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RESEARCH ARTICLE

Histone locus bodies: a paradigm for how nuclear biomolecular condensates control cell cycle regulated gene expression

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ABSTRACT

Histone locus bodies (HLBs) are biomolecular condensates that assemble at replication-dependent (RD) histone genes in animal cells. These genes produce unique mRNAs that are not polyadenylated and instead end in a conserved 3′ stem loop critical for coordinated production of histone proteins during S phase of the cell cycle. Several evolutionarily conserved factors necessary for synthesis of RD histone mRNAs concentrate only in the HLB. Moreover, because HLBs are present throughout the cell cycle even though RD histone genes are only expressed during S phase, changes in HLB composition during cell cycle progression drive much of the cell cycle regulation of RD histone gene expression. Thus, HLBs provide a powerful opportunity to determine the cause-and-effect relationships between nuclear body formation and cell cycle regulated gene expression. In this review, we focus on progress during the last five years that has advanced our understanding of HLB biology.

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Composition and function of histone locus bodies

RD histone genes do not contain introns, and thus the only pre-mRNA processing step necessary to produce a mature RD histone mRNA is cleavage of the pre-mRNA 4–5 nucleotides downstream of the 3' stem loop [1–4]. Like cleavage/polyadenylation, this cleavage reaction occurs between two cis elements in the pre-mRNA, the stem loop, which binds stem loop binding protein (SLBP), and a sequence (called the histone downstream element or HDE) 3' of the stem loop that base pairs with the 5' end of U7 snRNA of the U7 snRNP particle (Figure 1). Because the U7 snRNP concentrates only in the HLB, the HLB was originally identified and distinguished from Cajal Bodies (CBs) by detection of the U7 snRNP-specific proteins Lsm10 and Lsm11 in *Drosophila* tissues [5]. The HLB now is primarily defined by a large, mostly unstructured Cyclin E/Cdk2 substrate protein that in *Drosophila* is called Mxc and in humans is called NPAT, which is found at the promoters of all human RD histone genes [6–9]. NPAT/Mxc concentrates only in HLBs and is essential for HLB assembly and RD histone gene expression [8,10,11]. The C terminus of NPAT binds directly to the C terminus of the essential pre-mRNA processing factor FLASH [12], which like NPAT is required for HLB assembly [13,14]. Similarly, the C terminal region of Mxc interacts with the C terminal region of *Drosophila* FLASH, although the specific determinants of this interaction are slightly different than in humans [15]. NPAT/Mxc and FLASH initiate the HLB assembly pathway because in their absence no other HLB factors are recruited to and concentrated at RD histone genes, whereas the absence of other factors (e.g. U7 snRNP) does not affect the ability of NPAT/Mxc and FLASH to form an HLB [8,13,16,17]. The molecular details of this assembly pathway remain incompletely understood, although a critical step is likely NPAT/Mxc

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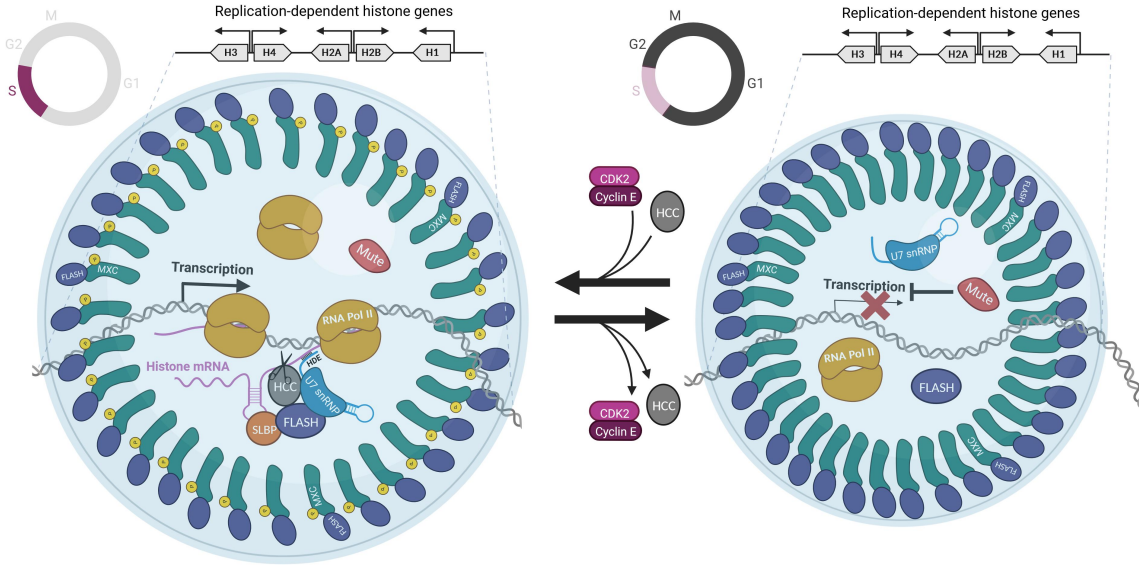

Figure 1. Model of HLB assembly and function. HLBs are primarily organized by NPAT/Mxc and FLASH, which bind to the C terminus of NPAT/Mxc. HLBs in S phase (left) are larger than HLBs in other phases of the cell cycle (right), likely because Cyclin E/Cdk2 phosphorylation of NPAT/Mxc induces HLB reorganization and/or because mRNA synthesis via transcription and pre-mRNA processing is occurring within the HLB. Whereas some RD histone pre-mRNA processing factors like U7 snRNP and FLASH are constitutive residents of the HLB, other critical factors like the HCC are recruited only when histone genes are active. The assembly of the active cleavage complex (left) may utilize a pool of FLASH (and perhaps other factors) that is distinct from the pool of FLASH that binds to NPAT/Mxc and organizes the HLB. RNA pol II is enriched in the HLB, including in HLBs that are not actively synthesizing RD histone mRNA (J. Kemp and R. Duronio, unpublished). Whether concentrating RNA pol II in the HLB is functionally important and whether all the RNA pol II in the HLB is engaged in transcription during S phase are interesting open questions. Negative regulators of RD histone transcription like *Drosophila* Mute (GON4L/YARP in humans) are found concentrated only in the HLB, and likely modulate histone gene expression during the cell cycle in coordination with Cyclin E/Cdk2 activity. Note that the gene cluster at the top of the diagram is based on the arrangement of RD histone genes in *Drosophila melanogaster*, but conceptually applies to other RD histone gene clusters, which associate together in 3D space within the nucleus. Image created with BioRender.com.

oligomerization, as point mutations in the N-terminal LisH domain of Mxc that prevent Mxc self-interaction abolish HLB assembly and RD histone gene expression *in vivo* [16]. The Coilin protein also uses N-terminal domain multimerization to drive CB assembly [18], suggesting common molecular strategies underlie nuclear body formation. *Drosophila* embryonic HLBs demonstrate several hallmarks of liquid droplets, suggesting that phase separation, perhaps resulting from multivalent interactions driven by Mxc multimerization, also contributes to HLB assembly [19].

How NPAT and Mxc recognize RD histone genes is not understood. Neither protein has sequence-specific DNA binding activity, and thus may rely on other proteins to become located to RD histone loci. In mammalian cells, the H4-gene-specific transcription factor HiNF-P binds NPAT [20]. However, HiNF-P is not required for expression of RD histone genes other than H4 or for HLB formation [21], suggesting that other factors recruit NPAT to the other RD histone genes. In addition, 3D genome mapping approaches revealed that NPAT but not HiNF-P binds to a putative regulatory sequence found in association with the human chromosome 6 histone gene cluster [22]. Thus, NPAT can interact with the genome independently of HiNF-P, suggesting perhaps that it interacts with other sequence specific DNA binding proteins. In *Drosophila*, a possible candidate for a protein that assists Mxc in recognizing RD histone genes and initiating HLB assembly is the zinc finger protein CLAMP [23]. CLAMP is enriched in the HLB and binds a GAGA repeat element between the divergently transcribed *H3-H4* promoters [23], a ~300 bp region that drives activation of all five RD histone genes [24]. Depletion of CLAMP reduces both accessibility of chromatin at the *Drosophila*

RD histone locus and RD histone gene expression [23]. However, CLAMP binds to many other GAGA repeats throughout the genome, particularly those on the X chromosome necessary for sex chromosome dosage compensation [25], indicating that other factors modulate where CLAMP interacts with the genome [26]. In addition, CLAMP can be recruited to the HLB when RD histone genes lack the GAGA repeat between the H3 and H4 promoters [27], suggesting that CLAMP recruitment to the HLB can be mediated by protein-protein interactions even without binding to DNA. Consequently, CLAMP binding DNA is not essential for HLB assembly [27]. A new bioinformatics study using publicly available ChIP seq data sets provides evidence for the localization of several DNA binding factors to the *Drosophila* histone locus [28]. Among these is the homeotic transcription factor Ubx, which is necessary for maintaining cell identity at specific positions along the anterior/posterior body axis during development. This observation suggests possible cell-type-specific coordination between developmental signaling and cell cycle control of RD histone gene expression. This new list of factors expands the number of potential HLB components and regulators of histone mRNA synthesis, but experimental evidence is needed to determine their potential roles. Thus, how the key HLB assembly factor NPAT/Mxc becomes associated with RD histone genes remains an interesting open question.

From in vitro biochemical experiments, we know a great deal about the molecular mechanism of RD histone mRNA 3' end formation, including a striking cryo EM structure of RD histone pre-mRNA bound to the active site in CPSF73 of the cleavage complex [29]. The N-terminus of FLASH interacts with the Lsm11 protein of U7 snRNP, forming a molecular surface that recruits the Histone Cleavage Complex (HCC) (Figure 1) [30]. The HCC is identical to mCF, a subcomplex of CPSF containing the HEAT domain protein Symplekin, which serves as a scaffold for the CPSF100/CPSF73 nuclease that cleaves RD histone pre-mRNA between the stem loop and HDE and then degrades the 3' RNA product via a concerted exonuclease reaction [31]. Interestingly, Symplekin/CPSF100/CPSF73 also is responsible for cleavage of all other mRNAs prior to polyadenylation [4]. Thus, one function of the HLB may be to exclude polyadenylation factors that do not participate in the RD histone pre-mRNA cleavage reaction. A caveat to this model is that in the absence of SLBP, FLASH, or U7 snRNP, *Drosophila* RD histone pre-mRNAs are polyadenylated as directed by cryptic poly A signals downstream of each RD histone gene [32]. In mammalian cells lacking SLBP some RD histone mRNA also becomes polyadenylated [33–35], a situation that can lead to genomic instability [36].

The RD histone pre-mRNA cleavage reaction has been reconstituted in vitro using a combination of recombinant U7 snRNP and a 200 amino acid N-terminal fragment of FLASH, with nuclear extract as a source of the HCC [37], providing an experimental platform for detailed studies of the cleavage reaction. Other biochemical studies have revealed additional molecular interactions that could govern HLB assembly or be modulated by the biophysical properties of the HLB. A particularly interesting example is provided by GON4L/YARP [38], the human homolog of the *Drosophila* Mute protein (encoded by the *muscle wasted* gene) which functions as a repressor of RD histone gene expression [39]. GON4L/YARP and Mute bind directly to the C terminal region of NPAT and Mxc, respectively. Interestingly, GON4L/YARP binds to the same 31 amino acids at the very C terminus of NPAT that FLASH does. GON4L/YARP and FLASH use similarly structured SANT/Myb domains containing a bundle of three alpha helices that each binds to NPAT in a slightly different orientation [40]. NMR structural studies indicate that these binding events are mutually exclusive, setting up an interesting possible scenario in vivo in which multiple different NPAT complexes with different functional roles reside with the HLB. Alternatively, perhaps at the end of S phase, a GON4L/YARP repressor complex displaces the active HCC/U7 snRNP from the C terminus of NPAT by binding in place of FLASH, thereby terminating RD histone mRNA biosynthesis both by preventing 3'end formation and down regulating transcription.

In addition to trans-acting factors, a key component of HLB organization is the RD histone genes themselves. As noted above, our knowledge of the cis acting elements that drive HLB assembly is limited, but it is clear from work in *Drosophila* that transcriptionally active histone genes provide a 'seed' for HLB assembly and growth [19,24]. Recent work in mammalian cells applying high-throughput sequencing techniques that probe 3D genome organization has provided insight into the arrangement of RD histone genes within HLBs. Each histone protein is encoded by multiple genes in eukaryotic cells, and in metazoans, these gene copies are clustered. For instance, the human and mouse genomes contain two major RD histone loci, each of which contain clusters of multiple RD histone genes (totaling about a dozen for each histone) [41]. These loci independently form an HLB, resulting in two large and two small HLB foci that are readily detected by NPAT staining [10,11,13,42]. At the human chromosome 6 locus, three sub-clusters of histone genes that are interrupted along the length of the chromosome by non-histone genes are located closely together in 3D space, as revealed by HiC and RD-SPRITE analysis [7,22,43]. A fourth sequence within the cluster that lacks RD histone genes but binds NPAT is also closely associated with the three histone gene sub clusters in 3D space and may act as a regulatory element [22]. Thus, one possibility is that oligomerization of NPAT bound at the promoters of each of these histone genes drives the aggregation of each of these genomic regions into one biomolecular condensate, perhaps via liquid–liquid phase separation.

The situation is a bit different in *Drosophila melanogaster*, where ~100 copies of a 5 kb gene cluster containing each of the RD histone genes (Figure 1) are organized into a ~0.5 Mb tandem array at a single locus on chromosome 2 [44]. Homologous chromosomes pair in *Drosophila melanogaster*, thus most diploid cells typically have one HLB because the homologous HLBs fuse into a single HLB when the chromosome 2s pair with one another [19]. In early embryonic development prior to homologous chromosome pairing, two HLBs are readily detected [19]. Interestingly, other fly species like *D. virilis* naturally contain two histone gene clusters like humans [23], each of which can form an HLB that is independently active in histone mRNA transcription [45]. The association of RD histone genes at different locations in the genome into a single HLB may provide a means of coordinating expression of the full complement of histone genes located a different genomic loci to achieve the correct level and stoichiometric amounts needed for proper genome assembly during S phase of the cell cycle [27,45].

Regulation of histone locus body formation

The beginning of *Drosophila* embryogenesis provides an excellent opportunity to analyze the *de novo* appearance of HLBs during animal development. The initial stages of *Drosophila* embryogenesis are driven by gene products deposited into the egg by the mother and consist of 13 rapid nuclear division cycles that occur in a syncytium and lack G1 and G2 phases. Zygotic transcription is not required for embryonic development until the 14th cycle when the cellular blastoderm forms and most maternal mRNAs are destroyed. Maternally provided gene products include RD histone mRNA and protein [46], which in flies [47] as well as zebrafish [48] require SLBP function for deposition into the egg and for normal progression through the early embryonic cycles. In addition, the amount of maternal histone influences the timing of onset of zygotic transcription in *Drosophila* embryos [49].

Although zygotic genome function in *Drosophila* is not required until nuclear cycle 14, there is a wave of zygotic transcription that begins earlier. This wave includes the initiation of zygotic RD histone gene expression precisely in nuclear cycle 11, which is also when HLBs are first observed [8,16,50]. A combination of quantitative live cell imaging and mathematical modeling revealed interesting properties of these newly formed HLBs during the nuclear cycles. The HLBs are disassembled during each mitosis and then appear and grow during each interphase immediately after the completion of mitosis [19]. HLB assembly can be modeled as a phase-separation processes driven by a single component (i.e. Mxc) in which HLB assembly is seeded by active histone genes and final HLB size is

determined by the number of active histone genes, or the seed size [19]. HLB growth in the syncytial *Drosophila* embryo is modulated by Cyclin E/Cdk2, which acts to elevate the nuclear concentration of Mxc likely via direct phosphorylation of Mxc. Reducing Cyclin E/Cdk2 activity results in a failure of normal RD histone pre-mRNA processing, suggesting a link between HLB size and/or organization and efficient mRNA biosynthesis in *Drosophila*. Human HLBs also increase in size as cells enter S phase [13], an observation that also suggests HLB size increases the efficiency of RD histone mRNA production (Figure 1).

The concomitant appearance of HLBs and RD histone transcription in early fly embryos suggests that the two events are coupled, although whether nucleation of Mxc triggers RD histone gene transcription or vice versa remains unclear. Nevertheless, several experiments clearly show that transcription of RD histone genes is necessary for the growth of HLBs to their full size: blocking transcription either with alpha amanitin or genetically results in very small foci of Mxc [19,24]. Moreover, the presence of these small foci indicates that Mxc can locate to RD histone genes in the absence of transcription. The mechanisms by which RD transcription promotes HLB growth are not clear, but an interesting possibility is raised by the observation that the Cyclin L/Cdk11 complex acts to phosphorylate Ser 2 of the RNA pol II tail specifically at RD histone genes in human cells [33]. Cyclin L/Cdk11 is enriched near the 3' end of RD histone genes and functions to promote RNA pol II elongation and the coupling of transcription to 3' end processing. Cyclin L/Cdk11 also is required for accumulation of FLASH, which it binds to and phosphorylates. Thus, perhaps the action of Cdk11 on transcribed histone genes triggers the phosphorylation and retention of FLASH in the HLB, promoting HLB growth.

A recent striking observation of HLBs is that they accumulate large amounts of RNA polymerase II, so much so that in *Drosophila* the 1 micron diameter HLB can readily be distinguished simply by staining for RNA pol II [15, 51–54] (Figure 2). Interestingly, recruitment of RNA pol II to embryonic HLBs in *Drosophila* occurs independently of Zelda [52,55], a Zn finger domain pioneering transcription factor necessary for the initiation of zygotic transcription of much of the rest of the genes in the fly genome [56]. Thus, the activation of transcription of *Drosophila* RD histone genes in the early embryo occurs through a mechanism that is distinct from other RNA pol II transcribed genes [55]. RNA pol II condensates have also been reported to associate with HLBs in human cells using live cell imaging approaches [57]. Whether all the RNA pol II present in HLBs is actively engaged in transcription is unclear, but one possibility is that the high concentration of RNA pol II in the HLB helps trigger rapid activation of RD histone gene expression, particularly in the fast G1-less cycles of early fly development where S phase occurs immediately after the completion of mitosis.

Other factors involved in mRNA synthesis can be enriched in the HLB, including *Drosophila* Prp40, which is best known for participating in spliceosome assembly [58], as well as subunits of the human Mediator complex, which promotes transcription [59]. Prp40 is needed for maximal histone gene expression but is not required for RD histone pre-mRNA processing [60]. In some transformed human cell types, HLBs are sometimes found in association with CBs, and this association is reduced upon depletion of Mediator subunits which localize to the HLB/CB interface [59]. Because Lsm11 of the U7 snRNP is also enriched at this interface, Suzuki et al. proposed that CBs associating with HLBs may promote RD histone mRNA 3' end processing [59]. However, not all HLBs associate with CBs and prior studies in flies and mice clearly indicate that RD histone gene expression and development do not require CBs [61,62]. Nevertheless, these types of cell biological observations suggest that the high rate of histone mRNA biosynthesis that occurs during S phase requires high local concentration of factors involved in RNA biosynthesis and/or metabolism. Evidence for this idea came from genetic analyses in *Drosophila*, indicating that efficient RD histone 3' end formation requires FLASH to be enriched in the HLB [63].

Like many other nuclear bodies, super-resolution imaging revealed that the spherical *Drosophila* HLB acquires a core/shell arrangement when histone genes are transcribed. Ser5 phosphorylated RNA pol II resides in the core together

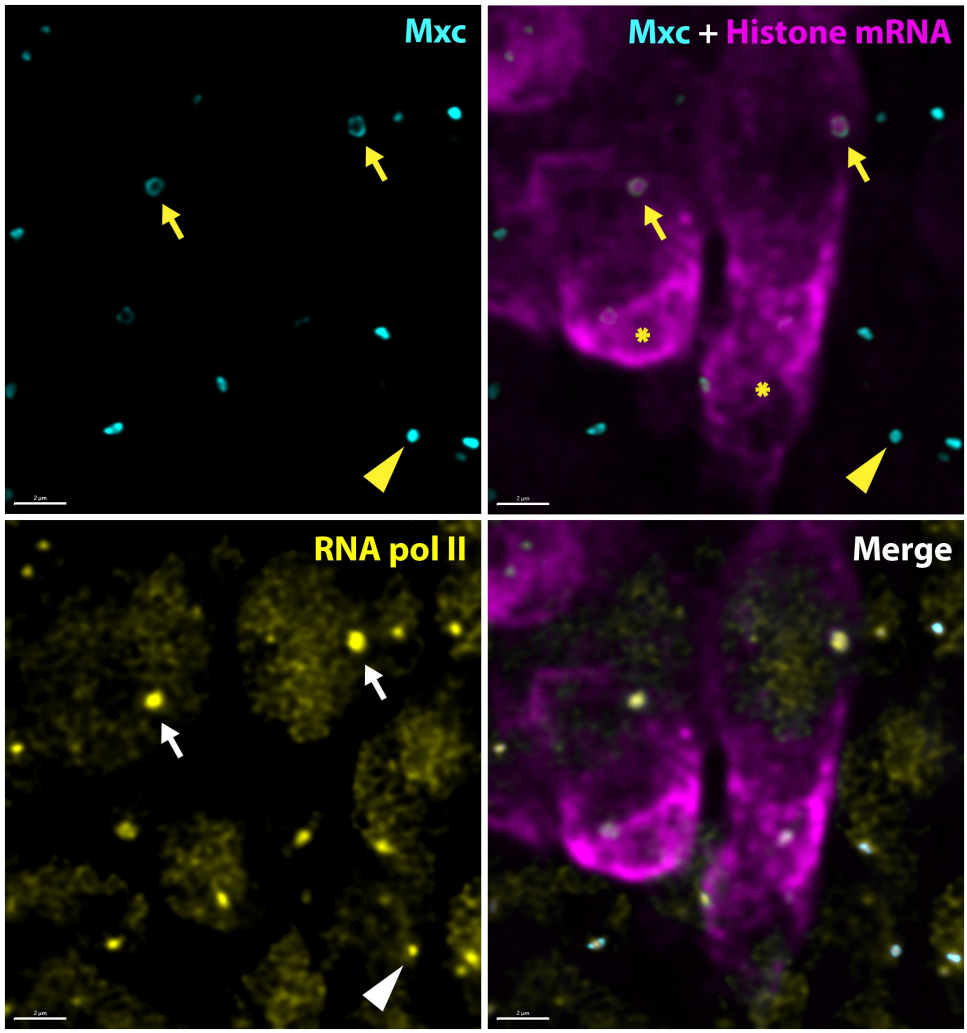

Figure 2. Active *Drosophila* HLBs display a core shell arrangement and are enriched in RNA polymerase II. High-resolution confocal images of *Drosophila* neuronal cells in the embryonic ventral nerve cord stained for Mxc (cyan), histone mRNA (magenta), and RNA polymerase II (yellow). The arrows indicate the HLB of two neuroblast stem cells in S phase as indicated by high level of cytoplasm histone mRNA (asterisks). Note the focus of nascent histone mRNA coincident with high amounts of RNA pol II that are both surrounded by a shell of Mxc protein. The arrowhead indicates an HLB in a quiescent cell that displays a more closed configuration of Mxc. Note that this HLB has RNA pol II, even though it is not in S phase, as indicated by the lack of RD histone mRNA. Scale bar = 2 microns.

with nascent histone mRNA and the N-terminus of Mxc [15] (Figure 2). The shell is enriched with the C-terminus of Mxc as well as the FLASH protein (Figure 1). This observation is somewhat counter-intuitive since the N-terminal region of FLASH is necessary for processing nascent RD histone mRNAs, which are enriched in the core domain. Small amounts of FLASH may dynamically occupy the center of the HLB where mRNA synthesis is taking place (Figure 1), or processing of the transcripts might occur at the core/shell interface. Such a situation happens in the nucleolus where rRNA transcription occurs at the interface between the fibrillar center (FC) and the dense fibrillar component (DFC), the two innermost phase separated compartments of the nucleolus, with rRNA modification and processing steps taking place as the nascent RNA radiates into the DFC [64].

Cell cycle regulation of the HLB

Live imaging of human cells has provided new insight into how HLB regulation occurs in canonical G1-S-G2-M cells cycles rather than the rapid,

specialized cycle of early development. RD histone mRNA accumulates to high levels during S phase, and this results from a combination of the activation of histone pre-mRNA processing and transcription at the G1-S transition [65]. A critical step in activation of RD histone gene expression is phosphorylation of NPAT/Mxc by Cyclin E/Cdk2 [8,66] (Figure 1). Careful single cell imaging of live human cells revealed that RD histone transcription begins to increase prior to S phase at the restriction point, a time late in G1 when cells activate Cyclin E1/Cdk2 and commit to entering S phase [42]. Cyclin E1/Cdk2 phosphorylates NPAT [9], and this phosphorylation is required for transcription of RD histone genes [66]. Accordingly, addition of a potent Cyclin E1/Cdk2 inhibitor rapidly shuts off histone transcription [13]. HLB assembly and growth, as measured by co-recruitment of NPAT and FLASH, are also initiated at the restriction point, but do not require Cyclin E/Cdk2 activity and HLBs are present even when RD histone genes are not transcribed [13]. Thus, as in *Drosophila*, there is a concomitant activation of histone gene expression and HLB formation, suggesting that the two are mechanistically coupled via Cyclin E/Cdk2 activity, and once fully formed, HLBs are maintained even after the cessation or inhibition of RD histone gene transcription. Interestingly, this mechanistic coupling can be bypassed in specialized developmental situations: late-stage *Drosophila* ovaries transcribe RD histone genes independently of S phase and Cyclin E/Cdk2 activity to load maternal RD histone mRNAs into the developing oocyte [46]. Finally, the human cell analyses also revealed a second wave of NPAT phosphorylation in G2 that is controlled by another Cdk kinase (likely Cyclin A2/Cdk2 or Cyclin A2/Cdk1). The function of this phosphorylation event is not known, but it could be involved in disassembling the HLB during mitosis.

Other signaling pathways may provide regulatory inputs into RD histone gene expression during the cell cycle via HLBs. For instance, the cAMP signaling modulator EPAC1 forms biomolecular condensates within the nucleus after cAMP synthesis [67]. Some (but importantly not all) of these condensates associate closely with HLBs and the chromosome 6 histone locus in human cells. Moreover, ectopic expression of EPAC1 coupled with cAMP-analog stimulated formation of EPAC1 condensates results in activation of histone gene expression independently of cell cycle progression. The biological role of this histone stimulatory pathway is unknown, but this report highlights the possibility that organizing histone genes into the HLB may permit cells to modulate histone gene expression by accessing different signaling modalities via biomolecular condensates that associate with HLBs.

HLBs and genomic instability and disease

Coordinated regulation of RD histone genes is critical to maintain genome stability and normal development. For instance, aberrant chromosome segregation occurs in the *Drosophila* male germ line when Mxc function is compromised [68], and *slbp* mutant zebrafish display defects in neuronal development in part due to disruption of cell proliferation [69,70]. Consequently, increasing evidence implicates mutations in HLB factors in human disease. NPAT mutations have been linked to both Hodgkin's lymphoma and ataxia disorder in humans [71,72]. In *Drosophila*, depletion of Mxc causes hyperplasia in larval lymph glands, which are sites of hematopoiesis in flies, and serves as a model for human leukemia [73]. This phenotype is also observed when other *Drosophila* HLB proteins such as Spt6 and Mute are knocked down in larval lymph glands or when ectopic expression of polyadenylated RD histone mRNA is induced, suggesting correct regulation of RD histone mRNA biosynthesis is necessary for proper lymph gland formation. Mxc was also shown to be required for proper division and differentiation of neural lineages in larval and adult brains [74]. This study found similar results when histone gene expression was knocked down, further suggesting that proper transcription and processing of RD histone mRNA is required for normal cell cycle progression and maintenance of neural lineages.

Other work suggested that the human protein Fused in Sarcoma (FUS) interacts with the U7 snRNP complex during S-phase and binds at RD histone gene promoters [75]. FUS has a wide range of roles in genome maintenance, RNA processing, and DNA recombination [76,77] and FUS gene mutations have been identified in familial

amyotrophic lateral sclerosis (ALS) patients [78,79]. Many of these mutations occur in the NLS of the FUS protein and lead to cytoplasmic aggregation of the typically nuclear protein. Interestingly, these cytoplasmic FUS aggregates sequester several RNA binding and processing proteins, most notably U7 snRNP [80]. Mutation or depletion of the FUS protein led to reductions of RD histone gene expression and an increase in misprocessed polyadenylated RD histone RNA [80].

The tumor suppressor p53 binding protein 1 (53BP1) is responsible for maintaining genome integrity in part through its promotion of the non-homologous end joining (NHEJ) DNA repair pathway. However, recent work found a p53 independent function for 53BP1 in maintaining genomic integrity through the activation of histone gene expression [81]. 53BP1 in conjunction with ATP citrate lyase (ACLY) begin a cascade which leads to acetylation of histone H3 and H4 residues, specifically at the promoter of the SLBP gene, which induces the production of the SLBP protein necessary for RD histone mRNA biosynthesis. Knockout of either 53BP1 or ACLY results in downregulation of SLBP and in turn lowered expression of RD histone genes and an increase in misprocessed poly-adenylated RD histone mRNA [81]. The decrease in RD histone expression due to SLBP downregulation led to delays in cell cycle progression and increased chromosomal rearrangements. Increased double-strand breaks due to lowered histone expression were also observed upon knock-down of *Drosophila* Mxc in larval lymph glands [74].

The common finding of these recent publications connecting HLB proteins, diseases, and genomic integrity can be distilled down to reduced expression of the replication-dependent histone genes and the increased presence of poly-adenylated histone mRNA. While the individual HLB proteins affected vary across these observed disease states and genomic perturbations, it is clear the proper regulation and production of histones is key to maintaining normal cell proliferation, differentiation, and genomic integrity. As such, the possible involvement of mutation or misexpression of HLB proteins in human disease is perhaps unsurprising. As we begin to look more deeply at the role of histone production in human malignancies, more connections between the proteins that govern HLB formation and RD histone mRNA biosynthesis are likely to arise. Furthermore, given the high evolutionary conservation of HLB components involved in histone mRNA biosynthesis, work in experimentally tractable organisms like *Drosophila* and zebrafish should continue to reveal concepts and mechanisms that are broadly applicable to our understanding of the formation of biomolecular condensates in the nucleus and their role in the control of gene expression.

Data accessibility statement

The data shown were generated at UNC Chapel Hill in the Duronio Lab and are available from the corresponding author [RJD] upon request.

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Disclosure statement

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