Abstract. The mddl mutation causes temperature-sensitive growth and defective transfer of nuclei and mitochondria into developing buds of yeast cells at the nonpermissive temperature. The MDM1 gene was cloned by complementation, and its sequence revealed an open reading frame encoding a potential protein product of 51.5 kD. This protein displays amino acid sequence similarities to hamster vimentin and mouse epidermal keratin. Gene disruption demonstrated that MDM1 is essential for mitotic growth. Antibodies against the MDM1 protein recognized a 51-kD polypeptide that was localized by indirect immunofluorescence to a novel pattern of spots and punctate arrays distributed throughout the yeast cell cytoplasm. These structures disappeared after shifting mddl mutant cells to the nonpermissive temperature, although the cellular level of MDM1 protein was unchanged. Affinity-purified antibodies against MDM1 also specifically recognized intermediate filaments by indirect immunofluorescence of animal cells. These results suggest that novel cytoplasmic structures containing the MDM1 protein mediate organelle inheritance in yeast.

The mdml mutation causes temperature-sensitive growth and defective transfer of nuclei and mitochondria into developing buds of yeast cells at the nonpermissive temperature. The MDM1 gene was cloned by complementation, and its sequence revealed an open reading frame encoding a potential protein product of 51.5 kD. This protein displays amino acid sequence similarities to hamster vimentin and mouse epidermal keratin. Gene disruption demonstrated that MDM1 is essential for mitotic growth. Antibodies against the MDM1 protein recognized a 51-kD polypeptide that was localized by indirect immunofluorescence to a novel pattern of spots and punctate arrays distributed throughout the yeast cell cytoplasm. These structures disappeared after shifting mddl mutant cells to the nonpermissive temperature, although the cellular level of MDM1 protein was unchanged. Affinity-purified antibodies against MDM1 also specifically recognized intermediate filaments by indirect immunofluorescence of animal cells. These results suggest that novel cytoplasmic structures containing the MDM1 protein mediate organelle inheritance in yeast.

The mdml mutant is defective for the transfer of nuclei and mitochondria into developing daughter buds during incubation at 37°C (McConnell et al., 1990). This mutant is one of several temperature-sensitive strains displaying conditional defects in mitochondrial inheritance and an uncoupling of mitochondrial movement from bud growth (McConnell et al., 1990; Stewart and Yaffe, 1991). mddl cells incubated at the nonpermissive temperature develop large buds devoid of nuclei and mitochondria. Successful nuclear division does occur in a fraction of mddl mutant cells incubated at 37°C; however, the mitotic spindle appears mis-oriented, resulting in two nuclei in the mother portion of the cell. Other cellular structures including the actin- and tubulin-based cytoskeletons appear normal in mddl cells. Genetic analysis demonstrated that the mutant phenotypes displayed by mddl cells are due to a single, recessive, nuclear mutation (McConnell et al., 1990). In the present study we report the isolation and analysis of the MDM1 gene. We demonstrate that MDM1 is an essential yeast gene encoding a protein localized to novel punctate structures distributed throughout the yeast cytoplasm.
Materials and Methods

Strains and Genetic Methods

The parent strain A364A (MATa ade2 ade2 ura3 his7 lys2 trpl gal1) and isolation of the mdm1 mutant has been described previously (McConnell et al., 1990). Strains MY290, MY291, and MY298 were described previously (Smith and Yaffe, 1991). Strain MY403 (MATa, mdm1, ura3, leu2, his3) was obtained from S. Emr (University of California, La Jolla, CA). Plasmid DNA was prepared in Escherichia coli strains DH5a and MHE4. Standard yeast techniques and genetic analysis were described as previously (Sherman et al., 1979).

Isolation of the MDM1 Gene

The MDM1 gene was isolated by genetic complementation of the mdm1 temperature-sensitive lethal mutation. The mdm1 strain MY403 was transformed with a yeast genomic plasmid library in the vector YEp3 (Broach et al., 1979) as described by Ito et al. (1983). Leu+ transformants were selected at 23°C and screened for growth at the nonpermissive temperature by two successive replica platings at 37°C. Complementing plasmids (YEp3-1, YEp3-2, and YEp3-3) were analyzed by restriction analysis. Portions of the complementing DNA were subcloned into plasmid YCp50 (Rose et al., 1987), and the ability of these constructs to complement the temperature-sensitive defect in mdm1 cells was analyzed.

Integrative Transformation

A 5.0-kbp HindIII-SphI fragment isolated from YEp3-1 was inserted into the yeast integrating plasmid YIp5 (Struhl et al., 1979) to yield plasmid YIp5-MDM1. This plasmid was linearized at the unique Kpnl site (see Fig. 1) and transformed into strain SEY6210. Stable Ura+ transformants were cross to strain MY403, and the diploids were sporulated. The meiotic progeny were tested for growth at 37°C and for growth on media lacking uracil. Analysis of this cross revealed no recombination between mdm1 and URA3 in any of the 40 tetrads tested. All tetrads were of the parental ditype (2 Ura+, 2 ts−; 2 Ura−, 2 ts+). These results indicated that the plasmid YIp5-MDM1 had integrated at a site within 1.2 centimorgans of the MDM1 locus.

Sequence Analysis of the MDM1 Gene

The complementing HindIII-XbaI fragment (see Fig. 1 B) was subcloned into the polylinker region of Bluescript SK (Stratagene, La Jolla, CA) to create plasmid BS-MDM1. Templates for sequencing were obtained using Exonuclease III to create a series of nested deletions (Henikoff, 1984) from the ORF. Portions of the complementing DNA were subcloned into plasmid YCp50 (Rose et al., 1987), and the ability of these constructs to complement the temperature-sensitive defect in mdm1 cells was analyzed.

MDM1 Gene Disruption

A 3.0-kbp SphI-Kpnl fragment containing the entire MDM1 gene (see Fig. 1 A) was isolated from plasmid YEp3-2 and ligated into the SphI and Kpnl sites of pUC19 creating the plasmid pUC19-MDM1. A 2.2-kbp Sall-XhoI fragment containing the LEU2 gene was isolated from YEp3 (Broach et al., 1979), the DNA ends were filled in with Klenow Polynucleotase, and the fragment was blunt-end ligated into the filled EcoRI and XhoI sites within the MDM1 gene (see Fig. 1 A) in plasmid pUC19-MDM1. The resulting plasmid pUC19-MDM1::LEU2 was cut with Sall and PvuI. The resulting Stul–PvuI fragment was used to replace one copy of MDM1 in the diploid strain MY2928. Leu+ transformants were isolated and the meiotic products from six independently isolated diploid colonies were analyzed at 23°C. Of 38 tetrads, 37 gave rise to only two viable spores, both of which were Leu+. One tetrad gave rise to only one viable spore which was Leu-. Southern analysis (Maniatis et al., 1982) of the Leu+ diploid strains, using a 1.9-kbp EcoRI probe (see Fig. 1), confirmed that one of the two copies of MDM1 had been replaced by the LEU2 gene (see Fig. 4). Growth of cells containing the gene disruption was rescued by transformation with a single wild-type copy of MDM1 in the plasmid YCP50. This complementary DNA extended from the SphI site, 350 bp upstream from the MDM1 ORF to an EcoRV site located 281 bp downstream from the ORF.

Preparation and Characterization of Antisera

Antibodies against the MDM1 protein were raised against a β-galactosidase-MDM1 fusion protein. Specifically, a 726-bp HaeIII fragment (Fig. 1 A) containing the COOH-terminal 418 bp of the L329-bp MDM1 ORF was isolated from plasmid BS-MDM1, the ends were filled using Klenow Polynucleotase, and the fragment was inserted into the filled BamIII site of the vector pTrBO (Burglin and DeRobertis, 1987). The fusion protein was expressed in the Escherichia coli strain 71-18, purified by SDS-PAGE and electro-elution, and used to immunize rabbits (Harlow and Lane, 1988).

Results

Cloning of the MDM1 Gene

Yeast cells with the recessive mdm1 mutation display temperature-sensitive defects in growth and in the transfer of both nuclei and mitochondria into developing buds (McCon nell et al., 1990). To determine the molecular basis of these defects, the wild-type MDM1 gene was cloned by complementation of the temperature-sensitive growth phenotype of mdm1 cells. Out of >8,000 Leu+ transformants, three were isolated which contained plasmids conferring growth at 37°C. These plasmids also complemented the mitochondrial inheritance defect of mdm1 cells. Restriction analysis
retrieved that the three plasmids contained inserts of yeast DNA which partially overlapped in their sequences. Subcloning and re-introduction of the yeast DNA fragments into\textit{mdm1} cells demonstrated that a 50-kbp HindIII-Sphl fragment from the plasmid YEpl3-1 (the complementing plasmid containing the smallest insert of yeast DNA) complemented the mutant phenotypes.

To verify that complementation by the isolated DNA reflected the presence of the wild-type\textit{MDM1} gene (rather than another gene that could suppress the\textit{mdm1}\textit{defect}), the 50-kbp HindIII-Sphl fragment of complementing DNA was inserted into the plasmid YIp5, integrated into the yeast genome, and the site of integration was mapped. This fragment directed integration of plasmid DNA to the\textit{MDM1}\textit{locus}, indicating that the plasmid contained the authentic\textit{MDM1}\textit{gene} (see Materials and Methods). Additionally, the\textit{MDM1}\textit{gene} was mapped to chromosome XIII by hybridization of a 3.7-kbp HindIII-XbaI probe to a yeast chromosomal OFAGE blot (data not shown).

**Sequencing of the\textit{MDM1}\textit{Gene}**

The nucleotide sequence of a 3.7-kbp HindIII-XbaI fragment from plasmid YEpl3-1 which complemented\textit{mdm1} was determined by dideoxy sequencing. A single, long open reading frame (ORF) was identified; however this ORF was initiated by a methionine located within adjacent vector sequences (Fig. 1\textit{B}). Nucleotide sequence analysis of the insert from a second complementing plasmid, YEpl3-2, revealed the full-length\textit{MDM1}\textit{gene} (Fig. 1\textit{A}). These results indicate that the HindIII-XbaI fragment from plasmid YEpl3-1 can complement the\textit{mdm1} mutation even with 49 amino acid residues from the amino terminus of the protein replaced by unrelated residues encoded in vector sequences (Fig. 1\textit{B}).

The full-length ORF of 1,329 bp encodes a putative protein of 443 amino acids with a predicted size of \(\sim 51.5\) kD (Fig. 2). The length of this ORF correlated well with the appearance of a single, 1.6-kbp band on Northern blots of isolated yeast RNA (probed with a 1.6-kbp Stul-PvuI fragment, data not shown). In addition, the ORF and adjacent sequences do not contain conserved elements characteristic of introns or spliced genes.

Comparison of the predicted protein sequence of\textit{MDM1} with sequences in the GENBANK database using the FASTA program (Lipman and Pearson, 1985) revealed no significant similarity to any previously described proteins. However, analysis with the SCANA program (Doolittle, 1986) indicated a relationship between the\textit{MDM1} protein and hamster vimentin (Quax-Jeuken et al., 1983) and mouse epidermal keratin (Steinert et al., 1983) with identities of 14 and 12\%, respectively, over the entire\textit{MDM1} protein sequence (Fig. 3). In addition,\textit{MDM1} and vimentin are essentially the same size (443 and 448 amino acids, respectively), show 32\% similarity of amino acid residues, share identical and similar residues throughout the length of their sequences, and possess very similar amino acid compositions. Keratin and\textit{MDM1} exhibit 22\% similarity, and the two proteins display 27\% similarity when the extremely glycine-rich amino- and carboxy-terminal domains of keratin are excluded from the comparison. Analysis of predicted secondary structure by two independent programs (Chou and Fasman, 1974; Garnier et al., 1978) suggested that the\textit{MDM1} protein possesses a high percentage of\( \alpha\)-helical structure (48 and 50\%, respectively). Sequence of the\textit{MDM1} protein revealed no hydrophobic domains typical of membrane spanning regions or characteristic targeting signals for intracellular protein sorting. These results demonstrate that\textit{MDM1} encodes a previously unidentified protein possessing modest sequence similarity to several vertebrate intermediate filament proteins.

**\textit{MDM1} is an Essential Gene**

Cells with the\textit{mdm1}\textit{mutation} show a temperature-sensitive
lethal phenotype, suggesting that \textit{MDM1} encodes an essential gene product or one that is needed for growth at elevated temperatures. To determine whether \textit{MDM1} is essential for cell viability at all temperatures, a null mutation in \textit{MDM1} was constructed. Most of the coding region of one of two copies of \textit{MDM1} in a diploid cell was replaced with the yeast \textit{LEU2} gene (see Materials and Methods and Fig. 1 \textit{A}). This gene replacement was confirmed by Southern analysis (Fig. 4 \textit{B}). When the resulting diploid cells were sporulated and the haploid progeny cultured at 23°C, only two spores from each tetrad developed into colonies (Fig. 4 \textit{A}). All viable spores were \textit{Leu}−, indicating the presence of an intact \textit{MDM1} gene. Additionally, the wild-type \textit{MDM1} gene, on a single-copy yeast plasmid (see Materials and Methods), res-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Sequence of the \textit{MDM1} gene and its predicted protein product. These sequence data are available from EMBL/GenBank/DDBJ under accession number X66371.}
\end{figure}
cued growth in haploid spores containing the gene disruption. These results demonstrate that MDM1 is a gene essential for normal mitotic growth and cell viability.

**The MDM1 Protein Is Localized in a Novel Cytoplasmic Pattern**

To determine the intracellular location and distribution of the MDM1 protein, antibodies were raised against a β-galactosidase-MDM1 fusion protein (see Materials and Methods). This antiserum specifically recognized a 51-kD polypeptide by Western blot analysis of total yeast proteins (Fig. 5). The level of the 51-kD band was increased in wild-type cells harboring a multicopy plasmid containing the MDM1 gene (Fig. 5), indicating that the antibody specifically recognized the

---

**Figure 3. Sequence comparison of MDM1 with vimentin and keratin.** Protein sequences were aligned as described by Feng and Doolittle (1987). Amino acids identical between MDM1 and hamster vimentin (VIMN) and between MDM1 and mouse epidermal keratin (KRTN) are boxed. Shown are the entire amino acid sequences of MDM1 (443 residues) and vimentin (448 residues), and the first 479 residues of keratin.
Figure 4. MDM1 is essential for cell viability. (A) One of two wild-type copies of MDM1 was replaced by the yeast LEU2 gene in the diploid strain MYY298 as described in the text. The diploid strain was sporulated, and the spores of eight tetrads were separated and cultured on an agar slab. The four spores from each tetrad (#1–8) were placed at positions a–d. (B) Southern analysis of diploid cells containing the MDM1 gene disruption. (Lane 1) Wild-type (MYY-298) chromosomal DNA cut with EcoRI and probed with a labeled fragment of MDM1 (see Materials and Methods). (Lane 2) Chromosomal DNA from a strain in which one copy of MDM1 was replaced with the yeast LEU2 gene, cut with EcoRI, and probed as in lane 1.

MDM1 protein. The 51-kD band also was specifically recognized by antibodies affinity purified on the fusion protein (not shown).

Western blotting of proteins extracted from subcellular fractions isolated from a yeast homogenate revealed the presence of MDM1 in all but the cytoplasmic fraction (data not shown). This finding suggested that the MDM1 protein exits in an aggregated or filamentous form, or is associated with membranous structures.

Distribution of the MDM1 protein in intact cells was characterized further by indirect immunofluorescence using affinity-purified antibodies. This approach revealed localization of MDM1 to a unique pattern of spots, sometimes appearing in linear arrays, distributed throughout the cytoplasm (Fig. 6). This punctate staining was most distinct and intense in unbudded cells, and appeared less abundant and less intense in cells with small daughter buds, particularly within the buds (Fig 6). Double-label indirect immunofluorescence analysis revealed no particular correlation of MDM1 spots and cytoplasmic organelles: MDM1 spots were distributed throughout the cytoplasm, some coincident with positions of nuclei and mitochondria while others localized in regions distinct from the organelles (not shown). Antibodies affinity-purified on either the β-galactosidase-MDM1 fusion protein (purified from E. coli) or the 51-kD band excised from a nitrocellulose blot of whole yeast proteins revealed an identical pattern of MDM1 distribution. Preimmune sera produced no specific cellular staining (data not shown).

Double-label microscopic analysis indicated that the pattern of MDM1 distribution is unrelated to the distribution of actin and tubulin (Fig. 7), major components of the two well-characterized cytoskeletal networks in yeast (Adams and Pringle, 1984; Kilmartin and Adams, 1984; Thomas et al., 1984). These results from microscopic analysis and subcellular fractionation demonstrate that the MDM1 protein is extensively distributed in the yeast cell cytoplasm in a novel pattern of punctate structures.

Antibodies to the MDM1 Protein Recognize the Intermediate Filament Network in Animal Cells

The pattern of MDM1 distribution detected by indirect immunofluorescence was unlike any previously reported for yeast proteins but suggested a cytoskeletal network. Since the cytoskeleton of animal cells has been highly characterized (Bershadsky and Vasiliev, 1988), we examined possible cross-reaction of anti-MDM1 antibodies with animal cell proteins by indirect immunofluorescence. When CV-1 (African green monkey kidney) cells were stained with affinity-purified anti-MDM1 antibody, a striking filamentous pattern was observed (Fig. 8, d and f; Fig. 9, d and f). The web-like fluorescent pattern was characteristic of that described previously for intermediate filaments (Ball and Singer, 1981; Bershadsky and Vasiliev, 1988), and was absent when preimmune sera subjected to affinity purification was employed (data not shown). In addition, a similar indirect immunofluo-
Figure 6. Distribution of the MDM1 protein in wild-type yeast cells. Wild-type cells (MYY290) were grown to mid-log phase in YPD medium at 30°C. Cells were fixed with formaldehyde and processed for indirect immunofluorescence using affinity-purified anti-MDM1 antiserum as described in Materials and Methods. Bar, 2 μm.

Figure 7. The MDM1 distribution is distinct from that of actin and tubulin. Wild-type yeast (MYY290) were grown at 30°C to mid-log phase and analyzed by microscopy as described for Fig. 6. Actin was detected with rhodamine-conjugated phalloidin, and microtubules and MDM1 were detected by indirect immunofluorescence. Cells were simultaneously stained for MDM1 (a) and microtubules (b) or for MDM1 (c) and actin (d). Bar, 2 μm.
Figure 8. Antibodies to the MDM1 protein localize to intermediate filaments in mitotic CV-1 cells. Double indirect immunofluorescence labeling was performed on mitotic CV-1 cells. Mitotic cells were harvested from supernatants of CV-1 cells grown as monolayers in tissue culture. Cells were fixed and processed for indirect immunofluorescence as described in Materials and Methods. Double-label analysis of single cells (a and b, c and d, e and f) are shown. (a) Microtubules; (b) vimentin intermediate filaments; (c) microtubules; (d) MDM1; (e) vimentin intermediate filaments; (f) MDM1. Bar, 40 μm.

rescence pattern was detected with anti-MDM1 antibody in CHO cells, XR-1 glial cells, and BHK-21 (baby hamster kidney) cells (data not shown).

The fluorescence pattern observed with anti-MDM1 antibodies resembled that of intermediate filaments, but in many animal cell types microtubules colocalize with intermediate filament during interphase (Ball and Singer, 1981; Bershadsky and Vasiliev, 1988). To determine whether anti-MDM1 labeled the microtubule or intermediate filament network, double-label indirect immunofluorescence analysis was performed on mitotic cells. In such cells, microtubules redistribute to form the mitotic spindle, while the intermediate filaments remain in an array extending throughout the cytoplasm (Lazarides, 1980; Bershadsky and Vasiliev, 1988). In the mitotic CV-1 cells, anti-MDM1 antibodies recognized a protein that colocalized with intermediate filaments and not with tubulin (Fig. 8). Control experiments in which cells were treated with only a single antibody revealed no crossover fluorescence (not shown).

To confirm that the cytoskeletal protein recognized by anti-MDM1 was associated with intermediate filaments, and not with microtubules, CV-1 cells were treated with nocodazole (to disrupt microtubules) before immunofluorescence analysis. Although microtubules were essentially absent after treatment with nocodazole (Fig. 9 c), filaments detected with the anti-MDM1 antibody persisted (Fig. 9 d). Furthermore, the anti-MDM1 pattern remained identical to the vimentin filament pattern (Fig. 9, e and f).

The pattern of anti-MDM1 fluorescence also was identical to that detected with antibodies against a second intermediate filament protein, cytokeratin (data not shown). This result is consistent with the colocalization of vimentin and cytokeratin in many cultured cells (Steinert and Roop, 1988; Lazarides, 1980). These results, taken together, demonstrate that antibodies against MDM1 recognize the intermediate filament network in a variety of animal cells.

MDM1 Distribution Is Greatly Altered in mdm1 Mutant Cells

Nuclei and mitochondria are not transferred into developing buds when mdm1 cells are shifted to 37°C (McConnell et al., 1990). To explore relationships between the mutant phenotype and the distribution of MDM1, indirect immunofluorescence was performed on mdm1 and wild-type cells that had been incubated at the nonpermissive temperature. Wild-type cells incubated at 37°C for 4 h retained the pattern of spots and punctate arrays (Fig. 10). However, this pattern was essentially absent from mutant cells exposed to the nonpermissive temperature (Fig. 10). Instead, weak, diffuse fluores-
Figure 9. Antibodies to the MDM1 protein localize to intermediate filaments in nocodazole-treated CV-1 cells. Cells were grown to semi-confluence, treated with nocodazole (Materials and Methods), and analyzed by indirect immunofluorescence microscopy as described for Fig. 8. Double-label analysis of single cells (a and b, c and d, e and f) is shown. Untreated panel: (a) microtubules; (b) vimentin intermediate filaments. Nocodazole-treated panel: (c) microtubules; (d) MDM1; (e) vimentin intermediate filaments; (f) MDM1. Bar, 40 μm.

Fluorescence was apparent throughout the mutant cell cytoplasm. Additionally, mdm1 cells grown at the permissive temperature displayed the characteristic wild-type pattern of MDM1 distribution (data not shown).

The possibility that the MDM1 protein was unstable in mdm1 cells incubated at 37°C was analyzed by Western analysis of extracted cellular proteins. Similar levels of the 51-kD polypeptide were present in mdm1 and wild-type cells at both room temperature and after a 4-h incubation at 37°C (Fig. 11). These results indicate that the mdm1 lesion leads to a

Figure 10. The MDM1 distribution is greatly altered in mdm1 mutant cells. Wild-type (MYY290) and mdm1 (MYY275) mutant cells were grown overnight at 23°C in YPD, diluted with fresh media, and incubated at 37°C for 4 h. Cells were fixed with formaldehyde, and processed for indirect immunofluorescence as described in Fig. 6. Wild-type panel shows MDM1 staining. mdm1 panel: (a) MDM1; (b) DAPI staining showing mitochondrial and nuclear DNA; and (c) microtubules. Bar, 2 μm.
temperature-dependent mislocalization of MDM1 or to instability or misassembly of the MDM1-containing structures.

Discussion

We have discovered a system of novel cytoplasmic structures that play a role in nuclear segregation and mitochondrial inheritance in yeast. This system was identified by indirect immunofluorescence using antibodies that recognize the product of the MDM1 gene. A mutation in this gene (mdm1) causes temperature-sensitive growth and defects in the transfer of nuclei and mitochondria into developing buds in yeast cells incubated at 37°C (McConnell et al., 1990). In addition, gene disruption experiments demonstrated that MDM1 is an essential gene, required for mitotic growth at all temperatures. After mdm1 cells were shifted to the nonpermissive temperature, the pattern of intense punctate staining was no longer detectable, although the MDM1 protein was still present at normal levels in the cells. Taken together, these results suggest a direct role for the MDM1-containing structures in organelle inheritance.

A number of observations suggest a relationship between the MDM1 protein and intermediate filament proteins of animal cells. First, affinity-purified antibodies against MDM1 recognize the intermediate filament network by indirect immunofluorescence in a number of different types of animal cells. Consistent with this observation, the affinity-purified antibodies react weakly with purified vimentin and with several unidentified species on immunoblots of total proteins from animal cells (S. McConnell, preliminary results). Second, protein sequence comparisons indicate a similarity of MDM1 to both vimentin and cytokeratin. Third, structural predictions suggest that the MDM1 protein contains a high a-helical content, a property shared by many filamentous proteins. Finally, the MDM1 protein demonstrates solubility properties similar to those described for intermediate filament proteins (Steinert and Roop, 1988; Lazarides, 1980). In particular, MDM1 remained largely in the insoluble fraction upon extraction of disrupted yeast cells with the detergent Triton X-100 (S. McConnell, unpublished observations). Additionally, the MDM1 protein in cellular protein extracts appeared to aggregate readily, resulting in the frequent appearance of material of high molecular weight recognized by the anti-MDM1 sera on Western blots (not shown). These results may suggest that MDM1 is a distant homologue of intermediate filament proteins of animal cells. Alternatively, MDM1 and intermediate filament proteins may be unrelated evolutionarily, but only share certain traits such as common epitopes or solubility characteristics. A third possibility is that animal cells contain a (as yet, unidentified) homologue of MDM1 which colocalizes with intermediate filaments. The future purification and biochemical analysis of the MDM1 protein should lead to a greater understanding of its structural properties.

The distribution of MDM1 is distinct from that of all previously described structures in the yeast cytoplasm. The MDM1 pattern is dramatically different from the cellular distributions of actin and tubulin, major components of the two well-characterized cytoskeletal networks in yeast (Adams and Pringle, 1984; Kilmartin and Adams, 1984; Thomas et al., 1984). Consistent with this observation, changes in the actin- or tubulin-based cytoskeletons have not been detected in mdm1 mutant cells at the nonpermissive temperature (McConnell et al., 1990), suggesting no direct involvement of these structures in the mutant phenotypes. Interestingly, MDM1 appears similar to actin in that both proteins are localized to largely punctate structures in the cytoplasm (Adams and Pringle, 1984; Kilmartin and Adams, 1984). However, the actin "dots" are concentrated in small buds or localized to the cell cortex, whereas the MDM1 spots are distributed throughout the cytoplasm and appear least abundant in small buds. Another structure extending through the cytoplasm, termed "cytoplasmic matrices," was described previously in yeast (Wittenberg et al., 1987). This complex network of filamentous material was detected after yeast spheroplasts were extracted with the detergent Triton X-100. The biological significance, composition, and presence of these matrices in untreated cells remain to be demonstrated, although it is possible that the MDM1 protein (with its similar solubilization profile) is associated with such structures. With respect to other yeast cellular structures, however, there is no evidence for a relationship between the MDM1 protein and the 10-nm filaments localized to a ring surrounding the bud neck (Byers and Goetsch, 1976). MDM1 displays no specific localization to the bud neck, and the MDM1 gene is distinct from four genes (CDC3, CDC10, CDC11, CDC12) whose function is required for the formation of the neck-associated structures (Kim et al., 1991).

The mechanistic role of the MDM1 protein in facilitating transfer of nuclei and mitochondria into developing buds remains to be determined. One model of the protein's function is that a network containing or composed of the MDM1 protein serves to organize the cytoplasm and provide a matrix along which organelles can be transported. Another role for this network might be to orient the mitotic spindle, a process defective in mdm1 cells incubated at 37°C (even though cytoplasmic microtubules extend into buds in these cells; see Fig. 10 and McConnell et al., 1990). Intriguingly, such functions have been proposed for the intermediate filament network in animal cells (Lazarides, 1980; Geiger, 1987; Skalli and Goldman, 1991). Future studies will focus on characterizing molecular details of MDM1 function, its assembly into complex structures, and on the identification of other cellular components with which the MDM1 protein interacts.
We are grateful to Russ Doolittle for advice concerning protein sequence comparisons; the Malhotra lab for assistance with growth and analysis of animal cells; and to Jon Singer, John Newport, Douglass Forbes, Vivek Malhotra, Mike Levine, and Leslie Stewart for critical reading of the manuscript and their valuable suggestions.

This work was supported by National Science Foundation grant 90-04719.

Received for publication 30 January 1992 and in revised form 15 April 1992.

References

Adams, R. J. 1982. Organelle movement in axons depends on ATP. Nature (Lond.). 297:327-329.
Adams, A. E. M., and J. R. Pringle. 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant Saccharomyces cerevisiae. J. Cell Biol. 98:934-945.
Adams, R. J., and T. D. Pollard. 1986. Propulsion of organelles isolated from Acanthamoeba along actin filaments by myosin-I. Nature (Lond.). 322:754-756.
Auderheide, K. J. 1977. Salatory motility of uninserted trichocytes and mitochondria in Paramecium tetraurelia. Science (Wash. DC). 198:299-300.
Ball, E. H., and S. J. Singer. 1981. Association of microtubules and intermediate filaments in neural fibroblasts and its disruption upon transformation by a temperature-sensitive mutant of Rous sarcoma virus. Proc. Natl. Acad. Sci. USA. 78:6986-6990.
Ball, E. H., and S. J. Singer. 1982. Mitochondria are associated with microtubules and not with intermediate filaments in cultured fibroblasts. Proc. Natl. Acad. Sci. USA. 79:123-126.
Bershadsky, A. D., and J. M. Vasiliev. 1988. Cytoskeleton. Plenum Press, New York. 298 pp.
Brosch, R. J., T. N. Straubern, and J. B. Hicks. 1979. Transformation in yeast: development of a hybrid vector and isolation of the CAN1 gene. Gene. 8:121-133.
Burglin, T. R., and E. M. DeRobertis. 1987. The nuclear migration signal of Xenopus laevis nuclearplasm. EMBO (Eur. Mol. Biol. Organ.) J. 6:2617-2625.
Byers, B., and L. Goetsch. 1976. A highly ordered ring of membrane-associated filaments in budding yeast. J. Cell Biol. 69:717-721.
Chen, L. B. 1988. Mitochondrial membrane potential in living cells. Annu. Rev. Cell Biol. 4:155-181.
Chou, P. Y., and G. D. Fasman. 1974. Prediction of protein conformation. Biochemistry. 13:222-245.
Daum, G., P. C. Bohn, and G. Schatz. 1982. Import of proteins into mitochondria. Cytochrome b2 and cytochrome c peroxidase are located in the intermembrane space of yeast mitochondria. J. Biol. Chem. 257:13028-13033.
Doolittle, R. F. 1986. Of Urfs and Orfs. University Science Books, Mill Valley, CA. 103 pp.
Deng, P. F., and R. F. Doolittle. 1987. Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J. Mol. Evol. 25:13028-13033.
Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. Proc. Natl. Acad. Sci. USA. 75:5034-5038.
Garnier, J., D. J. Osguthorpe, and B. Robson. 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. 120:97-120.
Geiger, B. 1987. Intermediate filaments. Looking for a function. J. Cell Biol. 100:1664-1675.
Kim, H. B., B. K. Haarer, and J. R. Pringle. 1991. Cellular morphology in the Saccharomyces cerevisiae cell cycle: localization of the CDC2 gene product and the timing of events at the budding site. J. Cell Biol. 112:535-544.
Kilmartin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast Saccharomyces. J. Cell Biol. 98:922-933.
Kim, H. B., B. K. Haarer, and J. R. Pringle. 1991. Intermediate filaments as mechanical integrators of cellular space. Nature (Lond.). 283:249-256.
Lee, C., and L. B. Chen. 1988. Dynamic behavior of endoplasmic reticulum in living cells. Cell. 54:37-46.
Lippman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. Science (Wash. DC). 227:1435-1441.
Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, 545 pp.
McConnell, S. J., L. C. Stewart, A. Tain, and M. P. Yaffe. 1990. Temperature-sensitive yeast mutants defective in mitochondrial inheritance. J. Cell Biol. 111:967-976.
Palade, G. 1983. Membrane biogenesis: an overview. Methods Enzymol. 96:29-40.
Pringle, J. R., A. E. M. Adams, D. G. Drubin, and B. K. Haarer. 1991. Immunofluorescence methods for yeast. Methods Enzymol. 194:565-601.
Quax-Israelen, W. Y., Quax, and H. Bloemendal. 1983. Primary and secondary structure of hamster vimentin predicted from the nucleotide sequence. Proc. Natl. Acad. Sci. USA. 80:3548-3552.
Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink. 1987. A Saccharomyces cerevisiae centromeric plasmid bank based on a centromere-containing shuttle vector. Gene. 60:237-243.
Russell, D. W., R. E. Jensen, M. J. Zoller, J. Burke, B. Errede, M. Smith, and I. Herskovitz. 1986. Structure of the Saccharomyces cerevisiae HO gene and analysis of its upstream regulatory region. Mol. Cell. Biol. 6:4281-4294.
Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463-5467.
Schafer, L. E., S. E. C. Gross, and R. W. Schekman. 1985. Inversional signal and mature sequence substitutions that delay intercompartamental transport of active enzyme. J. Cell Biol. 100:1664-1675.
Schroer, T. A., E. R. Steuer, and M. P. Sheetz. 1989. Cytoplasmic dynein is a minus end-directed motor for membranous organelles. Cell. 56:937-946.
Sherman, F. G. R. Fink, and J. B. Hicks. 1979. Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 98 pp.
Skalli, O., and R. D. Goldman 1991. Recent insights into assembly, dynamics, and function of intermediate filament networks. Cell Motil. Cytoskeleton. 19:67-79.
Smith, B. J., and M. P. Yaffe. 1991. A mutation in the yeast heat-shock factor gene causes temperature-sensitive defects in both mitochondrial protein import and the cell cycle. Mol. Cell. Biol. 11:2647-2655.
Steinert, P. M., and D. R. Roop. 1988. Molecular and cellular biology of intermediate filaments. Annu. Rev. Biochem. 57:593-625.
Steinert, P. M., R. H. Rice, D. R. Roop, B. L. Trus, and A. C. Streven. 1983. Complete amino acid sequence of a mouse epidermal keratin subunit and implications for the structure of intermediate filaments. Biochemistry. 26:351-359.
Vale, R. D. 1987. Intracellular transport using microtubule-based motors. Annu. Rev. Cell Biol. 3:347-378.
Wang, E., and R. D. Goldman. 1978. Functions of cytoplasmic fibers in intracellular movements in BHK-21 cells. J. Cell Biol. 79:708-726.
Wilson, E. B. 1925. The Cell in Development and Heredity. Macmilian, New York. 1232 pp.
Wittenberg, C., S. L. Richardson, and S. I. Reed. 1987. Subcellular localization of a protein kinase required for cell cycle initiation in Saccharomyces cerevisiae: evidence for an association between the CDC28 gene product and the insoluble cytoplasmic matrix. J. Cell Biol. 105:1257-1238.
Yaffe, M. P. 1991a. Analysis of mitochondrial function and assembly. Methods Enzymol. 194:627-643.
Yaffe, M. P. 1991b. Organelle inheritance in the yeast cell cycle. Trends Cell Biol. 1:160-164.