Open and Closed: The Roles of Linker Histones in Plants and Animals

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ABSTRACT Histones package DNA in all eukaryotes and play key roles in regulating gene expression. Approximately 150 base pairs of DNA wraps around an octamer of core histones to form the nucleosome, the basic unit of chromatin. Linker histones compact chromatin further by binding to and neutralizing the charge of the DNA between nucleosomes. It is well established that chromatin packing is regulated by a complex pattern of posttranslational modifications (PTMs) to core histones, but linker histone function is less well understood. In this review, we describe the current understanding of the many roles that linker histones play in cellular processes, including gene regulation, cell division, and development, while putting the linker histone in the context of other nuclear proteins. Although intriguing roles for plant linker histones are beginning to emerge, much of our current understanding comes from work in animal systems. Many unanswered questions remain and additional work is required to fully elucidate the complex processes mediated by linker histones in plants.

Key words: linker histone; histone H1; chromatin; gene regulation; development; differentiation; imprinting; posttranslational modifications; DNA methylation; high mobility group proteins.

INTRODUCTION

The nucleus is a complex organelle consisting of the DNA, proteins, and RNA, which together mediate the myriad functions that the cell must carry out. DNA and the proteins that package it are collectively called chromatin. The basic unit of chromatin is the nucleosome, which consists of ~150 bp of DNA wrapped around an octamer of ‘core’ histone proteins like wire around a spool (two of each: histone H2A, H2B, H3, and H4). Between nucleosomes is ~50 bp of DNA that can be bound by linker histones (H1). The density of chromatin packaging varies, with more or less dense regions referred to as heterochromatin and euchromatin, respectively. The degree of chromatin compaction is influenced by covalent modifications to both the DNA itself (e.g. cytosine methylation) and to packaging proteins, particularly the ‘tails’ of the core histones. These modifications can act to either facilitate or restrict the access of RNA polymerases and transcription factors to DNA, thereby promoting or inhibiting gene expression. Specific posttranslational modifications (PTMs) to the core histones are known to be associated with transcriptionally active euchromatin and transcriptionally repressed heterochromatin. While the role of core histones and their PTMs in chromatin packaging/gene expression are relatively well characterized, we understand far less about the importance of the linker histones, their functions, and their mechanisms of action, especially in plants. This review explores our current understanding of the role of linker histones in plants. We first summarize our current understanding of H1 in mammals, then discuss what is known about plant linker histones, using the mammalian system as a reference.

ANIMAL LINKER HISTONES

Domains and Families

Linker histones in animals (and plants) consist of three domains: a short, intrinsically disordered N-terminal domain (NTD), a conserved globular core, and a long intrinsically disordered C-terminal domain (CTD) (Figure 1). They are lysine- and proline-rich, and, like core histones, highly basic. In contrast to core histones, which are highly conserved across eukaryotes, linker histones are more variable. In addition to variation between species, many organisms are known to possess multiple linker histone variants (Kowalski and Palyga, 2012); exceptions include Drosophila, which has many copies of a single variant (Lifton et al., 1978). The expansion of the linker histone family in some animals has led to sub-functionalization. For example, just as some core histones are expressed...
during DNA replication while others are expressed constitutively (Talbert and Henikoff, 2010), linker histone variants can have divergent expression patterns. Humans possess 11 linker histones (Supplemental Table 1), five of which (H1.1–H1.5) are most highly expressed during replication (Godde and Ura, 2009; Happel and Doenecke, 2009). Linker histones are also developmentally regulated: H1.0–H1.5 and H1.X are the predominant variants in dividing cells, whereas H1.2, H1.X, and H1.0 are broadly expressed in differentiated cells. Additionally, four variants are expressed only in germline cells: testis-specific H1t, H1T2, and H1LS1, and oocyte-specific H1oo (Godde and Ura, 2009; Happel and Doenecke, 2009).

Chromatin Structure

In order to mediate complex patterns of gene expression, chromatin must be able to dynamically interconvert between transcriptionally active ( euchromatin) and silenced states (heterochromatin). PTMs of core histone tails have been shown to be a universal mechanism of gene regulation among eukaryotes (Zentner and Henikoff, 2013). The PTMs of core histones can affect chromatin structure directly, by altering inter-nucleosomal interactions, or indirectly, by serving as binding sites for other nonhistone proteins that modulate chromatin structure (Schrick et al., 2012). A well-characterized example is lysine acetylation of histone H4, a mark enriched at transcriptionally active sites. The tails of H4 facilitate compaction of chromatin by forming bridges with nearby nucleosomes. Acetylation of H4 lysine residues weakens these bridging interactions, facilitating a more open/accessible chromatin state. In addition to this direct effect on chromatin compaction, lysine acetylation can also be bound by histone mark ‘readers’, such as bromodomain proteins (Zeng and Zhou, 2002). Other histone modifications, such as lysine methylation, do not alter the charge of the histone tails and are thought to exert their effects on chromatin structure primarily by facilitating or inhibiting the binding of other proteins. For example, di-methylation of histone H3 at lysine 9 (H3K9me2) serves as a binding site for HETEROCHROMATIN PROTEIN 1 (HP1) (Lachner et al., 2001), which aids in the formation of heterochromatin through oligomerization (Nielsen et al., 2001). There are also cases where one PTM will prevent the addition of other PTMs. For example, H3K4me3 and H3K27me3 are antagonistic modifications, with H3K4me3 enriched in the promoters of transcriptionally active genes and H3K27me3 enriched at repressed loci. It was recently shown that the enzymes that catalyze the addition of H3K27me3 (the POLYCOMB REPRESSOR COMPLEX 2) cannot add K27 methylation to H3 proteins that already carry K4 methylation (Schmitges et al., 2011). Such mechanisms may help to restrict particular histone modifications to certain regions of chromatin, in this case by preventing repressive H3K27me3 from spreading into actively transcribed regions enriched in H3K4me3.

Our understanding of the interconversion between euchromatin and heterochromatin is not complete without considering linker histones, which play an essential role in the compaction of chromatin. The basic CTD of H1 binds to and neutralizes the negatively charged linker DNA on either side of the nucleosome, thereby facilitating compaction (Schrick et al., 2012). Like the PTMs of core histones, the PTMs of linker histones are known to affect chromatin structure both directly and indirectly. Phosphorylation of H1, which adds negative charge, directly increases its mobility by weakening the interaction between H1 and DNA (Misteli et al., 2000; Roque et al., 2008). In addition to affecting the strength of the electrostatic interaction between H1 and DNA, specific phosphorylation events are likely to affect H1 binding properties by eliciting conformational changes in H1 structure, particularly the intrinsically disordered CTD and NTD domains (Vila et al., 2001; Roque et al., 2008). Such phosphorylation-induced conformational changes may help to explain an apparent paradox: some phosphorylation events strengthen the binding of H1 to DNA, rather than weaken it (discussed below).

In addition to phosphorylation, mass spectrometry studies have identified acetylation and methylation of linker histones (Garcia et al., 2004; Wisniewski et al., 2007). One characterized modification is Lysine 34 acetylation of H1.4 (H1.4K34ac). Just as core histone PTMs are ‘read’ by specific proteins, H1.4K34ac is bound by the second bromodomain of TAF1, a subunit of the transcription factor TFIIID (Kamienniarz et al., 2012). Once bound to chromatin, TFIIID facilitates transcription by recruiting chromatin remodelers to move histones, including H1. Another such modification is H1.4K26me, which, like H3K9me2, is bound by HP1, thereby promoting heterochromatin formation (Daujat et al., 2005). Unrelated to a specific modification, it seems that HP1 also binds H1.5 in the C-terminal domain (CTD) (Hale et al., 2006). Interestingly, both cases appear to be ‘phosho switches’ (Fischle et al., 2003): phosphorylation near the HP1 binding sites of H1.4 and H1.5 eliminates HP1 binding. Thus, linker histones participate with the core histones in regulatory networks that control gene expression and chromatin structure.

Protein–Protein Interactions

The role of core histones in localizing additional proteins to sites along chromatin is well characterized, but the partners...
of H1 are just starting to be revealed. Only a handful of interacting partners had been identified (McBryant et al., 2010) until recently, when over 100 interacting partners for H1.0 were identified using mass spectrometry (LC–MS/MS) (Kalashnikova et al., 2013). These included core splicing factors and the FACT (Facilitates Chromatin Transcription) complex. Further work will be required to test the biological relevance of these interactions, but the results are intriguing. Though the function of the H1 N-terminal domain (NTD) is unknown, about two-thirds of the contacts are mediated by the NTD, suggesting that it may mediate protein–protein interactions. The remainder are mediated by the CTD, indicating that this domain also functions to recruit H1 binding partners. Among the H1.0 binding partners were also a number of proteins involved in rRNA biogenesis, suggesting a role in the nucleolus. This result is consistent with studies that have identified differential phosphorylation patterns of nucleolar linker histones (Zheng et al., 2010).

Though more specific studies are needed to determine the functional significance of these interactions, it is clear that H1 participates alongside the core histones in the regulation of gene expression and chromatin structure. In addition to the specific interaction with HP1 discussed above, H1 also contributes to repressive chromatin by other means. In mouse ES cells, H1 helps to recruit the DNA methyltransferases DNMT1 and DNMT3B, while physically excluding SET7/9 histone methyltransferases, which catalyze H3K4 methylation, a mark of active genes (Yang et al., 2013). In another example, Drosophila H1 also contributes to repression by recruiting the histone methylase Su(var)3–9, which catalyzes H3K9me2, thereby recruiting HP1 (Lu et al., 2009, 2013). Thus, cross-talk between H1 and other regulatory systems seems to be a common theme.

**Chromatin Dynamics**

Fluorescence Recovery After Photobleaching (FRAP) experiments have demonstrated that, while core histones are relatively stably associated with chromatin, linker histones are rapidly exchanged (Lever et al., 2000; Misteli et al., 2000; Phair and Misteli, 2000). Thus, the pool of H1 is dynamically shuttled between chromatin binding sites. The mobility of H1 is affected in part by chromatin remodelers but, even in their absence, H1 is relatively mobile and this mobility has been proposed to be essential for the dynamic nature of chromatin (Bustin et al., 2005; Catez et al., 2006). Interestingly, nondividing/differentiated cells have a higher ratio of H1 molecules per nucleosome compared with embryonic stem cells (Fan et al., 2003). In addition to overall levels of H1, certain variants are more mobile than others and the abundance of particular variants fluctuates with cell type (Talasz et al., 1998; Raghuram et al., 2009). Therefore, the intrinsic mobility of H1, its relative concentration, and the different characteristics of variants help to determine the properties of chromatin.

During transcription and other processes, chromatin remodelers displace histones to facilitate access to the underlying DNA. In the absence of competitors, however, histones would rapidly rebind and undo the work of the remodelers. High mobility group (HMG) proteins are small, abundant nuclear proteins that compete with H1 by binding DNA and nucleosomes transiently (Catez et al., 2002; Catez and Hock, 2010; Reeves, 2010). HMG proteins do not facilitate compaction of chromatin. Therefore, when HMG proteins transiently occupy linker histone binding sites, chromatin regions are kept ‘open’ longer, thereby facilitating prolonged access to the local region of DNA for transcription, repair, and replication (Reeves, 2010). Although H1 proteins (bound plus unbound) are more abundant than HMG proteins, HMG proteins are able to switch sites much more rapidly than H1 and so can effectively compete with H1 for unoccupied binding sites (Phair et al., 2004; Bustin et al., 2005; Catez et al., 2006). HMG proteins not only compete with histones, but also facilitate the formation of complexes through DNA bending and protein–protein interactions (Reeves and Beckerbauer, 2001; Catez and Hock, 2010; Reeves, 2010). Recent data have uncovered interactions with p53 and PCNA, suggesting that HMG protein–protein interactions are involved in a variety of nuclear processes (Postnikov et al., 2012; Rowell et al., 2012). Thus, while linker histones can be thought to compact chromatin, HMG proteins counter this action, resulting in a regulated, yet dynamic chromatin state.

**The Cell Cycle**

The progressive phosphorylation of linker histones is associated with cell-cycle stage. H1 phosphorylation is nearly absent in G1, but begins to increase during S-phase and reaches a peak during mitosis (Bradbury et al., 1974; Talasz et al., 1996). Based on this correlation, it has been proposed that the phosphorylation of linker histones during the cell cycle contributes to global changes in chromatin structure: decondensation during interphase and tight compaction during mitosis (Bradbury et al., 1974; Lewin, 1990; Roth and Allis, 1992; Maresca et al., 2005). Generally, phosphorylation of H1, especially in the CTD, increases mobility in vivo (Lever et al., 2000; Misteli et al., 2000) and weakens binding to chromatin (Horn et al., 2002; Contreras et al., 2003; Roque et al., 2008). The role of phosphorylation, however, cannot be adequately explained with a simple charge repulsion model. Early phosphorylation events in S-phase allow unfolding to facilitate DNA replication, whereas additional phosphorylation during mitosis is associated with tight compaction. This paradox (some phosphorylation decreases H1 binding, but further phosphorylation increases binding) has been resolved in part by the characterization of H1 as an intrinsically disordered protein (Roque et al., 2008). Intrinsically disordered proteins undergo binding-assisted folding and often have different folded states (Uversky and Dunker, 2010). Supporting this model, particular rather than nonspecific sites of
Recent work has demonstrated that H1 phosphorylation can play a regulatory role in the cell cycle (Figure 2). Moderate phosphorylation of linker histones during interphase causes global decondensation of chromatin (Sarg et al., 2006; Roque et al., 2008; Talasz et al., 2009; Green et al., 2011) and allows origins of replication to fire (Alexandrow and Hamlin, 2005; Hartl et al., 2007; Thiriet and Hayes, 2009). Further phosphorylation by Aurora B, Glycogen Sensitive Kinase 3, and Cyclin-Dependent Kinase 1 (CDK1) causes the global condensation of chromatin (Figure 2), thereby facilitating mitosis (Sarg et al., 2006; Happel et al., 2009; Talasz et al., 2009; Zheng et al., 2010; Hergeth, 2011). Curiously, it seems that one final phosphorylation by Protein Kinase A helps to reverse this condensation (Chu et al., 2011). To return the cell to G1, during which H1 is not phosphorylated, H1 is presumably dephosphorylated, though no phosphatases have yet been identified. An additional insight from these studies is that variant histones are differentially modified (Supplemental Table 1). Though many have a similar number of CDK consensus sites, not all are phosphorylated to the same extent during the cell cycle. For example, H1.5 and H1.4 are phosphorylated more than other variants, which affects their interaction with HP1 (Daujat et al., 2005; Hale et al., 2006).

The phosphorylation of linker histones may also play a role in variations on the cell cycle such as endocycling and meiosis, where DNA replication is uncoupled from cell division. Endocycling cells go through replication without mitosis, producing fully or partially endopolyploid cells, whereas, during meiosis, mitosis happens without replication (meiosis II), yielding haploid cells. The phosphorylation of linker histones provides a possible mechanism for this uncoupling—because different kinases mediate the progression into S-phase and mitosis by the phosphorylation of linker histones at different sites, S-phase can be decoupled from mitosis allowing endocycling, and mitosis can be decoupled from S-phase for meiosis. The association of H1 phosphorylation with endocycling has been demonstrated in Drosophila follicle cells, where gene amplification at the Chorion loci (a form of endocycling) was shown to be associated with H1 phosphorylation (Hartl et al., 2007). Checking the specific sites of phosphorylation during endocycling could test this hypothesis.

Development

As discussed above, the complement of linker histone variants within a cell changes during development (Godde and Ura, 2009; Happel and Doenecke, 2009). This is significant not only because the different linker histone variants have different binding properties, but also because their effect on the cell

Figure 2. Linker Histones Are Sequentially Phosphorylated during the Cell Cycle.

Indicated phosphorylation events were determined by mass spectrometry and immunolocalization studies of human cell cultures (Daujat et al., 2005; Sarg et al., 2006; Happel et al., 2009; Talasz et al., 2009; Zheng et al., 2010; Chu et al., 2011; Hergeth et al., 2011; Kamieniarz et al., 2012). All phosphorylation events for any H1 variant are shown. For H1 variant-specific phosphorylation, see Supplemental Table 1.
cycle is not equivalent. H1.4 and H1.5 contain a larger number of phosphorylation sites than H1.0–H1.3 (Supplemental Table 1). This may help to explain why H1.4 and H1.5 are relatively highly expressed in dividing cells, but their expression diminishes as differentiation progresses, whereas variants such as H1.0 show the opposite trend (Zlatanova and Doenecke, 1994; Terme et al., 2011). Furthermore, the overall quantity of linker histone changes during differentiation. Embryonic stem cells only contain approximately one linker histone per two nucleosomes, while differentiated cells contain ratios nearer to 4:5 (Fan et al., 2005; Woodcock et al., 2006). This may reflect a requirement for pluripotent stem cells to maintain a larger portion of the genome in an accessible state. This model is supported by experiments showing that knockdown of three major linker histone variants in mice impairs proper differentiation and interferes with the establishment of DNA methylation (Fan et al., 2005; Zhang et al., 2012). Finally, locus-specific binding of particular linker histone variants has been linked to differentiation. The transcription factors NANOG, OCT4, FOXA2, and SOX17 are master regulators involved in pluripotency and are highly expressed in stem cells, but rapidly down-regulated after the onset of differentiation. Coincident with this repression is an enrichment of H1.0 and H1.X in the regulatory regions of these pluripotency factors during differentiation (Shahhoseini et al., 2010; Terme et al., 2011). In conclusion, control of the complement of linker histones, their abundance, and their localization all play important roles in controlling differentiation in mammals.

PLANT LINKER HISTONES

Domains and Families
The linker histones of plants are more divergent from animals than their core histones. Conservation is stronger in the central globular domain, whereas the NTDs and CTDs are more divergent (Jerzmanowski et al., 2000). Although the sequences have diverged, the CTDs and NTDs share many of the same general characteristics; they are highly basic, and rich in lysine and proline. Further, like animal linker histones, the CTD also possesses CDK consensus sites. Another similarity with animals is that plants typically possess multiple linker histone variants, though the families are typically smaller than the 11 found in mammals. Arabidopsis, for example, has three linker histone variants: H1.1, H1.2, and H1.3. Interestingly, most plants possess at least one shorter linker histone variant that is induced during drought conditions (Wei and O’Connell, 1996; Ascenzi and Gantt, 1997; Bray et al., 1999; Scippa et al., 2000; Trivedi et al., 2012). In Arabidopsis, the drought-inducible variant (H1.3) has a distinct expression pattern (Figure 3A) and binds more tightly to chromatin (Ascenzi and Gantt, 1999b). On the basis of sequence homology, these ‘drought-inducible’ linker histones have been identified in both monocots and dicots (Jerzmanowski et al., 2000), suggesting that they have been retained throughout the evolution of higher plants. Despite their conservation, it is possible that drought resistance is not their only role. In some species, such as tomato, drought-inducible linker histones contribute to drought resistance by facilitating the down-regulation of stomatal conductance, transpiration, and net photosynthesis (Scippa et al., 2004). In Arabidopsis, H1.3 is drought-induced, but is not required for the induction of drought-resistance genes (Ascenzi and Gantt, 1999a). Finally, the two tobacco homologs of the ‘drought-inducible’ linker histones are not induced by drought at all (Przewloka et al., 2002). The fact that this class of linker histones is conserved in, but not always associated with, drought suggests that the so-called ‘drought-inducible’ linker histones may have other conserved functions in development, which we speculate on below.

Chromatin Structure and Dynamics
The general structure of chromatin is very similar between plants and animals. The core histones are particularly well conserved and the effects of specific PTMs on chromatin structure are also often similar between plants and animals.
For example, lysine acetylation of H4 is associated with transcriptionally active euchromatin, whereas heterochromatin is enriched in H3K9me2 and H3K27me3. Some differences, however, do exist. Plant nucleosomes are generally spaced closer together than mammals by ~10 bp or one turn of the double helix (Chodavarapu et al., 2010). Also, the plant homolog of HP1, called LhIKE HP1 (LHP1), binds to H3K27me3, rather than H3K9me2 as it does in animals (Turck et al., 2007; Zhang et al., 2007). In mammals, HP1 interacts with both H3 and H1 and these interactions are regulated by histone PTMs. Interactions between LHP1 and linker histones in plants have not yet been demonstrated, but seem plausible given the association of LHP1 with heterochromatin. Aside from a few sites identified in wheat, the specific PTMs of plant linker histones have not been characterized; however, the observation that H1 phosphorylation is regulated by many growth-related stimuli suggests that, as in animals, H1 phosphorylation will play a key role in regulating the cell cycle (discussed further below) (Sauter et al., 1995; Zhang et al., 1996; Slaninova et al., 2003; Kalamajka et al., 2010; Greer et al., 2012).

The mobility of linker histones in plants is very similar to that of animals, suggesting that, in both plants and animals, H1 is shuffled between chromatin binding sites (Misteli et al., 2000; Launholt et al., 2006). Competition for binding sites with HMG proteins also contributes to plant linker histone mobility (Misteli et al., 2000; Launholt et al., 2006). Some plant HMG proteins are similar to those found in animals, whereas others are plant-specific (Klosterman and Hadwiger, 2002; Pedersen and Grasser, 2010; Jerzmanowski and Kotlinski, 2011). An interesting plant-specific variant is similar to HMGa from animals, but with the addition of a H1 globular domain. In this case, the potential for competition with H1 for binding sites is obvious. As in animals, HMG proteins aid processes such as DNA repair, replication, and transcription through competition with H1 and by facilitating complex formation (Pedersen et al., 2011; Antosch et al., 2012).

**H1 and DNA Methylation**

In animals, the proteins that interact with H1 are beginning to be elucidated (McBryant et al., 2010; Kalashnikova et al., 2013). To date, only a few proteins have been shown to interact with H1 in plants, all of which are involved in DNA methylation. Methylated DNA is tightly bound by core histones to promote repression and is used extensively by plants for gene silencing. Heterochromatic DNA containing transposable elements (TEs), DNA repeats, and inactive ribosomal genes are often heavily methylated. Given the crosstalk in animals between H1 and other heterochromatic marks (see above), it is perhaps not surprising that H1 should play a role in DNA methylation in plants. Several lines of evidence connect linker histones with proper DNA methylation. H1 knockdown in *Arabidopsis* has been reported to cause stochastic changes in DNA methylation (both hypo- and hypermethylation) in a variety of gene contexts (Wierzbicki and Jerzmanowski, 2005). Recently, the connection between linker histones and DNA methylation has been at least partially explained by studies involving *Arabidopsis* plants defective in DEFICIENT IN DNA METHYLATION 1 (DDM1), which show a strong reduction in DNA methylation (Jeddeloh et al., 1999). The reason for the requirement of DDM1, a chromatin remodeler, in DNA methylation long remained a mystery. Recent work, however, showed that DNA methylation (particularly via small-RNA-directed DNA methylation) can be restored in a *ddm1* mutant by H1 knockdown (i.e. if linker histones are removed, DDM1 is no longer required for DNA methylation) (Zemach et al., 2013). This suggests a model in which DDM1 functions to remove linker histones in order to facilitate the access of DNA-methylation machinery.

A connection between linker histones and DNA methylation has also been uncovered at imprinted loci, which show parent-of-origin-specific expression. In *Arabidopsis* endosperm, only the maternal allele of the Polycomb gene MEDEA (MEA) is expressed. The activation of the maternal allele of MEA requires the DNA glycosidase DEMETER (DME). DME acts as a DNA demethylase through a base-excision pathway, in which 5-methylcytosine is removed and replaced with unmethylated cytosine. DME has been shown to physically interact with H1.2 (Rea et al., 2012). Further, maternal H1 is required for activation of MEA and several other imprinted loci (the paternal H1 is dispensable for activation) by DME. Thus, normal demethylation appears to be mediated through the interaction of DME with H1.2 This result, taken together with the DDM1 work described above, shows that linker histones play roles in both DNA methylation and demethylation.

**The Cell Cycle**

In mammals, progression through the cell cycle is mediated by the sequential phosphorylation of linker histones. As in animals, plant linker histones are also known to possess consensus sites in their C-terminal domains for CDK phosphorylation (Ascenzi and Gantt, 1997; Slaninova et al., 2003). Phosphorylation of H1 by CDKs during the cell cycle has been reported in rice, tobacco, corn, and algae (Sauter et al., 1995; Zhang et al., 1996; Zhao and Grafi, 2000; Slaninova et al., 2003). Phytohormones play central roles in regulating plant growth and development, so it is not surprising that CDK activity has been reported to be regulated by gibberellins, cytokinins, and auxins (Sauter et al., 1995; Zhang et al., 1996; Alatzas et al., 2008). As in animals, H1 variants vary in the number of CDK consensus sites (S/T)-P-(K/R) they possess (Supplemental Table 2). This may indicate that some variants play a more significant role in cell-cycle progression than others. Interestingly, drought-inducible linker histones consistently have either one or zero CDK consensus sites, suggesting that they are less likely to be involved in the cell cycle. Consistently with this model, the drought-inducible H1.3 is expressed at low levels in the apex and young leaves but, as leaves become older and cell division slows/ceases, H1.3
expression increases (Figure 3A). This suggests that, like mammals, plants may use a cell-cycle-incompetent variant to suppress cell division in differentiated cells. Applying this model to drought stress would predict that the drought-inducible linker histones inhibit cell-cycle progression because these linker histones cannot be phosphorylated (Figure 3B). It is interesting to note, however, that H1.3 does not appear to be the major variant in differentiated/nondividing cells (Ascenzi and Gantt, 1999a). Thus, if the expression of nonphosphorylatable H1 variants does indeed block cell division during drought stress, there must be other mechanisms to block cell division in nondividing cells under normal conditions.

Development

The relative fractions of linker histones at various stages of development are well characterized in animals: both H1 variant substitution and increased concentrations of H1 contribute to cellular differentiation (see above). In plants, this process is less well understood. Plants have a different life cycle than animals, albeit with some parallels. Similarly to animals, plant nuclei undergo changes in chromatin density during differentiation. Seed nuclei are very compact and lack heterochromatic chromocenters. During imbibition and germination, chromatin structure becomes less compact and heterochromatin becomes concentrated in chromocenters (Mathieu et al., 2003). Despite numerous studies aimed at determining the changes in H1 levels during this transition, it is still controversial whether the concentration of H1 relative to DNA changes (Grellet et al., 1977; Sugita et al., 1979; Yoshida et al., 1979; Faulde and Nagl, 1987; Baluška and Kubica, 1992; Dicorato et al., 1995; Alatzas et al., 2008). In at least some instances, changes in H1 abundance correlate with changes in overall chromatin compaction. In pollen grain nuclei of Lilium longiflorum, the chromatin of the vegetative nucleus, which facilitates pollen tube germination and growth, is much less dense than that of the generative nuclei, which participate in fertilization (Ueda and Tanaka, 1994; Tanaka, 1997; Tanaka et al., 1998). This difference in chromatin density is correlated with differential accumulation of H1 in the generative nuclei. Another example of H1 levels changing with differentiation was recently reported in Arabidopsis reproductive development. Prior to female meiosis, reprogramming of the megaspore mother cell is characterized by a transient reduction in H1 levels and chromatin decondensation (She et al., 2013). Thus, in these cases, H1 levels are likely to play a role in overall chromatin compaction during differentiation.

In addition to the total amount of H1, the expression of specific histone variants is associated with plant development and responses to the environment. Work in multiple species has shown tissue-specific expression of linker histone variants, and during leaf development and aging (Faulde and Nagl, 1987; Kosterin et al., 1994; Szekeres et al., 1995; Alatzas et al., 2008) (Figure 3). An interesting case of a developmentally regulated linker histone is a recently identified H1 variant in banana, which is induced by ethylene during fruit ripening (Wang et al., 2012). In addition to developmental regulation of H1 expression, knockdown experiments also implicate H1 variants in development. For example, several studies in tobacco have shown that knockdown of specific variants caused developmental phenotypes, including male sterility (Prymakowska-Bosak et al., 1999; Calikowski et al., 2000; Przewloka et al., 2002). Further research is needed to clarify the functional significance of plant linker histone variants and their roles in development.

A complete picture of the roles of H1 in plant development will likely require the inclusion of HMG proteins, which have been shown to be regulated in tandem with linker histones in some developmental processes. One example is grain filling in maize. In order to increase the copy number of genes encoding seed storage proteins, cells switch to endoreplication. During this process, HMG is hypo-phosphorylated, increasing its affinity for DNA, which may allow it to compete better with H1 for DNA binding sites (Zhao and Grafi, 2000; Kalamajka et al., 2010). Further, during endoreplication, the levels of H1 remain stable (despite the increase in DNA content), whereas HMG levels increase. Therefore, as the amount of DNA increases, the ratios of H1:DNA and H1:HMG both drop, allowing for a more open chromatin state and presumably more efficient DNA replication/gene expression (Zhao and Grafi, 2000). These first glimpses at the developmental roles of H1 and HMG proteins may be indicative of a more general mechanism by which plants regulate or modify H1 and HMG proteins to enact global genomic changes associated with different developmental pathways.

Other Roles

The most obvious roles for linker histones are those involved in chromatin architecture and gene expression. There are intriguing hints, however, that linker histones may have important DNA-independent functions. A handful of studies have linked microtubules to linker histones. This is particularly interesting for plants, because, unlike animals, plants lack microtubule organizing centers. In spite of this, microtubules still play complex roles in determining cell shape and growth (Ehrhardt and Shaw, 2006). Ongoing research is aimed at understanding how microtubules are organized without this centralized structure. Early work showed that sea urchin flagellar microtubules were stabilized by histone H1 (Multigner et al., 1992). In plants, altering linker histone content in cultured tobacco cells resulted in cells with abnormal microtubule organization (Calikowski et al., 2000). Subsequently, there have been two studies that suggest that linker histones are responsible for nucleating microtubules in tobacco cell culture (Hotta et al., 2007; Nakayama et al., 2008).

Future Challenges

Over the last 30 years, we have begun to unravel the contributions of linker histones to gene regulation, cell division, and development. Cell-cycle-competent linker histones are abundant in stem cells, whereas higher concentrations of
linker histones lacking CDK phosphorylation sites are found in nondividing cells. Changes in overall linker histone abundance and variant composition contribute to making differentiated cells more restricted in their gene expression than stem cells. Also, competition between linker histones and HMG proteins helps to give chromatin the dynamic quality necessary for transcription, DNA repair, and replication. Some outstanding questions regarding plant linker histones include: the identity and function of PTMs, the roles of linker histone variants (e.g. ‘drought-inducible’ variants), and the importance of competition between linker histones and HMG proteins. These questions and more still remain unanswered, but it is abundantly clear that linker histones are not merely general repressors of transcription; rather, they are an integral part of the dynamic network of nuclear proteins necessary for proper maintenance, regulation, and expression of eukaryotic genomes.

SUPPLEMENTARY DATA
Supplementary Data are available at Molecular Plant Online.

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