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Spotlight

Phase Separation as a Molecular Thermosensor

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How organisms sense temperature is a long-standing question. However, the identification of molecular thermosensors has been limited. Now, in a recent issue of *Nature*, Jung et al. demonstrate that phase separation of ELF3, a component of the circadian clock, acts as a thermosensor.

Temperature is a major environmental factor that affects every aspect of plant life. Molecular strategies have thus evolved to enable plants to register seasonal fluctuations in temperature and survive stressful temperature extremes. With respect to seasonal temperature, plants monitor daily peaks and troughs of temperature as well as longer-term averages (Zhao et al., 2020; Hepworth et al., 2018; Antoniou-Kourounioti et al., 2018). However, the molecular mechanisms that underpin temperature sensing are still largely unknown.

The circadian clock provides a mechanism for plants to align internal physiology with external environmental cues. At the hub of the circadian clock, an assembly of proteins called the evening complex (EC) directly coordinates gene expression with temperature information (Ezer et al., 2017). The evening complex is comprised of EARLY FLOWERING3 and EARLY FLOWERING4 (ELF3 and ELF4) and LUX ARRYTHMO (LUX). In a recent study in *Nature*, Jung et al. (2020) now address the molecular mechanism by which the EC mediates thermal responsiveness. When temperature increases, ELF3 phase separates to form molecular condensates in which ELF3 is inactive. This process can be reversed by decreasing temperature (Figure 1). These findings are exciting not only from the thermo-sensing perspective but also because they add to the growing realization that phase separation is a mechanism functionally relevant to many biological processes.

ELF3 contains a polyQ repeat, the length of which varies from 7 to 29 residues in natural *Arabidopsis* populations. Jung and colleagues first tested the association between polyQ length and ELF3 activity in response to higher temperature and found that this association was weak. In *Arabidopsis* ELF3, the polyQ repeat is embedded in a prion domain (PrD). This prompted the authors to hypothesize that regions surrounding the polyQ repeat may confer temperature responsiveness for ELF3. Indeed, they found that the PrD varies in size in plants that are adapted to different climates. In *Brachypodium distachyon*, a grass species adapted to warmer climates, ELF3 (BdELF3) does not contain a PrD, and in the potato *Solanum tuberosum*, which prefers moderate climates, ELF3 (StELF3) had a much smaller PrD than in *Arabidopsis*. The authors next sought to test the role of ELF3-PrD in temperature responsiveness. Overexpressing BdELF3 and StELF3 rescued the early-flowering phenotype of *Arabidopsis elf3-1* at normal (22°C), but not higher (27°C), temperatures. The chimeric ELF3-BdPrD, in which the PrD of *Arabidopsis* ELF3 was replaced with the corresponding sequence of BdELF3, was unable to fully support temperature-responsive flowering. These results unambiguously showed that the PrD is responsible for temperature responsiveness of ELF3.

Jung and colleagues next tested the function of ELF3-PrD at the molecular level. Previously, the Wigge group had shown that ELF3 is a transcriptional repressor that occupies and regulates its target genes in a temperature-dependent manner (Ezer et al., 2017). In the current work, the authors found that both BdELF3 and the chimeric ELF3-BdPrD lost the temperature responsiveness of binding and regulation of target genes. These results then led the authors to investigate whether temperature controls the activity of ELF3 directly. Surprisingly, they observed formation of nuclear speckles at higher temperature when expressing ELF3 fused with green fluorescent protein (ELF3-GFP) *in planta*. This behavior was dependent on PrD, as BdELF3 and chimeric ELF3-BdPrD were evenly distributed even at higher temperature. The ability to form thermo-responsive speckles is intrinsic to ELF3, because the authors observed the same phenomenon upon expressing ELF3 in *Saccharomyces cerevisiae*, a heterologous system lacking ELF3-related genes. PrDs are known to mediate phase separation of proteins (Franzmann et al., 2018), prompting the authors to analyze the behavior of ELF3 *in vitro*. The results showed that purified *Arabidopsis* ELF3 PrD, but not BdELF3 PrD, rapidly and reversibly formed liquid droplets in a temperature-dependent manner. These observations suggest that in response to high temperature ELF3 phase separates into molecular condensates in order to inactivate itself.

Within the EC, the small protein ELF4 is known to regulate the activity of ELF3 via an as-of-yet-unclear mechanism. The authors observed that overexpression of ELF4 largely abolished the thermal responsiveness of flowering and ELF3 binding to and regulation of its target genes, indicating that ELF4 is able to maintain ELF3 in an active state at high temperature.

Collectively, the elegant study of Jung et al. (2020) showed that *Arabidopsis* ELF3 can sense warm temperature through its PrD and adopt two conformations: an active diffused form and an inactive form in phase-separated condensates. These findings shed light on how temperature can be perceived and demonstrate that phase separation is functionally important. Yet, this work also raises many interesting questions that need further investigation. For example, as mentioned earlier, it remains an open question as to how the evening

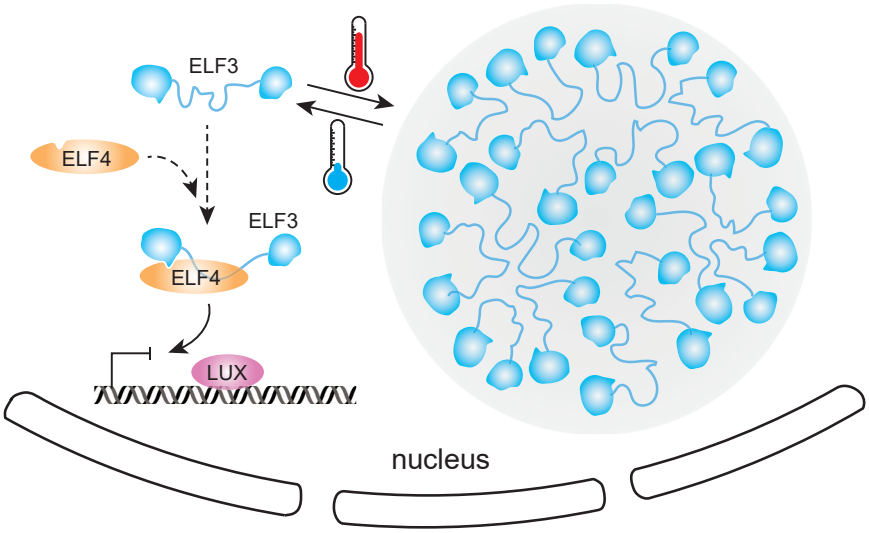

Figure 1. The Prion Domain (PrD) of *Arabidopsis* ELF3 Acts as a Molecular Thermosensor
When temperature increases, the PrD of ELF3 is affected by temperature, mediating the clustering of ELF3 molecules into condensates in which ELF3 is sequestered and/or inactivated. The ELF3 condensates can be reversed by lowering the temperature. The evening complex (EC) protein ELF4 stabilizes the activity of ELF3 likely by inhibiting ELF3 entry into the condensates.

complex component ELF4 modulates the temperature responsiveness of ELF3. ELF4 binds a region in ELF3 neighboring the PrD. It is possible that the binding of ELF4 tunes ELF3 structure as shown *in vitro* (Silva et al., 2020) or masks the PrD of ELF3, precluding ELF3 association with itself and the formation of condensates (Figure 1). However, this must be reconciled with a previous study showing that ELF4 caused ELF3 to form speckles in the nucleus (Herrero et al., 2012). Also, it is intriguing to know whether the speckles formed by ELF3 *in vivo* exhibit liquid-like behavior, and related to this, whether these speckles are reversible when the temperature drops. If reversible, how quickly do these speckles dissolve or disassemble, given that daily temperature fluctuates extensively (Hepworth et al., 2018)? As phase separation has been shown to be a mechanism for buffering noise in gene expression (Klosin et al., 2020), it would be valuable to explore the role of phase separation of ELF3 in buffering the consequences of daily temperature fluctuations.

Above all, the study from Jung and colleagues demonstrated that phase separation of a PrD-containing protein acts as a molecular thermosensor. About 500 *Arabidopsis* proteins harbor PrDs based on prediction (Chakrabortee et al., 2016). Therefore, the findings of this study might extend to other PrD-containing proteins, with other PrDs serving as sensors of different external stimuli.

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