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Reading oscillatory instructions: How cells achieve time-dependent responses to oscillating transcription factors

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Introduction

When writing a perspective about recent developments in oscillatory signaling, it is hard to ignore one oscillatory phenomenon that has affected our lives over the past two years: the COVID-19 pandemic, in which pulsatile spikes in case counts forced us to continuously adjust our behavior. Some adjustments occurred quickly, such as the implementation of lockdowns, while others took time to manifest, such as vaccine development; but with each wave, new information emerged that altered our response to subsequent waves.

Cells in an organism, like us, face the challenge of responding to oscillatory signals — and like us, their response depends not only on the level of a given signal at a moment in time, but also on the history of that signal. For instance, oscillating transcription factors (TFs), whose levels or sub-cellular localization change over time in a pulsatile pattern, often show time-varying patterns of target gene expression, with some that are transcribed quickly (akin to the stockpiling of hand sanitizer that occurs as soon as COVID cases rise), and others that turn on more slowly with each pulse (akin to school closures, which may lag in implementation). In addition to effects that occur with each pulse of a TF, epigenetic changes triggered by early pulses can affect transcriptional profiles in response to later pulses (akin to how vaccine development in response to the initial COVID wave altered mortality rates). Finally, transcription factors themselves may undergo changes over time — e.g. due to post-translational modifications or changes in available binding partners — that affect their function (akin to how the emergence of new COVID variants alters their impact).

In this review we will discuss recent advances in our understanding of how cells respond to oscillatory transcription factors, focusing on three general mechanisms that determine the kinetics of gene expression: (1) promoter activation kinetics and TF binding times; (2) target genes' mRNA and protein half-lives; and (3) chromatin modifications. We provide specific examples for each mechanism and highlight how they enable oscillatory transcription factors to induce temporally varying genetic programs that would be difficult to implement without pulsatile signaling.

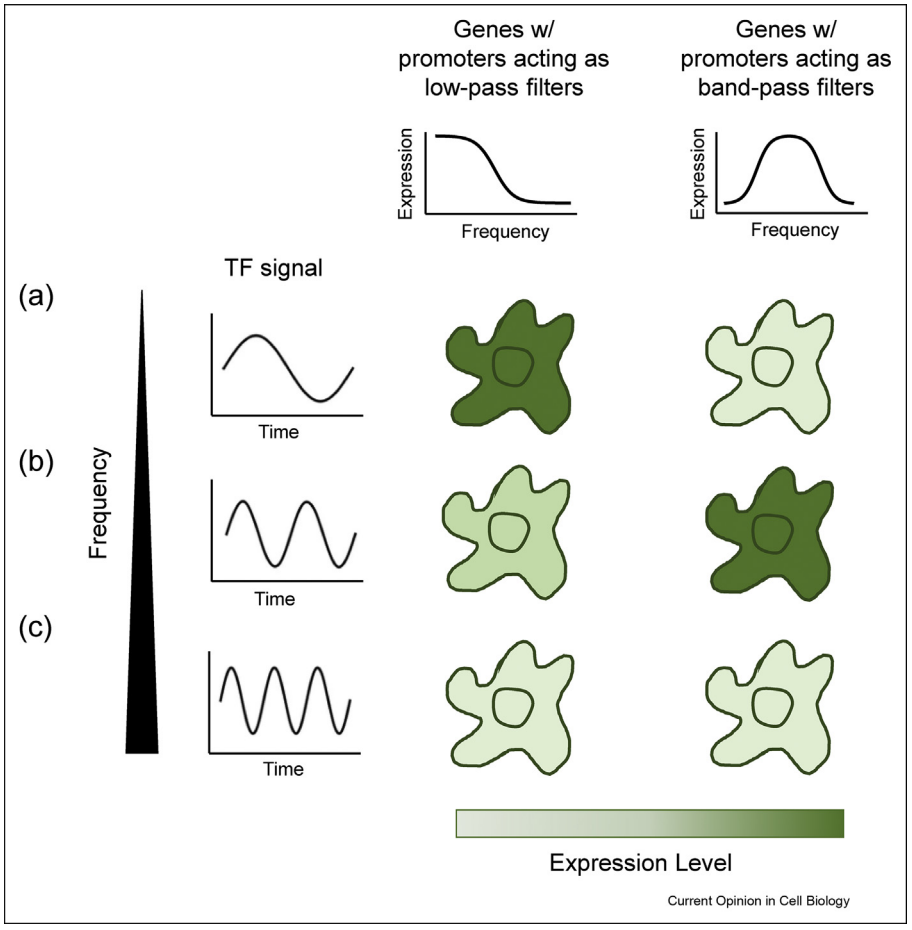
Transcription kinetics modulate the function of oscillatory TFs

The activation kinetics of different promoters in response to a TF can alter target gene dynamics in important ways. Promoters that turn on quickly can yield gene expression even under high-frequency pulsatile TF stimulation, while those that turn on slowly might require sustained or low-frequency pulsatile TF stimulation [1]. The tumor suppressor p53 is a TF whose levels oscillate following DNA damage with ionizing radiation [2,3]. Under these oscillatory dynamics, cells induced genes leading to cell cycle arrest and survival, while under sustained p53 expression (obtained by adding a pharmacological inhibitor of p53 degradation), cells induced genes promoting senescence [3]. When p53 pulse frequency was modulated, two target genes with comparable half-lives, *MDM2* and *CDKN1A*, responded differently to p53 inputs. The *CDKN1A* promoter acted as a low-pass filter (maximally expressing p21 with low-frequency p53 stimulation), and the *MDM2* promoter acted as a band-pass filter (maximally

expressing Mdm2 at the natural frequency of p53 oscillations) [4]. Thus, p53 can generate different response kinetics in its target genes depending on its oscillation frequency. By optogenetically controlling the nuclear localization of the yeast TF Crz1, it was found that Crz1 target gene promoters also differed in their responses to TF dynamics. Some showed higher expression under pulsatile dynamics, while others were more highly expressed under sustained Crz1. These differences were attributed in part to nucleosome occupancy and the kinetics of promoter remodeling [5]. Although TF dynamics were artificially perturbed in these studies, frequencies of TF oscillations can also vary in natural contexts (e.g. mouse p53 oscillates at a higher frequency than the human version) [6]. Figure 1 illustrates how gene expression can vary with TF signal frequency due to promoter kinetics. Further studies investigating target promoter activation under variable TF oscillation frequencies could reveal more about how TF oscillations cooperate with promoter properties to activate specific gene expression programs.

The dwell time of a TF on its binding site might also play a role in filtering oscillatory TF signals. Many TFs have dwell times on the order of seconds [7] to minutes [8], and a single TF can have different binding times at different genomic loci [8]. In addition, nucleosome occupancy can affect binding time as was shown for the TF Gal4, whose binding was reduced by nucleosomes, affecting levels of gene expression [9]. Short-term binding of TFs could allow for differential gene expression patterns between oscillatory and sustained signaling. In neural progenitor cells (NPCs), where the TF Ascl1 has a short dwell time, sustained but not oscillatory Ascl1 drives neuronal differentiation [10]. Recently, it was found that in nondividing oocytes, multiple TFs including Ascl1 remain bound to DNA with a residence time on the order of several hours or even days [11]. Long

Figure 1



The frequency of TF oscillations can alter the expression of target genes depending on their promoter properties. (a) Under low-frequency TF signaling, target genes with promoters that act as low-pass filters are maximally expressed. (b) Under intermediate-frequency TF signaling, target genes with promoters that act as band-pass filters are maximally expressed. (c) Under high-frequency TF signaling, all target genes have low expression.

dwell times (e.g. on the time-scale observed for Ascl1) could provide a mechanism for low-pass filtering of oscillatory TF signals. It will be interesting to investigate whether perturbing Ascl1 dwell time in NPCs, in a way that mimics the longer dwell times observed in oocytes, will impact differentiation in response to oscillatory Ascl1; specifically, whether increased dwell times will make the system insensitive to oscillations in total Ascl1 levels.

Stability of mRNA & protein impact gene expression programs in response to oscillatory TFs

The dynamics of proteins are strongly influenced by their mRNA and protein half-lives. For TFs with steady expression levels (e.g. non-oscillatory TFs), target genes with long half-lives will take longer to reach steady state than those with short half-lives, resulting in differential expression of target genes at early timepoints after TF induction. Such differences in expression kinetics are amplified for oscillatory TFs.

mRNA stability is an important factor governing the expression patterns of a TF's target genes; this effect has been demonstrated for the transcription factors p53 and NF-κB, each of which shows stimulus-dependent oscillatory dynamics [12–14]. An outstanding question in the field has been whether – and how – TF pulses are translated to target gene dynamics. In a recent study, genes activated by pulsatile p53 showed three distinct temporal expression patterns [15]. Some oscillated with the same dynamics as p53, while others showed increasing levels over time, with either slow or fast induction. The primary determinant of different transcription patterns was mRNA half-life. Genes with short mRNA half-lives acted as instantaneous readouts of p53 activity and oscillated, while genes with longer mRNA half-lives acted as integrators of p53 levels and rose continuously [15]. Similar trends were seen when the transcription factor NF-κB was induced to oscillate with pulses of TNF-alpha stimulation, with three clusters of activated genes identified under these conditions – those that oscillated, increased quickly, and increased slowly. A combination of experimental data and computational modeling demonstrated that faster mRNA degradation rates predicted oscillatory dynamics, while slower mRNA degradation rates predicted non-oscillatory increasing dynamics [16].

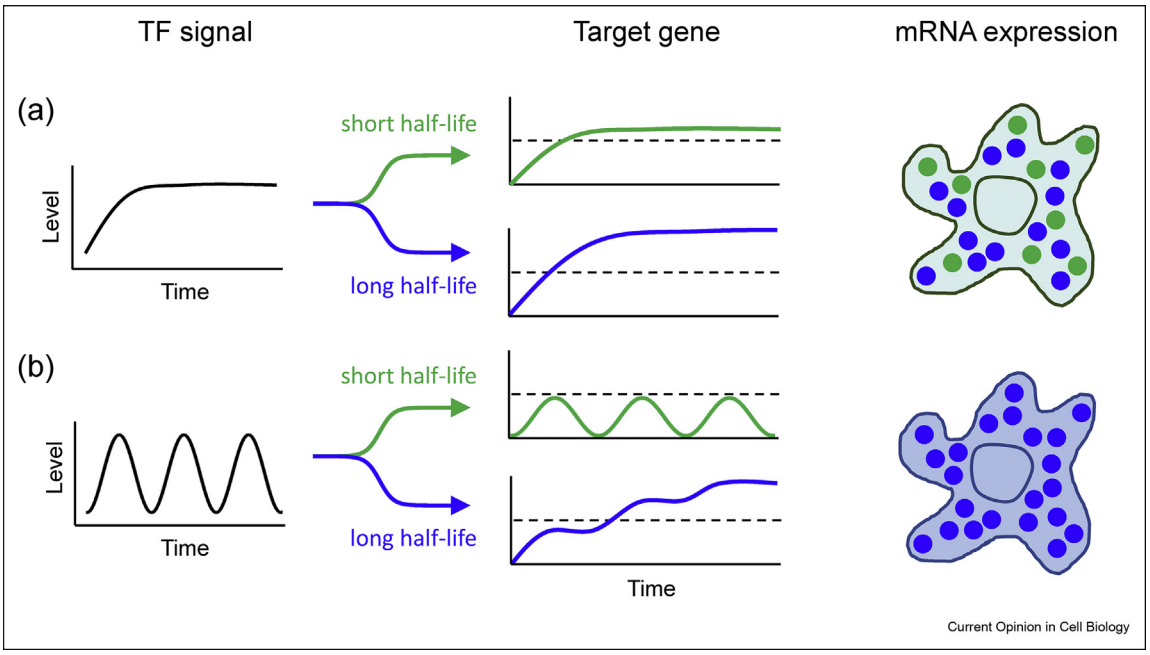
Protein stability also influences how an oscillating TF will affect the expression of its targets. Ordinary differential equation (ODE) models that incorporated mRNA and protein production and degradation rates of p53 target genes predicted that protein degradation played a major role in governing protein expression patterns [17]. For a pulsatile mRNA, short-lived proteins oscillated, whereas long-lived proteins rose continuously. Experimentally stabilizing the MDM2 protein, a p53 target with oscillatory protein expression, decreased its pulse frequency. In addition, pulsatile signaling allowed the largest variety of downstream gene expression kinetics. Specifically, pulsatile mRNAs gave rise to both pulsatile and continuously rising protein levels, whereas non-pulsatile mRNA could not give rise to an oscillating protein [17]. This work suggested a central role for protein degradation rates in determining the ultimate gene expression program induced by an oscillating TF. Figure 2 summarizes how variability in target half-lives can allow for different gene expression patterns between pulsatile and sustained TF signaling.

Epigenetic states modulate gene expression programs in response to oscillatory TFs

Open chromatin with activating modifications is necessary for gene expression, making chromatin state a major determinant of transcriptional output. Many TFs recruit chromatin modifying enzymes to ensure that transcriptional activation acts in concert with chromatin signals [18–20], and oscillations in chromatin state are observed at the target genes of pulsatile TFs [15,21,22]. The additional layer of signal processing afforded by chromatin state changes opens the possibility of executing different outputs at different timepoints (or at early vs late pulses) in response to a single oscillatory TF.

Transcriptional activation can generate both transient and stable epigenetic modifications [15,23]. These modifications can increase transcriptional efficiency, open new genes for transcription, or evolve to inhibit subsequent gene activation by the TF. In the case of an oscillatory TF, early pulses can trigger stable epigenetic modifications and influence gene expression during subsequent pulses. When levels of the TF C/EBPα were induced in a pulsatile manner, chromatin changes brought about by the initial expression of the TF C/EBPα led to more efficient transcription of certain target genes upon its re-expression. An initial pulse of C/EBPα resulted in a loss of H3K27me3 at enhancers that was sustained for 6 days as cells went through multiple divisions, and led to increased transcription of target genes upon restimulation with C/EBPα, consistent with the establishment of transcriptional memory. Increasing the duration of the initial pulse resulted in greater transcriptional memory [24], suggesting that in a naturally oscillating system, low-frequency oscillations could be more effective at inducing chromatin modifications. During hematopoietic differentiation, in contrast, chromatin modulation resulted in TF target genes becoming unresponsive to the TF over time. A model of differentiation was developed in which cells remained in an undifferentiated state when the TF Hoxa9 was expressed, and differentiated when it was inactivated

Figure 2



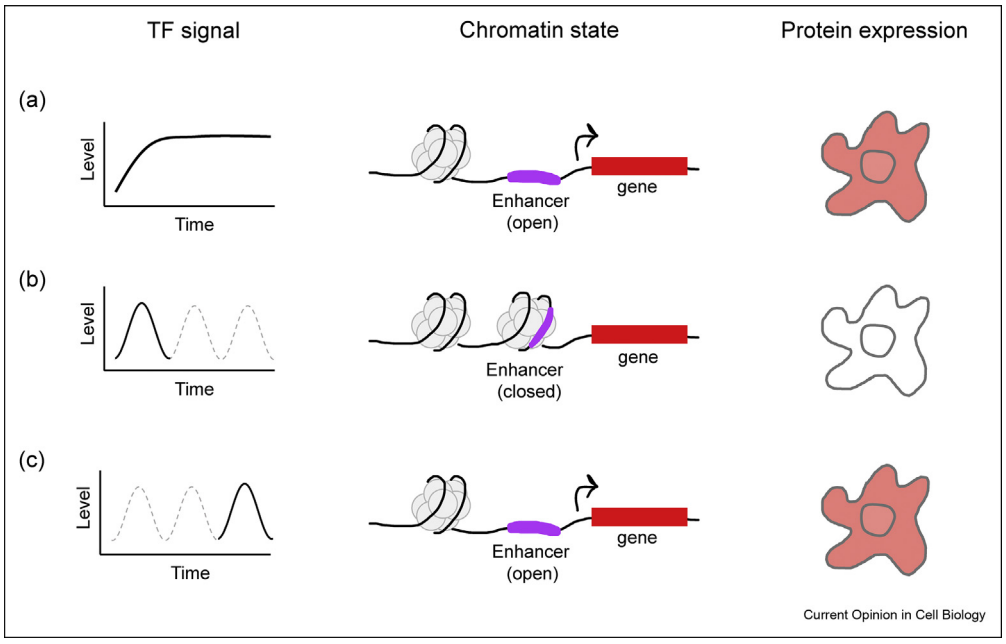
Oscillatory TFs can give rise to different gene expression dynamics depending on mRNA target stability. Targets with a short-mRNA-half-life (green) and long-half-life (blue) are expressed differently in response to oscillatory TF inputs. (a) Under sustained TF signaling, genes with different mRNA half-lives accumulate at a rate and to a maximum level that depends on their mRNA stability. (b) Under oscillatory TF signaling, genes with short mRNA half-life follow the dynamics of their TF and, depending on the frequency of oscillations, may not be able to accumulate. Similar behaviors are observed for oscillating mRNAs giving rise to long- and short-lived proteins.

[25]. Once differentiation was induced, the chromatin at most Hoxa9 target genes became closed and depleted of the activating H3K27ac mark, resulting in a lack of response to Hoxa9 re-expression [26]. Although in this case chromatin changes were induced by inactivating a non-oscillating TF, it is possible that chromatin changes induced in the first off-phase of a naturally-oscillating TF may inhibit expression of its target genes in the second pulse.

Multiple chromatin-based mechanisms were also shown to decode NF-κB dynamics, with chromatin remodeling influencing both the dynamics of gene expression as well as which genes are induced. LPS leads to sustained activity of NF-κB, which preferentially induces one subset of target genes (e.g. IL1α, IL1β, and IL-10), while TNF causes oscillations of NF-κB, which preferentially induces a different subset of target genes (e.g. Csf2 and IL-6) [27]. Such stimulus-specificity can be explained by either regulation of target genes' mRNA half-lives (with long half-lives correlating with LPS-specific expression) or regulation at the level of mRNA production (with higher levels of nascent mRNA associated with LPS-specific expression) [28]. A mathematical model incorporating both NF-κB-dependent chromatin opening and mRNA half-lives captured stimulus-specific gene expression better than a model based solely on mRNA half-lives [28]. A separate study focused on target gene expression in response to re-activation of NF-κB. Initial sustained expression of NF-κB remodeled chromatin at enhancers (Figure 3a), allowing the expression of new target genes upon NF-κB reactivation. Oscillatory NF-κB, however, failed to remodel chromatin at these enhancers or to stimulate expression of associated genes during reactivation [29]. Together, these recent studies show that chromatin remodeling facilitated by NF-κB is a major determinant of whether target genes are preferentially expressed under pulsatile or sustained conditions.

These studies collectively show how chromatin changes in response to TF activity can modulate the gene expression programs induced by the TF over time. For instance, chromatin modifications induced by the initial pulses of a TF can allow transcription of new genes in response to later pulses of that TF (Figure 3b and c). In addition, the duration of chromatin modifications could dictate how gene expression depends on TF pulse frequency, with genes harboring short-lived modifications being more sensitive to TF oscillation frequency, as activating epigenetic markers are lost between pulses. Chromatin based mechanisms could also explain how some genes act as low-pass filters of TF signals; if a TF needs to be present for a certain amount of time in order

Figure 3



Interplay between TF dynamics and chromatin states can modulate gene expression programs. Chromatin changes that depend on TF dynamical patterns and/or the duration of TF signaling can influence the choice and timing of target gene expression. (a) Under sustained TF signaling, chromatin remodeling can allow an enhancer (purple) or other regulatory region to be bound by that TF (or other TFs), leading to transcription of a gene (red). (b) At early time points under pulsatile TF signaling, chromatin remodeling may not take place (enhancer remains shielded by nucleosomes), preventing gene transcription. (c) At later time points under pulsatile signaling, chromatin remodeling may occur, allowing gene transcription. (b-c) Illustrate how gene expression programs governed by a TF can evolve over time.

to open chromatin for transcription, then the short TF pulses observed during high-frequency oscillations might not be sufficient to induce chromatin remodeling.

Future perspectives

In this review, we've highlighted several mechanisms that enable oscillatory TFs to induce temporally varying genetic programs that would be difficult to implement without pulsatile signaling. For example, when epigenetic modifications are caused by an oscillatory TF, they may alter the patterns of gene expression of that TF over time in a manner that depends on pulse amplitude and frequency. Investigating how modulating different parameters of a TF over time, e.g. frequency or amplitude, affects the epigenome will help elucidate how the history of a TF's signal impacts its future effects. Computational modeling can help identify situations in which filtering properties (e.g. high-, low-, or band-pass filters) cannot be fully explained by known mechanisms (e.g. mRNA half-lives and chromatin modifications); this could lead to the discovery of new potential mechanisms of pulse filtering.

TF function can be modulated by post-translational modifications or associated regulatory proteins, and these may vary between pulses of an oscillatory TF, allowing the system to re-evaluate the situation with each new pulse. For example, under sustained expression, p53 is sumoylated by its target protein TRIML2, resulting in reduced expression of genes promoting cell cycle arrest and increased expression of some pro-death genes [30]. Furthermore, activation of some p53 target genes initially depended on NM1, a chromatin regulator that binds with p53 at target gene promoters. Expression of these genes at later stages did not require NM1, suggesting a separate mechanism of gene activation at these later timepoints [31]. It is therefore possible that the selection of target genes activated by p53 may vary between its pulses, providing additional modes of regulating gene expression over time.

Oscillatory TFs might exist in many more systems than currently realized, and we may just be exploring the tip of the iceberg by focusing on known oscillatory transcription factors. Careful measurements at the individual cell level are required to identify additional potential oscillatory TFs. It might be of interest to modulate TF oscillation frequency for a library of TFs to identify those whose gene expression programs vary significantly with different oscillation frequency. Another outstanding question is the extent at which frequency-based encoding by TFs is required to enact specific genetic programs. New approaches combining machine learning with single-cell time-lapse microscopy and

RNA seq will allow us to better understand the extent to which oscillatory TFs influence downstream effectors, cellular decision making, and heterogeneity between individual cells.

As we start to understand COVID waves, and begin to appreciate how these waves affect us, we encounter many more questions: Will the next wave be similar to the previous one, or will it consist of some new variants? How have previous waves changed us to alter our responses to future waves? For TFs that exhibit waves, similar questions remain open. Moving forward, we aspire to gain a better understanding of why (and when) TF oscillations are necessary to induce gene expression patterns that are required for specific cellular functions and outcomes.

Conflict of interest statement

Nothing declared.

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* of special interest
** of outstanding interest

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