Published in final edited form as:
Biochim Biophys Acta. 2012 July ; 1819(7): 657–666. doi:10.1016/j.bbagrm.2012.03.004.

Complex Dynamics of Transcription Regulation

Diana A Stavreva[1,*], **Lyuba Varticovski**[1], and **Gordon L. Hager**[1]
[1]Laboratory of Receptor Biology and Gene Expression, Building 41, B507, 41 Library Dr. National Cancer Institute, NIH, Bethesda, MD 20892

Abstract

Transcription is a tightly regulated cellular function which can be triggered by endogenous (intrinsic) or exogenous (extrinsic) signals. The development of novel techniques to examine the dynamic behavior of transcription factors and the analysis of transcriptional activity at the single cell level with increased temporal resolution has revealed unexpected elements of stochasticity and dynamics of this process. Emerging research reveals a complex picture, wherein a wide range of time scales and temporal transcription patterns overlap to generate transcriptional programs. The challenge now is to develop a perspective that can guide us to common underlying mechanisms, and consolidate these findings. Here we review the recent literature on temporal dynamic and stochastic gene regulation patterns governed by intrinsic or extrinsic signals, utilizing the glucocorticoid receptor (GR)-mediated transcriptional model to illustrate commonality of these emerging concepts.

1. Introduction

Transcription studies over the years have produced a wide variety of data arguing for an assortment of temporal patterns, timescales and regulatory mechanisms for this pivotal cellular process. The existence of some of these patterns became apparent only after careful analysis of the transcription process in living cells by various microscopy methods, such as time-lapse microscopy, fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer (FRET), FCS (fluorescence correlation spectroscopy), and single-molecule fluorescence tracking (SMT). Using RNA fluorescent in situ hybridization (FISH) and live cell imaging of MS2- of PP7-tagged RNAs it was discovered at single cell level that some systems manifest a discrete, burst-like transcriptional activity during a restricted time period, which is frequently detected in a subpopulation of the examined cells [18,42,135,203]. However, transcription bursting is not a general phenomenon and stochastic, uncorrelated transcription events are observed on other promoters [90]. In some cases transcription responses become synchronous and are readily observed at the level of the entire cell population [104,156]. Underlying all these phenomena is the intrinsic dynamics of the transcription-associated regulatory proteins at DNA regulatory elements. We will review some of the examples that define the mechanisms governing the stochasticity and dynamics of transcriptional regulation.

[*]Corresponding Author: 301-402-4801, 301-496-4951 (Fax), stavrevd@mail.nih.gov.

2. Dynamics of transcription factors interaction with chromatin

In contrast to the earlier in vitro studies postulating that the interactions of transcription factors (TFs) with regulatory elements is stable and persist on a time scale of hours [128], recent live cell experiments have demonstrated that this process is inherently dynamic and that TFs are bound to chromatin only transiently with an average time of interaction measured in seconds [49]. Transient binding was first reported for the activated glucocorticoid receptor (GR), using FRAP analysis and a tandem array of GR-regulated promoters [103] (Fig. 1A).

TFs utilize diffusion to rapidly move throughout the cellular environment in an energy-independent manner, and the diffusion kinetics of a TF is believed to reflect its binding properties *in vivo* [113,164]. Diffusion within the nucleus also allows TFs a quick and efficient scan of the entire genome which could be completed in minutes. Calculation of the exact residence time for TFs *in vivo* has been challenging and is an area of active research [34,57,113,129,163,164]. The proper quantitative modeling and the extraction of the "on" and "off" rates for a TF are also evolving. Different approaches such as FRAP [112], fluorescence correlation spectroscopy (FCS) and temporal image correlation spectroscopy (TICS) [101] have been applied with somewhat different outcomes.

Many studies of transcription binding dynamics at promoter elements have utilized artificial promoter arrays [14,48,103,137,158] which has raised a concern about the physiological relevance of these studies. Therefore, it is important that such interactions were also reported at endogenous array structures at the ribosomal DNA genes in mammalian cells [29] and at the naturally occurring CUP1 gene array in the yeast [75].

Interactions of TFs with chromatin are associated with local changes in chromatin accessibility as assessed by sensitivity to DNase I or micrococcal nuclease [15,55,68,69,195,196]. Details about how DNase I differ from micrococcal nuclease in analysis of chromatin accessibility can be found elsewhere [185]. Some of the local chromatin reorganizations are transient ([139] and our unpublished data) contributing to the overall dynamics of the process. Interestingly, most of the DNase I hypersensitive sites (DHS) (many corresponding to regulatory elements) are located in distal sites, at a significant distance from the promoters[68]. These data suggest the existence of functional long-range interactions between distal regulatory sites and promoters [50,51], some of which could belong to a relatively stable part of the nuclear architecture, while others could be dynamic as suggested by earlier studies [126].

In addition to the TFs, other transcription-associated proteins, such as the GR interacting coactivator GRIP1[7]and chromatin remodeler BRG1 [71] also rapidly exchange with chromatin. Moreover, it has been recently discovered that the binding of one TF to its response element does not reduce the steady-state binding of another receptor that has affinity to the same site. In the case of this "assisted loading," prior interactions of a TF with DNA are required for the recruitment of another factor. Mathematical simulations assuming short residency times for the TFs and relatively long times between the chromatin binding events fatefully reproduce this noncompetitive state [190]. The emerging picture suggests that the interaction of TFs and other proteins with chromatin are highly dynamic. However, some TFs, including the HSF in Drosophila polytene chromosomes [198], Gal4 in yeast[119] and HIF-1 in human cells [200], appear to have a longer residence time on their target sites. These findings suggest that some promoters could be regulated in cell-type and/or tissue-specific manner by exploiting different residence times of their regulatory proteins for fine-tuning of the desired transcriptional response.

Transient TF interactions with the regulatory elements pose the question whether other components of the transcriptional machinery are also dynamic. FRAP experiments combined with mathematical modeling have studied Pol I [29] and Pol II [13,79,198] dynamics and elongation rates. By directly analyzing the *in vivo* dynamics of Pol II and its mRNA product as discernible by MS-2 tagging, it was determined that the rate of elongation for this system is typically 4.3 kb/min, and "pausing" decreases this process to 0.4 kb/min [22]. Thus, similarly to the highly dynamic interactions observed for TFs, the assembly of polymerase complexes also appears to be stochastic and dynamic. For more details on Pol II function and pausing see the reviews by DA Gilchrist et al., and D Larson et al. in this Special Issue.

The dynamic exchange of transcriptional machinery with the regulatory elements is also tightly regulated. It has been suggested that promoter complexes turnover due to their active ATP-dependent disassembly by various cellular activities like the remodeling complexes [37,74,75,118], chaperones [32,38] as well as proteolytic mechanisms [14,35,166]. This, in turn, suggests that fine-tuning and the dynamics of TFs and accessory proteins may act as a physiologically relevant mechanism in regulating gene expression *in vivo* [44,165,166].

3. Stochastic features of transcriptional regulation at a single cell level

Single cell studies have demonstrated that transcription frequently exhibits burst-like behavior where RNA syntheses consist of rounds of transcription with multiple molecules produced per round [18,41,134,203]. Transcription bursting has been observed for different genes in several systems, but does not seem to be a general phenomenon. For example, instead of a burst of transcription, a recent study on transcription initiation and elongation of endogenous yeast genes by single RNA microscopy demonstrated that there is no correlation between individual initiation events for these gene and that initiation events are stochastic and uncorrelated [90]. Another major finding from the single cell studies is the discovery of the inherent randomness of the single cell transcription manifested through considerable cell-to-cell variability of gene expression and protein levels [12,89,122,136,152,159,189,201].

Stochasticity is frequently attributed and could be traced to intrinsic and extrinsic sources [30,33,175]. Importantly, increasing the level of an activating signal does not correlate with an increase in transcription within the individual cell, but rather results in an increased fraction of responsive cells [12,18,42,76,81,127,134,146,153,162,170,194].

Single-cell data has been frequently analyzed in terms of the so called two-state (on/off) model. This is the most widely accepted model that provides important clues on promoter dynamics (reviewed in [88]). More recently, models focused on general stochasticity [127,149–151] or on single-gene stochastic expression as a result of the dynamic interplay between regulatory molecules and epigenetic factors [21], as well as models integrating the 'two-state model" with the idea of "promoter progression", [88] have also emerged.

4. Cyclic transcriptional regulations

Cyclic gene transcription activity has been observed in several systems and can be divided into two, sometimes overlapping, categories – intrinsic and extrinsic oscillations. Intrinsic oscillations are attributed to the cyclic nature of the transcription process itself. Some systems, like the circadian system, exhibit diurnal changes in clock gene transcription levels even in the absence of exogenous signals. In other cases, like NF-kB signaling, gene transcription is characterized by intrinsic oscillatory, wave–like activity that occurs in a much shorter timescale and is persistent upon continuous stimulation which will be discussed later. In contrast, periodic signaling events coming from the (cellular)

environment are characteristic for the extrinsically regulated transcriptional oscillations. Therefore, upstream regulatory processes also contribute to the transcription dynamics and regulation. For example, exogenous pulsatile hormone signals regulate glucocorticoid receptor (GR) activity, resulting in gene pulsing, an extrinsic oscillatory transcription behavior. By comparing transcriptional behavior of many genes over time in NIH3T3 and U2OS cells and the mouse liver, Hughes and colleagues [64] discovered sub-circadian transcriptional oscillations in the liver, which are largely lost in cell lines. Liver is an organ that receives and integrates many systemic cues and it is tempting to speculate that this loss of sub-circadian harmonics in cell lines is due to the lack of certain periodic systemic cues. Recent studies have also demonstrated that both, intrinsic and extrinsic signals contribute to the variability of gene expression [188]. Separating the two is not trivial and mathematical methods have been applied to achieve this [56,175]. We will examine some of the systems exhibiting intrinsic and extrinsic transcriptional behavior in more detail.

4.1. Intrinsic oscillations

Yeast “metabolic cycle” is an example of an intrinsic system which does not depend on cell synchronization or external signals [160,192]. It is documented for yeast cultures growing under continuous conditions at high cellular density, and is characterized by respiratory bursts, alternating with a non-respiratory reductive phase, accompanied by cyclic expression of specific genes [80,183]. Circadian clock is another autonomous system which can persist without any exogenous stimuli, however, in reality it is a subject of daily entrainment by exogenous cues like light and feeding [5,46,111,116,142,147,169]. In some isolated cases, like the *in vitro* reconstituted cyanobacterial clock [70] and the circadian rhythms of peroxiredoxins in the red blood cells [123,124] transcriptional activity is not required. However, in most cases circadian oscillations are based on a core feedback loop comprising complex positive and negative transcriptional/posttranslational interactions between the clock genes Clock, Bmal1, Per1, Per2, Cry1 and Cry2 and their products [125]. In addition, intercellular coupling in the suprachiasmatic nucleus (SCN) network generates circadian oscillations from a population of individually arrhythmic cells and confers synchrony and robustness to the master circadian pacemaker [109]. Recently more complex system-level approaches and mathematical modeling have been applied to unravel the complex biology of circadian clocks (reviewed in [61].

As described above, yeast metabolic cycle and circadian clock are endogenously driven oscillatory events that can proceed without any external stimuli. However, other systems exhibit distinct oscillatory features only upon exposure to an activating signal. In the latter case, steady state stimulation is transformed into a wave-like response which may be a better carrier for complex information with potential physiological significance. A good example, from yeast, is the strong 45-minutes transcription cycle of the CUP1 gene that is induced by copper [75].

Another system operating on a short (ultradian) timescale is the “segmentation clock” in mammals, in which the oscillations of RNA and protein levels of the helix-loop-helix gene Hes7 and the related gene Hes1 are critical for the somite segmentation of the presomitic mesoderm (PSM) and proper development of the embryo [2,25,59,73,131]. Many other factors have been demonstrated to also display oscillatory expression in PSM cells [25]. The importance of the oscillatory pattern of Hes7 expression is illustrated by the fact that both sustained expression of Hes7 and its suppression result in somite fusion. Hes7 oscillations are due to a short half-life of the Hes7 protein that represses its own transcription, and is degraded within one hour by the ubiquitin–proteasome pathway. In the absence of a functional Hes7 protein, the Hes7 gene is continuously transcribed in the PSM cells [10]. Thus, the negative regulation of Hes7 expression by Hes7 protein forms a negative feedback loop that is critical for maintaining oscillations with a period of about 2 h [58].

Mathematical simulations predict that Hes7 oscillations continue if the half-life of Hes7 protein is 20 min, but not 30 min. In the latter case, Hes7 oscillations are damped after three or four cycles [58]. Recent studies have also demonstrated the significance of intronic delays in dynamic Hes7 gene expression [176]. In addition, Fgf and Notch signaling cooperatively regulate Hes7 oscillations, and conversely, Hes7 oscillations couple and synchronize the oscillations of Fgf and Notch signaling. Furthermore, oscillations in the PSM become unstable when PSM cells are dissociated. These results indicate that individual PSM cells cannot maintain a stable oscillator without cell–cell communication [99,100]. Synchronization of these oscillations in PSM cells require Notch signaling which could provide the necessary long-distance coordination [62,67,141].

This system is not only important during development, but seems to also play a role in the tissues of a developed organism. It was demonstrated that serum stimulation of cultured mouse fibroblasts induces ultradian oscillations of the Hes1 gene expression as well as oscillations of Stat and Smad. Consequently, the loss of Stat oscillations leads to inhibition of Hes1 oscillations, which impairs cell cycle progression through G1 phase, suggesting that Hes1 oscillations with a defined ultradian frequency is required for efficient cell proliferation [199]. In spite of extensive studies of somite formation in the embryo since its discovery over ten years ago, the mechanisms responsible for the production of oscillatory gene activity are still not fully understood. Other oscillatory signaling activities during embryogenesis involved in development of the limbs, neurite progenitor maintenance, and others, may serve a more general function than previously thought [3]. Lack of synchrony in cellular oscillations can also result in divergence of stem cell fate and the degree of synchrony in rhythmic behaviors is intimately tied to a key question in developmental biology: how tissues become organized in time and space and how diverse cell types coordinate with each other.

Oscillations were also discovered for the cell's principle guardian of the genome, the tumor suppressor protein p53. The p53 and its master regulator Mdm2 are among the most intensely researched proteins due to their key role in the DNA damage-induced cell death and apoptosis [107,110,130,148,187]. However, p53 oscillations were only discovered after the introduction of single cell time-laps microscopy methods with fluorescently tagged p53 and Mdm2 proteins. Different numbers of p53 pulses were detected in genetically identical cells upon DNA damage, and a negative Mdm2 feedback loop was inferred. A model in which p53 molecules are released in quanta until DNA damage is resolved or apoptosis induced, was also proposed [40,85,86]. However, more recent studies demonstrated that the nature of p53 pulses is more complex than previously anticipated. It was shown that they are at least partially driven by cyclic activation of the upstream signaling kinases, ATM and Chk2. A complex feedback through ATM mediated by Wip1 is essential for the maintenance of p53 pulsatility [6].

NF-kB signaling system provides another example of oscillatory behavior upon stimulation [184]. NF-kB normally resides in the cytoplasm in a complex with IkB inhibitors. In the presence of inducing signal, such as tumor necrosis factor-alpha (TNF-alpha), the inhibitory IkB protein is phosphorylated and degraded by the proteasome. Released NF-kB than translocates to the nucleus and induces transcription from several downstream genes, including IkB, leading to its accumulation in the cytoplasm. If the activating signal is still present, IkB is degraded and NF-kB translocates again to the nucleus, as observed in living cells [14,120,173,174]. These oscillations are lost at the population level [60,77], where the cell desynchronisation is masking the oscillatory behavior of the individual cells. However, when the activating (THF-alpha) signal is applied in a pulsatile manner, a distinct oscillatory pattern and transcriptional activation from downstream genes is observed [1].

An interesting system in yeast is reported by Elowitz and colleagues [16], where the increasing concentrations of activating signal (Ca^{2+}) leads to an increase in the frequency of nuclear translocation of the transcription factor Crz1, and subsequent bursts of transcriptional activity. Notably, increased Ca^{2+} concentration did not affect the amplitude or duration of Crz1 nuclear translocation events. Frequency modulation of the activating signal, rather than its amplitude, seems to be favored in several cell signaling systems [16,20]. For example, varying the frequency (timing) of the consecutive NF-kB activation events is associated with cyclic differential regulation of downstream genes in mammalian cells [1]. TNF-induced signaling is a highly dynamic process resulting in rapid, coordinated, and repetitive changes in the RNA levels of hundreds of genes [171,172]. On the population level, the noise of each subsequent cycle increases as a result of the incomplete resetting of the components of the signal transduction machinery. Better synchronization and more uniform signaling of the transcription output is achieved when the necessary resetting period is taken into account during subsequent activation of the NF-kB signaling [1].

In some cases, multiple signaling components downstream of the TNF receptor, like three MAP kinases as well as p65, undergo oscillations [65]. In other cases, independent oscillatory pathways require simultaneous synchronous induction to produce biological responses [172].

The complexity of the intrinsic oscillatory systems is believed to be regulated by negative feedback loops with time delay [121,181]. These mechanisms have been proposed for a variety of cellular oscillatory systems, such as NF-kB [1,120], p53 [86], Erk2 [157] and the circadian clock [97]. Other studies suggest that transcription cycles may emerge from the fundamental kinetics of transcription initiation, elongation and termination [52,92,133]. In other words, intrinsic periodicity of the process itself is the basis of the cyclic nature of gene expression.

One of the best understood systems demonstrating intrinsic oscillatory properties is the estrogen-responsive pS2 promoter [104,138,156]. Addition of estrogen results in sequential cyclic interaction with the promoter not only of the estrogen receptor, but also of associated co-activators, co-repressors, chromatin remodelers, histone acetyl-transferases, histone decactylases, methyltransferases, proteasome complex, chaperones, and other transcription-related factors. The 45-minute cycles are revealed only after cell synchronization by prior hormone deprivation and use of inhibitors, like alpha-amanitin [104,105]. It is still to be determined how the slow (45 min) cycle, described for the pS2 promoter, is related to the fast exchange rate of the ER measured *in vivo* in mammalian cells [158,168]. Previous work in yeast cells described the existence of a “slow” and “fast” cycle for the binding of Ace1protein to the naturally occurring CUP1 array, and proposed that the fast cycling initiates transcription and the slow cycling regulates the quantity of mRNA synthesis [75]. However, it is still unclear how these cycles are temporally and functionally related in the ER signaling.

4.2. Oscillations governed by extrinsic signals

Several studies have addressed the responses of gene-regulatory networks and other cellular functions to extrinsic perturbations that mimic natural cellular habitats, and suggested that cellular systems have been optimized for robust and timely responses to changes in the environment [9,82,84,96,144,179]. It has been suggested that stochasticity or the “noise” in gene expression in response to the same environmental stimuli could provide cell populations with the flexibility needed to adapt to fluctuations in the environment [72,85]. On the other hand, it was proposed that “noise”-reduction may have benefited the evolution of eukaryotes and vertebrates [11]. Moreover, ageing and disease are frequently characterized by a loss of adaptive abilities and increased deregulation and stochasticity of

gene expression [45]. Examining the genes in the yeast galactose network, Singer and colleagues [39] demonstrated that transcriptional outputs of genes temporally induced by galactose are highly coordinated. In contrast, transcription of constitutive genes is not coordinated, presumably due to stochastic fluctuations. Therefore, dynamic balance between stochasticity and noise-reduction may be important for proper gene regulation especially in multicellular organisms [83].

Glucocorticoid receptor-mediated transcription is an example of an extrinsically regulated system integrating diverse range of timescales – from seconds to hours. As already mentioned, GR exchanges rapidly (in seconds) with its regulatory elements in living cells [103]. These seminal experiments were performed in a mouse cell line, 3617, containing an array of the GR-regulated mouse mammary tumor virus (MMTV) promoter followed by a reporter (ras-bpv) as well as a green fluorescent protein (GFP)-tagged GR under tetracycline regulation [191]. High number of GR binding elements at the MMTV array results in a local increase of the GFP-GR intensity above nucleoplasmic background allowing unambiguous array detection and a possibility to study the strength of GR interactions with its regulatory sites *in vivo* by photobleaching techniques such as FRAP (Fig. 1). Despite the fact that the GFP-GR-bound array appears as a stable and well defined bright structure (Fig. 1A), the resulting FRAP curve points to a fast rate of GR exchange with these sites which is counted in seconds (Fig. 1C). Fast FRAP recovery indicates that the photobleached GFP-GR molecules are replaced with fresh receptors in less than a minute. We refer to this fast GR exchange with regulatory elements as “chromatin cycle”. These observations pose a question on the function and mechanisms of the chromatin cycle. It has become increasingly clear that GR exchange at the array is slower than its exchange measured at any other place in the nucleus and this is attributed to the higher strength of GR binding at specific sites (Fig. 1D). Hormone withdrawal also results in a faster GR recovery curve (Fig. 1B, C) due to the loss of receptor affinity to the GR response elements (GREs) upon hormone dissociation (Fig. 1E). These changes in GR behavior in response to hormone availability are crucial for its role as a “sensor” for the hormone level fluctuations. It implies a low affinity of the natural hormone to its receptor and a short half-life of the GR-hormone complex, which has been supported by *in vitro* and *in vivo* studies [114,167]. However, when dexamethasone, a high affinity synthetic ligand which forms a relatively stable GR-hormone complex is used, receptor sensitivity to the hormone fluctuations is reduced [167] (Stavreva et al 2009). In addition to the chromatin cycle, GR interacts with nuclear chaperones in an ATP-dependent manner [63] and is involved in a “chaperone cycle”. This is driven by a mandatory association of the receptor with the nuclear chaperone machinery to restore its hormone binding affinity upon hormone dissociation [23,24,98,197] (Fig. 2A). The chaperone cycle recycles GR by reinstating its ability to bind hormone, when available, and together with the proteasome machinery serves as a quality control system removing damaged GR molecules by degradation (Fig. 2B).

Similarly to other proteins, GR movement throughout the volume of the nucleus is undirected, largely based on diffusion (Brownian motion), and does not require energy [113,164]. However, GR release from its GREs is an energy-dependent process and several cellular ATP-dependent complexes such as chromatin remodelers, chaperones, and proteolytic systems were found to be involved [32,37,38,118,166]. Multiple lines of evidence suggest that GR exchange rate may be an additional regulatory mechanism in gene expression and slower GR exchange at the MMTV array correlates with more mRNA syntheses [166]. A similar correlation has also been observed for RNA pol I - mediated transcription [44], suggesting this as a possible general regulatory mechanism.

A complex picture has emerged where the highly regulated fast GR chromatin cycle correlates with the level of transcriptional activity of the downstream responsive genes. In

this process, the slower (in order of minutes) chaperone cycle assists the GR chromatin cycle by providing transcriptionally competent GR molecules in the presence of the hormone. In addition, nuclear chaperones bind and sequester unliganded GR molecules to protect them from proteasome-mediated degradation in the absence of the hormone (Fig. 2B). The result is a tight coupling of transcriptional activity of the GR-regulated genes with the hormone availability, designated as gene pulsing [94,167]. The rationale for both chromatin and chaperone cycle is not apparent at the level of an individual cell. In other words, there is no apparent reason for an individual mammalian cell to develop these elaborate molecular mechanisms that allow fast responses to hormonal fluctuations in the extracellular environment, unless such changes are the norm, and not the exception in the life of the cells. Indeed, if instead of considering these mechanisms at a single cell level, we view them in the light of a whole body physiology, their rationale becomes evident. We will briefly review the temporal secretion pattern of glucocorticoids by the adrenal glands and relate this pattern to the previously described molecular events, namely the GR chaperone and chromatin cycles.

Naturally occurring GR ligands (cortisol in humans and corticosterone in rodents) are involved in a variety of cellular responses primarily by inducing tissue specific transcriptional regulation of multiple GR-responsive genes, but also through some less defined nongenomic mechanisms [140]. They synchronize the circadian cellular clocks in various peripheral tissues [4,78,117], induce GR interactions with the circadian co-regulators Cry1 and Cry2 [87], and are also involved in the regulation of the cell cycle and proliferation (reviewed in [26]). Glucocorticoids are released from the adrenal gland as a result of a dynamic interplay between of the circadian activity of hypothalamic-pituitary-adrenal axis and the rhythmicity of the adrenal gland itself, which is regulated by its own circadian clock [19]. Changes in plasma level of the glucocorticoids show a maximum before the beginning of the active phase (day for humans and night for rodents), and than declines to a minimum level in the early resting phase. The fact that glucocorticoid hormone in mammals is released in a circadian manner is well known and broadly accepted. Much less known and appreciated is the fact that the actual mode of hormonal release is highly pulsatile or ultradian (Fig.3A). This implies that GR-responsive tissues are subjected to periodic changes in hormonal concentration, which was recently confirmed experimentally [28] and these fluctuations are an integral part of normal mammalian physiology. Moreover, cells are well equipped to detect hormone changes and translate them into specific transcriptional responses.

To summarize, ultradian (hourly) fluctuations in hormone availability throughout the circadian cycle (Fig. 3A and B) are “detected” by the receptor which, with the help of the chaperone machinery (Fig. 2B), forms short-leaving GR-hormone complexes. These complexes engage in a dynamic exchange with the regulatory elements (Fig. 1D, Fig. 3C) and elicit pulsatile transcriptional responses as measured by nacent RNA synthesis (Fig. 3D). Altered patterns of hormone release or increased levels of glucocorticoids are associated with stress, certain pathological and disease states, as well as with various neuropsychiatric disorders [115,132,154,155]. The continuous and pulsatile stimulations elicit dramatically different transcriptional programs [94,102,167] that may have evolved to elicit divergent physiological responses. Indeed, correlation between the mode of hormone secretion and downstream physiological effects are well documented for other systems. For example, continuous or intermittent delivery of the gonadotropin releasing hormone (GnRH) elicit dramatically different downstream effects [8,43]. The exact mechanisms behind these effects are not completely understood, but some recent models suggest convergence of distinct pulsatile signals at the transcriptome [182]. Pulsatile GnRH administration is used clinically for treatment of infertility, while constant administration induces chemical castration and is used in therapy of prostate cancer [106]. It has also been demonstrated that

intermittent parathyroid hormone (PTH) administration has anabolic effects on the bone, improves bone mass density and counteracts glucocorticoid-induced osteoporosis [193], while continuous PTH administration has catabolic effects and induces bone resorption [47,145,180]. Some studies have shown that genes positively related to osteogenesis, BAALC (brain and acute leukemia cytoplasmic), are upregulated by intermittent but not by continuous PTH administration *in vivo* [143] while SOST gene, identified as potent negative regulator, is downregulated by the intermittent PTH [161]. Further studies are needed to fully understand the molecular nature of these differences in response to PTH delivery patterns.

Another example is growth hormone (GH) secretion which is characterized by an ultradian rhythmicity generated by the interplay of the stimulatory and inhibitory effects of GH releasing hormone (GHRH) and somatostatin (Ss), respectively. Cellular levels of Ss and GHRH mRNA in rat brain measured by in situ hybridization oscillate in ultradian manner [202], providing a mechanism for the observed GH oscillations. Ultradian GH release pattern [36,178] is more evident in the male [31]. These sex-specific differences in the GH release correlate with certain physiological responses such as body growth [66] and the activity of hepatic steroid metabolism enzymes [108]. DNA binding affinity of the STAT-5 [177] as well as the sex-specific DNA hypersensitive sites and corresponding gene transcription have been also shown to be temporally related to the GH pulses[91] shedding a light on some of the potential molecular mechanisms.

These examples show that the patterns of hormone release are crucial for the downstream physiological effects. The fact that many metabolic and endocrine signals reach their cellular targets in a pulsatile fashion suggests an intriguing possibility that cells may possess the means for extracting information encoded by the various physical parameters of these signals. For example, it has been suggested that the “calcium code” allows the cells to decode the frequency of Ca^{2+} oscillations [27,93]. It could be speculated that hormone oscillations may also carry additional information beyond the biochemical properties of the hormone. If this is correct, it will be important to understand how these different patterns are recognized by their targets, and how this information is “decoded”.

Based on our GR studies, we propose that common denominators of an extrinsically regulated oscillatory cascade include a highly responsive signal transduction pathway(s), transcription factor dynamics and cyclic changes in chromatin accessibility of regulatory elements leading to tight coupling of transcription and hormone level fluctuations. We also speculate that cellular pathways are fine-tuned to read and respond to differential “codes” of temporally delivered signals and adjust their transcriptional and other cellular responses for optimal physiological effects, and that this process is much more common than previously appreciated.

5. Perspectives

Transcription is one of the most studied cellular functions, yet our understanding of this key process is still evolving. Some of the most fascinating features of transcription are without a doubt its stochasticity, dynamic regulation and adaptability found in single cell organisms as well as in the cells of a multicellular organism. While the single cell must adapt to changes in the environment, individual cells in the multicellular organism respond to a web of intercommunicating processes and regulatory signals which are crucial for their function as a part of a tissue or an organ. Clearly, the dynamic features of transcriptional regulation could be best studied at single cell, single promoter or even a single molecule level. However, these properties are best understood in the light of a whole body physiology and

higher level system biology approach, where extracellular signals, either anticipated or not, are an integral part of dynamic transcriptional regulation.

The endocrine system is characterized by many periodic features with wide temporal range including seasonal and monthly reproductive cycles, circadian variations and high frequency (ultradian) fluctuations [17,53,54,95,186]. This is an excellent model for integrating and regulating diverse transcriptional responses leading to specific physiological outcomes. On every level of temporal hierarchy there is a necessity for coordination of diverse signals to maintain coherent system states. Dynamic and stochastic assembly of cellular transcription machinery at gene promoters is ideally suited to integrate elaborate intrinsic and extrinsic inputs with multiple periodicities and translate them to an appropriate gene expression program.

From hormonal secretion to gene expression, cellular dynamics are rich in oscillatory regulation. When organized in time and space, they can give rise to long-range coordination of gene expression. The literature reviewed here suggests that transcriptional output is a result of the dynamic interplay of diverse regulatory mechanisms, many of which remain to be discovered.

Acknowledgments

The authors would like to thank T. Miranda for critical reading of the manuscript.

Reference List

1. Ashall L, Horton CA, Nelson DE, Paszek P, Harper CV, Sillitoe K, Ryan S, Spiller DG, Unitt JF, Broomhead DS, Kell DB, Rand DA, See V, White MR. Pulsatile stimulation determines timing and specificity of NF-kappaB-dependent transcription. Science. 2009; 324:242. [PubMed: 19359585]
2. Aulehla A, Herrmann BG. Segmentation in vertebrates: clock and gradient finally joined. Genes Dev. 2004; 18:2060. [PubMed: 15342488]
3. Aulehla A, Pourquie O. Oscillating signaling pathways during embryonic development. Curr Opin Cell Biol. 2008; 20:632. [PubMed: 18845254]
4. Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, Schutz G, Schibler U. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science. 2000; 289:2344. [PubMed: 11009419]
5. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. Science. 2010; 330:1349. [PubMed: 21127246]
6. Batchelor E, Mock CS, Bhan I, Loewer A, Lahav G. Recurrent initiation: a mechanism for triggering p53 pulses in response to DNA damage. Mol Cell. 2008; 30:277. [PubMed: 18471974]
7. Becker M, Baumann CT, John S, Walker D, Vigneron M, McNally JG, Hager GL. Dynamic behavior of transcription factors on a natural promoter in living cells. EMBO Rep. 2002; 3:1188. [PubMed: 12446572]
8. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science. 1978; 202:631. [PubMed: 100883]
9. Bennett MR, Pang WL, Ostroff NA, Baumgartner BL, Nayak S, Tsimring LS, Hasty J. Metabolic gene regulation in a dynamically changing environment. Nature. 2008; 454:1119. [PubMed: 18668041]
10. Bessho Y, Hirata H, Masamizu Y, Kageyama R. Periodic repression by the bHLH factor Hes7 is an essential mechanism for the somite segmentation clock. Genes Dev. 2003; 17:1451. [PubMed: 12783854]
11. Bird AP. Gene number, noise reduction and biological complexity. Trends Genet. 1995; 11:94. [PubMed: 7732579]

12. Blake WJ, Kaern M, Cantor CR, Collins JJ. Noise in eukaryotic gene expression. Nature. 2003; 422:633. [PubMed: 12687005]
13. Boireau S, Maiuri P, Basyuk E, de la MM, Knezevich A, Pradet-Balade B, Backer V, Kornblihtt A, Marcello A, Bertrand E. The transcriptional cycle of HIV-1 in real-time and live cells. J Cell Biol. 2007; 179:291. [PubMed: 17954611]
14. Bosisio D, Marazzi I, Agresti A, Shimizu N, Bianchi ME, Natoli G. A hyper-dynamic equilibrium between promoter-bound and nucleoplasmic dimers controls NF-kB-dependent gene activity. EMBO J. 2006; 25:798. [PubMed: 16467852]
15. Boyle AP, Davis S, Shulha HP, Meltzer P, Margulies EH, Weng Z, Furey TS, Crawford GE. High-resolution mapping and characterization of open chromatin across the genome. Cell. 2008; 132:311. [PubMed: 18243105]
16. Cai L, Dalal CK, Elowitz MB. Frequency-modulated nuclear localization bursts coordinate gene regulation. Nature. 2008; 455:485. [PubMed: 18818649]
17. Chadwick, D.; Goode, JA. Mechanisms and Biological Significance of Pulsatile Hormone Secretion. John Wiley & Sons Ltd; New York, NY: 2000.
18. Chubb JR, Trcek T, Shenoy SM, Singer RH. Transcriptional pulsing of a developmental gene. Curr Biol. 2006; 16:1018. [PubMed: 16713960]
19. Chung S, Son GH, Kim K. Adrenal peripheral oscillator in generating the circadian glucocorticoid rhythm. Ann N Y Acad Sci. 2011; 1220:71. [PubMed: 21388405]
20. Cohen-Saidon C, Cohen AA, Sigal A, Liron Y, Alon U. Dynamics and variability of ERK2 response to EGF in individual living cells. Mol Cell. 2009; 36:885. [PubMed: 20005850]
21. Coulon A, Gandrillon O, Beslon G. On the spontaneous stochastic dynamics of a single gene: complexity of the molecular interplay at the promoter. BMC Syst Biol. 2010; 4:2. [PubMed: 20064204]
22. Darzacq X, Shav-Tal Y, de Turris V, Brody Y, Shenoy SM, Phair RD, Singer RH. In vivo dynamics of RNA polymerase II transcription. Nat Struct Mol Biol. 2007; 14:796. [PubMed: 17676063]
23. DeFranco DB. Role of molecular chaperones in subnuclear trafficking of glucocorticoid receptors. Kidney Int. 2000; 57:1241. [PubMed: 10760049]
24. DeFranco DB, Csermely P. Steroid receptor and molecular chaperone encounters in the nucleus. Sci STKE. 2000; 2000:e1-file.
25. Dequeant ML, Glynn E, Gaudenz K, Wahl M, Chen J, Mushegian A, Pourquie O. A complex oscillating network of signaling genes underlies the mouse segmentation clock. Science. 2006; 314:1595. [PubMed: 17095659]
26. Dickmeis T, Foulkes NS. Glucocorticoids and circadian clock control of cell proliferation: at the interface between three dynamic systems. Mol Cell Endocrinol. 2011; 331:11. [PubMed: 20833224]
27. Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. Nature. 1998; 392:933. [PubMed: 9582075]
28. Droste SK, de Groote L, Atkinson HC, Lightman SL, Reul JM, Linthorst AC. Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. Endocrinology. 2008; 149:3244. [PubMed: 18356272]
29. Dundr M, Hoffmann-Rohrer U, Hu Q, Grummt I, Rothblum LI, Phair RD, Misteli T. A kinetic framework for a mammalian RNA polymerase in vivo. Science. 2002; 298:1623. [PubMed: 12446911]
30. Dunlop MJ, Cox RS III, Levine JH, Murray RM, Elowitz MB. Regulatory activity revealed by dynamic correlations in gene expression noise. Nat Genet. 2008; 40:1493. [PubMed: 19029898]
31. Eden S. Age- and sex-related differences in episodic growth hormone secretion in the rat. Endocrinology. 1979; 105:555. [PubMed: 572295]
32. Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL, DeFranco DB. Molecular chaperones function as steroid receptor nuclear mobility factors. Proc Natl Acad Sci USA. 2004; 101:2876. [PubMed: 14978266]
33. Elowitz MB, Levine AJ, Siggia ED, Swain PS. Stochastic gene expression in a single cell. Science. 2002; 297:1183. [PubMed: 12183631]

34. Farla P, Hersmus R, Trapman J, Houtsmuller AB. Antiandrogens prevent stable DNA-binding of the androgen receptor. J Cell Sci. 2005; 118:4187. [PubMed: 16141232]
35. Ferdous A, Sikder D, Gillette T, Nalley K, Kodadek T, Johnston SA. The role of the proteasomal ATPases and activator monoubiquitylation in regulating Gal4 binding to promoters. Genes Dev. 2007; 21:112. [PubMed: 17167105]
36. Finkelstein JW, Roffwarg HP, Boyar RM, Kream J, Hellman L. Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. J Clin Endocrinol Metab. 1972; 35:665. [PubMed: 5071338]
37. Fletcher TM, Xiao N, Mautino G, Baumann CT, Wolford RG, Warren BS, Hager GL. ATP-dependent mobilization of the glucocorticoid receptor during chromatin remodeling. Mol Cell Biol. 2002; 22:3255. [PubMed: 11971959]
38. Freeman BC, Yamamoto KR. Disassembly of transcriptional regulatory complexes by molecular chaperones. Science. 2002; 296:2232. [PubMed: 12077419]
39. Gandhi SJ, Zenklusen D, Lionnet T, Singer RH. Transcription of functionally related constitutive genes is not coordinated. Nat Struct Mol Biol. 2011; 18:27. [PubMed: 21131977]
40. Geva-Zatorsky N, Rosenfeld N, Itzkovitz S, Milo R, Sigal A, Dekel E, Yarnitzky T, Liron Y, Polak P, Lahav G, Alon U. Oscillations and variability in the p53 system. Mol Syst Biol. 2006; 2:2006. [PubMed: 16773083]
41. Golding I, Cox EC. Eukaryotic transcription: what does it mean for a gene to be ‘on’? Curr Biol. 2006; 16:R371–R373. [PubMed: 16713947]
42. Golding I, Paulsson J, Zawilski SM, Cox EC. Real-time kinetics of gene activity in individual bacteria. Cell. 2005; 123:1025. [PubMed: 16360033]
43. Gore, AC. GnRH: The Master Molecule of Reproduction. Kluwer Academic Publishers; Boston, MA: 2002.
44. Gorski SA, Snyder SK, John S, Grummt I, Misteli T. Modulation of RNA polymerase assembly dynamics in transcriptional regulation. Mol Cell. 2008; 30:486. [PubMed: 18498750]
45. Gravina S, Vijg J. Epigenetic factors in aging and longevity. Pflugers Arch. 2010; 459:247. [PubMed: 19768466]
46. Green CB, Takahashi JS, Bass J. The meter of metabolism. Cell. 2008; 134:728. [PubMed: 18775307]
47. Greenfield EM. Anabolic Effects of Intermittent PTH on Osteoblasts. Curr Mol Pharmacol. 2011
48. Grontved L, Hager GL. Impact of chromatin structure on PR signaling: Transition from local to global analysis. Mol Cell Endocrinol. 2011 in press.
49. Hager GL, McNally JG, Misteli T. Transcription dynamics. Mol Cell. 2009; 35:741. [PubMed: 19782025]
50. Hakim O, Sung MH, Hager GL. 3D Shortcuts to Gene Regulation. Curr Opin Cell Biol. 2010; 22:305. [PubMed: 20466532]
51. Hakim O, Sung MH, Voss TC, John S, Splinter E, Sabo PJ, Thurman RE, Stamatoyannopoulos JA, de Laat W, Hager GL. Diverse gene reprogramming events occur in the same spatial clusters of distal regulatory elements. Genome Res. 2011; 21:697. [PubMed: 21471403]
52. Harper CV, Finkenstadt B, Woodcock DJ, Friedrichsen S, Semprini S, Ashall L, Spiller DG, Mullins JJ, Rand DA, Davis JR, White MR. Dynamic analysis of stochastic transcription cycles. PLoS Biol. 2011; 9:e1000607. [PubMed: 21532732]
53. Haus E. Chronobiology in the endocrine system. Adv Drug Deliv Rev. 2007; 59:985. [PubMed: 17804113]
54. Haus E, Smolensky MH. Biologic rhythms in the immune system. Chronobiol Int. 1999; 16:581. [PubMed: 10513884]
55. Hesselberth JR, Zhang Z, Sabo PJ, Chen X, Sandstrom R, Reynolds AP, Thurman RE, Neph S, Kuehn MS, Noble WS, Fields S, Stamatoyannopoulos JA. Global mapping of protein-DNA interactions in vivo by digital genomic footprinting. Nat Methods. 2009; 6:283. [PubMed: 19305407]
56. Hilfinger A, Paulsson J. Separating intrinsic from extrinsic fluctuations in dynamic biological systems. Proc Natl Acad Sci U S A. 2011; 108:12167. [PubMed: 21730172]

57. Hinow P, Rogers CE, Barbieri CE, Pietenpol JA, Kenworthy AK, DiBenedetto E. The DNA binding activity of p53 displays reaction-diffusion kinetics. Biophys J. 2006; 91:330. [PubMed: 16603489]
58. Hirata H, Bessho Y, Kokubu H, Masamizu Y, Yamada S, Lewis J, Kageyama R. Instability of Hes7 protein is crucial for the somite segmentation clock. Nat Genet. 2004; 36:750. [PubMed: 15170214]
59. Hirata H, Yoshiura S, Ohtsuka T, Bessho Y, Harada T, Yoshikawa K, Kageyama R. Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop. Science. 2002; 298:840. [PubMed: 12399594]
60. Hoffmann A, Levchenko A, Scott ML, Baltimore D. The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science. 2002; 298:1241. [PubMed: 12424381]
61. Hogenesch JB, Ueda HR. Understanding systems-level properties: timely stories from the study of clocks. Nat Rev Genet. 2011; 12:407. [PubMed: 21556016]
62. Horikawa K, Ishimatsu K, Yoshimoto E, Kondo S, Takeda H. Noise-resistant and synchronized oscillation of the segmentation clock. Nature. 2006; 441:719. [PubMed: 16760970]
63. Hu LM, Bodwell J, Hu JM, Orti E, Munck A. Glucocorticoid receptors in ATP-depleted cells. Dephosphorylation, loss of hormone binding, HSP90 dissociation, and ATP-dependent cycling. J Biol Chem. 1994; 269:6571. [PubMed: 8120009]
64. Hughes ME, DiTacchio L, Hayes KR, Vollmers C, Pulivarthy S, Baggs JE, Panda S, Hogenesch JB. Harmonics of circadian gene transcription in mammals. PLoS Genet. 2009; 5:e1000442. [PubMed: 19343201]
65. Iqbal J, Zaidi M. TNF-induced MAP kinase activation oscillates in time. Biochem Biophys Res Commun. 2008; 371:906. [PubMed: 18384751]
66. Jansson JO, Eden S, Isaksson O. Sexual dimorphism in the control of growth hormone secretion. Endocr Rev. 1985; 6:128. [PubMed: 2861084]
67. Jiang YJ, Aerne BL, Smithers L, Haddon C, Ish-Horowicz D, Lewis J. Notch signalling and the synchronization of the somite segmentation clock. Nature. 2000; 408:475. [PubMed: 11100729]
68. John S, Sabo PJ, Johnson TA, Sung MH, Biddie SC, Lightman SL, Voss TC, Davis SR, Meltzer PS, Stamatoyannopoulos JA, Hager GL. Interaction of the glucocorticoid receptor with the global chromatin landscape. Mol Cell. 2008; 29:611. [PubMed: 18342607]
69. John S, Sabo PJ, Thurman RE, Sung MH, Biddie SC, Johnson TA, Hager GL, Stamatoyannopoulos JA. Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. Nat Genet. 2011; 43:264. [PubMed: 21258342]
70. Johnson CH, Egli M, Stewart PL. Structural insights into a circadian oscillator. Science. 2008; 322:697. [PubMed: 18974343]
71. Johnson TA, Elbi C, Parekh BS, Hager GL, John S. Chromatin remodeling complexes interact dynamically with a glucocorticoid receptor regulated promoter. Mol Biol Cell. 2008; 19:3308. [PubMed: 18508913]
72. Kaern M, Elston TC, Blake WJ, Collins JJ. Stochasticity in gene expression: from theories to phenotypes. Nat Rev Genet. 2005; 6:451. [PubMed: 15883588]
73. Kageyama R, Yoshiura S, Masamizu Y, Niwa Y. Ultradian oscillators in somite segmentation and other biological events. Cold Spring Harb Symp Quant Biol. 2007; 72:451. [PubMed: 18419304]
74. Karpova TS, Chen TY, Sprague BL, McNally JG. Dynamic interactions of a transcription factor with DNA are accelerated by a chromatin remodeller. EMBO Rep. 2004; 5:1064. [PubMed: 15514679]
75. Karpova TS, Kim MJ, Spriet C, Nalley K, Stasevich TJ, Kherrouche Z, Heliot L, McNally JG. Concurrent fast and slow cycling of a transcriptional activator at an endogenous promoter. Science. 2008; 319:466. [PubMed: 18218898]
76. Karttunen J, Shastri N. Measurement of ligand-induced activation in single viable T cells using the lacZ reporter gene. Proc Natl Acad Sci U S A. 1991; 88:3972. [PubMed: 1902576]
77. Kearns JD, Basak S, Werner SL, Huang CS, Hoffmann A. IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. J Cell Biol. 2006; 173:659. [PubMed: 16735576]

78. Kiessling S, Eichele G, Oster H. Adrenal glucocorticoids have a key role in circadian resynchronization in a mouse model of jet lag. J Clin Invest. 2010; 120:2600. [PubMed: 20577050]
79. Kimura H, Sugaya K, Cook PR. The transcription cycle of RNA polymerase II in living cells. J Cell Biol. 2002; 159:777. [PubMed: 12473686]
80. Klevecz RR, Bolen J, Forrest G, Murray DB. A genomewide oscillation in transcription gates DNA replication and cell cycle. Proc Natl Acad Sci U S A. 2004; 101:1200. [PubMed: 14734811]
81. Ko MS, Nakauchi H, Takahashi N. The dose dependence of glucocorticoid-inducible gene expression results from changes in the number of transcriptionally active templates. EMBO J. 1990; 9:2835. [PubMed: 2167833]
82. Kruse K, Julicher F. Oscillations in cell biology. Curr Opin Cell Biol. 2005; 17:20. [PubMed: 15661515]
83. Kupiec JJ. A Darwinian theory for the origin of cellular differentiation. Mol Gen Genet. 1997; 255:201. [PubMed: 9236778]
84. Kussell E, Leibler S. Phenotypic diversity, population growth, and information in fluctuating environments. Science. 2005; 309:2075. [PubMed: 16123265]
85. Lahav G. The strength of indecisiveness: oscillatory behavior for better cell fate determination. Sci STKE. 2004; 2004:e55.
86. Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB, Alon U. Dynamics of the p53-Mdm2 feedback loop in individual cells. Nat Genet. 2004; 36:147. [PubMed: 14730303]
87. Lamia KA, Papp SJ, Yu RT, Barish GD, Uhlenhaut NH, Jonker JW, Downes M, Evans RM. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. Nature. 2011; 480:552. [PubMed: 22170608]
88. Larson DR. What do expression dynamics tell us about the mechanism of transcription? Curr Opin Genet Dev. 2011; 21:591. [PubMed: 21862317]
89. Larson DR, Singer RH, Zenklusen D. A single molecule view of gene expression. Trends Cell Biol. 2009; 19:630. [PubMed: 19819144]
90. Larson DR, Zenklusen D, Wu B, Chao JA, Singer RH. Real-time observation of transcription initiation and elongation on an endogenous yeast gene. Science. 2011; 332:475. [PubMed: 21512033]
91. Laz EV, Sugathan A, Waxman DJ. Dynamic in vivo binding of STAT5 to growth hormone-regulated genes in intact rat liver. Sex-specific binding at low- but not high-affinity STAT5 sites. Mol Endocrinol. 2009; 23:1242. [PubMed: 19423653]
92. Lemaire V, Lee CF, Lei J, Metivier R, Glass L. Sequential recruitment and combinatorial assembling of multiprotein complexes in transcriptional activation. Phys Rev Lett. 2006; 96:198102. [PubMed: 16803143]
93. Lewis RS. Calcium oscillations in T-cells: mechanisms and consequences for gene expression. Biochem Soc Trans. 2003; 31:925. [PubMed: 14505450]
94. Lightman SL, Conway-Campbell BL. The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration. Nat Rev Neurosci. 2010; 11:710. [PubMed: 20842176]
95. Lightman SL, Wiles CC, Atkinson HC, Henley DE, Russell GM, Leendertz JA, McKenna MA, Spiga F, Wood SA, Conway-Campbell BL. The significance of glucocorticoid pulsatility. Eur J Pharmacol. 2008; 583:255. [PubMed: 18339373]
96. Lipan O, Wong WH. The use of oscillatory signals in the study of genetic networks. Proc Natl Acad Sci U S A. 2005; 102:7063. [PubMed: 15883385]
97. Liu AC, Lewis WG, Kay SA. Mammalian circadian signaling networks and therapeutic targets. Nat Chem Biol. 2007; 3:630. [PubMed: 17876320]
98. Liu J, DeFranco DB. Chromatin recycling of glucocorticoid receptors: implications for multiple roles of heat shock protein 90. Mol Endocrinol. 1999; 13:355. [PubMed: 10076993]
99. Maroto M, Dale JK, Dequeant ML, Petit AC, Pourquie O. Synchronised cycling gene oscillations in presomitic mesoderm cells require cell-cell contact. Int J Dev Biol. 2005; 49:309. [PubMed: 15906246]

100. Masamizu Y, Ohtsuka T, Takashima Y, Nagahara H, Takenaka Y, Yoshikawa K, Okamura H, Kageyama R. Real-time imaging of the somite segmentation clock: revelation of unstable oscillators in the individual presomitic mesoderm cells. Proc Natl Acad Sci U S A. 2006; 103:1313. [PubMed: 16432209]
101. Mazza D, Stasevich TJ, Karpova TS, McNally JG. Monitoring dynamic binding of chromatin proteins in vivo by fluorescence correlation spectroscopy and temporal image correlation spectroscopy. Methods Mol Biol. 2012; 833:177. [PubMed: 22183595]
102. McMaster A, Jangani M, Sommer P, Han N, Brass A, Beesley S, Lu W, Berry A, Loudon A, Donn R, Ray DW. Ultradian cortisol pulsatility encodes a distinct, biologically important signal. PLoS ONE. 2011; 6:e15766. [PubMed: 21267416]
103. McNally JG, Mueller WG, Walker D, Wolford RG, Hager GL. The glucocorticoid receptor: Rapid exchange with regulatory sites in living cells. Science. 2000; 287:1262. [PubMed: 10678832]
104. Metivier R, Penot G, Hubner MR, Reid G, Brand H, Kos M, Gannon F. Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell. 2003; 115:751. [PubMed: 14675539]
105. Metivier R, Reid G, Gannon F. Transcription in four dimensions: nuclear receptor-directed initiation of gene expression. EMBO Rep. 2006; 7:161. [PubMed: 16452926]
106. Mezo G, Manea M, Szabi I, Vincze B, Kovacs M. New derivatives of GnRH as potential anticancer therapeutic agents. Curr Med Chem. 2008; 15:2366. [PubMed: 18855666]
107. Michael D, Oren M. The p53-Mdm2 module and the ubiquitin system. Semin Cancer Biol. 2003; 13:49. [PubMed: 12507556]
108. Mode A, Gustafsson JA, Jansson JO, Eden S, Isaksson O. Association between plasma level of growth hormone and sex differentiation of hepatic steroid metabolism in the rat. Endocrinology. 1982; 111:1692. [PubMed: 7128531]
109. Mohawk JA, Takahashi JS. Cell autonomy and synchrony of suprachiasmatic nucleus circadian oscillators. Trends Neurosci. 2011 in press.
110. Momand J, Wu HH, Dasgupta G. MDM2--master regulator of the p53 tumor suppressor protein. Gene. 2000; 242:15. [PubMed: 10721693]
111. Morin LP. The circadian visual system. Brain Res Brain Res Rev. 1994; 19:102. [PubMed: 7909471]
112. Mueller F, Karpova TS, Mazza D, McNally JG. Monitoring dynamic binding of chromatin proteins in vivo by fluorescence recovery after photobleaching. Methods Mol Biol. 2012; 833:153. [PubMed: 22183594]
113. Mueller F, Wach P, McNally JG. Evidence for a common mode of transcription factor interaction with chromatin as revealed by improved quantitative fluorescence recovery after photobleaching. Biophys J. 2008; 94:3323. [PubMed: 18199661]
114. Munck A, Foley R. Kinetics of glucocorticoid-receptor complexes in rat thymus cells. J Steroid Biochem. 1976; 7:1117. [PubMed: 1025357]
115. Murgatroyd C, Spengler D. Epigenetic programming of the HPA axis: early life decides. Stress. 2011; 14:581. [PubMed: 21854166]
116. Myers MP, Wager-Smith K, Rothenfluh-Hilfiker A, Young MW. Light-induced degradation of TIMELESS and entrainment of the Drosophila circadian clock. Science. 1996; 271:1736. [PubMed: 8596937]
117. Nader N, Chrousos GP, Kino T. Circadian rhythm transcription factor CLOCK regulates the transcriptional activity of the glucocorticoid receptor by acetylating its hinge region lysine cluster: potential physiological implications. FASEB J. 2009; 23:1572. [PubMed: 19141540]
118. Nagaich AK, Walker DA, Wolford RG, Hager GL. Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. Mol Cell. 2004; 14:163. [PubMed: 15099516]
119. Nalley K, Johnston SA, Kodadek T. Proteolytic turnover of the Gal4 transcription factor is not required for function in vivo. Nature. 2006; 442:1054. [PubMed: 16929306]
120. Nelson DE, Ihekwaba AE, Elliott M, Johnson JR, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG, Edwards SW, McDowell HP, Unitt JF, Sullivan E, Grimley R, Benson

N, Broomhead D, Kell DB, White MR. Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science. 2004; 306:704. [PubMed: 15499023]

121. Nelson DE, See V, Nelson G, White MR. Oscillations in transcription factor dynamics: a new way to control gene expression. Biochem Soc Trans. 2004; 32:1090. [PubMed: 15506974]
122. Newlands S, Levitt LK, Robinson CS, Karpf AB, Hodgson VR, Wade RP, Hardeman EC. Transcription occurs in pulses in muscle fibers. Genes Dev. 1998; 12:2748. [PubMed: 9732272]
123. O'Neill JS, Reddy AB. Circadian clocks in human red blood cells. Nature. 2011; 469:498. [PubMed: 21270888]
124. O'Neill JS, van OG, Dixon LE, Troein C, Corellou F, Bouget FY, Reddy AB, Millar AJ. Circadian rhythms persist without transcription in a eukaryote. Nature. 2011; 469:554. [PubMed: 21270895]
125. Okamura H. Clock genes in cell clocks: roles, actions, and mysteries. J Biol Rhythms. 2004; 19:388. [PubMed: 15534319]
126. Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, Goyenechea B, Mitchell JA, Lopes S, Reik W, Fraser P. Active genes dynamically colocalize to shared sites of ongoing transcription. Nat Genet. 2004; 36:1065. [PubMed: 15361872]
127. Pedraza JM, Paulsson J. Effects of molecular memory and bursting on fluctuations in gene expression. Science. 2008; 319:339. [PubMed: 18202292]
128. Perlmann T, Eriksson P, Wrange O. Quantitative analysis of the glucocorticoid receptor-DNA interaction at the mouse mammary tumor virus glucocorticoid response element. J Biol Chem. 1990; 265:17222. [PubMed: 2170368]
129. Phair RD, Misteli T. High mobility of proteins in the mammalian cell nucleus. Nature. 2000; 404:604. [PubMed: 10766243]
130. Piette J, Neel H, Marechal V. Mdm2: keeping p53 under control. Oncogene. 1997; 15:1001. [PubMed: 9285554]
131. Pourquie O. The segmentation clock: converting embryonic time into spatial pattern. Science. 2003; 301:328. [PubMed: 12869750]
132. Pryce CR, Aubert Y, Maier C, Pearce PC, Fuchs E. The developmental impact of prenatal stress, prenatal dexamethasone and postnatal social stress on physiology, behaviour and neuroanatomy of primate offspring: studies in rhesus macaque and common marmoset. Psychopharmacology (Berl). 2011; 214:33. [PubMed: 20809212]
133. Rabani M, Levin JZ, Fan L, Adiconis X, Raychowdhury R, Garber M, Gnirke A, Nusbaum C, Hacohen N, Friedman N, Amit I, Regev A. Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. Nat Biotechnol. 2011; 29:436. [PubMed: 21516085]
134. Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA synthesis in mammalian cells. PLoS Biol. 2006; 4:e309. [PubMed: 17048983]
135. Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA synthesis in mammalian cells. PLoS Biol. 2006; 4:e309. [PubMed: 17048983]
136. Raj A, van OA. Nature, nurture, or chance: stochastic gene expression and its consequences. Cell. 2008; 135:216. [PubMed: 18957198]
137. Rayasam GV, Elbi C, Walker DA, Wolford RG, Fletcher TM, Edwards DP, Hager GL. Ligand specific dynamics of the progesterone receptor in living cells and during chromatin remodeling in vitro. Mol Cell Biol. 2005; 25:2406. [PubMed: 15743833]
138. Reid G, Gallais R, Metivier R. Marking time: the dynamic role of chromatin and covalent modification in transcription. Int J Biochem Cell Biol. 2009; 41:155. [PubMed: 18805503]
139. Reik A, Schutz G, Stewart AF. Glucocorticoids are required for establishment and maintenance of an alteration in chromatin structure: induction leads to a reversible disruption of nucleosomes over an enhancer. EMBO J. 1991; 10:2569. [PubMed: 1678348]
140. Revollo JR, Cidlowski JA. Mechanisms generating diversity in glucocorticoid receptor signaling. Ann N Y Acad Sci. 2009; 1179:167. [PubMed: 19906239]
141. Riedel-Kruse IH, Muller C, Oates AC. Synchrony dynamics during initiation, failure, and rescue of the segmentation clock. Science. 2007; 317:1911. [PubMed: 17702912]

142. Ripperger JA, Schibler U. Circadian regulation of gene expression in animals. Curr Opin Cell Biol. 2001; 13:357. [PubMed: 11343908]

143. Robinson JA, Susulic V, Liu YB, Taylor C, Hardenburg J, Gironda V, Zhao W, Kharode Y, McLarney S, Bai Y, Malone DP, Murrills R, Bex F. Identification of a PTH regulated gene selectively induced in vivo during PTH-mediated bone formation. J Cell Biochem. 2006; 98:1203. [PubMed: 16514668]

144. Ronen M, Botstein D. Transcriptional response of steady-state yeast cultures to transient perturbations in carbon source. Proc Natl Acad Sci U S A. 2006; 103:389. [PubMed: 16381818]

145. Rosen CJ. What's new with PTH in osteoporosis: where are we and where are we headed? Trends Endocrinol Metab. 2004; 15:229. [PubMed: 15223053]

146. Ross IL, Browne CM, Hume DA. Transcription of individual genes in eukaryotic cells occurs randomly and infrequently. Immunol Cell Biol. 1994; 72:177. [PubMed: 8200693]

147. Rusak B, Robertson HA, Wisden W, Hunt SP. Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus. Science. 1990; 248:1237. [PubMed: 2112267]

148. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol. 2001; 13:332. [PubMed: 11343904]

149. Saiz L, Vilar JM. Stochastic dynamics of macromolecular-assembly networks. Mol Syst Biol. 2006; 2:2006. [PubMed: 16738569]

150. Sanchez A, Garcia HG, Jones D, Phillips R, Kondev J. Effect of promoter architecture on the cell-to-cell variability in gene expression. PLoS Comput Biol. 2011; 7:e1001100. [PubMed: 21390269]

151. Sanchez A, Kondev J. Transcriptional control of noise in gene expression. Proc Natl Acad Sci U S A. 2008; 105:5081. [PubMed: 18353986]

152. Schule R, Rangarajan P, Yang N, Kliewer S, Ransone LJ, Bolado J, Verma IM, Evans RM. Retinoic acid is a negative regulator of AP-1-responsive genes. Proc Natl Acad Sci USA. 1991; 88:6092. [PubMed: 1648728]

153. Scott M, Hwa T, Ingalls B. Deterministic characterization of stochastic genetic circuits. Proc Natl Acad Sci USA. 2007; 104:7402. [PubMed: 17446275]

154. Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab. 2007; 3:479. [PubMed: 17515892]

155. Seckl JR, Meaney MJ. Glucocorticoid "programming" and PTSD risk. Ann N Y Acad Sci. 2006; 1071:351. [PubMed: 16891583]

156. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell. 2000; 103:843. [PubMed: 11136970]

157. Shankaran H, Ippolito DL, Chrisler WB, Resat H, Bollinger N, Opresko LK, Wiley HS. Rapid and sustained nuclear-cytoplasmic ERK oscillations induced by epidermal growth factor. Mol Syst Biol. 2009; 5:332. [PubMed: 19953086]

158. Sharp ZD, Mancini MG, Hinojos CA, Dai F, Berno V, Szafran AT, Smith KP, Lele TT, Ingber DE, Mancini MA. Estrogen-receptor-alpha exchange and chromatin dynamics are ligand- and domain-dependent. J Cell Sci. 2006; 119:4101. [PubMed: 16968748]

159. Sigal A, Milo R, Cohen A, Geva-Zatorsky N, Klein Y, Liron Y, Rosenfeld N, Danon T, Perzov N, Alon U. Variability and memory of protein levels in human cells. Nature. 2006; 444:643. [PubMed: 17122776]

160. Silverman SJ, Petti AA, Slavov N, Parsons L, Briehof R, Thiberge SY, Zenklusen D, Gandhi SJ, Larson DR, Singer RH, Botstein D. Metabolic cycling in single yeast cells from unsynchronized steady-state populations limited on glucose or phosphate. Proc Natl Acad Sci U S A. 2010; 107:6946. [PubMed: 20335538]

161. Silvestrini G, Ballanti P, Leopizzi M, Sebastiani M, Berni S, Di VM, Bonucci E. Effects of intermittent parathyroid hormone (PTH) administration on SOST mRNA and protein in rat bone. J Mol Histol. 2007; 38:261. [PubMed: 17549589]

162. Singh A, Razooky B, Cox CD, Simpson ML, Weinberger LS. Transcriptional bursting from the HIV-1 promoter is a significant source of stochastic noise in HIV-1 gene expression. Biophys J. 2010; 98:L32–L34. [PubMed: 20409455]

163. Sprague BL, McNally JG. FRAP analysis of binding: proper and fitting. Trends Cell Biol. 2005; 15:84. [PubMed: 15695095]
164. Sprague BL, Pego RL, Stavreva DA, McNally JG. Analysis of binding reactions by fluorescence recovery after photobleaching. Biophys J. 2004; 86:3473. [PubMed: 15189848]
165. Sprouse RO, Karpova TS, Mueller F, Dasgupta A, McNally JG, Auble DT. Regulation of TATA-binding protein dynamics in living yeast cells. Proc Natl Acad Sci U S A. 2008; 105:13304. [PubMed: 18765812]
166. Stavreva DA, Muller WG, Hager GL, Smith CL, McNally JG. Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. Mol Cell Biol. 2004; 24:2682. [PubMed: 15024059]
167. Stavreva DA, Wiench M, John S, Conway-Campbell BL, McKenna MA, Pooley JR, Johnson TA, Voss TC, Lightman SL, Hager GL. Ultradian hormone stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. Nat Cell Biol. 2009; 11:1093. [PubMed: 19684579]
168. Stenoien DL, Patel K, Mancini MG, Dutertre M, Smith CL, O'Malley BW, Mancini MA. FRAP reveals that mobility of oestrogen receptor-alpha is ligand- and proteasome-dependent. Nat Cell Biol. 2001; 3:15. [PubMed: 11146621]
169. Stephan FK. The "other" circadian system: food as a Zeitgeber. J Biol Rhythms. 2002; 17:284. [PubMed: 12164245]
170. Stevense M, Muramoto T, Muller I, Chubb JR. Digital nature of the immediate-early transcriptional response. Development. 2010; 137:579. [PubMed: 20110323]
171. Sun L, Yang G, Zaidi M, Iqbal J. TNF-induced gene expression oscillates in time. Biochem Biophys Res Commun. 2008; 371:900. [PubMed: 18384746]
172. Sun L, Yang G, Zaidi M, Iqbal J. TNF-induced oscillations in combinatorial transcription factor binding. Biochem Biophys Res Commun. 2008; 371:912. [PubMed: 18384744]
173. Sung MH, Salvatore L, De Lorenzi R, Indrawan A, Pasparakis M, Hager GL, Bianchi ME, Agresti A. Sustained oscillations of NF-kappaB produce distinct genome scanning and gene expression profiles. PLoS One. 2009; 4:e7163. [PubMed: 19787057]
174. Sung MH, Simon R. In silico simulation of inhibitor drug effects on nuclear factor-kappaB pathway dynamics. Mol Pharmacol. 2004; 66:70. [PubMed: 15213297]
175. Swain PS, Elowitz MB, Siggia ED. Intrinsic and extrinsic contributions to stochasticity in gene expression. Proc Natl Acad Sci U S A. 2002; 99:12795. [PubMed: 12237400]
176. Takashima Y, Ohtsuka T, Gonzalez A, Miyachi H, Kageyama R. Intronic delay is essential for oscillatory expression in the segmentation clock. Proc Natl Acad Sci U S A. 2011; 108:3300. [PubMed: 21300886]
177. Tannenbaum GS, Choi HK, Gurd W, Waxman DJ. Temporal relationship between the sexually dimorphic spontaneous GH secretory profiles and hepatic STAT5 activity. Endocrinology. 2001; 142:4599. [PubMed: 11606424]
178. Tannenbaum GS, Martin JB. Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. Endocrinology. 1976; 98:562. [PubMed: 1261487]
179. Thattai M, van OA. Stochastic gene expression in fluctuating environments. Genetics. 2004; 167:523. [PubMed: 15166174]
180. Thomas T. Intermittent parathyroid hormone therapy to increase bone formation. Joint Bone Spine. 2006; 73:262. [PubMed: 16563840]
181. Tiana G, Krishna S, Pigolotti S, Jensen MH, Sneppen K. Oscillations and temporal signalling in cells. Phys Biol. 2007; 4:R1. [PubMed: 17664651]
182. Tsaneva-Atanasova K, Mina P, Caunt CJ, Armstrong SP, McArdle CA. Decoding GnRH neurohormone pulse frequency by convergent signalling modules. J R Soc Interface. 2011
183. Tu BP, Kudlicki A, Rowicka M, McKnight SL. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science. 2005; 310:1152. [PubMed: 16254148]
184. Turner DA, Paszek P, Woodcock DJ, Nelson DE, Horton CA, Wang Y, Spiller DG, Rand DA, White MR, Harper CV. Physiological levels of TNFalpha stimulation induce stochastic dynamics of NF-kappaB responses in single living cells. J Cell Sci. 2010; 123:2834. [PubMed: 20663918]

185. van Holde, KE. Chromatin. Springer-Verlag; Heidelberg: 1988.

186. Van CE. Diurnal and ultradian rhythms in human endocrine function: a minireview. Horm Res. 1990; 34:45. [PubMed: 1965834]

187. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000; 408:307. [PubMed: 11099028]

188. Volfson D, Marciniak J, Blake WJ, Ostroff N, Tsimring LS, Hasty J. Origins of extrinsic variability in eukaryotic gene expression. Nature. 2006; 439:861. [PubMed: 16372021]

189. Voss TC, Schiltz RL, Sung MH, Johnson TA, John S, Hager GL. Combinatorial probabilistic chromatin interactions produce transcriptional heterogeneity. J Cell Sci. 2009; 122:345. [PubMed: 19126674]

190. Voss TC, Schiltz RL, Sung MH, Yen PM, Stamatoyannopoulos JA, Biddie SC, Johnson TA, Miranda TB, John S, Hager GL. Dynamic exchange at regulatory elements during chromatin remodeling underlies assisted loading mechanism. Cell. 2011; 146:544. [PubMed: 21835447]

191. Walker D, Htun H, Hager GL. Using inducible vectors to study intracellular trafficking of GFP-tagged steroid/nuclear receptors in living cells. Methods (Companion to Methods in Enzymology). 1999; 19:386.

192. Wang J, Liu W, Mitsui K, Tsurugi K. Evidence for the involvement of the GTS1 gene product in the regulation of biological rhythms in the continuous culture of the yeast Saccharomyces cerevisiae. FEBS Lett. 2001; 489:81. [PubMed: 11231018]

193. Weinstein RS, Jilka RL, Almeida M, Roberson PK, Manolagas SC. Intermittent parathyroid hormone administration counteracts the adverse effects of glucocorticoids on osteoblast and osteocyte viability, bone formation, and strength in mice. Endocrinology. 2010; 151:2641. [PubMed: 20410195]

194. White MR, Masuko M, Amet L, Elliott G, Braddock M, Kingsman AJ, Kingsman SM. Real-time analysis of the transcriptional regulation of HIV and hCMV promoters in single mammalian cells. J Cell Sci. 1995; 108(Pt 2):441. [PubMed: 7768992]

195. Wu C, Bingham PM, Livak KJ, Holmgren R, Elgin SC. The chromatin structure of specific genes: I. Evidence for higher order domains of defined DNA sequence. Cell. 1979; 16:797. [PubMed: 455449]

196. Wu C, Wong YC, Elgin SC. The chromatin structure of specific genes: II. Disruption of chromatin structure during gene activity. Cell. 1979; 16:807. [PubMed: 455450]

197. Yang J, Liu J, DeFranco DB. Subnuclear trafficking of glucocorticoid receptors in vitro: chromatin recycling and nuclear export. J Cell Biol. 1997; 137:523. [PubMed: 9151662]

198. Yao J, Ardehali MB, Fecko CJ, Webb WW, Lis JT. Intranuclear distribution and local dynamics of RNA polymerase II during transcription activation. Mol Cell. 2007; 28:978. [PubMed: 18158896]

199. Yoshiura S, Ohtsuka T, Takenaka Y, Nagahara H, Yoshikawa K, Kageyama R. Ultradian oscillations of Stat, Smad, and Hes1 expression in response to serum. Proc Natl Acad Sci U S A. 2007; 104:11292. [PubMed: 17592117]

200. Yu P, Kodadek T. Dynamics of the hypoxia-inducible factor-1-vascular endothelial growth factor promoter complex. J Biol Chem. 2007; 282:35035. [PubMed: 17916562]

201. Yunger S, Rosenfeld L, Garini Y, Shav-Tal Y. Single-allele analysis of transcription kinetics in living mammalian cells. Nat Methods. 2010; 7:631. [PubMed: 20639867]

202. Zeitler P, Tannenbaum GS, Clifton DK, Steiner RA. Ultradian oscillations in somatostatin and growth hormone-releasing hormone mRNAs in the brains of adult male rats. Proc Natl Acad Sci U S A. 1991; 88:8920. [PubMed: 1681547]

203. Zenklusen D, Larson DR, Singer RH. Single-RNA counting reveals alternative modes of gene expression in yeast. Nat Struct Mol Biol. 2008; 15:1263. [PubMed: 19011635]

Highlights

- Transcription is a dynamic and stochastic, nevertheless tightly regulated cellular function
- Intrinsic and extrinsic signals generate diverse transcriptional responses and affect physiology
- Regulatory mechanisms driving specific transcriptional programs remain to be discovered

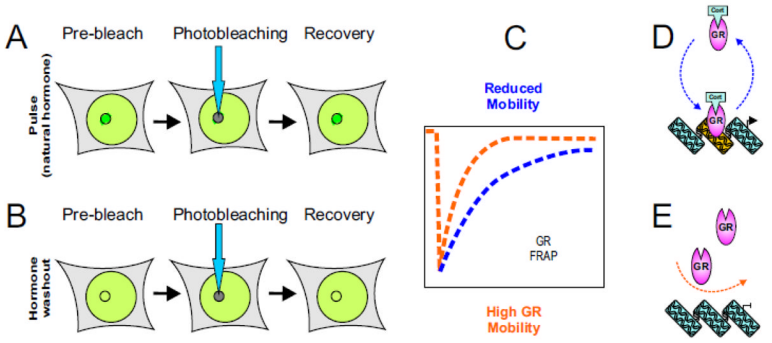

Fig. 1. Chromatin cycle – transient interactions of glucocorticoid receptor (GR) with chromatin revealed by fluorescent recovery after photobleaching (FRAP) method

A) Fast recovery upon laser photobleaching of the GFP-tagged GR (GFP-GR) associated with its respective regulatory elements demonstrates the transient nature of these interactions. **B**) Even faster GFP-GR recovery is observed upon hormone withdrawal. **C**) GFP-GR recovery profile in the presence and absence of hormone reflect differential affinity of the hormone-bound and hormone-free receptor to its regulatory elements. **D**) Schematic representation of the transient, nevertheless slower exchange of the hormone-activated receptor with the regulatory elements is reflected in the reduced mobility of the FRAP curve in C. **E**) Hormone-free receptor loses its affinity to specific sites and is extremely mobile as demonstrated by its FRAP curve in C.

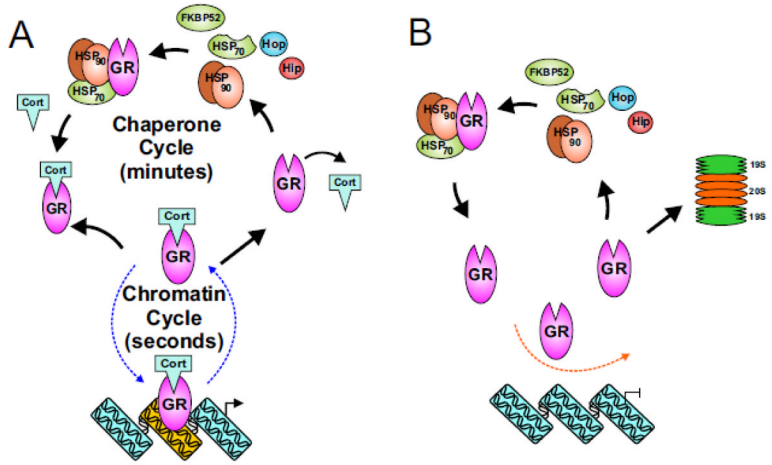

Fig. 2. Chaperone cycle

A) Upon dissociation from regulatory elements, GR will either re-enter the chromatin cycle or lose its hormone. Hormone–free GR is incapable of binding hormone on its own, and regains its hormone-binding affinity only after association with nuclear chaperone machinery. **B**) In the absence of hormone, GR association with chaperone machinery protects it from proteasome-mediated degradation. Chaperone cycle and proteasome machinery recycle or degrade GR molecules, respectively, serving as a GR quality control system.

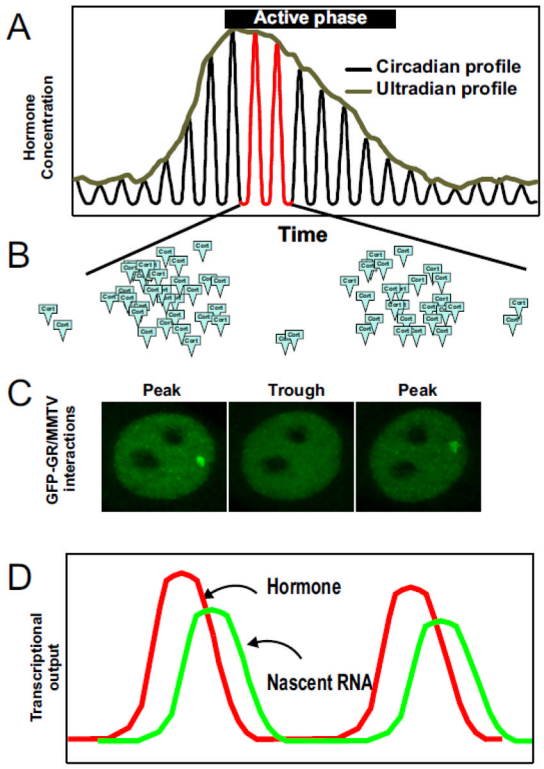

Fig. 3. Ultradian hormone fluctuations induce cyclic release of nascent RNA from GR-regulated genes

A) Glucocorticoids are released from the adrenal glands in circadian, as well as ultradian manner. **B**) Schematic representation of the hormone level fluctuations. **C**) During the ultradian peak GFP-GR accumulates at the MMTV array locus, while the ultradian trough is accompanied with a redistribution of the GFP-GR molecules due to the loss of the affinity of hormone-free receptors to their regulatory elements. Photos are adapted from [167]. **D**) Hormone level fluctuations lead to pulsatile release of nascent RNA from GR-regulated genes, or gene pulsing.