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Functional Roles of Pulsing in Genetic Circuits

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Abstract

A fundamental problem in biology is to understand how genetic circuits implement core cellular functions. Time-lapse microscopy techniques are beginning to provide a direct view of circuit dynamics in individual living cells. Unexpectedly, we are discovering that key transcription and regulatory factors pulse on and off repeatedly, and often stochastically, even when cells are maintained in constant conditions. This type of spontaneous dynamic behavior is pervasive, appearing in diverse cell types from microbes to mammalian cells. Here, we review recent work showing how pulsing is generated and controlled by underlying regulatory circuits and how it provides critical capabilities to cells in stress response, signaling, and development. A major theme is the ability of pulsing to enable time-based regulation analogous to strategies used in engineered systems. Thus, pulsatile dynamics is emerging as a central, and still largely unexplored, layer of temporal organization in the cell.

How inherently dynamic are individual living cells? Conventionally, we assume that in a constant external condition, the cell maintains a correspondingly constant internal state. In this view, the concentrations and activities of key cellular regulatory molecules, such as the transcription factors that control gene expression, generally remain steady over time or fluctuate stochastically around fixed mean values. Processes whose intrinsic dynamics are essential for their function, such as the cell cycle and circadian clock, or neural action potentials, are considered the exception rather than the rule. Recently, however, single-cell experiments have begun to reveal a very different picture of cellular regulation. In this view, many genetic circuits actively and spontaneously generate dynamic pulses in the activity of key regulators, and these pulses temporally organize critical cellular functions. Increasingly, it appears that even in constant conditions, cells behave like the proverbial duck, maintaining a calm appearance above the surface, while paddling furiously below.

Recent insights into the temporal organization of cellular regulatory activities have emerged from quantitative time-lapse microscopy and fluorescent reporter genes, which together allow researchers to accurately track the dynamic behavior of specific proteins over time in individual living cells. A recurring theme from these studies is that many regulatory factors undergo continual, repetitive pulses of activation. Each of these pulses involves the coherent activation and deactivation of the regulator, through changes in its concentration,

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modification state, or localization, on time scales ranging from minutes to hours (Fig. 1) (1–6). Pulsing is generated by genetic circuits that activate and deactivate key regulators and modulate pulse characteristics, such as frequencies and amplitudes. Pulsing is thus distinct from transcriptional bursting, which results from the stochastic nature of gene expression (7). Here, we use the term “pulsing” to denote a broad spectrum of repetitive phenomena that range from irregular and stochastic to more uniform and periodic dynamics.

Pulsing has previously gone undetected even in well-studied systems. Because pulses are typically unsynchronized between cells, they have been difficult to detect with traditional techniques that average over large cell populations. Pulsatile dynamics can produce “long-tailed” distributions in static measurements based on flow cytometry and microscopy snapshots. However, time-lapse movies that track molecular activities over time in individual living cells are required to definitively reveal pulses (Fig. 1A).

The discovery of pulsing in core regulatory systems provokes several fundamental questions: How widespread is pulsatile regulation? What cellular functions does it enable? And, what genetic circuit mechanisms does the cell use to generate and regulate pulsing? In this review, we first survey the growing list of pulsatile phenomena in diverse cellular systems. We next explain how pulsing facilitates specific cellular functions that could be more difficult to achieve with static regulation. In particular, we highlight the regulatory flexibility that comes from independently controlling the timing and amplitudes of pulses. We then discuss the circuit mechanisms that enable cells to generate and control pulsatile dynamics. Finally, we suggest additional ways, not yet discovered, in which pulsatile regulation could potentially enhance cellular capabilities. Owing to space limitations, we will focus primarily on more recently discovered pulsatile systems, rather than other beautiful and well-studied examples such as the cell cycle, circadian rhythm, calcium dynamics, and multicellular phenomena based on coordinated pulsing (8).

Pulsatile Regulatory Dynamics Pervade the Cell

Pulsing has been observed in many types of proteins, from alternative bacterial sigma factors to mammalian tumor suppressors like p53, and has been shown to function in diverse processes, from stress response to signaling to differentiation (Fig. 1B). To appreciate the pervasiveness of pulsing, consider the soil bacterium, *Bacillus subtilis*, for which many stress responses have been analyzed with time-lapse movies. In this species, pulsing occurs in at least three systems: genetic competence, which allows cells to take up DNA (9); sporulation initiation, which controls transformation of cells into dormant spores (10); and the general stress response pathway (1). Similarly, in yeast, pulsing has been observed in two distinct stress response pathways, mediated by the transcription factors Msn2/4 and Crz1 (2, 11).

Mammalian cells exhibit many pulsatile factors. The stress response pathways mediated by p53, which controls the DNA damage response (3, 12, 13), and nuclear factor κB (NF-κB), which is involved in immune responses (5, 14, 15), both pulse. Similarly, signaling pathways such as extracellular signal–regulated kinase (ERK) mitogenactivated protein kinase (MAPK), which responds to growth factors and regulates cell proliferation (6, 16–

18), nuclear factor of activated T cells 4 (NFAT4) (4), and transforming growth factor β (TGF-β) (19) have all been shown to pulse in response to activation by extracellular signals. Finally, the developmental transcription factor Hes1 also pulses in multiple cell types (20–22). The prevalence of pulsatility across these pathways raises the likely but generally unexplored possibility that multiple pulsatile systems coexist, and interact, in individual cells.

Functions of Pulsatile Cellular Dynamics

The power of pulsatile regulation arises from the ability to independently regulate pulse amplitudes, frequencies, and durations (Fig. 2A). By controlling these features in different ways, individual systems implement diverse functions, some of which may be challenging to achieve through less dynamic regulation.

Frequency Modulation Enables Coordination

Several pulsatile systems are controlled through frequency-modulated (FM) pulsing (1, 11, 17). In yeast, the transcription factor Crz1, which mediates the calcium response, is activated in a series of stereotyped pulses, even when cells are maintained in constant conditions (11). During each pulse, Crz1 molecules simultaneously localize to the nucleus, where they can activate target genes, remain there for ∼2 min, and then return to the cytoplasm. Extracellular calcium concentration modulates the frequency, but not the amplitude or duration, of these pulses. In a similar way, the ERK kinase can also be activated in FM pulses (17). Even under constant environmental conditions and ligand concentrations, ERK activates in a series of pulses of ∼30-min duration, whose frequency is modulated by extracellular levels of the epidermal growth factor (EGF) (17). Finally, in *B. subtilis*, the σ^B alternative sigma factor, which mediates the general stress response, is also activated in a series of stochastic pulses lasting about 1 hour each (1). Energy stresses that reduce cellular adenosine 5′-triphosphate concentrations modulate the frequency of these pulses.

What functional capabilities could FM pulsing provide for cells? One possibility is that it allows the cell to control the fraction of time that a regulator is active, rather than controlling the concentration of active regulator (Fig. 2B). Such time-based regulation is analogous to “bang bang” control, a well-known design principle in engineering, which similarly involves modulating the fraction of time that a system is on. Transcription factors activate multiple target genes, but may do so with varying strength, affinity, and degrees of nonlinearity. As a result, increasing the concentration of a transcription factor by a given amount will affect different target promoters to different extents. By contrast, in FM regulation, a given percentage change in transcription factor pulse frequency causes the same percentage change in the fraction of time that the transcription factor activates each of its targets. Expression levels of target genes are then proportional to pulse frequency, and also proportional to one another, as indicated schematically in Fig. 2B (bottom right). This coordination function was demonstrated experimentally for Crz1, which activates many targets in yeast (11). Coordination could be a useful function of pulsing in other systems and contexts as well, whenever diverse targets need to be regulated in fixed ratios.

Amplitude Modulation and Phasing Facilitates a Developmental Timer

Developmental timers enable cells to defer their response to signals for multiple cell cycles. In such systems cells detect a signal, proliferate for a defined amount of time or number of cell cycles, and then differentiate. A classic example is oligodendrocyte differentiation, where progenitors divide up to eight times before differentiating (23). Recently, pulsing was discovered in a developmental timer in B. subtilis, which, under some conditions, responds to sudden nutrient limitation by transforming into a dormant spore after five cell cycles have been completed (10) (Fig. 2C). During this period, the master transcription factor that controls sporulation, Spo0A, is phosphorylated (activated) once per cell cycle in a sequence of pulses with progressively growing amplitudes until it reaches a level high enough to initiate sporulation (24). Each pulse of Spo0A phosphorylation activates expression of a cognate kinase, which in turn increases the amplitude of subsequent Spo0A phosphorylation pulses (Fig. 2C). Thus, the system is based on a pulsed positive-feedback loop.

What role does pulsing play in this timer? Creating a multi—cell-cycle deferral time with continuous (nonpulsatile) genetic circuits is challenging in proliferating cells, as dilution of cellular components during cell growth forces most protein concentrations to relax to their steady-state values over a time scale of about one cell cycle or faster. Positive-feedback loops can help to solve this problem, by compensating for dilution with increased protein production. However, mathematical modeling suggests that continuous positive feedback is extremely sensitive to parameters, continually compounding small errors, making it infeasible for a timer operating in individual cells. By contrast, a pulsed positive-feedback loop can help overcome this problem. By effectively breaking up the positive-feedback loop in time, so that Spo0A phosphorylation and kinase accumulation occur in distinct temporal intervals, pulsing makes the timer circuit less sensitive to changes in circuit parameters. It thereby provides a more robust mechanism for implementing a cell-autonomous developmental timer (10).

Dynamic Signal Processing Expands Signaling Pathway Capabilities

Signal transduction pathways face the challenge of internally representing, or encoding, the identity, amplitude, and timing of many different external signals. Cells address this challenge, at least in part, by dynamic multiplexing, which encodes information about the stimulus in the dynamics of a regulator. Dynamic multiplexing systems typically encode stimulus information in the frequency, amplitude, and duration of pulses of pathway activation (1, 2, 5, 13, 25, 26) (Fig. 2D), and also in the presence or absence of pulses themselves.

One of the best-studied examples is the tumor suppressor p53 (3, 12, 13). Both γ-irradiation and ultraviolet (UV) irradiation activate p53, but cause distinct cellular responses: cell cycle arrest (and survival) and apoptosis, respectively (3). The two responses are related to the different dynamics of p53 activation: γ-irradiation produces a series of p53 activity pulses, whose number is controlled by the radiation dosage, whereas UV irradiation generates a sustained nonpulsatile response, whose amplitude and duration depend on dose (Fig. 2D) (13). If p53 dynamics are controlled directly through chemical manipulation, converting pulsatile dynamics into sustained dynamics is sufficient to switch the cell fate (3). Evidently,

the cell uses p53 dynamics to encode distinct input signals and decodes these dynamics to determine cell fates.

In the immune system, NF-κB displays repeated pulses of nuclear localization in response to constant amounts of its input, tumor necrosis factor alpha (TNFα) (5, 14, 15). Cells encode TNFα concentration in the probability of activating NF-κB in a cell, and the number of pulses once activated (5). Pulse characteristics in turn control the differential expression of genes of various immune response stages (5, 15). Thus, NF-κB uses dynamic multiplexing to represent the amplitude of its input.

Complex dynamic multiplexing is not specific to multicellular organisms. In yeast, the general stress response pathway, mediated by the Msn2/4 transcription factors, encodes stimulus information using diverse nuclear localization dynamics (2). Glucose limitation generates repeated stochastic Msn2 localization pulses, several minutes each, at a frequency modulated by glucose concentration. In contrast, oxidative stress induced by H_2O_2 generates a single, adaptive Msn2 localization pulse whose amplitude is modulated by H_2O_2 concentration. These dynamics may allow the cell to activate target genes in a stress type and intensity-dependent manner. The decoding of Msn2 dynamics appears to depend on the interaction kinetics between Msn2 and its target promoters (2, 27).

Even bacteria can multiplex. In *B. subtilis*, the alternative sigma factor σ^B is activated posttranslationally both by energy stresses mentioned above (1), and also by environmental stresses such as salt and ethanol (26). Unlike the FM pulsing observed in response to energy stresses, the response to environmental stresses depends on the rate at which the stress increases. That is, a sudden increase in stress generates much more σ^B activity than a gradual ramp to the same stress level (26). This allows a rapidly increasing stress to induce not only its specific targets, but also, through σ^B, many other stress targets as well, effectively anticipating potential future stresses (26). Rate-encoding schemes like this may similarly be observed in other general stress pathways where rate of change of stress can be experimentally manipulated (28). Thus, the type of stress (energy versus environmental), the magnitude of stress, and the rate of change of environmental stress are all dynamically encoded in this pathway.

Together these examples demonstrate that pulsatile responses, by modulating pulse amplitude, frequency, and duration, dynamically encode information in many pathways [see also (25)].

Pulsatile Gene Expression Implements Bet-Hedging

Fast pulses may occur many times in a cell cycle, but some systems show slower pulses, with durations of one cell cycle or longer. Here, pulses could generate something resembling a transient alternative cell state. Single-cell studies have revealed that individual microbial cells use such slow pulsing to produce a repertoire of transient phenotypic states, even when grown in constant conditions (9, 29). In many cases, slow pulses are initiated in a probabilistic manner, effectively implementing a bet-hedging strategy whereby cells randomize their individual states to adapt to uncertain future environmental changes (29, 31, 32). A classic example of microbial bet-hedging is antibiotic persistence, where individual

bacterial cells stochastically switch between an antibiotic-sensitive state of rapid growth, and an antibiotic-resistant state with no or slow growth (29). Even a small fraction of persister cells can ensure the survival of a microbial population in response to antibiotic treatment, with important biomedical consequences (33).

Several systems are known to implement slow pulsing as a bet-hedging strategy. For example, in *B. subtilis*, at any given time, a fraction of the population enters a state of genetic competence for ∼10 hours and then returns to vegetative growth (9). In the competent state, cells can take up extracellular DNA and recombine it with their own genome, a gamble that adaptive genetic material may be available in the environment. These transient competence events are controlled by an excitable genetic circuit (see below), which generates stereotyped pulses of the master competence regulator ComK, affecting many aspects of cell state.

Mycobacterium smegmatis uses a stochastic pulsing strategy that affects persistence in response to isoniazid (INH) (34), an antibiotic that requires activation by bacterial catalase-peroxidase KatG. The normal role of KatG is to catabolize and inactivate toxic reactive oxygen species. However, when INH is present, KatG appears to play a second, opposite, role, promoting killing of cells by activating INH (34). By analyzing individual cells in time-lapse movies, the authors observed that cells express katG in low-frequency stochastic pulses (34). These pulses effectively increase cell-to-cell variability in KatG concentration and may be advantageous to population survival, by assuring that at least some cells survive in both the presence and absence of INH. The role of pulsing has also been computationally investigated in the mar antibiotic resistance regulon of *Escherichia coli* (35). Here, a mixture of positive and negative feedback allows *marA* expression to stochastically pulse in low-stress conditions, allowing the population to bet hedge against sudden antibiotic appearance while retaining the ability to fully induce MarA in response to antibiotic stress. These examples suggest generally that dynamic, pulsatile gene regulation may be involved in other bacterial persistence contexts.

The Role of Pulsing in Cellular Differentiation

A fundamental question in development is how transcription factors control cell fate decisions. In some cases, the role of an individual transcription factor can be complex, promoting multiple, seemingly conflicting, cellular behaviors. Recent single-cell studies indicate that some of these transcription factors activate in a pulsatile fashion and suggest that this pulsatility may function to balance their conflicting activities (20). For example, during neural development, to ensure an adequate population of neurons, neural progenitors divide multiple times before differentiating into postmitotic neurons. This maintains their developmental potential while proliferating. Developmental regulators, including Hes1, play a critical role in this process. High concentrations of Hes1 maintain the progenitor state by repressing proneural genes. But they also block proliferation. In contrast, low amounts of Hes1 allow proliferation but permit differentiation away from the progenitor state. How do neural progenitors use Hes1 to juggle the conflicting tasks of maintaining the progenitor state, while allowing proliferation?

A potential resolution of this apparent paradox is provided by the observation that Hes1 expression oscillates in neural progenitors with a period of 2 to 3 hours (20). Hes1 oscillations might dynamically balance periods in which cell division is possible (low Hes1) with other periods in which the progenitor state is maintained (high Hes1), effectively causing the cells to alternate between two behaviors, rather than choosing one or the other. Hes1 also functions in an intercellular feedback loop mediated by Notch-Delta signaling, which may play a role in these oscillations (20).

Recently, optogenetic approaches have begun to enable forward experimental tests of the role of pulsing in fate determination by allowing direct manipulation of transcription factor dynamics. For example, optogenetic control of the transcription factor Ascl1, a Hes1 target that also oscillates in neural progenitors, revealed that the dynamics of Ascl1 can influence cell fate (22). More specifically, oscillatory Ascl1 expression maintained the neural progenitor state, while sustained high expression promoted neural differentiation (22). These observations are now provoking further questions about how developmental regulator dynamics control cell fate decisions in this and other systems.

The Mechanisms Behind Pulsatile Cellular Dynamics

How do gene circuits generate pulses and modulate their characteristics? Mechanisms for pulsing and oscillation have begun to be elucidated in a few cases, revealing both shared features and qualitative differences across systems.

In *B. subtilis*, transient differentiation into the competent state involves pulses of the master transcriptional regulator ComK. ComK directly activates its own transcription, and indirectly turns itself off, through a slower negative-feedback loop (9, 30). This circuit is excitable, meaning that any fluctuations (noise) that enable ComK to cross a threshold and begin to turn itself on trigger stereotyped pulses of ComK activation, with a relatively uniform duration (9) (Fig. 3A). The excitability of this circuit enables the system to independently tune the frequency and duration of these pulses (9).

Excitability might seem like an ideal property for any pulse-generating system. However, although the B. subtilis σ^B system also pulses, it does so with a different, nonexcitable circuit architecture (1). This system converts levels of energy stress into the frequency of pulses. In the absence of stress, σ^B is sequestered in an inactive form by its cognate anti-sigma factor RsbW. It can be released from this complex by the anti-anti-sigma factor RsbV. RsbV is normally phosphorylated, and thereby inactivated, by RsbW, but it can be dephosphorylated, and activated, by the phosphatase RsbQP, an energy stress sensor.

σ^B pulses are generated in two stages: First, the RsbV phosphoswitch responds ultrasensitively to RsbQP activity, causing RsbV to be suddenly dephosphorylated in response to a thresholdcrossing fluctuation in RsbQP activity. Dephosphorylation, in turn, activates σ^B, initiating a pulse. Tuning basal RsbQP expression can modulate pulse frequency by changing the likelihood of RsbQP fluctuations tripping the phosphoswitch. Second, active σ^B transcriptionally activates its own operon, amplifying the pulse, but also upregulating RsbW, which, because of its kinase activity, eventually shuts the phosphoswitch back off, terminating the pulse (Fig. 3B). Through these two stages, the

system effectively implements a simple “DC to AC” pulse frequency encoder. In contrast to the excitable dynamics of the competence circuit, which generate stereotyped pulses, the σ^B system generates a long-tailed distribution of pulse sizes at any stress level (Fig. 3, A and B). It remains unclear why nonexcitable dynamics are present in the σ^B system.

In mammalian cells, the p53 pulse generator has been studied extensively through experiments and mathematical modeling. Its mechanism appears to be based on repeated activation of an upstream stress sensor (12, 13). The kinase ataxia telangiectasia mutated (ATM) repeatedly initiates pulses by phosphorylating p53 in response to γ-irradiation and subsequent DNA double-strand breaks. These pulses are transmitted directly, through modification of p53 protein, and indirectly (through the kinase Chk2) to activate p53, initiating a pulse. The pulse terminates because p53 transcriptionally activates its negative regulator Mdm2, which targets p53 for degradation. p53 also resets itself in another way, by negatively regulating ATM through the phosphatase Wip1. Thus, the recurrent activation of the upstream sensor, together with multiple negative-feedback loops, appears to enable repeated p53 pulses as long as DNA damage persists (12, 13) (Fig. 3C).

Control of NF-κB nuclear localization provides another example of pulse generation. In resting cells, NF-κB is found in the cytoplasm as an inactive complex with its inhibitor IκB (inhibitor of κB). In response to a constant amount of TNFα, NF-κB exhibits repeated nuclear localization pulses (5, 14, 15). These pulses occur in a “digital” fashion, in that they are all-or-none at the level of individual cells (5). In this case, pulses are generated by a circuit containing multiple negative-feedback loops (Fig. 3D). One negative-feedback loop, which engages at a faster time scale, involves the transcriptional activation of IκB by NF-κB, which in turn antagonizes NF-κB. The second feedback loop, which is engaged over longer time scales, involves transcriptional activation of the A20 protein, which blocks activation of kinase IKK that in turn activates NF-κB (36). The way in which these two feedback loops work together to generate the dynamic responses of NF-κB observed in individual cells, and where the stochastic variability arises, are not fully understood.

Several features appear common to many pulsatile circuits. First, negative feedback loops occur in all examples. A delayed negative-feedback may be essential for both allowing pulses to build up and also ensuring that they ultimately terminate. In general, this is facilitated by separation of time scales, whereby negative feedback can be slow compared to processes that initiate or amplify pulses. However, positive feedback loops have been identified in bacterial, but not mammalian, circuits. This may reflect incomplete knowledge, or qualitatively different pulse-generation mechanisms, as positive-feedback loops in oscillators can have a strong impact on circuit behavior (37). Second, noise helps generate pulses. For both bacterial circuits, pulsing was reduced or eliminated when cells were placed in a filamentous state with reduced noise (1, 9). Moreover, the bacterial competence circuit displayed more variability in pulse durations than a rewired variant, suggesting that variable durations may play an adaptive role (30). The mammalian circuits are also variable, most notably in the example of digital activation in NF-κB. Finally, some dynamics, such as those of ERK activation and NF-κB activity at high input levels, become increasingly periodic. Indeed, theoretical models have demonstrated that circuits with mixed feedback loops can exhibit a variety of dynamical behaviors including multistability, oscillations, and pulsing,

depending on parameter regimes (38), so this feature may not be sufficient to guarantee pulsatility. It will be interesting to understand experimentally how these circuits transition between stochastic pulsing and oscillatory behaviors.

Potential Functions for Pulsing

Pulsing offers a flexible mode of regulation that can be adapted to many cellular contexts. More pulsatile systems and corresponding functions likely remain to be discovered. Here we discuss some potential additional functions that pulsatile systems might provide in the cell.

Pulsing Could Enable Time-Based Combinatorial Regulation

So far, most pulse systems have been analyzed in isolation. But gene regulation is frequently combinatorial, and pulsing transcription factors likely co-regulate targets with other regulators. For example, the NFAT signaling pathway responds to changes in the intracellular concentration of calcium by nuclear localization of NFAT1 and NFAT4 transcription factors (4). NFAT4 localizes to the nucleus in rapid, repeated stochastic bursts on a time scale of minutes. In contrast, NFAT1 localizes to the nucleus in a slower and nonpulsatile fashion. Thus, NFAT4 can be activated by brief calcium pulses, whereas NFAT1 filters them out. The combination of both isoforms could enable more advanced signalprocessing functions. For example, a promoter activated by both isoforms could respond faithfully to rapid activation, via NFAT4, while filtering out brief drops in calcium, via NFAT1.

Can multiple pulsing signaling pathways interact with each other? The relative timing of pulses between two different transcription factors could affect cellular regulation. In neurobiology, the relative timing of action potentials at pre- and postsynaptic neurons controls the strengthening or weakening of synaptic connections through spike timing—dependent plasticity (STDP) (39). Analogously, inputs might modulate the time interval between pulses of two transcription factors that cooperatively regulate a common target gene, such that transcription occurs only when both bind the promoter at the same time. In this hypothetical scheme, in one condition, unsynchronized pulses of the two factors might produce relatively little temporal overlap between the two pulses, and therefore generate low target transcription (Fig. 4A, left). By contrast, in another condition, pulsing might synchronize between two factors, enabling them to more productively activate target expression (Fig. 4A, right).

Pulsing Could Help Cells Share Limited Resources

Pulsing and oscillation could also help manage conflicting or incompatible physiological processes, enabling cells to alternate between conflicting regulatory programs by separating them in time (Fig. 4B). A classic example occurs in some cyanobacteria, which temporally alternate between incompatible nitrogen fixing and respiration phases (40). The yeast metabolic cycle provides another example of this strategy. In chemostat cultures, yeast cells undergo respiratory cycles of 4 to 5 hours, switching between reductive and oxidative phases (41). This temporal compartmentalization separates energyintensive processes such

as protein translation from oxidative damage–sensitive processes such as DNA replication, and may also occur in unsynchronized single cells (42).

Pulsing Can Randomize Sequences of Cellular States

In previous examples, cells switch among cellular states in a well-defined order, just as the eukaryotic cell cycle steps sequentially through distinct phases. By contrast, stochastic pulsing systems could permit a nondeterministic sequence of states (Fig. 4C). This may be advantageous in the context of bet-hedging, by allowing cells to dynamically control the distribution of states within a cell population.

Summary and Outlook

Our traditional view of cellular regulation as a largely steady-state process is ceding ground to a more dynamic picture. Evidently, cells are controlled by regulatory factors that show repetitive, pulsatile, and often stochastic dynamics even under constant conditions. Time-based control provides many capabilities in electrical circuits, so perhaps it is not surprising that cells have evolved related dynamics, despite their very different physical substrates and functional constraints. Understanding both the similarities and differences in the use of temporal dynamics represents an exciting challenge. Despite the fascinating discoveries of the last few years, several fundamental questions about dynamic cellular regulation remain to be answered.

A first challenge will be to develop reporters that can be tracked over time in single living cells and are sensitive to diverse molecular activities. Such reporters could enable the discovery of otherwise hidden dynamics. For example, the new membrane potential sensor PROPS (proteorhodopsin optical proton sensor) quickly led to the discovery of rapid, repeated pulsatile membrane voltage dynamics in bacteria (43).

A second critical challenge is determining the biological functions provided by different pulsing systems. In most cases, we lack a clear understanding of the functional capabilities pulsing dynamics provide and why they have been selected over other alternatives. One possible solution is to control a regulator's dynamics directly—for example, via pharmacological or optogenetic techniques—allowing a comparison between functional outcomes of time-based and concentration-based regulation. These techniques are already beginning to reveal different roles for pulsing and static regulation in the p53 stress response pathway, ERK MAPK regulation of cell proliferation, the neural developmental regulator Ascl1, and the yeast stress response regulator Msn2 (3, 18, 22, 27).

Third, from the standpoint of synthetic biology, the pervasiveness of pulsing raises the question of what forms of information processing and control are best suited to the cellular milieu (44). Most synthetic biology efforts have been based on continuous circuit design paradigms such as layered feed-forward logic circuits (45). Incorporating pulsatile dynamics into engineered circuits may more effectively address or exploit specific features of the cellular environment, such as noise, protein turnover, and shared regulatory resources (46). The relatively small number of components necessary for several pulse systems (1, 47) suggests that it may be feasible to integrate pulsatility into synthetic circuits and potentially

take advantage of regulatory schemes previously unexplored by traditional engineering disciplines.

The behavior of genetic circuits, and their response to pharmacological perturbations, critically depends not just on their connectivity but also on their dynamics. A deeper understanding of the prevalence, functions, and mechanisms of these dynamics in cells will open up new ways of analyzing and controlling cells and help to inform our understanding of the basic design principles of genetic circuits.

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References and Notes

1. Locke JC, Young JW, Fontes M, Hernández Jiménez MJ, Elowitz MB. Science. 2011; 334:366–369. [PubMed: 21979936]
2. Hao N, O'Shea EK. Nat Struct Mol Biol. 2011; 19:31–39. [PubMed: 22179789]
3. Purvis JE, et al. Science. 2012; 336:1440–1444. [PubMed: 22700930]
4. Yissachar N, et al. Mol Cell. 2013; 49:322–330. [PubMed: 23219532]
5. Tay S, et al. Nature. 2010; 466:267–271. [PubMed: 20581820]
6. Shankaran H, et al. Mol Syst Biol. 2009; 5:332. [PubMed: 19953086]
7. Raj A, van Oudenaarden A. Cell. 2008; 135:216–226. [PubMed: 18957198]
8. Goldbeter, A. Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of Periodic and Chaotic Behaviour. Cambridge Univ. Press; Cambridge: 1997.
9. Süel GM, Kulkarni RP, Dworkin J, Garcia-Ojalvo J, Elowitz MB. Science. 2007; 315:1716–1719. [PubMed: 17379809]
10. Levine JH, Fontes ME, Dworkin J, Elowitz MB. PLOS Biol. 2012; 10:e1001252. [PubMed: 22303282]
11. Cai L, Dalal CK, Elowitz MB. Nature. 2008; 455:485–490. [PubMed: 18818649]
12. Batchelor E, Mock CS, Bhan I, Loewer A, Lahav G. Mol Cell. 2008; 30:277–289. [PubMed: 18471974]
13. Batchelor E, Loewer A, Mock C, Lahav G. Mol Syst Biol. 2011; 7:488. [PubMed: 21556066]
14. Nelson DE, et al. Science. 2004; 306:704–708. [PubMed: 15499023]
15. Ashall L, et al. Science. 2009; 324:242–246. [PubMed: 19359585]
16. Cohen-Saidon C, Cohen AA, Sigal A, Liron Y, Alon U. Mol Cell. 2009; 36:885–893. [PubMed: 20005850]
17. Albeck JG, Mills GB, Brugge JS. Mol Cell. 2013; 49:249–261. [PubMed: 23219535]
18. Aoki K, et al. Mol Cell. 2013; 52:529–540. [PubMed: 24140422]
19. Warmflash A, et al. Proc Natl Acad Sci USA. 2012; 109:E1947–E1956. [PubMed: 22689943]
20. Shimojo H, Ohtsuka T, Kageyama R. Neuron. 2008; 58:52–64. [PubMed: 18400163]
21. Kobayashi T, et al. Genes Dev. 2009; 23:1870–1875. [PubMed: 19684110]
22. Imayoshi I, et al. Science 10.1126/science. 2013 1242366.
23. Temple S, Raff MC. Cell. 1986; 44:773–779. [PubMed: 3948247]
24. Vishnoi M, et al. Mol Microbiol. 2013; 90:181–194. [PubMed: 23927765]
25. Purvis JE, Lahav G. Cell. 2013; 152:945–956. [PubMed: 23452846]

26. Young JW, Locke JC, Elowitz MB. Proc Natl Acad Sci USA. 2013; 110:4140–4145. [PubMed: 23407164]
27. Hansen AS, O'Shea EK. Mol Syst Biol. 2013; 9:704. [PubMed: 24189399]
28. Muzzey D, Gómez-Uribe CA, Mettetal JT, van Oudenaarden A. Cell. 2009; 138:160–171. [PubMed: 19596242]
29. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Science. 2004; 305:1622–1625. [PubMed: 15308767]
30. Çağatay T, Turcotte M, Elowitz MB, Garcia-Ojalvo J, Süel GM. Cell. 2009; 139:512–522. [PubMed: 19853288]
31. Acar M, Mettetal JT, van Oudenaarden A. Nat Genet. 2008; 40:471–475. [PubMed: 18362885]
32. Wolf DM, Vazirani VV, Arkin AP. J Theor Biol. 2005; 234:227–253. [PubMed: 15757681]
33. Lewis K. Nat Rev Microbiol. 2007; 5:48–56. [PubMed: 17143318]
34. Wakamoto Y, et al. Science. 2013; 339:91–95. [PubMed: 23288538]
35. Garcia-Bernardo J, Dunlop MJ. PLOS Comput Biol. 2013; 9:e1003229. [PubMed: 24086119]
36. Werner SL, et al. Genes Dev. 2008; 22:2093–2101. [PubMed: 18676814]
37. Tsai TYC, et al. Science. 2008; 321:126–129. [PubMed: 18599789]
38. Rué P, Garcia-Ojalvo J. Annu Rev Biophys. 2013; 42:605–627. [PubMed: 23527779]
39. Bi GQ, Poo MM. J Neurosci. 1998; 18:10464–10472. [PubMed: 9852584]
40. Berman-Frank I, et al. Science. 2001; 294:1534–1537. [PubMed: 11711677]
41. Tu BP, Kudlicki A, Rowicka M, McKnight SL. Science. 2005; 310:1152–1158. [PubMed: 16254148]
42. Silverman SJ, et al. Proc Natl Acad Sci USA. 2010; 107:6946–6951. [PubMed: 20335538]
43. Kralj JM, Hochbaum DR, Douglass AD, Cohen AE. Science. 2011; 333:345–348. [PubMed: 21764748]
44. Nandagopal N, Elowitz MB. Science. 2011; 333:1244–1248. [PubMed: 21885772]
45. Moon TS, Lou C, Tamsir A, Stanton BC, Voigt CA. Nature. 2012; 491:249–253. [PubMed: 23041931]
46. Eldar A, Elowitz MB. Nature. 2010; 467:167–173. [PubMed: 20829787]
47. Stricker J, et al. Nature. 2008; 456:516–519. [PubMed: 18971928]

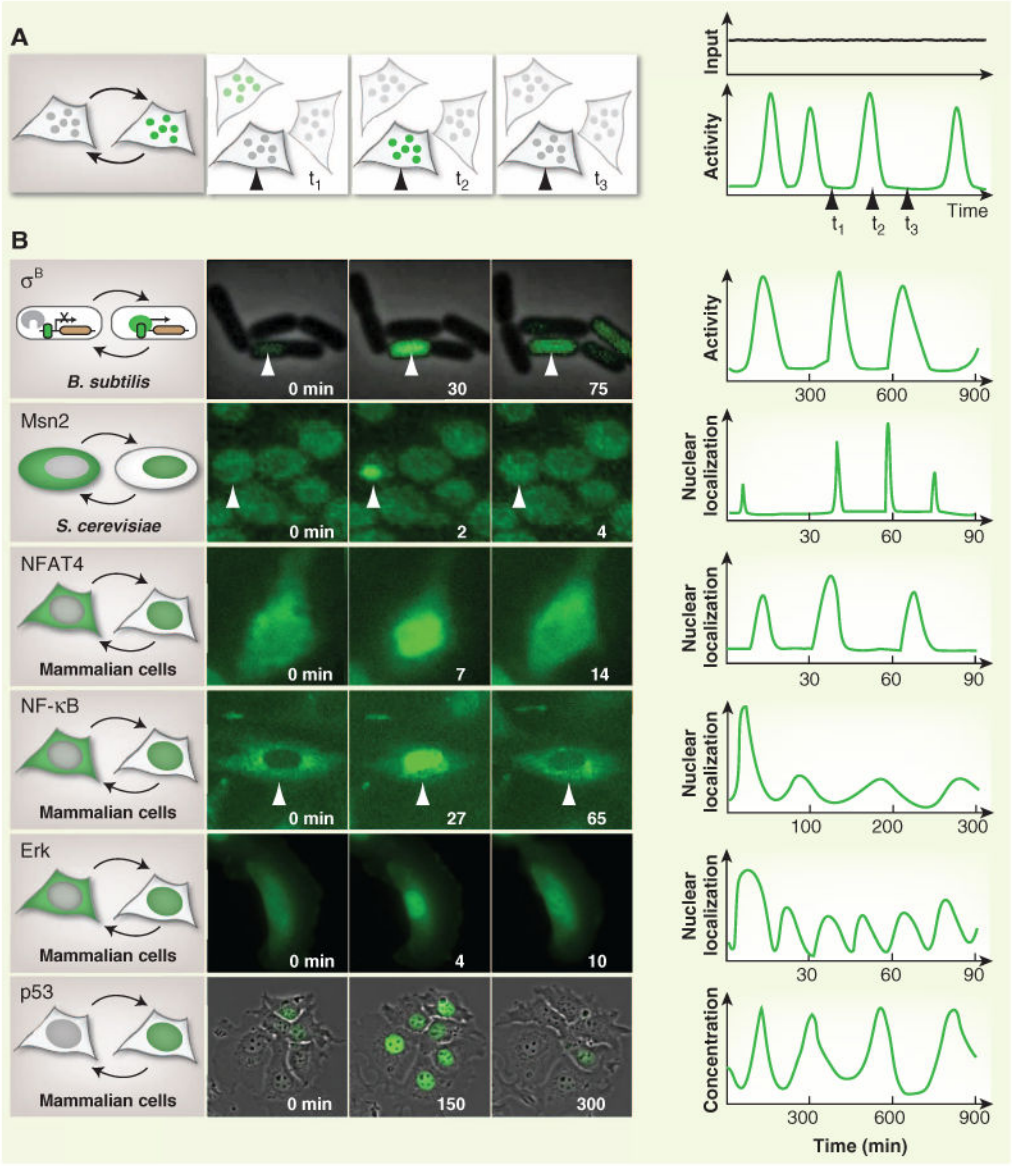

Fig. 1. Pulsing is ubiquitous in cellular regulation

(**A**)Pulsatile dynamics involve the transient, simultaneous activation of many molecules of a given type (circles), even under constant environmental conditions. Cells pulse asynchronously, making pulsing difficult to detect with static snapshots and necessitating tracking of cell lineages over time (right, schematic). (**B**) Pulsing occurs in a diverse array of pathways, molecular types, organisms, and time scales (1–6). For each example, a schematic of the type of regulation is shown at left, a typical filmstrip is shown at center, and a qualitative schematic plot of typical dynamics is shown at right.

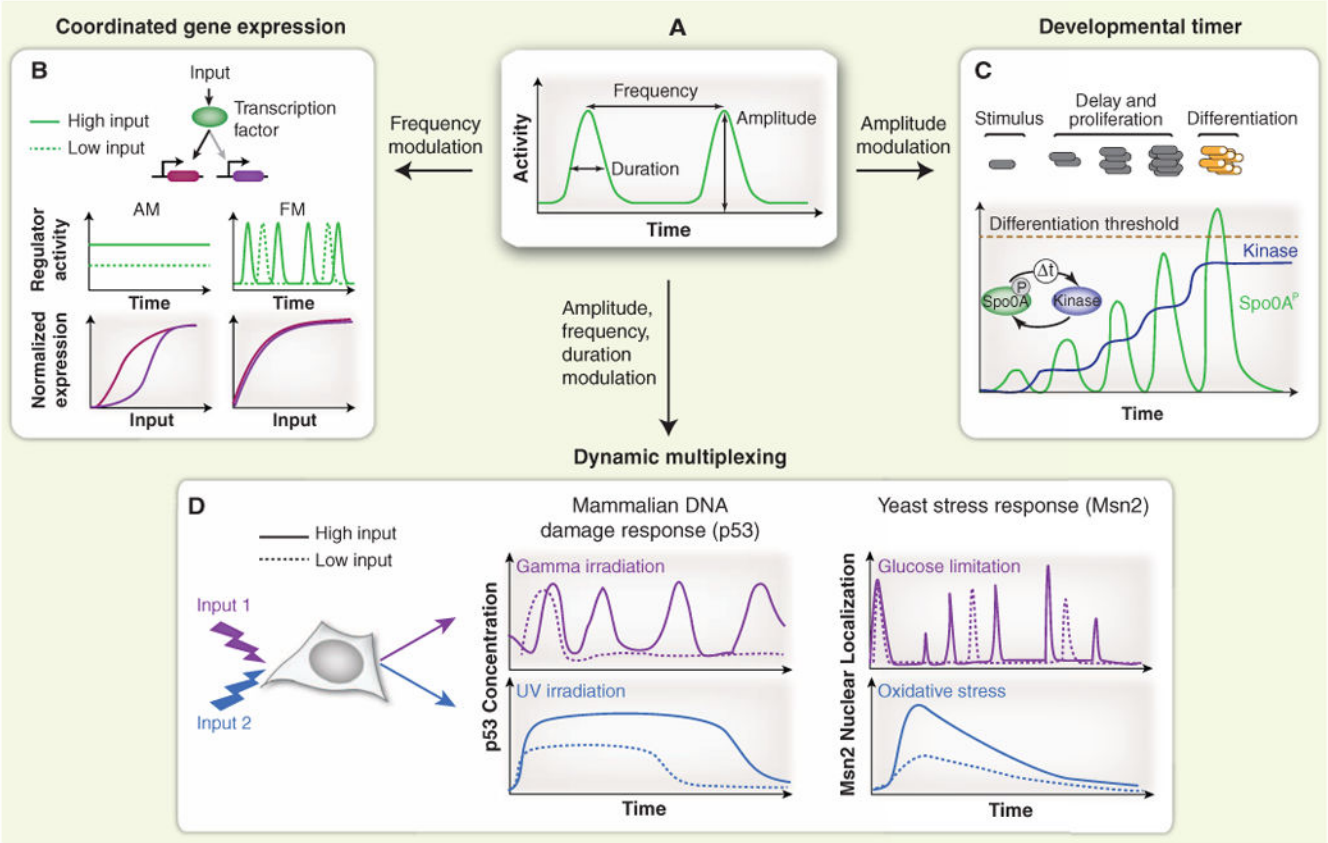

Fig. 2. Pulsing enables diverse cellular functions

(**A**) Cells modulate pulse characteristics, including amplitude, frequency, and duration, to implement diverse regulatory functions. (**B**) A transcription factor (green) may activate different target promoters at different thresholds or with different affinities (light and dark arrows). Concentration-based regulation (amplitude modulation, AM) would therefore lead to different, nonproportional, response profiles (bottom left). In contrast, frequency-modulated (FM) pulsing, by effectively controlling the fraction of time that all target genes are expressed, leads to expression of targets in fixed proportions (bottom right), indicated by overlap of expression curves (each is normalized to its own maximum) (11). (**C**) Pulsed regulation functions in a developmental timer. *B. subtilis* respond to sudden nutrient limitation by proliferating for multiple cell cycles before sporulating (schematic). A model of the underlying circuit (inset) is based on a positive-feedback loop (arrows) with a hypothesized time delay (Δ*t*). This circuit can generate progressive growth in pulses of phosphorylation of the sporulation master regulator Spo0A (green trace), via steplike growth in the kinase concentration (blue trace). The timer terminates when a threshold level of Spo0A is reached (dashed line) (10). (**D**) Examples in which dynamic multiplexing enables a single pathway to transmit multiple signals (2, 13). In each case, distinct types and levels of inputs generate distinct dynamic activation patterns for the indicated regulatory protein.

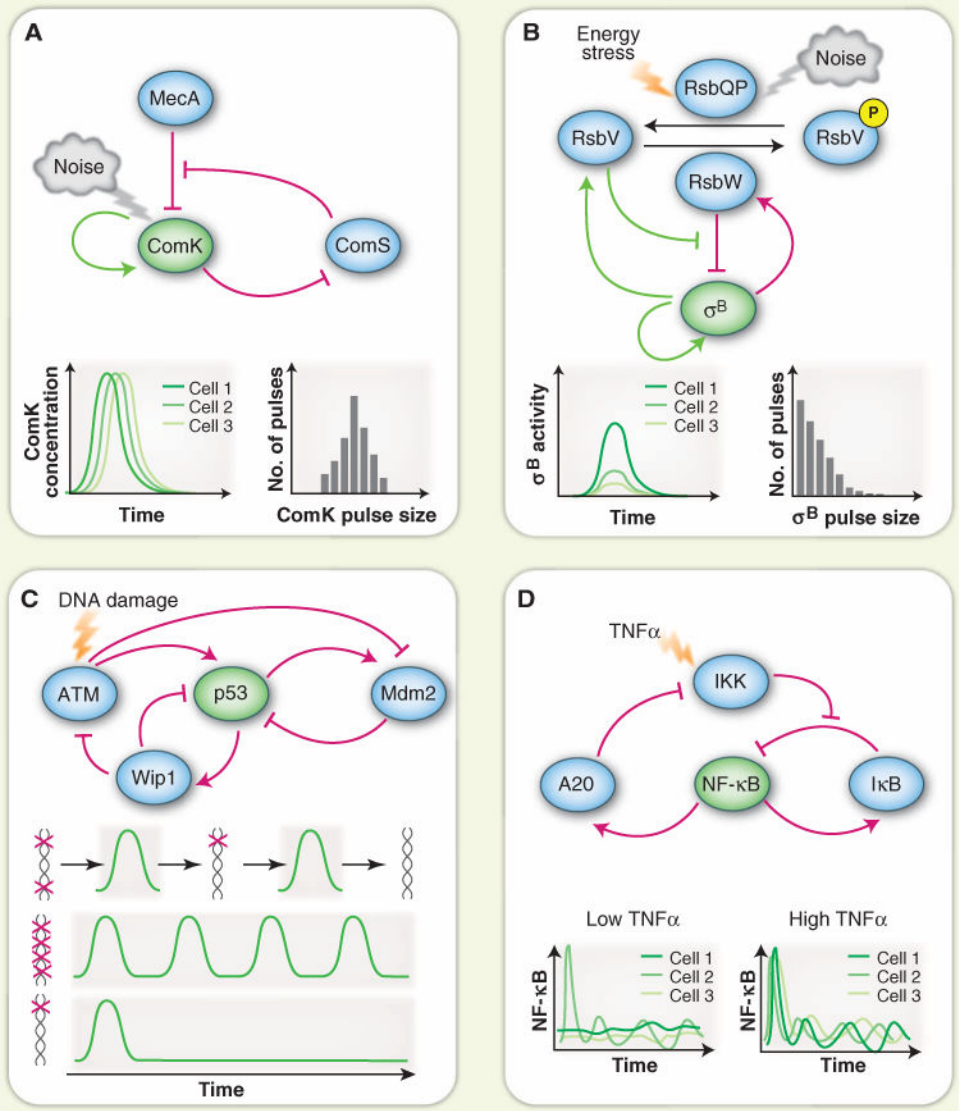

Fig. 3. Circuit mechanisms of pulse generation and modulation. (A)

The *B. subtilis* competence circuit generates stereotyped pulses of ComK activation (9). Green and red arrows represent positive and negative feedbacks, respectively. Pulses are stereotyped, as indicated by three identical traces (bottom left) and unimodal pulse size distribution (bottom right). (**B**) The *B. subtilis* general stress response (σ^B) circuit combines transcriptional feedback and an ultrasensitive phosphoswitch, and produces a nonstereotyped distribution of pulse amplitudes (bottom right) (1). (**C** and **D**) Mammalian cell pulse-generation mechanisms use multiple negative feedbacks. (C) In the p53 circuit, doublestranded DNA damage activates p53 pulses through ATM kinase. Pulses may lead to subsequent DNA repair (12). (D) NF-κB pulse mechanism. This circuit displays digital activation behavior, with the fraction of cells that pulse depending on the level of stimulus (5).

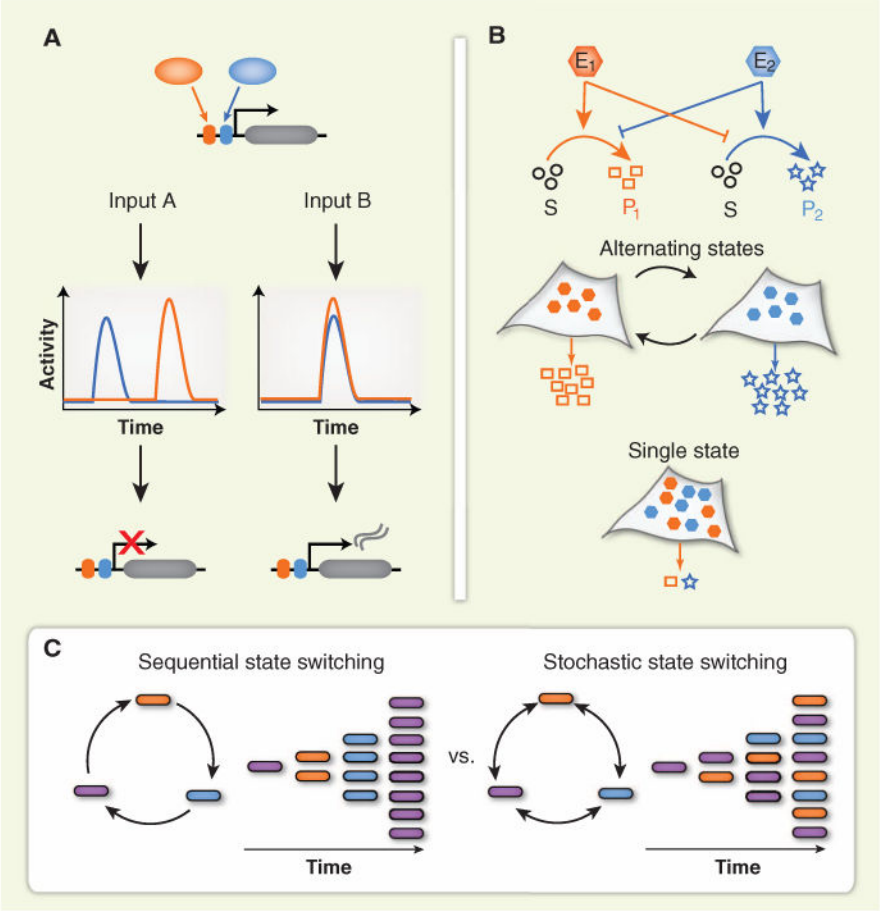

Fig. 4. Other potential regulatory functions of pulsing

(**A**) Regulation could occur through modulation of the relative pulse dynamics of two factors that co-regulate a common target. Inputs could modulate the relative level of synchronization of the regulator pulses. Here, we assume a target promoter activated only when both factors are present simultaneously. (**B**) Pulsing may reduce conflicts between incompatible pathways by temporally alternating between states in which only one or the other pathway is active (middle), rather than by simultaneously expressing conflicting programs (bottom). (**C**) Stochastic pulsing systems enable random switching between cellular states. In contrast to sequential switching, stochastic state switching allows cells to diversify cellular states on the time scale of about one cell cycle when the pulse duration is similar to the cell cycle time.