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Article:
Ronald, James Andrew and Davis, Seth Jon (2019) Focusing on the nuclear and subnuclear dynamics of light and circadian signalling. Plant, Cell and Environment. ISSN: 0140-7791

https://doi.org/10.1111/pce.13634

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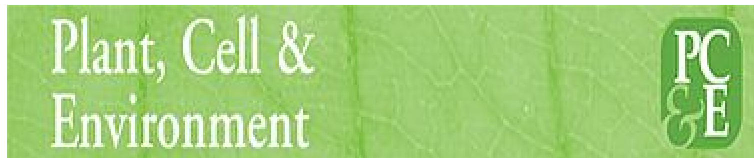

Focusing on the nuclear and subnuclear dynamics of light and circadian signalling

Journal:	*Plant, Cell & Environment*
Manuscript ID	PCE-19-0544.R1
Wiley - Manuscript type:	Special Issue
Date Submitted by the Author:	n/a

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Environment Keywords:	circadian, light quality
Physiology Keywords:	growth, proteome
Other Keywords:	

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.

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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.

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

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13 **Abstract**

14 Circadian clocks provide organisms the ability to synchronise their internal physiological
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16 the perception of internal and external stimuli. As with other organisms, in plants the
17 perception of light is a critical for the entrainment and sustainment of circadian rhythms. Red,
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25 signalling, including to the oscillator. We will also highlight recent progress that has been
26 made in understanding the regulation of the nuclear and subnuclear localisation of
27 components of the plant circadian clock.
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29 **Introduction**

30 The daily rotation of the Earth generates approximately 24-hour cycles of light and
31 temperature. To coordinate their internal physiological responses to match the predicted
32 external environment, most eukaryotic and some prokaryotic organisms have evolved a
33 molecular timekeeping mechanism termed a circadian clock (Cohen & Golden, 2015,
34 McClung, 2019, Takahashi, 2017). In plants, the circadian clock controls a diverse array of
35 processes including photosynthesis, thermomorphogenesis, hormone signalling, the
36 response to biotic and abiotic stress and flowering time (Sanchez & Kay, 2016).
37
38 The plant circadian oscillator is composed of a series of interlocking transcriptional-
39 translational feedback loops (TTFLs). At the centre of these TTFLs are the morning
40 expressed transcription factors *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)* and *LATE*
41 *ELOGNATED HYPOCOTYL (LHY)*, and the evening phased *TIMING OF CAB1* (TOC1, also
42 known as PRR1) which mutually repress each other's expression (Gendron, Pruneda-Paz,
43 Doherty, Gross, Kang & Kay, 2012, Más, Alabadí, Yanovsky, Oyama & Kay, 2003,
44 Mizoguchi, Wheatley, Hanzawa, Wright, Mizoguchi, Song, Carre & Coupland, 2002, Nagel,
45 Doherty, Pruneda-Paz, Schmitz, Ecker & Kay, 2015). The expression and activity of
46 CCA1/LHY and TOC1 is subsequently controlled by further morning and evening loops
47 (Figure 1). *PRR9/7/5* are sequentially expressed throughout the day starting at mid-morning
48 to repress *CCA1/LHY* expression (Nakamichi, Kiba, Henriques, Mizuno, Chua & Sakakibara,
49 2010, Nakamichi, Kita, Ito, Yamashino & Mizuno, 2005). The evening complex (EC)
50 composed of EARLY FLOWERING3, ELF4 and LUX ARRYTHMO (LUX) repress the
51 expression of *PRR9* and *PRR7* from dusk, while TOC1 and PRR5 are degraded in the
52 evening through their interaction with ZEITLUPE (ZTL) and GIGANTEA (GI) (Herrero,
53 Kolmos, Bujdoso, Yuan, Wang, Berns, Uhlworm, Coupland, Saini, Jaskolski, Webb,
54 Gonçalves & Davis, 2012, Kim, Fujiwara, Suh, Kim, Kim, Han, David, Putterill, Nam &
55 Somers, 2007, Kolmos, Nowak, Werner, Fischer, Schwarz, Mathews, Schoof, Nagy, Bujnicki
56 & Davis, 2009, Nusinow, Helfer, Hamilton, King, Imaizumi, Schultz, Farré & Kay, 2011). For
57 a detail discussion of the plant circadian oscillator, we point readers to recent reviews
58 (McClung, 2019, Ronald & Davis, 2017).
59
60 The synchronization of internal oscillations to mirror external time occurs through a process
61 termed entrainment. A wide range of entraining signals (termed *zeitgebers*) have been
62 discovered; these include environmental stimuli such as light and temperature, but also
63 internal signals, such as sucrose availability and hormone signalling (Millar, 2004, Oakenfull
64 & Davis, 2017, Webb, Seki, Satake & Caldana, 2019). Light signals are transmitted to the
65 oscillator through at least four classes of photoreceptors: CRYPTOCHROMEs (CRYs) detect

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66 blue light and UV-A, LOV-KELCH DOMAIN proteins also perceive BL, PHYTOCHROMES
67 (phys) primarily detect red (RL) and far-red light (FRL), while UV-B RESISTANCE8 (UVR8)
68 detects UV-B light (Oakenfull & Davis, 2017). Photoreceptors signal to the oscillator at the
69 transcriptional and post-translational level. However, unlike the mammalian system where
70 photoreceptors are essential for circadian rhythms, no single plant photoreceptor family is
71 required for the generation or sustainment of circadian rhythms (Devlin & Kay, 2000, Millar,
72 2004). For a detailed review of the role of photoreceptors in mediating entrainment of the
73 oscillator see (Oakenfull & Davis, 2017).
74
75 The intersection between light and circadian signalling mostly occurs in the nucleus (Herrero
76 & Davis, 2012). The nucleus is the site within the cell that is responsible for DNA replication,
77 transcription, ribosomal synthesis and RNA processing. The nucleus is a highly ordered
78 structure. Surrounding the nucleus is a double membrane nuclear envelope in which nuclear
79 pore complexes (NPCs) are embedded. The NPCs regulates the trafficking of proteins and
80 RNA from the nucleus to the cytoplasm (Kaiserli, Perrella & Davidson, 2018, Lamond &
81 Sleeman, 2003). Chromosomes typically packaged as chromatin are localised throughout
82 the nucleoplasm. In metazoans, each chromosome occupies a distinct space within the
83 nucleoplasm called chromosome territories (Lamond & Sleeman, 2003). In *Arabidopsis*
84 *thaliana* and the related *Arabidopsis lyrata*, chromosome territories are not observed and
85 chromatin is mostly randomly dispersed (Berr, Pecinka, Meister, Kreth, Fuchs, Blattner,
86 Lysak & Schubert, 2006, Berr & Schubert, 2007, Pecinka, Schubert, Meister, Kreth, Klatte,
87 Lysak, Fuchs & Schubert, 2004). The nucleus also contains a series of substructures called
88 nuclear bodies (Lamond & Sleeman, 2003). The formation of these subnuclear structures
89 are proposed to promote and enhance protein activity by condensing proteins, DNA and
90 RNA together (Matera, Izaguire-Sierra, Praveen & Rajendra, 2009). Some of these nuclear
91 bodies are conserved throughout eukaryotic nuclei. These include the nucleolus, cajal
92 bodies and speckles, which mediates ribosome synthesis, RNA processing and splicing
93 respectively. However, some of the nuclear substructures are kingdom specific. For
94 example, the plant nucleus contains photobodies, while the animal nucleus contains
95 promyelocytic leukemia protein (PML) bodies (Kaiserli *et al.*, 2018, Lamond & Sleeman,
96 2003).
97
98 In recent years, our understanding of the importance of subnuclear structures in mediating
99 downstream photoreceptor signalling activity has increased. In this review we will focus on
100 how photoreceptors localise to the nucleus and the mechanisms regulating their association
101 to subnuclear structures. We will also highlight the roles nuclear bodies have in facilitating
102 photoreceptor activity, including the signalling from photoreceptors to the circadian clock.

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

105

106 **Red and Far-Red Light**

107 The signalling of RL and FRL to the circadian clock occurs primarily through phys. In
108 Arabidopsis, there are five phys: the light liable phyA, and the light stable phyB-E (Clack,
109 Mathews & Sharrock, 1994). All phys are composed of a N-terminus photosensory domain
110 that is covalently attached to a tetrapyrrole bilin chromophore and a C-terminal region
111 required for downstream signalling and photobody formation (Rockwell, Su & Lagarias,
112 2006). Aside from phyC, all phys can associate as homodimers and the light stable phys can
113 also form heterodimers. A pulse of red light promotes the conversion from the Pr to the Pfr
114 conformer, while a pulse of FRL converts Pfr back to Pr (Rockwell *et al.*, 2006). Additionally,
115 temperature and prolonged darkness can promote the conversion of Pfr to Pr (Legris, Klose,
116 Burgie, Rojas, Neme, Hiltbrunner, Wigge, Schafer, Vierstra & Casal, 2016, Rockwell *et al.*,
117 2006).

118

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

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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).

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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

174 affected by these transient changes in light quality. Warm temperatures (27ºC) also repress
175 photobody formation by promoting the conversion of Pfr phyB to Pr phyB (Legris *et al.*,
176 2016).
177
178 Alongside environmental factors, proteins that co-localise with phyB in photobodies regulate
179 photobody formation. The first of these proteins to be characterised was HEMERA (HMR,
180 also known as pTAC12), a protein that functions in the nucleus and chloroplast (Chen,
181 Galvão, Li, Burger, Bugea, Bolado & Chory, 2010). In the absence of HMR, phyB either fails
182 to form photobodies or can only localise to small photobodies (Chen *et al.*, 2010, Qiu, Li,
183 Kim, Moore & Chen, 2019). PHOTOPERIODIC CONTROL OF HYPOCOTYL1 (PCH1) and
184 its homolog PCHL also regulate phyB photobody morphogenesis (Huang, Yoo, Bindbeutel,
185 Goldsworthy, Tielking, Alvarez, Naldrett, Evans, Chen & Nusinow, 2016). Unlike the *hmr*
186 mutant, phyB can still localise to large photobodies in the *pch1* background albeit at a
187 slightly reduced level. However, these large photobodies are less stable than in WT and
188 disassemble more rapidly in the dark (Huang *et al.*, 2016). This effect is further enhanced in
189 the *pch1/pchl* double mutant (Enderle, Sheerin, Paik, Kathare, Schwenk, Klose, Ulbrich, Huq
190 & Hiltbrunner, 2017, Huang *et al.*, 2016). Interestingly, HMR and PCH1 have both been
191 recently shown to be required for the temperature sensing role of phyB, indicating that the
192 function of photobodies may extend beyond light signalling (Huang, McLoughlin, Sorkin,
193 Burgie, Bindbeutel, Vierstra & Nusinow, 2019, Qiu *et al.*, 2019).
194
195 The importance of photobodies in phyB signalling has been debated since their discovery.
196 Currently, photobodies are thought to possess multiple non-mutually exclusive functions
197 (Figure 2). Firstly, photobodies may act as storage sites of Pfr phyB that preserve or stabilise
198 PFr phyB from converting back to the Pr state (Van Buskirk *et al.*, 2014) (Figure 2A). This
199 process is supported by the association of PCH1/PCHL to phyB within photobodies (Enderle
200 *et al.*, 2017, Huang *et al.*, 2019, Huang *et al.*, 2016). Secondly, photobodies are required for
201 some aspects of phy signalling (Figure 2B-D). After a pulse of light, phyA and phyB
202 associate to transient photobodies along with PIF3 (Bauer *et al.*, 2004). The localisation of
203 PIF3 to photobodies is associated with multi-site phosphorylation and subsequent
204 ubiquitination and degradation of PIF3 (Al-Sady, Ni, Kircher, Schafer & Quail, 2006, Dong,
205 Ni, Yu, Deng, Chen & Wei, 2017, Ni, Xu, Chalkley, Pham, Guan, Maltby, Burlingame, Wang
206 & Quail, 2013) (Figure 2B). Kinases that promote the phosphorylation of PIF3 co-localise
207 with PIF3 in nuclear foci, suggesting that phosphorylation may occur at photobodies (Ni, Xu,
208 González-Grandío, Chalkley, Huhmer, Burlingame, Wang & Quail, 2017). It is unclear
209 whether the ubiquitin machinery can also co-localise to photobodies. Other PIFs negatively

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210 regulated by phys are also phosphorylated prior to degradation but whether this occurs
211 within photobodies is unknown (Lorrain, Allen, Duek, Whitelam & Fankhauser, 2008).
212
213 Photobodies may also acts as sites to sequester or seclude proteins to inhibit their activity.
214 Both phyA and phyB can interact with SUPPRESSOR OF *phya-105 1* (SPA1) within nuclear
215 bodies in a light dependent manner (Lu, Zhou, Xu, Luo, Lian & Yang, 2015, Sheerin, Menon,
216 zur Oven-Krockhaus, Enderle, Zhu, Johnen, Schleifenbaum, Stierhof, Huq & Hiltbrunner,
217 2015) (Figure 2C). This interaction secludes SPA1 from interacting with CONSTITUTIVE
218 PHOTOMORPHOGENIC1 (COP1), inhibiting the ability of COP1 to promote the degradation
219 of transcriptional regulators that promote light signalling (Hoecker, 2017). Photobodies are
220 also sites of gene regulation. The transcription factor TANDEM ZINC-FINGER-PLUS3 (TZP)
221 co-localises to photobodies with phyB under RL to activate gene expression (Kaiserli, Paldi,
222 O'Donnell, Batalov, Pedmale, Nusinow, Kay & Chory, 2015) (Figure 2D). Other transcription
223 factors such as LONG AFTER FAR RED LIGHT1 (LAF1) and LONG HYPOCOTYL IN FAR-
224 RED1 (HFR1) also co-localises within photobodies (Ballesteros, Bolle, Lois, Moore, Vielle-
225 Calzada, Grossniklaus & Chua, 2001, Sheerin *et al.*, 2015). However, photobodies are
226 dispensable for phy signalling. The expression of a N-terminal fragment that fails to form
227 photobodies was sufficient in mediating phyB photosensory activity (Matsushita *et al.*, 2003).
228 Therefore, photobodies are important but may not be essential for phytochrome signalling.
229
230 The nuclear translocation of phys is essential for phy mediated entrainment of the oscillator
231 (Jones, Hu, Litthauer, Lagarias & Harmer, 2015). Phys have multiple entry points to the
232 oscillator at the transcriptional and post-translational level. phyB and phyA are both required
233 for red light mediated activation of *PRR9* and *CCA1* expression (Ito, Matsushika, Yamada,
234 Sato, Kato, Tabata, Yamashino & Mizuno, 2003, Rausenberger *et al.*, 2011, Wang & Tobin,
235 1998). phys also regulates the transcription of *ELF4*, although there are currently conflicting
236 reports on whether this is dependent on a RL or FRL signalling pathway (Li, Siddiqui, Teng,
237 Lin, Wan, Li, Lau, Ouyang, Dai, Wan, Devlin, Deng & Wang, 2011, Siddiqui, Khan, Rhodes
238 & Devlin, 2016). At the post-translational level, phyB physically interacts with ELF3, LUX,
239 CCA1, LHY, TOC1 and GI *in planta* (Yeom, Kim, Lim, Shin, Hong, Kim & Nam, 2014). The
240 interaction between phyB and ELF3 has been reported to stabilise ELF3, but separate work
241 has suggested that phyB could be repressing ELF3 function within the oscillator (Herrero *et*
242 *al.*, 2012, Kolmos, Herrero, Bujdoso, Millar, Toth, Gyula, Nagy & Davis, 2011, Nieto, Lopez-
243 Salmeron, Daviere & Prat, 2015). The outcome of the interaction between the other
244 circadian components and phyB remains unknown, but it has been proposed that some of
245 these proteins may facilitate the shuttling of phyB to the nucleus (Klose, Viczian, Kircher,
246 Schafer & Nagy, 2015).

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247
248 The role of photobodies in the entrainment of the oscillator has yet to be clearly established.
249 In the dark, the oscillations of most circadian genes rapidly dampen until they become
250 arrhythmic. However, the constitutively active allele of phyB (YHB) can maintain circadian
251 oscillations under constant darkness similar to what is observed under constant light (Jones
252 *et al.*, 2015). When this YHB allele is placed into the *pchl1* mutant background, YHB can no
253 longer form large photobodies and fails to sustain circadian rhythms in constant darkness
254 (Huang *et al.*, 2019). This would therefore suggest that photobodies are vital in the
255 entrainment of the oscillator. Supporting this, previous work has highlighted that under WL
256 the N-terminal fragment of phyB, which cannot form photobodies, is incapable of entraining
257 the oscillator (Palágyi, Terecskei, Adám, Kevei, Kircher, Mérai, Schäfer, Nagy & Kozma-
258 Bognár, 2010). However, this N-terminal fragment can sufficiently entrain the oscillator when
259 seedlings are entrained exclusively under RL. Therefore, photobodies might have a light-
260 dependent role in the entrainment of the oscillator and may act as points of convergence of
261 separate photoreceptor signalling pathways.
262
263 **Blue Light Signalling**
264 Blue light is transmitted to the oscillator through three classes of photoreceptors, LOV-
265 KELCH domain proteins, CRYs and phyA. As phyA has already been discussed, we will not
266 discuss it further. We also highlight the role of PHOTROPHINS (PHOTs) in controlling the
267 diurnal activity of photosystem II (Litthauer, Battle, Lawson & Jones, 2015). However, no role
268 for PHOT1 or PHOT2 has been described in the entrainment of nuclear circadian rhythms
269 (Litthauer, Battle & Jones, 2015) and therefore will not be discussed here.
270
271 LOV-KELCH
272 The LOV-KELCH domain family of protein has three members in Arabidopsis: ZTL, FLAVIN
273 BINDING KELCH REPEAT, F-BOX1 (FKF1) and LOV KELCH PROTEIN2 (LKP2).
274 ZTL/FKF1/LKP2 are composed of a N-terminal LOV domain, a F-box motif and six tandem
275 KELCH repeats (Ito, Song & Imaizumi, 2012). The LOV domain is required for blue light
276 perception and the interaction with GI, PRR5 and TOC1. The F-box domain regulates the
277 interaction with ARABIDOPSIS SKP1 LIKE (ASK1), a component of the SCF E3 ligase
278 complex (Han, Mason, Risseeuw, Crosby & Somers, 2004). The KELCH repeats provides a
279 further protein-protein interaction interface and also facilitates hetero-dimerisation of the
280 LOV-KELCH family (Ito *et al.*, 2012). The activity of ZTL is promoted by GI and HSP90 which
281 form a ternary chaperone complex to promote the maturation and stabilisation of ZTL (Cha,
282 Kim, Kim, Zeng, Wang, Lee, Kim & Somers, 2017). Similar post-translational mechanisms

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283 are thought to regulate FKF1, while it is unknown if LKP2 is post-translationally regulated by
284 HSP90/GI (Kim, Kim, Fujiwara, Kim, Cha, Park, Lee & Somers, 2011).
285
286 Within the circadian clock, ZTL, FKF1 and LKP2 redundantly promote the ubiquitination of
287 TOC1 and PRR5 through the SCF complex (Más *et al.*, 2003) (Baudry, Ito, Song, Strait,
288 Kiba, Lu, Henriques, Pruneda-Paz, Chua, Tobin, Kay & Imaizumi, 2010). Recently, ZTL was
289 shown to promote the ubiquitination of CCA1 HIKING EXPEDITION (CHE) (Lee, Feke, Li,
290 Adamchek, Webb, Pruneda-Paz, Bennett, Kay & Gendron, 2018, Sanchez & Kay), a
291 transcription factor that interacts with TOC1 to regulate *CCA1* expression (Pruneda-Paz,
292 Breton, Para & Kay, 2009). It is currently unknown whether FKF1 or LKP2 also promote
293 CHE degradation. ZTL also regulates circadian rhythms by sequestering GI to the cytoplasm
294 (Kim, Geng, Gallenstein & Somers, 2013a). Again, it is unknown if FKF1 or LKP2 can
295 sequester GI to the cytoplasm to suppress GI activity.
296
297 The activity of the LOV-KELCH domain family within the circadian clock is not thought to
298 occur within the nucleus. ZTL is exclusively localised to the cytoplasm, while FKF1 and
299 LKP2 are localised in the cytoplasm and nucleus (Zoltowski & Imaizumi, 2014). Within the
300 nucleus, LKP2 has been reported to co-localise to cajal bodies, while the sub-nuclear
301 localisation of FKF1 is not yet known (Fukamatsu, Mitsui, Yasuhara, Tokioka, Ihara, Fujita &
302 Kiyosue, 2005). However, the nuclear and sub-nuclear localisation of LKP2 and FKF1 is
303 unlikely to be important for the signalling of the LOV-KELCH family to the oscillator. Of ZTL,
304 FKF1 and LKP2, only *ztl* mutants have a circadian phenotype (Baudry *et al.,* 2010).
305 Therefore, the degradation of TOC1, PRR5 and CHE and any other circadian function of the
306 LOV-KELCH family is likely to be restricted to the cytoplasm.
307
308 <u>Cryptochromes</u>
309 In Arabidopsis there are three CRY genes: CRY1, CRY2 and CRY3. CRY3 is structurally
310 and functionally distinct from CRY1 and CRY2 and will not be discussed further (Yu, Liu,
311 Klejnot & Lin, 2010). CRY1 and CRY2 share a photosensory N-terminal domain that is non-
312 covalently bound to a flavin co-factor and a C-terminal effector domain (Yu *et al.*, 2010). The
313 C-terminal domain varies in size between CRY1 and CRY2, reflecting differences in
314 functional activity and the stability of the two proteins. CRY1 and CRY2 associate as
315 homodimers *in vivo* to facilitate their functional activity (Rosenfeldt, Viana, Mootz, von Arnim
316 & Batschauer, 2008, Wang, Wang, Han, Liu, Gu, Yang, Su, Liu, Zuo, He, Wang, Liu, Matsui,
317 Kim, Oka & Lin, 2017). There is no report of heterodimerisation between CRY1 and CRY2.
318

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319 CRY1 localises in the cytoplasm and nucleus to perform unique functions in the separate
320 compartments (Wu & Spalding, 2007, Yang, Wu, Tang, Liu, Liu & Cashmore, 2000). The
321 nucleocytoplasmic distribution of Arabidopsis CRY1 has also been observed in the rice
322 CRY1 and wheat CRY1a proteins, but no NLS has been identified in these proteins
323 (Matsumoto, Hirano, Iwasaki & Yamamoto, 2003, Xu, Xiang, Zhu, Xu, Zhang, Zhang, Zhang
324 & Ma, 2009). The N-terminus and C-terminus of the wheat and rice CRY1 orthologs are
325 intrinsically capable of localising to the nucleus, suggesting that multiple non-conventional
326 NLS signals may promote CRY1 localisation (Matsumoto *et al.*, 2003, Xu *et al.*, 2009). Rice
327 and Arabidopsis CRY1 also have a nuclear export signal (NES) in the N and C-terminus
328 respectively, while no NES has been identified in the wheat CRY1a ortholog (Matsumoto *et*
329 *al.*, 2003, Wu & Spalding, 2007, Xu *et al.*, 2009). In contrast to CRY1, CRY2 functions
330 exclusively in the nucleus before being degraded in a light dependent manner (Guo, Duong,
331 Ma & Lin, 1999, Yang *et al.*, 2000). The localisation of CRY2 to the nucleus is not dependent
332 on light and requires an NLS signal within the C-terminus (Guo *et al.*, 1999, Kleiner, Kircher,
333 Harter & Batschauer, 1999). Mutations within this NLS inhibit CRY2 nuclear localisation
334 (Zuo, Meng, Yu, Zhang, Feng, Sun, Liu & Lin, 2012). Once in the nucleus, Arabidopsis
335 CRY1 and CRY2 localises to nuclear bodies, which we will term cry-bodies to avoid
336 confusion with phy photobodies (although there is some overlap discussed below) (Gu,
337 Zhang & Yang, 2012, Yu, Sayegh, Maymon, Warpeha, Klejnot, Yang, Huang, Lee, Kaufman
338 & Lin, 2009). For CRY2, the formation of these cry-bodies occurs within 30 seconds of
339 exposure to blue light (Yu *et al.*, 2009). These number and size of the CRY2 cry-bodies is
340 also responsive to the intensity and length of BL exposure (Yu *et al.*, 2009). Recent work has
341 shown that BLUE-LIGHT INHIBITOR OF CRYPTOCHROME1 (BIC1) and its homolog BIC2
342 are negative regulators of CRY2 cry-body formation (Wang, Zuo, Wang, Gu, Yoshizumi,
343 Yang, Yang, Liu, Liu, Han, Kim, Liu, Wohlschlegel, Matsui, Oka & Lin, 2016). BIC1/2 directly
344 interact with CRY2 to inhibit CRY2 homodimerisation, suppressing the ability of CRY2's to
345 localise to cry-bodies (Wang *et al.*, 2016). It is unknown if similar mechanisms regulate
346 CRY1 cry-body formation.
347
348 The role of cry-bodies in CRY signalling is less established than with phys. CRY1 and CRY2
349 both localise with SPA1 within cry-bodies in a blue light dependent manner (Lian, He, Zhang,
350 Zhu, Zhang, Jia, Sun, Li & Yang, 2011, Zuo, Liu, Liu, Liu & Lin, 2011). The interaction
351 between CRY1 and SPA1 promotes the dissociation of SPA1 from COP1, suppressing
352 COP1 activity (Lian *et al.*, 2011) (Figure 3A). Separately the CRY2-SPA1 interaction results
353 in the association of COP1 to CRY2 to inhibit COP1 mediated degradation of CONSTANTS
354 (CO) (Zuo *et al.*, 2011). However, this association between SPA1-CRY2-COP1 also
355 promotes the degradation of CRY2 (Weidler, zur Oven-Krockhaus, Heunemann, Orth,

356 Schleifenbaum, Harter, Hoecker & Batschauer, 2012). The degradation of CRY2 is
357 dependent on its ability to associate to cry-bodies where it is phosphorylated prior to
358 degradation (Yu *et al.*, 2009) (Figure 3B). The degradation of CRY2 is promoted by phyA
359 and the SPA family, although it is unknown if phyA mediates this process by associating to
360 nuclear bodies with CRY2 and SPA (Weidler *et al.*, 2012). It has been recently shown that
361 the PPK kinases are responsible for CRY2 phosphorylation (Liu, Wang, Deng, Wang, Piao,
362 Cai, Li, Barshop, Yu, Zhou, Liu, Oka, Wohlschlegel, Zuo & Lin, 2017). In separate work,
363 these kinases were shown to with interact phyB to promote the phosphorylation of PIF3 (Ni
364 *et al.*, 2017). This paper reported that PIF3/PPK co-localises within nuclear bodies though
365 this remains to be confirmed. Therefore, PPKs could co-localise with SPA1 and phyA within
366 nuclear bodies to promote CRY2 degradation (Liu 2017). As with photobodies, cry-bodies
367 also act as sites for transcriptional regulation. CRY1 and CRY2 interacts with HBI1
368 (HOMOLOG OF BEE2 INTERACTING WITH IBH1) within cry-bodies to repress the
369 transcriptional activity of HBI1 (Wang, Li, Xu, Lian, Wang, Xu, Mao, Zhang & Yang, 2018)
370 (Figure 3C). Separately, CRY1 and CRY2 have also been shown to regulate the
371 transcriptional activity of PIFs and CRYPTOCRHOME-INTERACTING BASIC-HELIX-LOOP-
372 HELIX1 (CIB1) in the nucleus but it is unknown if they co-associate within nuclear bodies
373 (Liu, Yu, Li, Klejnot, Yang, Lisiero & Lin, 2008, Ma, Li, Guo, Chu, Fang, Yan, Noel & Liu,
374 2016, Pedmale, Huang, Zander, Cole, Hetzel, Ljung, Reis, Sridevi, Nito, Nery, Ecker &
375 Chory, 2016). Therefore, cry-bodies may have a similar function to photobodies in the
376 regulation of transcription and proteolytic degradation.
377
378 The mechanisms facilitating CRY-mediated entrainment of the oscillator and the cellular
379 location of this activity has remained unclear. In more recent work it was revealed that
380 ELOGNATED HYPOCOTYL5 (HY5) and its homolog HY5-HOMOLOG (HYH) are a key
381 signal integrator for BL-mediated entrainment of the oscillator (Hajdu, Dobos, Domijan,
382 Balint, Nagy, Nagy & Kozma-Bognar, 2018) (Figure 3D). HY5/HYH is a transcription factor
383 that acts as hub in the transduction of light signals (Gangappa & Botto, 2016). HY5 was
384 shown to associate to the promoter of most clock genes *in vivo* and this is association was
385 enhanced by BL and to a lesser extent by RL (Hajdu *et al.*, 2018). HY5 directly regulates the
386 expression of *PRR5*, *LUX* and the *LUX* sister gene *BOA* (BROTHER OF LUX ARRYTHMO)
387 and is predicted to also regulate CCA1 post-translationally. HY5 has also been separately
388 shown to promote the expression of *ELF4* through the transcription factors FAR-RED
389 ELONGATED HYPOCOTYL3 (FHY3) and FAR-RED-IMPAIRED RESPONSE (FAR1) (Li *et*
390 *al.*, 2011). HY5/HYH associates to nuclear bodies in a COP1 dependent manner and this
391 results in the proteolytic degradation of HY5/HYH (Ang, Chattopadhyay, Wei, Oyama,
392 Okada, Batschauer & Deng, 1998). COP1 mediated degradation of HY5 is suppressed by

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393 the light dependent association of CRYs and phys to these nuclear bodies (Lian *et al.*, 2011,
394 Sheerin *et al.*, 2015, Wang, Ma, Li, Zhao & Deng, 2001, Zuo *et al.*, 2011). Separate work
395 has shown that CRY2 can associate with phyB in photobodies to regulate the pace of the
396 oscillator (Más, Devlin, Panda & Kay, 2000). Therefore, CRY2 and phyB may co-localise
397 within nuclear bodies to inhibit COP1 mediated degradation of HY5 to facilitate the
398 entrainment of the oscillator. Such a mechanism would explain why the phyB N-terminal
399 mutants fail to entrain the oscillator under WL, as this construct would be incapable of
400 associating into nuclear bodies with CRY2 to promote HY5 stability (Palágyi *et al.*, 2010).
401

402 **UV-B**

403 So far, the sole UV-B receptor uncovered in plants is UVR8. In the absence of UV-B, UVR8
404 is localised to the cytoplasm as an inactive homodimer maintained by a salt-bridge
405 interaction between two UVR8 monomers (Rizzini, Favory, Cloix, Faggionato, O'Hara,
406 Kaiserli, Baumeister, Schafer, Nagy, Jenkins & Ulm, 2011). Conserved tryptophan residues
407 within the UVR8 protein serve as a chromophore for UV-B. The perception of UV-B light
408 weakens the salt bridge interaction, releasing monomeric UVR8 to interact with COP1
409 (Christie, Arvai, Baxter, Heilmann, Pratt, O'Hara, Kelly, Hothorn, Smith, Hitomi, Jenkins &
410 Getzoff, 2012, Rizzini *et al.*, 2011). In contrast to its traditional antagonistic role in red or blue
411 light signalling, COP1 is a positive factor in UV-B signalling and has a critical role in
412 facilitating UVR8 function (Oravecz *et al.,* 2006; Favory *et al.,* 2009). Once activated by UV-
413 B, the UVR8 monomers are rapidly reverted to their homodimeric ground state (Heijde &
414 Ulm, 2013, Heilmann & Jenkins, 2013). This process is promoted by two related proteins
415 REPRESSOR OF UV-B PHOTOMORPHOGENESIS1 (RUP1) and RUP2 (Heijde & Ulm,
416 2013).
417

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

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430 nuclear bodies (Favory, Stec, Gruber, Rizzini, Oravecz, Funk, Albert, Cloix, Jenkins,
431 Oakeley, Seidlitz, Nagy & Ulm, 2009). However, separate work in Arabidopsis and more
432 recent work in a Tobacco failed to identify UVR8 nuclear bodies (Kaiserli & Jenkins, 2007,
433 Yang, Liang, Zhang, Shao, Gu, Shang, Shi, Li, Zhang & Liu, 2018).
434
435 The HY5 TF has a critical role in facilitating UV-B signalling downstream of UVR8. The
436 expression of *HY5* and its homolog *HYH* is induced in response to UV-B light in a
437 UVR8/COP1 dependent manner (Binkert, Kozma-Bognár, Terecskei, De Veylder, Nagy &
438 Ulm, 2014, Oravecz, Baumann, Máté, Brzezinska, Molinier, Oakeley, Adám, Schäfer, Nagy
439 & Ulm, 2006). HY5 is required to regulate the expression of genes responsive to UV-B light
440 and mutations in *hy5* result in plants becoming hypersensitive to UV-B (Oravecz *et al.*, 2006,
441 Ulm, Baumann, Oravecz, Mate, Adam, Oakeley, Schafer & Nagy, 2004). However, it is
442 unclear how UVR8/COP1 signals to HY5. Originally, UVR8 was proposed to associate to
443 the promoter of *HY5* and promote *HY5* expression (Brown, Cloix, Jiang, Kaiserli, Herzyk,
444 Kliebenstein & Jenkins, 2005), but recent work has questioned the ability of UVR8 to bind to
445 chromatin (Binkert, Crocco, Ekundayo, Lau, Raffelberg, Tilbrook, Yin, Chappuis, Schalch &
446 Ulm, 2016). UVR8 can indirectly promote the expression of *HY5* by inhibiting the repressive
447 effect of WRKY DNA BINDING PROTEIN36 (WRKY36) on *HY5* expression (Yang *et al.*,
448 2018). UVR8 also promotes HY5 activity by enhancing HY5 stability through interactions
449 with COP1 and SPA proteins (Huang, Ouyang, Yang, Lau, Chen, Wei & Deng, 2013). The
450 mechanisms for how UV-B signals to the oscillator is unknown. UV-B induces the expression
451 of *CCA1* and *LHY*, but this is not dependent on HY5 or HYH (Feher, Kozma-Bognar, Kevei,
452 Hajdu, Binkert, Davis, Schafer, Ulm & Nagy, 2011). This study did highlight a role for UVR8
453 and COP1 in UV-B mediated entrainment of the oscillator, but the downstream targets of
454 UVR8/COP1 and whether this is a transcriptional or post-translational effect remains
455 unknown.
456
457 **Circadian Nuclear Dynamics**
458 So far, we have only discussed the nuclear and subnuclear dynamics of photoreceptors and
459 how these might influence circadian clock. In this section we will summarise recent
460 advances made in the understanding the nuclear dynamics of circadian components.
461
462 Excluding ZTL (discussed earlier), most of the known plant circadian-clock components are
463 transcription factors (TOC1, LUX, PRR5/7/9, CCA1 and LHY) or co-factors that aide
464 transcription factors (ELF4, GI and ELF3). Accordingly, all have been shown to display
465 nuclear localisation either in transient expression systems or in stable Arabidopsis lines
466 (Carré & Kim, 2002, Herrero *et al.*, 2012, Nakamichi *et al.*, 2005, Wang, Fujiwara & Somers,

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467 2010, Yakir, Hilman, Kron, Hassidim, Melamed-Book & Green, 2009). Of these components,
468 only the nuclear dynamics of CCA1, TOC1, PRR5, GI and ELF3 have so far been
469 characterised.
470
471 CCA1 intrinsically localises to the nucleus and this occurs rapidly upon translation (Yakir *et*
472 *al.*, 2009). The kinetics of CCA1 localisation does not changed in plants exposed to light or
473 kept in the dark, suggesting that CCA1 nuclear dynamics are not influenced by light (Yakir *et*
474 *al.*, 2009). However, the authors only used white light, so a red or blue light specific effect
475 cannot be ruled out. There was also no report of CCA1 localising to nuclear foci in this
476 report. TOC1 also intrinsically localises to the nucleus through a NLS in the C-terminus of
477 the protein (Wang *et al.*, 2010). TOC1 nuclear localisation is enhanced by PRR5 mediated
478 phosphorylation of TOC1. This effect is unique to PRR5; neither PRR3, PRR7 or PRR9 was
479 found to promote TOC1 phosphorylation or nuclear abundance (Wang *et al.*, 2010). PRR5
480 intrinsically localises to nuclear bodies, while TOC1 when expressed alone displays a diffuse
481 nuclear localisation. However, when *TOC1* and *PRR5* are co-expressed TOC1 co-localises
482 with PRR5 in nuclear bodies. It is unknown what role these nuclear bodies have in facilitating
483 TOC1 or PRR5 activity (Wang *et al.*, 2010).
484
485 ELF3 is a multifunctional scaffold protein that is divided into three regions termed the N, M
486 and C (Liu, Covington, Fankhauser, Chory & Wagner, 2001) (Figure 4A). In Arabidopsis,
487 ELF3 contains a NLS signal within the C-terminus and accordingly fragments of ELF3-C
488 intrinsically localise to the nucleus. However, fragments expressing the ELF3-M region
489 without an NLS are still capable of localising to the nucleus albeit more weakly (Herrero *et*
490 *al.*, 2012). The recruitment of ELF3-M to the nucleus is promoted by ELF4, an unrelated
491 protein that directly binds to the middle domain of ELF3 (Herrero *et al.*, 2012). When ELF4
492 and ELF3-M are co-expressed in transient or stable Arabidopsis lines the nuclear pool of
493 ELF3-M increases (Herrero *et al.*, 2012). In accordance with ELF4 promoting the nuclear
494 localisation of ELF3, mutations/natural-variants within the ELF4 binding site of ELF3 cause a
495 reduction in the nuclear accumulation of ELF3 (Anwer, Boikoglou, Herrero, Hallstein, Davis,
496 Velikkakam James, Nagy & Davis, 2014, Kolmos *et al.*, 2011).
497
498 How ELF4 promotes the nuclear accumulation of ELF3 is unknown. ELF4 intrinsically
499 localises to the nucleus (Herrero *et al.*, 2012), raising the possibility that ELF4 shuttles ELF3
500 to the nucleus like phyA/FYH/FHL and the proposed COP1/UVR8 shuttling mechanism, but
501 this remains to be confirmed. In the nucleus ELF3 can associate to nuclear bodies called foci
502 (Figure 4B). In transient systems ELF4 co-localises with ELF3 within foci, but this
503 colocalisation is not confirmed for Arabidopsis (Herrero *et al.*, 2012). The dynamics

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504 regulating ELF3 foci formation is still unclear. ELF4 has been proposed to promote ELF3 foci
505 formation as ELF3 allelic variants with weaker ELF4 binding are reported to produce fewer
506 foci (Anwer *et al.*, 2014). However, foci formation may not solely be regulated by ELF4. In
507 the absence of the N-terminus, ELF3 can still localise to the nucleus but does not form foci
508 (Herrero *et al.*, 2012) (Figure 4B). The N-terminus mediates the binding of phyB to ELF3,
509 suggesting that phyB may also promote ELF3 foci formation (Liu *et al.*, 2001). Supporting
510 this, recent work has revealed that ELF3 co-localises with TZP within nuclear bodies.
511 (Kaiserli *et al.*, 2015). The formation of TZP nuclear bodies occurs in a phyB red light
512 dependent manner and is associated with transcriptional activity. Separately, the C-terminal
513 fragment of ELF3 which cannot interact with ELF4 or phyB exclusively localises to large
514 nuclear bodies (Herrero *et al.*, 2012) (Figure 4B). However, as the ELF3C fragment fails to
515 recapture any of the *elf3* loss of function mutant phenotype these foci are not thought to be
516 functional and instead could be protein aggregates (Herrero, 2012 #22). The function of the
517 foci formed by ELF3F remains unknown.
518
519 GI also forms nuclear bodies. The formation of these nuclear bodies is under diurnal control,
520 with peak accumulation of nuclear bodies occurring at or just after dusk in long-day
521 photoperiods (Kim, Lim, Yeom, Kim, Kim, Wang, Kim, Somers & Nam, 2013b). The diurnal
522 accumulation of GI foci is dependent on ELF4. In *elf4* mutants, GI foci formation is strongly
523 reduced and is instead localised diffusely within the nucleus. The foci of GI did not co-
524 localise with markers of chromatin, DNA, the spliceosome or cajal bodies in Arabidopsis
525 nuclei, suggesting these foci facilitate a function independent of these processes (Kim *et al.*,
526 2013b). Previous work in transient systems suggested that GI associated to nuclear bodies
527 with COP1 and ELF3 and that this facilitated the proteolytic degradation of GI and ELF3 (Yu,
528 Rubio, Lee, Bai, Lee, Kim, Liu, Zhang, Irigoyen, Sullivan, Zhang, Lee, Xie, Paek & Deng,
529 2008). Separate work showed that ELF4 recruits GI to nuclear bodies to sequester GI from
530 binding to the CO promoter (Kim *et al.*, 2013b). Therefore, the nuclear bodies of GI are likely
531 to be antagonistic to GI function. It is unknown if GI, ELF4, ELF3 and COP1 all co-localise
532 within the same bodies at the same time.
533

534 **Concluding Remarks and Perspectives**

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

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541 transcriptional regulation, catalysing the initial stages of protein degradation and
542 sequestering proteins (Figure 2, 3). Photobodies have been shown to be highly responsive
543 to environmental stimuli, with light quality and quantity, and temperature all influencing the
544 formation and morphology of these structures. The formation of photobodies and cry-bodies
545 is also regulated internally by proteins, which interact and co-localise with phys and crys
546 (Chen *et al.*, 2003, Huang *et al.*, 2019, Legris *et al.*, 2016, Qiu *et al.*, 2019, Xu *et al.*, 2009,
547 Yu *et al.*, 2009). Together, this suggest that photobodies/cry-bodies may act as a central
548 processing unit within the cell where external stimuli and internal factors are integrated
549 together to facilitate among other processes photomorphogenesis, thermomorphogenesis
550 and flowering time. Whether internal signals such as photosynthates or hormones can also
551 be integrated into these central processing units by regulating the size, morphology or
552 function of these photobodies/cry-bodies remains to be seen.
553
554 In contrast to light signalling, our understanding of the sub-nuclear dynamics of circadian
555 signalling is still largely in the dark. Though most known components of the circadian clock
556 localise to the nucleus, so far only the nuclear dynamics of CCA1, GI, TOC1 and ELF3 have
557 been investigated to some degree. Of those four, GI, TOC1 (with PRR5) and ELF3 have
558 been described to form subnuclear structures. However, the mechanisms regulating the
559 formation of these subnuclear structures and the influences of these subnuclear structures
560 on circadian rhythms are largely unknown. The localisation of ELF3 to sub-nuclear structures
561 is associated with an increased repressive effect on circadian period (*i.e* period lengthens),
562 but it is unknown how these sub-nuclear structures aide ELF3 repressive activity (Herrero *et*
563 *al.*, 2012, Nieto *et al.*, 2015). ELF3 co-localises to nuclear bodies with ELF4, suggesting that
564 these foci could be sites of transcriptional activity. However, LUX, the TF component of the
565 EC, has not yet been shown to co-localise with ELF3 or ELF4 in foci (Herrero *et al.*, 2012).
566 Separately, ELF3 co-localises with GI and COP1 in nuclear bodies to facilitate the
567 degradation of GI (Yu *et al.*, 2008). Whether ELF3 forms different species of nuclear bodies
568 that are regulated in a spatio-temporal fashion, or if these foci are like photobodies/cry-
569 bodies and perform multiple independent functions is yet to be investigated. In contrast to
570 the positive effect of foci on ELF3 activity, the localisation of GI to nuclear bodies has been
571 proposed to repress GI function, while the role of nuclear bodies in TOC1/PRR5 activity
572 remains unclear. Further work is needed to understand how the nuclear and sub-nuclear
573 dynamics of the circadian components influence the parameters of the circadian clock.
574
575 The crosstalk between light and the circadian clock is critical for the entrainment of the plant
576 circadian oscillator. In Arabidopsis this is not exclusively a nuclear event, but the nucleus is a
577 key site for the intersection between photoreceptors and the circadian clock. Emerging

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

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590 **Figures**

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

602

603 Figure 2: Photobodies perform multiple functions in phytochrome signalling. (A) Photobodies
604 act as a storage site for phytochromeB (phyB) in the biologically active Pfr conformer to
605 protect against thermal reversion. The formation of these photobodies are promoted
606 independently by HEMERA (HMR) and PHOTOPERIODIC CONTROL OF HYPOCOTYL1
607 (PCH1). (B) Photobodies are also sites for the degradation of PHYTOCHROME
608 INTERACTING FACTOR3 (PIF3). phyB, PIF3 and PROTEIN PHOSPHATE KINASE (PPK)
609 co-localise within photobodies, resulting in the phosphorylation of PIF3. PIF3 is subsequently
610 ubiquitinated and degraded. (C) phyA and phyB co-localises with SUPPRESSOR OF PHYA-
611 1 (SPA1) within photobodies to seclude SPA1 from COP1, suppressing COP1 activity. (D)
612 Photobodies are sites of transcriptional activity. The transcription factor TANDEM ZINC-
613 FINGER PLUS3 (TZP) co-localises with phyB within photobodies and this co-localisation is
614 associated with transcriptional activity.

615

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

625

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

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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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643

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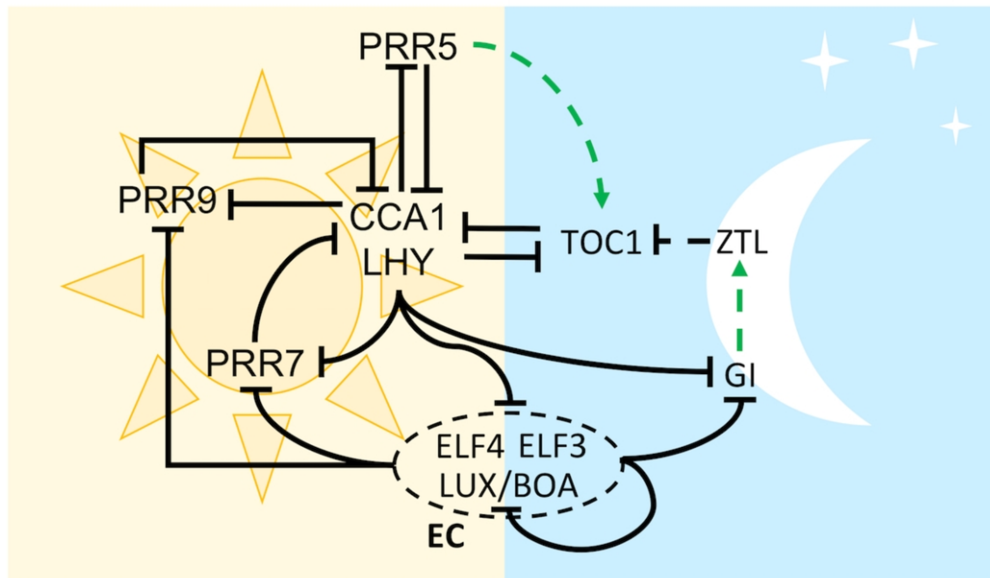

Figure 1: The current model of the Arabidopsis circadian clock.

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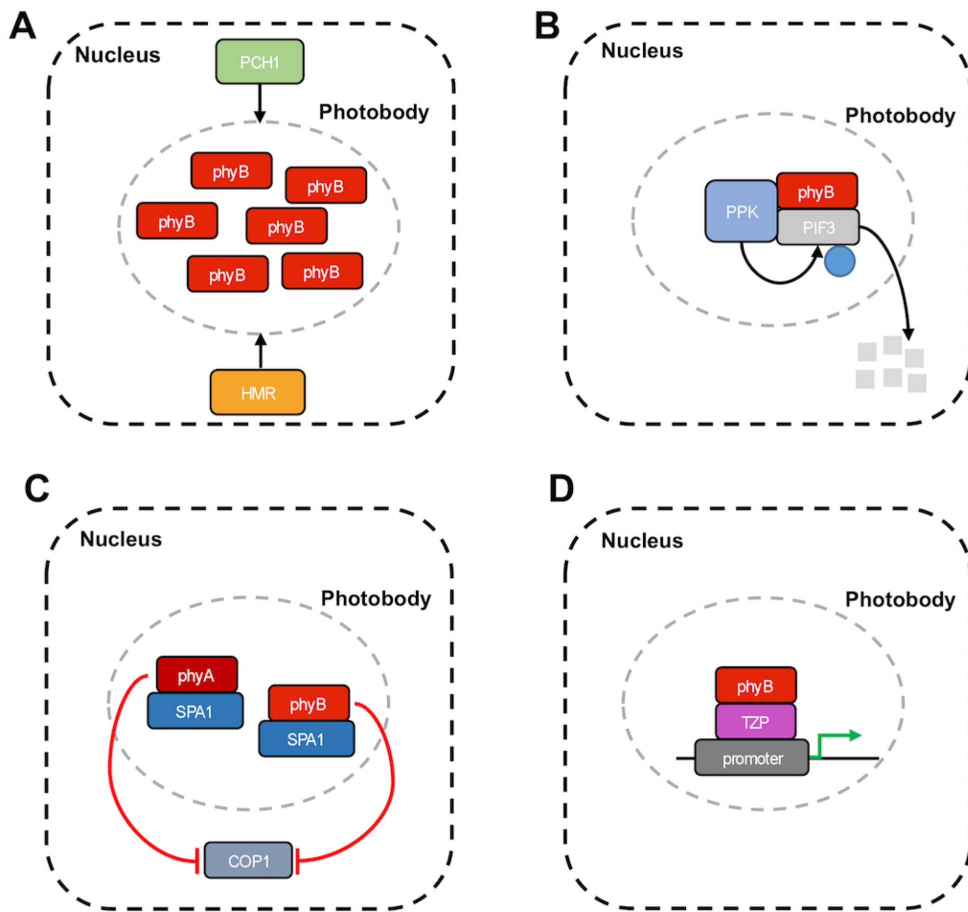

Figure 2: Photobodies perform multiple functions in phytochrome signalling.

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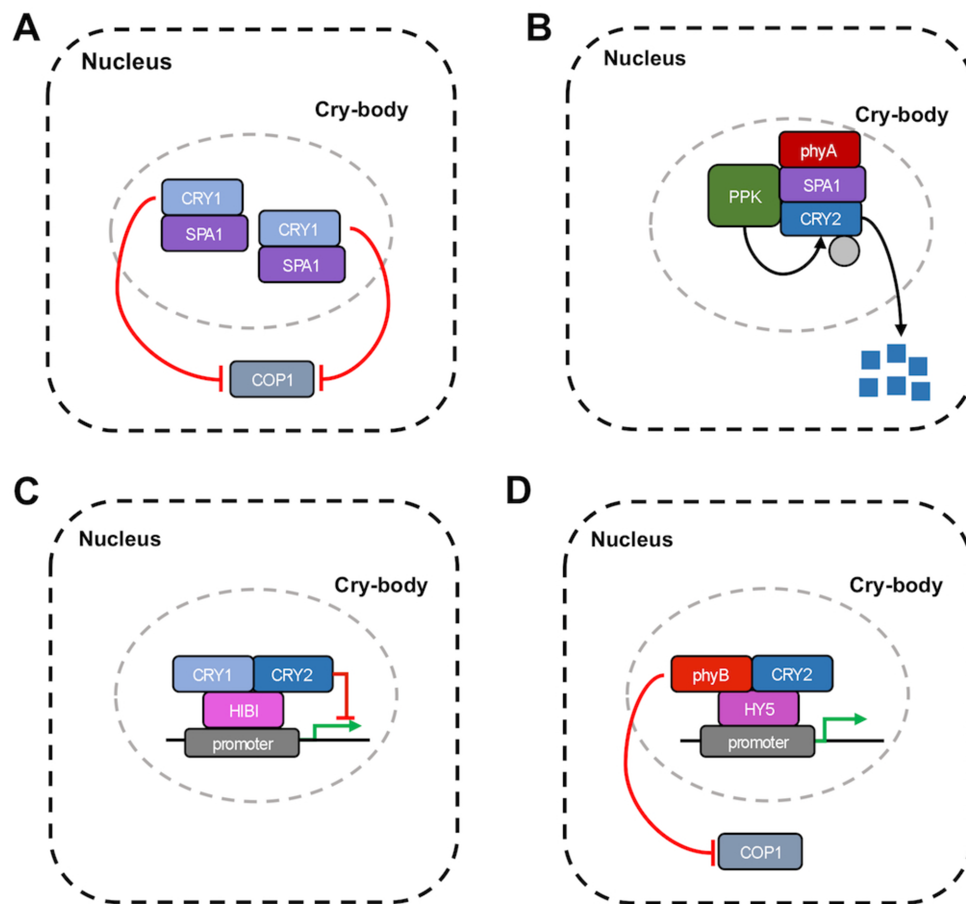

Figure 3: Nuclear bodies perform multiple functions in cry signalling.

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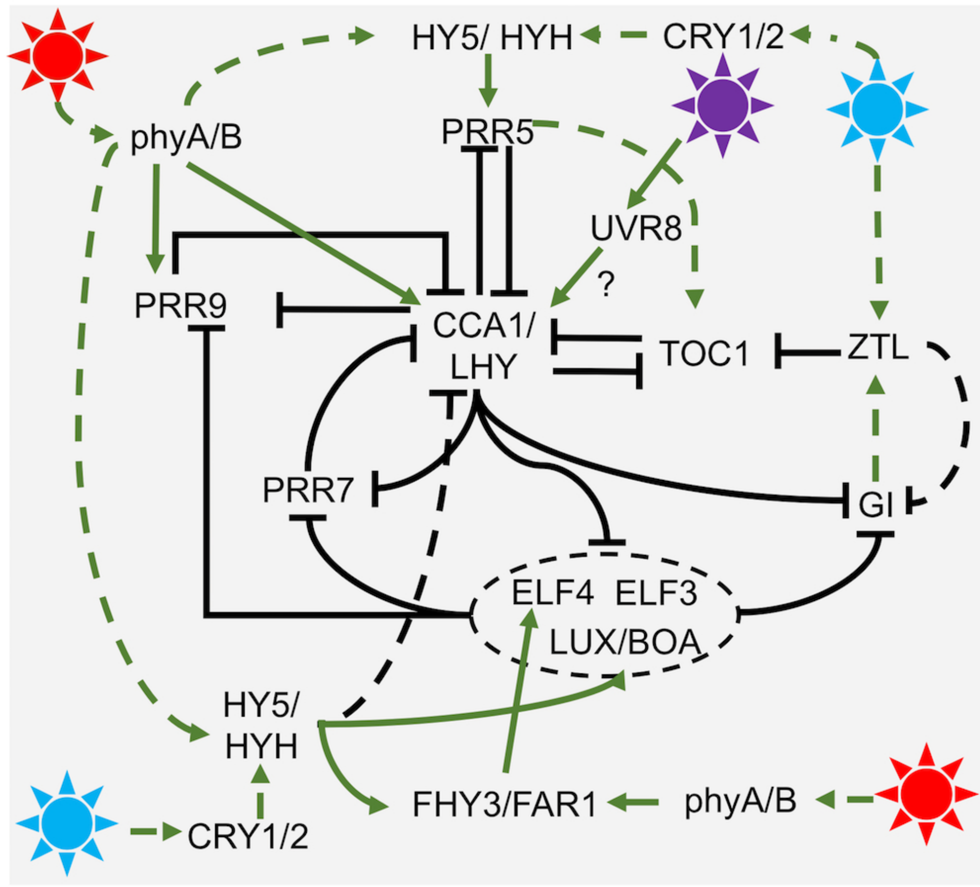

Figure 4: Light has multiple entry points to the plant circadian oscillator.

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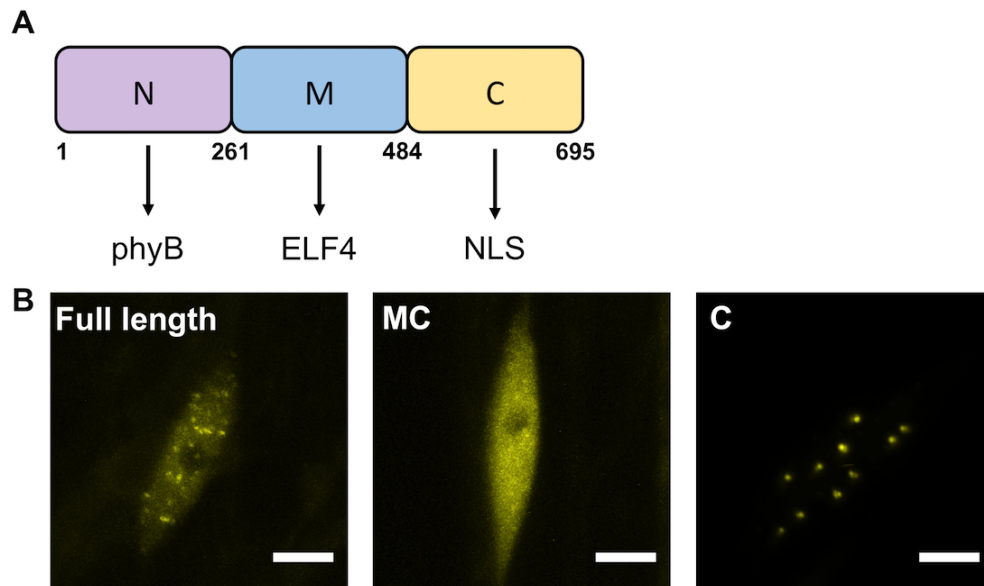

Figure 5: ELF3 fragments have different sub-nuclear structures.

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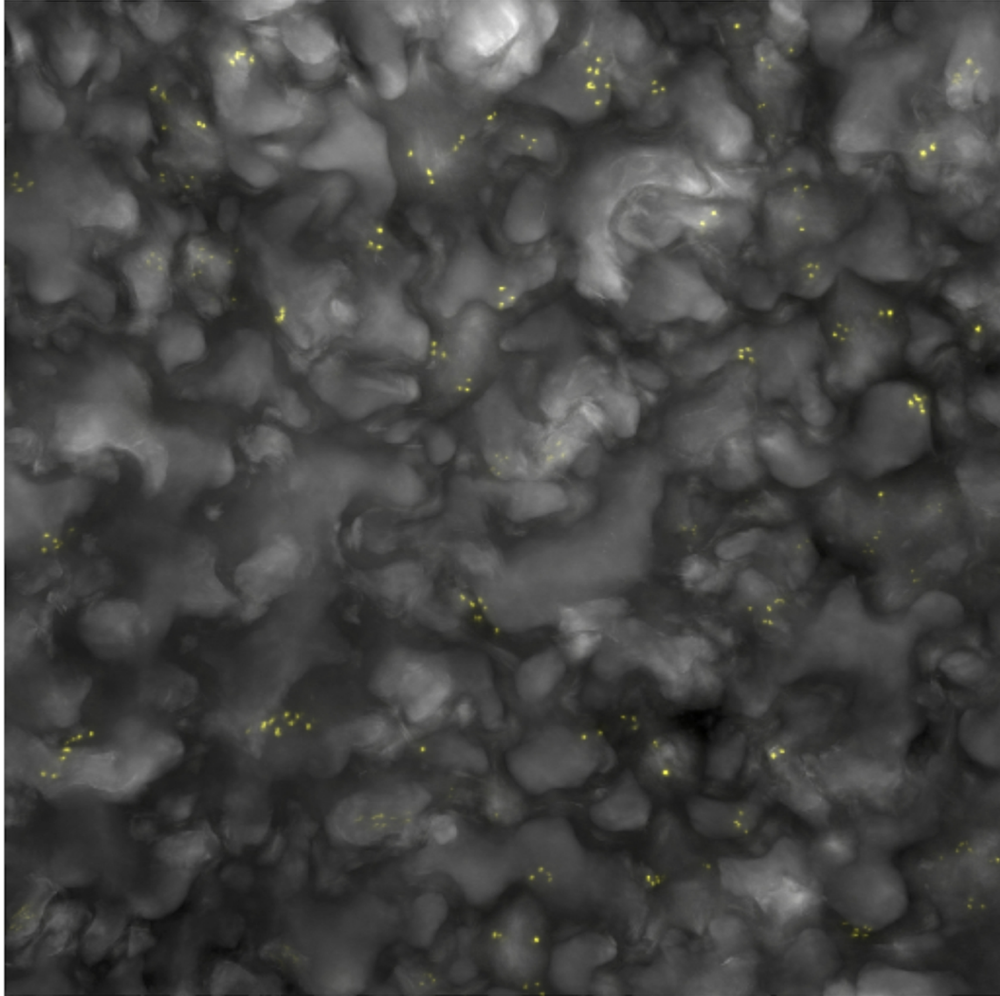

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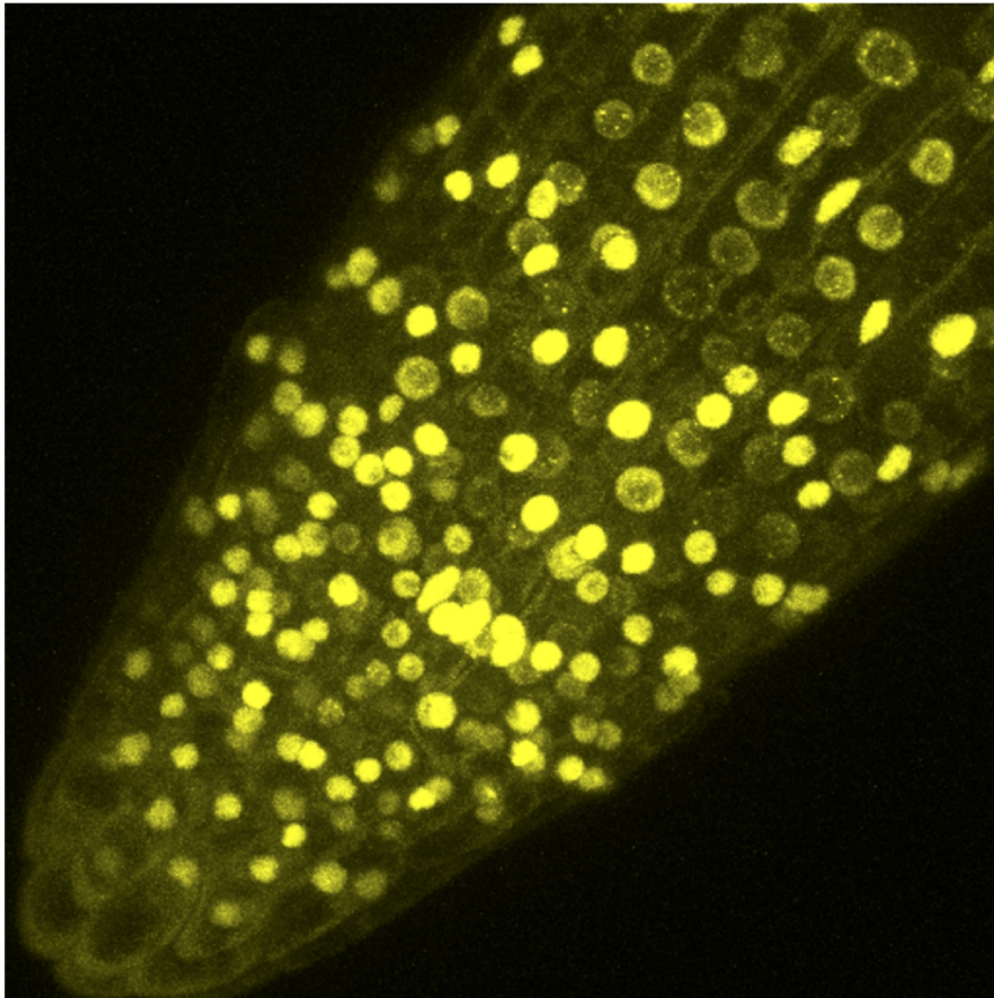

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