competent virus is extremely small in elite controllers. Furthermore, one participant in Jiang and colleagues' study had no detectable replication-competent HIV at all, even though the authors thoroughly analysed more than one billion cells from this person. Whether HIV has been completely eradicated from this individual's body will be hard to demonstrate, but their case is certainly reminiscent of previous reports of HIV cure[13,14].

Elite controllers represent only a small proportion of people living with HIV. Nonetheless, Jiang and colleagues' work has several implications for the rest of this population. It suggests that deeply latent proviruses could preferentially persist after years of viral suppression with ART, particularly in individuals who have maintained immune responses against HIV. Perhaps continuous immune pressure over years would select a small reservoir from which HIV replication would be less likely to reignite. But whether deep-sleeping viral genomes could be reactivated and contribute to viral rebound during ART interruption remains to be determined.

Either way, the results of this study imply that both the intactness and the activation potential of viral genomes should be assessed when measuring the magnitude of the persistent HIV reservoir that can cause viral rebound. Assays that are currently used to estimate the size of the viral reservoir generally measure either the number of intact HIV genomes or their ability to generate RNA or proteins *in vitro*. Jiang and colleagues' work suggests that combining both measures could be necessary, because many intact genomes might not be easily reactivated. A combination measure could provide researchers and clinicians with a better predictor of viral rebound following ART interruption.

The study indicates that a continuous and prolonged cellular-immune pressure might substantially reduce the size of the HIV reservoir over time, by selecting a small pool of cells containing hard-to-reactivate HIV genomes. This, in turn, suggests that immune-cell therapies — including therapies based on CAR T cells, which are currently being developed to control HIV reservoirs[15] — might not only control viral rebound during ART interruption, but also shrink the viral reservoir to a pool of deeply latent proviruses. Whether this could result in a long-term remission of HIV infection remains, of course, to be determined.

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Cell biology

The plant response to heat requires phase separation

Simon Alberti

Temperature determines the geographical distribution of plants and their rate of growth and development, but how they sense high temperatures to mount a response was unclear. Now a process underlying this responsiveness is known. **See p.256**

Unlike animals, plants cannot move to escape harsh conditions. Consequently, they must continuously monitor their environment and, when exposed to high temperatures, quickly adjust their expression of developmental and growth-related genes. On page 256, Jung *et al.*[1] describe a molecular process that might underlie this temperature responsiveness.

The expression of developmental and growth-related genes in animals and plants typically occurs in a rhythmic fashion over a 24-hour cycle. Such daily oscillations are controlled by a molecular loop of protein activity that provides what is termed the circadian clock. Clock-induced transcriptional changes enable plants to anticipate daily environmental changes.

In the model plant species *Arabidopsis thaliana*, one component of the circadian clock is a protein assembly called the evening complex. It is maximally active at dusk and represses the expression of many genes important for plant development. The evening complex comprises the transcription-factor protein ELF3 (Fig. 1), a small peptide known as ELF4 and a protein called LUX. Plants with mutations that disable the gene encoding ELF3 flower earlier than normal during development and grow long embryonic stems termed hypocotyls, suggesting that ELF3 has a key developmental role.

Temperature fluctuations are known to affect the circadian rhythm of plants. The growth of *A. thaliana* at 22°C is normally restricted to the period around dawn, because of the repressive action of the evening complex at other times of day[2]. However, at 27°C, this growth repression is relieved[2], and plants show accelerated flowering and rapid hypocotyl elongation compared with growth at 22°C. Yet the mechanism underlying such temperature-regulated growth has remained a mystery. Jung and colleagues propose that a physical process called phase separation is at the heart of plant responsiveness to heat.

To investigate, the authors focused on ELF3. They engineered *A. thaliana* so that ELF3 was replaced with a related version from two plants that do not show temperature-accelerated flowering: *Solanum tuberosum* (potato) and *Brachypodium distachyon* (a grass). The resulting *A. thaliana* plants were indistinguishable from wild-type *A. thaliana* at moderate temperatures, but were unable to accelerate flowering at a higher temperature, suggesting that ELF3 has a key role in temperature responsiveness.

To investigate further, Jung and colleagues focused on a region of ELF3 that is enriched in polar (hydrophilic) amino-acid residues, depleted of charged residues and predicted to be intrinsically disordered. Such protein regions are known as prion-like domains (PrDs), and have been proposed to mediate environmental responses in budding yeast (*Saccharomyces cerevisiae*)[3,4]. Jung *et al.* engineered *A. thaliana* to express a chimaeric protein, in which its normal PrD was replaced with the corresponding region of ELF3 from *B. distachyon*. The authors report that the engineered plant did not display temperature-accelerated flowering, indicating that this ELF3 domain might have a key role in establishing temperature responsiveness.

The PrD of *A. thaliana* contains continuous stretches of the amino acid glutamine that are called polyglutamine (polyQ) repeats. The authors note a correlation between plant species that have long polyQ repeats in this domain and accelerated growth at warm

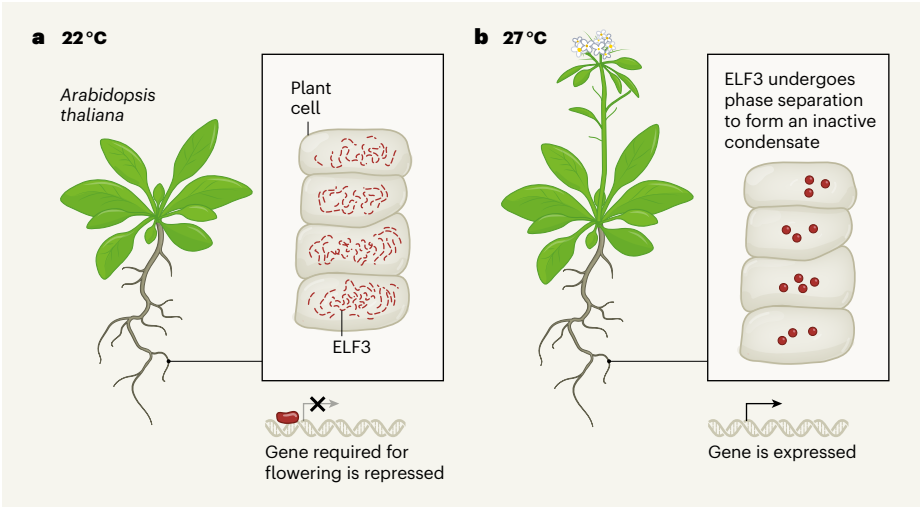

Figure 1 | A mechanism that enables plants to respond to high temperatures. In the model plant *Arabidopsis thaliana*, the protein ELF3 inhibits the expression of certain developmental genes, including some involved in flowering[2]. However, this transcriptional repression is relieved at high temperatures[2]. Jung *et al.*[1] show how this switch in ELF3 activity occurs. **a**, At 22°C, ELF3 is dispersed in the cell in a diffuse pattern, and binds to DNA to block transcription. **b**, At 27°C, ELF3 assembles into 'dots' (also called puncta). The authors suggest that this represents temperature-driven phase separation of ELF3 to form a discrete condensate. This would presumably prevent ELF3 from binding to its target genes, thereby inactivating ELF3 and enabling those genes to be expressed, promoting growth and flowering.

temperatures, suggesting that the polyQ repeats modify ELF3 temperature responsiveness. Because repeat expansions generally evolve rapidly compared with non-repetitive sequences, this correlation suggests a potential way for plants to adapt to the predicted higher temperatures arising from global warming.

To investigate the molecular changes underlying ELF3 temperature responsiveness, the authors used a range of biochemical, biophysical and cell-biological tests. They observed that, at low temperatures, ELF3 was diffusely distributed inside the cell, but when the temperature rose it assembled into microscopically visible 'dots' called puncta. This outcome depended on the presence of the PrD, and the number of observed puncta increased with the length of the polyQ repeats. Crucially, the formation of these puncta was reversed if the temperature fell, suggesting that this represents a normal assembly mechanism in response to heat, rather than an irreversible protein-aggregation event.

Previous work[3–5] led to the proposal that PrD-containing proteins in budding yeast undergo a stimulus-dependent phenomenon called phase separation. This is a process by which a well-mixed protein solution 'demixes' into a dense phase (or condensate) and a dilute phase, comparable to the way that oil and water are partitioned into different phases[6,7]. To test whether the ELF3 PrD forms condensates, the authors performed *in vitro* experiments using a fragment of *A. thaliana* ELF3 containing the PrD. Indeed, this fragment showed temperature-dependent phase separation with a threshold for condensate formation at approximately 28°C. By contrast, the corresponding fragment of ELF3 from *B. distachyon* did not form condensates under the same conditions. This indicates that the *A. thaliana* ELF3 PrD forms condensates *in vitro* in a temperature-dependent manner. However, whether the heat-induced assemblies observed in cells are condensates of inactive ELF3 remains to be established.

Next, the authors focused on ELF4, which binds to ELF3 in the vicinity of its PrD. Jung *et al.* found that ELF4 inhibits the temperature responsiveness of ELF3: plants that were engineered to express higher-than-normal levels of ELF4 were unable to respond to warm temperatures with accelerated flowering. This suggests that the binding of ELF4 to ELF3 modulates condensate assembly by ELF3. The regulation of phase separation by the action of a binding ligand is a widespread phenomenon termed polyphasic linkage[8]. However, more *in vitro* and *in vivo* experiments will be needed to determine whether polyphasic linkage of ELF3 and ELF4 underlies the inhibition of temperature-accelerated flowering.

"A physical process called phase separation is at the heart of plant responsiveness to heat."

The polyQ repeats modify the temperature responsiveness of ELF3, but alone they are probably insufficient to drive this responsiveness, and the identity of the amino-acid residues responsible for driving this property of ELF3 is unknown. Work so far in other systems[9] to understand phase separation of PrD-containing proteins has focused mainly on those that undergo phase separation on cooling, such as a human protein called FUS. Amino-acid residues that are aromatic (those that contain a benzene ring, or an analogue thereof), polar or basic provide cohesive forces for intramolecular interactions in FUS that enable phase separation[9]. ELF3 undergoes phase separation when the temperature rises, rather than falls, and previous studies[5,10] suggest that heat-induced phase separation of elastomeric proteins (flexible proteins with biomechanical functions) often depends on hydrophobic amino-acid residues. Indeed, the ELF3 PrD contains several hydrophobic amino-acid residues, such as methionine, but their role in condensate assembly is unknown.

Another crucial point to establish is how polyQ repeats modify condensate assembly. One possibility is that these repeats alter ELF3 solubility, thus shifting the temperature at which phase separation occurs.

This study raises some exciting questions for the future. For example, what are the properties and composition of these ELF3 condensates in plant cells? Can the ELF3 PrD respond to signals besides temperature, such as other physico-chemical cues? How widespread is this mechanism in plants, and do organisms other than plants regulate components of their circadian clocks through stimulus-dependent phase separation? Repeats of the amino acids threonine and glycine in a transcription-factor protein modulate the temperature responsiveness of the circadian clock in the fruit fly *Drosophila melanogaster*[11]. This suggests that phase separation could have a much broader role in coupling environmental inputs to biological rhythms than had been thought.

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