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Modeling oscillatory control in NF-κB, p53 and Wnt signaling

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Oscillations are commonly observed in cellular behavior and span a wide range of timescales, from seconds in calcium signaling to 24 hours in circadian rhythms. In between lie oscillations with time periods of 1–5 hours seen in NF-κB, p53 and Wnt signaling, which play key roles in the immune system, cell growth/death and embryo development, respectively. In the first part of this article, we provide a brief overview of simple deterministic models of oscillations. In particular, we explain the mechanism of saturated degradation that has been used to model oscillations in the NF-κB, p53 and Wnt systems. The second part deals with the potential physiological role of oscillations. We use the simple models described earlier to explore whether oscillatory signals can encode more information than steady-state signals. We then discuss a few simple genetic circuits that could decode information stored in the average, amplitude or frequency of oscillations. The presence of frequency-detector circuit downstream of NF-κB or p53 would be a strong clue that oscillations are important for the physiological response of these signaling systems.

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Introduction

Living cells continuously adjust gene expression patterns in response to changing environmental conditions. A simple way of encoding the presence of a stress or stimulus is to shift the concentration of a signaling molecule from one steady-state level to another. This scheme has potential disadvantages including the cost of continuous production of signaling molecules at a high level and unwanted cross-talk between pathways. It is not surprising, therefore, that cells often encode information about environmental changes in complex time-varying signals. This review deals with one subclass of such systems: those that exhibit oscillations.

The most obvious examples of periodic behavior are circadian rhythms [1,2] and cell cycles [3]. Much more rapid oscillations are seen in the levels of cellular calcium [4]. Many hormones also show intermittently periodic behavior and pulsatile secretion [5]. Figure 1 shows three more systems where oscillations have been observed: the NF-κB [6••,7••,8,9], p53 [10••,11••] and somitogenesis systems [12,13••,14], which are important for the immune response, cell growth/death and embryo development, respectively.

The purpose of this review is to provide a brief conceptual overview of deterministic mathematical models of such oscillations, and suggest how they can be used to explore the potential physiological role of oscillatory signals. Our emphasis will be on simplified models and the quintessential understanding they provide [15–17], rather than complex models which aim to reproduce experimental data in detail.

Modeling oscillations

Minimum ingredients for generating oscillations

The minimum requirement for oscillations is a negative feedback loop with a time delay [18]. A feedback loop is a closed cycle of nodes, representing genes, proteins, mRNA, etc. (henceforth: 'regulators'), each affecting the concentration or activity of the next node through activatory or inhibitory links (Figure 2a). A negative feedback loop is the one with an *odd* number of inhibitory links. A small perturbation of one regulator will perturb the next one in the loop, which will increase or decrease the concentration of the next regulator, and so on, until the signal returns to the original regulator. The original perturbation will be cancelled if there are an odd number of inhibitory links. Thus, negative feedback tends to produce homeostasis. The faster the signal goes around the loop, the quicker the perturbations are nullified and therefore the tighter the homeostasis. However, if the signal goes around sufficiently slowly, that is, with a distinct time delay, then this homeostasis can be broken. Negative feedback will still try to counteract perturbations, but the delay can make the regulator concentrations repeatedly overshoot their homeostatic levels and so oscillate (Figure 2b).

There are several ways to obtain an effective time delay [18], which will be elaborated upon in the next section:

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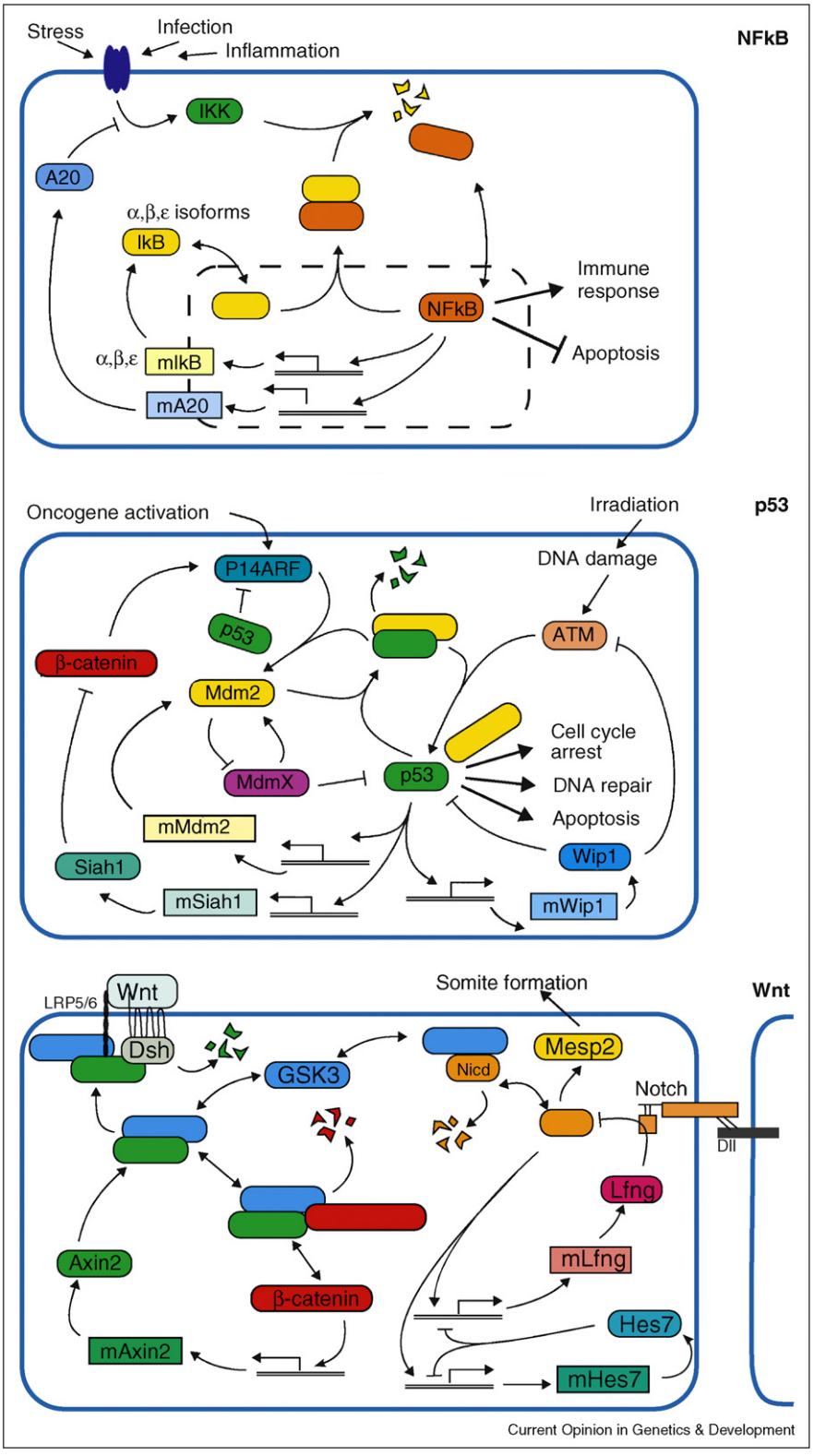
Figure 1



The NF-κB, p53 and Wnt signaling systems. Schematic figures of some of the important proteins (rounded rectangles), mRNA (rectangles), genes (double lines), and interactions (activatory: ordinary arrows; inhibitory: barred arrows) in the three systems. Ordinary arrows are also used to represent conversion and transport between cellular compartments, and merging arrows indicate complex formation. More details of each network can be found in Refs. [59,60] (NF-κB), [61,62,53] (p53), [63,64] (Wnt). Experiments showing sustained and damped oscillations in these systems can be found in Refs. [6••,7••,8,9] (NFkB), [10••,11••] (p53), [13••,14] (Wnt).

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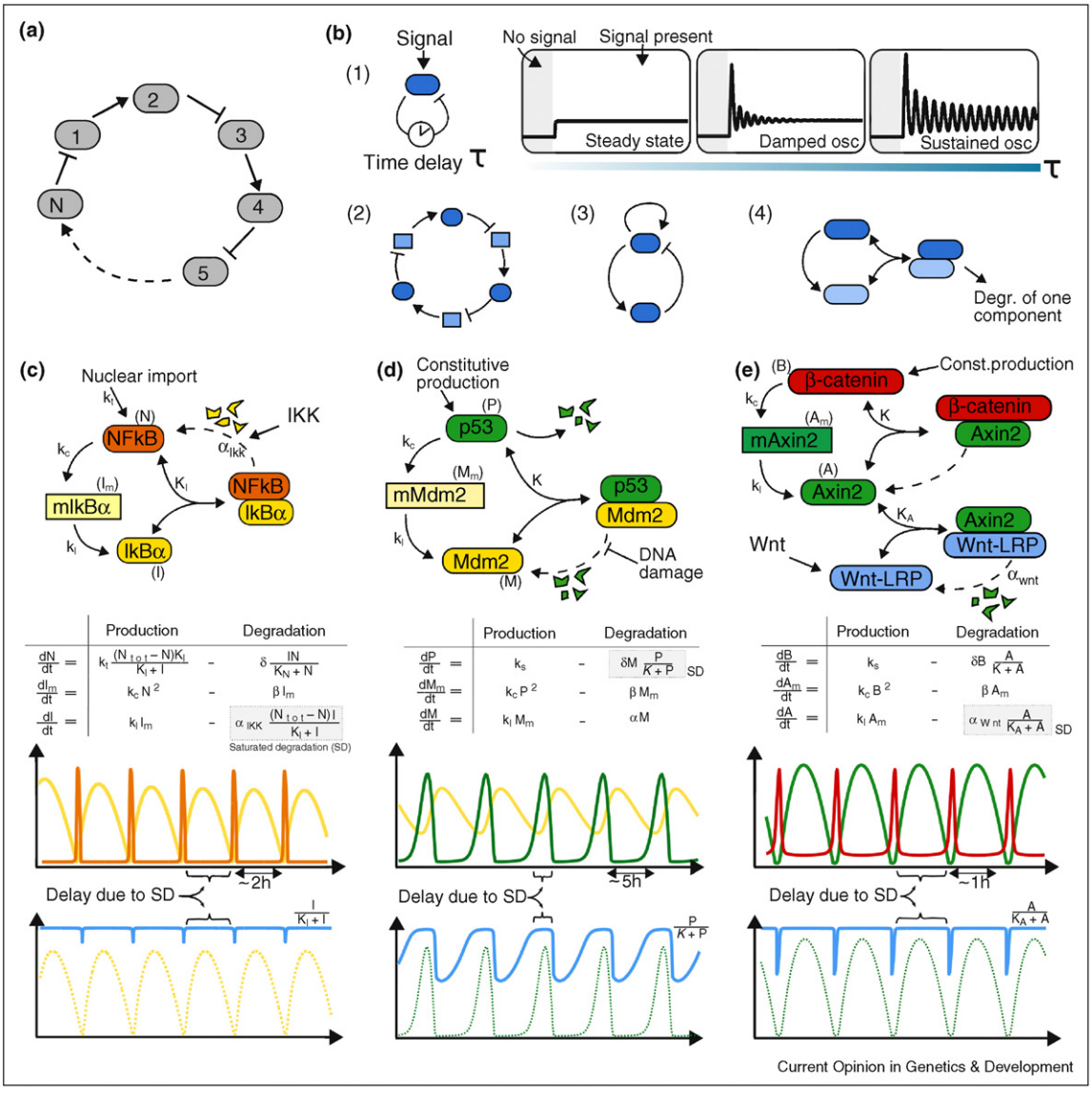
Figure 2



Modeling negative feedback and time delay. (**a**) A general feedback loop containing activatory (ordinary arrows) and inhibitory links (barred arrows). Negative feedback loops are those that contain an odd number of inhibitory links. (**b1**) The simplest negative feedback loop: a single regulator inhibiting its own production/activity with an explicit time delay described by the equation $dx/dt = \alpha/(K + x(t-\tau)^h) - \gamma x$. As the time delay in this loop is increased, the behavior shifts from homeostasis to damped to sustained oscillations. (**b2** –**b4**) Different ways of obtaining a time delay: more intermediate steps in the loop, an additional positive feedback which produces a switch-like response, and saturated degradation. (**c** –**e**) Core feedback loops generating oscillations in the NF-κB, p53 and Wnt systems. Rectangles denote mRNA and rounded rectangles represent proteins. Also shown are equations of three-variable models for each system. The NF-κB model is taken from Ref. [39]. The p53 and Wnt models have been simplified from the models of Refs. [42–44] by assuming all complexes are in quasi-equilibrium. Capital letters signify protein/mRNA concentrations, Greek letters are degradation rates, subscripted *k*'s denote maximal production rates, and subscripted *K*'s are dissociation constants. All these models use saturated degradation (terms in shaded boxes) to generate the sustained oscillations shown in the lower panels. The times when the relevant complexes are saturated are indicated by the flat peaks of the blue curves (bottom panels) that plot: (**c**) $I/(K_I + I)$, (**d**) $P/(K + P)$, (**e**) $A/(K_A + A)$. For a selection of more detailed deterministic models of these three systems see [65,66] (NFkB), [67,68] (p53), [69,70] (Wnt).

1. **Processes that take a minimum amount of time**. For example, transcription and translation (Figure 2b.1).
2. **Many intermediate steps**, that is, a long feedback loop. Each step adds to the overall time delay (Figure 2b.2).
3. **Switch-like responses**, where a regulator must reach a threshold concentration before it acts on the next in the loop (Figure 2b.3).
4. **Saturated degradation**, where the degradation of a regulator is delayed by saturated complex formation (Figure 2b.4).

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Modeling negative feedback and time delay

The model of Hes oscillations in [19] is an example of case (1) above, where an explicit time delay in the production of Hes mRNA (which is inhibited by Hes protein) represents the amount of time taken for transcription, translation and then nuclear import of Hes protein (the identical model was published again in [20]). Models using explicit time delay have been widely used to model oscillations, for example, in respiration patterns and hematopoiesis [21], p53 [22•], somitogenesis [23], insulin secretion [24], and the hypothalamic-pituitary-adrenal axis [25]. In our opinion, however, using explicit delays is somewhat ad hoc and we find it more satisfying to model the specific molecular processes that produce delay.

The conceptually simplest way to do this is case (2) above. For example, an oscillator formed by a loop of six nodes, involving three proteins, and their mRNA, each protein inhibiting transcription of the next (Figure 2b.2), has been modeled and experimentally realized in Ref. [26•]. Another synthetic circuit that oscillates due to many intermediate steps was constructed in [27]. More complex is case (3) where one regulator affects only the next when it reaches a threshold concentration. The delay arises from the time taken for the threshold to be reached. Simple models of oscillations have implemented this using either a highly cooperative interaction with a large ($\geq$8) Hill coefficient [28] or positive feedback loops [29•,30]. Both large Hill coefficients and positive feedback result in sigmoidal, switch-like responses and hence a threshold concentration below which the response is essentially zero. Combining positive and negative feedback has the advantage of making oscillations more robust and yet tunable [29•,30], and has been used to model many phenomena, including cell cycles [31,32], circadian rhythms [2,16,33], division site localization in *Escherichia coli* [34], and p53 oscillations [10••,35,36], as well as to design synthetic oscillators [37,38,27]

The next section elaborates on case (4), saturated degradation, which we find particularly interesting as it is seen in NF-κB, p53 and Wnt signaling. Note however that these time-delay mechanisms are not mutually exclusive. Systems typically use several of these mechanisms, each contributing to the overall delay.

Saturated degradation models of NF-κB, p53 and Wnt signaling

The key negative feedback loop underlying sustained and damped oscillations in NF-κB, in both wild-type and genetically modified cells, involves the inhibitor protein IκBα. The feedback loop has two legs (Figure 2c): first, NF-κB activates IκBα production, second, IκBα inhibits NF-κB by binding to it and sequestering it in the cytoplasm. Leg (i) is active when there is little IκBα, so most NF-κB is free to enter the nucleus, causing IκBα levels to rise. Free NF-κB levels then fall rapidly as it gets bound to newly synthesized IκBα. In the model of Figure 2c [39], the amount of NF-κB–IκBα complex has a Michaelis–Menten form: $N_c I/(K_I + I)$, where I is the IκBα concentration and N_c is the total cytoplasmic NF-κB concentration. The binding is strong, that is, K_I is small, so IκBα levels quickly become large enough to saturate NF-κB, at which point the amount of complex becomes equal to N_c and independent of I. This is leg (ii) of the feedback. Now there is no further production of IκBα, so its levels will eventually fall. However, IκBα molecules that are bound to NF-κB are more susceptible to IKK-dependent degradation (due to stabilization of IκBα by NF-κB [40]) so the degradation rate (second term in the dI/dt equation in Figure 2c) depends not on the amount of IκBα present, but on the amount of the complex. Because the complex is saturated and equal to N_c most of the time (see blue curve in Figure 2c, bottom panel) we call this 'saturated degradation'. If, instead, IKK-inducing stimuli led to equal degradation of both free and complexed IκBα, then the degradation rate would be proportional to I, which would make I fall exponentially fast. By contrast, with saturated degradation I falls slower than exponentially, resulting in the more rounded shape of I vs. time seen in Figure 2c (green curve). This provides a sufficient time delay to generate oscillations. The model of Figure 2c is of course a simplified one and it is important to check whether the assumptions made in simplifying the system are reasonable. For example, in wild-type cells, free IκBα is also degraded but the model ignores this. This could be included in the model as an additional degradation term, that is, proportional to I leading to an exponential, non-saturated, decrease of IκBα levels, which may neutralize the time delay provided by the saturated degradation pathway. To see which pathway of degradation is more important, one must compare the half-life of free IκBα with the rate of the NF-κB-IκBα complex formation. Using numbers for wild-type cells from Ref. [6••,41], we find the average time for complex formation is of the order of tens of seconds, whereas the half-life of free IκBα is several minutes. Thus, we expect that saturated degradation is an important source of time delay in the NF-κB-IκBα feedback loop despite the presence of other non-saturated degradation pathways.

The p53-Mdm2 feedback loop (Figure 2d) is very similar: first, p53 activates Mdm2 production, second, Mdm2 inhibits p53 by binding to it. Mdm2 also causes poly-ubiquitination and thereby degradation of p53, again resulting in saturated degradation. Here, it is the transcription factor (TF) that has saturated degradation, rather than the inhibitor. This is the opposite to what happens with NF-κB, but the model of [42], a simplified version of which is shown in Figure 2d, shows that it does not matter for generating oscillations — the

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time delay in this model occurs when p53 levels are high and fall relatively slowly, rather than when the inhibitor levels are high (see Figure 2d). Because p53 levels remain high for longer, Mdm2 levels also rise much higher than they would without saturated degradation. The extra time required for Mdm2 levels to fall in leg (ii) of the feedback provides an addition delay that helps oscillations. The half-life of p53 in the absence of Mdm2 is of the order of hours, whereas the average time for p53-Mdm2 complex formation is of the order of seconds (see [42] and references therein). So again, even though the p53-Mdm2 interaction is quite transient, we expect the saturated complex formation to be the dominant pathway for p53 degradation, rather than non-saturated pathways.

A third system where saturated degradation has been used to model oscillations is Wnt signaling (Figure 2e) [43,44]. Wnt and β-catenin are upstream controllers of all oscillating signals in the presomitic mesoderm [45] so it is useful to study the negative feedback loop involving Wnt, β-cetenin and Axin2. The model of this loop in Figure 2e demonstrates an interesting variation on the ones in Figure 2c,d, showing that saturated degradation need not arise from the same complex that results in inhibition of the TF. In the model, Axin2 binds to the TF β-catenin and separately to the Wnt-activated LRP receptor complex. The former provides the negative feedback but the binding is weak and not saturated. The latter complex has a much smaller dissociation constant, that is, larger binding strength, and results in saturated degradation of Axin2. Thus, negative feedback and saturated degradation are separate ingredients that can be implemented independently in an oscillator.

Other simple models of oscillations using saturated degradation can be found in Refs. [46•,47–49]. It is interesting that saturated degradation typically produces quite spiky oscillations, which have the advantage that the signaling molecule can achieve high levels without having to be produced at a high rate all the time. This brings us to the question of what these oscillations are useful for.

Potential physiological role of oscillations

In some systems periodicity is an obvious requirement. A periodic spatial pattern is clearly necessary for proper somite spacing and temporal oscillations in Wnt and Notch targets are a way of generating the spatial periodicity [50•,51]. Circadian clocks in cyanobacteria are useful for entraining metabolism, photosynthesis, cell division and global gene expression to the day–night cycle [52]. However, in NF-κB and p53 it is not obvious that the oscillations per se are important for the physiological response. For example, it has been suggested that p53 pulses might be a byproduct of pulsatility in ATM, an upstream regulator of p53 required for proper DNA damage repair [53].

From the opposite angle, what benefit could oscillations provide in helping NF-κB and p53 produce gene expression patterns specific to distinct stimuli? One idea is that signals with complex temporal variation contain more information than steady-state signals and therefore can control downstream genes more subtly [39,54,53]. We elaborate on this below.

Encoding information in oscillatory signals

Steady-state signals have a single adjustable characteristic, the level of the signal, while oscillations have many — average, amplitude, time period, spikiness, spike width, spike symmetry (see Figure 3a). Oscillations in NF-κB or p53 could thus encode more information than steady-states about which stimulus was triggering the system provided: first, different stimuli affect different parameters, and second, changing different parameters affects oscillation characteristics differently.

(i) In the p53 system, we know that different stimuli affect different sets of parameters. DNA damage affects Mdm2 activity and stability, hypoxia additionally alters the transcription rate of Mdm2, while other triggers like Nutlin change only the binding strength between p53 and Mdm2 (see references in [42]). In the NF-κB model, many triggers act through the IKK level which affects the degradation rate of IκBα, but different stimuli produce different profiles of IKK and thereby NF-κB [55,8].

(ii) Figure 3b–e show that for the simple model of NF-κB described above, changing different parameters does indeed affect oscillation characteristics differently. The plots show that there are parameter regimes where one of the three characteristics, time period, average and peak, can vary while the other two remain constant. However, not all characteristics can be independently varied because there are correlations. For example, spikiness is correlated with larger time periods, lower averages and asymmetry of the spike shape. Experiments have also shown that characteristics of the temporal profile of nuclear NF-κB concentration, for example, the steepness of the initial increase and the later decline, are under the control of different regulators [56••].

Similar behavior is seen in the p53 and Wnt models, so one can conclude that an oscillatory signal produced from a simple negative feedback loop with saturated degradation can indeed encode more information than steady-state signals.

Decoding information from oscillatory signals

Next, it is necessary that different genes should respond to different characteristics of the oscillations. Consider

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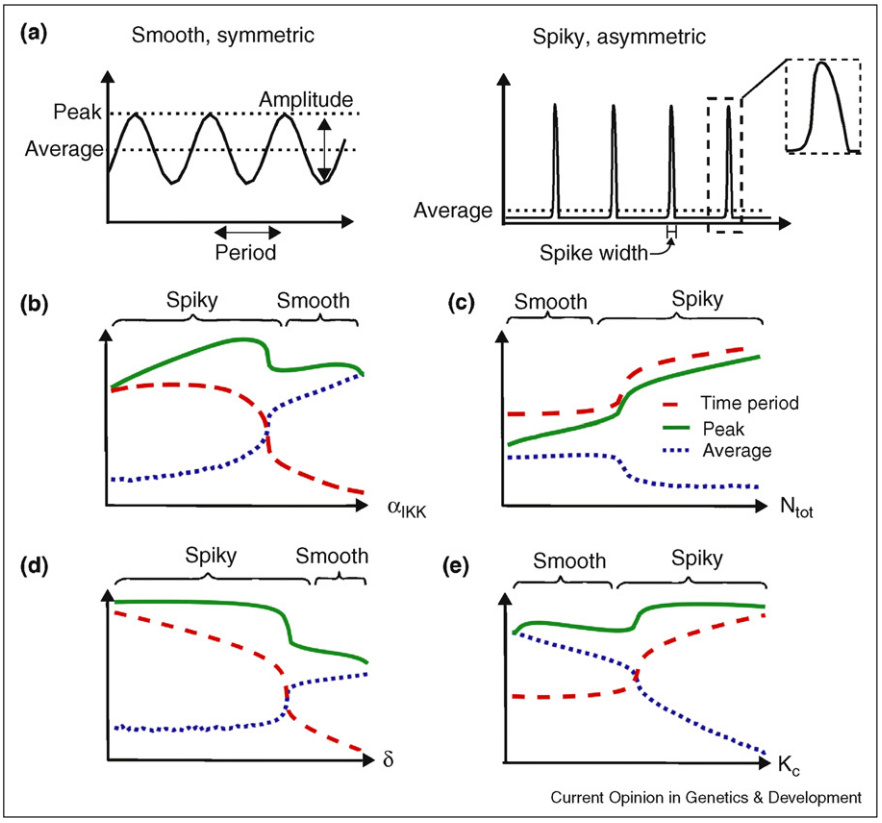
Figure 3



Encoding information in oscillations. (**a**) Characteristics of oscillatory signals that could potentially be used to encode information about external stimuli. (**b** –**e**) Time period (red dashed line), average (blue dotted line) and peak levels (green solid line) of NF-κB oscillations as selected parameters are varied in the model of NF-κB from Figure 2c. The braces mark the parameter ranges that produce smooth and spiky oscillations. Peak levels can be varied independently of time period and average by changing N_{tot} when oscillations are smooth. Similarly, average levels can be varied independently of the other two by changing k_c when oscillations are smooth. Finally, time period can be varied while average and peak remain constant by changing δ when oscillations are spiky. For all the parameter ranges shown, we also found that increased spikiness is correlated with larger time periods, lower averages and asymmetry of the spike shape.

the simplest case where an oscillating TF binds to a single operator site (Figure 4a). Two parameters describe the binding: the association (k_{on}) and dissociation (k_{off}) rate constants. Figure 4a shows that the expression of a gene, G_{fast}, with sufficiently large k_{on} and k_{off}, will closely follow the oscillations. If the stimulus changes the peak level of the oscillations, the expression of this gene will follow that change; G_{fast} is a peak-detector. By contrast, the activity of G_{slow}, which has a much smaller k_{off}, will not follow the oscillations because its expression has not time to decline much before the next spike occurs. Thus, this gene's expression averages over many spikes. G_{slow} is therefore an average detector. Note that if we look at the concentration of the proteins encoded by G_{fast} and G_{slow}, then we also have to take into account their half-lives and those of the mRNAs. Thus, for example, if G_{fast} produces a very long lived mRNA or protein then the protein concentration would follow changes in the average of the input oscillations rather than the peak. By contrast, even if G_{slow} produces a short-lived mRNA or protein it would remain an average detector.

The slightly more complex circuit of Figure 4b is a frequency detector. Two genes with twofold different k_{off} values respectively activate and inhibit a third, output gene. The average steady-state expression of this output gene has a maximum for a certain 'resonance' frequency (Figure 4b). Away from this resonance, especially for larger frequencies, the steady-state output falls dramatically. Here, the protein level will also show a similar resonance irrespective of its half-life because, assuming there is no complex translational or post-translational regulation, the average steady-state concentration of a protein is proportional to the average steady-state expression level of its gene. Interestingly, the spiky nature of the input oscillations is very important for this frequency resonance. Smooth oscillations with exactly the same time period and amplitude show a much weaker

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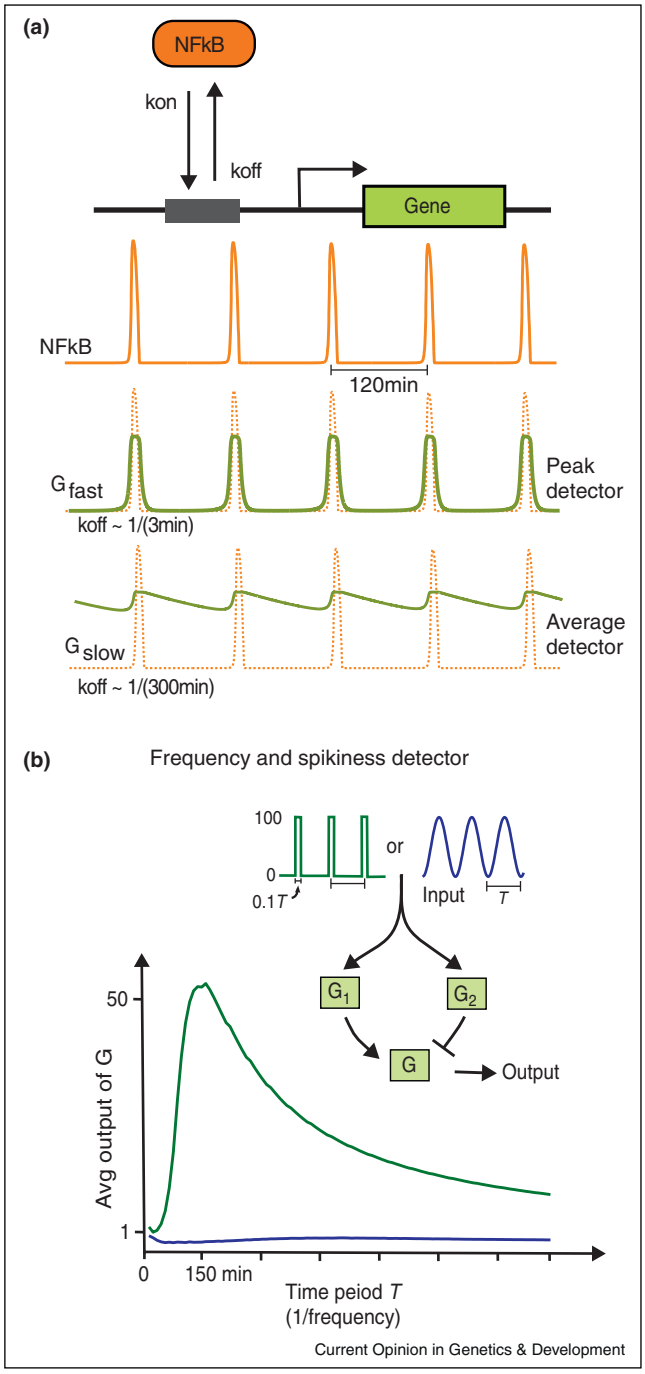
Figure 4



Decoding information from oscillatory signals. (**a**) Regulation of gene expression by a transcription factor (TF) that associates with a single operator site with a rate constant k_{on} and dissociates with a rate constant k_{off}. We make the (conservative, for NF-κB and p53) assumption that the maximal concentration of the TF is 100-fold the operator concentration. Gene G_{fast} has $k_{on} = 0.1$ min^{-1} per operator site, $k_{off} = 0.3$ min^{-1} and follows the oscillations of the TF closely (green). Therefore the peak of G_{fast} expression follows changes in the peak level of oscillations. G_{slow} has $k_{on} = 0.1$ min^{-1} per operator site, $k_{off} = 0.003$ min^{-1} and its peak expression follows the average level of the oscillations. Note the vastly different responses in the two cases. (**b**) G_1 ($k_{on} = 0.1$ min^{-1} per operator site, $k_{off} = 0.03$ min^{-1}) and G_2 ($k_{on} = 0.1$ min^{-1} per operator site, $k_{off} = 0.06$ min^{-1}) respectively activate and inhibit a third, output gene G: $dG/dt = k(G_1/(1 + G_1/K_1))(1/(1 + (G_2/K_2)^2)) - \gamma G$. For this circuit, we can find parameter values for the dG/dt equation such that the average expression of G has a maximum ('resonance') when the time period is around $T = 150$ min, when the input is spiky square-pulse oscillations (green curve). The position of this maximum can be tuned by varying k_{off} values of G_1 and G_2. With the same parameter values, when the input is smooth sine-wave oscillations of the same amplitude the response is much weaker (blue curve). See [47,71] for other frequency-detector circuits, involving protein phosphorylation.

steady-state response. This circuit is therefore a spikiness-detector as well.

Outlook

Some of the interesting questions this discussion raises for future research on oscillatory control are:

(A) Relating to encoding information in oscillations:

- Which parameters, and which characteristics of oscillations, do different stimuli affect in the NF-κB and p53 systems?
- Can additional feedback loops enhance the encoding abilities of oscillations?

(B) Relating to decoding information from oscillations:

- Can other decoding circuits be constructed to count, say, the number of spikes in a signal, or distinguish between symmetry and asymmetry, or other characteristics of oscillations?
- Do any such circuits exist downstream of NF-κB or p53?

The ideal experiment to evaluate the necessity of oscillations would require being able to control the frequency, number and width of spikes produced when NF-κB or p53 is triggered, and to see how this affects the physiological response. Exactly such experiments have shown that varying the frequency of oscillations in calcium signaling [4] and hormone secretion [5] changes the physiological response. Similar experiments on the NF-κB system are just beginning to be actualized, and have reported some dependence of gene expression on frequency of oscillations [57••]. Other experiments have shown the opposite, that expression of some NF-κB targets is unaffected in non-oscillating mutants [58]. These results are not necessarily contradictory, but until more comprehensive experiments become feasible for the NF-κB and p53 systems it might be useful to examine more carefully the genetic circuits downstream of these TFs. A frequency-detector circuit downstream of NF-κB or p53 would be a strong clue that oscillations are important for the physiological response.

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