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Phase separation at the heart of "heat sensing

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Phytochrome B is known as a receptor for both light and temperature signals. In this issue of *Molecular ell*, Chen et al. 2022) show how these two environmental signals are perceived distinctly by a single photoreceptor through liquid-liquid phase separation LLPS).

Phytochromes are red/far-red light (600–750 nm) photoreceptors universally present in plants and also present in fungal and bacterial kingdoms (Cheng et al., 2021). Phytochromes perceive not only light signals but also ambient temperature (Casal and Balasubramanian, 2019) and regulate a wide range of developmental programs throughout the life cycle of plants. In a pivotal study, Sakamoto and Nagatani changed the prevailing dogma of phytochrome function from a cytoplasmic origin to a nuclear localized signaling mechanism by showing that phyB is localized into nucleus in response to light (Sakamoto and Nagatani, 1996). A number of studies follow this discovery and show that all five *Arabidopsis* phytochromes translocate into the nucleus in response to light with varying kinetics and fluence rate specificity (Van Buskirk et al., 2012). Coinciding with these discoveries, the identification of nuclear localized signaling partners, especially phytochrome interacting factors (PIFs), firmly established the focus of the phytochrome signaling mechanism within the nucleus (Leivar and Quail, 2011). These early crucial reports showed not only nuclear localization of phytochromes and their signaling partners, but also the formation of the subnuclear foci "speckles later termed "photobodies (PBs) (Van Buskirk et al., 2012). PBs have been extensively characterized in plant cells, including kinetic analysis, studies of fluence rate-dependent dynamic behavior, fluorescence recovery after photobleaching (FRAP) assays to show liquid-like behavior, and even studies of PB formation in heterologous systems in a fragmented manner. However, the paper by Chen et al. (2022) takes the PB research to a new height by extensively characterizing the biophysical properties of PBs and domains necessary for PB formation, demonstrating PB formation in a heterologous system (HEK293T cells), and characterizing both light- and temperature-dependent PB dynamics and phyB oligomerization.

What sets this paper apart from previous stories are the following: Chen et al. firmly established that phyB PBs are liquid-liquid phase-separated (LLPS) droplets by characterizing the biophysical properties, including spherical behavior, fusion properties, and fluidity of phyB-GFP by FRAP assays, and even by calculating the approximate volume of PBs and the number of phyB molecules in each PB (Chen et al., 2022). They performed all of these studies in both *Arabidopsis* and a heterologous (HEK293T) system. Their structure-function analyses show that the N-terminal extension (NTE) is an intrinsically disordered region (IDR) and that the C-terminal output module (phyBC/OPM) is an oligomerization domain. The NTE is sufficient to drive LLPS of a fusion protein, NTE-mCherry-CRY2, under blue light, while the phyBC can spontaneously oligomerize even in the absence of the photosensory module (PSM). Moreover, both NTE and phyBC are necessary for LLPS of phyB. Taken together, these data suggest that phyB PBs display all the hallmark characteristics of LLPS.

Whether PBs are simply a storage site for phyB or a "signaling hub has been extensively debated in the field, mainly due to conflicting data correlating the functional significance of PBs, or lack thereof, with biological activity of phyB (Van Buskirk et al., 2012). One important photobiological contribution from a pioneer, Harry Smith, highlights the importance of PB formation. Smith and Jackson showed that photoconversion of phyB to the Pfr (biologically active) form is saturated at 3,000 $\mu molm^{-2}$ of total red light (Smith and Jackson, 1987). Yet the hypocotyl lengths of *Arabidopsis* seedlings continue to reduce over a broad range of red light, as shown in Figure 1 (Van Buskirk et al., 2012). Thus, phyB activity is proportional to the higher fluence rates of red light where PBs are formed, suggesting that PB formation is enhancing the phyB function beyond 3,000 $\mu molm^{-2}$. Chen et al. provide experimental evidence in support of this hypothesis. They show that NTE phyB (deletion of N-terminal 90 amino acids) does not form PBs under red light and that the transgenic plants expressing NTE phyB failed to rescue the *phyB* phenotype under red light. Moreover, it is well established that phyB PBs colocalize with a host of signaling factors, including PIFs, as confirmed here (Figure 1) (Chen et al., 2022; Van Buskirk et al., 2012), supporting the hypothesis that PBs are "signaling hubs for phyB.

A paradigm-shifting contribution of this story is defining the mechanism of temperature perception by phyB. phyB has been shown to function as a temperature sensor and regulates plant development in response to high ambient temperature (Casal and Balasubramanian, 2019). phyB functions like a dimmable light switch where increasing the amount of red light turns on phyB by promoting the formation of higher amounts of the Pfr (biologically active) form. By contrast,

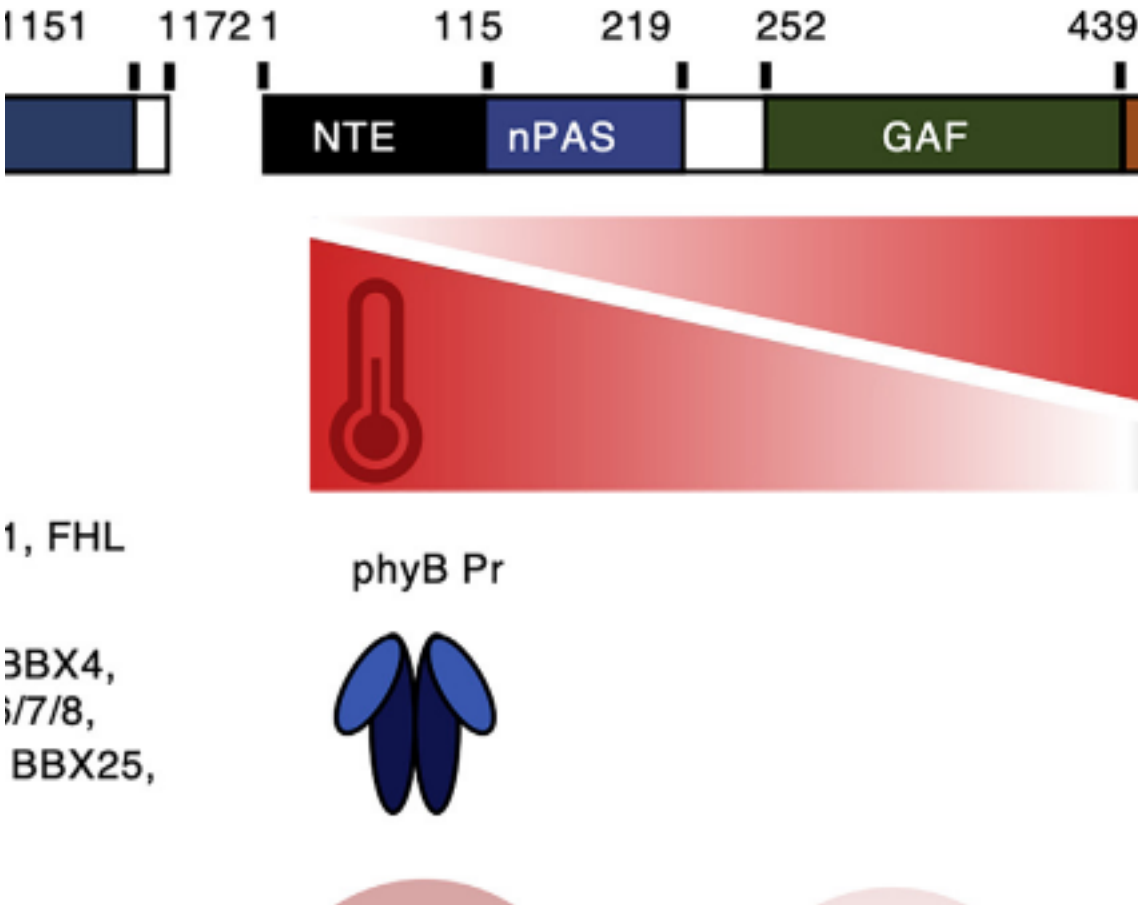

Figure 1. Liquid-liquid phase separation of phyB integrates light and temperature perception

Top: Domain structure of phyB containing the photosensory (PSM) and output module (OPM). Bottom left: phyB perceives light and temperature signals distinctly. Red light promotes photoconversion of phyB to the active Pfr form, promotes liquid-liquid phase separation of phyB into photobodies (PBs), and induces photomorphogenesis by shortening the length of the embryonic stems of seedlings. High ambient temperature perceived by the PBs through N-terminal extension (NTE) along with the PSM module reduces the size and number of PBs and/or completely dissolves them, resulting in increased length of the embryonic stem of seedlings. Bottom right: phyB interacts and colocalizes with a diverse class of signaling factors within PBs.

increasing the amount of far-red light turns off phyB by converting it into the Pr (biologically inactive) form. This later step of inactivation of phyB is thermally sensitive, where phyB Pfr can slowly convert to Pr in a thermal relaxation process (Klose et al., 2020). The prevailing understanding is that this thermal relaxation provides phyB the capacity to perceive ambient temperature. However, if this is the mechanism of temperature perception by phyB, how can phyB distinguish high ambient temperature from far-red light or shade conditions? The clue that the perception mechanisms might be different came from a recent study that showed that warm temperature and shade induce distinct PB dynamics (Hahm et al., 2020). Here, Chen et al. cleverly used the constitutively active phyB variant (Y276H point mutation, YHB) to show that the temperature signal is perceived directly through NTE to modulate the LLPS of phyB. The PBs of YHB, but not NTE YHB, sensitively respond to and can be reversibly regulated by temperature changes. NTE is necessary but not sufficient for temperature perception. NTE along with the PSM module together perceive the temperature signal. Thus, phyB directly senses and induces physiological responses to ambient temperature through the formation and disassembly of PBs.

While the PB field has come a long way, we still do not know whether the light-induced PB formation is an intrinsic property of phyB. *In vitro* assays for PB formation with purified phyB in a light-dependent manner are necessary to answer this question. phyB interacts and colocalizes with a diverse class of signaling partners, including transcription factors, kinases, E3 ubiquitin ligases, splicing factors, and others (Figure 1) (Cheng et al., 2021). Are all of these factors present in the same PB, or are there separate PBs for different processes? Although this story shows that PBs are the sites of temperature perception through NTE, there are conditions where phyB is in Pfr form without forming PBs. Does thermal relaxation of phyB Pfr form to Pr form also contribute to temperature perception under these conditions? In addition, perception of light and temperature in an organ/tissue-specific manner is still an open question.

Recently, a growing list of plant proteins, including the blue light photoreceptor

CRY2, early flowering 3 (ELF3), and others, have been shown to undergo LLPS (Emenecker et al., 2020; Wang et al., 2021). This story will contribute greatly to our understanding of how IDR-induced LLPS plays a central role in sensing and responding to environmental signals in plants.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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PYGBacking on glycogen metabolism to fuel early memory T cell recall responses

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Zhang et al. 2022) show that TCR signaling promotes the phosphorylation and activation of glycogen phosphorylase B PYGB) in CD8⁺ memory T Tmem) cells. PYGB-dependent glycogen mobilization provides a carbon source to support glycolysis and early Tmem cell recall responses.

Following antigen stimulation and appropriate co-stimulation, naive T cells become activated, expand, and differentiate into effector T (Teff) cells. Upon pathogen clearance, Teff cell populations contract, leaving behind a small population of long-lived, antigen-specific memory T (Tmem) cells with the capacity for rapid expansion upon re-infection. This dynamic behavior is facilitated by widespread changes in cellular metabolism, which is tightly linked to T cell function. Consistent with the metabolism of non-proliferating cells, resting naive and Tmem cells maintain low rates of glycolysis and predominantly derive their energy from oxidative phosphorylation. When stimulated by antigen, both naive and Tmem cells increase their uptake and metabolism of glucose; however, Tmem cells mount faster and stronger effector responses to antigen stimulation than naive T cells. Scientists have hypothesized that distinct metabolic programming of Tmem cells underlies their rapid recall ability. To this end, there is growing interest in characterizing not only the metabolic differences between T cells in different differentiation states, but also the dynamic changes in metabolism that drive transitions between these states. In this issue of *Molecular Cell*, Zhang et al. demonstrate that, in CD8⁺ Tmem cells, the initial energy requirements of the early recall response are supported in part by the mobilization of stored glycogen, which fuels a rapid glycolytic switch in response to antigen stimulation (Zhang et al., 2022) (Figure 1).

Gubser et al. previously described the importance of glycolysis for the acquisition of effector function in recalled CD8⁺ Tmem cells (Gubser et al., 2013); however, the different nutrients that could fuel this early and rapid glycolytic switch remained to be explored. In their recent study, Zhang et al. set out to address this gap in knowledge. To generate

