Evolution of cell size control is canalized towards adders or sizers by cell cycle structure and selective pressures

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Abstract

Cell size is controlled to be within a specific range to support physiological function. To control their size, cells use diverse mechanisms ranging from 'sizers', in which differences in cell size are compensated for in a single cell division cycle, to 'adders', in which a constant amount of cell growth occurs in each cell cycle. This diversity raises the question why a particular cell would implement one rather than another mechanism? To address this question, we performed a series of simulations evolving cell size control networks. The size control mechanism that evolved was influenced by both cell cycle structure and specific selection pressures. Moreover, evolved networks recapitulated known size control properties of naturally occurring networks. If the mechanism is based on a G1 size control and an S/G2/M timer, as found for budding yeast and some human cells, adders likely evolve. But, if the G1 phase is significantly longer than the S/G2/M phase, as is often the case in mammalian cells *in vivo*, sizers become more likely. Sizers also evolve when the cell cycle structure is inverted so that G1 is a timer, while S/G2/M performs size control, as is the case for the fission yeast *S. pombe*. For some size control networks, cell size consistently decreases in each cycle until a burst of cell cycle inhibitor drives an extended G1 phase much like the cell division cycle of the green algae *Chlamydomonas*. That these size control networks evolved such self-organized criticality shows how the evolution of complex systems can drive the emergence of critical processes.

Introduction

Cell size is fundamental to cell physiology and function because it sets the scale of subcellular compartments, cellular biosynthetic capacity, metabolism, mechanical properties, surface-to-volume ratios, and molecular transport (Chan & Marshall, 2010; Ginzberg et al., 2015; Neurohr et al., 2019; Zatulovskiy & Skotheim, 2020). While different types of cells vary enormously in size to perform their functions, cells within a particular type are generally uniform in size indicating that cell growth may be accurately coupled to division and differentiation processes.

On a phenomenological level, there are many commonalities in how cells regulate their size even though the molecules controlling cell division vary across the tree of life with the most striking differences separating eukaryotes and bacteria. This extreme molecular diversity in the regulatory proteins raises the question as to what are the common features of the control systems that evolved to implement size control.

Most generally, cell size control can be viewed as a return map where the division size is a function of the cell size at birth. The examination of proliferating cells in laboratory conditions has revealed a variety of size control phenomena that can be characterized quantitatively by plotting the size of a cell at birth against the amount of mass added before it divides (Amir, 2014; Facchetti et al., 2017; Jun & Taheri-Araghi, 2015). A 'sizer' has a slope of -1 so that all variation in cell mass at birth is compensated for in one cell cycle, while an 'adder' has a slope of 0 so that each cell adds the same amount of mass during the cell cycle regardless of initial size. In the case of an adder, control is weaker so that multiple cell cycles are required for a particularly large or small cell to return to the average cell size. Importantly, the slope relating size at birth with the amount of growth in the cell cycle is a metric that quantifies the amount of size control occurring in a particular condition.

Studies of cell size control have revealed a diverse set of phenomena. Fission yeast and mouse epidermal stem cells exhibit 'sizers', and most bacteria, archaea, and cultured human cell lines exhibit behavior closer to adders (Cadart et al., 2018; Eun et al., 2018; Jun et al., 2018; Sveiczer et al., 1996; Westfall & Levin, 2017; Willis & Huang, 2017; Xie & Skotheim, 2020). Thus, while diverse size control behaviors have been observed, adders have been observed more often than sizers. This raises the question of why adders are more frequently observed if sizers, by definition, are more effective at controlling cell size?

To address the question of why adders are the most often observed form of cell size control, we used evolutionary algorithms (Holland, 1992) to identify commonalities between networks evolved to control cell size. Evolutionary algorithms are a class of machine learning techniques aiming at mimicking evolutionary processes (François & Hakim, 2004; François, 2014; Xiong et al., 2019; Crombach, 2021). Because of the nature of evolution, results of evolutionary computations are often more efficient and more creative than expected (Lehman et al., 2020). Furthermore, solutions found by evolutionary algorithms are more constrained by their evolutionary paths followed and present similar characteristics to biologically evolved systems (Schaerli et al., 2018). We start with a simple model of the cell cycle with a timer for an S/G2/M phase (Fig. 1) that we evolve to optimize homeostatic cell size control. We discovered that different control mechanisms could perform cell size control based on protein quantity or concentration. Simulations in which size control takes place in G1 phase converge towards an adder mechanism for the entire cell cycle and identified an active quantity sensing mechanism similar to dilution-based mechanisms previously identified experimentally (Chen et al., 2020; Qu et al., 2019; Schmoller et al., 2015). The relative duration of G1 and S/G2/M were important in determining size control properties. A relatively shorter S/G2/M phase favors sizer mechanisms, while longer S/G2/M phases favor adders. Moreover, inverting the model, so that cell size rather than time controls the S/G2/M, like in fission yeast, results in more size-like control. Thus, we

anticipate adders arise when cell size is controlled at a point intermediate in the cell cycle, like the G1/S transition, while sizers will appear when cell size regulates a point later in the cell cycle, as is the case when G1 is proportionally longer, or control takes place at the transition to mitosis. While there is no one-to-one correspondence between a specific size control mechanism and a given evolutionary pressure, our work identifies clear evolutionary principles that shed light on the diverse cell size control phenomena previously observed experimentally.

Materials and Methods

Mathematical formalism

To model gene networks, we follow a standard ODE based formalism, where we simulate dynamics of the concentrations of proteins. We use Hill functions for transcriptional interactions, and standard mass action kinetics for protein-protein interactions. We also assume that all proteins are degraded at a constant rate. For most of the simulations presented in the paper, we use deterministic ODEs for simplicity. Importantly, cell-to-cell variability arises from the precise timing of cell cycle progression events (see below). This allows for a natural way to generate noise on cell volume that should then be compensated for by the evolved network. In the last part of the paper, we explicitly include Langevin noise for biochemical reactions that are modeled using a classical tau-leaping formalism (Gillespie, 2007). Thus, each biochemical reaction takes place with a Poisson rate that corresponds to the deterministic rate, to which we add one white Gaussian noise with a variance equal to that rate.

In general, there are two classes of mechanisms that cells use to control their size that can be separated in terms of whether cell division or cell growth *per se* is regulated by cell size. Note that in this work, we will use mass, size, and volume interchangeably. In the first class, it is crucial that the growth rate per unit mass of a cell depends on cell size so that cells further from the optimum cell size grow slower (Cadart et al., 2019; Ginzberg et al., 2018; Miettinen & Björklund, 2016; Nordholt et al., 2020; Tzur et al., 2009). Such slower growing cells are then outcompeted by cells closer to the optimum size even when divisions occur purely by chance (Conlon & Raff, 2003). While size-dependent growth mechanisms exist and do support size homeostasis, such mechanisms rely on inefficient growth in all the cells away from the optimum size. To avoid such inefficient growth, many types of cells use active size control mechanisms to accelerate progression through the cell cycle in larger cells (Zatulovskiy & Skotheim, 2020). In our simulations, we keep cell growth rates constant over a physiological range of cell sizes. This allows us to focus on the common features of the molecular networks in which increasing cell size drives changes in molecular activities to trigger cell division. We assume that cell volume V grows at a rate $\lambda(V) \times V$, so that growth is exponential when λ is a constant. Volume is divided by 2 at each division after which we follow one of the two daughter cells. The growth rate sets the time scale for the system dynamics as it defines the doubling time $\tau = ln\,(2)/\lambda$. Any interdivision time shorter than τ will see the cell volume shrink at the next generation while any

interdivision time larger than τ will see the cell volume grow. We also use the chemistry square bracket convention such that any protein X's *concentration* is denoted by $[X]$. Correspondingly, its *quantity* is denoted by X only and is defined as $X = [X] \times V$.

Volume influences protein dynamics in three ways. First, protein production rates are generally proportional to cell volume so that proteins reach and maintain a constant concentration that is independent of the cell volume (Chen et al., 2020; Elliott & McLaughlin, 1978; Newman et al., 2006; Swaffer et al., 2021). Thus, the only deterministic influence of volume on concentration dynamics is on the dilution rate, which is proportional to the cell growth rate $\lambda(V)$ (see details in Supplement). At cell division, we also assume that proteins are equally partitioned between the daughter cells, *i.e.*, the concentration is the same before and after division. The cell volume also contributes to stochastic effects, which are larger in smaller cells with fewer molecules. For stochastic simulations, we therefore include a multiplicative $\frac{1}{\sqrt{V}}$ contribution to the added Gaussian noise term (see more complete description in the Supplement). Note that we scale all our variables so that a concentration of one arbitrary unit corresponds roughly to 1000 proteins in a 100fL cell (Milo et al., 2010).

Evolutionary procedure

To evolve networks regulating cell size, we use the □-evo software (Henry et al., 2018) with a modified numerical integrator accounting for volume dynamics and volume dependencies as described above (Fig. 1B). □-evo simulates the Darwinian evolution of a population of gene networks. A network is encoded with the help of a bipartite graph connecting biochemical species (typically proteins) and interactions between them (we use a custom-made Python library). Networks are converted into an ensemble of stochastic differential equations using a Python to C interpreter. This code is then compiled and integrated on the fly to compute behavior of the network.

Each selection step in the algorithm is referred to as an *epoch* rather than the more commonly used term *generation* because we use the term generation to refer to cell divisions in the simulations. At each epoch of the algorithm, each gene network is simulated, and its fitness computed. Based on the fitness function(s) (see below), half of the networks are selected and duplicated, while the other half is discarded to maintain a constant population size. The duplicated networks are then randomly mutated. From the most to least probable, mutations consist in random changes of parameters of the network, random removal of interactions, and random additions of interactions or new proteins. All of this is easily made with our customized Python library encoding networks. For more details on technical aspects and implementations of the □-evo software, we refer the reader to (Henry et al., 2018).

Realistic evolutionary processes select for multiple phenotypes in parallel. While trade-offs between those phenotypes are non-trivial, it has been observed that phenotypes typically define an evolutionarily Pareto front (Shoval et al., 2012; Warmflash et al., 2012). We thus perform network selection using a Pareto mode (Warmflash et al., 2012), in which 2 distinct fitness

functions are computed. For the selection step, networks are first Pareto ranked. For example, consider two networks A and B and two fitness functions f^1 and f^2. f^1_A refers to the fitness of network A calculated with function f^1. Assuming fitness functions are to be maximized, we say network A Pareto-dominates network B if both $f^1_A > f^1_B$ and $f^2_A \geq f^2_B$. Rank 1 networks are networks which are not dominated by any other networks, Rank 2 networks are networks dominated only by Rank 1 networks, and Rank 3 networks are only dominated by Rank 1 and Rank 2 networks and so on. The algorithm then selects half of the population of Rank 1 networks using a fitness sharing algorithm to maximize population diversity (see details in (Warmflash et al., 2012)). One advantage of Pareto selection is the increased flexibility of the evolutionary process. Multiple fitness functions can provide different optimization paths in parameter space, which prevents the selection process from getting stuck in a local optimum of a single fitness function. We also perform a few simulations with only one fitness function, in which case networks are simply ranked based on their fitness.

We impose two evolutionary selection pressures in the form of two fitness functions. The first fitness function is simply the number of cell divisions during a long period, which we call N_{Div}. This is consistent with the classical definition of fitness as optimizing the number of offspring and is to be maximized by the algorithm. The second fitness function is the coefficient of variation of the volume distribution at birth for those N_{Div} generations, which we call CV_{Birth} and is to be minimized by the algorithm. This penalizes broad distributions of volume at birth, which are detrimental to cell size homeostasis. We also present results for additional fitness functions that we explored in the Supplement. These include a least-square residual function to minimize volume variation about a target size, and the fitted slope of the amount of volume added at each cycle to be minimized to drive models toward being a sizer.

Initial cell-cycle model

We initialize our network evolution simulations with a very simplified model of the cell-cycle. We model two independent phases of a symmetrically dividing cell, G1 and S/G2/M, separated by a commitment point at the end of G1 and division at the end of S/G2/M (Fig. 1A) (Chandler-Brown et al., 2017). We encode this cell cycle state information via a binary switch variable we call 'S/G2/M Switch' that is 0 in G1 and 1 in S/G2/M. In most simulations, we follow an inhibitor model (Heldt et al., 2018; Schmoller et al., 2015; Zatulovskiy et al., 2020) and assume that the probability of passing the G1/S transition is controlled by the *quantity* of a transcription regulator . A lower quantity of this inhibitor means a higher probability of a G1/S transition at the current time step of the simulation (Fig. S2). Like all other proteins, the quantity is produced with a rate proportional to volume, degraded at a constant rate, diluted by cell growth, and equally partitioned between mother and daughter cells at division. We found that due to the volume scaling assumption, $[I]'s$ concentration alone was largely independent of volume and could not trigger a size-dependent G1/S transition, which is why we opted for the quantity of I instead (Fig. S3). Upon passing the G1/S transition, we assume cells are committed to division and there is a fixed time delay before they divide thus modeling S/G2/M as a timer. We fixed the timer duration to be roughly equal to 50% of the doubling time τ with some uniform noise such

that G1 and S/G2/M durations would be the same at equilibrium. Regulation of the quantity during the cell cycle thus controls the precise timing of the G1/S transition, but it is not always perfect since the transition is probabilistic. This, along with the noise in S/G2/M timer duration, creates natural cell to cell variability in volume that needs to be compensated for by the evolved mechanism. We note that we initialized most of our simulations with one added interaction in which production of [] is activated by the S/G2/M Switch variable to reset its concentration to a higher level before the next generation. We initially ran simulations without this specific interaction but found that it systematically appears in the initial stages of evolution simulations. We therefore included it in the initial network to speed up our simulations. We refer the reader to the supplementary information for more details.

Typical dynamics of this simple cell-cycle model are represented in Figure 1. These dynamics are similar to models of cell cycles based on relaxation oscillators (Cross, 2003; Tsai et al., 2008). The left and right slow branches correspond to G1 and S/G2/M, respectively, and the fast horizontal branches represent G1/S and division. An intermediate fictitious nullcline is shown that connects the typical concentration [] at G1/S and at division. Starting with cell birth, the system goes down the left-G1 branch because of degradation, then jumps to the right-S/G2/M branch below the threshold for the G1/S transition, stays there while moving up due to production by the S/G2/M Switch, until it jumps back to the left branch at the end of the timer phase. We note that there is no explicit volume control in this initial model since the only control comes from the quantity of which does not initially depend in any way on the volume. This initial quantity sensing oscillator does not perform size control and instead results in unstable growth where size deviations are amplified at each generation instead of being corrected (Fig. S3). Thus, the network needs to evolve some other interactions and/or parameters to go beyond a simple G1 inhibitor driven by production in S/G2/M to create a viable cell lineage.

We also ran evolutionary simulations with different cell cycle structures. For evolutionary simulations where the G1/S transition was controlled by the concentration of [], we simply change the probability to pass the G1/S transition to depend on concentration [] instead of its quantity . For evolutionary simulations with a cell cycle structure similar to that found in the fission yeast *S. pombe*, we invert the network structure. In this case, quantity controls division and the Switch is turned on for a fixed amount of time in G1. In terms of the relaxation oscillator, this means that the left branch is now S/G2/M and the right branch is G1.

Results

Evolution of quantity-based size control mechanisms

To examine how networks controlling cell size could evolve, we ran an evolutionary algorithm that optimizes both the number of divisions N_{Div} and the coefficient of variation of the size distribution at birth CV_{Birth} (see Methods for algorithm details). Fig. 2A illustrates the behavior

and results of a typical evolutionary run. An almost linear Pareto front evolves as a function of the number of epochs, and eventually converges towards a flat horizontal line. This flat Pareto front corresponds to a set of networks able to control cell volume as indicated by the low CV_{Birth}. The optimal networks, at the right most end of this line, both maximize N_{Div} and minimize CV_{Birth}.

Evolution simulations are in part reproducible and most often lead to similar network topologies (Fig. 2B; Model A1 is a typical example). The network leading to this type of cell size control is very simple. One gene, , is added to the seed network and is both repressed and titrated by forming the network motif known as a Mixed Feedback Loop (François & Hakim, 2005). The mixed feedback loop network that reproducibly evolved is arguably the simplest network that can achieve size control within our formalism. This can be understood intuitively as follows. [] represses [], which is thus only produced when [] is low, *i.e.,* when the cell is close to the G1/S transition and in early S/G2/M. But, since the *quantity* = [] × is fixed at G1/S by design, we get that the *concentration* [] is inversely proportional to the volume of the cell at the G1/S transition ($V_{G1/S}$) as shown in our simulations (Fig. 2C). This means that the synthesis rate and the concentration of [] become (linear) functions of the volume of $V_{G1/S}$ (Fig. 2C). This provides an explicit volume sensing mechanism via [] and allows the cell to retain memory of its volume at G1/S. Subsequently, titration of [] by [] provides negative feedback that regulates the total *quantity* of inhibitor produced during S/G2/M, so that cells are now born with a *quantity* of inhibitor that is independent of volume (Fig. 2D). Note that because of the timer in S/G2/M, volume at birth remains proportional to $V_{G1/S}$, the volume that set the scale for the concentration of [] at G1/S and throughout S/G2/M. After division, in the absence of production by the S/G2/M Switch, [] is further titrated in G1 by the remaining [] from the previous generation (still proportional to $V_{G1/S}$). This ensures that daughter cells reach the quantity threshold of G1/S earlier if they were born larger. When we examine the change in quantity of as a function of time spent in G1 and of $V_{G1/S}$ we see the slope of these two quantities is volume-dependent. This confirms that the active titration mechanism is regulated by volume to provide a size control mechanism in G1 (Fig. 2E).

Our typical evolved size control networks, while relying on *quantity* sensing, are conceptually similar to the budding yeast network relying on *concentration* sensing of the cell cycle inhibitor Whi5 since there is a constant quantity of present right after division (just like Whi5). In budding yeast, the Whi5 protein is passively diluted in G1 to increase the stochastic rate of progression through the G1/S transition. The time spent in G1 depends on the initial concentration of Whi5 at birth, which scales as 1/V, to promote a sizer mechanism. Here, the concentration of [] at birth scales as 1/V, but so does the threshold concentration of [] regulating the G1/S transition. This is precisely why an active titration mechanism is required to obtain G1 size control in our setup. Such homeostatic control ensures cell size returns to its steady state distribution following an artificial perturbation as soon as a volume deviation is detected at G1/S (Fig. 2F). When we plot the amount of volume added ΔV for different phases of the cell cycle as a function of the initial volume at the beginning of these phases, we find an approximate adder over the whole cycle that results from weak sizer in G1 followed by a timer in S/G2/M as has been found in budding yeast (Chandler-Brown et al., 2017; Soifer et al., 2016) (Fig. 2G).

While we chose one simple model to illustrate the control mechanism common to our set of evolved networks (Model A1 shown in Fig. 2B), other evolved networks were more elaborate but illustrated a similar principle. For example, Model A2 contains extra interactions for the volume sensing gene , where [] is repressed by the S/G2/M Switch (meaning its production is completely shut down in S/G2/M). Furthermore, [] represses the synthesis of [], adding another layer of repression to promote size control (Fig. 2H). If we perturb cell size to examine the dynamics of the return to steady state and look at the added volume during the cell cycle, we again see overall a weak adder behavior similar to that found in Model A1 (Fig. 2I-J).

Quantifying size control

To study the mechanisms implicated in cell size control, we modify the control at G1/S and introduce the control volume . This control variable is independent from the biochemical network and is maintained fixed allowing us to disconnect the actual cell volume from the biochemical network and by forcing the G1/S transition to be triggered once $_{C}$ = [] × is low enough. We then numerically integrate the differential equations of the model and measure the period () of the simulated cell-cycle for this control volume. This allows us to break the size feedback system and distinguish its input, $_{G1/S}$, from its output, the induced cycle period T (Angeli et al., 2004). We compute () for Models A1 and A2 and compare their response with the analytical curves of the archetypical timer, adder, and sizer (Fig. 3A,C). Those curves intersect at the point where the induced period is exactly equal to the population doubling time (ln(2)/λ), which defines the equilibrium volume V_{eq} achieved by our cell size control network. Examination of the size control in different cell cycle phases indicates the contributions of G1 and S/G2/M to the overall system behavior (Fig. 3B,D). We note that we later examine statistics of ensembles of evolved models but that Models A1 and A2 are both typical examples of evolved feedback-based models.

Quantifying precisely how the cell cycle period depends on the control volume at G1/S allows us to see that the () for Model A1 overlaps with the theoretical adder curve over a broad range of volumes. In contrast, Model A2 behaves as a sizer for volumes smaller than the equilibrium volume. However, at higher volumes it behaves more like an adder/timer similar to Model A1. Model A2's equilibrium is thus 'tuned' by evolution to be in a regime corresponding to the minimum of the ΔV_{Cycle} curve extrapolated from the () as shown in Fig. 3D, precisely when the system transits from a sizer at small volumes to an adder-like behavior at higher volumes. Similar behavior was observed experimentally in budding yeast (see Fig. 4C in (Chandler-Brown et al., 2017)). Thus, both Models A1 and A2 approximate an adder near the equilibrium size, but their behavior differs further from equilibrium where for smaller volumes, Model A1 is still an adder while Model A2 is a sizer.

Modulating cell cycle structural constraints selects for adders or sizers

So far, our evolutionary algorithm selects networks that implement adders rather than sizers near the equilibrium size. This is surprising because sizers are in principle be better able to control cell size and reduce the CV of the size distribution at birth, which is one of our fitness functions. That adders are more frequently observed in nature than sizers (Cadart et al., 2018; Eun et al., 2018; Jun et al., 2018; Westfall & Levin, 2017; Willis & Huang, 2017; Zatulovskiy & Skotheim, 2020), is consistent with our evolution simulations, but adds to the mystery as to why this takes place.

To gain insight into the underlying reason for the prevalence of adders, we considered what might be exceptional in the cases where sizers occur. The best studied, and highly accurate sizer, is found in the fission yeast *S. pombe* (Fantes, 1977; Sveiczer et al., 1996). In contrast to budding yeast and human cells, where the size control takes place largely in G1 phase, fission yeast exerts size control later in the cell cycle at the G2/M transition. That size control took place later in the cell cycle in fission yeast suggested that this structural feature of its cell cycle might be responsible for its stronger size control. To test this, we inverted our seed network so that the G1 phase was a timer, while cell size control could evolve in S/G2/M (see Methods above). We compare these results to the evolutionary simulations starting with the 'control' seed network with G1 size control and an S/G2/M timer (Fig. 2).

To determine how the seed network structure influences the subsequent evolution of cell size control, we performed 60 independent evolutionary simulations for the two network structures initialized with the Model A1 topology and parameters (Fig. 4). 30 simulations were performed using Pareto optimization and another 30 simulations were performed using individual fitness optimization based on the number of cell divisions N_{Div}. For each simulation's fittest model after 500 epochs, we calculate the CV of the volume distribution at birth, CV_{Birth}, and the slope of the linear fit of the volume added in the entire cell cycle as a function of the cell size at birth, Slope ΔV_{Cycle} (we remind the reader that a slope of -1 corresponds to a sizer, 0 to an adder, and 1 to a timer). Model A1's initial CV_{Birth} and Slope ΔV_{Cycle} before evolution are indicated by the dashed black line. In the control experiment where G1 is a sizer and S/G2/M is a timer, most evolutionary simulations with two fitness functions (Pareto) yield models close to the adder regime with a low CV_{Birth}. However, when only the number of divisions (N_{Div}) is used as a fitness, the evolutionary simulations are closer to the sizer regime, albeit with a slightly higher CV_{Birth} (Fig. 4A, Welch's *t*-test $p < 10^{-6}$). When the cell cycle structure is inverted so that G1 is a timer and S/G2/M is a sizer like it is in the fission yeast *S. pombe*, we found that more sizer-like networks evolve than in the control experiment (Fig. 4B, Welch's *t*-test on agglomerated data $p = 0.07$). This shows that having a network structure like the fission yeast *S. pombe* promotes sizers, while having the size control portion of the cell cycle earlier, as in the budding yeast *S. cerevisiae*, promotes adders. Thus, performing simulations using cell cycle network structures of these two yeasts results in the evolution of size control mechanisms that reflects those that are naturally occurring.

The general notion that having cell size control in G1 results in adder-like mechanisms, while control later in the cell cycle results in more sizer-like mechanisms fits most observations of human cell lines grown in culture, budding yeast, and fission yeast. However, a recent study

examining mouse epidermal stem cells growing and dividing in the skin found both a strong sizer and that this sizer was largely due to the size-dependent regulation of G1 (Mesa et al., 2018; Xie & Skotheim, 2020). This raised the question as to how a network performing size control in G1 could result in a sizer for the entire cell cycle. One important difference between mammalian cells grown in culture and the mouse epidermal stem cells growing in an animal (*in vivo*) is the change in the relative durations of the G1 and S/G2/M phases of the cell cycle. While the S/G2/M phase of the cell cycle is similar in duration in cultured cells and the epidermal stem cells *in vivo* at ~12 hours, the G1 phase extends ~5-fold from ~10 hours in culture to ~50 hours *in vivo* (Cadart et al., 2018; Xie & Skotheim, 2020). This suggests the hypothesis that the overall size control behavior can be dominated by the relatively longer cell cycle phase, as is likely the case for the G1 phase of epidermal stem cells. To test this hypothesis, we performed evolutionary simulations with size control in G1 and a timer in S/G2/M but where we changed the duration of the S/G2/M timer phases of the cell cycle to be significantly shorter or longer than the G1 phase at equilibrium. When a timer in S/G2/M is relatively shorter compared to G1, we generally see more sizer-like behavior can evolve (Fig. 4C, Welch's *t*-test on agglomerated data $p = 0.06$), while when it is relatively longer, we see more adder-like or even timer-like behavior (Fig. 4D, Welch's *t*-test on agglomerated data $p < 10^{-9}$).

Taken together, our simulations show how the structural features of the cell cycle are important for determining what type of size control ultimately evolves. G1 control is more conducive to the evolution of adders, while G2 control is more conducive to sizers. Moreover, size control can be dominated by the cell cycle phase of longest duration and is modulated by the specific selection criteria.

A two-step evolutionary pathway for cell size control

From our series of evolution simulations, we found a somewhat paradoxical inverse correlation between the added volume slope quantifying the degree of size control (sizer vs. adder) and the CV_{Birth} (Fig. 4B-C). This was surprising because it means that there is a broader distribution of volumes in the sizer regime where control should be more effective in theory. To better understand this inverted correlation between Slope ΔV_{Cycle} and CV_{Birth}, we revisited our evolutionary simulations to examine the evolutionary pathways through which the networks progressed through the simulated epochs.

A typical evolutionary pathway for a Pareto simulation of the *S. pombe*-like network structure presented in Fig. 4B is shown in detail in Fig. 5. Here, Model A1 topology is conserved throughout evolution although individual parameter values change. In the early stages of the evolution (epoch 650), we typically see dynamics where small cell size triggers an overshoot to a large cell size, which is then reduced through a series of rapid divisions (Fig. 5A). Because of this small-size-triggered overshoot, the system behaves more like a sizer when the average behavior is analyzed (Fig. 5B). However, the high degree of variability in the cell cycles also leads to a broad distribution of volumes (Fig. 5C). The variability in volume is attenuated in later epochs where there are fewer and smaller volume overshoots triggered by small cell size (Fig. 5 D-I). Since small cell size no longer triggers a dramatic amount of cell growth, the slope of

ΔV_{Cycle} is increased and the system converges towards a weak adder in which the distribution of volume at birth is more Gaussian and the CV_{Birth} is lower (Fig. 5H-I). Taken together, these analyses suggest a two-step evolutionary pathway, consistent with the evolutionary dynamics first seen in Fig. 2A. First, a strong sizer mechanism evolves, but because of noise in the system, small variations in volume lead to a dramatic overcorrection and overshoot of the target volume. The variability in volume produced by this overshoot is then reduced by attenuating the strength of the size control response. Indeed, the overall weaker size control allows the system to respond more mildly to size deviations and yields a lower CV_{Birth}, which we select for. Thus, selecting for a smaller CV_{Birth}, *i.e.*, better size control, can end up selecting for adders rather than sizers. This paradoxical result is consistent with the fact that when we select only for the number of cell divisions (N_{Div}), one sees that more sizer-like behavior can evolve (Fig. 4A-C).

Fluctuation sensing and the evolution of self-organized criticality

One of the main features of smaller cells is that they have fewer proteins and mRNA. If some aspects of protein synthesis and degradation are subject to Poisson fluctuations, we expect such fluctuations to produce larger concentration fluctuations in smaller cells. For example, if we consider the synthesis and degradation of the quantity of a generic protein as a Poisson process with parameter $\frac{\rho V}{\delta}$, where ρ is the synthesis rate in number of proteins per unit of time for a reference volume of 1, *V* is the volume, and δ is the degradation rate. The average concentration of this protein will be $\frac{\rho}{\delta}$, which is independent of the cell volume V as expected from the production rate scaling. However, following the Bienaymé formula, the variance in the concentration is $\frac{\rho V}{\delta} \times \frac{1}{V^2} = \frac{\rho}{\delta V}$ (Fig. 6A), which decreases with volume. This result makes intuitive sense because bigger cells have to produce more proteins to keep concentrations constant, so that the fluctuations in the relative number of proteins (and thus concentration) are smaller. Thus, if the cell could sense the size of *concentration fluctuations* in some way, it would be able to harness the cell size-dependence of such stochastic Poisson fluctuations to regulate cell division and control cell size.

To test if we can evolve networks that control cell size through sensing Poissonian fluctuations, we first initialized our simulation with a network similar to that shown in Fig. 1C, but with an added activating gene that can activate the production of the inhibitor. We then ran evolutionary simulations using the cell cycle structure of a size controlling G1 and timer in S/G2/M, but where the G1/S transition is regulated by the *concentration* [] instead of its *quantity.* We also used Pareto fitness optimization of and of . Importantly, we use the stochastic version of our equations with the molecular noise modeled using a Langevin noise term with a variance inversely proportional to the volume as explained above. We then extracted the most fit network and optimized it further using Pareto optimization of and of the Δ slope to push the models towards the sizer regime. The most fit network of those combined evolutionary simulations is presented in Fig. 6B and demonstrates that we can indeed evolve fluctuation-based cell size control (Model B).

The mechanism for size control that evolved based on size-dependent fluctuation sensing is remarkably similar to what we observed for models without size-dependent fluctuations (Fig. 5A). For large volumes, the cycle has a constant period which corresponds to approximately 85% of the doubling time τ. This ensures that in the high-volume regime, the system shrinks over time. When the volume is small however, fluctuations allow the concentration [] to cross the threshold of the highly non-linear transcriptional activation of [] by []. This results in a massive increase of [] that needs to be degraded to progress further into the cell cycle. Thus, the low volume regime occasionally leads to a considerable increase in G1 length and a correspondingly very large cell at division. These very large cells then reliably and deterministically re-enter multiple, rapid cell cycles with short G1 until the cell is small again and the concentration fluctuations again become large enough to trigger the activation of [] by []. This mechanism thus appears very similar to the sizer mechanism observed in other quantity sensing simulations shown in Fig. 5. However, here the mechanism is based only on size-dependent fluctuations in protein concentration and the overall behavior is closer to an adder (Fig. 6E).

The system dynamics that evolved to perform fluctuation-based cell size control produce volume distributions that are long-tailed due to the stochastic occurrence of occasional exceptionally long G1 phases. Interestingly, the probability distribution of G1 durations follows a power law (Fig. 6F), which is due to the very broad distributions of cell cycle duration at lower cell volumes. A more controlled analysis specifying the initial conditions showed that cell cycles get increasingly long and their distributions wider with decreasing control volume (Fig. 6G). Such non-Gaussianity is the hallmark of critical behavior, suggesting that the evolution of fluctuation-based cell size control is based on self-organized criticality (SOC). SOC is defined as a system where an order parameter feeds back on a control parameter (Sornette et al., 1995; Vidiella et al., 2021). The canonical example of SOC is the sandpile to which grains of sand are added on top. As the sand accumulates, the slope steepens, and the angle of the pile (control parameter) increases. Eventually, this triggers avalanches (order parameter) that feedback to dramatically reduce the angle of the pile. This ensures that the system dynamically tunes itself at the critical value of the angle of the pile where avalanches can occur.

We conclude that our evolved size control network exhibits SOC based on several observations. Starting from a high volume, multiple divisions at a rate faster than it takes to double the biomass reduce cell volume V just like the addition of grains of sand gradually increase the slope of the pile. Then, for small enough volumes, bursts of [] drive an extended G1 that greatly increases cell size, which, like the sandpile avalanches, resets the system's control parameter (volume of the cell or angle of the sandpile). Interestingly, evolution tuned the system to be near a bifurcation (Fig. 6H). If we consider the deterministic regime, in which the fluctuations in [] are small, the cycle is unperturbed and oscillates with a period roughly equal to 85% of the doubling time (Fig. 6I). In contrast, if we consider the noisy regime, in which the fluctuations in [] are large, the cycle disappears, and the system stays locked in a prolonged G1 state with a high value of []/[] which is akin to a bifurcation destroying the cycle (Fig. 6H). This bifurcation takes place because the position of the G1 attractor for [] becomes larger with increasing [] and eventually overcomes the concentration required to induce the stochastic G1/S transition (Fig.

6H inset). Then, the system remains stuck in a state where G1/S cannot be triggered, and cells effectively exit the cycle. As growth occurs, noise dies down and so does the position of the G1 attractor, eventually becoming smaller than the [] concentration required to induce the G1/S transition which allows cells to re-enter the cell cycle. Thus, the system is critical from a dynamical systems standpoint and also fits the general observation that SOC systems tune themselves to be right at the point where the order parameter is non zero, but infinitesimal (Sornette et al., 1995). In our case, the bifurcation corresponds exactly to the point where [] can sufficiently activate the production of [] to prevent the G1/S transition.

Discussion

The last decade saw an explosion of time lapse microscopy studies measuring how cells control their size. These studies revealed diverse phenomena that are characterized by the correlation between cell size at birth and cell size at division. Size control ranged from sizers, where the size at division is uncorrelated from the size at birth, to adders, which add a constant volume in each cell division cycle, to timers, whose cell cycle duration is size-independent (Cadart et al., 2018; Eun et al., 2018; Jun et al., 2018; Willis & Huang, 2017; Wood & Nurse, 2015; Zatulovskiy & Skotheim, 2020). The presence of these diverse phenomena raises the question as to why the underlying control networks evolve one rather than another type of cell size control?

To explore the evolution of cell size control networks subject to distinct selection pressures, we used computational evolution simulations. We initially examined the evolution of a seed cell cycle model consisting of G1 and S/G2/M phases of similar duration, where the G1 phase was free to evolve size-dependence, but the S/G2/M phase was constrained as a timer. Our simulations reliably evolved a control mechanism based on a Mixed Feedback Loop (François & Hakim, 2005) (Fig. 2). This network is centered on a cell cycle regulator () that inhibits the G1/S transition in proportion to its quantity. [] is titrated away into an inactive complex by an increasing amount of another protein [] that is synthesized in proportion to cell size. This results in a size-dependent decrease in the effective cell cycle inhibitor (free []). Thus, our evolved network implements an effective dilution of a cell cycle inhibitor that is conceptually similar to the well-described inhibitor dilution models of budding yeast, human cells, and *Arabidopsis* plants (D'Ario et al., 2021; Schmoller et al., 2015; Xie & Skotheim, 2020; Zatulovskiy et al., 2020). We note that we did not allow the synthesis of our proteins, such as [], to be size-independent as has been found for budding yeast (Chen et al., 2020; Schmoller et al., 2015; Swaffer et al., 2021) as this could result in a one-step implementation of cell size control through the pure dilution of a cell cycle inhibitor. It is therefore interesting that given the constraint that all proteins be made in proportion to cell size, the network still evolved an effective 'dilution' of the active form of the cell cycle inhibitor molecule [].

Our evolution simulations gave insight into factors that bias evolution towards sizer or adder type control mechanisms (Fig. 4). First, it is worth noting that our evolution simulations were not deterministic. There was no one-to-one correspondence between a given evolutionary pressure and any one specific cell size control mechanism. Rather, our claims represent an average

behavior observed over the course of many simulations. In the selection of a size controlling G1 network followed by a timer in S/G2/M as described above, we observed a prevalence of adders that is consistent with the prevalence of adders reported in the literature. While fewer in number, sizers have also been observed. That the most accurate sizers have been observed in the fission yeast *S. pombe* (Fantes, 1977; Sveiczer et al., 1996; Wood & Nurse, 2015), and that this organism performs cell size control at G2/M rather than at G1/S led us to explore the effect of cell cycle structure on the evolution of cell size control. We found that controlling cell size later in the cycle in S/G2/M biases evolution away from adders and towards sizers. In retrospect, this result can be rationalized since any size deviations incurred earlier during the timer period can be compensated for by the end of the cycle with the sizer. However, when the order is inverted, any size deviations escaping a G1 control mechanism would only be amplified by exponential volume growth during the S/G2/M timer period. A second recent case exhibiting sizer control was found in mouse epidermal stem cells, which exhibit a greatly elongated G1 phase and a relatively short S/G2/M phase (Mesa et al., 2018; Xie & Skotheim, 2020). We found that if we increased the relative duration of G1 in our simulations by shortening the S/G2/M timer, we also see a bias towards sizer control. Taken together, these simulations suggest the principle that having size-dependent transitions later in the cell cycle selects for sizers, while having such transitions earlier selects for adders.

In addition to identifying cell cycle structural features that canalize evolution towards sizers and adders as described above, we also observed an intriguing mechanism relying on Poissonian molecular fluctuations. In this case, small cells would trigger an abnormally long G1 that would result in very large cells that decrease in size through a series of rapid cell divisions. This type of size control is reminiscent of that found in the green algae *Chlamydomonas* where a series of rapid, size-reducing cell divisions cease when cells go below a target size (Heldt et al., 2020). In our case, small size results in larger concentration fluctuations due to Poisson noise in the number of molecules. These concentration fluctuations, when large enough, are then able to trigger a burst of G1/S inhibitor that leads to an extended G1 phase and massive cell size growth before another series of rapid cell divisions is initiated (Fig. 6). Due to the occasional massive volume growth at small birth sizes, there is a strong inverse correlation between the birth size and the amount of cell growth in the cell cycle. This mechanism thus results in a weak adder that displays high variability in cell size. However, the system still performs statistical size control over many generations. Intriguingly, this size control mechanism exhibits hallmarks of self-organized criticality (SOC). Just like adding grains of sand to a pile eventually triggers avalanches, the consistent decrease of cell size in the rapid division cycles eventually triggers a greatly extended G1 phase. To our knowledge, this is the first example where self-organized criticality is obtained in artificially evolved models of gene networks performing a well-defined function and is consistent with the idea that evolution of complex systems can favor the emergence of critical processes.

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Conflict of interest

The authors declare no conflict of interest.

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Figures

Figure 1

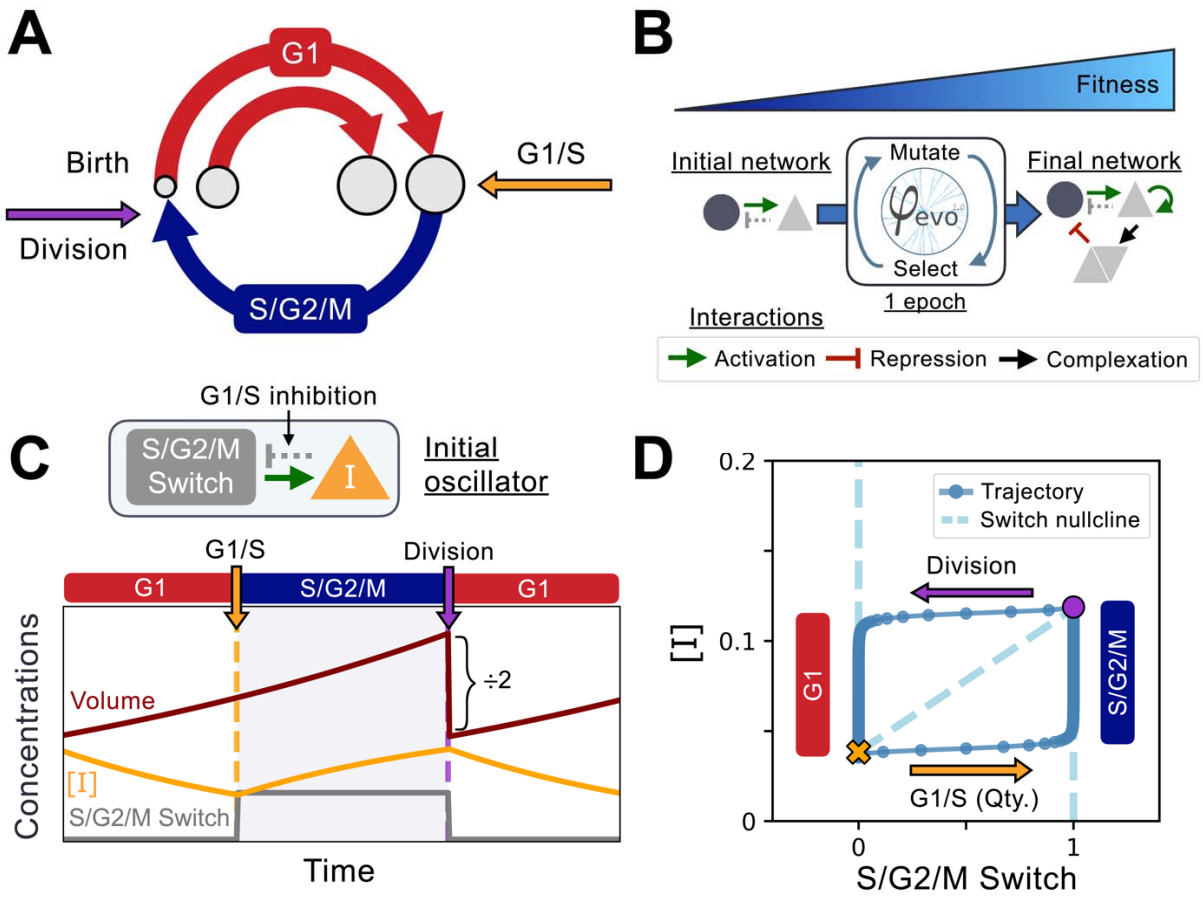

Figure 1 - Implementation of the cell cycle seed network and evolution algorithm.
A) Schematic representation of the coupling between cell size and cell cycle progression. The transition between G1 (red) and S phases of the cell cycle at the G1/S transition (orange) can evolve to depend on cell size, while the duration of the S/G2/M (blue) phase is independent of size. Cell division takes place instantaneously following mitosis (purple).
B) Schematic representation of the φ-evo algorithm implementation. The network generating tool takes an initial network topology as its starting point for evolution as well as a user-defined fitness function. φ-evo then goes through successive epochs of mutation and selection to extract a final optimized network. At each selection step, the fittest half of the networks are retained and duplicated for evolution in the subsequent epoch. Permitted interactions allowed by φ-evo include transcriptional activation (green arrow), transcriptional repression (red arrow) and protein-protein interactions responsible for complex formation (black arrow).
C) Schematic of our seed network topology implementing a simplified relaxation oscillator. Cell cycle state (G1 or S/G2/M) is encoded via a binary switch called *'S/G2/M Switch'* that is 0 in G1 and 1 in S/G2/M. Transition between G1 and S is controlled by the quantity of an inhibitor of the G1/S transition that we call . The lower the quantity , the higher the chance of progression through the G1/S transition. This interaction is represented as the grey arrow in the network

topology. After progressing through G1/S, cells enter S/G2/M which we model as a pure timer of fixed duration with some uniform noise. Cell volume grows exponentially and is divided symmetrically following mitosis. We then follow one of the daughter cells and disregard the other one.

D) Phase-space representation of the initial relaxation oscillator. The X-coordinate shows the S/G2/M Switch variable, and the Y-coordinate shows the concentration of $[I]$. The oscillator runs counterclockwise with the left branch (x=0) corresponding to G1 and the right branch (x=1) corresponding to S/G2/M. The G1/S transition and division events are instantaneous in our simulations but are smoothly represented here for visualization purposes. From these transitions, we can extract the approximate shape of the $[I]$ nullcline that we plot under the oscillator with a dashed line. We note that the position of the G1/S transition in phase-space will vary as a function of the volume of the cell as it depends on the *quantity* of $I = [I] \times V$ rather than its *concentration* $[I]$.

Figure 2

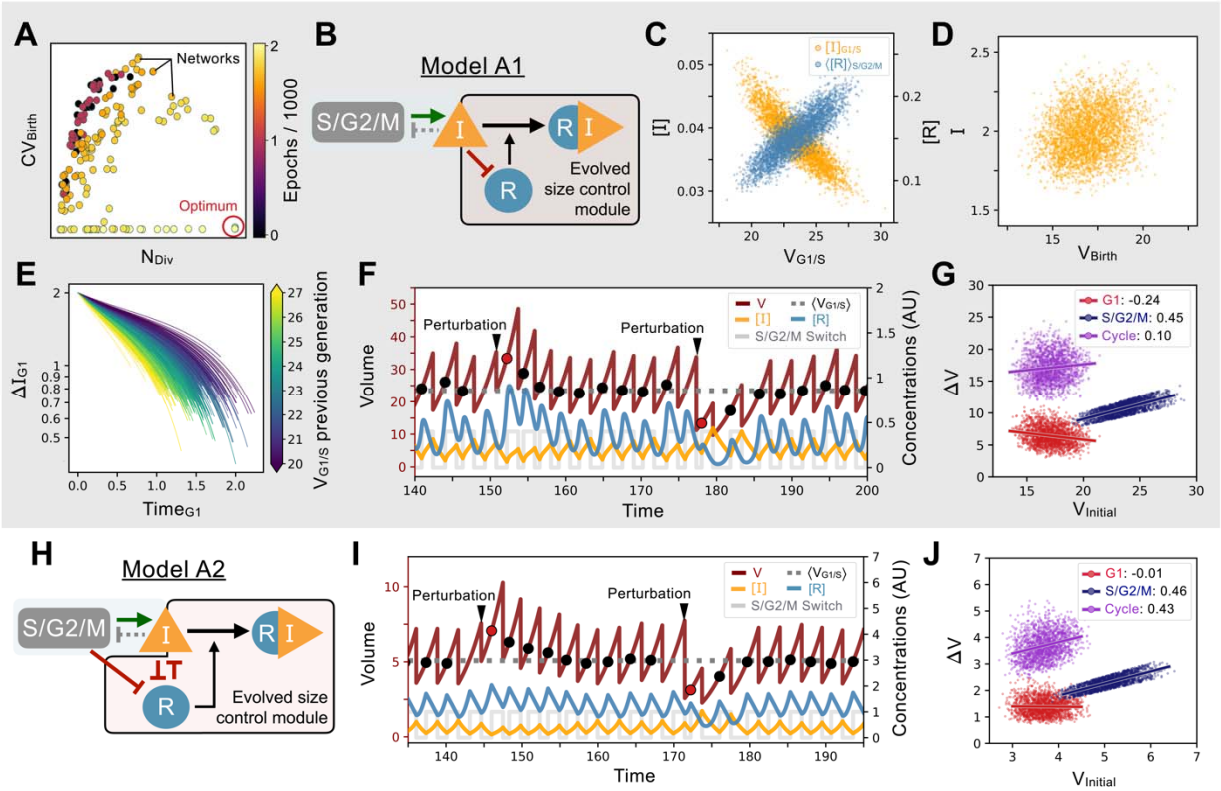

Figure 2 - Evolution of feedback-based size control:

A) Typical 2D fitness trajectory for an evolutionary run showing the number of divisions of a cell lineage during a time interval of fixed length (N_{Div}, X-coordinate) and the coefficient of variation of the volume distribution at birth (CV_{Birth}, Y-coordinate). Optimal model behavior is located in the bottom right corner of the figure where networks produce cell lineages with many offspring and strong size control. Individual networks are dots color coded by their epoch within the evolutionary trajectory.

B) Core network topology of the evolved Model A1 network that employs a feedback-based mechanism described in detail in panels C-F.

C) Concentration of at the G1/S transition (Y-coordinate, left axis, orange) and average concentration of the repressor protein in S/G2/M (Y-coordinate, right axis, light blue) as a function of the volume of the cell at G1/S (X-coordinate), *i.e.*, the beginning of S phase. We see here that acts as a direct size sensor of the volume at G1/S.

D) *Quantity* of inhibitor at birth as a function of volume of the cell at birth, which is independent of size due to titration by during S/G2/M.

E) Trajectories of quantity of inhibitor in G1 as a function of time. Trajectories are color coded as a function of the volume at G1/S during the previous generation's cell cycle. For visualization purposes, trajectories are offset vertically to all begin at the average *quantity* of at birth (t=0) shown to be on average independent of volume in panel D. Larger cell volumes lead to greater titration of in G1 by . In turn, this ensures that G1 duration of the daughter cell cycle is shorter, which underpins the size control mechanism.

F) Characteristic dynamics of Model A1. Trajectories of $[I]$, $[R]$ and S/G2/M Switch are rescaled with arbitrary units (AU) for visualization purposes. Circles indicate volume at G1/S. Extrinsic perturbations are applied to the model by temporarily changing the division ratios which kicks the system out of equilibrium at the subsequent cycle. The volume relaxation back to its homeostatic value takes ~2-3 generations regardless of if the perturbation is applied towards higher or lower volumes.

G) Amount of volume added (ΔV) in G1 (red), S/G2/M (dark blue), and over the whole cycle (purple) as a function of their initial volume at the beginning of these phases, *i.e.,* birth for G1 and cycle, and G1/S for S/G2/M, with the slope of linear fits indicated in legend. Slope of 1 corresponds to a Timer, slope of -1 to a Sizer and slope of 0 to an Adder.

H) Network topology of Model A2, a second evolved network that is similar to Model A1 albeit with different kinetic parameters and 2 additional interactions.

I) Typical dynamics of Model A2. Concentrations are rescaled with arbitrary units as in panel F.

J) Amount of volume added for different periods of the cell cycle for Model A2.

Figure 3

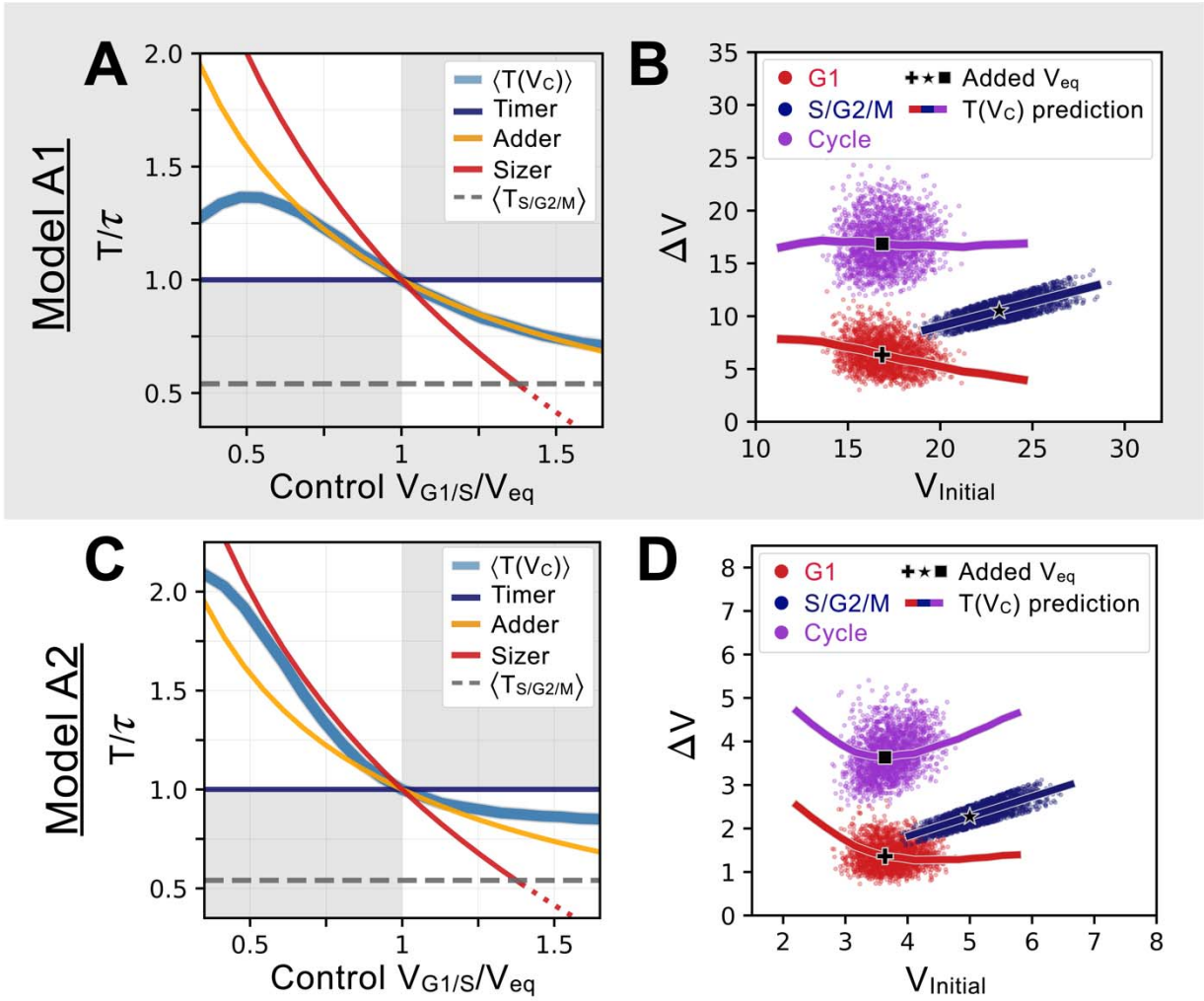

Figure 3 - Characterizing and comparing evolved size control mechanisms:
A) Average period of the oscillator of Model A1 as a function of the control volume at the G1/S transition. Period is normalized by the doubling time of the cell, and volume is rescaled by , the control volume such that . Periods for the sizer (red), adder (orange) and timer (dark blue) are shown for comparison. Model A1 follows approximately the adder archetype over a large range of control volumes.
B) Added volumes for different phases of the cell cycles for simulations of Model A1. Individual dots correspond to different cell cycles. The full line corresponds to the extrapolation from the curve shown in A. The black cross, star and square indicate the added volumes corresponding to when the system senses a volume corresponding to at the G1/S transition. We see that the model follows an adder over a large range of volumes.
C) Average period of the oscillator of Model A2 with archetypes shown for comparison. We note that the curve of this model is closer to the sizer at lower volumes and closer to a weak adder/timer at higher volumes relative to .

D) Added volumes ΔV for different phases of the cell cycles for simulations of Model A2, with similar conventions as panel B. Here we see the sizer behavior at lower volumes and the weak adder/timer behavior at higher volumes.

Figure 4

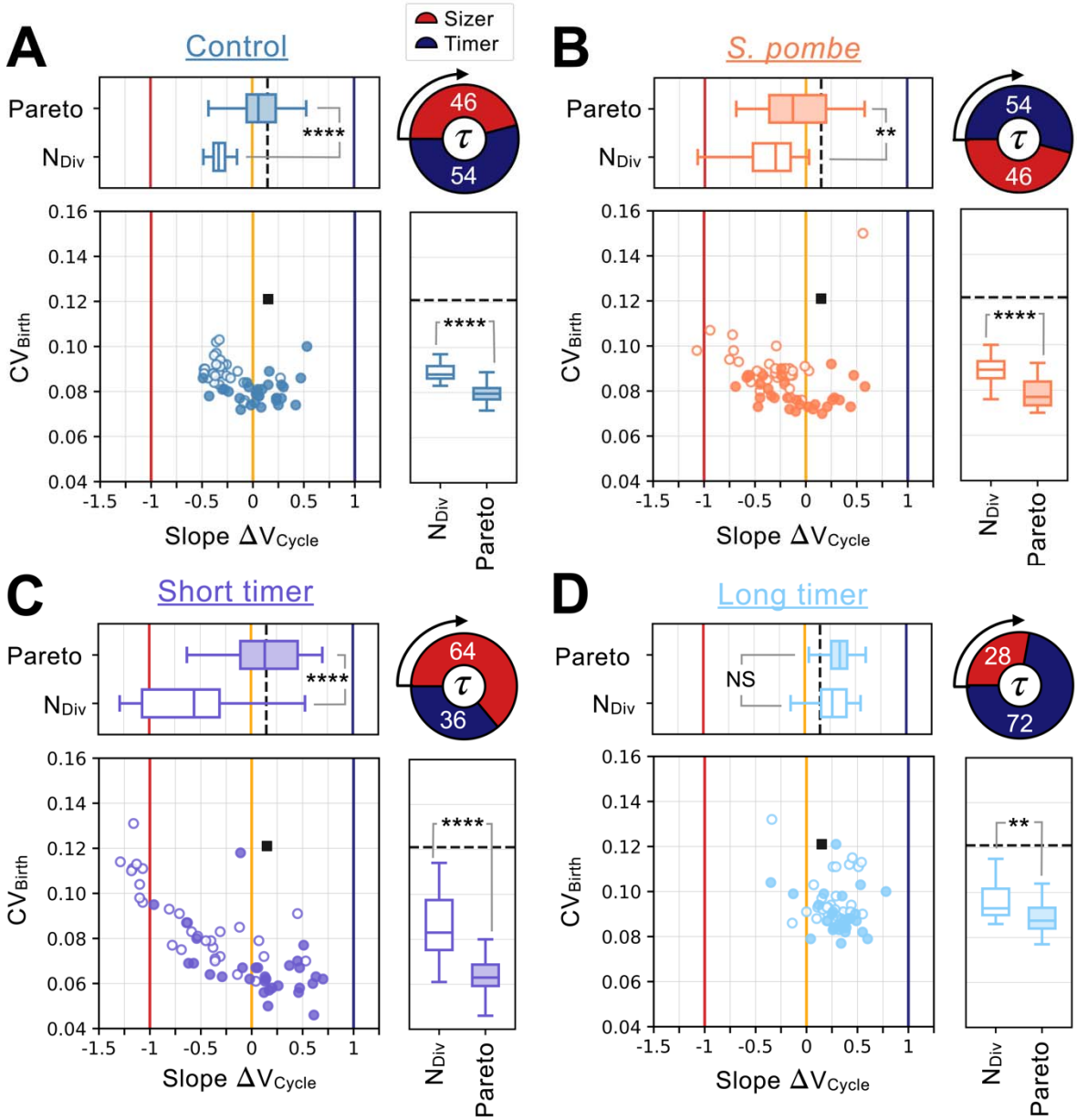

Figure 4 - Distinct network constraints and selection pressures bias size control evolution towards adders or sizers. Summary statistics for evolutionary simulations each having 500 epochs. Model A1 shown in Fig. 2 A) - G) was used as the initial seed network. 30 simulations were performed using Pareto optimization of the number of divisions ( ) and the CV of cell size at birth ( ), are labeled *Pareto* and are shown in full colors. 30 more simulations were performed using only the number of divisions as the fitness function, are labeled N_{Div} and are shown in colored outlines only. Scatter plots show the as a function of the fitted added volume slope over the whole cycle as a function of volume at birth (Slope) for the most fit models evolved during each of the 60 independent simulations. Horizontal box plots above the scatter plots display the distributions of the added volume slopes

for the *Pareto* and N_{Div} simulations. Timer (dark blue), adder (orange) and sizer (red) slopes are shown respectively at 1, 0, and -1 for comparison. Vertical box plots on the right of the scatter plots show the distributions of CV_{Birth} for the *Pareto* and N_{Div} simulations. Asterisks represent p-values for the Welch's t-Test between the distributions. The fitness value of N_{Div} and CV_{Birth} for the initial seed Model A1 are shown as a black square in the scatter plot or as a dashed black line in the box plots. Each panel explores different cell cycle structures which are summarized by the pie charts. Cycles begin on the left of the pie charts and rotate clockwise, indicating the order of the sizer (red) and timer (dark blue) phases. The labels indicate each phase's duration at equilibrium as a percentage of the doubling time τ.

A) Identical evolutionary parameters as for Model A1 evolution shown in Fig. 2A-G. G1 performs size control and has a duration 0.46τ at equilibrium and S/G2/M is a timer of duration 0.54τ. Welch's *t*-test $p < 10^{-7}$ for Slope ΔV_{Cycle}, $p < 10^{-6}$ for CV_{Birth}.

B) Evolution results for a cell cycle structure where the sizer and the timer phases of the cell cycle are inverted akin to *S. pombe*. G1 is a timer of duration 0.54τ and S/G2/M performs size control and has duration 0.46τ at equilibrium. Welch's *t*-test $p < 10^{-2}$ for Slope ΔV_{Cycle}, $p < 10^{-4}$ for CV_{Birth}.

C) Evolution results for a G1 size control of average duration 0.64τ at equilibrium where S/G2/M is a timer of duration 0.36τ. Welch's *t*-test $p < 10^{-5}$ for Slope ΔV_{Cycle}, $p < 10^{-5}$ for CV_{Birth}.

D) Evolution results for a G1 size control of average duration 0.28τ at equilibrium where S/G2/M is a timer of duration 0.72τ. Welch's *t*-test $p > 0.2$ for Slope ΔV_{Cycle}, $p < 10^{-2}$ for CV_{Birth}.

Figure 5

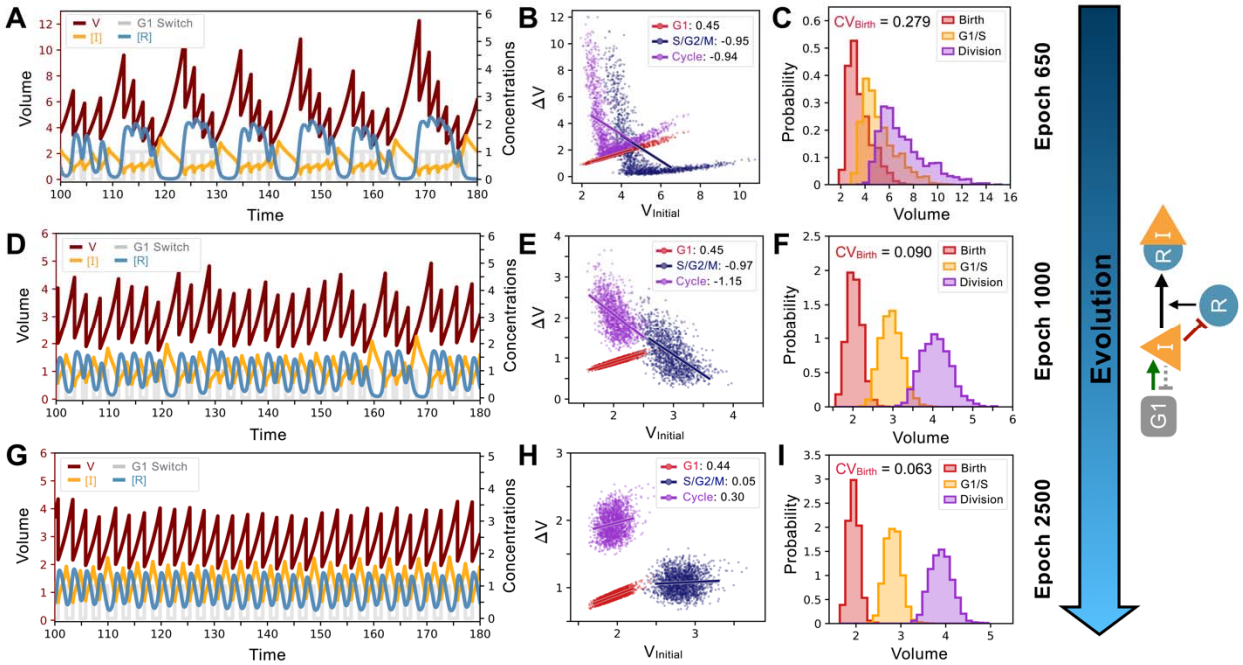

Figure 5 - System and evolutionary dynamics of cell size control networks.
Snapshots of an evolutionary simulation of 2500 epochs initialized with the Model A1 network topology along with an *S. pombe*-like cell cycle structure with a timer in G1 followed by a sizer in S/G2/M (see Fig. 4B). Pareto fitness optimization was performed using and as fitness functions. Rows indicate simulation results for the fittest networks from evolutionary epochs 650 (Panels A-C), 1000 (Panels D-F), and 2500 (Panels G-I). Network topology remains the same throughout the evolutionary simulation and is shown on the right.

A) Typical dynamics of the most fit model from epoch 650.

B) Added volumes for different phases of the cell cycle for the most fit model from epoch 650. Fitted slopes are indicated in the legend.

C) Size distributions at birth (red), G1/S (orange), and division (purple) for the most fit model from epoch 650. The coefficient of variation of the volume distribution at birth .

D) Typical dynamics of the most fit model from epoch 1000.

E) Added volumes for different phases of the cell cycle for the most fit model from epoch 1000 Fitted slopes are indicated in the legend.

F) Size distributions at birth (red), G1/S (orange), and division (purple) for the most fit model from epoch 1000. The coefficient of variation of the volume distribution at birth .

G) Typical dynamics of the most fit model from epoch 2500.

H) Added volumes for different phases of the cell cycle for the most fit model from epoch 2500. Fitted slopes are indicated in the legend.

I) Size distributions at birth (red), G1/S (orange), and division (purple) for the most fit model from epoch 2500. We see here that the sizer behavior from epoch 1000 was abandoned for a weaker adder overall yielding lower .

Figure 6

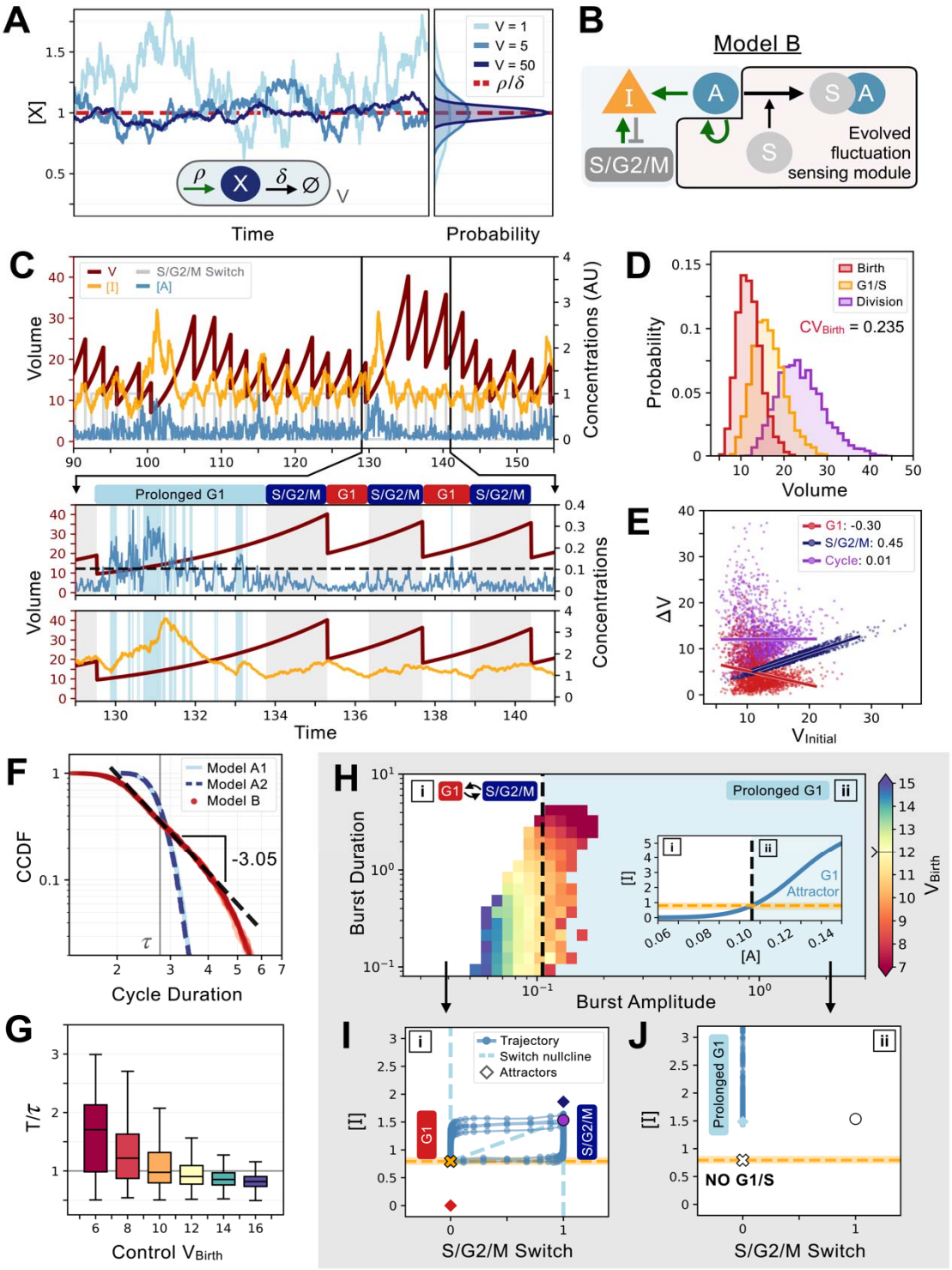

Figure 6 - An evolved concentration fluctuation sensing size control model exhibits self-organized criticality.

A) Size-dependent molecular noise arises due to Poissonian fluctuations in molecule number. Consider a protein production-degradation scheme for protein *quantity X* with production rate

and degradation rate δ contained in a volume *V*. At equilibrium, the concentration of $[X]$ will be given by a distribution with mean ρ/δ and variance $\propto 1/V$. The effect of *V* on the molecular noise is shown on the time trajectories for simulations in a constant volume of *V* = 1 (light blue), *V* = 5 (medium blue), and *V* = 50 (dark blue). Corresponding concentration distributions are shown on the right-hand side of the panel.

B) Network topology of Model B which evolved to sense fluctuations. Here, the G1/S transition is controlled by the *concentration* of $[I]$ and not its *quantity*.

C) Characteristic cell cycle dynamics of Model B. Trajectories of $[I]$, $[A]$, and S/G2/M Switch are rescaled with arbitrary units (AU) for visualization purposes. Below, we zoom-in on 3 cycles to show how a low-volume induced burst in $[A]$ leads to a massive production of $[I]$ inducing a temporarily prolonged G1 phase. Subsequently, cells become bigger and display lower molecular noise inducing a comparatively shorter G1 phase.

D) Volume distributions at birth (red), G1/S (orange), and division (purple) for Model B. The coefficient of variation of the volume distribution at birth $CV_{Birth} = 0.235$.

E) Amount of volume added (ΔV) in G1 (red), S/G2/M (dark blue), and over the whole cycle (purple) as a function of their initial volume at the beginning of these phases, *i.e.,* birth for G1 and cycle, and G1/S for S/G2/M, with the slope of linear fits indicated in legend.

F) Complementary cumulative distribution functions of the cycle duration (CCDF; probability that the cell cycle duration is larger than the value on the X-axis) for three models discussed in the main text: Model A1 (light blue), Model A2 (dashed dark blue), and Model B (red). The light grey line indicates the doubling time τ. We see that Model B exhibits a long tail past the doubling time, which is consistent with a power-law scaling of the cycle duration probability. We find a criticality indicative scaling exponent of -3.05 for the CCDF after fitting the tail of the distributions of 5 independent realizations of the dynamics of Model B.

G) Box plots of the cycle length distributions as a function of control volume V_C at birth. Cycle lengths are normalized by the doubling time τ. Here, V_C sets the molecular noise level to be equivalent to that of an exponentially growing cell born at V_C but whose volume is reset to V_C at each division. Note the very long tail of the distributions at small V_C.

H) Activator protein $[A]$ is synthesized in bursts whose amplitude and duration are a function of volume. We define the burst duration as the total time during which $[A]$>*0* for a cycle. The burst amplitude corresponds to the average level of $[A]$ during each G1 phase. Each burst is then color-coded as a function of the birth volume of the cell that induced it. We use a divergent colormap whose center value (light yellow) corresponds to the average volume of the cells at birth and is indicated by a notch on the colorbar. The black dashed line corresponds to the level of $[A]$ which triggers a transition between two modes of growth and division as shown in the inset by the position of the G1 attractor for $[I]$ becoming equal to the concentration required to induce the G1/S transition. Here, (i) corresponds to the deterministic regime when volume is high and (ii) corresponds to the noisy regime when volume is low. Note that the average volume of the cells at birth is positioned close to the black dashed line.

I) Phase-space representation of the relaxation oscillator in the deterministic regime (i). The X-coordinate shows the S/G2/M Switch variable, and the Y-coordinate shows the concentration of $[I]$. Here, when volume is high, the position of the G1 attractor is *below* the $[I]$ concentration at which the G1/S transition happens. Thus, G1/S takes place and cells are in the cell cycle with a period of ~0.85τ.

J) Phase-space representation of the noisy regime (ii). Here, when volume is low, the position of the G1 attractor becomes *greater* than the $[I]$ concentration at which the G1/S transition happens, and cells remain temporarily stuck in a prolonged G1 state and are unable to trigger the G1/S transition.