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The *alm1*$^+$ gene from *Schizosaccharomyces pombe* encodes a coiled-coil protein that associates with the medial region during mitosis

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Abstract We have isolated a cDNA that encodes a 142-kDa protein by immunoscreening of a *Schizosaccharomyces pombe* expression library with a new antibody, mAb8, that reveals spindle poles and equatorial ring-like structures in several organisms. This cDNA encodes a putative protein which we termed Alm (for abnormal long morphology). The protein is predicted to be a coiled-coil protein, containing a central α-helical domain flanked by non-helical terminal domains. Immunofluorescence analysis showed that Alm1 is localized in the medial region of the cell from anaphase to the end of cytokinesis. Cells carrying an *alm1*::*ura4*$^+$ disruption are viable and exhibit an elongated morphology. Homozygous *alm1*::*ura4*$^+$ diploids sporulated normally but the spores did not germinate. Spores that have inherited the disruption allele from a heterozygous *alm1*$^+$/*alm1*::*ura4*$^+$ diploid germinated but generated smaller colonies. We propose that Alm1 participates in the structural organization of the medial region in *S. pombe*.

Key words *Schizosaccharomyces pombe* · Mitosis · Medial region · Coiled-coil protein

Introduction

Cell division is an essential step in cell reproduction. In eukaryotic organisms it involves the spatial and temporal coordination of nuclear and cytoplasmic division. Due to its suitability for genetic manipulation the fission yeast *Schizosaccharomyces pombe* is being widely used to study cell division. In *S. pombe*, as in higher eukaryotes, cell partitioning is carried out by an actin-based contractile ring that bisects the cell into two identical daughter cells. In contrast to higher eukaryotes, in which the ring assembles at late anaphase (Schroeder 1972, 1973; Satterwhite and Pollard 1992), in *S. pombe* it begins to form during early mitosis before nuclear division occurs (Marks and Hyams 1985). In *S. pombe*, the site of formation of the contractile ring is apparently determined by the position of the nucleus (Chang and Nurse 1996), in contrast with what happens in higher eukaryotes – in which the mitotic spindle apparatus determines the position of the contractile ring (Rappaport 1986) – or with *Saccharomyces cerevisiae* which uses a cortical marker (Chant 1996).

In addition to actin, several other proteins participate in the formation of the contractile ring, among them myosin, which is believed to generate the forces needed for contraction of the ring (for review see Mabuchi 1986; Satterwhite et al. 1992). In *S. pombe* several genes implicated in the formation of the contractile ring have been characterized (for review see Nurse et al. 1976; Balasubramanian et al. 1997; Gould and Simanis 1997). Mutants in these genes are conditional and form elongated, multinucleate cells that are inviable.

From what it is known in other organisms it is likely that additional proteins are implicated in the formation of the contractile ring. It is also a reasonable assumption that essential proteins involved in cell division are conserved among different organisms and might display a similar distribution in the cell during division. With this in mind, we used the antibody mAb8, which, in the nematode *Parascaris univalens*, in *Drosophila melanogaster* and in mammalian cells, labels the spindle poles and equatorial ring-like structures during cell division (Jiménez and Goday, unpublished results) to detect proteins in *S. pombe* that might display a similar pattern of localization. Using this antibody against a *S. pombe* expression library, we have cloned a gene, *alm1*$^+$, whose

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product spans the whole equatorial region of the cells from anaphase until the end of cytokinesis. The putative Alm1 protein has a long coiled-coil central domain and shares similarities with the Mlp1 protein (myosin-like protein) of *S. cerevisiae* (Kölling et al. 1993) and with myosin heavy chains. Disruption of the *alm1*$^+$ gene resulted in the production of a high percentage of elongated mononucleate cells, suggesting that loss of the gene function causes a delay in entering mitosis.

Materials and methods

Strains, genetic methods and growth conditions

The *S. pombe* strains used in this study and their origins are described in Table 1. Growth media and genetic manipulations were as described by Moreno et al. (1991). TPY73 is a strain carrying the *alm1*$^+$::*ura4*$^+$ disruption allele described below. TPY79 is a diploid obtained from a cross between TPY73 and TPY39. TPY76 was derived from a spore of TPY79. TPY80 is a homozygous *alm1*$^+$::*ura4*$^+$ disruptant that was segregated from a cross between TPY73 and TPY76.

Indirect immunofluorescence analysis

The antibody mAb8 used in the experiments described below is one of a panel of monoclonal antibodies obtained against embryonic mitotic protein extracts of the nematode *P. univalens*, as described elsewhere (Esteban et al. 1998). In higher eukaryotes mAb8 stains spindle poles and equatorial ring-like structures (Jiménez and Goday, unpublished results). The following procedure was used to immunostain fission yeast cells. Samples (20 ml) from *S. pombe* cultures in the mid-exponential phase of growth were filtered through Whatman GF/C glass-fibre filters. For mAb8 staining the cells were fixed with methanol at –20° C for 10 min and washed three times with PBS. For antitubulin staining cells were fixed with 3% formaldehyde as described (Hagan and Hyams 1988). In both cases, after fixation, the cell wall was digested with 0.5 mg/ml each of NovoZym234 (Novo Industri A/S) and Zymolyase 20T (Seikagaku Kogyo) in PBS with 1.2 M sorbitol. The mixture was incubated for 20–40 min at room temperature, washed three times in PBS, resuspended in PBS with 1% BSA and 100 mM lysine hydrochloride (PBAL) and placed on a rotating wheel for 1 h at room temperature (Moreno et al. 1991). For immunostaining with mAb8, cells were then incubated with mAb8 (diluted 1/5 in PBAL) overnight at 18° C on a rotating wheel. They were washed three times for 10 min each with PBAL, resuspended in FITC-conjugated goat anti-mouse IgM secondary antibody (diluted 1/50 in PBAL) and incubated for 1 h at room temperature. They were then washed again three times with PBAL and resuspended in 50 ml of PBS. For immunostaining with anti-tubulin, cells were incubated overnight at 18° C on a rotating wheel with the anti-tubulin antibody YOL 1/4 (a generous gift of Dr. J. Andreu; Kilmartin et al. 1982), diluted 1/50 in PBAL. FITC-conjugated goat anti-rabbit secondary antibody was used at a dilution of 1/100 and incubated for 1 h at room temperature. Staining of F-actin with rhodamine-conjugated phalloidin was performed as described (Chang et al. 1996).

For microscopic observation, cells were air-dried onto coverslips coated with a solution of poly-L-lysine (1 mg/ml) and inverted onto a drop of antifading medium (9:1 glycerol/PBS pH 8) containing 1 mg/ml *o*-phenylene diamine and 5 mg/ml 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI).

Fluorescence microscopy

Observations were made using a computer-controlled Zeiss Axioscope epifluorescence microscope equipped with a cooled charge-coupled device (CCD) camera (Photometrics). FITC, Rhd and DAPI fluorescence, detected using specific filter combinations, were recorded separately as gray-scale images and, when needed, digitally pseudocolored and merged. Confocal microscopy was performed with an MRC System (BioRad) and the Zeiss Axioscope epifluorescence inverted microscope. Standardized conditions were used for image scanning, averaging and processing.

Identification of cDNA clones by expression library screening

A *S. pombe* λZAP II cDNA library, in which inserts are directionally cloned between an *Eco*RI site at the 5′ end and an *Xho*I site at the 3′ end, (a generous gift of Drs. A. Pidoux and Z. Cande) was screened with mAb8. About 500,000 plaque-forming units (pfu) were plated and transferred to nitrocellulose filters impregnated with IPTG (isopropylthio-β-D-galactoside). After blocking with 3% BSA in PBS, the filters were incubated with mAb8 (1/5) overnight at 4° C. They were washed in PBS with 0.5% Tween 20 and incubated for 2 h at room temperature with a secondary antibody goat anti-mouse IgM conjugated to alkaline phosphatase (1/10000) (Southern Biotechnology Associates). The filters were washed and developed with BCIP/NBT (bromochloroindolyl phosphate/nitro blue tetrazolium) in alkaline phosphatase buffer (100 mM TRIS-HCl pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) as described by Harlow and Lane (1988). One positive clone was isolated and excised into pBluescript SKII plasmid in vivo by superinfection with helper phage (Short et al. 1988). This clone, named 8Sp-I, contained a 3.6-kb cDNA insert. A 600-bp fragment situated in the 5′ region of the insert (see Results) was amplified by PCR using the following primers: 5′-GCCCTTGAATGCGATG-3′and 5′-CTCATTAGTCTCTCGC-3′. The PCR conditions were: 4 min at 94° C, followed by 30 cycles of 1 min at 94° C, 1 min at 55° C and 3 min at 72° C. This DNA fragment was labeled with digoxigenin (DIG DNA labeling and Detection kit, Boehringer) and used to rescreen the same library by DNA hybridization. Phages were lysed by treatment with 0.5 N NaOH and 1.5 M NaCl. After neutralization in 0.5 M TRIS-HCl pH 7.5, 1.5 M NaCl, phage DNA was immobilized on nylon filters by crosslinking with UV light. Hybridization with the digoxigenin-labeled DNA

Table 1 *S. pombe* strains used

Strain	Genotype/genetic background	Source
TPY38	*h*$^+$ *ade6-M216 leu1.32 his 3*.Δ2 *ura* 4.Δ18	K.L. Gould
TPY39	*h*$^-$ *ade6-M210 leu1.32 his 3*.Δ2 *ura* 4.Δ18	K.L. Gould
TPY73	*h*$^+$ *ade6-M216 leu1.32 his 3*.Δ2 *ura* 4.Δ18 *alm1*::*ura 4*$^+$	This study
TPY76	*h*$^-$ *ade6-M210 leu1.32 his 3*.Δ2 *ura* 4.Δ18 *alm1*::*ura 4*$^+$	This study
TPY79	*h*$^-$/*h*$^+$ *ade6-M210*/*ade6-M216 leu1.32*/*leu1.32 his 3*.Δ2/*his*3.Δ2 *ura* 4.Δ18/*ura* 4.Δ18 *alm*1$^+$/*alm1*::*ura 4*$^+$	This study
TPY80	*h*$^-$/*h*$^+$ *ade6-M210*/*ade6-M216 leu1.32*/*leu1.32 his 3*.Δ2/*his3*.Δ2 *ura* 4.Δ18/*ura* 4.Δ18 *alm*1::*ura 4*$^+$/*alm1*::*ura 4*$^+$	This study
cdc25-22	*h*$^-$ *cdc25-22 leu1.32*	P. Thuriaux
cdc4-8	*h*$^-$ *cdc4-8*	P. Nurse
cdc7-24	*h*$^-$ *cdc7-24 leu1.32*	P. Nurse

was carried out in 0.5% SDS, 5 × SSC, 5 × Denhart's, 12.5 mM phosphate buffer pH 6.8, 50% formamide and 200 mg/ml fragmented salmon sperm DNA at 42° C for 16–20 h. After hybridization the filters were washed three times in 2 × SSC, 0.1% SDS for 20 min at room temperature, once at 50° C and twice in 0.5 × SSC, 0.1% SDS at 50° C. For detection, filters were blocked in Buffer 1 (0.1 M maleic acid, 0.15 M NaCl pH 7.5) with 3% BSA for 1 h. Alkaline phosphatase-conjugated anti-DIG antibody was added at a dilution of 1/10000. After incubation at room temperature for 1 h, filters were washed three times at room temperature in Buffer 1 with 0.3% Tween. The chemiluminescence substrate CPD-*Star* (Boehringer) was used at a dilution of 1/100 in Detection Buffer (0.1 M TRIS-HCl, 0.1 M NaCl pH 9.5) before exposure to Kodak X-OMAT S film at room temperature for 5 min. Following the DNA hybridization screening, clone 8Sp-II was isolated and the insert excised into pBluescript SKII as described above.

DNA sequencing

Sequencing reactions were performed using the dideoxynucleotide termination method (Sanger et al. 1977) on an Applied Biosystems 377 automated DNA sequencer (Perkin Elmer). Sequences were obtained from both cDNA strands. The Accession No. of the sequenced DNA in the EMBL databank is AF010473. Database searches were performed with BLAST (Altschul et al. 1990) and FASTA (Pearson and Lipman 1988). ORF analysis was performed with DNA Strider. Prediction of secondary structure was performed using a program based on Garnier or Chou-Fasman algorithms (Chou and Fasman 1974; Garnier et al. 1978; Rost and Sander 1993).

Disruption and overexpression of *alm1*$^+$

To disrupt the *alm1*$^+$ gene, the internal 2.5-kb *Hin*dIII fragment (see Fig. 5A) was replaced by a 1.8-kb *Hind*III fragment from plasmid pREP4 (Maundrell 1993) containing the *ura4*$^+$ gene. The resulting plasmid was cut with *Eco*RI and *Xho*I and used for gene disruption by homologous recombination (Rothstein 1983). Transformation was done using lithium acetate as described by Bröker (1987). Stable *ura4*$^+$ transformants were analyzed for the presence of the *alm1*$^+$::*ura4*$^+$ disruption by Southern hybridization. Genomic DNA was obtained as described by Hoffman and Winston (1987).

To overexpress the gene *alm1*$^+$ we placed it in a multicopy plasmid under the control of the *nmt1*$^+$ promoter, which is repressed by thiamine (Maundrell 1993). DNA from clone 8Sp-I was digested with *Kpn*I, blunted and digested with *Sal*I. The 3.1-kb fragment obtained was inserted into pREP3X (Maundrell 1993) digested with *Sal*I and *Sma*I, to give plasmid pMER2. DNA from clone 8Sp-II was digested with *Xho*I and *Bgl*II and the 0.9-kb fragment was inserted into pMER2. The resulting plasmid was used to overexpress *alm1*$^+$.

Results

Immunolocalization of mAb8 in *S. pombe* cells

The distribution of mAb8 with respect to the position of the nucleus in wild-type *S. pombe* cells over the course of the cell cycle is shown in Fig. 1. The mAb8 antigen localizes to the medial region of the cell from the anaphase stage through cytokinesis (Fig. 1, cells 3–6). Staining with mAb8 is still visible immediately after septation (Fig. 1, cell 6) but antibody binding is not detectable in interphase or metaphase cells (Fig. 1, cells 1–2).

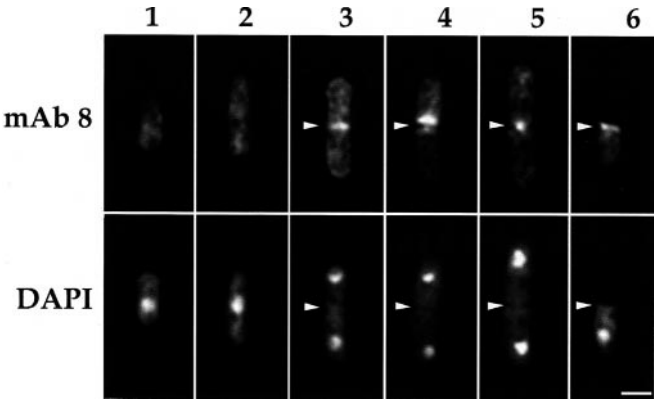

Fig. 1 Indirect immunofluorescence staining of the mAb8 antigen and DAPI staining of nuclei during the cell cycle in wild-type *S. pombe* TPY38 cells. During interphase (panel 1) and metaphase (2), no mAb8 staining is observed. From anaphase to late mitosis and cytokinesis (3–6) mAb8 labels the medial region of the cells. All the cells are shown at the same magnification. *Bar* 5 μm

To investigate further the subcellular distribution of the antigen recognized by mAb8, confocal microscopy was performed (Fig. 2). The results confirmed that the mAb8 antigen associates with the medial region of mitotic cells (Fig. 2, cells 2–4) but not of interphase cells (Fig. 2, cell 1). Moreover, the analysis revealed that the mAb8 antigen spans the whole equatorial region of the cell and is apparently not distributed in a ring-like fashion (Fig. 2, cell 2).

To determine the time at which the mAb8 antigen first appears, we used the mutants *cdc25-22*, *cdc4-8* and

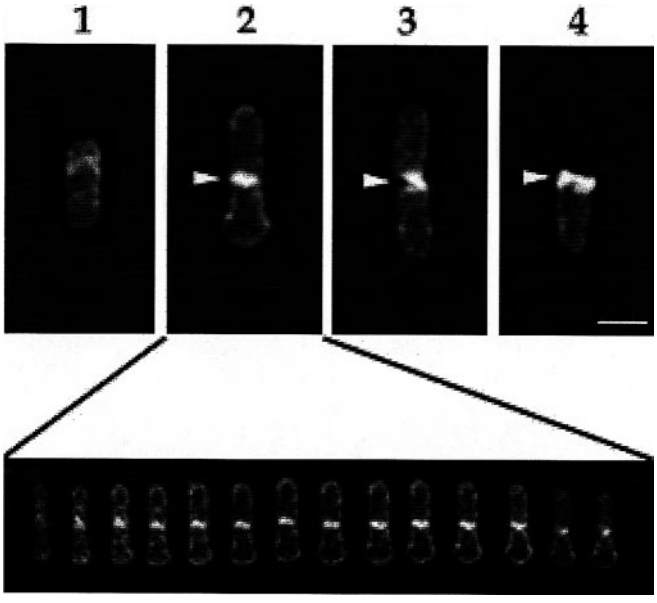

Fig. 2 Confocal microscopy of *S. pombe* cells stained for the mAb8 antigen. Whole-cell images were generated by digital summation of multiple 200-nm optical sections. Interphase cells (panel 1) display no mAb8 staining, while in cells undergoing division (2–4), mAb8 spans the whole equatorial region. The serial sections corresponding to cell 2 are shown at the bottom. *Bar* 5 μm

cdc7. *cdc25-22* mutants are affected in entry into mitosis, *cdc4-8* in contractile ring formation and *cdc*7 cells are defective in septum formation (for review see Le Goff et al. 1999). A block and release experiment using *cdc25-22* showed that the mAb8 signal first appears when the nuclei are clearly separated, i.e. during mid- to late anaphase (Fig. 3). No mAb8 signal was detected in *cdc4* or *cdc7* mutant cells (data not shown).

Isolation and molecular characterization of the cDNA encoding the mAb8 antigen in *S. pombe*

To isolate the DNA encoding the mAb8 antigen, immunoscreening of a *S. pombe* λZAP expression library was performed, using mAb8 as a probe. A positive clone (8Sp-I) containing a 3.6-kb insert, was isolated from a total of 500,000 recombinant phages screened. DNA from this insert contained a putative ORF lacking the N terminal region; we therefore rescreened the same cDNA library by DNA hybridization using a probe corresponding to the 5′ region of the cloned cDNA (see Materials and methods). A second clone (8Sp-II) that overlapped with 8Sp-I and contained a long 5′ noncoding region was isolated (Fig. 4A). Figure 4B shows the sequence of the cDNA and the deduced amino acid sequence of the putative ORF. An ORF of 3699 bp encoding 1233 amino acids was found. The predicted molecular mass of the putative protein product is 142 kDa. A consensus sequence for polyadenylation (AATAAA) followed 15 bases downstream by a poly(A) tail is found 65 bases downstream of the stop codon. We have termed the gene encoding this putative ORF *alm1*$^{+}$. The predicted amino acid sequence contains a low proportion – about 17% – of charged amino acids distributed along the whole molecule. Secondary-structure predictions (Chou and Fasman 1974; Garnier et al. 1978; Rost and Sander 1993) suggest that the putative protein has three structural domains, with two terminal globular segments separated by a discontinuous 1123-amino acid α-helical region (see Fig. 4C). Using the BLAST and FASTA algorithms (Pearson and Lipman 1988; Altschul et al. 1990) the protein was found to show 52% similarity to the myosin-like protein Mlp1 from *S. cerevisiae* (Kölling et al. 1993) and about 35% similarity to myosin heavy chains and the human nucleoprotein *tpr* (translocated promoter region) (Mitchell and Cooper 1992). Similarities were found mostly along the α-helical regions in all these cases (data not shown). These proteins are predicted to form long coiled-coil regions formed by the dimerization of the α-helical domains of two molecules through hydrophobic interactions. This hydrophobic interaction is based on the heptad periodicity along the primary amino acid sequence of the α-helix: in each of the heptads the first (a) and the fourth (d) residues are predominantly hydrophobic (Cohen and Parry 1986, 1990). The analysis of the Alm1 protein sequence revealed that the molecule is also organized in heptad repeats. A high level of hydrophobic amino acids (75%) is found at the first and fourth positions of the heptad, while very few charged residues are present (14%). Moreover, the deduced sequence for Alm1 may be divided into four α-helical segments based on the presence of short regions containing prolines, which can disrupt α-helical domains (Fig. 4C). In addition, two repeats of a hypothetical leucine-zipper sequence (Landshulz et al. 1988) are present at positions 218–239 and 902–923, respectively. Taken together our results suggest that Alm1 is most likely to be a coiled-coil protein.

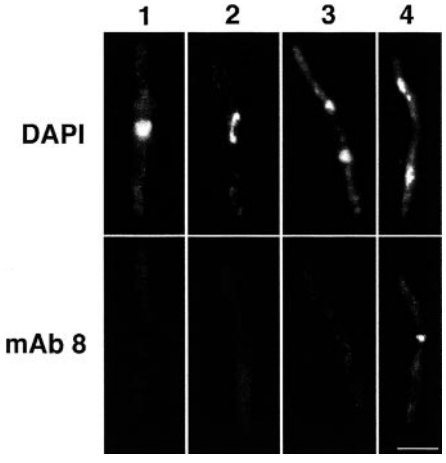

Fig. 3 Indirect immunofluorescence staining of the mAb8 antigen and DAPI staining of nuclei in *S. pombe cdc25-22* cells. The *cdc25-22* cells were shifted to non-permissive temperature (37° C) for 90 min and then back to the permissive temperature (30° C). Samples were taken and fixed at different times as described in Materials and methods. In metaphase (panel 1) and early anaphase (2 and 3), no mAb8 staining is observed. mAb8 staining in the medial region of the cell becomes visible as the distance between the nuclei increases (4). *Bar* 10 μm

Several recognition motifs for protein kinases are also present in Alm1. Interestingly, two potential phosphorylation sites for p34^{cdc2} kinase (S/TPXZ; where X is a polar amino acid and Z is generally basic) (Moreno and Nurse 1990) are located in the proline domains (amino acids 594, 596 and 877). This suggest that Alm1 protein may be phosphorylated in a cell cycle-dependent manner. A putative GTP-binding site (Robinshaw et al. 1986) is located at position 823. The predicted Alm1 protein shows two potential nuclear localization signals, KKXK (Chelsky et al. 1989), located at positions 636 and 1170; however, we were not able to detect the protein in the nucleus.

Disruption of *alm1*$^{+}$ and analysis of the mutant phenotype

We disrupted the *alm1*$^{+}$ gene by replacing an internal 2.5-kb *Hind*III-fragment with the *ura4*$^{+}$ gene of *S. pombe* as described in Materials and methods (Fig. 5A). Correct integration of the *ura4*$^{+}$ marker was confirmed by Southern analysis (Fig. 5B).

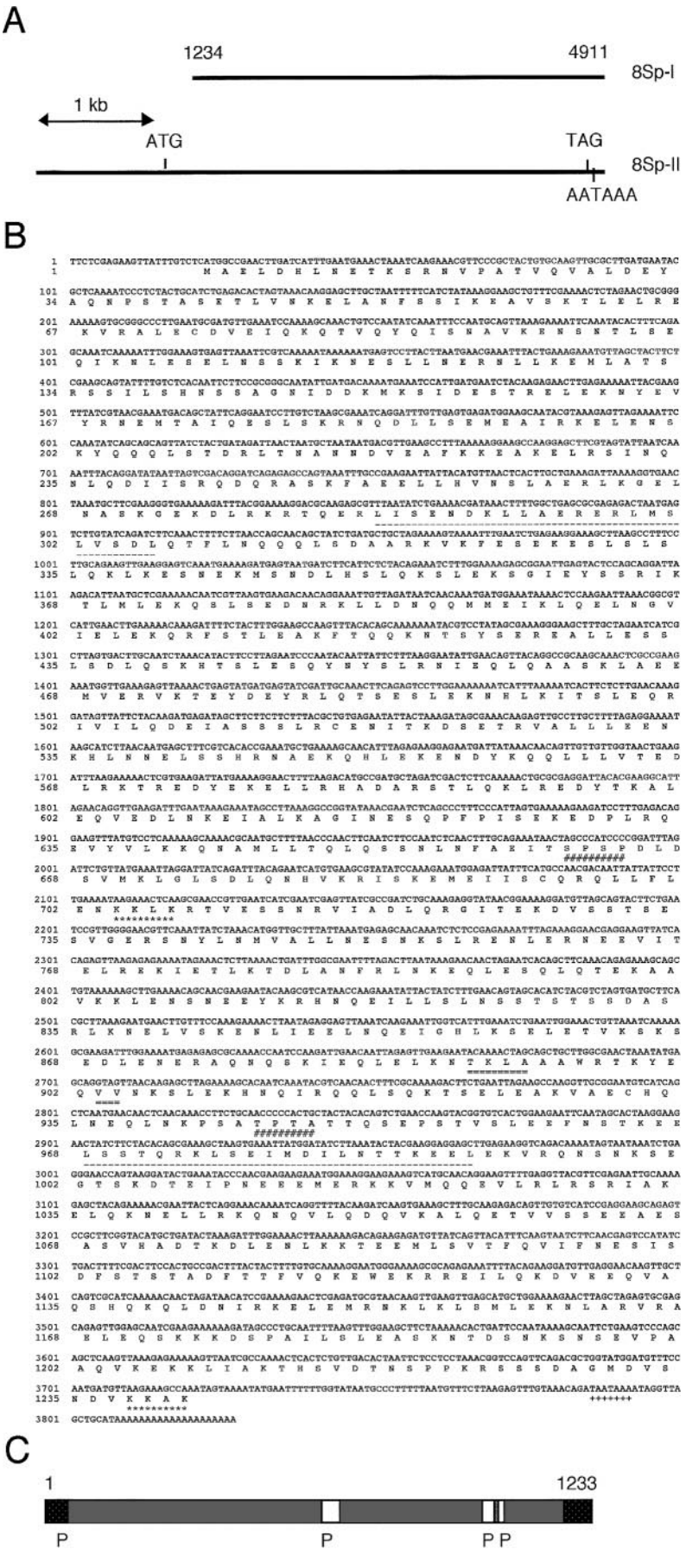

Fig. 4A–C Schematic representation of the *alm1* cDNA clones, cDNA and deduced amino acid sequences, and diagram of the predicted secondary structure of Alm1 protein. **A** Schematic drawing of the two overlapping cDNA clones that span the entire length (4911 bp) of the *alm1*$^{+}$ gene. The positions of the putative translation start and stop codons, and the polyadenylation signal sequence (AATAAA) are indicated. **B** Nucleotide sequence of the *alm1*$^{+}$ gene and predicted amino acid sequence of the putative protein product. The 3′ polyadenylation signal is indicated by plus signs; leucine zipper domains by *broken underlines*; the GTP binding domain by the *double broken underline*; the nuclear localization signals by *asterisks*; and the consensus phosphorylation sites for p34^{cdc2} kinase by ####. **C** Schematic diagram of the Alm1 protein indicating the positions of the globular domains (*black regions*) and the α-helical coiled-coil domains (*stippled regions*). Interruptions of the helical domain by proline (P) residues are indicated by the *white boxes*. The sequence is available from the EMBL GenBank under Accession No. AF010473

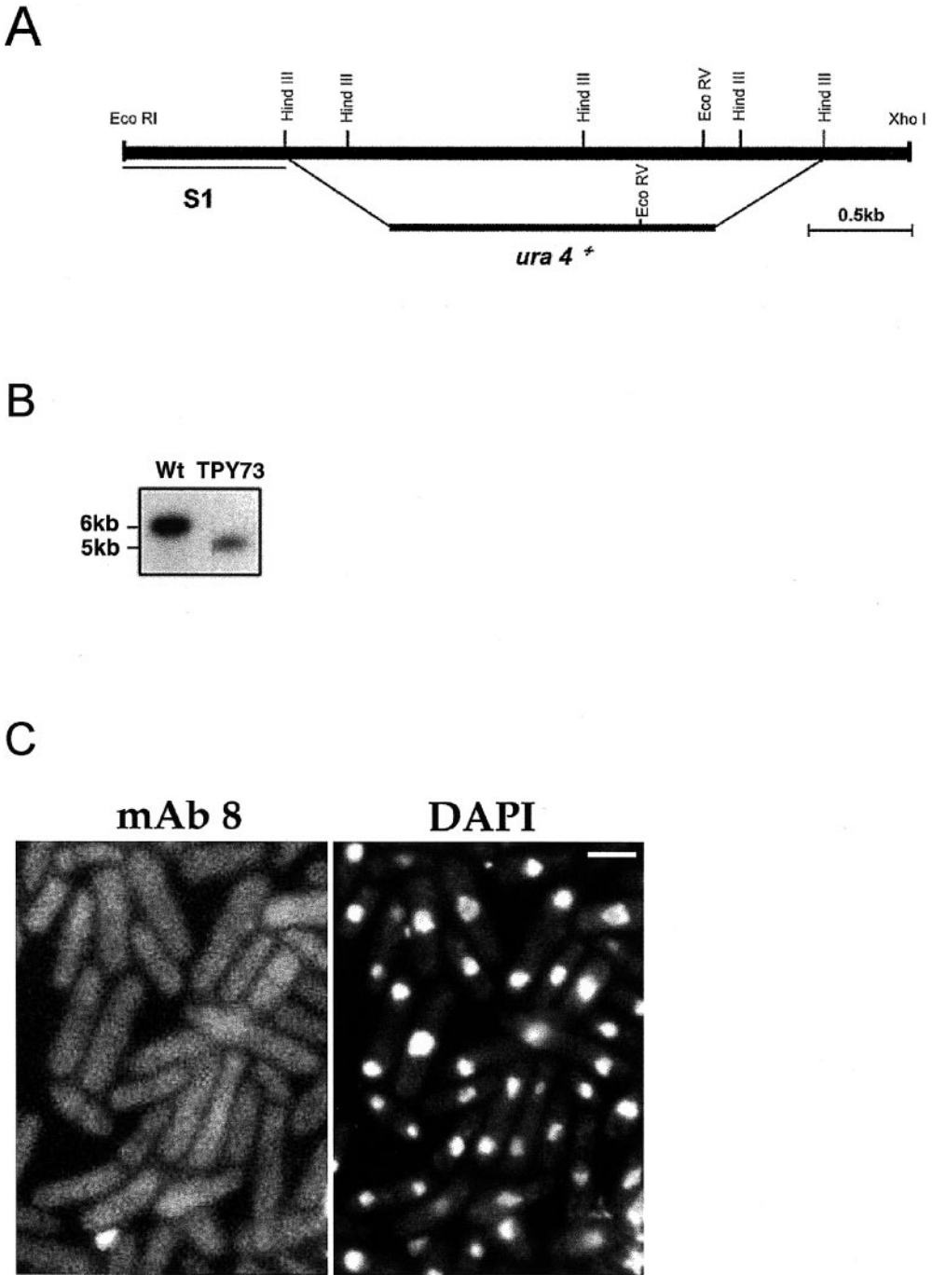

Fig. 5A Disruption of the *alm1*$^+$ gene. The 2.5-kb *Hin*dIII-fragment was replaced by the *ura4*$^+$ gene from *S. pombe* as described in Materials and methods. **B** Confirmation of the disruption by genomic Southern analysis. DNA was prepared from the wild type and the haploid disruptant TPY73 strain and digested with *Eco*RV. The 0.8-kb *Eco*RI-*Hin*dIII fragment (S1) was used as probe. The 6-kb band corresponds to the *alm1*$^+$ gene and the 5.2-kb band corresponds to the *alm1*$^+$::*ura4*$^+$ allele. **C** Indirect immunofluorescence staining of the mAb8 antigen and DAPI staining of *alm1*::*ura4*$^+$ *S. pombe* TPY73 cells, showing the absence of medial region staining by mAb8. *Bar* 5 μm

Cells carrying the disrupted *alm1*$^+$::*ura4*$^+$ gene were viable but reached a larger size than wild-type cells (Fig. 6). This "abnormal long morphology" gives the gene its name. Homozygous *alm1*::*ura4*$^+$ diploids sporulated normally, indicating that Alm1 is not involved in meiosis. However, release of the spores from the asci was delayed, relative to those derived from wild-type diploids and the spores did not initiate division (very occasionally two to three divisions could be observed). In contrast, spores from an *alm1*$^+$/*alm1*::*ura4*$^+$ heterozygous diploid germinated without problems, although those carrying the disrupted allele grew more slowly (data not shown). These results suggest that, while *alm1*$^+$ is important but not essential for mitotic cell division, it is essential for spore germination. No mAb8

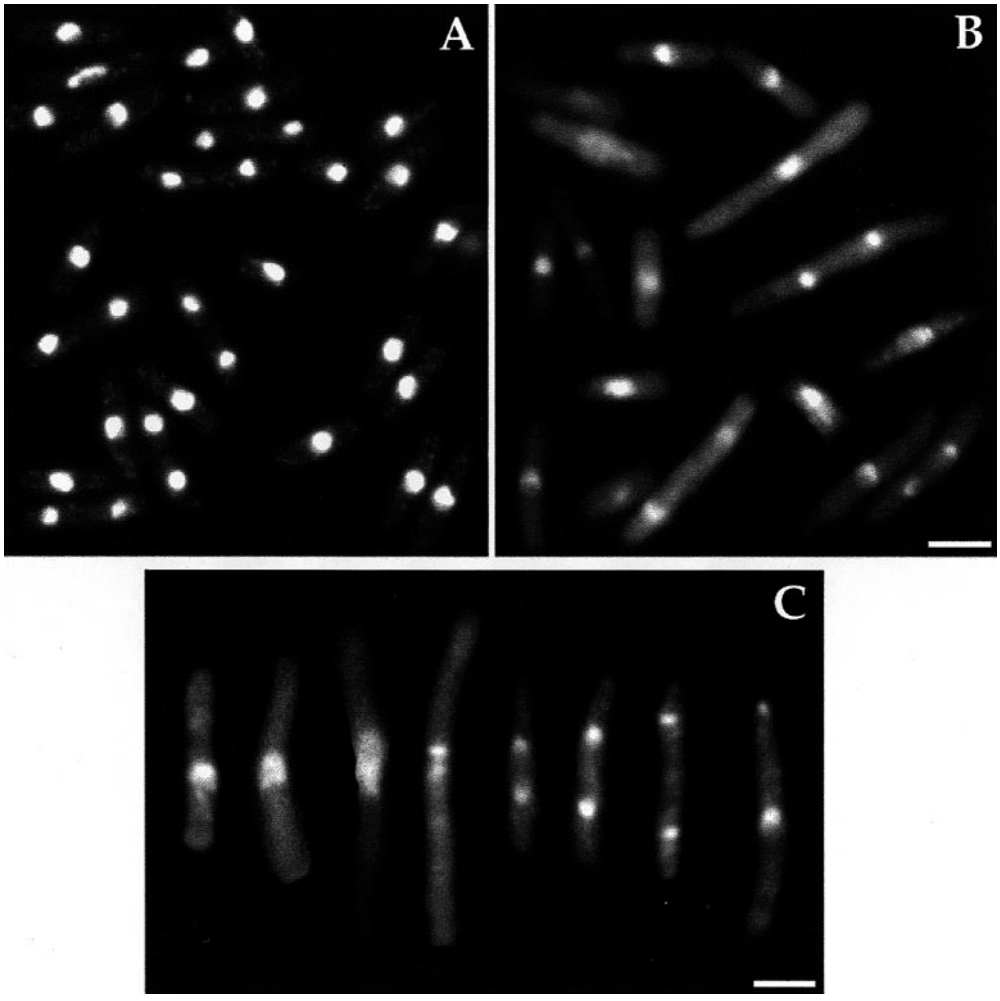

Fig. 6A–C DAPI staining of diploid wild-type and homozygous *alm1*::*ura4*$^+$/*alm1*::*ura4*$^+$ *S. pombe* cells. Homozygous diploid cells TPY80 bearing the *alm1*$^+$::*ura4*$^+$ disruption (**B**) are much longer than wild-type diploid cells (**A**). Examples of elongated cells undergoing nuclear division are shown in **C**. All the cells are shown at the same magnification. *Bar* 10 μm

staining was observed in the medial region of either TPY73 (Fig. 5C) or TPY80 cells (data not shown) undergoing division, confirming the absence of the epitope recognized by mAb8. The homozygous *alm1*$^+$::*ura4*$^+$ diploid TPY80 showed normal nuclear morphology in interphase cells and also in cells undergoing nuclear division (Fig. 6B, C).

Formation of the actin ring was detected in haploid and homozygous diploid *alm1* mutants. As shown in Fig. 7, cells of different lengths that are undergoing division exhibit actin ring formation. Most of the elongated cells containing a single nucleus showed a normal interphase actin pattern (Marks and Hyams 1985). Spindle organization was analyzed using anti-tubulin antibodies and was apparently normal in cells of different lengths undergoing nuclear segregation (Fig. 8). In addition, as in the case of actin staining, the elongated cells containing a single nucleus exhibited the microtubular pattern corresponding to interphase (Hagan and Hyams 1988). These results indicate that the mutant cells, or at least most of them, are able to perform nuclear division and cytokinesis irrespective of their size. Moreover, the appearance of this phenotype may reflect a delay in entering mitosis.

Reintroduction of a full-length *alm1*$^+$ clone under the control of the *nmt1* promoter rescued the mAb8 signal in Δ-*alm1* cells. The overexpression of *alm1*$^+$ did not cause any change in the distribution of the mAb8 antigen during the cell cycle of *S. pombe* (data not shown).

Discussion

We have identified a protein in fission yeast which we have named Alm1, for abnormal long morphology, which associates with the medial region of the cells and displays a cell cycle-dependent distribution. This protein belongs to a large group of proteins, similar to myosin and other fibrous proteins, that have a high content of potentially coiled-coil structures. According to our cytological observations Alm1 is a cytoplasmic protein that colocalizes with the medial region of the cells during

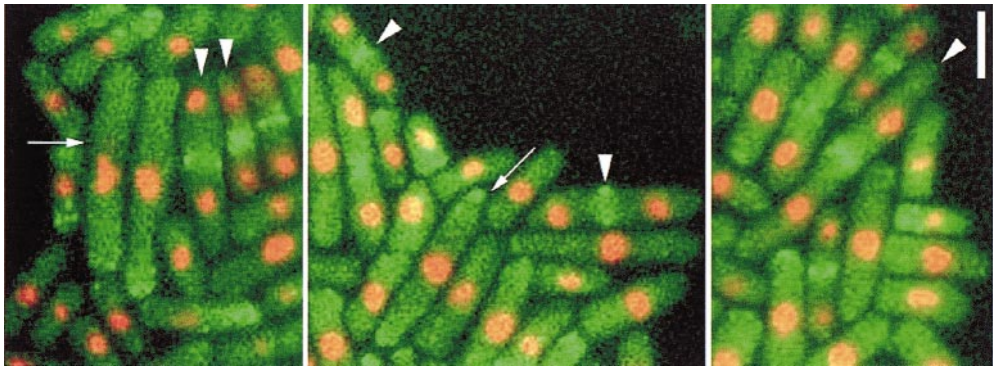

Fig. 7 Double staining of *alm1*::*ura4*$^{+}$ *S. pombe* cells (strain TPY73) with rhodamine-labeled phalloidin (*green*) and DAPI (*red*). Examples of undivided elongated cells that exhibit the actin distribution pattern typical of interphase are indicated by the *arrows*. Cells undergoing nuclear division show, irrespective of their size, actin ring formation in the medial region (*arrowheads*). *Bar* 5 μm

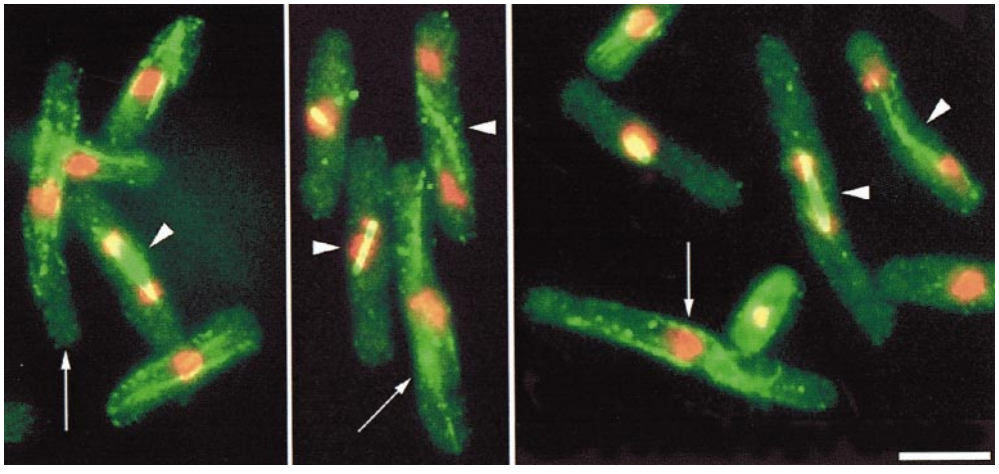

Fig. 8 Double staining of diploid *S. pombe* cells (strain TPY80) homozygous for the *alm1*::*ura4*$^{+}$ disruption allele with anti-tubulin antibodies (*green*) and DAPI (*red*). Examples of elongated cells containing a single nucleus and showing a microtubule pattern characteristic of interphase are indicated by the *arrows*. *Arrowheads* indicate cells undergoing mitosis. *Bar* 10 μm

nuclei separation at anaphase. The protein is found in the equatorial region of the cells, where the F-actin contractile ring is known to direct cytokinesis in *S. pombe* (Chang and Nurse 1996). However, the Alm1 pattern differs from that described for F-actin throughout the *S. pombe* cell cycle (Marks and Hyams 1985). Unlike F-actin, Alm1 is apparently not associated with the nucleus in early mitosis and is not detected as dots in cells at the end of interphase. The cellular localization of Alm1 during the cell cycle poses the question of whether this protein could become a constituent of the contractile ring at the onset of anaphase. From our cytological analysis, this seems unlikely, since Alm1 distributes throughout the central equatorial region and its accumulation in this medial cell region is maintained until the end of cytokinesis. In agreement with this, in *cdc25* cells Alm1 is not observed at the medial region until late anaphase. Moreover, the lack of Alm1 signal in *cdc4* and *cdc7* cells suggests that Alm1 localization in *S. pombe* requires the formation of the contractile ring and the septum. It is conceivable that Alm1 is present in the membrane underlying the growing septum. That Alm1 is not a constituent of the F-actin contractile ring is consistent with its lack of any of the previously described actin-binding motifs characteristic of the actin-binding proteins. Furthermore, the cytological analysis of Δ*alm1* cells showed that the elongated cells exhibited F-actin ring structures and were able to perform cytokinesis. The elongated cell morphology observed in the mutant may be due to a delay in entering mitosis. The results from the germination studies suggest that Alm1 could be required to initiate cell division in a dose-dependent manner (data not shown).

It is curious that the Alm1 function is not essential. This contrasts with the situation for other gene products that are required for contractile ring formation whose absence also results in elongated cells (for review see Nurse et al. 1976; Chang and Nurse 1996). This may indicate that other proteins may at least partially substitute for the function of Alm1. In fact, we have found in the databases a putative ORF in a cosmid which shows homology with Alm1 (EMBL Accesion number Z70690, ORF SPAC1F3.06c).

Our analysis indicated that Alm1 contains potential coiled-coil structures similar to those found in myosin and other fibrous proteins. Alm1 shows the highest degree of similarity with a 220-kDa myosin-like protein encoded by the *MLP1* gene of *S. cerevisiae* (Kölling et al. 1993). Mlp1, in turn, is similar to Nuf2, another potential coiled-coil protein, that associates with the spindle pole body (SPB) and is required for nuclear division in yeast (Osborne et al. 1994). As already mentioned, in higher eukaryotes, the antibody mAb8 binds to equatorial structures and the spindle poles. We cannot dismiss the

idea that in *S. pombe* Alm1 might also localize to the SPB, in addition to its localization in the equatorial region.

Like other coiled-coil proteins (Cohen and Parry 1986, 1990) two Alm1 molecules could form dimers due to hydrophobic interaction between their α-helical segments. Moreover, the nonhelical segments could be involved in interactions with other proteins, as well as in forming higher-order structures of Alm1 dimers. The two leucine-zipper domains found in the central α-helical region of Alm1 could facilitate protein dimerization, as has been described in other cases (Landshulz et al. 1988). None of the basically charged regions critical for DNA binding are located upstream of the zipper sequences (Bush and Sassone-Corsi 1990). This is consistent with the observed lack of intranuclear staining by mAb8. Several recognition sequences for phosphorylation by different kinases are found in the Alm1 sequence. This is noteworthy in view of the importance of phosphorylation in the assembly of fibrous coiled-coil proteins like myosins and intermediate filaments (for review see Yang et al. 1992). In particular, two potential sites for $p34^{cdc2}$ kinase, located in the proline domains, suggest that Alm1 may be phosphorylated in a mitosis-specific fashion.

Taken together, our data indicate that Alm1 is a new component that participates in the structural organization of the medial region in fission yeast. They also suggest a possible involvement of this protein in the cellular mechanisms underlying nuclear segregation during mitosis.

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