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Complementation of Human and Arabidopsis mod5 in Saccharomyces cerevisiae and induction of the prion form of Human and Arabidopsis MOD5

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Arts in Biochemistry and Molecular Biology and the Honors Program

by

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May, 2014
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entitled

Complementation of Human and *Arabidopsis mod5* in *Saccharomyces cerevisiae* and induction of the prion form of Human and *Arabidopsis MOD5*

be accepted in partial fulfillment of the requirements for the degree of

Bachelor of Biochemistry & Molecular Biology

Dr. Susan Liebman, Ph. D., Thesis Advisor

Tamara Valentine, Ph.D., Director, Honors Program

May 2014
Abstract:
The gene *MOD5* is highly conserved across all species from bacteria to mammals. Thus far, it is known to form prions [MOD+] only in yeast. The aim of this work is to test if human and *Arabidopsis MOD5* can form prions in yeast. This is done utilizing the properties of two yeast strains (MT-8, white color and ADE+ if complements) and *mod5Δ trm1Δ* (5-FU resistant if they complement) after transforming them with human and *Arabidopsis MOD5*. If the foreign *MOD5* is found to be complementary to that of yeast, then their prion form can be induced by exposure to other prions in presence of high concentrations of Fluconazole. Initial tests show that both human and *Arabidopsis MOD5* are complementing in yeast to some extent, *Arabidopsis* more so than human. Therefore an attempt at inducing the prion form of the foreign *MOD5* can be started.
Acknowledgments

I would like to thank my mentor Vydehi Kanneganti and my PI Dr. Liebman as well as everyone else in the Liebman lab.
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Introduction:
Prions are a unusual phenomenon that occur in living systems. A prion is a misfolded protein, typically due to the conversion of $\alpha$-helical structures to $\beta$-sheet structures. This causes the protein to lose function and a tendency to form aggregates. Due to their ability to recruit healthy proteins to the prion form, they can cause phenotypic shifts in the system without modifying the genetic code, or in other words, it is responsible for non-Mendelian inheritance (Paushkin et al., 1996) (Suzuki et al., 2012). Prion phenomena have been observed in yeast and filamentous fungi (Wickner, 1994; Maddelein et al., 2002), and fungal prion proteins share common characteristics with mammalian prion proteins. All of the yeast prion proteins identified thus far contain aggregation-prone Gln/Asn-rich domains that are critical for the formation of the self-propagating amyloid. A number of Gln/Asn-rich proteins in yeast have the potential to behave as prions (Alberti et al. 2009) for example the proteins Sup35 and Ure2 can form the prions [PSI+] and [URE3] respectively (Derkatch et al., 2001).

In this work, we focused on [MOD+], prion form of protein MOD5, a tRNA isopentenyl transferase that catalyzes the transfer of an isopentenyl group to A$^{37}$ in the anticodon loop (Dihanich et al., 1987), because MOD5 did not contain Gln/Asn-rich or repeat domains but acted as a PIN factor (inducible to [PSI+]) factors whose aggregation facilitates the de novo appearance of [PSI+]). As mentioned before, MOD5 is an isopentenyl transferase and it transfers a dimethylallyl group from dimethylallyl pyrophosphate (DMAPP) to certain variations of tRNA, specifically tRNA$^{Tyr}$, tRNA$^{Phe}$, and tRNA$^{Ser}$. The protein is highly conserved across all species (Pratt-Hyatt et al., 2013). DMAPP is also a substrate for Erg20 in the sterol biosynthetic pathway (Benko et al., 2000). Thus, a decrease in the tRNA modification by less soluble (functional) MOD5 in [MOD+] boosts the ergosterol synthesis, leading [MOD+] yeast to acquire resistance
against antifungal agents such as fluconazole, ketoconazole, and clotrimazole that inhibit ergosterol biosynthesis (Suzuki et al., 2012).

The function of MOD5 is to modify tRNA so it can attach the proper amino acid residues to new proteins. It has been found that yeast cells that have certain deletions, particularly those involved with tRNA modification (e.g. \( \text{trm1} \Delta \) and \( \text{trm10} \Delta \)), show increased sensitivity to the compound 5-fluorouracil (5-FU) at higher temperatures such as 38°C. 5-FU inhibits several tRNA modifying enzymes by forming covalent complexes between the enzyme and 5-FU-substituted tRNA. Combination of the deletions of \( \text{mod5} \) and \( \text{trm1} \) showed higher synthetic lethality on 5-FU plates due to tRNA destabilization, which can occur at room temperatures as well (Gustavsson and Ronne, 2008). The gene \( \text{TRM1} \) codes for another tRNA modifying protein that is responsible for the \( \text{m}^2\text{G}26 \) modification (Hopper et al., 1982). Cells with decreased MOD5 activity, for example yeast exhibiting [MOD+], also show 5-FU sensitivity in the presence of a deletion of \( \text{trm1} \) (Suzuki et al., 2012).

MOD5 is conserved across the species and it was isolated from humans (Golovko et al., 2000) and also higher plants like \textit{Arabidopsis} (Golovko et al., 2002) and show 53% overall similarity. We can utilize the function of MOD5 in the \textit{S. cerevisiae} strain, MT-8 (MAT\( \alpha \) SUP7 ura3-1 his5-2 leu2-3,112 ade2-1 trp1 lys1-1 lys2-1 can1-100 mod5::TRP1) (Gillman et al., 1991) to check the functional complementarity (isopentenyl transferase activity) of human and \textit{Arabidopsis} MOD5 to yeast MOD5. In the yeast strain MT-8 the MOD5 gene is completely inactivated by a TRP1 insertion (Gillman et al., 1991). This prevents the \( \text{i}^6\text{A} \) modification of cytoplasmic and mitochondrial tRNAs, including the nuclear-encoded suppressor tRNA SUP7 (Gillman et al., 1991). The lack of the \( \text{i}^6\text{A} \) modification renders SUP7 unable to suppress certain nonsense mutations, such as those in the ade2-1, can1-100 and lys2-1 alleles (Zoladek et al.,
Cells failing to suppress the ade2-1 mutation cannot grow on media lacking adenine and accumulate a red pigment when grown on rich media, such as YPD. The cells unable to suppress the can1-100 mutation lack arginine permease and are able to grow in the presence of canavanine, a toxic analog of arginine.

In this present work, we exploited both the characteristics of MT-8 cells (SUP7 suppression by MOD5) and 5-FU sensitivity of mod5Δ and trm1Δ to check if human and Arabidopsis MOD5 can complement yeast MOD5. We could use both the systems to check the complementation, and use mod5Δ trm1Δ to check for [MOD+] status of fluconazole resistant cells (MT-8 transformed with human or Arabidopsis MOD5 and induced with 50 µg/ml of fluconazole) human and Arabidopsis MOD5.

Methods:

1. Complementation Assay of human and Arabidopsis MOD5 in S. cerevisiae

Plasmid purification from Escherichia coli

Plasmids were purified using the Qiagen Miniprep Kit. The directions were followed as written except the final extraction was performed with pre heated water instead of elution buffer. The list of plasmids can be found in Table 1.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFL61-hMOD5</td>
<td>-ura</td>
</tr>
<tr>
<td>pFL61-Arabidopsis-MOD5</td>
<td>-ura</td>
</tr>
<tr>
<td>pRS416 (empty vector)</td>
<td>-ura</td>
</tr>
<tr>
<td>pRS426-ycfMOD5</td>
<td>-ura</td>
</tr>
<tr>
<td>pRS426-MOD5-GFP</td>
<td>-ura</td>
</tr>
<tr>
<td>pRS416-MOD5-M1-GFP</td>
<td>-ura</td>
</tr>
<tr>
<td>pRS426-MOD5-M2-GFP</td>
<td>-ura</td>
</tr>
</tbody>
</table>

Table 1: List of plasmids used for transformation of yeast for toxicity assay

This table shows the plasmids used for the various yeast transformations performed.
**Yeast Transformation with plasmids**

Yeast cells were grown in YPD culture overnight at 30°C at 200 rpm. The cells were centrifuged at 5000 rpm for five minutes at room temperature. The supernatant was decanted and the cells were resuspended in 10 mL of 1x LiOAc in TE buffer and incubated at room temperature for at least thirty minutes. After incubation the solution was once again centrifuged at 5000 rpm for five minutes at room temperature. The supernatant was decanted and the cells were resuspended in the remaining LiOAc. In an eppendorf tube, 3 μL of purified plasmid was mixed with 5 μL of ssDNA. To this, 50 μL of the suspended cells was added to the mixture of plasmid and ssDNA. To this, 500 μL of 50% PEG (polyethylene glycol) in 1x TE was added. The mixtures were vortexed to form a homogenous solution and placed in a 30°C incubator for 40 minutes. After this, the solutions were heat shocked in a 42°C hot water bath for 20 minutes. The solutions were centrifuged at 3000 rpm for three minutes at room temperature. The supernatant was aspirated via vacuum. The cells were washed with 1ml of 1x TE, followed by centrifugation at 3000 rpm for 3 minutes. The pellet was resuspended in 100 μL of 1x TE and subsequently plated onto uracil deficient plates (-ura) plates. The plates were placed in a 30°C incubator and allowed to grow for 3-4 days. After sufficient growth, colonies were patched onto another –ura plate. The yeast strains that were transformed can be found in Table 2.
Yeast strain genotype       | Strain name
---------------------------------|-------------------------
\(\text{MAT}^a/\alpha\ \text{ade}1-14\ \text{trp}1-289\ \text{his}3-200\ \text{ura}3-52\ \text{leu}2-3, 112, \Delta\text{mod}5::\text{kanMX4}, \Delta\text{trm}1::\text{CgHIS3}\) | mod5\(\Delta\) trm1\(\Delta\)
\(\text{MAT}^a/\alpha\ \text{ade}1-14\ \text{trp}1-289\ \text{his}3-200\ \text{ura}3-52\ \text{leu}2-3, 112, \text{MOD5}-\text{GFP}::\text{kanMX4}, \Delta\text{trm}1::\text{CgHIS3}\) | MOD5-GFP trm1\(\Delta\)

Table 2: List of yeast strains transformed for complementation of human and *Arabidopsis* MOD5

This is the list of yeast strains transformed during the various experiments to check complementation of the human and *Arabidopsis* mod5. The plasmids used to transform these strains were all strains listed in Table 1, or: Human MOD5, *Arabidopsis* MOD5, pRS416, YcfMOD5, pRS426-MOD5-GFP, pRS416-MOD5-M1-GFP, and pRS426-MOD5-M2-GFP.

**Serial dilution and drop test**

Serial dilution was performed using a 96 well plate by suspending the yeast colonies picked from the –ura plates in the first well followed by the serial dilution done according to the schematic as seen in Figure 1. Using a spotting tool the dilutions were spotted onto YPD, YPG, and YPD+5FU plates. The YPD+5FU plates have a 5-FU concentration of 100 \(\mu\text{g/mL}\). The plates were scanned after the plates were incubated at 30 °C for 3-4 days.
Figure 1: Schematic of serial dilution
This figure shows an example of serial dilution. Picked colonies were transferred to a well containing 100 μL of 1x TE and mixed. From this 10 μL were transferred to the next well containing 90 μL of 1x TE. This was repeated four additional times, for a final relative concentration of 10^-5 from the original well.

2. Functional Complementation Assay of human and Arabidopsis MOD5 in S. cerevisiae

Plasmid purification from Escherichia coli
Plasmid purification was performed as previously described. The plasmids used can be seen in the following table (Table 3).

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFL61–hMOD5</td>
<td>-Ura</td>
</tr>
<tr>
<td>pFL61–Arabidopsis-MOD5</td>
<td>-Ura</td>
</tr>
<tr>
<td>pRS416 (empty vector)</td>
<td>-Ura</td>
</tr>
<tr>
<td>YcfMOD5</td>
<td>-Ura</td>
</tr>
</tbody>
</table>

Table 3: List of plasmids used in transformation of yeast for functional complementation assay
Yeast Transformation with plasmids
MT-8 yeast cells (MATa SUP7 ura3-1 his5-2 leu2-3,112 ade2-1 trp1 lys1-1 can1-100 mod5::TRP1) were used for the transformation with the plasmids as previously described in Table 3.

Serial dilution and drop test
Serial dilution was performed as described above. However, the drop test was done using the following plates: YPD, YPG, adenine deficient plates (-ade), -ura plates, and ½ YPD plates. Other than this, the protocol was the same as previously described.

3. Confirmation of toxicity of human and Arabidopsis MOD5 in yeast cells.

Plasmid purification from Escherichia coli
Plasmid purification was performed as previously described. The plasmids purified are found in Table 3.

Yeast Transformation with plasmids
MT-8 yeast cells (MATa SUP7 ura3-1 his5-2 leu2-3,112 ade2-1 trp1 lys1-1 can1-100 mod5::TRP1) were used for the transformation with the plasmids as previously described in Table 3.

Serial dilution and drop test
Serial dilution was performed as described above. However, the drop test was done using the following plates: YPD, YPG, adenine deficient plates (-ade), -ura plates, lysine deficient plates (-lys), and ½ YPD plates. Other than this, the protocol was the same as previously described.

Normalization of concentration and cell count
Yeast cells were cultured in –ura liquid overnight at 30°C at 200 rpm. The solution was then analyzed using a spectrophotometer tuned to 600 nm to determine cell concentration. The concentrations were then normalized. Differing concentrations were then grown on different plates as shown in the table below (Table 4). Images were taken of plates after 2-3 days of incubation at 30°C.
Concentration | $10^3$ cells/mL | $10^2$ cells/mL | $10^1$ cells/mL
---|---|---|---
Plates | -ura(2x), -ade(2x) | ½ YPD(1x), YPD(1x) | ½ YPD(1x), YPD(1x)

**Table 4:** Concentrations and plates used to determine toxicity of human and *Arabidopsis MOD5* in yeast

4. **Induction of human and *Arabidopsis* [MOD+] in yeast cells.**

**Plasmid purification from *Escherichia coli***

Plasmid purification was performed as previously described. The plasmids used can be seen in the following table (Table 5).

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>pFL61–hMOD5</td>
<td>-Ura</td>
</tr>
<tr>
<td>pFL61- Arabidopsis-MOD5</td>
<td>-Ura</td>
</tr>
<tr>
<td>pRS416 (empty vector)</td>
<td>-Ura</td>
</tr>
<tr>
<td>YCF-MOD5 (Yeast MOD5)</td>
<td>-Ura</td>
</tr>
</tbody>
</table>

**Table 5:** List of plasmids used in transformation of yeast for determination of [MOD+] formation of foreign proteins

**Yeast transformation with plasmids**

Yeast transformation was performed as described above with the plasmids as given in Table 5 with the following strains.

<table>
<thead>
<tr>
<th>MATa/a ade1-14 trp1-289 his3-200 ura3-52 leu2-3, 112, Δmod5::kanMX4, Δtrm1::CgHIS3 [psi-] [pin-]</th>
<th>mod5Δ trm1Δ [psi-] [pin-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATa/a ade1-14 trp1-289 his3-200 ura3-52 leu2-3, 112, Δmod5::kanMX4, Δtrm1::CgHIS3 (V.H.) [PIN+]</td>
<td>mod5Δ trm1Δ Very High (V.H.) [PIN+]</td>
</tr>
<tr>
<td>MATa/a ade1-14 trp1-289 his3-200 ura3-52 leu2-3, 112, Δmod5::kanMX4, Δtrm1::CgHIS3 Strong (S.) [PSI+]</td>
<td>mod5Δ trm1Δ Strong (S.) [PSI+]</td>
</tr>
<tr>
<td>MATa/a ade1-14 trp1-289 his3-200 ura3-52 leu2-3, 112, Δmod5::kanMX4, Δtrm1::CgHIS3 High (H.) [PIN+]</td>
<td>mod5Δ trm1Δ High (H.) [PIN+]</td>
</tr>
</tbody>
</table>

**Table 6:** List of yeast strains for determination of [MOD+] formation of foreign proteins

The above strains were created by the cytoduction of *mod5Δ trm1Δ* with yeast strains carrying psi- pin-, Very high [PIN"], High [PIN"] and strong [PSI"].
Induction of [MOD+] 

All the above yeast strains were transformed with the plasmids as stated in the table 4. Individual colonies from above transformants were inoculated in 10ml YPD and grown at 30°C overnight. The cells were spun down and re-suspended in 10ml of YPD containing 50 µg/ml Fluconazole to induce the formation of human and or Arabidopsis [MOD+] in the presence or absence of [PIN+] and [PSI+] variants (for cross seeding) for 6hrs before plating 10⁶ cells onto YPG+Fluconazole plates (20, 30 and 50 µg/ml fluconazole). The plates were incubated at 30°C for 6-7 days before counting the fluconazole resistant colonies.

Results:

1. Complementation Assay of human and Arabidopsis mod5 in S. cerevisiae

After transformation the drop test was performed as described. Both strains, mod5Δ trm1Δ and MOD5-GFP trm1Δ, were grown and imaged, however, only the data from the mod5Δ trm1Δ is shown since all the different transformants of MOD5-GFP trm1Δ grow on YPD+5FU because of functional Yeast MOD5.

![Figure 2: Drop test of transformed mod5Δ trm1Δ yeast strain on YPD, YPG, and YPD+5FU](image-url)

YPD and YPG plates serve as positive controls, while YPD+5FU plates are the experimental plates. MOD5-GFP trm1Δ and ycfMOD5 serve as positive controls. mod5Δ trm1Δ, [MOD+] trm1Δ, and pRS426 (empty vector) serve as negative controls. Human MOD5 shows growth greater than that of the negative controls. Arabidopsis shows significant growth greater than the negative controls. Human-2 shows growth similar to that of the negative controls.
2. Functional Complementation Assay of human and *Arabidopsis MOD5* in *S. cerevisiae*

After transformation of the MT-8 strain the drop test was performed as described. The results of the drop test are shown below, Figure 3 showing the growth of transformed colonies on –ade plates, and Figure 4 showing the color testing of the grown colonies.

![Figure 3: Drop test of transformed MT-8 yeast strain on –ura and -ade](image)

The –ura plate is the control plate for this portion of the functional assay. On the –ade plate, empty vector serves as a negative control and YCF-MOD5 serves as a positive control. There is visible growth of *Arabidopsis* transformants. It is not visible on this figure, but there was limited growth of human transformants as well.
Figure 4: Drop test of transformed MT-8 yeast strain on YPD and ½ YPD
Empty vector transformants serves as a negative control and YCF-MOD5 transformants serves as a positive control. There are white sector colonies in the *Arabidopsis* transformants. There are also white sector colonies of the human transformants, but are less prevalent.

3. Confirmation of toxicity of human and *Arabidopsis* MOD5 in yeast cells.
   After transformation of the MT-8 strain of cells the drop test was performed as described. The results are shown below in Figure 5.
Figure 5: Confirmation of toxicity on –ura plates and functional assay results

Empty vector serves as a negative control and YCF-MOD5 serves as a positive control. ½ YPD and YPG plates serve as positive controls for growth. On the ½ YPD plates white sector colonies are seen in the Arabidopsis and human transformants. There is no growth from anything except YCF-MOD5 on –lys plate. The growth of –ade plate is consistent with Figure 3. On the –ura plate there is less growth on the Arabidopsis and human transformants than that of the empty vector and YCF transformants, however showing more growth on the ½ YPD plate.

On the –ura plate there is less growth in Figure 5, which can also be seen in Figure 3. To test if it was indeed toxicity due to the foreign gene cells were cultured and normalized which was then grown on YPD, ½ YPD, and –ura plates.

Figure 6: Illustration of toxicity on –ura plates

On each of the ½ YPD plates there are a similar number of colonies across all transformants while on the –ura plates there are much fewer colonies on the Arabidopsis and human transformants.

Most of the plates were contaminated. Too many colonies appeared on YPG+fluconazole 20 μg/ml plates. Micro colonies appeared only after 2 weeks on YPG+Fluconazole-50 μg/ml plates. The experiment could not be pursued further.

Discussion:

1. Complementation Assay of human and Arabidopsis mod5 in S. cerevisiae

By utilizing the susceptibility of the mod5Δ trm1Δ yeast strain to 5-FU it was shown that the human and Arabidopsis MOD5 are complementary in yeast to some extent. As shown in Figure 2 there is growth of the human and Arabidopsis MOD5 transformants, which is indicative of the foreign MOD5 being active in the yeast. Both the human and Arabidopsis transformants have shown more growth than the negative controls, as well as the strain expressing [MOD+]. The Arabidopsis transformant shows more growth than the human transformants, implying it is more complementary than the human. In addition to showing that the human and Arabidopsis mod5 is complementary in yeast, this also shows that the susceptibility to 5-FU can be used to test if the human and Arabidopsis MOD5 can from prions.

2. Functional Complementation Assay of human and Arabidopsis MOD5 in S. cerevisiae

By utilizing the characteristics of the MT-8 yeast strain (Sup7 repression by [MOD+] or deletion of MOD5) it was shown that human and Arabidopsis MOD5 are functional in yeast to some extent. Figure 3 illustrates the growth portion of the functional assay. Colonies with a functioning MOD5 will grow on an –ade plate. In Figure 2 the Arabidopsis transformant has visible growth. Although not well shown in the figure, the human transformants did have superior growth to that of the empty vector transformants. Figure 4 depicts the results of the color portion of the functional assay. Colonies with functional MOD5 will grow white while those
without will grow red. Both the human and *Arabidopsis* transformants have white sector colonies, showing that the foreign MOD5 is functional in both, to an extent. In both cases, the *Arabidopsis* transformant showed white sectored colonies than that of the human transformant. This shows that the *Arabidopsis* MOD5 is more functional than that of the human MOD5 in yeast.

3. Confirmation of toxicity of human and *Arabidopsis* MOD5 in yeast cells.

Throughout the various experiments there has been a pattern of the human and *Arabidopsis* transformants growing less effectively on –ura than yeast transformed with a plasmid YCF MOD5 containing Yeast MOD5 or an empty vector. This is significant because the growth on –ura should be uniform when compared to the growth of cells on YPD, across the different transformants. This implies that the foreign MOD5 is toxic to the yeast to some degree.

To confirm this, another drop test was performed by transforming a separate MT-8 strain with plasmids previously used. This is shown in Figure 5, which in addition to showing the lesser growth on the –ura plate with the human and *Arabidopsis* transformants, also supports the results from Figures 2 and 3. To show the toxicity of the foreign MOD5 in another way yeast was grown, and then subsequently normalized to ensure the plating of the same number of colonies. The results are shown in Figure 6. The ½ YPD plates all show a similar number of colonies grown across each transformant. On the –ura plates there is a severe difference in growth between the empty vector and YCF MOD5 transformants from the human and *Arabidopsis* transformants. The lack of growth on the –ura plates of the human and *Arabidopsis* transformants shows the toxicity of the genes in yeast.
4. General Discussion

There are several techniques to determine the functionality of MOD5 in yeast, and those techniques were used here to determine the ability of the human and Arabidopsis forms to function in the yeast, such that the proteins can be studied. The use of the MT-8 strain of yeast shows that both human and Arabidopsis MOD5 are slightly functional in yeast; the Arabidopsis form more so than that of the human. The sensitivity of the mod5Δ trm1Δ to 5-FU was exploited to show that the human and Arabidopsis MOD5 is complementary to the native MOD5; Arabidopsis MOD5 once again more so than that of human MOD5.

Despite the evidence showing that the human and Arabidopsis MOD5 allows for the survival of the yeast its ability to be studied for the prionization of the human and Arabidopsis MOD5 may be hampered by the toxicity of those genes. It was shown that the genes are toxic as evident form the results of current work (in Fig 5 and Fig 6).
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