in contrast to the situation in wild-type cells, remained at a low level even after 240 min at the elevated temperature (compare lanes 1 to 3 with lanes 4 to 6). Conversely, at least one protein whose synthesis was transiently increased after transfer to 37°C continued to be synthesized at an elevated level in both cdc68-1 and spt16-197 mutant cells even 240 min after transfer to the restrictive temperature (Fig. 4 and data not shown). We tentatively identified this protein as Hsp82; the product of the HSP82 gene (2, 12, 14), on the basis of its apparent molecular mass (approximately 90 kDa) and induction upon heat shock. A virtual wild-type pattern of labelled proteins was restored by the introduction of a wild-type CDC68 gene (45) on the 2μm-based vector YEp24 (Fig. 4, lanes 10 to 12) but not by YEp24 itself (Fig. 4, lanes 7 to 9). These results suggest that CDC68 gene function is required for the appropriate synthesis of many proteins following transfer to 37°C, proteins whose synthesis is either induced (like Hsp82) or decreased in response to the stress of heat shock.

The HSP82 gene is expressed at low basal levels at 23°C but is rapidly and transiently induced upon transfer of cells from 23 to 37°C. This induction has been shown to be mediated by a 320-base-pair (bp) element located in the left arm of the HSP82 open reading frame (Fig. 3) and is responsive to the heat-inducible promoter (2). To pursue this observation, we confirm that gene expression under the regulation of HSP82 sequences is altered in cdc68-1 mutant cells. For this purpose we transformed cells with a plasmid-borne fusion gene in which HSP82 sequences are fused to codon 8 of the lacZ gene from E. coli. Previous studies have demonstrated that expression of lacZ when fused to HSP82 sequences accurately reflects regulation of the endogenous HSP82 gene (15).

The pattern of newly synthesized proteins in transformants showed that the fusion gene in wild-type and cdc68-1 mutant cells directs the synthesis of a new 116-kDa polypeptide in a manner which parallels that of the 90-kDa polypeptide in Fig. 4 (data not shown). These data supported the identification of the 90-kDa polypeptide as the product of the HSP82 gene. Abundance of HSP82 transcripts is increased in cdc68-1 mutant cells. To examine more directly the cdc68-1-mediated effects on HSP82 gene expression, we determined the abundance of HSP82 mRNA in both wild-type and cdc68-1 mutant cells transferred from 23 to 37°C. For this determination we had to take into consideration that in addition to HSP82, S. cerevisiae contains a second closely related gene, HSC82. HSP82 and HSC82 are regulated differently: HSP82 is expressed at a low level at 23°C but is induced upon heat shock, whereas HSC82 exhibits higher basal expression and is induced to a lesser extent upon heat shock (2). To distinguish between the HSP82 and HSC82 transcripts, an HSP82-specific oligonucleotide probe was used; the specificity of this probe was confirmed by Northern hybridization to RNA extracted from strains harboring disrupted copies of each member of this two-gene family (for details, see Methods and Materials). Transcripts of approximately 2.4 kb were detected by this probe in RNA extracted from both wild-type and mutant cells (Fig. 2D), consistent with the size of the HSP82 open reading frame (12). HSP82 mRNA levels increased, as expected, in both wild-type and cdc68-1 mutant cells in response to the imposition of heat shock conditions. However, in cdc68-1 mutant cells, HSP82 mRNA abundance remained elevated compared with that in wild-type cells for as long as 5 h after transfer to the
restrictive temperature (Fig. 2D, compare lanes 1 to 5 with lanes 6 to 10). Similar results were obtained when the abundance of transcripts from the HSP82-lacZ fusion gene described above, in this case integrated in single copy at the ura3-52 locus, was determined by Northern blot analysis (data not shown).

The continued presence of significant levels of HSP82 transcripts in cdc68-1 mutant cells parallels the continued synthesis of the 90-kDa protein in Fig. 4. The persistent synthesis of this protein probably reflects persistent transcription of the HSP82 gene, since we have shown that transcripts from the endogenous HSP82 gene and both transcripts and protein from a heterologous fusion gene under the control of the HSP82 promoter are all present at elevated levels at this time.

DISCUSSION

The studies described here extend our previous work in which we identified a new temperature-sensitive START mutant, cdc68-1, and cloned the wild-type CDC68 gene (45). This paper presents a number of new results and molecular evidence suggest that the product of the CDC68 gene plays a general role in transcription. The Cdc68 protein is required for continued transcription of a variety of otherwise unrelated genes, including the genes encoding three yeast G1 cyclins, and is required to repress HSP82 expression after induction upon heat shock. Thus, the CDC68 gene product has both positive and negative effects on gene expression.

Ty and solo δ-insertion mutations have provided an effective selection scheme for the identification of a variety of trans-acting proteins, many involved directly in the regulation of transcription (5, 11, 13, 63, 64). The independent identification of the CDC68 (SPT16) gene by this suppressor approach supports the argument that the altered transcript levels that we have observed in cdc68-1 mutant cells are the result of transcriptional changes. Indeed, in the accompanying report Malone et al. (32) demonstrate that a temperature-sensitive CDC68 allele, spt16-197, alters transcription initiation at the lys2-1286 locus. Further support for a transcriptional role for the Cdc68 protein comes from the finding that mutation of the CDC68/SPT16 gene allows derepression of the glucose-repressible gene SUC2 in the absence of upstream regulatory sequences and at least partially suppresses a null mutation in SNF2, a gene required for the expression of several diversely regulated genes, including SUC2 (32).

The predicted amino acid sequence of the Cdc68 protein contains an extremely acidic carboxyl-terminal region which is also rich in serine residues. Acidic regions necessary for transcriptional activation have been identified in a number of transcriptional activators, including GCN4 (24) and GAL4 (31). The Cdc68 protein may function as an acidic activator for transcription, a role that may be facilitated by an as-yet-unidentified DNA-binding domain within the Cdc68 protein or by association of the Cdc68 protein with other sequence-specific DNA-binding factors.

Malone et al. (32) have demonstrated that both overexpression (by increased copy number) and mutation of the CDC68/SPT16 gene result in suppression of δ-element insertion mutations. In this respect the CDC68/SPT16 gene is similar to the SPT5 (60) and SPT6/SSN20/CRE2 genes (6, 59). Both SPT5 and SPT6 encode essential nuclear proteins with extremely acidic N termini and have been suggested to influence gene expression through a function in chromatin assembly or modification. Indeed, other proteins thought to interact with chromatin have similarly acidic regions (10). Furthermore, the gene dosage effects for SPT5 and SPT6 are similar to those for histone genes (5). The similarities among the mutant phenotype, dosage effects, and structural features of the CDC68, SPT5, and SPT6 genes are consistent with a role for the Cdc68 protein in chromatin conformation. In this model the function of the Cdc68 C-terminal acidic region might be to interact with the basic carboxyl-terminal histones. The gene dosage effects seen with the CDC68 gene further suggest that the role of the Cdc68 protein may require interaction of the Cdc68 protein with other proteins to form a complex. A preliminary analysis of this possibility by the isolation of unlinked suppressor mutations suggests that such proteins may be identified by a genetic approach (67).

The experiments described here also demonstrate that the inhibition of HSP82 gene expression after heat shock induction requires the Cdc68 protein; cdc68-1 mutant cells display the expected induction of the HSP82 transcript upon heat shock but, unlike wild-type cells, are unable to repress HSP82 gene expression and, as a result, exhibit prolonged HSP82 expression after heat shock. This prolonged expression is not simply a result of a SPT16 allele in place of the nonwild-type allele; other START mutations do not show this phenotype (1). The prolonged HSP82 expression therefore results from cdc68 effects at the HSP82 locus. Transcriptional regulation of HSP82 is complex. Recent experiments by McDaniel et al. (37) have demonstrated that the TATA-proximal heat shock element is required for basal expression of the HSP82 gene, implying that heat shock transcription factor is involved in basal-level expression of HSP82 as well as induction by heat shock. Recent analyses (25, 41, 54) of the heat shock transcription factor have identified physically separable activation domains that are regulated by conformational changes and perhaps also by interactions with other proteins. The Cdc68 protein may be one such protein that affects heat shock transcription factor activity.

An additional transcript that exhibited decreased abundance in cdc68-1 mutant cells transferred to the restrictive temperature was the CDC68 transcript itself. This result suggests that transcription of the CDC68 gene requires its own gene product, the Cdc68 protein. Although unusual, this situation is unlikely to be unique, since other genes encoding components of the basic transcription machinery such as RNA polymerase II subunits and transcription initiation factors are thought to be transcribed by complexes of proteins which must include their own gene products.

In addition to effects on transcript abundance, mutations in the CDC68 gene affect proliferation and bring about a regulated arrest at the cell cycle regulatory step, START. Recent investigations have revealed that the performance of START involves periodic activation of a protein kinase referred to as p34cdc28 (38, 65). In turn, activation of the p34cdc28 protein kinase requires periodic accumulation of G1 cyclin proteins, the products of three genes, CLN1, CLN2, and CLN3 (47, 66). Use of heterologous promoters to prevent cyclin gene expression leads to a rapid, first-cycle arrest of proliferation with cells blocked at the START event (8, 48). Conversely, the introduction of hyperactive or hyperstable alleles of CLN2 or CLN3 prevents cell cycle arrest at START (7, 17, 40). The physiological state of cells arrested at START as a result of cyclin depletion resembles the phenotype of arrests with cyclin mutants; both arrest conditions allow continued cell growth and mating competence (7, 45). Under these arrest conditions, cdc68-1 mutant cells contain dramatically decreased levels of all three CLN transcripts.
The findings described above provide a mechanism through which effects on transcription can result in START arrest. Under nonpermissive conditions, the short half-lives of the cyclin proteins (65, 66), coupled to an increase in the cdc68-1-mediated decrease in CLN transcript levels, preclude accumulation of sufficient cyclin proteins to activate START. Such cells thus become incapable of initiating a new cell cycle. We conclude that the Cdc68 protein is required for continued cyclin gene expression and that in the absence of an active Cdc68 protein, cyclin levels are insufficient to activate START. The mechanism of cdc68-1-mediated inhibition of cyclin gene expression is not yet established. However, an inappropriate activation of the mating-response pathway is unlikely, because the effects on cyclin transcript levels in cdc68-1 mutant cells do not resemble the pattern of transcript abundance observed after α-factor treatment. Upon activation of the mating-response pathway by α-factor, only CLN1 and CLN2 transcript levels decrease (66) and those of the CLN3 gene actually exhibit a moderate increase (40).

The CLN2-1 hyperactive allele alleviates cdc68-1-mediated START arrest, presumably by providing a more stable Cln2 protein. However, double-mutant cells harboring both the cdc68-1 and CLN2-1 mutations remain temperature sensitive for cell proliferation. Assessment of nuclear morphology showed that the cell cycle does not become blocked at any particular position. Therefore, the continued temperature sensitivity of cdc68-1 CLN2-1 double-mutant cells reflects additional effects of the cdc68-1 mutation on gene expression, but not on that of genes whose expression is rate limiting in the post-START cell cycle.

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