On the role of mechanical feedback in synchronous to asynchronous transition during embryogenesis

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Experiments have shown that during the initial stage of Zebrafish morphogenesis a synchronous to asynchronous transition (SAT) occurs, as the cells divide extremely rapidly. In the synchronous phase, the cells divide in unison unlike in the asynchronous phase. Despite the wide spread observation of SAT in experiments, a theory to calculate the critical number of cell cycles, n^*, at which asynchronous growth emerges does not exist. Here, using a model for the cell cycle, with the assumption that cell division times are Gaussian distributed with broadening, we predict n^* and the time at which the SAT occurs. The theoretical results are in excellent agreement with experiments. The theory, supplemented by agent based simulations, establish that the SAT emerges as a consequence of biomechanical feedback on cell division. The emergence of asynchronous phase is due to linearly increasing fluctuations in the cell cycle times with each round of cell division. We also make several testable predictions, which would further shed light on the role of biomechanical feedback on the growth of multicellular systems.

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Time dependent synchronous behavior is pervasive across biological systems. Numerous examples of synchronous behaviors exist, from networks of neurons in the circadian pacemaker[1,2], and insulin-secreting pancreatic cells[3] at the cellular scale, to the chirping of crickets[4], and synchronous flashing of a colony of fireflies[5,6]. A particularly fascinating example of tissue scale synchronous behavior occurs during the development of multicellular organisms, which proceeds through rapid and synchronous cleavage divisions that subdivide the fertilized egg into a large population of nucleated cells[7,8]. For instance, a frog egg, divides into 37,000 cells in $\sim$ 43 hours, while the Drosophila embryo proliferates to 50,000 cells in about 12 hours[8]. The early development of many animals is characterized by fast, synchronized divisions that occur are almost independent on inter cell interactions. This is followed by the midblastula transition accompanied by the loss of synchrony in cell division and a lengthening of the cell cycle[7,9].

The observation of spontaneous synchronization dates back to the $17th$ century when Christiaan Hyugens reported the synchronization of pendulums, due to transfer of energy from one to another. However, the reverse process of how asynchronous behavior emerges in a synchronous system is a fascinating question, with relevance to understanding morphogenesis in living systems. Given that cell collectives generate geometric order, driven predominantly by cell divisions and traction forces[10], a better understanding the transition between synchronous to asynchronous cell division during early development of many animals could provide important insights into this process. Based on the analysis of the experimental data, coupled with theory and simulations, we show that the synchronous to asynchronous transition (SAT), during zebrafish morphogenesis, provides vital clues about how cells integrate biochemical and mechanical signals in regulating cell division.

Regulation of cell division is necessary for robust tissue growth and morphogenesis, with rapid cell proliferation being an integral phenotype characterizing early development[11,12]. In cells that undergo cleavage divisions, maturation promoting factor (MPF) undergoes cyclical changes that drive mitosis[8,13]. Biochemically, the cyclical activity of MPF is driven by the protein kinase, cyclin B, that accumulates during the DNA synthesis phase of the cell cycle, which is degraded once the cells reach the mitotic phase[8,14,15]. In combination with the biochemical factors that regulate the cell behavior, changes in the embryonic physical properties can feed back onto biochemical dynamics[16–18]. In particular, mechanical pressure, built up due to rapid cell divisions may function as a feedback signal regulating cell division[19].

Here, we discover that the SAT transition commonly observed during embryo development serves as a powerful example in dissecting the contribution of mechanical forces in controlling cell division. By analyzing experimental data, and developing theory and simulations of 3D multicellular collectives, we show that pressure experienced by cells due to mechanical interaction with their neighbors *quantitatively* explains the onset of the SAT. Furthermore, we predict that the lengthening of the cell cycle is a consequence of mechanical feedback on cell division.

Experimental Motivation: Our study is motivated by experimental data on time-dependent pattern of cell division during the early stages of Zebrafish morphogenesis. We start with a brief description of the experiment[20], which tracks the number of cell nuclei (a proxy for the number of cells), $N(t)$, as a function of time (t) using high-speed laser light-sheet fluorescence microscopy. Fig. 1a shows a plot of $N(t)$ as a function of t observed in the experiments. The cell divisions occur on the animal pole of the Zebrafish embryo (see inset showing three distinct division cycles), roughly every ~ 20 mins[7,20].

We focus on two distinctive features of the data. First, in the initial regime ($100\ min \leq t \leq 185\ min$), $N(t)$ exhibits a clear step-like pattern (see arrows in Fig 1a). Time intervals, with constant N, are interspersed with time regimes where the cell number increases (see the shaded regions). The step like pattern is a consequence of synchronous cell division, which is punctuated by finite time regimes where there are no cell division events. Second, at later times ($t \geq 185\ min$), the staircase pattern in $N(t)$ disappears, signaling the onset of the synchronous to asynchronous transition (SAT), which occurs at $n_E^* \sim 5$ rounds of cell division. Here, the subscript "E" denotes experiments. These interesting features of cell division raise the following questions: what sets the time scale at which the SAT transition occurs? and what, if any role, does biomechanical feedback plays on cell division in the SAT transition? These questions motivated us to first develop the theoretical framework.

Increasing fluctuations in cell division times drive the SAT: The experimental data[20] in Fig. 1a makes clear that the SAT occurs after $n_E^* \sim 5$ (the subscript E denotes experiments) rounds of cell division. Our objective is to provide a theory for predict the number of rounds, n_T^* (T stands for theory), it takes for the Zebrafish to exhibit SAT.

Let the nth round of division for an individual cell occur at time, t_n. Assuming that t_n is

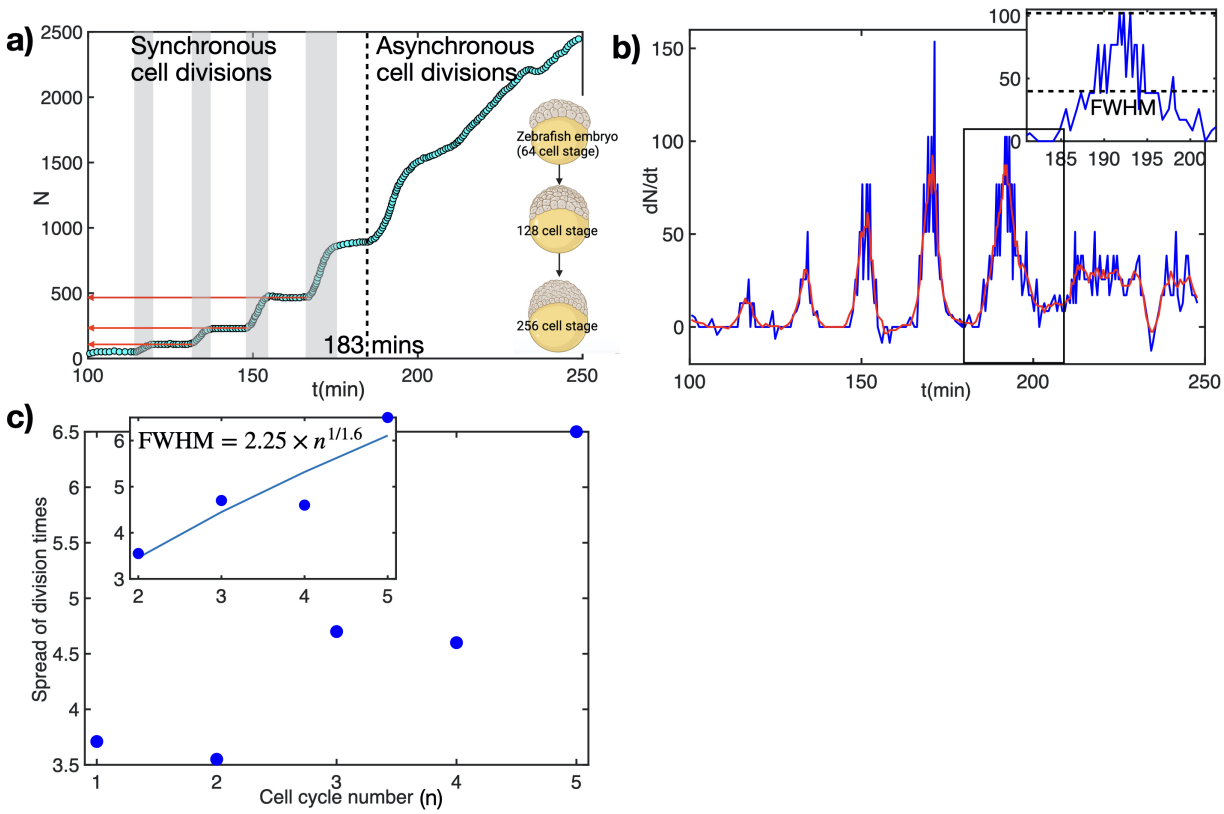

FIG. 1: **Synchronous to asynchronous transition in cell division during embryo development** **(a)** Experimental data for the number of nuclei in the animal hemisphere of the Zebrafish embryo from the 64-cell stage to 2,000+ cells. Initially, cell divisions are highly synchronized (shaded portion) exhibiting staircase-like pattern in the time dependence of cell number growth. After roughly five cell division rounds, the asynchronous behavior sets in (at times past the dashed line), where the cell number increases essentially continuously, without the staircase pattern observed at early times. **(b)** The time derivative of $N(t)$ (growth rate) exhibits an oscillatory pattern where peaks indicate rapid cell division events and valleys represent time points where divisions do not occur. Peak widths, shown in the inset, is a measure of the heterogeneity in the cell cycle times. **(c)** Heterogeneity in the cell cycle time extracted from the full width at half maximum (FWHM) of the peaks associated with division events in **(b)**. The FWHM increases as a function of the cell cycle number. The inset shows the fit to the data.

a random variable, we write it as,

$$t_n = \sum_{i=1}^{n} \tau_i, \tag{1}$$

where τ_i is the cell cycle time for the ith round of cell division (see Fig. 2 in the SI). We assume that all the τ_i's are independent and identically distributed random numbers with

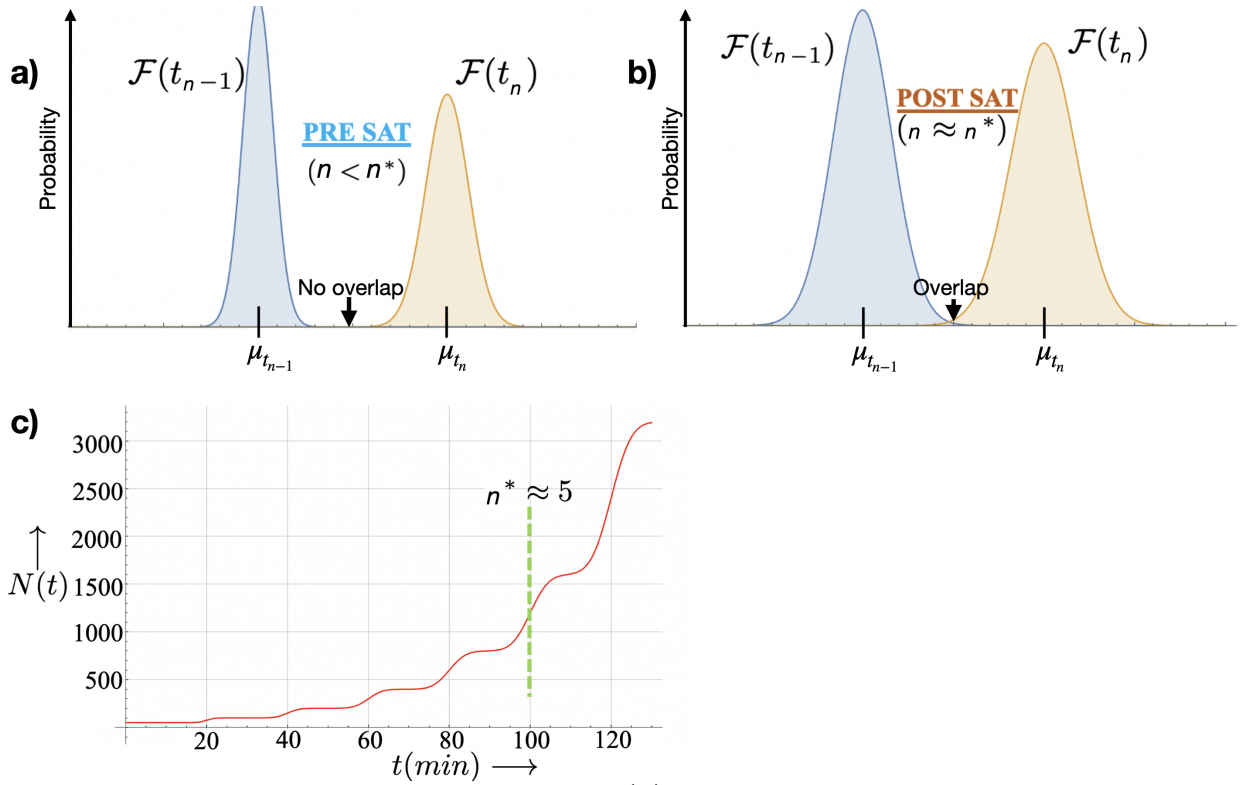

FIG. 2: **Variation in cell division times**. **(a)** Criteria used in the theory to predict the onset of the SAT in cell division. In the pre-SAT regime, the probability distributions, $\mathcal{F}(t_{n-1})$ and $\mathcal{F}(t_n)$ of the times at which cells divide, do not overlap, which implies that cells divide independently. **(b)** In the post-SAT regime, we propose that the distributions, $\mathcal{F}(t_{n-1})$ and $\mathcal{F}(t_n)$, begin to overlap, signaling the importance of cell-cell interactions. **(c)** Staircase-like pattern in N vs t curve calculated using theory. The plot shows the number of cells, $N(t)$, as a function of time (t) for multiple cell division rounds (n). The green dashed line demarcates the time at which SAT occurs resulting in $n^* \approx 5$. We reproduce the experimentally observed $N(t)$ vs t curves in Zebrafish Morphogenesis and the number of cell division cycles necessary for the onset of SAT[9].

the statistics of τ_i's being given by $\tilde{\mathcal{P}}(\tau)$ as derived in section I in the SI. From Eqn.(1), we can readily obtain the statistics of t_n. We will focus on the mean (μ_{t_n}) and the variance ($\sigma^2_{t_n}$) because they can be compared to the experimental data[20,21]. The mean, μ_{t_n}, is given by

$$\mu_{t_n} = \sum_{i=1}^{n} \mu_{\tau,i} = n\mu_\tau, \tag{2}$$

and the variance, $\sigma^2_{t_n}$, is given by

$$\sigma^2_{t_n} = \sum_{i=1}^{n} \sigma^2_{\tau,i} = n\sigma^2_\tau. \tag{3}$$

Here, μ_τ and σ_τ are the mean and standard deviation of a single cell cycle round (see SI section I for details). As can be seen from Eqs. (2) and (3), both the mean and the fluctuations (variance) increase linearly with the number of cell cycle rounds, n. Also, we predict that the probability distribution for t_n, $\mathcal{F}(t_n)$ given by

$$\mathcal{F}(t_n) = \mathcal{N}(t_n; n\mu_\tau, \sqrt{n}\sigma_\tau), \tag{4}$$

where $\mathcal{N}$ is a normal distribution with mean $n\mu_\tau$, and standard deviation $\sqrt{n}\sigma_\tau$.

To test the theoretical predictions, we quantified the statistics of cell cycle times from the experimental data. If our model is correct, we expect that the fluctuations in cell division times should increase at later cell division cycles. Remarkably, as predicted by theory (see Fig. 1) the variance increases as n increases. In Fig. 1b, we plot $\frac{dN}{dt}$ versus t, which allows us to extract the spread in cell division times for different n from the full width at half maximum (FWHM) of each peak (see Fig. 1b and inset). The spread in cell division times scales as $\sigma_{t_p} \propto n^{\frac{1}{1.6}}$, which is close to the theoretical prediction ($\sigma_{t_p} \propto n^{\frac{1}{2}}$). The modest deviation from theory might be due to poor statistics, suggesting that robust experiments should be performed to test the functional dependence of fluctuations with time. We note that Eq. (4) can be interpreted as a particle undergoing Brownian motion advected by a constant "velocity" μ_τ. Variability in cell cycle duration has been observed long ago, but its origin is unclear[22,23]. Our theory suggests that it is driven by fluctuations in cell division times.

With the ansatz for $\mathcal{F}(t_n)$, we can estimate n^* where SAT occurs. The onset of synchronous to asynchronous transition (SAT) corresponds to the absence of a plateau in the N as a function of t curves between successive cell division epochs. This is realized when the fluctuations in t_{n^*} are so large that the cell division time probabilities at two consecutive cell cycles - $\mathcal{F}(t_{n^*-1})$ and $\mathcal{F}(t_{n^*})$ - begin to overlap (see Figure 2a and 2b). Mathematically, this is equivalent to writing,

$$\mu_\tau \approx \lambda\big[\sigma_{t_{n^*-1}} + \sigma_{t_{n^*}}\big], \tag{5}$$

where λ controls the strength of the overlap between two successive distributions, $\mathcal{F}$'s. We assume that the two distributions start to overlap at three standard deviations from the

mean, $\lambda = 3$ (see Fig. 2b). Note that λ can vary depending on the strength of the overlap between the cell division time probability distributions. Using $\lambda = 3$, we can calculate n^*, the cell cycle round when SAT occurs given by,

$$n^* = \left(\frac{\kappa^2 + 1}{2\kappa}\right)^2, \tag{6}$$

where $\kappa = \frac{\mu_\tau}{3\sigma_\tau}$. In the experiments, $\sigma_\tau = 1.6\ min$ and $\mu_\tau = 20\ min$. Therefore, $n^* = 4.85 \approx 5$ from Eqn. (6) (refer to Figure 3 in SI for dependence of n^* on λ), close to the experimental data in Figure 1a, where the transition occurs after five cell division rounds. The theory also predicts that at the transition, $\sigma_{t_{n^*}} = \sqrt{5}\sigma_{t_{n=1}} = 3.6\ min$, which is close to the experimentally observed value of 2.8 min. The choice of $\lambda = 3$ is justified *a posteriori*.

Step-like pattern in cell number growth is recapitulated in theory: The theory also captures the step-like pattern in the growth of $N(t)$ observed in experiments (see Figure 1a). We estimate that $N(n,t)$ which denotes the number of cells at time t after n rounds of cell division from the following relation,

$$N(n,t) = N(n-1,t)\big[1 + \xi(n,t)\big], \tag{7}$$

where $\xi(n,t)$ is the probability that a cell has undergone n rounds of cell division until time t. In particular, $\xi(n,t)$ is the cumulative distribution function (CDF) associated with $\mathcal{F}(t_n)$ is given by,

$$\xi(n,t) = \int_{-\infty}^{t} \mathcal{N}[s; \mu_{t_n}, \sigma_{t_n}]ds = \frac{1}{2}[1 + erf\left(\frac{t - \mu_{t_n}}{\sqrt{2}\sigma_{t_n}}\right)], \tag{8}$$

where erf is the error function. In the above equation, the lower integration limit is from $-\infty$ because the support of the normal distribution is from $[-\infty, \infty]$. However, since the standard deviation, σ_{t_n}, is small compared to μ_{t_n}, the majority of the contribution to integral in Eq. 8 arises from $[\mu_{t_n} - 3\sigma_{t_n}, \mu_{t_n} + 3\sigma_{t_n}]$. Substituting the expression for $\xi(n,t)$ into Eq. 7, we obtain the recursive relation,

$$N(n,t) = N(n-1,t) + \frac{N(n-1,t)}{2}\left[1 + erf\left(\frac{t - \mu_{t_n}}{\sqrt{2}\sigma_{t_n}}\right)\right]. \tag{9}$$

Since the initial condition, $N(n = 0, t = 0)$ is known, we can calculate $N(n,t)$ for any n and t. Remarkably, Figure 2c, which shows the plot for $N(n,t)$ as a function of t using Eqn. (9), reproduces the step-like pattern observed during Zebrafish morphogenesis (compare to Figure 1a and Figure 2c).

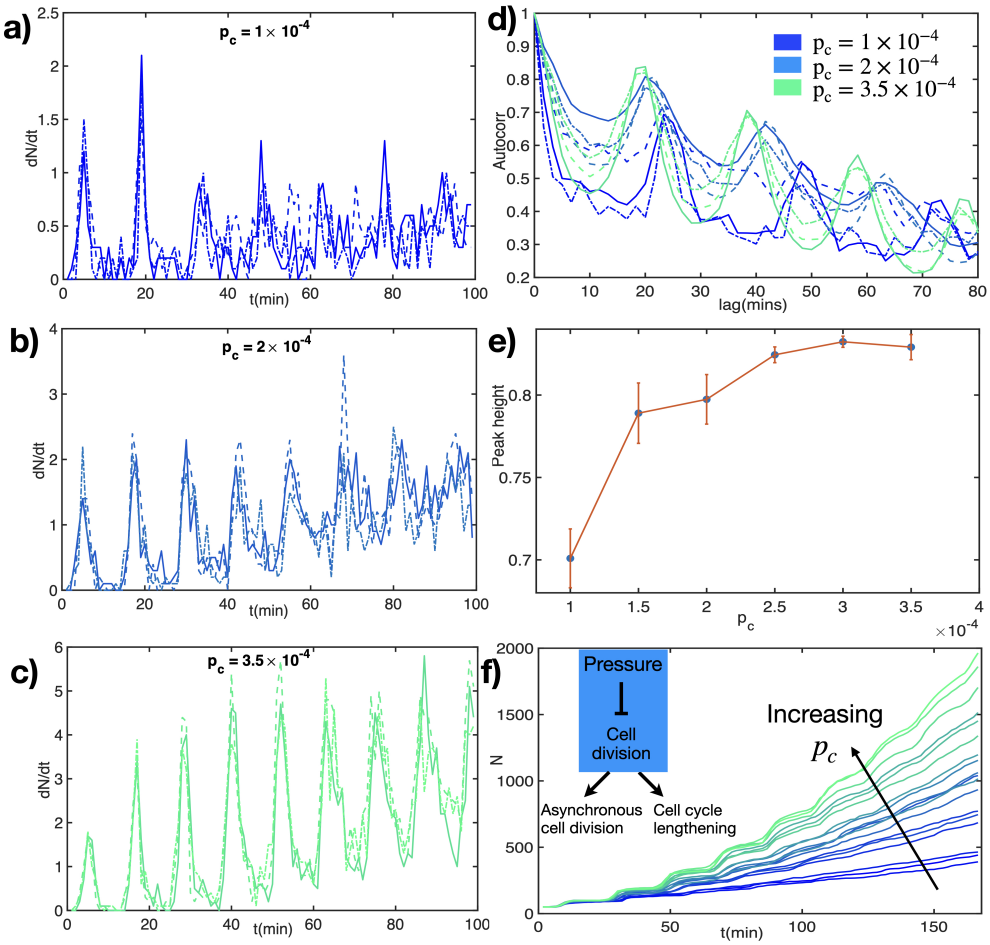

FIG. 3: **Mechanical feedback determines synchronous cell division** **(a)** Division rate as a function of time at low $p_c = 1 \times 10^{-4} MPa$. Low p_c value corresponds to heightened cell sensitivity to mechanical feedback on cell growth and division. **(b-c)** Division rate as a function of time at intermediate and high $p_c = 2.5 \times 10^{-4} MPa$ and $p_c = 3.5 \times 10^{-4} MPa$, respectively. Higher p_c value, corresponding to lesser cell sensitivity to mechanical feedback on cell growth and division, leads to highly synchronous cell division for a longer time range. **(d)** Autocorrelation of cell division rate at 3 p_c values. The height and time point of the first peak determine the strength of periodicity and the interval between repeated peaks in cell divisions. **(e)** Peak height of the autocorrelation function at varying sensitivity to mechanical feedback through pressure. Strength of periodic division rate increases with p_c and saturates at higher values. **(f)** Number of cells vs time as generated from simulations incorporating mechanical feedback on cell divisions. Inset: Schematic illustrating how increasing pressure limits cell divisions, leading to cell cycle lengthening and asynchronous cell divisions.

An essential question in cell biology is whether cell cycle control is deterministic or stochastic[24], and whether the observed variability in division times originate mainly from stochasticity, or from deterministic processes that appear to be random[23,25]. Although the theory shows that synchronous cell division may be quantitatively described without considering interactions between cells, the onset of asynchronous cell division, with increasing cell numbers, points to the importance of the local physical forces experienced by cells upon division[19,26,27]. Indeed, seminal studies have demonstrated that variations in cell density control cell proliferation rate through contact inhibition of proliferation[28]. Contact inhibition is thought to restrict cell proliferation when space is limited with dense spatial arrangements of cells leading to strong suppression of cell division[29,30]. To determine whether such mechanical effects influence SAT, we turn to a three-dimensional (3D) computational model for cell divisions.

Biomechanical feedback controls the SAT: Cells that are constantly in contact with their neighbors experience compressive forces that limit their ability to progress through the cell cycle. The cumulative effect of such compressive forces from the cell microenvironment can characterized at the multicellular scale through pressure experienced by the cells. Such cell-cell contact-induced pressure is expected to decrease the rate of division[19,21,27]. The possibility of mechanical feedback on cell division has significant implications, because cell cycle regulation is important in stem cell biology as pluripotent cells divide slowly over long periods but can initiate growth on demand. To capture these effects, we performed particle-based simulations[27,31–36], to investigate how cell cycle progression, controlled via feedback based on local inter-cellular mechanical interactions, impacts the ability of cells to grow and divide. The cell cycle time during each round of cell division is μ_τ. Cells may divide only if pressure experienced by a cell is below a critical pressure ($p < p_c$). This implies that synchronous divisions occur during zebrafish morphogenesis before t mechanical feedback is operative. The universality of SAT can be tested by conducting high temporal resolution cell cycle experiments on different cell types[23,37].

In our model, cells are either in the dormant (D) or in the growth (G) phase. Even though cells in the experiment do not grow, we consider physical cell growth as a proxy for cell cycle progression. We track the sum of the normal pressure, p_i that a particular cell i

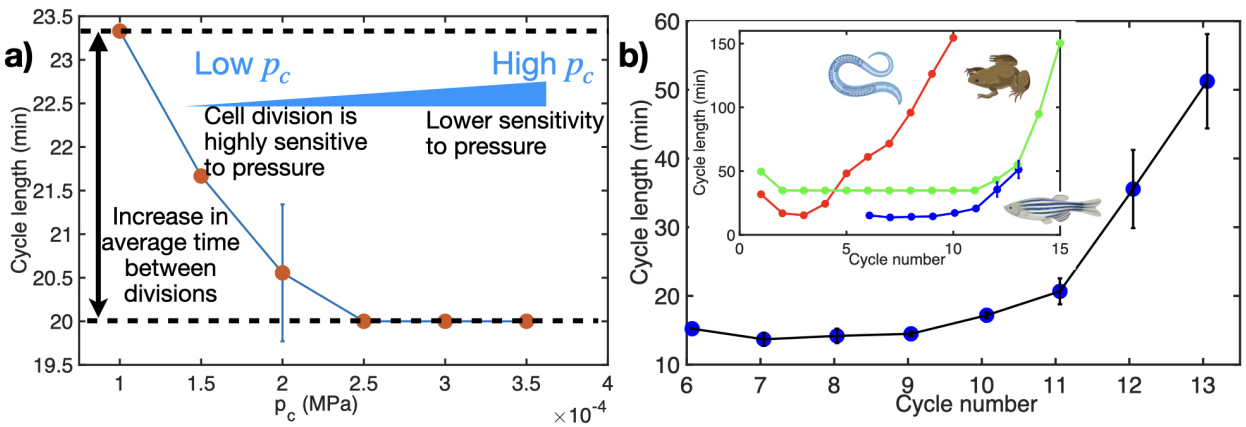

FIG. 4: **Mechanical feedback elongates cell cycle (a)** Average cell cycle length increases with stronger mechanical feedback. Cell cycle length is quantified from the time of the first peak in the growth rate autocorrelation function. **(b)** Experimental data extracted from Ref.[7] for cell cycle length during cycle numbers 6-13 for Zebrafish development. Inset shows similar behavior that is conserved across multiple species - *C. elegans* (roundworm), *X. laevis* (frog) in addition to *D. rerio* (zebrafish).

experiences due to contact with its neighbors, using

$$p_i = \Sigma_{j \in NN(i)} \frac{|\vec{F}_{ij} \cdot \vec{n}_{ij}|}{A_{ij}}, \tag{10}$$

where $\vec{F}_{ij}$ is the force that i^{th} cell experiences from its nearest neighbors (NN) j, $\vec{n}_{ij}$ is the unit vector that points from the center of cell j to the center of cell i, and the contact surface area between cells i and j is $A_{ij} = \pi h_{ij} R_i R_j / (R_i + R_j)$. The nearest neighbors of a cell is determined through positive overlap between cells through the h_{ij}, defined as $\max[0, R_i + R_j - |\vec{r}_i - \vec{r}_j|]$. Importantly, once the pressure exceeds the critical pressure p_c the cell stops growing and enters a dormant phase where cell division cannot occur. Lower p_c implies that cells enter the dormant phase with high probability while higher p_c values would make the cells less sensitive to feedback on division due to mechanical interactions with its neighbors. By varying p_c, we predict that higher sensitivity to mechanical feedback on cell growth leads to faster onset of asynchronous cell divisions. The growth rate of the cell collective closely recapitulate experimental data where clear peaks with bursts of cell division events followed by time regimes with absence of division events (see Figs. 3a-c). Notably, the synchronous division events are more persistent at higher p_c and are maintained for longer times as compared to lower p_c where the clear peaks in division rate are quickly washed out

(compare Figs. 3a and c).

To quantify the synchronous nature of the cell division events, we evaluated the autocorrelation of the division rate to verify the presence of cycles and determine their duration. The autocorrelation function of a periodic signal has the same periodic characteristics, evaluated using,

$$C(t_\gamma) = \langle \Sigma_{\alpha,\beta} \frac{dN(t_\alpha)}{dt} \times \frac{dN(t_\beta)}{dt} \delta(t_\gamma - (t_\alpha - t_\beta)) \rangle, \tag{11}$$

where, t_γ is the lag time between time points t_α, t_β, and $\delta(z) = 1$ if $z = 0$ and 0 otherwise. The autocorrelation function is normalized to ensure that $C(t_\gamma = 0) = 1$. We focus on the first peak in the autocorrelation function in order to quantify the peak height and peak location as a function of p_c. The peak height measures the strength of the oscillatory division patterns. The peak height increases and saturates at high p_c, as shown in Fig. 3e, which is indicative of more persistent synchronous division patterns with weak mechanical feedback. (see Fig. 3f).

Mechanical feedback lengthens the cell cycle: So far we have focused on the collective effect of coordination of cell division events between different cells. We now delve into the single cell scale to verify our prediction that cell cycle lengthening is due to mechanical feedback. Many embryos exhibit a period of rapid cell division followed by a lengthening of cell division times during early development[38]. The embryo developmental processes are crucially linked to the speed of cell division as well as the cell cytoskeleton during mitosis is engaged in building the mitotic spindle[38,39]. As many of the morphogenetic events require cytoskeletal arrangements, they must wait for cell divisions to slow down[38]. As such slowing of the cell division time, associated with the mid blastula transition, is an important and conserved feature of embryo development.

We anticipate that pressure-dependent feedback on cell division may lead to the lengthening of the cell cycle. Recently, we showed that pressure experienced by cells near the core of multicellular spheroids is elevated compared to those at the periphery, setting up emergent variations in the cell division rate between the core and periphery[27,32,40]. Moreover, there is a the coupling between cell growth rate and pressure through intracellular molecular crowding was[41]. From the autocorrelation in cell growth rate (see Eq. 11), the lag time at which the peak is located is a measure of the time difference between two division events, which corresponds to the average cell cycle time. Our simulations show that the cell cycle time is inversely proportional to the value of the threshold pressure, p_c (see Fig 4a). Lower values of

p_c correspond to higher sensitivity to cell division because mechanical forces exerted by cells on one another could easily reach a lower value of p_c (see Fig. 4b inset). Our quantification shows that higher sensitivity to mechanical feedback lead to a longer cell division time. We next turned to the experimental data to assess whether cell cycle lengthening is observed during Zebrafish development. Indeed, as shown in Fig. 4b, the cell cycle lengthens markedly as cells progress through repeated division cycles. Experimental data, therefore, shows that SAT is followed by an increase in the mean cell cycle[9] during Zebrafish morphogenesis. The data in this regime can be fit using $\mu_\tau(p, p_c) = \mu_\tau e^{\frac{p}{p_c}}$, where the exponential increase in cell cycle times is a consequence of mechanical feedback.

Conclusion The finding that synchronous cell division depends on the strength of mechanical feedback points to an interesting manifestation of the cross-talk between mechanical forces and cell cycle regulation during embryo development. Here, we showed that coordination between cells during cell cycle progression is regulated by whether cells experience strong or weak mechanical feedback. A direct implication of our study is that suppression of mechanical feedback, which may be realized by partial knockout of E-Cadherin, would prolong the synchronous phase. Given our prediction that asynchronized cell division emerge during the course of embryo development at the cusp of the mid-blastula transition, corresponding to the activation of transcription, SAT in cell division could be a signal for cells to trigger their own transcription programs as a function of increasing cell density. Cells that grow under strong mechanical feedback, transition rapidly into asynchronous cell division patterns even at very values of small pressure. In contrast, for cells growing under weak mechanical feedback, synchronous division patterns persist for a longer period of time.

We have shown that mechanical feedback drives the lengthening of the cell cycle time. Cells experiencing strong mechanical feedback elongate their cell cycle. On the other hand, cells under weak mechanical feedback progress through the cell cycle more rapidly and more uniformly. The proposed feedback mechanism on growth is therefore a 'mechanical' intercellular signal that enables cells to measure density and the timing of development.

The observation that embryos develop autonomously under a predetermined schedule is striking[42–44]. Studies based on molecular biology revealed that various genetic factors control the periodicity of the development of somites in which a network of genes whose expression oscillates synchronously and regulate the timing of development. As tissue mechanics was recently shown to be an important factor in the formation of body segments, formerly

thought to be based on segmentation clock[45], we propose that mechanical feedback on cell growth serves as a clock (timing device) that mediates asynchronization of cell division, and therefore, the onset of the transcriptional machinery during the mid-blastula transition.

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