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Determining the function of IFIT1 using experimental evolution in *S. cerevisiae*

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Roxanne Archer

Committee in charge:

Professor Matthew Daugherty, Chair
Professor Rachel Dutton, Co-Chair
Professor Amy Pasquinelli

2018

The Thesis of Roxanne Archer is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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2018

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ABSTRACT OF THE THESIS

Determining the function of IFIT1 using experimental evolution in *S. cerevisiae*

by

Roxanne Archer

Master of Science in Biology

University of California San Diego, 2018

Professor Matthew Daugherty, Chair
Professor Rachel Dutton, Co-Chair

IFIT (interferon-induced proteins with tetratricopeptide repeats) proteins are important components of the innate immune system that are strongly upregulated when the cell detects viral invasion. IFITs act to inhibit the translation of viral mRNA by binding to the 5' cap, preventing translation initiation from occurring. Even though IFITs are vital in preventing viral infection, the mechanism of how IFIT1 binds specifically to viral mRNA remains to be

poorly understood. Although IFIT1 evolved to inhibit the replication of viruses, it can also inhibit the growth of other organisms like budding yeast (*S. cerevisiae*) due to their relative lack of mRNA modifications. To examine how IFIT1 inhibits translation, I performed a genetic screen to select for yeast that grew despite the expression of IFIT1. After several rounds of selection, the mutant yeast strains were tested in spotting assays and have developed the ability to evade the growth inhibition effects of IFIT1. The mutant strains show variability in their ability to grow in the presence a plasmid containing IFIT1 and results from cross-testing the strains against IFIT2 suggest that the phenotypes seen in the spotting assays are specific to IFIT1. Sequencing these yeast strains could provide information about which genes can be altered to prevent IFIT1 from inhibiting translation and that information can then be used to determine what the mechanism of 'non-self' sensing and translation inhibition by IFIT1.

INTRODUCTION:

The innate immune system is responsible for the body's immediate response to pathogen detection, acting to prevent pathogens from entering and infecting the cell (Daugherty & Malik, 2012; Fensterl & Sen, 2015). The cytokine interferon (IFN) is an important part of the innate immune system and it signals for cells to turn on genes that defend against the invasion of pathogens (Fensterl & Sen, 2015; Schneider, Chevillotte, & Rice, 2014; Schoggins, 2014). The interferon family of proteins are categorized into classes I, II, and III based on which cell-surface receptors they bind, with type I and type III acting as the primary antiviral interferons (Fensterl, Chattopadhyay, & Sen, 2015; Schneider et al., 2014). Interferon molecules bind to receptors, starting the Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signaling cascade and resulting in the upregulation of a vast array of interferon-stimulated genes (ISGs) (Fensterl & Sen, 2015; Schneider et al., 2014). The upregulation of these ISGs lead to a profound antiviral state in the cell, with many of the ISGs directly inhibiting important stages of the viral life cycle—such as blocking entry into the cell, preventing production of viral proteins by inhibiting translation, or preventing viral egress (Schneider et al., 2014; Schoggins, 2014). For example, the protein tetherin prevents enveloped viruses like HIV from budding by anchoring the envelope to the cell membrane (Schneider et al., 2014). Although hundreds of genes are upregulated in response to IFN, the functions of the majority of the ISGs remain to be poorly understood (Schoggins, 2014).

Interferon induced proteins with tetratricopeptide repeats (IFITs) are among the most highly expressed ISGs and act to inhibit viral infection by preventing the translation of viral RNA by binding to 'non-self' markers that are not present on host mRNA (Fensterl & Sen,

2010). As the name suggests, IFITs are usually only expressed after interferon stimulation or viral infection and can be found in the cytoplasm, although expression can be induced by other signals like retinoic acid (Diamond, 2014). The tetratricopeptide repeat (TPR) motifs in IFITs, which are made of 34 amino acid sequences that have a helix-turn-helix structure, mediate their interaction with other proteins as well as with RNA (Diamond, 2014). Specifically, within the TPR structure of IFIT1 is a positively-charged cavity that allows the protein to interact with the 5' cap of mRNA and interrupt translation by blocking translation initiation factors from binding to the viral mRNA (Diamond, 2014; Fensterl et al., 2015; Fensterl & Sen, 2015). However, the exact chemical signal that distinguishes 'self' from 'non-self' RNA and allows IFITs to specifically bind to viral mRNA is not yet understood.

Mouse IFIT1B (previously known as mouse IFIT1) has been used as a model for understanding the function and mechanism of IFITs in general. Previous work showed that this protein specifically inhibits viruses that are unmethylated on the 2' position of the ribose sugar of the first transcribed nucleotide (Daffis et al., 2010). While all mammalian mRNAs have a 2'-O-methyl group in this position, known as a Cap1 structure, certain viruses lack this methylation and have what is known as a Cap0 structure. Thus, Cap1 methylation is the critical 'self' mark that allows mouse IFIT1B to inhibit translation of mRNAs from some viruses (Cap0) while allowing translation of host (Cap1) mRNAs (Diamond, 2014). From this work, it was assumed that human IFIT1 would function with similar biochemical and antiviral specificity. However, it was recently discovered that mouse IFIT1B is more evolutionarily related to human IFIT1B, a protein shown that lacks IFN inducibility and is likely non-functional, than human IFIT1 (Daugherty, Schaller, Geballe, & Malik, 2016). This discovery explains previous observations that mouse IFIT1B and human IFIT1 inhibit different viruses;

mouse IFIT1B specifically inhibits only Cap0-containing viruses, while human IFIT1 has been reported to inhibit many other types of viruses (Diamond, 2014). For example, human IFIT1 suppresses the replication of parainfluenza virus 5 (PIV5) (Andrejeva et al., 2013) and vesicular stomatitis virus (VSV) (Daugherty et al., 2016), two viruses that have Cap1-methylated mRNA. Overall, very little is known about the molecular pattern that allows human IFIT1 to inhibit translation or about what types of viruses can be expected to be susceptible to IFIT1 despite the fact that it appears to be important in establishing an antiviral state in cells.

It was previously observed that when IFITs are expressed in yeast cells (*Saccharomyces cerevisiae*), they can dramatically inhibit yeast growth. This is likely due to the fact that yeast lack many of the mRNA modifications that are present in mammalian mRNAs, so IFITs recognize the yeast mRNA as 'non-self' and inhibit its translation (Banerjee, 1980). Mouse, African green monkey, and gibbon IFIT1B behave as expected in the model yeast system because all three inhibit the yeast growth due to the lack of Cap1 modification on the mRNA, but the inhibition can be reversed by co-expressing Cap1 methyltransferase (Daugherty et al., 2016). These results recapitulate what occurs in the mammalian antiviral response: a virus that encodes an active Cap1 methyltransferase is resistant to inhibition by IFIT1B while a virus that lacks Cap1 methyltransferase activity is sensitive to IFIT1B (Daugherty et al., 2016). These data suggest the yeast model system behaves in a similar manner to the human system and that further data obtained from the yeast model system would be applicable *in vivo*. Due to the fact that human IFIT1 also inhibits yeast growth this genetic system could be used to examine molecular factors that are important to IFIT1's activity. Specifically, the genetic screen could be used to determine the

'non-self' pattern that IFIT1 recognizes or identify cellular co-factors that IFIT1 interacts with in order to inhibit the translation of mRNA.

IFIT1's ability to function similarly in both a yeast model system and its native mammalian system is not unique: protein kinase R (PKR), an antiviral factor, also acts in yeast as it does in mammalian cells (Dever et al., 1993). Even though PKR is not native to yeast, much like IFIT1, it functions in both systems to phosphorylate the translation initiation factor eIF2α upon sensing double-stranded RNA in order to prevent translation (Dever, Dar, Sicheri, 2007). The validation of the yeast system for another antiviral factor and suggests that the system should be useful for studying translational control in the antiviral immune system. It is especially promising because IFIT1 also interacts with a translation initiation factor, eIF3 (Fensterl & Sen, 2010), that is conserved in yeast like eIF2α is for PKR, so IFIT1 should still be able to interact with its native co-factors.

One way to use the yeast system to try to elucidate the many aspects of IFIT1 that are still poorly understood is to generate mutant yeast that are resistant to IFIT1's inhibitory effects. Conveniently, it was previously observed that some yeast expressing IFIT1 spontaneously showed restored growth, likely due to mutations in the yeast genome that specifically allowed them to block the action of IFIT1 (M. Daugherty, personal communication). Therefore, selecting for these mutant yeast and then examining which genes were changed can help to elucidate how IFIT1 prevents the translation of 'non-self' mRNA, help to predict which pathogens might be inhibited by IFIT1 based on knowledge of how their mRNA is processed, and may provide insights into the mechanism of other proteins that regulate gene expression at the level of translation.

MATERIALS AND METHODS:

GENETIC SCREEN:

Throughout the experiment, a plasmid (pSB819) was used that expressed human IFIT1 under the control of the inducible gal promoter and URA3 was used as a selectable marker. First, the "wildtype" yeast (BY4741, not a true type of yeast found in the wild but the lab equivalent of a wildtype strain) were transformed with the plasmid containing IFIT1 and allowed to grow on uracil dropout media with glucose for two days. Twelve individual colonies were picked and grown in overnight cultures. For each culture, approximately $1x10^7$ yeast cells were plated onto 15cm uracil dropout plates with galactose to make 12 independent replicates and allowed to grow for six days. From these 12 large plates, up to six individual, medium-sized colonies were selected. Medium colonies were used because it was assumed that small colonies had not accumulated beneficial mutations to evade the action of IFIT1 and large colonies had simply eliminated the plasmid. Any strains that were contaminated were removed. These strains were then plated on media containing 5-FOA (5-Fluoroorotic Acid). Yeast cells can only grow on 5-FOA when they lack the URA3 gene. This step therefore results in selection of yeast that have lost the original pSB819 plasmid. These 'cured' strains were then glycerol stocked to use for later experiments.

The strains were named after which of the original 12 replicates they came from and then which of the six colonies from that were used. For example, strain 2.3 would be the third colony picked from the second replicate.

SPOTTING ASSAY:

After the strains were cured of the plasmid, they were individually retransformed with IFIT1 or empty plasmid, two colonies from the transformations were selected and then tested

in a spotting assay. This involved 10-fold serial diluting two colonies from each transformation in a 96-well plate and using a frogger to spot this onto two glucose (uninduced control) and two galactose (induced IFIT1 expression) plates for technical replicates. This resulted in four comparisons for every selected strain: yeast with IFIT1 on galactose media, yeast with IFIT1 on glucose media, yeast with an empty plasmid on galactose media, and yeast with an empty plasmid on glucose media. It was expected that any strains that had acquired mutations that allowed them to evade the inhibition of IFIT1 would have greater growth on galactose than the wildtype yeast strain. The plates were monitored for five days with pictures taken daily. The strains that rescued against IFIT1, meaning that they had increased growth compared to parental yeast on galactose media, were then cross-tested against human IFIT2 (in the same manner as before) to test if the mutations generated in the screen are specific to IFIT1 or function against IFITs or plasmids in general.

PREPARATION FOR SEQUENCING:

Twenty strains were selected for sequencing as representative examples of each of the replicates and then two strains that failed to rescue against IFIT1. The genomes of the strains were prepared using a kit and then the sequencing library was prepared by Candace Todd.

RESULTS:

Multiple controls were instituted in the protocol of the genetic screen to increase the likelihood of obtaining strains that had meaningful mutations that inhibited the effects of IFIT1. First, twelve independent replicates were used to guarantee that at least twelve separate mutations were made to the yeast. Then, to ensure that the yeast had made mutations in their genomes and not the plasmid, the strains were cured of the plasmid using 5-FOA and then individually re-transformed with the plasmid again. Spotting assays were done using two transformants from each strain to determine if the strain had mutations in its genome that allowed it to evade IFIT1, and, if so, how well that mutation helped to increase the yeast's growth.

There were many potential ways for the strains to have increased growth before or during the spotting assays. Strains could have dealt with the pressure of IFIT1 by eliminating the plasmid or creating mutations in the plasmid that allowed them to have increased growth. In the spotting assays, these strains would have growth similar to wildtype because they would lack any genomic mutations that would block the action of IFIT1. It is also possible that some strains would evade the system that was used to expressed IFIT1, a phenotype that will be discussed later. Strains that successfully created mutations that block the action of IFIT1 could have mutations in genes that could allow the yeast to mimic 'self' markers or, more likely, alter co-factors that IFIT1 binds to. In order to inhibit translation, it has been suggested that IFIT1 binds to eIF3 (conserved in yeast), but the details of this and downstream co-factors remain poorly characterized (Fensterl & Sen, 2010). These co-factors could be altered in the mutant strains to allow them to prevent IFIT1 from inhibiting their growth. Here PKR might be able to act as an example of what to expect because it has been

shown to act in a manner similar to IFIT1. If this screen was used against PKR, it might be expected that eIF2α to be altered because PKR interacts with it in order to inhibit yeast mRNA (Dever et al., 2007).

Out of the 42 strains generated in the genetic screen, only 11 strains failed to rescue, meaning that those strains did not have increased growth when compared to wildtype (Table 1). The remaining 31 strains could be categorized into three broad phenotypic groups based on if the strain grew 10-fold, 100-fold, or 1,000-fold better than wildtype (Table 1). These phenotypes were estimated based on how many more 10-fold dilutions further the strain grew in relation to the wildtype strains. The most common phenotype throughout the whole dataset was for mutant strains to grow 100-fold better than the wildtype yeast, such as with strains 8.1, 10.4, and 14 other strains (Table 1, Fig. 1). The next most common phenotype belonged to strains that grew 1,000-fold better than the wildtype yeast like strains 5.1, 8.5, and eight other strains did (Table 1, Fig. 2). Only five strains, like 4.2 and 9.4, showed relatively low growth compared to the majority of the mutant strains (Table 1, Fig. 1).

Several replicates contained strains that belonged in two or more phenotypic categories. Typically, these replicates had some strains with relatively weak rescue phenotypes while other strains from the same replicate had up to 100-fold more growth than the strains with weaker rescue phenotypes (Table 1). Replicate 4, like replicates 8 and 11, was one such replicate with several different phenotypes. Four of the strains (4.2, 4.3, 4.5, and 4.6) had very similar, weak rescue phenotypes (10-fold more growth than wildtype) while two other strains (4.1 and 4.4) had similar, strong rescue phenotypes (100-fold more growth than wildtype) (Fig. 2, Table 1). Although some replicates had variation in phenotypes, other

replicates, such as 6 and 12, appeared to have only a single phenotype (Table 1). Both types of replicates were common in the dataset.

The strains that rescued when tested against IFIT1 were cross-tested against IFIT2 to see if the mutant yeast also had resistance to the inhibitory effects of IFIT2. Out of the 20 strains that have currently been tested, all have shown increased growth when compared to wildtype (Table 1). IFIT2 appeared to have a weaker inhibitory effect than IFIT1 did on wildtype yeast, so wildtype with IFIT2 grew approximately 10-fold better than wildtype with IFIT1 (Fig. 3). Only two broad classes of phenotypes were observed in the spotting assays against IFIT2: strains that grew 10-fold better than wildtype and strains that grew 100-fold better than wildtype (Table 1). The strains that grew 100-fold better against IFIT2 had varied rescue strengths against IFIT1. For example, strains 4.6 (grew 10-fold better against IFIT1) and 8.5 (grew 1,000-fold better against IFIT1) had similar rescue strengths against IFIT2 (Fig. 3, Table 1). Only 5.1 and 6.2 grew 10-fold better than wildtype and these two strains were also the only strains that had a stronger rescue phenotype in the assay against IFIT1 (Table 1), an example of which can be seen in Figure 3.

Interestingly, two strains, 2.2 and 11.3, had what we called a ‘gal defect’ (Table 1). It was named this because the strains grew normally on glucose media but had unusual growth on galactose media regardless of if an IFIT was being expressed. Typically, strains grew like wildtype did on both glucose media and galactose media with strains transformed with an empty plasmid (WT and 2.6 in Fig. 4), but the gal defect strains had identical growth on both empty and IFIT1 galactose plates (2.2 in Fig. 4). Strain 2.2 grew as much as wildtype on glucose media (no IFIT1 or 2 expression), appeared to rescue on galactose media (induced IFIT1 or 2 expression), but had nearly the same growth on galactose media with only an

empty plasmid (Fig. 4). None of the strains from the same replicates as the gal defect strains showed any sign of having the same phenotype. Unsurprisingly, this pattern of growth remained consistent regardless of if the yeast expressed IFIT1 or 2.

After the strains were tested in the spotting assay against IFIT1, 20 strains, as well as the wildtype BY4741 strain, were selected and prepared for sequencing. The strains were selected so that every replicate was represented by strains that showed the diversity of rescue phenotypes from that replicate. Two of the strains prepared for sequencing failed to rescue against IFIT1 and acted as a control while the other 18 rescued against IFIT1(Table 1). Unfortunately, while the samples were being prepared for sequencing, nine had their concentrations drop below the usable threshold and so they were not sequenced (Table 1), although we could prepare these samples again and sequence them in the future. The raw sequencing data has been returned but computational protocols for how to process the data have not been prepared, and so the sequencing data is not yet ready for full analysis. We anticipate these data will be processed and mutations will be identified in the next several months.

DISCUSSION:

The results of the spotting assays suggest that the genetic screen was successful in generating mutants that are resistant to IFIT1. The majority of the strains, 31 out of 42 total strains that were generated in the screen, were able to grow despite the expression of IFIT1. This suggests that the yeast did have mutations in their genomes that allowed them to block the action of IFIT1 or otherwise allowed them to evade the expression of IFIT1 (Table 1). It was an important step to cure the selected strains of the original plasmid and retransform them, as this allowed us to eliminate cases where the yeast genome itself did not have the mutation desired. Such was the case with strains 1.5 and 5.2, among others (Table 1), which grew as poorly as wildtype yeast in the spotting assay (failed to rescue) after retransformation with IFIT1. Assuming that the genetic screen created mutations in the yeast that specifically block the action of IFIT1, strains that have a stronger rescue phenotype than others would have a mutation that is more effective at inhibiting IFIT1. Hopefully, the variation in the rescue phenotypes mean that the strains evolved unique ways of handling the pressure put on them by IFIT1.

Although the strains had variable phenotypes overall, some individual replicates had lower levels of variation. In some replicates, such as replicates 6 and 12, all the strains appeared to have identical phenotypes (Table 1). Given that all strains from any replicate came from a single colony that had been exposed to IFIT1, it is more likely that the shared phenotype in these replicates is due to a mutation that was present in that colony, was passed down, and was shared by all the strains. The alternative and less probable explanation is that of multiple strains separately developed mutations that all resulted in the same phenotype. Several other replicates, such as replicates 4 and 8, had some strains that appeared to have

identical phenotypes while one or two other strains had stronger phenotypes (Table 1, Fig. 2). Once again, the strains that share a common phenotype likely also share a common mutation that weakly blocks the action of IFIT1 and the other strains have gained an additional mutation that more effectively blocks IFIT1, either on its own or in conjunction with the common mutation.

Several unrelated strains in the data set had nearly identical phenotypes, such as 8.1 and 10.4 (Fig. 1). The similarities between these strains might suggest that multiple different mutations have comparable effects on the phenotypes or comparable effects on. It is possible, that separate replicates had mutations in the same gene, but this is unlikely due to the fact that there are numerous genes that could have been changed. Therefore, strains with similar phenotypes but from different replicates most likely also had different mutations. So, strains like 5.1 and 8.5, which both grew 1,000-fold better than wildtype (Table 1, Fig. 1), probably have different mutations that convey similar fitness advantages to the yeast.

The results from cross-testing the strains against IFIT2 currently provide the best evidence that the results seen in the spotting assays are due to IFIT1 and not the yeast's response to a detrimental protein or plasmid. This is indicated by the apparent specificity of the rescue phenotypes to IIFT1 and dissimilar rescue phenotypes against IFIT2. All but two strains had virtually identical rescue phenotype against IFIT2 regardless of the variation present in the rescue phenotypes against IFIT1, so disparate strains like 4.6 (10-fold phenotype) and 8.5 (1,000-fold phenotype) had the same phenotype against IFIT2 (Fig. 3). The fact that rescuing against IFIT1 does not predict how a strain will rescue against IFIT2 suggests that the strains have not simply evolved in a way that makes them resistant to any IFIT or poisonous plasmid. If they were simply responding to a plasmid, we would expect the

results to be exactly the same for IFIT1 and 2 or that there would be a clear pattern that could predict one phenotype from the other (such as all strains that rescue against IFIT1 rescue 10-fold better against IFIT2). Because this is not how the results appear, it can be reasonably assumed that the response is specific to IFIT1.

Despite the fact that many of the strains have different responses to IFIT2 than IFIT1, just over one half of the strains tested against IFIT2 have the same phenotype when tested against IFIT1 (Table 1). It is possible that these strains had mutations that responded to IFITs in general or the presence or a detrimental protein. For instance, we expect that IFIT1 and IFIT2 may have different 'non-self' recognition patterns and may have different interactions with cellular factors, but both proteins have been described to inhibit translation (Fensterl & Sen, 2015). Thus, a shared rescue phenotype against IFIT1 and IFIT2 could be general rescue against any toxic protein or through a specific translation rescue, with either result being potentially interesting mechanistically. This could be tested by repeating the spotting assays again with another protein that inhibits mRNA translation (e.g. PKR) or some other protein that inhibits yeast growth through other mechanisms (e.g. production of a drug or toxin). Depending on the outcome of these assays, it can be concluded how yeast have evolved to resist IFIT1. While the general translation rescue, or rescue against any toxic protein, is not of interest for IFIT1 specific functions, these mechanisms of resistance may not have been previously characterized so might be quite informative.

One extreme example of the general rescue was seen with the strains that showed a gal defect. This phenotype, observed in two of the strains, appears to be the result of the system used to perform the genetic screen and not evidence that the screen failed. The contents of the plasmid do not have an effect on the strange phenotype, which is demonstrated by the fact that

the same growth on galactose media was seen both with an empty plasmid, a plasmid with IFIT1, and a plasmid with IFIT2 (Fig. 4). The defect was seen in galactose media but not glucose media, so the phenotype is likely induced by galactose or the lack of glucose. Given that IFIT1 expression was driven by a gal promoter, it is possible that the two strains with the gal defect found a way to bypass the expression of IFIT1 by altering their growth on galactose. This caused a strong 'rescue' phenotype, but also caused the strains to not grow as well in the presence of galactose. None of the strains from the same replicate had similar phenotypes as the gal defect strains, so it is likely that this mutation was acquired in both strains after the replicates had been split up into individual strains. Overall, evidence around the gal defect strains suggests that this phenotype is not a true rescue against IFIT1 but simply an artifact of the alternative mechanism the yeast used to block the action of IFIT1. While we can predict what mutations might lead to such a phenotype, our selection may reveal additional mutations that can control the gal system, highlighting the power of an unbiased screen to identify interesting routes of evolution.

Although many pieces of evidence suggest that the genetic screen had the desired effect, without sequencing data, it is not truly possible to confirm that the yeast strains have created mutations that specifically block the action of IFIT1. It is entirely possible that the yeast have simply created ways of degrading the IFIT1 mRNA or protein in order to prevent it from harming the yeast. This hypothesis could be easily tested by measuring the levels of IFIT1 mRNA and protein in the mutant strains and wildtype yeast. If the mutant strains have decreased levels of either the mRNA or protein of IFIT1, that suggests that the spotting assay results from that strain are at least partially due to the degradation of IFIT1 before it was able to act to inhibit the translation of the yeast mRNA. It is possible that mutations that directly

inhibit the action of IFIT1 and mutations that increase the degradation of IFIT1 could play a part in the results seen in the spotting assays.

Overall, the data suggest that the genetic screen was likely successful at creating strains of yeast that have mutations in their genomes that specifically allow the action of IFIT1 to be blocked in some manner. The variation of rescue strengths both between and within replicates indicates that there are likely several different mechanisms behind the varying abilities to inhibit the effects of IFIT1. Unfortunately, the data from the spotting assays alone are not sufficient to address the question of what 'non-self' marker IFIT1 recognizes, but this question can be more fully addressed once the sequencing results are analyzed. Genes that were found to have mutated in comparison to the wildtype strain can be identified as genes of interest that might have an effect on how IFIT1 can function. If these genes have mammalian equivalents, the same mutations can be put into mammalian cells and a virus that is susceptible to IFIT1 inhibition can be used to test if the mutant gene is able to block the effects of IFIT1. If the introduction of the mutant gene increases viral replication, then it would confirm the results seen in the spotting assay and this information could then contribute to the understanding of how IFIT1 acts to inhibit the translation of viral mRNA.

Despite the fact that the success of the genetic screen cannot yet be determined, the effects of the mutations can be speculated about. There are two types of modifications that might have been made: changes in co-factors that IFIT1 interacts with or changes in the 5' cap so that IFIT1 can't bind to it. However, little is currently known about IFIT1's co-factors beyond the fact that it might interact with eIF3, so possible mRNA modifications are easier to speculate about (Fensterl & Sen, 2010). Recent crystal structures of IFIT1 indicate that the positively charged cavity, the part of IFIT1 that is responsible for interactions with mRNA,

can have relatively flexible interactions with the 5' cap (Abbas et al., 2017). Additionally, it has been discovered that both cap1 and cap2 methylation of mRNA can interfere with IFIT1 binding, but cap1 methylation alone is not sufficient to prevent IFIT1 from binding (Abbas et al., 2017; Young et al., 2016). Some other studies show that the addition of a guanosine nucleoside to the mRNA cap can increase IFIT1 binding affinity, suggesting that it might play a role in how IFIT1 recognizes foreign mRNA (Young et al., 2016). It has even been suggested that IFIT3 interacts with the C-terminus of IFIT1 to form a heterodimer that stabilizes IFIT1 and increases IFIT1's binding affinity to cap0 mRNA (Fleith et al., 2018; Johnson et al., 2018). The many different modifications and interactions that have been implicated in IFIT1's interactions with mRNA suggests that IFIT1 recognizes more than one modification on mRNA. If this is true, the strains from the genetic screen would need to create or mimic multiple signals on the mRNA that IFIT1 would recognize as 'self' in order to prevent IFIT1 from inhibiting translation. The strains that most effectively get rid of 'non-self' signals would be strains that have strong rescue phenotypes because they would be the ones most able to block IFIT1's inhibitory effects.

FIGURES AND TABLES:

Table 1. Summary of spotting assay results for all strains. For each assay, at least two independent transformants of each strain were tested in technical duplicates as described in Methods. Strains that successfully rescued were cross-tested against IFIT2. For each strain, an estimate for the increased growth of the strain when compared to wildtype was made. Strains that failed to rescue are marked by a line (—) and strains that were not tested are left blank in the appropriate columns. All strains with an (*) were sequenced while all strains with a (^) were prepared for sequencing but were not sequenced due to low concentrations.

Strain	IFIT1 Rescue	Est. Fold Increase in Growth (IFIT1)	Gal Defect	IFIT2 Rescue	Est. Fold Increase in Growth (IFIT2)	Strongest Rescue with which IFIT
1.1	No	———	No	———	———	———
1.3*	Yes	1,000	No	Yes	100	same
1.5	No	———	No	———	———	———
1.6	No	———	No	———	———	———
2.1	No	———	No	———	———	———
2.2^	Yes	1,000	Yes	Yes	100	same
2.6*	Yes	100	No	Yes	100	same
4.1*	Yes	100	No	Yes	100	IFIT2
4.2	Yes	10	No	Yes	100	IFIT2
4.3	Yes	100	No	Yes	100	IFIT2
4.4*	Yes	100	No			
4.5^	Yes	10	No	Yes	100	IFIT2
4.6	Yes	10	No	Yes	100	IFIT2
5.1*	Yes	1,000	No	Yes	10	IFIT1
5.2*	No	———	No	———	———	———
5.3	No	———	No	———	———	———
6.1^	No	———	No	———	———	———
6.2*	Yes	1,000	No	Yes	10	IFIT1
6.3	Yes	1,000	No	Yes	100	same
6.4	Yes	1,000	No	Yes	100	same
6.5	Yes	1,000	No			
6.6	No	———	No	———	———	———
7.1*	Yes	1,000	No	Yes	100	same
7.3	No	———	No	———	———	———
7.4	Yes	100	No	Yes	100	same
8.1	Yes	100	No			
8.2	Yes	100	No			
8.3*	Yes	100	No	Yes	100	same
8.5*	Yes	1,000	No	Yes	100	same
8.6^	Yes	100	No	Yes	100	same
9.3^	Yes	100	No	Yes	100	same
9.4	Yes	10	No	Yes	100	IFIT2
9.6	No	———	No	———	———	———
10.1	No	———	No	———	———	———
10.4^	Yes	100	No	Yes	100	IFIT2
10.6	Yes	100	No			
11.1^	Yes	10	No	Yes	100	
11.2^	Yes	100	No	Yes	100	
11.3^	Yes	1,000	Yes	Yes	100	
12.1*	Yes	100	No	Yes	100	
12.2	Yes	100	No	Yes	100	
12.5	Yes	100	No	Yes	100	

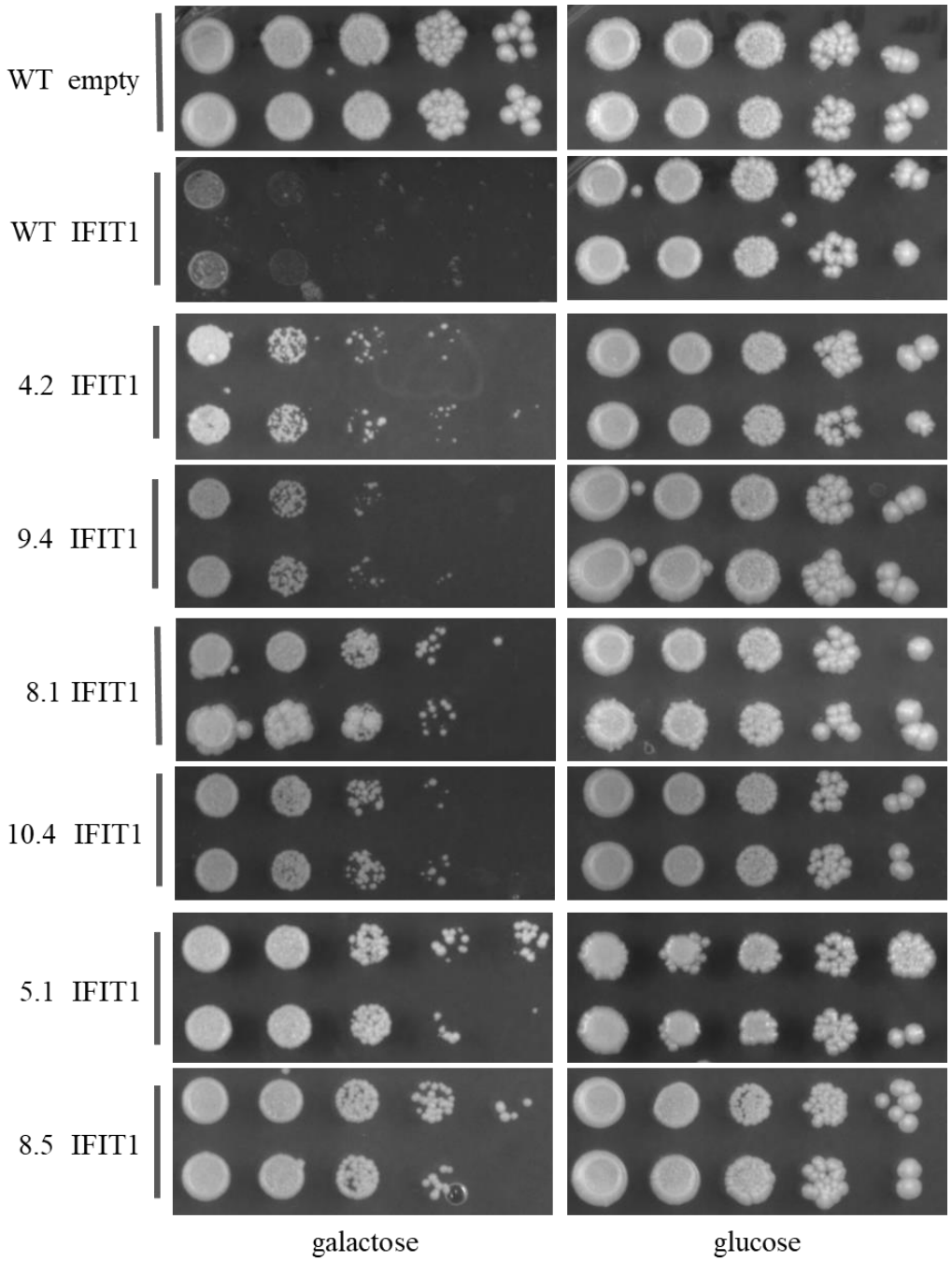

Figure 1. Variation of rescue phenotypes between replicates of mutant *S. cerevisiae* strains tested against IFIT1. Strains of yeast generated in the genetic screen and re-transformed with IFIT1 and wildtype transformed with IFIT1 are 10-fold serial diluted and then spotted on to plates that induce IFIT expression (galactose) or do not induce IFIT1 expression (glucose). Each row represents an independent transformant. Only one of the technical replicate plates is shown. The strains are roughly organized in order of increasing growth when compared to wildtype.

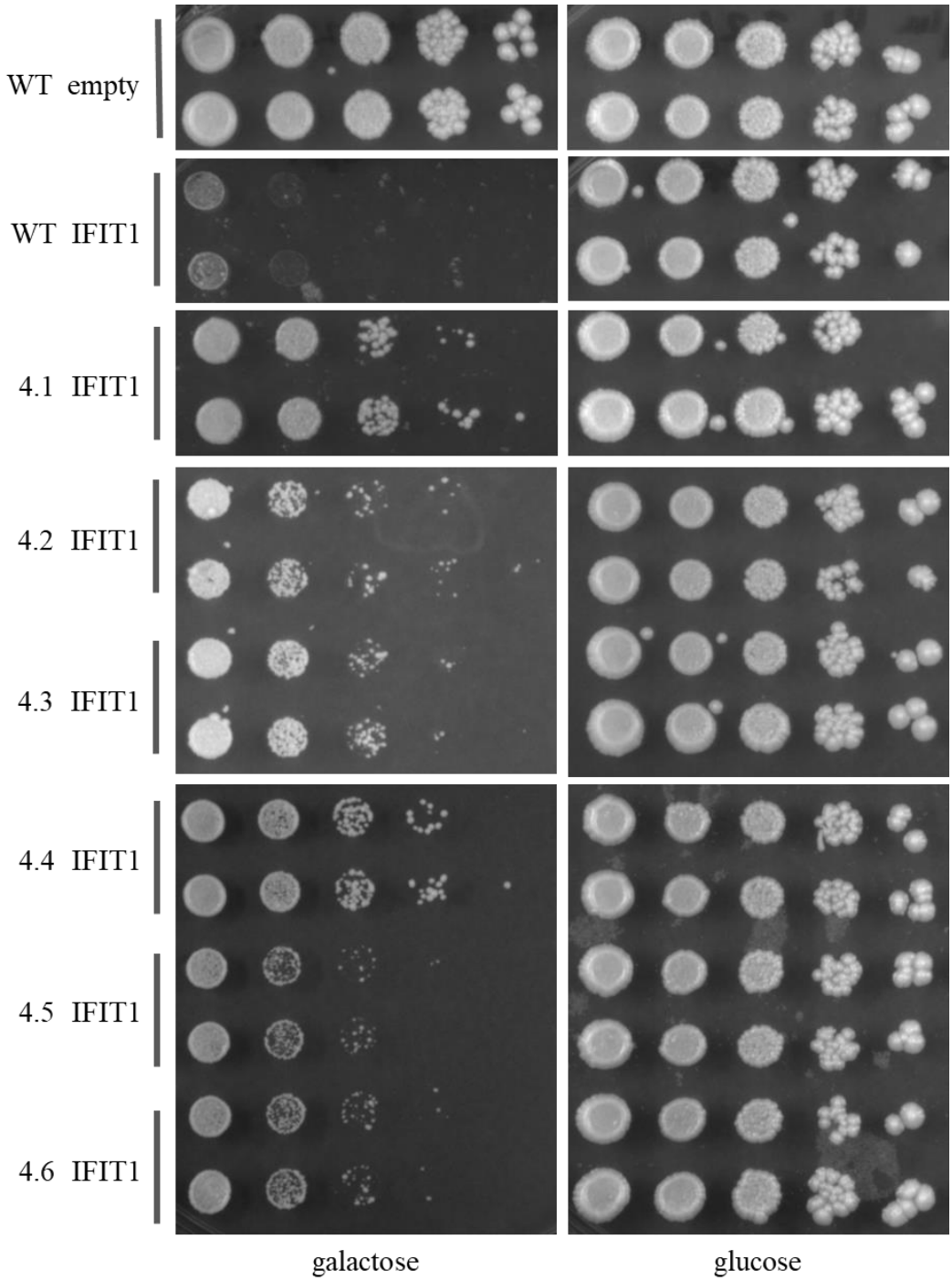

Figure 2. Variation of rescue phenotypes within replicates of mutant *S. cerevisiae* strains tested against IFIT1. Spotting assays were performed as described in Figure 1. All strains from replicate 4 are shown to demonstrate the phenotypic variation present within replicates.

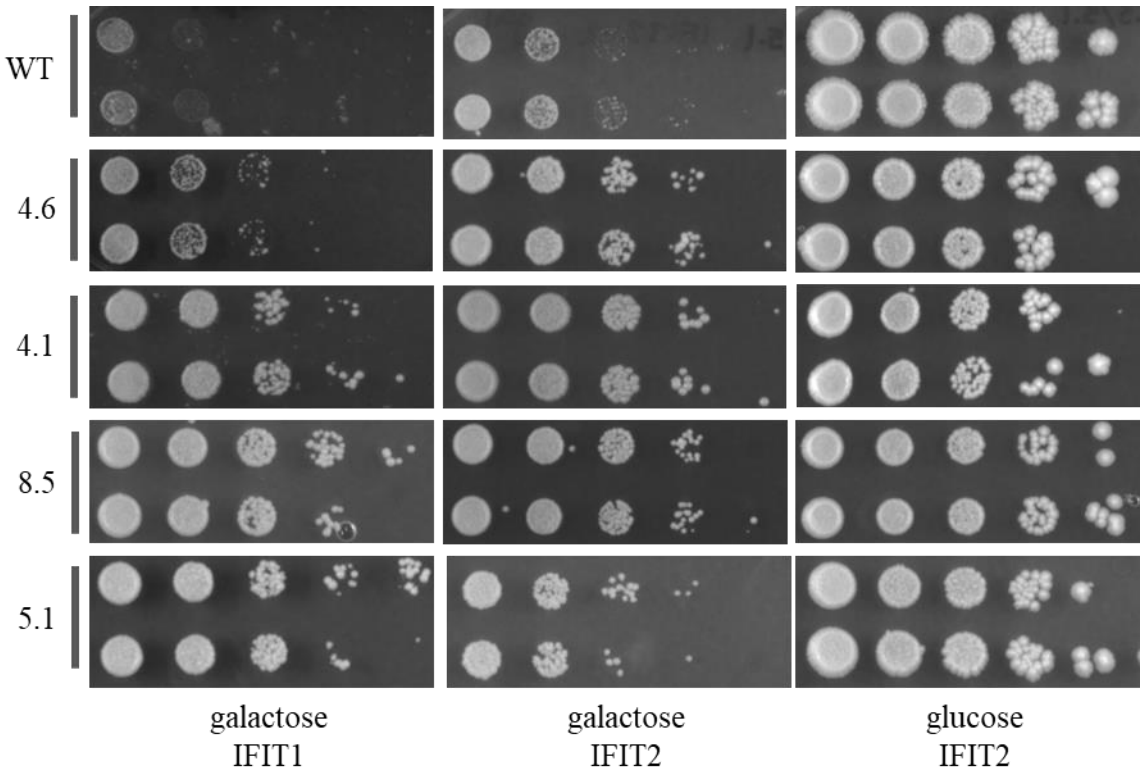

Figure 3. Examples of rescue phenotype comparison for IFIT1 and 2 spotting assays with mutant *S. cerevisiae* strains. Spotting assays performed as in Figure 1 for the images on the left. For the two panels on the right, spotting assay were performed as in Figure 1 except IFIT2 is used instead of IFIT1. Strains with varied IFIT1 rescue phenotypes are shown next to the cross-testing results against IFIT2. The strains are roughly organized in order of increasing growth in IFIT1 spotting assays.

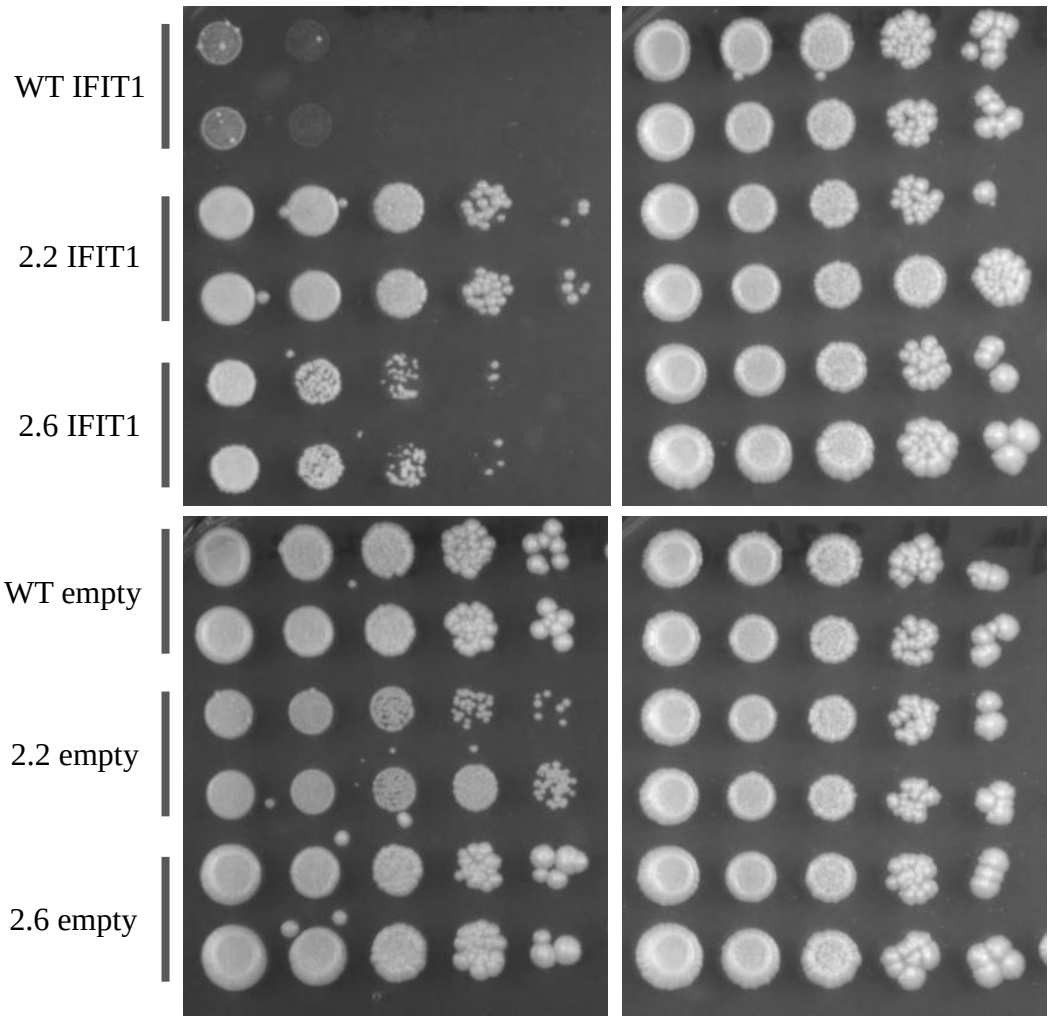

Figure 4. Gal defect decreases growth on galactose media regardless of IFIT expression. Spotting assay were performed as in Figure 1, plates with empty plasmid are shown at bottom to demonstrate the growth effect of the gal defect. The gal defect strains like 2.2 have normal growth on glucose plates but the same decreased growth on galactose plates with an empty plasmid or with IFIT1.

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