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Oscillatory Expression of Hes1, p53, and NF-κB Driven by Transcriptional Time Delays

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Summary

Feedback inhibition of gene expression is a widespread phenomenon in which the expression of a gene is downregulated by its protein product. Feedback in eukaryotic cells involves time delays resulting from transcription, transcript splicing and processing, and protein synthesis. In principle, such delays can result in oscillatory mRNA and protein expression [1]. However, experimental evidence of such delay-driven oscillations has been lacking. Using mathematical modeling informed by recent data, I show that the observed oscillatory expression and activity of three proteins is most likely to be driven by transcriptional delays. Each protein (Hes1, p53, and NF-κB) is a component of a short feedback inhibition loop [2–4]. The oscillatory period is determined by the delay and the protein and mRNA half-lives, but it is robust to changes in the rates of transcription and protein synthesis. In contrast to nondelayed models, delayed models do not require additional components in the feedback loop. These results provide direct evidence that transcriptional delays can drive oscillatory gene activity and highlight the importance of considering delays when analyzing genetic regulatory networks, particularly in processes such as developmental pattern formation, where short half-lives and feedback inhibition are common.

Results and Discussion

Recent data from cultured mammalian cell lines have revealed striking oscillatory dynamics of the expression or activity of three transcription factors (Hes1, p53 and NF-κB) that are components of short negative feedback loops [2–4]. In each case, transient stimulation of the cells initiates oscillatory gene expression with a period in the range of 2–3 hr. While circadian gene expression is well documented [5], such short period (ultradian) transcriptional oscillations are quite novel. Circadian oscillations are driven by feedback loops in which the expression of a gene is regulated by its own protein product via multiple slow steps [6]. The new data raise the question of whether ultradian gene expression is also driven by genetic feedback loops.

Oscillatory gene expression driven by negative feedback loops was first predicted by Goodwin [7]. In Goodwin's model, interpreted in terms of gene expression, feedback must involve at least one intermediate component in addition to the oscillating mRNA and protein [8]. Variants of this model have been proposed to account for the oscillatory dynamics of Hes1, p53, and NF-κB [2–4]. The models for Hes1 and p53 both require an unidentified intermediate in the feedback loop; without these intermediates, sustained oscillations cannot be generated [2, 3]. These models assume that the elongation, processing, and export of primary gene transcripts are instantaneous processes, assumptions that are almost universal in models of gene regulation [9, 10]. However, in reality, the coupled processes of transcript elongation, splicing, processing, and export are complex and time consuming [11–13] (an extreme example is furnished by the human dystrophin gene, which requires 16 hr to be transcribed [14]). Overall, there is an average delay of around 10–20 min between the action of a transcription factor on the promoter of a gene and the appearance of the corresponding mature mRNA in the cytoplasm [15]. Since the processes are coupled and there is little, if any, degradation of intermediate transcript states [11, 13], I will refer to this overall delay as the *transcriptional delay*. Similarly, synthesis of a typical protein from mRNA takes around 1–3 min and results in a translational delay.

General mathematical models incorporating delayed feedback have been studied in some detail (for example, [1, 16, 17]). In many such models, oscillations are generated if the delays surpass a critical value; in such cases, the delays can be considered as driving the oscillations. Since feedback is very common in intercellular and intracellular signaling, delay-driven oscillatory gene expression could, in principle, be widespread. However, delayed feedback drives oscillations only if the relevant mRNA and protein half-lives are sufficiently small relative to the delay. Given that typical half-lives in eukaryotic cells are of the order of a few hours, the question arises of whether transcriptional and translational time delays can be expected to have a significant impact on the dynamics of gene expression. The recent data [2–4] allow this question to be addressed in well-defined biological contexts.

The best-characterized system centers on the induction of oscillatory expression of the basic helix-loop-helix (bHLH) transcription factor Hes1 in cultured murine cell lines stimulated by serum [2]. Hes1 represses the transcription of its own gene through direct binding to regulatory sequences in the *hes1* promoter ([2], see Figure 1). Taking into account the transcriptional time delay, and denoting the concentration at time t of *hes1* mRNA by $M(t)$ and Hes1 protein by $P(t)$, this system can be represented by a simple model:

$$\begin{aligned} dM/dt &= \alpha_m G[P(t-\tau)] - \mu_m M(t) \\ dP/dt &= \alpha_p M(t) - \mu_p P(t), \end{aligned} \quad (1)$$

where μ_m and μ_p are the rates of degradation of mRNA and protein, respectively, α_m is the basal rate of transcript initiation in the absence of Hes1 protein, α_p is the

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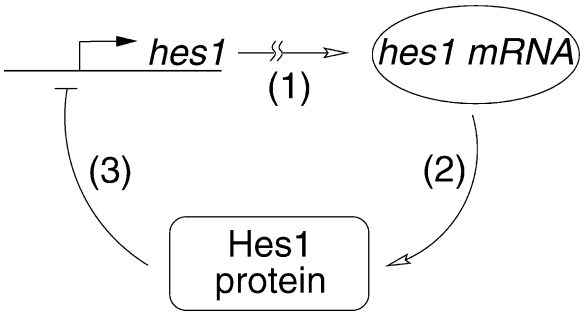

Figure 1. Schematic Representation of the Delayed Hes1 Feedback Loop

(1): Transcript elongation, splicing, processing, and export from the nucleus to the cytoplasm. (2): Synthesis of Hes1 protein by translation of *hes1* mRNA. A translational delay can be absorbed into the transcriptional delay. (3): Repression of transcript initiation from the *hes1* gene, through the binding of Hes1 dimers to the promoter.

rate at which Hes1 protein is produced from *hes1* mRNA, and $G[P(t-\tau)]$ is a monotonic decreasing function representing the delayed repression of *hes1* mRNA production by Hes1 protein. G takes the general form

$$G[P(t-\tau)] = \frac{1}{1 + (P(t-\tau)/P_0)^n}, \qquad (2)$$

where P_0 is the concentration of Hes1 that reduces the rate of initiation of *hes1* transcripts to half of its basal value (the *repression threshold*), and n is a Hill coefficient that determines the steepness of G (i.e., the degree of cooperativity in the repressive interaction). The delay τ represents the sum of the transcriptional and translational time delays (see the Supplemental Data available with this article online).

Rescaling these equations reveals how the dynamics of the system depend on the model parameters. In terms of the rescaled variables $m = M/\alpha_m$, $p = P/\alpha_m\alpha_p$, and $p_0 = P_0/\alpha_m\alpha_p$, the model equations, and thus the dynamics of the system, depend on only five parameters: the degradation rates μ_m and μ_p, the delay τ, the normalized repression threshold p_0, and the Hill coefficient n (see the Supplemental Data). In murine cell lines, *hes1* mRNA and Hes1 protein half-lives are approximately 24 min and 22 min, respectively, corresponding to first order degradation rates of 0.03/min [2]. Specific data on τ, p_0, and n are not available. However, typical transcript elongation and processing rates would result in a time delay of around 15–20 min [12, 13, 15]. Reasonable estimates for p_0 and n are $10 \leq p_0 \leq 100$ and $2 \leq n \leq 10$ (see the Supplemental Data).

Figure 2 shows a simulation of the delay model (1) with the measured values of μ_m and μ_p and with p_0, n, and τ taking values in the estimated ranges. The model exhibits pronounced oscillations in *hes1* mRNA and Hes1 protein expression. Both the period of the oscillations (2 hr) and the phase lag between mRNA and protein expression (20 min) are in excellent agreement with experimental data [2]. Figure 3 shows how the oscillatory period depends on the model parameters. As expected, the period is an increasing function of the delay and the mRNA and protein half-lives. Most strikingly, the period is essentially independent of p_0 and is thus remarkably robust to changes in the basal mRNA and protein synthesis rates. If the protein half-life is increased to several hours, and if the other parameters remain unchanged, the mRNA level peaks 1 hr after stimulation and then falls essentially to zero by 3 hr; the protein level rises to a plateau by 2 hr (see the Supplemental Data). These results are in excellent agreement with the behavior of cells treated with proteasome inhibitor [2]. These simulation results are supported by a mathematical analysis of the model equations (data not shown).

Given the measured mRNA and protein half-lives, the oscillations exhibit two notable dependencies on the other model parameters. Firstly, sustained oscillations with a period of 2 hr can be induced only if $n > 4$ (data not shown). Secondly, for $n = 5$, oscillations persist at a reasonably high amplitude for more than 6 hr only if the delay is greater than around 15 min; this minimal delay drives oscillations with a period of around 110 min (see Figure 3B). A Hill coefficient of $n > 4$ corresponds to the fact that there is significant nonlinearity, or cooperativity, in the regulation of *hes1* transcription by Hes1 protein. Hes1 acts as a dimer, which alone would suggest that $n = 2$; the requirement that $n > 4$ implies that further cooperative interactions must be involved. Interaction between the three binding sites for Hes1 that have been identified in the *hes1* promoter is a likely source of the additional cooperativity, but protein modification and nuclear import may also contribute. This

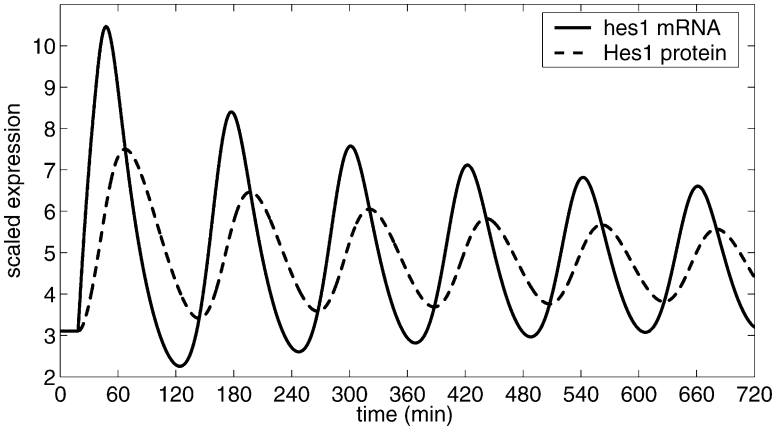

Figure 2. Oscillations in the Levels of *hes1* mRNA and Hes1 Protein in a Discrete-Delay Model

Simulation of the model system described by a rescaled form of (1). Protein oscillations lag mRNA oscillations by approximately 20 min. Expression levels are shown scaled by α_m (mRNA) and $\alpha_m\alpha_p/\mu_p$ (protein). Thus, to obtain the absolute expression levels (number of molecules per cell), the mRNA and protein levels must be multiplied by α_m and $\alpha_m\alpha_m/\mu_p$, respectively. The following parameters were used: $\mu_m = 0.03$/min, $\mu_p = 0.03$/min, $\tau = 18.5$ min, $p_0 = 100$, $n = 5$. The following initial conditions were used: *hes1* mRNA = 3 and Hes1 protein = 100 for $0 \leq t \leq 18.5$ min. Similar results are obtained for other initial conditions.

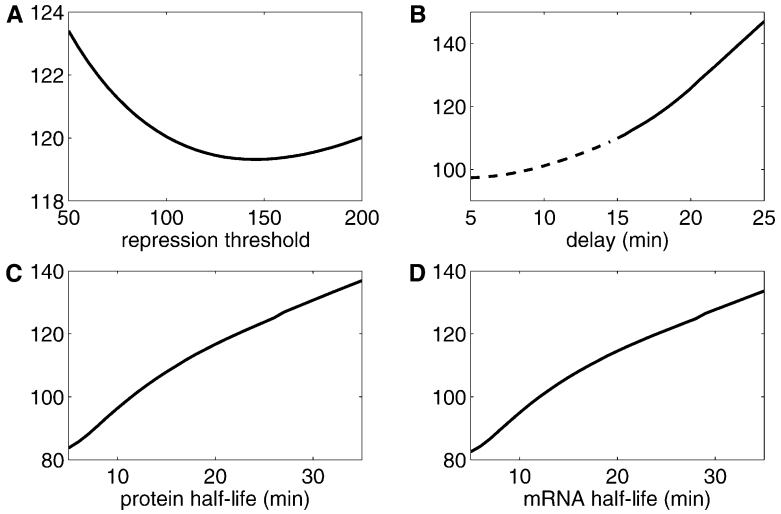

Figure 3. The Oscillatory Period Depends Primarily on the Delay and Half-Lives and Is Robust to Changes in the Rate of Protein Synthesis

Dependence of the oscillatory period (in minutes) on the model parameters. In each panel, all but one of the parameters are set as in Figure 2, while the remaining parameter is varied.

(A) The period depends only weakly on the normalized repression threshold p_0; a 4-fold change in p_0 results in a change in the period of less than 4%. The amplitudes of the oscillations do vary with p_0.

(B–D) The period increases with (B) increasing delay, (C) protein, and (D) mRNA half-lives. In (B), the dashed curve for delays of less than 15 min represents oscillations whose amplitude diminishes more rapidly over 6 hr than the observed Hes1 oscillations [2].

restriction on n is not a generic feature of the type of delay model considered, but it stems from the fact that in the Hes1 system, the mRNA and protein half-lives are greater than the transcriptional delay. Mathematical analysis of the model shows that oscillations are most easily obtained when the delay is greater than the half-lives; in this case, sustained oscillations can be obtained with $n = 2$ (data not shown.)

The Hes1 model equations encode three principal assumptions: (1) translation is nonsaturating; (2) movement of Hes1 protein between the cytoplasm and nucleus is neglected; (3) the delay takes a discrete, well-defined value, τ. Assumption (1) is likely to hold, and its relaxation has little impact on the dynamics of the model. The possible consequences of assumption (2) have been studied in general delayed models [1, 16, 17]. In principle, the time taken for Hes1 nuclear import is incorporated into the delay τ, and any associated nonlinearity is incorporated in the function G. Assumption (3) can potentially have serious dynamical consequences, and a more realistic assumption is that the delay for each transcript is drawn from some distribution. The model equations can be modified to include such a distributed delay; the behavior of this more biologically realistic distributed delay model is virtually indistinguishable from that of the discrete-delay model (see the Supplemental Data).

Oscillations in gene activity with a period similar to that of Hes1 have been reported recently in two other mammalian systems centered on the p53-Mdm2 and NFκB-IκBα feedback loops [3, 4]. Like the Hes1 system, both depend on a negative feedback loop involving a single transcriptional step; however, each loop involves the posttranslational regulation of a protein in addition to transcriptional regulation. The network of regulatory interactions underlying these oscillations is significantly more complex than the Hes1 network [18–20]. However, in both cases, a transcription factor (p53 or NF-κB) positively regulates the expression of a gene (*mdm2* or *IκBα*), the protein product of which acts to reduce the activity of the corresponding transcription factor (either by enhancing its degradation [p53-Mdm2] or by sequestering it in an inactive complex [NFκB-IκBα]). These interactions are believed to constitute core modules that act to keep in check the activity of potent transcription factors (p53 is a tumor suppressor; NF-κB is a central mediator of inflammatory and immune responses.)

Can the transcriptional delay drive oscillations in the p53-Mdm2 and NFκB-IκBα feedback systems? Relevant parameter values are not known with certainty in the context of the cell lines in which oscillations have been observed. However, delay models can be constructed by using reasonable parameter estimates. Nondelayed models have been studied in [3] and [4] and can reproduce essential features of the observed oscillations. In the p53 model, however, oscillations depend on the inclusion of an unspecified intermediate between the p53 and Mdm2 proteins [3]. The essential features of the p53-Mdm2 loop are shown schematically in Figure 4. A simple delay model encoding this scheme can reproduce the observed oscillatory behavior by using plausible parameter values (for model specification, see the Supplemental Data). Figure 5 shows oscillations in p53 and Mdm2 protein levels with a period of 3 hr, resulting from a delay of 15 min. Notably, the delay model does not require any unidentified components, and it exhibits robustness to basal mRNA and protein production rates.

A similar delay model can account for the observed oscillations with a period of 2 hr in the NFκB-IκBα system (data not shown). An important prediction stemming from parameter fitting in the nondelayed NFκB models reported in [4] is that rates of mRNA synthesis for IκB isoforms should be 7-fold lower in wild-type cells compared to knockout cells in which only a single IκB isoform is present. The fact that the oscillatory period is typically robust to changes in mRNA synthesis rates in delay models suggests that this prediction may be a specific feature of the nondelayed models. This illustrates the importance of incorporating delays in models; even if parameters can be chosen such that the predictions of a nondelayed model agree with experimental data, there is a significant chance that the numerical value of these parameters will be different in a (more realistic) delay model.

The recently reported oscillatory dynamics of the

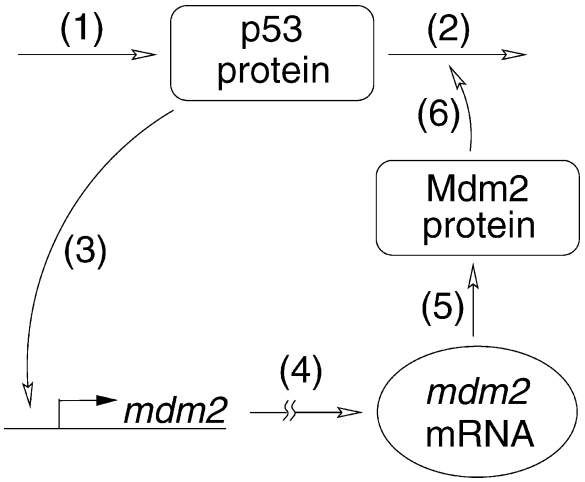

Figure 4. Schematic Representation of the p53-Mdm2 Feedback Loop

The p53-Mdm2 feedback loop depends on two key events: upregulation of *mdm2* transcription by p53, and destabilization of p53 by Mdm2 protein [19]. (1): Constitutive production of p53. (2) Degradation of p53. The degradation rate is enhanced markedly by Mdm2. (3) Upregulation of *mdm2* transcription by p53. (4) Delayed synthesis of mature *mdm2* mRNA. (5) Synthesis of Mdm2 protein. (6) Enhancement of the rate of degradation of p53 by Mdm2, acting through ubiquitination and subsequent proteolysis of p53.

Hes1, p53-Mdm2, and NFκB-IκBα systems [2–4], taken together with the mathematical models presented here, provide strong support for the hypothesis that time delays associated with mRNA and protein synthesis can drive oscillatory dynamics with periods in the range of 2–3 hr. For typical delays of 15–20 min, the generation of oscillations with these periods requires mRNA and protein half-lives of around 20 min. While these are at the lower end of the range of observed half-lives, they are not exceptional, particularly given that protein degradation in each of the systems depends on rapid proteolysis regulated by ubiquitination [2–4]. Since feedback regulation of transcription is a central feature of most cellular functions, delay-driven ultradian transcriptional oscillations could be widespread. If so, why have so few been observed? Detection is difficult, requiring measurements with high temporal and spatial resolution. In the reported systems, oscillations are detected by drawing samples of cells from a population [2–4]. This works only because the oscillations are approximately synchronous and phase locked throughout the population. However, if this is not the case, then single-cell measurements become necessary. New imaging technologies are rendering such measurements increasingly practical [21].

The results presented here show that delays can have significant impact both on the dynamical behavior of models and on numerical parameter prediction. They suggest that there is a pressing need for a wider assessment of the effects of transcriptional delays in systems where time scales are short and transcription is regulated by feedback. Such conditions are characteristic of developmental pattern formation. Somitogenesis and neurogenesis are prime examples of processes in which delays may play significant roles. Indeed, models of the neurogenic network based on the Notch-regulated transcription of *Delta* [22, 23] exhibit delay-driven oscillations and give new insight into the interactions underlying neurogenesis (N.A.M.M., S. Veflingstad, L. Gregory, and E. Plahte, unpublished data).

Supplemental Data

Supplemental Data including details of the models used and additional model simulations are available at http://www.current-biology.com/cgi/content/full/13/16/1409/DC1/.

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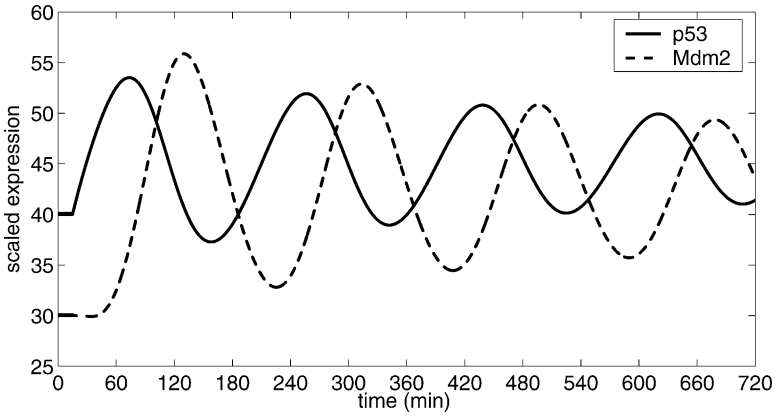

Figure 5. Oscillations in the Levels of p53 and Mdm2 in a Discrete-Delay Model

Simulation of a model of the p53-Mdm2 feedback loop incorporating transcriptional delay. As observed in irradiated mammalian cell lines, p53 levels first peak approximately 1 hr after irradiation, and Mdm2 oscillations lag p53 oscillations by approximately 1 hr. The following model parameters were used: *mdm2* transcriptional delay = 15 min, Mdm2 protein half-life = 20 min, *mdm2* mRNA half-life = 20 min, p53 half-life in the absence of Mdm2 = 100 min, minimal p53 half-life (in the presence of excess Mdm2) = 7 min, ratio of basal to maximal (p53 induced) rate of *mdm2* transcript initiation = 0.01, Hill coefficient for p53 activation of *mdm2* transcription = 4, normalized activation thresholds (for regulated *mdm2* transcription (3) and p53 degradation (6)) = 100. Model details can be found in the Supplemental Data.

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