Cell

Leading Edge
Review

Impulse Control: Temporal Dynamics in Gene Transcription

Nir Yosef[1,2] and Aviv Regev[1,3,*]
[1]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA
[2]Center for Neurologic Diseases, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
[3]Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: aregev@broad.mit.edu
DOI 10.1016/j.cell.2011.02.015

Regulatory circuits controlling gene expression constantly rewire to adapt to environmental stimuli, differentiation cues, and disease. We review our current understanding of the temporal dynamics of gene expression in eukaryotes and prokaryotes and the molecular mechanisms that shape them. We delineate several prototypical temporal patterns, including "impulse" (or single-pulse) patterns in response to transient environmental stimuli, sustained (or state-transitioning) patterns in response to developmental cues, and oscillating patterns. We focus on impulse responses and their higher-order temporal organization in regulons and cascades and describe how core protein circuits and *cis*-regulatory sequences in promoters integrate with chromatin architecture to generate these responses.

Introduction

The transcriptional program that controls gene expression in cells and organisms is remarkably flexible, constantly reconfiguring itself to respond and adapt to perturbations. These changes are apparent across a broad range of timescales, from rapid responses to environmental signals (i.e., minutes to hours) to slower events during development and pathogenesis (i.e., hours to days) (Lopez-Maury et al., 2008).

Dissecting these dynamic changes, both functionally and mechanistically, is a fundamental challenge in biology and raises several key questions. What is the scope of temporal patterns of gene expression in biological systems? What functions do different patterns serve? What molecular mechanisms underlie the formation of each pattern, and what is their capacity to process the temporal signal into a specific change in gene expression over time? Finally, are any principles, either functional or mechanistic, shared among temporal responses in distinct timescales?

Recent parallel advances in genomics and cell biology provide an unprecedented opportunity to map dynamic gene expression and decipher its underlying mechanisms. At the same time, live-cell imaging of fluorescent reporter proteins (Locke and Elowitz, 2009) allows us to study gene expression at fine temporal resolution and at the single-cell level. Such studies, when coupled with molecular manipulations and quantitative modeling, can identify basic mechanisms of temporal patterning. Further, genomic technologies provide global insights on the regulation of gene expression by allowing us to measure and perturb many aspects of the regulatory system, such as mRNA levels, protein-promoter interactions (Badis et al., 2009; Lee et al., 2002), or chromatin modification states (Wei et al., 2009; Whitehouse et al., 2007). Finally, emerging methods in synthetic biology, robotics, and microfluidics (Szita et al., 2010) are poised to transform our ability to manipulate cellular inputs and components at unparalleled temporal resolution.

Here, we review recent advances in our understanding of transcriptional dynamics, including the prototypical patterns of temporal mRNA expression and their underlying molecular mechanisms. We identify a small number of prominent temporal patterns, such as single pulse responses ("impulses"), sustained state-transitioning patterns, and oscillations. Focusing on impulse responses, we then present the molecular circuits that generate these patterns, highlighting the prominent role that transcription factor localization, integration of multiple inputs through *cis*-regulatory elements, and nucleosome occupancy play in tuning the response to a given stimulus. Finally, we discuss the prospect for a unified view of regulatory dynamics across timescales and systems, emphasizing critical directions for further research.

Prototypical Patterns of Temporal Dynamics

What capacity does a cell or organism have to generate temporal patterns of gene expression? Recent studies reveal several key classes of patterns (Figure 1). The first one, indefinite oscillators (Figure 1A), plays integral roles in homeostasis, such as the execution of the cell cycle or circadian rhythm. Other classes of temporal patterns follow an external stimulus. These include impulse (or single-pulse) patterns in response to environmental stimuli (Figures 1B–1D) and sustained (or state-transitioning) patterns in response to developmental stimuli (Figure 1E). Each of these patterns serves a set of interrelated functional goals, including optimizing the investment of cellular responses, temporally compartmentalizing antagonistic processes, and imposing order on the biogenesis of complex biological systems. On a systems-wide scale, the regulation of individual genes is commonly organized at a higher order into

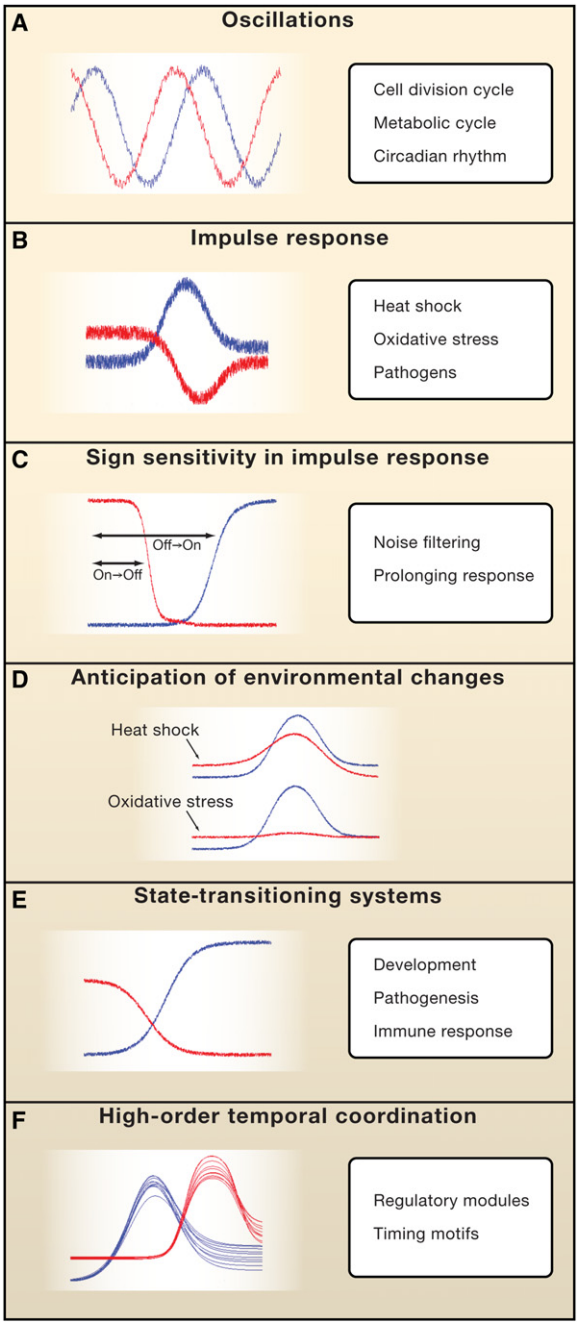

Figure 1. Prototypical Patterns of Temporal Dynamics of Gene Expression

Schematic views of gene expression levels (y axis; arbitrary units) over time (x axis) commonly found in cells in steady state or during a response to environmental, developmental, or pathogenic stimuli. Blue and red plots show possible profiles for different genes under each category. Common functions for these gene expression patterns are listed.

regulons, in which a group of genes are controlled by the same transcription factors and, thus, share the same gene expression patterns. In addition, genes can be organized into transcriptional cascades and other patterns in which expression is ordered sequentially. Here, we focus on the impulse-like pattern, specifically its function and integration within transcriptional programs.

Impulse (Single-Pulse) Responses to Environmental Signals

Changes in gene expression in response to perturbations of the surrounding environment, such as heat, salinity, or osmotic pressure, typically follow a characteristic "impulse"-like pattern (Chechik and Koller, 2009; Chechik et al., 2008). Transcript levels spike up or down abruptly following the environmental cue, sustain a new level for a certain period of time (which may or may not depend on the continuation of the cue), and then transition to a new steady state, often similar to the original levels (Figure 1B). Impulse patterns are prevalent in responses to environmental changes in all organisms, from bacteria to mammals (Braun and Brenner, 2004; Gasch et al., 2000; Litvak et al., 2009; Lopez-Maury et al., 2008; Murray et al., 2004).

One of the most extensively studied impulse systems is the environmental stress response (ESR) program in yeast. The ESR consists of ~900 genes that exhibit short-term changes in transcription levels in response to various environmental stresses (Gasch et al., 2000). The transient impulse pattern of the ESR likely represents an adaptation phase, during which the cell optimizes its internal protein milieu before resuming growth (Gasch et al., 2000). Indeed, many of the downregulated genes in the ESR are associated with protein synthesis, reflecting the characteristic transient suppression in translation initiation and growth (Gasch et al., 2000). The ESR is also associated with the brief induction of genes involved in specific response mechanisms, such as DNA-damage repair, carbohydrate metabolism, and metabolite transport (Capaldi et al., 2008; Gasch et al., 2000). A notable exception to the impulse-like stress response in yeast is the case of starvation, in which the cells initiate more sustained programs, such as quiescence, filamentation, or sporulation (Lopez-Maury et al., 2008).

Transient impulse patterns are also prevalent in mammalian cells (Foster et al., 2007; Litvak et al., 2009; Murray et al., 2004), extending beyond environmental stimuli. For example, when innate immune cells, such as macrophages (Gilchrist et al., 2006; Ramsey et al., 2008) or dendritic cells (Amit et al., 2009), respond to pathogens, expression changes in individual genes follow a clear impulse pattern. These patterns, however, are often coupled to each other, forming multistep transcriptional cascades, in which the products of genes that are induced early in a response affect the expression of downstream targets. These targets, in turn, may exhibit either an impulse pattern or a more sustained one that initiates a long-term change in the cell's state (Amit et al., 2007a; Murray et al., 2004).

Sign-Sensitive Delay and Persistence Detection in Impulse Responses

Impulse patterns can respond distinctly to the introduction of a signal versus its withdrawal. This differential response results in a "sign-sensitive delay" (Figure 1C), in which the speed of the cell's response to one "sign-shift" (e.g., from the presence to the absence of a nutrient) is different from that of the complementary shift (e.g., from the absence to the presence of a nutrient).

Sign-sensitive delays are common in responses of microorganisms to changes in nutrients. For example, consider the arabinose-utilization system of *E. coli*, in which cyclic adenosine monophosphate (cAMP) regulates transcription from the L-arabinose operon. The transcriptional response to an increase in cAMP (i.e., "on" sign) is much slower than to a cAMP decrease (i.e., "off" sign) (Mangan et al., 2003). One possible reason for this asymmetry is that, at least inside a mammalian host, the "on" state is common whereas the "off" state is maintained only during short and rare pulses of glucose. Consequently, although the cell can halt the production of L-arabinose genes soon after the introduction of glucose, it can tolerate slower commencement of their production when glucose levels decrease and cAMP is produced (Mangan et al., 2003). Alternatively, a sign-sensitive delay may reflect noise filtering; the cell refrains from activation of response pathways following spurious or transient signals. For the arabinose system, the "on" switch delay is approximately 20 min, comparable to the timescale of spurious pulses of cAMP in other natural settings (Alon, 2007).

Conversely, a delayed response to the "off" switch can prolong the effect of a transient stimulus. For example, the expression of flagella motor genes in *E. coli* persists for 1 hr after the biogenesis input signal is turned off, but no delay occurs during the on switch. Indeed, this delay time in shutting down is comparable to the time needed for the biogenesis of a complete flagella motor (Kalir et al., 2005).

Similar principles of signal processing in impulse responses have also been observed in mammalian systems. For instance, a small regulatory circuit that controls the expression of the gene encoding the proinflammatory cytokine interleukin-6 (IL-6) in mouse macrophages exhibits a delayed response to lipopolysaccharide (LPS) stimulation (the on switch) and discriminates between transient and persistent signals in the innate immune system (Litvak et al., 2009). Other "persistence detection" mechanisms have also been observed in transcriptional responses to DNA damage (Loewer et al., 2010), to epidermal growth factor (EGF) (Amit et al., 2007a), and to extracellular-signal-regulated kinase (ERK) signaling (Murphy et al., 2002).

Transcriptional Anticipation as an Adaptation to Dynamic or Noisy Environments

Most studies of environmental stimuli in the lab focus on one sustained signal at a time, but the natural environment to which cells are adapted is substantially more complex, noisy, and irregular (Lopez-Maury et al., 2008; Wilkinson, 2009). Impulse-like transcriptional programs reflect some strategies that cells employ to handle such temporally fluctuating environments.

Random fluctuations are optimally handled by sensing environmental changes and specifically responding by transcriptional changes in relevant genes, as described above (e.g., Capaldi et al., 2008; Gasch et al., 2000). In certain cases, a population of cells may respond stochastically; they activate different changes in gene expression in different cells of the same population, thus "hedging" their adaptive bets (Lopez-Maury et al., 2008).

When fluctuations are stable and predictable, bacteria and yeast cells may use an anticipatory strategy for gene regulation (Mitchell et al., 2009; Tagkopoulos et al., 2008). For example, when exposed to heat shock, yeasts induce an impulse response of genes needed for oxidative stress, although these genes are not directly necessary for adaptation to heat shock. Interestingly, yeast do not induce heat shock genes in response to oxidative stress (Mitchell et al., 2009). This asymmetry (Figure 1D) may reflect the predictable order of the two stresses under natural circumstances: oxidative respiration and accumulation of oxidative radicals follow a temperature increase during fermentation.

Notably, this anticipation strategy differs from symmetrical cross-protection (Kultz, 2005) through shared stress-response pathways (Gasch et al., 2000). Rather, it indicates that any optimization of transcriptional programs during evolution occurred in a complex adaptive landscape. Thus, a strategy that may appear "suboptimal" when considering only one stimulus in the lab may indeed be optimal in the presence of multiple simultaneous or sequential stimuli.

Higher-Order Temporal Coordination of Impulse Responses

A functional temporal program of gene expression requires appropriate temporal coordination between genes (Figure 1F). Studies reveal two main classes of temporal coordination: regulatory modules and timing motifs.

A regulatory module consists of genes that are coexpressed with the same temporal pattern or amplitude (FANTOM consortium et al., 2009; Gasch et al., 2000; Spellman et al., 1998). Regulatory modules serve to coordinate the production of proteins that are needed to perform relevant cellular functions in the given response. Regulatory modules are a hallmark of all known transcriptional programs and all known temporal patterns (Figure 1), including oscillatory patterns (e.g., Spellman et al., 1998), sustained responses (e.g., FANTOM consortium et al., 2009), and impulse responses (e.g., Chechik et al., 2008).

Complementing the tight temporal coincidence within regulons, timing motifs reflect a particular order of transcriptional events among genes or modules, such as a linear cascade of genes with sequentially ordered expression (Alon, 2007; Chechik et al., 2008; Ihmels et al., 2004). In microorganisms, such ordering is commonly observed among genes encoding metabolic and biosynthetic enzymes, and therefore, it can play an important role in achieving metabolic efficiency or avoiding toxic intermediates (Chechik et al., 2008; Ihmels et al., 2004; Zaslaver et al., 2004). For example, following deprivation of amino acids, *E. coli* induces the expression of amino acid metabolic genes in the same order that their encoded enzymes are present in the relevant amino acid biosynthetic pathway (Zaslaver et al., 2004). This "just-in-time" pattern (Zaslaver et al., 2004), which may optimize resource utilization, has also been observed in other bacterial processes, most notably flagellar biogenesis (Kalir et al., 2005).

A broader range of ordered patterns of expression onset, typically in impulse responses, is found in metabolic enzymes in yeast (Chechik et al., 2008; Ihmels et al., 2004). These include timing motifs with gene expression in the same order as the metabolic pathway (i.e., a just-in-time induction or shutoff of a pathway), as well as in the reverse order to the metabolic pathway. These reversed directions possibly contribute to the fast removal of an end metabolite that is either toxic or otherwise disruptive under the new condition (Chechik et al., 2008).

Coordinated timing motifs are also found at metabolic branch points (Chechik et al., 2008; Ihmels et al., 2004). For example, consider a metabolic funnel, where two enzymes (A, B) produce complementary metabolites that are together consumed by a third reaction (catalyzed by C). In the "funnel-same-time" motif, the genes that encode the three enzymes (A, B, and C) are often expressed simultaneously, thus optimizing metabolite use by coordinating the production or consumption of metabolites along codependent branches. Similar temporal coordination was found for the genes encoding enzymes in "forks," involving one enzyme producing two metabolites, which are then consumed by two separate reactions.

Ordered Impulse Responses within State-Transitioning Systems

Cell-fate decisions are typically associated with stable changes in gene expression that transition the regulatory system from one steady state to the next (Figure 1E). Such cell-fate decisions are prevalent in development (Basma et al., 2009; Nachman et al., 2007; Oliveri et al., 2008), pathogenesis (Iliopoulos et al., 2009), and immune responses (Amit et al., 2007a, 2009; Ramsey et al., 2008; Wei et al., 2009). State transitioning in cells involves sustained induction or repression of gene expression, stabilizing the cell on a new characteristic expression program, and disassociating it from its precursors. Nevertheless, processes that lead to such stable changes often involve a succession of impulse responses that promote transient effects necessary for achieving the transition.

Such a combination of transient and stable changes in transcription was observed during PMA (phorbol myristate acetate)-induced differentiation of myelomonocytic leukemia cells (THP-1) cells (FANTOM consortium et al., 2009). Sustained responses included repression of genes required for cell-cycle progression and DNA synthesis, which is consistent with the growth arrest associated with PMA-induced differentiation. In addition, genes that characterize the differentiated phenotype (e.g., immune response) were persistently induced. Conversely, transient, impulse-like, changes were associated with various transcription factors that play an important role early in the transition, promoting the differentiation program prior to repression of the factors that maintain the undifferentiated state. A similar pattern, specifically immediate early impulse responses of key regulators followed by stable changes of downstream genes, has been observed in many other mammalian systems, including responses to growth (Amit et al., 2007a), pathogens (Amit et al., 2009), and stress (Murray et al., 2004) signals.

Impulse responses are not limited to the immediate wave of transcription at the beginning of the state transition. Rather, a succession of impulses, forming a series of transcriptional "waves," has been observed in various state-transitioning responses (Amit et al., 2007a, 2009; Ramsey et al., 2008; Shapira et al., 2009). For instance, the response of immune dendritic cells to pathogens involves several waves of induction in which coregulated genes follow a simple impulse profile with distinct onset and offset times (Amit et al., 2007a). As in PMA-induced differentiation, the first wave is an immediate-early response enriched for genes that encode proteins with roles in transcriptional regulation. Then a subsequent transcriptional wave is enriched for genes that are required for extracellular signaling (e.g., interferon-beta 1 or IFNB1) and motility (e.g., chemokine ligand 3 or CCL 3) during that time interval (~2–4 hr post-stimulus) in the in vivo innate immune response. This temporal organization allows innate immune cells to activate the CCL3 ligand at the appropriate time, favoring the migration of activated cells to the draining lymph node to activate the adaptive immune response.

Long transcriptional cascades of ordered sequential regulation are also at the basis of many complex developmental processes (Davidson, 2010). For instance, in the sea urchin embryo, the transcriptional program of skeletogenic cell development in endomesoderm specification includes several layers of regulation that correspond to developmental phases (Oliveri et al., 2008). Progression through the phases is facilitated by a regulatory cascade in which transcription factors that are active during one phase (e.g., early micromere specification) activate genes in the next phase (e.g., late specification). Notably, transcriptional changes in genes encoding regulatory factors can also feedback and regulate the expression of their temporal "predecessors" (Amit et al., 2007a). Such mechanisms are used to shape both impulse responses and sustained responses, as we discuss below.

Mechanism of Temporal Control of Impulse Responses

What is the cell's capacity to "compute" a temporal pattern of mRNA expression? Are there canonical molecular mechanisms that underlie distinct types of patterns? Can a single mechanistic unit generate more than one pattern depending on the incoming signal or its downstream target? In this section, we focus on the molecular mechanisms that generate impulse responses at single genes, gene modules, and temporal motifs.

Network Architecture Can Be Decomposed into Characteristic Topological Motifs

Regulatory systems that control gene expression are often represented as networks (i.e., directed graphs) with the nodes corresponding to regulatory proteins (e.g., transcription factors) and the edges linking a DNA-binding protein to proteins encoded by genes it binds to and regulates (e.g., Hu et al., 2007; Lachmann et al., 2010; Shen-Orr et al., 2002). Such graphs have been assembled from many small-scale studies on regulation of individual genes and operons (Shen-Orr et al., 2002) or by systematic chromatin immunoprecipitation (ChIP), in vitro assays, and computational analysis of *cis*-regulatory sequence elements (Badis et al., 2009; Harbison et al., 2004; Hu et al., 2007; Lachmann et al., 2010; Lee et al., 2002).

Although network graphs appear highly complex, they can be effectively decomposed to putative functional units based on recurring topological patterns (Figure 2). These "network motifs" (Shen-Orr et al., 2002) are small subnetworks consisting of only a few nodes and edges with a topological pattern that is significantly overrepresented in the transcriptional graph.

Although the patterns themselves are static, they can be associated, analytically (Bolouri and Davidson, 2003; Goentoro et al., 2009; Kittisopikul and Suel, 2010; Mangan et al., 2003; Shen-Orr et al., 2002; Tyson et al., 2003) or experimentally (Basu et al., 2004; Cantone et al., 2009; Kaplan et al., 2008; Mangan et al., 2006; Rosenfeld et al., 2002), with different dynamic interpretations, thus relating the architecture of these network components with a functional capacity for generating temporal

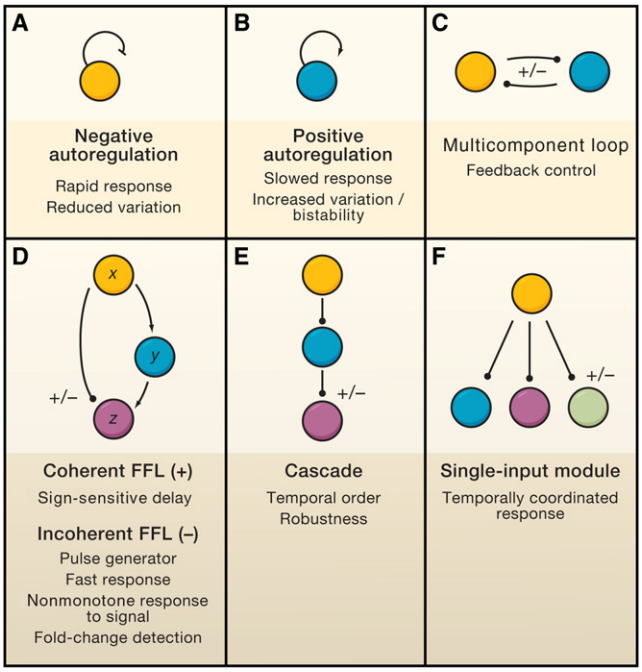

Figure 2. General Network Motifs in Transcriptional Regulatory Networks

General motifs found in transcriptional regulatory networks are shown. Nodes represent proteins; edges are directed from a DNA-binding protein to a protein encoded by a gene to which it binds and regulates. Arrows and blunt-arrows represent activation and repression, respectively; circle-ending arrows are either activation (+) or repression (−). Relevant functions for these motifs are listed.

responses (Figure 2). These responses include rapid or slowed responses (Figures 2A and 2B), feedback control (Figure 2C), sign-sensitive delays (Figure 2D), temporal ordering (Figure 2E), and temporal coordination in modules (Figure 2F).

Notably, the relation between the topology of a motif and its induced temporal pattern is far from unique and depends on the characteristics of the incoming signal and of the interacting molecules (Macia et al., 2009). For instance, protein production rate, protein degradation rate, or activation thresholds of regulators can each alter the dynamic transcriptional pattern generated by the motif (Lahav et al., 2004). Moreover, different motifs or combinations of motifs (Geva-Zatorsky et al., 2006) can induce similar behaviors. For a more thorough discussion of network motifs, we refer the reader to other extensive reviews (Alon, 2007; Davidson, 2009, 2010; Tyson et al., 2003).

Combinatorial Logic in the Feedforward Loop Generates Sign-Sensitive Delays

The feedforward loop (Figure 2D) is a major building block of combinatorial regulation (Amit et al., 2009). A feedforward loop has a unidirectional structure consisting of three nodes: an upstream regulator X that regulates a downstream regulator Y, which in turn regulates a downstream target Z (which is not necessarily a regulator). An additional edge is directed from X to Z, thus closing a unidirectional "loop." Each interaction can be suppressing or activating, resulting in eight distinct feedforward loop structures.

One commonly found structure in transcriptional networks (Alon, 2007) is the Type-1 coherent feedforward loop, in which all of the interactions are activating. This feedforward loop can generate a sign-sensitive time delay. The length of the delay and whether it occurs during the off or the on switch depends on the specific molecular parameters of the loop. The particular logic mediated by the loop largely depends on the organization of *cis*-regulatory elements in the promoter of the target gene ("Z"). For instance, when the two transcription factors in a coherent feedforward loop exhibit an "or" logic at the promoter of the downstream gene (i.e., only one transcription factor suffices to activate the gene), the resulting dynamics is usually a sign-sensitive delay with faster response to the on switch and a prolonged transcriptional response, as in flagellar biogenesis (Kalir et al., 2005). Conversely, an "and" logic for the two transcription factors (i.e., both factors are needed to activate the gene) is associated with a faster response to the off switch, as in the L-arabinose operon. This feedforward loop structure facilitates persistence detection (Mangan et al., 2003).

Another prevalent form of the feedforward loop is the incoherent variant (Figure 2D), in which Y acts as a repressor rather than an activator. Depending on its parameters, this motif can induce pulse-like responses (Basu et al., 2004), lead to a rapid (Mangan et al., 2006) or nonmonotone (Kaplan et al., 2008) response of the downstream target Z, or provide a mechanism for detecting fold-change (e.g., that a component's level changed by 2-fold rather than an absolute value) (Goentoro et al., 2009).

Single-Input Modules and Chromatin Architecture Coordinate Responses in Modules and in Just-in-Time Motifs

The single-input module (Figure 2F) motif occurs when a single regulator has multiple targets (Alon, 2007; Lee et al., 2002). This architecture, often associated with regulatory hubs ("master regulators"), can facilitate a temporally coordinated response of multiple genes in a module.

However, the activation of the downstream genes in a single-input module is not necessarily concurrent, and differences in their promoter properties can lead to ordered activation (Figure 3). Specifically, a transcription factor's affinity for a specific *cis*-regulatory sequence affects the fraction of time that it occupies a binding site (Bruce et al., 2009; Tanay, 2006). The stronger the binding affinity, the higher the probability that the transcription factor remains bound to a site and recruits the transcriptional machinery (Hager et al., 2009). Differential recruitment at different promoters results in a range of induction thresholds, allowing a single transcription factor with a temporally fluctuating level to generate an ordering of its target genes.

This principle was demonstrated in a recent study using a series of genetically modified promoters of the Pho5 gene during the response to phosphate starvation in yeast (Lam et al., 2008). In this system (Figure 3), promoters with high-affinity sites for the transcription factor Pho4 that are "open" (i.e., not occluded by nucleosomes) responded to weaker signals of slight phosphate deprivation (Figure 3B) and had a shorter response time (Figure 3A) to phosphate starvation compared to those with lower-affinity sites. Similar behavior was observed for synthetic promoter variants and for different targets of Pho4 that had similar promoter architecture.

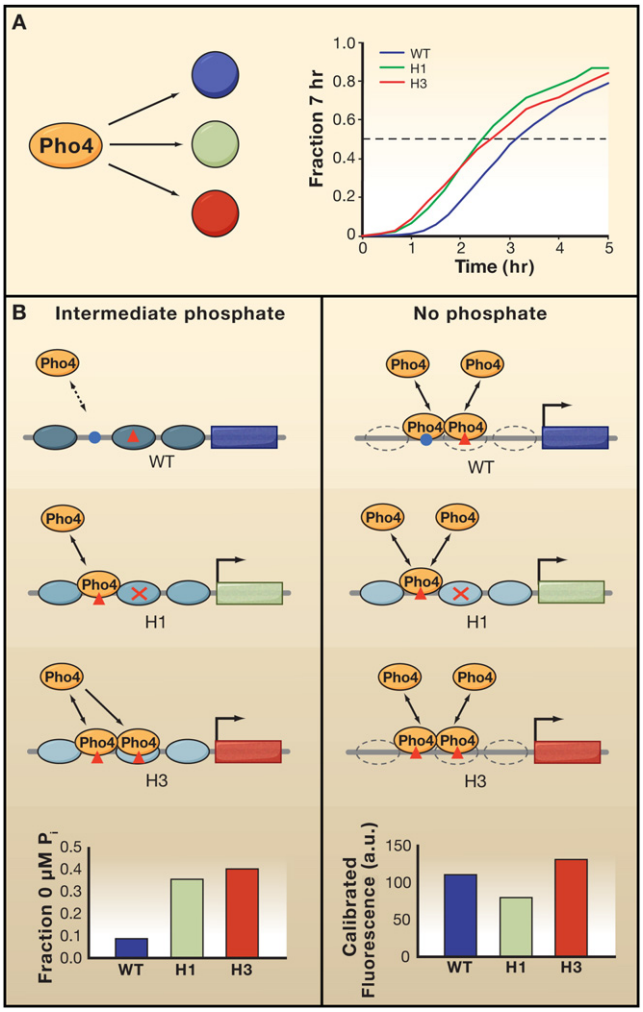

Figure 3. Promoter Regions and Nucleosome Positioning as Temporal Signal Processors

(A) The transcription factor Pho4 (orange oval) targets different variants of the Pho5 promoter following phosphate starvation in yeast cells (left). The purple (upper) promoter contains the wild-type Pho5 promoter sequence, whereas the green and red promoters (denoted as H1 and H3, respectively) are synthetic variants. Each target exhibits a different response time (right), depending on the affinity of Pho4 for its binding site when the site is unoccluded by nucleosomes (depicted in panel B). The y axis corresponds to median fluorescence levels, across separate measurements, scaled between the promoter-specific expression minimum at 0 hr and maximum at 7 hr after induction.

(B) Suggested mechanism for decoupling promoter induction threshold from dynamic range. These cartoons show occupancies of Pho4 and nucleosome at the three Pho4 promoter variants under mild (left) and acute (right) phosphate starvation. Gray-blue and yellow ovals represent nucleosomes and Pho4, respectively; dark blue circles and red triangles correspond to low-affinity and high-affinity binding sites, respectively; and X marks ablation of the Pho4-binding motif. Darker blue ovals represent more highly occupied nucleosomes (across a cell population). Under intermediate levels of phosphate (left), substantial Pho4 occupancy and subsequent transcriptional activity occurs only at promoters with exposed high-affinity sites. The plot at the bottom left shows the respective expression levels, divided for each variant by the maximum level at full starvation in arbitrary units (a.u.). In the absence of phosphate (right), Pho4 activity is saturated, resulting in nucleosome eviction and maximum expression at all promoters. The plot at the bottom right shows the respective maximal induction levels (a.u.). Reproduced from Lam et al. (2008), with permission from the authors.

Thus, graded binding affinities complement the single-input module motif in which a single transcription factor induces temporal ordering among its targets through differential binding affinity (Figure 3A). In the phosphate starvation responses, this results in tuning of the responding genes to the severity and duration of phosphate depletion. At intermediate phosphate levels (with intermediate levels of nuclear Pho4), first-response genes with exposed high-affinity sites like *PHO84* and *PHM4* allow the cell to take up environmental phosphate and mobilize internal reserves. Under starvation conditions, this initial response is followed by a second-order response such as upregulation of *PHO5* and other phosphate-scavenging components (Springer et al., 2003).

More generally, such graded affinities may explain the ordered timing of an impulse-like response of genes within metabolic pathways, in timing motifs such as just-in-time. In yeast, the timing of ordered activation in a timing motif was found to correlate with the affinity of the respective gene with its regulating transcription factor (Chechik and Koller, 2009; Chechik et al., 2008). Similar principles were also observed in *E. coli* (Zaslaver et al., 2004).

Nucleosome Positioning Contributes to Activation Timing

The position of nucleosomes in a gene promoter impacts the accessibility of transcription factors for their DNA-binding sites. Therefore, nucleosome positioning also affects the order of activations across several genes regulated by the same transcription factor. This effect was convincingly demonstrated in the Pho5 system (Lam et al., 2008). Most Pho4-binding sites are occluded under nucleosomes in normal conditions, but they become exposed when chromatin is dynamically remodeled in response to phosphate starvation (Figure 3B). The threshold of response, and hence a gene's onset time, is thus also affected by the chromatin architecture of the repressed state. Conversely, the dynamic range of the response is determined by the active state's architecture. Maximum transcriptional outputs of the Pho5 variants differed by up to 7-fold and correlated with the number, affinity, and placement of Pho4 sites, irrespective of their accessibility in the initial (pre-starvation) chromatin state. These results suggest a mechanism by which the cell decouples the determinants of promoter activation timing (site affinity and nucleosome positions) from the determinants of expression capacity (site affinity alone). Global studies on changes in nucleosome positions in response to environmental signals (Deal et al., 2010) support the generality of the Pho model, at least in yeast (Shivaswamy et al., 2008).

Protein Oscillators Generate Coordinated Impulse Responses across Regulons

Recent studies suggest that oscillations in the localization or activity of *trans*-regulators that control single-input modules play a substantial role in governing (nonoscillating) impulse transcriptional patterns. Most notably, coordinated impulse patterns across a regulon may often stem from limited oscillations in the nuclear localization of a regulatory factor controlling the target genes (Ashall et al., 2009; Cai et al., 2008). This has been suggested for the transcription factor Crz1 in yeast, which uses a "pulsing" mechanism to encode information about extracellular calcium levels (Figure 4) (Cai et al., 2008). When extracellular

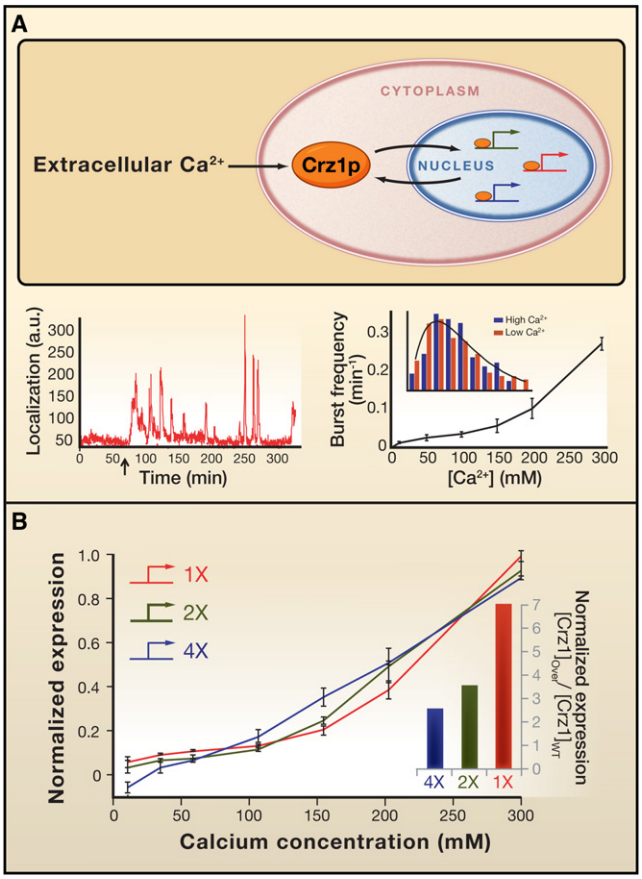

Figure 4. Coordinated Impulse Response Generated by Protein Oscillators

(A) In response to extracellular calcium, yeast cells initiate bursts of nuclear localization of the transcription factor Crz1. Bottom left: A single-cell time trace of the amount of phosphorylated Crz1 in the nucleus; the arrow indicates introduction of extracellular calcium. Bottom right: The frequency of bursts (y axis) rises with calcium levels (x axis). Error bars calculated by using different thresholds for burst determination (see Cai et al., 2008). Inset: A histogram of burst duration times under high (red) and low (blue) calcium levels indicates that burst duration is independent of calcium concentration.

(B) Expression levels of three synthetic Crz1-dependent promoters increase proportionally to extracellular calcium concentration (x axis). On the y axis, data are divided, for each variant, by the expression at maximum calcium level. The synthetic promoters have 1 (red), 2 (green), or 4 (blue) calcineurin-dependent response elements. Inset: A bar chart showing the fold-change of the different targets, following Crz1 overexpression. The targets exhibit different responses, probably due to their different numbers of Crz1-binding sites. Reproduced from Cai et al. (2008) with permission from the authors.

calcium increases, Crz1 is dephosphorylated and exhibits short bursts of translocation to the nucleus. At higher levels of calcium, the cells respond, not by increasing the amount of nuclear Crz1 in each translocation burst but rather by increasing the frequency of the bursts (Figure 4A).

Such "frequency modulation" may be important because of the nonlinearity (Yuh et al., 2001) and diversity (Kim et al., 2009) of the input functions associated with different target promoters. Because distinct Crz1 target promoters (Figure 4A) probably respond differently to changing levels of Crz1, amplitude modulation of Crz1 would not maintain their relative ratios (Figure 4B, inset). In contrast, modulation of the frequency of Crz1's nuclear localization can control the expression of multiple target genes in a more proportional manner and thus maintain more stable ratios of gene expression, regardless of the shapes of their input functions (Figure 4B, main graph). This behavior might be explained by the fact that a strong nonlinear component (i.e., dependence on Crz1 magnitude) is now kept relatively constant for different calcium levels, and the variable part is the amount of time the promoters are exposed to a fixed amount of nuclear Crz1.

Oscillations in the level or localization of transcription factors have been observed in diverse environmental responses, such as those involving NF-κB (nuclear factor K-light-chain-enhancer of activated B cells) (Ashall et al., 2009; Covert et al., 2005; Friedrichsen et al., 2006; Nelson et al., 2004; Tay et al., 2010) and the tumor suppressor p53 (Geva-Zatorsky et al., 2006; Loewer et al., 2010) (Ashall et al., 2009; Friedrichsen et al., 2006; Nelson et al., 2004; Tay et al., 2010) in mammals and the SOS response to DNA damage in bacteria (Friedman et al., 2005). In the p53 and SOS systems, monitoring with high temporal resolution revealed tightly regulated oscillations in the nuclear levels of the key regulators (e.g., p53) with variable amplitude but more precise timing.

Oscillations in regulatory proteins, which are driven by external stimuli, often lead to nonoscillatory, impulse transcriptional patterns. For example, the expression of p21, a p53-target gene, is induced in a nonoscillatory manner during DNA damage (Loewer et al., 2010). Similarly, oscillations in NF-κB localization and activity following TNF-α stimulation are coupled to impulse-like patterns in a host of early response genes, such as the NF-κB inhibitor Ik-Bα, even when assessed at the single-cell level (Tay et al., 2010).

Thus, protein oscillations in environmental response systems may play a general mechanistic role in regulating downstream impulse transcriptional changes. First, oscillation of transcription factor levels can maintain a steady response as long as the damage signal is present and constitutive supply of the downstream gene products is needed (as in the p53 response). Second, oscillations in transcription factor localization can underlie the induction of proportional responses through frequency modulation (as with Crz1). Finally, combinations of protein oscillators can generate various transcriptional kinetic patterns. For instance, activation of NF-κB in mouse embryo fibroblasts treated with LPS depends on two pathways, MyD88-dependent and MyD88-independent (Covert et al., 2005). Perturbing either one of these pathways and leaving the other one intact leads, in both cases, to oscillatory NF-κB activity. However, when both pathways are intact, both oscillators act upon LPS stimulation but with a relative phase shift of ~30 min, resulting in a stable, non-oscillatory pattern of NF-κB activity. It is likely that other combinations, as well as modulation of both amplitude and frequency, will play a role at encoding other complex patterns of transcriptional regulation at single genes and gene modules.

Attenuation and Ordering of Impulse Responses through Feedback and Cascades

Impulse patterns can be attenuated and ordered in more complex programs and through more elaborate regulatory architectures, most notably within developmental programs. In

particular, in the cascade motif (Figure 2E), regulators are ordered in layers, and proteins from one layer control ones in subsequent layers (Hooshangi et al., 2005; Rappaport et al., 2005). This pattern was observed in transcriptional networks during sea urchin development (Bolouri and Davidson, 2003; Davidson, 2009, 2010; Oliveri et al., 2008), state-transitioning systems in microorganisms (Chu et al., 1998), and environmental responses in mammalian cells (Amit et al., 2007a, 2009; Ramsey et al., 2008; Shapira et al., 2009). A cascade-like network topology entails an inherent temporal order of regulation events (Hooshangi et al., 2005). It was postulated to enable context-specific responses (Davidson, 2009) and to provide robustness both to spurious input signals (Hooshangi et al., 2005) and to noise in the rates of protein production (Rappaport et al., 2005).

Regulatory interactions between different layers in a cascade can form multicomponent loops in which genes in a late transcriptional wave regulate genes from earlier waves (Figure 2C). The ensuing feedback effect can contribute to the ultimate attenuation of impulse responses, even under a sustained signal (Amit et al., 2007b). For example, stimulation of human cell lines with EGF induces several ordered impulse responses (Amit et al., 2007a), including the induction of ''delayed early'' genes. Delayed early genes are primarily induced by transcription factors that were themselves induced as ''immediate early'' genes. Delayed early genes encode a large number of signaling proteins and RNA-binding proteins that attenuate RNA levels and protein activity of the initial response pathways. Such negative transcriptional feedback mediated through a transcriptional cascade is common in environmental responses in yeast as well (Segal et al., 2003).

A more basic form of feedback is the autoregulatory loop by which a transcription factor regulates its own gene. Negative autoregulation (Figure 2A) facilitates a rapid transcriptional response of the autoregulating gene. It has been associated with the induction of a rapid impulse response to EGF stimulation in human cells (Amit et al., 2007a) and to DNA damage in *E. coli* (Camas et al., 2006). Conversely, the positive autoregulatory loop (Figure 2B) is associated with the opposite effect because it results in a slow response time (Alon, 2007). Positive loops, with either one or more components (Figure 2B or Figure 2C, respectively), can lead to substantial variation between isogenic cells, due to stochastic effects, and can play an important role in maintaining stability after state transitioning (Davidson, 2009; Kim et al., 2008; Macarthur et al., 2009; Oliveri et al., 2008).

Perspective

Diverse mechanisms drive impulse-like changes in mRNA levels, which can occur on a broad range of timescales, from rapid environmental stress responses to slower and more elaborate developmental processes. What can we learn by comparing these processes across timescales? The emerging picture supports a few basic principles. Just-in-time responses and sign sensitivity optimize process efficiency, whereas the organization of the impulse responses in functional waves and cascades provides temporal compartmentalization and order to gene expression.

Although in this Review, we have made convenient distinctions between impulse responses, state transitions, and oscillators, most biological systems intertwine these temporal patterns. For example, oscillations in protein levels or localization can also lead to impulse responses, and ordered impulses are important in generating sustained responses through cascades. Furthermore, many of the underlying molecular mechanisms driving these temporal patterns can be intimately linked. For example, a gene may be poised for transcription with a preinitiation complex in anticipation of both developmental and environmental stimuli.

Similarly, the mechanistic regulatory building blocks surveyed here are typically embedded within a wider network context. First, many responses, especially in metazoans, involve a large number of inputs into a single promoter during both environmental and developmental responses (Amit et al., 2009; FANTOM consortium et al., 2009). In addition, transcriptional cascades are often combined with other motifs, such as negative feedbacks (Amit et al., 2007a), feedforward loops (Basu et al., 2004; Shen-Orr et al., 2002), and single-input modules (Shen-Orr et al., 2002). Such elaborate loops (Figure 2C) and cascades (Figure 2E) are essential to generate temporal order and stable cell states in developmental systems (Davidson, 2009; Hooshangi et al., 2005; Kim et al., 2008; Lee et al., 2002; Li et al., 2007; Macarthur et al., 2009; Oliveri et al., 2008; Rappaport et al., 2005). Furthermore, multiple *cis*-regulatory elements and sequences affecting nucleosome positions are integrated within more complex *cis*-regulatory functions in both yeasts (Gertz et al., 2009; Raveh-Sadka et al., 2009) and metazoans (Kaplan et al., 2009; Yuh et al., 2001; Zinzen et al., 2009).

Both computational studies and synthetic molecular circuits (Cantone et al., 2009) have provided additional insights into the crosstalk between motifs (Ishihara et al., 2005; Ma et al., 2004) and into the dynamics of complex networks (Walczak et al., 2010) that integrate multiple motifs. Nevertheless, the correspondence between simple subnetworks and motifs and the observed temporal patterns of mRNA levels (Alon, 2007; Davidson, 2009, 2010) suggests a substantial degree of modularity in the operation of regulatory systems.

Most of the mechanisms driving mRNA concentrations described in this Review, and that have been deciphered in detail so far, are transcriptional, but other pathways also affect mRNA levels, including mRNA processing, transport, and degradation. Although recent studies (Shalem et al., 2008) suggest that such mechanisms can play a substantial role in shaping temporal profiles of mRNA levels, these mechanisms are still far less understood than transcription regulation. Indeed, the scarcity of experimental methods to monitor these processes has hampered progress in this area. However, we anticipate that recent advances in massively parallel cDNA sequencing (RNA-Seq) (Mortazavi et al., 2008) will help advance this front.

More generally, deciphering circuitry and understanding the capacity of molecular mechanisms to encode complex signals and decode them into specific responses will require tight integration between experiments, analysis, and computation, in particular for temporal responses. First, there is a substantial need for direct manipulation of both signals, for example using microfluidic devices, and of individual components, by manipulation of either *trans*-components (Amit et al., 2009; Costanzo et al., 2010; FANTOM consortium et al., 2009) or *cis*-sequences

(Gertz et al., 2009; Patwardhan et al., 2009). Monitoring temporal responses in segregating populations (Eng et al., 2010) can provide a complementary means for testing the effect of many simultaneous genetic perturbations. Analytical methods and computational models can guide the design of these perturbations to a search space that is maximally informative and biologically relevant. For example, sequence models of gene regulation (Gertz et al., 2009; Raveh-Sadka et al., 2009) can help investigators make relevant promoter variants to test, whereas provisional models of *trans*-regulation (Amit et al., 2009) can help narrow down targets for gene silencing or disruption.

Improving the ability to monitor a larger number of circuit components over time in living cells is important for broadening the scope of single-cell studies and for deepening our understanding of population-level phenomena observed with genomics profiling technologies. Recent advances in simultaneously monitoring in vivo multiple types of RNA (Kern et al., 1996; Muzzey and van Oudenaarden, 2009) or proteins (Bandura et al., 2009) are promising. Notably, although the difference between a single-cell and population view is a recurring theme of recent studies, reconciling the two is important for a functional understanding of a response, especially in multicellular organisms (Simon et al., 2005). For example, a recent study of the NF-κB response to TNF-α stimulation showed that the observed cellular heterogeneity may be optimal for achieving a functional population (or mean) response for paracrine cytokine signaling (Paszek et al., 2010).

Computational analysis of time course data presents several challenging problems. These include, among others, identifying differentially expressed genes, grouping them into clusters of similar temporal patterns, and inferring their regulatory interactions. Recent studies have shown that a useful algorithmic starting point is to derive a continuous representation of transcriptional profiles by fitting to a particular mathematical function (Chechik and Koller, 2009; Storey et al., 2005). Specifically, impulse responses fit well to a certain class of sigmoid "impulse-like" functions, which have a small number of biologically interpretable parameters (e.g., onset time) (Chechik and Koller, 2009; Chechik et al., 2008). The fitted continuous representations can then be used in conjunction with the original expression values, aiming to provide a more robust analysis, particularly for differential expression (Storey et al., 2005) and clustering (Chechik and Koller, 2009; Chechik et al., 2008).

Despite these advances and the vast amount of research on the more advanced task of regulatory network inference (Bansal et al., 2007; Karlebach and Shamir, 2008), there is still much to be accomplished. The emerging complexity of regulatory mechanisms and the expected availability of more diverse and refined temporal data leave substantial room for developing more refined mechanistic models of gene regulation, which account for both *cis* and *trans* elements and their integration in time.

Finally, advances in synthetic biology promise the ability not only to manipulate biological entities but also to design systems to aid the development and interpretation of analytical models with increasing complexity. This would be particularly critical to decipher the complex web of interactions and the multiplicity of inputs that determine temporal changes in gene regulation in living cells.

REFERENCES

Alon, U. (2007). Network motifs: theory and experimental approaches. Nat. Rev. Genet. *8*, 450–461.

Amit, I., Citri, A., Shay, T., Lu, Y., Katz, M., Zhang, F., Tarcic, G., Siwak, D., Lahad, J., Jacob-Hirsch, J., et al. (2007a). A module of negative feedback regulators defines growth factor signaling. Nat. Genet. *39*, 503–512.

Amit, I., Wides, R., and Yarden, Y. (2007b). Evolvable signaling networks of receptor tyrosine kinases: relevance of robustness to malignancy and to cancer therapy. Mol. Syst. Biol. *3*, 151.

Amit, I., Garber, M., Chevrier, N., Leite, A.P., Donner, Y., Eisenhaure, T., Guttman, M., Grenier, J.K., Li, W., Zuk, O., et al. (2009). Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. Science *326*, 257–263.

Ashall, L., Horton, C.A., Nelson, D.E., Paszek, P., Harper, C.V., Sillitoe, K., Ryan, S., Spiller, D.G., Unitt, J.F., Broomhead, D.S., et al. (2009). Pulsatile stimulation determines timing and specificity of NF-kappaB-dependent transcription. Science *324*, 242–246.

Badis, G., Berger, M.F., Philippakis, A.A., Talukder, S., Gehrke, A.R., Jaeger, S.A., Chan, E.T., Metzler, G., Vedenko, A., Chen, X., et al. (2009). Diversity and complexity in DNA recognition by transcription factors. Science *324*, 1720–1723.

Bandura, D.R., Baranov, V.I., Ornatsky, O.I., Antonov, A., Kinach, R., Lou, X., Pavlov, S., Vorobiev, S., Dick, J.E., and Tanner, S.D. (2009). Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. Anal. Chem. *81*, 6813–6822.

Bansal, M., Belcastro, V., Ambesi-Impiombato, A., and di Bernardo, D. (2007). How to infer gene networks from expression profiles. Mol. Syst. Biol. *3*, 78.

Basma, H., Soto-Gutierrez, A., Yannam, G.R., Liu, L., Ito, R., Yamamoto, T., Ellis, E., Carson, S.D., Sato, S., Chen, Y., et al. (2009). Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology *136*, 990–999.

Basu, S., Mehreja, R., Thiberge, S., Chen, M.T., and Weiss, R. (2004). Spatiotemporal control of gene expression with pulse-generating networks. Proc. Natl. Acad. Sci. USA *101*, 6355–6360.

Bolouri, H., and Davidson, E.H. (2003). Transcriptional regulatory cascades in development: initial rates, not steady state, determine network kinetics. Proc. Natl. Acad. Sci. USA *100*, 9371–9376.

Braun, E., and Brenner, N. (2004). Transient responses and adaptation to steady state in a eukaryotic gene regulation system. Phys. Biol. *1*, 67–76.

Bruce, A.W., Lopez-Contreras, A.J., Flicek, P., Down, T.A., Dhami, P., Dillon, S.C., Koch, C.M., Langford, C.F., Dunham, I., Andrews, R.M., et al. (2009). Functional diversity for REST (NRSF) is defined by in vivo binding affinity hierarchies at the DNA sequence level. Genome Res. *19*, 994–1005.

Cai, L., Dalal, C.K., and Elowitz, M.B. (2008). Frequency-modulated nuclear localization bursts coordinate gene regulation. Nature *455*, 485–490.

Camas, F.M., Blazquez, J., and Poyatos, J.F. (2006). Autogenous and nonautogenous control of response in a genetic network. Proc. Natl. Acad. Sci. USA *103*, 12718–12723.

Cantone, I., Marucci, L., Iorio, F., Ricci, M.A., Belcastro, V., Bansal, M., Santini, S., di Bernardo, M., di Bernardo, D., and Cosma, M.P. (2009). A yeast synthetic network for in vivo assessment of reverse-engineering and modeling approaches. Cell *137*, 172–181.

Capaldi, A.P., Kaplan, T., Liu, Y., Habib, N., Regev, A., Friedman, N., and O'Shea, E.K. (2008). Structure and function of a transcriptional network activated by the MAPK Hog1. Nat. Genet. *40*, 1300–1306.

Chechik, G., and Koller, D. (2009). Timing of gene expression responses to environmental changes. J. Comput. Biol. *16*, 279–290.

Chechik, G., Oh, E., Rando, O., Weissman, J., Regev, A., and Koller, D. (2008). Activity motifs reveal principles of timing in transcriptional control of the yeast metabolic network. Nat. Biotechnol. *26*, 1251–1259.

Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O., and Herskowitz, I. (1998). The transcriptional program of sporulation in budding yeast. Science *282*, 699–705.

Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E.D., Sevier, C.S., Ding, H., Koh, J.L., Toufighi, K., Mostafavi, S., et al. (2010). The genetic landscape of a cell. Science *327*, 425–431.

Covert, M.W., Leung, T.H., Gaston, J.E., and Baltimore, D. (2005). Achieving stability of lipopolysaccharide-induced NF-kappaB activation. Science *309*, 1854–1857.

Davidson, E.H. (2009). Network design principles from the sea urchin embryo. Curr. Opin. Genet. Dev. *19*, 535–540.

Davidson, E.H. (2010). Emerging properties of animal gene regulatory networks. Nature *468*, 911–920.

Deal, R.B., Henikoff, J.G., and Henikoff, S. (2010). Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science *328*, 1161–1164.

Eng, K.H., Kvitek, D.J., Keles, S., and Gasch, A.P. (2010). Transient genotype-by-environment interactions following environmental shock provide a source of expression variation for essential genes. Genetics *184*, 587–593.

FANTOM consortium, Suzuki, H., Forrest, A.R., van Nimwegen, E., Daub, C.O., Balwierz, P.J., Irvine, K.M., Lassmann, T., Ravasi, T., Hasegawa, Y., et al. (2009). The transcriptional network that controls growth arrest and differentiation in a human myeloid leukemia cell line. Nat. Genet. *41*, 553–562.

Foster, S.L., Hargreaves, D.C., and Medzhitov, R. (2007). Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature *447*, 972–978.

Friedman, N., Vardi, S., Ronen, M., Alon, U., and Stavans, J. (2005). Precise temporal modulation in the response of the SOS DNA repair network in individual bacteria. PLoS Biol. *3*, e238.

Friedrichsen, S., Harper, C.V., Semprini, S., Wilding, M., Adamson, A.D., Spiller, D.G., Nelson, G., Mullins, J.J., White, M.R., and Davis, J.R. (2006). Tumor necrosis factor-alpha activates the human prolactin gene promoter via nuclear factor-kappaB signaling. Endocrinology *147*, 773–781.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell *11*, 4241–4257.

Gertz, J., Siggia, E.D., and Cohen, B.A. (2009). Analysis of combinatorial cis-regulation in synthetic and genomic promoters. Nature *457*, 215–218.

Geva-Zatorsky, N., Rosenfeld, N., Itzkovitz, S., Milo, R., Sigal, A., Dekel, E., Yarnitzky, T., Liron, Y., Polak, P., Lahav, G., et al. (2006). Oscillations and variability in the p53 system. Mol. Syst. Biol. *2*, 0033.

Gilchrist, M., Thorsson, V., Li, B., Rust, A.G., Korb, M., Roach, J.C., Kennedy, K., Hai, T., Bolouri, H., and Aderem, A. (2006). Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4. Nature *441*, 173–178.

Goentoro, L., Shoval, O., Kirschner, M.W., and Alon, U. (2009). The incoherent feedforward loop can provide fold-change detection in gene regulation. Mol. Cell *36*, 894–899.

Hager, G.L., McNally, J.G., and Misteli, T. (2009). Transcription dynamics. Mol. Cell *35*, 741–753.

Harbison, C.T., Gordon, D.B., Lee, T.I., Rinaldi, N.J., Macisaac, K.D., Danford, T.W., Hannett, N.M., Tagne, J.B., Reynolds, D.B., Yoo, J., et al. (2004). Transcriptional regulatory code of a eukaryotic genome. Nature *431*, 99–104.

Hooshangi, S., Thiberge, S., and Weiss, R. (2005). Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. Proc. Natl. Acad. Sci. USA *102*, 3581–3586.

Hu, Z., Killion, P.J., and Iyer, V.R. (2007). Genetic reconstruction of a functional transcriptional regulatory network. Nat. Genet. *39*, 683–687.

Ihmels, J., Levy, R., and Barkai, N. (2004). Principles of transcriptional control in the metabolic network of Saccharomyces cerevisiae. Nat. Biotechnol. *22*, 86–92.

Iliopoulos, D., Hirsch, H.A., and Struhl, K. (2009). An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell *139*, 693–706.

Ishihara, S., Fujimoto, K., and Shibata, T. (2005). Cross talking of network motifs in gene regulation that generates temporal pulses and spatial stripes. Genes Cells *10*, 1025–1038.

Kalir, S., Mangan, S., and Alon, U. (2005). A coherent feed-forward loop with a SUM input function prolongs flagella expression in Escherichia coli. Mol. Syst. Biol. *1*, 0006.

Kaplan, N., Moore, I.K., Fondufe-Mittendorf, Y., Gossett, A.J., Tillo, D., Field, Y., LeProust, E.M., Hughes, T.R., Lieb, J.D., Widom, J., et al. (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. Nature *458*, 362–366.

Kaplan, S., Bren, A., Dekel, E., and Alon, U. (2008). The incoherent feed-forward loop can generate non-monotonic input functions for genes. Mol. Syst. Biol. *4*, 203.

Karlebach, G., and Shamir, R. (2008). Modelling and analysis of gene regulatory networks. Nat. Rev. Mol. Cell Biol. *9*, 770–780.

Kern, D., Collins, M., Fultz, T., Detmer, J., Hamren, S., Peterkin, J.J., Sheridan, P., Urdea, M., White, R., Yeghiazarian, T., et al. (1996). An enhanced-sensitivity branched-DNA assay for quantification of human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. *34*, 3196–3202.

Kim, H.D., Shay, T., O'Shea, E.K., and Regev, A. (2009). Transcriptional regulatory circuits: predicting numbers from alphabets. Science *325*, 429–432.

Kim, J., Chu, J., Shen, X., Wang, J., and Orkin, S.H. (2008). An extended transcriptional network for pluripotency of embryonic stem cells. Cell *132*, 1049–1061.

Kittisopikul, M., and Suel, G.M. (2010). Biological role of noise encoded in a genetic network motif. Proc. Natl. Acad. Sci. USA *107*, 13300–13305.

Kultz, D. (2005). Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. *67*, 225–257.

Lachmann, A., Xu, H., Krishnan, J., Berger, S.I., Mazloom, A.R., and Ma'ayan, A. (2010). ChEA: transcription factor regulation inferred from integrating genome-wide ChIP-X experiments. Bioinformatics *26*, 2438–2444.

Lahav, G., Rosenfeld, N., Sigal, A., Geva-Zatorsky, N., Levine, A.J., Elowitz, M.B., and Alon, U. (2004). Dynamics of the p53-Mdm2 feedback loop in individual cells. Nat. Genet. *36*, 147–150.

Lam, F.H., Steger, D.J., and O'Shea, E.K. (2008). Chromatin decouples promoter threshold from dynamic range. Nature *453*, 246–250.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison, C.T., Thompson, C.M., Simon, I., et al. (2002). Transcriptional regulatory networks in Saccharomyces cerevisiae. Science *298*, 799–804.

Li, J., Liu, Z.J., Pan, Y.C., Liu, Q., Fu, X., Cooper, N.G., Li, Y., Qiu, M., and Shi, T. (2007). Regulatory module network of basic/helix-loop-helix transcription factors in mouse brain. Genome Biol. *8*, R244.

Litvak, V., Ramsey, S.A., Rust, A.G., Zak, D.E., Kennedy, K.A., Lampano, A.E., Nykter, M., Shmulevich, I., and Aderem, A. (2009). Function of C/EBPdelta in a regulatory circuit that discriminates between transient and persistent TLR4-induced signals. Nat. Immunol. *10*, 437–443.

Locke, J.C., and Elowitz, M.B. (2009). Using movies to analyse gene circuit dynamics in single cells. Nat. Rev. Microbiol. *7*, 383–392.

Loewer, A., Batchelor, E., Gaglia, G., and Lahav, G. (2010). Basal dynamics of p53 reveal transcriptionally attenuated pulses in cycling cells. Cell *142*, 89–100.

Lopez-Maury, L., Marguerat, S., and Bahler, J. (2008). Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat. Rev. Genet. *9*, 583–593.

Ma, H.W., Kumar, B., Ditges, U., Gunzer, F., Buer, J., and Zeng, A.P. (2004). An extended transcriptional regulatory network of Escherichia coli and analysis of its hierarchical structure and network motifs. Nucleic Acids Res. *32*, 6643–6649.

Macarthur, B.D., Ma'ayan, A., and Lemischka, I.R. (2009). Systems biology of stem cell fate and cellular reprogramming. Nat. Rev. Mol. Cell Biol. *10*, 672–681.

Macia, J., Widder, S., and Sole, R. (2009). Specialized or flexible feed-forward loop motifs: a question of topology. BMC Syst. Biol. *3*, 84.

Mangan, S., Zaslaver, A., and Alon, U. (2003). The coherent feedforward loop serves as a sign-sensitive delay element in transcription networks. J. Mol. Biol. *334*, 197–204.

Mangan, S., Itzkovitz, S., Zaslaver, A., and Alon, U. (2006). The incoherent feed-forward loop accelerates the response-time of the gal system of Escherichia coli. J. Mol. Biol. *356*, 1073–1081.

Mitchell, A., Romano, G.H., Groisman, B., Yona, A., Dekel, E., Kupiec, M., Dahan, O., and Pilpel, Y. (2009). Adaptive prediction of environmental changes by microorganisms. Nature *460*, 220–224.

Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L., and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods *5*, 621–628.

Murphy, L.O., Smith, S., Chen, R.H., Fingar, D.C., and Blenis, J. (2002). Molecular interpretation of ERK signal duration by immediate early gene products. Nat. Cell Biol. *4*, 556–564.

Murray, J.I., Whitfield, M.L., Trinklein, N.D., Myers, R.M., Brown, P.O., and Botstein, D. (2004). Diverse and specific gene expression responses to stresses in cultured human cells. Mol. Biol. Cell *15*, 2361–2374.

Muzzey, D., and van Oudenaarden, A. (2009). Quantitative time-lapse fluorescence microscopy in single cells. Annu. Rev. Cell Dev. Biol. *25*, 301–327.

Nachman, I., Regev, A., and Ramanathan, S. (2007). Dissecting timing variability in yeast meiosis. Cell *131*, 544–556.

Nelson, D.E., Ihekwaba, A.E., Elliott, M., Johnson, J.R., Gibney, C.A., Foreman, B.E., Nelson, G., See, V., Horton, C.A., Spiller, D.G., et al. (2004). Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science *306*, 704–708.

Oliveri, P., Tu, Q., and Davidson, E.H. (2008). Global regulatory logic for specification of an embryonic cell lineage. Proc. Natl. Acad. Sci. USA *105*, 5955–5962.

Paszek, P., Ryan, S., Ashall, L., Sillitoe, K., Harper, C.V., Spiller, D.G., Rand, D.A., and White, M.R. (2010). Population robustness arising from cellular heterogeneity. Proc. Natl. Acad. Sci. USA *107*, 11644–11649.

Patwardhan, R.P., Lee, C., Litvin, O., Young, D.L., Pe'er, D., and Shendure, J. (2009). High-resolution analysis of DNA regulatory elements by synthetic saturation mutagenesis. Nat. Biotechnol. *27*, 1173–1175.

Ramsey, S.A., Klemm, S.L., Zak, D.E., Kennedy, K.A., Thorsson, V., Li, B., Gilchrist, M., Gold, E.S., Johnson, C.D., Litvak, V., et al. (2008). Uncovering a macrophage transcriptional program by integrating evidence from motif scanning and expression dynamics. PLoS Comput. Biol. *4*, e1000021.

Rappaport, N., Winter, S., and Barkai, N. (2005). The ups and downs of biological timers. Theor. Biol. Med. Model. *2*, 22.

Raveh-Sadka, T., Levo, M., and Segal, E. (2009). Incorporating nucleosomes into thermodynamic models of transcription regulation. Genome Res. *19*, 1480–1496.

Rosenfeld, N., Elowitz, M.B., and Alon, U. (2002). Negative autoregulation speeds the response times of transcription networks. J. Mol. Biol. *323*, 785–793.

Segal, E., Shapira, M., Regev, A., Pe'er, D., Botstein, D., Koller, D., and Friedman, N. (2003). Module networks: identifying regulatory modules and their condition-specific regulators from gene expression data. Nat. Genet. *34*, 166–176.

Shalem, O., Dahan, O., Levo, M., Martinez, M.R., Furman, I., Segal, E., and Pilpel, Y. (2008). Transient transcriptional responses to stress are generated by opposing effects of mRNA production and degradation. Mol. Syst. Biol. *4*, 223.

Shapira, S.D., Gat-Viks, I., Shum, B.O., Dricot, A., de Grace, M.M., Wu, L., Gupta, P.B., Hao, T., Silver, S.J., Root, D.E., et al. (2009). A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. Cell *139*, 1255–1267.

Shen-Orr, S.S., Milo, R., Mangan, S., and Alon, U. (2002). Network motifs in the transcriptional regulation network of Escherichia coli. Nat. Genet. *31*, 64–68.

Shivaswamy, S., Bhinge, A., Zhao, Y., Jones, S., Hirst, M., and Iyer, V.R. (2008). Dynamic remodeling of individual nucleosomes across a eukaryotic genome in response to transcriptional perturbation. PLoS Biol. *6*, e65.

Simon, I., Siegfried, Z., Ernst, J., and Bar-Joseph, Z. (2005). Combined static and dynamic analysis for determining the quality of time-series expression profiles. Nat. Biotechnol. *23*, 1503–1508.

Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D., and Futcher, B. (1998). Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. Mol. Biol. Cell *9*, 3273–3297.

Springer, M., Wykoff, D.D., Miller, N., and O'Shea, E.K. (2003). Partially phosphorylated Pho4 activates transcription of a subset of phosphate-responsive genes. PLoS Biol. *1*, E28.

Storey, J.D., Xiao, W., Leek, J.T., Tompkins, R.G., and Davis, R.W. (2005). Significance analysis of time course microarray experiments. Proc. Natl. Acad. Sci. USA *102*, 12837–12842.

Szita, N., Polizzi, K., Jaccard, N., and Baganz, F. (2010). Microfluidic approaches for systems and synthetic biology. Curr. Opin. Biotechnol. *21*, 517–523.

Tagkopoulos, I., Liu, Y.C., and Tavazoie, S. (2008). Predictive behavior within microbial genetic networks. Science *320*, 1313–1317.

Tanay, A. (2006). Extensive low-affinity transcriptional interactions in the yeast genome. Genome Res. *16*, 962–972.

Tay, S., Hughey, J.J., Lee, T.K., Lipniacki, T., Quake, S.R., and Covert, M.W. (2010). Single-cell NF-kappaB dynamics reveal digital activation and analogue information processing. Nature *466*, 267–271.

Tyson, J.J., Chen, K.C., and Novak, B. (2003). Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. Curr. Opin. Cell Biol. *15*, 221–231.

Walczak, A.M., Tkacik, G., and Bialek, W. (2010). Optimizing information flow in small genetic networks. II. Feed-forward interactions. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. *81*, 041905.

Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., Cui, K., Kanno, Y., Roh, T.Y., Watford, W.T., et al. (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating $CD4^+$ T cells. Immunity *30*, 155–167.

Whitehouse, I., Rando, O.J., Delrow, J., and Tsukiyama, T. (2007). Chromatin remodelling at promoters suppresses antisense transcription. Nature *450*, 1031–1035.

Wilkinson, D.J. (2009). Stochastic modelling for quantitative description of heterogeneous biological systems. Nat. Rev. Genet. *10*, 122–133.

Yuh, C.H., Bolouri, H., and Davidson, E.H. (2001). Cis-regulatory logic in the endo16 gene: switching from a specification to a differentiation mode of control. Development *128*, 617–629.

Zaslaver, A., Mayo, A.E., Rosenberg, R., Bashkin, P., Sberro, H., Tsalyuk, M., Surette, M.G., and Alon, U. (2004). Just-in-time transcription program in metabolic pathways. Nat. Genet. *36*, 486–491.

Zinzen, R.P., Girardot, C., Gagneur, J., Braun, M., and Furlong, E.E. (2009). Combinatorial binding predicts spatio-temporal cis-regulatory activity. Nature *462*, 65–70.