Translational components driving heart morphogenesis in *Drosophila*: Implications for Congenital Heart Disease (CHD)

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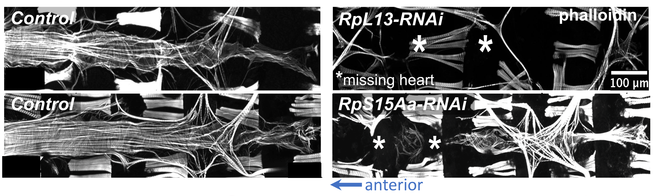
Despite the seemingly generic function of TRANSLATIONAL COMPONENTS, we uncovered spatial and temporal specific effects disrupting cardiac morphogenesis that led to varied and significant adult fly heart phenotypes

Ribosomal Subunits: RpL13, RpS15Aa [1]

Heart phenotypes suggest SPATIAL specificity in activity of individual ribosomal subunits

A

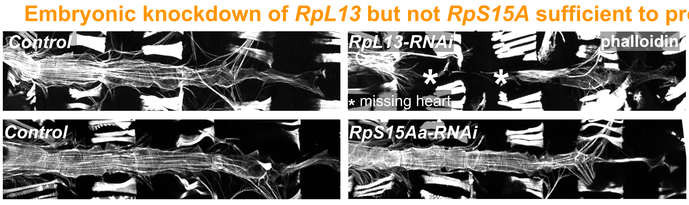


Top: RpL13 knockdown (KD) in the fly heart throughout development (Hand4.2Gal4) resulted in a 'no heart' phenotype in adults. *Bottom: RpS15Aa* KD resulted in a partial heart phenotype, where the anterior region is absent and the posterior region is maintained. The differences in phenotype suggest differential requirements in the activity of the two ribosomal subunits in different regions of the heart.

TEMPORAL specificity in the activity of ribosomal subunits

Embryonic knockdown of *RpL13* but not *RpS15A* sufficient to produce heart phenotype

B

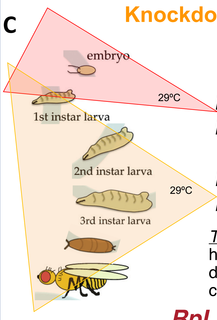


Top: Embryonic KD of *RpL13* (TinDGal4) was sufficient to induce a no heart phenotype, *Bottom*: Embryonic KD of *RpS15Aa* was not sufficient to induce the partial heart phenotype, indicating differential temporal requirement of ribosomal subunit activity.

C

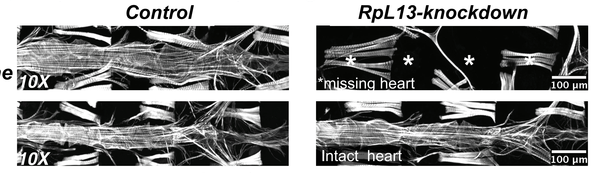
Knockdown of *RpL13* after embryonic stages does not induce heart loss

Cardiac-Specific, Temperature-driven temporal regulation of **Knockdown**



RpL13 Knockdown: **Embryo → L1 Larvae**

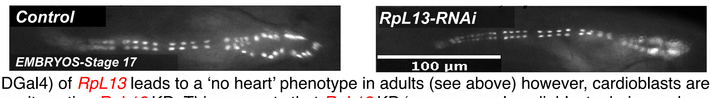
RpL13 Knockdown: **L1 Larvae → Adult**



Top: Temperature induced KD of *RpL13* (Hand4.2TubGAL80^{ts}(2x)) in embryos was sufficient to induce a no heart phenotype. *Bottom*: Temperature induced KD starting at L1 larval to adult stages did not cause gross defects in heart structure or function, which suggests that *RpL13* function is temporally specific during early cardiac developmental stages.

D

RpL13 knockdown 'pre-programs' cardioblasts for later lysis



Embryonic KD (TinDGal4) of *RpL13* leads to a 'no heart' phenotype in adults (see above) however, cardioblasts are present in stage 17 embryos (left) despite active *RpL13* KD. This suggests that *RpL13* KD 'preprograms' cardioblasts during embryonic stages that induces cell lysis later in development.

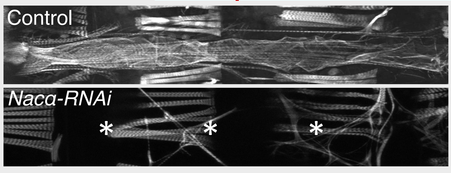
Hypothesis: RpL13 partakes in cardioblast programming & differentiation

Nascent Polypeptide Associated Complex-alpha (Nac-α) [2,3]

regulates translation by binding a subset of nascent polypeptides emerging from ribosomes and targeting their localization and expression

Heart-specific KD of Nac-α leads to a 'no heart' phenotype

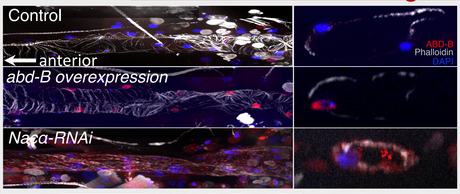
E



Left: Knockdown (KD) of *Nac-α* in the heart throughout development led to a 'no heart' adult phenotype. The defects were developmental as adult only KD does not lead to gross cardiac defects (data not shown). Specifically, the heart completely histolyzes during metamorphosis during cardiac remodeling, a process dependent on *HOX* gene expression (data not shown) and therefore we explored this interaction.

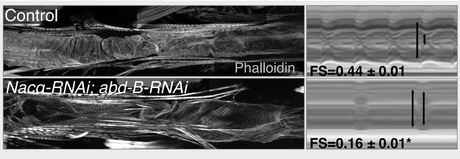
Nac-α interacts with *Hox* gene *abd-B during cardiac remodeling*

F



Left, Top: In early pupae, the *HOX* gene *abdB (red)* is normally expressed only in the most posterior cardiomyocytes, and these cells histolyze during metamorphosis and are absent in adults. *Middle*: Overexpression of *abdB* in the heart leads to accumulation in the nuclei of all cardiomyocytes and causes histolysis of the entire heart during metamorphosis. *Bottom:* KD of *Nac-α* led to a misexpression of *abdB*, where *abdB* is increased throughout the heart prior to cardiac remodeling, possibly driving complete histolysis of the heart.

G



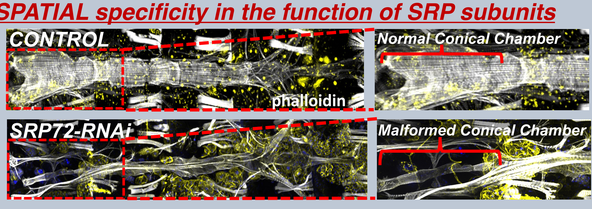
Because ABDB is increased when *Nac-α* is KD, we tested whether *abdB* KD could rescue the phenotype. *Left*: Indeed, KD of both *Nac-α* and *abdB* resulted in a partial rescue of the heart structure (left) and function (right) suggesting that *Nac-α* and *abdB* genetically interact.

Genetic Interaction of Nac-α and abd-B suggests function of Nac-α is cardiac specific

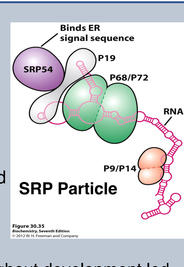
Signal Recognition Particle (SRP)

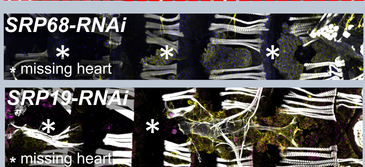
SPATIAL specificity in the function of SRP subunits

H



Right: SRP (Signal Recognition Particle) targets the translocation of a subset of nascent polypeptides destined for the membrane or secretion to the ER.





Left: Heart-specific knockdown (KD) of *SRP72* throughout development led to a malformed conical chamber (ventricle-like) and a largely intact heart. KD of *SRP68* led to complete loss of the heart, whereby histolysis occurred during metamorphosis. KD of *SRP19* led to a partial heart phenotype, with the absence of the anterior region and maintenance of the posterior. KD of some subunits did not produce a phenotype. This suggests that SRP subunits may have regional/cell type specific effects leading to varied heart phenotypes when KD.

Determining nascent polypeptide targets of SRP would uncover its role in heart morphogenesis

Translational genes *RpL13, RpS15A, Nacα and SRP* signaling components are associated with CHD in humans and should be explored as targeted drivers and regulators of heart development and pathogenesis

References: 1 Schroeder AM, *et al.* 2019. Model System identification of novel congenital heart disease gene candidates: focus on RpL13. *Hum Mol Genet. 28:3954-69.*
2 Van der Harst, *et al.* 2016. 52 Genetic Loci Influencing Myocardial Mass. *J Am Coll Cardiol. 68:1435-48.*
3 Liu L, *et al.* 2017. Whole exome sequencing identifies novel mutation in eight Chinese children with isolated tetralogy of Fallot. *Oncotarget.* 8:106976-88.