Protein Analysis Report of wild type yeast *Saccharomyces cerevisiae* and mutant *MGA2*

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ABSTRACT

Protein from wild-type *Saccharomyces cerevisiae* and mutant *MGA2* strains were evaluated semi-quantitatively using a variety of biological techniques. Proteins were assayed using a bovine serum albumin (BSA) standard curve, loaded onto sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed for tubulin protein using western immunoblotting. The BSA standard curve showed protein concentration extractions of 2.9 mg/mL for wild-type and 2.2 mg/mL for the mutant. SDS-PAGE showed that 25µg wild-type and *MGA2* protein bands were darker than 15µg bands. Western immunoblotting revealed tubulin at approximately 50 kDa in both wild-type and *MGA2* strains, with the same overall banding pattern for wild-type and *MGA2*; thus showing *MGA2* had no effect on tubulin production. Unexpected double-banding patterns were seen in *MGA2* and wild-type lanes, as well as the positive control lane.

INTRODUCTION

Proteins are fundamental to all organisms. They are the macromolecule responsible for various tasks ranging from growth to regulation and providing appropriate immune responses. Recently, there has been an increased interest in the total protein abundance of *S. cerevisiae*. The absolute quantification of protein provides the values for models in system biology and control analysis (von der Haar, 2007). The purpose of this experiment is to determine possible differences in overall protein and tubulin protein compositions between wild type *S. cerevisiae* strain BY4741 and mutant *MGA2,* which could indicate that Mga2p is involved in cell tubulin processes. *MGA2* was grown in the background of BY4741 with the single MGA2 gene knocked out. *MGA2* is a transcription factor gene of the *OLE1* membrane protein gene, which produces delta-9 fatty acid desaturase that converts fatty acyl-CoA's into monounsaturated fatty acids, oleic acid and palmitoleic acid (Chellappa et al, 2001). By comparison of the two strains using protein analysis techniques SDS-PAGE and

Western Immunoblot, we can gain more insight into *MGA2*'s protein composition through qualitative evaluation (Andersen et al, 1989). We expect overall protein compositions to be comparable based on the *MGA2* mutation resulting in the absence of two proteins, *MGA2* and *OLE1*. In addition, when immunoblotting for tubulin, the binding patterns produced should be very similar, if not the same, because our mutant does not directly alter tubulin concentrations so it should be present in the same quantity as the wild type. Differences in absolute protein composition in the wild type and *MGA2* may also contribute to understanding its transcription for other genes, or other cellular differences that the *MGA2* mutation may result in. Such qualitative differences in absolute protein values and tubulin values will be observed from SDS-PAGE and western blot analysis.

METHODS

Protein Extraction

Approximately 2 mL cultures of wild type (BY4741a) and knock out mutant (*MGA2*) were inoculated at exponential growth phase. ~1mL aliquots of cultures were successively spun down in a centrifuge for 5 minutes at 12,000 rpm. The collected pellets were suspended in 1x Sample Buffer (SB) and ~ 100 mL glass beads. The pellets were further vortexed for 5 minutes, then chilled for 5 minutes. Samples were then incubated in a 95 °C water bath for 10 minutes. Cultures were lightly spun down again, and cell extracts were removed and kept on ice.

Protein Assay

The Pierce 660 nm procedure explained by Antharavally *et al*. (2008) was used to create a standard curve with duplicates of bovine serum albumin (BSA) and 1x sample buffer (SB) blanks. Wild type and *MGA2* mutant cultures were diluted 10 fold originally and further diluted 2 fold to lie within the standard curve (total 20 fold dilution). Serial dilutions of BSA (500 µg/µL, 250 µg/µL, 125 µg/µL, 62.5 µg/µL), blanks, and wild type and mutant cultures were pipetted in volumes of 10 µL into microplates. 150 µL of

Pierce 660 nm Protein Assay Reagent was added to each well. The microplate was covered and mixed on a plate shaker for 1 minute, then left to rest for 5 minutes before being read by the spectrophotometer at 660 nm.

SDS-PAGE gel electrophoresis

Extracted proteins from both mutant and wild type were analyzed using SDS-PAGE gel electrophoresis in accordance with Laemmili (1970). Bromophenol blue was forgotten and not added to the samples. A total of 8 lanes were used in the experiment. 10 µg and 20 µg of BSA, 15 µg and 25 µg of wild type, 15 µg and 25 µg of *MGA2* mutant protein, and 2 protein standards were inserted into the wells. Precision Plus Protein Kaleidoscope Standards #161-0375 was used as the protein standard. The standard BSA curve was used to determine the volume of sample needed in each lane for the wild type and mutant samples. The gel was run until bromophenol blue reached 1 cm above the bottom of the glass plate. The second gel was produced in the same manner as above, but with the addition of positive control (a wild type extract tested against the antibody). Gels were stained with Coomassie blue for 24 hours, then destained and developed.

Western blot analysis

The same samples as indicated above, in addition to the positive control was repeated with gel electrophoresis in accordance with Laemmili (1970). The resulting gel was used for ECL Western Blotting. The gel was transferred to polyvinylidene fluoride (PVDF) at 15V for 30 minutes outlined by the Bio-Rad Trans-blot semi-dry Transfer Cell manual (Catalog Number 170-3940, pg 5-9). The membranes were marked and stained with Ponceau S to determine if proteins were successfully transferred. The membrane was then placed in staining solution and gently shaken for 3 minutes, followed by being distained with distilled water. The membrane was air dried and stored at 4 °C for a week. Membranes were subjected to western blot analysis as outlined by Towbin et al. (1979) with sheep anti-alpha/beta tubulin (1:1000) and donkey anti-sheep HRP conjugates (1:3000). The membrane was developed using ECL (Enhanced

ChemiLuminescence) and X-ray film. 2 exposures of 15 seconds and 30 seconds were carried out.

RESULTS

Protein Extraction and Quantification

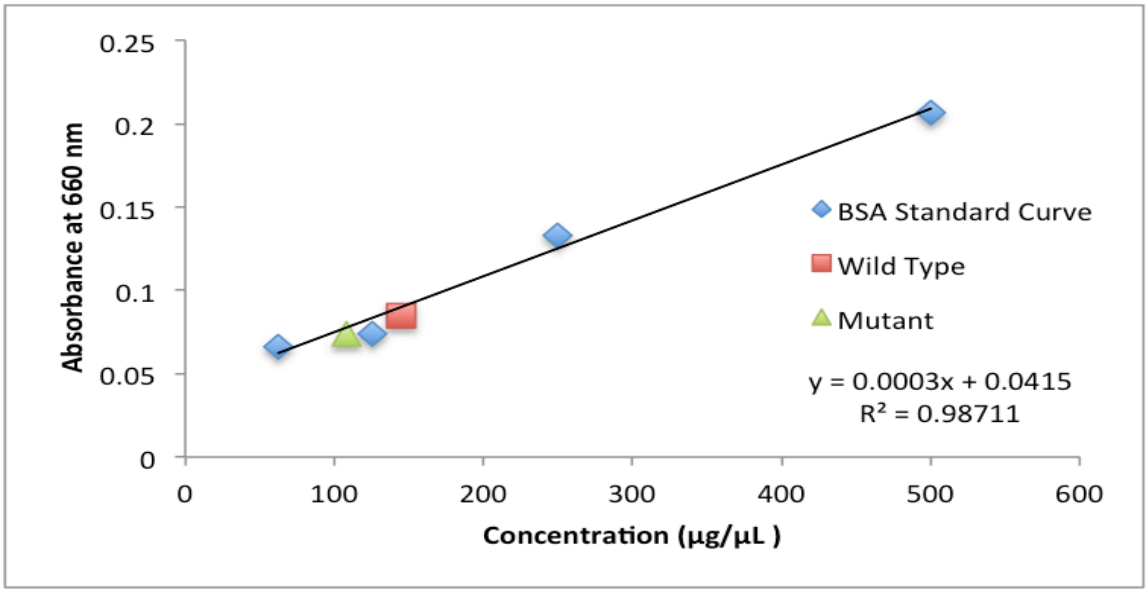

Figure 1. Relationship of optical density at 660 nm and cell extract concentration. Absorbance at 660 nm is plotted against different known concentrations of BSA to create a standard curve. 500 µg/µL, 250 µg/µL, 125 µg/µL, 62.5 µg/µL of BSA resulted in absorbance values of 0.207, 0.133, 0.074, 0.066 respectively. The equation of the line of best fit and the R^2 value are displayed for reference. Wild type, indicated by the red square, and the *MGA2* mutant, indicated by the green triangle, had absorbance values of 0.085 and 0.074 respectively at 660 nm. Protein concentrations within the wild type and mutant were determined through the standard curve determined by the different absorbances and cell concentrations of BSA. Total protein concentration for the wild type was 2.9 mg/mL and 2.2 mg/mL for the mutant.

Approximately 100 µL of crude cell extract was recovered from our extraction method. Averaged spectrophotometer data for BSA minus the SB blanks were 0.207, 0.133, 0.074, 0.066 for 500 µg/µL, 250 µg/µL, 125 µg/µL, 62.5 µg/µL respectively.

Through analysis of the spectrophotometer data, a relationship between Absorbance at 660 nm and concentration of BSA was determined to have a best fit line of y=0.0003x+0.0415 (*Figure 1*). Our initial wild type and mutant readings gave us absorbance values that lied outside of the standard curve, therefore the samples were read again at a 2 fold dilution (20 fold dilution resulted from the initial 10 fold and the subsequent 2 fold dilution). The averaged readings minus the SB blanks were 0.085 and 0.074 for the wild type and the mutant respectively. The undiluted concentration of proteins extracted from our samples were approximately 2.9 mg/mL for the wild type and 2.2 mg/mL for the MGA2 mutant, as determined from the BSA standard curve.

SDS-PAGE gel electrophoresis

SDS-PAGE gel electrophoresis was run twice - once to observe the gel itself and once to transfer the proteins onto PVDF to observe tubulin by ECL western blot analysis. The first gel was used to observe differences in our *MGA2* mutant and the wild type in terms of overall proteins present and concentration of specific sizes of proteins. Banding patterns observed between the mutant and the wild type are very similar (*Figure 1*). The lane with higher protein concentration, lane 3, appears darker than the lane with lower protein concentration, lane 2 (no comparison could be made between lanes 4 and 5 since lane 5 was improperly loaded). The banding patterns in these lanes were heavily concentrated around 10 kDa, moderately concentrated between 25-37 kDa, and lightly concentrated above 37 kDa. Both concentrations of BSA have the same banding pattern at approximately 65 kDa. An unexpected band was seen at approximately 65 kDa in lane 8. Bromophenol Blue was not added to the samples of the first gel and the 25 µg mutant sample (lane 5) was lost during loading. During the first run, the gel was running unevenly due to low running buffer volume, but eventually evened out once more running buffer was introduced.

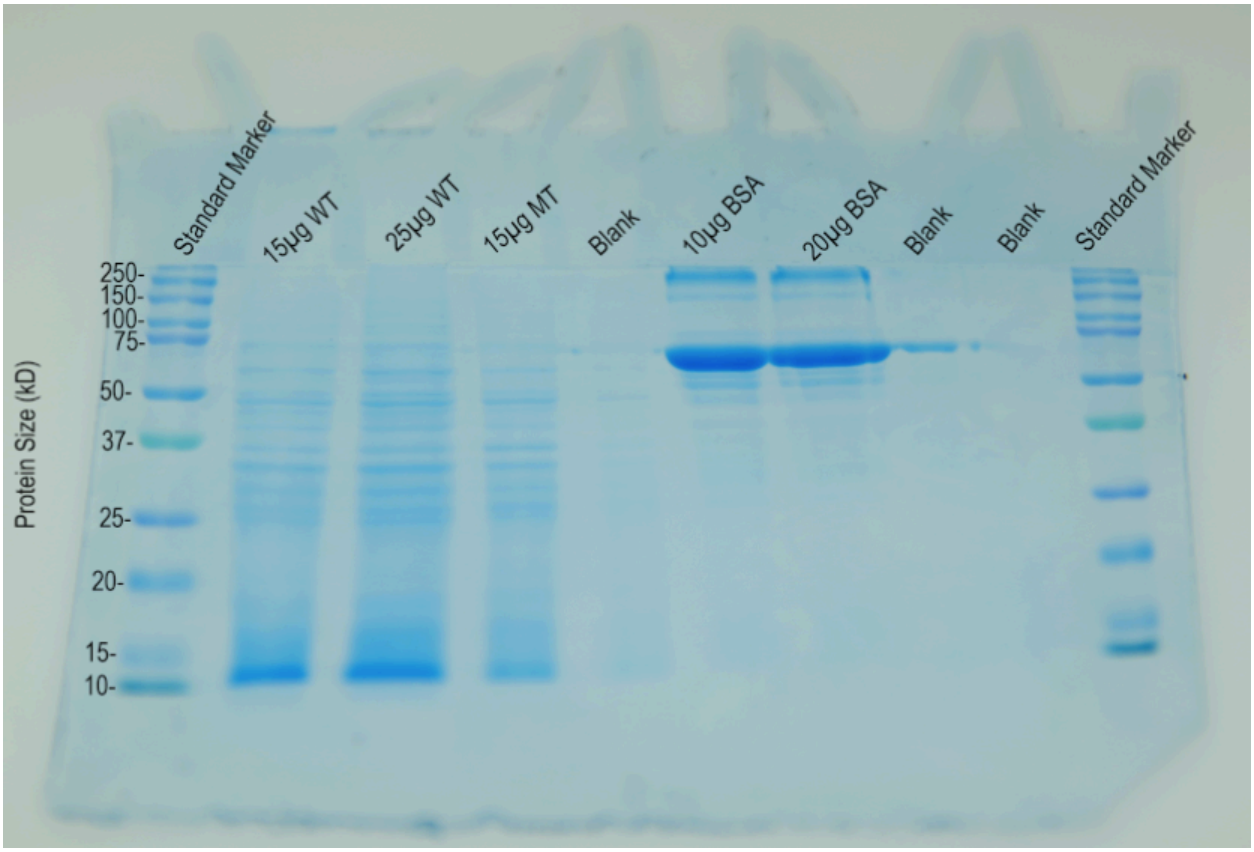

Figure 2. Result from SDS-PAGE gel electrophoresis, stained with Coomassie Blue. Lane 1 and 10 were loaded with pre-stained standard markers. Lane 2 and 3 were loaded with 15 µg and 25 µg of wild type cell extracts respectively. Lane 4 was loaded with 15 µg of the mutant cell extract. Lane 6 and 7 were loaded with 10 µg and 20 µg of BSA respectively. Lane 5 , 8, and 9 were left blank.

The second run of samples included the Bromophenol Blue and the 25 µg mutant sample. This gel was immediately transferred onto a PVDF membrane for western blot analysis (results below). After staining and destaining, this gel showed a lighter banding pattern across the wild type and mutant lanes (lanes 2-5), but lanes containing BSA (lanes 6 and 7) appeared to not have changed much as compared to the first gel (*Figure 3*). Once again, the higher concentrated samples had darker banding patterns.The bands were more evenly distributed in the wild type and mutant wells, both concentrations of BSA have the same banding pattern at approximately 65 kDa, and the positive control showed faint banding patterns most concentrated at approximately 50 kDa and 37 kDa (lane 8). The dark bands seen at approximately 10 kDa in the first gel were absent in the second gel.

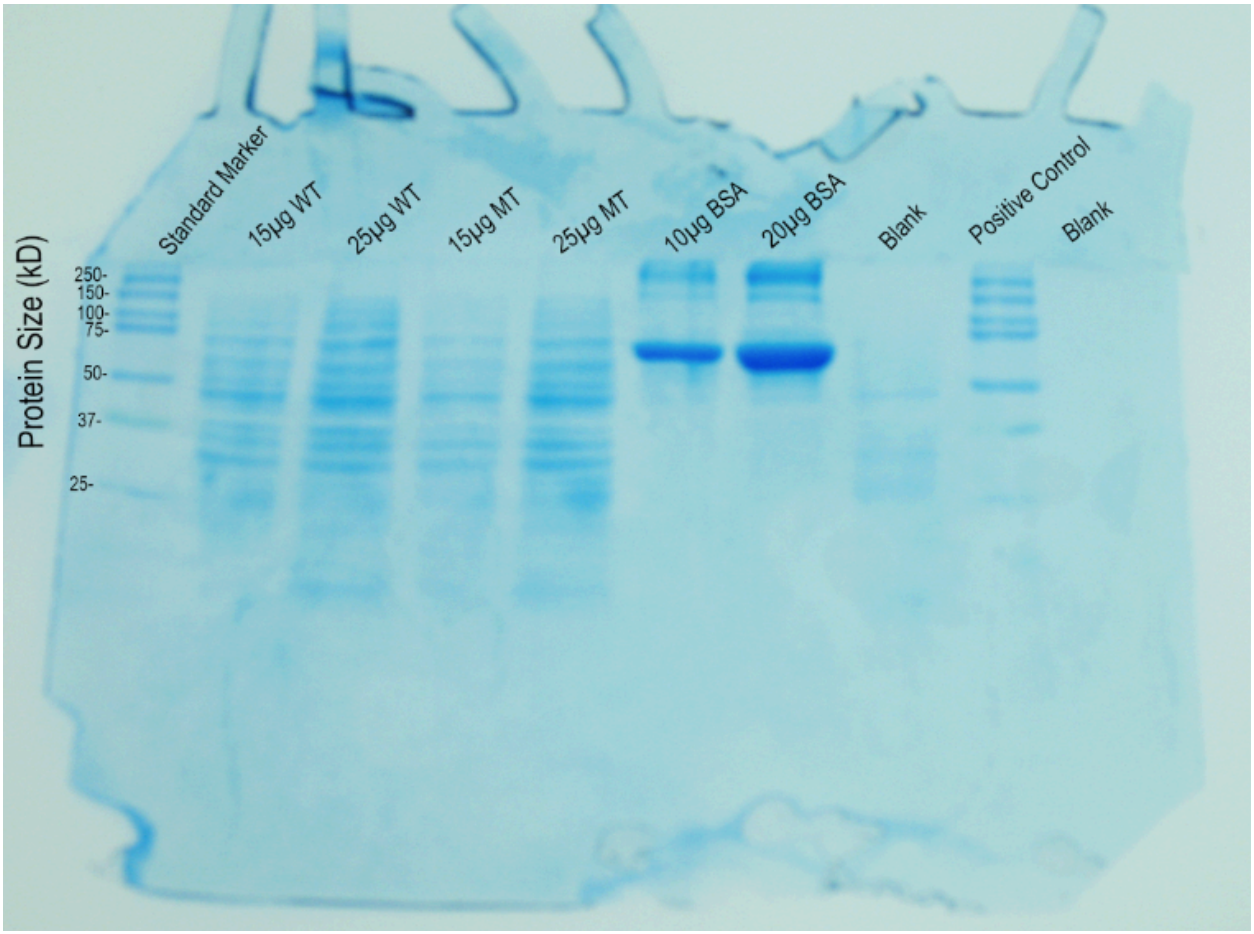

Figure 3. Result from SDS-PAGE gel electrophoresis, stained with Coomassie Blue. Lane 1 and 9 were loaded with pre-stained standard markers. Lane 2 and 3 were loaded with 15 µg and 25 µg of wild type cell extracts respectively. Lane 4 and 5 were loaded with 15 µg and 25µg of the mutant cell extract respectively. Lane 6 and 7 were loaded with 10 µg and 20 µg of BSA respectively. Lane 8 was loaded with the positive control and Lane 10 was left empty.

Western blot analysis

Before western blot analysis was carried out, our PVDF membrane was stained with Ponceau S. However, no protein transfer was detected. The protein standard was observed on the transfer membrane. As shown in *Figure 4*, exposure for 15 seconds showed 2 distinct bands at approximately 50 kDa across wild type, mutant and the positive control lanes (lanes 2-5 and lane 8). Exposure for 30 seconds also showed 2 distinct bands at approximately 50 kDa for wild type, mutant and the positive control (*Figure 5*). No bands were detected in lanes 6 and 7, which contained BSA. Exposure for 30 seconds picked up more background noise compared to the 15 second exposure.

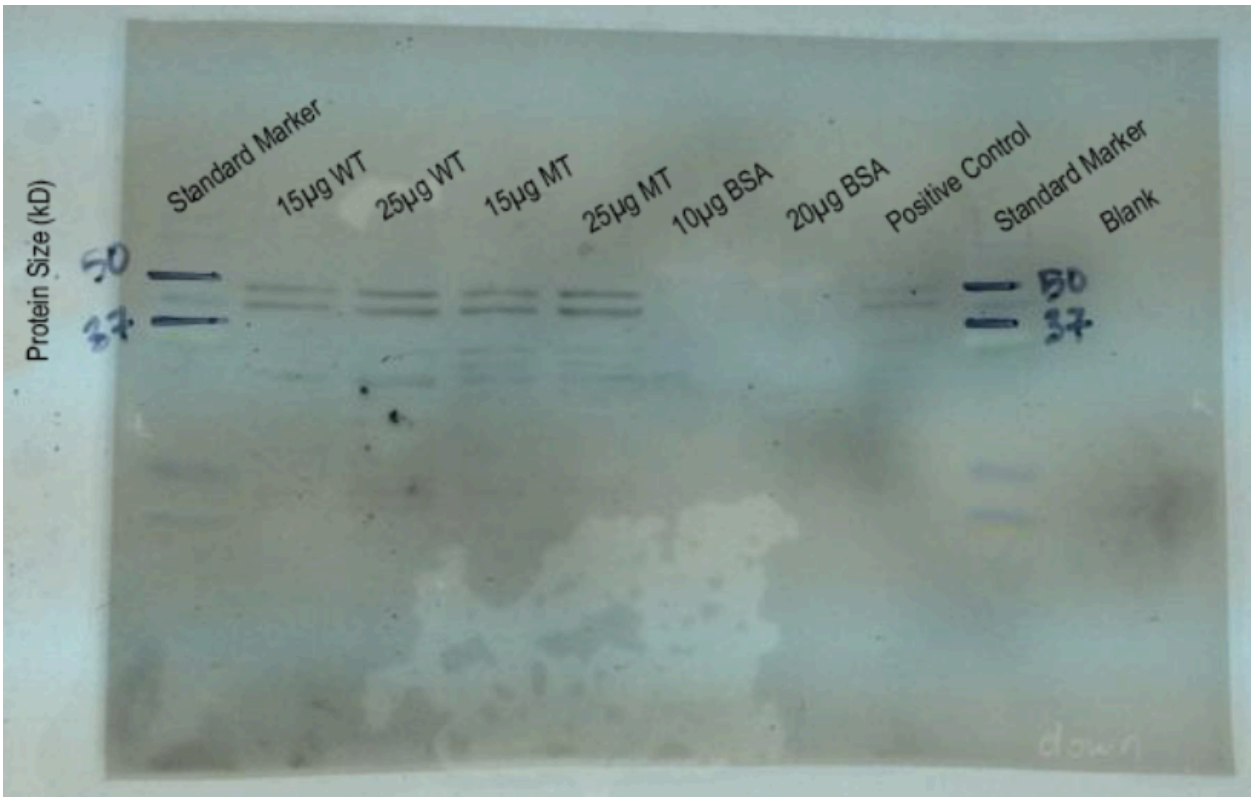

Figure 4. X-ray film showing result from immunoblotting, carried out to detect tubulin (55kD). The film was exposed for 15 seconds. Lane 1 and 9 were loaded with pre-stained standard markers. Lane 2 and 3 were loaded with 15 µg and 25 µg of wild type cell extracts respectively. Lane 4 and 5 were loaded with 15 µg and 25µg of the mutant cell extract respectively. Tubulin appeared in all four lanes containing the wild type and mutant strains, as indicated by the bands showing up around 50kD. The doublet observed cLane 6 and 7 are the negative controls, loaded with 10 µg and 20 µg of BSA respectively. Since BSA does not contain tubulin, the absence of bands in lane 6 and 7 indicates that the negative control worked. Lane 8 was loaded with the positive control and Lane 10 was left empty. The faint bands in lane 8 shows that the positive control worked.

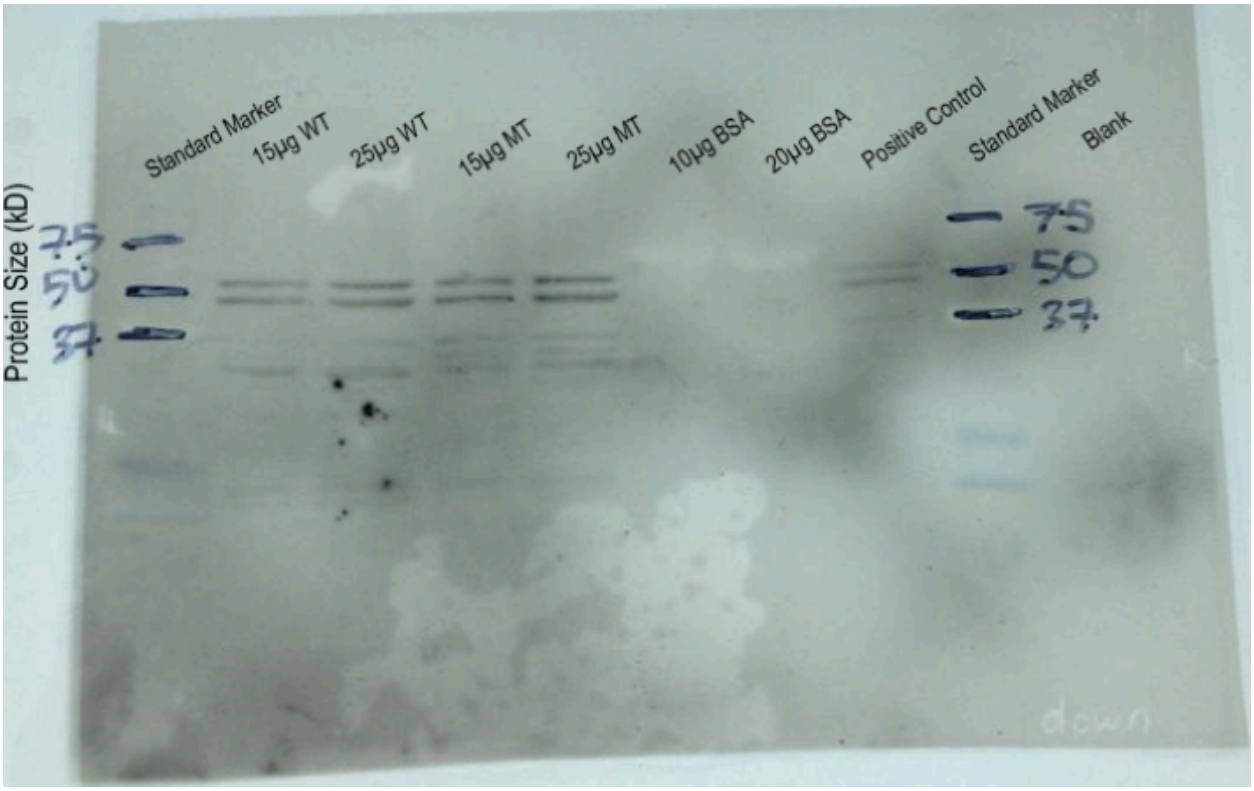

Figure 5. X-ray film showing result from immunoblotting, carried out to detect tubulin. The film was exposed for 30 seconds. Lane 1 and 9 were loaded with pre-stained standard markers. Lane 2 and 3 were loaded with 15 µg and 25 µg of wild type cell extracts respectively. Lane 4 and 5 were loaded with 15 µg and 25µg of the mutant cell extract respectively. Tubulin appeared in all four lanes containing the wild type and mutant strains, as indicated by the bands showing up around 50kD. Lane 6 and 7 are the negative controls, loaded with 10 µg and 20 µg of BSA respectively. Since BSA does not contain tubulin, the absence of bands in lane 6 and 7 indicates that the negative control worked. Lane 8 was loaded with the positive control and Lane 10 was left empty. The faint bands in lane 8 shows that the positive control worked.

DISCUSSION

Banding patterns in SDS-PAGE gel show the mixture of proteins present in each loaded lane. The similar banding patterns of the wild type *Saccharomyces cerevisiae* and mutant *MGA2* protein extractions in *Figure 1* tell us that the protein composition of the two cultures are highly similar. These patterns are consistent with the fact that our mutant strain *MGA2* is only missing one gene, *MGA2* which causes the mutant to lack

Mga2p and Ole1p, compared to the wild type (Chellappa et al, 2001). Because the protein composition of the wild type and the mutant differ by only two proteins, the banding patterns should show high resemblance.

In both wild type and mutant protein cultures, the more concentrated lanes (lanes 3 and 5) appear darker than their corresponding lower concentrated lanes (lanes 2 and 4). The darker the band, the more proteins are present. BSA was loaded as a negative control (lanes 6 and 7 in both *Figure 1* and *Figure 2*). It has a molecular weight of approximately 65 kDa. In the second gel, we can see that the protein band in lane 7 is slightly darker than in lane 6, due to the fact that lane 7 has a higher concentration of BSA loaded. However, the differences in BSA band width are generally minimal because BSA is a purified protein, making differentiation of concentrations difficult. Furthermore, the unexpected bands at the top of the BSA wells could be due to impurities in the BSA or BSA dimers. Lane 8 and 9 of the first gel (*Figure 2*) was not loaded with any samples. However, a faint band at around 65 kDa appeared in lane 8. This is likely due to a spillover that occurred when loading lane 7 with BSA. The second gel shows inconsistent and less concentrated banding patterns. This is due to the transfer of proteins to the membrane prior to staining that give the second gel an altered appearance, even though the same samples were loaded as the first gel. Lane 8 of the second gel (*Figure 3)* was loaded with a positive control with no added bromophenol blue. Faint detection of protein was concentrated at approximately 50 kDa and 37 kDa for the yeast extract. This is consistent with a wild type yeast extract sample because the most concentrated proteins detected are the most concentrated proteins seen in the wild type sample. Yeast extract contains fractions of the yeasts solids therefore this pattern is to be expected (Osbourne and Wakeman, 1919).

Immunoblotting was performed on our second gel. Banding patterns only appeared in lanes 2, 3, 4, 5, and 8, excluding the standard lanes (lane 1 and 9) (*Figure 3)*. Lanes 6 and 7 containing BSA showed no banding patterns for immunoblotting, since BSA is a protein that doesn't contain tubulin. Lane 8, the positive control, shows the presence of tubulin at around 50 kDa. All our yeast strains, both wild type and

mutant, show the same pattern (lanes 2-5), meaning that all our yeast strains contain tubulin. Because there is very minimal protein and tubulin differences between the wild type and the mutant yeast strains, results from the SDS-page and immunoblotting were highly similar. An unexpected double-banding pattern observed in all of lanes 2, 3, 4, 5, and 8 suggests an experimental error or broken down tubulin could be present in the samples. This may be due to faulty operation with the ECL system (3 out of 4 lab groups showed these results) or broken down tubulin could also cause a separate banding pattern from the 50 kDa expected.

Lane 5 of our first SDS-gel shows no banding pattern because the protein extraction was loaded improperly. Observations regarding 25ug mutant strain are therefore made using the second gel only. Initially, the first gel ran unevenly due to an insufficient amount of running buffer used during electrophoresis. However, after topping off the inner chamber with running buffer, the banding patterns leveled out. Furthermore, even though bromophenol blue was not added to the first SDS-gel, banding patterns still appeared after staining with Coomassie blue. Initially, staining of the PVDF membrane with Ponceau S stain detected no transfer of proteins from the gel onto the membrane. This may be because the amount of protein/tubulin is minimal in the cultures. Additionally, unidentified white stains were present on the membrane, likely due to improper handling of the materials.

For more accurate data collection, procedures should be conducted more carefully in the future. Because everyone was inexperienced with the lab equipment, numerous human errors were made, such as improper loading of SDS-PAGE gels and incorrect operation of the Bio-Rad tetra cell. In order to minimize human errors, more practice with the lab equipment is required for everyone for better familiarity. In order to increase the validity of our results, more replicates of the samples should be used in future experiments.

In the future, the standard curve, produced by measuring different concentrations of BSA and its absorbance values, can serve as a useful tool for easy measurement of

protein concentrations of *S. cerevisiae.* The best fit equation (y= 0.003x + 0.0415) can extrapolate the protein concentrations of either wild type or mutant yeast so only the optical density of the culture at 660 nm will need to be determined. Also, we can perform more specific SDS-PAGE or western blot analysis for other proteins of interest. In terms of western blotting, changing the protein of interest would require similar procedures besides using a different antibody and secondary antibody depending on that protein of interest. Therefore, these techniques are conveniently replicable and beneficial in further studying our mutant.

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