Calmodulin Concentrates at Regions of Cell Growth in *Saccharomyces cerevisiae*

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**Abstract.** Calmodulin was localized in *Saccharomyces cerevisiae* by indirect immunofluorescence using affinity-purified polyclonal antibodies. Calmodulin displays an asymmetric distribution that changes during the cell cycle. In unbudded cells, calmodulin concentrates at the presumptive site of bud formation ~10 min before bud emergence. In small budded cells, calmodulin accumulates throughout the bud. As the bud grows, calmodulin concentrates at the tip, then disperses, and finally concentrates in the neck region before cytokinesis. An identical staining pattern is observed when wild-type calmodulin is replaced with mutant forms of calmodulin impaired in binding Ca$^{2+}$. Thus, the localization of calmodulin does not depend on its ability to bind Ca$^{2+}$ with a high affinity. Double labeling of yeast cells with affinity-purified anti-calmodulin antibody and rhodamine-conjugated phalloidin indicates that calmodulin and actin concentrate in overlapping regions during the cell cycle. Furthermore, disrupting calmodulin function using a temperature-sensitive calmodulin mutant delocalizes actin, and act1-4 mutants contain a random calmodulin distribution. Thus, calmodulin and actin distributions are interdependent. Finally, calmodulin localizes to the shmoo tip in cells treated with α-factor. This distribution, at sites of cell growth, implicates calmodulin in polarized cell growth in yeast.

**CALMODULIN** is a small, highly conserved, Ca$^{2+}$-binding protein present in all eukaryotic cells. It has been characterized as the Ca$^{2+}$-dependent regulator of cyclic nucleotide metabolism, muscle contraction, glycogen breakdown, and neurotransmitter release (for review see Cohen and Klee, 1988). Calmodulin is also required for cell proliferation in *Saccharomyces cerevisiae* (Davis et al., 1986), *Schizosaccharomyces pombe* (Takeda and Yamamoto, 1987), and *Aspergillus nidulans* (Rasmussen et al., 1990).

The role of calmodulin in cellular proliferation is not known. Several studies suggest that calmodulin interacts with microfilaments. Calmodulin localizes with actin based stress filaments in 3T3 cells (Luby-Phelps et al., 1985; Welsh et al., 1978). This association with actin is probably not direct since binding between F-actin and calmodulin has not been detected in a gel overlay assay or by sedimenting F-actin in the presence of calmodulin (Piazza and Wallace, 1985). In red blood cells, calmodulin regulates shape through an interaction with spectrin–protein 4.1–actin complexes. Calmodulin enhances the destabilizing effect of Ca$^{2+}$ on red blood cell membranes (Takakuwa et al., 1990) possibly through a conformational change of an apicalcalmodulin–protein 4.1 complex that occurs when calmodulin binds Ca$^{2+}$ (Tanaka et al., 1991). Furthermore, calmodulin associates with actin-binding proteins, including caldesmon (Sobue et al., 1981) and myosin I from brush border microvilli (Howe and Mooseker, 1983).

Information about actin function in *S. cerevisiae* is accumulating rapidly. The distribution of the actin cytoskeleton reflects the asymmetry of yeast cell growth (Adams and Kilmartin, 1984; Adams and Pringle, 1984). An unbudded yeast cell initiates growth from a particular site on the cell surface and grows in one direction due to the localized fusion of vesicles with the cell membrane (Sloat et al., 1981). Actin cables are aligned towards the bud in the mother cell, and actin cortical patches accumulate at the bud tip. Characterization of temperature-sensitive actin mutants confirmed that actin is essential in polarized cell growth (Novick and Botstein, 1985). Under nonpermissive conditions, actin mutants arrest as unbudded cells and enlarge uniformly rather than directing material to the bud. Furthermore, at least 10 different genes important for actin cytoskeletal function have been identified in yeast (Drubin, 1990) including actin-binding proteins similar to fimbrin (Adams et al., 1991), tropomyosin (Liu and Bretscher, 1989), profilin (Magdolen et al., 1988) conventional myosin (Watts et al., 1987), and unconventional myosin (Johnston et al., 1991).

In addition to interacting with microfilaments, calmodulin in higher eukaryotes is associated with microtubules. In plant and vertebrate cells, calmodulin localizes to kinesome microtubules (Vantard et al., 1985; Welsh et al., 1979). Consistent with the localization of calmodulin, expression of calmodulin anti-sense RNA in C127 cells results in a delay in metaphase of mitosis (Rasmussen and Means, 1989). The mechanism of calmodulin function in mitosis is unclear.

*S. cerevisiae* provides a simple system for analyzing the
function of calmodulin in cell proliferation. We previously demonstrated that the requirement for calmodulin in cellular proliferation does not depend on its ability to bind Ca\textsuperscript{2+} (Geiser et al., 1991). Mutant calmodulins with inactivated Ca\textsuperscript{2+}-binding loops substitute for wild-type calmodulin without affecting cell growth. The accompanying paper reports the characterization of a temperature-sensitive calmodulin mutant (Davis, 1992). Characterization of the phenotype of this mutant suggests calmodulin is involved in bud growth, cytokinesis, and chromosome segregation. In this report, we localized calmodulin by indirect immunofluorescence. The calmodulin distribution resembles that of proteins involved in polarized cell growth and overlaps actin throughout the cell cycle. To determine the role of calmodulin in establishing and maintaining cell polarity we analyzed calmodulin and actin distributions in temperature-sensitive calmodulin and actin mutants.

Materials and Methods

Strains and Plasmids

The strains used in this study are listed in Table I. Strains KWY231 and KWY474 are derived from S288C and were kindly provided to us by Ken Wertman (University of California, Berkeley, CA). All other strains are derivatives of W303 (Wallis et al., 1989). Strain TDY66 was constructed from strain TDY62-3D by a single step gene disruption (Rothstein, 1983) of the \textit{STTI} gene. The genotype of strains showing supersensitivity to \textit{a}-factor was confirmed by Southern blot analysis. Plasmids pG19 and pG26 contain CEN4, \textit{URA3}, ARS1, \textit{fl} origin, and the mutated yeast calmodulin gene, encoding 3E\textsuperscript{20V} or 3D\textsuperscript{20A}, respectively. Plasmid pG60 is similar except that it contains a vertebrate calmodulin cDNA in place of the yeast calmodulin gene (Geiser et al., 1991).

Antibody Purification and Immunoblot Analysis

Antibodies were raised in rabbits as previously described (Geiser et al., 1991 and 1991). MATa/MATa ade2-1oc/canl-lOO/his3A200/tub2-201/ura3-52

Table I. Strains

| Strain | Genotype | Reference |
|--------|----------|-----------|
| CRY1   | MATa ade2-1oc can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 | Robert Fuller |
| CRY2   | MATa ade2-1oc can1-100 his3-11,15,16 leu2-3,112 trp1-1 ura3-1 | Robert Fuller |
| JGY46  | MATa/MATa ade2-1oc ade2-1oc can1-100 his3-11,15 his3-11,15 leu2-3,112 leu2-3,112 trp1-1 ura3-1 ura3-1 | Davis, 1992 |
| JGY44-2A | MATa ade2-1oc can1-100 cmdl-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 | Geiser et al., 1991 and Davis, 1992 |
| TDY55-5D | MATa ade2-1oc ade3A can1-100 cmdlA::TRP1 his3-11,15 leu2-3,112 lys2A::HIS3 trpl-1 ura3-1 | Davis, 1992 |
| TDY66  | Mata ade2-1oc can1-100 cmdl-1 his3-11,15 leu2-3,112 lys2A::HIS3 trpl-1 ura3-1 | Davis, 1992 |
| TDY72  | Mata ade2-1oc can1-100 his3-11,15 leu2-3,112 srp1::LEU2 | This study |
| TDY72  | Mata ade2-1oc can1-100 his3-11,15 leu2-3,112 srp1::LEU2 | This study |
| KWT231 | Mata ade2-1oc can1-100 his3-11,15 leu2-3,112 srp1::LEU2 | Ken Wertman |

| Strain | Genotype | Reference |
|--------|----------|-----------|
| KWTY474 | Mata ade2-1oc can1-100 his3-11,15 leu2-3,112 srp1::LEU2 | Ken Wertman |
Davis, unpublished observation) and (b) the affinity-purified anti-calmodulin antibody has a lower affinity for the mutant proteins than for the wild-type protein as judged by immunoblot analysis (data not shown). Stained cells were viewed with an Axioplan fluorescent microscope (Carl Zeiss, Thornwood, NY) and photographed using TMAX 400 professional film (Eastman Kodak Co., Rochester, NY). Cells were printed using different exposure times to show optimum detail. The intensity difference between cells in different prints is not a measure of differences in calmodulin concentrations.

**Isolation of Unbudded Cells**

Strain CRY1 was grown to ~400 Klett units in YPD. 4 ml of cells were pelleted, resuspended in 0.5 ml YPD, sonicated for 5 s, and then loaded on a 37 ml 12-18% gradient of ficoll (Sigma Chemical Co.) dissolved in YP. Cells were sedimented in an HB-4 swinging bucket rotor in a high speed centrifuge (RC-2B; Sorvall Instruments, Wilmington, DE) for 5 min at 750 rpm (100 g). Fractions (0.5 ml) were collected and analyzed by phase contrast microscopy. Fractions 8 and 9 contained >95% unbudded cells. Cells in fractions 8 and 9 were washed, resuspended in 5.0 ml YPD, and incubated at 30°C until ~30% of the culture was budded. The cells were then fixed and prepared for indirect immunofluorescence as described above.

**Synchronization Using α-Factor**

TDY72-5D was grown in 20 ml YPD at 30°C. At 20 Klett units (7 x 10^6 cells/ml), α-factor was added to a final concentration of 30 ng/ml. Greater than 95% of the cells had formed a shmoo by 1.5 h. Cells were harvested by centrifugation after 30 h, resuspended in 30 ml fresh YPD, and incubated with shaking at 30°C. Aliquots (2.0 ml) were removed and fixed every 5 min for the first 30 min and then every 20 min. The fraction of unbudded and budded cells was determined by phase contrast microscopy. Cells were prepared for indirect immunofluorescence as described above. Over 200 cells were counted for each time point. Three separate experiments gave similar results.

**Results**

**Specificity of Anti-Calmodulin Antibodies**

Rabbit polyclonal antibodies were made against yeast calmodulin expressed in E. coli (Geiser et al., 1991) and the antibody was affinity-purified as described in Materials and Methods. On an immunoblot, the affinity-purified anti-calmodulin antibody reacted primarily with wild-type calmodulin in a crude yeast extract (Fig. 1, lane B). The antibody did not interact with any protein with the same mobility as calmodulin in a yeast extract containing vertebrate instead of yeast calmodulin (Fig. 1, lane A). Thus, although vertebrate calmodulin can functionally replace yeast calmodulin in yeast cells (Davis and Thorner, 1989; Ohyya and Anraku, 1989; Persechini et al., 1991), these two calmodulins do not share the epitopes recognized by the antibody used in this study. High molecular weight bands were barely detectable in the extract containing vertebrate calmodulin and the extract containing vertebrate calmodulin (Fig. 1, lanes A and B).

The immunoblot analysis suggested that the affinity-purified antibody identified, albeit weakly, several proteins in addition to calmodulin. However, the results of two other experiments convinced us that the immunofluorescent signal was specific to calmodulin. First, if the affinity-purified antibody was preincubated with a three-fold molar excess of yeast calmodulin prepared from E. coli for 30 min before labeling yeast cells, no immunofluorescent staining was visible (data not shown). This result indicated that yeast calmodulin, free of any other yeast protein, was sufficient to compete away the immunofluorescent signal seen in yeast. However, this result did not establish whether the signal was due to an antigenically related protein.

To verify that the immunofluorescent signal was due solely to the presence of calmodulin, we stained yeast cells containing vertebrate instead of yeast calmodulin with the affinity-purified anti-yeast calmodulin antibody. As shown above, the affinity-purified anti-yeast calmodulin antibody did not interact with vertebrate calmodulin. Thus, if all the signal seen in yeast cells is due to calmodulin, cells containing vertebrate instead of yeast calmodulin should not be stained. When cells containing vertebrate calmodulin were stained with affinity-purified anti-yeast calmodulin antibodies, no staining over background was detected (Fig. 2 A). DAPI staining (Fig. 2 B) aided in determining the location of the cells. In contrast, cells containing wild-type yeast calmodulin were brightly stained by affinity-purified anti-calmodulin antibody and had a distinct staining pattern (Fig. 2 C and Fig. 3). From these results, we concluded that our affinity-purified anti-calmodulin antibody specifically localizes yeast calmodulin.

**Immunofluorescent Localization of Calmodulin**

Calmodulin was localized in an asynchronously growing
Figure 2. Anti-yeast calmodulin staining of yeast cells expressing vertebrate calmodulin or wild-type yeast calmodulin. Indirect immunofluorescence was done as described in Materials and Methods. Cells were simultaneously stained with DAPI to see the nuclear DNA. (A) Strain TDY55-5D (cdmlΔ::TRPI) containing pJG60 (see Fig. 1) stained with affinity-purified anti-yeast calmodulin antibody. (B) DAPI stain of the same cells as in A. (C) Strain CRY1 (CMD1) stained with affinity-purified anti-yeast calmodulin antibody. Bar, 6 μm.

culture by indirect immunofluorescence as described in Materials and Methods. The calmodulin distribution was asymmetric and changed as cells progressed through the cell cycle (Fig. 3). In 70% of 322 unbudded cells, calmodulin concentrated in a patch (Fig. 3 A). 25% did not have a visible patch of calmodulin. The remaining 5% had two patches. In small budded cells calmodulin concentrated throughout the bud (Fig. 3 B). As the bud grew, calmodulin accumulated at the
Figure 3. Localization of calmodulin in an asynchronous wild-type culture. Strain JGY46 was stained with affinity-purified anti-calmodulin antibody as described in Materials and Methods. Cells were simultaneously stained with rhodamine-conjugated phalloidin (*middle*) which binds F-actin, and DAPI (*bottom*) to observe the position of the nuclear DNA. Some FITC fluorescence is visible when viewing for DAPI fluorescence. (A) unbudded yeast cell; (B) small budded yeast cell; (C) medium budded yeast cell; (D) large budded cell in mitosis (see DAPI); and (E) large budded cell during cytokinesis. CaM, calmodulin. Bar, 6 μm.
Calmodulin Localization in Synchronized Cells

Calmodulin was localized in a synchronous culture to determine when its distribution becomes polarized in unbudded cells and to determine the percent of budded cells with an asymmetric distribution. Yeast cells were synchronized in G1 by treatment with the mating pheromone α-factor. After release from α-factor arrest, aliquots were taken at various times for 1.5 generations, fixed, counted to determine per-

Yeast cells also exhibit polarized growth during shmoo formation. To determine whether calmodulin concentrates at the shmoo tip during shmoo formation, we localized calmodulin in cells treated with α-factor. Cells treated with α-factor stained brightly for calmodulin. A patch similar to that observed in unbudded cells in an asynchronous culture was present at the shmoo tip (Fig. 4).

Calmodulin Localization in Synchronized Cells

Calmodulin was localized in a synchronous culture to determine when its distribution becomes polarized in unbudded cells and to determine the percent of budded cells with an asymmetric distribution. Yeast cells were synchronized in G1 by treatment with the mating pheromone α-factor. After release from α-factor arrest, aliquots were taken at various times for 1.5 generations, fixed, counted to determine per-

Figure 4. Calmodulin localization in cells treated with α-factor. TBY72-5D was grown in 7 ml YPD to 20 Klett units (7 × 10⁶ cells/ml), and then α-factor was added to a final concentration of 30 ng/ml. The cells were then prepared for indirect immunofluorescence as described in Materials and Methods except 15 μg/ml zymolyase instead of 5 μg/ml was used to permeabilize cells. Bar, 9 μm.
same in both cultures. Calmodulin localization in wild-type cells \((n = 408)\) grown at 36°C was indistinguishable from that seen in the same wild-type strain grown at 21° or 30°C; 94% of the wild-type cells stained with affinity-purified anti-calmodulin antibodies and 72% of these had a polarized calmodulin distribution.

After incubation at the nonpermissive temperature, the cells containing \textit{cmdl-1} differed from the wild-type culture in two respects. First, only 67% of the mutant cells \((n = 448)\) grown at 36°C stained for calmodulin. Second, of those cells that stained, only 30% had a polarized calmodulin distribution. In 70% of the stained cells, calmodulin appeared diffusely distributed throughout the mother cell and bud (Fig. 5, B and C). The bud often stained more faintly than the mother cell. In contrast to wild-type cells, a diffuse distribution of calmodulin was observed at stages of the cell cycle other than G1 and mitosis as judged by the position of DAPI staining material and by the bud size. Furthermore, while 85% of wild-type cells showed a polarized actin distribution (Fig. 5 A), only 30% of the stained \textit{cmdl-1} cells showed a polarized actin distribution. In many \textit{cmdl-1} cells, the disrupted actin cytoskeleton was characterized by mother cells containing few if any actin cables and containing many more cortical dots than usual along the cell periphery (Fig. 5 C). Other mutant cells contained a diffuse actin distribution (Fig. 5 B). Thus calmodulin appears necessary for maintaining and/or establishing a polarized actin distribution. Calmodulin and actin localization in \textit{cmdl-1} containing cells grown at permissive temperature was indistinguishable from that seen in wild-type cells (data not shown).

Next, we analyzed whether disrupting actin function affected the calmodulin distribution. We examined the calmodulin and actin distributions in a strain containing the temperature-sensitive actin mutation \textit{actl-4} and in a congenic wild-type strain. Under nonpermissive conditions, large unbudded cells accumulate in a strain containing \textit{actl-4} (Dunn and Shortle, 1990). The results described above indicate that the distribution of calmodulin is diffuse in many unbudded cells. To ensure that the observed difference in calmodulin polarization between \textit{actl-4} and a wild-type strain was not just due to the increase in unbudded cells in the mutant culture, we separately compared mutant and wild-type unbudded and budded cells. After 75 min at the restrictive temperature, the actin distribution was polarized in the wild-type strain and most (79%) of the cells stained with affinity-purified anti-calmodulin antibodies (Fig. 6 A). Of the stained cells, 65% of the budded cells \((n = 105)\) and 42% of the unbudded cells \((n = 146)\), contained a polarized calmodulin distribution. We noticed that fewer unbudded cells contained a polarized calmodulin distribution in this wild-type strain than in the wild-type strains described above. This difference may be because the strain containing the \textit{actl-4} mutation and the corresponding wild-type strain are in a different genetic background than all other strains used in this study (see Materials and Methods).

In contrast to the congenic control strain, actin was not...
polarized in the act1-4 containing strain after incubation under nonpermissive conditions (Fig. 6 B). Furthermore, only 30% of the cells containing the act1-4 mutation stained with anti-calmodulin antibodies. Of the stained unbudded cells (n = 101), only 5% had a polarized distribution of calmodulin. Of the stained budded cells (n = 100), none had a polarized calmodulin distribution (Fig. 6 B). Thus calmodulin and actin have a reciprocal effect on establishing and/or maintaining the distribution of each other. However, disrupting actin function has a more dramatic effect on the distribution of calmodulin than disrupting calmodulin has on the polarization of actin.

**Discussion**

Localization of calmodulin by indirect immunofluorescence revealed that calmodulin concentrates at sites of cell growth. Calmodulin and actin concentrate in overlapping regions during the cell cycle. In unbudded cells, calmodulin concentrates in a patch ~10 min before bud emergence shortly after actin. The timing of patch formation and the fact that actin concentrates in the same region indicates that calmodulin is accumulating at the nascent bud site. Calmodulin concentrates throughout the bud in small budded cells and remains in the tip as the bud grows. During mitosis, calmodulin is dispersed throughout the cell and bud, and then moves to the neck during cytokinesis. In cells treated with α-factor, calmodulin concentrates at the shmoo tip. This distribution implicates calmodulin in polarized cell growth in S. cerevisiae.

The actin network in yeast cells is required for proper localization of material to the bud. Mutations in actin lead to

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**Figure 6.** Immunofluorescent localization of actin and calmodulin in an actin mutant. Log phase cultures (6 x 10^6 cells/ml) of strain KWY474 (ACT1) and strain KWY231 (act1-4) grown in YPD were shifted from 21° to 36°C for 75 min. Cells were then fixed and prepared for indirect immunofluorescence as described in Materials and Methods. (A) KWY474; (B) KWY231; CaM, calmodulin. Bar, 12 μm.
Figure 7. Immunofluorescent localization of mutant calmodulins impaired in binding Ca²⁺. An asynchronous culture of strain TDY55-5D containing (A) pJG19 (3E→V), or (B) pJG26 (3D→A), was fixed and prepared for indirect immunofluorescence as described in Materials and Methods except that cells containing pJG19 were incubated with anti-calmodulin antibody diluted 1:1,000. Bar, 4 μm.

A

B

a disruption of the actin cytoskeleton and a subsequent defect in polarized cell growth (Novick and Botstein, 1985). To determine the role of calmodulin in polarized cell growth and the relationship between calmodulin and actin in this process, we analyzed the dependence of these molecules on each other for proper localization. We found that the polarized distributions of actin and calmodulin are interdependent: a mutation in actin disperses calmodulin, and conversely, disruption of calmodulin function disorganizes the actin network in most cells. From this result, a strain containing a temperature-sensitive form of calmodulin is predicted to be defective in polarized cell growth under nonpermissive conditions. However, as presented in the accompanying paper, the cmdl-1 mutant exhibits only a mild defect in bud growth and dies as it proceeds through mitosis. Unlike mutations in actin which severely disrupt the cytoskeleton and completely inhibit bud growth, mutations in calmodulin apparently only partially disrupt the actin cytoskeleton. Thus, calmodulin may facilitate but not be essential for bud growth. Alternatively, the calmodulin function in bud growth may be incompletely inactivated in the cmdl-1 mutant whereas the function in mitosis is completely abolished.

Mutations in actin-binding proteins also reorganize the actin cytoskeleton but do not prevent bud growth. Yeast strains deleted for the gene encoding the yeast homolog to fimbrin, SAC6, are viable at 23°C although they contain few actin cables and the cortical patches are not limited to the bud (Adams et al., 1991). Disruption of the gene encoding tropomyosin leads to a loss of actin cables but does not prevent bud growth (Liu and Bretscher, 1989). Calmodulin shares characteristics with actin-binding proteins, but unlike the distributions of actin and actin-binding proteins, calmodulin, and actin distributions overlap but are mostly not coincident. This suggests that the interaction between calmodulin and actin is indirect. Consistent with this possibility, a direct interaction between mammalian calmodulin and actin has not been detected although diverse assays have been used (Luby-Phelps et al., 1985; Piazza and Wallace, 1985).

The distribution of Spa2p and calmodulin in yeast cells is strikingly similar (Gehrung and Snyder, 1990; Snyder, 1989; Snyder et al., 1991) suggesting that these two proteins may interact. However, Spa2p and calmodulin appear to play significantly different roles in the polarization of yeast cells. Neither actin nor Spa2p are necessary for maintaining the localization of the other. Actin mutants properly localize Spa2p (Snyder et al., 1991) and the majority of cells in a culture of a spa2 mutant have a normal distribution of actin (Gehrung and Snyder, 1990). Thus, although Spa2p and calmodulin share a similar distribution, current knowledge of their functions suggests that they are not components of the same complex.

Chitin (Hayashibe and Katohda, 1973) and the components of the 10-nm filaments, Cdc3p and Cdc12p, accumulate at the nascent bud site shortly before bud emergence as does calmodulin (Haarer and Pringle, 1987; Kim et al., 1991). Distinct from calmodulin, chitin, and the components of the 10-nm filaments remain at the neck between the mother and the bud during the cell cycle. The 10-nm filaments are assembled early in the cell cycle but are not required until cytokinesis (Haarer and Pringle, 1987; Kim et al., 1991). The temperature-sensitive calmodulin mutant displays a severe defect in cytokinesis if shifted to the nonpermissive temperature from early G1 but not if shifted to the nonpermissive temperature from later G1 or G2 (Davis,
suggesting that calmodulin is required for the arrangement of the 10-nm filaments. In the absence of calmodulin in early G1, the 10-nm filaments may either not form or assemble incorrectly resulting in an inhibition of cytokinesis.

Results of a genetic analysis suggest that an unconventional myosin encoded by the MYO2 gene acts as the molecular motor to transport secretory vesicles along actin cables to the site of bud development. Under nonpermissive conditions, a strain containing myo2-66 arrests as a large unbudded cell with an abnormal actin distribution and an accumulation of secretory vesicles (Johnston et al., 1991). The unconventional myosin is a likely target for calmodulin action since it has six putative calmodulin-binding sites. Furthermore, a related myosin from bovine brain, P190, has been shown to bind calmodulin in a Ca2+-independent manner (Johnston et al., 1991; Larson et al., 1988). In yeast, mutant calmodulins in which the Ca2+-binding sites have been inactivated show a distribution very similar to wild-type calmodulin. Thus, Ca2+-binding is not required for a polarized distribution of calmodulin. One model is that calmodulin binds directly to Myo2p in a Ca2+-independent manner and facilitates bud growth.

The characterization of the temperature-sensitive mutant reported in the accompanying paper and the immunolocalization reported here highlight different aspects of calmodulin function. The temperature-sensitive mutant shows a severe defect in chromosome segregation and cannot survive even a single mitosis at the nonpermissive temperature (Davis, 1992). The concentration of calmodulin at sites of cell growth shown in this paper does not suggest a mitotic function. In fact, calmodulin appears to be less abundant in the nucleus than in the cytoplasm. We have thought of two possible explanations for these seemingly contradictory results. One is that calmodulin performs an unknown cytoplasmic function that affects chromosome segregation. Alternatively, the mitotic function may require calmodulin in the nucleus at concentrations below our level of detection. In higher eukaryotic organisms, calmodulin localizes to kinetochore microtubules (Vantard et al., 1985; Welsh et al., 1979). Since yeast have only one kinetochore microtubule per chromosome, as opposed to many in higher eukaryotes (Peterson and Ris, 1976), a similar distribution in yeast might be difficult to detect.

In conclusion, calmodulin localizes at sites of cell growth and is involved in the process of polarized growth in yeast. Calmodulin depends on actin for proper localization and disruption of calmodulin function delocalizes actin. Calmodulin may be interacting with the unconventional myosin, Myo2p, recently identified. Future studies will continue to examine the relationships between the proteins involved in polarized cell growth.

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