Shedding light on the chromatin changes that modulate shade responses

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Perception of vegetation proximity or plant shade informs of potential competition for resources by the neighboring vegetation. As vegetation proximity impacts on both light quantity and quality, perception of this cue by plant photoreceptors reprograms development to result in responses that allow plants to compete with the neighboring vegetation. Developmental reprogramming involves massive and rapid changes in gene expression, with the concerted action of photoreceptors and downstream transcription factors. Changes in gene expression can be modulated by epigenetic processes that alter chromatin compaction, influencing the accessibility and binding of transcription factors to regulatory elements in the DNA. However, little is known about the epigenetic regulation of plant responses to the proximity of other plants. In this manuscript, we review what is known about plant shade effects on chromatin changes at the cytological level, that is, changes in nuclear morphology and high order chromatin density. We address which are the specific histone post-transcriptional modifications that have been associated with changes in shade-regulated gene expression, such as histone acetylation and histone methylation. Furthermore, we explore the possible mechanisms that integrate shade signaling components and chromatin remodelers to settle epigenetic marks at specific loci. This review aims to be a starting point to understand how a specific environmental cue, plant shade, integrates with chromatin dynamics to implement the proper acclimation responses.

Abbreviations – B, blue; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; cry2, cryptochrome 2; DAPI, 4′,6-diamidino-2-phenylindole; DET1, DEETIOLATED1; FR, far-red; GCN5, GENERAL CONTROL NONDEREPRESSIBLE 5; HAT, histone acetyltransferases;

HAF2, HISTONE ACETYLTRANSFERASE OF THE TAFII250 FAMILY 2; HAG4, HISTONE ACETYLTRANSFERASE OF THE GNAT FAMILY 4; HAM1, HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1; HDAC, histone deacetylase; HDA6, histone deacetylase 6; HDA15, histone deacetylase 15; HDA19, histone deacetylase 19; HDAC, histone deacetylases; HY5, ELONGATED HYPOCOTYL 5; IAA3, INDOLE-3-ACETIC ACID INDUCIBLE 3; MRG2, MORF RELATED GENE 2; PAR, photosynthetic active radiation; PHYB, PHYTOCHROME B; PIF, PHYTOCHROME INTERACTING FACTOR; R, red; PRE1, PACLOBUTRAZOL RESISTANCE 1; RBCS-1A, RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN 1A; SDG8, SET DOMAIN GROUP 8; YUC8, YUCCA8.

Light not only provides energy for photosynthesis but also information to adapt plant growth and development to a variable environment. In the high planting densities found in natural and agronomic settings, light availability to drive photosynthesis can be strongly reduced and compromised by the close proximity of other plants. Indeed, photosynthetic tissues absorb specifically blue (B, 400-500 nm) and red light (R, around 660-670 nm), thus photosynthetic active radiation (PAR, from 400 to 700 nm) of sunlight found below a plant canopy (i.e., plant shade) is strongly impoverished in these colors of the spectrum, while most far-red light (FR, around 720-730 nm) is transmitted or reflected. Consequently, the resulting R to FR ratio (R:FR) that impacts plants growing underneath a vegetation canopy is also strongly reduced. In contrast with plant or canopy shade, proximity of vegetation does not decrease the amount of light but specifically reflects FR, hence also lowers the R:FR that impacts in a non-shaded neighboring plant. Therefore, while both conditions result in a decrease in the R:FR, only canopy shade decreases the amount of light. In this review we will refer to both low light and low R:FR as components of plant shade.

Reduction of the R:FR that accompanies the proximity of vegetation in nature acts as an early and reliable cue of the presence of vegetation that potentially might compete for light and other resources. Early detection of this signal is key to plant survival and triggers acclimation responses to optimize plant development. Among these responses were found the promotion of stem or petiole elongation and hyponastic growth to outgrow neighboring plants. The low R:FR ratio and the reduction of PAR light (low light) mediates the activity (amount, conformation and localization) of the phytochromes, the photoreceptors able to sense R and FR wavelengths. Under low light, the B-sensing cryptochromes also participate in the perception. Both types of photoreceptors mediate rapid changes in gene expression and developmental processes (Yu et al. 2010, Casal 2012, Roig-Villanova and Martinez-Garcia 2016).

There is a synergistic interaction between low B and low R:FR, as the combination of both factors has more severe effects than the single ones (de Wit et al. 2016). Because of the anticipatory and reliable nature of the low R:FR signal indicating the presence of vegetation proximity, how phytochrome signaling works has received significant attention. Based mostly on studies using *Arabidopsis thaliana*, it has been established that low R:FR-triggered inactivation of phytochromes decreases their interaction with PHYTOCHROME INTERACTING FACTORs (PIFs), a group of bHLH transcription factors. This results in the accumulation and/or activation of PIFs to regulate the expression of several light-responsive genes. Among the eight PIFs identified, the most relevant in regulating shade responses is PIF7, although PIF4 and PIF5 also participate at a certain extent (Li et al. 2012). Accumulation and/or activation of these PIFs in low R:FR releases the transcription of hundreds of genes that trigger

different aspects of the shade developmental program. The best known of these responses is hypocotyl elongation, which is promoted by the action of PIFs, and takes place because of rapid and reversible changes in the expression of dozens of genes.

Although many of the genetic components that regulate the shade response of the hypocotyl have been elucidated, little is known about how this process is transcriptionally and epigenetically orchestrated. To address this point, it is necessary to understand not only how chromatin organizes the genetic material but also how it is regulated and whether the environmental cues further modulate this regulation.

Chromatin is a dynamic structure that responds to environmental changes

Chromatin is a nuclear multicomponent complex composed of DNA, RNA and proteins. The basic unit of chromatin is the nucleosome, a structure conserved in all eukaryotes that is formed by DNA wrapped around an octamer of histone 3 (H3), H4, H2A and H2B, sealed by the H1 linker histone. Chromatin can have different degrees of compaction that regulates the accessibility of genes to the transcriptional machinery. How this occurs is basic in order to understand transcriptional regulation.

Some of the most important mechanisms that regulate changes in chromatin organization involve DNA methylation and histone post-translational modifications and variants, which are highly diverse and dynamic. The various modifications in either DNA or histones are commonly referred to as epigenetic marks. It is assumed that different combinations of marks, or epigenetic status, modulate chromatin compaction impacting either positively or negatively on transcription. When the environment changes, chromatin needs to be modified and restructured to translate the environmental signals into an acclimation response at the transcriptional level (Probst and Mittelsten Scheid 2015). Therefore, the ability of rapidly and reversibly altering the epigenetic status could be a key component to plant plasticity to respond to the environment.

Chromatin-level regulation has been reported as an essential component of light signaling (Barneche et al. 2014, Kim et al. 2015, Perrella and Kaiserli 2016). Little attention has been given, however, to the chromatin dynamics associated to shade (low light and/or low R:FR) responses. The purpose of this review is to address the current state of the art regarding epigenetic regulation in shade response, with specific focus in the model plant *Arabidopsis thaliana*. Two different aspects will be considered: chromatin compaction at the nuclear level and histone modifications that affect specific gene expression.

Nucleus structure changes after low light and low R:FR exposure

As mentioned, chromatin presents different compaction levels. From this point of view, chromatin can be classified in two major domains: euchromatin and heterochromatin. Each of these chromatin states are enriched in specific histone variants and post-translational modifications to form either transcriptionally active (euchromatin) or silent (heterochromatin) chromatin domains (Fransz et al. 2003). Heterochromatin can be distinguished from euchromatin in Arabidopsis nuclei by analyzing the chromatin density in the interphase nuclei after DNA counterstaining with DAPI (4′,6-diamidino-2-phenylindole), reflecting that they conform large-scale compartments (Fig. 1A). Heterochromatin clusters in highly stained speckles called chromocenters, which are formed by the centromeric and pericentromeric regions, satellite repeats, ribosomal DNA genes and nucleolar organizer regions (NORs) repetitive sequences (Del Prete et al. 2014, Simon et al. 2015). Less condensed loops of active euchromatin emanate from the chromocenters (Fransz et al. 2002), which are enriched in transcriptionally active genes. Euchromatic regions are poorly stained with DAPI (Fig. 1A).

The two contrasting chromatin states are not static but highly dynamic, with their degree of compaction changing under certain conditions. Quantifying the size and number of chromocenters allows the assessment of wide-range chromatin density that correlates with chromatin state (Tessadori et al. 2007). Using this approach, chromatin decondensation has been detected during developmental switches and also upon environmental changes or stress, including dark-to-light transitions (Exner and Hennig 2008, Bourbousse et al. 2015, Probst and Mittelsten Scheid 2015). Regarding the exposure of seedlings to shade, either low light or low R:FR results in a reduction of chromatin compaction (chromatin decondensation) assessed by a decrease in the amount and size of chromocenters (Tessadori et al. 2009) (Fig. 1B). Maximum decondensation in response to low light is reached at 4 days after the treatment. The reversion of this state once the plant is shifted to normal light levels takes similar times (van Zanten et al. 2010). It is unknown, however, if the rate of chromatin decondensation observed in response to low R:FR is similar to the one caused after exposure to low light, but the chromocenter kinetics in other light processes (such as dark-to-light transitions) suggests that they will have a similar behavior.

To find regulators of chromatin density in different light environment, 21 Arabidopsis accessions from different locations and habitats were used. This study showed that compaction of chromatin appeared to be dependent on light intensity, as the southernmost Arabidopsis accession Cvi-0 presents the lowest chromatin compaction (Tessadori et al. 2009), a finding that

agrees with independent observations indicating that accessions closer to the equator are less sensitive to light (Maloof et al. 2001). QTL mapping using the Cvi-0 and L*er* accessions, which present different levels of chromatin compaction, identified *PHYTOCHROME B* (*PHYB*) and *HISTONE DEACETYLASE-6* (*HDA6*) genes as positive regulators of light-dependent chromatin compaction (Fig. 1B). Accordingly, *phyB* mutants display lower chromatin density than wild-type plants in similar light conditions (Tessadori et al. 2009). Interestingly, phyB reduces nucleus size (area and perimeter) independent of its positive role in heterochromatin condensation (chromocenter formation and/or maintenance) (Snoek et al. 2017). Therefore, phyB has a central role in the coregulation of the nucleus phenotype in low light. So far, the effect of low R:FR on nuclear size has not been tested. Nevertheless, considering the decrease in chromocenter number induced by low R:FR, and the fact that a clear positive correlation between nucleus size (area and perimeter) and increased chromocenter values has been demonstrated (Snoek et al. 2017), it is very likely that nuclear size is also increased under low R:FR.

Besides phyB, the blue-light receptor cryptochrome 2 (cry2) has also been found to participate in the low-light reduced chromatin compaction (van Zanten et al. 2010). The mechanisms of action of phyB and cry2 photoreceptors could be direct, as both are nuclear proteins known to interact (Mas et al. 2000), or indirect, as phyB controls cry2 protein levels (van Zanten et al. 2010). The latter authors propose a model where phyB and cry2 control a chromatin remodeler complex responsible for chromatin compaction, in which CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) might participate (Fig. 1B). COP1 is a member of the E3 ligase complex that interacts with cryptochromes and phytochromes (Yi and Deng 2005). COP1, together with DE-ETIOLATED 1 (DET1), maintain chromatin decondensation in etiolated cotyledons (Bourbousse et al. 2015); after light exposure chromatin condensates. The ubiquitination machinery of this protein complex could be important for the cell fate either by inducing chromatin changes, as DET1 binds to H2B (Benvenuto et al. 2002), or reducing the stability of transcription factors. However, there is no information available on whether *cop1* or *det1* mutants have defective nuclear phenotypes in shade. Another component of this hypothetical chromatin remodeler complex controlled by photoreceptors can be HDA6 (Fig. 1B), which was also found in the same QTL analysis as phyB (Tessadori et al. 2009). HDA6 is involved in histone deacetylation, which is expected to compact chromatin (Fig. 2A). Consistently, the compaction of heterochromatin in the *hda6* mutant is reduced. Importantly, the *hda6* mutant phenotype can be partially restored by higher irradiance (Tessadori et al. 2009), that is sensed by cryptochromes and phytochromes.

Besides histone acetylation, many other epigenetic processes have been related with large-scale chromatin relaxation. Recently, the linker histone H1 has been also related to regulation of several developmental transitions, e.g., from vegetative to flowering development (Rutowicz et al. 2019). Interestingly, the H1.3 variant is stress inducible. After 3-4 days of low light exposure, H1.3 protein accumulates, and within 1-2 days after moving plants back to standard light conditions, *H1.3* expression returns to normal levels, matching the timing with the low-light induced chromatin decondensation. Moreover, the levels of low light-induced *H1.3* expression in the *cry2* and *phyB* mutants are slightly enhanced (Rutowicz et al. 2015). These results make H1.3 one of the possible candidates linking chromatin condensation and photoreceptor activity (Fig. 1B).

We still have a lot to understand on chromocenter dynamics and higher order chromatin compaction. In addition, it is unclear how sensing environmental changes through phyB and cryptochromes integrate in these processes.

Histone marks at specific loci as a mean to control shade-regulated gene expression

Chromatin changes in specific genes can be studied by analyzing the occurrence of epigenetic marks in determined regions of the genome, such as those corresponding to genes involved in the shade-response regulation. Among the most important epigenetic marks are DNA methylation and histone post-translational modifications. We will discuss in here changes in histone modifications, as so far, only these marks have been related to shade responses. In addition to controlling the higher order chromatin structure, as addressed in the previous section, the presence or absence of specific histone marks at the locus scale can also be directly linked to gene regulation. These epigenetic modifications play an important role in transcription factor binding to DNA regulatory sequences and target gene transcription. When understanding light-regulation of gene expression, several epigenetic marks have been studied (Barneche et al. 2014, Kim et al. 2015, Perrella and Kaiserli 2016) but only a few have been related to shade. Below we will describe three examples of histone marks over specific loci and the mechanisms that modulate shade-regulated gene expression.

Case 1: Histone acetylation of specific genes is induced in response to shade

Histone acetylation is a post-translational modification that consists in the transfer of acetyl groups to Lys residues of histones. The addition of the acetyl group (1) neutralizes the Lys positive charge that counteracts the negative charged DNA, eventually provoking the relaxation of the chromatin, and/or (2) provide recognition sites for factors involved in the activation of gene expression (Carrozza et al. 2003). Acetylation is catalyzed by histone acetyltransferases

(HATs), whereas histone deacetylation is catalyzed by histone deacetylases (HDACs) (Fig. 2A). These enzymes are encoded by multiple genes with functional redundancy, with at least 12 HAT and 18 HDAC genes in Arabidopsis (Pandey et al. 2002).

In shade, histone acetylation levels play an important role in chromatin compaction at the chromocenter level, as previously discussed (Fig. 1B). The HDAC activity of HDA6 is required for the deacetylation that leads to chromatin compaction. Several studies show that histone acetylation and deacetylation mutants have defects in hypocotyl elongation when grown in monochromatic FR and R conditions, pointing to the importance of acetylation in the phyA- and phyB-mediated responses of hypocotyl elongation. One of the HATs that has been found to play a role in R, FR and B light response pathways is HISTONE ACETYLTRANSFERASE OF THE TAFII250 FAMILY 2 (HAF2, also known as TAF1) (Bertrand et al. 2005). Although the *haf2* mutant does not show significant changes in hypocotyl growth in R and FR conditions, when combined with the *ELONGATED HYPOCOTYL 5* (*HY5*) deficient mutant showed an enhancement of the *hy5* hypocotyl elongation. HY5 is a light-responsive positive transcription factor that, when mutated, results in hyposensitivity (i.e., long hypocotyls) to several light conditions (Oyama et al. 1997). This enhancement suggests that the HAF2-mediated acetylation interacts with the HY5 pathway downstream of the phytochrome signaling. Another HAT that has a role in the R and FR perception is GENERAL CONTROL NONDEREPRESSIBLE 5 (GCN5, also known as HAG1). In contrast with the *haf2* single mutant, the single *gcn5* mutant shows a long hypocotyl phenotype under different light conditions. Moreover, *gcn5 haf2* double mutant shows an additive hypocotyl phenotype. Although both HATs share target genes, the lack of effect on hypocotyl elongation in the *gcn5 hy5* double mutant, in contrast with the *haf2 hy5*, suggests that they still regulate different pathways (Fig. 2B) (Benhamed et al. 2006).

The role of HATs is counterbalanced by the action of HDACs (Fig. 2A). In addition to HDA6, two other HDACs have been reported to regulate hypocotyl growth in R and FR conditions, HDA19 and HDA15. HDA19 (also known as HD1) is a positive regulator of gene expression and hypocotyl growth in FR (and slightly also in R), showing the opposite role than GCN5, as can be concluded from the *hda19* mutant short hypocotyl phenotype compared to wild-type (Benhamed et al. 2006). The GCN5/HDA19 pair might act as an antagonistic system to control histone acetylation levels to switch gene expression. But this opposite HAT/HDAC activity is not always as clear. HDA15, which belongs to the same HDAC family than HDA19, plays an opposite role to HDA19 in the regulation of hypocotyl elongation. Under R and FR, *hda15 hda19* double mutant presents an intermediate hypocotyl phenotype compared to the *hda15* (long hypocotyls) and *hda19* (short hypocotyls) single mutants (Liu et al. 2013). Therefore, different HDACs may have distinct (even opposite) functions in hypocotyl elongation.

The complexity of the regulation by acetylation can be understood not only by the different HATs or HDACs that participate, but also by (1) the specific genes targeted by the same HAT or HDAC, (2) which histones (H3 or H4) are modified, (3) the particular Lys residues that are acetylated, and (4) the position of the acetylation mark within the gene (e.g., promoter vs. coding region). Some studies have addressed the acetylation levels of specific genes in various light conditions, such as different light wavelengths, low light and low R:FR. In different monochromatic lights and in low light, levels of acetylated H3 in Lys 9 (H3K9ac) rise or fall together with the expression levels of four tested genes in wild-type seedlings: light induces *PSII LIGHT HARVESTING COMPLEX GENE 1.4* (*LHCB1.4*) and *RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN 1B* (*RBCS-1B*) and represses *PEROXIDASE 21* and *CITRATE SYNTHASE 3* (*CSY3*) (Guo et al. 2008). Interestingly, changes of acetylation levels were regulated through HY5, which agrees with the long hypocotyl phenotype of *hy5 haf2* double mutant. When analyzing specific genes, *haf2* mutant has lower H3 acetylation levels in the light-induced genes *RBCS-1A*, *CHLOROPHYLL A/B-BINDING PROTEIN 2* (*CAB2*) although does not affect the acetylation in the *INDOLE-3-ACETIC ACID INDUCIBLE 3* (*IAA3*) (Bertrand et al. 2005, Benhamed et al. 2006). Acetylation of H3 and H4 in these three genes is also altered in the *gcn5* (reduced acetylation) and *hda19* (enhanced acetylation) mutants (Benhamed et al. 2006). The acetylated residues differ among these mutants; both GCN5 and HAF2 acetylate Lys 9 and Lys 27 in H3 (H3K9 and H3K27, respectively), but these marks are regulated differently depending on the target gene. Taking this also into consideration, *RBCS-1A* seems to be regulated by GCN5, HAF2 and HDA19, whereas *IAA3* is only dependent on GCN5 and HDA19 (Fig. 2B). To add more complexity, not only the specific target genes differ but also the distribution of the acetylation mark along the gene. The location of the reduced acetylation levels in the *gcn5* mutant do not correspond completely with those hyperacetylated in the *hda19* mutant. HDA19 is involved the regulation of *RBCS-1A* and *IAA3* with an effect on histone acetylation that seems to be operating in a large range of promoters and both upstream and at the core promoter regions (i.e., the one that contains the TATA box) (Benhamed et al. 2006). The specificity of the acetylation location can be explained by the mode of action of the HATs and by the mechanisms that target them to the specific genes: GCN5 binds directly to the *RBCS-1A* and *CAB2* promoters and HAF2 may do it through the transcription factor HY5 (Fig. 2B).

It seems likely that these mechanisms and/or epigenetic components might also have a role in low R:FR regulated gene expression. Indeed, it has been recently demonstrated that histone acetylation also changes in low R:FR ratio (Fig. 2C). Histone acetylation is promoted after just one hour of simulated shade in the auxin biosynthetic gene *YUCCA* 8 (*YUC8*). It is reported that

shade increases the levels of H3K9ac, H3K27ac and H4K5ac, but not those of H3K14ac or H3K36ac (Peng et al. 2018). Among the different marks, increase in H4K5 acetylation has been proposed to happen through the HAT activity encoded by *HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1* / *HISTONE ACETYLTRANSFERASE OF THE GNAT FAMILY 4* (*HAM1*/*HAG4*) and *HAM2*/*HAG5* (Earley et al. 2007, Xiao et al. 2013), and the other marks, H3K9ac and H3K27ac, have to be regulated by other HAT, possibly GCN5 and HAF2.

In summary, the variety of the Lys residue acetylated, the distribution of the mark along the genome, together with the different photomorphogenic hypocotyl phenotypes, exemplifies the complexity of how transcriptional control of the different shade-induced genes is exerted by the epigenetic mark imposed by HATs and HDACs. It seems likely that enhanced acetylation of histones might have an impact on the expression of the specific locus either directly or indirectly: if placed in the promoter region, it might facilitate binding of transcription factors to DNA regulatory sequences; alternatively, if placed in the gene body (the region containing introns and exons), it might also facilitate transcription by reducing compaction of nucleosomes.

Case 2: Histone methylation is involved in gene regulation in response to low R:FR

Methylation of histones consists in the addition of one, two or three methyl groups to a Lys residue. Unlike acetylation, the methyl group is neutral and does not affect the positive charge of the Lys. Methylation marks can be related to transcriptional activation or repression depending on the modified residue. In general, H3 trimethylation of the Lys 4 (H3K4me3) and Lys 36 (H3K36me3) are associated with activation and H3 dimethylation of Lys 9 (H3K9me2) and trimethylation of Lys 27 (H3K27me3) with repression of genes (Pontvianne et al. 2010).

The H3K4me3 and H3K36me3 activating marks have been described in the 5'-end of the gene body of *YUC8* and their presence have an important role in recruiting, by direct binding, the MORF RELATED GENE 2 (MRG2) histone methylation reader (Peng et al. 2018). Accordingly, the reduced levels of those H3 marks in the H3K4-methyltransferase mutant *homologue of trithorax 1-2* (*atx1-2*) and the H3K36-methyltransferase mutant *set domain group 8-2* (*sdg8-2*) results in a reduction of MRG2 binding to the modified histones (Peng et al. 2018) (Fig. 2C). Although histone methylation levels of these marks are not affected by simulated shade, their reduction in the *atx1-2* and *sdg8-2* mutant results in a slightly shorter hypocotyl. This supports a basal role of H3K4me3 and H3K36me3 in the response of seedlings to low R:FR. The methylation reader *mrg2* mutant does not exhibit a phenotype, but when the other member of the family *MRG1* is also mutated, hypocotyl elongation is drastically reduced in shade (Peng et al. 2018). Interestingly, binding of MRG2 to *YUC8*, *IAA19* and *PACLOBUTRAZOL RESISTANCE 1* (*PRE1*) is enhanced in shade and dependent on PIF7. Peng

and collaborators found that PIF7 mediates MRG2 binding to the genes by direct interaction and that the recruitment of MRG2 leads to the acetylation of H4K5 probably by the known interaction of MRG2 with the HAM1/HAM2 HATs (Xu et al. 2014) (Fig. 2C). This case illustrates how different types of marks, histone methylation and acetylation, crosstalk to modulate shade-regulated transcription.

Case 3. H3K27me3 could modulate gene regulation in response to shade

A histone methylation mark associated with gene repression, H3K27me3, has been also indirectly linked to the regulation of the seedling response to shade through the role of LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), also known as TERMINAL FLOWER 2 (TFL2) (Valdés et al. 2012). LHP1 is a chromatin-associated protein recruited to H3K27me3-rich regions that is proposed to be responsible for the stabilization of those repressive regions (Exner et al. 2009). When de-etiolated under R and FR (but not under B), *lhp1* mutant seedlings present a strong hypersensitive response as hypocotyl elongation is strongly inhibited. The *lhp1* de-etiolation phenotype is dependent on phyA and phyB, as double mutants with *lhp1* have the same phenotype of the *phyA* and *phyB* single mutants in FR and R, respectively. This indicates that LHP1 acts downstream of phytochromes. Importantly, *lhp1* mutant seedlings are almost unresponsive to low R:FR. Because LHP1 has been related to auxin biosynthesis, which is reduced in the *lhp1* mutant (Rizzardi et al. 2011), the authors explored the possibility of an impaired auxin-mediated shade response. The addition of external auxin cannot rescue the *lhp1* phenotype but potentiates the shade-induced gene expression of the Aux/IAA members *IAA5* and *IAA19*. As *lhp1* is not responsive to auxin, although the Aux/IAAs expression levels are increased, it is expected to act downstream of Aux/IAA, hypothesis reinforced by the direct binding of LHP1 with IAA5, IAA6 and IAA19 proteins (Valdés et al. 2012). This case highlights the role of LHP1 in regulating the seedlings respond to shade probably via the H3K27me3 mark. How LHP1 is acting at the chromatin level and the role of phytochromes and Aux/IAAs in this process is still unknown.

Future perspectives and open questions

When the environment changes, chromatin is reshaped. Chromatin relaxation likely allows access to the transcriptional machinery to sustain changes in the expression of a broad range of genes that are instrumental in plant's acclimation to the new environment. From this perspective, few works have been conducted till date in the shade response field. What aspects need further studies?

(1) Global changes in nucleus structure are clearly detected after a few days of shade exposure (van Zanten et al. 2010), when changes in gene expression affect a group of late (indirect) target genes belonging to Gene Ontology (GO) categories related to diverse stresses (Leivar et al. 2012) (Fig. 3). Consistently, a global chromatin decondensation occurs in a wide variety of abiotic changes and stress situations (Probst and Mittelsten Scheid 2015) and responses to shade are not an exception. How a wide variety of environmental changes converge in a common chromatin decondensation process? Shade also provokes early changes in gene expression within one hour of treatment, which are mostly directed by PIF binding (Kohnen et al. 2016) (Fig. 3). Are nuclear structure changes required for this early transcriptional reprogramming induced by shade? To link chromosome structure and gene expression, higher resolution techniques, like chromosome conformation capture (Kempfer and Pombo 2019), might be needed.

(2) Chromatin structural changes induced by an environmental cue may persist to make genes more responsive to future changes in the same signal. This "memory" can be useful to allow the plant to give faster acclimation responses (Fig. 3) (Bruce et al. 2007). Addressing the dynamics of shade-induced histone marks might be required to define their possible role in providing a memory.

(3) So far, shade-triggered epigenetic modifications at specific loci have been studied in a few genes, which makes difficult to propose general mechanisms to regulate shade responses. This can be addressed by using a genome-wide approach of different epigenetic marks in response to shade.

(4) Current studies have focused only in a limited number of epigenetic marks. When searching for new players in shade-response, we can look into the epigenetic regulation of thermomorphogenesis, a process known to share many phenotypic and regulatory similarities with that regulated by shade (Legris et al. 2017). Two components known to mediate the thermosensory response in plants emerge in this search: the histone variant H2A.Z (Kumar and Wigge 2010), and the ATP-dependent chromatin remodeling factor PICKLE, also shown to negatively control photomorphogenesis (Zha et al. 2017).

(5) How are the epigenetic and transcriptional mechanisms integrated? We can envisage at least two non-exclusive possibilities: (a) transcription factor binding capacity can be modulated by chromatin structure and (b) the transcription factor itself can act as a pioneer attracting chromatin remodelers to specific loci. Indeed, the latter possibility has been already suggested

in a few cases, such as the HDA15-PIF3 (Liu et al. 2013) and the MRG2-PIF7 (Peng et al. 2018).

Author contributions

JM-R and JFM-G developed the draft of the review, JM-R wrote the draft of the manuscript, and JM-R and JFM-G reviewed, edited and approved the final manuscript.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

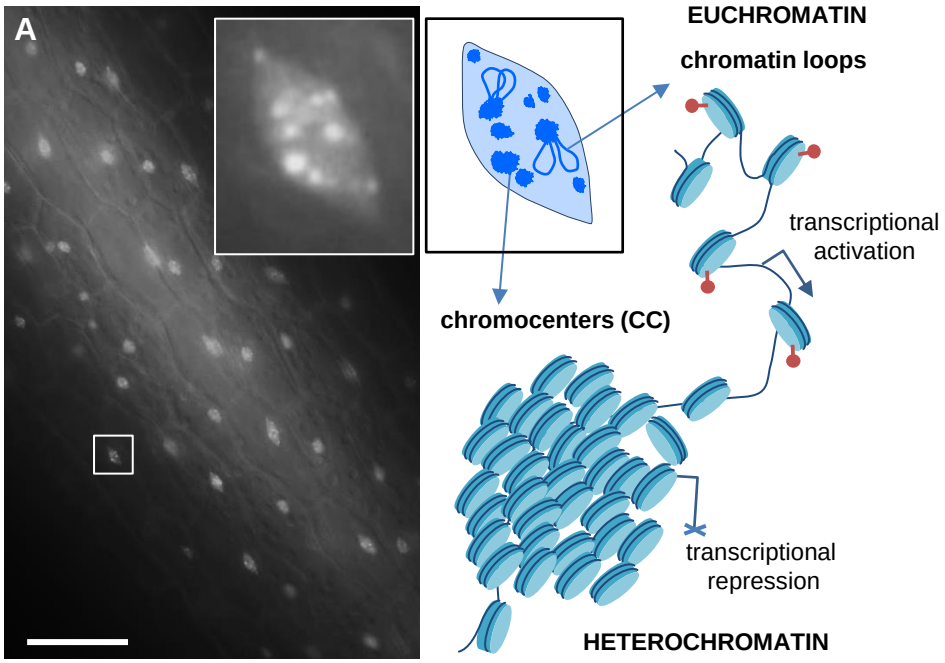
FIGURE LEGENDS

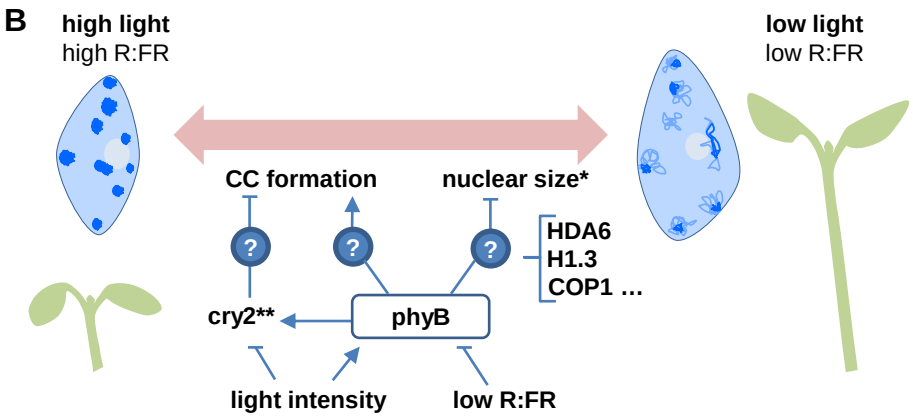
Fig. 1. Nuclear structure changes in response to low light and low R:FR. (A) Chromatin is heterogeneously distributed in the nucleus. This can be observed after DAPI staining, where nuclei present highly stained speckles called chromocenters (CC). The left panel corresponds to a hypocotyl stained with DAPI and the magnification of a selected nucleus. On the right, a simplified outlined version of this nucleus is represented, highlighting the CC and the surrounding nucleoplasmic area. These CC are formed by densely packed heterochromatin with very low transcriptional activity. From them, chromatin loops emanate occupying the less stained regions of the nucleus. This fraction called euchromatin is less packed (more open) and more accessible to transcriptional machinery to bind, which therefore allows genes to be expressed. Bar corresponds to 50 µm. (B) Shade (low light and/or low R:FR) promotes decondensation of the chromatin, reducing the number and size of CC. Low light also increases nuclear size. So far, it is known that both responses are controlled by the light receptors phyB and cry2. Shade (low R:FR) and low light inhibits phyB activity, that reduces the promotion of CC and increases nuclear size. As phyB, cry2 is a regulator of CC formation. In high light, cry2 is degraded and CC is promoted. Low light directly induces cry2 accumulation and indirectly increases its abundance by phyB activity, that results in less induction of CC formation. Both

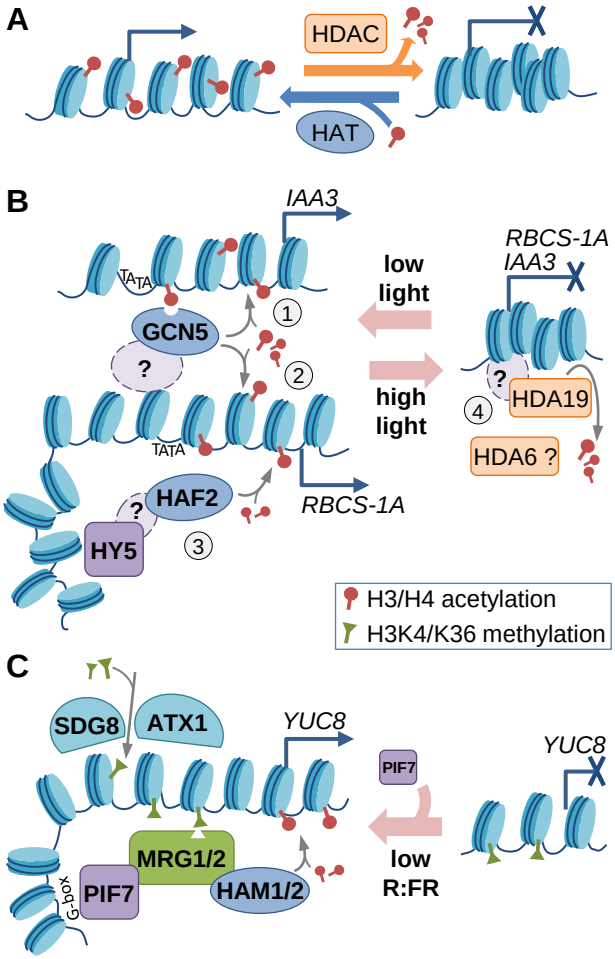
receptors can modulate chromatin structure either directly or indirectly through a chromatin remodeler complex, which can include HDA6, H1.3 (that has been related to chromatin condensation in shade) or members of the ubiquitination machinery (as COP1). * Changes in nuclear size have not been reported in low R:FR. ** The participation of cry2 in CC formation has been reported only in low light conditions, not in low R:FR.

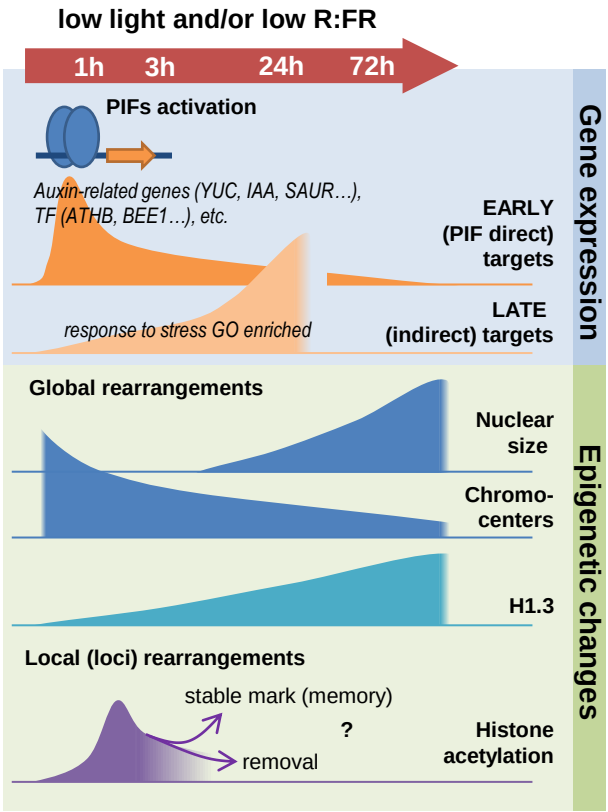
Fig. 2. Histone acetylation changes in low light and low R:FR. (A) Histone acetylation mediated by HAT promotes chromatin decondensation and allows gene expression, while deacetylation by HDAC provokes chromatin compaction that leads to transcriptional repression. (B) HAT activity targets specific genes. The HAT GCN5 is able to either (1) directly bind to the core promoter (where the TATA element is located) of some genes like *IAA3* to acetylate histones (this binding is enhanced when histones are acetylated) and/or (2) indirectly through an unknown protein like in the *RBCS-1A* gene. This protein could be a transcription factor like in the case of HAF2 (3) that is indirectly guided by HY5 to the regions in the *RBCS-1A* promoter to be acetylated. HAF2 and GCN5 can act synergistically to control *RBCS-1A* expression, whereas GCN5 binds alone to promote *IAA3* expression. The HDAC activity (4) of HDA19 seems to globally de-acetylate many genes. It is still unknown if the binding is direct (for instance, via a TF) or indirect. The participation of HDA6 at the gene level is unknown but likely as it affects nuclei chromatin density. (C) Another mechanism to acetylate histones is linked to the presence of other histone marks, as it occurs in the control of *YUC8* expression. The H3K4/K36me mark deposited by the action of SDG8/ATX1 is read by MRG1/2 only when interacts with PIF7. MRG1/2 are responsible to bind the HAT HAM1/2 to acetylate the gene and promote its expression. Acetylation only occurs when PIF7 is activated by low R:FR.

Fig. 3. Changes in gene expression and epigenetic marks in low light and low R:FR take place at different paces. Gene expression is induced rapidly after low R:FR (within 15 min in some cases) via PIF activity. Many of these genes belong to the auxin biosynthesis pathway or are TFs that trigger the expression of other genes. Increased auxin levels and activation of TFs results in the late activation or repression of PIF-indirect secondary genes. These latter genes belong to GO categories related to different stresses. In addition to gene expression reprogramming, epigenetic changes occur. Some are mid-long term (increase of nuclear size, decrease of chromocenters or the accumulation of the H1.3 variant) and others are faster (increase of acetylation already after one hour of the signal). It is unknown if these rapid changes in acetylation are maintained or not in later time points.


A
EUCHROMATIN
chromatin loops
transcriptional
activation
chromocenters (CC)
transcriptional
repression
HETEROCHROMATIN


B
high light
high R:FR
low light
low R:FR
CC formation
nuclear size*
HDA6
H1.3
COP1 …
cry2**
phyB
light intensity
low R:FR


A
HDAC
HAT
B
IAA3
TATA
GCN5
?
1
2
TATA
HAF2
?
HY5
3
RBCS-1A
low
light
high
light
RBCS-1A
IAA3
?
4
HDA19
HDA6 ?
H3/H4 acetylation
H3K4/K36 methylation
C
SDG8
ATX1
YUC8
MRG1/2
PIF7
HAM1/2
G-box
PIF7
low
R:FR
YUC8


low light and/or low R:FR
1h
3h
24h
72h
PIFs activation
Auxin-related genes (YUC, IAA, SAUR…),
TF (ATHB, BEE1…), etc.
EARLY
(PIF direct)
targets
response to stress GO enriched
LATE
(indirect) targets
Gene expression
Global rearrangements
Nuclear
size
Chromo-
centers
H1.3
Local (loci) rearrangements
stable mark (memory)
?
removal
Histone
acetylation
Epigenetic changes