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Forum

HELLS: the transcriptional sentinel

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The role of the chromatin remodeler HELicase Lymphoid Specific (HELLS) has been historically associated with DNA methylation and DNA damage repair. However, recent studies have shed light on an unexpected, multimodal, and direct participation of HELLS in transcriptional regulation. This forum article aims to discuss how, through different and context-specific mechanisms, HELLS modulates the expression of functionally related genes favoring transcriptional plasticity and phenotypic adaptation, ultimately safeguarding the genome organization and stability.

HELLS meets transcription

Genome integrity is constantly threatened by the execution of its functions. Processes such as transcription, replication, and DNA repair often coexist within the same genomic regions, causing tensions in DNA [1]. This favors the emergence of breaks or lesions. In particular, massive transcription, like that necessary to support cancer cell growth, generates severe mechanical DNA helix distortions and DNA tensional stress, posing a significant challenge to safeguarding the genome [1]. Transcriptionally active genes are typically associated with nucleosome-deprived regions (NDRs) where RNA polymerase (RNAP) and transcription factors (TFs) can bind [2,3]. The current model posits that pioneering TFs recruit ATP-dependent chromatin remodelers to evict or slide nucleosomes, facilitating RNAP binding. The rapid and dynamic cycle of nucleosome loading and eviction is essential for the correct execution of complex transcriptional programs, preserving cellular identity and function [2,3]. Disruptions in this delicate equilibrium, as seen in diseases like cancer, result in altered chromatin structure driving tumorigenesis [2]. In this scenario, proper chromatin remodeling function is indispensable in spatially modulating the timing of the transcriptional programs by preserving the chromatin architecture [4].

Human HELLS – also known as lymphoid-specific helicase (Lsh), SMARCA6, or PASG – stands out as a unique member of the SNF2 family of chromatin remodeling proteins [5]. HELLS is characterized by the presence of two ATPase domains which confer on it its peculiar ATPase activity and nucleosome remodeling ability [5]. While primarily expressed in proliferating lymphoid tissues and stem cells, HELLS mutations contribute to the rare immunodeficiency–centromeric instability–facial anomalies (ICF) syndrome, characterized by immunodeficiency, developmental defects, and mental retardation [5]. As occurs for many chromatin remodelers, precise regulation of HELLS is lost in most types of cancers, becoming a hallmark of solid and hematological malignancies [5].

For a long time, HELLS has been considered the guardian of heterochromatin due to its ability to promote DNA methylation during development and to serve as a docking or signaling site for DNA repair pathways [5]. However, recent evidence expanded this view, showing a direct and multimodal participation of HELLS in the transcriptional process. On the one hand, HELLS reshapes chromatin, influencing the binding of RNAP and specific TFs, fine-tuning the expression of cell-identity-associated transcriptional programs. On the other hand, by removing R-loops, HELLS supports efficient elongation at the level of essential and fitness-associated genes, ensuring their robust expression and eventually protecting the genome from structural stresses.

Herein, these novel aspects have been integrated into the established framework of HELLS genomic function, emphasizing the diverse mechanisms by which HELLS regulates transcription and their subsequent effects on the execution of distinct and context-specific expression programs.

HELLS: shaping chromatin dynamics

Nucleosome positioning during transcription is dynamically regulated by ATP-dependent chromatin remodeling enzymes [4]. These enzymes can evict histone octamers, exchange histones, and manipulate nucleosome structure through sliding and distortion [4]. Taking advantage of its ATP-dependent remodeling properties, HELLS alters the contacts between histones and DNA into the nucleosomes, catalyzing the incorporation of macroH2A1 and macroH2A2 histones into single nucleosomes [6,7]. MacroH2A incorporation is usually linked to transcriptional repression, and HELLS utilizes this mechanism primarily in repetitive sequences and distal and proximal developmental enhancers where it exerts its silencing effects [5–7]. However, this is not the only modality through which HELLS remodels the chromatin. When HELLS arrives at a nucleosome, in addition to incorporating specific histone variants, it can also slide nucleosomes [8]. This mechanism has been elegantly demonstrated in embryonic stem cells, where HELLS-dependent sliding affects CpG sites exposed to DNA methyltransferase activity, contributing to the formation of pericentromeric heterochromatin foci [8]. HELLS can slide nucleosomes in only a single direction and in the presence of the cell division cycle associated 7 (CDCA7) protein, which is necessary for the full functionality of HELLS [8]. Despite the presence of two helicase ATPase domains, HELLS harbors an autoinhibitory domain that restricts its intrinsic nucleosome remodeling activity *in vitro* [8,9]. It is plausible that when CDCA7 binds HELLS, conformational changes occur to enhance the ATPase and chromatin remodeling activities of HELLS [8]. In addition to its repressive role, HELLS can also promote transcription [10], and specific remodeling mechanisms employed by HELLS are now emerging. A recent study has demonstrated that HELLS enhances promoter accessibility by creating NDRs [10]. These NDRs serve as binding platforms for RNA polymerase and transcription factors, thereby guaranteeing transcriptional initiation [2].

HELLS interactions with other remodeling complexes, nucleosomes, and other transcriptional regulators are cell-type-specific [5]. These findings support the hypothesis that remodeling functions of HELLS and, consequently, its ability to repress or promote transcription may be context-dependent.

HELLS and transcriptional priming

Tens of thousands of regulatory elements undergo a dynamic shift between active and inactive states during development and differentiation, driving a concerted change in chromatin structure [3].

HELLS pervasively occupies the mammalian genome and directly influences the accessibility of enhancers and promoters, contributing to the overall distribution of nucleosomes and the stability of chromatin states [5]. During embryonic development, the regulation of enhancer–promoter communication is crucial for lineage commitment. This interplay serves to orchestrate RNAP recruitment, thereby priming transcription and ultimately contributing to the activation of specific genes [3]. TFs play a crucial role in this communication, not only by recognizing specific DNA sequences and influencing local chromatin accessibility, but also by recruiting chromatin remodelers that shape the three-dimensional topological organization of the genome [2,4]. During the early stages of embryogenesis, HELLS limits the accessibility of distal and proximal developmental enhancers and reduces the engagement of lineage-specific TFs such as GATA binding protein 3 (GATA3), myogenic differentiation 1 (MYOD), SRY-box transcription factor 6 (SOX6) and achaete–scute family basic helix–loop–helix (BHLH) transcription factor 1 (ASCL1) [11]. During development, HELLS shuts down the expression of pluripotency genes such as a subset of homeobox (Hox) and POU class 5 homeobox 1 (Oct4) [5,11]. In these contexts, HELLS alters the chromatin landscape in part by catalyzing the incorporation of macroH2A1 and macroH2A2 histones [6,7] (Figure 1A). This creates an inaccessible chromatin landscape that acts as a highly energetic barrier to RNAP passage. It is plausible that HELLS' ability to cooperate with DNA methyltransferases [8] contributes to the faster propagation of these repressed chromatin states.

Nevertheless, HELLS has been shown to cooperate directly with different transcription factors to modulate specific gene expression programs [5,12,13] (Figure 1B and Box 1). This relationship is frequently restrained in cancer [5,12,13] (Box 1), where the shifts in chromatin organization drive tumorigenesis leading to a globally dysregulated state [4]. A growing number of studies have revealed that HELLS is abnormally expressed in human cancers [5]. Also in these settings, HELLS has been largely associated with active transcriptional elements such as enhancers and promoters, which fuel the increased overall transcription needed for tumor cell proliferation [5]. A recent study revealed that HELLS modifies chromatin accessibility by creating NDRs at immune-related promoters in T cell lymphoma cells [10]. While the exact mechanism remains elusive, this process appears to facilitate RNAP recruitment and TF binding, ultimately leading to enhanced transcriptional activity (Figure 1B). The promoters of immune-related genes are associated with enriched levels of H3K4me3 [10]. This mark is normally found around the transcriptional starting site of actively transcribed genes [3], further indicating that changes in the entire chromatin structure are necessary for their precise gene expression. Notably, this set of genes, like those of cell identity during the differentiation, can be activated and transcribed within minutes after stimulation. Recruitment of RNAP at the target promoter is a crucial step for these genes. A delay in this step has catastrophic consequences for the cell, with a delay in primary response and the execution of transcriptional programs.

HELLS and R-loops: a dynamic duo in the regulation of RNAP elongation

Transcription is a primary source of genome instability. The unwinding of DNA by RNAP during elongation generates torsional stress and is a major source of DNA supercoiling throughout the genome [1]. Stable RNA–DNA hybrids formed between the nascent RNA and the DNA upstream of the RNAP, known as R-loops, can hinder both the elongation and the stability of the chromatin template [1].

When massive transcription is required, such as in sustaining the proliferation of cancer cells, the elongating RNAP alone is not able to transcribe through the gene body due to the structural impediments imposed by the R-loops [1]. Thus, RNAP passage is likely to require assistance in moving through nucleosomes without unscheduled obstacles [1,3]. A recent study has demonstrated that HELLS co-localizes with R-loops, and its dysregulation results in the accumulation of R-loops nearby or at the same sites where RNAP stalls [10]. This phenomenon also induces the accumulation of damaging events on the same genomic sites affected by R-loop

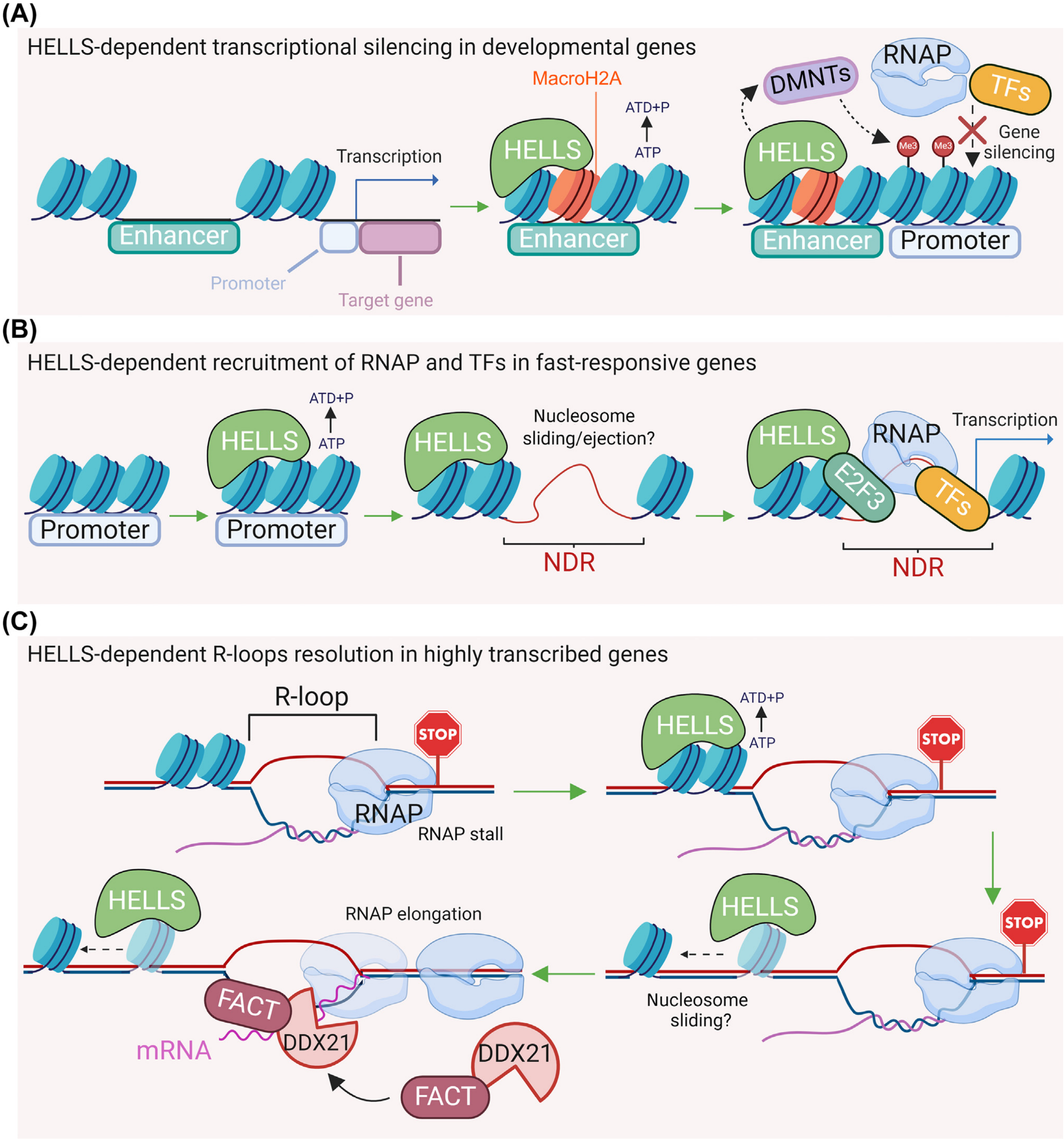

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Figure 1. Transcriptional mechanisms regulated by helicase lymphoid specific (HELLS). HELLS regulates transcription through different modalities and in a context-dependent manner. During the establishment of cell identity and cell fate, HELLS directly influences the accessibility of chromatin by depositing the histone variant MacroH2A through its ATP-binding domain. The ability of HELLS to cooperate with DNA methyltransferases (DNMTs) may facilitate the rapid propagation of repressed chromatin states. This mechanism leads to the silencing of developmental enhancers and the control of enhancer–promoter communication (A). During early cellular transformation and subsequent tumor progression, HELLS drives aberrant and excessive transcription by altering chromatin accessibility in an ATP-dependent

(Figure legend continued at the bottom of the next page.)

Box 1. HELLS and TFs: a complex dance in gene regulation

HELLS can act as a potent transcriptional co-activator, physically interacting with TFs to enhance their recruitment and DNA-binding affinity at target gene promoters. This function as a co-activator has been exemplified, for the first time, by the interaction with E2F transcription factor 3 (E2F3) [13]. This occurs through the E2F3-coiled-coil domain and the interaction is stabilized by the E2F3-marked box. HELLS–E2F3 interaction aids the induction of E2F-target genes and cell-cycle re-entry, establishing a proliferative circuit [13]. This network is also restrained in tumors with sustained cell proliferation and tumor aggressiveness [13]. Von Eyss *et al.* showed that 74% of all HELLS-bound promoters display an E2F-like motif and that a massive surge of E2F3A at E2F promoters cannot compensate for the loss of HELLS in the target gene and S-phase induction [13] (Figure 1B in the main text). Although the direct association with RNAP occupancy has not been investigated, most HELLS-bound promoters overlap with the active marker of transcription H3K4me3, further indicating the creation of a permissive environment for RNAP recruitment.

Recent findings demonstrated that HELLS' repertoire extends beyond E2Fs. HELLS also promotes YY1 transcription factor (YY1) transcriptional activity by facilitating its recruitment to target genes [12]. This interaction is essential for ensuring the aberrant expression of cytoskeleton-associated genes and plays a role in tumor aggressiveness [12]. The loss of HELLS leads to YY1 displacement from DNA in lymphoma cells, indicating a precise hierarchy that selectively drives gene programs [12]. Collectively, HELLS gains access to lineage-specific factors to their respective binding sites, ensuring the execution of precise transcriptional programs. The interplay between HELLS and TFs offers potential therapeutic targets for cancer and other diseases. Targeting HELLS interactions with transcriptional factors could disrupt selective transcriptional networks and halt disease progression.

persistence, leading to genomic instability in lymphoma [10]. HELLS creates a permissive chromatin landscape in which the RNAP can successfully elongate, increasing the transcript level [10]. This specific regulation occurs in selected target genes central to cancer cell structure maintenance, such as Rho-GTPase and cytoskeleton organization [10,12]. Due to their structural nature, these genes are required to be transcribed at high levels and in a continuum manner to guarantee cellular support. The molecular mechanisms underlying HELLS involvement in resolving R-loops are likely to be multitiered and remain to be elucidated. Based on its pleiotropic activities, HELLS may help resolve R-loops through different mechanisms. HELLS may remodel nucleosome positioning in R-loop-flanking regions, thus making chromatin accessible to RNA-helicase DExD-box helicase 21 (DDX21) and the facilitates chromatin transcription (FACT) complex, which generally resolves R-loops in those regions [14] (Figure 1C). Alternatively, based on ATP-binding domain sequence homology with its ortholog in plant decrease in DNA methylation 1 (DDM1) [15], HELLS could clear co-transcriptional R-loops directly. Given the importance of HELLS in development and tumorigenesis, establishing the exact principles of HELLS-mediated resolution of R-loops is important for understanding the mechanisms of cancer propensity and identifying new therapy targets.

Concluding remarks

Elucidating the precise mechanisms of HELLS action remains a key challenge, and several open questions emerge from these insights. The first includes understanding its recruitment to target genes. As a chromatin remodeler, HELLS lacks DNA sequence specificity. Presumably, beyond CDCA7, other pioneering TFs act as interpreters, providing the specificity of HELLS in establishing cell identity and responding to environmental cues. Second, what is the precise mechanism through which HELLS makes chromatin accessible to RNAP and TFs? HELLS could exert this function by the eviction or sliding of nucleosomes favoring the binding of locus-specific TFs priming the transcription.

By virtue of its central and multifunctional functions in tumors [5], there is substantial interest in identifying compounds capable of inhibiting HELLS activity. Such pharmacological inhibitors hold promise not only as monotherapy but also in synergistic combination with conventional and targeted therapies. HELLS downregulation could potentially circumvent resistance to DNA-damaging agents by hindering repair mechanisms and disrupting critical gene expression networks.

Further research efforts are warranted to develop effective and clinically translatable strategies for HELLS inhibition.

Author contributions

S.M., G.G., A.C., and V.F. wrote the manuscript. All the authors read and approved the final version of the manuscript.

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manner. HELLS induces the formation of nucleosome-depleted regions (NDRs) at promoters of tumor cells, facilitating the recruitment of RNA polymerase (RNAP) and transcription factors (TFs) to target genes. HELLS employs this mechanism in E2F transcription factor 3 (E2F3)-dependent G1/S cell cycle genes and in immune-related genes that require rapid transcriptional activation upon stimulation (B). In genes essential for maintaining cancer cell structure, characterized by high transcriptional activity, HELLS restricts the formation of co-transcriptional R-loops. This may be achieved through nucleosome remodeling in the vicinity of R-loops, thereby facilitating access to the RNA helicase DDX21 and the facilitates chromatin transcription (FACT) complex. Consequently, HELLS promotes efficient RNAP elongation, contributing to tumor aggressiveness (C). Figure created with BioRender.

Declaration of interests

The authors declare no competing interests.

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