Switching genes to silent mode near DNA double-strand breaks

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Transcription is tightly regulated in response to DNA damage. Rapid and transient pausing of RNA polymerase II (RNAPII) is indeed critical to restrict the production of aberrant transcripts from damaged loci and to prevent deleterious collisions between transcription and repair machineries. Yet, how DNA lesions signal to the transcription machinery to coordinate DNA repair with transcriptional silencing is not fully elucidated. In this issue of *EMBO Reports*, Awwad *et al* [1] bring a new piece to the puzzle by identifying the negative transcription elongation factor NELF as a critical player in this process. They demonstrate that NELF is recruited to DNA double-strand breaks (DSBs) near transcriptionally active genes in a poly(ADP-ribose)- and RNAPII-dependent manner to promote transcriptional repression and facilitate DSB repair.

See also: **SW Awwad *et al*** (May 2017)

Transcription is a potential source of genome instability and needs to be turned off during repair of DNA damage [2]. While bulky DNA lesions directly interfere with the progression of the transcription machinery, other lesions, like DSBs, inhibit transcription through complex and not yet fully elucidated mechanisms that involve the DNA damage sensor kinases ataxia telangiectasia mutated (ATM) [3] and DNA-dependent protein kinase (DNA-PK) [4], as well as poly(ADP-ribose) polymerase (PARP) [5]. These enzymatic activities promote transcription silencing at DSBs through the recruitment of repressive chromatin modifying complexes [5–8]. However, it is still unclear how DSBs signal to the transcription machinery to trigger silencing.

In this issue of *EMBO Reports*, Awwad *et al* [1] uncover a hitherto unknown role for the negative elongation factor NELF in blocking transcription of genes close to DSBs in human cells. NELF is a four subunit protein complex, comprising NELF-A, B, C/D, and E, which cooperates with DRB sensitivity-inducing factor (DSIF) to repress transcription elongation by RNAPII [9]. Focusing on one of the essential subunits for transcriptional pausing, NELF-E, Awwad *et al* [1] take three complementary approaches for assessing the role of NELF in transcriptional silencing upon DSBs in human cells: a global strategy consisting in labeling nascent transcripts upon cell exposure to ionizing radiation, and two targeted approaches for analyzing gene transcription upon site-specific DSB induction. For this, they make use of a previously established reporter cell line, allowing them to study the effect of DSB induction by the I-SceI endonuclease upstream of a tetracycline-responsive MS2 gene. In addition, they establish a novel Cas9-based system for inducing a sequence-specific DSB upstream of the A20 gene, whose transcription is induced in the presence of tumor necrosis factor- α . Combining these approaches with NELF-E knock-down, they demonstrate that NELF-E is required for transient transcriptional silencing in response to DSBs and more specifically for repressing the transcription of genes lying next to DSBs. These results indicate that NELF-E likely contributes to short-range transcriptional silencing in cis to DSBs. Future work will characterize the possible role of DSIF in this silencing pathway, to determine whether this repressive factor cooperates with NELF to silence the activity of RNAPII in response to DSBs as observed in unchallenged conditions.

This first set of data suggests that NELF-E acts locally to switch off genes close to DSBs. In line with this hypothesis, Awwad *et al* [1] investigate NELF-E recruitment to damaged DNA, using once again three complementary approaches: laser-induced damage, I-SceI cut, and Cas9-mediated DSB. Thus, they establish that both NELF-E and NELF-A relocate to DSBs. Interestingly, they do not observe the recruitment of other NELF subunits, suggesting the existence a NELF subcomplex at damage sites that is fully functional for transcription inhibition. Furthermore, by employing deletion mutant analyses, they demonstrate that NELF-E localizes to laser- and nuclease-induced DSBs through its amino-terminal leucine zipper domain. Mechanistic studies further establish that NELF-E recruitment to DSBs is independent of ATM but requires PARP1 activity, as shown both by inhibiting and knocking-down PARP1. Accordingly, PARP inhibition phenocopies NELF-E knock-down by alleviating transcriptional repression at DSBs. Also consistent with a dependency on PARP, NELF-E recruitment to DSBs is rapid and transient and relies on PAR binding by NELF-E amino-terminal domain. This domain contains a putative PAR-binding motif identified by *in silico* analyses. Further studies will be needed to test the relevance of this motif for NELF-E recruitment to DSBs. Importantly, while NELF-E recruitment to damaged DNA is mediated by an interaction with PARylated proteins, PARylation of NELF-E itself, which inhibits its transcription pausing activity [10], is dispensable.

Remarkably, further exploitation of the reporter cell lines in which DSBs are induced upstream of inducible genes reveals that NELF-E is preferentially recruited to DSBs

Figure 1. Model for NELF-mediated transcriptional silencing near DSBs.

Upon DSB induction near a transcriptionally active gene, PARP1 modifies RNAPII (1), which in turn recruits a NELF subcomplex comprising NELF-A and E subunits (2). NELF blocks transcription elongation (3), thus facilitating DSB repair (4).

close to transcriptionally active genes. Moreover, NELF-E recruitment requires the presence of RNAPII, and NELF-E association with RNAPII large subunit is disrupted upon PARP inhibition, which raises the possibility that PARylated RNAPII recruits NELF-E to DSBs. However, the contribution of other PARylated targets cannot be excluded.

Rescue experiments with wild-type and truncated mutants of NELF-E show that both NELF-E recruitment to DSBs and its repressive activity are required for transcriptional silencing at damage sites. Awwad *et al* also shed light on a possible interplay between different silencing mechanisms at DSBs. Epistasis analyses indicate that ATM, PARP, and NELF-E may function in the same pathway, with PARP also regulating the recruitment of additional silencing factors like

Nucleosome Remodeling Deacetylase and Polycomb Group proteins to DSBs [1]. It will be important to further clarify potential cross talks between ATM-, DNA-PK-, and PARP-mediated silencing at DNA breaks, to understand in which context these signaling pathways are activated and fully grasp the complexity of the process.

Finally, Awwad *et al* investigate the physiological relevance of NELF-mediated silencing in response to DNA damage by assessing the importance of NELF-E for DSB repair. NELF-E knock-down moderately increases cell sensitivity to ionizing radiation and impairs DSB repair by homologous recombination and non-homologous end-joining [1]. These results suggest that NELF-E silencing activity at DSBs may be critical to promote repair, although this still needs to be formally demonstrated. Whether NELF-E

facilitates repair of only a subset of DSBs, as shown for other silencing factors [8], is an interesting hypothesis that deserves further investigation.

Altogether, the results put forward NELF as a new player in the response to DSBs, a subcomplex of NELF-A and NELF-E being recruited to DSBs close to transcriptionally active genes to repress transcription and facilitate DSB repair (Fig 1). This study thus brings a new piece to the puzzle of transcription regulation following DNA damage and also provides mechanistic insights into a possible tumor suppressive function for NELF.

It is worth pointing out that, in addition to DSB-induced transcription silencing, small non-coding RNAs are generated from the damaged locus in mammalian cells, promoting damage signaling and repair [2]. It will be of major interest to investigate how silencing pathways activated in response to DSBs still allow the production of non-coding transcripts at damage sites to shed new light on the complex relationship between transcription and the DNA damage response.

References

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