***Schizosaccharomyces pombe* Mop1–Mcs2 is related to mammalian CAK**

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The cyclin-dependent kinase (CDK)-activating kinase, CAK, from mammals and amphibians consists of MO15/CDK7 and cyclin H, a complex which has been identified also as a RNA polymerase II C-terminal domain (CTD) kinase. While the *Schizosaccharomyces pombe* *cdc2* gene product also requires an activating phosphorylation, the enzyme responsible has not been identified. We have isolated an essential *S.pombe* gene, *mop1*, whose product is closely related to MO15 and to *Saccharomyces cerevisiae* Kin28. The functional similarity of Mop1 and MO15 is reflected in the ability of MO15 to rescue a *mop1* null allele. This suggests that Mop1 would be a CDK, and indeed Mop1 associates with a previously characterized cyclin H-related cyclin Mcs2 of *S.pombe*. Also, Mop1 and Mcs2 can associate with the heterologous partners human cyclin H and MO15, respectively. Moreover, the rescue of a temperature-sensitive *mcs2* strain by expression of *mop1*⁺ demonstrates a genetic interaction between *mop1* and *mcs2*. In a functional assay, immunoprecipitated Mop1–Mcs2 acts both as an RNA polymerase II CTD kinase and as a CAK. The CAK activity of Mop1–Mcs2 distinguishes it from the related CDK–cyclin pair Kin28–Ccl1 from *S.cerevisiae*, and supports the notion that Mop1–Mcs2 may represent a homolog of MO15–cyclin H in *S.pombe* with apparent dual roles as a RNA polymerase CTD kinase and as a CAK.

Keywords: CAK/CDK/cell cycle/CTD kinase/MO15/*mop1*/*Schizosaccharomyces pombe*

Introduction

Cyclin-dependent kinases (CDKs) are key molecules for eukaryotic cell cycle control. Events such as response to growth factors, DNA replication, transcription and mitosis are regulated by different CDKs. The CDK activity oscillations are crucial for the cell cycle to proceed from one step to the other, the consequence of an elaborate regulation which includes the association with a regulatory cyclin subunit. CDK activity can be inhibited by the association of specific small molecular weight CDK inhibitors (reviewed in Morgan, 1995). The central role of cyclins, CDKs and their inhibitors in cell growth control is also reflected by the implication of some of these effectors in human malignancies (reviewed in Hartwell and Kastan, 1994; Hunter and Pines, 1994).

In addition, most CDKs contain conserved inhibitory and activating phosphorylation sites (Morgan, 1995). The inhibitory phosphorylation on Tyr15 in human p34^{cdc2} is mediated by the Wee1 kinase (Piwnicka-Worms *et al.*, 1991), and has to be dephosphorylated by the Cdc25 phosphatases (Gautier *et al.*, 1991) to obtain an active CDK. The activating phosphorylation on Thr161 in human Cdc2 is mediated by the CDK-activating kinase, CAK (Solomon *et al.*, 1992). In mammalian and amphibian cells, CAK has been found to consist of a CDK kinase, MO15 (Fesquet *et al.*, 1993; Poon *et al.*, 1993; Solomon *et al.*, 1993) and a cyclin subunit, cyclin H (Fisher and Morgan, 1994; Labbé *et al.*, 1994; Mäkelä *et al.*, 1994). Thus, the CAK cyclin–CDK directly activates a number of other CDKs, including *cdc2*, CDK2, CDK3 and CDK4 (Fisher and Morgan, 1994; Labbé *et al.*, 1994; Matsuoka *et al.*, 1994).

Intriguingly, MO15–cyclin H was recently identified as part of the basal transcription–DNA repair factor TFIIF (Roy *et al.*, 1994; Serizawa *et al.*, 1995; Shiekhatter *et al.*, 1995). MO15–cyclin H in TFIIF is thought to phosphorylate the C-terminal domain (CTD) of RNA polymerase II, thereby regulating transcription (reviewed in Drapkin *et al.*, 1994). Similarly, the *Saccharomyces cerevisiae* MO15-related kinase Kin28 (Simon *et al.*, 1986) was identified in TFIIF (Feaver *et al.*, 1994). Kin28 has a cyclin partner, Ccl1 (Valay *et al.*, 1993), and was shown to be required for both RNA polymerase II-mediated transcription and phosphorylation of CTD (Cismowski *et al.*, 1995; Valay *et al.*, 1995).

p34^{cdc2} in the yeast *Schizosaccharomyces pombe* appears to be regulated in much the same way as in mammalian species (Nurse, 1994). Thus, Thr14 and Tyr15 dephosphorylation is required for p34^{cdc2} kinase activity (Gould and Nurse, 1989). Thr167 phosphorylation is required for p34^{cdc2} kinase activity at mitosis and is involved for the association of p34^{cdc2} with the cyclin B encoded by *cdc13* (Booher and Beach, 1986; Gould *et al.*, 1991). The kinase activity responsible for Thr167 phosphorylation in *S.pombe*, Cdc2, or for the corresponding Thr169 in *S.cerevisiae*, Cdc28, has not been characterized in detail (Deshaies and Kirschner, 1995), but might be expected to resemble the mammalian CAK.

TFIIF in *S.cerevisiae* contains Kin28 which is homologous to MO15, but Kin28 is not a component of CAK of CDC28 (Solomon, 1994). In *S.pombe* the Mcs2 cyclin (Molz *et al.*, 1989; Molz and Beach, 1993) is structurally related to both Ccl1 and cyclin H, and it was identified by virtue of its genetic interaction with a *cdc2* allele (Molz *et al.*, 1989). Mcs2 was also found to associate with a kinase activity (Molz *et al.*, 1989). Thus Mcs2 is a good candidate for a *S.pombe* CAK cyclin, but the Mcs2-associated CDK has not been identified. Interestingly, the kinase encoded by *csk1* (which shares limited homology

with both MO15 and Kin28) was isolated as a high copy number suppressor of a *mcs2* mutation. However, no physical interaction between Mcs2 and Csk1 has been demonstrated (Molz and Beach, 1993).

In a search for new CDKs in *S.pombe*, we have identified *mop1*, which encodes a serine–threonine kinase essential for cell proliferation. Mop1 is homologous to MO15 and Kin28, and associates physically and genetically with the Mcs2 cyclin. The Mop1–Mcs2 complex can function *in vitro* both as a CAK and as an RNA polymerase II CTD kinase. In contrast to the *S.cerevisiae* Kin28, Mop1 is able to activate CDK2. These results indicate that Mop1 is a MO15-related CDK, and that Mop1–Mcs2 may have functions similar to those of MO15–cyclin H in *S.pombe*.

Results

Cloning of the *S.pombe* *mop1* cDNA

To isolate novel CDKs in *S.pombe*, we designed degenerate primers corresponding to amino acid residues conserved among members of the Cdc2 protein family (GEGTYV and TLWYRAPE). *S.pombe* genomic DNA was amplified by PCR, identifying two major DNA fragments of ~480 and 650 bp by agarose gel electrophoresis. Sequence analysis revealed that the 650 bp product corresponded to the *S.pombe* *cdc2*. The 480 bp fragment contained an open reading frame (ORF), which was predicted to encode a polypeptide highly similar to the *S.cerevisiae* Kin28 (Simon *et al.*, 1986) and to the human (Levedakou *et al.*, 1994), mouse (Matsuoka *et al.*, 1994) and *Xenopus laevis* MO15 proteins (Shuttleworth *et al.*, 1990). This fragment was used to isolate a full-length cDNA with an ORF encoding a 335 amino acids long polypeptide with a predicted M_r of 38.5 kDa (Figure 1A) containing the domains conserved among serine–threonine protein kinases (Hanks *et al.*, 1988). The deduced protein was found to be 50.3% identical to the *S.cerevisiae* KIN28 gene product, and 46.0% identical to the human, mouse and *X.laevis* MO15 proteins (Figure 1B and C). Due to these homologies, this novel gene was named *mop1* for MO15 *S.pombe*.

mop1 is an essential gene

To investigate the role of *mop1* in cell cycle control, we constructed a null allele of *mop1* using gene replacement (Rothstein, 1983). One locus of the *mop1* coding sequence was interrupted by introducing the *ura4⁺* gene (Grimm *et al.*, 1988) in a diploid strain (Figure 2). Transformants were grown on selective medium and proper replacement of a *mop1* locus was verified by Southern blot. This diploid was sporulated, and tetrads were analyzed. In each tetrad, only two *Ura⁻* spores could form colonies. The other two spores were able to germinate and would undergo a few divisions. Therefore, the lethal phenotype results from the loss of the *mop1* gene product, and it was concluded that *mop1* is essential for cell proliferation. To analyze further the terminal phenotype of the *mop1* null allele, the *mop1/mop1::ura4⁺* diploid strain was transformed with a *LEU2*-based vector bearing the *mop1* cDNA under the control of the thiamine-repressible *nmt1* promoter (Maundrell, 1990, 1993). After tetrad analysis, the *Ura⁺/Leu⁺* segregants were selected and revealed that the *mop1* cDNA rescues the *mop1::ura4⁺* allele and

allows colony formation on medium lacking thiamine (Figure 2B). Whenever the cells were streaked on medium supplemented with thiamine, they were unable to form colonies after two passages of 48 h of this growth regimen. Microscopic observation of the poorly growing colonies revealed the terminal phenotype of the *mop1* null allele (Figure 2C). Cells arrested proliferation with a heterogeneous phenotype and aberrant cell morphology.

Human MO15/CDK7 rescues the *mop1* null allele

The *mop1/mop1::ura4⁺* diploid strain was transformed with a *LEU2*-based vector bearing the human MO15 cDNA under the control of the thiamine-repressible *nmt1* promoter (Maundrell, 1990, 1993). Further genetic analysis as described above (Figure 2B) showed that *Ura⁺ Leu⁺* segregants could form colonies, revealing that the human MO15 cDNA rescues the *mop1* null allele (Figure 2D). This result shows a functional similarity between *mop1* and MO15.

The Mop1 kinase physically associates with a *S.pombe* cyclin Mcs2

The closest relatives of Mop1 are two CDKs; Kin28 and MO15, which associate with their cognate cyclins Ccl1 and cyclin H in *S.cerevisiae* and mammalian cells, respectively. As the *S.pombe* cyclin Mcs2 is closely related to both Ccl1 and cyclin H, we investigated whether Mop1 would associate with Mcs2. Furthermore, since previous genetic results suggested that Mcs2 might interact with the Csk1 kinase, we included Csk1 in our studies, although Mop1 is clearly more similar to the MO15 protein family than is Csk1 (Figure 1C). We also included the human MO15 and cyclin H as controls for association.

To investigate these interactions, we used the two-hybrid system in *S.cerevisiae* as described (Gyuris *et al.*, 1993). The kinase subunits (Mop1, MO15 and Csk1) were expressed as DNA binding *lexA* fusions, and the cyclin subunits (Mcs2 and human cyclin H) as transactivating VP16 fusions. Interactions between the kinases and the cyclins were scored by the growth of the cells in medium lacking leucine as well as by the appearance of a blue color due to the activation of the *LacZ* gene (Gyuris *et al.*, 1993). No growth was detected on selective plates when each plasmid was co-transformed with the relevant vector control (data not shown). Specific interactions between Mop1 and Mcs2, as well as between the human MO15 and cyclin H proteins, were observed (Figure 3). Interestingly, Mop1 also interacts with human cyclin H, and human MO15 interacts with Mcs2. No interactions between Csk1 and Mcs2 or cyclin H were detected. It was concluded that the Mop1 kinase and Mcs2 cyclin associate efficiently in a manner indistinguishable from that of MO15 and cyclin H. In addition, these results indicate that the structurally similar proteins from these very different species can interact functionally with a heterologous partner.

Kinase activity of the Mop1–Mcs2 complex

The complementation of the *mop1* null allele by the human MO15 gene and the similar physical interaction between Mop1–Mcs2 and MO15–cyclin H complexes suggested that Mop1–Mcs2 might function as a kinase with substrate specificities similar to those described for MO15–cyclin

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-120  tgttgtatagcttcactaccataccatcactgctatacaaacctgtcctcaatatta
-120  ctaccactgtattcttaagtagctctgtaattaatagtaggggttaaggtttctcggt
1    ATGGATATCGAAAGTCTGACAAGTGGACGTATGTGAAGGAGCGCAAAGTTGGTGAAGGA
1    M D I E K S D K W T Y V K E R K V G E G
61   ACGTATCCGTCGTGTTCTCGGGTCGCCAAAAAGAACTAATCGCGCTGTGGCTATTAAA
21   T Y A V V F L G R Q K E T N R R V A I K
121  AAGATTAAAGTGGCCAGTTCAAAAGTGGTATCGATATAAGTGCTTTACGAGAGATCAAA
41   K I K V G Q F K D G I D I S A L R E I K
181  TTTCTTCGGAGTCAAGGCACGACAATGTCATCGAGTAGTTGATGATTTCTCCACAAAG
61   F L R E S R H D N V I E L V D V F S T K
241  TCCAACCTCAACATCATCTTGGAAATCTCGGACAGTGATTGGAAATGTCATCAAGAC
81   S N L N I I L E F L D S D L E M L I K D
301  AAATTCATCGTTTTTCAACCTGCCACATCAAAATCGTGGATGGTATGTTGTACGCGGT
101  K F I V F Q P A H I K S W M V M L L R G
361  TTGATCACATTCATCTCGCTTCATCCTTCATCGAGATTGAAGCCTAATAACCTTTTA
121  L H H I H S R F I L H R D L K P N N L L
421  ATTTTCGAGTGACGGTGTCTAAAATTTGGCTGATTTTGGCTTTCTCGTGATTTTGGCACC
141  I S S D G V L K L A D F G L S R D F G T
481  CCAAGCCACATGAGTCATCAGGTATTACACGATGGTACAGGCTCCAGAGCTATTATG
161  P S H M S H Q V I T R W Y R P P E L F M
541  GGTGTGCAAGCTATGGCACCAGTGTGATATGTGGAGTGTAGGCTGCAATTTTTCAGAG
181  G C R S Y G T G V D M W S V G C I F A E
601  TTGATGCTTAGGACCCCGTATTGGCAGGCGAATCTGATCTGGATCAGCTTAACGTAATT
201  L M L R T P Y L P G E S D L D Q L N V I
661  TTCCGCGCTCTTGAACCTCTGAACAGAGGTCAATAAAGCATGCAACAGCTTCCCAAC
221  F R A L G T P E P E V I K S M Q Q L P N
721  TACGTTGAGATGAAACACATTCCTCCTCAACGGTGGTATGAGGCTCTTTTTCGGCA
241  Y V E M K H I P P P N G G M E A L F S A
781  GCTGCCATGAAGAAATGATTTACTCAAGATGATGTTGATTACAATCCATATCGTCGT
261  A G H E E I D L L K M M L D Y N P Y R R
841  CCCACTGCTCAACAGCCCTTGAGCATCATTTTCTGCCCTTCTAAACCCACACAT
281  P T A Q Q A L E H H Y F S A L P K P T H
901  CCTTCTTCTCCACGCAAGGGGTGAGGAAGGAATCAAGCAGCTCTAGTGACCTG
301  P S L L P R K G G E E G I K H V S S D L
961  CAGCGACAAAACATTTCCAATGCGTCAAAATATTAAATTTGTTTAAatgattgatcggtg
321  Q R Q N N F P M R A N I K F V * 335
1021 tgctagtagtcacctttaactatataaccattcttttctcattcatgtttccctacagaaa
1081 aattggaatgatgtgactgtatgctggatctttctgtttagataattgtcattctattttt

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B

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1  M A L D V K S R A K R Y E K L D F L G E G Q F A T V Y V K A R D K N T N Q I V A I K K I K L G H R S E A H.s. MO15
1  M K V N M E - - - - Y T K E K K V G E G T Y A V V Y L G C Q H S T G R K I A I K E I K T S - - - E F S.c. KIN28
1  M D I E K S D K - W T Y V K E R K V G E G T Y A V V F L G R Q K E T N R R V A I K K I K V G - - - Q F S.p. mop1

52  K D G I N R T A L R E I K L L Q E L S H P N I I G L L D A F G H K S N I S L V F D F M E T D L E V I I H.s. MO15
44  K D G L D M S A I R E V K Y L Q E M Q H P N V I E L I D I F M A Y D N L N L V L E F L P T D L E V V I S.c. KIN28
48  K D G I D I S A L R E I K F L R E S R H D N V I E L I D I V F S T K S N L N I T L E F L D S D L E M L I S.p. mop1

103 K D N S L V L T P S H I K A Y M L M T L Q G L E Y L H Q H W I L H R D L K P N N L L L D E N G V L K L H.s. MO15
95  K D K S I L F T P A D I K A W M L M T L R G V Y H C H R N F I L H R D L K P N N L L F S P D G Q I K V S.c. KIN28
99  K D K F I V F Q P A H I K S W M V M L L R G L H H I H S R F I L H R D L K P N N L L I S S D G V L K L S.p. mop1

154 A D F G L A K S F G S F N R A Y T H Q V V T R W Y R A P E L L F G A R M Y G V G V D M W A V G C I L A H.s. MO15
146 A D F G L A R A I P A P H E I L T S N V V T R W Y R A P E L L F G A K H Y T S A I D I W S V G V I F A S.c. KIN28
150 A D F G L S R D F G T P S H - M S H Q V I T R W Y R P P E L F M G C R S Y G T G V D M W S V G C I F A S.p. mop1

205 E L L L R V F F L P G D S D L D Q L T R I F E T L G T P T E E Q W P D M C S L P D Y V T F K S F P G I H.s. MO15
197 E L M L R I P Y L P G Q N D V D Q M E V T F R A L G T P T D R D W P E V S S F M T Y N K L Q I Y P P P S.c. KIN28
200 E L M L R T P Y L P G E S D L D Q L N V I F R A L G T P E P E V I K S M Q Q L P N Y V E M K H I P P P S.p. mop1

256 P - - L H H I F S A A G D D L L D L I Q G L F L F N P C A R I T A T Q A L K M K Y F S N R P G P T P H.s. MO15
248 S R D E L R K R F I A A S E Y A L D F M C G M L T M N P Q K R W T A V Q C L E S D Y F K E L P P P S D S.c. KIN28
251 N - G G M E A L F S A A G H E E I D L L K M M L D Y N P Y R R P T A Q Q A L E H H Y F S A L P K P T H S.p. mop1

304 G C Q L P R P N C P V E T L K E Q S N P A L A I K R K R T E A L E Q G G L P K K - - L I F H.s. MO15
299 P S S I K I R N S.c. KIN28
301 P S L L P R K G - - - - - G E E G I K H V S S D L Q R Q N N F P M R A N I K F V S.p. mop1

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C

Fig. 1. Structure of the *mop1* cDNA. (A) Nucleotide and predicted amino acid sequence of the *mop1* cDNA. Numbering of both nucleotide and amino acid sequences starts at the translation initiation site. The asterisk marks the stop codon. The *Clal* site used for the gene disruption is underlined. The DDJ, EMBL and GenBank databases accession number of this sequence is L47353. (B) Sequence comparison of MO15-related polypeptides: human MO15 (H.s. MO15), *S.cerevisiae* Kin28 (S.c. Kin28) and *S.pombe* Mop1 (S.p. Mop1). Identical amino acids are boxed. Mop1 is 46 and 50.3% identical to MO15 and Kin28 respectively, Kin28 and MO15 are 45.1% identical. (C) Phylogenetic tree between the human and yeast *cdc2* and MO15 protein families and the *S.pombe* *csk1* gene product. H.s. = *Homo sapiens*; S.p. = *S.pombe*; S.c. = *S.cerevisiae*.

H. To study this, we used the fusion proteins expressed in the two-hybrid interaction experiments described above. The Mcs2 and cyclin H fusions contained a hemagglutinin

(HA) epitope tag, which allowed us to immunoprecipitate Mcs2- or cyclin H-associated polypeptides with a monoclonal HA antibody. Such immunoprecipitates from *S.cere-*

Fig. 2. (A) Schematic of the construct used to disrupt *mop1*. The restriction map with selected restriction sites of the *S.pombe* *mop1* cDNA is shown at the top. The white box marks the open reading frame with the start (ATG) and stop (TAA) codons indicated. Integration of the 1.8 kbp *ura4* gene (black arrow) into the unique *ClaI* site is shown schematically below. (B) Experimental scheme to identify the *mop1* null allele terminal phenotype and its rescue by either *mop1* or human MO15 expression. The *mop1*/*mop1::ura4*⁺ diploid strain was transformed with a *LEU2*-based vector bearing either the *mop1*⁺ cDNA or human MO15 cDNA under the control of the thiamine-repressible promoter. The diploid transformants were streaked on sporulation medium. After tetrad dissection, the haploid Ura⁺ Leu⁺ segregants were selected and streaked on medium with and without thiamine, promoter off and on respectively. (C) Cells carrying *mop1*⁺ under the control of the *nmt1* promoter were grown in the absence (promoter on) or presence of thiamine (promoter off). Cells were examined with dark phase contrast microscopy. (D) MO15-expressing cells grown in the absence of thiamine were stained with DAPI and calcofluor.

visiae cells expressing *mcs2* alone or together with either *mop1* or *csk1* were analyzed for kinase activities towards CDK (CAK activity) or toward the large subunit of RNA polymerase II CTD. For this purpose, either a recombinant GST-CDK2 or a CTD peptide were used as substrates. Both of these substrates have been used widely to measure

and identify CTD or CAK activities (Kato *et al.*, 1994; Poon *et al.*, 1994; Roy *et al.*, 1994; Serizawa *et al.*, 1995).

As observed in other systems, the cyclin H-associated MO15 kinase displayed activity toward both CDK2 and CTD (Figure 4A, lane 1). Immunoprecipitates from cells expressing *mcs2* alone had low levels of kinase activity

Fig. 3. Interaction of Mop1 with Mcs2 and cyclin H in a two-hybrid assay. *S.cerevisiae* strain EGY048 was transformed with plasmid combinations expressing the indicated fusion proteins. The *mop1*, MO15 and *csk1* kinases were cloned into the pEG202 vector, and *mcs2* and cyclin H cyclins into the galactose-inducible pJG4-5 vector. Transformants were restreaked on selective medium containing either galactose or glucose to select for growth of cells expressing a combination of a bait and prey associating with each other.

Fig. 4. Kinase activity of *mop1-mcs2* immunocomplexes. (A) Mop1-Mcs2 phosphorylates CDK2 and RNA Pol II CTD. *S.cerevisiae* cells expressing the indicated combinations of CDKs expressed from the pEG202 vector and HA-cyclin fusion proteins expressed from the pJG4-5 vector were used to prepare anti-HA immunocomplexes for separate *in vitro* kinase assays with either GST-CDK2 or GST-CTD substrates. (B) Mop1-Mcs2 phosphorylates CDK2 on Thr160 and CTD on Ser5. MO15-cyclin H (lanes 1–4) or Mop1-Mcs2 (lanes 5–8) immunocomplexes were used for kinase assays employing wild-type or mutant CDK2 (CDK2-T160A) or CTD (CTD-S5A) substrates, as indicated above the lanes.

(Figure 4A, lane 4). This low activity probably reflects the association of an endogenous *S.cerevisiae* kinase (e.g. Kin28) with the Mcs2 cyclin, and was not significantly altered in cells expressing *csk1* together with *mcs2* (Figure 4A, lane 5). However, when *mop1* was co-expressed with *mcs2*, both the CTD and CDK2 kinase activities were increased >10-fold (Figure 4A, lane 3). Taken together with the results from the interaction experiments, this indicates that Mcs2 and Mop1 form a tightly associated cyclin-CDK pair with kinase activity toward both CDK2 and RNA Pol II CTD *in vitro*.

To investigate whether the sites phosphorylated by Mop1-Mcs2 on CDK2 and CTD were identical to those phosphorylated by MO15-cyclin H, mutant substrates for both CDK2 and CTD were prepared. The GST-CDK2-T160A has an alanine substitution at Thr160 and has been used previously to control CAK activity (Brown *et al.*, 1994). As the Pol II CTD is a multimeric repeat of the sequence YSPTSPS where Ser5 is phosphorylated by MO15 (Roy *et al.*, 1994), any single point mutation would not be sufficient to inhibit phosphorylation of the substrate. This problem was circumvented by using CTD peptides with three repeats of either the wild-type sequence or with

an alanine substitution at Ser5 (YSPTAPS) as described (Roy *et al.*, 1994). Using the wild-type substrates, both MO15-cyclin H and Mop1-Mcs2 phosphorylated the GST-CDK2 as well as the triple CTD peptide (Figure 4B, lanes 1, 3, 5 and 7). When the mutant substrates were used, no phosphorylation was observed (Figure 4B, lanes 2, 4, 6 and 8), confirming that Mop1-Mcs2 phosphorylated the GST-CDK2 on the activating Thr160 site, and the CTD on Ser5. These results indicate that Mop1-Mcs2, like MO15-cyclin H, can function as a CAK and as a CTD kinase.

mop1* interacts genetically with *mcs2

As *csk1*, whose gene product is a far member of the MO15-Mop1 family (Figure 1C), was isolated by virtue of its ability to rescue the temperature-sensitive strain *mcs2-75 cdc2-3w* and because of the physical association between Mop1 and Mcs2, we tested if *mop1* could rescue the *mcs2* mutant strain. The *mop1*⁺ cDNA under the control of the *nmt1* promoter was introduced into the *mcs2-75 cdc2-3w* strain. Transformants were streaked on plates with and without thiamine. Cells grown in the absence of thiamine (promoter on) could form colonies at

Fig. 5. Genetic interaction of *mop1* and *mcs2*. The temperature-sensitive *mcs2-75 cdc2-3w* strain was transformed with a thiamine-repressible *mop1*⁺ plasmid. Transformants were streaked on medium without (A) or with thiamine (B) and incubated at the restrictive temperature, where the *mcs2-75 cdc2-3w* strain has a terminal elongated cell phenotype (B) rescued by *mop1*⁺ (A). Cells were examined by dark phase microscopy.

the restrictive temperature, while cells grown in the presence of thiamine (promoter off) could barely form colonies. Microscopic observations of the cells showed that the cells not expressing *mop1* had the elongated cell phenotype characteristic of the strain (Molz and Beach, 1993) (Figure 5). The *mop1* suppression of the temperature-sensitive phenotype associated with *mcs2-75* confirmed the *in vivo* interaction between *mop1* and *mcs2*.

Physical association between Mop1 and Mcs2 in *S.pombe*

To validate the physical association of Mop1 and Mcs2 in *S.pombe* cells, we constructed a Myc epitope-tagged Mop1. The Myc epitope was fused to the Mop1 N-terminus and is referred to as Myc:mop1. The episomic fusion protein is under the control of the *nmt1* promoter. To study the interaction, we used a strain in which the *mcs2* locus has been replaced by a genomic HA-tagged *mcs2*. The strain is referred to as HA:mcs2. The HA:mcs2 strain was transformed with the conditional Myc:mop1-carrying vector or with a control vector. Transformants were grown in the absence of thiamine in order to express *mop1*. The Myc and HA epitopes allowed us to immunoprecipitate Mop1-Myc- or Mcs2-HA-associated polypeptides and to use Western blotting to analyze whether we could detect the Mcs2-HA or Mop1-Myc, respectively, in these complexes. Using this immunoprecipitation-Western blotting approach, a 39 kDa doublet corresponding to Mcs2-HA was detected in anti-Myc immunoprecipitates only in cells expressing both Mcs2-HA and Mop1-Myc (Figure 6A). In a similar fashion, a 37 kDa doublet corresponding to Mop1-Myc was observed in anti-HA immunoprecipitates (Figure 6B). Both the Mcs2-HA and Mop1-Myc polypeptides appeared as doublets, possibly due to phosphorylation, which in the case of Mop1 may represent phosphorylation on the presumed activating phosphorylation site as observed with MO15 (Labbé *et al.*, 1994). Taken together, these results confirmed the physical interaction between Mop1 and Mcs2, and demonstrated that these proteins form a complex *in vivo* in *S.pombe*.

Mop1-Mcs2 as an RNA polymerase II CTD kinase and as a CAK in *S.pombe*

In view of the physical association of Mop1 and Mcs2 in *S.pombe* cells, we analyzed the kinase activities associated

Fig. 6. Physical association of Mop1 and Mcs2 in *S.pombe*. The HA-tagged *mcs2*-expressing strain was transformed with the Mop1-Myc-expressing vector or a control vector. (A) Anti-Myc or control IgG immunoprecipitates from the indicated strains were detected by Western blotting with an anti-HA antibody. (B) Anti-HA or control IgG immunoprecipitates were detected by Western blotting with an anti-Myc antibody.

with the Mop1-Mcs2 complex (Figure 7). For this purpose, we used anti-Myc immunoprecipitates from cells expressing Mop1-Myc (lane 3) or a control vector (lane 4) in kinase reactions with several substrates. Human CAK (lane 1) or kinase reactions with substrates alone (lane 2) were used as controls. Mop1-Mcs2 phosphorylated both an RNA polymerase II CTD peptide and a recombinant CDK2 efficiently. Phosphorylation of the GST-CDK2 was confirmed to represent the activating phosphorylation on Thr160 by the inability of the T160A mutant of CDK2 to be phosphorylated. In addition, when a cyclin A-CDK2 complex was used as a substrate for human CAK or Mop1-Mcs2, an increased activity on histone H1 was subsequently observed (Figure 7, bottom panel). These results demonstrate that the *S.pombe* Mop1-Mcs2 closely resembles the mammalian MO15-cyclin H in its ability to phosphorylate both a Pol II CTD and act as a CAK. This is in clear distinction from the activity of the *S.cerevisiae* Kin28 which, using a similar assay, does not function as a CAK (Cismowski *et al.*, 1995).

Discussion

The number of identified CDKs in mammalian cells and in budding yeast is rapidly expanding (Espinoza *et al.*, 1994; Measday *et al.*, 1994; Liao *et al.*, 1995; reviewed

Fig. 7. CDK2 and CTD kinase activities and CAK activity associated with Mop1-Mcs2 in *S.pombe*. Anti-Myc immunoprecipitates from a Mop1-Myc-expressing *S.pombe* extract (lane 3) or a control vector extract (lane 4) and human MO15-cyclin complexes (lane 1) or no immunoprecipitates (lane 2) were tested for kinase activities towards CTD peptide, recombinant CDK2, recombinant mutant CDK2-T160A and for histone H1 kinase activity associated with Mop1-activated CDK2-cyclin A as described (Mäkelä *et al.*, 1994).

in Morgan, 1995). In *S.pombe*, Cdc2 has been the only known CDK, and it has been shown to interact with various cyclins during the mitotic and meiotic cell cycles (Forsburg, 1994; Nurse, 1994). Here we have identified a second *S.pombe* CDK gene named *mop1*. Mop1 is closely related to the mammalian and amphibian MO15 as well as *S.cerevisiae* Kin28, and probably represents a *S.pombe* homolog of these kinases. Mop1 shares conserved domains with the MO15 protein kinase family not maintained in other CDKs; Mop1 contains a DISALRE motif similar to the NRTALRE sequence of human MO15, and different from the PSTAIRE region specific to Cdc2 proteins. However, other regions in Mop1 and MO15 are not conserved. Thr17 and Tyr18 in Mop1 correspond to similar sites found in Cdc2, Cdc28 and Kin28, but not in MO15. These residues are the targets of inhibitory phosphorylation in Cdc2, suggesting that the activity of Mop1 and Kin28, unlike MO15, is dependent on the phosphorylation state of these sites. In addition, the sequence surrounding the conserved activating phosphorylation site (Thr161 in human cdc2) in Mop1 is not closely related to those of either Cdc2 or MO15. Mop1, unlike Kin28 but like the metazoan MO15 homologs, also contains an amino acid extension at its C-terminal end.

According to our gene disruption experiments, *mop1* is an essential gene. The terminal phenotype of the *mop1::ura4⁺* was heterogeneous and did not give rise to a uniform cell cycle arrest. The *mop1* null allele can be rescued by the ectopic expression of *mop1⁺* and human MO15 cDNAs, while the *KIN28* temperature-sensitive mutants cannot be rescued by the *Xenopus* MO15 (Cismowski *et al.*, 1995). This rescue reveals a functional similarity between Mop1 and MO15, and identifies fundamental differences between Mop1 and Kin28.

The Mop1-associated cyclin Mcs2 has also been shown

to be essential (Molz *et al.*, 1993). Interestingly, the closely related *S.cerevisiae* CDK Kin28 and cyclin Ccl1 are also essential for cell division (Simon *et al.*, 1986; Valay *et al.*, 1993). These findings suggest that members of these gene families play key roles in cell growth. However, the lethal phenotype does not differentiate between functions in cell cycle regulation or in transcription regulation. In *S.cerevisiae* *CCL1* was identified by virtue of its ability to suppress a temperature-sensitive allele of *KIN28*. Similarly, in *S.pombe*, the expression of *cdc2* rescued the temperature-sensitive alleles of *cdc13* which encodes a B-type cyclin (Booher and Beach, 1987). With a similar approach, we showed that overexpression of *mop1⁺* rescues the *mcs2-75 cdc2-3w* strain, hence demonstrating a genetic interaction between *mop1* and *mcs2*.

The genetic interaction between *mop1* and *mcs2* and the similarities to the human MO15-cyclin H were strongly suggestive of a physical interaction of Mop1 and Mcs2 proteins. Indeed, such an interaction was demonstrated and it was also observed that the Mop1-Mcs2 and MO15-cyclin H kinase-cyclin pairs can form functional interspecies complexes. These data, combined with the genetic interaction between *mop1* and *mcs2*, argue strongly for a partnership between Mop1 and Mcs2 *in vivo*.

Functional characterization of *S.pombe* Mop1-Mcs2 indicated that the activity of this complex closely resembles that of the mammalian MO15-cyclin H, and is clearly distinct from *S.cerevisiae* Kin28-Ccl1. Thus, while all three kinases show high specific activity toward RNA polymerase II CTD, only the *S.pombe* and mammalian kinases can also phosphorylate CDK2. Moreover, both MO15 and Mop1 immunoprecipitates are capable of activating CDK2-cyclin A complexes to phosphorylate histone H1, directly demonstrating that these kinases function as CAKs, whereas Kin28 does not have CAK activity. The observed differences between Kin28 and Mop1 represent an additional fundamental difference between the yeasts *S.cerevisiae* and *S.pombe*.

These results suggest that the Mop1-Mcs2 may have dual role as a CAK and as a TFIIF kinase in *S.pombe* cells. Clearly, they indicate that the Mop1 kinase has characteristics very similar to those of mammalian MO15, including activation of CDKs.

Materials and methods

Strains

Schizosaccharomyces pombe: *h⁺N/h⁺N ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18, h⁺N/h⁺N leu1-32/leu1-32 ade6-M210/ade6-M216 ura4-D18/ura4-D18 mop1/mop1::ura4⁺, h⁺N leu1-32 ura4-D18 ade6-M216 mop1::ura4⁺ [pREP41-mop1], h⁺N leu1-32 ura4-D18 ade6-M216 mop1::ura4⁺ [pREP3-Myc:MO15], h⁺N mcs2::HA-mcs2 leu1-32 and h⁺S mcs2-75 cdc2-3w leu1-32 (Molz and Beach, 1993).*

Saccharomyces cerevisiae: EGY048: *MATa ura3 his3 trp1,6 leu2 lexAop::LEU2* (Gyuris *et al.*, 1993).

Cloning of the *S.pombe* *mop1* cDNA

Two degenerate oligonucleotides, 5'-TCN GGN GCN C(G/T)(A/G) TAC CAN A(A/G)N GT-3' and 5'-GGN GA(A/G) GGN ACN TA(C/T) GGN GTN GT-3', corresponding to the conserved regions GEGTYGVV and TLWYRAPE of the cdc2-like protein family, were used to amplify *S.pombe* genomic DNA. The PCR consisted of 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min. Two major PCR products of ~480 and 650 bp were separated on agarose gel, cloned (TA cloning system, Invitrogen, USA) and sequenced. The 480 bp DNA fragment was ³²P-labeled (NEBlot kit, New England Biolabs, USA), and used to

screen an *S.pombe* cDNA library (Damagnez *et al.*, 1995). Hybridizations were performed in a solution containing 7% SDS, 250 mM NaP pH 7 and 10 mM EDTA pH 7, overnight at 45°C. Filters were washed in a buffer containing 2× SSC and 0.1% SDS at 45°C.

Phagemids *in vivo* excised using the manufacturer's protocol (Stratagene, USA). The resulting plasmid containing the complete *mop1* ORF was named pmop1.1.

Sequencing data were obtained from pBSII (Stratagene, USA) double-stranded plasmid templates using the ABI (Applied Biosystems, USA) sequencing kit and DNA sequencer according to the manufacturer's instructions. Sequence analysis and comparisons were done using the DNA Star package (San Diego, USA) with the CLUSTAL alignment method (Higgins *et al.*, 1992) and the BLAST1.7 program (Altschul *et al.*, 1990).

Cloning into the two-hybrid system

mop1

Two oligonucleotides, 5'-GGG TTA AGG TGG ATC CTT ATG GAT ATC GAA AAG TCT G-3' and 5'-GCA CAC GCT CGA GCA TTA AAC AAA TTT AAT ATT TGC-3', containing a *Bam*HI restriction enzyme site (underlined) and the *S.pombe mop1* ATG (bold), and a *Xho*I restriction enzyme site (underlined) and the *mop1* stop codon (bold), respectively, were used to amplify the full length *mop1* cDNA.

csk1

Two oligonucleotides, 5'-GAA TTT ATA GAA TTC ATG AAA TCA GTC GGT CAT TTC G-3' and 5'-CAA TCC CTC GAG TAA ATT TTA TGC ATA TTG TGA AAG C-3', containing a *Eco*RI restriction enzyme site (underlined) and the *S.pombe csk1* ATG (bold), and a *Xho*I restriction enzyme site (underlined) and the *csk1* stop codon (bold), respectively, were used to amplify *S.pombe* cDNA.

mcs2

Two oligonucleotides, 5'-ACT TAC ATT GAA TTC ATG TCT GCC GAT AAA TTT CG-3' and 5'-TCA AAG CTC GAG TCA TTC AAA CGG ATT TTT ATC C-3', containing an *Eco*RI restriction enzyme site (underlined) and the *S.pombe mcs2* ATG (bold), and a *Xho*I restriction enzyme site (underlined) and the *mcs2* stop codon (bold), respectively, were used to amplify *S.pombe* cDNA.

The PCR program consisted of 30 cycles of 94°C for 1 min, 61°C for 1 min and 72°C for 1 min. PCR products were analyzed on an agarose gel, cloned into the pCRTMII vector (Invitrogen, USA) or pCRscript vector (Stratagene, USA), and the integrity of their sequences was verified. Resultant plasmids were digested with the corresponding restriction enzymes, and their gel-purified inserts were subsequently cloned into the pEG202 vector (*mop1* and *csk1*) or pJG4-5 vector (*mcs2*).

The human MO15 and cyclin H were cloned into pEG202 and pJG4-5, respectively (Mäkelä *et al.*, 1994).

Cloning of *mop1* and human MO15 under the control of the *S.pombe* thiamine-repressible *nmt1* promoter

A pCRTMII plasmid containing the *mop1* ORF was digested with *Bam*HI, and the isolated DNA fragment was cloned into the unique *Bam*HI site of pREP41 or pREP3 (Maundrell, 1990, 1993). pREP41-*mop1* and pREP3-*mop1* carry the *mop1* cDNA in the correct orientation.

To construct and clone a *myc*-tagged *mop1*, two oligonucleotides, 5'-GGG TCG ACA CCA TGG AAC AGA AGC TGA TCA GCG AAG AGG ACC TGG ATA TCG AAA AGT CTG-3' and 5'-GCA CAC GGA TCC TTA ACC AAA TTT AAT ATT TGC-3', containing a *Sal*I restriction enzyme site (underlined), a *myc* tag (bold), and a *Bam*HI restriction enzyme site (underlined) and a *mop1* stop codon (bold) were used to amplify the full length *mop1* cDNA. The PCR program consisted of 30 cycles of 94°C for 1 min, 61°C for 1 min and 72°C for 1 min. The purified PCR product was cloned into a pCRTMII vector and the integrity of the *mop1* sequence was verified. This plasmid was subsequently digested with *Sal*I and *Bam*HI, and the *myc*-tagged *mop1* DNA fragment was cloned into pREP3 digested with *Sal*I and *Bam*HI.

A plasmid containing a *Myc*-tagged MO15 (Mäkelä *et al.*, 1994) was digested with *Eco*RI and *Xba*I, the 1.1 kb DNA fragment containing human *Myc*-tagged MO15 was blunt ended with Klenow enzyme, and cloned in pREP3 digested with *Sma*I. The plasmid pREP3-MO15 contained the *Myc*-tagged MO15 in the correct orientation.

Yeast transformation

Saccharomyces cerevisiae and *S.pombe* were transformed using the lithium acetate high efficiency transformation protocol (Becker and Lundblad, 1988–1995). We transformed the *S.cerevisiae* strain EGY048

carrying the pSH18-34 plasmid with all different combinations of plasmids.

***mop1* gene disruption**

The plasmid pmop1.1 was digested with the *Clal*I restriction enzyme, blunt ended with Klenow enzyme, and treated with shrimp alkaline phosphatase (United States Biochemicals). pKS-*ura4*, a plasmid containing the *S.pombe ura4*⁺ gene, was digested with *Hind*III, and the purified 1.8 kb *ura4* DNA fragment was blunt ended with Klenow enzyme, and subsequently cloned into pmop1.1. The resulting plasmid, pmop1::*ura4*, was digested with *Xmn*I and *Pst*I, and the 3 kbp DNA fragment containing the *mop1* gene interrupted with the *ura4* gene was purified and used to transform the diploid *S.pombe* strain *h*⁺*N*⁺*ade6*⁻*M210/ade6*⁻*M216 leu1-32/leu1-32 ura4-D18/ura4-D18*.

Immunoprecipitation and kinase assays

Five ml cultures of yeast strains were grown overnight in galactose-containing media. Cell pellets were washed once in phosphate-buffered saline, and disrupted in lysis buffer (150 mM NaCl, 50 mM HEPES pH 7.5, 5 mM EDTA, 1% NP-40 with 10 mg/ml aprotinin and leupeptin, 0.2 mM PMSF, 10 mM β-glycerophosphate and 5 mM NaF). Following disruption, lysates were adjusted to 0.5% NP-40 for immunoprecipitation with the 12CA5 monoclonal antibody (Boehringer Mannheim, Germany). Immunoprecipitates on protein A-agarose beads were washed four times with lysis buffer containing 0.1% NP-40 and once with kinase buffer [20 mM Tris, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 2.5 mM MnCl₂, 1 mM dithiothreitol (DTT)]. Kinase reactions were performed for 30 min at 30°C in 20 mM Tris, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 2.5 mM MnCl₂, 1 mM DTT, 10 mM ATP supplemented with 10 μCi of [γ-³²P]ATP using the following substrates as indicated in the figures: 1 μg of GST-CTD (a kind gift of Dr David Chao); 1 μg of GST-CDK2 [actually GST-CDK2/D155N, a kinase-deficient mutant of CDK2 (T.P.M., unpublished), was used due to low intrinsic kinase activity of a bacterially purified GST-CDK2 substrate; the original GST-CDK2 was a kind gift of the Harlow laboratory]; 1 μg of GST-CDK2/D155N/T160A (a mutant of CDK2 with no CAK site; the original CDK2-T160A was a kind gift of Katsumi Yamashita and Tim Hunt); 1 μg of a biotinylated wild-type CTD peptide (YSPTSPSYSPSYSPSYSPSYSPS); and 1 μg of a biotinylated mutant CTD peptide (YSPTAPSYSPSYSPSYSPSYSPS). For the CAK assay in Figure 7, 2 μg of a GST-CDK2/cyclin A complex prepared as described (Mäkelä *et al.*, 1994) was used. Phosphorylated substrates were analyzed by 6% SDS-PAGE followed by autoradiography. Western blotting was done according to the manufacturer's instructions (Amersham).

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References

- Altschul,S.F., Gish,W., Miller,W., Myers,E.W. and Lipman,D.J. (1990) *J. Mol. Biol.*, **215**, 403–410.
- Becker,D.M. and Lundblad,V. (1988–1995) In Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidman,J.G., Smith,J.A. and Struhl,K. (eds), *Current Protocols in Molecular Biology*. John Wiley and Sons, Inc., Media, PA, pp. 13.7.1–13.7.10.
- Booher,R. and Beach,D. (1986) *Mol. Cell. Biol.*, **6**, 3523–3530.
- Booher,R. and Beach,D. (1987) *EMBO J.*, **6**, 3441–3447.
- Brown,A.J., Jones,T. and Shuttleworth,J. (1994) *Mol. Biol. Cell*, **5**, 921–932.
- Cismowski,M.J., Laff,G.M., Solomon,M.J. and Reed,S.I. (1995) *Mol. Cell. Biol.*, **15**, 2983–2992.
- Damagnez,V., Rolfe,M. and Cottarel,G. (1995) *Gene*, **155**, 137–138.
- Deshaies,R.J. and Kirschner,M. (1995) *Proc. Natl Acad. Sci. USA*, **92**, 1182–1186.
- Drapkin,R., Sancar,A. and Reinberg,D. (1994) *Cell*, **77**, 9–12.
- Espinoza,F.H., Ogas,J., Herskowitz,I. and Morgan,D.O. (1994) *Science*, **266**, 1388–1391.

- Feaver,W.J., Svejstrup,J.Q., Henry,N.L. and Kornberg,R.D. (1994) *Cell*, **79**, 1103–1109.
- Fesquet,D. *et al.* (1993) *EMBO J.*, **12**, 3111–3121.
- Fisher,R.P. and Morgan,D.O. (1994) *Cell*, **78**, 713–724.
- Forsburg,S.L. (1994) *Curr. Biol.*, **4**, 828–830.
- Gautier,J., Solomon,M.J., Booher,R.N., Bazan,J.F. and Kirschner,M.W. (1991) *Cell*, **67**, 197–211.
- Gould,K. L. and Nurse,P. (1989) *Nature*, **342**, 39–45.
- Gould,K.L., Moreno,S., Owen,D.J., Sazer,S. and Nurse,P. (1991) *EMBO J.*, **10**, 3297–3309.
- Grimm,C., Kohli,J., Murray,J. and Maundrell,K. (1988) *Mol. Gen. Genet.*, **215**, 81–86.
- Gyuris,J., Golemis,E., Chertkov,H. and Brent,R. (1993) *Cell*, **75**, 791–803.
- Hanks,S.K., Quinn,A.M. and Hunter,T. (1988) *Science*, **241**, 42–52.
- Hartwell,L.H. and Kastan,M.B. (1994) *Science*, **266**, 1821–1828.
- Higgins,D.G., Bleasby,A.J. and Fuchs,R. (1992) *CABIOS*, **8**, 189–191.
- Hunter,T. and Pines,J. (1994) *Cell*, **79**, 573–582.
- Kato,J.Y., Matsuoka,M., Strom,D.K. and Sherr,C.J. (1994) *Mol. Cell Biol.*, **14**, 2713–2721.
- Labbé,J.-C. *et al.* (1994) *EMBO J.*, **13**, 5155–5164.
- Levedakou,E.N., He,M., Baptist,E.W., Craven,R.J., Cance,W.G., Welsh,P.L., Simmons,A., Naylor,S.L., Leach,R.J., Lewis,T.B., Bowcock,A. and Liu,E.T. (1994) *Oncogene*, **9**, 1977–1988.
- Liao,S.M., Zhang,J., Jeffery,D.A., Koleske,A.J., Thompson,C.M., Chao,D.M., Viljoen,M., van Vuuren,H.J. and Young,R.A. (1995) *Nature*, **374**, 193–196.
- Mäkelä,T.P., Tassan,J.-P., Nigg,E.A., Frutiger,S., Hughes,G.J. and Weinberg,R.A. (1994) *Nature*, **371**, 254–257.
- Matsuoka,M., Kato,J.Y., Fisher,R.P., Morgan,D.O. and Sherr,C.J. (1994) *Mol. Cell Biol.*, **14**, 7265–7275.
- Maundrell,K. (1990) *J. Biol. Chem.*, **265**, 10857–10864.
- Maundrell,K. (1993) *Gene*, **123**, 127–130.
- Measday,V., Moore,L., Ogas,J., Tyers,M. and Andrews,B. (1994) *Science*, **266**, 1391–1395.
- Molz,L. and Beach,D. (1993) *EMBO J.*, **12**, 1723–1732.
- Molz,L., Booher,R., Young,P. and Beach,D. (1989) *Genetics*, **122**, 773–782.
- Morgan,D.O. (1995) *Nature*, **374**, 131–134.
- Nurse,P. (1994) *Cell*, **79**, 547–550.
- Piwnica-Worms,H., Atherton-Fessler,S., Lee,M.S., Ogg,S., Swenson,K.I. and Parker,L.L. (1991) *Cold Spring Harbor Symp. Quant. Biol.*, **56**, 567–576.
- Poon,R.Y., Yamashita,K., Adamczewski,J.P., Hunt,T. and Shuttleworth,J. (1993) *EMBO J.*, **12**, 3123–3132.
- Poon,R.Y.C., Yamashita,K., Howell,M., Ershler,M.A., Belyavsky,A. and Hunt,T. (1994) *J. Cell Sci.*, **107**, 2789–2799.
- Rothstein,R. (1983) *Methods Enzymol.*, **101**, 202–211.
- Roy,R., Adamczewski,J. P., Seroz,T., Vermeulen,W., Tassan,J.-P., Schaeffer,L., Nigg,E.A., Hoeijmakers,J.H.J. and Egly,J.-M. (1994) *Cell*, **79**, 1093–1101.
- Serizawa,H., Mäkelä,T.P., Conaway,R.C., Conaway,J.W., Weinberg,R.A. and Young,R. (1995) *Nature*, **374**, 280–282.
- Shiekhhattar,R., Mermelstein,F., Fisher,R.P., Drapkin,R., Dynlacht,B., Wessing,H.C., Morgan,D.O. and Reinberg,D. (1995) *Nature*, **374**, 283–287.
- Shuttleworth,J., Godfrey,R. and Colman,A. (1990) *EMBO J.*, **9**, 3233–3240.
- Simon,M., Seraphin,B. and Faye,G. (1986) *EMBO J.*, **5**, 2697–2701.
- Solomon,M.J. (1994) *Trends Biochem. Sci.*, **19**, 496–500.
- Solomon,M.J., Lee,T. and Kirschner,M.W. (1992) *Mol. Biol. Cell*, **3**, 13–27.
- Solomon,M.J., Harper,J.W. and Shuttleworth,J. (1993) *EMBO J.*, **12**, 3133–3142.
- Valay,J.-G., Simon,M. and Faye,G. (1993) *J. Mol. Biol.*, **234**, 307–310.
- Valay,J.-G., Simon,M., Dubois,M.-F., Bensaude,O., Facca,C. and Faye,G. (1995) *J. Mol. Biol.*, **249**, 535–544.

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