A Role for Unsaturated Fatty Acids in Mitochondrial Movement and Inheritance

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Abstract. Yeast cells with the *mdm2* mutation display temperature-sensitive growth and defective intracellular mitochondrial movement at the non-permissive temperature. The latter phenotype includes both an absence of mitochondrial transfer into daughter buds of mitotically growing cells and an aberrant mitochondrial distribution in cells exposed to mating pheromone. The wild-type *MDM2* gene was cloned by complementation, and DNA sequence analysis revealed a large open reading frame encoding a putative protein of 58.4 kD. The predicted protein sequence is identical to that reported for the yeast *OLE1* gene encoding fatty acid desaturase. Unsaturated fatty acid levels are substantially decreased in *mdm2* cells after a prolonged incubation at the non-permissive temperature. The addition of oleic acid complements the temperature-sensitive growth and mitochondrial distribution defects of the mutant cells. These results indicate that *mdm2* is a temperature-sensitive allele of *OLE1* and demonstrate an essential role for unsaturated fatty acids in mitochondrial movement and inheritance.

An essential component of cell division is the distribution of cytoplasmic organelles to daughter cells. This intracellular distribution process ensures a continuance of the cytoplasmic organellar population, since mitochondria and other subcellular structures proliferate by the growth and division of pre-existing organelles (Palade, 1983; Attardi and Schatz, 1988). The mechanisms by which cytoplasmic organelles are distributed during mitosis are poorly understood. Observations in a number of cell types have suggested roles for cytoskeletal components in organellar movement and position in (Ball and Singer, 1982; Hirokawa, 1982; Aufderheide, 1977; Vale, 1987; Wang and Goldman, 1978; Kachar and Reese, 1988), but evidence for the function of these structures in organellar inheritance is lacking. Additionally, structural features of mitochondria and other organelles that are important for organellar distribution have yet to be identified.

We have undertaken a genetic approach using *Saccharomyces cerevisiae* in order to identify molecular mechanisms responsible for the delivery of mitochondria to daughter cells during mitosis. We reported previously (McConnell et al., 1990) the isolation of several mitochondrial distribution and morphology (*mdm*) mutants which display temperature-sensitive growth and a failure to transfer mitochondria into a growing bud during incubation at the non-permissive temperature. The *mdm* mutations are single, nuclear lesions which uncouple mitochondrial movement from bud formation and growth. One of these mutants, *mdm2*, is highly specific for mitochondria: the nuclei and vacuoles are faithfully transmitted to developing buds, secretion, nuclear division, and cytokinesis processed normally, and the structure and function of the actin and tubulin cytoskeletons appear similar to those in wild-type cells.

In the present investigation, we report the isolation and characterization of the *MDM2* gene. We demonstrate that it encodes the Δ9 fatty acid desaturase, and further show that a product of the desaturase, oleate, will complement all of the mutant phenotypes of *mdm2* cells. These findings indicate a critical role for unsaturated fatty acids in intracellular mitochondrial movement.

Materials and Methods

Strains and Growth Conditions

Yeast strains used in this study (Table I) were grown in YPD (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose), YPG (1% Bacto-yeast extract, 2% Bacto-peptone, 3% glycerol), or in minimal medium (Sherman et al., 1979) supplemented with 20 mg/l uracil. Oleic acid was added to YPD or YPG as a 95:5 mixture of oleic acid/Tween 40. Oleate was removed from cultures by successive washing of cells with 1% Tween 40, 0.5% Tween, and H_2O, followed by resuspension in unsupplemented media.

A spontaneous revertant of *mdm2* was obtained by plating 3×10^8 cells of strain MYY276 on YPD medium and selecting colonies capable of growth at 37°C. One potential revertant was crossed to strain A364A-1 and the resulting diploid sporulated. All meiotic progeny from this cross displayed only wild-type phenotypes, suggesting that the original isolate was a true revertant.

Plasmid DNA isolated directly from yeast was propagated through *Escherichia coli* strain MH6. Otherwise, *E. coli* strain DH5α was used for all manipulations of plasmids. Standard procedures (Maniatis et al., 1982) were used for the preparation and analysis of plasmid DNA.

Table I. Yeast Strains

Strain	Genotype	Source or reference
A364A-1	*MATa, ura1, his7, lys2, tyr1, gal1, ade1, ade2*	C. McLaughlin
A364A-2	*MATα, ura1, his7, lys2, tyr1, gal1, ade1, ade2*	C. McLaughlin
MYY274	*MATa, mdm1*, A364A background	McConnell et al. (1990)
MYY278	*MATa, mdm2*, A364A background	McConnell et al. (1990)
MYY276	*MATα, mdm2*, A364A background	McConnell et al. (1990)
MYY402	*MATa, mdm2, ura3, leu2*	This study
KD115	*MATα, ole1*	Yeast Genetic Stock Center
HR125	*MATa, ura3, leu2, trp1, his3*	Russell et al. (1986)
SEY6210	*MATα, ura3, leu2, his3, trp1, ade2, suc2*	S. Emr

Isolation of the MDM2 Gene

The *MDM2* gene was cloned by complementation of the temperature-sensitive growth phenotype of the *mdm2* mutant. Strain MYY402 containing the *mdm2* and *ura3* mutations was constructed by crossing strain MYY276 with strain HR125. Strain MYY402 was transformed with a yeast genomic library constructed in YCp50 (Rose et al., 1987) by the lithium acetate method (Ito et al., 1983). Seven Ura^+ transformants were found to be capable of growth on YPD at 37°C. Plasmid DNA was isolated from these candidates and amplified through *E. coli* strain MH6.

DNA Sequencing and Homology Analysis

The complementing HindIII-XhoI fragment was subcloned into the polylinker region of both plasmid pUC19 and plasmid pBluescript KS^+. Overlapping, nested deletions were constructed in both directions using Exonuclease III as previously described (Henikoff, 1984). The nucleotide sequence was determined by the dideoxy chain termination method (Sanger et al., 1977) using a Sequenase 2 DNA sequencing kit (United States Biochemical, Cleveland, OH). The deduced MDM2 protein sequence was compared to sequences in GENEBANK using the FastA program (Lipman and Pearson, 1985).

Integrative and Chromosomal Mapping

The complementing HindIII-XhoI fragment was subcloned into the yeast integrating plasmid YIP5 (Struhl et al., 1979) to yield plasmid pLSint. This plasmid was linearized by digesting with KpnI (which cuts within the *MDM2* gene; see Fig. 2) and transformed into the *ura3* strain SEY6210. Ura^+ transformants were selected and crossed to strain MYY402, and the diploids were sporulated. In 40 out of 43 tetrads analyzed, there was a 2:2 patter of segregation of Ura^+ ts^-:Ura^- ts^+. The other three tetrads each possessed three ts^- spores (two of which were Ura^+), suggesting that gene conversion events had taken place. These results indicated that the plasmid pLSint had integrated into a site within 1.2 cM of the *MDM2* gene.

The *MDM2* gene was localized to a specific chromosome by hybridization of a SalI-EcoRV probe to a blot of yeast chromosomes separated by OFAGE electrophoresis (purchased from Clonetech). The probe was labeled by random priming (Maniatis et al., 1982), hybridization was performed at 42°C, and radioactive bands were detected by autoradiography.

Fluorescence Microscopy

DASPMI staining of cells and fluorescence microscopy was as previously described (McConnell et al., 1990).

Fatty Acid Analysis

Wild-type strain A3464A-1 and strain MYY276 were grown overnight at room temperature in YPD medium to an OD_{600} of 1.0. Samples of each culture were shifted to 37°C for 6–7 h (or 1–2 h). Cells were collected by centrifugation, washed with water, and transferred to methanol containing butylated hydroxytoluene. Total cellular lipids were isolated by extracting cells once with methanol and twice with chloroform-methanol (1:1 vol/vol) as described by Hubbard and Brody (1975). Conversion of the lipids to the corresponding methyl-ester derivatives, and analysis by gas chromatography was as described by Roeder et al. (1982).

Results

mdm2 Cells Are Defective in the Intracellular Movement of Mitochondria

The *mdm2* mutant was originally identified as a temperature-sensitive strain which failed to deliver mitochondria to the buds of mitotically growing cells during incubation at the non-permissive temperature (McConnell et al., 1990). An additional phenotype of this mutant is an altered mitochondrial morphology characterized by extensive clumping and aggregation (McConnell et al., 1990). This aggregation and clumping of mitochondria is observed even in unbudded cells not undergoing mitosis.

The *mdm2* phenotype was characterized further by examining another type of mitochondrial movement: distribution of the organelles into the elongated cytoplasmic projections produced during exposure of haploid cells to mating pheromone. Mitochondria were found to migrate into such projections in wild-type cells and in cells containing another mutation affecting mitochondrial inheritance, *mdm1* (McConnell et al., 1990), during prolonged incubation of cells of mating type a (*MATa*) with α-factor at 37°C (Fig. 1). In *mdm2* cells, however, the mitochondria were absent from the projections following the incubation at the non-permissive temperature. This defect was observed in >50% of *mdm2* cells that had formed elongated projections. These observations indicate that mitochondrial movement is generally defective in *mdm2* cells at the non-permissive temperature.

Cloning of the MDM2 Gene

The wild-type *MDM2* gene was cloned by complementation of the temperature-sensitive growth phenotype of *mdm2* cells. Out of ~11,000 Ura^+ transformants, seven were found to be capable of growth at 37°C. DNA isolated from these transformants was analyzed by restriction mapping. The seven isolates represented two unique genomic inserts which overlapped over a 6-kb region. Retransformation of each of these plasmids into *mdm2* cells resulted in full complementation of the *mdm* and temperature-sensitive growth phenotypes. Subcloning revealed a 4.5-kb HindIII-XhoI fragment to be the smallest complementing fragment (Fig. 2).

The isolated fragment of genomic DNA was shown to contain the authentic *MDM2* gene (rather than an extragenic suppressor) by integrative mapping. The complementing HindIII-XhoI fragment integrated to a chromosomal position within 1.2 cM of the *MDM2* locus (see Materials and

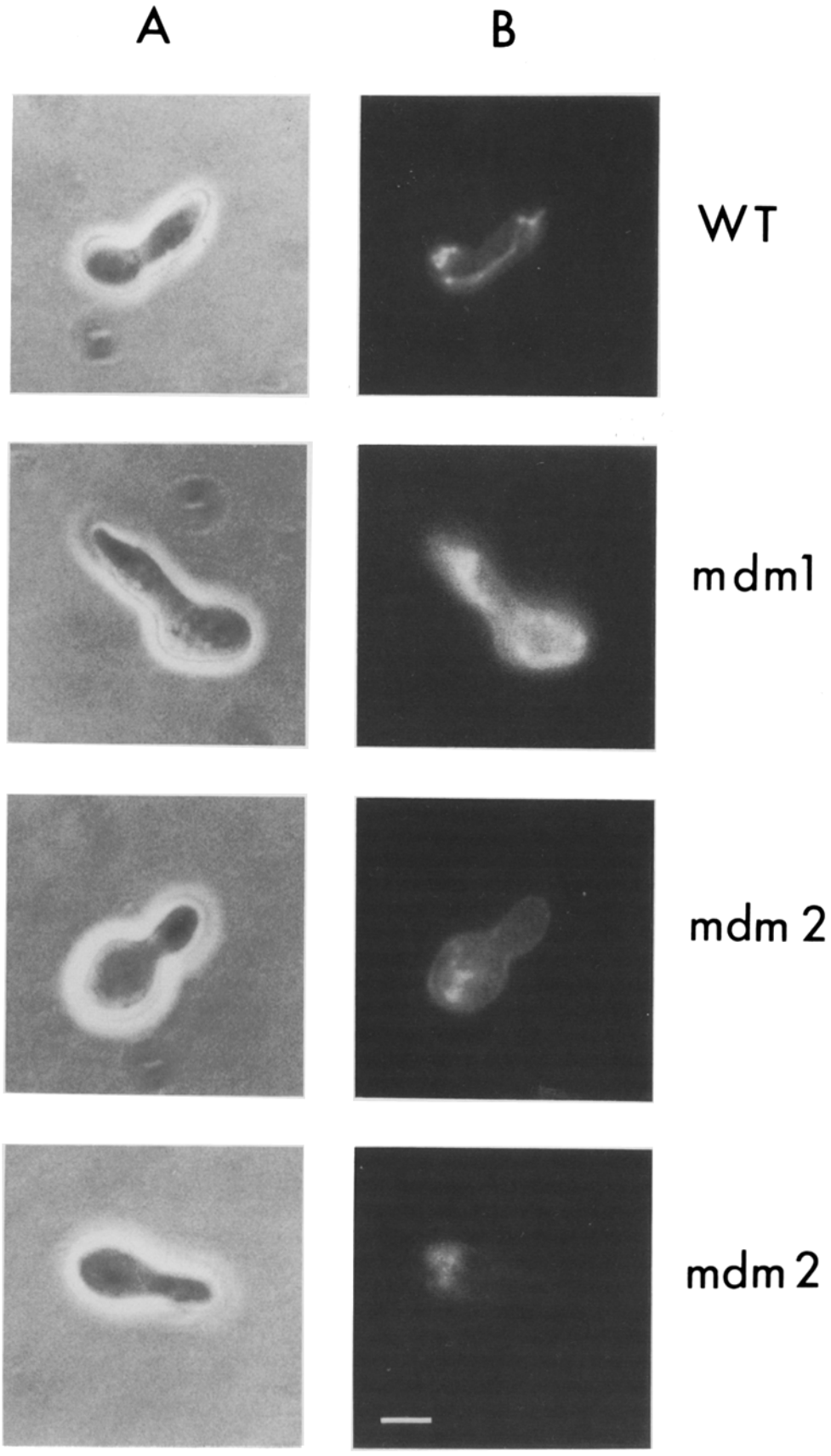

Figure 1. Mitochondrial distribution in cells treated with mating pheromone. Cultures of wild-type (A364A-1), *mdm1* (MYY274), and *mdm2* (MYY-278) cells (all mating-type *a*) were grown overnight at 23°C to an OD_{600} of 1.0 and then incubated at 37°C in the presence of 5 μg/ml of α-factor mating pheromone. After two hours, additional α-factor was added to a total, final concentration of 10 μg/ml. Cells were incubated for another 3–4 h and then stained for mitochondria with the fluorescent dye DASPMI (McConnell et al., 1990). Panels on the left show phase contrast image of cells; panels on right show mitochondrial staining. Bar, 2 μm.

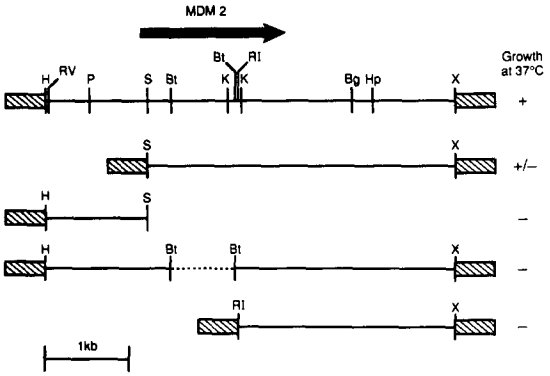

Figure 2. Localization of the MDM2 gene. The restriction map for several different enzymes was determined for the complementing HindIII-XhoI DNA fragment. Fragments shown below the map were tested for their ability to complement the temperature-sensitive growth phenotype of *mdm2* cells. The dashed line indicates a region deleted from the fragment. Location of the *MDM2* open reading frame is indicated by the arrow. ▨, vector sequences. Restriction sites: *Bg*, BglII; *Bt*, BstEII; *H*, HindIII; *Hp*, HpaI; *K*, KpnI; *P*, PstI; *RI*, EcoRI; *RV*, EcoRV; *S*, SalI; *X*, XhoI.

Methods). Additionally, the *MDM2* gene was mapped to chromosome VII by hybridization of a SalI-EcoRV probe to a yeast chromosomal OFAGE blot (see Materials and Methods).

Sequence of the MDM2 Gene

The nucleotide sequence of the complementing 4.5-kb HindIII-XhoI fragment was determined by dideoxy sequencing using templates generated by ExoIII digestion. A single, long open reading frame of 1,530 bp was identified beginning just upstream of the SalI site and extending 478 bp beyond the second KpnI site (Fig. 2). The length of this ORF correlated well with the appearance of a single, ~1.5-kb band on Northern blots of isolated yeast RNA (using a SalI-EcoRV probe, data not shown), and its location is in agreement with the complementation data of Fig. 2. The ORF encoded a putative protein product of 510 amino acids with a predicted size of 58.4 kD (data not shown).

Comparison of the predicted protein sequence of MDM2 with sequences in the GENBANK database revealed homology to rat stearoyl CoA-Δ9 desaturase (Strittmatter et al., 1988) with 35% identity over a 274 amino acid region. Alignment of the hydropathy profiles of the rat and yeast sequences revealed extensive similarity in the distribution of hydrophobic and hydrophilic regions throughout most of the protein's sequence (data not shown). The nucleotide sequence of *MDM2* also was found to be identical to the sequence of the yeast Δ9 fatty acid desaturase gene, *OLE1*, which was recently described by Stukey et al. (1990). Therefore, *mdm2* is a mutant allele of *OLE1*.

Levels of Unsaturated Fatty Acid Are Lowered in mdm2 Cells upon Prolonged Shift to 37°C

The original *ole1* mutant was characterized by its inability to grow in the absence of added unsaturated fatty acids at all temperatures (Wisnieski et al., 1970). Following a shift of *ole1* cells to medium depleted of unsaturated fatty acids, the levels of both 16:1 and 18:1 unsaturated fatty acid species were reported to decline by 10- and sevenfold, respectively (Stukey et al., 1989). To determine whether the levels of unsaturated fatty acids were actually depleted after prolonged shift to the non-permissive temperature in *mdm2* cells, we analyzed the composition of total cellular lipids from the mutant strain MYY276 and its wild-type parental strain A364A-1 after both growth at 23°C and incubation at 37°C. Both mutant and wild-type strains contain a predominance of 16 carbon fatty acids over 18 carbon chain lipids at both temperatures (Fig. 3), and we did not find evidence for detectable levels of either 14 carbon or >18 carbon-chain unsaturated fatty acids. The fatty acid levels detected in the wild-type strain were very similar to those reported previously for other strains of yeast (Stukey et al., 1989) and showed only slight changes between 23 and 37°C (Fig. 3). In *mdm2* cells incubated at 37°C, however, there was a decline of approximately threefold in the amount of 16:1 unsaturated fatty acids over the levels in cells grown at 23°C (Fig. 3). A similar decline of ~2.5-fold was detected in the level of 18:1 species. A concomitant increase in the levels of precursor 16:0 and 18:0 fatty acids was also observed; these increases most likely resulted from the defective desaturation in the *mdm2* cells at 37°C.

Unsaturated fatty acid levels were analyzed also in cells shifted to 37°C for only 90 min, a time when *mdm* phenotypes first begin to appear in the cells. During this incubation total unsaturated fatty acid levels dropped 10–15% in *mdm2* cells. In wild-type cells, a similar incubation resulted in a decrease of ~5% in unsaturated fatty acid levels. Therefore, the *mdm* phenotype appeared in cells after only a modest decrease in unsaturated fatty acid levels.

The mdm2 Phenotypes Are Reversed by Addition of Unsaturated Fatty Acid

The identity of *MDM2* with *OLE1* and the decreased levels of unsaturated fatty acids in the mutant cells following incubation at 37°C suggested that the mutant phenotypes resulted from inadequate amounts of unsaturated fatty acid. To ex-

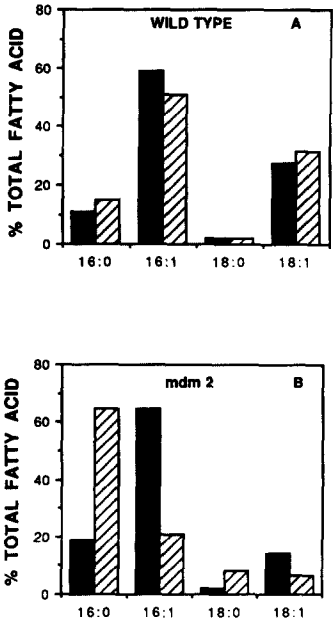

Figure 3. Unsaturated fatty acid levels are decreased in *mdm2* cells after prolonged incubation at 37°C. Wild-type (*A*) and *mdm2* (*B*) cells were grown overnight at 23°C to an OD_{600} of 1.0. Portions of each culture were either held at 23°C or incubated at 37°C for 6–7 h. Total cellular lipids were extracted and analyzed by gas chromotography. ■, cells incubated at 23°C; ▨, cells incubated at 37°C. 16:0, 16:1, 18:0, and 18:1 refer to the fatty acid species palmitic acid, palmitoleic acid, stearic acid, and oleic acid, respectively.

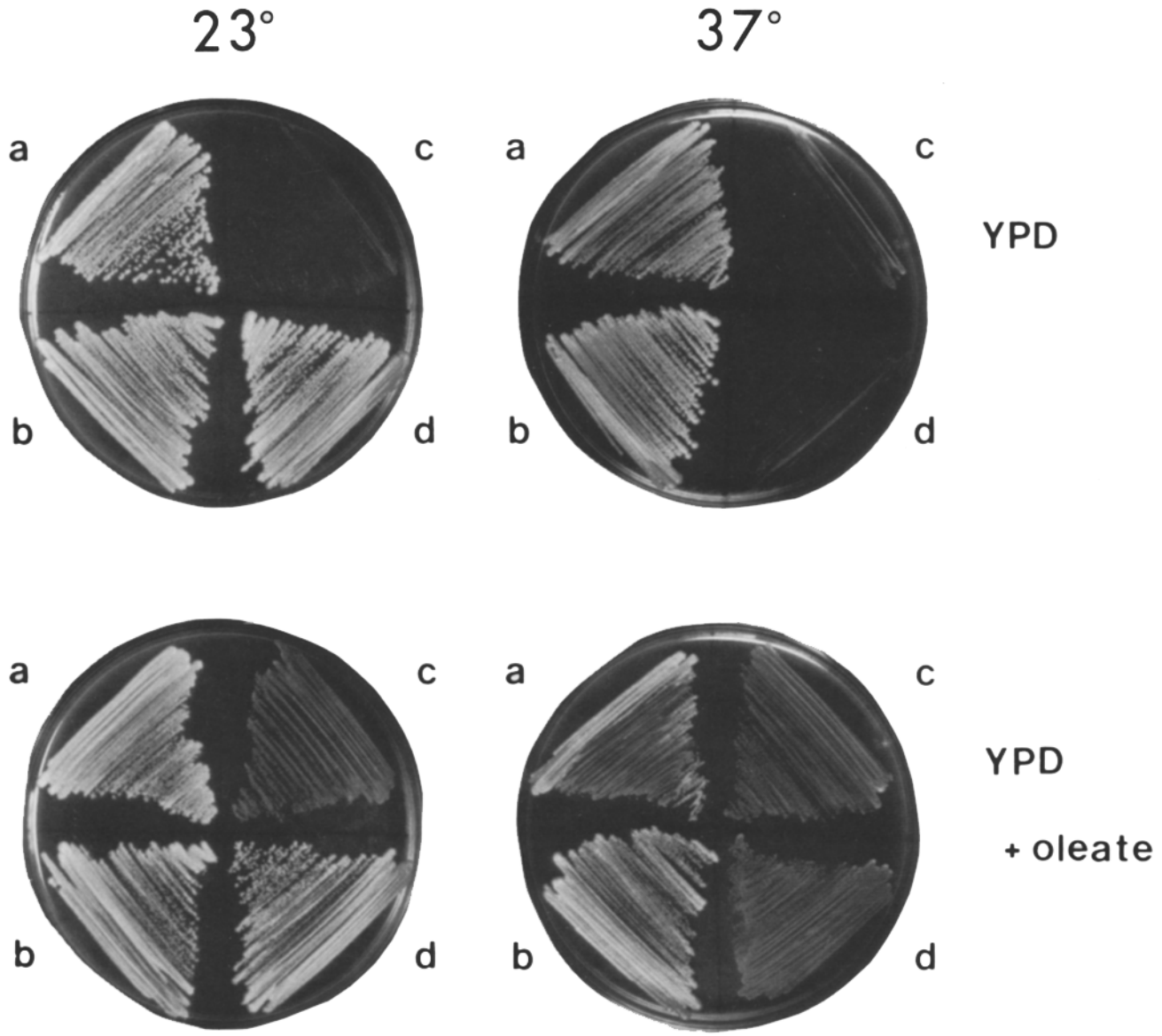

Figure 4. Unsaturated fatty acids complement the temperature-sensitive growth of *mdm2* cells. Cells were grown for 4 d at 23 or 37°C on agar media composed of either YPD (*top*) or YPD containing 1 mM oleic acid (*bottom*). *a*, wild type (A364A-2); *b*, a spontaneous revertant of *mdm2* (see Materials and Methods); *c*, *ole1* (KD115); *d*, *mdm2* (MYY276).

plore this possibility directly, the effect of the addition of unsaturated fatty acid on the properties of *mdm2* cells was examined. The addition of 1 mM oleic acid (18:1) to growth medium supported *mdm2* growth at 37°C (Fig. 4). In contrast, the *ole1* auxotroph (strain KD115) required oleic acid supplementation for growth at either 23 or 37°C. Both *mdm2* and *ole1* cells growing on oleate-supplemented medium varied from the wild-type (and *mdm2* at 23°C) in color and opaqueness (Fig. 4), however colony size was similar for all strains under these growth conditions.

The addition of oleate also corrected defects in mitochondrial inheritance, movement, and morphology. The distribution and appearance of mitochondria in *mdm2* cells shifted to the non-permissive temperature were completely wild type in the presence of 1 mM oleate (Fig. 5). This effect was observed regardless of whether the culture of *mdm2* cells was grown initially at 23°C in the presence or absence of added oleate. Additionally, the addition of oleate reversed the defective distribution of mitochondria in cytoplasmic projections formed in response to mating pheromone in *mdm2* cells at 37°C (Fig. 6). The presence or absence of oleate had no effect on mitochondrial distribution in cytoplasmic projections of wild-type cells (data not shown).

The growth and *mdm* phenotypes were analyzed for *mdm2* cells incubated over a range of oleate concentrations. Above 500 μM oleate cells displayed wild-type phenotypes of growth and mitochondrial distribution. At oleate concentrations below 50 μM cells showed *mdm* phenotypes and failed to grow on either plates or in liquid media. As exogenous oleate levels were decreased from 500 to 50 μM increasing numbers of cells in a population displayed aggregated mitochondria and an absence of mitochondria in buds. The cells also showed progressively lower growth rates.

To further characterize the reversal of mutant phenotypes

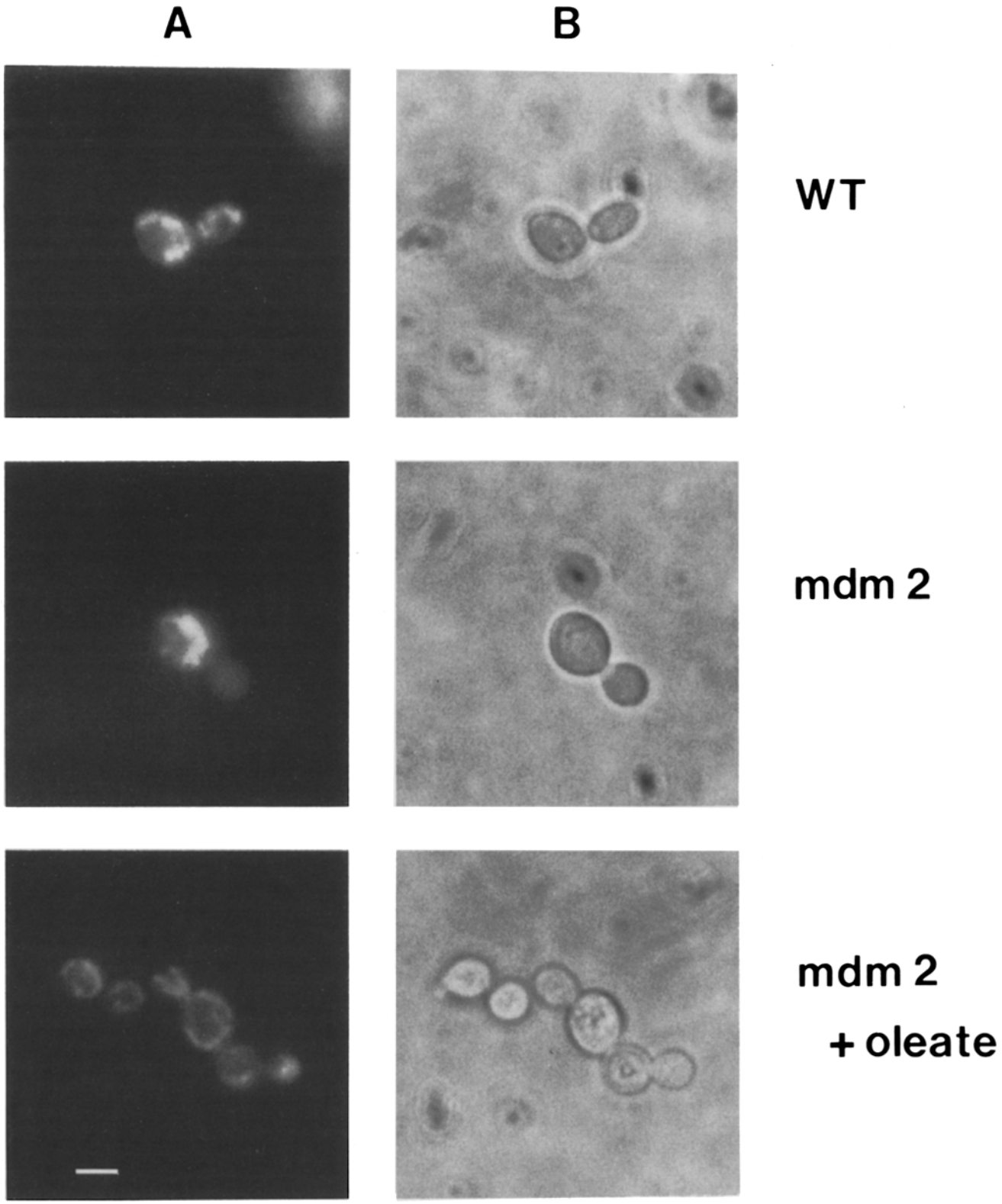

Figure 5. Unsaturated fatty acids reverse the aberrant mitochondrial distribution in *mdm2* cells. Cultures of wild-type cells were grown overnight in YPD and shifted to 37°C for 4 h. Cultures of *mdm2* cells were grown overnight in YPD and then shifted to 37°C in the presence or absence of 1 mM oleate. *A* shows mitochondrial staining with DASPMI; *B* represents phase contrast images of cells. Bar, 2 μm.

by oleate addition, a culture of *mdm2* cells was synchronized with α-factor to form a uniform population of unbudded cells and then shifted to 37°C (YPD-no oleate) for 90 min. Following this treatment, the cells possessed small, empty buds with aggregated mitochondria in the mother portion of the cell (data not shown). Oleic acid was added to a concentration of 1 mM and the phenotype of the cells followed with time. Approximately 2 h after the addition of oleate, the mitochon-

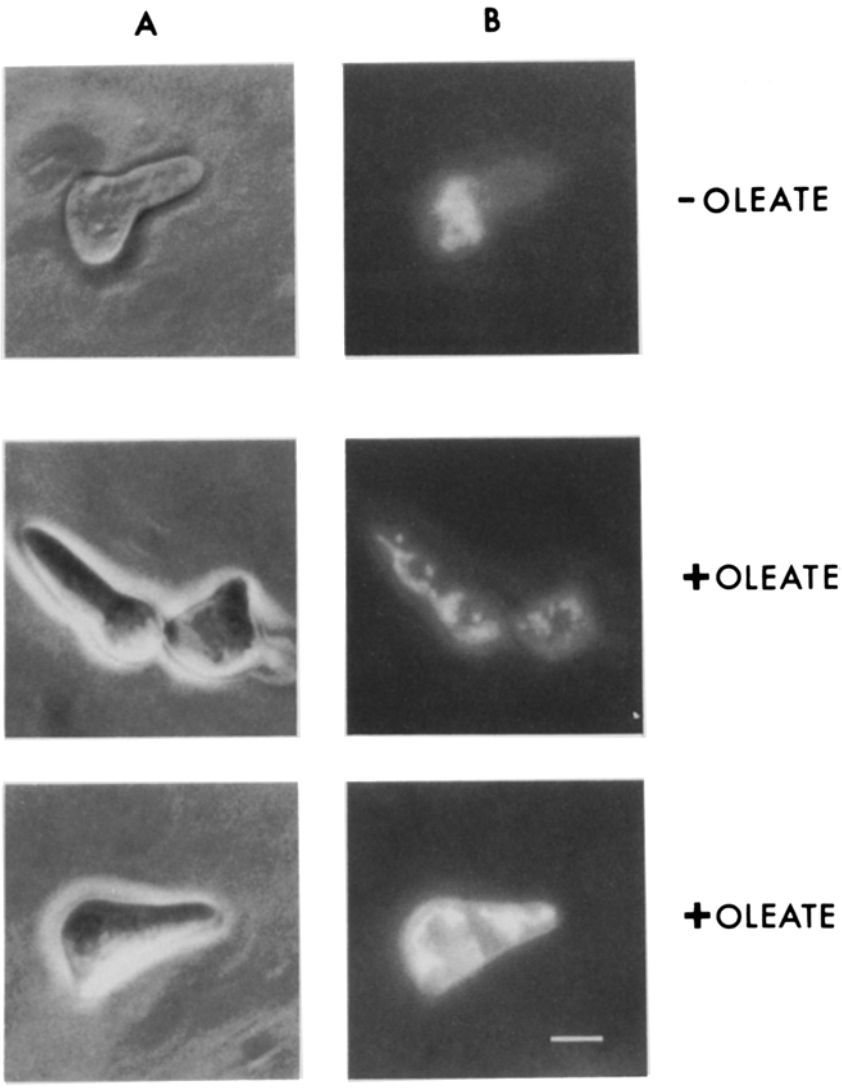

Figure 6. Unsaturated fatty acids reverse the aberrant mitochondrial distribution in *mdm2* cells treated with mating pheromone. Cultures of *mdm2* (MYY278) cells were grown at 23°C and incubated in the presence or absence of 1 mM oleate at 37°C with α-factor as described for Fig. 1. Mitochondria were stained with the fluorescent dye DASPMI (McConnell et al., 1990). *A*, phase contrast image of cells; *B*, mitochondrial staining. Bar, 2 μm.

drial distribution of the cells looked uniformly wild type (data not shown), indicating that the *mdm2* phenotype was reversed by unsaturated fatty acids.

The *mdm2* mutant is not auxotrophic for oleate at room temperature (Fig. 4); only at 37°C is the defect in mitochondrial inheritance apparent. To assess the role of unsaturated fatty acids in mitochondrial inheritance at room temperature, we examined the effects of oleate starvation on the unsaturated fatty acid auxotroph *ole1* (KD115). Initially, cultures of these cells were grown overnight in the presence of 1 mM oleate and subsequently shifted to unsupplemented media. Even after prolonged incubation, a slight aggregation of mitochondria in both the bud and mother portions of these cells was the only change observed microscopically (data not shown). Because of the possibility that *ole1* cells might be capable of accumulating reserves of unsaturated lipids when grown in the presence of excess unsaturated fatty acid, we attempted the same experiment using *ole1* cultures grown overnight in the presence of a reduced level of oleate (50–100 μM). Wisnieski et al. (1970) reported that strain KD115 grown in the presence of 10 μM oleate displays an eightfold slower doubling time than cells grown with 100 μM oleate, suggesting that oleate becomes limiting for growth in this concentration range. Following the addition of unsupplemented medium, a gradual change in mitochondrial morphology was observed (data not shown). After ~8 h, the mitochondria were distinctly aggregated and clumped relative to wild-type controls. Occasional cells lacking mitochondrial staining in buds were observed, although this phenotype was represented in <1% of the cells examined.

Discussion

We have described the isolation and characterization of the wild-type *MDM2* gene. Sequence analysis of *MDM2* revealed its identity to the recently described *OLE1* gene, which encodes the yeast Δ9-fatty acid desaturase (Stukey et al., 1990). We have further demonstrated that levels of unsaturated fatty acids were substantially decreased in *mdm2* cells following prolonged incubation at 37°C (Fig. 3). Additionally, the supplementation of culture medium with a product of the desaturase, oleic acid, was found to cure the defects in mitochondrial distribution, mitochondrial morphology, and temperature-sensitive growth in *mdm2* cells (Figs. 4, 5, and 6). These results demonstrate that *mdm2* is a temperature-sensitive allele of *OLE1*.

The phenotype of the *mdm2* mutant cells indicates an essential requirement for unsaturated fatty acids in mitochon-

drial movement into both developing buds (McConnell et al., 1990) and cytoplasmic projections formed in response to mating pheromone (Fig. 1). The complementation of mutant phenotypes by oleic acid indicates that it is, indeed, the unsaturated fatty acids rather than some specialized activity of the desaturase enzyme that is required for mitochondrial movement. The mutant phenotypes appear within the first cell cycle after a shift to the non-permissive temperature (McConnell et al., 1990) suggesting either a sensitivity of mitochondrial movement to small changes in the levels of unsaturated fatty acids or a specific role for "new" unsaturated fatty acids. The requirement is also highly specific: other organelles and cellular structures appear to be distributed normally, processes such as bud growth, nuclear division, and cytokinesis are uninterrupted, and mitochondrial energy production continues at the non-permissive temperature for (at least) a number of hours. This specificity of the *mdm2* phenotype does not appear to reflect a mitochondrial bias in the concentration of unsaturated fatty acids, since the fatty acid composition of the mitochondrial membranes mirrors that of the cell as a whole (Stukey et al., 1989), and mitochondrial lipids are largely in rapid equilibrium with those of other cellular membranes (Yaffe and Kennedy, 1983; Daum, 1985).

What is the role of unsaturated fatty acids in mitochondrial movement? One possibility is that mitochondrial movement is extremely sensitive to changes in fluidity of the mitochondrial membranes and that fatty acid alterations in *mdm2* cells at 37°C lead to such fluidity changes. However, data associating membrane fluidity in yeast with modest changes in levels of unsaturated fatty acids are lacking. A second possibility is that unsaturated fatty acids are needed for the production of some structure on the mitochondrial surface or to induce the correct conformation of a mitochondrial membrane protein. This structure or protein might serve as a site for the interaction of mitochondria with other cellular components (e.g., cytoskeletal elements), and its properties might be influenced by either the local lipid environment or covalent modification by addition of unsaturated fatty acids. A third possible role for unsaturated fatty acids is that these species are needed for mitochondrial division or changes in mitochondrial shape. These processes are poorly understood (Attardi and Schatz, 1988), but such morphological alterations might be essential for mitochondrial movement. While gross morphological changes were not apparent in *mdm2* mitochondria examined by EM (McConnell et al., 1990), these organelles might possess more subtle structural modifications or be incapable of certain conformational changes.

Our identification of the importance of the yeast Δ9 fatty acid desaturase in the intracellular movement of mitochondria is interesting in light of previous findings of lipid requirements for organelle movement in the secretory pathway. The yeast *SEC14* gene product, which is essential for transfer of secretory proteins beyond the Golgi apparatus, has been identified as the phosphatidylinositol/phosphatidylcholine transfer protein (Bankaitis et al., 1990). This protein is thought to adjust the membrane lipid composition of secretory vesicles exiting the Golgi apparatus, and its function can be bypassed by alterations in phospholipid biosynthesis (Cleves et al., 1991). Additionally, Pfanner et al. (1989) have described a requirement for fatty acyl-CoA in the budding of transport vesicles from Golgi compartments.

The *mdm2* allele of *OLE1* reveals an essential role for unsaturated fatty acids in mitochondrial movement. The *mdm2* lesion is likely to alter some property of the mitochondria themselves, preventing their distribution into buds and throughout the cytoplasm. The isolation of extrageneic suppressors of the *mdm2* mutation may reveal additional proteins involved in the intracellular movement of mitochondria and should further clarify the role of unsaturated fatty acids in this essential process.

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References

Attardi, G., and G. Schatz. 1988. Biogenesis of mitochondria. *Ann. Rev. Cell Biol.* 4:289–333.

Aufderheide, K. J. 1977. Saltatory motility of uninserted trichocysts and mitochondria in *Paramecium tetraurelia*. *Science (Wash. DC).* 198:299–300.

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.

Bankaitis, V. A., J. R. Aitken, A. E. Cleves, and W. Dowhan. 1990. An essential role for a phospholipid transfer protein in yeast Golgi function. *Nature (Lond.).* 347:561–562.

Cleves, A. E., T. P. McGee, E. A. Whitters, K. M. Champion, J. R. Aitken, W. Dowhan, M. Goebl, and V. A. Bankaitis. 1991. Mutations in the CDP-choline pathway for phospholipid biosynthesis bypass the requirement for an essential phospholipid transfer protein. *Cell.* 64:789–800.

Daum, G. 1985. Lipids of mitochondria. *Biochim. Biophys. Acta.* 822:1–42.

Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene.* 28:351–359.

Hirokawa, N. 1982. Cross-linker system between neurofilaments, microtubules, and membranous organelles in frog axons revealed by the quick-freeze, deep-etch method. *J. Cell Biol.* 94:129–142.

Hubbard, S. C., and S. Brody. 1975. Glycerophospholipid variation in choline and inositol auxotrophs of *Neurospora crassa*. *J. Biol. Chem.* 250:7173–7181.

Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163–168.

Kachar, B., and T. S. Reese. 1988. The mechanism of cytoplasmic streaming in Characean algal cells: sliding of endoplasmic reticulum along actin filaments. *J. Cell Biol.* 106:1545–1552.

Lipman, 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. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.

McConnell, S., L. Stewart, A. Talin, 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.

Pfanner, N., L. Orci, B. S. Glick, M. Amherdt, S. R. Arden, V. Malhotra, and J. E. Rothman. 1989. Fatty acyl-coenzyme A is required for budding of transport vesicles from Golgi cisternae. *Cell.* 59:95–102.

Roeder, P.E., M. L. Sargent, and S. Brody. 1982. Circadian Rhythms in *Neurospora crassa*: oscillations in fatty acids. *Biochemistry.* 21:4909–4916.

Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink. 1987. A *Saccharomyces cerevisiae* genomic 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. Herskowitz. 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.

Sherman, F., G. R. Fink, and J. B. Hicks. 1979. Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 98 pp.

Strittmatter, P., M. A. Thiede, C. S. Hackett, and J. Ozols. 1988. Bacterial synthesis of rat stearyl-CoA desaturase lacking the 26-residue amino-terminal amino acid sequence. *J. Biol. Chem.* 263:2532–2535.

Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis. 1979. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. *Proc. Natl. Acad. Sci. USA.* 76:1035–1039.

Stukey, J. E., V. M. McDonough, and C. E. Martin. 1989. Isolation and characterization of *OLE1*, a gene affecting fatty acid desaturation from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 264:16537–16544.

Stukey, J. E., V. M. McDonough, and C. E. Martin. 1990. The *OLE1* gene of *Saccharomyces cerevisiae* encodes the Delta 9 fatty acid desaturase and can be functionally replaced by the rat stearoyl-CoA desaturase gene. *J. Biol. Chem.* 265:20144–20149.

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.

Wisnieski, B. J., A. D. Keith, and M. A. Resnick. 1970. Double-bond requirement in a fatty acid desaturase mutant of *Saccharomyces cerevisiae*. *J. Bacteriol.* 101:160–165.

Yaffe, M. P., and E. P. Kennedy. 1983. Intracellular phospholipid movement and the role of phospholipid transfer proteins in animal cells. *Biochemistry.* 22:1497–1507.