Biohelikon: Cell Biology(ISSN: 2348-3741), 2014 2:a18

Review

The Negative Elongation Factor Complex – a poorly understood multi-faceted transcript-processing complex

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Abstract

With the advent of whole genome screens, promoter-proximal pausing of RNA polymerase II (RNAPII) has been shown to play a much more significant role in eukaryotic systems than previously thought. This type of transcription inhibition is dependent on the binding of the Negative Elongation Factor (NELF) complex which is composed of four sub-units, presenting unique domains and consequently fulfilling different functions. Numerous questions still surround the mechanism by which NELF is recruited, stabilised and dissociated from the RNAPII complex. Furthermore, not much is known about which other transcription stages the NELF complex is involved in and through which sub-units it carries out such functions. Based on the current knowledge of the role of NELF in transcription pausing, it is hypothesised that different interaction partners are required to direct context-specific functions of the NELF complex. This review covers some of the known roles and contexts in which NELF acts in an attempt to identify key questions for future NELF-dependent transcriptional research.

Keywords
Transcription inhibition, Negative Elongation Factor complex, promoter-proximal pausing, transcription regulation model, transcription factors

Highlights

1. The Negative Elongation Factor (NELF) is a multi-subunit transcription elongation factor
2. The great majority of human protein coding genes exhibit promoter-proximal pausing of RNA Polymerase II (RNAPII) as a result of NELF action
3. NELF can only bind to a pre-formed 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) Sensitivity-Inducing Factor (DSIF)/RNAPII complex
4. NELF is involved in multiple mRNA processing steps during transcription by interacting with different factors
5. Transcription factors e.g. GAGA factor can recruit proteins involved in promoter-proximal pausing of RNAPII

The NELF complex

The Negative Elongation Factor (NELF) complex, originally isolated through its suppression of transcription elongation, is a multi-subunit transcription elongation factor composed of four sub-units: NELF-A (66kDa), NELF-B (62kDa), NELF-C (60kDa) or NELF-D (59kDa), and NELF-E (46kDa) [1-4]. NELF induces a process called promoter-proximal pausing of RNA polymerase II (RNAPII) [5-7].

NELF-A is encoded by the *WHSC2* gene, a candidate gene for Wolf-Hirschhorn syndrome – characterised by a distinctive craniofacial phenotype, growth and mental retardation, seizures and cardiac abnormalities [3,8-10]. Interestingly, the N-terminal segment of NELF-A shows sequence similarity to the hepatitis delta antigen (HDAg), which binds to RNAPII and activates transcription elongation, indicating that NELF-A mediates the interaction between NELF and RNAPII [3,7].

NELF-B (also known as cofactor of BRCA1 – COBRA) has been reported to physically interact with the product of breast/ovarian cancer susceptibility gene, BRCA1 and computer analysis has shown that this interaction occurs between a coiled-coil structure in NELF-B and a leucine zipper motif in NELF-E [1,11]. It has been shown that NELF-B inhibits the growth of oestrogen receptor α (ERα) positive breast cancer cells *in vitro* [12] and that NELF-B expression is reduced in several established breast cancer cell lines [13]. A study of a cohort of breast ductal carcinoma samples from patients with known clinical outcomes revealed that a low level of NELF-B mRNA is associated with metastatic breast cancer [14]. In a different context, mice homozygous for a mutation in NELF-B have an inner cell mass deficiency and die at the time of implantation, providing evidence as to the critical role of NELF-B in early mouse embryogenesis [15].

NELF-C and NELF-D are the least studied of the NELF sub-units. It is known that they arise from a common mRNA species through the use of alternative start codons [1] and that they are highly similar in sequence to the *Drosophila* protein TH1, which as yet has no known function. However no

other functional information has been reported in the literature.

The structure of NELF-E is characterised by an N-terminal leucine zipper motif, a central domain rich in arginine and aspartic acid (Arg-Asp or R-D) dipeptide repeats (the RD motif), and a C-terminal RNA recognition motif (RRM). RRMs, which typically encompass 80 to 90 amino acids with two highly conserved elements called RNP1 and RNP2, often bind to RNA in a sequence- or structure-specific manner [16,17]. The RRM of NELF-E is capable of binding to various nascent RNA transcripts synthesised by RNAPII, and in so doing, repressing transcription [4].

Promoter-Proximal Pausing

In yeast, RNAPII is typically distributed roughly uniformly across transcription units, suggesting a context in which RNAPII is not regulated after transcription is initiated [18]. However, in higher eukaryotes, RNAPII-binding is concentrated near the Transcription Start Site (TSS) of many genes, implying the action of a control process referred to as promoter-proximal pausing.

Promoter-proximal pausing of RNAPII is a mechanism that finely controls the levels of gene expression, as opposed to, switching gene expression on and off. This is brought about by the physically binding of 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) Sensitivity-Inducing Factor (DSIF) to RNAPII, without affecting the catalytic activity of the latter, but creating a scaffold for NELF to bind to this pre-formed RNAPII/DSIF complex [2,4,7]. Transcription inhibition of RNAPII is alleviated by the recruitment of chromatin-specific transcription elongation factor Facilitates Chromatin Transcription Complex (FACT), together with the protein kinase Positive Transcription Elongation Factor b (P-TEFb), which through its Cyclin-dependent kinase 9 (Cdk9) sub-unit binds to and phosphorylates the C-terminal repeat domain (*CTD*) of RNAPII and DSIF. Upon phosphorylation, DSIF is converted from a negative to a positive regulator of transcription, NELF is removed and RNAPII can proceed to produce full-length transcripts (Fig. 1) [2,7,20-25].

In *Drosophila*, promoter-proximal pausing of RNAPII is present in approximately 10% of genes in early embryos and 20% of genes in adult S2 cells [26,27], acting as a checkpoint to regulate transcription [28,29]. Even more so, in human embryonic stem cells, approximately 75% of protein coding genes exhibit promoter-proximal pausing of RNAPII, while only about half of these genes produce detectable full-length transcripts [30].

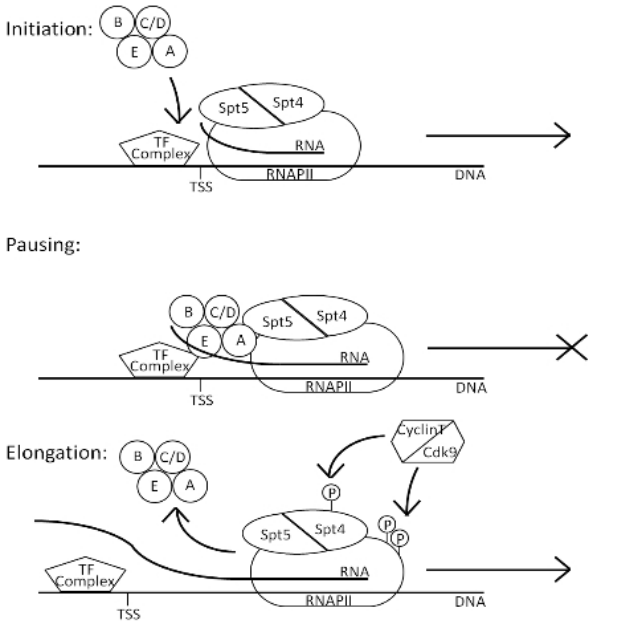

Figure 1 Initiation – The transcription factor complex (TF Complex) binds to the promoter, recruiting RNA Polymerase II (RNAPII) to the transcription start site (TSS). DSIF, composed of two sub-units (Spt4 and Spt5), then binds to RNAPII and a short RNA strand begins to form. Pausing – The negative elongation factor complex made up of the sub-units Nelf-A, Nelf-B, Nelf-C/D, Nelf-E (A, B, C/D, E) is recruited to the TSS and hypothesised to be stabilised by binding to the TF complex. The RNA strand formed binds to the RMM domain of Nelf-E, blocking the elongation process. Elongation – A stimulus brings about recruitment of P-TEFb (CyclinT and Cdk9), which phosphorylates RNAPII and DSIF causing the dissociation of the negative elongation factor complex (A, B, C/D, E) and allowing the extension of the RNA strand, forming a full transcript.

Biological significance of NELF

The importance of NELF and promoter-proximal pausing of RNAPII is evident in response to intracellular and extracellular stimuli e.g. heat shock, lipopolysaccharide (LPS) and oestrogen [31,37] NELF localises with RNAPII just downstream of the TSS of heat shock genes when they are not induced, where it collaborates with DSIF to establish promoter-proximal pausing of RNAPII, by associating with nascent RNAs, 20–60 nucleotide long [6,38,39]. Upon heat shock, activating factors trigger the release of RNAPII from its paused state, and there is a rapid production of full-length transcripts from Heat Shock Protein (HSP) genes[6,38,39]. A loss of NELF however, does not affect the rate of heat shock gene induction but instead delays the time taken for HSP transcription to decrease down to basal levels after the heat shock stimulus has ceased, implying that the presence of NELF somehow facilitates the dissociation of the transcription factor Heat Shock Factor (HSF) [40].

NELF activity is also important during development e.g. in early *Drosophila* development, mutant clones lacking NELF-A or NELF-E either stopped developing before the blastoderm stage (displaying abnormal nuclear morphology) or during germ-band retraction (exhibiting head defects and incomplete dorsal closure). However, it appears that abnormal NELF has no effect on segmentation genes (e.g. *eve*, *fushi tarazu*, and *sloppy paired 1*) at the blastoderm stage [41]. NELF has also been shown to

play a critical role in the neuronal development of zebra fish embryos [42-44].

Other contexts involving NELF regulation include the attenuation of transcription levels of oestrogen-responsive genes following stimulation [12], the attenuation of transcription levels of the immediate-early genes and the transcription factor junB, both before and after induction [5], as well as the activation of paused genes involved in the immune response pathways in *Drosophila* in response to an immune challenge [45].

Another set of external and internal stimuli to which promoter-proximal pausing might be applicable, but has not been studied so far, is that involved in reactive oxygen species (ROS) production. The redox systems involving thioredoxin (TXN) and glutathione (GSH) are linked to the transcription of a multitude of genes through their redox effect on transcription factors [46]. When cells experience oxidative stress due to excessive ROS, the response has to be fast and well-co-ordinated and having primed gene transcripts, as observed for the HSP genes, appears to be quite plausible.

NELF and cancer

NELF has so far been implicated in some forms of gastrointestinal and breast cancers. In cancers of the upper digestive system, both NELF-B and NELF-E have been found to be overexpressed [47,48]. In certain breast cancers, NELF-B plays a critical role, as it binds both BRCA1 and ERα. The NELF complex is recruited to the ERα-responsive promoters by ERα through binding with NELF-B.This results in the NELF complex attenuating ERα-responsive gene activation and oestrogen-dependent proliferation of breast cancer cells.Recruitment of NELF to endogenous ERα-responsive promoters has been shown to be greatly stimulated upon oestrogen treatment [12]. Thus NELF can be affected by dysregulated BRCA1, which is a co-repressor of ERα [49-51] and in turn affect the ERα–oestrogen signalling pathway in breast cancer development [52-54].

Role of NELF in other transcription steps

With the emergence of a transcription regulation model, in which the mRNA processing steps of capping, splicing, and poly-adenylation are inter-related and occur co-transcriptionally, rather than independently and sequentially [55], NELF-induced promoter-proximal pausing of RNAPII is thought to provide the conditions for co-ordinating proper transcript processing [6,12,55-56] in a step called elongation checkpoint control [57,58]. As a consequence of the intercalation of mRNA processing steps, certain proteins may play a role in different steps as is the case of RNAPII through changes in its phosphorylation state during capping, splicing, and poly-adenylation [59].

The involvement of NELF in multiple mRNA processing steps during transcription is supported by the report that NELF associates with almost 50% of the most highly expressed *Drosophila* genes, but paused RNAPII is only found is a sub-set of these genes, indicating that NELF does not only fulfil the role of repressor of gene expression [60]. This is achieved through interaction with different factors.

One protein which displays a similar functional-flexibility to NELF is the nuclear cap binding complex (CBC), which was originally found to bind to the 5' cap structure of maturing RNA and facilitates its export [61]. However, it also influences the rate of RNA backbone cleavage steps during splicing [61] and has an additional role in 3' end processing [55,62]. Another similar example of a multi-functional factor is Cleavage and Poly-adenylation Specificity Factor (CPSF) [55]. NELF appears to interact with certain factors related to the mRNA 5' cap, since NELF-mediated repression of transcription is released following the recruitment of capping enzyme to the elongation complex [63].

On the other hand, NELF and CBC appear to function together in the 3' end processing of histone mRNAs, since the knock-down of either NELF or CBC leads to the accumulation of a poly-adenylated (poly-A) form of the mRNAs of replication-dependent histones. This is highly unusual, as replication-dependent histone mRNAs, unlike replication-independent histone mRNAs and other general mRNAs do not normally end in poly-A tails [64]. In this process, NELF has been shown to physically associate with histone gene loci and forms distinct intra-nuclear foci, which often overlap with Cajal bodies and cleavage bodies. NELF interacts with CBC, most likely through association with the histone stem-loop binding protein (SLBP), preventing the aberrant production of poly-adenylated histone mRNAs [65].

NELF appears to be also involved in the elongation phase. In fact, NELF can associate with the oestrogen receptor as well as other nuclear receptors to attenuate the level of induction of target genes indicating that NELF may be actively recruited by DNA-binding proteins to the elongation complex [12,66].

NELF and the resulting paused RNAPII have been implied in the maintenance of histone modifications typically associated with active transcription, while preventing the formation of repressive chromatin both by excluding nucleosomes from the promoter region and by maintaining patterns of histone modification that are conducive to activation [67].

NELF interaction with transcription factors

In addition to the basic complex, both *trans*-acting factors and *cis*-acting elements control transcription elongation by RNAPII [68,69]. Transcription elongation factors such as transcription factor IIF (TFIIF), TFIIS and elongin interact with RNAPII to prevent its pausing or to reactivate it from a paused state, while *cis*-acting elements are mainly located on nascent RNA transcripts. Some RNA elements cause RNAPII to pause or arrest without the aid of protein factors by forming structures that destabilise RNAPII-DNA-RNA complexes [68,70].

The activity of transcription factors to recruit proteins involved in promoter-proximal pausing of RNAPII is exemplified by the recruitment of P-TEFb to gene promoters by the transcription factors Myc and NF-κB [71,72], in addition to the existence of other mechanisms involving indirect association with acetylated histones via the chromatin reader, bromodomain-containing protein 4 (Brd4) [34], or association with a mediator kinase like cyclin-dependent protein kinase 8 (Cdk8) [73].

The involvement of transcription factors in the promoter-proximal pausing of RNAPII by NELF (and DSIF) is best characterised in the association of GAGA factor with 39% of the *Drosophila* gene promoters that are bound by NELF [60]. Transcription control of the *Drosophila HSP70* gene is one of the best studied[6,74] . Induction of the *HSP70* gene after heat shock, relies on the binding of the transcription factors, GAGA factor and HSF, upstream of the core promoter region [75-77]. This mechanism involves the binding of GAGA factor before heat shock, which results in the pausing of primed RNAPII, and then following heat shock, HSF is induced into binding with the primed *HSP70* promoter [36,75,76]. This leads to the recruitment of P-TEFb to the *HSP70* promoter, which removes the NELF-mediated inhibition [33]. Interestingly, the HSF activation domain appears to be unable to activate transcription without the prior action of the GAGA factor [78].

It has been proposed that during initiation the GAGA factor acts by recruiting TFIID, (and possibly the chromatin nuclear remodelling factor (NURF), and the histone chaperone FACT) and establishing a nucleosome-free region that allows access of the transcription machinery to the promoter [79-81]. The resultant promoter-proximal pausing of RNAPII following GAGA factor initiation is hypothesised to be a default outcome of the inability of this transcription factor to overcome the action of NELF, since it is not able to recruit P-TEFb to the RNAPII complex [60].

In higher eukaryotes, it might be possible for transcription factors recognising a similar motif to fulfil such a role. One possible candidate might be the E26 Transformation-Specific (ETS) protein family, which is characterised by a DNA-binding winged-helix-turn-helix domain that binds to purine-rich sequences containing a 5'-GGAA/T-3' core motif [82,84]. ETS transcription factors act at specific promoters via protein-protein interactions, and as a result can function as either activators or repressors of transcription. Of this family, one of the members that might be of special interest is E4TF1-60, due to its ubiquitous presence, action on TATA-less promoters and unique characteristic of forming obligate heterodi- or tetramers with E4TF1-53 depending on the presence of single or tandem ETS consensus motifs within promoters [82-87]. One way this transcription factor obtains promoter specificity is through the positioning of single E4TF1-binding sites adjacent to the binding sites of other transcription factors such as Sp1 [88]. Post-translational modifications further modulate the transcriptional activity by affecting stability or binding strength (Baron, 2014b). Thus, such a transcription factor or the complexes it forms could either recruit or stabilise the NELF complex through interaction with one of the sub-units, enabling promoter-proximal pausing of a response-specific group of genes.

Conclusion

The available data suggests that the NELF complex is crucial in the priming of genes that respond to various stimuli through promoter-proximal pausing of RNAPII, which thus allows for a rapid response to changes in cellular conditions such as in the case of heat shock. This opens a whole new perspective into different groups of response genes and of particular interest is the oxidative-stress context since this might help provide insight into the response of cancers to increased intracellular ROS levels. However, as part of the transcription regulation model, NELF, like other transcription-related factors plays multiple roles and participates in multiple steps throughout the process of transcription. Each sub-unit has unique protein domains which can fulfil different functions in distinct stages of transcription. Moreover, NELF interacts with other factors, to specifically target such processes. As shown by GAGA factor in *Drosophila*, ubiquitous mammalian transcription factors such as ETS family transcription factors might be involved in the recruiting or stabilisation of the NELF complex during transcription suppression.

Perspectives

Multiple questions arise from the current knowledge on the NELF complex: Does the NELF-E RRM encode an important function other than RNA binding? How is the NELF complex recruited to the TSS of specific genes? To what extent do transcription factors control the initial association of the NELF complex with the elongation complex? Are transcription factors involved in promoter clearance of NELF in response to context-dependent signals/stimuli? Can oxidative stress lead to the release of NELF and initiation of transcription activation in a subset of genes? Is NELF regulation achieved by the association of one or more of the NELF sub-units with *trans*-acting factors or *cis*-acting RNA elements? With more focus on the interactions, domains and activities of the separate NELF sub-units, a better understanding of the roles of these proteins in the transcription process can be achieved. This has significance both academically and clinically as transcription control can be a therapeutic target for various cancers.

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