



ELSEVIER

Biochimica et Biophysica Acta 1448 (1998) 236–244

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

The fork head transcription factor Hcm1p participates in the regulation of *SPC110*, which encodes the calmodulin-binding protein in the yeast spindle pole body

Gefeng Zhu¹, Trisha N. Davis^{*}

Department of Biochemistry, University of Washington, Seattle, WA 98195, USA

Accepted 13 October 1998

Abstract

We previously identified *HCMI* as a dosage-dependent suppressor of a calmodulin temperature-sensitive mutant (*cmd1-1*). Calmodulin performs multiple functions in yeast. Here we demonstrate that the effects of *HCMI* are specific to the role of calmodulin at the spindle pole body. Overexpression of *HCMI* fully suppresses the temperature sensitivity of a calmodulin mutant (*cmd1-3*) that only has defects in assembly of the spindle pole body but does not suppress the temperature sensitivity of a calmodulin mutant (*cmd1-8*) that only affects other functions of calmodulin. The DNA binding specificity of Hcm1p was determined by a selection, amplification and binding protocol. The consensus sequence for an Hcm1p binding site is WAAYAAACA AW. Mutations in the DNA binding domain of Hcm1p abolish the ability of Hcm1p to specifically recognize this binding site and abolish the ability of Hcm1p to act as a suppressor of calmodulin mutants. The promoter of *SPC110* contains a match to the consensus binding site. Deletion of *HCMI* does not affect the basal level of *SPC110* transcription, but reduces the induction that occurs late in G₁ of the cell cycle. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Calmodulin; Fork head protein; Spindle pole body; Centrosome; Yeast

1. Introduction

Since the initial discovery that calmodulin is essential for the growth of budding yeast cells [1], two essential functions of calmodulin have been identified. Calmodulin acts as a light chain for the class V myosin, Myo2p, and calmodulin is required for the proper assembly of the spindle pole body component Spc110p [2–6]. Characterization of temperature-sensitive mutants of calmodulin suggests that two additional functions may exist but the calmodulin targets responsible for performing these functions have not yet been identified [7]. We found that *HCMI*, which encodes a protein with homology to the fork head DNA binding domain, is a dosage-dependent suppressor of a calmodulin temperature-sensitive mutant. The mutant displays defects in spindle pole assembly and in bud emergence, but overexpression of *HCMI* only suppresses the mitotic defects [8,9]. *HCMI* is not an essential gene but deletion of the gene (*hcm1Δ*) exacerbates the phenotype of the calmodulin temperature-sensitive mutant. The specificity of the suppression suggested that Hcm1p had a

sitive mutants of calmodulin suggests that two additional functions may exist but the calmodulin targets responsible for performing these functions have not yet been identified [7]. We found that *HCMI*, which encodes a protein with homology to the fork head DNA binding domain, is a dosage-dependent suppressor of a calmodulin temperature-sensitive mutant. The mutant displays defects in spindle pole assembly and in bud emergence, but overexpression of *HCMI* only suppresses the mitotic defects [8,9]. *HCMI* is not an essential gene but deletion of the gene (*hcm1Δ*) exacerbates the phenotype of the calmodulin temperature-sensitive mutant. The specificity of the suppression suggested that Hcm1p had a

^{*} Corresponding author;
E-mail: tdavis@u.washington.edu

¹ Present address: Mayo Clinic, Department of Immunology, Rochester, MN 55905, USA.

function related to the role of calmodulin at the spindle pole body. However, the most obvious hypothesis, that Hcm1p regulated the levels of the *CMD1* transcript, was disproved because deletion of *HCM1* affects neither the levels of the transcript nor the levels of calmodulin [9]. Here we demonstrate that overproduction of Hcm1p specifically suppresses defects in the spindle pole body caused by defective interactions between calmodulin and Spc110p. We identify a consensus for the DNA recognition sequence of Hcm1p. Consistent with the demonstration that Hcm1p does not regulate transcription of *CMD1*, the *CMD1* promoter does not contain a match to the consensus. However, *SPC110*, the gene encoding the target of calmodulin at the spindle pole body, contains an Hcm1p binding site in the promoter. Moreover, Hcm1p regulates the transcription of *SPC110*.

2. Materials and methods

2.1. Strains and media

Media LB, YPD, SD, and SpoIII were as described previously [8,10]. Yeast transformants were selected on minimal medium SD with appropriate supplements [9]. *Escherichia coli* strain XL1-blue [11] was used routinely as a plasmid host. *E. coli* strain S1540 (*dam*⁻, *dcm*⁻, gift from Colin Manoil, source Stanley Brown) was used to make demethylated plasmids. Fusion proteins were expressed in *E. coli* strain GM1 [12]. *E. coli* strain CJ236 was used for site-directed mutagenesis ([13] and Bio-Rad). All yeast strains used in this study are listed in Table 1 and are derived from W303 [14] except ELW55-7A.

2.2. Plasmids

Plasmids are listed in Table 2. Only constructions that are not simple subclonings are described. Plasmid pGF57 containing *hcm1-3* and pGF58 containing *hcm1-1* were made by site-directed mutagenesis using pGF10 as the template. pGF57 was mutagenized again to make pGF59, containing *hcm1-4*. The *SalI-NotI* fragments containing *HCM1*, *hcm1-3*, *hcm1-1*, or *hcm1-4* from plasmids pGF10, pGF57,

pGF58, or pGF59 were ligated into pGF29 to make plasmids pGF54, pGF60, pGF61, or pGF62. In pGF80, two copies of an oligo encoding Flag, an 8-residue peptide with sequence of DYKDDDDK [15,16], were introduced into the *NcoI* site of pGF11 by annealing two primers GZ-41 (CATG-GACTACAAAGACGATGACGATAAAGG) and GZ-42 (CATGCCTTTATCGTCATCGTCTTTGT-AGTC) followed by ligation. The *BamHI* site in vector pGF80 was removed by digesting pGF80 with *SmaI* and *NotI* followed by filling in the *NotI* site with Klenow and religating, which generated pGF83. The *EagI-BamHI* fragment of pGF83, which carried a short fragment of Hcm1p (amino acids 1–138) and two copies of Flag sequences fused in frame to Hcm1p was isolated and ligated into the same sites of pGF54, pGF60, pGF61, and pGF62 to replace the non-tagged Hcm1p fragments to make plasmids pGF84, pGF85, pGF86, and pGF87.

Plasmid pGF25 encoding GST-Hcm1p was made by ligating a 855 bp *NcoI-ClaI* DNA fragment encoding the fork head domain of Hcm1p (amino acids 1–284) to pGEX-2T digested with *BamHI* and *EcoRI*. The 5' overhang left by the digestion was filled in using the Klenow fragment of DNA polymerase. Plasmids expressing GST-Hcm1-1p and GST-Hcm1-4p were constructed in two steps. The 1043 bp *SacI-EcoRI* fragments of pGF58 and pGF59 were ligated to pGF25 digested with *SacI-EcoRI* to create pGF191 and pGF192. The extra 677 bp *ClaI-EcoRI* fragment was then removed by digesting pGF191 and pGF192 with *BspDI* and *EcoRI* followed by filling in with the Klenow and religating to make plasmids pGF197 and pGF198.

Plasmid pMM78 was constructed by ligating an *NcoI-SalI* fragment containing the *BglII-EcoRV* fragment of *MET3* promoter region [17] into pMM48, a pRS315 derivative carrying the *CMD1* gene. An *NcoI* site was added to the *EcoRV* site of *MET3* for convenient cloning, which resulted in removing 66 bp upstream from the start codon of *MET3*. In pMM78, the *CMD1* gene is under the control of the *MET3* promoter. To fuse the *MET3* promoter with genes of interest, the *CMD1* gene was removed from the *NcoI* site and replaced by a new gene containing *NcoI* or *BspHI* at the start codon. Plasmid pGF129 containing *MET3-PDS1* was con-

Table 1
Yeast strains

Strain	Genotype ^a	Source or Ref.
BCY4	<i>MATa cmd1-8</i>	[4]
CRY1	<i>MATa</i>	Robert Fuller
GZY21-3B	<i>MATa ade3Δ-100 cmd1-1 hcm1Δ::TRP1 cyh2? his3?</i>	[9]
GZY21-6C	<i>MATa ade3Δ-100 hcm1Δ::TRP1 cyh2? his3?</i>	[9]
GZY35-9D	<i>MATa hcm1Δ::TRP1 sst1Δ::LEU2</i>	This study
JGY41	<i>MATa cmd1-3</i>	[10]
JGY44-2A	<i>MATa cmd1-1</i>	[8]
JGY46	<i>MATa/MATα</i>	[10]
TDY72-5D	<i>MATa sst1Δ::LEU2</i>	[37]
ELW55-7A	<i>MATα cdc31-2 ADE2 CAN1 HIS3 trp1Δ ura3-52</i>	

^aAll strains contain the following markers except as noted: *ade2-1oc can1-100 his3-11, 15 leu2-3, 112 trp1-1 ura3-1*.

structed by ligating pMM78 *NcoI-SpeI* fragment with a 1.38 kb fragment containing *PDS1/ESP2* amplified by PCR from plasmid *ESP2/YEp351* (gift from Breck Byers). Plasmid pGF136 containing *MET3-SPC110* was created by ligating pMM78 *NcoI-NotI* fragment with a *NcoI-NotI* fragment carrying *SPC110* isolated from pMM80, a pRS305 derivative containing *MET3-SPC110*. pGF145 was made by cloning a 2.2 kb *BspHI-BamHI* fragment containing *CIK1* amplified by PCR from genomic DNA into pMM78 *NcoI-BamHI* sites. Plasmid pGF148 containing *MET3-ESP1* was made in several steps. A 340 bp *NcoI-PstI* fragment was PCR amplified from plasmid CP1 [18]. This fragment was ligated into the pGEM-T vector to give pGF127. Then a *NcoI-NotI* fragment from pGF127 was ligated into pMM78 to form pGF134. pGF134 was digested with *PstI-SacII*, and ligated with a *PstI-SacII* fragment containing *ESP1* (1064–3820) isolated from pGF132 to form pGF135. Plasmid pGF132 is pBluescriptII KS– with the *PstI-SpeI* fragment containing an internal piece of *ESP1* (1064–3820). The third piece of *ESP1* (3616–5961) isolated from CP1 by digestion with *HindIII* and *EcoRI* was cloned into pBluescriptII KS– to give pGF131. The *SpeI* site in pBluescript of pGF131 was removed by digesting the plasmid with *BamHI* and *NotI* followed by religation (pGF131-2). Finally, a *SpeI-SacI* fragment containing *ESP1* (3820–5961) from pGF131-2 was ligated to pGF135 *SpeI-SacI* sites to form pGF148.

All the PCR products used for cloning were amplified by a high-fidelity thermophilic DNA polymerase Vent DNA polymerase (New England Biolabs).

2.3. Preparation of fusion proteins

GST-Hcm1p^{1–285}, GST-Hcm1-1p^{1–285}, GST-Hcm1-4p^{1–285} fusion proteins were expressed from plasmids pGF25, pGF197, and pGF198 respectively in *E. coli* strain GM1 [12]. Cells were grown to 30 Klett units in LB medium containing 100 µg/ml ampicillin and then IPTG was added to a final concentration of 1 mM. After 3 h induction, cells were harvested and lysed with a French Press as described [19]. The fusion proteins were affinity purified as described (Pharmacia). The proteins were dialyzed, lyophilized, dispensed into aliquots and stored at –80°C.

2.4. Selection of DNA sequences recognized by Hcm1p

The DNA recognition sites of Hcm1p were identified using a modified SAAB protocol as described [20]. The degenerate oligonucleotide used in the site selection was GZ-16 with sequence of CTAGATATCCCTGGATCCTAA(N)₁₅GAATTCAGGCTCAAAGCTCAC. Primers GZ-17 (GTGAGCTTTGAGCCTGAATTC) and GZ-18 (CTAGATATCCCTGGATCCTAA) were used for PCR amplification of the DNA that bound to GST-Hcm1p^{1–285}.

2.5. Gel shift assay

The probes used for gel shift assays [21,22] were amplified by PCR using primers recognizing sequences flanking the N₁₅ sequences. One-tenth of the

PCR products was labeled with [γ - 32 P]ATP as described [23]. Affinity-purified GST or GST-fusion proteins (50 ng) were incubated with $1\text{--}3 \times 10^4$ cpm of probe in 20 μ l of gel shift buffer containing poly-(dI-dC) and 5 μ g bovine serum albumin. Fusion proteins were diluted in gel shift buffer containing 0.2% NP40. The DNA was incubated with protein at 21°C for 30 min and then subjected to electrophoresis as described [23].

2.6. Synchronization using α -factor

Strains TDY72-5D (*HCMI*, *sst1* Δ) and GZY35-9D (*hcm1* Δ , *sst1* Δ) were grown to 50 Klett units in 650 ml YPD at 30°C. α -Factor was added to the final concentration of 20 nM. Cells were harvested by filtration after 2 h, washed with pre-warmed YPD, and

resuspended in 650 ml conditioned medium [24] prepared from strain CRY1. Greater than 95% of the cells formed a shmoo. The cells were incubated with shaking at 30°C. Fifty ml aliquots were removed every 10 min for the first 70 min and then every 15 min for a total of 145 min. The aliquots were added to a tube containing 0.5 ml 10% sodium azide to stop cell growth and immediately chilled by transferring into a new tube containing 5 ml of frozen 0.2 M EDTA. Cells were centrifuged, washed with cold water, and the RNA isolated.

2.7. RNA isolation and Northern blot analysis

Preparation of total RNA and poly(A)-containing RNA and the Northern blot analysis were as described [9].

Table 2
Plasmids

Name	Parent	Relevant markers	Ref.
pGF10	pBluescriptII KS-	3.5 kb <i>PstI-SalI</i> fragment containing <i>HCMI</i>	[9]
pGF11	pGF10	<i>HCMI</i> with <i>NcoI</i> site at ATG	This study
pGF25	pGEX-2T	Hcm1p amino acids 1–285 fused to GST	This study
pGF29	pRS306	2 μ m origin, <i>URA3</i> , <i>EcoRI</i> fragment containing 2 μ m from YEp24 in pRS306 <i>AatII</i> site	[20]
pGF54	pGF29	3.4 kb <i>SalI</i> ^a - <i>PstI</i> fragment containing <i>HCMI</i>	This study
pGF57	pGF10	<i>hcm1-3</i>	This study
pGF58	pGF10	<i>hcm1-1</i>	This study
pGF59	pGF57	<i>hcm1-4</i>	This study
pGF60	pGF29	2 μ m, <i>URA3</i> , <i>hcm1-3</i>	This study
pGF61	pGF29	2 μ m, <i>URA3</i> , <i>hcm1-1</i>	This study
pGF62	pGF29	2 μ m, <i>URA3</i> , <i>hcm1-4</i>	This study
pGF80	pGF11	2 copies of Flag sequences inserted into <i>HCMI NcoI</i> site	This study
pGF83	pGF80	The vector <i>BamHI</i> site in pGF80 was removed	This study
pGF84	pGF29	2 μ m, <i>URA3</i> , Flag-tagged <i>HCMI</i>	This study
pGF85	pGF29	2 μ m, <i>URA3</i> , Flag-tagged <i>hcm1-1</i>	This study
pGF86	pGF29	2 μ m, <i>URA3</i> , Flag-tagged <i>hcm1-3</i>	This study
pGF87	pGF29	2 μ m, <i>URA3</i> , Flag-tagged <i>hcm1-4</i>	This study
pGF129	pMM78	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-PDS1</i>	This study
pGF134	pMM78	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-ESP1</i> (aa 1–114)	This study
pGF135	pGF134	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-ESP1</i> (aa 1–1065)	This study
pGF136	pMM78	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-SPC110</i>	This study
pGF197	pGEX-2T	Hcm1-1p fused to GST	This study
pGF198	pGEX-2T	Hcm1-4p fused to GST	This study
pGF145	pMM78	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-CIK1</i>	This study
pGF148	pMM78	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-ESP1</i>	This study
pMM78	pRS315	<i>CEN6</i> , <i>ARSH4</i> , <i>LEU2</i> , <i>MET3-CMD1</i>	This study
CPI	YEp13	2 μ m, <i>LEU2</i> , <i>ESP1</i>	[18]
ESP2/YE p351	YEp351	2 μ m <i>LEU2</i> , <i>ESP2</i>	Gift from Breck Byers

^aSite is within the multiple cloning site of the parent plasmid.

2.8. Expression of genes under control of the *MET3* promoter

Genes under the control of the *MET3* promoter were induced by growing cells containing *MET3*-gene constructs on minimal selective media without methionine. The genes were turned off by growing the cells on minimal selective media containing 0.25 mg/ml methionine.

3. Results

The *cmd1-1* allele confers defects in the assembly of the spindle pole body and delays in bud emergence [3,8]. Overexpression of *HCM1* overcomes the spindle pole body defects conferred by the *cmd1-1* allele but does not affect the delay in bud emergence [9]. This suggested that *HCM1* is specifically involved in the spindle pole body function of calmodulin. Overexpression of *HCM1* fully suppresses the temperature sensitivity conferred by *cmd1-3* (Table 3), an allele conferring only mitotic defects as demonstrated by the fact that it is fully suppressed by mutations in *SPC110* [2]. The specificity of the *HCM1* suppression is further shown by the demonstration that *HCM1* is not a dosage-dependent suppressor of the *cmd1-8* allele (Table 3), which interferes with bud growth but has no effect on the spindle pole body [4,25]. Moreover, the effects are specific to calmodulin because overexpression of *HCM1* has no effect on the temperature sensitivity conferred by a mutation in *CDC31*, which encodes a Ca²⁺-binding protein that

is related to calmodulin and is also associated with the spindle pole body (Table 3).

3.1. The DNA recognition site of *Hcm1p*

We identified the recognition sites of *Hcm1p* by a selection, amplification, and binding protocol exploiting GST-*Hcm1p* fusion proteins as described in Section 2. The visible gel shift bands were observed after four cycles for GST-*Hcm1p* (Fig. 1). Selected fragments were cloned and sequenced after eight cycles. Fourteen out of 23 sequences defined a *Hcm1p* binding consensus (Table 4).

3.2. The fork head DNA binding domain is required for *Hcm1p* to act as a dosage-dependent suppressor of *cmd1-1*

We mutagenized the DNA binding domain of *Hcm1p* to determine whether the ability to act as a dosage-dependent suppressor required the ability to bind DNA. By homology with the fork head protein HNF-3 γ , for which the three-dimensional structure is known [26], residues 150–163 of *Hcm1p* form the third helix that directly contacts DNA (Fig. 2). We mutated four residues in this helix: N155, H159, S162, and L163. These residues contact the DNA directly in the HNF-3 γ -DNA crystal structure. The first three residues are completely conserved between HNF-3 γ and *Hcm1p* and L163 is a conservative replacement. In *Hcm1-1p*, the only mutation is N155A; *Hcm1-3p* contains the three mutations H159A, S162A, and L163A, and *Hcm1-4p* contains

Table 3

Growth of cells carrying temperature-sensitive mutations in *CMD1* in the presence of multiple copies of *HCM1*^a

Allele	Mutations in <i>CMD1</i>	Vector	2 μ - <i>HCM1</i>
<i>CMD1</i>	none	38°C	38°C
<i>cmd1-1</i>	I100N, E104V	30°C	32°C
<i>cmd1-3</i>	D20A, E31V, D56A, E67V, D93A, E104V	34°C	37°C
<i>cmd1-8</i>	G113V	37°C	37°C
<i>cdc31-2</i>		25°C	25°C

^aCells carrying different *cmd1* alleles or *cdc31* were grown in YPD medium to exponential phase and transformed with the plasmids shown. The transformants were selected on SD-ura plates. The growth temperature shown is the highest temperature at which the strain could form colonies. The temperatures tested were 21°C, 25°C, 30°C, 32°C, 34°C, 37°C and 38°C. Transformants were allowed to grow for 3–4 h at 21°C before being incubated for 3 days at a higher temperature. Several different transformants of each strain and plasmid gave an identical phenotype. The strains were: *CMD1*, CRY1; *cmd1-1*, JGY44-2A; *cmd1-3*, JGY41; *cmd1-8*, BCY4; and *cdc31-2*, ELW55-7A. The plasmids used were: vector, pGF29 and *HCM1*, pGF54.

Table 4
Alignment of DNA binding sites recognized by Hcm1p

SAAB selected sequence
AAACAAACAAT (5) ^a
AAACAAACAAA (2)
AAATAACAAT (1)
AAATAACAAA (1)
TAACAAACAAA (2)
TAACAAACAAG (1)
CAATAACAAT (1)
CAATAACAAG (1)
Consensus ^b WAAYAAACAAW

^aNumber of selected oligonucleotides with this sequence.

^bPresent in greater than 80% of the selected sequences.

be regulated by Hcm1p. Four genes whose functions appeared to be related to the spindle pole body were chosen for further study. They were *ESPI* [18], *PDS1* [27] *CIK1* [28], and *SPC110* [2]. Northern blot analyses of RNA isolated from asynchronous cultures showed that these genes were expressed at similar levels in *HCM1* wild-type cells and in *hcm1Δ* cells (data not shown).

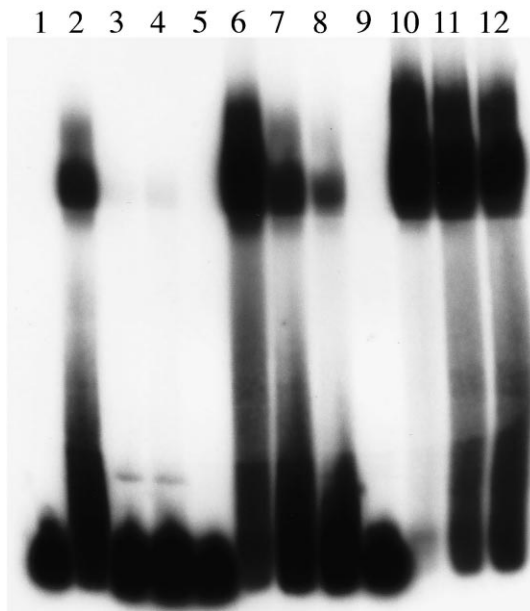


Fig. 3. Gel shift analysis of mutant Hcm1ps. An aliquot (100 ng) of GST (lanes 1, 5, 9), GST-Hcm1p (lanes 2, 6, 10), GST-Hcm1-1p (lanes 3, 7, 11), or GST-Hcm1-4p (lanes 4, 8, 12) were incubated with 60 pmole (4×10^5 cpm) of [γ -³²P]ATP labeled HCM1-23 (AAATAACAAA). Different amounts of dI-dC were included in the incubations: lanes: 1–4, 40 ng; 5–8, 8 ng, 9–12, 0.8 ng.

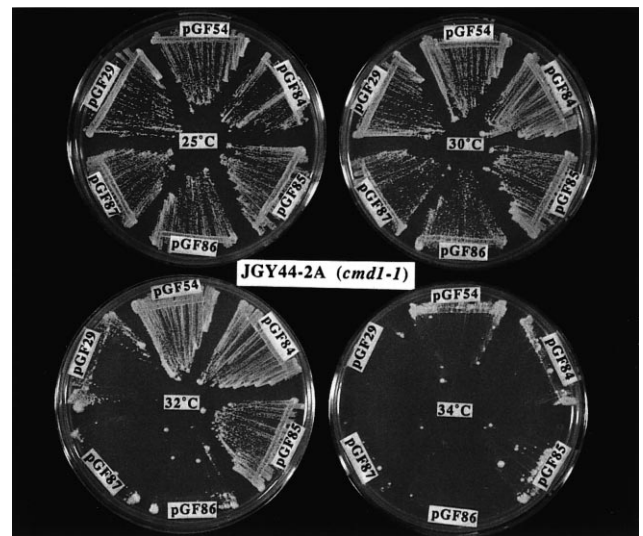


Fig. 4. Growth of cells carrying *cmd1-1* in the presence of multiple copies of wild-type and mutant *HCM1*. Strain JGY44-2A was transformed with the plasmids indicated. The Ura⁺ transformants were streaked on SD-ura plates, incubated at 21°C for 4 h, and shifted to the temperatures indicated. pGF29, vector; pGF54, Hcm1p; pGF84, Flag-Hcm1p; pGF85, Flag-Hcm1-1p; pGF86, Flag-Hcm1-3p; pGF87, Flag-Hcm1-4p.

We further examined the regulation of *SPC110* because it encodes the target of calmodulin at the spindle pole body. Transcription of *SPC110* is cell cycle regulated, being induced early in the cell cycle [29]. To determine whether Hcm1p plays a role in this cell cycle regulation, we isolated poly(A) containing RNAs from different time points after synchronizing *HCM1* wild-type cells and *hcm1Δ* cells with α -factor. In two separate experiments, *SPC110* expression was slightly decreased in *hcm1Δ* cells as compared to *HCM1* control cells. Deletion of *HCM1* decreased but did not abolish the cell cycle regulation of *SPC110* (Fig. 5).

We next tested whether overcoming the decreased expression of *SPC110* was sufficient to rescue the poor growth of the *cmd1-1*, *hcm1Δ* double mutant. Strain GZY21-3B carrying *cmd1-1*, *hcm1Δ* grows poorly at 21°C, while strain JGY44-2A carrying *cmd1-1* alone grows well at 21°C and grows poorly at 30°C. We reasoned if the decreased expression of *SPC110* caused by *hcm1Δ* was the only cause of the poor growth, then expression of *SPC110* under the control of a heterologous promoter should allow normal growth. We found that *SPC110* under the control of the *MET3* promoter allowed growth of

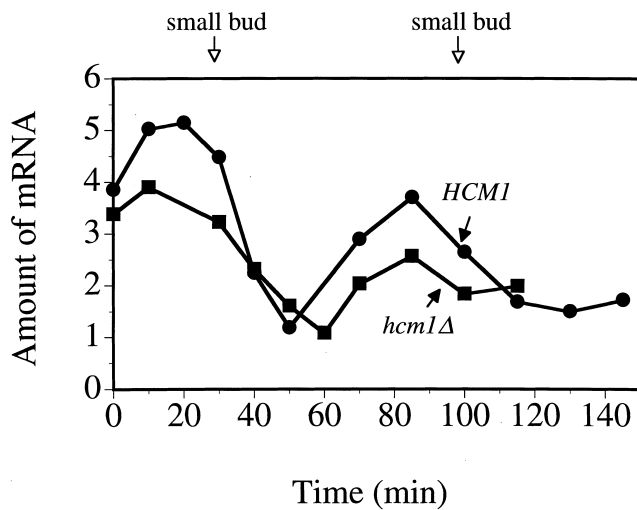


Fig. 5. Northern blot analysis of transcription of *SPC110*. Cultures of strain TDY72-5D (*HCM1*) and strain GZY35-9D (*hcm1Δ*) were synchronized by treatment with the mating pheromone α -factor. Poly(A) RNAs were isolated at the time points shown as described in Section 2. The levels of *SPC110* mRNA were quantified using a phosphoimager and normalized to that in a *TRX1* control. The probe for *SPC110* was a 2.5 kb *Hind*III fragment [29]. The probe for *TRX1* was a 0.5 kb *Nco*I-*Xho*I fragment [38]. Hybridization was carried out for 48 h at 65°C with both probes.

the *cmd1-1*, *hcm1Δ* double mutant strain up to 34°C. However, the same construct allowed the *cmd1-1* single mutant to grow well up to 37°C. Thus, deletion of *HCM1* decreases the growth rate of the *cmd1-1* mutant even in the presence of *MET3-SPC110*. We also showed that neither the *MET3-CIK1*, the *MET3-ESP1*, nor the *MET3-PDS1*, constructs suppressed the poor growth of the *cmd1-1*, *hcm1Δ* double mutant nor the temperature sensitivity of *cmd1-1*.

4. Discussion

Fork head proteins are a family of transcription factors implicated in development and differentiation in multicellular organisms [30]. All family members share a ‘winged helix’ domain or fork head domain, which was originally found in the protein encoded by the *Drosophila* gene *fork head* [31]. The ability of the fork head domain to bind DNA was recognized upon characterization of hepatocyte nuclear factors, HNF-3 α , HNF-3 β , and HNF-3 γ [32–34]. In the past 8 years, more than 80 family members have been

identified in almost all eukaryotic cells from yeast to human [35]. The yeast *Saccharomyces cerevisiae* contains four proteins that share homology with the fork head DNA binding domain. Fhl1p is involved in RNA maturation [36]. Fkh1p and Fkh2p act as repressors of pseudohyphal growth [20]. *HCM1* is a dosage-dependent suppressor of a temperature-sensitive calmodulin mutant [9].

In yeast, the genes regulated by the fork head transcription factors have been difficult to identify. Here we identify the DNA recognition sequence for Hcm1p and use that information to identify *SPC110* as one gene regulated by Hcm1p. *SPC110* encodes the essential calmodulin-binding protein at the spindle pole body. The sequence AAATAAA-CAAA lies 105 base pairs upstream from the ATG in *SPC110*. This sequence matches the consensus for an Hcm1p binding site and was one of the sequences actually identified in the selection for Hcm1p binding sites. In a wild-type strain, *SPC110* is induced 4–5-fold late in G₁ of the cell cycle. In the *hcm1Δ* strain, the basal level of transcription is not altered but the induction is reduced to only 2–3-fold over background. Thus, Hcm1p is required for full induction of *SPC110* late in G₁. Since some cell cycle regulation remains, other factors must also be involved in regulating *SPC110*. Hcm1p may work in concert with the factors that bind to the MluI cell cycle box found at position 144 upstream from the ATG in *SPC110* [29].

Deletion of *HCM1* worsens the phenotype of the *cmd1-1* mutant and decreases the expression of *SPC110*. Overcoming the decreased expression of *SPC110* by putting the gene under the control of a heterologous promoter partially rescues the poor growth of the *hcm1Δ*, *cmd1-1* double mutant. Since the rescue is not complete, Hcm1p must regulate additional genes important in the function of the spindle pole body. Regulation of several genes important in the activity of the spindle pole by a single transcription factor may ensure that a correct stoichiometry of spindle pole components is maintained.

Acknowledgements

We thank Douglas Crawford for reading the manuscript. This work was supported by Grant

GM-40506 to TND from the National Institute of General Medical Sciences.

References

- [1] T.N. Davis, M.S. Urdea, F.R. Masiarz, J. Thorner, *Cell* 47 (1986) 423–431.
- [2] J.R. Geiser, H.A. Sundberg, B.H. Chang, E.G.D. Muller, T.N. Davis, *Mol. Cell. Biol.* 13 (1993) 7913–7924.
- [3] H.A. Sundberg, L. Goetsch, B. Byers, T.N. Davis, *J. Cell Biol.* (1996).
- [4] S.E. Brockerhoff, R.C. Stevens, T.N. Davis, *J. Cell Biol.* 124 (1994) 315–323.
- [5] R.C. Stevens, T.N. Davis, *J. Cell Biol.* (1998) in press.
- [6] D.A. Stirling, T.F. Rayner, A.R. Prescott, M.J.R. Stark, *J. Cell Sci.* 109 (1996) 1297–1310.
- [7] Y. Ohya, D. Botstein, *Science* 263 (1994) 963–966.
- [8] T.N. Davis, *J. Cell Biol.* 118 (1992) 607–617.
- [9] G. Zhu, E.G. Muller, S.L. Amacher, J.L. Northrop, T.N. Davis, *Mol. Cell. Biol.* 13 (1993) 1779–1787.
- [10] J.R. Geiser, D. van Tuinen, S.E. Brockerhoff, M.M. Neff, T.N. Davis, *Cell* 65 (1991) 949–959.
- [11] W.O. Bullock, J.M.m. Fernandez, J.M. Short, *BioTechniques* 5 (1987) 376–378.
- [12] C. Coulondre, J.H. Miller, *J. Mol. Biol.* 117 (1977) 577–606.
- [13] T.A. Kunkel, J.D. Roberts, R.A. Zakour, *Methods Enzymol.* 154 (1987) 367–382.
- [14] J.W. Wallis, G. Chrebet, G. Brodsky, M. Rolfe, R. Rothstein, *Cell* 58 (1989) 409–419.
- [15] T.P. Hopp, K.S. Prickett, V.L. Price, R.T. Libby, C.J. Match, D.P. Cerretti, D.L. Urdal, P.J. Conlon, *Bio/Technology* 6 (1988) 1204–1210.
- [16] K.S. Prickett, D.C. Amberg, T.P. Hopp, *BioTechniques* 7 (1989) 580–589.
- [17] C. Korch, H.A. Mountain, A.S. Byström, *Mol. Gen. Genet.* 229 (1991) 96–108.
- [18] J.T. McGrew, L. Goetsch, B. Byers, P. Baum, *Mol. Biol. Cell* 3 (1992) 1443–1454.
- [19] M.J. Moser, S.Y. Lee, R.R. Klevit, T.N. Davis, *J. Biol. Chem.* 270 (1995) 20643–20652.
- [20] G. Zhu, D.K. Crawford, T.N. Davis, *Mol. Cell. Biol.* (1998) under revision.
- [21] M. Fried, D.M. Crothers, *Nucleic Acids Res.* 9 (1981) 6505–6525.
- [22] M.M. Garner, A. Revzin, *Nucleic Acids Res.* 9 (1981) 3047–3060.
- [23] F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.A. Smith, J.G. Seidman, K. Struhl, Greene Publishing Associates and Wiley-Interscience, 1987, p. 3.5.8. *Current Protocols in Biology*.
- [24] K. Nasmyth, G. Adolf, D. Lydall, A. Seddon, *Cell* 62 (1990) 631–647.
- [25] J.R. Geiser, *Genetic Analysis of Essential Calmodulin Functions in Saccharomyces cerevisiae*, University of Washington, Seattle, WA, 1993.
- [26] K.L. Clark, E.D. Halay, E. Lai, S.K. Burley, *Nature* 364 (1993) 412–420.
- [27] O. Cohen Fix, J.M. Peters, M.W. Kirschner, D. Koshland, *Genes Dev.* 10 (1996) 3081–3093.
- [28] B.D. Page, M.P. Snyder, *Genes Dev.* 6 (1992) 1414–1429.
- [29] J.V. Kilmartin, S.L. Dyos, D. Kershaw, J.T. Finch, *J. Cell Biol.* 123 (1993) 1175–1184.
- [30] E. Lai, K.L. Clark, S.K. Burley, J.E. Darnell, *Proc. Natl. Acad. Sci. USA* 90 (1993) 10421–10423.
- [31] D. Weigel, G. Jürgens, F. Küttner, E. Seifert, H. Jäckle, *Cell* 57 (1989) 645–658.
- [32] R.H. Costa, D.R. Grayson, J. James E Darnell, *Mol. Cell. Biol.* 9 (1989) 1415–1425.
- [33] E. Lai, V.R. Prezioso, E. Smith, O. Litvin, R.H. Costa, D.J.J.E., *Genes Dev.* 4 (1990) 1427–1436.
- [34] E. Lai, V.R. Prezioso, W. Tao, W.S. Chen, D.J.J.E., *Genes Dev.* 5 (1991) 416–427.
- [35] E. Kaufmann, W. Knöchel, *Mech. Dev.* 57 (1996) 3–20.
- [36] S.H.-L. Denmat, M. Werner, A. Sentenac, P. Thuriaux, *Mol. Cell. Biol.* 14 (1994) 2905–2913.
- [37] S.E. Brockerhoff, T.N. Davis, *J. Cell Biol.* 118 (1992) 619–629.
- [38] E.G.D. Muller, *J. Biol. Chem.* 266 (1991) 9194–9202.