Differential Roles of Broadly Expressed Transcription Factors in Early Cardiac Cell Specification

Shiva Abbasi†, Rie Conley, Theodora Koromila*

Department of Biology, University of Texas at Arlington, Arlington, TX

†sxa1511@mavs.uta.edu | *theodora.koromila@uta.edu

Background

- All developmental processes are well-orchestrated and coordinated by numerous developmentally associated genes that determine the various cell fates and tissue differentiation. In this study, we used *Drosophila* melanogaster as a model system to study heart cell specification.
- In this study, we focused on the role of Odd-paired (Opa/ZIC3)[13] and Suppressor of Hairless (Su(H)/RBPJ)[14,] in regulating cardiac-specific genes before the onset of gastrulation.
- We chose to focus on two genes that are involved in heart cell specification, *slp1* and *odd* genes.
- We coupled in situ hybridization technique with ChIP-seq, and ATAQ-seq meta-analysis to study the DNA accessibility and binding profile of the early-expressed transcription factors (TFs).

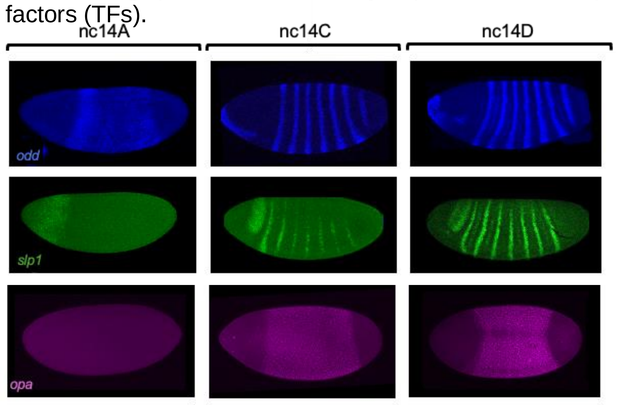

Fig. 1. *slp1* and *odd* expression patterns and levels before gastrulation.

- Most cardiac-specific genes are not significantly altered in *opa* knockdown embryos[3].

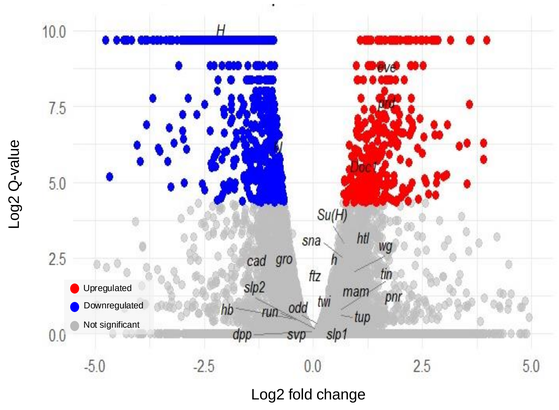

Fig. 2. Volcano plot of RNA-seq differential cardiac gene expression in *hs_opa* embryos.

Results

- A well-studied cis-regulatory elements identified in the *slp1* genomic region, distal early stripe element *DESE* is an Opa-dependent late stripe enhancer, as we see loss of *DESE* stripes in *opa* knockdown embryos at nc14D.

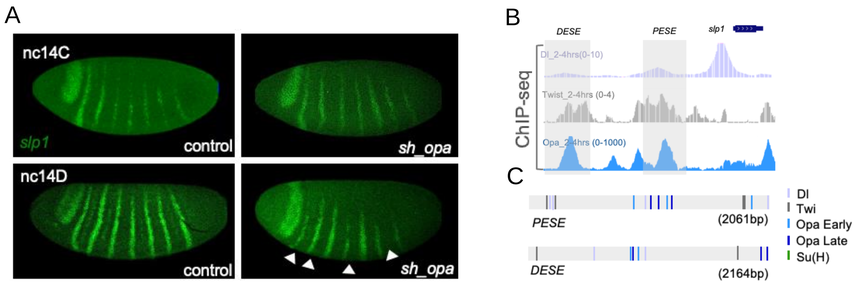

Fig. 3. A) *slp1* transcriptional dynamics before gastrulation. B) ChIP-seq of of TFs Dl, Twi, Opa[3-6] C) Schematic representation showing the binding sites of the TFs (Dl, Twi, Opa early, Opa late, Su(H)) in the genomic location of *DESE* and *PESE*.

- TFs such as Su(H) counterbalances Opa's activity during early cellularization. *odd* expression shows expedited phenotype in the absence of Opa, and wider expression stripes at nc14C in the absence of Su(H)

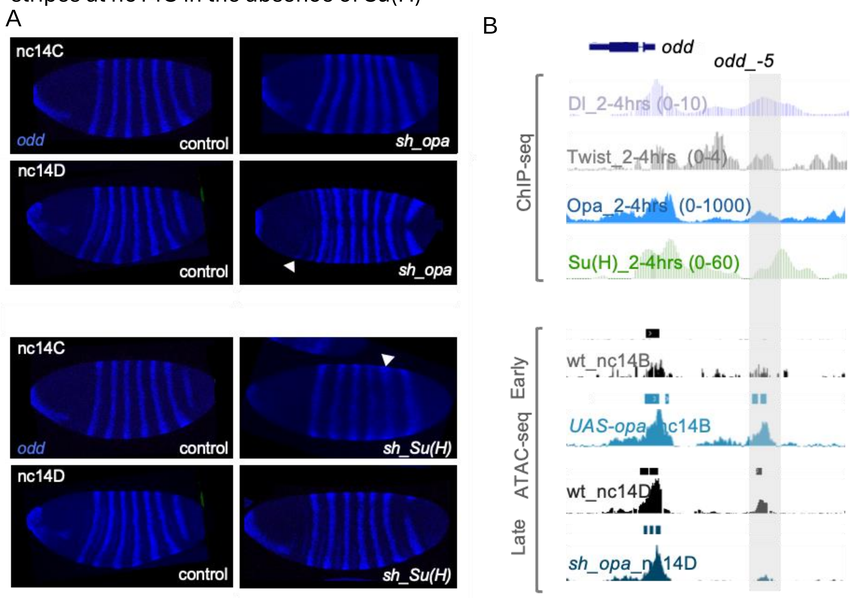

Fig. 4. A) In situ hybridization of *odd* in blue control (yw), *sh_opa,* and *sh_Su(H)* embryos. Arrowhead showing the changes in *odd* expression. B) ChIP-seq of Dl, Twi, Opa, and Su(H)[3-6]

- Opa, Su(H), and Twi showing overlap in their bound regions.

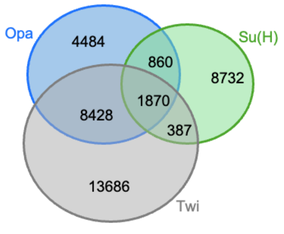

Fig. 5. Venn diagram created using publicly available ChIP-seq data showing overlapping and unique binding sites among , Twi (gray) and Opa (blue), and Su(H) (green).

Discussion

- The interplay between transcription factors prior to gastrulation is complex and crucial for proper developmental patterning.
- TFs regulate target genes through two mechanisms. Early in embryonic cellularization, when Opa levels are low and Su(H) levels are high, Su(H) suppresses primary gene expression (e.g., *odd*). Later, as secondary genes and enhancers become active, increased Opa levels enable it to act as a transcriptional activator. Without Opa, Su(H) cannot repress *odd*, allowing its progression to the next stage.

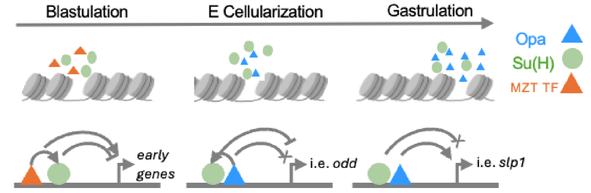

Fig 7. Regulation of cardiac-specific enhancer.

Future Directions

- Develop live-imaging reporter constructs (MS2/MCP) for enhancers regulated by both Su(H) and Opa.
- Perform mutagenesis experiments to introduce changes to the binding motifs of these transcription factors.
- Genome-Wide mapping of transcription factor binding Sites Using ChIP-Seq.
- Single-Cell Profiling of gene expression and chromatin accessibility for *sh_Su(H)* and *sh_opa*

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