Regulation of flowering time: a splicy business

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The mechanisms regulating flowering time at ambient temperatures are controversial. One hypothesis ascribes prominent roles to two competing protein isoforms of FLOWERING LOCUS M (FLM) which are produced by alternative splicing. Now, Capovilla et al. (2017) have demonstrated that alternative splicing of FLM is indeed important, but that competing protein isoforms are probably of much lesser relevance than originally thought.

Few decisions in life are more important than getting the time for reproduction right. If a bad choice is made one may not be able to find a suitable partner, conditions for producing progeny might be suboptimal or the progeny might be forced to grow up in an unsuitable environment. To prevent that, elaborate mechanisms have evolved in many species. In plants, the switch from vegetative to reproductive development is controlled by dozens of genes integrated in numerous well known pathways (Srikanth and Schmid, 2011). However, it was discovered only relatively recently that ambient temperature plays a critical role in controlling flowering time: many Arabidopsis ecotypes flower substantially earlier at 27°C than at the more usual growth temperature of 23°C (Balasubramanian et al., 2006). This ambient temperature pathway has been the subject of intense research over the past few years, not least because in times of global warming the analysis of genetic circuits responding to temperature changes has gained considerable importance.

One of the key genes identified was FLOWERING LOCUS M, which encodes a MADS-domain transcription factor (Scortecci et al., 2001; Balasubramanian et al., 2006). In Arabidopsis plants in which FLM is deleted, early flowering is observed no matter whether the plants are grown at 23°C or 27°C (Balasubramanian et al., 2006). This indicates that FLM is a repressor of early flowering at lower temperatures (Balasubramanian et al., 2006).

To exert its repressive effect, FLM interacts with another MADS-domain protein, SHORT VEGETATIVE PHASE (SVP) (Lee et al., 2013). Like FLM, SVP is also involved in regulating flowering time at ambient temperatures (Lee et al., 2007). The current view is that SVP/SVP homomeric and SVP/FLM heteromeric protein complexes delay flowering at lower temperatures by repressing genes that act as floral activators (Lee et al., 2013; Pose et al., 2013). But exactly how is this repression released at higher temperatures? In the case of SVP, one hypothesis is that the protein shows a marked decrease in stability at higher temperatures (Lee et al., 2013). FLM, on the other hand, is subject to alternative splicing, and the occurrence of specific splice forms is correlated to the growth temperature (Balasubramanian et al., 2006; Pose et al., 2013). The two major splice forms of FLM are designated FLM-β and FLM-δ (Scortecci et al., 2001). FLM-β is the predominant transcript at lower temperatures, whereas FLM-δ appears to be more enriched at higher temperatures (Pose et al., 2013). The FLM-β and FLM-δ proteins are capable of interacting with SVP. However, only FLM-β and not FLM-δ forms DNA-binding complexes with SVP (Pose et al., 2013). In addition, overexpression of FLM-β resulted in late flowering, whereas overexpression of FLM-δ led to earlier flowering in comparison to wild-type plants (Pose et al., 2013).

This gave rise to an appealing hypothesis: the FLM-β and FLM-δ proteins compete with each other in binding to SVP. However, only the FLM-β/SVP complex is capable of repressing flowering, because FLM-δ/SVP lacks DNA-binding activity. At lower temperatures, FLM-β/SVP and SVP/SVP complexes predominate and hence flowering is repressed. In contrast, with an increase in temperature, FLM-δ becomes more abundant, outcompetes FLM-β and SVP itself for SVP binding and hence flowering is activated (Pose et al., 2013).

FLM-δ under scrutiny

However, as exciting as the hypothesis of a dominant-negative protein variant generated by alternative splicing is (especially for those researchers with a strong interest in the biophysics of transcription factor functions), it has been challenged in recent years.

For example, although FLM-β transcripts decrease with increased temperatures, a concomitant increase in FLM-δ cannot always be detected (Sureshkumar et al., 2016). Those and other findings led to the competing hypothesis that the decrease in FLM-β transcripts (and with this FLM-β protein) is the main determinant of early flowering at higher temperatures and that the contribution of the FLM-δ protein is negligible (Lutz et al., 2015; Sureshkumar et al., 2016; Lutz et al., 2017).

Capovilla et al. (2017) take a fresh look at the role of FLM-δ in flowering-time regulation. Towards that goal, they produced Arabidopsis lines that are incapable of expressing either FLM-β or FLM-δ. To circumvent potential problems and artefacts associated with the expression of transgenic FLM versions, the genomic FLM locus was edited using CRISPR-Cas9, creating plants that lacked either the 2nd or 3rd exon of FLM. As the 2nd exon is part of the FLM-β but not of the FLM-δ transcript,
plants lacking it can produce FLM-δ but not FLM-β. Conversely, plants lacking the 3rd exon are incapable of producing FLM-δ but can produce FLM-β. This set-up makes it possible to more clearly distinguish whether the FLM-δ transcript (and protein) plays an important role in controlling flowering time: if the FLM-δ protein is a dominant-negative version that competes with FLM-β for binding to SVP (Pose et al., 2013), plants expressing only FLM-δ might be expected to flower earlier than flm loss-of-function mutants. This is because in those plants FLM-δ would prevent SVP homodimer formation (something that wouldn’t be possible in flm loss-of-function mutants) and hence flowering can proceed. This, however, was not observed. The ‘FLM-δ only’ plants flowered as early as full loss-of-function mutants, indicating that there is no substantial dominant-negative effect exerted by the FLM-δ protein. Thus, the results clearly favour the idea that decreasing levels of the FLM-β transcripts alone trigger flowering at elevated temperatures (Capovilla et al., 2017).

Splicing and the quest for the temperature signalling pathway in plants

One surprising result from the study of Capovilla et al. (2017) was that plants only capable of producing FLM-β and not FLM-δ flowered later than wild-type plants. This is initially not easy to reconcile with the idea that FLM-δ is a non-functional transcript. After all, if FLM-δ has no function, why should it matter whether the plant is capable of producing it? Transcript analysis provides an important clue here: the results of Capovilla et al. (2017) and others (Sureshkumar et al., 2016) indicate that the temperature-dependent variation in FLM-β levels is not achieved by different rates of transcription but by alternative splicing. At higher temperatures, splicing produces less FLM-β and more alternative transcripts, among them FLM-δ but also others, many of which contain premature stop codons and may be subjected to nonsense-mediated decay (Sureshkumar et al., 2016). Intriguingly, one of the major temperature-sensitive splice donor sites that is predominantly used at higher temperatures is that of intron 3, and exactly that site was removed in the ‘FLM-β only’ plants (Sureshkumar et al., 2016; Capovilla et al., 2017). So, what presumably happens in those plants is that more FLM-β is produced because the alternative splice site for producing FLM-β and other splice variants is missing. And more FLM-β leads to delayed flowering.

This highlights an important corollary of alternative splicing: the production of alternative, non-functional transcripts can be an important regulatory mechanism to reduce the amount of functional transcripts (Reddy et al., 2013). It is becoming increasingly clear that transcript abundance of many genes is regulated by this ‘alternative splicing nonsense-mediated decay’ pathway (Reddy et al., 2013).

Interestingly, many of the RNA-binding proteins implicated in alternative splicing are subject to alternative splicing themselves, and this splicing can also be susceptible to temperature variations (Lazar and Goodman, 2000; Duque, 2011; Capovilla et al., 2015). It is therefore tempting to speculate that the temperature-dependent variation in abundance and/or activity of splicing factors is responsible for temperature-dependent splicing of FLM (Box 1). An ‘alternative splicing–alternative splicing’ cascade may exist in which FLM utilizes a pre-existing circuit that is susceptible to temperature variations (Verhage et al., 2017). Importantly, it was recently discovered that Phytochromes act as temperature sensors in plants and that they possess RNA-binding activity (Jung et al., 2016; Legris et al., 2016; Reichel et al., 2016). One may therefore speculate that Phytochromes act as splicing factors that are involved in the temperature-dependent regulation of FLM (Køster et al., 2017).

In general, the secondary structure of RNA is also highly susceptible to temperature changes and an important factor that determines splicing patterns (Reddy et al., 2013; Vandivier et al., 2016). It does therefore also appear possible that the structure of the FLM pre-mRNA or the pre-mRNA structure of a splice factor acts as a sensor in the ambient temperature pathway (Box 1).

This would also explain why a more ‘common’ developmental mechanisms of transcript abundance regulation, like a temperature-dependent rate of transcription, is not observed for FLM: if the RNA of FLM or a splice factor is a temperature sensor, FLM has to be expressed to sense the temperature.

Rise and fall of a hypothesis

The achievements in unravelling the molecular mechanism of FLM function notwithstanding, the study by Capovilla et al. (2017) might be even more important from the perspective of how science is conducted. The original idea of FLM-δ acting as a dominant-negative protein version made perfect sense at the time it was published (Pose et al., 2013). Indeed, it soon became a standard example on the importance of protein isoforms produced by alternative splicing in plants (Staiger and Brown, 2013; Pajoro et al., 2014). But subsequent papers didn’t find evidence for an important role of the FLM-δ protein (Lutz et al., 2015; Sureshkumar et al., 2016; Lutz et al., 2017). So, the same group that developed the original hypothesis was also involved in studies contradicting it (Lutz et al., 2015), and with Capovilla et al. (2017) they themselves provided the last nail in the coffin of the FLM-δ hypothesis. This is the essence of how science should be conducted: formulating a hypothesis, testing it rigorously and, if necessary, rejecting it. However, we all know that this is not always easy. As Max Planck once put it (Planck, 1949): ‘A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it.’ Capovilla et al. (2017) prove that this is not always the case and that scientists can (and maybe more often should) change their mind in the light of new evidence.

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Box 1. Hypothetical scenario as to how FLM controls flowering time in response to temperature differences

At lower temperatures (left), the FLM pre-mRNA is correctly spliced, yielding the FLM-β transcript. The FLM-β protein represses flowering. At higher temperatures (right), splicing is disturbed, triggering nonsense-mediated decay through premature stop codons (red octagon). Consequently, less (or no) FLM-β protein is produced and flowering is activated. FLM-δ is very likely also produced, but is not shown here as the protein presumably has no role in flowering-time regulation.

The presence/absence of splicing factors (SF) may contribute to alternative splicing. It is not yet entirely clear how plants sense temperature differences, but one possibility is that temperature-dependent RNA structures (symbolized by the presence/absence of the stem-loop structure) are involved.
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