Screen of post-translational modifying enzymes for their effects on translation

Elliot Martin, Siu Wah Wong-Deyrup, Gaby Fuchs, and Prashanth Rangan

Abstract

The ribosome has been demonstrated to play an active role in regulating translation. We hypothesized that post-translational modifications on ribosomal proteins might modulate the activity of the ribosome. Therefore, we attempted to screen for enzymes known to deposit post-translational modifications with the goal of identifying genes that play a role in regulating translation using a dual-luciferase assay which monitors the relative amount of Internal Ribosome Entry Site (IRES) translation compared to cap-based translation. We identified several genes that had a significant effect on the translation of the dual luciferase reporter, however, these affects were modest. We also found that ovaries enriched for stem cells and stem cell daughters had significantly higher relative IRES translation, however, we did not observe the expected result when we knocked down RpS25, which is known to be required for efficient IRES translation. Due to these limitations, we did not pursue these findings further.

Introduction

In the past, ribosomes have been considered as passive players in the process of translation. However, evidence has begun to accumulate that ribosomes can play a regulatory role in translation [@goscinskaEvolvingRoleRibosomes2020]. There are several mechanisms through which ribosomes have been found to regulate translation. One example of this is Rack1 which is a core ribosomal protein [@gerbasiYeastAsc1pMammalian2004]. Despite the role of Rack1 as a core ribosomal protein, it is dispensable for cell viability, but required for efficient Internal Ribosome Entry Site (IRES) translation [@coyleDirectLinkRACK12009; @lafontaine2020ribosomal; @majzoubRACK1ControlsIRESMediated2014]. Furthermore, Rack1 activity can be regulated through post-translational phosphorylation which can be exploited during viral infection to allow for translation initiation on polyA-leader sequences [@jhaTranskingdomMimicryUnderlies2017].

Other mechanisms of translation control involving the ribosome have not been conclusively demonstrated but show promise. For instance, under stress conditions, ribosomal proteins have been shown to carry different post-translational modifications than under basal conditions [@jhaTranskingdomMimicryUnderlies2017; @mukhopadhyayDAPKZIPKL13aAxisConstitutes2008; @simsekEmergingRoleRibosome2017]. The modifications in some cases can alter the propensity of the ribosome to translate certain mRNAs, which could allow the cell to better adapt to varying conditions [@jhaTranskingdomMimicryUnderlies2017].

There are two main modes of translation initiation, canonical cap-based translation and cap-independent translation. Cap-based translation is a complex many-stepped process in which the 43S ribosome pre-initiation complex (43S PIC) is recruited to an mRNA through interacting with cap-binding protein (eIF4E) as well as other components [@jacksonMECHANISMEUKARYOTICTRANSLATION2010]. This initiates scanning where the 43S PIC moves along the mRNA until a suitable start codon is identified and translation begins once a 60S ribosomal subunit is recruited [@jacksonMECHANISMEUKARYOTICTRANSLATION2010]. Translation can also be initiated cap-independently [@jacksonMECHANISMEUKARYOTICTRANSLATION2010]. Several mechanisms can allow translation to occur without the presence of eIF4E, but of interest to this work is IRES-based initiation [@shatskyCapIndependentTranslationWhat2018]. IRESs are secondary structures of RNA of which several classes have been described which are classified based on the initiation factors they require as well as their secondary structure [@mailliotViralInternalRibosomal2018]. IRESs bypass at least the use of eIF4E and can bypass the use of any initiation factors at all, requiring only the 40S and 60S ribosomal subunits to perform initiation [@mailliotViralInternalRibosomal2018; @shatskyCapIndependentTranslationWhat2018]. In general, IRESs are used at a higher rate under stress conditions such as those initiated by viral infection, but also under other stress conditions [@mailliotViralInternalRibosomal2018; @shatskyCapIndependentTranslationWhat2018]. However, how cellular stress changes the usage of IRES translation vs cap-based translation is not fully understood.

We performed a screen to attempt to identify enzymes that post-translationally modify proteins and have the potential to alter the translation landscape. To perform this screen, we used the *Drosophila* ovary as it has several advantages. First, *Drosophila* have thousands of available RNAi lines that can be used to knock down genes of interest [@delvallerodriguezPowerToolsGene2012]. Second, many of the genes we chose to screen are histone modifying enzymes that may be required for cell survival, by using the ovary as our tissue of interest, we were able to deplete those genes in the germline without affecting the viability of the flies. The last major advantage to working in the *Drosophila* ovary in this case is that endogenous control elements have been identified allowing for expression of a reporter specifically in the germline [@rorthGal4DrosophilaFemale1998; @seranoElementTransformationVectors1994]. This in combination with germline specific RNAi allowed us to assay for changes in translation only in the germline tissue.

Results and Conclusions

To assess the status of translation we adapted a dual luciferase assay commonly used to monitor translation status. This reporter contains two ORFs encoded in a single transcript which allowed us to assay for changes in translation while eliminating confounding differences in transcription. The reporter transcript was under the control of a Nos promoter, which is only expressed in the germline. The reporter had a Nos 5’UTR and a K10 3’UTR which have both been demonstrated to have a neutral effect on translation [@gavisLocalizationNanosRNA1992; @gavisLocalizationNanosRNA1992; @seranoElementTransformationVectors1994]. The first ORF encodes *Renilla* luciferse under the control of the Nos 5’UTR and its production monitors for cap-based translation, this ORF is followed by hairpins to prevent ribosomal read-through. The second ORF encodes a firefly luciferase under the control of the Cricket Paralysis Virus intergenic (CrPV) IRES . These luciferases can be monitored sequentially as they require different conditions in order to produce luminescence and in this way can be used to monitor changes in the relative amount of IRES to cap-based translation.

We performed a screen of 35 genes known to perform post-translational modification to determine if any of them played a role in regulating IRES vs cap-based translation. We also screened several control genes to ensure our reporter system was working as intended. We knocked down RpS25 which is known to be required for IRES, but not cap-based translation, however, we did not find a statistically significant difference between the knockdown and control ovaries [@hertzRibosomalProteinS252013; @landryRPS25EssentialTranslation2009]. There are a several explanations of this finding. First, knockdown of RpS25 had a severe phenotype, which was unexpected as previous work indicated that RpS25 is required for IRES, but not cap-based translation [@landryRPS25EssentialTranslation2009]. Therefore, it could be that developmental differences between the control and RpS25 RNAi masked any change in relative IRES translation. Second, it is possible RpS25 is not efficiently depleted or that the RNAi line used has off-target effects. Because of this lack of a result from RpS25 RNAi, our positive control, the remainder of our findings must be taken in context. Several genes were found to have a significant affect on the amount of relative IRES translation, including Not, HDAC3, Lsd1, nsl-1, and Set8.

We additionally included two developmental controls to determine if a phenotype which enriched for stem cells (UAS-*tkv*) or stem cell daughters (*bam* RNAi) would alter the relative IRES translation . Indeed, we found that the relative IRES translation was slight, but significantly elevated. This could suggest that IRES translation occurs more in the stem cells and stem cell daughters of the ovary.

Lastly, we identified five genes that when knocked down resulted in significantly lower relative IRES translation, including *Set8*, *nsl-1*, *Lsd1*, *HDAC3*, and *Not*. However, the affect size observed when these genes were knocked down was modest, with Set8 having the most dramatic affect with a 23.6% reduction in the relative amount of IRES translation. However, it should also be noted that as is widely accepted, no multiple test-correction was performed as for screens multiple-test correction leads to a high degree of type I error and screens typically are followed by confirmatory studies [@gausInterpretationStatisticalSignificance2015]. In this case the results have not been followed-up for the aforementioned limitations regarding RpS25 as well as how the subtle changes in relative IRES translation were.

As one of the largest changes to relative IRES translation was in *bam* RNAi, which increased relative IRES translation by 23%, it could indeed be that IRES translation is regulated over the course of stem cell differentiation. Future work should be aimed at this aspect of these findings. A spatial version of this reporter could be made using fluorescent reporters to assay the relative IRES translation over stem cell differentiation in the ovary.

Methods

Flylines

Generated by this work: *nosGAL4*; *dLuciferase*

RNAi lines used in screen:

|  |  |
| --- | --- |
| Protein Target | Line Number |
| Gpp | v110264 |
| D12 | v29954 |
| Art8 | v100228 |
| Su(z)12 | 33402 |
| Sgf29 | 36637 |
| Atac2 | 32890 |
| CG2051 | 34730 |
| chm | 32484 |
| Tip60 | 28563 |
| Set1 | 33704 |
| chm | 32484 |
| Atac2 | 53918 |
| Lsd1 | V16235 |
| Ada3 (dik) | 28905 |
| Caf1 | 31714 |
| CG10289 | 35597 |
| Spt7 (comr) | 42552 |
| E(z) | 36068 |
| Enok | 29518 |
| faf | 35728 |
| Gcn5 | 33981 |
| Hdac3 | 31633 |
| Jarid2 | 32891 |
| Ubp8 (not) | 28725 |
| nsl1 (wah) | 32561 |
| Polybromo | 32840 |
| Set8 | 35322 |
| Su(var)205 | 33400 |
| Sxc | 50909 |
| Trr | 29563 |
| Wda | 31125 |
| Rps25 | V101342 |
| LRRK | 32457 |
| S6K | 41895 |
| S6KII | 41895 |
| TKV | 36537 |

Methods

Cloning

A gBlock corresponding to a codon optimized version of *Renilla* and firefly luciferase as well as the readthrough block and CrPV IGR IRES were purchased from IDT. This gBlock was cloned using Gibson cloning according to manufacturer’s instructions (#E2611S) into pCasper2 containing a Nos promoter and K10 3’UTR. Colonies were picked, cultured, and plasmids purified and sequenced by Eton Bioscience Inc. to conform the correct sequence had been cloned. A midi-prep scale of the plasmid was prepared using standard methods and plasmids were sent to BestGene Inc. for microinjection.

Dual luciferase assay

Ovaries were dissected on 1X PBS in microcentrifuge tubes. Excess PBS was aspirated and ovaries were stored at -20°C. Ovaries were lysed in 25 µl of passive lysis buffer (Promega) using a plastic pestle. 20 µl of lysate was added to a white 96 well plate and luminance was measured using a Glomax 20/20 luminometer (Promega) using the dual luciferase assay reagent (Promega #E1910). At least three biological replicates for Firefly/*Renilla* luminescence values of each RNAi line were generated.