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The impact of transcriptional cycling on gene regulation

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Research of the past decade showed that transcriptional regulation could be a highly dynamic and cyclical process. Many transcription factors and their co-regulators cyclically associate with a periodicity of 30–75 min with regulatory chromatin regions resulting in dynamically changing chromatin marks and cyclical activities of RNA polymerase II in mRNA synthesis.

Key words: transcription, gene regulation, nuclear receptor, chromatin, stochastic modeling

Abbreviations: ChIP, chromatin immunoprecipitation; CoA, coactivator; CoR, co-repressor; HAT, histone acetyltransferase; HDAC, histone deacetylase; PDK4, pyruvate dehydrogenase kinase 4; TF, transcription factor; TSS, transcription start site

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The process of transcriptional regulation is of central importance for all fundamental decisions in development and an organism's responses to environmental challenges. In eukaryotes transcription is impaired by a repressive chromatin environment of gene regulatory regions. Therefore, prior to transcription initiation, repressive complexes, including co-repressor (CoR) proteins and histone deacetylases (HDACs), have to be replaced by coactivator (CoA) complexes, which directly affect chromatin structure via histone acetyltransferase (HAT) activity. DNA-binding transcription factors (TFs) serve as adaptors between gene regulatory regions and these chromatin modifying enzyme complexes. In this way, TFs mark those genes that are supposed to change their transcriptional level in response to an environment signal. Moreover, via mediator CoA complexes, TFs link to the basal transcription machinery with RNA polymerase II as its core.

Historically, transcriptional regulation was considered as a continuous process, since most fundamental observations were based on static biochemical investigations. However, the development during the last decade of novel techniques, such as in vivo imaging, chromatin immunoprecipitation (ChIP) and chromosome conformation capture assays, allowed observations that indicate that transcriptional regulation could be a cyclical process. Using time-resolved ChIP, Shang et al.[1] and, later, Métivier et al.[2] demonstrated that several CoAs were recruited in a cyclical fashion to the estrogen receptor responding chromatin region of the human *trefoil factor 1* (also called *pS2*) gene. Similar observations were made with the androgen receptor on the human *kallikrein 3* (also called *PSA*) gene,[3] with the thyroid hormone receptor on the human *dio1* gene,[4] with the vitamin D receptor on the human genes *24-hydroxylase*[5,6] and *CDKN1A* (also called *p21*),[7] and with the peroxisome proliferator-activated receptor δ on the human *pyruvate dehydrogenase kinase 4* (*PDK4*) gene.[8] Interestingly, all these TFs are members of the nuclear receptor superfamily and are inducible by small lipophilic molecules.[9] These studies indicated a cyclical nature of TF, RNA polymerase II and co-regulator association with the regulatory regions and the transcription start site (TSS) showing a periodicity of 30 to 75 min. Comparable periodicities were also observed concerning looping between the regulatory regions and the TSS and for mRNA accumulation.

In a first, more simplified analysis this suggests that transcriptional cycling depends on stimulus availability, association and dissociation of the TF with and from its specific DNA binding sites, and, finally, a possible removal of the TF and its co-regulators through proteasomal degradation. These steps first generate transcriptional competence followed by productive transcription and finally limit transcription through the clearance of

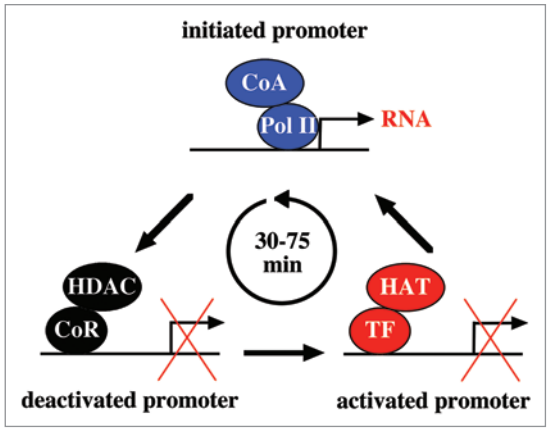

Figure 1. Model of transcriptional cycling. The model depicts the three phases of transcriptional cycling, of which only the initiation phase results in the synthesis of mRNA. Please note that only the core proteins of the respective complexes are shown, we assume that each protein complex contains a dozen or more components.

regulatory regions.[10,11] However, these models are too simple to explain transcriptional cycling with a periodicity of 30–75 min.[8] Therefore, we introduced a model based on stochastic modeling explaining how individual cycling cells can be synchronized in a population of cells to produce oscillating patterns of mRNA accumulation. The specific binding of individual TFs and their co-regulators to regulatory chromatin regions is relatively short (approximately 100 s),[12] while the association of protein complexes to these regions seems to be in the order of 10–20 min. Therefore, we assumed that at least 30 proteins and 6 irreversible (i.e., energy consuming) steps participate in each transcription cycle of in average 60 min. This is in agreement with a recent publication indicating that cycling indeed required energy consumption.[13] In principle, the recruitment and assembly of these complexes could occur in a random fashion, in a partially random fashion (partially determined order) or in a uniquely defined sequential order. In addition, the complexes could already be preformed in the solution of the nucleoplasm or assembled on the DNA. Based on physiologically relevant protein concentrations, on/off rates and equilibrium constants, we found that only the models based on sequential or partially determined orders of transcription complex assembly produce outputs that are consistent with the kinetics of our experimental observations.

We distinguish three phases per transcription cycle (**Fig. 1**): (i) an activation phase in which TFs and HATs are recruited to the regulatory regions in order to locally open chromatin, (ii) an initiation phase in which RNA polymerase II and mediator proteins bind and mRNA transcription starts and (iii) a deactivation phase where HDAC and CoR association lead to chromatin condensation. Hierarchical clustering analysis of the ChIP association profiles of TFs, co-regulators and chromatin modifications on the *PDK4* gene confirmed this subdivision.[8] Our model suggests that only an assembly of protein complexes on the DNA and not in solution can reproduce the experiments. The order of the proposed phases has to be strict, because otherwise cyclic behavior would not be observed in the experiments, such as time-resolved ChIP. In contrast, the cycling of mRNA seems to occur by a different mechanism, namely: the gene cycles between an active state, during which mRNA is synthesized and degraded, and an inactive state, during which only mRNA degradation occurs (**Fig. 1**). In this way, the complexes on the regulatory regions reflect the information on the transcription activation and repression status of the respective genes. Moreover, our stochastic transcription model for single cells predicted that on the population level the transcription cycles would fade out 5 h after stimulation, which we confirmed in a long time course experiment of *PDK4* mRNA accumulation.[8]

Not every gene will show transcriptional cycling on the level of mRNA accumulation. Transcriptional cycling requires a burst of transcription, which in most cases is the result of the activation of an inducible TF, such as a member of the nuclear receptor superfamily, which must have a dominant role on the activation of the respective gene. At the example of the *PDK4* gene we showed that during the initiation phase approximately 18 mRNA molecules per gene are generated.[8] This is in accordance with single-cell studies on transcription that display transcriptional bursts.[14-17] In fact, bursty transcription is a quite general phenomenon employing a vast array of mechanisms, such as nuclear translocation and oscillations of second messengers (Ca^{2+}, cAMP).[14,18] Moreover, the lifetime of the initiation phase, which determines the size and duration of a transcription burst, is modulated and influenced by epigenetic changes of the involved chromatin regions. The half-life of the induced transcript should be lower than the periodicity of one transcription cycle, i.e., less than 60 min in average. This reduces the list of genes that show transcriptional cycling to those that encode TFs, kinases and other short-lived regulatory proteins. Furthermore, in order to also see transcriptional cycling on a cell population level, cells have to be synchronized in their individual cycles. The stimulation with a nuclear receptor ligand was shown to be sufficient for a population level synchronization of cells; although in some studies[1,2] a pre-treatment with the RNA polymerase II inhibitor α-amanitin was applied.

Oscillations are a widespread phenomenon in cell biology, including those in glycolysis,[19] calcium signaling[20] and signal transduction.[21] Such oscillations arise from mechanisms that can be understood in terms of nonlinear dynamics. The periodic phenomenon of transcriptional cycling is of a different nature. Any single molecule will display periodic dynamics in its state progression, if its state diagram is cyclic. The modus operandi of reusable factors, such as TFs and their co-regulators, and of epigenetic changes of the chromatin status is intrinsically cyclic, since they act as catalysts or scaffolds. Ensembles of such systems can subsequently display

synchronized cycles depending on the stochastic distribution functions of their cycling time.

There were also other forms of transcriptional cycling observed. Low frequency stimulations of cells with tumor necrosis factor were shown to induce cycling of the abundance of the TF NFκB in the nucleus.[22] Moreover, pulsative entrainment of cells with ultradian release of cortisol induced transcriptional cycling of the glucocorticoid receptor,[23] which is another member of the nuclear receptor superfamily. Interestingly, these ligand-induced transcriptional cycles are not observed when the synthetic glucocorticoid receptor ligand dexamethasone, which stabilizes the receptor for longer periods than the natural ligand cortisol,[23] is used. We made similar observations when using constant (i.e., non-pulsative) stimulation experiments with the potent synthetic vitamin D receptor agonist Gemini. Gemini failed to induce transcriptional cycling of the human *IGFBP3* gene, while the natural ligand does.[24] These observations may have implications for the therapeutic application of synthetic nuclear receptor ligands and may explain some of their side effects.

Why do genes show transcriptional cycling? The most obvious answer to this question is that it allows better control of gene transcription. When a gene has to confirm every 60 min if its transcription is still required it can be silenced far quicker than without this control mechanism. In this way, the system is reset every 60 min and can respond more accurately to a new incoming signal. However, this concept has to be experimentally validated. In parallel, transcriptional cycling can dampen the overall response of the system and helps to avoid overboarding effects. The transcription cycle can be stopped in several ways, such as by lack of the inducing signal for the TF or by changes to the chromatin activation status.

For a more general answer to the sense of transcriptional cycling, one has to consider that transcription is a dynamic process where TFs and co-regulators have high mobility[25] and relatively short times of contact with their specific chromatin binding sites.[12] Moreover, chromatin activation and repression states rapidly change and nucleosomes slide along genomic DNA. This multitude of events therefore indicates that gene transcription is stochastic in nature, in other words, it is noisy.[15,26] This raises the question of whether transcriptional cycling could be the result of noisy transcription. The answer to this question depends on whether the processes in transcriptional cycling are controlled by negative feedback mechanisms, which in most cases reduce the effect of noise, or by positive feedback processes, which are able to enhance noise.[27] Several biochemical oscillations, such as circadian rhythms, show resistance to noise; they are entrained by periodic exposure to signals, such as sunlight, but are capable of "free running" without any external signals. These oscillations display a remarkable fidelity in their duration from cycle to cycle, but the source of this reliability is still unclear and may depend on properties of the network used to implement the oscillator[27] and on cell-cell communications.[28] We assume a similar situation for transcriptional cycling, which is entrained by the stimulus of the respective TF.

The concept of transcription factories[29] suggests that transcription does not take place in a uniform fashion throughout the genome but is concentrated in transcriptional factories to which active genes are recruited.[30] A limited number of these factories (100–200) are responsible for most mRNA transcription in the cell. This raises the question of whether only a limited number of transcription factories are cycling and are responsible for all bursty expressions of mRNAs with short half-lives or whether these genes are evenly handled by all transcription factories. The latter case is more likely, since there is no indication that the transcription of mRNAs with short half-lives is concentrated on certain regions of the nucleus. This would suggest that all transcription factories are cycling and that this cycling may affect all transcribed genes. Moreover, under these conditions the periodicity of cycling would be the same for all genes of a given cell, while it may vary between different cell types. Interestingly, the pre-mRNAs of all genes have short half-lives, i.e., all the ones that show bursty transcription may cycle. We are presently investigating this question.

In summary, the observation of transcriptional cycling and its explanation by stochastic modeling provides a novel mechanistic insight into gene transcription. This offers an integrative framework of the basal architecture of transcription that accounts for similar observations for different genes across various cell types and eukaryotic species. Deeper insight into the kinetic mechanism of transcription initiation can probably primarily be gained through single-cell studies.

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