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Cycling in the nucleus: regulation of RNA 3′ processing and nuclear organization of replication-dependent histone genes

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The histones which pack new DNA during the S phase of animal cells are made from mRNAs that are cleaved at their 3′ end but not polyadenylated. Some of the factors used in this reaction are unique to it while others are shared with the polyadenylation process that generates all other mRNAs. Recent work has begun to shed light on how the cell manages the assignment of these common components to the two 3′ processing systems, and how it achieves their cell cycle-regulation and recruitment to the histone pre-mRNA. Moreover, recent and older findings reveal multiple connections between the nuclear organization of histone genes, their transcription and 3′ end processing as well as the control of cell proliferation.

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Introduction

''And the end and the beginning were always there

Before the beginning and after the end.''

(T.S. Eliot, ''Burnt Norton'', 1935)

By reminding us of the importance of ends (or endings) and of the cyclic nature of many aspects of life, this quote from Eliot's 'Four Quartets', sets an appropriate stage for this review. In the RNA World, transcription termination and 3′ end processing are as important as transcription initiation for RNA biogenesis. For mRNAs, the rate and site of 3′ end processing also play important roles in regulating their biological functions. Moreover, early determinants of gene expression, for example, chromatin structure and transcription, are interconnected with later ones, as RNA processing or the cytoplasmic fate of the mRNAs. Such connections have been well described for the bulk of mRNAs that acquire their 3′ ends by cleavage/polyadenylation (CPA; reviewed in [1–3]). The aim of this review is to discuss new aspects of the regulation of the animal replication-dependent (RD) histone genes whose mRNAs are generated by a different 3′ processing mechanism and which are expressed in a cyclic manner during the cell cycle. We particularly discuss the regulation of the so-called heat-labile processing factor and its relation to the CPA machinery. Additionally, we highlight how the organization of the RD histone genes in specific subnuclear domains termed histone locus bodies allows for cross-talks between transcription, 3′ processing, chromatin structure, cell proliferation and disease.

The main points of this review are summarized in Table 1.

The animal replication-dependent histone genes

In multicellular animals (metazoans), the synthesis of histones during the S phase of the cell cycle is ensured by a particular class of genes, termed RD histone genes. These are usually present in multiple copies. In humans, ~50 genes for the five histone types are clustered in two major loci on chromosomes 6 and 1 [4]. These genes do not contain introns, and their mRNA 3′ ends are produced by cleavage of longer precursors but do not get polyadenylated. This unique mode of RNA processing is important to ensure that adequate amounts of histone proteins are produced to pack the new DNA synthesized during S phase and that the expression of these genes is low in other cell cycle phases. In mammalian cells, RD histone transcription increases ~5-fold during the G1 to S phase transition, and processing is enhanced ~8-fold, resulting in an overall 30–40-fold increase of mature histone mRNAs [5]. At the end of S phase, the half-life of these histone mRNAs drops dramatically in response to a destabilization of the specific 3′ ends (see 'Cell cycle regulation of SLBP'). Another class of histone transcripts which are expressed constitutively at a basal level throughout the cell cycle (encoding so-called replacement histones) contain introns and are polyadenylated [6].

Table 1

Overview of replication-dependent histone gene regulation

Process	Entity	S phase	Non-S phases	References
Histone gene transcription	NPAT	**Phosphorylated**	Unphosphorylated	[38–40]
	FLASH	**Abundant in HLBs**	Less abundant in HLBs	[34,43,59]
	U7 snRNP	No interaction with hnRNP UL1	*Interaction with hnRNP UL1*	[51••]
	FUS/TLS	**Interaction with NPAT and histone promoters**	*Interaction with hnRNP UL1*	[52••]
	HLB staining	Strong (4 HLBs)	Weak (2 HLBs)	[34,43,59]
Histone RNA 3′ processing	SLBP concentration	**High**	Low	[19]
	CstF64 concentration	**High**	Low	[23••]
	HLF	**Active**	Inactive	[20]
	U7 snRNP/Lsm11	**Interaction with FLASH in HLBs**	No interaction with FLASH	[33••]
	FUS/TLS	**Interaction with U7 snRNP**		[52••]

Transcription and RNA 3′ end processing of the animal replication-dependent histone genes are minimal in G1 phase and activated upon entry into S phase of the cell cycle. The table summarizes the regulatory events involved in this regulation as discussed in this review. These are coordinated in histone locus bodies (HLBs) which are specialised subnuclear structures containing the histone gene clusters and their expression machinery. Stimulatory and inhibitory mechanisms are highlighted by**bold** and *italic* lettering, respectively.

Unique histone RNA 3′ end processing mechanism

RD histone RNA processing (RHP; reviewed in [7,8]) requires two *cis*-acting elements which are recognized by histone mRNA-specific factors (Figure 1). A highly conserved stem-loop (or hairpin) structure upstream of the cleavage site constitutes the binding site for the stem-loop-binding or hairpin-binding protein (SLBP/HBP) [9,10]. The second so-called histone downstream element (HDE) is bound by the RNA moiety of the U7 small nuclear ribonucleoprotein (snRNP) [11,12]. This minor snRNP (present in few thousand copies per cell) has a specific protein composition consisting of five Sm proteins that are also present in spliceosomal snRNPs and two U7-specific Sm-like proteins named Lsm10 and Lsm11 [13,14]. Three further proteins associate with the U7 snRNP: a zinc finger protein of 100 kDa (ZFP-100, ZNF473) that connects the U7 snRNP to SLBP [15], FLASH (short for FLICE-Associated Huge Protein, also named Caspase 8 Associated Protein 2, CASP8AP2) [16] and the 68 kDa subunit of mammalian cleavage factor I (CFI_m68, CPSF6) [17]. Additional *trans*-acting proteins that contribute to RHP are organized in the so-called heat-labile factor (HLF) [18]. Two of the RHP factors have been shown to be cell cycle-regulated: SLBP [19] and the HLF [20]. These regulations, in particular that of the HLF, will be discussed in more detail below.

Interestingly, most if not all RD histone genes contain at least one polyadenylation signal downstream of the U7-dependent site (Figure 1). When RHP is disturbed experimentally, these downstream signals are used to produce polyadenylated transcripts [21,22]. These read-through transcripts are present in low amounts compared to the canonical histone mRNAs and can also be detected in unperturbed cells, especially outside of S phase when RHP is less efficient [23••]. Most of them remain nuclear and appear to be rapidly degraded by the nuclear exosome. However, a small fraction can be detected on polysomes, indicating that they can function in protein synthesis [23••]. This may be a way to ensure that the cell can produce small amounts of histones outside of S phase or during proliferative arrest to replace lost histones, for example, during chromatin remodeling and/or DNA damage events. This back-up mechanism may also prevent transcription from overrunning the neighboring genes. A more pronounced case of this behavior is exemplified by the replication-independent H2A.X gene which is processed to polyadenylated mRNA in G1 phase but selects an upstream U7-dependent cleavage site in S phase [24].

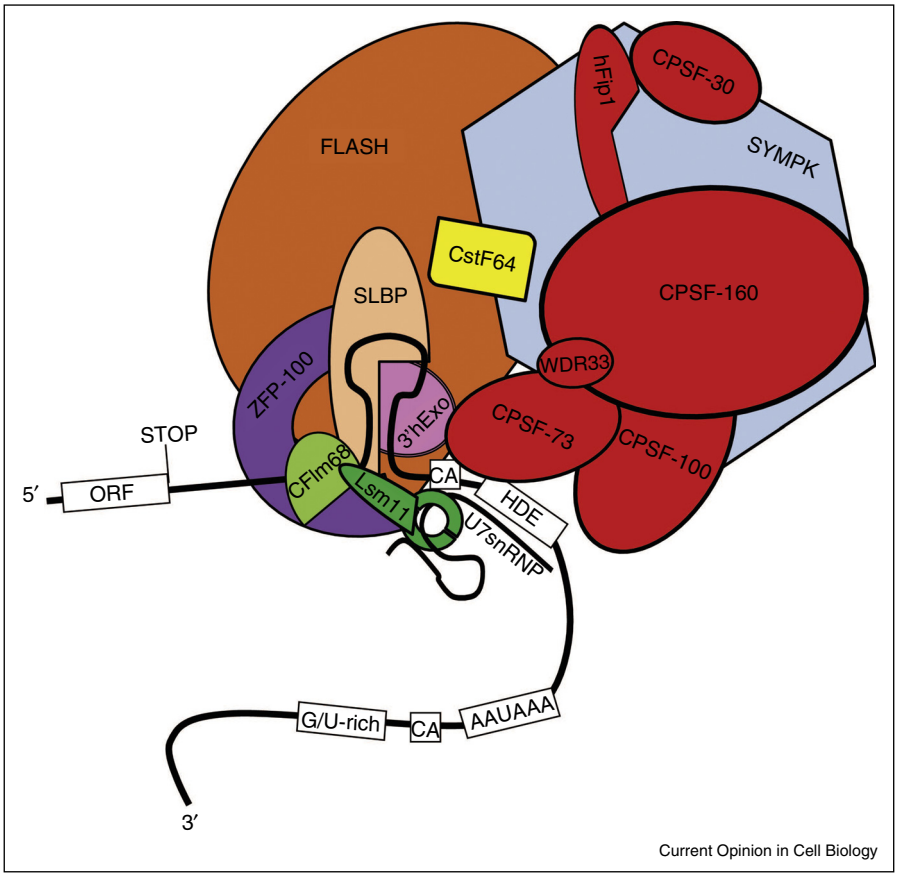
Cell cycle regulation of SLBP

The SLBP levels fluctuate during the cell cycle in parallel with those of the histone mRNAs [19]. The upregulation of SLBP during the G1/S phase transition is brought about by an activation of its translation, and its down-regulation after S phase is a consequence of an accelerated degradation by the proteasome. Besides being involved in RHP (Figure 1), SLBP protects histone mRNAs from degradation and is a positive effector of their translation [25–27]. Its degradation at the end of S phase leads to a stop of histone translation and to the rapid elimination of histone mRNAs from the cell [19].

The cell cycle-regulated heat-labile factor plays a pivotal role between RHP and CPA

The other regulated RHP factor, HLF, is composed of symplekin, the complete Cleavage and Polyadenylation Specificity Factor (CPSF) and the 64 kDa subunit of Cleavage Stimulation Factor (CstF64) [28]. Although symplekin was shown to be the heat-labile component [28], it has, until recently, remained unclear how the activity of HLF is regulated nor how it gets recruited to the histone pre-mRNA. Moreover, the fact that all its components are shared with the CPA machinery raised the question how the cell manages the assignment of these components to the two 3′ processing systems. Based on work in *Drosophila* cells, it has been proposed that a

Figure 1

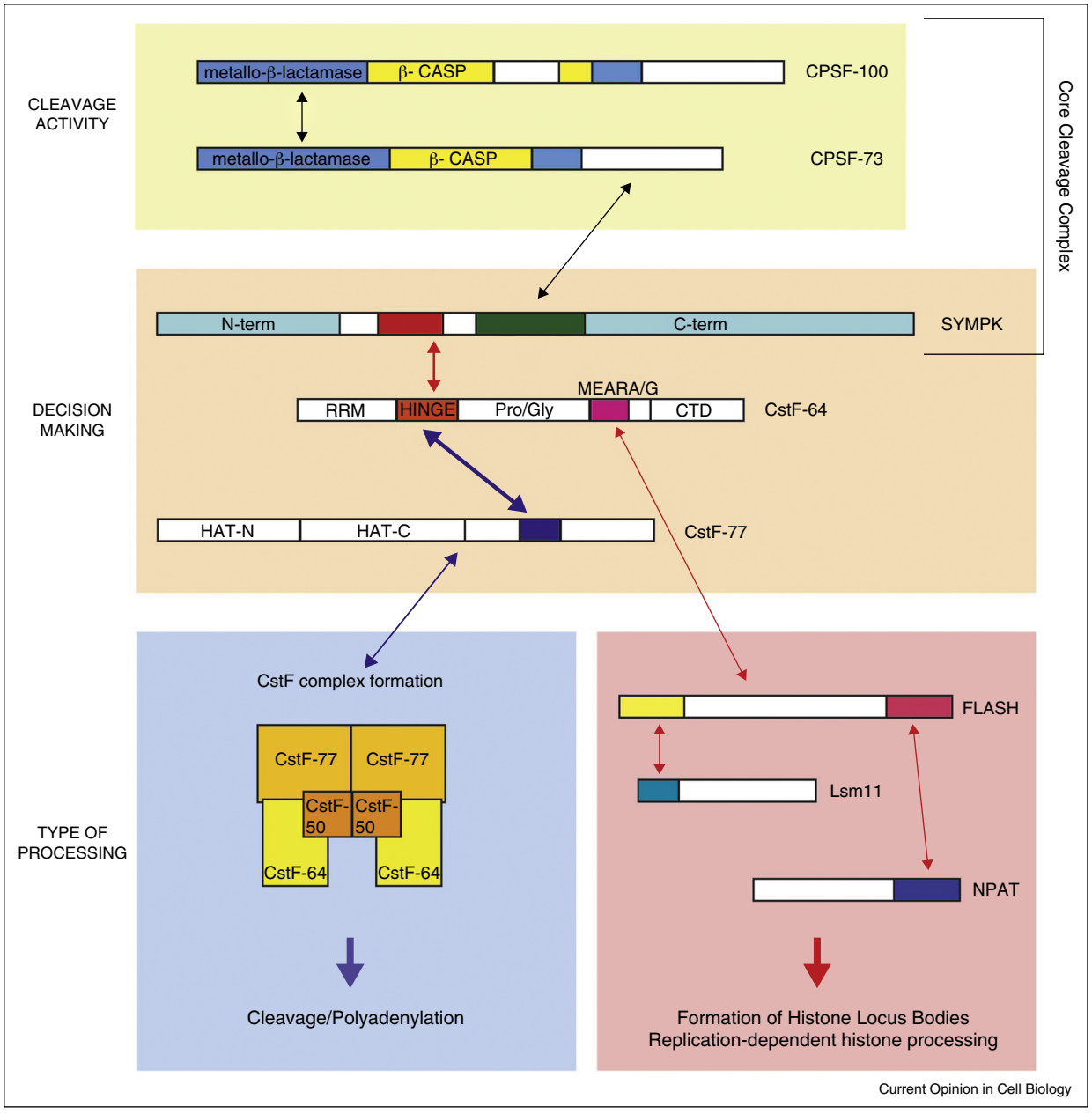


Schematic view of the replication-dependent histone RNA processing complex. Within ~50 nucleotides of the stop codon, histone pre-mRNAs contain two conserved processing signals, the stem-loop element and the purine-rich histone downstream element (HDE). These elements are recognized by stem-loop binding protein (SLBP) and the U7 snRNP, respectively. A 100 kDa zinc finger protein (ZFP-100) forms a connection between these two factors and stabilizes the complex. The U7 snRNP contains a ring-like structure composed of five Sm proteins and the U7-specific Sm-like proteins, Lsm10 and Lsm11. A N-terminal extension of Lsm11 additionally binds the 68 kDa subunit of mammalian cleavage factor I (CFIm68) and FLASH. Together, Lsm11 and FLASH provide a docking platform for the Heat Labile Factor (HLF) which consists of proteins that are also involved in cleavage/polyadenylation (CPA): symplekin (SYMPK), Cleavage Stimulation Factor 64 kDa subunit (CstF64) and all six subunits of Cleavage and Polyadenylation Factor (CPSF). CPSF-73 is the endonuclease that cleaves the pre-mRNA after a CA dinucleotide. While SLBP binds to the 5′ side of the hairpin, the 3′ side is occupied by 3′hExo, an exonuclease that trims the 3′ end of the mRNA after cleavage and may also be involved in histone mRNA degradation. If the canonical histone processing site is not recognized, a downstream polyadenylation signal can be used by the CPA machinery, resulting in polyadenylated histone transcripts.

subcomplex of the HLF, composed of CPSF73, CPSF100 and symplekin (termed core cleavage complex (CCC)), is operational in both RHP and CPA [29,30•] (Figure 2). Moreover, CstF64 is known to undergo mutually exclusive interactions with CstF77 and symplekin [31,32], and recent work showed that the CstF64-symplekin interaction is essential for RHP [23••,32]. These mutually exclusive interactions may determine whether CstF64 gets incorporated into the CstF complex used in CPA or the HLF involved in RHP. However, it is not known whether and how this binding to CstF77 or symplekin is regulated and whether HLF exists throughout the cell cycle or has to be assembled during the G1/S phase transition. As CstF64 levels also show a slight cell cycle regulation, its increasing amounts during S phase might favor its incorporation into the HLF, whereas the lower amounts present in other cell cycle phases might be incorporated preferentially into CstF complexes [23••]. However it is also possible that other events, for example, posttranslational modifications, play a role in HLF assembly and/or activation.

Concerning the recruitment of the HLF to the histone pre-mRNA, it has been shown that the U7 component Lsm11 and FLASH are essential for RHP [14,16] and, by interacting with each other, provide a platform for binding

Figure 2



Molecular interactions guiding the formation of cleavage/polyadenylation (CPA) and replication-dependent histone RNA processing (RHP) complexes. A core cleavage complex common to RHP and CPA is formed by the interaction of the C-termini of CPSF-73 and CPSF-100 with a CPSF-binding segment (green) of symplekin (SYMPK). Mutually exclusive interactions of the hinge domain of CstF-64 with either CstF-77 or SYMPK are decisive for the formation of the CstF complex involved in CPA or the heat-labile factor (HLF) active in RHP. The HLF is tethered to FLASH by interactions of the MEARA/G domain of CstF-64 and a yet undefined region of SYMPK with FLASH. FLASH, in turn, makes contact with NPAT (leading to the formation/stabilization of HLBs and stimulation of histone gene transcription) and with the U7 snRNP component Lsm11 to allow the recruitment of the HLF to the histone pre-mRNA.

the HLF [33••]. On the other hand, two of the HLF components can bind to FLASH: CstF64 does this through its MEARA/G domain, and symplekin with a not yet characterized domain [23••]. Additionally, CstF64 and symplekin must bind to each other to fully tether the HLF to FLASH and the histone pre-mRNA [23••,32] (Figures 1 and 2).

Another possibility which is also compatible with the above interactions is that HLF does not get assembled

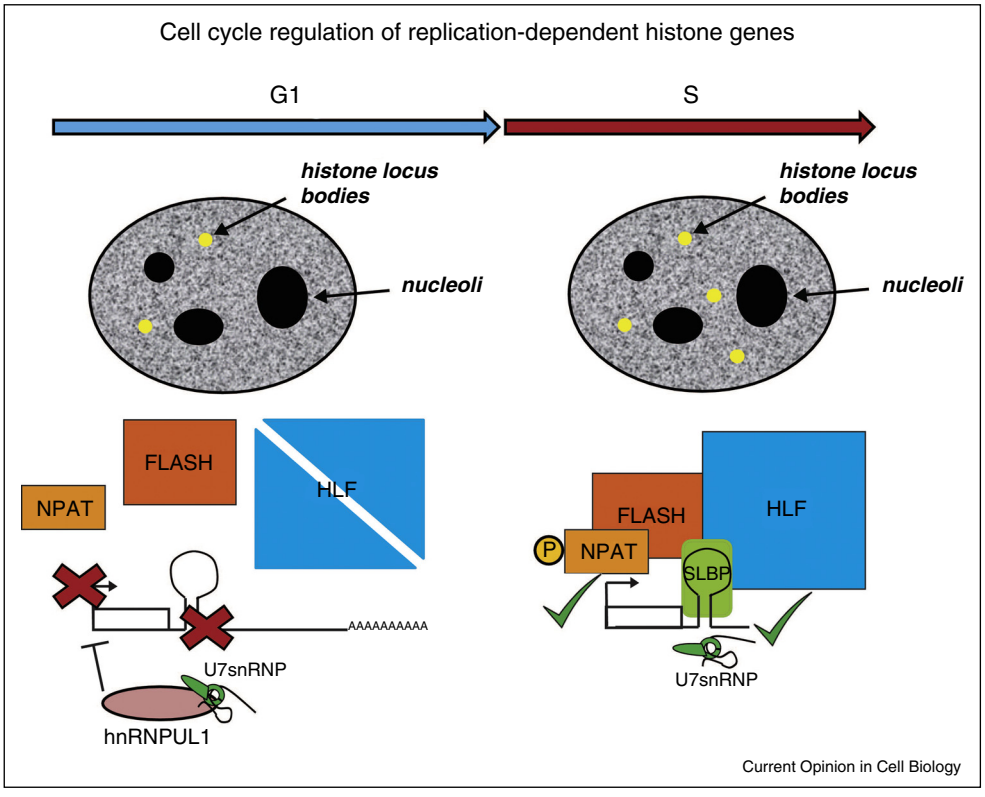
as a separate entity, but rather directly on the U7 snRNP/FLASH platform. An argument against this is the fact that the original affinity-purified HLF did not contain U7 snRNP components or FLASH [28], but this might have been a purification artefact.

Nuclear 3D organization and the co-regulation of histone gene transcription and processing

The repetitive nature and genomic clustering of the RD histone genes and the formation of specialized subnuclear structures at these loci, called histone locus bodies (HLBs) [34,35], are important features in the cell cycle control of histone gene expression. HLBs are enriched in transcription factors and 3′ processing components needed for histone gene expression, and their formation follows a hierarchical protein recruitment that appears to be nucleated by transcription [36,37]. By immunostaining of HLB components, two foci, corresponding to the largest cluster on chromosome 6, can be visualized in G1 phase cells, whereas the two additional HLBs corresponding to the minor cluster on chromosome 1 only appear during the transition to S phase (Figure 3).

NPAT (Nuclear Protein, Ataxia-Telangiectasia Locus) is the main factor responsible for the S-phase-specific transcriptional activation of all RD histone genes. It gets activated by cyclin E/Cdk2-dependent phosphorylation which most likely is the primary event that triggers a cascade of protein recruitments to the HLBs at the G1/S phase boundary [35,38–41]. This phosphorylation activates histone transcription and promotes the recruitment of FLASH (by direct interaction with NPAT) to the HLBs [42], thus reinforcing the formation of active HLBs [34,43,44] (Figure 3). Subsequently, FLASH makes contacts with the U7 component Lsm11 to provide a platform for the recruitment of the HLF [33••] while transcription

Figure 3



Nuclear organization and cell cycle-dependent regulation of replication-dependent histone genes. The expression of RD histone genes is tightly regulated during the cell cycle at transcriptional, post-transcriptional and translational levels (see text). This figure depicts nuclear events occurring during the G1 to S phase transition. Histone locus bodies (HLBs) are subnuclear sites containing the clustered RD histone genes and components of the corresponding gene expression machinery. In G1, only two HLBs corresponding to the major histone cluster on chromosome 6 can be stained by antibodies against HLB marker proteins such as FLASH. As NPAT is not phosphorylated, the interaction with FLASH is weak. The level of SLBP is low, and the U7 snRNP, interacting with hnRNP UL1, inhibits histone transcription. As a consequence, transcription is minimal and RNA 3′ processing is inefficient so that most transcripts from the RD histone genes become polyadenylated at cryptic polyA signals downstream from their regular cleavage sites. In S phase, NPAT gets phosphorylated by cyclinE/Cdk2 which stabilizes the interaction with FLASH. The concentration of factors in HLBs increases, and two more HLBs corresponding to the minor cluster on chromosome 1 can be detected. SLBP is up-regulated, and FLASH interacts with the U7 snRNP, hence providing a platform for the recruitment of the now activated HLF. Thus, RD histone gene transcription and RNA 3′ processing at the regular sites are stimulated ~5-fold and ~8-fold, respectively.

occurs. This may involve the non-coding Y3** RNA which was recently shown to promote the accumulation of CPSF into HLBs and to stimulate RHP [45•].

Even though there is no direct evidence for an association of processing factors with the C-terminal domain (CTD) of RNAP II as for CPA, several lines of evidence indicate that the transcription machinery for the RD histone genes has special features which contribute to both the cell cycle regulation and the particular mode of RNA 3′ processing. Studies on *Drosophila* polytene chromosomes indicate that the TATA-less H1 gene promoter utilizes TBP (TATA-box-binding protein)-related factor 2 (TRF2) [46]. By contrast, the core histone promoters appear to use TBP and TFIIA but neither TFIIB nor the complete TFIID complex [47•]. Even though both types of promoters show an S phase-specific activity, their actual kinetics within the S phase are different. Moreover, the phosphorylation of the CTD at Thr-4 has been reported to be important for RHP in mammalian cells [48•]. However, Thr-4 phosphorylation is also more generally enriched at the 3′ ends of genes that generate polyadenylated mRNAs, and its suppression generates global elongation defects [49,50].

Another interesting connection between RHP and histone gene transcription is that the U7 snRNP interacts with the hnRNP protein U-like 1 (hnRNP UL1) and represses histone gene transcription in growth-arrested cells [51••] (Figure 3). The mechanism has not been investigated in detail and it is not clear whether this phenomenon also occurs in cycling cells. Nevertheless, it is likely that this feed-back inhibition co-operates with the NPAT-dependent transcriptional activation to regulate histone gene transcription. Furthermore, recent work has suggested that FUS/TLS (fused in sarcoma/translocated in liposarcoma) may play a role in histone gene regulation [52••]. FUS was shown to interact with NPAT in S phase and to positively affect transcription and 3′ end processing of histone mRNAs. Additionally, through interactions with the U7 snRNP and hnRNP UL1, FUS also seems to be involved in the transcriptional repression of the RD histone genes outside of S phase.

Connections with chromatin, DNA damage and cell proliferation

In addition to its role in RD histone gene transcription, NPAT interacts with Cdk9 and thereby regulates chromatin modifications through a complex network of phosphorylations and protein interactions which ultimately also affect RHP [53••]. This recruitment of Cdk9 to histone genes increases the phosphorylation of Ser2 on the RNAP II CTD, which is essential for the enrollment of the multifunctional RNA Polymerase II Associated Factor (PAF). PAF in turn stimulates the activity of the E3 ubiquitin ligase complex RNF20/40 that catalyzes H2B monoubiquitination (H2Bub1). On the histone loci, H2Bub1 levels are specifically elevated near the 3′ cleavage sites. H2Bub1 can recruit the ASH2L or SET2D methyltransferase complex that methylates the lysine 4 position of histone H3 (H3K4me) [54]. This modification serves as docking site for the chromodomain helicase DNA binding protein 1 (CHD1) that can recruit spliceosomal components, in particular the U2 snRNP [55]. This sequence of events is most likely relevant for RHP, since histone open reading frames (ORFs) contain a conserved 22nt binding site for the U2 snRNP and this binding stimulates RHP [56]. Furthermore, one component of the PAF complex, the tumor suppressor Cdc73, has been shown to associate with CPSF and CstF and to contribute to the 3′ end maturation of polyadenylated [57] and RD histone mRNAs [58].

RHP is also affected by the DNA damage response. When DNA damage occurs during DNA replication, FLASH relocates from HLBs to the cytoplasm leading to the disruption of HLBs and to a cell cycle block in S/G2 phase [59,60]. Another component of HLBs is ARS2 (Arsenite Resistance Protein 2, SRRT), a component of the cap-binding complex (CBC) that interacts with FLASH through its central domain. A disruption of this interaction results in proliferation defects [42]. Moreover, a depletion of SLBP leads to defects in chromatin condensation and to cell death [61]. Thus RHD is not only cell cycle-regulated but also important for a normal chromatin structure and cell proliferation.

Outlook

Even though the major players in the actual histone RNA processing reaction are known, it is conceivable that additional components may be found, especially ones that link RHP to other nuclear events. Concerning the cell cycle regulation of RHP components, that of SLBP is fairly well understood, but the regulation of the HLF is still in an early phase of investigation. To fully understand how the cell manages the assembly of this complex composed of proteins used in both CPA and RHP, how it regulates its activity and how the HLF integrates into the RHP complex will require a detailed study of all possible interactions and post-translational modifications of these components. Moreover, for most of the connections between the nuclear organization of RD histone genes in HLBs, their transcription and processing and other nuclear events discussed here, the underlying mechanisms remain to be characterized in detail. Finally, the fact that histone gene expression, and in particular FLASH, may be involved in the regulation of cell proliferation [59,60] suggests that RHP may be related to diseases. There is emerging evidence for a role of the polyadenylated histone read-through transcripts in cancer [62–64]. Moreover, partial deletions of the histone gene cluster on chromosome 6 have been linked to Down syndrome acute lymphoblastic leukemia [65]. Thus,

future studies of the metabolism of RD histone genes are likely to yield further interesting discoveries.

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