Biochimica et Biophysica Acta 1829 (2013) 937–945

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

Transcriptional Control by NF-κB: Elongation in Focus

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ARTICLE INFO

Article history:
Received 28 January 2013
Received in revised form 15 April 2013
Accepted 17 April 2013
Available online 25 April 2013

Keywords:
NF-κB
transcription elongation
P-TEFb
Brd4
DSIF

ABSTRACT

The NF-κB family of transcription factors governs the cellular reaction to a variety of extracellular signals. Following stimulation, NF-κB activates genes involved in inflammation, cell survival, cell cycle, immune cell homeostasis and more. This review focuses on studies of the past decade that uncover the transcription elongation process as a key regulatory stage in the activation pathway of NF-κB. Of interest are studies that point to the elongation phase as central to the selectivity of target gene activation by NF-κB. Particularly, the cascade leading to phosphorylation and acetylation of the NF-κB subunit p65 on serine 276 and lysine 310, respectively, was shown to mediate the recruitment of Brd4 and P-TEFb to many pro-inflammatory target genes, which in turn facilitate elongation and mRNA processing. On the other hand, some anti-inflammatory genes are refractory to this pathway and are dependent on the elongation factor DSIF for efficient elongation and mRNA processing. While these studies have advanced our knowledge of NF-κB transcriptional activity, they have also raised unresolved issues regarding the specific genomic and physiological contexts by which NF-κB utilizes different mechanisms for activation.

1. Introduction

The transcription elongation stage and regulatory factors

Transcription by RNA polymerase II (Pol II) is divided into three mechanistically distinct phases: initiation, elongation and termination. The initiation phase involves promoter recognition, assembly of the pre-initiation complex, formation of an open DNA duplex, and synthesis of the first few nucleotides. Elongation begins after Pol II clears the promoter and enters into the productive synthesis of the mRNA. Termination occurs after recognition of the poly-A termination signal and is followed by the release of the nascent transcript and dissociation of Pol II from the DNA. An important regulatory module for all these transcriptional steps is the C-terminal domain (CTD) of the largest subunit of Pol II. This domain is composed of tandem heptad repeats enriched with phosphate-acceptor amino acids with the consensus sequence YSPTSPS. The Pol II CTD is conserved among all eukaryotes and undergoes extensive phosphorylation [1]. The CTD not only controls transcription but also transcription-coupled mRNA processing [2]. During transcription the CTD undergoes dynamic changes in phosphorylation [3]. The hypophosphorylated form of Pol II CTD is preferentially recruited to promoters where it is subsequently phosphorylated on serine 5 by the TFIIH-associated kinase CDK7, potentiating the transition from initiation to elongation (Fig. 1). As Pol II elongates, serine 2 is increasingly phosphorylated by P-TEFb, while serine 5 phosphorylation is gradually removed by phosphatases (Fig. 1). Thus the serine 5 phosphorylation is usually seen around the transcription start site (TSS), and serine 2 phosphorylation is usually detected in the middle and the end of actively transcribed genes (for review, see [4,5]). However, as discussed below, the situation is more complex than this generalized model and there are several exceptions to this regulatory mode, in particular within the NF-κB pathway. In addition P-TEFb itself is highly regulated and exists in two distinct forms: the active one which consists of the protein kinase Cdk9 and regulatory subunit cyclin T (T1, T2a or T2b); and the catalytically inactive form which is bound and inhibited by the 7SK snRNP complex (consisting of 7SK snRNA, HEXIM1 and additional proteins) [6].

The transcription elongation factors, DRB sensitivity-inducing factor (DSIF, a heterodimer composed of Spt5 and Spt4 subunits) and negative elongation factor (NELF) can function either together with P-TEFb or in a manner independent of it. In many genes, the two factors associate with Pol II shortly after transcription initiation and pause the elongation downstream from the TSS [7,8]. The release of Pol II from pausing occurs when P-TEFb phosphorylates DSIF, NELF and the Pol II CTD leading to dissociation of NELF from the paused Pol II [9–11]. Interestingly, a significant portion of all genes seems to have a paused Pol II downstream from their TSS [12–15]. Pausing is thought to constitute a mechanism to obtain rapid and coordinated transcription during development and in response to external stimuli [16–18].

NF-κB transcription factors

NF-κB was first discovered in 1986 in the lab of David Baltimore as a transcription factor that regulates immunoglobulin kappa light chain in B cells [19,20]. Since then NF-κB has emerged as a family of transcription factors that is activated in response to a variety of

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http://dx.doi.org/10.1016/j.bbagrm.2013.04.007

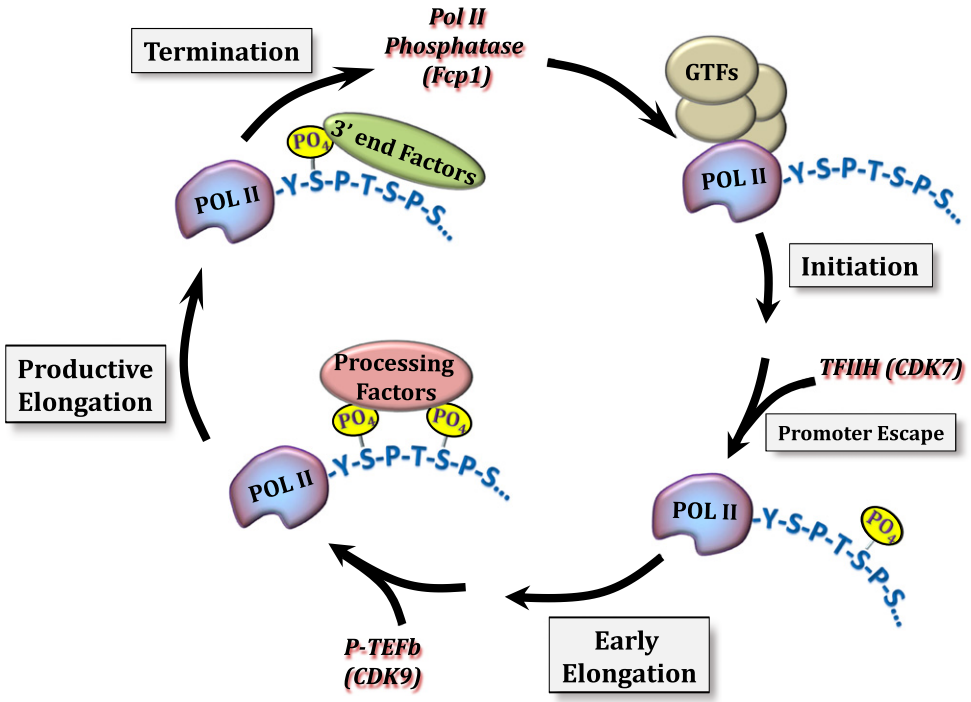

Fig. 1. Pol II CTD phosphorylation cycle. During transcription the C-terminal domain of Rbp1, the RNA Polymerase II large subunit, undergoes dynamic changes in the pattern of its phosphorylation. The hypo-phosphorylated form of Pol II CTD is preferentially recruited to promoters where it is subsequently phosphorylated on Serine 5 by the TFIIH-associated kinase CDK7, correlating with the transition from initiation to elongation. When paused near the promoter, Pol II is predominately phosphorylated on Serine 5. As Pol II elongates, Serine 2 is increasingly phosphorylated by P-TEFb, while Serine 5 phosphorylation is gradually removed by phosphatases, such as downstream of the polyadenylation site only Serine 2 phosphorylation is detected. These phosphorylations promote co-transcriptional recruitment of mRNA processing factors (such as splicing, export and termination factors) to the CTD.

extracellular signals such as inflammatory cytokines, infections and multiple stress situations. Activated NF-κB induces the expression of genes affecting diverse biological processes including development, immunity, tissue homeostasis, inflammatory and stress responses, cell survival and proliferation [21–25]. A subset of genes activated by NF-κB encodes for its own negative regulators thereby forming negative feedback loops. Consequently, the activation of the NF-κB pathway is generally a transient process, lasting up to several hours. However, in certain cancer and inflammatory diseases NF-κB activity becomes abnormally persistent and directly contributes to the pathology [26].

The family of NF-κB is composed of structural homologs that in mammals include NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, and c-Rel. These proteins share a highly conserved DNA-binding and dimerization domain called Rel homology domain (RHD). NF-κB proteins bind to specific DNA sequences called κB sites. All Rel proteins can form homodimers or heterodimers, except for RelB, which can only form heterodimers. This combinatorial diversity contributes to the regulation of distinct, but overlapping sets of genes since individual dimers display variable affinity towards a collection of related κB sites. Moreover, the relative abundance of different NF-κB proteins varies between different cell types with the exception of p50-RelA heterodimer, which is ubiquitous and the most readily detected complex in most cells [27].

The activity of different NF-κB dimers is primarily regulated by interaction with inhibitory proteins that hold these complexes in the cytoplasm in an inactive form. These inhibitors include p105, p100 and IκB α, β, γ and proteins, which interact with NF-κB through an ankyrin repeat domain. When a cell receives an NF-κB activating signal, the interaction between IκB and NF-κB is disrupted enabling NF-κB to rapidly enter the nucleus [28]. Most of the NF-κB activating signals converge on the activation of a high molecular weight complex that contains an IκB kinase (IKK) which stimulates NF-κB in two distinct pathways, the canonical and non-canonical [29]. In the canonical pathway, the signal-activated IKK complex phosphorylates two specific serine residues at IκBα N-terminus, which then trigger IκBα ubiquitination and degradation by the 26S proteasome. In the non-canonical pathway, the p100-RelB complex is activated by phosphorylation of the C-terminal region of p100, which also leads to ubiquitination and degradation of the p100 IκB-like C-terminal sequences to generate p52-RelB. In both of these pathways NF-κB complex is released from IκB proteins and translocates into the nucleus to activate target gene transcription [30].

2. NF-κB-mediated transcription activation

Among the NF-κB family only the RelA, c-Rel and RelB subunits contain transcription activation domains (TAD) in their C-termini, which are conserved among different species but less conserved between family members [31]. The two other NF-κB proteins p50 and p52 are processed from precursor inhibitory proteins p105 and p100, respectively, to shorter proteins containing the RHD but lacking transcriptional activity of their own [32]. However p50 and p52 can form transcriptionally active heterodimers in association with p65, c-Rel, and RelB. Moreover, p52 can activate transcription as a complex with Bcl-3, an IκB-like molecule with coactivator functions [33]. The TADs of NF-κB proteins are responsible for the recruitment of various co-regulators to their target genes such as co-activators, chromatin modifying factors and components of the basal transcriptional machinery [34] (Fig. 2).

The most abundant and well-characterized NF-κB dimer consists of the ubiquitously expressed p65/RelA and p50. Since p65/RelA is responsible for the transcriptional activity of this complex, the mechanism underlying its transcriptional activity has been explored in great details. p65/RelA has a composite TAD within its C-terminal 120 amino acids which is responsible for transcriptional activation. p65/RelA TAD is divided into two distinct transactivation sub-domains, TA1 and TA2 (Fig. 3), both of which are required for the full transcriptional activity