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Supplementary information

Left–right symmetry of zebrafish embryos requires somite surface tension

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Supplementary Note

Mechanical model of AP length adjustment of a newly formed somite

Here we construct and discuss the coarse-grained mechanical model of a newly-formed somite. Following the experimental observations in the main text, in the model the somite is considered as a homogeneous cuboid with dimensions L_{AP}, L_{ML} and L_{DV} (Extended Data Fig. 7A(a)) oriented along the main animal axes throughout the relevant time window. In such a cuboid, the velocity gradient tensor is diagonal and the shape changes are related to the corresponding velocity gradient components

$$v_{AP} = \frac{\partial_t L_{AP}}{L_{AP}} \tag{1}$$

$$v_{ML} = \frac{\partial_t L_{ML}}{L_{ML}} \tag{2}$$

$$v_{DV} = \frac{\partial_t L_{DV}}{L_{DV}} \tag{3}$$

Following experimental observation that the volume is conserved, we describe somites as incompressible so that the volume

$$V = L_{AP} L_{ML} L_{DV} \tag{4}$$

is constant. We can express the incompressibility as

$$v_{AP} + v_{ML} + v_{DV} = 0 \tag{5}$$

The shear rate tensor is also diagonal and due to incompressibility, the diagonal components are equal to those of velocity gradients

$$\tilde{v}_{AP} = v_{AP} - \frac{1}{3}\left(v_{AP} + v_{ML} + v_{DV}\right) = v_{AP} \quad , \tag{6}$$

$$\tilde{v}_{ML} = v_{ML} - \frac{1}{3}\left(v_{AP} + v_{ML} + v_{DV}\right) = v_{ML} \quad , \tag{7}$$

$$\tilde{v}_{DV} = v_{DV} - \frac{1}{3}\left(v_{AP} + v_{ML} + v_{DV}\right) = v_{DV} \quad . \tag{8}$$

Stresses acting on somite boundaries arise from somite surface tension Γ, which originates from a fibronectin-rich extracellular matrix, epithelial cells of the somite and from contact with surrounding tissues. Surface tension stresses acting on each cuboid surface arise from surface tension forces in the neighbouring surfaces acting on the surface boundaries (Extended Data Fig. 7A(b)) and therefore,

$$\sigma_{\Gamma,AP} = -2\Gamma\left(\frac{1}{L_{ML}} + \frac{1}{L_{DV}}\right) \quad , \tag{9}$$

$$\sigma_{\Gamma,ML} = -2\Gamma\left(\frac{1}{L_{AP}} + \frac{1}{L_{DV}}\right) \quad , \tag{10}$$

$$\sigma_{\Gamma,DV} = -2\Gamma\left(\frac{1}{L_{ML}} + \frac{1}{L_{AP}}\right) \quad . \tag{11}$$

PSM ablation experiments established the presence of a compressive stress σ_a on the posterior somite surface (Extended Data Fig. 8A). In principle, σ_a could depend on L_{AP} and contribute to the adjustment mechanism of somite lengths. Although we cannot directly test this dependence, we rely on: i) the fact that explant relaxation time-scales are comparable and smaller than *in vivo* suggests that σ_a is not very sensitive to L_{AP}, and ii) even if the PSM were responding elastically to L_{AP}, it is much longer than the somite so that observed variations of L_{AP} create only a tiny relative deformation of the PSM. We thus expect that σ_a does not depend significantly on L_{AP}. Therefore, we consider the PSM to act as a stress boundary condition, imposing a dynamic but L_{AP} independent normal stress σ_a on the somite posterior surface. To balance the forces acting on the somite along the AP direction, we include normal stress σ_a on both the anterior and posterior surfaces of the somite (Extended Data Fig. 7A(c)). To describe the contact between the somite and neural plate on the dorsal side and with yolk on the ventral side, we introduce a time-dependent constraint on $L_{DV} \leq l(t)$, where $l(t)$ is the distance between the yolk and neural plate (Extended Data Fig. 7A(d)). Convergence-extension flow drives the extension of the somite in the DV direction to fill the available space and the constraint can be written as $L_{DV} = l(t)$, as long as stress between the somite and the neural plate is compressive: $\sigma_{DV} - \sigma_{\Gamma,DV} < 0$, which we discuss below.

Following the results of lateral mesoderm ablation experiments (Extended Data Fig. 8B), we do not consider explicitly contact stresses with neighbour-

ing tissues in the mediolateral direction. If such contribution exists it would appear in the model similarly to σ_a. In the outcome of the model we can consider σ_a to account for this contribution as well and not including it explicitly would not change our conclusions.

In the model, we do not consider frictional forces between the somite and its environment and the force balance thus requires stresses in the somite to be constant [1]. We now write the boundary conditions as

$$\sigma_{AP} = \sigma_{\Gamma,AP} + \sigma_a \tag{12}$$

$$\sigma_{ML} = \sigma_{\Gamma,ML} \tag{13}$$

$$L_{DV} = l(t) \tag{14}$$

Developing somites undergo a significant permanent shape change, which suggests that stresses acting on it are beyond the proposed yield stress value [1] and that the somite tissue is in a plastic regime where it flows under stress. Furthermore, our observation of rounding of explanted somites (Fig. 3C and Extended Data Fig. 6) suggests that surface tension stresses are sufficiently above the yield stress value. Taken together, we can write linear constitutive equations relating the somite shear rate and shear stress as

$$\tilde{v}_{AP} = \frac{1}{\eta}\left(\sigma_{AP} + P\right) + \frac{1}{\eta}\zeta_{AP} \quad , \tag{15}$$

$$\tilde{v}_{ML} = \frac{1}{\eta}\left(\sigma_{ML} + P\right) + \frac{1}{\eta}\zeta_{ML} \quad , \tag{16}$$

$$\tilde{v}_{DV} = \frac{1}{\eta}\left(\sigma_{DV} + P\right) + \frac{1}{\eta}\zeta_{DV} \quad , \tag{17}$$

where η is somite viscosity and tensor $\boldsymbol{\zeta}$ describes anisotropy of internal stresses coming from the active anisotropic stresses that drive convergence-extension flows [2, 3]. The somite viscosity contains contributions from stochastic part of active stresses generated by cells as well as mechanical noise due to changes in the cellular structure such as cell rearrangements and cell divisions. Note that $\boldsymbol{\zeta}$ is traceless by definition: $\zeta_{AP} + \zeta_{ML} + \zeta_{DV} = 0$. Furthermore, if the somite tissue is a yield stress material such as Bingham plastic, in the flowing regime the magnitude of the shear flow follows

[1] The usual compatibility condition is assumed, which ensures that the displacement field remains continuous.

$\tilde{v} = (\tilde{\sigma} - \sigma_y)/\eta_{\text{plastic}}$, where $\tilde{\sigma}$ is the magnitude of shear stress tensor, σ_y is the value of the yield stress beyond which the tissue plastically flows and η_{plastic} is the viscosity of the plastic flow. Direction of the shear flow is determined by the direction of the shear stress so that for each of the diagonal components $XY = AP, ML, DV$ we can write $\tilde{v}_{XY} = \tilde{v}\tilde{\sigma}_{XY}/\tilde{\sigma} = \tilde{\sigma}_{XY}/\eta_{\text{plastic}} - (\sigma_y/\eta_{\text{plastic}}))$. Therefore, in a uniformly flowing plastic tissue where the direction of the shear stress does not change significantly, the yield stress term $(\sigma_y/\eta_{\text{plastic}})(\sigma_{XY}/\tilde{\sigma})$ is constant and can be absorbed effectively into the active stress term $\boldsymbol{\zeta}$. Therefore, our results are applicable to somites that have a yield stress, as long as they are in a (uniformly) flowing state.

To find the dynamical equation for L_{AP} we subtract Eqs. 15 and 16 and use Eq. 5 to express v_{ML} in terms of v_{AP} and v_{DV}. Finally, using the boundary conditions Eqs. 12, 13 and 14, as well as Eq. 1, we obtain

$$\frac{1}{L_{AP}}\partial_t L_{AP} = -\frac{1}{2l(t)}\partial_t l(t) + \frac{1}{2\eta}\sigma_a(t) + \frac{1}{2\eta}\left(\zeta_{AP} - \zeta_{ML}\right) - \frac{\Gamma}{\eta}\left(\frac{l(t)L_{AP}}{V} - \frac{1}{L_{AP}}\right) \tag{18}$$

Solving this equation would require knowledge of time dependence of $l(t)$, contact stress $\sigma_a(t)$ and possibly active stress tensor $\boldsymbol{\zeta}$. However, in this work we are interested in somite size adjustment and an extensive study of how L^0_{AP} is set is beyond our current scope. Therefore, we assume that a well defined value of L^0_{AP} exists, as observed in experiments, which we approximate as a constant in time defined by a combination of time-varying contributions

$$L^0_{AP} = \frac{V\eta}{2\Gamma l(t)}\left(\sqrt{s(t)^2 + \frac{4\Gamma^2}{\eta^2}\frac{l(t)}{V}} + s(t)\right) \quad , \tag{19}$$

where $s(t) = -\partial_t l(t)/(2l(t)) + \sigma_a(t)/(2\eta) + (\zeta_{AP} - \zeta_{ML})/2$. Now, given a constant value of L^0_{AP}, we proceed to test its stability by considering a small perturbation $\delta L_{AP} = L_{AP} - L^0_{AP}$. To the lowest order, the perturbation follows

$$\partial_t \delta L_{AP} = -\frac{\Gamma}{L^0_{AP}\eta}\left(1 + \frac{(L^0_{AP})^2 l(t)}{V}\right)\delta L_{AP} \quad . \tag{20}$$

Therefore, any variation of L_{AP} from L^0_{AP} will be reduced in time. The relaxation rate is time-dependent through $l(t)$. However, for observed typical

values of $L_{DV}(0) \approx 39\ \mu m$, $L_{DV}(1hr) \approx 53\ \mu m$, $V \approx 1.2 \cdot 10^5\ \mu m^3$, and $L^0_{AP} \approx 53\ \mu m$, the variable term changes from 0.91 at initial time to 1.24 at 1 hr. Therefore, the relaxation rate changes by about 16% and for simplicity we approximate it with its mean value

$$\partial_t \delta L_{AP} \approx -\frac{2.1\Gamma}{L^0_{AP}\eta}\delta L_{AP} \quad . \tag{21}$$

The relaxation time-scale is then

$$\tau \approx \frac{\eta L^0_{AP}}{2.1\Gamma} \tag{22}$$

In experiments the somite AP length is measured at the time of formation and at 1 and 2 hours later. In order to determine the time-scale τ from experimental data we write the Eq. 21 as

$$\partial_t L_{AP} = -\frac{1}{\tau}\left(L_{AP} - L^0_{AP}\right) \quad , \tag{23}$$

which can be solved for

$$L_{AP}(t) = e^{-\frac{t}{\tau}} L_{AP}(0) + \left(1 - e^{-\frac{t}{\tau}}\right) L^0_{AP} \quad . \tag{24}$$

Therefore, the time-scale τ can be determined by performing a linear fit to the experimental data $L_{AP}(1hr)$ vs. $L_{AP}(0hr)$ and $L_{AP}(2hr)$ vs. $L_{AP}(0hr)$. Then, $\tau = -t/\ln a$ where a is the slope coefficient of the linear fit. It is interesting to point out that even in a more general case where L^0_{AP} is not constant but relaxes from initial value at the moment of the somite formation to a final equilibrium value this procedure still provides a good measure of τ. Namely, for $L^0_{AP}(t) = L_0 + \Delta e^{-t/\tau_0}$ dynamics of somite AP length follows

$$L_{AP}(t) = e^{-\frac{t}{\tau}} L_{AP}(0) + \left(1 - e^{-\frac{t}{\tau}}\right) L_0 + \left(e^{-\frac{t}{\tau_0}} - e^{-\frac{t}{\tau}}\right) \Delta \quad , \tag{25}$$

which gives the same coefficient of proportionality between $L_{AP}(t)$ and $L_{AP}(0)$.

Explanted somites relax over time from an irregular shape to a sphere. We quantified the relaxation time-scale of the explants τ_e, as shown in the main text. In order to compare τ_e with relaxation time-scale τ of an *in vivo* somite, we approximate the former with an analytical expression for relaxation of a fluid prolate ellipsoid under surface tension [4]

$$\tau_e \approx \frac{19}{20}\frac{\eta R}{\Gamma} \quad , \tag{26}$$

where R is the final radius of the sphere and viscosity of the surrounding solution is neglected in comparison to the tissue viscosity η. For the mean volume estimate mentioned above, we find $R = \sqrt[3]{3V/(4\pi)} \approx 31\ \mu m$ so that $\tau_e \approx 29\eta/\Gamma\ \mu m$. Using the measured value $\tau_e = 1.1 \pm 0.4\ hr$ we can estimate $\eta/\Gamma \approx 0.04 \pm 0.01\ hr/\mu m$. For *in vivo* somites, we use Eq. 22 to quantify the same quantity $\eta/\Gamma \approx 0.063 \pm 0.004\ hr/\mu m$. Although not exactly the same the two measured values are similar, which suggests that surface tension is indeed a major contributor to somite size robustness. Differences between *in vivo* and explant values could stem from additional viscous and/or frictional dissipation occurring *in vivo*.

Interestingly, we find that explanted somites relax to a spherical shape, whereas in the presence of anisotropic internal stresses the final shape would be anisotropic. It is therefore likely that the organisation of CE flows is perturbed in explanted somites. However, since the physical mechanisms of CE flows is currently not understood it is difficult to immediately know how the loss of organised CE flow would affect mechanical properties such as viscosity of the somite tissue. To illustrate this point we discuss several qualitatively different scenarios that at the moment cannot be directly ruled out: i) The organised component of active stresses (responsible for CE flows) disappears but the noisy component of active stresses is not affected and viscosity does not change, ii) All active stresses, including the noisy component, are reduced and viscosity increases, iii) An organisational cue setting the somite scale pattern of active stresses disappears and the previously organised component of active stresses becomes noisy, increasing the overall active noise and therefore reducing the viscosity. Given the similarity of the relaxation times of explants and *in vivo* somites, we think it is reasonable to assume that surface tension and the effective somite viscosity might have changed only slightly. Namely, a large change in any of the two quantities would require a finely tuned (or coupled) change in the other in order to maintain a constant relaxation time. Since it is not clear that loss of CE flows would directly affect surface tension, we propose that even if CE flows are lost in the explants it is unlikely that somite viscosity is significantly affected. This discussion illustrates the importance of obtaining a solid biophysical description of CE flows during somitogenesis.

Finally, we note that the condition $\sigma_{DV} - \sigma_{\Gamma,DV} < 0$, required for the DV

constraint $L_{DV} = l(t)$ to be fulfilled, can be expressed as

$$\frac{3}{2}\eta\left(\frac{1}{L_{DV}}\partial_t L_{DV}\right) - \frac{3}{2}\zeta_{DV} - 2\Gamma\frac{1}{L_{DV}} + \Gamma\frac{1}{L_{AP}} + \Gamma\frac{1}{L_{ML}} < 0 \qquad (27)$$

The somites that we observe remain in contact with the neural plate during the experiment. Over that time L_{DV} grows and typically overcomes both L_{AP} and L_{ML}, which suggest that q_{DV} is positive and sufficiently large to maintain this condition. It is interesting to further remark that in the absence of mechanical constraints on the DV surfaces, we would have $\sigma_{DV} = \sigma_{\Gamma,DV}$ and the dynamics of DV somite dimension would be determined by equating left hand side of Eq. 27 with 0, which could be relevant for description of tail somites that may have no mechanical contacts at DV boundaries.

Supplementary References

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