The impact of chromatin dynamics on plant light responses and circadian clock function

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Abstract

Research on the functional properties of nucleosome structure and composition dynamics has revealed that chromatin-level regulation is an essential component of light signalling and clock function in plants, two processes that rely extensively on transcriptional controls. In particular, several types of histone post-translational modifications and chromatin-bound factors act sequentially or in combination to establish transcriptional patterns and to fine-tune the transcript abundance of a large repertoire of light-responsive genes and clock components. Cytogenetic approaches have also identified light-induced higher-order chromatin changes that dynamically organize the condensation of chromosomal domains into sub-nuclear foci containing silenced repeat elements. In this review, we report recently identified molecular actors that establish chromatin state dynamics in response to light signals such as photoperiod, intensity, and spectral quality. We also highlight the chromatin-dependent mechanisms that contribute to the 24-h circadian gene expression and its impact on plant physiology and development. The commonalities and contrasts of light- and clock-associated chromatin-based mechanisms are discussed, with particular emphasis on their impact on the selective regulation and rapid modulation of responsive genes.

Key words: Chromatin, circadian clock, gene expression, histone modifications, light signalling, plant.

Introduction

Research on the functional properties of nucleosome structure and composition dynamics has improved our understanding of the chromatin-based mechanisms that influence gene expression. In eukaryotes such as plants, these processes overlay with genome sequence information to control gene expression. Combinations of histone and DNA modifications organize chromatin into transcriptionally active or inert regions, most notably by affecting nucleosome positioning and controlling the access of trans-acting factors such as RNA polymerases and chromatin-binding proteins (Berger, 2007; Smith and Shilatifard, 2010). Dynamic changes of histone modifications can act sequentially or in combination to influence transcriptional outcomes and to respond in a coordinated fashion to developmental and external signals by selectively and synchronously modulating gene expression in multiple cells (Arney and Fisher, 2004; Kouzarides, 2007; Law and Jacobsen, 2010). Chromatin marks, notably those qualified as epigenetic, create a source of variation in

Abbreviations: ChIP, chromatin immunoprecipitation; HAT, histone acetyltransferase; H2Bub, histone H2B monoubiquitination; HDAC, histone deacetylase; HKMT, histone methyltransferase acting on lysine; PRMT, histone methyltransferase acting on arginine; SAR, shade-avoidance response; TSS, transcription start site.

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plant populations while having the potential to be reverted through the action of replication-dependent or -independent enzymatic activities. This property can potentiate flexible and dynamic processes as well as evolutionary changes when they are heritable (Richards, 2006; Rando and Verstrepen, 2007; Bossdorf et al., 2008; Weigel and Colot, 2012; Zhang et al., 2013). Consequently, deciphering the impact of epigenetic and chromatin-based mechanisms in plant development and adaptive responses has gained an enormous interest in recent years (reviewed in Berger and Gaudin, 2003; Crevillon and Dean, 2010; Kerr et al., 2011; He et al., 2011; Grimaneli and Roudier, 2013; Patel and Wang, 2013).

As sessile organisms, plants display a high capacity for phenotypic adaptations to environmental cues, notably because development continues during their lifetime and morphology can be optimized to suit local variable environments. In particular, adaptations to ambient light conditions are of particular importance for plant growth and fitness, especially because light is the energy source for photosynthesis and therefore is essential for phototrophy (Schmitt et al., 2003; Mathews, 2006; Franklin, 2008; Sultan, 2010; von Wetberg et al., 2012). The first evidence that chromatin-based mechanisms associate with light responses arose more than two decades ago when (i) histone acetylation and reduction in nucleosome density at the PetE photosynthetic gene promoter was shown to associate with increased transcription in green shoots (Chua et al., 2001), (ii) DE-ETIOLATED 1 (DET1), a major repressor of photomorphogenesis (Chory et al., 1989; Pepper et al., 1994), was found to bind histone H2B proteins with differing affinity as a function of their acetylation state (Benvenuto et al., 2002) and (iii) the circadian activation of the central clock oscillator gene TIMING OF CAB EXPRESSION 1/PSEUDO RESPONSE REGULATOR 1 (TOC1/PRR1) was shown to correlate with clock-controlled cycles of histone acetylation at its promoter to favour transcriptionally permissive chromatin states (Perales and Más, 2007). Altogether these early studies highlighted histone acetylation as a regulatory element of photomorphogenesis and clock function. Since then, an ever-increasing number of light- and circadian-driven processes have been shown to involve chromatin-based mechanisms for developmental responses but also for transient adaptations to changing light conditions and for the control of circadian clock function (reviewed in Fisher and Franklin, 2011; He et al., 2011; Li et al., 2012; van Zanten et al., 2012). Of particular note, several light-driven pathways control flowering time through the convergent activity of chromatin-based processes to the FLOWERING LOCUS C (FLC) regulatory gene. These aspects have been reviewed (Crevillon and Dean, 2010), as have the general properties of histone-modification mechanisms (Hennig and Derkacheva, 2009; Grimaneli and Roudier, 2013; Law and Jacobsen, 2010; Molitor and Shen, 2013; Teixeira and Colot, 2010).

Here, we compare the chromatin-based regulatory mechanisms associated with light signalling and circadian clock function in plants. We report recently identified functional links between these two interconnected pathways and novel elements contributing to selective regulation of light-responsive and clock genes through chromatin dynamics, and we discuss potential directions for future efforts aimed at deciphering the impact of chromatin-based regulatory mechanisms on rapid modulation of gene expression in these two contexts.

**Light responses and circadian clock function**

The physical properties of light can be sensed by a battery of dedicated nuclear and cytosolic photoreceptors independently from the light-harvesting activity of plastid-localized photosynthetic antennae. Such signals are further integrated by transduction pathways and effectors such as transcription factors to continuously influence plant development and morphogenesis as well as physiological responses (Chen et al., 2004; Jiao et al., 2007; Franklin and Quail, 2010; Kami et al., 2010; Casal, 2013). Five classes of photoreceptor have been characterized in Arabidopsis. The phytochrome photoreceptor family (phyA, B, C, D, and E) mediates red and far-red light perception (Sharrock and Quail, 1989; Clack et al., 1994; Rockwell et al., 2006) whereas UVA/blue light is mostly perceived by three types of photoreceptor: the cryptochromes (cry1 and cry2) (Ahmad and Cashmore, 1993; Lin et al., 1996), the phototropins (phot1 and phot2) (Huala et al., 1997; Kagawa et al., 2001), and members of the Zeilupe family [ZTL (ZEITLUPE) (Somers et al., 2000) FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) (Nelson et al., 2000) and LOV KELCH PROTEIN 2 (LKP2) (Schultz et al., 2001)]. Finally, UVB wavebands are perceived and transduced from UV RESISTANCE LOCUS 8 (UVR8), a recently identified photoreceptor acting atypically as a β-propeller protein homodimer that monomerizes upon UVB irradiation absorbed by specific tryptophans acting as a chromophore (Rizzini et al., 2011; Christie et al., 2012; Wu et al., 2012).

Plant photoreceptors display either specific or redundant roles for triggering plant responses. Genome-wide transcriptomic analyses have revealed that most of them act in concert to regulate the expression of a common set of genes sequentially during de-etiolation (Ma et al., 2001; Tepperman et al., 2006; Lopez-Juez et al., 2008; Peschke and Kretsch, 2011) and also for circadian clock function. In particular, photosensory perception determines the timing of most developmental transitions during the plant life cycle, including seed development, germination, photomorphogenesis (including de-etiolation), and the transition from vegetative to reproductive phases. Photoreceptors also control more subtle or reversible adaptations such as phototropism, shade-avoidance response (SAR), chloroplast movements, and the entrainment of the circadian clock (reviewed in Sullivan and Deng, 2003). Indeed, the diurnal changes in light quality and intensity play a major role in clock entrainment (Wijnen and Young, 2006). The photoreceptor families of phytochromes, cryptochromes, and Zeilupe integrate the red/far-red and blue light information to ultimately modulate transcription of several oscillator genes (Somers et al., 1998; Devlin and Kay, 2000). The circadian clock uses the predictable changes in the environmental
signals to keep track of time and to coordinate many biological functions to the most appropriate time of day (McClung, 2011). In *Arabidopsis thaliana*, the circadian clock plays a key role in the regulation of many key processes such as growth, flowering time, stomata opening, leaf movement, and metabolism as well as the regulation of many light-responsive genes (Harmer et al., 2000; Doherty and Kay, 2010). In fact, the circadian clock also modulates the expression of several light-regulated genes by gating the transcription abundance of photosynthesis- and phytohormone-related genes to the proper time of day (Harmer et al., 2000; Michael et al., 2008). For example, the *EARLY FLOWERING 4* (*ELF4*) gene displays both clock and light-signalling regulatory elements in its promoter region, which altogether delimit its light-mediated transcriptional activation to the end of the day (Herrero and Davis, 2012). More generally, recent transcriptome analyses have shown that about 70% of the light-inducible genes respond at least twice as strongly to a light pulse given in the middle of the subjective day than during subjective night, a direct consequence of circadian regulation (Rugnone et al., 2013). The light-inducible and clock-regulated (*LNK*) gene family seems to play a key role in this process by integrating the early light signals with the daily rhythms of genes expressed in the afternoon (Rugnone et al., 2013). Therefore, molecular genetic, and physiological evidence points to a direct connection between the light and the circadian signalling pathways.

**Contribution of chromatin-based mechanisms to light responses**

**Light-driven developmental adaptations impacted by chromatin dynamics**

Knowledge on the role played by chromatin-based mechanisms in plant light responses principally comes from a series of studies on photomorphogenesis and more particularly on the de-etiolation process. This developmental switch occurs when etiolated seedlings germinated in darkness reach the soil surface and perceive light for the first time. The shift from skoto- to photomorphogenesis involves the repression of embryonic stem (hypocotyl) elongation, the expansion of the embryonic leaves (cotyledons), and the generation of the photosynthetic machinery and ultimately allows both vegetative and reproductive development. Photomorphogenesis is therefore not established through a defined embryonic program but relies on continuous light-signalling regulatory mechanisms. De-etiolation involves massive reprogramming of genome expression in the cotyledons with many genes being expressed for the first time (Ma et al., 2005; Lopez-Juez et al., 2008). This developmental window is fast and initially occurs without cell division. For these reasons it constitutes an excellent system for dissecting the kinetics of transcriptional responses to light exposure and the associated chromatin dynamics. Two epigenomic studies that compare chromatin marks along the genome sequence in etiolated (dark-grown) and de-etiolated (upon a shift to white light for 1 or 6 h) seedlings have shown that transcriptional changes are significantly associated with local variations of specific histone post-translational modifications (Charron et al., 2009; Bourbousse et al., 2012). This allowed the elucidation of precise chromatin changes along hundreds of genes but also the establishment of general principles governing chromatin changes associated with light-driven developmental adaptations (Fig. 1; Table 1). These are massive and rapid, and therefore involve both *de novo* deposition as well as active removal of histone modifications.

Recent advances have now revealed that chromatin dynamics are associated to other types of light response, such as the SAR (van Zanten et al., 2010). SAR is a developmental adaptation to unfavourable light conditions under a canopy or a dense plant environment characterized by a relative decrease of red and blue versus far-red and green wavelengths. The specific decrease in the red/far-red ratio generates a signal for phyB activity, which triggers important modifications of morphogenesis in several plant species including enhanced cell elongation in stems and petioles as well as leaf hyponasty to compete for light availability (Casal, 2013). Transcriptome analyses have also uncovered massive changes of genome expression during this process (Devlin et al., 2003; Hornitschek et al., 2012; Leivar et al., 2012; Ciolfi et al., 2013). Detailed analyses using mutant plants affected in photoperception and in histone modifications, gene silencing, and transcriptional co-repression have now allowed the determination of a set of chromatin-based regulatory mechanisms impacting the regulation of light-driven gene expression (Table 1).

**Histone acetylation**

Histone acetylation usually influences transcriptional activity positively, most probably because the addition of an acetyl group on histone lysine residues relaxes the chromatin structure by neutralizing the lysine positive charges (Lee and Workman, 2007). This ‘open’ conformation may facilitate the accessibility of the transcriptional machinery and of effectors to the DNA. A precise regulation of histone acetylation through the opposite actions of various classes of histone acetyltransferases (HATs) and of histone deacetylases (HDACs) (Lee and Workman, 2007; Shahbazian and Grunstein, 2007) is important for the control of many plant biological processes. Acetylation can occur on multiple lysine residues. It has notably been detected on histone H3 (such as K9, 14, 18, 23, 27, and 56), histone H4 (K5, 8, 12, 16, and 20), and histone H2B isoforms (e.g. K6, 11, 27, 32, 38, 39, and 145 for HTB9) in *Arabidopsis* (Johnson et al., 2004; Bergmuller et al., 2007; Earley et al., 2007; Zhang et al., 2007a).

A landmark work analysing chromatin states at the endogenous plastocyanin *PetE* gene in etiolated and in green pea shoots was the first to reveal that light-induced transcriptional activity associates with hyperacetylation of histones H3 and H4 at enhancer/promoter regions and is preceded by a decrease in nucleosome density (Chua et al., 2001). The latter effect was detected in shoots and not in roots, indicating that both light- and organ-specific variations of chromatin
states may contribute to regulate PetE gene expression. Using a PetE promoter domain to drive expression of the uidA (GUS) reporter gene in transgenic tobacco, the authors further showed that artificially increasing histone acetylation through chemical inhibition of HDAC activities enhances the GUS signal 4-fold (Chua et al., 2003). This set of observations provided some of the first evidence that histone acetylation enhances transcriptional activity in plants.

A series of analyses in Arabidopsis and other plant species have shown that light-regulated changes in expression correlate with histone H3 and H4 acetylation levels on various light-responsive genes such as the model light-inducible gene chlorophyll A/B-binding protein 2, LIGHT HARVESTING CHLOROPHYLL A/B-BINDING PROTEIN 1.1 (CAB2/LHCB1.1) under white or monochromatic UVB, blue, red, and far-red light (Bertrand et al., 2005; Benhamed et al., 2006; Offermann et al., 2006; Casati et al., 2008; Cloix and Jenkins, 2008; Guo et al., 2008; Jang et al., 2011; Liu et al., 2013). Reciprocally, PHYA induction in light-to-dark shifts is accompanied by increased H3 acetylation at K9/K14 and K27 and histone H4 hyperacetylation (Jang et al., 2011). H3K9ac levels at a few light-responsive genes in dark- versus light-grown seedlings were shown to be quantitatively correlated with light intensity and to rise or fall together with the respective mRNA levels (Guo et al., 2008). Such correlative analyses have now been completed by a genome-wide survey of histone H3 acetylation changes at K9 and K27 during de-etiolation (Charron et al., 2009). This revealed rapid and massive variations of acetylated domains at light-regulated genes for these two marks. Photosynthetic genes were usually marked by H3K9ac and H3K27ac, implying a possible genome-wide effect of histone acetylation to activate this pathway (Charron et al., 2009).
Table 1. Chromatin modifications and related factors associated with expression of light-responsive genes

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<thead>
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<th>Process</th>
<th>Histone mark</th>
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<th>References</th>
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* Indicates a direct functional relevance on chromatin states or gene expression; D vs L, compares dark- and light-grown seedlings; L-to-D and D-to-L shifts, compares adult plants transferred from light to dark conditions or vice versa; ac, acetylation; me3, trimethylated; GWS, Genome-Wide Study; CUF-1, CAB upstream factor 1; DtRE, DET1-Dark Response Element (Maxwell et al., 2003); nd, not determined.

The functional relevance of HAT and HDAC activities on photomorphogenesis has now been illustrated by a series of studies that associate dynamic changes of acetylation levels on light-responsive genes with defective expression levels of the corresponding genes in histone HAT/HDAC mutant plants or upon a treatment with HDAC-inhibitory drugs. For instance, the HAT activity of TATA-BINDING PROTEIN ASSOCIATED FACTOR 1 (TAF1), a component of the TFIID complex in human and Drosophila cells, was proposed to function as an activator capable of transducing light signals to the transcription basal machinery. Using a promoter:uidA reporter-gene approach in transgenic Arabidopsis plants TAF1 was demonstrated to be required for efficient CAB2 gene induction (Bertrand et al., 2005), thereby providing another evidence for a role of histone acetylation in light-driven transcriptional activation. Furthermore, the evolutionarily conserved HAT GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) associates with chromatin on promoter elements of the IAA3 and RBCSI-A light-inducible genes. This association is functionally required for acetylation at various residues of histones H3 and H4 (notably K14/K27 and K8/K12, respectively) on their promoter region and for their efficient induction by light (Benhamed et al., 2006). GCN5 is part of the SAGA complex in yeast, a transcriptional co-activator that promotes gene expression at a post-initiation step (Weake and Workman, 2008; Spedale et al., 2012), a function that seems to be conserved in Arabidopsis (Vlachonasios et al., 2003; Servet et al., 2010).

At least two HDACs counterbalance acetylation levels on histone H3 and H4 on light-regulated genes, HISTONE DEACETYLASE 1/19 (HD1/HDA19) and HDA15, in addition to HDA6 that impacts light-triggered heterochromatin dynamics. HD1 is required for reducing histone H3K9
acetylation levels on several light-induced genes such as RBCS-1A, CAB2, and LHCB1.4 and several light-repressed genes such as Peroxidase 21 and PHYHA (Benhamed et al., 2006; Guo et al., 2008; Jang et al., 2011). Kinetic analyses comparing the reversibility of histone mark dynamics during de-etiolation and light-to-dark shifts further showed that repression of PHYA gene is delayed in the absence of HD1, therefore uncovering a functional role of this HDAC at this locus (Jang et al., 2011). Similarly, HDA15 has recently been reported as a transcriptional repressor of several chlorophyll biosynthetic and photosynthetic genes in etiolated seedlings, with contrasted effects on photomorphogenesis (Liu et al., 2013). Interestingly, HDA15 was found to physically associate with the light-signalling PHYTOCHROME INTERACTING FACTOR 3 (PIF3) transcription factor in vitro, in vivo, and in a yeast heterologous system, and to require PIF3 for binding to target gene promoters. The association of HDA15- and PIF3-tagged versions with some target genes in etiolated seedlings is released upon exposure to red light (Liu et al., 2013). This study therefore provides a model for selective regulation of some light-responsive genes through dynamic regulation of acetylation levels under the control of phytochrome photoperception.

The biological output of the different HAT and HDAC activities on light adaptations seems to be complex and might involve both redundant and antagonistic effects, presumably depending on the targeted genes. GCN5 and HD1 loss-of-function plants display opposite effects on photomorphogenesis under white, red, and most notably under far-red light; gcn5 and hd1 mutant plants are hypo- and hypersensitive to light with respect to hypocotyl elongation, respectively (Benhamed et al., 2006). This allows the generation of a simple model in which GCN5 and HD1 antagonistically regulate a common set of genes, GCN5 activating and HD1 repressing light-mediated transcription (Fig. 1). In contrast, hypocotyl elongation under red or far-red light seems to be negatively regulated by HDA15 and positively regulated by HD1, and accordingly hda15/hd1 double mutant plants display intermediate phenotypes (Liu et al., 2013). These data may indicate that these two HDACs have antagonistic effects on this process and may target different gene sets.

**Histone methylation marks associated with permissive chromatin**

Histone methylation is dynamically regulated by several histone methyltransferases acting on lysine or arginine residues (HKMTs and PRMTs, respectively) and the opposite activity of histone demethylases (Zhang and Reinberg, 2001). One, two, or three methyl groups can be covalently added, most notably at lysine 4, 9, 27, and 36 of H3 and lysine 20 of H4. The residue and the degree of methylation determine the functional outcome, notably through the recruitment of several chromatin readers (Patel and Wang, 2013), which can in turn regulate chromatin activities and gene expression (Berger, 2007). Broadly speaking, H3K4me3, H3K36me/me2/me3, and H3K9me3 are permissive to transcription and usually associate with active genes. They accumulate around the transcription start site (TSS) and along a variable portion of the 5′ region of the gene body (Pfluger and Wagner, 2007; Roudier et al., 2009; Liu et al., 2010). The genome-wide distribution of H3K9me3 has been determined during seedling de-etiolation. Similarly to histone acetylation, more than 3000 genes were found to be differentially marked, although the mark appeared to be weakly correlated with gene expression changes (Charron et al., 2009). In contrast, dynamic gain of H3K4me3 around the TSS was found to be highly correlated with the induction of LHCB1.4, LHCB1.5, HCF173, TZZ, and other genes (Guo et al., 2008; Bourbousse et al., 2012). In a reciprocal manner, loss of H3K4me3 on the PHYA gene during its repression in dark-to-light transitions is rapid (within 1 h), dependent on a functional phyB signalling pathway under red light and independent of the clock function (Jang et al., 2011). This last study further revealed that hd1 mutant plants are impaired in H3K4me3 demethylation on the PHYA gene during de-etiolation, possibly uncovering an interesting crosstalk between histone H3 acetylation and methylation at this locus that may relate to the one recently described in human cells (Tang et al., 2013).

To our knowledge, the precise impact of H3K4me3 or other permissive histone H3 methylation marks on transcription has not been assessed so far in plants. The Arabidopsis genome encodes about 37 SET-domain-containing proteins with potential redundant activities in histone methylation (Thorstensen et al., 2011), and several of them might be at play in this process.

**Histone ubiquitination and transcriptional activation**

Ubiquitination consists of the covalent attachment of the ubiquitin moiety, a highly conserved 76-amino acid polypeptide (Hochstrasser, 1996). Unlike poly-ubiquitination that mediates the formation of ubiquitin chains on histones H3 and H4 in the context of DNA repair (Wang et al., 2006) and is usually associated with targeted proteolytic degradation (Hicke, 2001; Zhang, 2003; Smalle and Vierstra, 2004), monoubiquitination is a stable post-translational modification. This histone modification can be removed by deubiquitinating enzymes (DUBs), which are also known as ubiquitin proteases (UBPs) (Zhang, 2003).

Histone H2B monoubiquitination (H2Bub) is part of a general mechanism that influences transcriptional activity positively (Weake and Workman, 2008). H2Bub was found to facilitate RNA Pol II processivity by favouring DNA accessibility, by helping to recruit the histone chaperone Facilitates Chromatin Transcription (FACT) and/or ensuring nucleosome reassembly upon RNA Pol II elongation (Belotserkovskaya et al., 2003; Pavri et al., 2006; Fleming et al., 2008; Xin et al., 2009; Fierz et al., 2011). In Saccharomyces cerevisiae a transcription-coupled cyclic process involves H2Bub by the Rad6-Brc1 ubiquitin ligase and subsequent deubiquitination by the SAGA complex. SAGA combines the two histone-modifying activities of ubiquitin Protease 8 (Ubp8) and of the GCN5 acetyltransferase.
In *Arabidopsis* histone H2B is ubiquitinated on lysine 145 (Bergmüller et al., 2007) by the heterodimeric HISTONE MONO-UBIQUITINATION 1/2 (HUB1/HUB2) Brc1 homologue (Fleury et al., 2007). *Arabidopsis hub1/2* mutant plants represent excellent tools since modification of just one histone mark is abrogated and because null alleles do not display pleiotropic seedling phenotypes (Fleury et al., 2007; Cao et al., 2008; Gu et al., 2009; Xu et al., 2009). In a recent study, spatial and temporal H2Bub dynamics have been investigated by integrating transcriptomic responses in wild-type and *hub1-3* mutant seedlings and genome-wide H2Bub distribution at three time points during de-etiolation (Fig. 2). This revealed that 177 genes are marked *de novo* by the first hour of illumination (and 272 genes after 6 h), illustrating wide and fast H2Bub variations (Bourbousse et al., 2012). For a majority of genes, light-triggered upregulation associates with increased H2Bub levels, while they generally remain stable during downregulation. Functionally, *hub1-3* mutant plants are affected in the kinetics of gene expression changes: both up- and downregulation are globally weaker or slower than in wild-type seedlings. Accordingly, the series of genes potentially impacted directly by H2Bub-dependent regulation comprises master regulators of chloroplast biogenesis (e.g. four genes of the *HIGH CHLOROPHYLL FLUORESCENT* family) and genes with fast and transient light induction such as the clock components *TOC1*, *PSEUDO RESPONSE REGULATOR 7* (*PRR7*), and *GIGANTEA* (*GI*). Based on these findings, H2B ubiquitination has been proposed to constitute part of a transcription-coupled chromatin-based mechanism to rapidly modulate gene expression (Bourbousse et al., 2012).

The H2Bub mark is distributed along gene bodies and not on promoters in the *Arabidopsis* genome (Roudier et al., 2011), in accordance with its proposed role in transcription elongation in various organisms (Shilatifard, 2006; Berger, 2007). This also fits with the observation that lack of H2Bub in *hub1-3* mutant plants has a more pronounced effect on the induction of long genes (>4 kb) rather than short genes (<1 kb) during de-etiolation (Bourbousse et al., 2012), suggesting that long genes may be particularly dependent on H2Bub to promote RNA PolII processivity through nucleosomal physical barriers.

In *S. cerevisiae*, histone H2B monoubiquitination is a prerequisite for H3K4 and H3K79 trimethylation (Sun and Allis, 2002). Whereas the H3K79me3 mark has not been detected in plants, the reduction of H3K4me3 levels in the *FLC* coding region in *hub1* mutant plants, impaired in H2Bub deposition, first suggested a conservation in *Arabidopsis* of this trans-histone crosstalk triggered by COMPLEX ASSOCIATED TO SET1 (COMPASS) activity in yeast (Shilatifard, 2012). Accordingly, several core COMPASS subunits are conserved in *Arabidopsis* (Jiang et al., 2009, 2011). However, H3K4me3

Fig. 2. Schematic representation of H2Bub epigenomic changes during early de-etiolation. (A) Phenotypic changes of an *Arabidopsis* seedling during early de-etiolation. Photos were taken at time 0 (Dark) and upon 1 and 6 h of exposure to light. (B) Genome browser screenshots representing H2Bub levels detected by chromatin immunoprecipitation using a promoter fragments array (ChIP chip) analyses at the three time points during de-etiolation. The top panel shows H2Bub signals over chromosome 1 tiles. H2Bub exclusively marks gene bodies in euchromatin, and chromosome-level patterns are globally similar between the three time points. The blow-up shows a genomic locus encompassing the light-induced gene *HCF173* (At1g16720) with significant gain of H2Bub during de-etiolation (lower panels). Positions of gene models and transposable element models (TEs) are given by blue and red boxes, respectively. Green bars represent significant H2Bub signals; grey bars represent non-significant signals. Epigenomic data are from Bourbousse et al. (2012).
abundance is globally unaffected in plants lacking H2Bub (Cao et al., 2008; Gu et al., 2009). Moreover, kinetic analyses of H3K4me3 levels along the HCF173, TZP, and SPA1 genes showed a similar gain of H3K4me3 around their TSS during light induction in wild-type and in null hub1 mutant plants (Bourbousse et al., 2012). In Arabidopsis, as in human cells (Tang et al., 2013), H3K4me3 can therefore be deposited by H2Bub-dependent and -independent mechanisms for optimal gene expression changes.

**Polycomb-associated marks**

In plants, as in other multicellular organisms, two types of Polycomb Repressive Complex (denoted PRC1 and PRC2) are thought to stably maintain specific developmental programmes or cell identity by catalysing histone H2A monoubiquitination (Bratzel et al., 2010) and H3K27 trimethylation (Hennig and Derkacheva, 2009), respectively, for potent repression of transcription. Profiling of H3K27me3 has identified that almost 2000 genes gain H3K27me3 marking when exposing etiolated seedlings to light for 6 h (Charron et al., 2009). For example, repression of the PHYA gene during de-etiolation is associated with a 3-fold increase of H3K27me3 levels around its TSS (Jang et al., 2011). This histone mark is the hallmark of PRC2-mediated gene repression (Exner et al., 2009), and therefore it is likely that this type of Polycomb activity has a prominent role in the regulation of gene expression patterns linked to photomorphogenesis establishment.

A potential impact of PRC1-like activity is less clear. Arabidopsis LIKE-HETEROCROMATIN PROTEIN 1 (LHP1; also known as TERMINAL FLOWER 2 or TFL2) binds H3K27me3 in vitro, and the genome-wide distributions of these two marks match co-extensively (Turck et al., 2007; Zhang et al., 2007b). The observation that LHP1 is required for maintaining the repression of some PRC2 targets led to the proposal that LHP1 is part of a PRC1-like complex that binds H3K27me3 chromatin domains (Molitor and Shen, 2013). Arabidopsis itshl loss-of-function seedlings are impaired in hypocotyl elongation under red and far-red lights and under light conditions triggering a SAR (Valdes et al., 2012). In this study, LHP1 was found to be required for repression of the IAA5 and IAA19 auxin-related genes under different light conditions, in agreement with the role of the auxin pathway in seedling development. Altogether, LHP1 may act in combination with PRC2 activity during photomorphogenesis. Further studies are required to determine the possible impact of PRC1 and of H2Aub histone mark in plant light signalling.

**Light-induced higher-order chromatin changes**

Dynamic chromatin states and regulatory functions can also be studied by determining higher-order chromatin compaction through cytogenetic approaches. The characterization of interphase nuclei phenotypes provides valuable information about the relationships between nuclear organization, gene regulation, and plant cell identity (Koornneef et al., 2003; Pavlova et al., 2010). The analysed parameters notably include the proportion and the distribution of DNA methylation and histone modifications in the nucleus, but can also relate to small-scale aspects such as local chromatin condensation or gene position (Fransz and de Jong, 2011). DNA fluorescence in situ hybridization (FISH) analyses of individual Arabidopsis chromosomal fragments in leaf and cotyledon nuclei have revealed that large heterochromatin domains are organized as so-called chromocenters, which contain the majority of dispersed pericentromeric repeats, including transposons and non-transposon repeats (Fransz et al., 2006; van Zanten et al., 2011; Benoit et al., 2013).

The compaction of chromocenters is dynamically regulated in response to variations in light conditions and also during light-driven developmental adaptations. First, a 10-fold reduction in light intensity induces a dosage-dependent and reversible large-scale decrease of heterochromatin compaction, which is dependent on cry2 and phyB photoreceptors (van Zanten et al., 2010). A similar effect is observed when exposing plants to SAR-inducing conditions, indicating that massive gene expression changes occurring during this adaptation associate with higher-order chromatin rearrangements (van Zanten et al., 2010). Based on these findings, it has been proposed that light signals are integrated to control heterochromatin condensation (van Zanten et al., 2012). Such a subtle mechanism is likely to be highly relevant in the wild, as demonstrated by the discovery that natural polymorphism in phytochrome protein sequence in Arabidopsis accessions from diverse geographical locations affects global heterochromatin compaction levels (Tessadori et al., 2009; van Zanten et al., 2010). This functional correlation indicates that modulation of light signalling readily influences chromatin-based processes in a natural context and is subject to evolutionary constraints.

Not surprisingly, massive disruption of chromocenter structures also accompanies dramatic changes of plant cell identity, as first shown during the artificial dedifferentiation of mesophyll cells into prooplasts (Tessadori et al., 2007a), but also in leaf mesophyll cells during a short period at plant bolting, the switch from vegetative to reproductive development. Interestingly, this latter effect also relies on a functional cry2 blue-light photoreceptor (Tessadori et al., 2007b). Based on these findings, a model has been proposed in which blue and red light sensed by cry2 and phyB photoreceptors converge on chromatin-modifying activities responsible for regulating heterochromatin compaction (van Zanten et al., 2010).

The molecular mechanism driving these chromatin dynamics on repeat sequences are still poorly understood. As observed for other transient changes of heterochromatin compaction and silencing (Pecinka et al., 2010; Tittel-Elmer et al., 2010), no significant decrease of heterochromatin marks such as DNA cytosine methylation and H3K9me2 could be detected on the decondensed chromosomal fragments in this context. A first hint came from the isolation of HDA6 as a positive regulator of heterochromatin compaction that is subjected to natural variation under latitudes with different light conditions (Tessadori et al., 2009) like phyB (Filiall et al., 2008). HDA6 is a member of the HDA1 Arabidopsis HDAC family (Pandey et al., 2002) and is involved in maintaining chromatin compaction at various heterochromatin loci such as ribosomal genes, retrotransposons (Lippman et al., 2003; Probst et al., 2004;
genes (Charron et al., 2006) and presumably also some genes such as FLC (Gu et al., 2011). Its HDAC activity notably reduces levels of H3K14ac, H4K5ac, and H4K12ac (Earley et al., 2006). As mentioned above histone acetylation is a central mechanism in chromatin-based regulation of gene expression, and HDA6 may work in concert with other HDACs such as HD1 (Benhamed et al., 2006) and HDA15 (Alinsug et al., 2012; Liu et al., 2013) to regulate acetylation levels of light-responsive loci, including photomorphogenic genes. The impact and the possible functional relevance of such heterochromatin dynamics on the euchromatin compartment and in particular on light regulations remain to be established.

From light signals to chromatin modifications and remodelling

Although most photoreceptor-based signalling converges to establish photomorphogenesis, specific pathways seem to modulate chromatin modifications under different light conditions. This is notably revealed by the observation that gen5, tafl, hdl, and hda15 loss-of-function plants display photomorphogenic phenotypes or gene expression defects under specific monochromatic light conditions. For example, the analysis of hypocotyl length relationships between tafl, gen5, hdl, hy1-1 and hy4-1 (cry1) mutant alleles in red/far-red or in blue light, respectively, have revealed that TAF1 functions downstream from both the phytochrome and cryptochrome signalling pathways (Bertrand et al., 2005). Consistent with these phenotypic analyses, H3K9ac levels on the LHCBI.4 gene promoter are lower in phytochrome and cryptochrome photoreceptor mutant plants than in wild-type plants under red/far-red and blue lights, respectively (Guo et al., 2008), and several chromatin modifications associated with PHYA gene repression under red light require a functional phyB signalling pathway (Jang et al., 2011). Noteworthy is the expression of several light-signalling components themselves are influenced by chromatin-based mechanisms, such as the PHYA and ELONGATED HYOCOTYL 5 (HY5) genes (Charron et al., 2009; Jang et al., 2011). More precisely, repression of PHYA gene upon exposure to red light seems to require active deacetylation of H3K9/14ac and H3K27ac by HD1 on its promoter region, which is controlled by phyB photoreceptor signalling (Jang et al., 2011).

HY5 and HY5-HOMOLOG (HYH) are two major transcription factors for establishing photomorphogenesis under all light conditions (Chattopadhyay et al., 1998), and they also seem to be central for signalling events driving histone acetylation dynamics. Notably, analyses of GCN5–DNA physical associations by chromatin immunoprecipitation (ChIP) using a promoter fragments array (ChIP chip) suggested that GCN5 might be more prevalently recruited to early light-responsive regulatory elements (Benhamed et al., 2008). Further analysis of the potential GCN5 binding fragments revealed significant overlap with HY5 target genes (Lee et al., 2007). These observations are in agreement with a model in which a significant portion of light-regulated genes depend on both GCN5 and HY5 for optimal expression (Benhamed et al., 2008), suggesting the elegant possibility that the HY5 transcription factor may allow recruitment of a SAGA-like co-activator complex on targeted genes through sequence-specific binding (Fig. 1). Accordingly, genetic analyses of gen5/hy5 double mutant plants have shown epistatic interactions (Benhamed et al., 2006).

A direct link between HY5 and a chromatin-bound regulator has recently been uncovered through a genetic screen for mutant seedlings displaying hypersensitive responses to red light exposure (Jing et al., 2013). This study uncovered PICKLE, a known ATP-dependent chromatin remodeler of the CHD3 family (Ho and Crabtree, 2010), as a novel actor in the control of photomorphogenesis through physical interaction with HY5. In the proposed model, PICKLE requires both HY5 and HYH to be recruited to cell elongation-related genes such as IAA19 and EXP2 and for promoting their expression by binding (through a chromodomain) and modifying chromatin at their promoter regions. PICKLE activity could notably result in preventing the deposition of the H3K27me3 Polycomb-mediated repressive histone mark. Consistent with its proposed function, PICKLE is more highly expressed in etiolated seedlings and is repressed under light conditions through phyA, phyB, and cry1 photoreceptor pathways (Jing et al., 2013).

The regulation of gene transcription can also involve cofactors (activators or repressors) capable of transducing signals from enhancer-bound specific transcription factors to RNA polymerases, which usually act by recruiting chromatin remodelling or modification activities such as HATs and HDACs (Eberharter and Becker, 2002). One possible example is DET1, an evolutionarily conserved factor in multicellular organisms acting as a major repressor of photomorphogenesis in Arabidopsis (Chory et al., 1989; Pepper et al., 1994). Viable det1-1 mutation induces a series of developmental defects with pronounced effects on light-inducible genes, altogether misregulating 2200 nuclear genes and many plastid genes in light-grown seedlings (F. Barneche et al., unpublished data). Most remarkably, det1-1 mutation induces constitutive photomorphogenesis in darkness (COP phenotype) with ectopic development of plastids in the roots, which correlates with gene expression profiles significantly mimicking those triggered by light (Schroeder et al., 2002; Ma et al., 2003). The molecular mechanisms underlying DET1 function remain poorly understood after 20 years (Chory, 2010; Lau and Deng, 2012), but a significant step was made when its direct interaction with histone H2B was identified (Benvenuto et al., 2002), and when it was demonstrated that it acts molecularly as a transcriptional repressor in planta and in heterologous systems (Maxwell et al., 2003; Lau et al., 2011). Since DET1 exhibits stronger affinity for non-acetylated histone H2B tails, it is likely that DET1 locks gene expression through a chromatin-based mechanism (Benvenuto et al., 2002). DET1 is part of the so-called CDD complex together with the E ubiquitin-conjugase variant CONSTITUTIVE PHOTOMORPHOGENIC 10 (COP10) (Wei et al., 1994) and DAMAGED-DNA BINDING PROTEIN 1 (DDB1), a CULLIN4-associated WD40-repeat protein (Schroeder et al., 2002). In addition to DDB1 direct binding, DET1 was subsequently found to associate with CULLIN4-DDB1 ubiquitin ligase activities
(Bernhardt et al., 2006; Chen et al., 2006). All three DET1 complex subunits are therefore connected to the ubiquitination pathway (Yanagawa et al., 2004), and accordingly its role in the inhibition of photomorphogenesis has been reported to rely on ubiquitination-dependent protein degradation of the HY5 transcription factor (Osterlund et al., 2000). Other recent work has reported that DET1 facilitates UV-damaged DNA repair through a mechanism that involves CULLIN4-DDB1, also possibly linking DET1-associated ubiquitination activities to chromatin during the nucleotide excision repair process (C Castells et al., 2011). Altogether, a unifying function for DET1 may integrate a role for CULLIN4, DDB1, and COP10 in regulating the abundance of chromatin-modifying machineries at UV-damaged or light-regulated genes (Fig. 1).

Finally, several lines of evidence suggest that some photoreceptors themselves may physically associate with chromatin. First, a GFP-CRY2 fusion protein was demonstrated to nicely decorate mitotic chromosomes (Cutler et al., 2000). Cry2 photoreceptor has also been proposed to mediate heterochromatin decondensation under low light conditions (van Zanten et al., 2010). This indicates that cry2 could perceive light and influence DNA or histone-based processes directly or through connections with transcription factors such as HY5 or CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HEXILX (CIBs) (van Zanten et al., 2010).

The UVB photoreceptor UVR8 has also been reported to physically associate with histones in vitro and in immunoprecipitation experiments, with a higher affinity for histone H2B (Cloix and Jenkins, 2008). UVR8 was further reported to associate in vivo with various HY5 gene regions including its promoter region, as well as with other genes in a UVB-independent manner (Brown et al., 2005). The functional relevance of direct associations of UVR8 with histones remains to be fully understood. Chromatin dynamics such as changes in histone H3 and H4 acetylation levels at UVB-responsive genes do occur and might directly be influenced by UVR8 upon UVB-induced conformation changes to promote their transcription (Casati et al., 2008; Cloix and Jenkins, 2008).

The molecular inner workings of the circadian oscillator

Chromatin dynamics, including histone acetylation and methylation, have been also shown to regulate the Arabidopsis circadian clock. At its basis, circadian rhythmicity is generated by a central core mechanism or circadian oscillator comprising a complex network of transcriptional feedback loops (Nagel and Kay, 2012). Two morning-expressed MYB transcription factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) (Wang and Tobin, 1998) and LATE ELONGATED HYOCOTYL (LHY) (Schaffer et al., 1998), together with the evening-expressed TOC1/PRR1 (Strayer et al., 2000; Makino et al., 2002) are the components of the first transcriptional loop identified in the Arabidopsis circadian system (Alabadi et al., 2001). CCA1 and LHY bind to an Evening Element motif (EE) at the TOC1 gene promoter and repress its expression. In turn, TOC1 represses the expression of CCA1 and LHY genes by binding to their promoters (Gendron et al., 2012; Huang et al., 2012). CCA1 and LHY expression is induced by light in the morning and the accumulation of CCA1 and LHY represses not only TOC1 but also other evening oscillator components such as PRR5 (Matsushika et al., 2000), LUX ARRHYTHMO/PHYTOCLOCK 1 (LUX/PCL1) (Hazan et al., 2005), ELF4 (Doyle et al., 2002), and GI (Park et al., 1999).

The PRR/TOC1 family members are consecutively expressed with approximately 2 h of delay, from the morning to the evening (PRR9, PRR7, PRR5, PRR3, and TOC1) (Matsushika et al., 2000). In agreement with the timing of their expression, PRR9, PRR7, PRR5, and TOC1 repress CCA1 and LHY from early morning to dusk (Nakamichi et al., 2010; Huang et al., 2012). TOC1 is not only responsible for the repression of CCA1 and LHY but also for other morning- and evening-expressed oscillator genes (PRR9, PRR7, GI, LUX, and ELF4) (Huang et al., 2012). The evening complex (EC), composed of LUX, ELF4, and ELF3 proteins, also functions at dusk repressing PRR9 (and probably PRR7) through a mechanism that might indirectly activate CCA1 and LHY transcription (Dixon et al., 2011; Helfer et al., 2011; Nusinow et al., 2011).

Light input to the clock

The light–dark transitions that occur during the day and night cycle are major environmental cues entraining the plant circadian clock. In particular, the phytochrome and cryptochrome pathways integrate the red/far-red and blue light information to ultimately modulate transcription of several oscillator genes (Somers et al., 1998; Devlin and Kay, 2000). The members of the Zeitlupe family (ZTL, FKF1, and LKP2) also perceive and transduce the blue light information to the core of the oscillator. In addition to the LIGHT OXYGEN OR VOLTAGE (LOV) domain required for photoreception, ZTL, FKF1, and LKP2 also contain an F-box domain involved in proteolytic degradation and a C-terminal Kelch repeat, which facilitates protein–protein interactions (Ito et al., 2012). The Zeitlupe family modulates the circadian clock by regulating TOC1 and PRR5 proteosomal degradation (Mas et al., 2003; Baudry et al., 2010). Blue light induces ZTL interaction with GI; the interaction stabilizes ZTL and prevents binding of ZTL to its protein targets. During the night, the affinity of ZTL for GI decreases and this leads to TOC1 and PRR5 degradation by ZTL (Kim et al., 2007). Recent studies have also shown that FKF1 and LKP2 play a similar role in the regulation of TOC1 and PRR5 stability (Baudry et al., 2010).

Histone acetylation and deacetylation at the core of the clock

Histone acetylation/deacetylation is intimately linked with the core of the oscillator in Arabidopsis. Indeed, histone acetylation directly correlates with the transcriptional activation of
several clock components (Fig. 3). The circadian rhythms of TOCI expression are preceded by the oscillatory accumulation of H3 acetylation (H3ac) at the TOCI promoter (Perales and Más, 2007). H3ac accumulation also parallels the expression of nearly all the oscillator components, including CCA1, LHY, PRR9, PRR7, LUX, and TOCI (Malapeira et al., 2012). Detailed ChIP combined with quantitative PCR analyses revealed that H3K9ac, H3K14ac, and H3K56ac associate with active clock gene transcription (Table 2) (Hemmes et al., 2012; Malapeira et al., 2012). In contrast, H4ac accumulation does not seem to be correlated with their expression (Song and Noh, 2012).

The importance of histone acetylation for proper expression of clock genes was further supported by pharmacological inhibition of acetylation. Plants treated with the inhibitors showed a reduction in H3ac accumulation that correlated with low expression of oscillator genes (Malapeira et al., 2012). These results are consistent with histone acetylation/deacetylation functioning as an important transcriptional regulator at the heart of the Arabidopsis oscillator. The control of H3ac accumulation on central clock genes also plays an important role in the regulation of other circadian systems. For instance, the mammalian clock genes Period 1 (Per1) and Period 2 (Per2) are subject to the rhythmic accumulation of H3K9ac at their promoters, which strongly correlates with their expression (Etchegaray et al., 2003). The regulation of H3K9ac at the Per1 and Per2 promoters depends on the HAT activity of the oscillator component CIRCADIAN LOCOMOTOR OUTPUT CYCLES KAPUT (CLOCK) (Doi et al., 2006) and the HDAC activity of SIRTUIN 1 (SIRT1) (Asher et al., 2008; Nakahata et al., 2008).

The detailed characterization of the H3ac dynamics at the TOCI promoter uncovered an interesting regulatory mechanism. Indeed, the analysis of plants that overexpress CCA1 showed a reduction in H3ac, which correlated with reduced TOCI expression. Consistently, analysis of the cca1/lhy double mutant plants showed increased H3ac at the TOCI promoter. These observations suggest that CCA1 might regulate TOCI expression by rhythmic binding to its promoter, a binding that is antiphasic to the rhythms of acetylated histone H3 and the TOCI mRNA circadian waveform (Perales and Más, 2007).

Plants treated with the HDAC inhibitor trichostatin A showed

Fig. 3. Schematic model depicting the dynamic chromatin changes at the core of the clock. The model shows known chromatin modifications at the promoters of clock genes during the circadian cycle. Ovals indicate the enzymes that modify histones and rectangles indicate transcriptional repressors. Clock gene expression is mostly induced by H3ac and H3K4me3 accumulation at the promoters. H3ac seems to function by relaxing the chromatin structure while H3K4me3 prevents the binding of transcriptional repressors. SDG2/ATXR3 is the major HKMT responsible for the accumulation of H3K4me3 at the oscillator gene promoter. The HAT required for histone H3 acetylation remains to be discovered. After the circadian peak of expression, H3ac is removed by an unknown HDAC activity, presumably increasing chromatin compaction. During the declining phase H3K4me3 is demethylated, leading to accumulation of H3K4me2, which might permit the binding of transcriptional repressors.
higher amplitude of \( TOC1 \) at dusk, just after \( TOC1 \) circadian peak of expression. These results indicate that a HDAC activity might contribute to the declining phase of \( TOC1 \). The results also open the possibility that the repressing activity of CCA1 might rely, at least in part, on the recruitment of HDACs at the \( TOC1 \) promoter (Perales and Más, 2007).

Another factor contributing to chromatin changes at the \( TOC1 \) promoter is the MYB transcription factor \( REVEILLE 8/\) LHY CCA1-LIKE 5 (RVE8/LCL5), which plays an antagonistic role with CCA1 in the control of \( TOC1 \) expression. Indeed, RVE8/LCL5 binds to the \( TOC1 \) promoter and increases H3ac accumulation, which facilitates the rising phase of \( TOC1 \). It has been proposed that RVE8/LCL5 and CCA1 antagonistic function might contribute to precisely fine-tune \( TOC1 \) circadian waveform (Farinas and Más, 2011). Interestingly, the chromatin-driven regulation of \( TOC1 \) expression is distinctively modulated by different photoperiodic conditions and plays an important role in the control of hypocotyl elongation and flowering time (Perales and Más, 2007).

**Circadian clock regulation by histone methylation**

Recent studies have demonstrated that rhythms in H3K4 trimethylation (H3K4me3) correlate with the oscillation of gene expression at the core of the clock (Table 2) (Hemmes et al., 2012; Malapeira et al., 2012; Song and Noh, 2012). The relevance of H3K4me3 for oscillator gene activation was analysed by using pharmacological inhibitors that block H3K4me3 accumulation. The circadian expression of \( CCA1 \) and \( TOC1 \) in plants treated with the inhibitors showed reduced amplitude, a delayed phase, and a long period phenotype, indicating that H3K4me3 is necessary for the proper expression of the oscillator genes (Malapeira et al., 2012). A time-course analysis of H3K4me3 oscillation at the promoters of the core clock genes showed a phase delay compared to the waveforms of gene expression and H3ac accumulation (Malapeira et al., 2012). This finding led to the hypothesis that H3K4me3 might have a different function from that of H3ac, which precisely correlates with gene expression. Indeed, reduction of H3K4me3 correlated with increased clock repressor binding at the promoters of the oscillator genes. The H3K4me3 mark controls the timing of repressor binding, thus preventing an advanced phase of transcriptional inhibition (Malapeira et al., 2012). The SET domain HKMT SET DOMAIN GROUP 2/ARABIDOPSIS TRITHORAX RELATED 3 (SDG2/ATXR3) is responsible for H3K4me3 accumulation at the core of the clock (Fig. 3). Analysis of heterozygous \( sdg2/\)atxr3 mutant plants showed reduced accumulation of H3K4me3 at the promoters of the oscillator genes and reduced clock gene expression. SDG2/ATXR3 over-expression on the other hand, induced H3K4me3 accumulation and promoted transcriptional activity of the core genes (Malapeira et al., 2012). These results indicate that SDG2/ATXR3 regulates clock gene expression most likely by modulating H3K4me3 at the promoters of the clock genes.

Accumulation of H3K4me2 at the promoters of the oscillator genes might negatively correlate with their circadian expression (Table 2) (Malapeira et al., 2012). An interesting hypothesis consistent with this observation is that dimethylation could be permissive to repression, facilitating transcriptional inhibition through clock repressor binding. The accumulated H3K4me2 marks might serve as a substrate for a yet-to-be discovered histone demethylase that might contribute to the accumulation of H3K4me2. This way, the ‘break’ imposed by H3K4me3 might be liberated, facilitating clock repressor binding and thus the circadian declining phase (Fig. 3).

Another histone mark that might negatively correlate with clock gene expression is H3K36me2 (Table 2). The accumulation of H3K36me2 at the promoters of \( TOC1 \), \( CCA1 \), and \( LHY \) seems to be associated with transcriptional repression (Song and Noh, 2012). Although H3K36me2 or H3K36me3 are mostly associated with transcription elongation regions of expressed genes, it has been proposed that H3K36me2 may act as a negative regulator when located at the promoter regions.
Histone demethylases might also have a key function at the heart of the circadian system. For instance, the expression of JUMONJI DOMAIN CONTAINING 5/30 (JMJD5/JMJ30) is regulated in a circadian manner with a peak at dusk. The evening-phased circadian regulation of JMJD5/JMJ30 is controlled by both CCA1 and LHY, which repress JMJD5/JMJ30 by directly binding to its promoter (Jones et al., 2010; Lu et al., 2011b). In addition, jmjd5/jmj30 mutants show reduced expression of CCA1 and LHY under high-intensity red light, which suggests that JMJD5/JMJ30 might form a negative feedback loop with CCA1 and LHY under these particular light conditions (Jones et al., 2010). Both JMJD5/JMJ30 over-expression and loss-of-function mutants lead to a short period phenotype, which suggests a complex mechanism underlying JMJD5/JMJ30 function in the clock (Lu et al., 2011b). A short period phenotype has also been observed in mammalian cells that lack the JMJD5/JMJ30 orthologue. The circadian phenotype can be rescued by the Arabidopsis JMJD5/JMJ30 and vice versa, suggesting that JMJD5/JMJ30 might have a conserved function in both circadian systems (Jones et al., 2010).

Histone ubiquitination and circadian clock function

Monoubiquitination of histone H2B also pervades the plant circadian clock with a clear effect in the expression of the oscillator genes. The hub1-1 mutation, which drastically decreases H2Bub deposition, reduces the amplitude in the oscillation of CCA1 and ELF4, and phase-advances LHY expression (Table 2). In contrast, hub1-1 mutation seems to enhance TOC1 expression, most likely through the decrease of CCA1-repressing activity (Himanen et al., 2012). In de-ethylated seedlings, when several clock genes are first induced by light, H2Bub seems to act as a positive regulator of TOC1, PRR7, and GI expression. The transient induction by light of these three oscillator genes is associated with a rapid gain of H2Bub, an increment that is significantly reduced in hub1-3 mutant plants. These observations fit with the general observation that H2Bub-based chromatin mechanisms are required for the rapid modulation of gene expression changes, which are likely to impact a large set of clock components whose mRNA abundance is tightly regulated by sharp oscillations (Bourbousse et al., 2012).

Role of co-repressors at the core of the clock

Chromatin-related co-repressor activity is emerging as a key regulatory mechanism controlling circadian gene expression. Consistent with this notion, a recent study has identified a physical and functional association between DET1 and CCA1 and LHY repressor activity (Lau et al., 2011). Indeed, DET1 binds CCA1 and LHY through its N-terminal domain and this interaction leads to DET1 rhythmic association with the TOC1 and GI promoters (Table 2). The det1-1 loss-of-function mutants display a short period of TOC1 and GI oscillation similar to the one observed in cca1 and lhy mutants. In addition, the det1-1 mutation was able to partially rescue the arrhythmic expression of TOC1 caused by CCA1 over-expression. These observations suggest that DET1 is required for CCA1 and LHY full repressor activity (Lau et al., 2011). Although the actual molecular function of DET1 remains to be elucidated, it is possible that DET1 represses morphogenic and clock gene expression through mechanisms involving the physical association with histone H2B (Benvenuto et al., 2002). In this context, it would be interesting to investigate the potential role of other CDD components and of CULLIN4 in this process.

Another recent report has identified the interaction between the Groucho/TUP1 repressor family, TOLESS (TPL)/TOLESS RELATED PROTEINs (TPRs) (Causier et al., 2012) with members of the PRR family. PRR9, PRR7, and PRR5 bind to the TPL/TPR family members and recruit the TPL/TPR repressors to the CCA1 and LHY promoters to inhibit their expression (Table 2). The TPL/TPR members are required for proper circadian function as their depletion leads to a long-period phenotype. The tpl-1 mutation also increases CCA1 expression similarly to prr9/prr7/prr5 triple mutations. The fact that trichostatin A treatments prevent the increased expression of CCA1 in tpl-1 plants suggests that HDAC activity is required for TPL repressor activity. Notably, an interaction between PRR9, TPL, and HDA6 has been demonstrated in vivo, suggesting that removal of histone acetylation might be part of CCA1 repressor activity (Wang et al., 2013) as previously suggested (Perales and Mä's, 2007). Additional studies might provide further insights into the emerging theme of chromatin-based repressing mechanisms at the core of the clock.

Conclusions and perspectives

Recent advances using cytogenetic, genetic, molecular, and epigenomic approaches have placed chromatin-related mechanisms as essential contributors to light signalling and circadian networks, two interconnected pathways that rely on extended transcriptional control. In these two contexts, changes in transcript abundance are tightly regulated, a process that requires optimal transcriptional induction and also a tight control of mRNA processing and half-life. At the chromatin level this is notably achieved through the sequential and combinatorial actions of histone acetylation at enhancer/promoter regions that favour DNA accessibility, H3K4me3 enrichment around TSS for recruiting chromatin readers, and also with H2Bub deposition along the gene body for facilitating RNA Pol II elongation (Fig. 1). Both the histone H2B ubiquitin ligase HUB1/HUB2 as well as GCN5, the HAT subunit of SAGA, seem to be required for a wide range of plant responses to environmental cues and deeply impact light
responses. Also, orthologous subunits of yeast COMPASS are conserved in Arabidopsis and associate with methyltransferase activities triggering H3K4me3 deposition (Jiang et al., 2011). This increasing knowledge suggests that many histone marks (except H3K79me3) and chromatin-associated factors linked with transcription in yeast and mammalian systems are conserved in plants and contribute to optimal transcriptional regulatory processes. They presumably act through coordinated chromatin changes to trigger a transcription-coupled mechanism to rapidly modulate gene expression for light responses and clock function. Several other subunits and factors such as the missing HATs and HDACs triggering clock-controlled histone acetylation dynamics remain to be identified.

The shift between transcriptionally permissive and repressive chromatin states might rely on various mechanisms. Diurnal decrease of activating chromatin marks is too rapid to depend solely on passive dilution through DNA replication and might mostly depend on active mechanisms, possibly modulated by the activity of histone demethylases and/or deacetylases (Fig. 3; Perales and Más, 2007; Jones et al., 2010; Lu et al., 2011b). As suggested by the role of the FACT complex on TOC1 gene oscillation (Perales and Más, 2007), rapid changes of chromatin states might also rely on precisely controlled histone turnover, nucleosome exchange, and/or replacement of canonical histones by histone variants. In the context of de-etiolation, gene downregulation does not seem to be systematically correlated with the erasure of activating chromatin marks. With the notable exception of the PHYA gene that displays rapid loss of H3K9/14ac and H3K4me3 during light-driven repression (Jang et al., 2011), no decrease of H3K9ac has been observed on genes downregulated during de-etiolation (Guo et al., 2008). Also, H2Bub domains are rapidly gained on many light-induced genes but are globally stable upon downregulation (Bourbouze et al., 2012). Although the evolution of chromatin states during cell lifetime should be determined thoroughly, these observations are in agreement with a relative stability of permissive chromatin states acquired upon transcriptional activation during photomorphogenesis. Notwithstanding, the mechanisms involved are likely to be different for each gene and each type of light response.

In agreement with these observations, it is likely that repression of gene expression is actively and massively mediated through H3K27me3/PRC2-based mechanisms during photomorphogenesis. H3K27me3 is part of a major gene-repression system in seedlings (Turck et al., 2007; Zhang et al., 2007b) and several hundred genes are subjected to dynamic changes of H3K27me3 marking during de-etiolation (Charron et al., 2009). Such Polycomb-mediated activity might have profound impacts on the establishment and/or the maintenance of cell differentiation during this process and other developmental light adaptations. Whether a PRC1-like activity is also involved remains to be determined. So far, PRC1-mediated H2Aub deposition has only been shown to impact short and specific developmental windows, such as the switch from embryonic to postgerminative phases (Yang et al., 2013). Altogether, it is conceivable that photomorphogenesis involves PRC2 as well as PRC1 activity.

Circadian changes in H3K27me3 accumulation at the oscillator gene promoters were not detected (Hemmes et al., 2012; Malapeira et al., 2012). Although a H3K27me3 demethylase activity does exist in Arabidopsis (Lu et al., 2011a), diurnal repression of central oscillator genes seems to rely on the removal of activating histone marks through HDAC and histone demethylase activities rather than the deposition of repressive marks. The clock might anticipate and gate the mechanism for establishing transcriptionally permissive chromatin states on targeted genes. Altogether, an emerging picture suggests that photomorphogenesis involves the relative stability of chromatin states over cell divisions while clock-associated chromatin changes mostly rely on dynamic and tightly controlled activating histone marks.

The molecular mechanisms underlying these different processes and their occurrence during other types of light response and notably transient responses will be an interesting aspect to decipher in future studies. For example, the diurnal distribution of Polycomb-mediated chromatin domains could be assessed on a genome-wide scale. High-throughput analyses assessing the impact of chromatin dynamics on the entire set of central clock genes from the different regulatory loops and their output genes might reveal interesting knowledge in the future, as recently shown in mammals (Koike et al., 2012). Tissue-specific or cell-specific analyses will be required, because light-responses trigger different developmental and physiological outputs in different organs, and therefore likely involve diverse transcriptional and chromatin-based changes in different cell types. In contrast, the clock presumably involves the synchronization of most, if not all, cells of a plant individual and might not be subjected to these limitations.

Future efforts will also require determining the primary effects triggered by physical associations between chromatin modifiers and their target genes. DET1 was recently shown to represent a functional link between light signalling and circadian clock core components and to be rhythmically recruited by CCA1 and LHY onto evening-expressed genes such as TOC1 in order to repress their transcription (Lau et al., 2011). Although the relevance of such mechanisms for the repression of light-inducible genes remains to be tested, it indicates that DET1 can be directed to target genes to drive their transcriptional repression through a third party carrying DNA sequence-specificity. These observations are in line with the recent discovery that HDA15 and PICKLE physically associate with the PIF3 and HY5 transcription factors, respectively (Jing et al., 2013; Liu et al., 2013). These studies strengthen a simple and elegant model explaining gene selectivity, and raise the possibility that activating and repressive effects of transcription factors could rely on chromatin-based processes. It is therefore possible that DET1 might be targeted by HY5- or PIF-related transcription factors onto DET1-Dark Responsive Elements (DiREs) or other loci to repress them by forming or controlling the abundance of a repressor complex to prevent the formation of acetylated chromatin states favourable to transcription (Fig. 1). These examples might represent the tip of the iceberg, and genome-wide as well as biochemical studies of transcription factor-binding sites during circadian and light response kinetics might allow the
identification of missing elements in the signalling pathways that link photoreceptors to chromatin modifications.

Light adaptations and clock regulation have wide impacts on genome expression profiles, with as much as one-third of the genes being potentially circadian-regulated (Covington et al., 2008) and one-third of the genes being differentially expressed between dark- and light-grown seedlings (Ma et al., 2001). Many of those genes are impacted by chromatin dynamics to modulate their expression changes during de-etiolation (Bourbousse et al., 2012). The co-occurrence of massive chromatin changes with genome expression reprogramming is a recurrent theme in plant developmental transitions (Berger and Gaudin, 2003; Feng et al., 2011; Fransz and de Jong, 2011). Large-scale studies using epigenomics and cytogenetics also indicate that intrinsic physical properties such as gene length can also influence the impact of chromatin modifications (Bourbousse et al., 2012; Shu et al., 2012). The functional relevance of higher-order chromatin dynamics on such regulatory processes has not yet been established. Gene-rich euchromatin loops emanate from the chromocenters (Fransz et al., 2002) and therefore the subnuclear localization and the expression of many genes might be influenced by nuclear organization and heterochromatin compaction (Fransz and de Jong, 2011). Such effects may overlay with gene-specific regulatory mechanisms for massive reprogramming of gene expression during light-driven developmental switches such as de-etiolation and SAR. Although this remains to be determined, they may eventually influence the accessibility of gene sets to the chromatin-modifying/transcriptional machineries. Despite this increasing knowledge, much remains to be done to identify the molecular actors that directly modulate chromatin states at specific loci and their relationships with the basal transcriptional machinery, the mechanisms allowing selective regulation of responsive genes, the stability of newly established chromatin states, and the mechanisms controlling chromatin dynamics on the numerous genes regulated by both clock and light-signalling pathways.

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