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The transcription elongation factors NELF, DSIF and P-TEFb control constitutive transcription in a gene-specific manner

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ABSTRACT

We examined whether transcription elongation factors control constitutive transcription of the *histone H1(0)* and *GAPDH* genes. Chromatin immunoprecipitaion demonstrated positive transcription elongation factor b (P-TEFb) and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) present together with RNA polymerase II (pol II) throughout the *histone H1(0)* gene, whereas negative elongation factor (NELF) was confined to the 5′ region. Contrarily, DSIF, NELF and pol II were confined to the 5′ region on the *GAPDH*. Inhibition of those factors affected the constitutive transcription of the *histone H1(0)* gene but not the *GAPDH* gene. Thus, NELF, DSIF and P-TEFb control constitutive transcription in a gene-specific manner.

1. Introduction

The three main steps of transcription are: initiation, elongation and termination. All three steps may become rate-limiting and thus determine mRNA output. Although initiation is the most highly regulated step, recent work highlights the crucial regulation of transcription elongation controlling mRNA levels. Regulation of transcription elongation not only controls the continuous and ubiquitous expression of immediate early genes (IEGs) but also the expression of a large number of genes transcription of which may be arrested in particular rapidly reversible circumstances such as starvation [1–4]; furthermore, transcription elongation is directly linked to transcript maturation (capping, splicing, polyadenylation) [2–4].

How is the transcription elongation step controlled? Recent biochemical studies have addressed two major machineries: (i) phosphorylation of the C-terminal domain (CTD) of a large subunit of RNA polymerase II (pol II) and (ii) regulation by transcription elongation factors [4–6]. Predominant phosphorylation of the 2nd and 5th serines in the YSPTSPS repeat in the CTD (CTD Ser-2 and CTD Ser-5, respectively) occurs concomitantly with pol II elongation and initiation, respectively. The CTD Ser-2 phosphorylation is thus likely to be a key for pol II elongation, and principally positive transcription elongation factor b (P-TEFb) phosphorylates the CTD Ser-2 [7,8]. On the other hand, some transcription elongation factors directly modulate processive pol II elongation. For example, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) and negative elongation factor (NELF) cooperatively stall initiated pol II at promoter-proximal regions [9–13]. Once P-TEFb phosphorylates the CTD Ser-2 of the pol II and the C-terminal repeats of Spt5, a subunit of DSIF, the promoter-proximal pausing is overcome, pol II resuming elongation [10,14–16]. The regulatory importance of these two mechanisms controlling transcription elongation machineries has been demonstrated in vitro and in vivo particularly on inducible IEGs (e.g. *c-fos*, MAP kinase phosphatase-1 (*MKP-1*), *junB*, heat shock genes) [9–22].

In *Drosophila* cells promoter-proximal pausing of pol II has been reported also on constantly transcribed house keeping genes including the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene [19,23]. A recent study using chromatin immunoprecipitation (ChIP) analysis combined with microarray technique (ChIP on chip) indicated that approximately 12% of *Drosophila* genes possess disproportionate accumulation of pol II around promoter-proximal regions [24]. These observations show that

Abbreviations: ChIP, chromatin immunoprecipitation; CDK, cyclin-dependent kinase; CTD, C-terminal domain; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; DSIF, DRB sensitivity inducing factor; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; IEG, immediate early gene; *MKP-1*, MAP kinase phosphatase-1; NELF, negative elongation factor; pol II, RNA polymerase II; P-TEFb, positive transcription elongation factor b

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promoter-proximal pausing of pol II soliciting transcription elongation factors NELF, DSIF and P-TEFb may not be restricted to IEGs.

Paradoxically, constitutive transcription also requires some regulatory control, because if it was simply ruled by mass action, it would vary with the general transcription activity as a function of ⟨free⟩ pol II. Indeed, transcription of the *GAPDH* gene is likely to be regulated by promoter-proximal pausing [19,22,23]. Constitutive association of pol II with the *GAPDH* gene renders its transcription insensitive to changes in available pol II. NELF, DSIF and P-TEFb appropriately control promoter-proximal pausing of pol II on IEGs [15,16]. However, knock-downs of NELF and of DSIF hardly affect *GAPDH* transcription [25]. It thus remains unclear whether those factors also regulate the transcription elongation of constitutively transcribed genes. We herein examined whether and how the transcription elongation of a constitutively transcribed gene coding for *histone H*1(0), a lysine-rich member of the linker *histone H*1 family, was regulated directly by NELF, DSIF and P-TEFb.

2. Materials and methods

2.1. Cells and cell culture

Pituitary nueroendocline GH4C1 cells and previously established GH4C1 RNAi cells (NELF-E-RNAi, Spt5-RNAi and control cells [25]) were maintained as reported previously [15,25]. GH4C1 cells incubated in serum-free medium (SFM) for 24 h were stimulated for indicated time with 100 nM thyrotropin-releasing hormone (TRH) (Roche, Indianapolis, IN, USA), 10 nM epidermal growth factor (EGF) (Sigma, St. Louis, MO, USA), 10 ng/mL tumor necrosis factor α (TNFα) (R&D Systems, Minneapolis, MN, USA), or 20 mM KCl. For inhibition of transcription and of cyclin-dependent kinase 9 (CDK9) activity, GH4C1 cells incubated in SFM for 24 h were treated with 30 μM actinomycin D (Sigma) and 30 or 60 μM DRB (Sigma), respectively.

2.2. Chromatin immunoprecipitation (ChIP) assays

ChIP assays were performed as described previously [22]. An anti-cyclin T1 polyclonal antibody (H-245) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), an anti-Spt5 monoclonal antibody (BD Bioscience, Lexington, KY, USA), an anti-NELF-A polyclonal antibody (A-20) (Santa Cruz Biotechnology) and an anti-pol II polyclonal antibody (N-20) (Santa Cruz Biotechnology) were used. The primers and TaqMan probes used in this experiment are as follows: *H*1(0) 5′, forward primer 5′-GGACCACCCCAAGTATTCA-3′, reverse primer 5′-GCCGGCGCGGTTCT-3′, TaqMan probe 5′-FAM-CGTGGCTGCCATCCAGGCAGA-TAMRA-3′; *H*1(0) 3′, forward primer 5′-TAGGAGGACGTTGTTCGTTTCC-3′, reverse primer 5′-GAACT-GAAGTGGCACCAAGCA-3′, TaqMan probe 5′-FAM-TCCCCTCTTCCT-GTGTAAGATGTGGCA-TAMRA-3′; *GAPDH*5′, forward primer 5′-CTCTCTGCTCCTCCCTGTTCTA-3′, reverse primer 5′-CTGGCACTGC-ACAAGAAGA-3′; *GAPDH*3′, forward primer 5′-GGGCAGCCCAGAA-CATCA-3′, reverse primer 5′-CCGTTCAGCTCTGGGATGAC-3′, TaqMan probe 5′-FAM-CCCTGCATCCACTGGTGCTGCC-TAMRA-3′.

2.3. RNA preparation and quantitative RT-PCR

RNA preparation and quantitative RT-PCR were performed as described previously [26]. The primers and TaqMan probes used in this experiment are as follows: *H*1(0), forward primer 5′-CGGACCACCCCAAGTATTCA-3′, reverse primer 5′-GCCGGCGCGG-TTCT-3′, TaqMan probe 5′-FAM-CGTGGCTGCCATCCAGGCAGA-TAMRA-3′; *GAPDH*, forward primer 5′-ATGGTGAAGGTCGGTGT-GAAC-3′, reverse primer 5′-GAAGGCAGCCCTGGTAACC-3′.

2.4. Western blotting

Preparation of whole cell lysates and Western blotting were performed as reported previously [25]. An anti-*histone H*1(0) monoclonal antibody (Abcam, Cambridge, UK), an anti-*GAPDH* polyclonal antibody (FL-335) (Santa Cruz Biotechnology) and an anti-actin polyclonal antibody (Sigma) were used.

3. Results

3.1. Constant levels of histon H1(0) transcripts in spite of rapid turnover

Transcription of the *histone H*1(0) gene has been reported to be constant during cellular proliferation, but *histone H*1(0) mRNA levels rise during cellular differentiation [27,28]. In pituitary neuroendocrine GH4C1 cells *histone H*1(0) mRNA levels were constant and insensitive to a variety of stimuli to which these cells respond (TRH, EGF, TNFα and KCl) (Fig. 1A). Such stability indeed reflects a stable rate of transcription since we confirmed the relatively short half-life of *histone H*1(0) transcripts in GH4C1 cells. mRNA levels of the *histone H*1(0) gene decreased after blocking transcription with actinomycin D, reaching 50% of the control between 60 and 120 min (Fig. 1B). We subsequently verified the half-life of *GAPDH* transcripts, which are also maintained at constant levels in GH4C1 cells [22,25]. Similarly to murine EL-4 cells [29], the half-life in GH4C1 cells was around 24 h (data not shown), much longer than the half-life of *histone H*1(0) transcripts.

3.2. Gene-specific distribution of NELF, DSIF and P-TEFb

We next examined by chromatin immunoprecipitation (ChIP) assays whether the transcription elongation factors NELF, DSIF and P-TEFb were associated with the *histone H*1(0) and the *GAPDH* genes in GH4C1 cells. We designed two primer sets for each gene to monitor in vivo association of those three factors at the promoter-proximal (5′) and 3′ regions (Fig. 2A). The ChIP assay with the anti-pol II (N-20) antibody demonstrated that both regions on the *histone H*1(0) gene were well occupied by pol II. The fact that pol II occupancy of the 5′ region at steady state was larger could be entirely accounted for by increased speed of elongation when pol II progresses towards the 3′ region of the *histone H*1(0) gene. In contrast, relatively less pol II was found at the 3′ region on the *GAPDH* gene when compared to the 5′ region (Fig. 2B). The pol II distribution patterns are consistent with the proposal that pol II features promoter-proximal pausing on the *GAPDH* but not on the *histone H*1(0) gene [19,22,23]. We previously observed similar pol II distribution patterns also in the ChIP assay with another anti-pol II antibody (8WG16) [22]. Additional ChIP experiments with specific antibodies against the three transcription elongation factors showed confined occupancy of NELF at the 5′ region (Fig. 2C) and distribution patterns similar to total pol II of DSIF and P-TEFb on the *histone H*1(0) gene (Fig. 2D and E). Thus, these results suggest that most of pol II elongates together with P-TEFb and DSIF toward the 3′ region on the *histone H*1(0) gene while NELF functions specifically at the 5′ region. In contrast, on the *GAPDH* gene both NELF and DSIF were confined to the 5′ region (Fig. 2C). P-TEFb occupancy of the *GAPDH* gene was low and did not correlate with the occupancy by pol II and DSIF, in contrast to P-TEFb occupancy of the *histone H*1(0) gene (Fig. 2D). Thus, consistent with the relative absence of P-TEFb, the pol II complex would be confined together with NELF and DSIF to the 5′ region on the *GAPH* gene.