CDC33 Encodes mRNA Cap-Binding Protein eIF-4E of Saccharomyces cerevisiae

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The bcyl mutation makes the cdc33 start mutant arrest at random points in the cell cycle instead of only at G1. We cloned and sequenced CDC33. This coding sequence is identical to that of the gene encoding the Saccharomyces cerevisiae 24-kilodalton mRNA cap-binding protein, eIF-4E.

The mitotic cycle of the budding yeast Saccharomyces cerevisiae is initiated in the G1 phase of the cell cycle, at the stage called start. Start events are controlled by several cell division cycle (CDC) genes (12). According to the terminal phenotype shown by conditional cdc mutants, two classes of start mutants have been identified (13). Upon shifting to the nonpermissive temperature, class I mutants (cdc28, cdc36, cdc37, and cdc39) resemble mating-pheromone-arrested cells, while class II mutants (cdc25, cdc33, and cdc35) resemble nutritionally arrested cells. The CDC35 gene is allelic with CYR1 and codes for the catalytic subunit of adenylate cyclase (3, 8), whereas the CDC25 gene product appears to be involved in positive control of adenylate cyclase activity (4). Cyclic AMP (cAMP) exerts its effect by binding regulatory subunits (BCY1 gene product) of cAMPdependent protein kinase, thereby freeing active catalytic subunits (9). bcyl mutations render cAMP-dependent protein kinases cAMP independent. Thus bcyl can suppress the growth defect of cdc25 and cdc35 (cyrl) (4, 9). In addition, bcyl cells fail to arrest in the G1 phase of the cell cycle upon nutrient starvation (7). These findings demonstrate that cAMP-dependent protein phosphorylation is required for cell cycle initiation. To investigate the role of other genes involved in the control of start, we have chosen to study CDC33.

The lengths of the G1 phase in cdc33-1 and $CDC33^+$ strains were compared (Table 1). Since differences in the genetic backgrounds of strains have some effect on the kinetics of the cell cycle, such as the percentage of unbudded cells and doubling time, isogenic strains which differed only at the CDC33 locus were constructed. From the proportions of unbudded (G1) and budded (S+G2+M) cells in exponentially growing cultures of cdc33-1 and $CDC33^+$ cells, the length of the G1 phase of the total cell cycle time was calculated by the equation of Rivin and Fangman (14). The G1 period of exponentially growing cdc33-1 cells was approximately twice as long as that of CDC33⁺ cells. The duration of S+G2+M in cdc33-1 cells was slightly longer than that in $CDC33^+$. These results indicate that even at a permissive temperature, the cdc33-1 mutation affected the G1 phase. To test the effect of bcyl on cdc33-1, we constructed the isogenic strain CB103 (bcy1::URA3 cdc33-1). At the permissive temperature, the length of the G1 period in bcy1::URA3 cdc33-1 cells was restored to that of CDC33⁺.

However, bcy1::URA3 cdc33-1 cells had the same length of S+G2+M as *cdc33-1* cells. These results suggest that *bcy1* suppresses the defect of cdc33-1 within the G1 phase. When exponentially growing cultures of cdc33-1 and bcy1::URA3 cdc33-1 cells were shifted from 25 to 35°C, the number of cells increased approximately twofold (data not shown). Upon cessation of growth, cdc33-1 cells were arrested in the G1 phase of the cell cycle, whereas bcy1::URA3 cdc33-1 cells stopped growth but the number of unbudded cells did not increase under the same conditions (Table 1). These results confirm that the bcyl mutation suppressed G1 arrest caused by cdc33-1 but did not suppress the growth defect of cdc33-1 at the restrictive temperature. These results suggest that CDC33 plays an essential role in the G1 phase that can be overcome by constitutive activation of cAMP-dependent protein kinase but that it has another function essential for growth that is not specific for the G1 phase.

To address the function of the CDC33 gene product, we cloned CDC33. Plasmids containing the CDC33 gene were isolated by selection for complementation of a temperaturesensitive mutation. Strain CB101 (a cdc33-1 leu1 ura3 trp1) was transformed with a plasmid library carrying yeast genomic DNA averaging 8 kilobases (kb) inserted into the YCpN1 vector (10). Cells were plated at 37°C on medium selective for Trp^+ . A total of 29 Trp^+ transformants were obtained at 37°C, two of which, when analyzed further. displayed coincident loss of the TRP1 marker and the ability to grow at 37°C, thereby establishing that these markers were plasmid borne. Physical mapping of two clones with restriction enzymes revealed that they share a 3-kb Clal restriction fragment. The 3-kb DNA fragment (Fig. 1). maintained in either YCpN1 or YEp24, complemented the growth defect of the cdc33-1 mutation. Southern blot analysis of yeast genomic DNA cut with several restriction enzymes and probed with the 3-kb ClaI fragment showed that this fragment is unique in the genome (data not shown).

Proof that the cloned gene is actually *CDC33* depended on establishing that its genomic position coincides with that of *CDC33*. A 2-kb *ClaI-Hind*III fragment was cloned into YIp5 (Fig. 1). The resulting plasmid was linearized with *HpaI* within the insert and targeted to its homologous genomic site by integrative transformation of CB101. A mitotically stable Ura⁺ transformant was obtained at 37°C (CB102). To determine the site of integration relative to the *CDC33* gene, CB102 was crossed with KMY2-6B (a *CDC33⁺ ura3 his3 leu2*). Fifteen tetrads of this diploid gave 4+:0- segregation

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		Characteristics of exponential growth phase at 25°C ^a				
Strain	Genotype	% Unbudded	Doubling	Length (h) of phase(s):		% Unbudded cells when shifted to 35°C ^b
		cells	time (h)	G1	S+G2+M	
CB102	CDC33 ⁺ BCY1 ⁺	48	2.5	1.0	1.5	-
CB101	cdc33-1 BCY1+	61	3.8	2.0	1.8	93
CB103 ^c	cdc33-1 bcy1::URA3	44	3.0	1.1	1.9	42

 TABLE 1. Effect of the cdc33-1 mutation on the cell cycle

^a Cells were grown in YPD (1% yeast extract, 2% Bacto-Peptone [Difco Laboratories], 2% glucose) medium. The length of the G1 period was calculated from populations of unbudded cells in exponentially growing cultures at 25°C by the equation by Rivin and Fangman (14), $T[1 - log(2 - F \cdot unbud)/log 2]$, in which T is the doubling time and F \cdot unbud is the fraction of unbudded cells.

^b Exponentially growing cultures in YPD at 25°C were shifted to 35°C and incubated for 6 h, after which the population of unbudded cells was determined. ^c CB103 (α cdc33-1 bcy1::URA3 leu1 ura3 trp1) was generated by transforming CB101 (α cdc33-1 leu1 ura3 trp1) with a BamHI DNA fragment containing BCY1 disrupted with URA3 (19).

for temperature sensitivity and 2+:2- segregation for Ura. In addition, the diploid formed by mating CB102 with KMY65-1D (a cdc33-1 ura3 his3 leu2 trp1) was analyzed. The temperature sensitivity and Ura segregations were 2+:2- in 16 tetrads; all non-temperature-sensitive segregants were Ura⁺ and all temperature-sensitive segregants were Ura⁻. These results indicate that plasmid YIp5 carrying the ClaI-HindIII fragment had integrated at the cdc33-1 locus and that we had cloned the wild-type CDC33 gene.

Transcription of the *CDC33* gene was analyzed by using the 1.3-kb *ClaI-Eco*RI fragment (Fig. 1) as a radioactively labeled hybridization probe. Yeast $poly(A)^+$ RNA was separated by gel electrophoresis and blotted to nitrocellulose filters. A single band of about 900 bases was detected (Fig. 2).

The nucleotide sequence of the 2-kb ClaI-HindIII fragment (Fig. 1) that contains the CDC33 gene was determined (Fig. 3), revealing that the CDC33 gene contains a 639-basepair open reading frame. This open reading frame would encode a 24-kilodalton polypeptide of 213 amino acid residues. Recently, Altmann et al. (1) purified protein synthesis initiation factor eIF-4E from S. cerevisiae. They subsequently cloned the gene that encodes this protein (2). The predicted CDC33 protein sequence was found to be identical to that of the yeast eIF-4E.

In the present study, we show that CDC33 is identical to the gene encoding the yeast mRNA cap-binding protein eIF-4E (2). This result indicates that the regulation of start may involve translational initiation. Since protein synthesis is required not only for the G1-to-S transition but also through the cell cycle, it is interesting to ask why cdc33mutants arrest at the G1 phase. Iida and Yahara (6) have compared protein synthesis among cdc25, cdc33, cdc35, and wild-type cells and have shown that at least nine proteins are synthesized specifically in the resting state. Six of these proteins have been identified as heat shock proteins. In support of the idea that certain proteins may be required for G1 arrest, we have recently demonstrated that the polyubi-

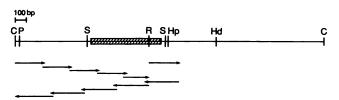


FIG. 1. Restriction enzyme map of and sequencing strategy for *CDC33*. C, *Cla*I; P, *Pst*I; S, *Spe*I; R, *Eco*RI; Hp, *Hpa*I; Hd, *Hind*III. 🖾, Position of the open reading frame. Transcription proceeds from left to right.

quitin gene (UBI4) is required for G1 arrest because ubi4 disruption mutants are defective in the processes of the G1 phase: sporulation, resistance to starvation, and morphological G1 arrest (18). To test the effect of cdc33-1 on the level of UBI4 expression, β -galactosidase activities produced by CDC33⁺ and cdc33-1 cells carrying GAL7-lacZ or UBI4lacZ fusion plasmids were compared. These fusion genes contain the promoters and sequences coding for the first 133 and 79 amino acids of GAL7 and UBI4, respectively, linked to Escherichia coli β-galactosidase (17, 18). GAL7 encodes galactose-1-phosphate uridyltransferase and is induced in the presence of galactose (17). This gene was chosen as a control. Since the cdc33-1 mutation affected the G1 phase at the permissive temperature, the enzyme activity from cultures growing at 25°C was measured. CDC33⁺ cells expressed a twofold higher activity from the GAL7-lacZ fusion than did cdc33-1 cells in the presence of galactose (CDC33⁺ cells, 960 U per optical density of cells at 660 nm [OD₆₆₀]; cdc33-1 cells, 460 U/OD₆₆₀). On the other hand, cdc33-1 cells expressed a 1.8-fold higher activity from the UBI4-lacZ fusion than did CDC33⁺ cells (CDC33⁺ cells, 80 U/OD₆₆₀; cdc33-1 cells, 140 U/OD₆₆₀). These results indicate that the cdc33-1 mutation did not cause the uniform reduction of all protein synthesis but may have led to preferential synthesis of certain proteins, such as polyubiquitin, required for G1 arrest. The result that bcy1::URA3 suppressed G1 arrest caused by cdc33-1 is consistent with the facts that expression of UBI4 is repressed by cAMP-dependent protein phosphorylation and that UBI4 expression is required for G1 arrest (18).

On the basis of the result that CDC33 encodes eIF-4E, it is possible that in cdc33-1 mutants a distinct and differential effect on the production of particular polypeptides required for G1 arrest could occur if the 5' termini of a class(es) of

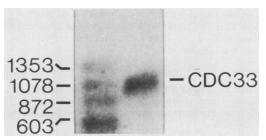


FIG. 2. Northern (RNA) blot of *CDC33*. Lanes: left, end-labeled, denatured *HaeIII* digest of $\phi X174$ RF DNA; right, 5 µg of poly(A)⁺ RNA from KMY125 (a/ α wild type). The blot was probed with the 1.3-kb *ClaI-EcoRI* fragment of *CDC33*. Size markers are indicated in nucleotides.

TAATCACTAACGAAAGTTCTAA ¹ GAAAAGCTCAGAGCGGAAGCTCAGCAGGGGGAATAAGAAGATCATGGACAAGTTTGGATACTGGTATTGTGACTCTTGTAAGAAGAAGAATACAT CTTGTGTTCTATGTGAAAGACCATTAAAGAACTACCAGGGGCATCCCCCCGTGGGACACGAAGGTCCTTCCAGTGCATACCAGGATGGTTTCTCGATGGAGAATGAACAAGAATGTCCCC GGCGGTTGCCCCCGGTGTTGCATTCATCTAGGTTCTCCACCATAGTTAAGTTAAACTATACATAC	ATCGATACGAACTCTTCGGCATTG <u>CTGCAG</u> ACGTCTTAAAATACTGCCCATTCGAAGATATCATGGGTTCTGAGGGTGACCAATCTTCCATTCGGTTGTTTTGCGAACGCTGTGGTGAGT	120
t t	TAATCACTAACGAAAGTTCTAAAGAAAAGCTCAGAGCGGAAGCTCAGCAGACGGGCAATAAGAAGATCATGGACAAGTTTGGATACTGGTATTGTGACTCTTGTAAGAAGAAGAAGAATACAT	245
AATTTTATTACCCTCTCCGAAAAGAAAATTTTTTCGTCGTCAATAGAGTTTAATGCAATACCTGATAAAGAGAGTTTTACATTGCAAGAGGTAGTGTTAATTCTGGATT <mark>ATT</mark> TTGAC ATATGTGTTTGGTTAGTGCTTGAGTACTTCCTAAGAAGTTTTACGAAAAATAAAAAGCATTTTTGTCTGAAA <u>ACTAGT</u> GGAAAGGAAAGAAAAATGTCCGGTGAAAAAAGAAGATTAGCAAGAAAGTTAGCAAGAAAGTTAGCAAGAAAGTTT MetSerValGuGluGuValSerLysLysPhe 19 GAAGAAAACGTTTCAGTCGATGATACCACAGCTACTCCCAAAGACTGTTTAAGTGACAGTGCTCACTTCGAATGCAAGCACCCCATTGAACACCCAATGGACTTTATGGTACACAAAGCCA GluGluAsnValSerValAspAspThrThrAlaThrProLysThrValLeuSerAspSerAlaHisPheAspValLysHisProLeuAsnThrLysTrpThrLeuTrpTyrThrLysPro 59 GCCGTCGATAAATCTGAGTCGTGGTCTGATCTATTACGTCCCGTCACTTCAATTCCAAACTGTTGAAGAATTTTGGGCTATCATTCAAAATATTCCTGAGCCAACGAACTACCATTGAAA AlaValAspLysSerGluSerTrpSerAspLeuLeuArgProValThrSerPheGInThrValGIuGIuPheTrpAlaIIeIIeGInAsnIIeProGluProHisGluLeuProLeuLys 99 TCCAGATTACCACGTCTTCCGTAATGACGTTAGACCTGAATGGGAAAGACGCCAATGCTAAAGGTGGTAAATGGTCTTTCCAACTTAGAGGAAAAGGTGCTGATATTGGAACTAATGG SerAspTyrHisValPheArgAsnAspValArgProGluTrpGluAspGluAlaAsnAlaLysGlyGlyLysTrpSerPheGInLeuArgGIyLysGlyAlaAspIIeAspGluLeuTrp 139 CTAAGAACTTTACTAGCAGTTATTGGTGGAAACAATTGATGAAGACGACTCCCCAAATTAACGGTGTGGTTTTAAGAAAAGGTGGTAACAAGTTGCCTTATGGACTAAATTGAAGAAACCACTTATGGAGGAAAAGGTGGTAACAAGTTGCCTTATGGACAAAATTGAAGAACAATTGATAATTAACGGTGGTGCGTTTTAAGAAAAGGTGGTAACAAGTTGCCTTATGGACAAAATTGATGAAACAATTGATGAAGACGACTCCCCAAATTAACCGGTGTCGTTTTAAGCATTAGAAAAAGGTGGTAACAAGTTGCCTTATGGAACAAATTGATGAAGAAGACGACTCCCCAAATTAACGGTGGTGCGTTTTAAGCATTAGAAAAAGGTGGTAACAAGTTGCCTTATGGACAAACAA	CTTGTGTTCTATGTGAAAGACCATTAAAGAAACTACCATGGTCATCCTCCCCTGTGGACACGAAGGTCCTTCCAGTGCATACAAGAATGGTTTCTCGATGAGAATGAACAAGAATGTCCC	368
ATATGTGTTTGTGTTAGTGCTTGAGTACTTCCTAGGAGTTTTACGAAAAATAAAAGCATTTTTGTCTGAAA <u>ACTAGT</u> GAAAGGAAGAAAATGTCCGTTGAAGAAGTTAGCAAGAAGTTA MetSerValGluGluValSerLysLysPhe 18 GAAGAAAACGTTTCAGTCGATGCTACCACAGCTACTCCAAAGACTGTTTTAAGTGACAGTGCTCACTTCGAAGTGCCAAGCAACCCAATGGACTTTATGGTAGAAAAGCCA GluGluAsnValSerValAspAspThrThrAlaThrProLysThrValLeuSerAspSerAlaHisPheAspValLysHisProLeuAsnThrLysTrpThrLeuTrpTyrThrLysPro 58 GCCGTCGATAAATCTGAGTCGTGGTCTGATCTATTACGTCCCGTCACTTCCATTCCAAACTGTTGAAGAACTTTGGGCTATCATTCAAAATATTCCTGAGCCAACGAACTACCAATGGACACTACCATTGAA AlaValAspLysSerGluSerTrpSerAspLeuLeuArgProValThrSerPheGInThrValGIuGluPheTrpAlaIleIleGinAsnIleProGluProHisGluLeuProLeuLys 98 TCAGAATTACCACGTCTTCCGTAATGACCTTAGACCTGAATGGGAAAGATGAAGCCAATGCTAAAGGTGGTAAATGGTCTTTCCAACTATGGAGAAAAGGTGGCTGATATTGGAGAAATTATGG SerAspTyrHisValPheArgAsnAspValArgProGluTrpGluAspGluAlaAsnAlaLysGlyGlyLysTrpSerPheGInLeuArgGIyLysGlyAlaAspIleAspGluLeuTrp 138 CTAAGAACTTTACTAGCAGTTATTGGTGAAACAATTGATGAAGACGACCCCCCAAATTAACGGTGTGCTTTTAAGCATTAAGAGTGGTAAACGGTGGAAAAGGTGGCAAAGCTCACCTAATTCGAA 1299 CTAAGAACTTTACTAGCAGTTATTGGTGAAACAATTGATGAAGACGACCCCCCAAATTAACGGTGTGCGTTTTAAGCATTAGGAGGAAAAGGTGGTAAACAGTTGCCTTATGGACTAATCTGAA 1299 CTAAGAACTTTACTAGCAGTTATTGGTGGTAAATTGATGAAGACGACCCCCCCAAATTAACGGTGTGCGTTTTAAGCATTAGAAAAGGTGGTAACAAGTTGCCTTATGGACTAAATTCAAA 1299 CTAAGAACCACTTATTGAGAAATTGGTGGTAAAATTGATGAAGACGACCCCCCAAATTAACGGTGTGCGTTTTAAGCATTAGAAAAGGTGGTAACAAGTTGCCTTATGGACTAAATCTGAA 1299 GACAAAGAACCACTTATTGAGAATTGGTGGTAAATTCAAGCAAG	GGCGGTTGCCCCGGTGTTGCATTCATCTAGGTTCTCCACATAATGTATAGTTTAACATATCATCACCACTTGTTTAGTTAAATCGTTTAGAGTAATATTACCCGTCAAAAAGGTCGGGTAA	48 0
MetSerValGluGluValSerLysLysPhe 19 GAAGAAAACGTTTCAGTCGATGATACCACAGCTACTCCAAAGACTGTTTTAAGTGACAGTGCTCACTTCGATGTCAAGCACCCATTGAACACCAAATGGACTTTATGGTACACAAAGCCA 849 GluGluAsnValSerValAspAspThrThrAlaThrProLysThrValLeuSerAspSerAlaHisPheAspValLysHisProLeuAsnThrLysTrpThrLeuTrpTyrThrLysPro 58 GCCGTCGATAAATCTGAGTCGTGGTCTGATCTATTACGTCCCGTCACTTCATTCCAAACTGTTGAAGAATTTTGGGCTACATTCAATAATATTCCTGAGCCAACGAACCAACC	AATTTTATTACCCTCTCCGAAAAGAAAATTTTTTCGTCGTCAATAGAGTTTAATGCAATACCTGATAAAGAGAGTTTTACATTGCAAGAGGTAGTGTTAATTCTGGATT <mark>TATA</mark> TTGTAC	688
GluGluAsnVa ISerVa IAspAspThrThrAlaThrProLysThrVa ILeuSerAspSerAlaHisPheAspVa ILysHisProLeuAsnThrLysTrpThrLeuTrpTyrThrLysPro 59 GCCGTCGATAAATCTGAGTCGTGGTCTGATCTATTACGTCCCGTCACTTCATTCCAAACTGTTGAAGAATTTTGGGCTATCATTCAAAATATTCCTGAGCCAACGAACTACCATTGAAAA 968 AlaVa IAspLysSerGluSerTrpSerAspLeuLeuArgProVa IThrSerPheGInThrVaIGIuGluPheTrpAlaIleIleGInAsnIleProGluProHisGluLeuProLeuLys 96 TCAGATTACCACGTCTTCCGTAATGACCGTGAATGGGAAGATGAAGCCAATGCTAAAGGTGGTAAATGGTCTTTCCAAATTAAAGGTGGTAAAAGGTGGTGATATTGATGAATTATGG 1888 SerAspTyrHisVaIPheArgAsnAspVaIArgProGIuTrpGIuAspGIuAlaAsnAlaLysGIyGIyLysTrpSerPheGInLeuArgGIyLysGIyAlaAspIleAspGIuLeuTrp 138 CTAAGAACTTTACTAGCAGTTATTGGTGAAACAATTGATGAAGACGACTCCCAAAATTAACGGTGTGCGTTTTAAGCATTAGAAAAAGGTGGTAAAAAGGTGGTAACAAGTTTGGACTAAATCTGAA 1296 CTAAGAACTTTACTAGCAGTTATTGGTGAAACAATTGATGAAGACGACTCCCCAAATTAACGGTGTGCGTTTTAAGCATTAGAAAAAGGTGGTAACAAGTTTGGACTAAATCTGAA 1296 CTAAGAACTTTACTAGCAGGTGATATTGGTGGAAACAATTGATGAAGAAGACGACTCCCCAAATTAACGGTGTGCGTTTTAAGCATTAGGAAAAGGTGGTAACAAGTTGGCCTAATGGACAACCAATTGGTGATAATCGAAGACGACTCCCCAAAGTAATCTGAA 1296 CTAAGAACCACTATCGCGGGAAATTGGTGGTAAATTCAAGCAAG		
A laVa lAspLysSerGluSerTrpSerAspLeuLeuArgProValThrSerPheGInThrValGluGluPheTrpAlaIleIleGInAsnIleProGluProHisGluLeuProLeuLys * TCAGATTACCACGTCTTCCGTAATGACGTTAGACCTGGAATGGGAAGATGAAGCCAATGCTAAAGGTGGTAAATGGTCTTTCCAACTTAGAGGAAAAGGTGCTGATATTGATGAATTATGG SerAspTyrHisValPheArgAsnAspValArgProGluTrpGluAspGluAlaAsnAlaLysGlyGlyLysTrpSerPheGInLeuArgGIyLysGlyAlaAspIleAspGluLeuTrp 138 CTAAGAACTTTACTAGCAGTTATTGGTGAAAACAATTGATGAAGACGACTCCCCAAATTAACGGTGTCGTTTTAAGCATTAGGAAAAGGTGGTAACAAGTTTGCCTTATGGACTAAATCTGAA 1296 CTAAGAACTTTACTAGCAGTTATTGGTGAAAACAATTGATGAAGACGACTCCCCAAATTAACGGTGTGCGTTTTAAGCATTAGGAAAAGGTGGTAACAAGTTTGCCTTATGGACTAAATCTGAA 1296 CTAAGAACCTATTGCAGAAGTTATTGGTGGTAAAACAATTGATGAAGACGACCCCCCAAATTAACGGTGGTGCGTTTTAAGCATTAGGAAAGGTGGTAACAAGTTTGCCTTATGGACTAAATCTGAA 1296 CTAAGAACCACTATTGAGAAATTGGTGGTAAATTCAAGCAAG	GAAGAAAACGTTTCAGTCGATGATACCACAGCTACTCCAAAGACTGTTTTAAGTGACAGTGCTCACTTCGATGTCAAGCACCCATTGAACACCAAATGGACTTTATGGTACACAAAGCCA GIuGIuAsnVaISerVaIAspAspThrThrAIaThrProLysThrVaILeuSerAspSerAIaHisPheAspVaILysHisProLeuAsnThrLysTrpThrLeuTrpTyrThrLysPro	
SerAspTyrHisValPheArgAsnAspValArgProGluTrpGluAspGluAlaAsnAlaLysGlyGlyLysTrpSerPheGlnLeuArgGlyLysGlyAlaAspIleAspGluLeuTrp 138 CTAAGAACTTTACTAGCAGTTATTGGTGAAACAATTGATGAAGACGACTCCCAAATTAACGGTGTCGTTTTAAGCATTAGAAAAGGTGGTAACAAGTTTGCCTTATGGACTAAATCTGAA 1298 LeuArgThrLeuLeuAlaValIleGlyGluThrIleAspGluAspAspSerGlnIleAsnGlyValValValLeuSerIleArgLysGlyGlyAsnLysPheAlaLeuTrpThrLySerGlu 178 GACAAAGAACCACTATTGAGGAATTGGTGGTAAATTCAAGCAAG		
LeuArgThrLeuLeuAIaVaIIIeGIyGIuThrIIeAspGIuAspAspSerGInIIeAsnGIyVaIVaIVaILeuSerIIeArgLysGIyGIyAsnLysPheAlaLeuTrpThrLysSerGIu 179 GACAAAGAACCACTATTGAGAATTGGTGGTAAATTCAAGCAAG		
AspLysGluProLeuLeuArgIleGlyGlyLysPheLysGlnValLeuLysLeuThrAspAspGlyHisLeuGluPhePheProHisSerSerAlaAsnGlyArgHisProGlnProSer 210 ATCACCTTGTAAGATAGTCTGAATTTTTCTTAAGATAATTGTTATTGTTATTTAATCAAAATTATATAATATAATATAATATATAT		
IleThrLeu ● 213		
ТСАТ <u>GTTAAC</u> ACAGAAGGAAAAGTCAAAATGAAGAAAGAAAAAAAAAA		- · · -
	ТСАТ <u>GTTAAC</u> ACAGAAGGAAAAAGTCAAAAATGAAGAAAGAAAAAAAAAA	1 586

FIG. 3. Sequence of *CDC33* genomic DNA (1,500 nucleotides of the coding strand are shown). Restriction sites shown in Fig. 1 are underlined. Differences between this sequence and that of Altmann et al. (2) are indicated by arrows. The TATA box is boxed. An asterisk indicates the serine at position 55 which may correspond to serine 53 of human eIF-4E, which has been shown to be phosphorylated (16).

mRNAs have altered affinity for mutated eIF-4E or if these mRNAs are uncapped. Translational discrimination has been reported in the case of mammalian eIF-4F, a capbinding protein complex composed of p220, eIF-4A, and eIF-4E. It has been reported that inactivation of eIF-4F in lysates of heat-shocked cells is responsible for a decreased rate of protein synthesis and for the preferential translation of heat shock mRNAs (11). In addition, eIF-4E is a phosphorylated protein and is dephosphorylated upon heat shock (5). These findings suggest that eIF-4E phosphorylation may play a role in the regulation of initiation. Since the sequence surrounding the phosphorylation site of human eIF-4E (15, 16) is highly conserved in yeast eIF-4E (Fig. 3), yeast eIF-4E may also be phosphorylated. It will be interesting to investigate the phosphorylation state of yeast eIF-4E and the functional significance of this with regard to heat shock, translation, and cell cycle control.

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LITERATURE CITED

- 1. Altmann, M., N. Edery, N. Sonenberg, and H. Trachsel. 1985. Purification and characterization of protein synthesis factor eIF-4E from the yeast *Saccharomyces cerevisiae*. Biochemistry 24:6085–6089.
- Altmann, M., C. Handschin, and H. Trachsel. 1987. mRNA cap-binding protein: cloning of the gene encoding protein synthesis initiation factor eIF-4E from Saccharomyces cerevisiae. Mol. Cell. Biol. 7:998–1003.
- 3. Boutelet, F., A. Petitjean, and F. Hilger. 1985. Yeast cdc35 mutants are defective in adenylate cyclase and are allelic with

cyrl mutants, while CASI, a new gene, is involved in the regulation of adenylate cyclase. EMBO J. 4:2635–2641.

- Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler. 1987. The S. cerevisiae CDC25 gene product regulates the RAS/adenylate cyclase pathway. Cell 48:789-799.
- 5. Duncan, R., S. C. Milburn, and J. W. B. Hershey. 1987. Regulated phosphorylation and low abundance of HeLa cell initiation factor eIF-4F suggest a role in translational control. J. Biol. Chem. 262:380-388.
- Iida, H., and I. Yahara. 1984. Durable synthesis of high molecular weight heat shock protein in G₃0 cells of the yeast and other eukaryotes. J. Cell Biol. 99:199–207.
- Matsumoto, K., I. Uno, and T. Ishikawa. 1983. Control of cell division in *Saccharomyces cerevisiae* mutants defective in adenylate cyclase and cAMP-dependent protein kinase. Exp. Cell Res. 146:151-161.
- Matsumoto, K., I. Uno, and T. Ishikawa. 1984. Identification of the structural gene and nonsense alleles for adenylate cyclase in Saccharomyces cerevisiae. J. Bacteriol. 157:277-282.
- Matsumoto, K., I. Uno, Y. Oshima, and T. Ishikawa. 1982. Isolation and characterization of yeast mutants deficient in adenylate cyclase and cyclic AMP dependent protein kinase. Proc. Natl. Acad. Sci. USA 79:2355-2359.
- 10. Nakayama, N., A. Miyajima, and K. Arai. 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. 4:2643-2648.
- Panniers, R., E. B. Stewart, W. C. Merrick, and E. C. Henshaw. 1985. Mechanism of inhibition of polypeptide chain initiation in heat shocked Ehrlich cells involves reduction of eukaryotic initiation factor 4F activity. J. Biol. Chem. 260:9648–9653.
- 12. Pringle, J. R., and L. H. Hartwell. 1982. The Saccharomyces cerevisiae cell cycle, p. 97–142. In J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast Saccharomyces cerevisiae: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 13. Reed, S. I. 1980. The selection of S. cerevisiae mutants defec-

tive in the start event of cell division. Genetics 95:561-577.

- Rivin, C. J., and W. L. Fangman. 1980. Cell cycle phase expansion in nitrogen-limited cultures of Saccharomyces cerevisiae. J. Cell Biol. 85:96-107.
- Rychlik, W., L. L. Domier, P. R. Gardner, G. M. Hellmann, and R. E. Rhoads. 1987. Amino acid sequence of the mRNA cap-binding protein from human tissues. Proc. Natl. Acad. Sci. USA 84:945-949.
- 16. Rychlik, W., M. A. Russ, and R. E. Rhoads. 1987. Phosphorylation site of eukaryotic initiation factor 4E. J. Biol. Chem. 262:

10434-10437.

- 17. Tajima, M., Y. Nogi, and T. Fukasawa. 1985. Primary structure of the Saccharomyces cerevisiae GAL7 gene. Yeast 1:67-77.
- 18. Tanaka, K., K. Matsumoto, and A. Toh-e. 1988. Dual regulation of the expression of the polyubiquitin gene by cyclic AMP and heat shock in yeast. EMBO J. 7:495-502.
- Yamano, S., K. Tanaka, K. Matsumoto, and A. Toh-e. 1987. Mutant regulatory subunit of 3',5'-cAMP dependent protein kinase of yeast Saccharomyces cerevisiae. Mol. Gen. Genet. 210:413-418.