Journal of Cell Science 111, 1717-1727 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JCS7215

The *S. cerevisiae CLU1* and *D. discoideum cluA* genes are functional homologues that influence mitochondrial morphology and distribution

Stephen D. Fields, Michael N. Conrad and Margaret Clarke*

Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA
*Author for correspondence

Accepted 28 March; published on WWW 27 May 1998

SUMMARY

The *cluA* gene, encoding a novel 150 kDa protein, was recently characterized in *Dictyostelium discoideum*; disruption of *cluA* impaired cytokinesis and caused mitochondria to cluster at the cell center. The genome of *Saccharomyces cerevisiae* contains an open reading frame (*CLU1*) that encodes a protein that is 27% identical, 50% similar, to this *Dictyostelium* protein. Deletion of *CLU1* from *S. cerevisiae* did not affect cell viability, growth properties, sporulation efficiency, or frequency of occurrence of cells lacking functional mitochondria. However, in *clu1Δ* cells the mitochondrial reticulum, which is normally highly branched, was condensed to one side of the cell. Transformation of *cluA*$^-$ *Dictyostelium* mutants with the yeast *CLU1* gene yielded amoebae that divided normally and had dispersed mitochondria. The mitochondria in *cluA*$^-$ *Dictyostelium* cells complemented with *CLU1* were not as widely scattered as in *cluA*$^+$ *Dictyostelium* cells, but formed loose clusters throughout the cytoplasm. These results indicate that the products of the *CLU1* and *cluA* genes, in spite of their limited homology, are functional homologues.

Key words: *S. cerevisiae*, *D. discoideum*, Mitochondrion

INTRODUCTION

Mitochondria supply most of the cellular ATP in eukaryotic cells. The accumulation of mitochondria near sites of high ATP consumption (i.e. near flagellar basal bodies and between myofibrils) suggests that mitochondrial positioning is important for efficient cellular metabolism. Furthermore, even cells that can survive anaerobically still require mitochondria (Gbelska et al., 1983; Yaffe and Schatz, 1984), because these compartments play an essential role in biosynthesis of pyrimidines, phospholipids, nucleotides, folate enzymes, sterols, and some amino acids (Shimizu et al., 1973; Zelikson and Luzzati, 1977; Goewert et al., 1981). Mitochondrial membranes cannot arise de novo, so proper transport, division, and partitioning of mitchondria to daughter cells is essential. The mechanisms by which mitochondria are replicated, transported, and partitioned are not well understood and may vary among different cell types.

The three major cytoskeletal elements, microtubules, microfilaments and intermediate filaments, act to varying degrees as the scaffolding along which mitochondria are transported (see Yaffe, 1996, for an extensive review of mitochondrial movement). Mitochondrial distribution has been linked most consistently to microtubules, with numerous studies demonstrating that mitochondrial position correlates with and/or depends upon the microtubular array (Heggeness et al., 1978; Ball and Singer, 1982; Couchman and Rees, 1982; Summerhayes et al., 1983; Yaffe et al., 1996). In axons, anterograde transport of mitochondria takes place along microtubules, suggesting that kinesin is the motor protein (Morris and Hollenbeck, 1995). Several microtubule-based, kinesin-like motor proteins specific for mitochondrial transport have been identified, including KIF1B in frog neurons (Nangaku et al., 1994; Hirokawa, 1996) and KLP67A in mitotic cells of *Drosophila* (Pereira et al., 1997).

Intermediate filaments and microfilaments have also been implicated in mitochondrial distribution. Mitochondria co-distribute with intermediate filaments by immunofluorescence (Mose-Larsen et al., 1982; Summerhayes et al., 1983), and crossbridges between the outer mitochondrial membrane and intermediate filaments have been observed by electron microscopy (David-Ferreira and David-Ferreira, 1980). The lack of direct evidence for organelle movement along intermediate filaments may indicate that these filaments serve as scaffolding for other cytoskeletal elements or as anchorage points for mitochondria, rather than participating directly in mitochondrial transport.

Microfilaments have been shown to play an active role in mitochondrial transport in some cell types. In axons, mitochondria are transported slowly along microfilaments in a net retrograde direction, contrasting with the fast microtubule-based anterograde movement (Morris and Hollenbeck, 1995). In *S. cerevisiae*, mitochondria localize to actin cables in vivo and move along actin filaments in vitro (Drubin et al., 1993; Lazzarino et al., 1994; Simon et al., 1997). Disruption of actin cables leads to defects in

mitochondrial motility and delays transfer of mitochondria to bud cells (Simon et al., 1997; Hermann et al., 1997). The presence of an actin-based, ATP-dependent motor activity on the mitochondrial surface (Lazzarino et al., 1994) and the finding that mutations in the myosin-binding site of the *ACT1* gene of *S. cerevisiae* result in abnormally organized mitochondria (Simon et al., 1995), both suggest that a myosin-like protein acts as a motor for the transport of this organelle. However, mutations in each of the yeast myosin genes (or pairs of those genes) produce no significant effect on mitochondrial movement or arrangement (Simon et al., 1995), so the identity of this presumptive actin-based motor is uncertain.

Although motor and linker proteins involved specifically in yeast mitochondrial movement have not been characterized, a number of studies in *S. cerevisiae* have identified mutants that are deficient in mitochondrial inheritance, movement, and morphology (reviewed by Yaffe, 1996). The yeast gene *MMM1* encodes a protein that localizes to the outer mitochondrial membrane and is involved in maintaining proper mitochondrial morphology and inheritance. Burgess et al. (1994) have proposed that Mmm1p attaches the mitochondrion to the cytoskeleton, thereby helping to maintain the shape and perhaps aiding in organizing and moving the mitochondrion. Lack of the protein encoded by *MDM10* results in giant spherical mitochondria as well as a defect in mitochondrial inheritance; Mdm10p is found in the outer mitochondrial membrane (Sogo and Yaffe, 1994). Another mitochondrial membrane protein, the product of the *MDM12* gene, is also required for proper mitochondrial distribution and inheritance (Berger et al., 1997). Disruption of *MDM2*, a gene involved in the synthesis of unsaturated fatty acids, also impairs mitochondrial transmission to buds (McConnell et al., 1990); this defect can be complemented by addition of oleic acid to *mdm2* mutants (Stewart and Yaffe, 1991). The gene *MGM1* encodes a protein with homology to dynamin; this gene is required for the propagation of functional mitochondria in yeast, apparently through an effect on the replication or partitioning of mitochondrial DNA (Jones and Fangman, 1992; Guan et al., 1993). In the absence of Mgm1p, the distribution of mitochondria is also abnormal. The gene *MDM1* has multiple functions in organelle inheritance. This gene encodes an intermediate filament-like protein that localizes to punctate arrays throughout the cytoplasm of yeast cells (McConnell and Yaffe, 1992). Disruption of *MDM1* results in failure to transfer both mitochondria and nuclei to buds (McConnell et al., 1990); these two functions have been separated in different mutant alleles (Fisk and Yaffe, 1997). Overall, these data indicate that both cytoskeletal elements and components of mitochondrial membranes contribute to mitochondrial morphology and inheritance.

In the present report, we describe a new yeast gene, *CLU1*, whose product affects mitochondrial morphology. The predicted sequence of Clu1p is 27% identical (50% similar) to the product of the *cluA* gene of *Dictyostelium discoideum*, whose disruption results in clustering of mitochondria near the cell center (Zhu et al., 1997). We show here that the *CLU1* gene of *S. cerevisiae* is a functional homologue of *Dictyostelium cluA* and that the lack of Clu1p affects the morphology of the mitochondrial network in yeast cells.

MATERIALS AND METHODS

Strains, culture media and genetic methods

S. cerevisiae strains used in this study are listed in Table 1. Yeast culture media were prepared, and standard genetic manipulations were carried out, as described by Rose et al. (1990). Media with non-fermentable carbon sources included YPG (3% glycerol), YPE (3% ethanol) and YPL (2.5% lactic acid). YPD contained 2% dextrose. *D. discoideum* strains were grown in HL5 medium. Growth conditions, transformation by electroporation, and selection in G418-containing medium were as previously described (Liu and Clarke, 1996).

Disruption of *CLU1*

The *S. cerevisiae CLU1* gene was deleted in the diploid strains MCY387 and MDD1 by one-step gene disruption, using the technique of Baudin et al. (1993). The shuttle vector pRS304, which contains the *TRP1* gene (Sikorski and Hieter, 1989), was used as template for polymerase chain reaction (PCR). The forward primer was identical to bases 25,801-25,853 of YM8270.16 (*Saccharomyces* Genome Database) with the addition at the 3′-end of the bases 5′-CAGAGCAGATTGTACTGAGAGTCGACC-3′, which are homologous to the pRS304 region upstream of *TRP1*. The reverse primer was an oligonucleotide complementary to bases 29,615-29,670 of YM8270.16, plus an additional 3′ sequence (5′-GCATCTGTGCGGTATTTCACACCG-3′) homologous to the pRS304 region downstream of *TRP1*. MCY387 and MDD1 cells (both *trpΔ*) were transformed by the lithium acetate method with the PCR disruption cassette, and transformants were selected as tryptophan prototrophs. Several transformants from each strain were sporulated and tetrads dissected. The resulting haploid segregants were screened by PCR to verify disruption of *CLU1*, as described in Results. The segregants were subjected to standard genetic analyses (Sherman, 1991).

Complementation methods

For complementation studies, the *CLU1* gene was synthesized by PCR with Deep Vent polymerase (New England Biolabs) using as template the phage clone λPM-6114 (ATCC #70677), which contains the *CLU1* gene. Primers were positioned 300 bp upstream of the 5′-end and 100 bp downstream of the 3′-end of the gene. The forward primer was 5′-GGATCCCTGAGAAACTTGTGCATGATTTG-3′, and the reverse primer was 5′-GGTACCTGCATTTATGATGATTTGTG-3′. The ends of the 4.2 kb PCR product were phosphorylated with T4 polynucleotide kinase, and the product was cloned into the *Sma*I site of the yeast centromeric vectors, YCp*lac111* and YCp*lac33*, containing the *LEU2* and *URA3* selectable markers, respectively (Gietz and Sugino, 1988). Haploid *clu1Δ* strains carrying the *leu2* or *ura3* auxotrophic markers were transformed with the appropriate plasmids as above, and transformants were selected for leucine or uracil prototrophy.

The *S. cerevisiae CLU1* gene was also used to complement a *cluA*$^-$ mutant of *D. discoideum* (Zhu et al., 1997). The yeast *CLU1* gene was synthesized by PCR from λPM-6114 using primers that inserted a *Kpn*I site just upstream of the translation start codon and a *Bam*HI site at the 3′ end of the gene (see Fig. 2B). The forward primer was 5′-GGTACCAGCGAGAAAAAAGAAGAAGTTA-3′, and the reverse primer was 5′-GGATCCATGCTTCTTTTTATTGTTGG-3′. The PCR product was then cloned in-frame into the *Kpn*I/*Bam*HI sites of the *Dictyostelium* expression vector pDXA-HC (Manstein et al., 1995). The *cluA*$^-$ *Dictyostelium* strain was co-transformed with the recombinant plasmid pDXA-*CLU1* and with pREP, a plasmid encoding a *trans*-acting gene product that is required for replication of the expression vector (Manstein et al., 1995). Transformants were selected for resistance to G418 (5 μg/ml) as previously described (Zhu et al., 1997).

Microscopy

Indirect immunofluorescence of mitochondria in fixed yeast cells was carried out according to the method of Hill et al. (1996) using a monoclonal antibody against yeast mitochondrial porin (Molecular Probes). Briefly, mutant and wild-type strains were grown overnight in YPG at 30°C to approximately 3×10^6 cells/ml. Formaldehyde (37%) was added directly to the culture to a final concentration of 4%. Cells were fixed for 2 hours at room temperature, spheroplasted, and allowed to adhere to polyethylenimine-coated coverslips. Spheroplasts on coverslips were pretreated in methanol (–20°C) for 6 minutes, rinsed with PBS (150 mM NaCl in 20 mM sodium phosphate buffer, pH 7.5), blocked with PBS containing 1% bovine serum albumin and 0.05% NP-40, and incubated with the anti-porin monoclonal antibody (12.5 μg/ml) for 1.5 hours at room temperature, followed by a 1 hour incubation at room temperature with CY3-conjugated anti-donkey IgG (Jackson Laboratories).

Fluorescence localization of mitochondria in living yeast cells was accomplished with the vital dye $DiOC_6$ according to the method of Koning et al. (1993), but with the following modifications. Overnight cultures were grown in YPG at 30°C to a density of $1\text{-}3\times10^6$/ml. Cells were washed once and resuspended in PBS, pH 7.5, so that the cell density was 1×10^7/ml. Ethanolic $DiOC_6$ was added to a final concentration of 0.1 μg/ml, and an aliquot of the cell suspension was immediately mixed with an equal volume of 4% low melt agarose (US Biochemical) at 42°C. 10 μl was placed on a glass slide, a coverslip applied, and the agarose allowed to solidify at 22°C for several minutes. Cells were viewed immediately with FITC cutoff filters. Complemented yeast cells were grown overnight in selective medium then switched to YPG for 3 hours before staining with $DiOC_6$. Mitochondria in *Dictyostelium* cells were visualized by staining with 4′,6-diamino-2-phenylindole (DAPI) as previously described (Zhu et al., 1997).

Assays for growth, sporulation, and other properties

Growth of the haploid *clu1Δ* and wild-type strains was compared in liquid YPD, YPG, YPE, and YPL media at 30°C. Cells from overnight cultures were counted and inoculated into 25 ml of prewarmed medium to a starting density of approximately 7×10^5 cells per ml and agitated at 300 rpm. Cultures were sampled every 90-120 minutes and culture density determined by hemacytometer or OD_{600} readings. Growth rate (k), expressed as divisions per hour, was determined with the equation $k=\log(N_2/N_1)(3.322/t)$, where N_1 is the initial cell density, N_2 is the ending cell density, and t is the interval (in hours) between collections.

Spore viability was determined by dissecting tetrads from a diploid homozygous deletion mutant grown on sporulation medium. Sensitivity to high osmotic conditions was tested by replica plating dissected tetrads to YPD containing either 0.9 M NaCl or 1.5 M sorbitol. Susceptibility to the lipophilic dye $DiOC_6$ was tested in liquid YPG containing concentrations of 0.2, 1.0, 2.5, and 5.0 μg/ml of the dye. An initial concentration of 1×10^6 cells was incubated overnight at 30°C, and the presence or absence of growth was noted. $DiOC_6$ sensitivity was also tested by replica-plating segregants to YPD plates containing 1, 5, or 10 μg/ml $DiOC_6$. Sensitivity to calcofluor was tested by replica-plating segregants to YPD containing 20 μg/ml calcofluor white.

To test susceptibility to heat shock, overnight cultures of SFY225B, SFY565D, MCY433 and SFY200 grown in YPD at 30°C were diluted to 2×10^6 cells/ml in fresh YPD. 1 ml aliquots of each diluted strain were transferred to 13 mm × 100 mm test tubes and placed at 30°, 42° or 50° for one hour. Heat-treated samples were diluted with sterile H_2O and plated to YPD. Percentage viability was relative to growth at 30°C.

Biochemical methods

SDS polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970) using gels containing 7.5% polyacrylamide. Each lane was loaded with the lysate from 1×10^6 *Dictyostelium* cells. Gels were either stained with Coomassie Brilliant Blue to visualize total protein, or proteins were transferred to nitrocellulose membrane for immunoblotting. The histidine-tagged Clu1p was stained with anti-(His)6-peroxidase-conjugated antibodies (Boehringer Mannheim) diluted to 125 mU/ml in PBS. Transfer conditions and visualization of peroxidase-conjugated antibodies were as described previously (Liu and Clarke, 1996).

RESULTS

CLU1 is not required for growth in *S. cerevisiae*

A search of the GenEMBL database detected a single open reading frame in the *S. cerevisiae* genome with significant homology to the *cluA* gene of *D. discoideum*. This open reading frame, now designated *CLU1*, is located on chromosome XIII, locus YM8270.16 *(Saccharomyces* Genome Database ORF YMR012w and GenBank accession number Z48613). Comparison of the predicted amino acid sequences of the *D. discoideum* and *S. cerevisiae* proteins showed 27% identity between these two proteins, with only scattered short stretches of highly conserved sequence (Fig. 1). As had been found for the *Dictyostelium* protein, the only homology between Clu1p and known proteins in the database was to a series of 42-residue imperfect repeats present in the kinesin light chain; these repeats are thought to function in protein-protein interaction (Gindhart and Goldstein, 1996).

Table 1. Genotypes of yeast strains used in this study

Strain	Genotype	Source
MCY387	*MAT**a**/MATα CLU1/CLU1 his4::HIS3/HIS4 ade2-101/ADE2 his3Δ200/his3Δ200 leu2/leu2 lys2/lys2 trp1Δ1/ trp1Δ1 ura3-52/ura3-52*	Conrad et al. (1997)
SFY200B	*MATα CLU1 his leu2 lys2 trp1Δ1 ura3-52*	Segregant from MCY387
SFY225A	*MAT**a** clu1::TRP1 ade2-101 his leu2 lys2 trp1Δ1 ura3-52*	Segregant from MCY387 transformant
SFY225B	*MATα clu1::TRP1 his leu2 lys2 trp1Δ1 ura3-52*	Segregant from MCY387 transformant
SFY290	*MAT**a**/MATα clu1::TRP1/clu1::TRP1 ADE2/ade2-101 his/his leu2/leu2 lys2/lys2 trp1Δ1/trp1Δ1 ura3-52/ura3-52*	SFY225A × SFY225B
MDD1	*MAT**a**/MATα CLU1/CLU1 ADE2/ade2 ADE5/ade5 his7-1/his7-2 leu1-c/leu1-d lys2-2/lys2-1 met13-c/met13-d trp1-63/ trp1-63 ura3-13/ura3-13 tyr1-2/tyr1-1*	Dresser et al. (1997)
MDY433	*MATα CLU1 ade5 his7 leu1-12 lys2-2 met13-d trp1-63 tyr1-1 ura3-13*	Dresser et al. (1997); used to construct MDD1
SFY562B	*MATα CLU1 his7 leu1 lys2 met13 trp1-63 tyr1 ura3-13*	Segregant from MDD1 transformant
SFY561A	*MATα clu1::TRP1 his7 leu1 lys2 met13 trp1-63 tyr1 ura3-13*	Segregant from MDD1 transformant
SFY563B	*MAT**a** clu1::TRP1 ade5 his7 leu1 lys2 met13 trp1-63 tyr1 ura3-13*	Segregant from MDD1 transformant
SFY565D	*MATα clu1::TRP1 ade5 his7 leu1 lys2 met13 trp1-63 tyr1 ura3-13*	Segregant from MDD1 transformant

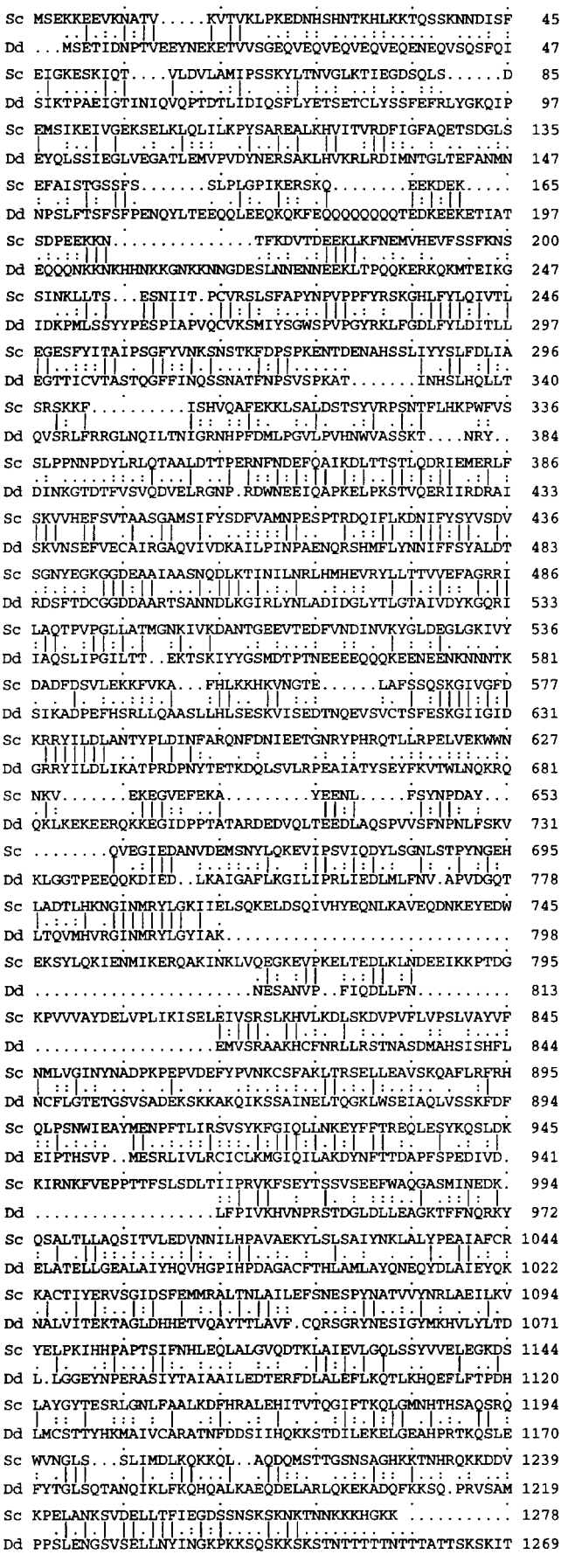

Fig. 1. Comparison of deduced amino acid sequences of proteins encoded by *S. cerevisiae CLU1* and *Dictyostelium cluA*. The figure was generated with the GAP program of the University of Wisconsin Genetics Computer Group package. The upper line shows the yeast sequence (Sc) and the lower line the *Dictyostelium* sequence (Dd). Vertical bars connecting the sequences mark identical residues, and dots indicate similar ones. Gaps introduced during the alignment are shown by dots in the sequence line. The default parameters of the GAP program calculated 27% identity and 50% similarity for the two proteins.

Using standard methods (see Materials and Methods) we disrupted the *CLU1* gene (*clu1::TRP1*) in two different diploid strains. Homologous recombination between the disruption cassette and the chromosomal *CLU1* gene was expected to result in deletion of all but 7 bases at the 5′-end and 66 bases at the 3′-end of *CLU1*, with insertion of the *TRP1* gene at the site of the deletion. Transformants were screened by PCR using the strategy shown in Fig. 2 to verify that this homologous recombination event had occurred, and a number of *clu1Δ* transformants were identified. Diploid transformants containing a disrupted copy of *CLU1* were sporulated. The tetrads produced four viable spores, 2 Trp+ and 2 Trp–. PCR analysis (Fig. 2) confirmed that the Trp+ spores were *clu1Δ*. All other relevant markers displayed normal segregation as well (data not shown). A homozygous diploid *clu1Δ* strain, SFY290, was generated by mating of appropriate haploid segregants, and the sporulation efficiency of this strain was determined. 95% of the spores from SFY290 were viable. The diploid parental strain, MCY387, displayed similar spore viability.

The growth properties of four haploid *clu1Δ* segregants (SFY225B, SFY561A, SFY563B, and SFY565D) and two *CLU1* segregants (SFY562B and SFY200B) were examined (Table 2). Growth of *clu1Δ* segregants resembled that of wild-type cells under all conditions tested. The growth rates of normal and mutant cells were similar using non-fermentable carbon sources (glycerol, lactate, or ethanol) as well as glucose. Even under extreme temperature conditions, the growth rate on different carbon sources remained unaffected by deletion of the *CLU1* gene. Deletion of *CLU1* also did not impair growth under conditions of high osmolarity, even at restrictive temperatures. Finally, viability after heat shock was similar for wild-type and mutant cells. Thus, within the limits of these assays, *clu1Δ* had no effects on normal growth.

Possible defects in the ability to synthesize the cell wall were tested by exposure of cells to calcofluor white (Ram et al., 1994). Neither mutant nor wild-type strains displayed sensitivity to concentrations of 20 μg/ml calcofluor in agar plates. Both mutant and wild-type strains also exhibited the same degree of susceptibility to the fluorescent lipophilic dye $DiOC_6$, which is accumulated by mitochondrial membranes.

CLU1 is not required for inheritance of functional mitochondria

The frequency of defects in mitochondrial inheritance was determined using the tetrazolium overlay method (Ogur et al., 1957; Newlon et al., 1979). Haploid wild-type and *clu1Δ* cells were spread on YPD plates with 5% glucose and incubated for 3 days at 30°C. Colonies were then overlaid with agar containing 0.1% 2,3,5-triphenyl tetrazolium chloride (TTC). In this colony-sectoring assay, cells with normal mitochondrial activity reduce

the TTC to a crimson product, while those cells lacking mitochondria or mitochondrial function ('petite' cells) do not show a color change. Petites were detected at a similar frequency in wild-type and mutant strains. Half-sectored colonies comprised less than 1% of the population in four wild-type strains and in three of the four *clu1Δ* haploid segregants that were tested. In the fourth mutant, SFY225B, nearly 4% of the colonies were half-sectored, presumably owing to the presence of some other marker carried by this segregant. Observation of mitochondria by fluorescence microscopy, as described in the next section, also indicated that mitochondria were present in buds.

CLU1 affects mitochondrial morphology

The most obvious difference between *clu1Δ* and wild-type cells was the morphology of their mitochondria, which was visualized by fluorescence microscopy using an antibody against the mitochondrial porin protein. Wild-type cells taken from log phase growth on YPG (density ~2×10^6 cells/ml) had a reticulated mitochondrial network that was dispersed around the cell periphery (Fig. 3A). A portion of this network was visible in all focal planes, but its branching structure was most clearly seen in the focal planes corresponding to the top and bottom of the cells. In contrast, *clu1Δ* cells possessed a more condensed mitochondrial mass, usually found at one side of the cell (Fig. 3B, most cells), and occasionally appearing as a ring (Fig. 3B, cell at far left). Fluorescence microscopy revealed that buds contained mitochondrial protein (Fig. 3B, arrows), indicating that the aggregated state of the mitochondrial network did not significantly impair its transmission to daughter cells. This finding is consistent with the tetrazolium overlay results and with the similar growth rates of mutant and wild-type cells on non-fermentable carbon sources.

Mitochondria were also visualized by staining living yeast cells with the mitochondrion-specific dye, DiOC6. Fig. 4 shows higher magnification views of representative wild-type (A,B) and mutant (C,D) cells stained with this vital dye. The mitochondrion of a typical mutant cell, with staining restricted to one side of the cell, is shown in C, while the less common ring-type staining, which probably represents an en face view, is shown in D. The ring-type staining of mutant cells was restricted to a single focal plane and was tightly compacted, distinguishing it from the open cortical reticulum of wild-type cells, which was visible at both the upper and lower cell surfaces. Observation of late log phase (5×10^7 cells/ml) and stationary phase *clu1Δ* cells indicated that the aggregated mitochondria became fragmented and dispersed under these growth conditions, as did the mitochondria of wild-type cells (not shown).

Transformation of *clu1Δ* yeast strains with a centromeric plasmid carrying the *CLU1* gene resulted in restoration of the normal mitochondrial network in log phase cells (Fig. 4E and F), while cells transformed with the empty vector remained mutant in appearance (not shown). Mitochondrial fragmentation during stationary phase also occurred normally in the complemented cells.

CLU1 can complement *cluA*$^-$ *Dictyostelium* cells

The *cluA*$^-$ *Dictyostelium* mutant (Zhu et al., 1997) was transformed with an expression vector, pDXA (Manstein et al., 1995), that carried either *S. cerevisiae CLU1* or *Dictyostelium cluA*. Total cell lysates from the two types of transformants were examined by polyacrylamide gel electrophoresis (Fig. 5). The pDXA-*CLU1* transformant (lane a) contained a new polypeptide of ~145 kDa, in agreement with the predicted size of Clu1p (predicted molecular mass, 145,164), while the pDXA-*cluA* transformant (lane c) contained a slightly larger polypeptide, confirmed by antibody staining (not shown) to correspond to the ~150 kDa product of the *Dictyostelium cluA* gene (predicted molecular mass, 148,916). In both cases, the polypeptides expressed in the pDXA transformants were more abundant than the endogenous polypeptide of wild-type cells, which was not detectable by Coomassie staining (lane b). Because an antibody to yeast Clu1p is not yet available, we verified the identity of the presumptive Clu1p polypeptide by staining an immunoblot with an antibody that recognizes the (His)6 tag added to Clu1p upon cloning *CLU1* into pDXA (lane d).

As previously shown (Zhu et al., 1997), *cluA*$^-$ *Dictyostelium* cells are commonly large and multinucleated,

Table 2. Comparison of the growth properties of *clu1Δ* and wild-type cells

Growth parameter	Result	Notes
Carbon source	No difference in utilization of acetate, glycerol, and glucose	Tested by replica-plating
Growth rate	YPD: wt = 1.2 hours; mt = 1.1 hours YPG: wt = 3.5 hours; mt = 3.6 hours YPL: wt = 2.6 hours; mt = 2.7 hours YPE: wt = 3.4 hours; mt = 3.6 hours	Doubling times not significantly different
Temperature sensitivity (17°C, 30°C, 37°C)	No temperature sensitivity on YPD or YPG	Tested by replica-plating
$DiOC_6$ sensitivity	Wild type and mutant sensitive to >0.2 μg/ml in YPG. Not sensitive to 10 μg/ml in YPD	Tested in liquid YPG or replica-plated on YPD
Calcofluor sensitivity	wt and mt strains not sensitive to 20 μg/ml	Tested by replica-plating
High osmolarity	wt and mt strains not sensitive to 0.9 M NaCl or 1.5 M sorbitol	Tested by replica-plating
Spore viability	95% viability for SFY290 and MCY387	SFY290 is a homozygous *clu1Δ* diploid
Heat shock viability	wt: 42ºC-55%, 50ºC<1% mt: 42ºC-58%, 50ºC<1%	Percentages based on cell viability at 30ºC
Petite formation (% half-sectored colonies)	wt (4 strains): <1% (*n*=4,332) mt (3 strains): <1% (*n*=1,730) mt (1 strain): 3.6% (*n*=2,058)	Tetrazolium overlay

Abbreviations: wt; wild type; mt, mutant; YPD, rich medium with dextrose; YPE, rich medium with ethanol; YPG, rich medium with glycerol; YPL, rich medium with lactic acid.

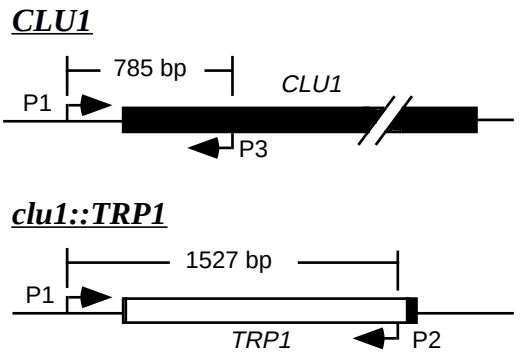

Fig. 2. Identification of strains with a clu1::TRP1 gene disruption. Diploid transformants were analyzed by PCR using genomic DNA as template with the indicated primers. Primer P1 is a sequence upstream of *CLU1* on the chromosome, and P3 is within *CLU1*. (P1 and P3 correspond to bases 2,5516-2,5541 and 2,6283-2,6301, respectively, of locus YM8270.16 in the Saccharomyces Genome Database.) Primer P2 is 5′-GCATCTGTGCGGTATTTCACACCG-3′, a sequence found in the pRS304 vector immediately flanking the 3′-end of the *TRP1* marker. For a wild-type copy of *CLU1*, primers P1 and P3 should yield a 785 bp PCR product. For a *clu1::TRP1* disrupted gene, primers P1 and P2 should yield a 1,527 bp product. Haploid segregants from diploid transformants that carried *clu1::TRP1* were tested with both sets of primers. Each segregant yielded only one of these products, and tetrads displayed 2:2 segregation.

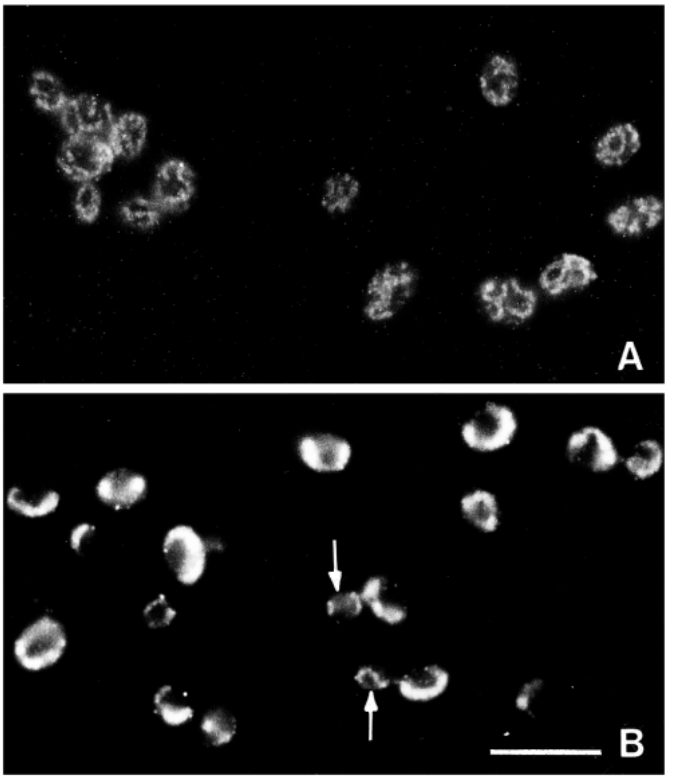

Fig. 3. Mitochondrial morphology of *CLU1* and *clu1Δ* cell populations. Cells were harvested from log phase growth on glycerol-containing medium (YPG). Mitochondria were visualized by staining fixed cells with anti-porin monoclonal antibodies followed by fluorescently-labeled secondary antibodies. Wild-type (*CLU1*) cells displayed normal branched mitochondrial morphology (A). No cells in the mutant population (B) had this appearance. Mitochondrial staining of mutant (*clu1Δ*) cells was commonly found at one side of the cell, or occasionally in a ring. The arrows mark budding cells, with mitochondria extending into the bud. Bar, 10 μm.

and their mitochondria are found in a single cluster. Transformation of *cluA*$^-$ cells with a plasmid expressing either *Dictyostelium cluA* or yeast *CLU1*, yielded transformants that were small and mostly mononucleated (Fig. 6), as are wild-type *Dictyostelium* cells. Thus, the *Dictyostelium* and yeast gene products were equally effective in correcting the cytokinesis defect of *cluA*$^-$ cells. However, the degree of mitochondrial dispersal in the complemented *Dictyostelium* cells depended on which protein was being expressed (Fig. 7). Those cells complemented with the *Dictyostelium cluA* protein resembled wild-type *Dictyostelium* cells in that the mitochondria were well-dispersed and had no sign of clustering. For cells expressing yeast Clu1p, the mitochondria were no longer tightly clumped, but were scattered throughout the cytoplasm as small, loose aggregates. These aggregates sometimes appeared to be linked by a single elongated mitochondrion or possibly a line of interconnected mitochondria. Because Clu1p only partially restored normal mitochondrial distribution in *cluA*$^-$ *Dictyostelium* cells, and because the phenotypic consequences of *CLU1* disruption in yeast cells were relatively subtle, we did not undertake the reciprocal complementation experiment, expression of *cluA* in *clu1Δ* yeast cells.

DISCUSSION

Consequences of *CLU1* deletion

In order to gain further insight into the function of *cluA* and its homologues, we have disrupted the *CLU1* gene of *S. cerevisiae*, which encodes a protein approximately 50% similar to the *Dictyostelium* protein in primary structure. As had been found in *Dictyostelium*, the lack of Clu1p had minimal consequences on cell growth. The *clu1Δ* yeast cells grew at normal rates on both glucose and non-fermentable carbon sources, did not manifest temperature sensitivity, and survived as well as wild-type cells exposure to stress conditions such as heat shock and osmotic shock. They did not exhibit elevated sensitivity to calcofluor white, a dye that binds to nascent chitin and glucan chains and interferes with cell wall synthesis; mutants with weakened cell walls are hypersensitive to this dye (Ram et al., 1994). Cell wall growth, like other aspects of morphogenesis, is affected by defects in the actin cytoskeleton (Cid et al., 1995). They were also no more sensitive than wild-type cells to $DiOC_6$. This lipophilic dye is accumulated by mitochondrial membranes in a manner dependent on the high *trans*-membrane potential of functional mitochondria (Johnson et al., 1981). High concentrations of the dye are toxic to cells growing on a non-fermentable carbon source such as glycerol (Koning et al., 1993). Thus, one might expect cells whose mitochondrial function had been compromised to be more sensitive to the dye; this was not the case for *clu1Δ* cells. In summary, a variety of assays detected no impairment of cell physiology in general and mitochondrial function in particular

as a consequence of the disruption of *CLU1*. Nonetheless, as in *Dictyostelium*, there were clear changes in the morphology of the mitochondrial network in *clu1Δ* cells.

Other mutations affecting mitochondrial morphology

As described in the Introduction, there are many types of mutations known to affect the distribution of mitochondria in *S. cerevisiae* (also see review by Yaffe, 1996). Two general classes of mutations have been found, those that affect proteins of mitochondrial membranes and those that affect cytoskeletal elements with which the mitochondria interact. The identifying phenotype of both types of mutations has been an impaired transmission or absence of mitochondria in the bud; commonly, mitochondrial morphology is also aberrant. Few mutations have been identified that alter the morphology of the mitochondrial reticulum without affecting mitochondrial inheritance. Other than *CLU1*, we are aware of only DNM1. It was recently reported that disruption of DNM1, which encodes a dynamin-like protein (Gammie et al., 1995), causes the mitochondrial network to collapse to a tubular morphology, but does not impair its function or inheritance (Shaw et al., 1997). The basis of this effect is not yet known. Although *CLU1* has no significant homology to DNM1, the similar phenotypes raise the intriguing possibility of a common pathway or a genetic interaction between the products of these genes.

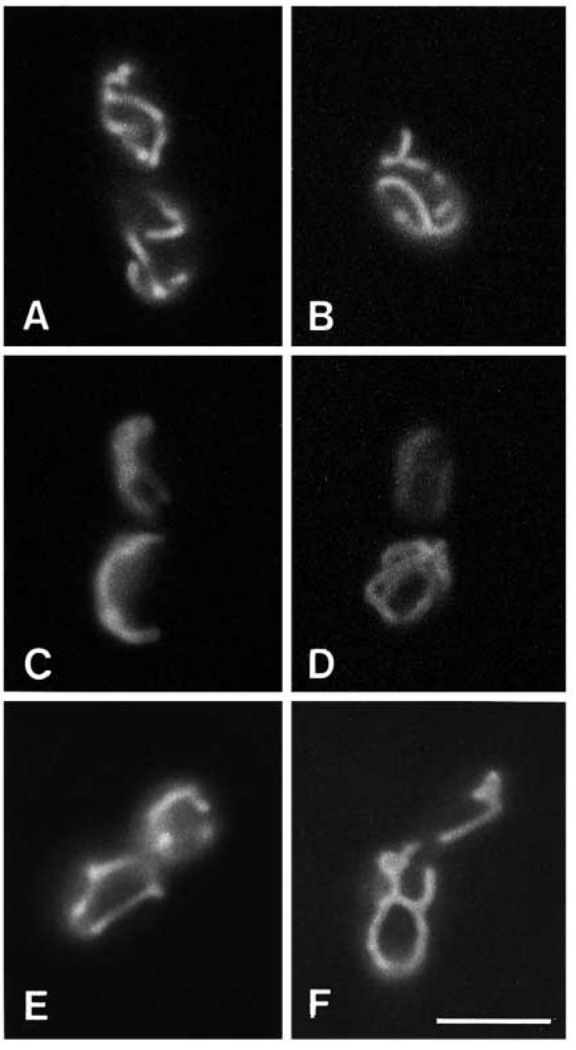

Fig. 4. Visualization of mitochondria in living *CLU1*, *clu1Δ*, and complemented *clu1Δ* yeast strains. Cells in log phase growth on YPG were stained with $DiOC_6$ and observed by standard fluorescence microscopy. (A-B) Wild-type *CLU1* yeast cells (SFY562B). (C-D) *clu1Δ* yeast cells (SFY561A). (E-F) *clu1Δ* cells (SFY561A) complemented with YCp*CLU1*. Bar, 5 μm.

Complementation analysis

Complementation studies have demonstrated that *CLU1* encodes a functional homologue of the *Dictyostelium cluA* gene. This homology has interesting implications, especially in light of the many differences between these two organisms. *Dictyostelium* cells are highly motile amoebae, while yeast cells, encased by a cell wall, are nonmotile. *Dictyostelium* cells contain an extensive array of cytoplasmic microtubules, and, although this has not been directly demonstrated, it is likely that these function in organelle transport much as they do in mammalian cells (see Introduction). In yeast cells, microtubules are not required for transport of mitochondria into buds (Huffaker et al., 1988). The mitochondria in growing *Dictyostelium* cells are in the form of dozens of small, individual units distributed throughout the cytoplasm. In *S. cerevisiae*, exponentially growing cells possess a reticular, essentially continuous mitochondrial structure that surrounds the cell just beneath the plasma membrane; the mitochondrial mass is greater when cells are grown on a non-fermentable

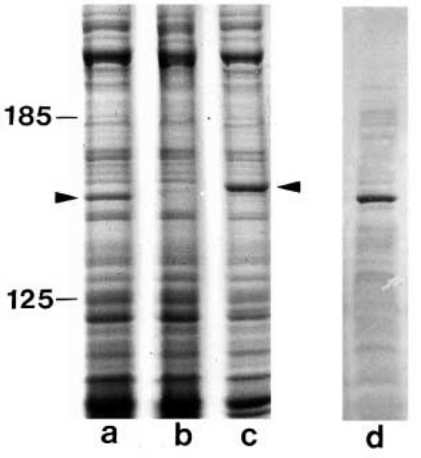

Fig. 5. SDS polyacrylamide gel showing expression in *cluA*$^-$ *Dictyostelium* cells of proteins encoded by plasmid-borne copies of *S. cerevisiae CLU1* and *Dictyostelium cluA*. Total cell lysates from 1×10^6 cells were denatured and electrophoresed on an SDS gel containing 7.5% polyacrylamide; proteins in the first three lanes were visualized by staining with Coomassie Brilliant Blue. The cells were (a) *cluA*$^-$ cells transformed with pDXA-*CLU1*, (b) *cluA*$^-$ cells, (c) *cluA*$^-$ cells transformed with pDM2 (= pDXA-*cluA*). The positions of the prestained molecular mass markers (Life Technologies) and their apparent size in kDa are indicated to the left, and the new polypeptides observed in lanes a and c are marked with arrowheads. Lane d, an immunoblot of pDXA-*CLU1* cells stained with peroxidase-conjugated antibody to (His)6. The antibody stained a single polypeptide, which co-migrated with the band marked in a, confirming its identity as Clu1p.

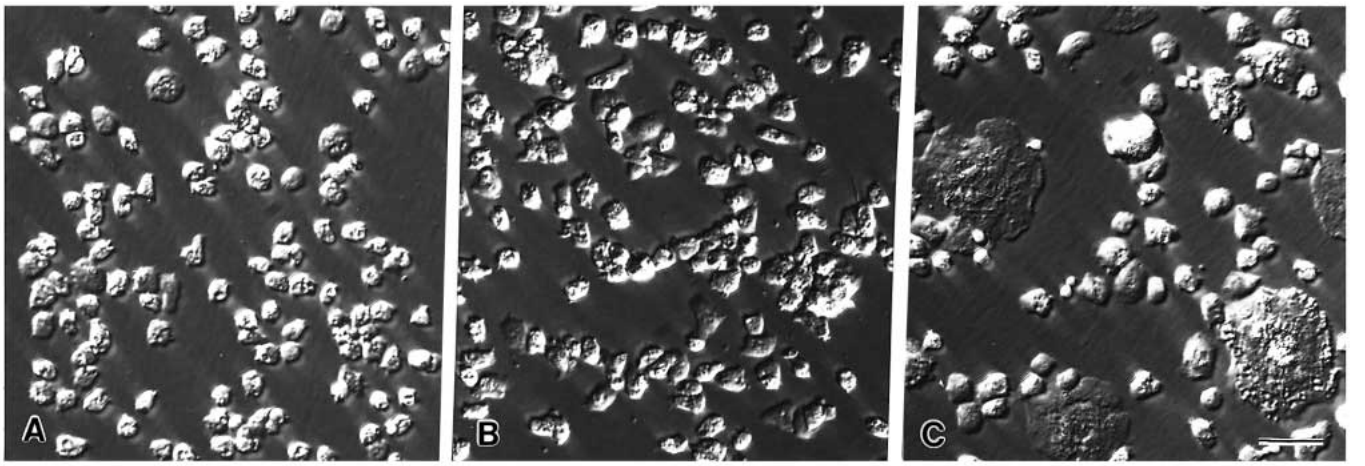

Fig. 6. Appearance of *cluA*$^-$ and complemented *Dictyostelium* cells growing on a surface. Living cells growing on tissue culture plates in HL5 were viewed with Hoffman Modulation Contrast optics. (A) *cluA*$^-$ cells complemented with the yeast *CLU1* gene. (B) *cluA*$^-$ cells complemented with the *Dictyostelium cluA* gene. (C) *cluA*$^-$ cells. Note the presence of giant cells in the *cluA*$^-$ population, and the uniformly small size of cells in both of the complemented populations. Bar, 20 μm.

carbon source (Hoffman and Avers, 1973; Stevens, 1981). The branching interconnections of the mitochondrial reticulum are continually being rearranged by ongoing fusion and fission (Nunnari et al., 1997).

In spite of these substantial differences between the two cell types, disruption of *cluA* in *Dictyostelium* or *CLU1* in yeast produces a similar phenotype, clustering of mitochondria on the one hand, and compression of the mitochondrial mass on the other. In *Dictyostelium cluA*$^-$ cells, the dozens of individual mitochondria became collected into a single aggregate. In yeast *clu1Δ* cells, the reticular mitochondrion became condensed to one side of the cell. Importantly, transformation of *cluA*$^-$ *Dictyostelium* cells with yeast *CLU1* resulted in complementation of the phenotypic defects in the *Dictyostelium* mutant, indicating that the two gene products serve similar functions in their respective organisms. This result suggests that the aspect of mitochondrial structure or interaction with other cellular components that depends on *cluA*/*CLU1* is common to both cell types.

Remarkably, complementation extended to an area in which the effect of the mutations differs in *Dictyostelium* and yeast cells. The *cluA*$^-$ *Dictyostelium* cells are impaired in cytokinesis, yielding a population that contains many giant, multinucleated cells (Fig. 6C; also Zhu et al., 1997). No comparable defect was exhibited by yeast *clu1Δ* cells, which grew normally and were able to separate the bud from the mother cell. Nonetheless, expression of yeast Clu1p in *cluA*$^-$ *Dictyostelium* cells restored normal cytokinesis. This result is consistent with the possibility that the problem in cytokinesis in *Dictyostelium* is indirect, i.e. the contractile ring may sometimes be physically obstructed by the large mass of mitochondria, blocking completion of cleavage. If this were the case, dispersal of the mitochondrial aggregate would suffice to correct the problem.

In *cluA*$^-$ *Dictyostelium* cells complemented with the *Dictyostelium* protein, the mitchondria were completely dispersed, as in wild-type cells. However, in cells complemented with yeast Clu1p, the dispersal was less complete, and the cells often contained loose clusters of mitochondria scattered throughout the cytoplasm. In both types of transformants, the gene carried by pDXA was overexpressed, and the protein accumulated to higher levels than found in wild-type cells. For the *Dictyostelium* protein, we have monitored protein levels on a cell-by-cell basis, either by staining fixed cells with a monoclonal antibody specific for the *cluA* protein (Zhu et al., 1997), or by observing living cells expressing a fusion of this protein with green fluorescent protein (unpublished data). Both methods have shown that there is great cell-to-cell variation (even in clonal populations) in the level of the *cluA* protein expressed from pDXA, and both methods have revealed a normal dispersed pattern of mitochondria in cells whose staining intensities ranged from undetectable (as is true of wild-type cells) to extremely bright. Those rare cells with clustered or only partially dispersed mitochondria displayed no detectable staining, arguing that such phenotypes resulted from inadequate levels of the *cluA* protein. Thus, the incomplete dispersal observed in *cluA*$^-$ cells expressing high levels of yeast Clu1p is unlikely to be a consequence of overabundance of the yeast protein; instead, this result suggests that the yeast protein acts somewhat less effectively than the *Dictyostelium* protein in *Dictyostelium* cells. This result also raises an interesting possibility, to be explored in future ultrastructural studies, that the mitochondria in *Dictyostelium* cells expressing Clu1p may be interconnected, with a pattern of organization intermediate between that of *Dictyostelium* and that of yeast cells.

Partitioning of mitochondria at cell division

In general, cells use one of two mechanisms, stochastic or ordered, for distributing mitochondria at cell division (Warren and Wickner, 1996). For a cell that contains a large number of well-dispersed mitochondria, cleavage into two equally-sized daughter cells is sufficient to ensure that each daughter receives an approximately equal complement of mitochondria. This is normally the case for *Dictyostelium* amoebae. However, in

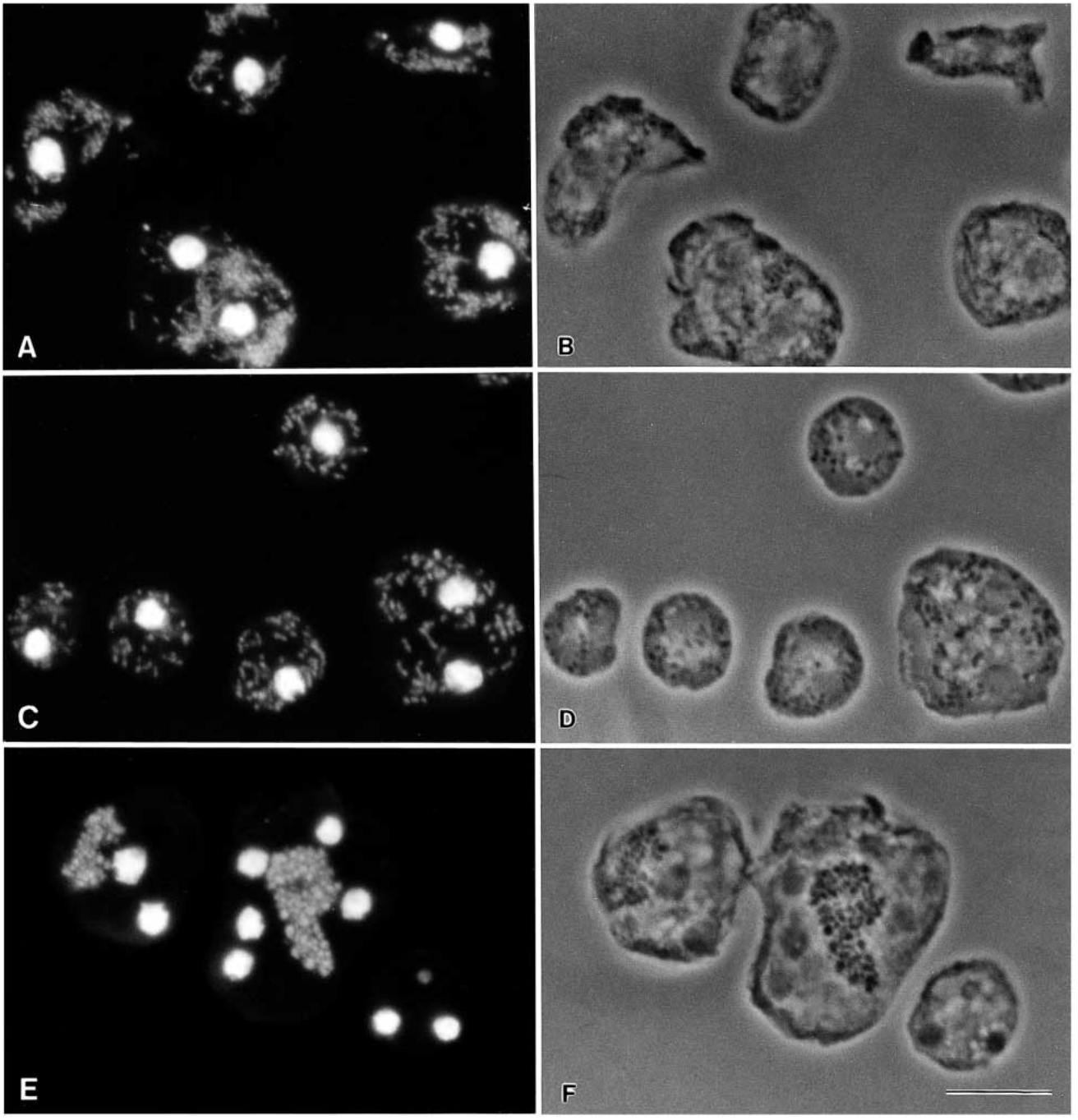

Fig. 7. Distribution of mitochondria in mutant and complemented *Dictyostelium* cells. *Dictyostelium* were fixed and stained with DAPI, which stains the DNA of both mitochondria and nuclei. Fluorescence and phase contrast images are shown. (A,B) *cluA*$^-$ cells complemented with yeast *CLU1* gene. (C,D) *cluA*$^-$ cells complemented with *Dictyostelium cluA* gene. (E,F) *cluA*$^-$ cells. In E, note the presence of multiple nuclei in the largest *cluA*$^-$ cell and the paucity of mitochondria in the smallest one. Bar, 10 μm.

cluA$^-$ *Dictyostelium* cells, the stochastic mechanism fails because the mitochondria are clustered into a single aggregate, and the cleavage plane (even when cleavage is successful) may not divide this aggregate into similar portions. Some daughters receive few (Fig. 6E) or no mitochondria, and others an overabundance. A cell that receives no mitochondria is probably not viable, but less extreme imbalances are presumably corrected during the cell cycle by the normal, albeit unidentified, regulatory mechanisms.

S. cerevisiae employs an ordered mechanism to ensure that the daughter cells receive an appropriate complement of mitochondria. A portion of the mitochondrial reticulum is actively transported into the bud, where it moves rapidly back and forth through the bud neck until cleavage occurs (Stevens,

1981). This process appears to remain intact in *clu1Δ* cells, since mitochondrial inheritance is not impaired. (The dynamics of mitochondrial movement have not yet been analyzed to determine whether kinetic differences exist between mutant and wild-type cells.) An implication of the normal transmission of mitochondria to buds is that the association of the yeast mitochondrion with components required for its movement, such as cytoskeletal elements and motor proteins, is still functional in the mutant. This hypothesis can be tested by in vitro assays of mitochondrial movement (Simon et al., 1995) and by time lapse observation of the behavior of mitochondria in living cells (see Nunnari et al., 1997).

In conclusion, although we still do not understand the mechanism of action of the proteins encoded by *cluA* and *CLU1*, a comparison of the effects of gene disruption in *Dictyostelium* and yeast cells and of the properties of *cluA*$^-$ *Dictyostelium* cells complemented with these two proteins, have suggested directions for future research. In addition, the present study has shown that the *Dictyostelium* and yeast proteins, while only 50% similar in primary structure, are functional homologues. Searches of databases containing expressed sequences have detected possible homologues of this protein in organisms ranging from insects to mammals. The conservation of function between *Dictyostelium* and yeast (in spite of their differences in mitochondrial structure and transport mechanisms) and the apparent presence of this protein in a diversity of species, argue that this protein family will be found to play an important and similar role in all eukaryotes.

We thank Dr Michael Dresser for yeast strains, reagents, and invaluable discussions. This work was supported by grants from the National Science Foundation to M.C. (MCB-9317286 and 9728403) and the Oklahoma Center for the Advancement of Science and Technology to S.D.F. (H97-069).

REFERENCES

Ball, E. H. and Singer, S. J. (1982). Mitochondria are associated with microtubules and not with intermediate filaments in cultured fibroblasts. *Proc. Nat. Acad. Sci. USA* **79**, 123-126.

Baudin, A., Ozier-Kalogeropoulos, O., Denoul, A., Lacroute, F. and Cullin, C. (1993). A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucl. Acids Res.* **21**, 3329-3330.

Berger, K. H., Sogo, L. F. and Yaffe, M. P. (1997). Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol.* **136**, 545-553.

Burgess, S. M., Delannoy, M. and Jensen, R. E. (1994). *MMM1* encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol.* **126**, 1375-1391.

Cid, V. J., Duran, A., del Rey, F., Snyder, M. P., Nombela, C. and Sanchez, M. (1995). Molecular basis of cell intergrity and morphogenesis in *Saccharomyces cerevisiae*. *Microbiol. Rev.* **59**, 345-386.

Conrad, M. N., Dominguez, A. M. and Dresser, M. E. (1997). Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. *Science* **276**, 1252-1255.

Couchman, J. R. and Rees, D. A. (1982). Organelle-cytoskeleton relationships in fibroblasts: mitochondria, golgi apparatus and endoplasmic reticulum in phases of movement and growth. *Eur. J. Cell Biol.* **27**, 47-54.

David-Ferreira, K. L. and David-Ferreira, J. F. (1980). Association between intermediate-sized filaments and mitochondria in rat Leydig cells. *Cell Biol. Int. Rep.* **4**, 655-662.

Dresser, M. E., Ewing, D. J., Conrad, M. N., Dominguez, A. M., Barstead, R., Jiang, H. and Kodadek, T. (1997). *DMC1* functions in a *Saccharomyces cerevisiae* meiotic pathway that is largely independent of the RAD51 pathway. *Genetics* **147**, 533-544.

Drubin D. G., Jones, H. D. and Wertman, K. F. (1993). Actin structure and function: Roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. *Mol. Biol. Cell* **4**, 1277-1294.

Fisk, H. A. and Yaffe, M. P. (1997). Mutational analysis of Mdm1p function in nuclear and mitochondrial inheritance. *J. Cell Biol.* **138**, 485-494.

Gammie, A. E., Kuirhar, L. J., Vallee, R. B. and Rose, M. D. (1995). *DNM1*, a dynamin-related protein, participates in endosomal trafficking in yeast. *J. Cell Biol.* **130**, 553-566.

Gbelska, Y., Subic, J., Svoboda, A., Goffeau, A. and Kavac, L. (1983). Intramitochondrial ATP and cell functions: yeast cells depleted of intramitochondrial ATP lose the ability to grow and multiply. *Eur. J. Biochem.* **150**, 281-286.

Gietz, R. and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. *Gene* **74**, 527-534.

Gindhart, J. G. and Goldstein, L. S. B. (1996). Tetratrico peptide repeats are present in the kinesin light chain. *Trends Biochem. Sci.* **21**, 52-54.

Goewert, R. R., Sippel, C. J., Grimm, M. F. and Olson, R. E. (1981). Identification of 3-methoxy-4-hydroxy-5-hexaprenylbenzoic acid as a new intermediate in ubiquinone biosynthesis by *Saccharomyces cerevisiae*. *Biochemistry* **20**, 5611-5616.

Guan, K., Farh, L., Marshall, T. K. and Deschenes, R. J. (1993). Normal mitochondrial structure and genome maintenance in yeast requires the dynamin-like product of the *MGM1* gene. *Curr. Genet.* **24**, 141-148.

Heggeness, M. H., Simon, M. and Singer, S. J. (1978). Association of mitochondria with microtubules in cultured cells. *Proc. Nat. Acad. Sci. USA* **75**, 3863-3866.

Hermann, G. J., King, E. J. and Shaw, J. M. (1997). The yeast gene, *MDM20*, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. *J. Cell Biol.* **137**, 141-153.

Hill, K. L., Catlett, N. L. and Weisman, L. S. (1996). Actin and myosin function in directed cell movement during cell division in *S. cerevisiae*. *J. Cell Biol.* **135**, 1535-1549.

Hirokawa, N. (1996). Organelle transport along microtubules – the role of KIFs. *Trends Cell Biol.* **6**, 135-141.

Hoffman, H. P. and Avers, C. J. (1973). Mitochondrion of yeast: ultrastructural evidence for one giant, branched organelle per cell. *Science* **181**, 749-751.

Huffaker, J. C., Thomas, J. H. and Botstein, D. (1988). Diverse effects of β-tubulin mutations on microtubule formation and function. *J. Cell Biol.* **106**, 1997-2010.

Johnson, L. V., Walsh, M. L. and Chen, L. B. (1981). Monitoring of relative membrane potential in living cells by fluorescence microscopy. *J. Cell Biol.* **88**, 526-535.

Jones, B. A. and Fangman, W. L. (1992). Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. *Genes Dev.* **6**, 380-389.

Koning, A. J., Lum, P. Y., Williams, J. M. and Wright, R. (1993). $DiOC_6$ staining reveals organelle structure and dynamics in living yeast cells. *Cell Motil. Cytoskel.* **25**, 111-128.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lazzarino, D. A., Boldogh, I., Smith, M. G., Rosand, J. and Pon, L. A. (1994). Yeast mitochondria contain ATP-sensitive, reversible actin-binding activity. *Mol. Biol. Cell* **5**, 807-818.

Liu, T. and Clarke, M. (1996). The vacuolar proton pump of *Dictyostelium discoideum*: Molecular cloning and analysis of the 100 kDa subunit. *J. Cell Sci.* **109**, 1041-1051.

Manstein, D. J., Schuster, H. P., Morandini, P. and Hunt, D. M. (1995). Cloning vectors for the production of proteins in *Dictyostelium discoideum*. *Gene* **162**, 129-134.

McConnell, S. J., Stewart, L. C., Talin, A. and Yaffe, M. P. (1990). Temperature-sensitive yeast mutants defective in mitochondrial inheritance. *J. Cell Biol.* **111**, 967-976.

McConnell, S. J. and Yaffe, M. P. (1992). Nuclear and mitochondrial inheritance in yeast depends on novel cytoplasmic structures defined by the *MDM1* protein. *J. Cell Biol*. **118**, 385-395.

Morris, R. L. and Hollenbeck, P. J. (1995). Axonal transport of mitochondria along microtubules and F-actin in living vertebrate neurons. *J. Cell Biol.* **131**, 1315-1326.

Mose-Larsen, P., Bravo, R., Fey, S. J., Small. J. V. and Celis, J. E. (1982).

Putative association of mitochondria with a subpopulation of intermediate-sized filaments in cultured human skin fibroblasts. *Cell* **31**, 681-692.

Nangaku, M., Sato-Yoshitake, R., Okada, Y., Noda, Y., Takemura, R., Yamazaki, H. and Hirokawa, N. (1994). KIF1B, a novel microtubule plus end-directed monomeric motor protein for transport of mitochondria. *Cell* **79**, 1209-1220.

Newlon, C. S., Ludescher, R. D. and Walter, S. K. (1979). Production of petites by cell cycle mutants of *Saccharomyces cerevisiae* defective in DNA synthesis. *Mol. Gen. Genet.* **169**, 189-194.

Nunnari, J., Marshall, W. F., Straight, A., Murray, A., Sedat, J. W. and Walter, P. (1997). Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. *Mol. Biol. Cell* **8**, 1233-1242.

Ogur, M., St John, R. and Nagai, S. (1957). Tetrazolium overlay technique for population studies of respiration deficiency in yeast. *Science* **125**, 928-929.

Pereira, A. J., Dalby, B., Stewart, R. J., Doxsey, S. J. and Soldstein, L. S. B. (1997). Mitochondrial association of a plus end-directed microtubule motor expressed during mitosis in *Drosophila*. *J. Cell Biol.* **136**, 1081-1090.

Ram, A. F. J., Wolters, A., Hoopen, R. T. and Klis, F. M. (1994). A new approach for isolating cell wall mutants in *Saccharomyces cerevisiae* by screening for hypersensitivity to calcofluor white. *Yeast* **10**, 1019-1030.

Rose, M. D., Winston, F. and Hieter, P. (1990*). Methods in Yeast Genetics: A Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shaw, J. M., Otsuga, D., Keegan, B., Hermann, G. and Bleazard, W. (1997). The dynamin-like GTPase, Dnm1p, is required for maintenance of yeast mitochondrial network morphology. *Mol. Biol. Cell* **8S**, 425a.

Sherman, F. (1991). Getting started with yeast. *Meth. Enzymol.* **194**, 3-21.

Shimizu, I., Nagai, J., Hatanaka, H. and Katsuki, H. (1973). Mevalonate synthesis in the mitochondria of yeast. *Biochim. Biophys. Acta* **296**, 310-320.

Sikorski, R. S. and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19-27.

Simon, V. R., Swayne, T. C. and Pon, L. A. (1995). Actin-dependent mitochondrial motility in mitotic yeast and cell-free systems: Identification of a motor activity on the mitochondrial surface. *J. Cell Biol.* **130**, 345-354.

Simon, V. R., Karmon, S. L. and Pon, L. A. (1997). Mitochondrial inheritance: Cell cycle and actin cable dependence of polarized mitochondrial movements in *Saccharomyces cerevisiae*. *Cell Motil. Cytoskel.* **37**, 199-210.

Sogo, L. F. and Yaffe, M. P. (1994). Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* **126**, 1361-1373.

Stevens, B. (1981). Mitochondrial structure. In *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance* (ed. J. N. Strathern, E. W. Jones and J. R. Broach), pp. 471-504. Cold Spring Harbor Laboratory, New York.

Stewart, L. C. and Yaffe. M. P. (1991). A role for unsaturated fatty acids in mitochondrial movement and inheritance. *J. Cell Biol.* **115**, 1249-1257.

Summerhayes, I. C., Wong, D. and Chen, L. B. (1983). Effect of microtubules and intermediate filaments on mitochondrial distribution. *J. Cell Sci.* **61**, 87-105.

Warren, G. and Wickner, W. (1996). Organelle inheritance. *Cell* **84**, 395-400.

Yaffe, M. P. and Schatz, G. (1984). Two nuclear mutations that block mitochondrial protein import in yeast. *Proc. Nat. Acad. Sci. USA* **81**, 4819-4823.

Yaffe, M. P. (1996). The division and inheritance of mitochondria. *Advan. Mol. Cell Biol.* **17**, 341-350.

Yaffe, M. P., Harata, D., Verde, F., Eddison, M., Takashi, T. and Nurse, P. (1996). Microtubules mediate mitochondrial distribution in fission yeast. *Proc. Nat. Acad. Sci. USA* **93**, 11664-11668.

Zelikson, R. and Luzzati, M. (1977). Mitochondrial and cytoplasmic distribution in *Saccharomyces cerevisiae* of enzymes involved in folate-coenzyme-mediated one-carbon-group transfer. *Eur. J. Biochem.* **79**, 285-292.

Zhu, Q., Hulen, D., Liu, T. and Clarke, M. (1997) The *cluA*$^-$ mutant of *Dictyostelium* identifies a novel class of genes required for dispersion of mitochondria. *Proc. Nat. Acad. Sci. USA* **94**, 7308-7313.