Oscillations in transcription factor dynamics: a new way to control gene expression

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Abstract
Oscillations in second-messenger signalling (e.g. calcium) have previously been shown to be important in the control of transcription. More recently, oscillations in localization and absolute levels of transcription factors and their regulators have been identified. Here we discuss the role of network motifs such as the negative feedback loop and their role in oscillatory signalling, and how oscillations in components of the nuclear factor κB signalling pathway are important to the dynamic control of transcription in response to a cytokine stimulus.

Introduction
A stone thrown into water gives rise to oscillating ripples leading to a lasting perturbation of the smooth surface, long after the stone’s initial impact. Similarly, an initial biological stimulus can propagate oscillating intracellular signals, providing an additional way to encode information. Oscillations in cellular localization and/or concentration of second-messenger molecules and transcription factors are increasingly being recognized as features of signalling pathways [1–4]. By encoding information within both the amplitude and period, oscillations can provide increased complexity to a simple signal, thereby increasing the range and intricacy of control to the downstream targets.

Control of transcription by oscillations of second messengers
Some intracellular second messengers, such as calcium, are well known to exhibit oscillatory behaviour. Stimulation by a neurotransmitter or a hormone leads to the onset of repetitive calcium spikes, sparks, puffs and waves [5]. These oscillatory intracellular calcium signals in turn mediate cellular processes such as gene expression, secretion, proliferation etc. One decoder of these oscillations is the calcium-calmodulin-dependent kinase protein II [6]. It has also been demonstrated in live cells that artificial Ca²⁺ oscillations differing in frequency or amplitude can differentially control downstream transcription regulators such as c-Jun N-terminal protein kinase, NF-κB (nuclear factor κB) and nuclear factor of activated T cells [7,8]. In addition, spontaneous oscillatory pulses of the second-messenger cAMP are generated with a periodicity of 7 min during the development of Dictyostelium cells and they occur in phase with pulses of mitogen-activated protein kinase activation [9]. These self-regulatory systems use robust oscillatory circuits to encode information.

Key words: gene expression, NF-κB, oscillations, transcription

Abbreviations used: IκB, inhibitor κB; IKK, IκB kinase; NF-κB, nuclear factor κB.

Role of the delayed negative feedback loop in transcription factor oscillations
Transcription factor pathways typically contain various network motifs. These motifs, such as the feedforward motif [10], can either ensure rapid response or delayed response of downstream target genes. The negative feedback loop is one of the more common network motifs, and operates by increasing the rate of synthesis or activation of an inhibitor when the pathway is activated, thereby down-regulating its own activity. The time required to either synthesize or activate the inhibitor may generate a delay in the system. This delayed negative feedback loop is a pre-requisite for oscillating transcription factor pathways and, for example, is central to the operation of the circadian clock, which regulates the 24 h biological dark–light or sleep–wake cycle. The circadian clock is responsive to external inputs, such as light levels, but for transcription factor oscillations to be useful in response to signals arriving at irregular intervals, oscillations should be of lower periodicity (i.e. faster). Signals such as cytokines and other cellular stresses are examples of stimuli which cells receive at irregular times, and shorter oscillations are seen in response to these in ultradian oscillator pathways such as p53 and NF-κB [3,4].

The NF-κB pathway
NF-κB transcription factors are composed of homodimers or heterodimers, the most common among them being the RelA:p50 heterodimer. NF-κB transcription factors are critical to the control of response to cellular stress, and are also involved in the regulation of cell-cycle/growth, survival, apoptosis, inflammation and immunity [11]. NF-κB transcriptional activity is regulated by IκB (inhibitor κB) proteins. IκBs repress NF-κB-dependent transcription by both reducing NF-κB DNA-binding affinity and by anchoring NF-κB within the cytoplasm. NF-κB activity can be induced by a variety of stimuli, including the cytokine TNFα (tumour necrosis factor α). Upon stimulation, the IKK (IκB kinase) target IκBs for degradation, liberating NF-κB [12].
Figure 1 | The NF-κB negative feedback loop

Diagrammatic representation of the NF-κB feedback loop: (1) the IKK complex is activated, (2) IKK phosphorylates NF-κB dimer bound IκBα at Ser-32 and Ser-36, (3) IκBα is degraded by the 26 S proteasome, (4) free NF-κB dimers translocate to the nucleus activating transcription of NF-κB-dependent genes, (5) transcription of the IκBα gene is upregulated, (6) newly synthesized free IκBα translocates to the nucleus, and (7) IκBα binds DNA-complexed NF-κB, promoting IκBα:NF-κB cytoplasmic relocalization.

Free NF-κB dimers then translocate to the nucleus, bind NF-κB response elements and regulate gene transcription. Significantly, transcription of IκBα is up-regulated by NF-κB [13]. This completes the delayed negative feedback loop, with synthesis of IκBα proteins acting as the time delay in this system (Figure 1).

Oscillations in NF-κB signalling

NF-κB oscillations were first observed by Hoffmann et al. [3] using electromobility shift assays in population studies of IκBβ−/− embryonic fibroblasts and simulated in a computational model. Bulk cell analysis techniques (e.g. Western
blotting and electro-mobility shift assays) only measure the average temporal response of cell populations. Therefore it was not clear from these results whether asynchronous single-cell oscillations occur in wild-type cells following NF-κB stimulation [14]. In a recent study [15], oscillations in the cellular concentration and nuclear-cytoplasmic (N:C) localization of IκBα and p65-fluorescent fusion proteins respectively were observed in single living cells in response to TNFα. The duration, amplitude and damping of the oscillations were dependent on the cell type and had consequences on the dynamics of gene transcription. The use of luciferase as a reporter in living cells showed that the dynamics of NF-κB-dependent transcription from consensus κB-binding sites and phosphorylation of IκBα correlated with the duration of p65 oscillations. Unlike circadian clocks, which are limit-cycle oscillators (oscillations of consistent/regular amplitude), the NF-κB pathway produces damped oscillations of IκBα protein expression and p65 localization. Therefore, as NF-κB transcription factor oscillations vary in amplitude over time, such as calcium signalling [2], NF-κB could be an oscillating system that uses period and amplitude changes to regulate transcription differentially.

Oscillators, such as the circadian clock, segmentation clock or the cell cycle, are now well-characterized biological oscillators. The combination of functional fluorescent fusion proteins and time-lapse fluorescence microscopy of individual living cells is helping to uncover new ultradian oscillators involving second messengers and transcription factors. Such oscillators encode intracellular signalling information with extreme precision through the frequency of response, the number of discrete pulses and their size and shape. These exciting new observations raise the possibility that many regulatory circuits might be described as oscillating or ringing.

References

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