Light-induced protein condensation regulates chlorophyll homeostasis

Here we show that photoexcited blue light receptor cryptochrome 2 (CRY2) mediates blue light-induced liquid–liquid phase separation (LLPS) of CRY2–SPA1–FIO1 trimolecular complexes. This activates the N^6-methyladenosine (m^6A) writer FIONA1 (FIO1) to methylate mRNAs that encode chloroplast proteins, which are required for maintaining chlorophyll homeostasis and photosynthesis in response to light.

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The problem

Light regulates chlorophyll homeostasis and photosynthesis through various molecular mechanisms in plants. Although regulation of the transcription and protein stability of nuclear-encoded chloroplast proteins by light have been extensively studied, the effects of light on mRNA metabolism and the abundance of nuclear-encoded chloroplast proteins regulating chlorophyll homeostasis remain poorly understood.

m^6A is the most abundant internal modification of eukaryotic mRNAs that regulates mRNA splicing, nuclear export, degradation and translation[1]. In plants, m^6A methylation is catalysed by two evolutionarily conserved eukaryotic RNA methyltransferases, the m^6A writer complexes containing MTA or FIO1[2]. Cryptochromes (CRYs) are blue light receptors that regulate photomorphogenesis and photosynthesis in plants[3]. We have previously shown that blue light positively regulates mRNA methylation and that CRYs mediate blue light-responsive mRNA methylation through light-induced LLPS of the CRY2–MTA heterodimer[4,5]. However, it is not understood how CRYs regulate FIO1 to affect mRNA methylation, protein expression and photosynthesis.

The discovery

We discovered that the *Arabidopsis thaliana* mutant *fio1*, but not the *mta* mutant, exhibits a low-chlorophyll phenotype similar to that of the *cry1cry2* double mutant. Additional genetic analyses indicated that CRYs mediate light regulation of FIO1 to maintain chlorophyll homeostasis (Fig. 1a).

We analysed the transcriptomes, m^6A epitranscriptomes, translatomes and proteomes of wild-type plants, *cry1cry2* double mutants, and *fio1* and *mta* single mutants. However, we did not detect any chlorophyll synthesis enzymes (CSE) that show a CRY- and FIO1-dependent but MTA-independent change in both mRNA and protein expression that would explain the genotype-specific low-chlorophyll phenotypes. Instead, we identified six chlorophyll homeostasis regulator (CHR) genes that show a CRY- and FIO1-dependent but MTA-independent change in mRNA m^6A methylation, translation and protein abundance. Five of the six CHR genes identified in this study have been previously shown to be required for maintaining chlorophyll homeostasis under various conditions. We further showed that the CRY2 signalling protein SUPPRESSOR OF PHYTOCHROME A (SPA1) acts as a nuclear chaperone to facilitate light-induced LLPS of the CRY2–SPA1–FIO1 complex. Importantly, the intrinsically disordered CCE domain of CRY2 (which is required for light-induced LLPS of CRY2) and the WD domain of SPA1 (which interacts with both FIO1 and the CCE domain of CRY2) co-activate the m^6A methyltransferase activity of FIO1 in vitro.

These results are consistent with an epitranscriptomic mechanism that regulates chlorophyll homeostasis in response to light. According to this model, photoexcited CRY2 interacts with SPA1 to co-condense FIO1, forming CRY2–SPA1–FIO1 trimolecular condensates. CRYs and SPA1 co-activate FIO1 within these CRY2–SPA1–FIO1 condensates, facilitating deposition of m^6A markers and increased translation of *CHR* mRNAs required for maintaining chlorophyll homeostasis and photosynthesis in response to light (Fig. 1b).

The implications

Most known CRY signalling proteins, such as SPA1, show altered binding affinity to the CRY photoreceptor in response to blue light[3]. Other CRY signalling proteins, such as MTA, undergo light-induced LLPS without altering their binding affinity to the photoreceptor[4]. This study implies that the two seemingly distinct photoresponsive mechanisms may work together to mediate photoresponses in plants. Our study also implies that CRYs regulate essential metabolic pathways, such as chlorophyll homeostasis, by multiple mechanisms. For example, CRYs may regulate transcription of the CSE genes to directly affect chlorophyll synthesis through previously described mechanisms, such as the CRY2–SPA1–COP1-mediated light regulation of proteolysis[3]. In addition, CRYs may regulate *CHR* gene expression to indirectly affect chlorophyll metabolism by an epitranscriptomic mechanism, such as the CRY2–SPA1–FIO1-mediated light regulation of methylation and translation of *CHR* mRNAs.

In the future, we will investigate the exact biochemical mechanisms underlying the CRY2–MTA- and CRY2–SPA1–FIO1-mediated blue light regulation of mRNA methylation and protein expression to better understand how light regulates photosynthesis.

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EXPERT OPINION

"This exciting study uncovers a new role of CRY2 condensates in RNA biology, that is for m^{6}A RNA methylation in blue light. What's particularly novel is that the co-condensate formation of CRY2 with the m^{6}A writer FIO1 requires a third protein, SPA1, which is a component of a ubiquitin ligase complex. It will be interesting to resolve the biochemical mechanism of the CRY2–FIO1–SPA1 co-action in future research." **Ute Hoecker, University of Cologne, Germany.**

FIGURE

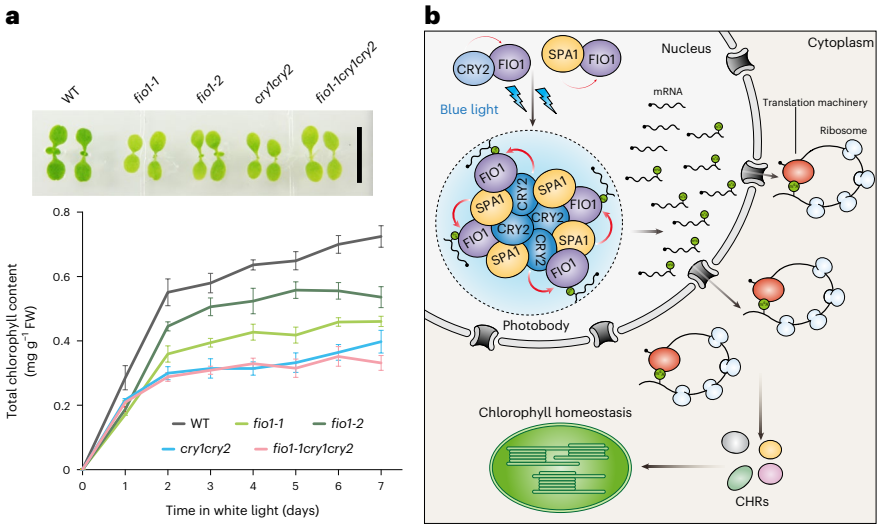

Fig. 1 | A regulatory mechanism of chlorophyll homeostasis. a, Seedling images and chlorophyll content of wild-type (WT) *A. thaliana* and mutants of the m^{6}A methyltransferase FIO1 and the cryptochromes CRY1 and CRY2 after six days in white light. FW, fresh weight. **b**, A diagram depicting blue light-induced LLPS of CRY2, the chaperone SPA1 and FIO1 in nuclear photobodies. This facilitates m^{6}A methylation (green circles on RNA molecules) and translation of mRNAs encoding CHRs to maintain chlorophyll homeostasis.

BEHIND THE PAPER

In 2017, one of the authors, Dr. Xu Wang, identified FIO1 as a CRY2-interacting protein in his systematic screen of CRY2-interacting proteins, prompting us to study the CRY2–FIO1 complex. However, our initial attempts failed to explain the genotype-specific low-chlorophyll phenotype of the *cry1cry2* and *fio1* mutants that was not present in the *mta* mutant. We initially hypothesized that light might induce co-condensation of the CRY2–FIO1 heterodimer to regulate mRNA methylation, stability and/or translation to alter abundance of CSE protein. Surprisingly, we did not identify an effect on *CSE* genes in our mutants that could explain the genotype-specific phenotypes, nor did we find the light-induced co-condensation of the CRY2–FIO1 heterodimer. In light of these 'failures', we performed additional experiments to test alternative hypotheses, which revealed CRY2–SPA1–FIO1-mediated LLPS that regulates expression of the *CHR* genes. **B.J. & C.L.**

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FROM THE EDITOR

"This study weaves together several important aspects of plant responses to external stimuli. The blue light photoreceptor CRY2 condensates in gel-like granules with the m^{6}A mRNA methylation modifier enzyme named FIO1. Together they control various genes linked with photosynthesis and through these, the plant's growth and development in response to light." **Guillaume Tena, Senior Editor, *Nature Plants*.**