Identification of cold-sensitive mutations in the *Schizosaccharomyces pombe* actin locus

Dannel McCollum[a,*], Mohan Balasubramanian[b], Kathleen Gould[c]

[a] *University of Massachusetts Medical Center, Department of Molecular Genetics and Molecular Biology, 377 Plantation Street, Worcester, MA 01605, USA*
[b] *Institute of Molecular Agrobiology, Singapore 118240, Singapore*
[c] *Howard Hughes Medical Institute and Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*

Received 26 March 1999; received in revised form 26 April 1999

Abstract In recent years, the actin cytoskeleton in *Schizosaccharomyces pombe* has become the subject of intense scrutiny. However, to date, only a single actin mutation has been identified. Described here is the isolation and characterization of four new cold-sensitive actin mutations. Sequence analysis of the mutant actin genes indicated that each of these mutations caused alterations in single amino acids that are conserved in all actin sequences. These mutants differ in their phenotypes. One of these mutations (*act1-48*) was identified as an extragenic suppressor of a mutation in the *cdc4* gene, which is required for actin ring formation and cytokinesis. Interestingly, when *act1-48* mutant cells were shifted to the restrictive temperature, actin patches were not detected but the actin ring formation and stability was unaffected. The three other mutations, *act1*-16, *act1*-32 and *act1*-67, primarily affected the actin ring formation or stability while F-actin patches did not seem to be substantially different in appearance. Given that the ultrastructural architectures of F-actin patches and the F-actin ring are presently unclear, these mutations, which affect one structure or the other, should be useful for future studies on the role of actin itself in the function of these F-actin-containing structures in *S. pombe*.

Key words: Fission yeast; Actin; Cytokinesis; Septum formation

1. Introduction

In eukaryotic cells, the actin cytoskeleton plays a fundamental role in numerous cellular processes including cell motility, cytokinesis, cell polarity and, in yeast, secretion and endocytosis [1]. The function of actin and its associated proteins in these processes is exceedingly complex. Simple genetic systems such as yeast have proven to be quite useful in dissecting complex cellular pathways. Considerable characterization of the actin cytoskeleton in the budding yeast *Saccharomyces cerevisiae* has been achieved. Many actin binding proteins have been identified in a large measure due to the large number of actin mutants available in this organism. These were created by direct mutagenesis of the *S. cerevisiae* actin gene [2,3]. They have been used for a number of different genetic screens to identify components that interact with the actin cytoskeleton [4–6].

Other studies suggest that the fission yeast *Schizosaccharomyces pombe* may also be a valuable model organism for the study of actin function [7]. The actin cytoskeleton of *S. pombe* contains three major types of F-actin structures which re-organize during the cell cycle. In the interphase, actin is found in patches localized at the growing ends of cells. Actin cables which run the length of the cell have also been observed. At the onset of mitosis, a ring of F-actin forms in the medial region of the cells which is believed to be analogous to the cleavage furrow of higher eukaryotes. During the anaphase, actin patches become concentrated adjacent to the actin ring rather than at the cell ends. After completion of the anaphase, the ring begins to constrict and the septum is deposited in the wake of the constricting ring. Upon exit from mitosis, actin patches once again localize to the growing ends of the cell. Numerous *S. pombe* mutants have been isolated that affect cytokinesis (reviewed in [8,9]). Many of the corresponding genes encode actin binding proteins. Also, numerous cell shape and growth polarity mutants of *S. pombe* have identified proteins that modulate the actin cytoskeletal architecture (reviewed in [10]). However, only a single actin allele has been identified in *S. pombe* [11] and no actin mutations have been identified that cause a loss of actin patches. In this paper, we describe the identification and characterization of four new cold-sensitive alleles of the actin gene, each with distinctive properties. Further study of these mutants should enable the role of actin in cellular processes to be evaluated and should provide a means to use genetic analysis to identify actin-interacting proteins.

2. Materials and methods

2.1. Strains, media and genetic and molecular biology methods

S. pombe strains used in this study are listed in Table 1. Growth media were as previously described [12]. Cells were grown in yeast extract (YE) medium or EMM minimal medium with appropriate supplements. Standard genetic and recombinant DNA methods [12,13] were used except where noted. Yeast transformations were performed using electroporation [14]. Because the *act1-16, act1-32* and *act1-67* strains mated poorly, genetic crosses involving these strains were performed by selecting for diploid cells using nutritional markers following standard crosses or cell fusion [12]. Diploid strains were then induced to sporulate by incubation in ME medium. All double mutant strains were constructed by tetrad dissection using a Singer MSM dissecting microscope. DNA was prepared from bacteria and isolated from agarose gels using Qiagen kits and from yeast cells as previously described [15].

2.2. Genetic screens

The *act1-48* strain was isolated in a screen for *cdc4-8* suppressors. Approximately 2×10^8 cells of a *cdc4-8 ura4-D18 leu1-32 h*$^-$ strain were mutagenized for 15 min with nitrosoguanidine (NTG) as described [12] and plated at 36°C. This screen yielded 400 colonies.

*Corresponding author. Fax: (1) (508) 856 8508.
E-mail: dannel.mccollum@ummed.edu

PII: S0014-5793(99)00619-5

Table 1
Strains used

Strain	Genotype	Source
JZ758	*mam2::LEU2, leu1*-32 *ura1 ade6*-M216, h^{90}	C. Shimoda
KGY439	*cdc4-8 ura4-D18 leu1-32 h*	Our stock
YDM105	*leu1-32 ura4-D18 ade6-210* h^-	Our stock
YDM106	*leu1-32 ura4-D18 ade6-210* h^+	Our stock
YDM107	*leu1-32 ura4-D18 ade6-216* h^-	Our stock
YDM108	*leu1-32 ura4-D18 ade6-216* h^+	Our stock
YDM53	*lys1-131* h^+	Our stock
YDM189	*act1-48 ura4-D18 leu1-32 lys1-131* h^-	This study
YDM190	*act1-48 ura4-D18 leu1-32 lys1-131* h^+	This study
FCS41	*act1-41 mam2::LEU2, leu1*-32 *ura1 ade6*-M216, h^{90}	This study
FCS127	*act1-127 mam2::LEU2, leu1*-32 *ura1 ade6*-M216, h^{90}	This study
YDM181	*act1-16 ura4-D18 leu1-32 ade6-216* h^-	This study
YDM184	*act1-32 ura4-D18 leu1-32 ade6-210* h^-	This study
YDM182	*act1-67 ura4-D18 leu1-32 ade6-216* h^-	This study
YDM93	*cdc4-8 act1-48 ura4-D18 leu1-32 lys1-131 h*	This study
KGY492	*cdc3-124 leu1-32 ura4-D18 ade6-216 h*	Our stock
KGY901	*cdc8-110 leu1-32 ura4-D18 ade6-210 h*	Our stock
KGY1003	*cdc12-112 leu1-32 ura4-D18 ade6-210 h*	Our stock
YDM28	*cdc4-31 ade6-210 leu1-32* h^+	Our stock
YDM227	*lys1-131, mei1-102*	P. Nurse

12 of these colonies were unable to grow at 19°C and were pursued further. These 12 strains were crossed to wild-type cells (YDM53) to determine if the suppressing mutation was intragenic or extragenic and whether the cold-sensitive phenotype was linked to the suppressor mutation. Four strains were identified as carrying extragenic cold-sensitive suppressor mutations and these were characterized further as described in Section 3. All mutants were then back-crossed at least three times to wild-type strains YDM53, YDM105, YDM106, YDM107 or YDM108 as appropriate.

A previously described strategy [16–19] was employed to screen for cold-sensitive mutants defective in cytokinesis. A *mam2::LEU2 ade6*-M216 *leu1*-32 *ura1*$^-$ h^{90} (JZ758, a kind gift of Dr C. Shimoda) strain was used as the starting strain for mutagenesis. The homothallic *mam2* null mutant is capable of mating with h^- cells but is incapable of mating with h^+ cells due to the fact that the *mam2*$^+$ gene encodes the plus factor receptor. However, a diploid strain of the genotype *mam2::LEU2/mam2::LEU2* h^{90}/h^{90} is capable of meiosis and sporulation since conjugation is not necessary under these circumstances. The genetic screen was carried out with the following rationale. JZ758 was mutagenized with NTG and cells were allowed to recover, after mutagenesis, at 30°C until cells began to cycle normally. The culture was then split three ways and shifted to 19°C for 8 h. Following the temperature shift, cells were washed three times with sporulation medium and incubated in the same medium for 3 days to allow meiosis and sporulation. Spores were prepared by digesting ascus and cell walls with glusulase and spores were suspended in water. Spores were plated on YE agar plates and incubated at 30°C until colonies appeared. Colonies were replica-plated to YE agar plates containing the vital dye phloxin B and incubated 2 days at 19°C. These plates were screened microscopically for phenotypes exhibited by cytokinesis mutants. Five mutant strains (*act1-41, act1-16, act1-32, act1-67* and *act1-127*) were chosen for further analysis and were back-crossed at least three times to wild-type strains YDM105, YDM106, YDM107 or YDM108 as appropriate.

2.3. Identification of act1 mutations

Genomic DNA was isolated from *S. pombe act1* mutant strains as described [15]. The *act1* encoding region of each strain was amplified by the polymerase chain reaction (PCR) using the oligonucleotides 5′-CCCTCAAAAGACAAGACC-3′ and 5′-CGTTACAAAAAGTAAGAGGAGC-3′. The resultant DNA fragments were sequenced directly using the Thermo Sequenase radiolabelled terminator cycle sequencing kit (Amersham) and the priming oligonucleotides. To complete the DNA sequence of each PCR fragment, the oligonucleotide 5′-GTTTTGTCTTTGTATGCC-3′ was used to prime the reactions. A single base substitution relative to wild-type *act1*$^+$ was detected in each PCR fragment. Oligonucleotide primers were obtained from Operon.

2.4. Microscopy

All fluorescence microscopy was performed using a Nikon 600 microscope and images were captured using a Dage CCD camera. DNA and septal material were visualized using DAPI and calcofluor, respectively, as described by Balasubramanian et al. (1997). Immunofluorescence was performed using methanol fixation as described previously [20]. Antibodies to Cdc4p [21] and actin (Amersham) were diluted 1:100 and detected with Texas red-tagged anti-rabbit IgG and Texas red-tagged anti-mouse IgG antibodies (Molecular Probes).

3. Results

3.1. Identification of an actin mutant as a suppressor of the cdc4-8 mutation

In order to identify genes important for the function of Cdc4p, an EF-hand protein with properties of a myosin light chain, we screened for mutations capable of suppressing the *cdc4-8* temperature-sensitive growth defect at 36°C. Strain KGY439 (*cdc4-8 ura4-D18 leu1-32* h^-) was mutagenized with NTG and incubated at 36°C. Colonies that arose were replica-plated to 19°C and assayed for growth. Strains which were unable to grow at 19°C were crossed to wild-type cells to determine if the *cdc4-8* suppression was extragenic and segregated with the cold-sensitive phenotype. Four such extragenic cold-sensitive suppressors were identified. Each suppressor strain was back-crossed to wild-type three times to create strains carrying the suppressor mutation on its own. Linkage analysis demonstrated that the four suppressor mutations defined a single genetic locus. In order to test for dominance, each suppressor strain was crossed to a *mei1-102* strain (*mei1-102* mutants will mate with h^- partners, but the resulting diploids will not sporulate) and stable diploids were obtained using complementation of auxotrophic markers. All suppressor mutations were found to be recessive for the cold-sensitive growth phenotype. To test whether these mutations were dominant in their ability to suppress the *cdc4-8* growth defect at 36°C, diploid strains homozygous for the *cdc4-8* mutation but heterozygous for the suppressing mutations were constructed. These diploid strains were capable of growth at 36°C, thus the suppressing mutations are dominant in their ability to suppress the *cdc4-8* mutation. One of these strains

(YDM189) was transformed with a genomic library and three plasmids were identified that were capable of rescuing the cold-sensitive growth defect. Molecular cloning and sequence analysis demonstrated that these plasmids contained the actin gene, *act1*$^+$. *act1*$^+$ has been shown previously to be tightly linked to the *his3* locus [11]. All four suppressor mutations were found to be extremely tightly linked to *his3* and a subsequent sequence analysis of the *act1* gene from the four mutants revealed that the same amino acid was altered in all four cases. The allele of *act1* defined by this mutation was designated *act1-48*.

3.2. Identification of other actin alleles

Several other actin mutants were isolated as part of a screen for cold-sensitive mutants defective in cytokinesis (see Section 2). We used a strategy which enriched for mutants that diploidize by one of the following routes: re-replication of chromosomal DNA, improper chromosome segregation or failure to undergo cytokinesis followed by nuclear fusion [16–19]. Colonies were visually screened for those which displayed morphologies characteristic of cytokinesis mutants such as elongated or branched cells that were often undergoing cell lysis. Colonies were further screened by staining with DAPI and calcofluor, which stain DNA and septal material, respectively, to identify those strains with multiple nuclei and either irregular septa or no septa at all. Five such strains were identified (*act1-16, act1-32, act1-41, act1-67* and *act1-127*) and found to be in the same linkage group. These mutants were also found to be tightly linked to *his3* and in the same linkage group as *act1-48*. Furthermore, sequence analysis identified mutations in the *act1* locus of each of these strains confirming that these strains were actin mutants. The *act1-16* and *act1-41* as well as the *act-32* and *act1-127* strains harbored identical mutations and thus, only one of each was characterized further (see below). The cold-sensitive growth defect of *act1-16* and *act1-67* mutants was determined to be recessive, whereas the *act1-32* cold-sensitive growth defect was found to be dominant. All four actin alleles were compared to wild-type for growth at 30°C and 19°C. The *act1-67, act1-48* and *act1-32* alleles grew similarly to wild-type at 30°C, but were unable to form colonies at 19°C. The *act1-16* mutant strain grew at 30°C but the cells had aberrant morphologies. At 19°C, it grew very poorly although it was capable of forming very small colonies.

3.3. Genetic analysis

As a way to gain insight into the molecular basis by which the *act1-48* mutation suppressed *cdc4-8*, we characterized the *act1-48* mutation using further genetic analysis. One mechanism of suppression could be that the *act1-48* mutation has a positive effect on medial ring formation in general. To test this possibility, we crossed *act1-48* to other mutants defective in medial ring formation such as *cdc3-124* (profilin) (KGY492) [22], *cdc8-110* (tropomyosin) (KGY901) [23] and *cdc12-112* (KGY1003) [24]. We found that the *cdc12-112* and *act1-48* mutations were synthetically lethal and both the *cdc3-124 act1-48* and *cdc8-110 act1-48* double mutants had more severe growth defects than either single mutant. Thus, it did not seem that the *act1-48* mutation generally promoted the process of actin ring formation. A second possibility was that the *act1-48* mutation enhanced the function of Cdc4p or actually weakly bypasses the requirement for Cdc4p. If this were the case, one would predict that the *act1-48* mutation might rescue other alleles *cdc4*. However, *act1-48* was not able to rescue the *cdc4-31* mutant strain (YDM28) even though this mutation is not as severe as the *cdc4-8* mutation. Finally, it could be imagined that any weakly inactivating mutation in the actin gene would rescue the *cdc4-8* mutation. To test this possibility, we crossed *act1-16, act1-32* and *act1-67* to *cdc4-8* to check if they were capable of rescuing it. This analysis showed that the *cdc4-8* mutation displayed synthetic lethality in combination with either the *act1-16* or *act1-32* mutation. Although some *cdc4-8 act1-67* double mutants were recovered, the *act1-67* mutation was not able to suppress the *cdc4-8* growth defect at 36°C. Together, these analyses suggest that the *act1-48* mutation specifically suppresses the defect in *cdc4-8* cells, although the mechanism of this suppression remains unclear.

3.4. Phenotypic characterization of mutants

Actin plays a central role in the formation of the division septum during cytokinesis. To examine the ability of the actin mutants to form septa at the restrictive temperature, cells were grown at 30°C and then shifted to 19°C for 6 h and stained with calcofluor which stains the septa and cell wall. The *act1-16* mutant cells were branched and formed multiple irregular septa at both 19°C and 30°C (Fig. 1B and data not shown). The *act1-32*, *act1-48* and *act1-67* mutant cells formed septa similar to wild-type at 30°C (data not shown). However, at 19°C, *act1-67* cells made irregular septa (Fig. 1E) and *act1-32* cells had only faint deposits of septal material (Fig. 1C). Interestingly, the *act1-48* mutant made relatively normal septa at 19°C but displayed irregular deposits of septal material at the ends of the cell (Fig. 1D).

Septal defects are usually indicative of defects in forming the medial actomyosin ring since the medial ring guides the placement of the septum [7,9]. Actin patches also play a role in septum formation as well as polarized growth [25]. To examine the effects of these actin mutations on actin patches

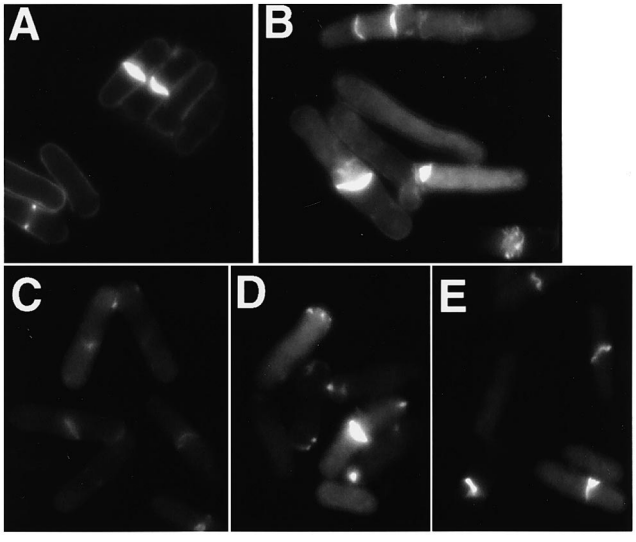

Fig. 1. Septum staining in the different actin mutants. Wild-type (strain YDM105, A), *act1-16* (strain YDM181, B), *act1-32* (strain YDM184, C), *act1-48* (strain YDM189, D) or *act1-67* (strain YDM182, E) cells growing exponentially at 30°C were shifted to 19°C for 6 h, then fixed and stained with calcofluor to visualize septal material.

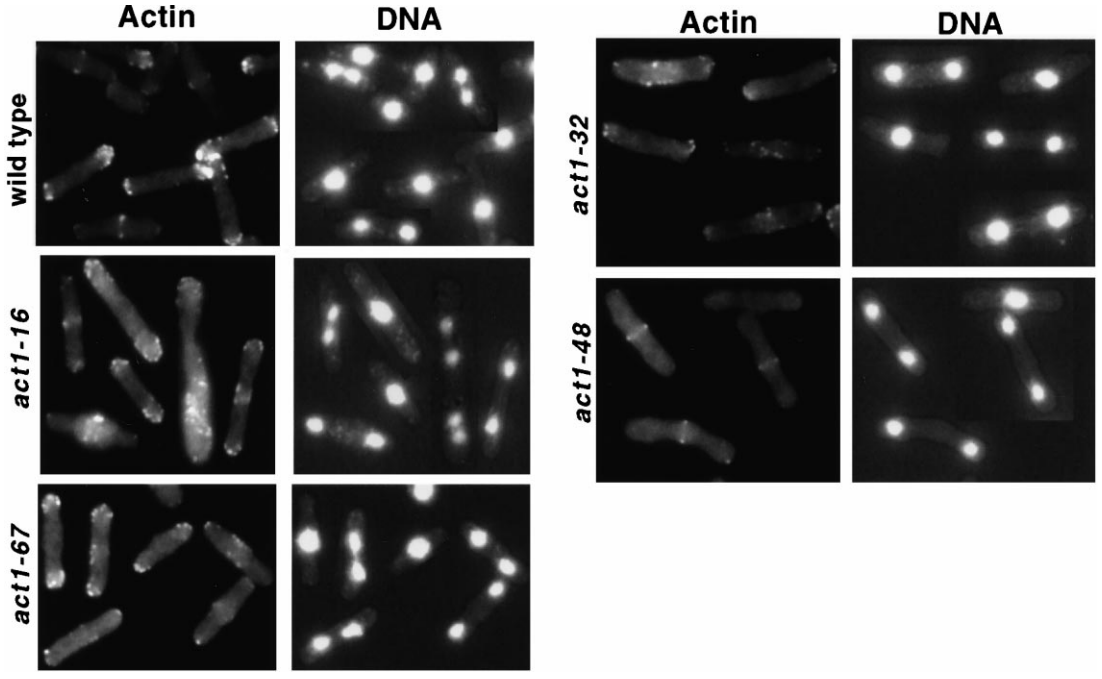

Fig. 2. Actin staining in actin mutant cells. Wild-type (strain YDM105), *act1-16* (strain YDM181), *act1-67* (strain YDM182), *act1-32* (strain YDM184) or *act1-48* (strain YDM189) cells growing exponentially at 30°C were shifted to 19°C for 6 h, then fixed and double-stained for actin and DNA.

and actin rings, the actin mutant strains were pre-cultivated as described above and then stained with antibodies against actin and the actin ring component Cdc4p. Cells were also stained with rhodamine-conjugated phalloidin which gave a similar staining pattern to that observed using actin antibodies (data not shown). The *act1-67* and *act1-16* mutant strains displayed actin patch staining similar to wild-type cells, but both strains seemed to make disorganized medial rings as judged by both actin and Cdc4p staining (Figs. 2 and 3). These strains also accumulated cells with multiple nuclei due to a failure in cytokinesis. Occasionally, normal looking medial rings were observed in the *act1-16* mutant which is consistent with this strain being able to grow, albeit poorly, at 19°C (Fig. 3, see arrow). The *act1-32* mutant cells displayed even more drastic defects in cytokinesis as seen by the accumulation of binucleate cells. Actin rings were never observed in *act1-32* mutant cells although actin patches did seem to localize to the medial region of the cell (Fig. 2). In addition, the actin patch staining was fainter than that observed in wild-type (Fig. 2 and data not shown). *act1-32* mutant cells

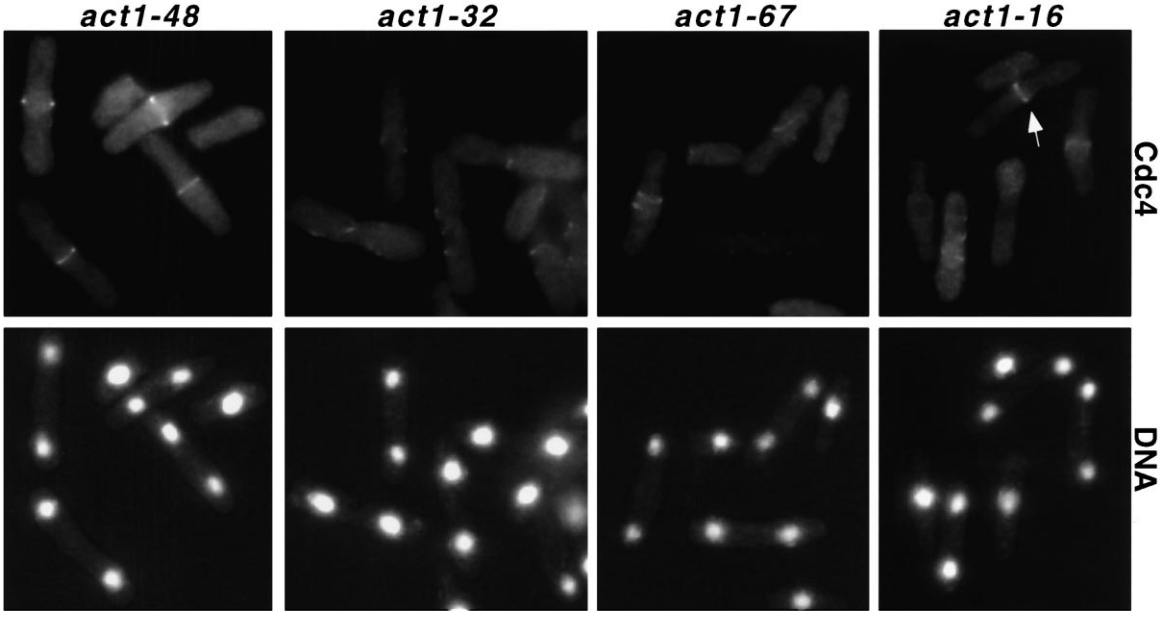

Fig. 3. Cdc4p staining in actin mutant cells. Wild-type (strain YDM105), *act1-16* (strain YDM181), *act1-67* (strain YDM182), *act1-32* (strain YDM184) or *act1-48* (strain YDM189) cells growing exponentially at 30°C were shifted to 19°C for 6 h, then fixed and double-stained for Cdc4p and DNA. Arrows indicate structures described in the text.

Table 2
Identification of *act1* alleles

Mutant allele	48	16/41*	67	32/127*
Wild-type sequence	GGT	CGT	ACC	GGT
	Gly-46	Arg-116	Thr-160	Gly-245
Mutant sequence	AGT	CAT	ATC	GAT
	Ser-46	His-116	Ile-160	Asp-245

*These pairs of alleles proved to be iso-allelic to one another.

contained short cables as well as punctate structures that contained Cdc4p in the medial region, but no clear Cdc4p rings (Fig. 3). The *act1-48* mutant displayed quite a distinct phenotype. Intense medial ring staining was observed in this mutant using antibodies against both actin (Fig. 2) and Cdc4p (Fig. 3). However, virtually no actin patches were seen in these cells. Actin patch staining was also faint in *act1-48* mutant cells at the permissive temperature of 30°C (data not shown).

3.5. Sequence of the mutants

To identify the mutations in the different actin alleles, the *act1* locus was amplified from each strain using PCR and the PCR products were sequenced directly. Each strain was found to contain a single mutation that resulted in an amino acid change (Table 2). The *act1-16* and *act1-41* as well as the *act1-32* and *act1-127* pairs of alleles were found to contain identical mutations. The *act1-48* (Gly-46 to Ser), *act1-16* (Arg-116 to His), and *act1-32* (Gly-245 to Asp) mutations are all in highly conserved residues. According to the model for the actin filament, the *act1-32* mutation lies in a region of the actin molecule proposed to be involved in actin/actin interaction and the *act1-48* and *act1-16* mutations are adjacent to regions of actin/actin contact [26]. The *act1-67* mutation is in the ATP binding pocket and, therefore, may be interfering with some aspects of nucleotide binding or hydrolysis.

4. Discussion

We have described, in this paper, the identification and characterization of four novel mutations of the *S. pombe* actin gene. All four are cold-sensitive mutations but each one has different properties. Not surprisingly, the three actin mutants isolated in the screen for mutants defective in cytokinesis (*act1-16, act1-32* and *act1-67*) all seem to be defective primarily in the formation of the medial actin ring. Hence, they arrest as primarily as binucleate cells with absent or abnormal septa. The *act1-16* and *act1-67* mutants continue to elongate and maintain actin patch staining at the restrictive temperature. In contrast, the *act1-32* mutant shows a reduced actin patch staining after prolonged incubation at the restrictive temperature and does not elongate to the same extent as the other two alleles, suggesting that the actin patch function may also be compromised to some extent in this mutant.

Interestingly, the fourth mutation, *act1-48*, which was isolated as a *cdc4-8* suppressing mutation, is not defective in actin ring formation, but does seem to be defective in the actin patch formation or stability. Two possible explanations could account for this differential defect in the actin patch structure among the mutants. First, the *act1-48* mutant protein could be defective in interacting with a component of patches that is not required for ring formation. Alternatively, there may be some intrinsic difference in actin dynamics between actin patches and the actin ring which renders actin patches more sensitive to the effects of the *act1-48* mutation. Consistent with its ability to affect actin patch dynamics, we have previously shown that the *act1-48* mutation was synthetically lethal in combination with mutations in the actin patch proteins Arp3p [25] and Sop2p [27]. However, we have now found that the *act1-48* mutation also displayed negative genetic interactions with mutations in genes required for actin ring formation, namely *cdc12*, *cdc8* and *cdc3-124*. Thus, the possibility remains that this mutant may have generalized defects in the actin function.

An intriguing result is the specificity by which the *act1-48* mutation suppresses the *cdc4-8* mutation. As mentioned above, the *act1-48* mutation will not suppress mutations in other genes required for actin ring formation or even a different allele of *cdc4*. Also, the other actin mutations identified here will not suppress *cdc4-8*. The sequence of the *act1-48* mutation has not provided insight into why this mutation behaves so differently than the others. The mutation is adjacent to a region involved in actin/actin interaction and could thus potentially affect the actin filament formation or stability. Clearly, more biochemical analysis of the *act1-48* mutant protein will be required to understand its properties.

The sequence of the *act1-32* mutation was somewhat more informative. The same mutation present in the *act1-32* allele was identified previously in the human actin gene from the transformed cell line HUT 14 [28]. When this mutant actin gene was overexpressed in an immortalized but not tumorigenic cell line, it caused the cells to become tumorigenic [29]. Expression of this corresponding mutant actin gene in *Drosophila melanogaster* flight muscles causes myofibrillar disruption [30]. Since non-mutant actin was present in both of these experiments, the G245D mutant actin must have been functioning in a dominant manner. Consistent with this interpretation, we found that the cold-sensitive phenotype of the *act1-32* mutation, unlike the other actin mutations identified here, was dominant. Because we identified the *act1-32* mutation based on its ability to induce an increase in the cell ploidy by causing a failure in the cytokinesis, it would be interesting to determine if expression of this mutant actin affects the cytokinesis in mammalian cells and as a result, their ploidy. Polyploidy is often associated with oncogenic transformation and thus, the association between expression of this mutant actin and a transition to a tumorigenic state observed by Leavitt and colleagues could be due to effects on cytokinesis.

Although the actin cytoskeleton in *S. pombe* has become the subject of intensive study in recent years (for review see Gould and Simanis [9]), until now, only one other actin mutation has been described in *S. pombe* [11]. The previously identified actin mutant, termed *cps8*, contained actin patches but formed disorganized rings and failed in cytokinesis. This phenotype is much like that of the *act1-16, act1-32* and *act1-67* mutants identified here, although the cytokinesis defect in the *act1-32* mutant appears to be tighter and *cps8* is not iso-allelic with the four mutations reported here [11]. Studies of the *S. pombe* actin cytoskeleton have been hampered by the lack of reagents and mutations that completely eliminate specific actin structures. This problem has been eliminated to some extent through the use of the drug latrunculin A which will completely eliminate both actin patches and actin rings in *S. pombe* [31]. However, latrunculin A is very expensive and has the drawback for certain experiments that it eliminates all actin structures and thus cannot be used to test the effects

of eliminating just actin patches or actin rings. The identification of the new actin alleles described here should be helpful in this regard since the *act1-32* and *act1-48* alleles most strongly effect the actin ring and actin patches, respectively. These new actin alleles should also be useful in genetic screens designed to identify other F-actin ring or F-actin patch components in *S. pombe*.

Acknowledgements: Many thanks to Dr C. Shimoda and Dr P. Nurse for strains. This work was supported by the National Institutes of Health Grant GM56730 to D.M. and by the Howard Hughes Medical Institute, of which K.L.G. is an Assistant Investigator. All experiments described here comply with the current laws of the USA.

References

[1] Ayscough, K.R. and Drubin, D.G. (1996) J. Annu. Rev. Cell Dev. Biol. 12, 129–160.
[2] Shortle, D., Novick, P. and Botstein, D. (1984) Proc. Natl. Acad. Sci. USA 81, 4889–4893.
[3] Wertman, K.F., Drubin, D. and Botstein, D. (1992) Genetics 132, 337–350.
[4] Novick, P., Osmond, B.C. and Botstein, D. (1989) Genetics 121, 659–674.
[5] Adams, E.M. and Botstein, D. (1989) Genetics 121, 675–683.
[6] Welch, M.D., Vinh, D.B.N., Okamura, H.H. and Drubin, D.G. (1993) Genetics 135, 265–274.
[7] Marks, J. and Hyams, J.S. (1985) Eur. J. Cell Biol. 39, 27–32.
[8] Chang, F. and Nurse, P. (1996) Cell 84, 191–194.
[9] Gould, K.L. and Simanis, V. (1997) Genes Dev. 11, 2939–2951.
[10] Mata, J. and Nurse, P. (1998) Trends Cell Biol. 8, 163–167.
[11] Ishiguro, J. and Kobayashi, W. (1996) FEBS Lett. 392, 237–241.
[12] Moreno, S., Klar, A. and Nurse, P. (1991) Methods Enzymol. 194, 795–823.
[13] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[14] Prentice, H.L. (1992) Nucleic Acids Res. 20, 621.
[15] Hoffman, C.S. and Winston, F. (1987) Gene 57, 267–272.
[16] Broek, D., Bartlett, R., Crawford, K. and Nurse, P. (1991) Nature 349, 388–393.
[17] Hayles, J., Fisher, D., Wollard, A. and Nurse, P. (1994) Cell 78, 813–822.
[18] Chang, F., Wollard, A. and Nurse, P. (1996) J. Cell Sci. 109, 131–142.
[19] Balasubramanian, M.K., McCollum, D., Wong, K.C.Y., Chang, L., Naqvi, N.I., He, X., Sazer, S. and Gould, K.L. (1998) Genetics 149, 1265–1275.
[20] Balasubramanian, M.K., McCollum, D. and Gould, K.L. (1997) Methods Enzymol. 283, 494–506.
[21] McCollum, D., Balasubramanian, M.K., Pelcher, L.E., Hemmingsen, S. and Gould, K.L. (1995) J. Cell Biol. 130, 651–660.
[22] Balasubramanian, M.K., Hirani, B.R., Burke, J.D. and Gould, K.L. (1994) J. Cell Biol. 125, 1289–1301.
[23] Balasubramanian, M.K., Helfman, D.M. and Hemmingsen, S.M. (1992) Nature 360, 84–87.
[24] Chang, F., Drubin, D. and Nurse, P. (1997) J. Cell Biol. 137, 169–182.
[25] McCollum, D., Feoktistova, A., Morphew, M., Balasubramanian, M. and Gould, K.L. (1996) EMBO J. 15, 6438–6446.
[26] Holmes, K.C., Popp, D., Gebhard, W. and Kabsch, W. (1990) Nature 347, 44–49.
[27] Balasubramanian, M., Feoktistova, A., McCollum, D. and Gould, K.L. (1996) EMBO J. 15, 6426–6437.
[28] Vandekerckhove, J., Leavitt, J., Kakunaga, T. and Weber, K. (1980) Cell 22, 893–899.
[29] Leavitt, J., Ng, S.Y., Varma, M., Latter, G., Burbeck, S., Gunning, P. and Kedes, L. (1987) Mol. Cell Biol. 7, 2467–2476.
[30] Sakai, Y., Okamoto, H., Yamada, T. and Hota, Y. (1990) J. Biochem. 107, 499–505.
[31] Bähler, J. and Pringle, J.R. (1998) Genes Dev. 12, 1356–1370.