Nuclear bodies: Built to boost

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The classic archetypal function of nuclear bodies is to accelerate specific reactions within their crowded space. In this issue, Tatomer et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201504043) provide the first direct evidence that the histone locus body acts to concentrate key factors required for the proper processing of histone pre-mRNAs.

The nonrandom organization of the cell nucleus significantly contributes to gene expression and genome maintenance (Hübner et al., 2013). Within the tightly packed nuclear interior, which lacks defining membranes, various structurally distinct compartments and nuclear bodies (NBs) exist. NBs are dynamic nuclear domains that contain an expanding list of enriched components with conserved functions across many species. These domains are frequently associated with specific gene loci, the activities of which appear to contribute to their biogenesis (Dundr and Misteli, 2010). However, NBs are not passive byproducts of specific gene activity but act principally as sites of defined biochemical reactions and of gene activation or repression. More specifically, it is believed NBs concentrate substrates, enzymes, and assembly intermediates within their confined space to boost certain reactions. NBs can positively or negatively regulate concentrations of essential factors by sequestering or releasing them as needed (Mao et al., 2011; Dundr, 2012).

However, the precise molecular mechanisms of NB formation and how they subsequently accelerate molecular reactions remain unclear. It is difficult to study the role of NBs directly and gain insight into their in vivo functions in the context of naturally occurring processes, posing a technical hurdle that has been challenging for the NB field to overcome. In most cells, NBs are continuously present in interphase. The creation of specialized microenvironments is an energetically favorable situation, and it has been suggested that an essential function of NBs is to expedite molecular events in a temporal and spatial manner. Although this presents an attractive idea, direct demonstrations of such a role are scarce. In this issue, Tatomer et al. use an elegant transgene system in *Drosophila melanogaster* to manipulate the localization and activity of specific NB components without NB disassembly. Using this system, they have dissected the roles of protein–RNA associations and the formation of a specialized nuclear microenvironment involved in the transcription and processing of histone pre-mRNAs.

In metazoans, the synthesis of histones is restricted to the point in the cell cycle when the genome is replicated (S phase). This process is regulated by the expression of replication-dependent (RD) histone genes. In humans, 55 histone RD genes, which encode variants of five histone genes, are grouped together at the major histone gene cluster, called *HIST1*, on chromosome 6p22, and at a minor histone cluster, *HIST2*, on chromosome 1q21, which contains 13 histone genes. In *Drosophila*, only one RD histone gene cluster exists, and it consists of ∼110 tandemly arranged uniform gene repeats, each comprising five histone genes (H1, H2A, H2B, H3, and H4) on chromosome 2. During S phase, the expression of histone mRNAs rapidly increases by ∼30-fold (Osley, 1991). In contrast to most protein-encoding genes, the majority of RD histone genes do not contain introns and are not polyadenylated but contain a unique 3′ end with a conserved stem-loop structure in the 3′ UTR. Thus, the production of histone pre-mRNA requires the coordination of a highly specialized and evolutionarily conserved system. This histone RNA production apparatus consists of multiple protein factors, as well as the U7 small nuclear RNA (snRNA), a component of the U7 snRNP complex, which is involved in cleaving these pre-mRNAs. This exceptional type of pre-mRNA synthesis ensures that adequate numbers of histone proteins are produced at the appropriate time for packaging newly replicated DNA into nucleosomes.

Two indispensable histone RNA production factors, FLASH and U7 snRNP, are required for the 3′ end of RD histone mRNAs to form. These proteins are concentrated in a NB called the histone locus body (HLB), which assembles in the G1 phase of the cell cycle, augments histone mRNA transcription during S phase, and persists until mitosis (Romeo and Schümperli, 2016). Upon histone gene transcription, U7 snRNP hybridizes to a specific binding site (the histone downstream element) located downstream of the cleavage site in histone pre-mRNAs (Fig. 1). The U7 snRNP contains a subunit called Lsm11 in the heptameric Sm ring that mediates the association of the complex with FLASH (Burch et al., 2011) and leads to the recruitment of a polyadenylation complex called the histone cleavage complex (HCC), which cleaves the 3′ end of histone pre-mRNAs. After each round of pre-mRNA processing, the downstream cleavage product is degraded, which dissociates the U7 snRNP from the histone downstream element. The U7 snRNP is then available for another round of histone pre-mRNA processing. In both mammals and *Drosophila*, the HLB is nucleated by the histone gene clusters, which are recognized by the histone transcription factors NPAT (in mammals) or Mxc (the NPAT orthologue in *Drosophila*). These components work

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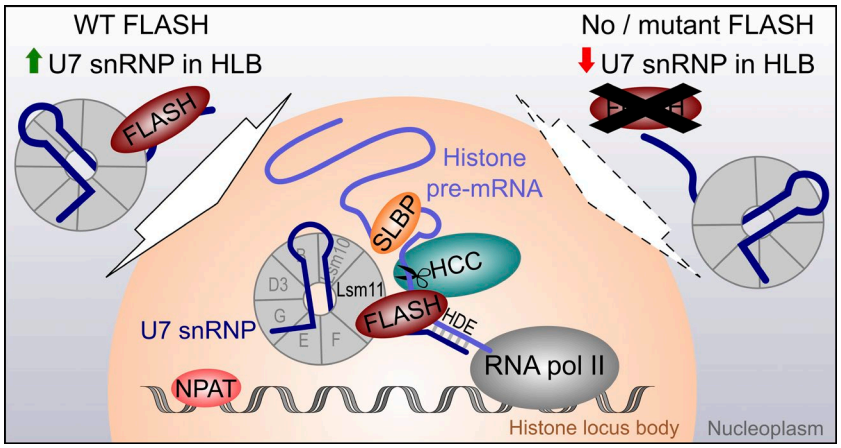

Figure 1. **FLASH–U7 snRNP association is essential for histone mRNA processing by the HLB.** The HLB forms at defined sites of histone mRNA synthesis (histone RD gene clusters *HIST1* on chromosome 6 and *HIST2* on chromosome 1 in humans). Tatomer et al. (2016) have shown that the HLB concentrates critical factors for histone pre-mRNA processing, such as the U7 snRNP, stem loop binding protein (SLBP), FLASH, and the HCC. U7 snRNP binding to FLASH via Lsm11 in the U7 snRNP heptameric Sm ring (other components of the Sm ring are annotated in gray) and subsequent enrichment in the HLB is essential for efficient histone pre-mRNA 3′ end processing. After U7 snRNP duplex formation with the histone downstream element (HDE), the HCC cleaves the histone mRNA 3′ end, which releases the transcript from the complex. Efficient processing and transport of stabilized histone mRNAs is also dependent on binding of SLBP to the histone mRNA stem loop element located in the 3′ UTR.

together to efficiently produce histone mRNAs, but the benefits of their cooperation are unclear.

In their study, Tatomer et al. (2016) perturb HLB composition by expressing functional mutants of FLASH that disrupt the association of U7 snRNP with histone pre-mRNAs and thus their cleavage, as well as mutations in Mxc, without the loss of HLB structures. They observed that a high concentration of FLASH in the HLB is required for U7 snRNP to accumulate in this body. Also, the efficiency of 3′ end histone mRNA formation is sensitive to the amount of FLASH activity in the HLB. They also found that failure to concentrate FLASH and U7 snRNP in the HLB led to the misprocessing of histone pre-mRNAs. As a result, they detected a small, but significant, level of histone mRNA polyadenylation via cryptic downstream polyadenylation signals, suggesting that some compensatory histone pre-mRNA processing occurs through the normal transcriptional machinery. Longer nascent read-through histone mRNA transcripts also accumulated at the histone gene locus array. This indicates that the processing of histone pre-mRNA is delayed, resulting in the uncoupling of RNA production and transcriptional termination.

What are the possible benefits of concentrating the components of histone pre-mRNA processing in HLBs? One possible benefit is to accumulate a limited essential factor, such as the U7 snRNP, at its site of activity; U7 snRNA is expressed at possibly <1,000 copies per cell in mammals (Marz et al., 2007). Other major spliceosomal snRNAs, such as U1 and U2, are cotranscriptionally processed by the Integrator complex at their extended 3′ end. These snRNAs are essential for defining 3′ and 5′ splice sites and the branch sequence (essential for intron excision) during pre-mRNA processing by the spliceosome. In contrast to U7 snRNA, these snRNAs (with broad targets across the genome) are expressed at between 10^5 and 10^6 copies per cell (Zieve and Sauterer, 1990).

Tatomer et al. (2016) establish that FLASH maintains high local concentrations of U7 snRNP in the HLB. This accumulation in the HLB, and not its overall availability in the nucleus, might be necessary for the efficient production of histone pre-mRNA in vivo. It is energetically favorable for cells to limit the overall concentration of U7 snRNP (which has no known functions outside of histone pre-mRNA processing during S phase) at sites of high demand. Without the HLB, the recycling of U7 snRNP in the nucleoplasm is insufficient for efficient histone pre-mRNA synthesis. To maintain normal levels of histone mRNA production in cells that theoretically lack HLBs, the total nuclear concentration of U7 snRNP available at a given time would need to be far higher. This would entail a highly inefficient energetic and organizational commitment for cells. Further work will be required to conclusively resolve this issue. Spatial regulation by NBs also aids nuclear function by sequestering key factors, such as FLASH (which is also of low abundance) and U7 snRNP to target genomic regions, lowering the risk of off-target effects or the spurious deregulation of other essential processes through noncanonical molecular functions. This is also true of other NBs, which similarly cluster specific target genes to their periphery in order to spatially regulate gene expression and RNA processing (Wang et al., 2016). Thus, the spatial organization of key factors required for specialized molecular events through NB assembly at target gene loci is of considerable benefit to the cell.

NBs are defined by several key characteristics, including their isolation from the surrounding nucleoplasm through phase separation, the accumulation of specific factors, and the augmentation of molecular processes. The work by Tatomer et al. (2016) is the first direct evidence of this augmenting role, as these authors' data demonstrate the increased processing of histone pre-mRNAs by the HLB. This represents an important step in our understanding of the HLB microenvironment. Accelerated pre-mRNA production by NBs is thought to be a consequence of increased macromolecular crowding within the NB microenvironment (Cho and Kim, 2012). This physical phenomenon is expected to stimulate multiple processes essential for protein function and stability. These include the promotion of correct protein folding, as well as enhanced protein–protein and protein–RNA assembly. The increased stability of correctly folded molecules, such as protein factors and RNA, in the HLB is expected to produce increased levels of active protein–RNA complexes. Macromolecular crowding also reduces diffusion rates, which is predicted to promote the channeling of the HCC toward the U7 snRNP–histone mRNA complex and to increase the rate of U7 snRNP rebinding after each round of pre-mRNA processing (Mourão et al., 2014). Macromolecular crowding is also expected to destabilize U7–histone RNA duplex formation, to enable the resampling of target sites on histone pre-mRNAs, and to prevent the U7 complex from being trapped at an incorrect mismatched target site. The observations of Tatomer et al. (2016) are aligned with the suggestion that concentrating crucial factors within the crowded HLB microenvironment boosts histone pre-mRNA processing. This is advantageous during S phase when a rapid rate of histone mRNA production is required.

Finally, an important question raised by Tatomer et al. (2016) is how we assess and validate the functionality of NBs. Stated in its simplest terms: Are all NBs created equally? The HLBs that contain these functional FLASH mutants are potentially representative of an inactive intermediate state, similar to those described by Novotný et al. (2015), who reported the appearance of de novo Cajal bodies (CBs) in human primary cells that normally lack CBs. These de novo CBs, which appeared after U4/U6.U5 tri-snRNP assembly blockade, were deficient in a small number of important CB components but otherwise resembled fully fledged CBs. Similarly, other studies have suggested CB formation to be involved in DNA damage repair but only coilin, the CB structural marker protein, colocalizes with sites of DNA damage, and not the SMN complex, which is required for snRNP biogenesis and translocation (Bártová et al., 2014). Other essential CB components, such as TCAB1/WRAP53, have also been implicated in DNA damage repair but it is unclear if this is related to CB formation (Rassoolzadeh et al., 2015). Can a compositionally identical NB be involved in several distinct cellular events or trapped in intermediate states? These studies suggest this to not be the case. Thus, the characterization of the overall molecular content of active and inactive intermediate NBs, as well as those involved in noncanonical functions, including both processing machinery and substrates, is necessary.

These observations build a strong argument for the importance of nuclear microenvironments for efficient cellular function. In contrast to the reported molecular role of another NB, the CB, these data suggest that the presence of functional HLBs during S phase is essential. In mammals, these two structures are frequently found to be associated. The CB catalyzes the transcription, extended 3′ end processing, and base modification of spliceosomal U snRNAs and hosts certain snRNP/small nucleolar RNP assembly steps (Bizarro et al., 2015). However, several cell types do not possess CBs, and these biosynthetic events still occur, if less efficiently, in the nucleoplasm (Klingauf et al., 2006; Nizami et al., 2010). The perturbation or loss of specialized nuclear domains, such as the HLB, with direct roles in essential processes, is detrimental to cell survival. However, other NBs, such as the CB and the promyelocytic leukemia NB (Borden, 2002), are perhaps only required in times of need under distinct cellular metabolic and proliferative demands. As such, the results presented by Tatomer et al. (2016) in this issue suggest that whereas some NBs are essential, others are not, depending on cell context. These data will be vital for exploring the underlying functional benefits of NB assembly and their contribution to genome function.

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