Specific Aims

Mitochondria-actin association is necessary for transmission of mitochondria to daughter cells during budding. Mitochondrial distribution and morphology (mdm) and mitochondrial morphology maintenance (mmm) mutants in *S. cerevisiae* fail to transmit mitochondria to daughter cells and demonstrate abnormal mitochondria morphology at non-permissive temperatures. Two proteins, Mdm10p and Mmm1p, have been identified as integral proteins of the outer mitochondrial membrane that interact with one or more peripheral mitochondrial actin-binding protein(s) (mABP). Mitochondria in *mdm10*Δ and *mmm1*Δ cells do not associate with actin cables as mitochondria in wild-type cells do, and subsequently fail to transmit mitochondria during budding. Recently, a new gene, MDM12, has been identified through genetic screens. Mdm12p is a 31 kDa transmembrane protein of the outer mitochondrial membrane. I propose Mdm12p is required for mABP docking, which is necessary for the proper binding of .

<u>Specific Aim 1:</u>

I will investigate the organization of mitochondria and actin in wild-type and *mdm12p*Δ cells in order to see if they co-localize. I will compare these results to mitochondria-actin association in *mdm12p*Δ cells with an Mdm12p expressing plasmid. I will also investigate the requirement of Mdm12p for association of mABP to the mitochondrial membrane. Finally, I will then determine if the mobility of mitochondria in *mdm12p*Δ cells is hampered, as in *mdm10*Δ cells.

<u>Specific Aim 2:</u>

I will investigate the association of Mdm12p with other factors using the two-hybrid system and . This may lead to the identification and characterization of novel proteins of the outer mitochondrial membrane, as well as one or more mitochondrial actin-binding proteins (mABP).

Background

Importance of Mitochondrial Inheritance

Mitochondria are essential organelles. Their primary function is oxidative phosphorylation, the production of ATP by five enzymatic complexes located in the inner mitochondrial membrane. They are also involved in a number of other cellular processes, such as fatty acid oxidation, the urea cycle, and heme synthesis. Mitochondria are also unique because they contain their own genome, mtDNA, a closed, circular molecule. mtDNA is essential to human cells because it encodes 13 of the approximately 100 subunits of the OxPhos complexes. It has also been shown that point mutations or deletions in mtDNA can cause serious diseases, involving cardiomyopathy, skeletal muscle dysfunction, deafness, and blindness [18-20]. Also, mitochondrial dysfunction in general has been implicated in the aging process and common neurodegenerative diseases such as Parkinson's disease [21 and 22].

Mitochondria, and therefore mtDNA molecules, are maternally inherited. An interesting feature of people affected by mitochondrial disease is the heteroplasmic nature of their mtDNA. A person must carry normal mtDNA as well as the mutant form of mtDNA in order to survive. The degree of heteroplasmy (amount of normal mtDNA vs. mutant mtDNA) can differ dramatically between mother and offspring, siblings, and even among tissues of a single affected person. Presumably, this phenomenon is caused by the inheritance of different mitochondria (and therefore mtDNA) that each egg cell receives from the mother. Very little is known about the mechanism by which mitochondria segregate in humans during cell division. Progress made toward discovering the secrets of mitochondrial inheritance could not only enhance our understanding of mitochondrial disease states, but also contribute to the important field of organelle inheritance in general. Using *S. cerevisiae* as a model system has allowed rapid advances to be made in the study of mitochondrial inheritance due to the ease of genetic screening. This grant focuses specifically on the mitochondria-cytoskeleton interactions that control mitochondria motility and segregation during budding in yeast.

Role of actin in mitochondrial inheritance

Asymmetric growth of the developing bud or daughter cell characterizes the process of cell division in yeast. Since organelles cannot be synthesized de novo, there must be mechanisms by which organelles are moved into the developing bud for assurance of a viable daughter cell. Inheritance of mitochondria in *S. cerevisiae* is a series of four cell-cycle dependent events: mitochondria (1) are polarized along the mother-bud axis towards the site of bud emergence in G1 phase; (2) move in a linear fashion to the developing bud during S phase; (3) become immobilized in the bud tip and accumulate there during S and G2 phases; (4) are released from the bud tip and redistribute throughout the bud during M phase [3].

This process is dependent on the actin cytoskeleton. In yeast, the actin cytoskeleton consists of two structures, actin patches and actin cables. Actin patches are invaginations in the plasma membrane enriched in F-actin (polymerized actin). Actin cables are collections of actin filaments that extend from the mother cell into the bud. Mitochondria have been shown to localize to actin cables in vivo [2, 10, and 11]. Also, studies have shown that disruption of actin cable polarization or stabilization by either

chemical or genetic means results in a complete lack of mitochondrial motility and defective mitochondrial inheritance [2, 4,10, and 11]. Additionally, in vitro studies indicate yeast mitochondria bind to the lateral surface of actin filaments. A protein or proteins on the mitochondrial surface is responsible for this binding activity, which is ATP-sensitive, reversible, and saturable [2 and 10]. Finally, a protein-based motor activity has been localized to the surface of yeast mitochondria, whose activity is ATP-driven and actin-dependent [4]. This motor activity displayed sensitivity to ATP concentrations in a manner similar to that of the myosin family of proteins; however, this motor activity was not encoded exclusively by any of the known myosin genes [4].

Collectivity, these findings support the model that mitochondria are moved along actin cables by a myosin-like motor activity in a polarized manner to the developing bud. Little is known, however, about the proteins or other factors that mediate the association of mitochondria to actin cables or to the motor itself. The projects proposed in this grant are designed to provide details about the mitochondrial-actin association in *S. cerevisiae*.

MDM and MMM genes and mitochondrial inheritance

Genetic screens have identified over thirty genes, called MDM (mitochondrial distribution and morphology) and MMM (maintenance of mitochondrial morphology), that are essential for mitochondrial inheritance [5, 6, 9, and 12]. At non-permissive temperatures, these mutants fail to distribute mitochondria to daughter cells. Only a handful of proteins encoded by mdm and mmm genes have been characterized. Mdm1p is an intermediate filament-like protein localized to punctate structures throughout the cytoplasm of the yeast cell, but its exact function with regard to mitochondrial inheritance is unknown [6-8]. Mdm20p is necessary for the stabilization of actin cables [13].

Mmm1p and Mdm10p are integral mitochondrial outer membrane proteins first identified by Burgess et al [12] and Sogo and Yaffe [9], respectively. Deletion of each of these genes produces a temperature-sensitive phenotype of giant spherical mitochondria and a high number of buds without mitochondria, demonstrating defective mitochondrial inheritance. Extensive characterization of the functions of these proteins was performed by Boldogh et al [2]. Specifically, they determined that two essential elements are necessary for actin-mitochondria interactions. (Figure 1) First, they found an actin binding activity they termed mitochondrial actin binding protein(s) (mABP), which is a peripheral protein(s) associated with the outer mitochondrial membrane that exhibits ATP-sensitive actin binding activity [2]. Second, they found integral outer mitochondrial membrane protein(s), i.e., Mmm1p and Mdm10p, were required for docking of mABP on the mitochondria [2]. However, the identity of mABP is still unknown, as is the exact mechanism by which Mmm1p and Mdm10p function in mitochondria-actin association.

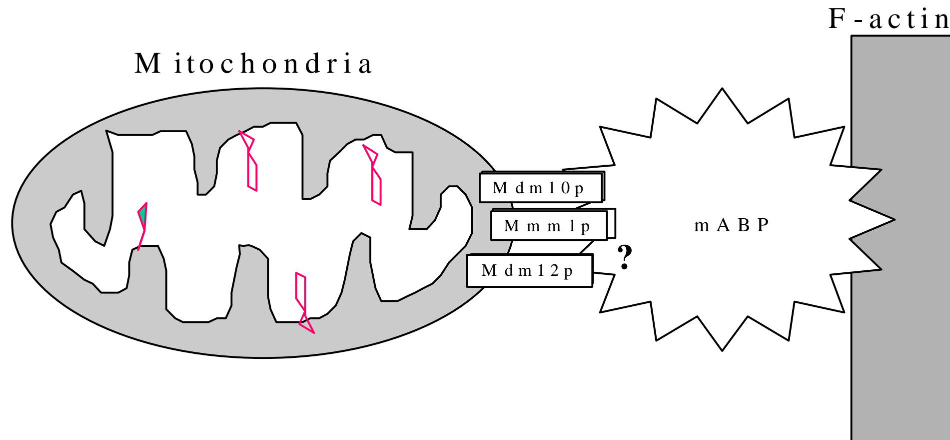

Figure 1. Model for mitochondrial binding to actin. The component(s) of mABP and the function of Mdm12p are unknown, and are the questions this grant is designed to answer.

Another gene, MDM12, has recently been identified by our lab through genetic screens [1]. MDM12 encodes a 31 kDa protein located in the outer mitochondrial membrane with a putative transmembrane domain of amino acids 23-42. Deletion of MDM12, or production of a mutant Mdm12p (termed Mdm12p-1) produces giant mitochondria and leads to defects in mitochondrial inheritance; a phenotype identical to that of *mmm1**D*** and *mdm10**D***[1, 9, and 12]. The similarity of the *mdm12**D*** strain to *mmm1**D*** and *mdm10**D*** strains suggest that Mdm12p may also function as a mediator of mABP docking, which is required for binding of mitochondria to actin.

The projects proposed in this grant are designed to investigate the role of Mdm12p in mitochondrial inheritance in yeast. Studies of Mdm12p and other *mdm* and *mmm* mutants, as well as identification of mitochondrial actin binding proteins (mABP), will fill in large gaps of knowledge in the field of mitochondrial inheritance. A greater understanding of these processes is essential before the molecular basis of segregation of mitochondria in human cells can be determined and studied effectively.

Preliminary Data

This data is taken (sometimes verbatim) from: Berger KH, LF Sogo, and MP Yaffe. 1997. Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol*. 136:545-553.

The MDM12 gene was first identified in our lab by screening temperature-sensitive strains of yeast for defective mitochondrial partitioning to buds when stained by the mitochondrial-specific dye DASPMI [5 and 9]. When viewed by fluorescence microscopy, wild-type mitochondria are tubular structures scattered throughout the cell, while mutant mdm12-1 cells exhibit one or two large round mitochondria, as is characteristic of mdm and mmm mutants (Figure 2).

We were able to clone the MDM12 gene by complementation analysis of the temperature-sensitive growth defect from a wild-type *S. cerevisiae* genomic DNA library. The MDM12 gene was found to contain an open reading frame of 813 bp encoding a putative polypeptide of ~31 kDa. To determine the phenotype of a mdm12-null mutation, the DNA corresponding to most of the coding sequence of MDM12 was replaced by a URA3 gene. The phenotype of the *mdm12-null* (*mdm12D*) cells was identical to that of the mdm12-1 cells: enlarged mitochondria that failed transmit to the bud. Adding back the MDM12 gene on a centromere-based plasmid to *mdm12D* cells fully complemented the defects.

The dysfunction in mitochondrial inheritance was obvious in *mdm12D* cells at both permissive (23°C) and non-permissive (37°C) temperatures. To quantify these inheritance defects, mitochondrial distribution in populations of mutant or wild-type cells was examined by fluorescence microscopy after incubation at 23°C and 37°C. At both temperatures, 100% of wild-type (MDM12) cells showed normal mitochondrial inheritance, but 84% (235/281) of *mdm12D* cells exhibited a defect in mitochondrial inheritance.

Mdm10p and Mmm1p are both integral proteins of the outer mitochondrial membrane. To investigate whether Mdm12p was also a mitochondrial protein, an antibody was raised against a peptide whose sequence corresponded to the carboxyl terminus of Mdm12p. Indirect immunofluorescence microscopy was used to visualize Mdm12p in wild-type cells. Mdm12p is localized to mitochondria in wild-type cells, but not in *mdm12D* cells.

To further pinpoint the exact location of Mdm12p in mitochondria, an immunoblot was done. (Figure 3A) This showed Mdm12p was associated with the outer mitochondrial membrane. To characterize this association, peripheral membrane proteins were separated from integral membrane proteins by extraction of isolated mitochondria with 0.1 M sodium carbonate and centrifugation. Immunoblot analysis showed Mdm12p was an integral membrane protein. (Figure 3B) It has a putative transmembrane domain located at amino acids 23-42, but no other motifs have been identified by computer analysis [23].

These studies show MDM12 is required for transmission of mitochondria from mother to daughter cell, and detail some basic characteristics of the Mdm12 protein. This grant is designed to further characterize Mdm12p and uncover the precise role of Mdm12p in mitochondrial inheritance.

Research Design

There are several ways in which loss of Mdm12p might cause defects in mitochondrial inheritance. First, mitochondria may fail to associate with the actin cytoskeleton. I will address this issue by investigating the association of mitochondria in *mdm12**D*** cells by direct immunofluorescence. Also, I will determine if Mdm12p is required for mABP binding in actin/mitochondria co-sedimentation assays. Given the similarities in phenotype of *mdm12**D**, mdm10**D**,* and *mmm1**D*** cells, I expect that mitochondria will not associate with actin cables in *mdm12**D*** cells. However, should my experiments show mitochondria are indeed localized to actin cables in *mdm12**D*** cells, this would not be detrimental to my research, but may in fact lead to the identification of an exciting new function for an mdm protein.

Mere association of mitochondria to actin cables is necessary but not sufficient for transmission of mitochondria to daughter cells. Also found to be required for proper transmission is a novel protein which acts as a motor to move mitochondria along actin cable "tracks" [4]. Therefore, even if mitochondria do localize with actin cables in mdm12Δ cells, there may be a disruption of some type which does not allow proper motor activity necessary to move mitochondria into daughter cells. I will investigate this possibility by determining the mobility of mitochondria in *mdm12**D*** cells using immunofluorescence. Another possibility is that Mdm12p is required to bind to a protein mediating either of the above activities (actin association or movement), which could be a component of mABP. I will look for binding of Mdm12p to other proteins using a two-hybrid screen, and use biochemical assays to verify any potential positives.

Specific Aim 1:

A. Verifying mitochondria-actin association in *mdm12D* cells by immunofluorescence

First, I will determine if the mitochondrial inheritance defect in the *mdm12**D*** strain is due to lack of association of mitochondria with actin cables. I will investigate the organization of mitochondria and actin in three strains of S. cerevisiae: strain MYY290 (wildtype Mdm12p), strain MYY623 (*mdm12**D***), and strain MYY999 (*mdm12**D*** with a centromere-based plasmid encoding the MDM12 gene, hereafter called *mdm12**D** R*). This will be done using immunofluorescence microscopy [4]. Briefly, fixed budding cells will be stained for mitochondria by indirect immunofluorescence using an antibody raised against mitochondrial outer membrane proteins. Cells will be stained for actin using rhodamine phalloidin, a ligand that binds specifically to actin polymers [15]. As mentioned previously, two other mdm and mmm mutants, *mdm10* and *mmm1* have mitochondria that do not co-localize with actin cables [2]. Given the similarity of phenotype of mdm12Δ cells to these other mutants, I expect the *mdm12**D*** strain to also display lack of co-localization. These results will be confirmed by in vitro assays, as explained in (B).

B. Requirement of Mdm12p for docking of mABP

Mitochondria-actin association is known to require mitochondrial actin binding protein(s) (mABP), which is one or more peripheral mitochondrial outer membrane proteins with ATP-sensitive actin binding activity (2). I propose that Mdm12 is required for proper docking of mABP. To test this hypothesis, I will use a sedimentation assay described by Lazzarino et al [11]. These experiments will provide a biochemical test of mitochondrial-actin association in vitro (compared to the in vivo visualization methods described above). This assay measures binding of mitochondria to F-actin. Briefly, mitochondria from wild-type and *mdm12**D*** cells will be isolated and incubated with phalloidin-stabilized yeast actin filaments. After a low speed centrifugation, actin found with the mitochondrial pellet is defined as mitochondria-bound actin. Previous studies have shown that this bind is ATP-sensitive; that is, F-actin will sediment with mitochondria only when ATP is absent [2].

mABP activity can be removed by washing the isolated mitochondria with 1 M KCL. This wash releases peripheral outer mitochondrial membrane proteins, but does not disrupt the integrity of the mitochondria. I will determine the actin association of untreated mitochondria in both wild-type and *mdm12**D*** cells as described above. Next, I will test the ability of salt-washed (SW) mitochondria in both strains to bind actin. The expected result is negative, due to the removal of mABP. Then, I will add back mABP and repeat the actin sedimentation assay to determine if mABP is necessary for mitochondria-actin binding in these strains of yeast. I expect wild-type and *mdm12**D*** SW mitochondria not to bind actin, but the addition of mABP to wild-type mitochondria will allow binding, as has been reported previously [2]. If adding back mABP to SW mitochondria does not rescue actin binding in mdm12D cells, it would suggest that Mdm12p is necessary for mABP binding, thereby supporting my hypothesis.

However, if addition of mABP does rescue actin binding in mdm12Δ mitochondria, it will weaken my hypothesis that Mdm12p is required for docking of mABP, but not necessarily disprove it. It is still unknown whether mABP consists of one or many proteins. Perhaps Mdm10p and Mmm1p, the two proteins identified as necessary for mABP binding interact with one (or more) of the mABP proteins directly involved in actin binding. On the contrary, Mdm12p may have another function, such as interaction with a protein that stabilizes the binding of the myosin motor to the mitochondria, or may be directly involved with motility itself. This may prove to be more exciting than the alternative, since it may lead to the identification of a new function for an Mdm protein. Experimental approaches that will be used to identify specific protein interactions will be described in Specific Aim 2.

C. Mitochondrial mobility in *mdm12D* cells

As discussed previously, actin-mitochondria association is not sufficient for inheritance of mitochondria in budding cells. Also required is a motor activity which moves mitochondria along actin cables into the bud. Therefore, regardless of my data regarding the association of actin with mitochondria, I will also investigate the mobility of mitochondria in budding *mdm12**D*** cells. The method followed will be that set forth by Boldogh et al [2]. Briefly, mitochondria of wild-type, *mdm12**D***, and *mdm12**D**R* cells will be labeled by creating a fusion protein consisting of citrate synthase (a protein localized to

the inner mitochondrial membrane) to green fluorescent protein (CS1-GFP). CS1-GFP will be expressed in cells under the control of a Gal1-10 promoter [16]. Movement of GFP-labeled mitochondria will be measured by viewing budding cells under a light microscope. NIH Image v1.60 will be used to determine the change in position of individual mitochondria per unit time. These velocities will be average to give a mean velocity per unit time.

Movement of GFP-labeled mitochondria in several wild-type strains has been shown to be fast and largely polarized (toward the direction of the bud) [2-4]. In *mdm10**D*** and *mmm1**D*** mutants, a complete lack of polarized movement of mitochondria has been shown; instead, random, short, oscillatory movement has been detected (2). This movement bears a striking resemblance to mitochondrial movement in budding cells treated with latrunculin-A (Lat-A), which causes the rapid loss of F-actin (polymerized actin) [17]. I will also investigate mitochondrial movement in Lat-A treated wild-type strains. If, as expected, mitochondrial mobility in Lat-A treated cells is very similar to that in *mdm12**D*** strains, this will further support my hypothesis that Mdm12p is required for association of mitochondria to the actin cytoskeleton.

CS1-GFP labeling is specific to mitochondria and should not have any effect on the parameters which I will be measuring. However, an alternative to GFP labeling is use of 3,3'-dihexyloxacarbocyanine iodide (DiOC6). DiOC6 is a membrane potential-sensing dye that can be visualized by fluorescence microscopy.

Specific Aim 2: Mdm12p-protein associations

In my second specific aim, I will further investigate the hypothesis that Mdm12p is required for specific mABP binding by identifying proteins with which Mdm12p interacts. Identification of the proteins with which Mdm12p binds will allow elucidation of its exact function, and hopefully will also lead to the discovery of novel mABP components.

I will first investigate the association of Mdm12p with other proteins by using a yeast two-hybrid screen that is well-established in our lab. A plasmid will be made that will fuses the cytoplasmic portion of Mdm12p to the DNA-binding domain of GAL4. This plasmid will be transformed into a two-hybrid reporter yeast strain, which contains two GAL4-regulated reporter genes, HIS3 and lacZ, that allow growth in the absence of histidine and a blue color in the presence of X-gal, respectively. A yeast GAL4 activation library (a genomic library with the GAL4 activation domain fused randomly to yeast genes in a LEU2 gene-containing plasmid) will also be transformed into this strain. Colonies that can grow in the absence of histidine and that are blue in the presence of X-gal (denoting a potential two-hybrid interaction) will be selected for further study. To help identify false positives, I will determine if each interactor can self-activate the reporter genes, and also test activation with the protein of interest using a standard test protein, such as p53. Positives will be sequenced, then the resulting sequences searched for matches on internet databases to help identify the genes involved.

One requirement for a meaningful two-hybrid screen is the expression of an active Mdm12p. To demonstrate that I do have an active, expressible form of Mdm12p, I will express the cytoplasmic portion of Mdm12p on a high-copy plasmid and investigate the effects on mitochondrial inheritance. Overexpression of mdm12p should have a dominant negative effect on budding yeast cells. That is, the expressed protein should compete with

the wild-type, integral outer mitochondrial membrane Mdm12p for its binding sites and show the same defects in mitochondrial inheritance as *mdm12Δ* cells do. This assay will also provide a means of confirming potential positives from the two-hybrid screen. I will over-express each interactor in the strain with the high-copy MDM12 plasmid. If the two proteins do bind, I would expect the dominant negative effect of Mdm12p expression to be reversed, due to binding of the newly identified interactor to the overexpressed Mdm12p.

Methods (Mostly taken from [2])

Visualization of Mitochondria and the Actin Cytoskeleton

Strains will be grown to mid-log phase in YPR at 22°C, then shifted to 37°C for 3 hours. Mitochondria will be visualized using a fusion protein consisting of the mitochondrial signal sequence of citrate synthase1 fused to green fluorescent protein. CS1-GFP will be expressed in DAUL1 cells using a centromere-based plasmid under control of the Gal 1-10 promoter. Cells expressing CS1-GFP will be grown to mid-log phase in a synthetic, galactose-based liquid media to mid-log phase at 30°C. Samples will be mounted on microscope slides and visualized by fluorescence microscopy. The actin cytoskeleton will be counterstained in fixed DAUL1 cells expressing CS1-GFP using rhodamine-phalloidin. This will be added to fixed samples, which will stand in the dark at 4°C for 16 hours.

Light Microscopy

Images will be collected with a Zeiss Axioplan II microscope. Image enhancement and analysis will be performed on a Macintosh Quadra 800 computer using the NIH Image v1.60.

Velocity Measurements

Velocities of mitochondrial movement will be determined from images recorded at 20-s intervals>10min of real time. In wild-type cells, only velocities of organelles undergoing linear movement for at least three consecutive frames (1 min of real time) will be measured. In mutants, velocities of random samples of organelles will be measured, due to lack of linear movement. NIH Image v1.60 will be used to determine the change in position (x and y coordinates) of mitochondria per unit time, and these will be averaged to give a mean velocity.

Sedimentation Assay for Binding of F-Actin to Mitochondria

F-actin will be prepared from blocks of baker's yeast according to Kron et al. [24]. Isolated mitochondria will be incubated with phalloidin-stabilized F-actin. After binding, mitochondria will be separated from unbound actin filaments by centrifugation through a sucrose cushion and 12, 500 *g* for 10 min. Proteins recovered in the mitochondrial pellet will be separated by SDS-PAGE and identified by Western blot analysis using polyclonal antibodies raised against the mitochondrial marker proteins cytochrome b_2 and porin and a monoclonal antibody raised against actin.

Preparation of Salt-washed Mitochondria and Mitochondrial Salt Extract

To remove salt-sensitive peripheral proteins from the outer membrane, mitochondria will be incubated in SM1 buffer (0.6 M sorbitol, 20 mM Hepes-KOH, pH 7.4, 1 M KCl, 2 mM MgCl2, 1 mM PMSF, and protease inhibitor cocktail) for 15 min on ice. Then, the mixture will be centrifuged at 10,000 *g* for 5 min at 4°C. Salt-washed

mitochondria will be recovered in this pellet. The salt-extracted peripheral proteins will be in the supernatent. To regenerate the actin binding activity of salt-washed mitochondria, the KCl concentration of the salt-extract will be diluted to 0.2 M, and will be incubated with salt-washed mitochondria for 15 min on ice. Mitochondria will then be separated from the mixture by centrifugation at 10,000 *g* at 4°C and then used for the sedimentation assay.

Two-hybrid Assay

A plasmid (pAS-mdm12) will be made that will fuse the cytoplasmic domain of Mdm12p to the GAL4 DNA-binding domain. This plasmid will be transformed into the two-hybrid reporter yeast strain (Y190), which contains two GAL4-regulated reporter genes, HIS3 and lacZ, that allow growth in the absence of histidine and a blue color in the presence of X-gal, respectively. A yeast GAL4 activation library (a genomic library with the GAL4 activation domain fused randomly to yeast genes in a LEU2 gene-containing plasmid) will be transformed into Y190(pAS-Mdm12p). Colonies that can grow in the absence of histidine, indicating activation of the HIS3 reporter gene, will be selected because that denotes a potential two-hybrid interaction. Positives will be sequenced, then the resulting sequences searched for matches on internet databases to help identify the gene involved.

Overexpression Assay

The cytoplasmic domain of Mdm12p will be expressed in a high-copy plasmid in a wild-type yeast strain. Effects of this overexpression on mitochondrial inheritance will be determined using immunofluorescence as described above. Also, putative positives from the two-hybrid assay will also be expressed in this strain (along with the Mdm12p cytoplasmic domain) to investigate any quenching effect the interactor may have.

References

[1] Berger KH, LF Sogo, and MP Yaffe. 1997. Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol.* 136:545-553.

[2] Boldogh I, N Vojtov, S Karmon, and LA Pon. 1998. Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. *J. Cell Biol*. 141:1371-1381.

[3] Simon VR, Karmon SL, and LA Pon. 1997. Mitochondrial inheritance: cell cycle and actin cable dependence of polarized mitochondrial movements in Saccharomyces cerevisiae. *Cell Motil. Cytoskeleton*. 37:199-210.

[4] Simon, VR, TC Swayne, and LA Pon. 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.

[5] McConnell SJ, LC Stewart, A Talin, and MP Yaffe. 1990. Temperature-sensitive yeast mutants defective in mitochondrial inheritance. *J. Cell Biol*. 111:967-976.

[6] McConnell SJ and MP Yaffe. 1992. Nuclear and mitochondrial inheritance in yeast depends on novel cytoplasmic structures defined by the MDM1 protein. *J. Cell Biol.* 118:385-395.

[7] McConnell SJ and MP Yaffe. 1993. Intermediate filament formation by a yeast protein essential for organelle inheritance. *Science*. 260:687-689.

[8] Fisk HA and MP Yaffe. 1997. Mutational analysis of Mdm1p function in nuclear and mitochondrial inheritance. *J. Cell Biol*. 138:485-494.

[9] Sogo LF and MP Yaffe. 1994. Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol*. 126:1361-1373.

[10] Drubin, DG, HD Jones, and KF Werman. 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.

[11] Lazzarino, DA, I Boldogh, MG Smith, J Rosand, and LA Pon. 1994. Yeast mitochondria contain ATP-sensitive, reversible actin-binding activity. *Mol. Biol. Cell*. 5:807-818.

[12] Burgess SM, M Delannoy, and RE Jensen. 1994. MMM1 encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol*. 126:1375-1391.

[13] Hermann JG, EJ King, and JM Shaw. 1997. the yeast gene, MDM20, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. *J. Cell Biol*. 137:141-153.

[14] Smith MG, VR Simon, H O'Sullivan and LA Pon. 1995. Organelle-cytoskeletal interactions: actin mutations inhibit meiosis-dependent mitochondrial rearrangement in the budding yeast Saccharomyces cerevisiae. *Mol. Biol. Cell* 6:1361-1373.

[15] Cooper JA. 1987. Effects of cytochalasin and phalloidin on actin. *J. Cell Biol.* 105:1473-1478.

[16] Zelenaya-Troitskaya O, SM Newman, K Okamoto, PS Perlman, and RA Butow. 1997. Functions of the High Mobility Group protein, Abf2, in mitochondrial DNA segregation, recombination and copy number in Saccharomyces cerevisiae. *Genetics*. 148:1763-1776.

[17] Spector I, NR Shochet, Y Kashman, and A Roweiss. 1983. Latrunculins: novel marine toxins that disrupt microfilament organization in cultured cells. *Science.* 219:493-495.

[18] Schon EA, E Bonilla, and S DiMauro. 1997. Mitochondrial DNA mutations and pathogenesis. *J. Bioenerg. Biomembr*. 29:131-49.

[19] Bohlega S, K Tanji, FM Santorelli, M Hirano, A al-Jishi, and S DiMauro. 1996. Multiple mitochondrial DNA deletions associated with autosomal recessive opthalmoplegia and severe cardiomyopathy. *Neurology* 46:1329-34.

[20] Fadic R and DR Johns. 1996. Clinical spectrum of mitochondrial disease. *Semin. Neurol*. 16:11-20.

[21] Parker WD Jr. and RH Swerdlow. 1998. Mitochondrial dysfunction in idiopathic Parkinson's disease. *Am. J. Human Genetics*. 62:758-62.

[22] Lenaz G. 1998. Role of mitochondria in oxidative stress and ageing. *Biochim. Biophys. Acta*. 1366:53-67.

[23] Bairoch A. 1992. PROSITE: a dictionary of sites and patterns in proteins. *Nucleic Acids Res.* 20:2013-2018.

[24] Kron SJ, DG Drubin, D Botstein, and JA Spudich. 1992. Yeast actin filaments display ATP-dependent sliding movement over surfaces coated with rabbit muscle myosin. *Proc. Natl. Acad. Sci*. 89:4466-4470.