Decoupling Mechanical and Biochemical Control of Gastruloid Symmetry Breaking

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1. Motivation and Gap

Large-scale tissue flows drive the emergence and stability of developmental order as a stem-cell aggregate breaks symmetry and elongates into an embryo-like structure [4]. Symmetry breaking and axial elongation mark the appearance of organized body-axis structure, yet a unified account of how mechanical and biochemical signals are sequenced during this transition remains absent.

Two competing frameworks have been proposed to explain this transition. In the first, feedback loops between mechanical force and biochemical patterning coordinate tissue flows, with junctional tension, adhesion and neighbor exchange linking local cellular rearrangements to larger-scale deformation [3]. In the second, morphogen-dependent patterning, modeled through reaction-diffusion dynamics, drives the formation of spatial asymmetry via signaling gradients, boundary conditions and polarity stabilization before tissue flow becomes visible [1]. The causal sequence connecting them remains unresolved and it is unclear whether mechanical control initiates symmetry breaking or is downstream of biochemical patterning.

A perturbation framework that decouples surface tension from morphogen patterning resolves this sequence by testing whether polarity, flow and elongation fail at the same developmental stage. The point where the sequence breaks down identifies the initiating mechanism and grounds a more integrated model of developmental order.

2. Background

Surface-tension gradients can drive Marangoni flow in fluids. In gastruloids, large-scale tissue recirculation can be interpreted as a Marangoni-like flow driven primarily by changes in effective tissue surface tension [4]. Gastruloids are an appropriate model for this question because they establish T/Brachyury polarity and elongate without extra-embryonic tissues or externally localized signals [9, 11].

The cellular source of surface-tension differences in gastruloids is still open. Work on tissue cohesion shows that adhesion alone is insufficient. Cadherin-mediated contacts, actomyosin contractility, cortical tension and boundary mechanical polarization can all contribute to tissue surface tension [8, 7, 6]. In the adhesion-tension model, a reduced form for the mechanical contribution is

$$\alpha = \max\left(0, \frac{\gamma/2 - \beta}{2P_{\text{hex}}}\right)$$

where γ is effective adhesion, β is cortical tension and P_{hex} is the perimeter of a compact hexagonal cell. When $\gamma/\beta < 2$, the differential adhesion hypothesis is approximately correct. When $\gamma/\beta > 2$, surface-cell stretching and cortical mechanics dominate [7]. This matters because changing E-cadherin or α-catenin also changes contractility. A perturbation that appears adhesive therefore alters the mechanical field that drives flow, so adhesion, contractility, motility and tissue-scale flow must be read out in the same developmental window.

3. Research Goal and Hypothesis

Tissue surface tension is either an upstream driver of gastruloid polarity or a downstream consequence of biochemical patterning, and this project resolves which. The central hypothesis is that surface-tension gradients organize the anterior-posterior axis: perturbing E-cadherin expression or actomyosin contractility reduces these gradients, impairs large-scale recirculating tissue flows and reduces T/Brachyury polarization. The co-occurrence of all three effects distinguishes a causal mechanical role from mere correlation between tension and polarity.

The falsifiability criterion is the linked mechanical chain: surface-tension gradient reduction, recirculating-flow dissipation, and reduced T/Brachyury dipole moment under the same perturbation and window. A break at any link, reduced gradients without flow loss or flow loss without polarity loss, localizes the initiating signal downstream of biochemical patterning rather than in the mechanical field.

4. Proposed Experimental Approach

The approach perturbs tissue surface tension in matched 3D gastruloids formed from mouse embryonic stem cells and reads out the mechanical chain in a single developmental window. Contractility is controlled with optoGEF-RhoA or a related optogenetic system that places RhoA-driven actomyosin tension under blue-light control [5, 10, 2, 12]. Parallel E-cadherin and α-catenin perturbations test the adhesion side of the same mechanical model.

Perturbations are applied during the 72 to 96 h symmetry-breaking window. One light pattern increases contractility near the emerging T/Brachyury-high pole and tests whether added tension sharpens flow and polarity. A uniform illumination condition reduces natural surface-tension contrast and tests whether flow and polarity weaken together. Controls are unstimulated gastruloids, illuminated cells lacking the optogenetic construct and unperturbed wild-type aggregates.

Live imaging and quantitative analysis measure the effect of each perturbation. Particle image velocimetry or optical flow quantifies tissue flow fields. T/Brachyury reporter or immunofluorescence images reduce to a polarization dipole,

$$p_T = \frac{\left|\sum_i I_i(\mathbf{r}_i - \mathbf{r}_{\mathrm{cm}})\right|}{\sum_i I_i}$$

where I_i is T/Bra intensity at position $\mathbf{r}_i$. Elongation and aspect ratio at 120 h measure later morphology. E-cadherin and phospho-myosin II localization report adhesion and contractility state. Aggregate fusion or micropipette aspiration estimates effective tissue surface tension. AFM aggregate compression, traction force microscopy and contact-angle measurements at T+/T- interfaces provide secondary checks on adhesion, cortical tension and flow interpretation. Rac1/Cdc42 perturbations and single-cell trajectories in disaggregated controls track cell motility, so a failed polarization phenotype attributes to adhesion, contractility or motility rather than to nonspecific developmental arrest.

5. Expected Outcomes and Significance

The mechanism is supported when contractility or adhesion perturbation dissipates Marangoni-like recirculating flows below control levels and reduces the T/Brachyury dipole moment or delays its approach to the control polarization level. This joint outcome establishes that surface-tension gradients are mechanistically necessary for posterior and anterior differentiation.

Alternative outcomes localize the mechanism. Reduced recirculating flows with preserved T/Bra polarization place biochemical signaling upstream as sufficient to initiate axis formation, while a slower but complete polarization places mechanical flow downstream as an accelerant rather than an initiator. Each dissociation assigns the initiating signal to a specific layer of the tension-flow-polarity chain.

Compensation after E-cadherin knockout, including up-regulation of other adhesion or contractility pathways, is the principal confound, and the optogenetic arm controls it by perturbing cortical tension directly and reversibly. The adhesion, contractility and motility assays separate a true surface-tension mechanism from general loss of tissue viability. A positive result connects mechanical self-organization to developmental patterning and explains axis-formation defects in terms of measurable physical variables rather than marker expression alone.

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