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Focusing on the nuclear and subnuclear dynamics of light and circadian signalling

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Abstract:	Circadian clocks provide organisms the ability to synchronise their internal physiological responses with the external environment. This process, termed entrainment, occurs through the perception of internal and external stimuli. As with other organisms, in plants the perception of light is a critical for the entrainment and sustainment of circadian rhythms. Red, blue, far-red and UV-B light is perceived by the oscillator through the activity of photoreceptors. Four classes of photoreceptors signal to the oscillator: phytochromes, cryptochromes, UVR8 and LOV-KELCH domain proteins. In most cases, these photoreceptors localise to the nucleus in response to light and can associate to subnuclear structures to initiate downstream signalling. In this review, we will highlight the recent advances made in understanding the mechanisms facilitating the nuclear and subnuclear localisation of photoreceptors and the role these subnuclear bodies have in photoreceptor signalling, including to the oscillator. We will also highlight recent progress that has been made in understanding the regulation of the nuclear and subnuclear localisation of components of the plant circadian clock.

Photoreceptors can associate to subnuclear structures to initiate signalling. Similarly many interacting clock proteins also exist in distinct sub-nuclear structures in a time-dependent manner. In this review, we highlight recent advances made in understanding the mechanisms facilitating their nuclear and subnuclear localisation.

Focusing on the nuclear and subnuclear dynamics of light and circadian signalling

James Ronald and Seth J. Davis

Department of Biology, University of York, York, UK

Corresponding E-mail: seth.davis@york.ac.uk

Abstract

Circadian clocks provide organisms the ability to synchronise their internal physiological responses with the external environment. This process, termed entrainment, occurs through the perception of internal and external stimuli. As with other organisms, in plants the perception of light is a critical for the entrainment and sustainment of circadian rhythms. Red, blue, far-red and UV-B light is perceived by the oscillator through the activity of photoreceptors. Four classes of photoreceptors signal to the oscillator: phytochromes, cryptochromes, UVR8 and LOV-KELCH domain proteins. In most cases, these photoreceptors localise to the nucleus in response to light and can associate to subnuclear structures to initiate downstream signalling. In this review, we will highlight the recent advances made in understanding the mechanisms facilitating the nuclear and subnuclear localisation of photoreceptors and the role these subnuclear bodies have in photoreceptor signalling, including to the oscillator. We will also highlight recent progress that has been made in understanding the regulation of the nuclear and subnuclear localisation of components of the plant circadian clock.

Introduction

The daily rotation of the Earth generates approximately 24-hour cycles of light and temperature. To coordinate their internal physiological responses to match the predicted external environment, most eukaryotic and some prokaryotic organisms have evolved a molecular timekeeping mechanism termed a circadian clock (Cohen & Golden, 2015, McClung, 2019, Takahashi, 2017). In plants, the circadian clock controls a diverse array of processes including photosynthesis, thermomorphogenesis, hormone signalling, the response to biotic and abiotic stress and flowering time (Sanchez & Kay, 2016).

The plant circadian oscillator is composed of a series of interlocking transcriptional-translational feedback loops (TTFLs). At the centre of these TTFLs are the morning expressed transcription factors *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)* and *LATE ELOGNATED HYPOCOTYL (LHY)*, and the evening phased *TIMING OF CAB1* (TOC1, also known as PRR1) which mutually repress each other's expression (Gendron, Pruneda-Paz, Doherty, Gross, Kang & Kay, 2012, Más, Alabadí, Yanovsky, Oyama & Kay, 2003, Mizoguchi, Wheatley, Hanzawa, Wright, Mizoguchi, Song, Carre & Coupland, 2002, Nagel, Doherty, Pruneda-Paz, Schmitz, Ecker & Kay, 2015). The expression and activity of CCA1/LHY and TOC1 is subsequently controlled by further morning and evening loops (Figure 1). *PRR9/7/5* are sequentially expressed throughout the day starting at mid-morning to repress *CCA1/LHY* expression (Nakamichi, Kiba, Henriques, Mizuno, Chua & Sakakibara, 2010, Nakamichi, Kita, Ito, Yamashino & Mizuno, 2005). The evening complex (EC) composed of EARLY FLOWERING3, ELF4 and LUX ARRYTHMO (LUX) repress the expression of *PRR9* and *PRR7* from dusk, while TOC1 and PRR5 are degraded in the evening through their interaction with ZEITLUPE (ZTL) and GIGANTEA (GI) (Herrero, Kolmos, Bujdoso, Yuan, Wang, Berns, Uhlworm, Coupland, Saini, Jaskolski, Webb, Gonçalves & Davis, 2012, Kim, Fujiwara, Suh, Kim, Kim, Han, David, Putterill, Nam & Somers, 2007, Kolmos, Nowak, Werner, Fischer, Schwarz, Mathews, Schoof, Nagy, Bujnicki & Davis, 2009, Nusinow, Helfer, Hamilton, King, Imaizumi, Schultz, Farré & Kay, 2011). For a detail discussion of the plant circadian oscillator, we point readers to recent reviews (McClung, 2019, Ronald & Davis, 2017).

The synchronization of internal oscillations to mirror external time occurs through a process termed entrainment. A wide range of entraining signals (termed *zeitgebers*) have been discovered; these include environmental stimuli such as light and temperature, but also internal signals, such as sucrose availability and hormone signalling (Millar, 2004, Oakenfull & Davis, 2017, Webb, Seki, Satake & Caldana, 2019). Light signals are transmitted to the oscillator through at least four classes of photoreceptors: CRYPTOCHROMEs (CRYs) detect

blue light and UV-A, LOV-KELCH DOMAIN proteins also perceive BL, PHYTOCHROMES (phys) primarily detect red (RL) and far-red light (FRL), while UV-B RESISTANCE8 (UVR8) detects UV-B light (Oakenfull & Davis, 2017). Photoreceptors signal to the oscillator at the transcriptional and post-translational level. However, unlike the mammalian system where photoreceptors are essential for circadian rhythms, no single plant photoreceptor family is required for the generation or sustainment of circadian rhythms (Devlin & Kay, 2000, Millar, 2004). For a detailed review of the role of photoreceptors in mediating entrainment of the oscillator see (Oakenfull & Davis, 2017).

The intersection between light and circadian signalling mostly occurs in the nucleus (Herrero & Davis, 2012). The nucleus is the site within the cell that is responsible for DNA replication, transcription, ribosomal synthesis and RNA processing. The nucleus is a highly ordered structure. Surrounding the nucleus is a double membrane nuclear envelope in which nuclear pore complexes (NPCs) are embedded. The NPCs regulates the trafficking of proteins and RNA from the nucleus to the cytoplasm (Kaiserli, Perrella & Davidson, 2018, Lamond & Sleeman, 2003). Chromosomes typically packaged as chromatin are localised throughout the nucleoplasm. In metazoans, each chromosome occupies a distinct space within the nucleoplasm called chromosome territories (Lamond & Sleeman, 2003). In *Arabidopsis thaliana* and the related *Arabidopsis lyrata*, chromosome territories are not observed and chromatin is mostly randomly dispersed (Berr, Pecinka, Meister, Kreth, Fuchs, Blattner, Lysak & Schubert, 2006, Berr & Schubert, 2007, Pecinka, Schubert, Meister, Kreth, Klatte, Lysak, Fuchs & Schubert, 2004). The nucleus also contains a series of substructures called nuclear bodies (Lamond & Sleeman, 2003). The formation of these subnuclear structures are proposed to promote and enhance protein activity by condensing proteins, DNA and RNA together (Matera, Izaguire-Sierra, Praveen & Rajendra, 2009). Some of these nuclear bodies are conserved throughout eukaryotic nuclei. These include the nucleolus, cajal bodies and speckles, which mediates ribosome synthesis, RNA processing and splicing respectively. However, some of the nuclear substructures are kingdom specific. For example, the plant nucleus contains photobodies, while the animal nucleus contains promyelocytic leukemia protein (PML) bodies (Kaiserli *et al.*, 2018, Lamond & Sleeman, 2003).

In recent years, our understanding of the importance of subnuclear structures in mediating downstream photoreceptor signalling activity has increased. In this review we will focus on how photoreceptors localise to the nucleus and the mechanisms regulating their association to subnuclear structures. We will also highlight the roles nuclear bodies have in facilitating photoreceptor activity, including the signalling from photoreceptors to the circadian clock.

Finally, we will discuss the nuclear and subnuclear dynamics of the circadian clock and how subnuclear structures may influence circadian protein activity.

Red and Far-Red Light

The signalling of RL and FRL to the circadian clock occurs primarily through phys. In Arabidopsis, there are five phys: the light liable phyA, and the light stable phyB-E (Clack, Mathews & Sharrock, 1994). All phys are composed of a N-terminus photosensory domain that is covalently attached to a tetrapyrrole bilin chromophore and a C-terminal region required for downstream signalling and photobody formation (Rockwell, Su & Lagarias, 2006). Aside from phyC, all phys can associate as homodimers and the light stable phys can also form heterodimers. A pulse of red light promotes the conversion from the Pr to the Pfr conformer, while a pulse of FRL converts Pfr back to Pr (Rockwell *et al.*, 2006). Additionally, temperature and prolonged darkness can promote the conversion of Pfr to Pr (Legris, Klose, Burgie, Rojas, Neme, Hiltbrunner, Wigge, Schafer, Vierstra & Casal, 2016, Rockwell *et al.*, 2006).

The activity of phytochromes is dependent on their localisation to the nucleus (Huq, Al-Sady & Quail, 2003, Matsushita, Mochizuki & Nagatani, 2003). In the dark, phys are predominantly, though not exclusively, localised to the cytoplasm and will translocate to the nucleus after a pulse of RL for phyB-E or BL, RL or FRL for phyA (Gil, Kircher, Adam, Bury, Kozma-Bognar, Schafer & Nagy, 2000, Kim, Kircher, Toth, Adam, Schäfer & Nagy, 2000, Nagatani, 2004). The movement of phyA and phyB-E to the nucleus is controlled through different mechanisms. phyA does not intrinsically localise to the nucleus and is dependent on FAR-RED ELONGATED HYPOCOTYL1 (FHY1) and FHY1-LIKE (FHL). FHY1/FHL interact with PFr phyA in the cytoplasm and rapidly shuttle phyA to the nucleus to initiate downstream signalling (Genoud, Schweizer, Tscheuschler, Debrieux, Casal, Schäfer, Hiltbrunner & Fankhauser, 2008, Hiltbrunner, Tscheuschler, Viczian, Kunkel, Kircher & Schafer, 2006). Once in the nucleus, phyA is either degraded in a light dependent manner or is re-shuttled back to the cytoplasm by FHY1/FHL in the Pr form (Rausenberger, Tscheuschler, Nordmeier, Wüst, Timmer, Schäfer, Fleck & Hiltbrunner, 2011). In contrast to phyA, phyB-E intrinsically localises to the nucleus through a nuclear localisation signal (NLS) present within the C-terminus of the protein (Chen, Tao, Lim, Shaw & Chory, 2005). When in the Pr conformer, the NLS is masked by an interaction between the N and C-terminus of the phyB protein. The absorption of RL promotes the phyB protein to undergo a conformational change to unmask the C-terminal NLS (Chen *et al.*, 2005). Separately, phyB may also

translocate to the nucleus through a physical interaction with PHYTOCHROME INTERACTING FACTORS (PIFs) (Pfeiffer, Nagel, Popp, Wüst, Bindics, Viczián, Hiltbrunner, Nagy, Kunkel & Schäfer, 2012). Similar dynamics are thought to control the translocation of the phyC-E, although phyE accumulates in the nucleus under much lower fluence rates of RL than phyB (Adam, Kircher, Liu, Merai, Gonzalez-Schain, Horner, Viczian, Monte, Sharrock, Schafer & Nagy, 2013).

In the nucleus all phytochromes can associate to nuclear bodies termed photobodies. In temporal terms, there are two species of photobodies. First to appear after light exposure are the transient photobodies. These photobodies form within minutes of RL (phyA or phyB) or FRL (phyA only) exposure but disappear after 30 to 60 minutes following the start of the light pulse (Bauer, Viczián, Kircher, Nobis, Nitschke, Kunkel, Panigrahi, Ádám, Fejes, Schäfer & Nagy, 2004, Casal, Davis, Kirchenbauer, Viczian, Yanovsky, Clough, Kircher, Jordan-Beebe, Schäfer, Nagy & Vierstra, 2002, Kircher, Gil, Kozma-Bognár, Fejes, Speth, Husselstein-Muller, Bauer, Ádám, Schäfer & Nagy, 2002). The second species of photobodies, termed stable photobodies, appear 2-3 hours after the start of constant RL (Kircher *et al.*, 2002). Unlike the first species of photobodies, these photobodies remain within the nucleus for up to 12 hours after the end of the RL pulse (Van Buskirk, Reddy, Nagatani & Chen, 2014). These secondary photobodies are likely dominated by phyB, as phyA is degraded under constant RL (Debrieux & Fankhauser, 2010). PhyC-E also associates to these stable photobodies, either through hetero-dimerisation with phyB or as homodimers (Adam *et al.*, 2013, Kircher *et al.*, 2002).

So far, most investigations on the dynamics of photobody formation have focused on stable phyB photobodies. The ability of phyB to associate to photobodies is dependent on the C-terminus of phyB and in the absence of the N-terminus the C-terminus will intrinsically localise to photobodies independently of light (Matsushita *et al.*, 2003). The wavelength, intensity and duration of light all influences photobody cellular morphology. RL promotes the formation of photobodies in an intensity dependent manner (Chen, Schwab & Chory, 2003). At intensities of RL lower than 0.5 μmol m^{-2} s^{-1} no photobodies will form, while small photobodies are detectable at 1 μmol m^{-2} s^{-1} and large photobodies at above 8 μmol m^{-2} s^{-1}. Between 1 and 8 μmol m^{-2} s^{-1} there is a mixture of small and large photobodies (Chen *et al.*, 2003). In contrast to RL, FRL promotes the rapid disablement of photobodies (Van Buskirk *et al.*, 2014) and BL inhibits large photobody formation (Trupkin, Legris, Buchovsky, Tolava Rivero & Casal, 2014). The formation of small photobodies is also promoted by a transient reduction in irradiance or the R:FR ratio (Trupkin *et al.*, 2014). Large photobodies are not

affected by these transient changes in light quality. Warm temperatures (27ºC) also repress photobody formation by promoting the conversion of Pfr phyB to Pr phyB (Legris *et al.*, 2016).

Alongside environmental factors, proteins that co-localise with phyB in photobodies regulate photobody formation. The first of these proteins to be characterised was HEMERA (HMR, also known as pTAC12), a protein that functions in the nucleus and chloroplast (Chen, Galvão, Li, Burger, Bugea, Bolado & Chory, 2010). In the absence of HMR, phyB either fails to form photobodies or can only localise to small photobodies (Chen *et al.*, 2010, Qiu, Li, Kim, Moore & Chen, 2019). PHOTOPERIODIC CONTROL OF HYPOCOTYL1 (PCH1) and its homolog PCHL also regulate phyB photobody morphogenesis (Huang, Yoo, Bindbeutel, Goldsworthy, Tielking, Alvarez, Naldrett, Evans, Chen & Nusinow, 2016). Unlike the *hmr* mutant, phyB can still localise to large photobodies in the *pch1* background albeit at a slightly reduced level. However, these large photobodies are less stable than in WT and disassemble more rapidly in the dark (Huang *et al.*, 2016). This effect is further enhanced in the *pch1/pchl* double mutant (Enderle, Sheerin, Paik, Kathare, Schwenk, Klose, Ulbrich, Huq & Hiltbrunner, 2017, Huang *et al.*, 2016). Interestingly, HMR and PCH1 have both been recently shown to be required for the temperature sensing role of phyB, indicating that the function of photobodies may extend beyond light signalling (Huang, McLoughlin, Sorkin, Burgie, Bindbeutel, Vierstra & Nusinow, 2019, Qiu *et al.*, 2019).

The importance of photobodies in phyB signalling has been debated since their discovery. Currently, photobodies are thought to possess multiple non-mutually exclusive functions (Figure 2). Firstly, photobodies may act as storage sites of Pfr phyB that preserve or stabilise PFr phyB from converting back to the Pr state (Van Buskirk *et al.*, 2014) (Figure 2A). This process is supported by the association of PCH1/PCHL to phyB within photobodies (Enderle *et al.*, 2017, Huang *et al.*, 2019, Huang *et al.*, 2016). Secondly, photobodies are required for some aspects of phy signalling (Figure 2B-D). After a pulse of light, phyA and phyB associate to transient photobodies along with PIF3 (Bauer *et al.*, 2004). The localisation of PIF3 to photobodies is associated with multi-site phosphorylation and subsequent ubiquitination and degradation of PIF3 (Al-Sady, Ni, Kircher, Schafer & Quail, 2006, Dong, Ni, Yu, Deng, Chen & Wei, 2017, Ni, Xu, Chalkley, Pham, Guan, Maltby, Burlingame, Wang & Quail, 2013) (Figure 2B). Kinases that promote the phosphorylation of PIF3 co-localise with PIF3 in nuclear foci, suggesting that phosphorylation may occur at photobodies (Ni, Xu, González-Grandío, Chalkley, Huhmer, Burlingame, Wang & Quail, 2017). It is unclear whether the ubiquitin machinery can also co-localise to photobodies. Other PIFs negatively

regulated by phys are also phosphorylated prior to degradation but whether this occurs within photobodies is unknown (Lorrain, Allen, Duek, Whitelam & Fankhauser, 2008).

Photobodies may also acts as sites to sequester or seclude proteins to inhibit their activity. Both phyA and phyB can interact with SUPPRESSOR OF *phya-105 1* (SPA1) within nuclear bodies in a light dependent manner (Lu, Zhou, Xu, Luo, Lian & Yang, 2015, Sheerin, Menon, zur Oven-Krockhaus, Enderle, Zhu, Johnen, Schleifenbaum, Stierhof, Huq & Hiltbrunner, 2015) (Figure 2C). This interaction secludes SPA1 from interacting with CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), inhibiting the ability of COP1 to promote the degradation of transcriptional regulators that promote light signalling (Hoecker, 2017). Photobodies are also sites of gene regulation. The transcription factor TANDEM ZINC-FINGER-PLUS3 (TZP) co-localises to photobodies with phyB under RL to activate gene expression (Kaiserli, Paldi, O'Donnell, Batalov, Pedmale, Nusinow, Kay & Chory, 2015) (Figure 2D). Other transcription factors such as LONG AFTER FAR RED LIGHT1 (LAF1) and LONG HYPOCOTYL IN FAR-RED1 (HFR1) also co-localises within photobodies (Ballesteros, Bolle, Lois, Moore, Vielle-Calzada, Grossniklaus & Chua, 2001, Sheerin *et al.*, 2015). However, photobodies are dispensable for phy signalling. The expression of a N-terminal fragment that fails to form photobodies was sufficient in mediating phyB photosensory activity (Matsushita *et al.*, 2003). Therefore, photobodies are important but may not be essential for phytochrome signalling.

The nuclear translocation of phys is essential for phy mediated entrainment of the oscillator (Jones, Hu, Litthauer, Lagarias & Harmer, 2015). Phys have multiple entry points to the oscillator at the transcriptional and post-translational level. phyB and phyA are both required for red light mediated activation of *PRR9* and *CCA1* expression (Ito, Matsushika, Yamada, Sato, Kato, Tabata, Yamashino & Mizuno, 2003, Rausenberger *et al.*, 2011, Wang & Tobin, 1998). phys also regulates the transcription of *ELF4*, although there are currently conflicting reports on whether this is dependent on a RL or FRL signalling pathway (Li, Siddiqui, Teng, Lin, Wan, Li, Lau, Ouyang, Dai, Wan, Devlin, Deng & Wang, 2011, Siddiqui, Khan, Rhodes & Devlin, 2016). At the post-translational level, phyB physically interacts with ELF3, LUX, CCA1, LHY, TOC1 and GI *in planta* (Yeom, Kim, Lim, Shin, Hong, Kim & Nam, 2014). The interaction between phyB and ELF3 has been reported to stabilise ELF3, but separate work has suggested that phyB could be repressing ELF3 function within the oscillator (Herrero *et al.*, 2012, Kolmos, Herrero, Bujdoso, Millar, Toth, Gyula, Nagy & Davis, 2011, Nieto, Lopez-Salmeron, Daviere & Prat, 2015). The outcome of the interaction between the other circadian components and phyB remains unknown, but it has been proposed that some of these proteins may facilitate the shuttling of phyB to the nucleus (Klose, Viczian, Kircher, Schafer & Nagy, 2015).

The role of photobodies in the entrainment of the oscillator has yet to be clearly established. In the dark, the oscillations of most circadian genes rapidly dampen until they become arrhythmic. However, the constitutively active allele of phyB (YHB) can maintain circadian oscillations under constant darkness similar to what is observed under constant light (Jones *et al.*, 2015). When this YHB allele is placed into the *pchl1* mutant background, YHB can no longer form large photobodies and fails to sustain circadian rhythms in constant darkness (Huang *et al.*, 2019). This would therefore suggest that photobodies are vital in the entrainment of the oscillator. Supporting this, previous work has highlighted that under WL the N-terminal fragment of phyB, which cannot form photobodies, is incapable of entraining the oscillator (Palágyi, Terecskei, Adám, Kevei, Kircher, Mérai, Schäfer, Nagy & Kozma-Bognár, 2010). However, this N-terminal fragment can sufficiently entrain the oscillator when seedlings are entrained exclusively under RL. Therefore, photobodies might have a light-dependent role in the entrainment of the oscillator and may act as points of convergence of separate photoreceptor signalling pathways.

Blue Light Signalling

Blue light is transmitted to the oscillator through three classes of photoreceptors, LOV-KELCH domain proteins, CRYs and phyA. As phyA has already been discussed, we will not discuss it further. We also highlight the role of PHOTROPHINS (PHOTs) in controlling the diurnal activity of photosystem II (Litthauer, Battle, Lawson & Jones, 2015). However, no role for PHOT1 or PHOT2 has been described in the entrainment of nuclear circadian rhythms (Litthauer, Battle & Jones, 2015) and therefore will not be discussed here.

LOV-KELCH

The LOV-KELCH domain family of protein has three members in Arabidopsis: ZTL, FLAVIN BINDING KELCH REPEAT, F-BOX1 (FKF1) and LOV KELCH PROTEIN2 (LKP2). ZTL/FKF1/LKP2 are composed of a N-terminal LOV domain, a F-box motif and six tandem KELCH repeats (Ito, Song & Imaizumi, 2012). The LOV domain is required for blue light perception and the interaction with GI, PRR5 and TOC1. The F-box domain regulates the interaction with ARABIDOPSIS SKP1 LIKE (ASK1), a component of the SCF E3 ligase complex (Han, Mason, Risseeuw, Crosby & Somers, 2004). The KELCH repeats provides a further protein-protein interaction interface and also facilitates hetero-dimerisation of the LOV-KELCH family (Ito *et al.*, 2012). The activity of ZTL is promoted by GI and HSP90 which form a ternary chaperone complex to promote the maturation and stabilisation of ZTL (Cha, Kim, Kim, Zeng, Wang, Lee, Kim & Somers, 2017). Similar post-translational mechanisms

are thought to regulate FKF1, while it is unknown if LKP2 is post-translationally regulated by HSP90/GI (Kim, Kim, Fujiwara, Kim, Cha, Park, Lee & Somers, 2011).

Within the circadian clock, ZTL, FKF1 and LKP2 redundantly promote the ubiquitination of TOC1 and PRR5 through the SCF complex (Más *et al.*, 2003) (Baudry, Ito, Song, Strait, Kiba, Lu, Henriques, Pruneda-Paz, Chua, Tobin, Kay & Imaizumi, 2010). Recently, ZTL was shown to promote the ubiquitination of CCA1 HIKING EXPEDITION (CHE) (Lee, Feke, Li, Adamchek, Webb, Pruneda-Paz, Bennett, Kay & Gendron, 2018, Sanchez & Kay), a transcription factor that interacts with TOC1 to regulate *CCA1* expression (Pruneda-Paz, Breton, Para & Kay, 2009). It is currently unknown whether FKF1 or LKP2 also promote CHE degradation. ZTL also regulates circadian rhythms by sequestering GI to the cytoplasm (Kim, Geng, Gallenstein & Somers, 2013a). Again, it is unknown if FKF1 or LKP2 can sequester GI to the cytoplasm to suppress GI activity.

The activity of the LOV-KELCH domain family within the circadian clock is not thought to occur within the nucleus. ZTL is exclusively localised to the cytoplasm, while FKF1 and LKP2 are localised in the cytoplasm and nucleus (Zoltowski & Imaizumi, 2014). Within the nucleus, LKP2 has been reported to co-localise to cajal bodies, while the sub-nuclear localisation of FKF1 is not yet known (Fukamatsu, Mitsui, Yasuhara, Tokioka, Ihara, Fujita & Kiyosue, 2005). However, the nuclear and sub-nuclear localisation of LKP2 and FKF1 is unlikely to be important for the signalling of the LOV-KELCH family to the oscillator. Of ZTL, FKF1 and LKP2, only *ztl* mutants have a circadian phenotype (Baudry *et al.,* 2010*)*. Therefore, the degradation of TOC1, PRR5 and CHE and any other circadian function of the LOV-KELCH family is likely to be restricted to the cytoplasm.

Cryptochromes

In Arabidopsis there are three CRY genes: CRY1, CRY2 and CRY3. CRY3 is structurally and functionally distinct from CRY1 and CRY2 and will not be discussed further (Yu, Liu, Klejnot & Lin, 2010). CRY1 and CRY2 share a photosensory N-terminal domain that is non-covalently bound to a flavin co-factor and a C-terminal effector domain (Yu *et al.*, 2010). The C-terminal domain varies in size between CRY1 and CRY2, reflecting differences in functional activity and the stability of the two proteins. CRY1 and CRY2 associate as homodimers *in vivo* to facilitate their functional activity (Rosenfeldt, Viana, Mootz, von Arnim & Batschauer, 2008, Wang, Wang, Han, Liu, Gu, Yang, Su, Liu, Zuo, He, Wang, Liu, Matsui, Kim, Oka & Lin, 2017). There is no report of heterodimerisation between CRY1 and CRY2.

CRY1 localises in the cytoplasm and nucleus to perform unique functions in the separate compartments (Wu & Spalding, 2007, Yang, Wu, Tang, Liu, Liu & Cashmore, 2000). The nucleocytoplasmic distribution of Arabidopsis CRY1 has also been observed in the rice CRY1 and wheat CRY1a proteins, but no NLS has been identified in these proteins (Matsumoto, Hirano, Iwasaki & Yamamoto, 2003, Xu, Xiang, Zhu, Xu, Zhang, Zhang, Zhang & Ma, 2009). The N-terminus and C-terminus of the wheat and rice CRY1 orthologs are intrinsically capable of localising to the nucleus, suggesting that multiple non-conventional NLS signals may promote CRY1 localisation (Matsumoto *et al.*, 2003, Xu *et al.*, 2009). Rice and Arabidopsis CRY1 also have a nuclear export signal (NES) in the N and C-terminus respectively, while no NES has been identified in the wheat CRY1a ortholog (Matsumoto *et al.*, 2003, Wu & Spalding, 2007, Xu *et al.*, 2009). In contrast to CRY1, CRY2 functions exclusively in the nucleus before being degraded in a light dependent manner (Guo, Duong, Ma & Lin, 1999, Yang *et al.*, 2000). The localisation of CRY2 to the nucleus is not dependent on light and requires an NLS signal within the C-terminus (Guo *et al.*, 1999, Kleiner, Kircher, Harter & Batschauer, 1999). Mutations within this NLS inhibit CRY2 nuclear localisation (Zuo, Meng, Yu, Zhang, Feng, Sun, Liu & Lin, 2012). Once in the nucleus, Arabidopsis CRY1 and CRY2 localises to nuclear bodies, which we will term cry-bodies to avoid confusion with phy photobodies (although there is some overlap discussed below) (Gu, Zhang & Yang, 2012, Yu, Sayegh, Maymon, Warpeha, Klejnot, Yang, Huang, Lee, Kaufman & Lin, 2009). For CRY2, the formation of these cry-bodies occurs within 30 seconds of exposure to blue light (Yu *et al.*, 2009). These number and size of the CRY2 cry-bodies is also responsive to the intensity and length of BL exposure (Yu *et al.*, 2009). Recent work has shown that BLUE-LIGHT INHIBITOR OF CRYPTOCHROME1 (BIC1) and its homolog BIC2 are negative regulators of CRY2 cry-body formation (Wang, Zuo, Wang, Gu, Yoshizumi, Yang, Yang, Liu, Liu, Han, Kim, Liu, Wohlschlegel, Matsui, Oka & Lin, 2016). BIC1/2 directly interact with CRY2 to inhibit CRY2 homodimerisation, suppressing the ability of CRY2's to localise to cry-bodies (Wang *et al.*, 2016). It is unknown if similar mechanisms regulate CRY1 cry-body formation.

The role of cry-bodies in CRY signalling is less established than with phys. CRY1 and CRY2 both localise with SPA1 within cry-bodies in a blue light dependent manner (Lian, He, Zhang, Zhu, Zhang, Jia, Sun, Li & Yang, 2011, Zuo, Liu, Liu, Liu & Lin, 2011). The interaction between CRY1 and SPA1 promotes the dissociation of SPA1 from COP1, suppressing COP1 activity (Lian *et al.*, 2011) (Figure 3A). Separately the CRY2-SPA1 interaction results in the association of COP1 to CRY2 to inhibit COP1 mediated degradation of CONSTANTS (CO) (Zuo *et al.*, 2011). However, this association between SPA1-CRY2-COP1 also promotes the degradation of CRY2 (Weidler, zur Oven-Krockhaus, Heunemann, Orth,

Schleifenbaum, Harter, Hoecker & Batschauer, 2012). The degradation of CRY2 is dependent on its ability to associate to cry-bodies where it is phosphorylated prior to degradation (Yu *et al.*, 2009) (Figure 3B). The degradation of CRY2 is promoted by phyA and the SPA family, although it is unknown if phyA mediates this process by associating to nuclear bodies with CRY2 and SPA (Weidler *et al.*, 2012). It has been recently shown that the PPK kinases are responsible for CRY2 phosphorylation (Liu, Wang, Deng, Wang, Piao, Cai, Li, Barshop, Yu, Zhou, Liu, Oka, Wohlschlegel, Zuo & Lin, 2017). In separate work, these kinases were shown to with interact phyB to promote the phosphorylation of PIF3 (Ni *et al.*, 2017). This paper reported that PIF3/PPK co-localises within nuclear bodies though this remains to be confirmed. Therefore, PPKs could co-localise with SPA1 and phyA within nuclear bodies to promote CRY2 degradation (Liu 2017). As with photobodies, cry-bodies also act as sites for transcriptional regulation. CRY1 and CRY2 interacts with HBI1 (HOMOLOG OF BEE2 INTERACTING WITH IBH1) within cry-bodies to repress the transcriptional activity of HBI1 (Wang, Li, Xu, Lian, Wang, Xu, Mao, Zhang & Yang, 2018) (Figure 3C). Separately, CRY1 and CRY2 have also been shown to regulate the transcriptional activity of PIFs and CRYPTOCRHOME-INTERACTING BASIC-HELIX-LOOP-HELIX1 (CIB1) in the nucleus but it is unknown if they co-associate within nuclear bodies (Liu, Yu, Li, Klejnot, Yang, Lisiero & Lin, 2008, Ma, Li, Guo, Chu, Fang, Yan, Noel & Liu, 2016, Pedmale, Huang, Zander, Cole, Hetzel, Ljung, Reis, Sridevi, Nito, Nery, Ecker & Chory, 2016). Therefore, cry-bodies may have a similar function to photobodies in the regulation of transcription and proteolytic degradation.

The mechanisms facilitating CRY-mediated entrainment of the oscillator and the cellular location of this activity has remained unclear. In more recent work it was revealed that ELOGNATED HYPOCOTYL5 (HY5) and its homolog HY5-HOMOLOG (HYH) are a key signal integrator for BL-mediated entrainment of the oscillator (Hajdu, Dobos, Domijan, Balint, Nagy, Nagy & Kozma-Bognar, 2018) (Figure 3D). HY5/HYH is a transcription factor that acts as hub in the transduction of light signals (Gangappa & Botto, 2016). HY5 was shown to associate to the promoter of most clock genes *in vivo* and this is association was enhanced by BL and to a lesser extent by RL (Hajdu *et al.*, 2018). HY5 directly regulates the expression of *PRR5*, *LUX* and the *LUX* sister gene *BOA* (BROTHER OF LUX ARRYTHMO) and is predicted to also regulate CCA1 post-translationally. HY5 has also been separately shown to promote the expression of *ELF4* through the transcription factors FAR-RED ELONGATED HYPOCOTYL3 (FHY3) and FAR-RED-IMPAIRED RESPONSE (FAR1) (Li *et al.*, 2011). HY5/HYH associates to nuclear bodies in a COP1 dependent manner and this results in the proteolytic degradation of HY5/HYH (Ang, Chattopadhyay, Wei, Oyama, Okada, Batschauer & Deng, 1998). COP1 mediated degradation of HY5 is suppressed by

the light dependent association of CRYs and phys to these nuclear bodies (Lian *et al.*, 2011, Sheerin *et al.*, 2015, Wang, Ma, Li, Zhao & Deng, 2001, Zuo *et al.*, 2011). Separate work has shown that CRY2 can associate with phyB in photobodies to regulate the pace of the oscillator (Más, Devlin, Panda & Kay, 2000). Therefore, CRY2 and phyB may co-localise within nuclear bodies to inhibit COP1 mediated degradation of HY5 to facilitate the entrainment of the oscillator. Such a mechanism would explain why the phyB N-terminal mutants fail to entrain the oscillator under WL, as this construct would be incapable of associating into nuclear bodies with CRY2 to promote HY5 stability (Palágyi *et al.*, 2010).

UV-B

So far, the sole UV-B receptor uncovered in plants is UVR8. In the absence of UV-B, UVR8 is localised to the cytoplasm as an inactive homodimer maintained by a salt-bridge interaction between two UVR8 monomers (Rizzini, Favory, Cloix, Faggionato, O'Hara, Kaiserli, Baumeister, Schafer, Nagy, Jenkins & Ulm, 2011). Conserved tryptophan residues within the UVR8 protein serve as a chromophore for UV-B. The perception of UV-B light weakens the salt bridge interaction, releasing monomeric UVR8 to interact with COP1 (Christie, Arvai, Baxter, Heilmann, Pratt, O'Hara, Kelly, Hothorn, Smith, Hitomi, Jenkins & Getzoff, 2012, Rizzini *et al.*, 2011). In contrast to its traditional antagonistic role in red or blue light signalling, COP1 is a positive factor in UV-B signalling and has a critical role in facilitating UVR8 function (Oravecz *et al.,* 2006; Favory *et al.,* 2009). Once activated by UV-B, the UVR8 monomers are rapidly reverted to their homodimeric ground state (Heijde & Ulm, 2013, Heilmann & Jenkins, 2013). This process is promoted by two related proteins REPRESSOR OF UV-B PHOTOMORPHOGENESIS1 (RUP1) and RUP2 (Heijde & Ulm, 2013).

Monomeric UVR8 rapidly localises to the nucleus in response to UV-B light (Kaiserli & Jenkins, 2007, Yin, Skvortsova, Loubéry & Ulm, 2016). The localisation of UVR8 to the nucleus is necessary for UVR8 function but the mechanism regulating the localisation of UVR8 to the nucleus is not clear. UVR8 does not have a *bona fide* NLS, but previous work revealed a twenty-three amino acid stretch within the N-terminus was required for UVR8 to localise to the nucleus (Kaiserli & Jenkins, 2007). These residues may not form an NLS but instead could contribute to the perception of UV-B, which is required for UVR8 to interact with COP1 (Yin *et al.*, 2016). COP1 has a NLS and NES and intrinsically localises to the nucleus (Stacey, Hicks & von Arnim, 1999). This has led to the proposal that COP1 could shuttle monomeric UVR8 to the nucleus as FHY/FHL does in phyA signalling. However, the presence of a cryptic NLS cannot be ruled out (Yin *et al.*, 2016). It is unclear if UVR8 localises to nuclear bodies. In transient work, UVR8 and COP1 were shown to co-localise to

nuclear bodies (Favory, Stec, Gruber, Rizzini, Oravecz, Funk, Albert, Cloix, Jenkins, Oakeley, Seidlitz, Nagy & Ulm, 2009). However, separate work in Arabidopsis and more recent work in a Tobacco failed to identify UVR8 nuclear bodies (Kaiserli & Jenkins, 2007, Yang, Liang, Zhang, Shao, Gu, Shang, Shi, Li, Zhang & Liu, 2018).

The HY5 TF has a critical role in facilitating UV-B signalling downstream of UVR8. The expression of *HY5* and its homolog *HYH* is induced in response to UV-B light in a UVR8/COP1 dependent manner (Binkert, Kozma-Bognár, Terecskei, De Veylder, Nagy & Ulm, 2014, Oravecz, Baumann, Máté, Brzezinska, Molinier, Oakeley, Adám, Schäfer, Nagy & Ulm, 2006). HY5 is required to regulate the expression of genes responsive to UV-B light and mutations in *hy5* result in plants becoming hypersensitive to UV-B (Oravecz *et al.*, 2006, Ulm, Baumann, Oravecz, Mate, Adam, Oakeley, Schafer & Nagy, 2004). However, it is unclear how UVR8/COP1 signals to HY5. Originally, UVR8 was proposed to associate to the promoter of *HY5* and promote *HY5* expression (Brown, Cloix, Jiang, Kaiserli, Herzyk, Kliebenstein & Jenkins, 2005), but recent work has questioned the ability of UVR8 to bind to chromatin (Binkert, Crocco, Ekundayo, Lau, Raffelberg, Tilbrook, Yin, Chappuis, Schalch & Ulm, 2016). UVR8 can indirectly promote the expression of *HY5* by inhibiting the repressive effect of WRKY DNA BINDING PROTEIN36 (WRKY36) on *HY5* expression (Yang *et al.*, 2018). UVR8 also promotes HY5 activity by enhancing HY5 stability through interactions with COP1 and SPA proteins (Huang, Ouyang, Yang, Lau, Chen, Wei & Deng, 2013). The mechanisms for how UV-B signals to the oscillator is unknown. UV-B induces the expression of *CCA1* and *LHY*, but this is not dependent on HY5 or HYH (Feher, Kozma-Bognar, Kevei, Hajdu, Binkert, Davis, Schafer, Ulm & Nagy, 2011). This study did highlight a role for UVR8 and COP1 in UV-B mediated entrainment of the oscillator, but the downstream targets of UVR8/COP1 and whether this is a transcriptional or post-translational effect remains unknown.

Circadian Nuclear Dynamics

So far, we have only discussed the nuclear and subnuclear dynamics of photoreceptors and how these might influence circadian clock. In this section we will summarise recent advances made in the understanding the nuclear dynamics of circadian components.

Excluding ZTL (discussed earlier), most of the known plant circadian-clock components are transcription factors (TOC1, LUX, PRR5/7/9, CCA1 and LHY) or co-factors that aide transcription factors (ELF4, GI and ELF3). Accordingly, all have been shown to display nuclear localisation either in transient expression systems or in stable Arabidopsis lines (Carré & Kim, 2002, Herrero *et al.*, 2012, Nakamichi *et al.*, 2005, Wang, Fujiwara & Somers,

2010, Yakir, Hilman, Kron, Hassidim, Melamed-Book & Green, 2009). Of these components, only the nuclear dynamics of CCA1, TOC1, PRR5, GI and ELF3 have so far been characterised.

CCA1 intrinsically localises to the nucleus and this occurs rapidly upon translation (Yakir *et al.*, 2009). The kinetics of CCA1 localisation does not changed in plants exposed to light or kept in the dark, suggesting that CCA1 nuclear dynamics are not influenced by light (Yakir *et al.*, 2009). However, the authors only used white light, so a red or blue light specific effect cannot be ruled out. There was also no report of CCA1 localising to nuclear foci in this report. TOC1 also intrinsically localises to the nucleus through a NLS in the C-terminus of the protein (Wang *et al.*, 2010). TOC1 nuclear localisation is enhanced by PRR5 mediated phosphorylation of TOC1. This effect is unique to PRR5; neither PRR3, PRR7 or PRR9 was found to promote TOC1 phosphorylation or nuclear abundance (Wang *et al.*, 2010). PRR5 intrinsically localises to nuclear bodies, while TOC1 when expressed alone displays a diffuse nuclear localisation. However, when *TOC1* and *PRR5* are co-expressed TOC1 co-localises with PRR5 in nuclear bodies. It is unknown what role these nuclear bodies have in facilitating TOC1 or PRR5 activity (Wang *et al.*, 2010).

ELF3 is a multifunctional scaffold protein that is divided into three regions termed the N, M and C (Liu, Covington, Fankhauser, Chory & Wagner, 2001) (Figure 4A). In Arabidopsis, ELF3 contains a NLS signal within the C-terminus and accordingly fragments of ELF3-C intrinsically localise to the nucleus. However, fragments expressing the ELF3-M region without an NLS are still capable of localising to the nucleus albeit more weakly (Herrero *et al.*, 2012). The recruitment of ELF3-M to the nucleus is promoted by ELF4, an unrelated protein that directly binds to the middle domain of ELF3 (Herrero *et al.*, 2012). When ELF4 and ELF3-M are co-expressed in transient or stable Arabidopsis lines the nuclear pool of ELF3-M increases (Herrero *et al.*, 2012). In accordance with ELF4 promoting the nuclear localisation of ELF3, mutations/natural-variants within the ELF4 binding site of ELF3 cause a reduction in the nuclear accumulation of ELF3 (Anwer, Boikoglou, Herrero, Hallstein, Davis, Velikkakam James, Nagy & Davis, 2014, Kolmos *et al.*, 2011).

How ELF4 promotes the nuclear accumulation of ELF3 is unknown. ELF4 intrinsically localises to the nucleus (Herrero *et al.*, 2012), raising the possibility that ELF4 shuttles ELF3 to the nucleus like phyA/FYH/FHL and the proposed COP1/UVR8 shuttling mechanism, but this remains to be confirmed. In the nucleus ELF3 can associate to nuclear bodies called foci (Figure 4B). In transient systems ELF4 co-localises with ELF3 within foci, but this colocalisation is not confirmed for Arabidopsis (Herrero *et al.*, 2012). The dynamics

regulating ELF3 foci formation is still unclear. ELF4 has been proposed to promote ELF3 foci formation as ELF3 allelic variants with weaker ELF4 binding are reported to produce fewer foci (Anwer *et al.*, 2014). However, foci formation may not solely be regulated by ELF4. In the absence of the N-terminus, ELF3 can still localise to the nucleus but does not form foci (Herrero *et al.*, 2012) (Figure 4B). The N-terminus mediates the binding of phyB to ELF3, suggesting that phyB may also promote ELF3 foci formation (Liu *et al.*, 2001). Supporting this, recent work has revealed that ELF3 co-localises with TZP within nuclear bodies. (Kaiserli *et al.*, 2015). The formation of TZP nuclear bodies occurs in a phyB red light dependent manner and is associated with transcriptional activity. Separately, the C-terminal fragment of ELF3 which cannot interact with ELF4 or phyB exclusively localises to large nuclear bodies (Herrero *et al.*, 2012) (Figure 4B). However, as the ELF3C fragment fails to recapture any of the *elf3* loss of function mutant phenotype these foci are not thought to be functional and instead could be protein aggregates (Herrero, 2012 #22). The function of the foci formed by ELF3F remains unknown.

GI also forms nuclear bodies. The formation of these nuclear bodies is under diurnal control, with peak accumulation of nuclear bodies occurring at or just after dusk in long-day photoperiods (Kim, Lim, Yeom, Kim, Kim, Wang, Kim, Somers & Nam, 2013b). The diurnal accumulation of GI foci is dependent on ELF4. In *elf4* mutants, GI foci formation is strongly reduced and is instead localised diffusely within the nucleus. The foci of GI did not co-localise with markers of chromatin, DNA, the spliceosome or cajal bodies in Arabidopsis nuclei, suggesting these foci facilitate a function independent of these processes (Kim *et al.*, 2013b). Previous work in transient systems suggested that GI associated to nuclear bodies with COP1 and ELF3 and that this facilitated the proteolytic degradation of GI and ELF3 (Yu, Rubio, Lee, Bai, Lee, Kim, Liu, Zhang, Irigoyen, Sullivan, Zhang, Lee, Xie, Paek & Deng, 2008). Separate work showed that ELF4 recruits GI to nuclear bodies to sequester GI from binding to the CO promoter (Kim *et al.*, 2013b). Therefore, the nuclear bodies of GI are likely to be antagonistic to GI function. It is unknown if GI, ELF4, ELF3 and COP1 all co-localise within the same bodies at the same time.

Concluding Remarks and Perspectives

The nucleus is not a disordered structure but one that is formed of many sub-structures. These sub-structures serve to condense DNA, RNA and proteins together to promote a diverse array of functions. Sub-nuclear structures are prevalent throughout light signalling, with phys, crys and LKP2 from the LOV-KELCH domain family localising to photobodies. In recent years the diverse functions these nuclear bodies perform have begun to be uncovered, with photobodies acting as sites for storing photoactivated photoreceptors,

transcriptional regulation, catalysing the initial stages of protein degradation and sequestering proteins (Figure 2, 3). Photobodies have been shown to be highly responsive to environmental stimuli, with light quality and quantity, and temperature all influencing the formation and morphology of these structures. The formation of photobodies and cry-bodies is also regulated internally by proteins, which interact and co-localise with phys and crys (Chen *et al.*, 2003, Huang *et al.*, 2019, Legris *et al.*, 2016, Qiu *et al.*, 2019, Xu *et al.*, 2009, Yu *et al.*, 2009). Together, this suggest that photobodies/cry-bodies may act as a central processing unit within the cell where external stimuli and internal factors are integrated together to facilitate among other processes photomorphogenesis, thermomorphogenesis and flowering time. Whether internal signals such as photosynthates or hormones can also be integrated into these central processing units by regulating the size, morphology or function of these photobodies/cry-bodies remains to be seen.

In contrast to light signalling, our understanding of the sub-nuclear dynamics of circadian signalling is still largely in the dark. Though most known components of the circadian clock localise to the nucleus, so far only the nuclear dynamics of CCA1, GI, TOC1 and ELF3 have been investigated to some degree. Of those four, GI, TOC1 (with PRR5) and ELF3 have been described to form subnuclear structures. However, the mechanisms regulating the formation of these subnuclear structures and the influences of these subnuclear structures on circadian rhythms are largely unknown. The localisation of ELF3 to sub-nuclear structures is associated with an increased repressive effect on circadian period (*i.e* period lengthens), but it is unknown how these sub-nuclear structures aide ELF3 repressive activity (Herrero *et al.*, 2012, Nieto *et al.*, 2015). ELF3 co-localises to nuclear bodies with ELF4, suggesting that these foci could be sites of transcriptional activity. However, LUX, the TF component of the EC, has not yet been shown to co-localise with ELF3 or ELF4 in foci (Herrero *et al.*, 2012). Separately, ELF3 co-localises with GI and COP1 in nuclear bodies to facilitate the degradation of GI (Yu *et al.*, 2008). Whether ELF3 forms different species of nuclear bodies that are regulated in a spatio-temporal fashion, or if these foci are like photobodies/cry-bodies and perform multiple independent functions is yet to be investigated. In contrast to the positive effect of foci on ELF3 activity, the localisation of GI to nuclear bodies has been proposed to repress GI function, while the role of nuclear bodies in TOC1/PRR5 activity remains unclear. Further work is needed to understand how the nuclear and sub-nuclear dynamics of the circadian components influence the parameters of the circadian clock.

The crosstalk between light and the circadian clock is critical for the entrainment of the plant circadian oscillator. In Arabidopsis this is not exclusively a nuclear event, but the nucleus is a key site for the intersection between photoreceptors and the circadian clock. Emerging

evidence suggests that photoreceptors and components of the oscillator may co-localise together in subnuclear structures and this could influence the pace and amplitude of circadian rhythms. phyB and ELF3 could co-localise together within photobodies, while the co-localisation of HY5, phyB and CRY2 in nuclear bodies could provide a mechanism for red and blue light entrainment of the oscillator (Kaiserli *et al.*, 2015, Wang *et al.*, 2017). The development of super-resolution microscopy coupled with high-throughput chromatin confirmatory capture (HI-C), chromatin precipitation and next-generation sequencing will provide further insights into the protein, DNA and possibly RNA composition of these sub-nuclear structures. By understanding their composition, we can begin to understand how light and other signalling pathways converge with the circadian oscillator in nuclear bodies to facilitate entrainment.

Figures

Figure 1: The current model of the Arabidopsis circadian clock. The Arabidopsis circadian clock is composed of day and night expressed components arranged into a series of interlocking loops. The position of the components does not reflect their phase of expression. Black arrows highlight a repressive effect, while green arrows highlight a positive effect. Dashed arrows indicate a post-translational effect, while full arrows highlight a transcriptional effect. CCA1: CIRCADIAN ASSOCIATED1, LHY: LATE ELONGATED HYPOCOTYL, TOC1: TIMING OF CAB1 EXPRESSION, ZTL: ZEITLUPE, GI: GIGANTEA, ELF3: EARLY FLOWERING3, ELF4: EARLY FLOWERING4, LUX: LUX ARRYTHMO, BOA: BROTHER OF LUX ARRYTHMO, PRR9: PSEUDO RESPONSE REGULATOR9, PRR7: PSEUDO RESPONSE REGULATOR7, PRR5: PSEUDO RESPONSE REGULATOR5 and EC: Evening Complex.

Figure 2: Photobodies perform multiple functions in phytochrome signalling. (A) Photobodies act as a storage site for phytochromeB (phyB) in the biologically active Pfr conformer to protect against thermal reversion. The formation of these photobodies are promoted independently by HEMERA (HMR) and PHOTOPERIODIC CONTROL OF HYPOCOTYL1 (PCH1). (B) Photobodies are also sites for the degradation of PHYTOCHROME INTERACTING FACTOR3 (PIF3). phyB, PIF3 and PROTEIN PHOSPHATE KINASE (PPK) co-localise within photobodies, resulting in the phosphorylation of PIF3. PIF3 is subsequently ubiquitinated and degraded. (C) phyA and phyB co-localises with SUPPRESSOR OF PHYA-1 (SPA1) within photobodies to seclude SPA1 from COP1, suppressing COP1 activity. (D) Photobodies are sites of transcriptional activity. The transcription factor TANDEM ZINC-FINGER PLUS3 (TZP) co-localises with phyB within photobodies and this co-localisation is associated with transcriptional activity.

Figure 3: Nuclear bodies perform multiple functions in cry signalling. (A) CRY1 co-localises with SPA1 within cry-bodies to suppress the activity of COP1. (B) CRYPTOCHROME2 (CRY2) co-localises to cry-bodies where it is phosphorylated by PPKs, resulting in the subsequent degradation of CRY2. This process is promoted by SPA1, which co-localises with CRY2 in cry-bodies. (C,D) Cry-bodies are also sites of transcriptional activity. (C) CRY1 and CRY2 co-localises with HOMOLOG OF BEE2 INTERACTING WITH IBH1 (HIBI) in cry-bodies to repress HIBI transcriptional activity. (D) CRY2 and phyB co-localise together within nuclear bodies. This co-localisation may facilitate blue and red light mediated entrainment of the oscillator by stabilising HY5 from COP1 mediated degradation.

Figure 4 : Light has multiple entry points to the plant circadian oscillator. The current model of the Arabidopsis circadian clock from figure 1 expanded to include the current known entry points of photoreceptors to the oscillator. Black arrows highlight a negative interaction, while green arrows highlight a positive interactions. Dashed lines indicate a post-translational effect, and full lines highlight transcriptional regulation. Red suns indicate red light, blue suns indicate blue light and purple suns highlights UV-B. It is currently unknown how UVR8 mediates UV-B signalling to the circadian oscillator but CCA1 and LHY are targets of UV-B signalling. HY5: ELONGATED HYPOCOTYL5, HYH: HY5 HOMOLOG, FHY3: FAR-RED ELONGATED HYPOCOTYL3, FAR1: FAR-RED IMPAIRED RESPONSE1, UVR8: UV-B RESISTANCE8.

636 Figure 5: ELF3 fragments have different sub-nuclear structures. (A) Cartoon of diagram of
637 ELF3 with its three described domains, N, M and C. phyB binds to the N-terminus, ELF4
638 binds to the M region and the NLS is within the C-terminus. Numbers below the diagram
639 indicate the amino acid positions of the division as defined in Herrero et al., 2012 (B) The
640 nuclei of full length ELF3, ELF3MC or ELF3C in stable Arabidopsis lines at ZT10 (short day
641 photoperiods). Scale bars indicate 5 μM.

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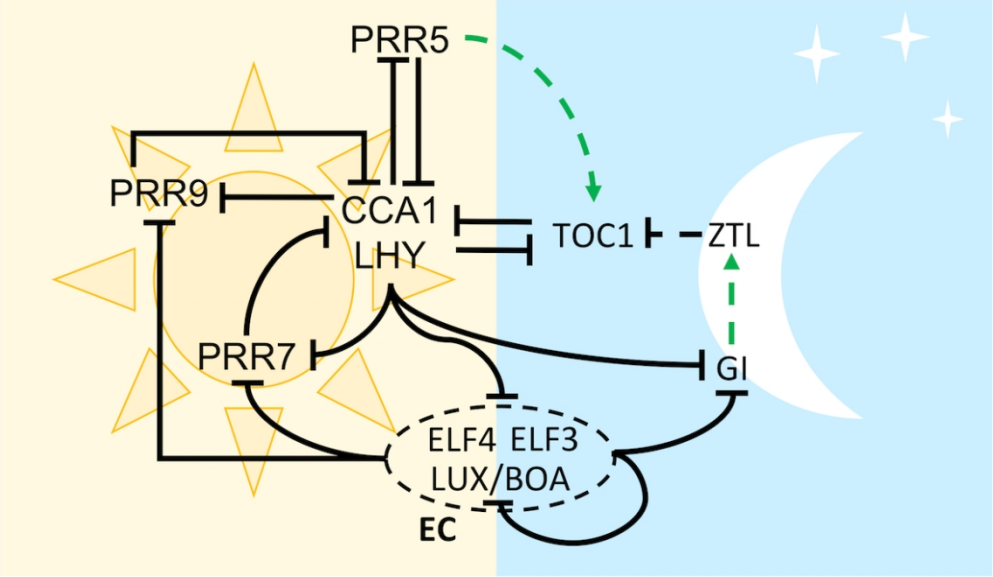

Figure 1: The current model of the Arabidopsis circadian clock.

100x59mm (300 x 300 DPI)

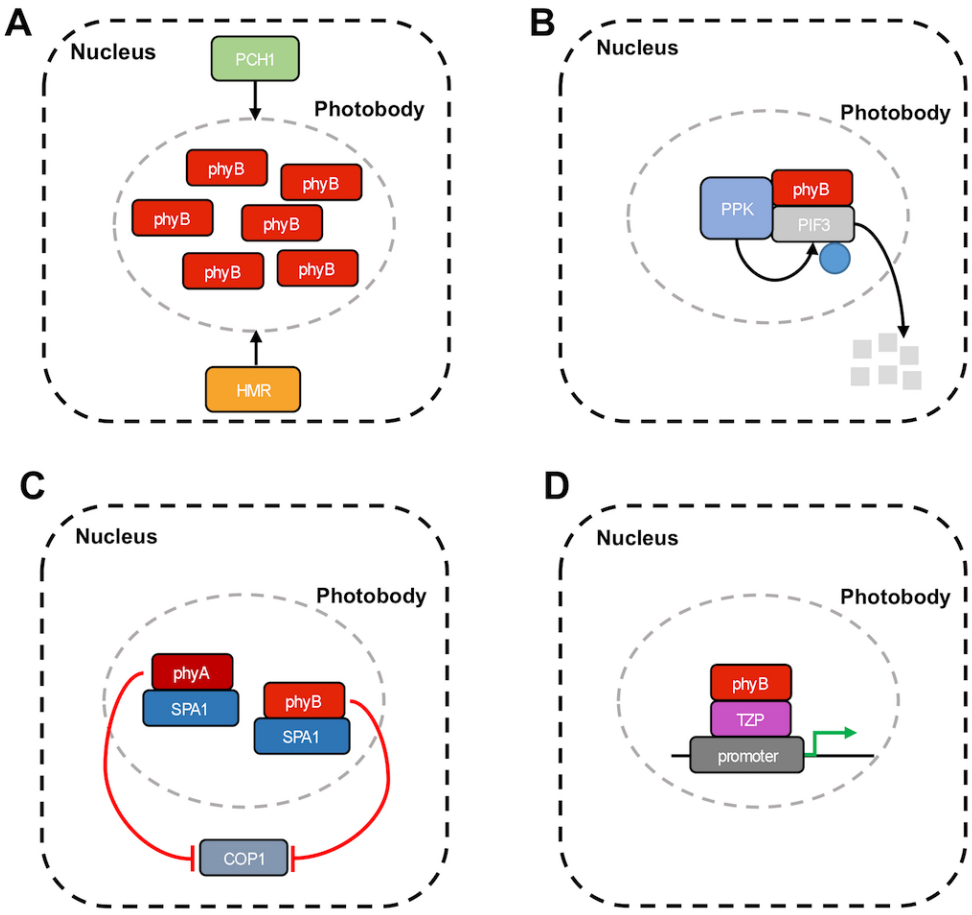

Figure 2: Photobodies perform multiple functions in phytochrome signalling.

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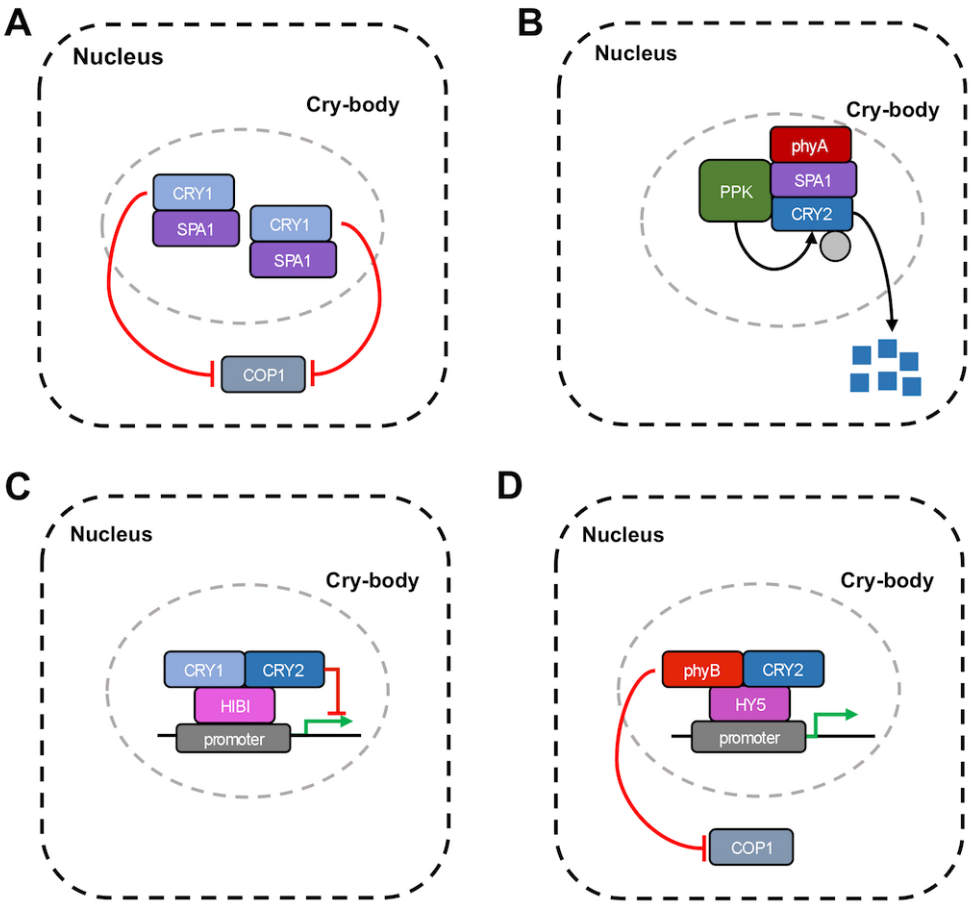


Figure 3: Nuclear bodies perform multiple functions in cry signalling.

88x81mm (300 x 300 DPI)

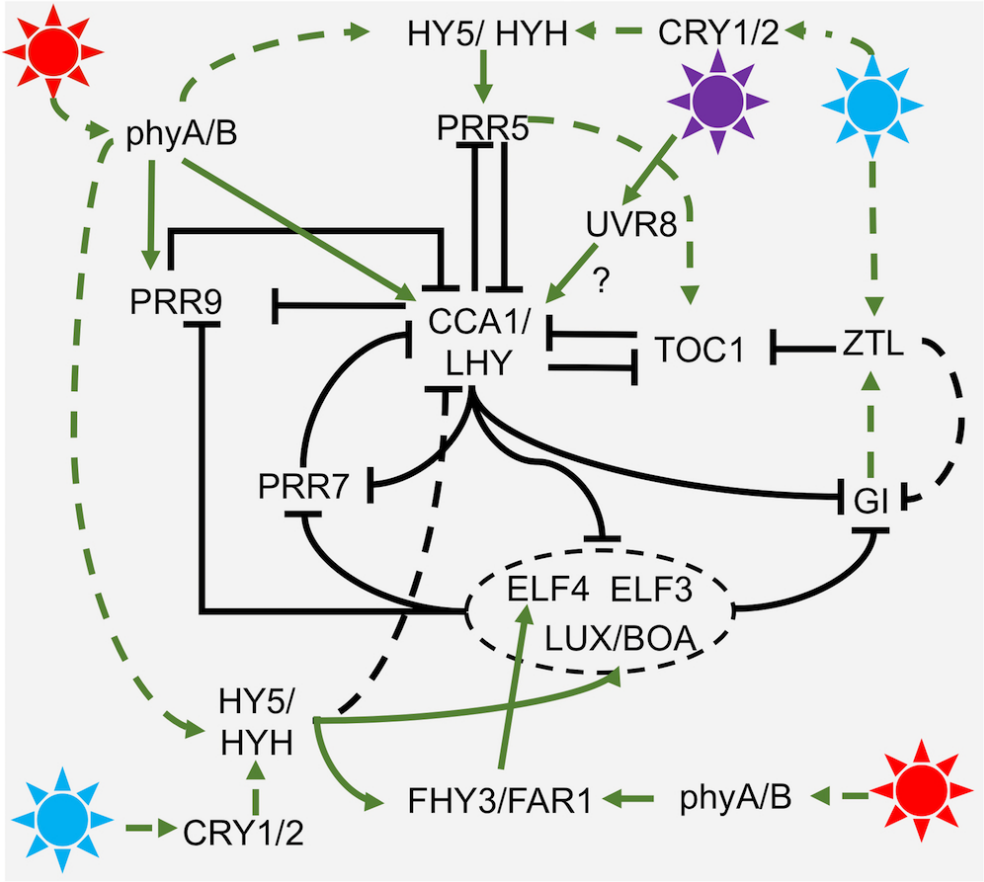


Figure 4: Light has multiple entry points to the plant circadian oscillator.

85x77mm (300 x 300 DPI)

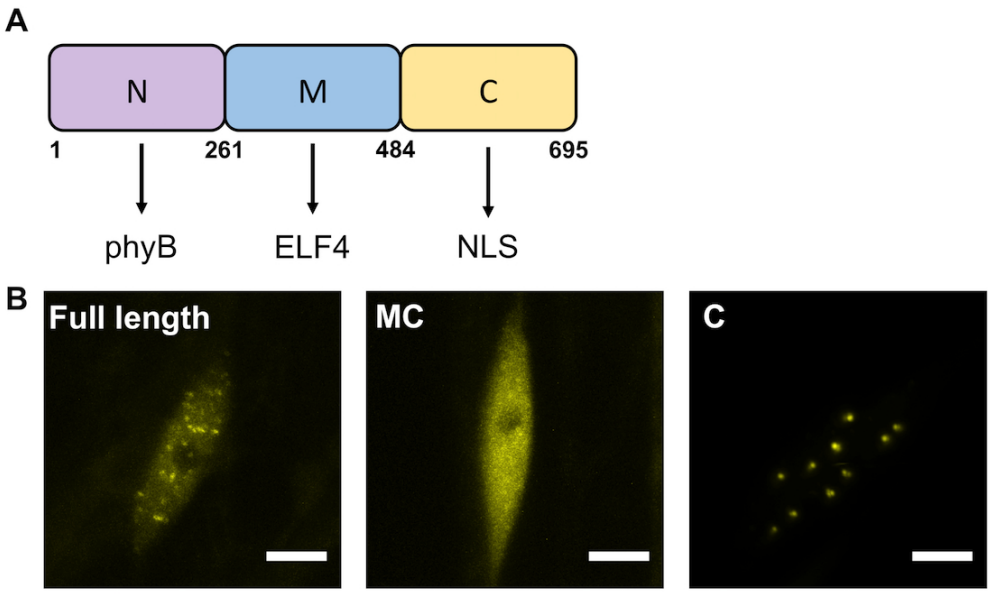
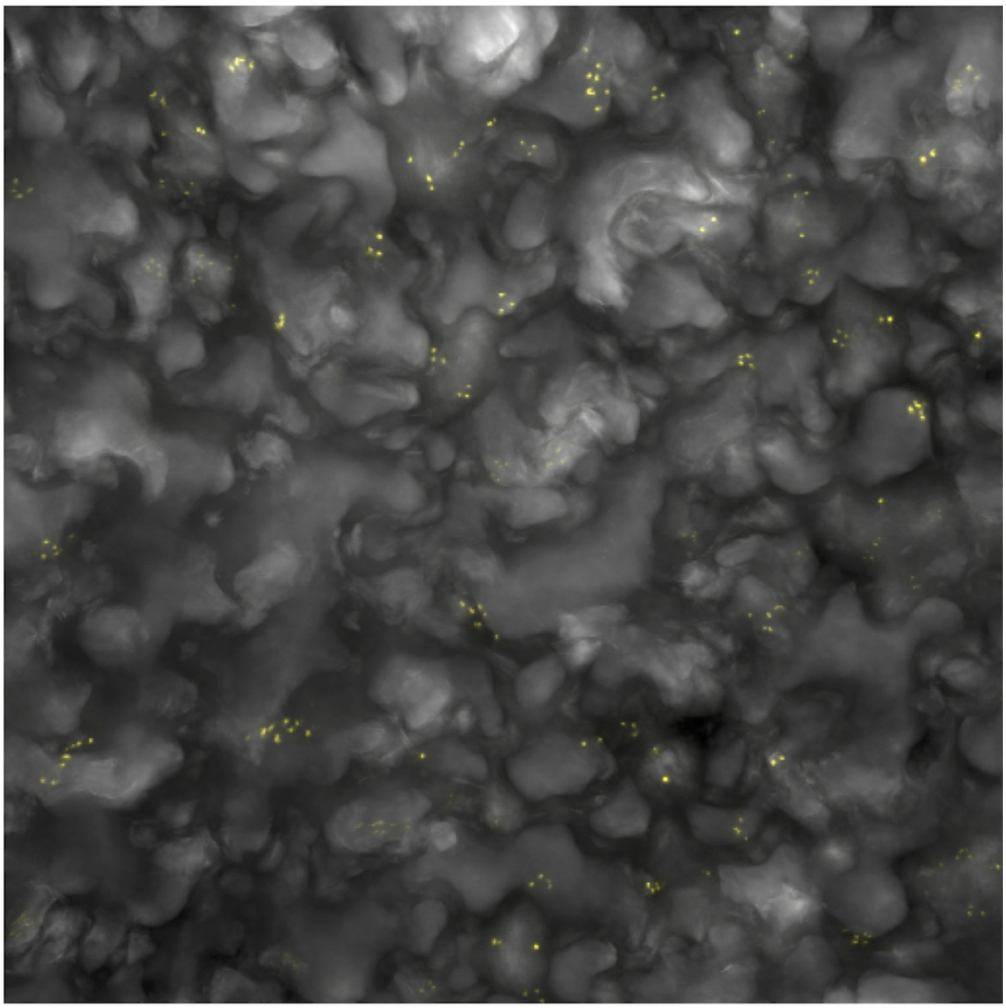


Figure 5: ELF3 fragments have different sub-nuclear structures.

93x56mm (300 x 300 DPI)



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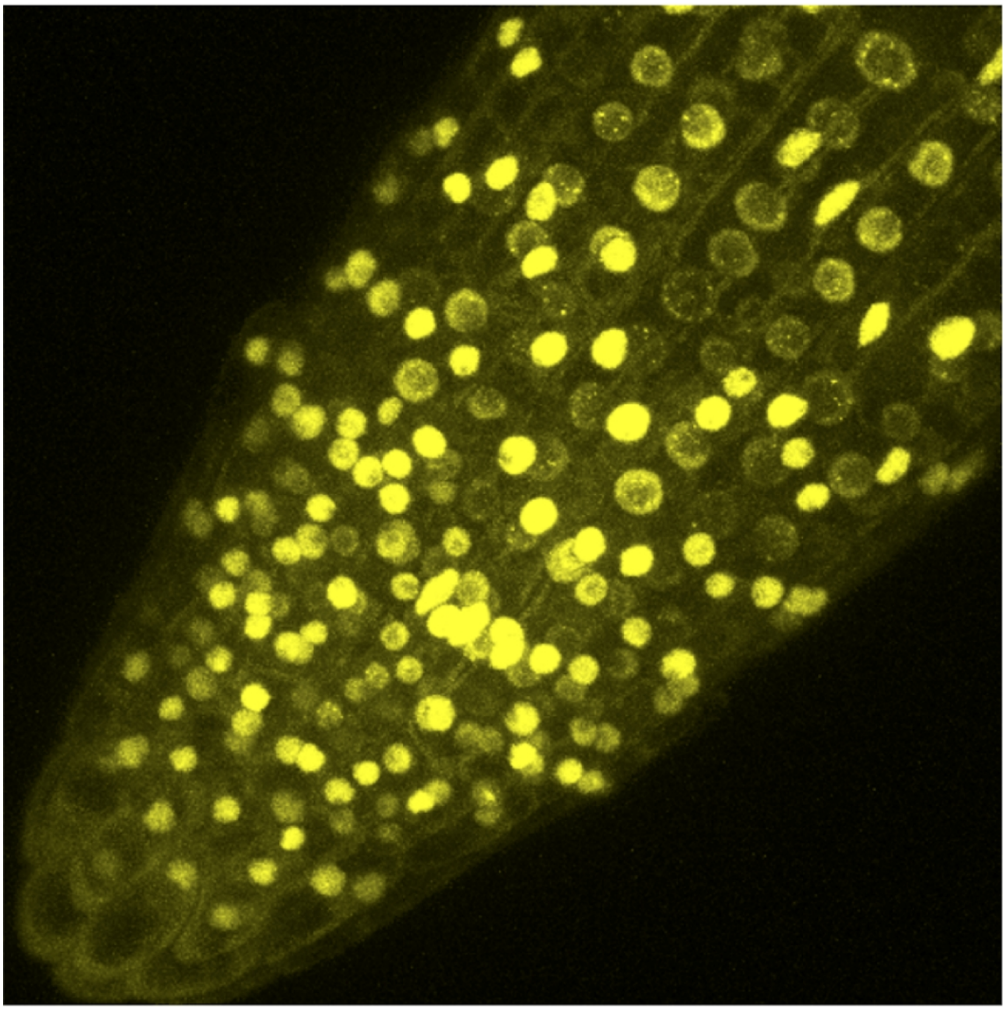

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