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TITLE: Translational Targets of Ribosomal Protein RPL13 as Novel Cardiac Drivers of Differentiation in Drosophila and Human iPSCs: Implications for CHD

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14. ABSTRACT

There is a need to identify novel genetic networks and pathways driving Congenital Heart Disease (CHD). Our research is aimed at utilizing an unconventional gene involved in translation, the large ribosomal subunit RpL13, to extract and identify novel players and mechanisms in heart development with implications for CHD. During year one, we performed preliminary staining of fly embryos using various cardiac markers and consistent with our hypothesis, we observed changes in the proportion of cell types, suggesting that cells are undergoing cell fate switches. We therefore refined our single-cell RNAseq protocol to collect and enrich for fly cardioblasts from controls and RpL13 knockdown flies and set parameters for FACS sorting. We created 10X Genomic libraries which are currently being sequenced. We will analyze the data as soon as we receive them. While we have not been able to move forward with experiments in human Multipotent Cardiac Progenitors due to lack of access, we have recently secured a new source for these cells and are excited to perform transcriptomic and proteomic analysis on these cells. We have also come up with an alternative approach that develops a new genetic tool in Drosophila that will enable us to capture the translatome with up to single-cell resolution. This would allow for better comparisons between transcriptomics and translatomic changes in fly cardioblasts.

15. SUBJECT TERMS
Congenital Heart Disease, RpL13, ribosome, translation, differentiation, cell identity, profiling, diagnosis, single-cell RNAseq, Drosophila, iPSCs

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1. **INTRODUCTION:** Our research is focused on identifying novel genes and pathways involved in Congenital Heart Disease pathogenesis by focusing on the role of the large ribosomal subunit *RpL13.* We had demonstrated that the knockdown of large Ribosomal Protein RpL13 in the *Drosophila* heart and human Multipotent Cardiac Progenitors (MCPs) led to cardiac-specific defects, and therefore, we hypothesized that *RpL13* could be used as an unconventional gene candidate to identify a novel genetic network regulating cardiac development and pathogenesis. This proposal aims to 1.) identify the consequences of *RpL13* knockdown on the transcriptome and 2.) to identify the translational targets of *RpL13.*

2. **KEYWORDS:** Congenital Heart Disease, RpL13, ribosome, translation, differentiation, cell identity, profiling, diagnosis, single-cell RNAseq, Drosophila, iPSCs

3. **ACCOMPLISHMENTS:**

o **What were the major goals of the project?**

The major goal under AIM1 is to obtain a transcriptomic profile of *Drosophila* cardioblasts (Major Task 1) and human Multipotent Cardiac Progenitors (Major Task 2) with single-cell resolution to track emerging cell identities that are altered by RpL13 knockdown. Analysis of the transcriptomic profiles will uncover altered expression of genes and pathways induced by RpL13 knockdown, resulting in changes in cell fates and heart morphogenesis.

The major goal of AIM2 is to use proteomic methods to identify the changes in overall translation (Major Task 3 and 4) and to identify the specific translational targets affected by RpL13 knockdown (Major Task 5). This will inform us of the selectivity of RpL13-bound ribosomes in targeting translation and will provide a snap shot of the resulting translatome/proteome.

Specific Aim 1: Single-cell transcriptomics for population mapping of FACS-sorted *Drosophila* cardioblasts and human Multipotent Cardiac Progenitors, to track emerging cell identities and how they are altered as a result of RPL13 knockdown.	**Timeline**	**Site 1**	**Progress (%)**
Major Task 1: Molecular Characterization of *Drosophila* cardioblasts by single cell-RNAseq	Months	SBP	
Subtask 1. **RNA-seq of Stage 16-17 embryonic cardioblasts.** To determine whether cardioblasts have undergone a transformation in molecular/cardiac identity following *RpL13* knockdown.	1-3	Dr. Schroeder	50%
Subtask 2. **Single-cell RNA-seq of Stage 16-17 embryonic cardioblasts.** Obtain molecular signatures of individual cells and determine whether subpopulations of cells respond differentially to the KD of *RpL13*.	2-5	Dr. Schroeder	100%
Subtask 3. **Computational Analysis of scRNA-seq data, generation of genetic map and verification of candidate genes by ISH and antibody staining.** Select differentially expressed genes as indicated by RNA-seq and visualize gene expression changes in the embryo.	5-9	Dr. Schroeder	75%
Milestone(s) Achieved: Identified pathways and genes that are altered in expression following *RPL13* KD in Drosophila cardioblasts.			
Major Task 2: Molecular characterization of cardiac progenitors in Multipotent Cardiac Progenitors by			

single cell-RNAseq			
Subtask 1. **Single-cell RNA-seq of Multipotent Cardiac Progenitors.** Time course following *RPL13* siRNA treatment to determine changes in the transcriptomic landscape of the heterogenous cell population.	6-10	Dr. Schroeder	70%
Subtask 2. **Computational Analysis of scRNA-seq data** and comparison of pathways affected by knockdown of *RPL13* between Drosophila and human MCPs.	10-14	Dr. Schroeder	0%
Milestone(s) Achieved: Identify various subpopulations of cells in MCP cultures and attach a molecular signature to each population. Identify key pathways altered by *RPL13* knockdown. Compare pathways between Drosophila and MCPs and look for parallels between species.			
Specific Aim 2: Polysome Profiling to identify direct and indirect translational targets of *RPL13* in human Multipotent Cardiac progenitors, leading to construction of a *RPL13*-centric genetic network driving cardiac differentiation.			
Major Task 3. Measure overall Protein Translation in MCP and *Drosophila* Cardioblasts following *RPL13* knockdown. Puromycin protein synthesis quantification assay to measure protein production following *RPL13* knockdown.	12	Dr. Schroeder	40%
Milestone Achieved: Determined how *RPL13* KD affected overall protein translation in MCPs and *Drosophila* cardioblasts.			
Major Task 4. **Ribosomal Protein Quantification in MCPs**			
Subtask 1. **Polysome isolation from MCP cells treated with RPL13 siRNA.** Optimize conditions and select appropriate markers/antibodies for FACS sorting.	13-14	Dr. Schroeder	0%
Subtask 2. **Quantification of ribosomal subunit levels and stoichiometry between controls and *RPL13* siRNA treated MCPs using liquid chromatography coupled to tandem mass-spectrometry LC-MS/MS).** Determine whether subunits are enriched in monosomes vs. polysome.	14-15	Dr. Schroeder	0%
Milestone(s) Achieved: Determined changes in the levels and stoichiometry of ribosomal proteins following *RPL13* knockdown. Determined enrichment of ribosomal proteins in the various polysome fractions.			
Major Task 5. **Polysome Profiling with RNA-seq in MCP cells** NOTICE: We have modified the methodology of this major task as described in previous progress report. In this reporting period, we have prepared much of the reagents and tools for these experiments not reflected in the percentages but described below.			

Subtask 1. Polysome Profiling to identify mRNAs targeted by ribosomes in MCPs and how the mRNA targets shift following RPL13 siRNA treatment. Timecourse following siRNA treatment. RNA-seq followed by data analysis and interpretation.	14-20	Dr. Schroeder	0%
Subtask 2. **Identify mRNA species that are directly bound by RPL13 loaded ribosomes.** Polysome fractionations will be subject to a pulldown using RPL13 antibody, to isolate ribosomes loaded with RPL13. Bound mRNA will be processed for RNA-seq to identify and analyze RPL13 targets.	18-22	Dr. Schroeder	0%
Subtask 3. **Gather RNA-seq data and perform Computation Analysis and gene network design.**	22-24		0%
Milestone(s) Achieved: Identified direct and indirect mRNA targets of *RPL13* in differentiating MCPs. Developed genetic maps by computational analysis that describe and predict *RPL13* involvement in cardiac differentiation pathways.			

- **What was accomplished under these goals?**

We successfully completed the proposed scRNAseq experiments on FACS-sorted Drosophila *embryonic cardioblasts following RpL13 knockdown. We worked on developing our computational abilities through training in programming/coding and familiarizing ourselves with the methods/protocols to analyze scRNAseq data. In our data sets, we isolated the cardioblast cell populations based on Transcription Factor expression and ascertained a significant downregulation of RpL13 in RpL13-RNAi expressing cardioblasts compared to several controls (**Fig.1**). Alongside our RpL13-RNAi and control scRNAseq experiment, we included two other control conditions: 1.) younger and 2.) older control cardioblasts to determine if we could differentiate these two populations. Indeed, based on spatial distribution of calculated UMAPs (Uniform Manifold Approximation and Projection for Dimension Reduction; **Fig.2**) we found that cardioblasts from the older more mature samples (**Fig2A;** purple) clustered distinctly from the younger, more immature sample with some overlap (**Fig2A**; red). In a sc-RNAseq experiment evaluating the role of large Ribosomal Protein 13 (RpL13) on cardiac differentiation (**Fig 2B**), the knockdown of RpL13 (**Fig.2B**; yellow) led to different spatial coverage on the UMAP compared to controls (**Fig.2B**; green). When we compared our RpL13 experiment (RP) samples with the young and old control samples, we found that control cardioblasts from the RP experiment clustered closer to control older/mature cardioblasts (**Fig 2C**), whereas RpL13 knockdown cardioblasts clustered*

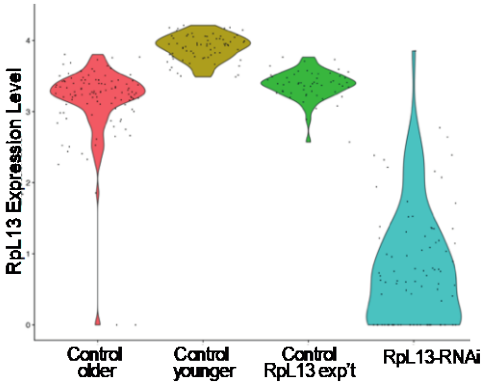


Fig. 1. RpL13 downregulation in experimental sample. *Following isolation of cardioblasts from scRNAseq data, we ascertained that RpL13 levels were significantly downregulated in the RPL13-RNAi sample compared to control samples verifying genotype.*

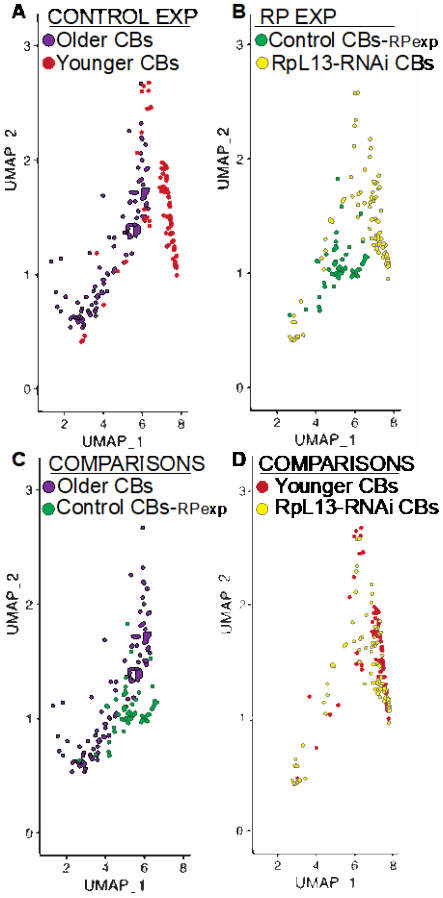


Figure 2: *sc-RNAseq of Drosophila cardioblasts (CBs). uMAPs compare gene expression similarities of individual cells **A**. CBs from older/mature (purple) sample are compared with a younger/immature (red) sample which showed separation in their distribution. **B.** In a separate Ribosomal Protein (RP) experiment, uMAPs of control CBs (green) have different spatial distribution vs RpL13 knockdown (yellow). **C.** Control CBs from the RP experiment (green) are more similar to older/mature CBs (purple from A) while **D.** distribution of RpL13 knockdown CBs (yellow) are more similar to younger/immature CBs (red from A), suggesting a deviation/block in CB differentiation.*

*closer to younger/immature cardioblasts (**Fig 2D**) suggesting that RpL13 knockdown led to deviations in or blocked differentiation of cardioblasts into a more mature state.*

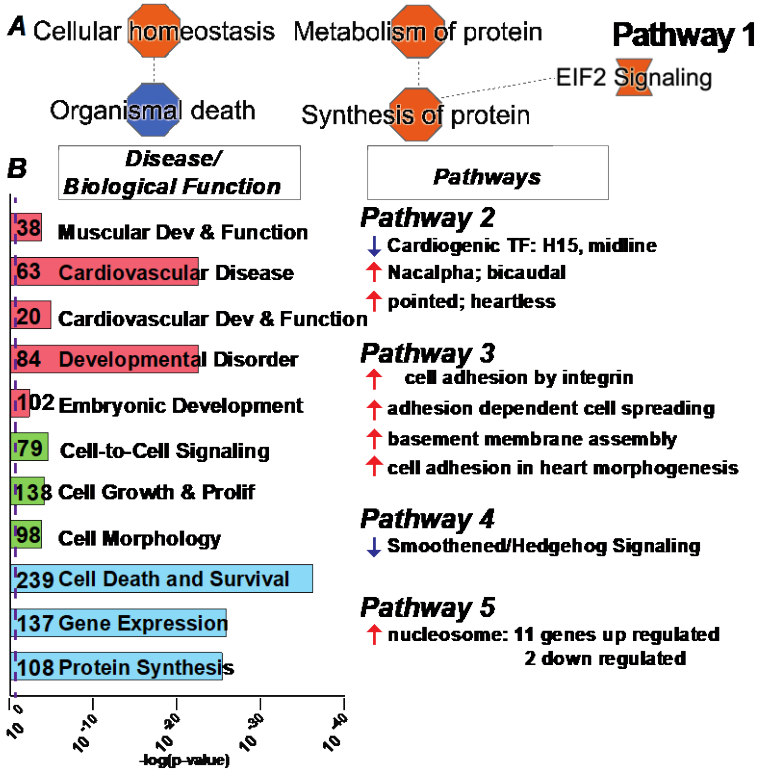


***Fig. 3.** Gene Ontology and IPA(Qiagen) analysis of sc-RNAseq data from RpL13 knockdown cardioblasts. **A.** Top networks altered by RpL13 knockdown. Blue-downregulation. Orange-upregulation **B.** Select biological functions altered by RpL13 knockdown. Top pathways and genes will be further explored.*

A total of 546 genes were differentially expressed (160 downregulated and 386 upregulated in RpL13-RNAi sample) between experimental controls and RpL13-RNAi. We initially hypothesized that RpL13 knockdown would alter the subunit composition of ribosomes. Based on transcriptomic data, we found strikingly that pathways involved in the synthesis/metabolism of protein were increased, which includes an upregulation of 78 other Ribosomal Subunits. Interestingly, in other cell types with verified reduction in RpL13 levels, including pericardial cells and skeletal muscles, changes in gene expression of ribosomal subunits were mixed, with most subunits unchanged while a handful were mildly downregulated or upregulated. This is an interesting observation, suggesting that working cardiomyocytes may respond distinctly to perturbations in ribosomal subunit composition compared to other cell types. When we have our Multipotent Cardiac Progenitor cultures working, we will attempt to measure changes in subunit composition and try to separate fibroblasts from cardiomyocytes.

*Importantly, Gene Ontology (Gene Ontology Resource) and network analysis (Ingenuity Pathway Analysis-Qiagen) of differentially expressed genes between RpL13 knockdown and control cardioblasts predicted that organismal/cell death pathways are downregulated while cellular homeostasis pathways are upregulated in knockdown hearts (**Fig. 3A**). These network predictions, including the increased transcription of ribosomal subunits in cardiomyocytes, suggest that RpL13 knockdown is not unequivocally leading to cell death and that protein synthesis is not blocked. Rather, increased ribosome subunit production compared to controls could be a marker for a progenitor-like state whereas, terminal differentiation throttles rRNA synthesis[33, 34]. This would be consistent with the UMAP clustering results, where RpL13-RNAi samples clustered closer to younger/immature control sample rather than the older/mature control sample (**Fig.2**). The analysis also indicated an increase in translation initiation EIF2 Signaling (**Fig.3A, Pathway 1**) suggesting a mechanism leading to a redirection of translation regulation that we plan to pursue. Other striking pathways include (**Fig.3B**) altered expression of cardiogenic factors (**Pathway 2**), an upregulation in basement membrane formation and Integrin signaling (**Pathway 3**), and down regulation in smoothened/Hedgehog signaling (**Pathway 4**), and altered expression of genes found within the nucleosome (**Pathway 5**).*

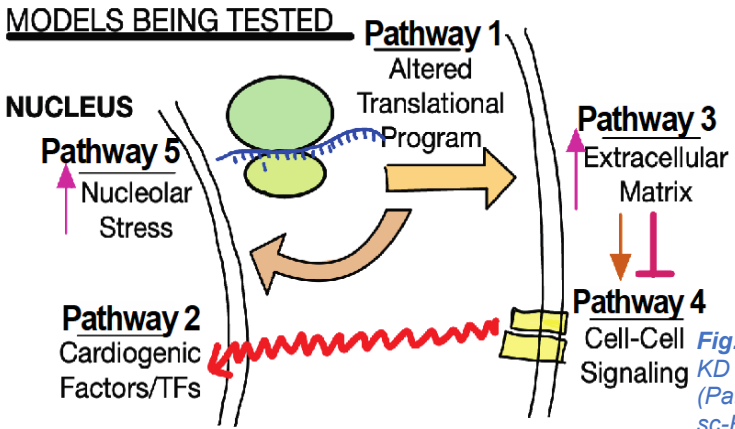


***Fig. 4.** Model on how combined dysregulation of pathways by RpL13 KD lead to altered cardiac differentiation. Alterations in translation (Pathway 1) increase deposition of ECM (Pathway3; as suggested by sc-RNAseq results). ECM disrupts cell signaling (i.e. smoothened/Hedgehog; Pathways 4) that feeds back into the cardiogenic transcription network (Pathways 2). Alternatively, nucleolar stress could be directly induced (Pathway 5).*

We hypothesize that the shift in translation as a result of RpL13 Knockdown is combinatorially altering cardiac-specific pathways (ECM, cell signaling, and cardiogenic factors) that redirect differentiation (Fig.4).

We have begun to examine these predicted pathways and test for genetic interactions of cardiogenic factors and differentially expressed genes with RpL13. In order to do this, we created a novel sensitized RpL13 line that combines a heart specific GAL4 Driver (HandGAL4^{GMRD88}) with a weaker RpL13 RNAi line (Kyoto Stock Center- 4651R1-II; ***Fig. 5A-C****). This line led to offspring where 80% of offspring have intact hearts (****Fig. 5B****) whereas 20% of the hearts were aberrant (****Fig. 5C****). Any improvement or exacerbation of phenotype when combined with a knockdown or heterozygous mutation of prioritized candidate genes from our predicted pathways, would indicate a genetic interaction with* RpL13. *We have crossed this line with various cardiac transcription factors mutants (dorsocross/DocA/TBX20,* tinman/NkX2-5, pnr/GATA4). All heterozygous mutants alone displayed minimal or no cardiac phenotypes on a structural level (example DocA mutant heterozygote; **Fig. 5D**). However, when combined with the RpL13-sensitized line, we saw an exacerbation of phenotype, where there was an increase in the number of aberrant hearts (**Fig. 5 E,F**), and in some cases, we found complete loss of the heart (**Fig. 5 F**), similar to a stronger RpL13 knockdown phenotype. Our scRNAseq, suggests that the transcription factor *pointed/pnt/ETS1 is upregulated with RpL13-knockdown. Interestingly, combined RpL13 and* pnt *knockdown reduced the number of aberrant hearts (data not shown), suggesting an improvement of phenotypes when combined, validating the scRNAseq, and providing another network relationship to further study.* In order to make the screening more efficient, we are introducing a heart-specific fluorescent marker (tdtk) that will make visualization through the cuticle possible, allowing us to score positive interactions quickly. We will then dissect and examine positive hits for more in depth characterization. We will also assess hearts functionally for another quantitative method to assess heart phenotypes. We have a number of RNAi lines and mutants ready to be test in the following weeks.

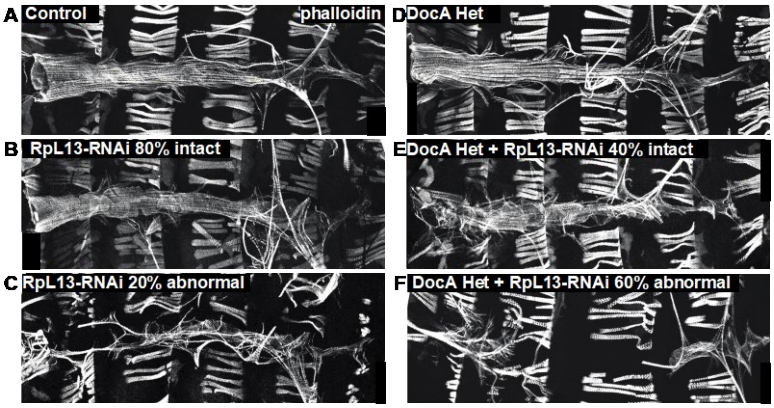


Fig. 5. Testing genetic interactions with RpL13. *Compared to controls (****A****), our sensitized RpL13 line results in 80% of offspring with intact hearts(****B****), while 20% resulted in aberrant/partial loss of hearts(****C****). In testing for genetic interactions with the cardiogenic transcription factor DocA/Dorsocross, the heterozygous mutant alone did not produce gross changes in heart structure(****D****). However, when the heterozygous mutation was combined with the RpL13-sensitized line, this produced an exacerbation of phenotype. 40% of hearts were intact (****E****), while 60% had abnormal or absent hearts (****F****). Similar trends are found with tinman/Nkx2.5 and pnr/GATA4 combinations. Suggesting that RpL13 may feed back onto the core cardiogenic network to disrupt differentiation.*

We are also staining the embryos for markers to validate the scRNAseq. For example, we stained the hearts with the antibody against the gene slit, which is a ligand-glycoprotein for Robo-receptor family signaling present in the heart lumen, where cardiomyocytes interface. Preliminary results show increased slit staining with RpL13 knockdown which we will further validate, but nonetheless may support the predicted pathways of a dysregulation in extracellular matrix (**Fig.6**). We are in the process of performing additional stainings of extracellular matrix (*nidogen*, pericardin-GFP, Laminins, etc.), cardiogenic factors (H15 and midline), and smo/hedgehog pathway genes, to further validate the scRNAseq results.

We have secured an alternative source of Multipotent Cardiac Progenitors (MCPs) for a scRNAseq time course experiment to follow cell fate changes caused by RpL13 knockdown. Upon receiving the MCPs, we established cell-culture capabilities in our own laboratory, developed new protocols and re-established the conditions for RpL13 knockdown and sample collection. We've determined the appropriate concentration of RpL13 siRNA (50nM) to elicit a decrease in cardiomyocyte population and an increase in fibroblast concentrations. We are now finalizing the protocol for cell recovery and nuclear extraction for 10X genomics scRNAseq pipeline.

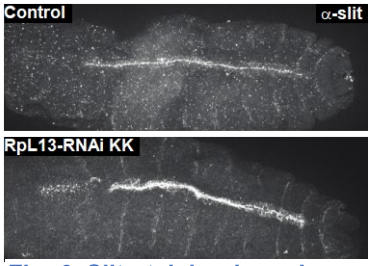


Fig. 6. **Slit staining in embryos.** Mesodermal *Knockdown (Twist-GAL4; 24B-GAL4) of RpL13 in embryos led to an increase in slit staining in the heart lumen, suggesting a possible disruption of the extracellular matrix.*

Because the results of the sc-RNAseq on fly cardioblasts were comprehensive and indicated that different cell-types responded uniquely to RpL13 ribosomal knockdown, we will be forgoing the bulk-RNAseq (Subtask 1) as bulk would not accurately reflect the biology.

We had trouble with puromycin incorporation and staining in Drosophila embryos, so we will attempt to capture overall translation by staining MCPs instead (see plans for next reporting period).

In our previous progress report, we described an alternative method to probe changes in the translatome in response to reduced RpL13 levels (**AIM 2**) using the Ribo-STAMP method developed by our collaborator (Dr. Gene Yeo). This was because of our lack of access to MCPs at the time. Despite this, we decided to pursue Ribo-STAMP,

a method that was demonstrated to identify sites of ribosome binding onto single-stranded mRNA with up to single-cell resolution. Briefly, *Ribo-STAMP tags ribosomal subunits with the APOBEC1 enzyme to modify ribosome-bound mRNA by conv*erting *Cytosine into Uracil*. *The mRNAs are sequenced by RNAseq and analyzed by SAILOR analysis which identifies the modifications on mRNA and detects enrichment of ribosome binding sites.* Our collaboration with the Yeo lab has led to the completion of plasmid constructs that fused fly ribosomal subunits (RpS2 and RpL13) with APOBEC1 (**Fig.7**). These constructs have been sent out to be injected into fly embryos. Plans are set to test the lines once we receive them (see plans to do during next reporting period; **Fig.8**).

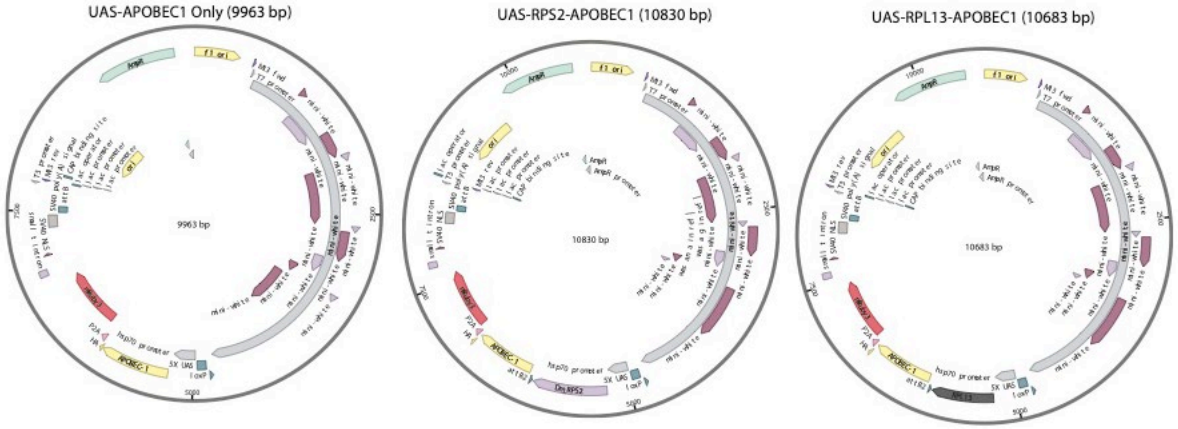


Fig. 7. Plasmid Constructs of APOBEC1, RPS2-APOBEC1, and RPL13-APOBEC1 constructs fused downstream of a UAS. *Plasmids have been sent out for embryo injection.*

- **What opportunities for training and professional development has the project provided?**

Nothing to Report.

- **How were the results disseminated to communities of interest?**

Nothing to Report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

During the no-cost-extension period of the grant we will accomplish the following goals.

AIM1*:*

Major Task 1: We will continue with the validation of genes that displayed changes in gene expression following RpL13 knockdown. We will recombine a heart-specific fluorescent marker (tdtk) to our sensitized RpL13 line that will allow us to more quickly screen through genetic interactors with RpL13 and continue to build a network of biological processes and pathways responsible for the no heart phenotype seen in RpL13. We will also use the fluorescent marker to assess functional changes in the heart that will provide more quantitative measures of heart phenotypes. These will help us determine whether those hearts that remain intact in our immunostaining, may in fact have functional defects induced by combined RpL13 and candidate gene knockdown. These methods are well-established in our lab.

Major Task 2: Following characterization of an alternative source of Multipotent Cardiac Progenitors cells, and re-establishing conditions for siRNA knockdown, we are in the final weeks of preparation for performing a scRNAseq time-course experiment on MCPs following RpL13 knockdown. Following which, we will perform Quantitative analysis (similar to Major Task 1) to identify changes in cell expression with single-cell resolution. We will compare these data with Drosophila results. Prioritize genes from MCP analysis will be tested in *Drosophila for validation of genetic interaction with RpL13 and characterize resulting phenotype in the heart.*

AIM2*:*

Upon receiving the newly generated APOBEC1 transgenic flies, we will sequence the lines for APOBEC to validate fly genotypes. We have three major experiments for this AIM. In

*experiment 1 (**Fig 8**), we will validate that APOBEC1 is modifying mRNA in embryonic cardioblasts. We will use a strong mesodermal (Twist- and 24B- GAL4) driver line expressing a cardiac-specific RFP tag, similar to the one used for the RpL13 sc-RNAseq experiment. This driver line will be crossed to 1.) GFP alone, 2.) APOBEC1 alone, and 3.) APOBEC1 tagged RpS2 or 4.) RpL13. We will compare overall mRNA expression of sorted embryonic RFP-labelled cardioblasts (Stage 14-18) from all conditions by RNAseq to determine if APOBEC1 or RP constructs induce any significant changes in the transcriptomic landscape. We will then look for increased C-to-U conversions of mRNA in the presence of APOBEC1 and compare the patterning of modifications. APOBEC1 alone will induce random C-to-U conversions in all mRNA, while Ribo-tagged APOBEC1 will selectively modify ribosome bound mRNA. We will also look for differences in the pattern of mRNA conversions between APOBEC1 tagged RpS2 and RpL13 lines. Differences in mRNA targets between two APOBEC1-Ribosome tagged lines would provide insights on the specific targets of RpL13 loaded ribosomes compared to other ribosomal subcompositions containing RpS2.*

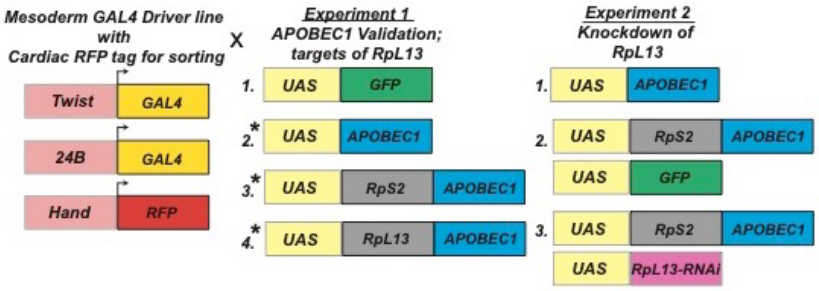


***Figure 8.** Constructs and genetic crosses for translatomic analysis. * Indicates the constructs we have created (see Fig.7) and are injecting in flies. Lines will be combined with existing UAS-GFP and UAS-RNAi to create specified genotypes.*

*After validation of Ribo-STAMP activity in flies, experiment 2 (**Fig.7**) will compare the translatome in sorted embryonic cardioblasts following mesodermal KD of RpL13. The strong dual mesodermal drivers should be sufficient to drive expression of both APOBEC1 and RNAi constructs. Modified mRNA will be identified and subject to network analysis to uncover enriched pathways that influence the differentiation process. We will compare the translatome and transcriptome (scRNAseq data) of RpL13 KD cardioblasts and look for consistencies in reprogramming, for example whether Extracellular Matrix genes overrepresented in the translatomic data. Also, we will look for striking genes whose expression levels are discordant on a transcriptomic and translatomic level which could provide insights on targets directly regulated by translational mechanisms. Computational abilities of the Yeo lab and SBP Bioinformatics Core will interrogate these RNAseq datasets and extract qualifying pathways and place them into biological context. Prioritized genes will be tested for genetic interactions with RpL13 in Drosophila hearts and heart phenotypes assessed.*

When we complete the scRNAseq on MCPs (from AIM1) we will proceed with Major Task 3 and 4 using MCPs where we will quantify overall protein translation using puromycin staining (Task 3) and quantify ribosome subunit composition (Task 4) by mass spec.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  Nothing to Report

- **What was the impact on other disciplines?**
  Nothing to Report

- **What was the impact on technology transfer?**
  Nothing to Report

- **What was the impact on society beyond science and technology?**
  Nothing to Report

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Because of a labor shortage, we had to establish a new source of MCPs from another collaborator and set up our own cell-culture equipment, reagents and tools in our own lab. Upon initial testing, we did not obtain the appropriate phenotypes or responses following establish RpL13 knockdown protocols. We therefore had to troubleshoot and determine the appropriate conditions to generate an appropriate response to RpL13 knockdown. We have most of the conditions worked out, and are working on nuclear isolation protocol for scRNAseq (instead of whole-cell due to cell size), which we believe will generate more reliable results. After this, we will proceed with the actual scRNAseq experiment and analyze the results (as in AIM1) and look for trajectories in gene expression that would explain changes in cell-fate choices among the MCP cells.

Establishing our MCP culture conditions will then allow us to extract sufficient protein to perform mass spec and measure changes in ribosome subunit composition.

- **Changes that had a significant impact on expenditures**.

Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**.

Nothing to Report

- **Significant changes in use or care of human subjects**.

Nothing to Report

- **Significant changes in use or care of vertebrate animals.**

Nothing to Report

- **Significant changes in use of biohazards and/or select agents**.

Nothing to Report

6. PRODUCTS:

- **Journal publications.**

Nothing to Report

- **Books or other non-periodical, one-time publications.**

Nothing to Report

- **Other publications, conference papers, and presentations.**

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	Analyne Schroeder
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-7537-458X
Nearest Person Month Worked:	7.3
Contribution to Project:	Dr. Schroeder has designed and performed the experiments, obtained necessary fly stocks and performed fly husbandry. Collected embryos, troubleshoot scRNA-seq experiments, acquired data and performed data analysis of immunostaining.
Funding Support:	N/A

Name:	Arthur Bautista
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest Person Month Worked:	1.1
Contribution to Project:	Assisted with the maintenance of fly stocks, preparation of reagents.
Funding Support:	N/A

Name:	Marco Tamayo
Project Role:	Lab Coordinator
Researcher Identifier (e.g. ORCID ID):	0000-0001-9891-0755
Nearest Person Month Worked:	2.3
Contribution to Project:	Assisted with the maintenance of fly stocks, embryo collections, preparation of reagents and constructs.
Funding Support:	N/A

- **What other organizations were involved as partners?**
  Nothing to Report

- **Organization Name:**

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report

9. APPENDICES

Appendix A - Active Support

PREVIOUS, CURRENT, AND PENDING SUPPORT

Name of Individual: Schroeder, Analyne
Current Appointments: 03/2023 Staff Scientist

Other Support – Project/Proposal

CURRENT
THIS AWARD

Title: **Translational Targets of Ribosomal Protein RPL13 as Novel Cardiac Drivers of Differentiation in Drosophila and Human iPSCs: Implications for CHD**

Major Goals: The main goal is to identify new CHD candidate genes and uncover pathways and mechanisms involved in CHD pathogenesis by focusing on an unconventional gene candidate involved in protein translation, the large ribosomal protein RPL13.

Specific Aims:
1. Single-cell transcriptomics for population mapping of FACS-sorted Drosophila cardioblasts and human Multipotent Cardiac Progenitors, to track emerging cell identities and how they are altered as a result of RPL13 knockdown.
2. Polysome Profiling to identify direct and indirect translational targets of RPL13 in human Multipotent Cardiac progenitors, leading to construction of a RPL13-centric genetic network driving cardiac differentiation.

Project Number: PR202454
Name of PD/PI: Schroeder, Analyne
Source of Support: Department of the Army
Project Performance Period: 03/01/2021 – 12/31/2023
Total Award Amount (including Indirect Costs):

Time Commitment per Budget Period:

YEAR (YYYY)	Person Months (##.##)
1. 2023	6.4 Calendar Months

Grants Management Officer: Abigail Strock; Phone: (301)-619-2342; Abigail.l.strock.civ@health.mil
Overlap: None

Title: **Genetic Control of Cardiac Development: Congenital Heart Disease Gene Discovery in Drosophila**

Major Goals: The overall goal is to use our heart assays as an efficient throughput in vivo discovery tool for systematically identifying the ~90% as of yet unknown CHD genes.

Specific Aims:	1. Define and functionally test cardiogenic gene regulatory networks identified by scRNAseq of embryonic Drosophila heart cells. 2. Determine the cardiogenic function of CHD gene candidates identified from patient-derived WGS data, focusing on ribosomal protein genes (RpL13) and MICOS complex genes.
Project Number:	R01 HL054732
Name of PD/PI:	Bodmer, Rolf
Source of Support:	NIH/NHLBI
Project Performance Period:	07/01/2021 – 06/30/2025
Total Award Amount (including Indirect Costs):	

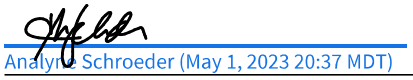
Time Commitment per Budget Period:

YEAR (YYYY)	Person Months (##.##)
1. 2023	5.6 Calendar Months
2. 2024	5.6 Calendar Months
3. 2025	5.6 Calendar Months

Grants Management Officer:	Charlene Schramm; Phone: (301) 435-0510; Schrammc@nhlbi.nih.gov
Overlap:	None

PI Certification

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and agree to update such disclosure at the request of the agency prior to the award of support and at any subsequent time the agency determines appropriate during the term of the award. I have been made aware of the requirements under Section 223(a) of the William M. (Mac) Thornberry National Defense Authorization Act. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: 
Analyne Schroeder (May 1, 2023 20:37 MDT)

Date: May 1, 2023

DOD Other Support Schroeder (05-01-2023)

Final Audit Report 2023-05-02

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