BBA - Gene Regulatory Mechanisms 1861 (2018) 178–189

Contents lists available at ScienceDirect

BBA - Gene Regulatory Mechanisms

journal homepage: www.elsevier.com/locate/bbagrm

One signal stimulates different transcriptional activation mechanisms

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ABSTRACT

Transcriptional activation is often represented as a “one-step process” that involves the simultaneous recruitment of co-activator proteins, leading to a change in gene status. Using Drosophila developmental ecdysone-dependent genes as a model, we demonstrated that activation of transcription is instead a continuous process that consists of a number of steps at which different phases of transcription (initiation or elongation) are stimulated. Thorough evaluation of the behaviour of multiple transcriptional complexes during the early activation process has shown that the pathways by which activation proceeds for different genes may vary considerably, even in response to the same induction signal. RNA polymerase II recruitment is an important step that is involved in one of the pathways. RNA polymerase II recruitment is accompanied by the recruitment of a significant number of transcriptional coactivators as well as slight changes in the chromatin structure. The second pathway involves the stimulation of transcriptional elongation as its key step. The level of coactivator binding to the promoter shows almost no increase, whereas chromatin modification levels change significantly.

1. Introduction

Transcriptional regulation is the main means of modulating protein expression levels in eukaryotic organisms. Genes encoding proteins of the transcriptional apparatus represent a significant segment of eukaryotic protein-coding genes [1]. A large group of transcriptional regulators encompassing the DNA-binding proteins is primarily responsible for the specificity of transcriptional activation/repression. Another large group of transcription-related proteins, named transcriptional coactivators, act by interacting with DNA-binding proteins and are involved in the regulation of a diverse array of genes [2,3].

The subunit composition of the majority of coactivator complexes was described several years ago [4]. Their molecular functions, including their roles in transcriptional activation, are the subject of many current investigations [5–7]. What is much more rarely studied is the functional and structural connections between different coactivator complexes. An understanding of these interactions is indispensable when one aims to obtain a comprehensive picture of the processes involved in transcriptional activation.

Most of the previously described relationships between coactivators involve the Mediator complex. As one of the key structural components of the promoter-bound protein complex, Mediator is a direct target of diverse transcriptional regulators [8,9]. The relationship between the chromatin modifying and chromatin remodelling complexes is another example of the tight functional connection between coactivators. Indeed, many chromatin remodelling complexes include protein subunits containing domains capable of reading histone modifications. A significant segment of chromatin modifying enzymes can govern the recruitment/removal or functional activation of chromatin remodelling complexes by generating covalent histone marks [10,11]. Much less is known regarding the precise roles played by a large number of coactivator complexes during transcriptional activation. Existing time-course studies have focused on the role of several well-described coactivators (e.g., CBP/p300, PCAF, SWI/SNF and Mediator) in transcriptional activation [12–14]. Most of these studies sought to determine coactivator behaviour on genes with delayed transcriptional responses (i.e., that take hours to be induced). Only a few studies have described the kinetics of coactivator recruitment, with resolution within minutes [15]. Molecular mechanisms of oestrogen- and progesterone-dependent transcription induction remain the best-known in the field, as these include the largest numbers of described coactivators [16–18].

For a long time, the recruitment of RNA polymerase II to the promoter was believed to be the main mechanism of transcriptional activation and the direct target of coregulatory proteins [19]. It has recently been shown that many eukaryotic genes have a substantial amount of RNA polymerase II associated with their promoters, yet still remain in an inactive state. The transcription of such genes is activated by forcing the RNA polymerase II transition to the coding region,

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https://doi.org/10.1016/j.bbagrm.2018.01.016
Received 28 August 2017; Received in revised form 10 December 2017; Accepted 15 January 2018
Available online 06 February 2018
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thereby stimulating elongation [20,21]. The marker of this process is an increase in the phosphorylation level of the RNA polymerase II. Phosphorylation of the C-terminal domain (CTD) of the Rpb1 subunit on Ser5 is believed to indicate the start of the elongation process, the completion of the mRNA capping process and the synthesis of a short 40–50 bp-long mRNA [22–24]. Phosphorylation of the Rbp1 CTD on Ser2 is believed to mark the formation of the RNA polymerase II complex, which is capable of efficient elongation. However, stimulation of transcriptional elongation has not traditionally been considered an important regulatory step; for this reason, the molecular mechanism underlying this process remains relatively poorly understood. NELF and DSIF complexes, as well as GAF and M1BP proteins, remain the most described participants of the RNA polymerase II pausing process; pTEFb is the most well-known driver, and its action is needed to stimulate transcription [25–30]. Recently, several of transcription complexes have come to be considered players in the pausing process, while others have been reported to act as stimulators of transcriptional elongation [25,31–36].

We believe that the study of the dynamics of transcriptional complexes may be beneficial for greater understanding of the mechanisms of transcriptional activation [5,37–39]. Indeed, ongoing analyses of transcriptional kinetics have shown a number of significant differences compared to the routine comparative study of genes in their active/inactive states. Kinetic studies can help identify additional participants of the transcriptional activation process (which are important for activation, but do not participate in the maintenance of the active state of the gene). Furthermore, tracing the recruitment/removal/functional dynamics of the coactivator may contribute to revealing the most important links between coactivators, as well as to defining the stages of the activation process they participate in.

Ecdysone signalling was recently suggested as a promising model system for the study of transcriptional activation [40,41]. The hormone ecdysone is the primary and almost exclusive driver of Drosophila development [42,43]. Although, the ecdysone cascade has been studied for decades, many important points still remain unclear [44–46]. One of them is the molecular mechanism of ecdysone-dependent transcriptional activation. In a previous study, we characterized several properties of the *dhr3* and *hr4* genes of the ecdysone cascade, whose transcription is rapidly and most efficiently induced in *Drosophila melanogaster* S2 Schneider cells treated with 20-hydroxyecdysone [41]. High activation rates (first changes detected within 10–20 min after hormone treatment) and mild non-stressful conditions under which the induction is performed are the main benefits of the described inducible system. Another advantageous characteristic of *dhr3* and *hr4* genes transcriptional activation is the length of the time interval between the exposure to the induction signal and the cell response, i.e., the activation of transcription. This time period is very suitable for the experimental study of transcription kinetics. An alternative is a well-described system of the heat-shock inducible genes; however, the interval between the induction and the response varies from one to two minutes and is too short for a kinetic study to be performed [47].

The main objective of the current work was to obtain comprehensive data concerning the initial stages of ecdysone-dependent transcriptional activation. Our ultimate goal was to describe the molecular mechanism of this process and to elucidate its functional aspects. For this reason, this work is concerned with the study of the kinetic changes of the RNA polymerase II state, chromatin modification, recruitment and functional activity of the main coactivator complexes, rather than with the analysis of the behaviour of individual DNA-binding proteins. We have concentrated here on the study of the promoter-associated processes, as we assumed that the changes in the coactivator recruitment to other regulatory elements and the changes in their functional activity would, over the long run, affect the promoter region. This hypothesis is based on the current model describing the activity of regulatory regions (which suggests that remote DNA sites are physically connected to the promoters of the genes they regulate) [48,49].

2. Results

2.1. Ecdysone-dependent transcriptional activation in S2 cells involves RNA polymerase II recruitment and phosphorylation

We have performed whole-genome analysis of the RNA polymerase II state at the promoters of S2 cells 1 h after ecdysone treatment. Antibodies against the Rpb1 subunit, phosphorylated on Ser2 or Ser5 of its CTD, and anti-Rpb3 antibodies were used in our ChIP-Seq experiments. We have previously performed whole-genome profiling of transcription induction in S2 cells 1 h after treatment with 20-hydroxyecdysone using RNA-Seq [41]. The current experiment sought to examine the RNA polymerase II state at the promoters of the previously described ecdysone-dependent genes (Fig. 1A and Table S1). All genes that demonstrated ecdysone-dependent transcriptional activation in S2 cells were subdivided into four groups according to the transcriptional activation level; the 1st group included genes with the highest activation levels, and the 4th group included genes with the lowest activation levels. We detected a correlation between the changes in the RNA polymerase II state at the promoter and the level of transcriptional activation in the corresponding group of genes. The first group, which comprised the most efficiently activated genes, also demonstrated the most significant increase in the RNA polymerase II binding level and phosphorylation level of Ser2 and Ser5 of the Rpb1 CTD. It is worth noting that overall changes in the RNA polymerase II binding level and phosphorylation state were rather weak, not exceeding three-fold for the most efficiently activated genes. This observation may be accounted for by the fact that a significant amount of pre-recruited RNA polymerase II has been detected on inactive ecdysone-dependent promoters, suggesting that these promoters do not require the additional recruitment of a large amount of RNA polymerase II during activation since they already have a significant level.

In accordance with the existing model of regulation of paused genes, activation of their transcription takes place by phosphorylation of RNA polymerase II, which is pre-recruited to their promoters. To verify the suitability of the activation mechanisms of ecdysone-dependent genes in the existing model, we divided the ecdysone-inducible genes into groups according to the amount of total RNA polymerase II bound to their promoters before the 20-hydroxyecdysone treatment (Fig. S1). Surprisingly, we found no tendency of promoters with greater amounts of polymerase to be activated only by its phosphorylation. All RNA polymerase II forms demonstrated an increase upon transcriptional activation in all groups. Moreover, genes with the highest RNA polymerase II-bound levels tended to further increase these amounts upon activation.

Nevertheless, as we wrote above, the degree of RNA polymerase II increase was found to correlate with the level of transcriptional induction of the gene (Fig. 1A). The increase in the RNA polymerase II phosphorylation level at promoters with the highest activation levels exceeded the respective changes in its general binding level. This effect was clearly demonstrated for the dhr3 and hr4 gene promoters, whose transcriptional levels were found in the RNA-Seq experiments to increase between 80 and 96 times yet became comparable after 1 h of transcriptional induction (Fig. S2) [41]. *dhr3* and *hr4* promoters showed a two-fold increase in the total amount of bound RNA polymerase II, as well as a minimally three-fold increase in its phosphorylation level (Fig. 1B). We hypothesized that the process of transcriptional activation of these two genes combined the stimulation of transcriptional initiation (or RNA polymerase II recruitment) and transcriptional elongation or transition of pre-recruited RNA polymerase II to the coding region of the gene (the marker of this process is an increase in the RNA polymerase II phosphorylation level). We have selected the *dhr3* and *hr4* genes as a model for further detailed study of the primary events in ecdysone-dependent transcriptional activation.

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