An actin point-mutation neighboring the 'hydrophobic plug' causes defects in the maintenance of cell polarity and septum organization in the fission yeast *Schizosaccharomyces pombe*

Junpei Ishiguro*, Wataru Kobayashi

Department of Biology, Faculty of Science, Konan University, Okamoto 8-9-1, Kobe 658, Japan

Received 18 June 1996

Abstract The fission yeast *cps8* mutation gives rise to abnormally enlarged and dispolarized cells, each of which contains several nuclei with aberrant multisepta. Molecular cloning and sequence analysis of the *cps8* gene indicated that it encodes an actin with an amino acid substitution of aspartic acid for glycine at residue 273 in the hydrophobic loop that is located between actin subdomains 3 and 4. Fluorescence microscopy using phalloidin and anti-actin antibody revealed changes in the F-actin structure and distribution in the mutant cells. These results indicate that the hydrophobic loop plays an essential role for creating normal F-actin structure, only by which cell polarity and the late mitotic events can be maintained properly.

Key words: Fission yeast; Actin mutation; *cps8*; Cell polarity; Septum organization; Spindle poison

1. Introduction

Over the past decade, yeast has been extensively used as model system for examining the regulatory mechanisms of the eukaryotic cell cycle. Because a variety of cytoskeletal mutants are available, yeast may also offer an advantage over other higher eukaryotes in studies of cytoskeletal functions during the cell cycle [1–4]. The yeast actin cytoskeleton has been shown to be involved, not only in late mitotic events such as septum formation and cytokinesis, but also in directing cell wall deposition at the growing region of the cell [5,6]. In the budding yeast *Schizosaccharomyces cerevisiae*, actin function is also required to maintain proper spindle orientation prior to the onset of anaphase [7]. All these events are, thus, essential for proper chromosome distribution, by which daughter cells inherit a complete set of genetic information required for the next cell cycle. Actin might function via interaction with a variety of its binding proteins, depending on the cell cycle stages. However, their structure/function relationships as well as the regulatory mechanisms of the interactions are poorly understood, although the three-dimensional structure of the actin molecule has been progressively refined in recent years [8–11]. To solve such problems, comparative analyses between changes in structure of functional molecules and in vivo phenotypes caused by mutations might be useful. In this report, we show that a point-mutation neighboring the hydrophobic plug of the actin molecule in the fission yeast *S. pombe* (called *cps8*) causes defects in the maintenance of cell polarity and late mitotic events, such as septum organization and cytokinesis.

*Corresponding author. Fax: (81) 78-435-2539

2. Materials and methods

2.1. Strains and culture conditions

The *S. pombe* strain, 972 (wild type) and CP18-15 (h^+ *cps8-188 leu1-32*) were described previously [12]. Routine growth (YE) and selective (EMM) media were as described in Alfa et al. [13]. Sporulation medium contains 1% glucose, 0.1% KH_2PO_4, 40.8 nM biotin, 2.1 μM calcium-pantothenate, 81.2 μM nicotinic acid and 55.5 μM inositol. The *Escherichia coli* strains, HB101 and MV1184, were used for routine propagation of plasmids.

2.2. Isolation and characterizations of cps8 gene

The mutant strain, CP18-15, was transformed with *S. pombe* genome library as described elsewhere [14]. A transformant capable of growing at 36°C was isolated and the plasmid recovered (called pA932). Three kinds of fragments, 4.0 kb *Hin*dIII–*Bam*HI, 3.0 kb *Bam*HI–*Hin*dIII and 4.3 kb *Sac*II–*Hin*dIII, generated from pA932, were subcloned into pAU-KS plasmid and tested for their ability to complement the mutant phenotype described above. Nested deletions were prepared from pUC119 subclones containing 2.0 kb *Sac*II–*Kpn*I fragment of pA932 by the modified method of Henikoff [15]. The nucleotide sequence of both DNA strands was determined by the dideoxy chain-termination method [16], using A.L.F. DNA Sequencer (Pharmacia). To determine if a cloned gene is authentic *act1*+, one-step gene disruption method was employed [17]. A diploid strain (h+/h− *ade6−M210/ade6−M216 ura4−D18/ura4−D18 cps8−118/+*) was transformed with a 3.4 kb *Sac*II–*Xho*I fragment that contains 1.8 kb *ura4*+ gene [18] at the *Bam*HI site, and several diploids capable of growing on a minimal medium (EMM) were isolated. Spore formation and asci dissection were carried out according to standard methods. For Southern analysis, DNAs prepared from a wild-type strain, a diploid transformant and cosmid clones 32H8c (ICRFc 60H0832), 29B5c (ICRFc 60B0529) were digested with *Hin*dIII followed by agar gel electrophoresis. Blotting analysis was carried out by DIG-ELISA method (Boehringer Mannheim), using 3.9 kb *Hin*dIII–*Kpn*I fragment as a probe. *Cps8* gene was cloned from the mutant genome by amplification with PCR [19]. Primer regions used are indicated by single-underlines in Fig. 2C. The amplified genes (1258 bp) were cloned into palsmid PCR®II (Invitrogen), and five independently isolated clones were sequenced.

2.3. Fluorescence microscopy

Log-phase cells grown in YE medium at 28°C were fixed with formaldehyde and stained with 4′,6-diamidino-2-phenylindole (DAPI), calcofluor and rhodamine-conjugated phalloidin as described elsewhere [20,21]. For indirect immunofluorescence microscopy, cell walls of the fixed cells were digested enzymatically and then stained with anti-actin or anti-α-tubulin monoclonal antiboty [22]. In the case of temperature-shift experiments, the cells, grown at 28°C, were sedimented and immediately transferred to a fresh YE medium prewarmed at 36°C, and then cultivated for 4 h at the restrictive temperature.

3. Results and discussion

3.1. Molecular characterizations of cps8 gene

Cps8 was originally identified as a mutation responsible for supersensitivity to the spindle poison, isopropyl *N*-3-chlorophenyl carbamate [12]. At permissive temperature (28°C), the

PII S0014-5793(96)00819-8

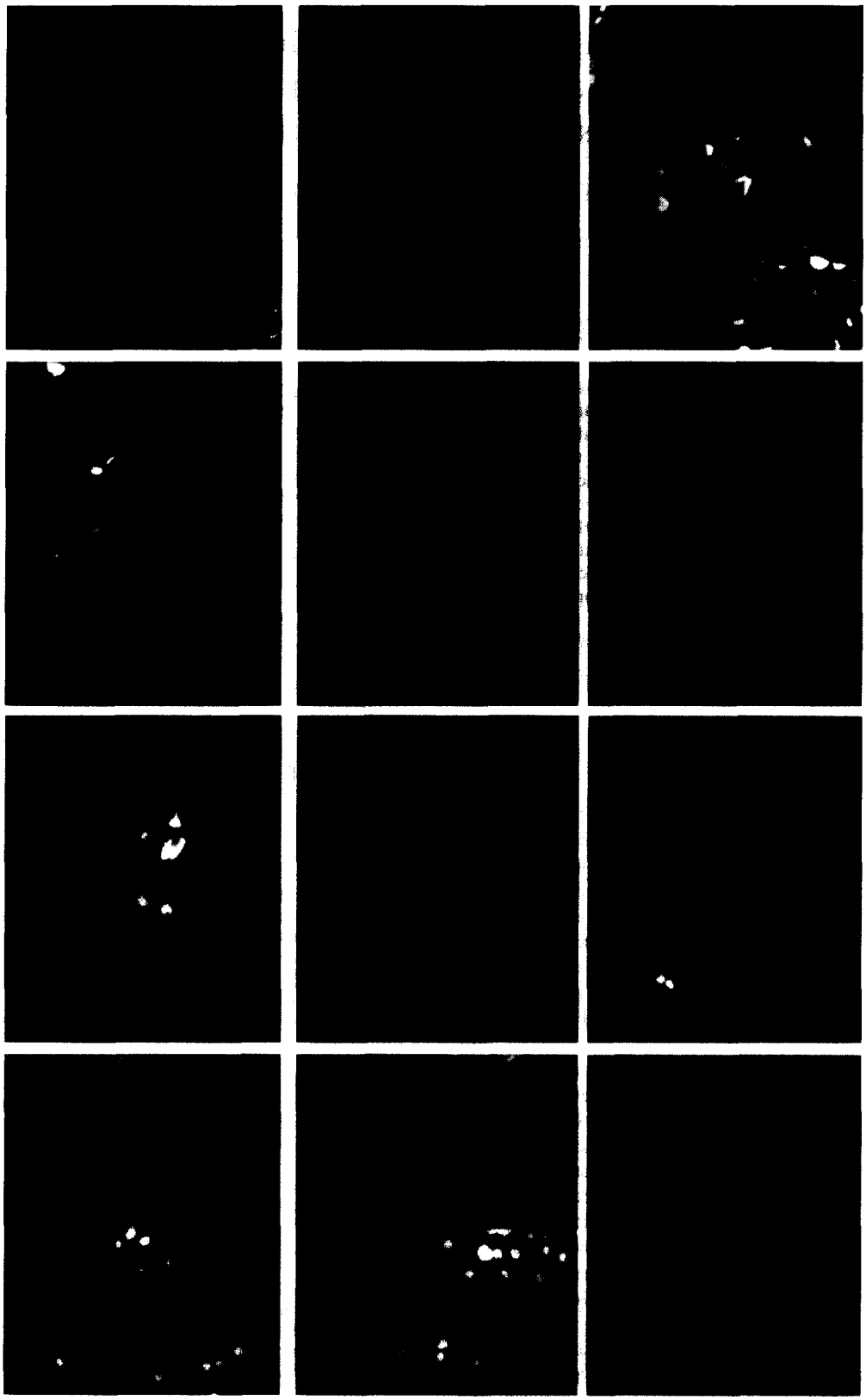
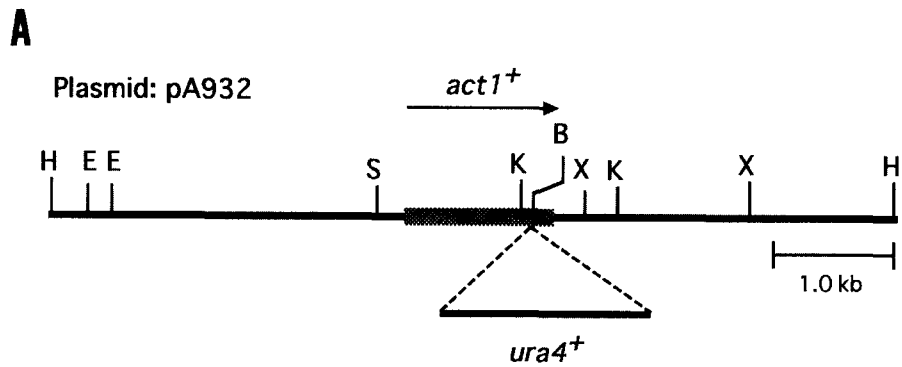
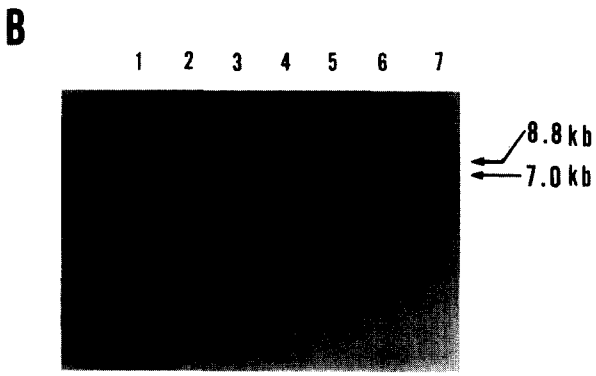


Fig. 1. Micrographs of the *S. pombe cps8* mutant cells. (A) Phase-contrast, *cps8* cells. (B) 4′,6-diamidino-2-phenylindole (DAPI) staining, *cps8* cells. (C) Calcofluor staining, *cps8* cells. (D) Calcofluor staining, *cps8* cells in shifted culture (36°C). (E) DAPI staining, *cps8* cells in shifted culture (36°C). Arrows indicate incompletely segregated nuclei. (F) Rhodamine-conjugated phalloidin staining, wild-type cells. (G) Rhodamine-conjugated phalloidin staining, *cps8* cells. (H) Anti-actin antibody staining, cps8 cells. (I) Anti-actin antibody staining (upper images) and DAPI staining (lower images) in the same field of *cps8* cells in shifted culture (36°C). (J) Anti-tubulin antibody staining (upper images) and DAPI staining (lower images) in the same field of wild-type cells. An arrow indicates mitotic spindle or nuclei. (K) Anti-tubulin antibody stainig (upper images) and DAPI staining (lower images) in the same field of *cps8* cells. An arrow indicates mitotic spindle or nuclei. (L) Anti-tubulin antibody staining (upper images) and DAPI staining (lower images) in the same field of *cps8* cells in shifted culture (36°C).





C

```
1     GCC GTG TTA TAA CTT ACC GTT TAC CAA CTA CAT TTT TTG TAA CGA ACC AAA AAA CCC TCA AAA GAC AAG ACC      72

73    ATG GAA GAA GAA ATC GCA GCG TTG GTT ATT GAT AAT GGC TCT GGT ATG TGC AAA GCC GGT TTC GCT GGA GAT     144
1     Met Glu Glu Glu Ile Ala Ala Leu Val Ile Asp Asn Gly Ser Gly Met Cys Lys Ala Gly Phe Ala Gly Asp      24

145   GAT GCC CCT AGA GCT GTA TTC CCC TCG ATT GTC GGT AGA CCC CGT CAC CAT GGT ATT ATG GTA GGT ATG GGA     216
25    Asp Ala Pro Arg Ala Val Phe Pro Ser Ile Val Gly Arg Pro Arg His His Gly Ile Met Val Gly Met Gly      48

217   CAA AAG GAT TCC TAC GTT GGT GAT GAA GCT CAA AGC AAG CGT GGT ATT TTG ACC TTG AAG TAC CCC ATT GAG     288
49    Gln Lys Asp Ser Tyr Val Gly Asp Glu Ala Gln Ser Lys Arg Gly Ile Leu Thr Leu Lys Tyr Pro Ile Glu      72

289   CAC GGT ATT GTC AAC AAC TGG GAT GAT ATG GAA AAG ATT TGG CAT CAC ACT TTC TAC AAC GAG CTT CGT GTT     360
73    His Gly Ile Val Asn Asn Trp Asp Asp Met Glu Lys Ile Trp His His Thr Phe Tyr Asn Glu Leu Arg Val      96

361   GCT CCT GAG GAG CAC CCT TGC TTG TTG ACT GAG GCT CCT TTG AAC CCC AAA TCC AAC CGT GAG AAG ATG ACT     432
97    Ala Pro Glu Glu His Pro Cys Leu Leu Thr Glu Ala Pro Leu Asn Pro Lys Ser Asn Arg Glu Lys Met Thr     120

433   CAA ATT ATT TTC GAA ACC TTT AAT GCA CCT GCC TTT TAT GTT GCT ATT CAA GCT GTT TTG TCT TTG TAT GCC     504
121   Gln Ile Ile Phe Glu Thr Phe Asn Ala Pro Ala Phe Tyr Val Ala Ile Gln Ala Val Leu Ser Leu Tyr Ala     144

505   TCT GGT CGT ACC ACT GGT ATC GTC TTG GAC TCT GGT GAT GGT GTT ACC CAC ACT GTT CCC ATT TAT GAG GGT     576
145   Ser Gly Arg Thr Thr Gly Ile Val Leu Asp Ser Gly Asp Gly Val Thr His Thr Val Pro Ile Tyr Glu Gly     168

577   TAT GCT CTT CCT CAT GCT ATC ATG CGT CTT GAT CTC GCC GGT CGT GAC TTG ACT GAC TAC CTT ATG AAG ATT     648
169   Tyr Ala Leu Pro His Ala Ile Met Arg Leu Asp Leu Ala Gly Arg Asp Leu Thr Asp Tyr Leu Met Lys Ile     192

649   CTC ATG GAG CGT GGT TAT ACT TTC TCT ACT ACC GCC GAA CGT GAA ATT GTT CGT GAC ATC AAG GAG AAA CTT     720
193   Leu Met Glu Arg Gly Tyr Thr Phe Ser Thr Thr Ala Glu Arg Glu Ile Val Arg Asp Ile Lys Glu Lys Leu     216

721   TGC TAC GTC GCT TTG GAC TTT GAG CAA GAA CTT CAA ACT GCT GCT CAA TCT TCC TCC CTT GAG AAA TCT TAT     792
217   Cys Tyr Val Ala Leu Asp Phe Glu Gln Glu Leu Gln Thr Ala Ala Gln Ser Ser Ser Leu Glu Lys Ser Tyr     240

793   GAG CTT CCT GAT GGT CAA GTT ATC ACT ATC GGA AAC GAG CGT TTC CGT GCC CCT GAA GCT CTT TTC CAA CCC     864
241   Glu Leu Pro Asp Gly Gln Val Ile Thr Ile Gly Asn Glu Arg Phe Arg Ala Pro Glu Ala Leu Phe Gln Pro     264

865   TCA GCT TTG GGT CTT GAA AAC GCC GAT ATT CAT GAG GCT ACT TAC AAC TCT ATC ATG AAA TGT GAT GTT GAT     936
265   Ser Ala Leu Gly Leu Glu Asn Ala Asp Ile His Glu Ala Thr Tyr Asn Ser Ile Met Lys Cys Asp Val Asp     288

937   ATT CGT AAA GAT TTG TAC GGT AAC GTT GTT ATG TCT GGT GGT ACC ACT ATG TAT CCC GGT ATT GCC GAT CGT    1008
289   Ile Arg Lys Asp Leu Tyr Gly Asn Val Val Met Ser Gly Gly Thr Thr Met Tyr Pro Gly Ile Ala Asp Arg     312

1009  ATG CAA AAG GAG ATT CAA GCC CTT GCT CCT AGC TCC ATG AAG GTC AAG ATT GTT GCT CCT CCT GAG CGT AAA    1080
313   Met Gln Lys Glu Ile Gln Ala Leu Ala Pro Ser Ser Met Lys Val Lys Ile Val Ala Pro Pro Glu Arg Lys     336

1081  TAC TCT GTC TGG ATT GGT GGA TCC ATT CTT GCT TCT CTT TCT ACT TTC CAA CAG ATG TGG ATC TCC AAG CAA    1152
337   Tyr Ser Val Trp Ile Gly Gly Ser Ile Leu Ala Ser Leu Ser Thr Phe Gln Gln Met Trp Ile Ser Lys Gln     360

1153  GAG TAT GAT GAG AGC GGA CCT GGT ATC GTT TAC CGT AAG TGC TTC TAA GCT CCT CTT ACT TTT TGT AAC GTT    1224
361   Glu Tyr Asp Glu Ser Gly Pro Gly Ile Val Tyr Arg Lys Cys Phe ***                                     376

1225  TTT TAC ATA CTT TTG AAT AAC ATC GAT TCT TCT G                                                      1258
```

Fig. 2. Isolation and characterization of the *cps8* gene. (A) Restriction map and strategy for one-step gene disruption. A 7.0 kb fragment that complements the temperature-lethal phenotype was cloned (plasmid pA932), and a *Sac*II–*Kpn*I region (1964 bp) was sequenced. A 1.8 kb *ura4*$^+$ gene was inserted into the *Bam*HI site in the ORF. (B) Southern blot analysis. lane 1, pA932; lanes 2 and 3, wild-type genome; lanes 4 and 5, disruptant genome (diploid); lane 6, 32H8c cosmid clone (*cdc2* flanking region); lane 7, 29B5c cosmid clone (control). (C) Nucleotide and deduced amino acid sequence of the *cps8* gene (Accession No. D84318). The nucleotide sequence was identical with an *act1*$^+$ except that guanine at position 890 of the isolated gene (818 of the ORF) was replaced by adenine, predicting a substitution of Asp for Gly^{273} at protein level (the position is indicated by a double-underline). Restriction sites: H, *Hin*dIII; E, *Eco*R1; S, *Sac*II; B, *Bam*H1; X, *Xho*1; K, *Kpn*1.

mutant cells become enlarged and often bend or branch in a horn-like fashion (Fig. 1A). The cell contains several nuclei (Fig. 1B), and is partitioned with multiple septa which are situated diagonally along the long cell axis at irregular positions (Fig. 1C). After being transferred to a fresh medium at 36°C, the multinucleate cells rapidly divide into nearly normal-shaped cells (Fig. 1D), in which nuclear segregation is often blocked, resulting in the formation of incomplete binucleate cells which are characterized by pairs of nuclei (Fig. 1E). Under these conditions, cells suffer high lethality ($\approx$50% of the cell population loses viability at 4 h of incubation [23]). In this study, a wild-type gene was cloned by rescue of the temperature lethal phenotype of the *cps8* mutant. The base sequence analysis showed that the gene is identical to an *act1*+ reported by Mertins and Gallwitz [24]. To determine whether *act1*+ is authentic *cps8*+ itself or it is acting as an extragenic multicopy suppressor of *cps8*, one-step gene disruption using *ura4*+ gene was employed (Fig. 2A). Several *ura4*+ diploids were isolated and subjected to spore formation followed by tetrad analysis. Only two spores in the tetrad were capable of growing and were always ura− and cps8+ in the eight asci examined. Southern blot analysis showed both 7.0 and 8.8 kilo-base (kb) bands in the diploid genome (Fig. 2B), indicating that the *cps8* gene was virtually disrupted by the *ura4*+ gene. Thus, $act1^+$ is an authentic $cps8^+$ or a locus very closely linked to it, and is essential for cell division. We have previously mapped *cps8* on chromosome II tightly linked to *cdc2* by tetrad analysis [23]. To confirm this, physical mapping was performed, using an ordered cosmid library prepared by Hoheisel et al. [25]. A 3.9 kb *Hin*dIII–*Kpn*I fragment of the cloned gene hybridized on a 7.0 kb band of *Hin*dIII digested cosmid clone, 32H8c (ICRFc 60H0832) (Fig. 2A,B), indicating again the linkage between *cps8* (*act1*) and *cdc2* on the telomere side. To determine the site of the mutation which occurred in the $act1^+$ gene, a *cps8* gene was cloned from the mutant genome by means of polymerase chain reaction (PCR). Sequence analysis of five independent clones obtained from amplified genes indicated that guanine at position 818 of ORF was changed to adenine (GGT→GAT) in all cases examined. This indicates that, at protein level, glycine 273 would be replaced by aspartic acid (Fig. 2C). Gly273 is located at the last position of a hydrophobic loop which is composed of ten residues (264 to 273), and which is between subdomains 3 and 4 of the actin molecule. Holmes et al. [8] have proposed that the loop may generate a finger-like structure protruding from the filament axis at a right angle, with four residues in the loop, Phe266, Ile267, Gly268 and Met269 (the 'hydrophobic plug') which might then be inserted into the hydrophobic pocket formed on the opposing actin strand. Such a hydrophobic interaction might greatly stabilize the F-actin structure. Based on this hypothesis, Chen et al. [26]. created a *S. cerevisiae* actin mutant in which Leu^{266} was replaced by Asp to increase hydrophilicity, and then analyzed the in vivo phenotype. Unexpectedly, no obvious altered phenotype, other than cold-temperature sensitive growth, has been observed. In contrast with these results, dramatic phenotypic alterations were observed in our case, the replacement of Gly^{273} by Asp, as described above. The glycine residue, located at the neck region of the loop, may be an important factor in bending the finger-like structure in a right direction, since a polypeptide chain can be easily bent at the position of a glycine residue because its side chain is a hydrogen. When the glycine is replaced by aspartic acid by mutation, the special orientation of the finger-like structure may be changed with the result that the 'plug' region cannot interact properly with the hydrophobic pocket of the opposing actin strand. This may explain why the substitution of Asp for Gly^{273} in *S. pombe* caused phenotypic alterations to a much greater extent than that of Asp for Leu^{266} in *S. cerevisiae*.

3.2. Structure and distribution of F-actin and microtubules in cps8 cells

In subsequent experiment, actin staining was carried out using rhodamine-conjugated phalloidin in order to determine F-actin structures and distributions in the mutant cells. Fig. 1F shows typical staining patterns of the wild-type cells [21]. Actin dots are localized at the growing cell tip or tips and transient actin rings appear at the region of septum deposition. In the mutant cells, however, the fluorescent phalloidin failed to detect any F-actin structure at both permissive and restrictive temperatures (Fig. 1G). This, however, may not necessarily mean that no filamentous actin exists within the cell. In *S. cerevisiae*, an actin having two altered residues (R177A, D179A) was reported to be assembled into F-actin filaments which could be visualized by anti-actin immunofluorescence but not by phalloidin [27]. To determine whether this was the case, anti-actin monoclonal antibody staining was carried out. The immunofluorescence of the *cps8* cells showed intensely stained actin dots which distribute rather randomly throughout the cell, although actin ring structure was not observed (Fig. 1H). These results suggest that *cps8* F-actin filaments are still present but in an altered conformation, rather than change in phalloidin-binding site(s) itself [27], to which phalloidin cannot bind. After being shifted to restrictive conditions, multinucleate cells divide rapidly into several cells that contain one, and often, two incompletely segregated nuclei. Actin-antibody immunofluorescence showed a substantial decline in both staining intensity and number of actin dots (Fig. 1I), suggesting that the mutant F-actin filaments collapsed to a significant extent under restrictive conditions. To examine the effects of the actin mutation on microtubules organization, tubulin-antibody staining was carried out. Fig. 1J shows a typical pattern of microtubules during the cell cycle [22,28]; cytoplasmic microtubules span the long cell axis in interphase, and the spindle microtubules, resembling a thick bar in appearance, are observed during mitotic phase. The mutant cells growing at 28°C showed both cytoplasmic and mitotic microtubules arrays nearly indistinguishable from those observed in wild-type cells (Fig. 1K). After being shifted to restrictive conditions, mitotic spindle disappeared, and instead, only cytoplasmic-like microtubules that appear to be shorter and thicker than normal ones were observed, in which two incompletely segregated nuclei were present (Fig. 1L). These observations suggest that a defect in spindle organization during mitosis occurs in the mutant cells under restrictive conditions. The multinucleate cell is capable of surviving when cell division does not take place (*cps8* cells survive completely in saline solution at 36°C with no cell division [23]). It should be noted that the mutant cells transformed with $act1^+$ gene displayed a significant recovery in cell morphology as well as nuclear division under either permissive and restrictive conditions. In view of these observations, it is reasonable to suspect that progression of the late mitotic events in the absence of functional cytoskeleton, including either actin fila-

ments or/and microtubules, might bring about rapid cell death at 36°C. It has been well known that the late mitotic events (from metaphase to anaphase) can be driven by switching cdc2 kinase activity off. In the normal cell cycle, checkpoint control operates to arrest the cell cycle via changes in cdc2 kinase activities, when mitotic apparatus is impaired [29–31]. Thus, it is entirely possible that cdc2 kinase activity might be affected, when cytoskeletal structures are significantly disturbed in certain ways, possibly due to changes in cellular localization of cdc2 kinase and/or its regulators (also see ref. [32]). This scenario is consistent with the previous observation that *cps8* temperature lethality was substantially suppressed in a presence of adh-cdc25+ (over-expression of $cdc25^{+}$) [23]. In conclusion, our results strongly support the model of Holmes et al. [8] concerning the role of the hydrophobic interstrand interaction of the actin molecules. In addition, the present study showed that the hydrophobic interaction is essential for creating normal F-actin structure, only by which cell polarity and the late mitotic events can be maintained properly.

Acknowledgements: We thank F. Chang (Univ. of California, Berkeley) for helpful comments particularly on actin staining, C. Shimoda (Osaka City University) and H. Okayama (Univ. of Tokyo) for kindly providing the plasmids. We are also grateful to T. Miyazawa (Konan Univ.) for comments on peptide structure and H. Nakamura (Konan Univ.) for supporting this work.

References

[1] Novick, P. and Botstein, D. (1985) Cell 40, 405–416.
[2] Johannes, F.-J. and Gallwitz, D. (1991) EMBO J. 10, 3951–3958.
[3] Wertman, K.F., Drubin, D.G. and Botstein, D. (1992) Genetics 132, 337–350.
[4] Brown, S.S. (1993) Curr. Opin. Cell Biol. 5, 129–134.
[5] Robinow, C.F. and Hyams, J.S. (1989) in: The Molecular Biology of the Fission Yeast (Nasim, A., Yound, P.G. and Johnson, B.F., eds.), pp. 273–331, Academic Press, New York.
[6] Welch, M.D., Holtzman, D.A. and Drubin, D.G. (1994) Curr. Opin. Cell Biol. 6, 110–119.
[7] Palmer, R.E., Sullivan, D.S., Huffaker, T. and Koshland, D. (1992) J. Cell Biol. 119, 583–593.
[8] Holmes, K.C., Popp, D., Gebhard, W. and Kabsch, W. (1990) Nature 347, 44–49.
[9] Bremer, A. and Aebi, U. (1992) Curr. Opin. Cell Biol. 4, 20–26.
[10] Lorenz, M., Popp, D., and Holmes, K.C. (1993) J. Mol. Biol. 234, 826–836.
[11] Kabsch, W. and Vandekerckhove, J.(1992) Annu. Rev. Biophys. Biomol. Struct. 21, 49–76.
[12] Ishiguro, J. and Uhara, Y. (1992) Jpn J. Genet. 67, 97–109.
[13] Alfa, C., Fantes, P., Hyams, J., McLeod, M. and Warbrick, E. (1993) Experiments with Fission Yeast, A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, New York.
[14] Ishiguro, J., Uhara, Y. and Kawahara, K. (1994) Jpn J. Genet. 69, 671–678.
[15] Henikoff, S. (1984) Gene 28, 351–359.
[16] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[17] Rothstein R.J. (1983) Methods Enzymol. 101, 202–211.
[18] Grimm, C., Kohli, J., Murray, J. and Maundrell, K. (1988) Mol. Gen. Genet. 215, 81–86.
[19] Saikai, R.K., Gelfand, D.H., Stoffel, S., Scharf, S., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) Science 239, 487–491.
[20] Moreno, S., Klar, A. and Nurse, P. (1991) Methods Enzymol. 194, 795–823.
[21] Marks, J. and Hyams, J.S. (1985) Eur. J. Cell. Biol. 39, 27–32.
[22] Hagan, I.M. and Hyams, J.S. (1988) J. Cell Sci. 89, 343–357.
[23] Ishiguro, J. and Yamada, N. (1993) Jpn J. Genet. 68, 265–276.
[24] Mertins, P. and Gallwitz, D. (1987) Nucl. Acid Res. 15, 7369–7379.
[25] Hoheisel, J.D., Maier, E., Mott, R., McCarthy, L., Grigoriev, A.V., Schalkwyk, L.C., Nizetic, D., Francis, F. and Lehrach, H. (1993) Cell 73, 109–120.
[26] Chen, X., Cook, R.K. and Rubenstein P.A. (1993) J. Cell Biol. 123, 1185–1195.
[27] Drubin, D.G., Jones, H.D. and Wertman, K.F. (1993) Mol. Biol. Cell 4, 1277–1294.
[28] Horio, T., Uzawa, S., Jung, M.K., Oakley, B.R., Tanaka, K. and Yanagida, M. (1991) J. Cell Sci. 99, 693–700.
[29] Hoyt, M.A., Totis, L. and Roberts, B.T. (1991) Cell 66, 507–517.
[30] Li, R. and Murray, A.W. (1991) Cell 66, 519–531.
[31] Li, X. and Nicklas, R.B. (1995) Nature, 373, 630–632.
[32] Alfa, C.E., Ducommun, B., Beach, D. and Hyams, J.S. (1990) Nature, 347, 680–682.