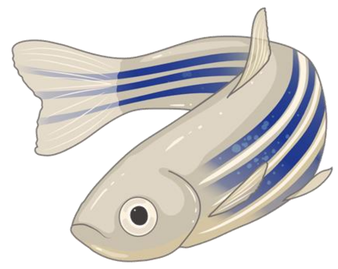
Mechanosensitive gene regulation that coordinates epiboly cell movements in zebrafish gastrulation

SCAN ME

Martínez-Vara, Andrea [1], García-Junco, Jesús [1], Aperador-Redondo, Juan [1], Mongera, Alessandro [2], Almuedo-Castillo, María [1]

[1] *Andalusian Center for Developmental Biology (CABD), Pablo de Olavide University CSIC/Junta de Andalucía, Seville, Spain,* [2] Department of Cell & Developmental Biology, University College London, London, UK

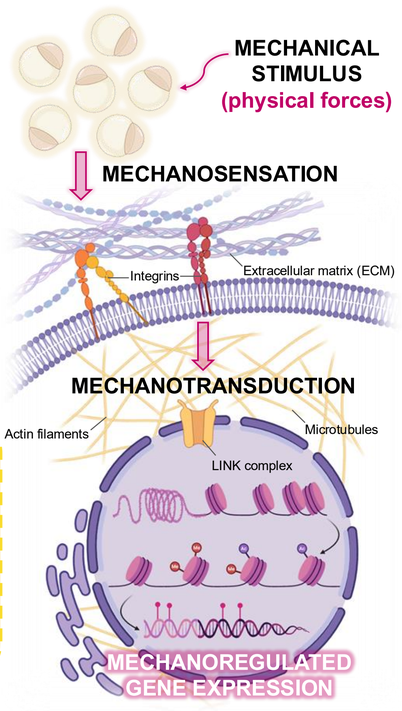


INTRODUCTION

During vertebrate embryogenesis, **mechanical and geometrical inputs** are integrated with **gene regulatory mechanisms to guide differentiation and organ formation**, however, the specific epigenetic programs and genetic mechanisms that translate these mechanical cues into tissue development remain poorly understood

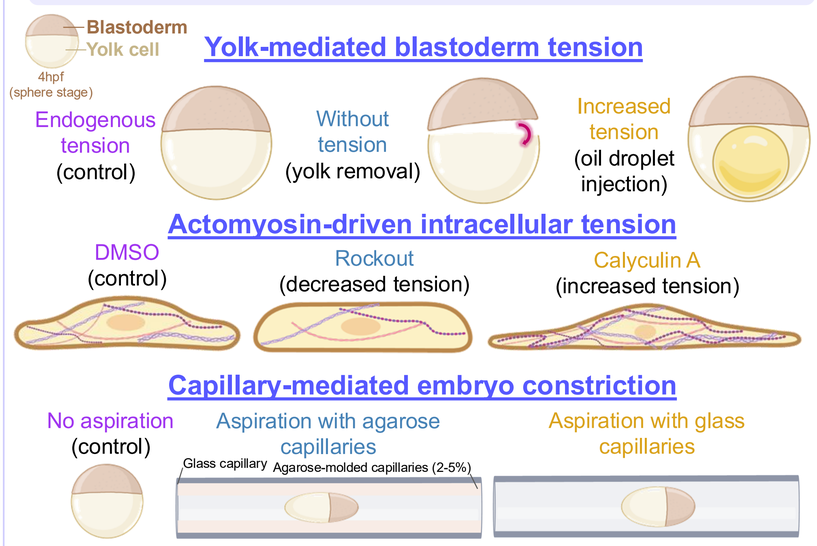
AIM OF THE STUDY

To uncover the crosstalk between **mechanical forces** and **epigenetic regulation of gene expression programs** during the movements of epiboly of the zebrafish **Enveloping Layer (EVL)**



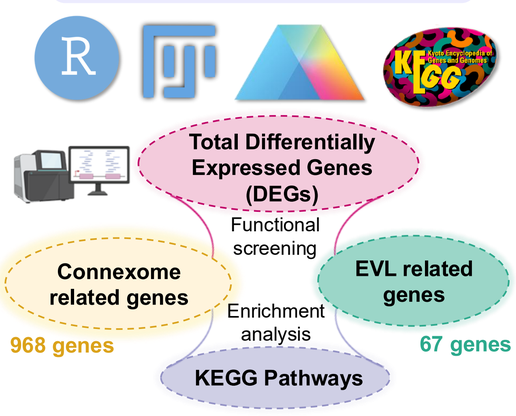
MATERIALS & METHODS

Mechanical tension modulation strategies



Morphological measurements

Morphological and bulk RNA-seq data analysis



RESULTS

1. Mechanical tension modulation alters embryonic morphology at 8 hpf (Fig. 1), leading to **differential impacts on epiboly progression depending on the mechanical strategy applied**

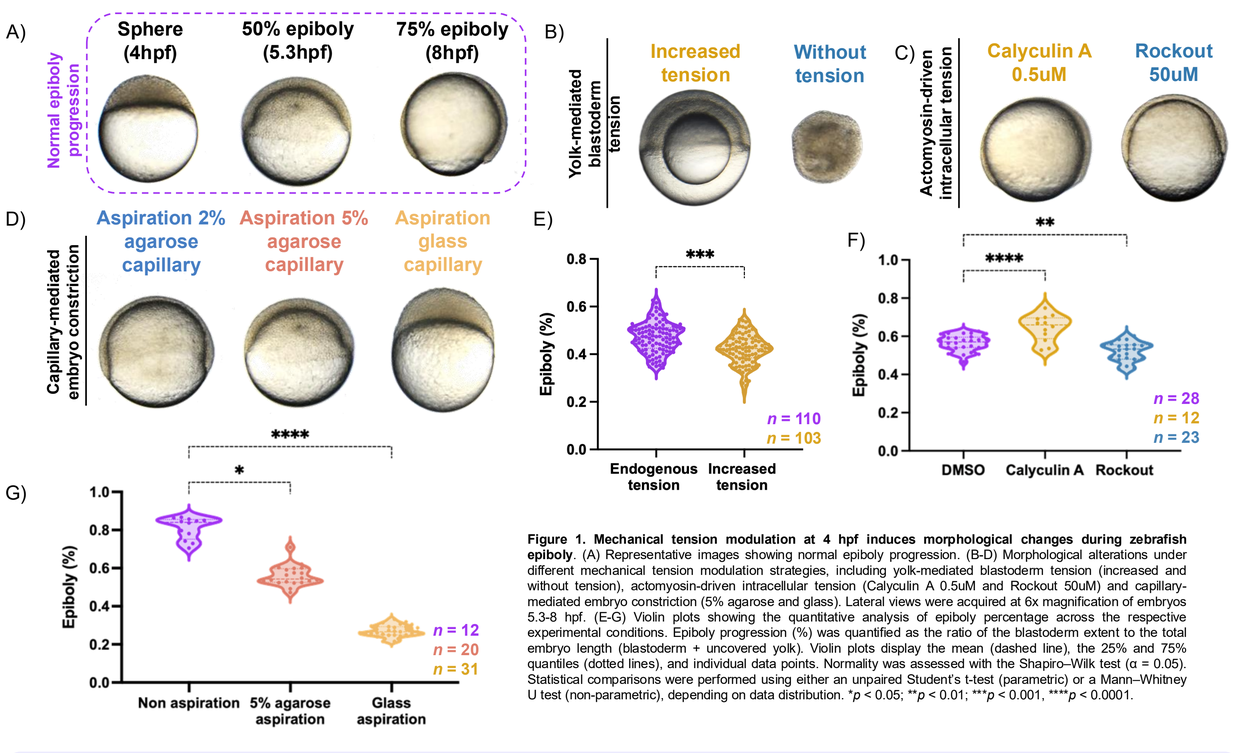


Figure 1. Mechanical tension modulation at 4 hpf induces morphological changes during zebrafish epiboly. (A) Representative images showing normal epiboly progression. (B-D) Morphological alterations under different mechanical tension modulation strategies, including yolk-mediated blastoderm tension (increased and without tension), actomyosin-driven intracellular tension (Calyculin A 0.5uM and Rockout 50uM) and capillary-mediated embryo constriction (5% agarose and glass). Lateral views were acquired at 6x magnification of embryos 5.3-8 hpf. (E-G) Violin plots showing the quantitative analysis of epiboly percentage across the respective experimental conditions. Epiboly progression (%) was quantified as the ratio of the blastoderm extent to the total embryo length (blastoderm + uncovered yolk). Violin plots display the mean (dashed line), the 25% and 75% quantiles (dotted lines), and individual data points. Normality was assessed with the Shapiro–Wilk test (α = 0.05). Statistical comparisons were performed using either an unpaired Student's t-test (parametric) or a Mann–Whitney U test (non-parametric), depending on data distribution. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$.

2. Global transcriptomic profiling reveals condition-specific PCA clustering (Fig. 2), confirming that **the alteration of embryonic mechanical forces drives changes in the transcriptome**

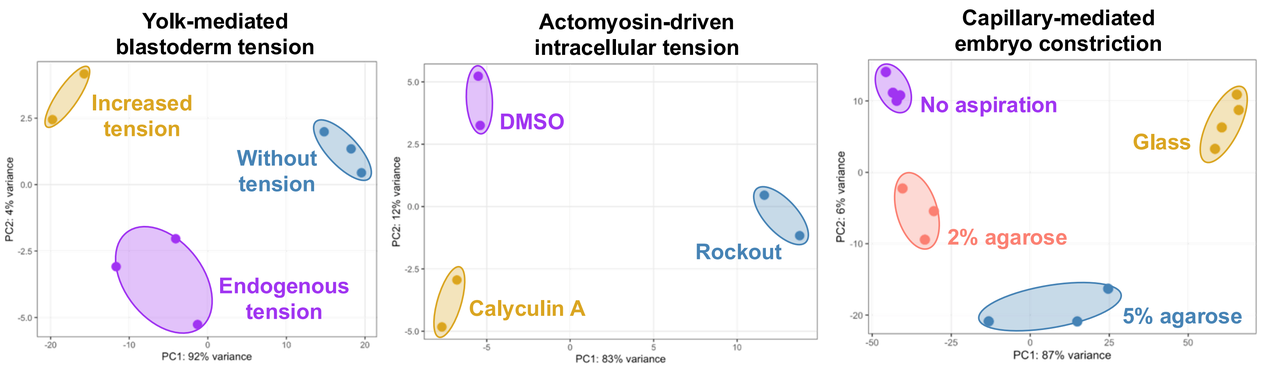


Figure 2. Mechanical tension modulates the transcriptomic landscape of the zebrafish embryo. Principal Component Analysis (PCA) for yolk-mediated blastoderm tension, actomyosin-driven intracellular tension, and capillary-mediated embryo constriction, showing distinct transcriptomic clustering by mechanical state.

3. Decreased mechanical tension triggers metabolic quieting and cellular stress, yet embryos actively initiate a robust transcriptomic program to remodel adhesion junctions (Fig. 3)

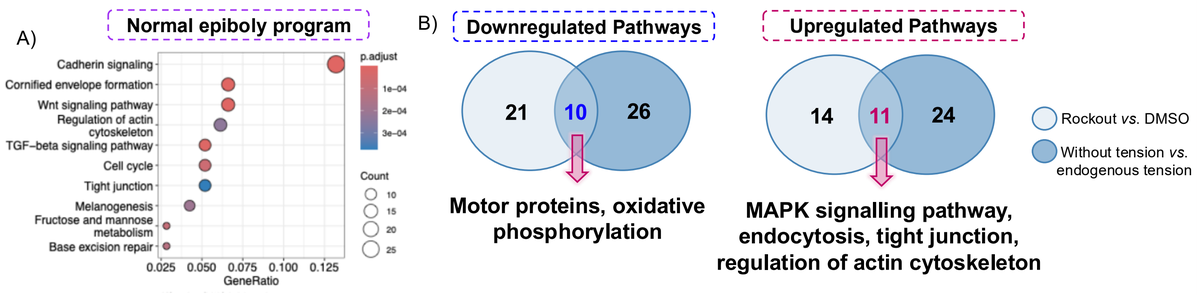


Figure 3. Transcriptomic response to decreased mechanical tension during gastrulation. (A) Dotplot representing significantly upregulated KEGG pathways during normal development (75% epiboly *vs.* sphere stage in non-modified embryos). (B) Venn diagrams showing the convergence of differentially expressed KEGG pathways between actomyosin-driven intracellular tension (Rockout *vs.* DMSO) and yolk-mediated blastoderm tension (Without tension *vs.* Endogenous tension). All enriched pathways displayed in both panels A and B were selected based on a threshold of |log2 FC|≥2 and padj < 0.05.

4. Although capillary constriction forces a deceptive arrest, **transcriptomics uncovers an active developmental drive** driven by cytoskeletal and stress pathways (Fig. 4)

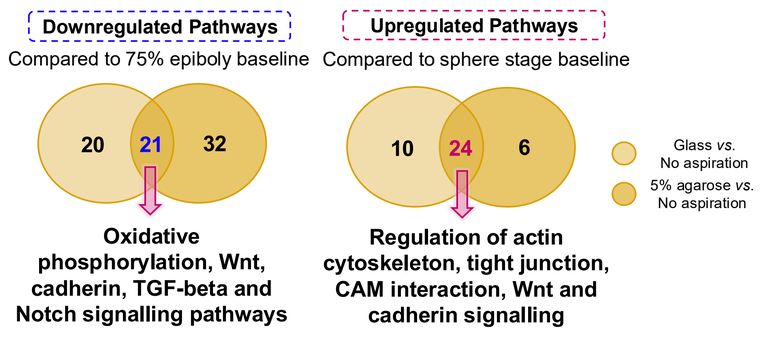


Figure 4. Transcriptomic drive during physical constriction. Venn diagrams showing the convergence of differentially expressed KEGG pathways between capillary-mediated embryo constriction. (Left) Downregulated pathways in glass and 5% agarose compared to 75% epiboly baseline. (Right) Upregulated pathways in glass and 5% agarose compared to sphere stage baseline. All enriched pathways displayed in both panels A and B were selected based on a threshold of |log2 FC|≥2 and padj < 0.05.

5. Stiffer capillary aspiration **upregulates core EVL and Connexome genes** (Fig. 5A), demonstrating an active molecular program. Despite morphological arrest, expression profiles of mechanically restricted embryos cluster with advanced controls, confirming **molecular progression** (Fig. 5B)

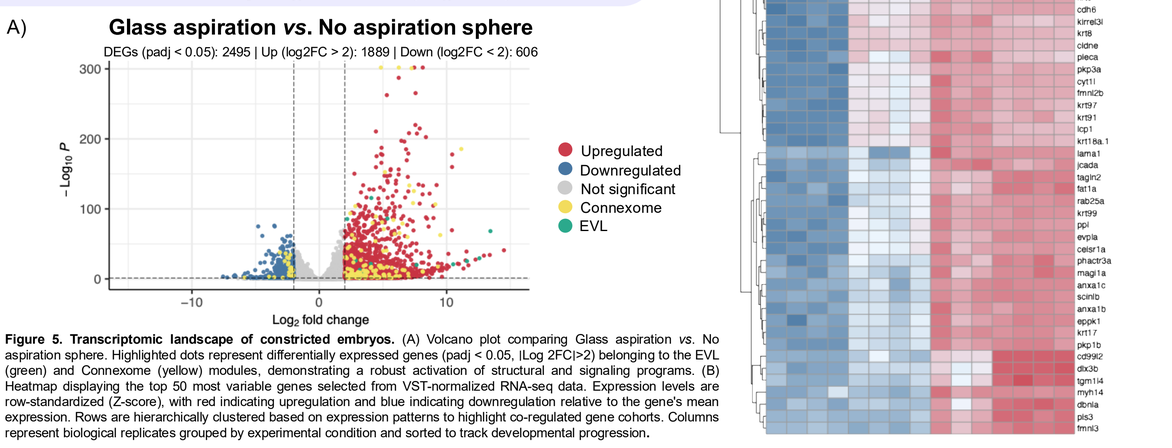


Figure 5. Transcriptomic landscape of constricted embryos. (A) Volcano plot comparing Glass aspiration *vs.* No aspiration sphere. Highlighted dots represent differentially expressed genes (padj < 0.05, |Log 2FC|>2) belonging to the EVL (green) and Connexome (yellow) modules, demonstrating a robust activation of structural and signaling programs. (B) Heatmap displaying the top 50 most variable genes selected from VST-normalized RNA-seq data. Expression levels are row-standardized (Z-score), with red indicating upregulation and blue indicating downregulation relative to the gene's mean expression. Rows are hierarchically clustered based on expression patterns to highlight co-regulated gene cohorts. Columns represent biological replicates grouped by experimental condition and sorted to track developmental progression.

CONCLUSIONS

1. Our study provides evidence that **mechanical forces are essential regulators of gene expression programs during zebrafish epiboly**, highlighting a direct coupling between tissue mechanic and transcriptional regulation in early development.
2. **Modulation of mechanical tension** in the enveloping layer (EVL) results in distinct condition-dependent effects on epiboly progression and embryonic morphology, indicating that **cells actively interpret mechanical inputs to coordinate gastrulation movements**.
3. Transcriptomic analyses reveal that **changes in mechanical tension leads specific and robust gene expression responses**, such as the activation of stress- and cytoskeleton-related pathways as well as the remodelling of adhesion-related gene networks. Interestingly, embryos execute coordinated developmental program even under mechanical constraints, suggesting that there are compensatory regulatory mechanisms that ensure developmental robustness.
4. All together, our resuts support a model in which mechanosensitive gene regulation integrates with biomechanical forces to orchestrate coordinated cell movements during gastrulation, providing new insights into the crosstalk between physical forces and gene regulatory networks in vertebrate embryogenesis.

FUTURE PERSPECTIVES

We aim also to perform integrative multiomic analysis using **10X Chromium Single Cell Multiome (scATAC-seq + scRNA-seq)** and **Whole Genome Bisulfite Sequencing (WGBS),** to **map mechanosensitive genes and correlate them with chromatin accessibility and DNA methylation patterns**

amarvar@alu.upo.es