The C-terminal region of Apq12 is potentially involved in the regulation of cell cycle progression and cell separation

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Every living eukaryotic cell requires a basic set of criteria in order to sustain life. The ability to replicate genetic information and pass it on to every offspring is arguably the most important aspect of the cell cycle. When this process goes wrong the cell is often in-viable and commits apoptosis. In some cases, the mutation is not serious enough to cause apoptosis, but can still cause sustainable diseases or a disruption in the normal mitotic process, which can cause the cell to become cancerous. In less severe cases a mutation can cause or mutations. The mutation of Apq12 is one of the latter mutations, causing defects in cellular morphology and separation. Such defects have been shown to include odd cell shape, delocalization of the NE, and/or misplaced or incomplete NPC's. To investigate the role of Apq12, an integral membrane protein of the yeast *Saccharomyces cerevisiae*, we performed a series of sited-directed mutagenesis experiments using the Quigen Quikchange kit. Sites were selected for point mutation in the Apq12 sequence, which was acquired from yeastgenome.org, based on where a point mutation would change the codon to a stop codon. By changing a codon to a stop codon in the middle of the Apq12 coding sequence we created a partial truncated version that was missing the C-terminal region. This allowed us to investigate if there was a specific domain of the Apq12 sequence that is necessary for ensuring it properly regulates the cell cycle processes and cell separation. Our findings indicated that while Apq12 is itself a dismissible gene with regards to cell viability, part of the gene is responsible for regulating the progression of the cell cycle and separation, with implications that it could be required for ensuring the development of WT cell morphology.

INTRODUCTION

The correct inheritance of chromosomes during the mitotic cell cycle requires reliable duplication of the genetic material followed by equal and complete separation of the sister chromatids. Malfunctions in chromosome duplication or separation occur when there is a defect in any number of cellular processes, including DNA replication, chromosome cohesion, chromosome-microtubule (MT) attachment, nucleocytoplasmic transport, and overall cell cycle control. Since these processes are directly involved in the cell cycle or cell separation, their regulatory mechanisms present a good starting point for analysis. For example, the proper timing and functioning of MT are necessary for ensuring that each daughter cell receives a single copy of each chromosome, making it a viable cell. Therefore, if abnormal cell cycle progression or cell shapes are observed it suggests the possibility that there is a malfunction with the MT. In order to understand what went wrong with the formation and functioning of the MT the origins of regulatory mechanism should be investigated.

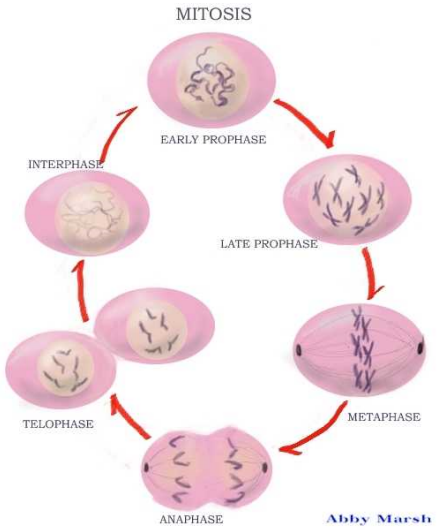


Figure 1.1 – Mitotic Cell Cycle Overview

Another potential and not well understood region of cell cycle control lies in the proteins of the NE. The NE is a bilayer membrane composed of hydrophilic phosphate heads which make up the outer surfaces, and hydrophobic lipid tails which make up the inner layers. The importance of this lipid bilayer is that it contains the DNA that is encoding to the information necessary to construct and control the cell. The NE separates and protects the contents of the nucleus from the contents of the cytoplasm so that no mixing can occur between the

fragile DNA and the harsh environment of the cytoplasm. The NE not only regulates the trafficking of macromolecules between the nucleoplasm and cytosol, but also provides anchoring sites for chromatin and the cytoskeleton. Chromatin is the complex of DNA and the proteins it wraps around, known as histones, which make up the contents of the nucleus.

In both yeast and human systems the (NE) contains all of the regions of MT organization, which are known as spindle pole bodies (SPBs) in *Saccharomyces cerevisiae* (Montpetit *et al.,* 2005). The SPBs, however, duplicate at the same time of DNA, creating a bipolar body to which the chromosomes may attach. The kinetochore, a large protein complex residing on the centromeric DNA, directs the attachment of the chromosomes to the MTs. Once all kinetochores have attached to their respective MTs, the metaphase to anaphase transition continues and chromosome disjunction occurs. Chromosome disjunction refers to the physical act of the two sister chromatids separating from each other during meiosis. The failure of even one chromosome attachment signals the cell cycle checkpoint machinery to temporarily stop the cycle. If such a cell cycle was allowed to continue it would lead to an abnormal number of chromosomes, or aneuploidy, a hallmark of many common diseases and cancers. Fortunately, a series of checkpoint proteins known as cyclins, closely regulate cell cycle progression by activating cyclin-dependent kinase (Cdk) enzymes.

The activation of these Cdk's triggers a signaling cascade that allows the cell cycle to enter the next phase. The two main cyclins responsible for this in both yeast and human models are G1 cyclin and mitotic cyclin. The G1 cyclin acts by binding to a specific CDK, which activates the kinase and triggers the DNA replication machinery. Similarly, the mitotic cyclin binds to its respective Cdk, but at another point of the cell cycle. The mitotic cyclin binding to the Cdk triggers the synthesis of M phase promoting factors that, in turn, trigger the formation of the mitosis machinery and the progression of mitosis itself (Alberts *et al.,* 1994). Through these interactions the NE helps regulate the progression of the cell cycle and cell separation.

Recent evidence suggests that the cell cycle is regulated by a wide variety of proteins found in several locations throughout the cell. Current research seeks to identify new proteins or locations of such proteins that are hypothesized to regulate the cell cycle. Recent evidence suggests that the cell cycle may be regulated in part by protein complexes residing in the NE (Antonin *et al.,* 2008*)*.

All materials transported between the nucleus and cytoplasm must pass through the NE or NPCs (Tran and Wente, 2006).These NPCs operate with extreme speed and efficiency while selectively transporting proteins, RNA, and other macromolecules across the nuclear membrane. In humans, for example, NPCs can transport more than one kilogram of material through our body every minute (Weis *et al.,* 2007). NPCs are believed to be very important to the vitality of the cell and development of disease because of their involvement with the large transfer of material throughout the body (Weis 2007). Another question is how the NPC retain selectivity when they are responsible for moving such a large quantity of material. One mechanism of control lies in the various proteins that are linked to the NPC. These proteins, known as nucleoporins (Nups), mediate which molecules cross through the NPCs. The NPCs receive a signal from the physical interaction between a component of the pore and the molecule that's trying to pass. A nuclear localizing signal (NSL) or nuclear export signal (NES) are sent to the NPC depending on whether a large molecule is trying to enter or exit the nucleus respectively. This process requires three distinct steps: first the molecule must bind with a carrier molecule, after which it binds to the NPC and is transported to the other side, where it is finally released at the target location and the carrier molecule continues its cycle (Stewart *et al.* 2007).

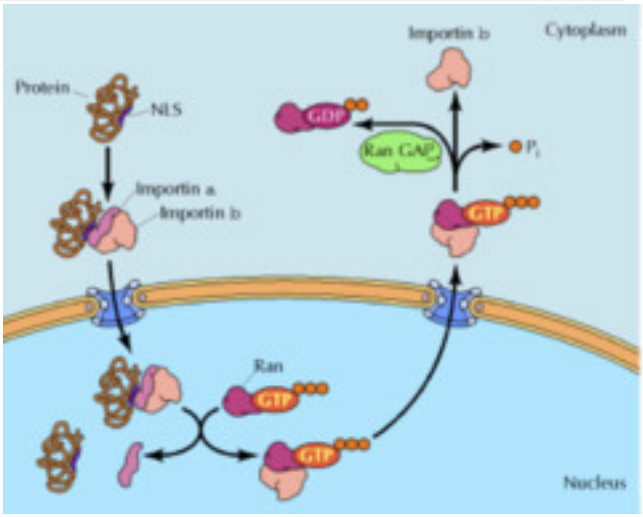


Figure 1.2 – Nuclear Import Process via NPCs

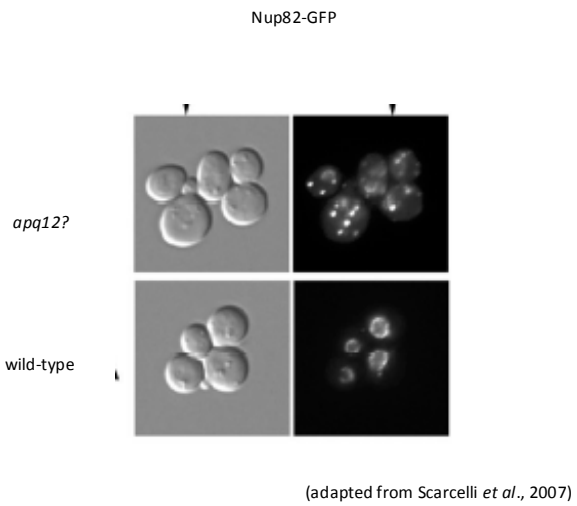
While the structure and function of NPCs have been the focus of many studies, the purpose and significance of other proteins located throughout the NE but not the NPC remains elusive. Among these NE proteins is Apq12, a protein known for potential involvement in cell cycle regulation.

Previous research has shown that Apq12 is required for appropriate progression of the cell cycle, NPC biogenesis, and nuclear envelope morphology in *Saccharomyces cerevisiae.* The deletion of Apq12 produced cells that were cold sensitive to growth (meaning they grew poorly at cooler temperatures), smaller overall cell size, and mislocalization of NPC components (Scarcelli *et al* 2007*)*. Other studies show that Apq12 is potentially involved with nuclear envelope morphology, nuclear pore complex localization, and mRNA export from the nucleus (Hodge *et al.,* 2010). Current evidence also suggests that Apq12 may affect nuclear transport through its ability to regulate nuclear membrane fluidity (Hodge *et al.,* 2010). Deletions of the Apq12 gene are suspected to disrupt the transport of mRNA and proteins between the nucleus and cytoplasm, with the potential to cause morphological irregularities in cellular and nuclear shape.

Here we investigate the role of Apq12, a non-essential integral membrane protein of the budding yeast *Saccharomyces cerevisiae*, in the regulation and progression of the cell cycle. Fluorescence microscopy was used in order to compare the cell shape, nuclear membrane formation, and NPC localization of wild-type cells (WT) to cells containing the mutant strains of Apq12. For this experiment we investigated three mutants along with our WT sample: one in which the indigenous Apq12 sequence was entirely deleted, a second in which the indigenous Apq12 was replaced with an over expressed version of the mutant with the partially truncated Apq12 sequence, and a third in which indigenous Apq12 was entirely deleted and replaced with an over expressed WT copy. Fluorescence was achieved by using green fluorescence protein (GFP), a protein isolated from the deep sea bioluminescent jellyfish *Aequorea Victoria.* The significant scientific application is that when this protein absorbs ultraviolet (UV) light, it emits a fluorescent green color. Therefore, the sequence encoding this protein can be attached to the coding sequence of a protein of interest and become an invaluable protein marker since anywhere that the target protein is being expressed will be fluoresce green and allow us to characterize its localization with fluorescence microscopy. To test Apq12's role in the regulation of the cell cycle and cell separation we performed a site directed mutagenesis experiment, which allowed us to design a series of mutations to Apq12, removing part of the C-terminus end. The purpose of performing partial mutations to Apq12, even though the entire gene is dispensable, is to investigate if a certain section of the Apq12 gene is vital to normal progression of the cell cycle and separation.

METHODS

Yeast Strains, Media, and Equipment. The Apq12 deletion yeast strain with green fluorescent protein (GFP) marking the nuclear pore Nup 60 or Nup 82 was kindly given to Dr. Michael Wolyniak by Charles Cole of the Department of Biochemistry at Dartmouth Medical School in Hanover, New Hampshire. Apq12 was also supplied on YEP195, a plasmid that can be expressed in multiple copies per cell, and thus allows for over expression of the gene. *S. cerevisiae* was grown on plates with rich synthetic complete medium and synthetic complete medium lacking Uracil. Yeasts were also grown in the standard rich YPD medium. All materials were acquired through the Hampden-Sydney College Biology Department. An Excella E24 shaking table incubator (New Brunswick Scientific, New Brunswick, NJ) was used for the bacteria transformants, which needed to be grown at 37C. Bacterial transformation is the process by which bacterial cells take up naked DNA with the purpose of manipulating the gene sequence. A rolling drum placed on the lab bench was used for growth of the liquid yeasts cultures. Qiagen Quikset DNA kits were used for extracting and separating the DNA (Valencia, CA). The yeast and bacteria transformants were stored in -20°C and -80°C freezers.



(adapted from Scarcelli *et al*., 2007)

Figure 1.3 - Apq12Δ cells expressing Nup82-GFP

Site Directed Mutagenesis of Apq12. Site directed mutagenesis was performed with the QuikChange XL Site-Directed Mutagenesis Kit (Agilent Technologies ,La Jolla, CA). Yeast strains were grown to midlog phase overnight at 23°C. Cells were washed twice in H_2O and washed once in 1x TE/Lithium acetate solution (LiAc). The cells were then resuspended in residual TE/LiAc solution and then incubated for 15 minutes at 30°C. While incubation was occurring, salmon sperm DNA (10mg/ml) was boiled for 5 minutes. The salmon sperm was immediately transferred to ice in order to snap-cool the DNA. The cells were dispensed 50µl per Eppendorf tube, and to those cells was added 5µl of boiled salmon sperm DNA. Roughly 100ng of *SIC1* plasmid DNA and 300µl of 40% polyethylene glycol/1x TE/LiAc were added to the cells. The tubes were then vortexed vigorously and Incubated for 30°C for 30 minutes followed by 42°C for 20 minutes (heat-shock phase). Following heat shock, the cells were centrifuged at 10,000rpm for 15 seconds, the supernatant was removed, and the cells were resuspended in 100µl of H_2O. The 100µl were spread on synthetic complete plates lacking leucine and incubated at 30°C for two to three days.

Microscopy. All microscopy and fluorescent microscopy was performed using an Olympus 1x70 inverted phase contrast fluorescent microscope (Olympus Inc., Center Valley, PA) equipped with a Photometric Coolsnap cf camera (Photometrics Inc., Tuscon, AZ) and Metamorph 6.3 computer analysis software (Molecular Devices, Sunnyvale, CA). Cells were prepared for microscopy by centrifuging and washing them with sterile water two times. Cells were re-suspended in 100 µl of sterile water. 10 µl of the cell mixture was pipetted onto a clean microscope slide, covered with a cover slip, and topped with a drop of immersion oil for the microscope's 100x Oil Objective lens. Each Apq12 variant was observed independently for cell shape (normal, oblong, or hyphae) and cell pairing or clustering. Normal yeast cells are characterized as circular in shape, oblong can range from oval shaped to tear drop-like cells, while hyphae is a cell exhibiting a mostly normal shape, but has a characteristic protrusion from one side of the cell.

Student T-test. Three replicates were done for each of the transformants for reproducibility and the totals were recorded in Microsoft Excel. A student T-test was used for statistical analysis of the collected data. A P-value less that 0.05 indicates that the findings are significant, and if the p-value is greater than 0.05 it indicates that any discrepancy in the data is likely due to chance and bears no significant impact.

RESULTS

We performed a general characterization of Apq12 to confirm previous findings that the Apq12 knock-out yeast cells exhibited abnormal cell shape and mislocalization of NPCs (Scarcelli *et al, 2007;* Figure 1).

As a preliminary test we grew wild-type (WT) yeast cells (Nup 60 GFP and Nup 82 GFP) and Apq12 knock-out yeast cells lacking GFP on separate plates in a 23°C incubator for two days, both in YPD media. Nup 60 and Nup 80 are both nucleoporins, however Nup 60 is located in the internal layers of the nuclear membrane, while Nup 82 is located on the surface edge of the nuclear membrane. We used mutants both with and without the GFP tag fused to them. A comparison of the plates confirmed Scarcelli's findings that the WT colonies achieved much fuller and abundant growth than did the Apq12 knock-out strain (Figure 5), strongly suggesting a role for Apq12 in the regulation of cell cycle progression.

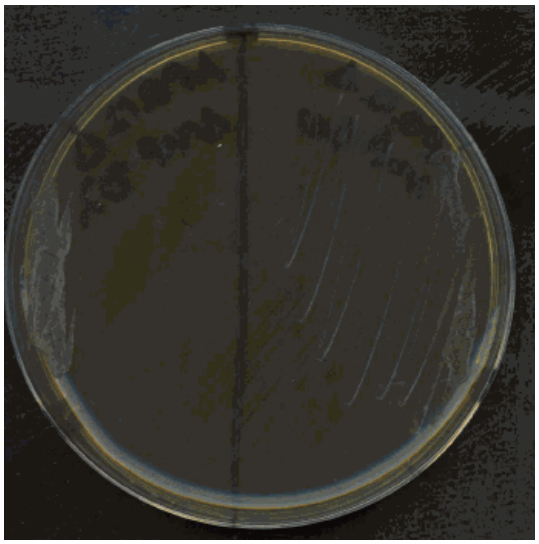


Figure 1.4 - Representative Cultures of Nup 82 GFP and Nup 60 GFP

Figure 1.5 – Representative Cultures of Apq12Δ Nup 60 and Apq12Δ Nup 82

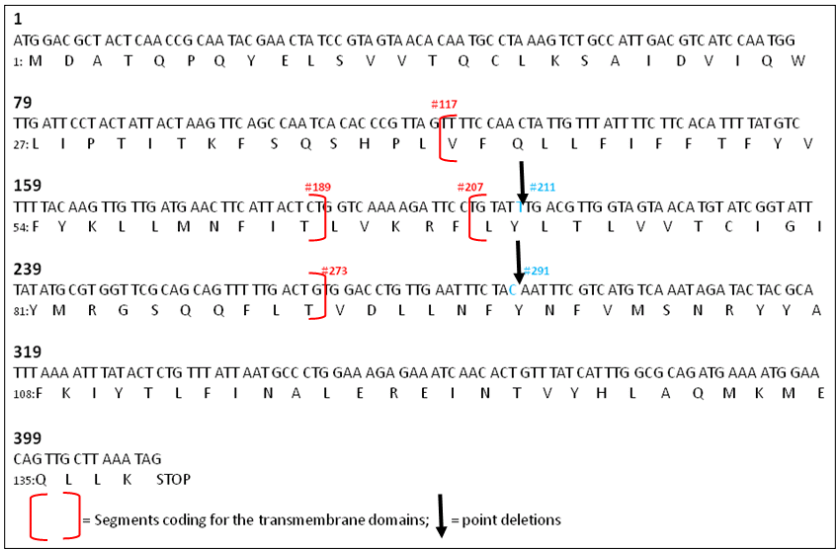


Figure 1.6 – DNA Sequence of Apq12

In order to investigate which domains of the Apq12 gene are essential to proper cell cycle progression and cell growth, we performed a site-directed mutagenesis experiment to create a series of Apq12 mutants that were deleted only for specific portions of the protein. These mutagenesis experiments were performed on a copy of *APQ12* residing on a plasmid, or circular piece of DNA, that also contains genes conferring ampicillin resistance and the ability to synthesize uracil. These two genes will allow for the selection of bacteria and yeast cells, respectively, that have taken up the plasmid.

In order to perform the site-directed mutagenesis, we first had to obtain the Apq12 DNA sequence from yeastgenome.org (Figure 1.6). We then analyzed the sequence with the purpose of locating the segments coding for transmembrane domains, as well as specific nucleotides that could be easily be converted into a stop codon. When a stop codon is encountered by the protein translation machinery it halts the translation and transcription process, resulting in a truncated protein coding sequence. By truncating the C-terminal region of Apq12, we hope to gain insight into the potential importance of this region to cell cycle progression.

The transmembrane domains (TMDs) are known to be coded by the sections of the Apq12 sequence from nucleic acids 117-189 and 207-273, denoted by the red brackets in Figure 5. In order to remove the transmembrane domains, we created specific primers upstream and downstream of each TMD. Each restriction site was specifically created for use with either XHOI or SAC II, enzymes that bind to specific sequences of DNA and cleave it at that site. The purpose of creating such restriction sites is to isolate a segment of DNA of interest for further investigation. By using enzymes that only cut at specific segments of DNA we will be able to ensure that our target DNA is digested only in that specific location, leaving the remaining portion intact. This target sequence retains the single stranded sticky ends of the restriction site that will be used to reconnect it to other pieces of DNA. This allows us to investigate different domains of the gene to determine specifically which parts of the gene are essential to proper protein formation, function, and ultimately cell cycle progression . We were successfully able to make the restriction sites for both XHOI and SAC II (Figure 7), as indicated by the ability of these enzymes to linearize circular plasmids by cutting at the created site. Current work is attempting to create the second restriction sites necessary for transmembrane region removal.

We also created a series of candidate truncation mutations, each of which removed varying portions of the Apq12 C-terminal end. The main two mutants in this experiment had nonsense truncation mutations at nucleic acids 211 and 291, removing the later portion of Apq12's C-terminus, respectively. A nonsense truncation refers to any point mutation that leads to the formation of a stop codon in a premature location of the transcribed mRNA, usually resulting in a truncated or incomplete protein, which can alter its function or efficiency.

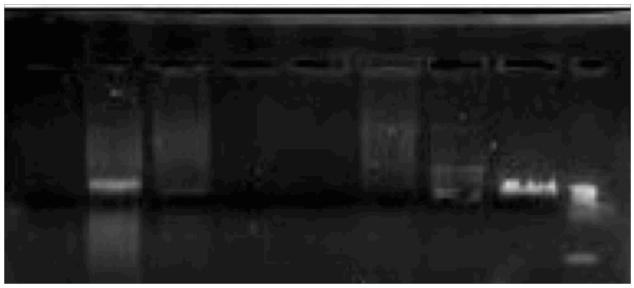


Figure 1.7 – Restriction digest showing digestion of Apq12 plasmid

These candidate mutants were transformed into yeast cells deleted for the *APQ12* gene and mutated for the *URA3* gene following the procedures laid out in the Stratagene QuikChange Site-Directed Mutagenesis Kit (Figure 1.8). The QuikChange site-directed mutagenesis kit is used to make point mutations, switch amino acids, and delete or insert single or multiple amino acids. The QuikChange site-directed mutagenesis method is performed using PfuTurbo™ DNA polymerase‡,ll and a thermal temperature cycler.‡ PfuTurbo DNA polymerase replicates both plasmid strands with high fidelity and without displacing the mutant oligonucleotide primers. The basic procedure utilizes a supercoiled double-stranded DNA (dsDNA) vector with an insert of interest and two synthetic oligonucleotide primers containing the desired mutation (see Figure 8). The oligonucleotide primers, each complementary to opposite strands of the vector, are extended during temperature cycling by using PfuTurbo DNA polymerase. Incorporation of the oligonucleotide primers generates a mutated plasmid containing staggered nicks. Following temperature cycling, the product is treated with Dpn I. The Dpn I endonuclease (target sequence: 5´-Gm6ATC-3´) is specific for methylated and hemimethylated DNA and is used to digest the parental DNA template and to select for mutation-containing synthesized DNA.7

DNA isolated from almost all *Escherichia coli* strains is dam methylated and therefore susceptible to Dpn I digestion. The nicked vector DNA incorporating the desired mutations is then transformed into *E. Coli*® XL1-Blue supercompetent cells‡. The small amount

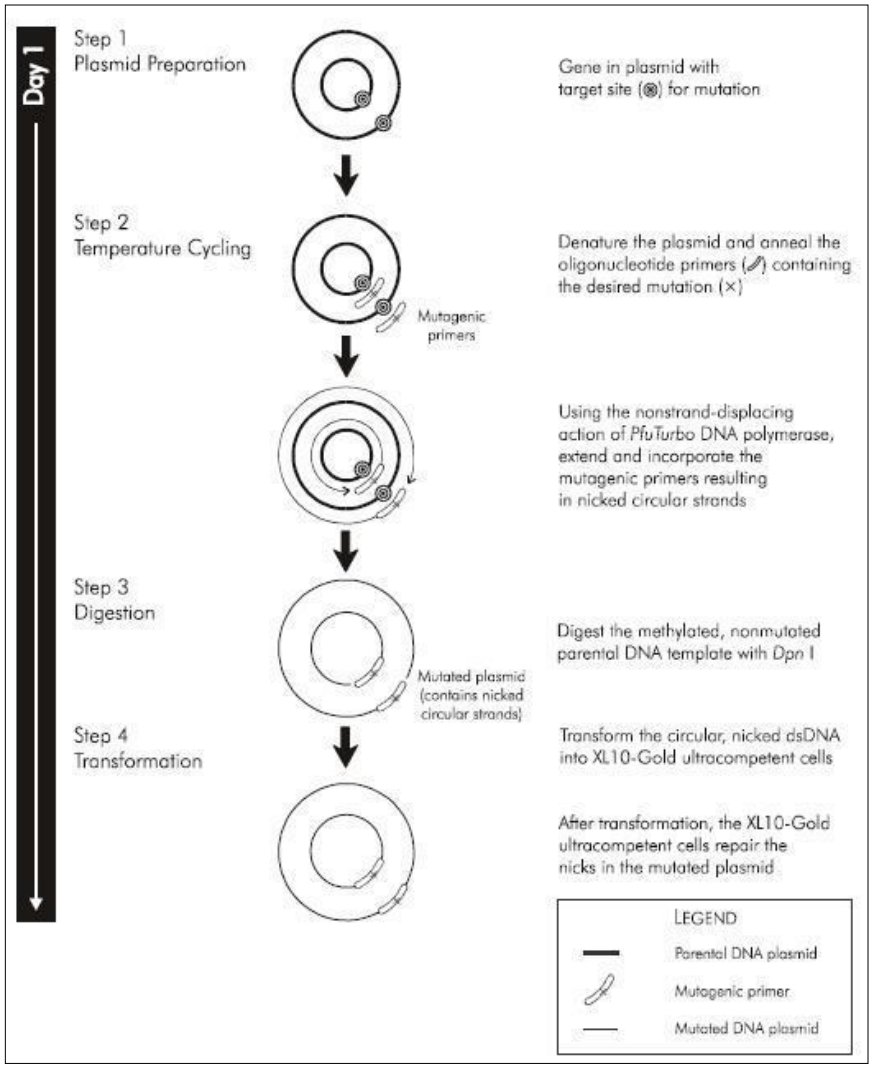


Figure 1.8 – Site-Directed Mutagenesis Experiment

of starting DNA template required to perform this method, the high fidelity of the PfuTurbo DNA polymerase, and the low number of PCR cycles all contributes to the high mutation efficiency and decreased potential for random mutations during the reaction (Stratagene).

Each candidate mutant was then grown separately on Ura- media plates to selectively grow only the colonies that successfully took up the candidate mutant plasmids. In order to better understand Apq12's function we compared the WT yeast strains (Nup 60 GFP and Nup 82 GFP) with mutant strains in which Apq12 was deleted (Δ=deleted; Apq12 Δ Nup 60 and Apq12 Δ Nup 82). WT strains were also compared with mutant strains in which the WT Apq12 was replaced with over expression of WT Apq12 (Apq12Δ Nup 60 + over expressed APQ12 and Apq12Δ Nup 82 + over expressed APQ12). Finally, they were compared with the mutants containing over expressed versions of the non-sense partial truncated versions. Observations were made about cell shape and budding for each of the Apq12 transformants and the relative numbers of cell morphology are displayed in Table 1.

Number	Sample	# Circular	# Oblong	# Hyphae
1	Nup 60 GFP (wild-type)	75	513	12
2	Nup 82 GFP (wild-type)	92	501	7
3	Apq12Δ Nup 60	34	524	22
4	Apq12Δ Nup 82	28	561	19
5	Apq12Δ Nup 60 + overexpressed Apq12 NS 211	97	493	10
6	Apq12 Nup 82 A + overexpressed Apq12 NS 291	22	555	7
7	Apq12 Nup 82 B + overexpressed Apq12 NS 291	31	559	0
8	Apq12Δ Nup 60 + overexpressed APQ12	115	382	3
9	Apq12Δ Nup 82 + overexpressed APQ12	184	412	3

Number	Sample	% Circular	% Oblong	% Hyphae
1	Nup 60 GFP (wild-type)	12.50	85.50	2.00
2	Nup 82 GFP (wild-type)	15.33	83.50	1.17
3	Apq12Δ Nup 60	5.86	90.34	3.79
4	Apq12Δ Nup 82	4.61	92.27	3.13
5	Apq12Δ Nup 60 + overexpressed Apq12 NS 211	16.17	82.17	1.67
6	Apq12 Nup 82 A + overexpressed Apq12 NS 291	3.77	95.03	1.20
7	Apq12 Nup 82 B + overexpressed Apq12 NS 291	5.25	94.75	0.00
8	Apq12Δ Nup 60 + overexpressed APQ12	23.00	76.40	0.60
9	Apq12Δ Nup 82 + overexpressed APQ12	30.72	68.78	0.50

Number	Sample	Pairs/Buds	Clumping
1	Nup 60 GFP (wild-type)	Yes	No
2	Nup 82 GFP (wild-type)	Yes	No
3	Apq12Δ Nup 60	Yes	Yes
4	Apq12Δ Nup 82	Yes	Few
5	Apq12Δ Nup 60 + overexpressed Apq12 NS 211	Yes	Few
6	Apq12 Nup 82 A + overexpressed Apq12 NS 291	Yes	No
7	Apq12 Nup 82 B + overexpressed Apq12 NS 291	Yes	No
8	Apq12Δ Nup 60 + overexpressed APQ12	Yes	Yes

Table 1 – Cell Count for Various Transformants

WT cells appear to have more defects in cell morphology than cells possessing the Apq12 over expression plasmid. For example, a comparison of Nup 82 GFP with Apq12Δ Nup 82 B + Apq12 NS 291 reveals a 10-15% increase in the number of normal cells in the over expression yeasts' than in the WT yeasts. The presence of Apq12 over expression seems to help correct or prevent abnormal cell shapes; however the C-terminus seems to be dispensable and not needed for correct cell development.

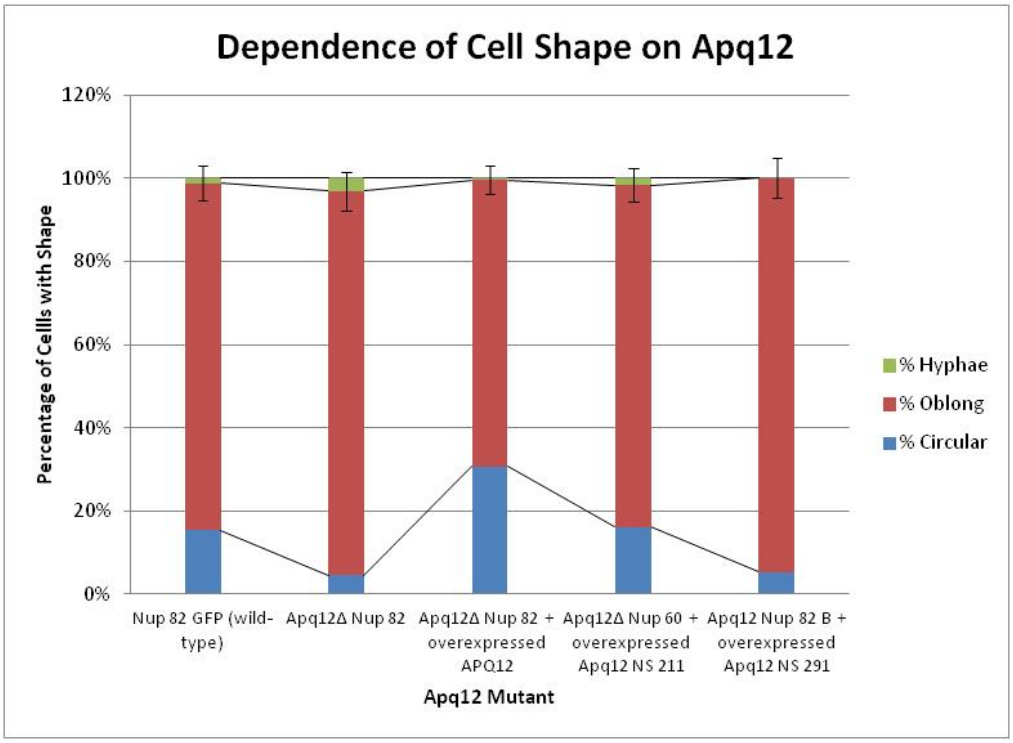


Figure 1.9 - Graphically representation of the percentage of cell shape in the WT and each of the mutants

Figure Notes:
The knockout of Apq12 (2nd column) reduces the number of normal shaped cells. The over-expression of WT Apq12 increases the number of normally shaped cells (middle column). The 4th and 5th columns represent over-expression of Apq12 without a portion of the C-terminus end. The 4th column (Apq12Δ Nup 60 + OE Apq12) removes more of the C-terminus than the 5th column. However, the mutant with more of the Apq12 gene removed has more normal shaped cells than the mutant with just the end tip of the Apq12 C-terminus removed. This suggests an antagonistic relationship between the sequence before nucleic acid 211 and the segment between 211 and 291.

DISCUSSION

One of the most critical life processes of cells is their ability to bud and produce identical daughter cells. We were able to successfully create point mutations in Apq12 that caused the premature truncation of the gene, usually resulting in a prematurely truncated and therefore partially functional protein. This is important because it allows for the analysis of different parts of the C-terminal region of Apq12 with an emphasis on which domains are necessary for proper cell morphology. The cell cycle and cell division processes are not random events, but rather are a highly regulated orchestration of many genes and proteins. This process is highly regulated because if a mutation causes a disruption in the normal progression of the cycle, it has the potential to cause catastrophic malfunctions in the cellular processes, disease, or even tumor genesis. Single nucleotide polymorphisms and point mutations are important for cell shape because they have the potential to change the genetic code which can alter the protein folding, and may disrupt cellular function altogether. Proteins interact with the cell cycle through signaling cascades and protein-to-protein interactions. By understanding how these proteins interact with each other we can gain further understanding into how this orchestra of proteins functions in regulating the cell cycle. Apq12 is one such protein, along with cyclins and cyclin-dependent kinases (Cdks), responsible for regulating the cell cycle progression.

The goal of our experiment was to determine if Apq12 is, in fact, a regulatory protein that functions on the order of cyclins or cyclin-dependent kinases (Cdks). While our results indicate that APq12 does not function in this manner, our data does suggest that it may play a role in determining cell morphology. Our findings suggest that a domain in the C-terminal portion of Apq12(nucleic acids (NA) 292-420) are antagonistic to the domain between nucleic acids (NA) 211-291. When the Apq12 sequence was truncated at NA 211 the number of cells displaying normal cell morphology increased compared to both the Apq12Δ Nup 82 knockout cells and the Apq12Δ Nup 82 B + OE Apq12 291, suggesting that this section of the gene is required for proper cell morphology (See Figure 9).

In fact, the NS 211 transformant closely resembles the WT yeast cells (Figure 9). However, if the Apq12 sequence was truncated closer to the C-

terminus end at NA 291, the protective effect of Apq12 was lost and the number of normal shaped cells decreased, while the number of oblong shaped cells increased. This suggests that the segment of the gene between NA 211 and 291 is an antagonist to the positive effects of NA 1-211. This transformant (Apq12 Nup 82 B + OE Apq12 NS 291) is missing the tip of the C-terminal end of the gene. When compared with the WT over expression transformant (Apq12Δ Nup 82 OE Apq12) it becomes apparent that the C-terminal end of the Apq12 gene (NA 291-420) is vital to the overall formation and function of the Apq12 protein in regulating the cell cycle. This is confirmed when the Apq12 Nup 82 B + OE Apq12 NS 291 transformant is compared with the transformant where the Apq12 gene was knocked-out altogether (Apq12Δ Nup 82) because both groups of yeast cells exhibited similar cell morphology with the lowest number of normal shaped cells. This suggests that the C-terminal portion of the Apq12 gene is required to achieve the full regulatory function of the cell cycle and cell separation.

Meticulous regulation of the cell cycle is essential to the proper formation and function of the cell. A disruption in the normal progression of the cycle can lead to defects such as non-disjunction of the chromosomes, incomplete formation of the cell, or incomplete cell separation, often leading to abnormal cell morphology. Cellular irregularities can result in cell clumping, which can be dangerous if it leads to clotting or tumorgenesis. This study concludes that Apq12, is an important player in the in the regulation and progression of the cell cycle and separation. We also conclude that it is possible to create specific primers to induce point mutations in a target gene prematurely truncating it, thereby changing its level of function or efficiency. Our results suggest that there are domains in the coding sequences of these integral membrane proteins that are responsible for regulating these highly orchestrated events and ensuring that the cell replicates and divides successfully.

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