Ambient temperature and alternative splicing in flowering time control

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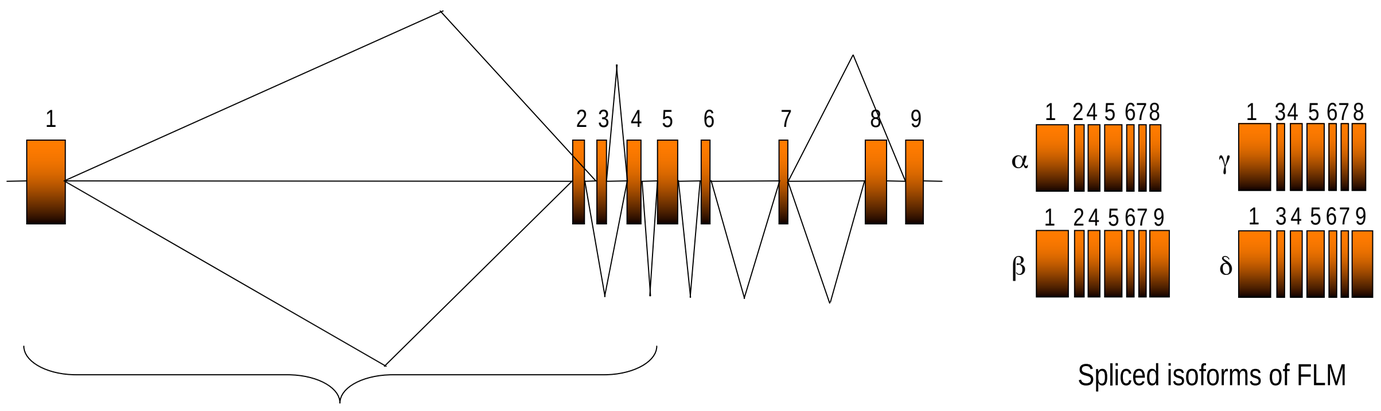
FLM, a MADS-box gene involved in regulation of flowering time

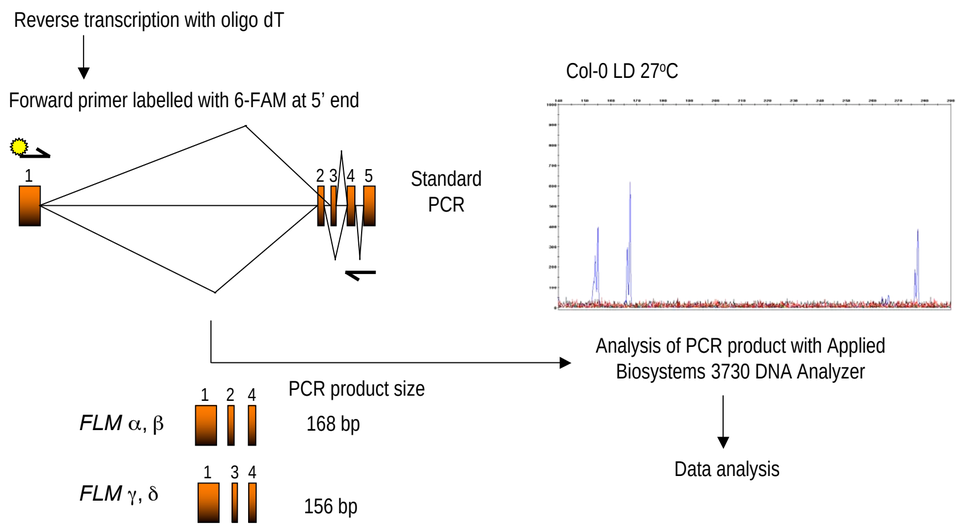
The transition to flowering is influenced by different environmental factors such as day-length and ambient temperature. Changes in the flowering time of many British plant species has provided some of the best biological evidence for recent climate change.
The MADS-box transcription factors *FLOWERING LOCUS M* (*FLM*) and *SHORT VEGETATIVE PHASE* (*SVP*) function to control Arabidopsis flowering in response to ambient temperature (1, 2, 3 and Fig.). *FLM* is related in sequence to the floral repressor *FLOWERING LOCUS C (FLC)* (4), and to a cluster of four genes located on Arabidopsis chromosome V (*MAF2* [*MADS AFFECTING FLOWERING2*] to *MAF5*) (5).
FLM shows mutually exclusive alternative splicing: Either the second or the third exon is spliced out in the different variants, but they do not exist together in the same mRNA. Despite the relatedness of the MAF genes, this mutually exclusive splicing appears to be unique to *FLM*. This may have a functional consequence for *FLM* activity as this alternative splicing influences the sequence of the I domain – a dimerisation domain of MADS box proteins that influences DNA binding.



Gene structure of *Flowering Locus M* (*FLM*) gene

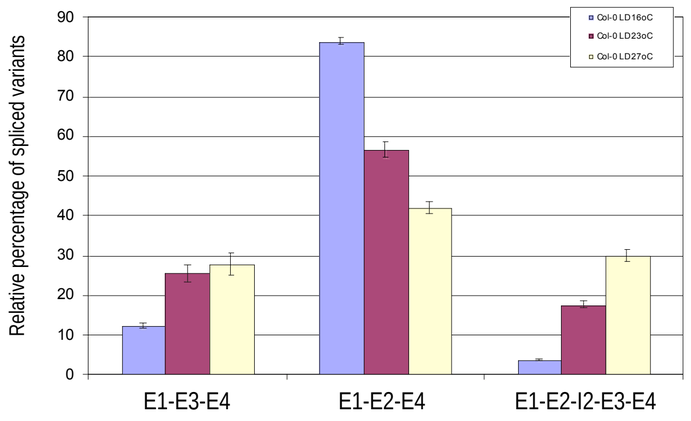
Identification of *FLM* splice variants

To identify the relative level of alternatively spliced variants of *FLM* RNA we used an RT-PCR based method which was developed by Craig Simpson, SCRI. The PCR products were analysed by Applied Biosystems 3730 DNA Analyzer.



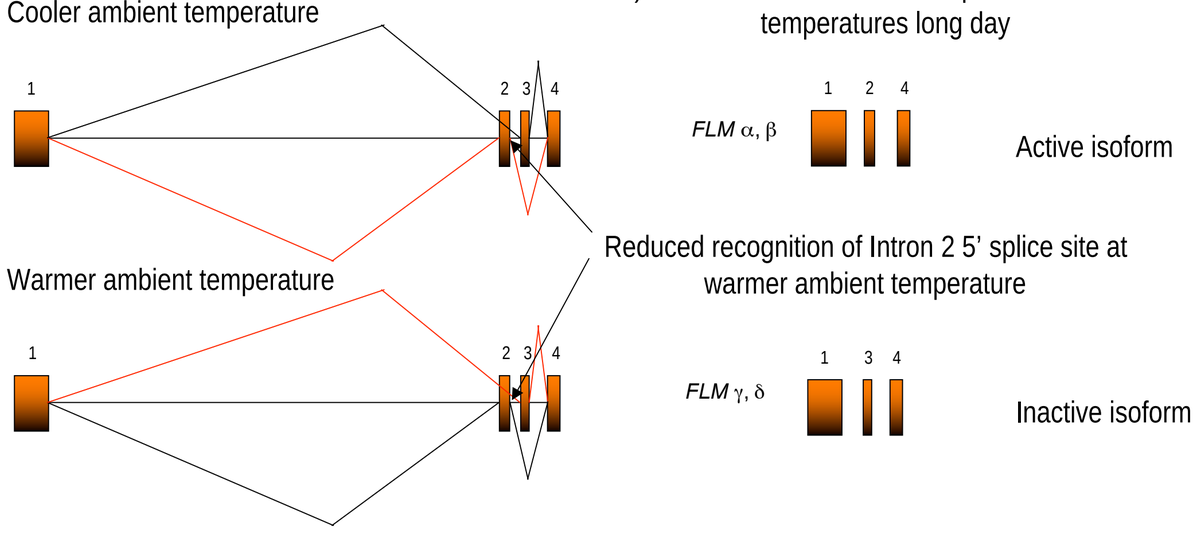
The effect of ambient temperature on *FLM* alternative splicing

Our results show that in Col-0 accession different ratio of alternatively spliced *FLM* variants could be found at different temperature (A). Increasing the ambient temperature the relative level of active *FLM* isoform (E1-E2-E4) decreases dramatically; the third exon incorporation into the mRNA is preferred (A). Working with Ler accession we found different ratio of *FLM* variants comparing to Col-0 (*fpa* mutant panel Fig. D)). This result might indicate a genotype specific accumulation ratio of alternatively spliced isoforms. This change clearly shows that at warmer ambient temperature the level of active isoform of *FLM* is lower; the blocking effect of *FLM* on flowering time is reverted, plants flower earlier.
Our findings demonstrate that the ambient temperature has a pronounced effect on *FLM* splicing. The mutually exclusive splicing of *FLM* results in less mRNA coding for the functional repressor isoform at warmer temperatures. The recognition of Intron 2 5' splice site might be reduced at higher temperature (B).



A) *FLM* isoform levels in Col-0 plants at different temperatures long day

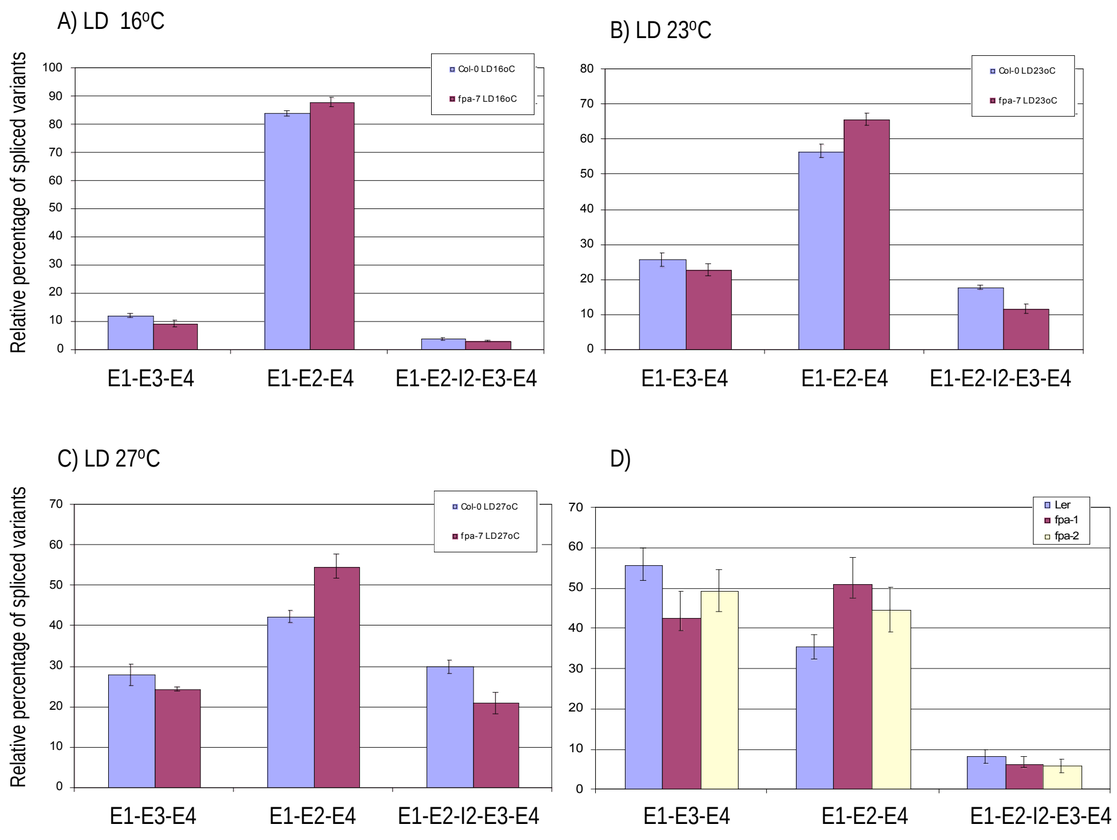
B)



FPA affects the ratio of alternatively spliced isoforms of *FLM*

The RNA binding protein *FPA* promotes flowering. Loss-of-function mutants exhibit elevated levels of *FLC* mRNA. Although *FPA* does not affect *FLM* mRNA levels, we have found that it affects the ratio of *FLM* mutually exclusive splicing. In *fpa-1*, *fpa-2* and *fpa-7* background there are changes in the mRNA levels of alternatively spliced *FLM* gene comparing to the wild type plants.

At all examined temperature on long day the level of active *FLM* isoform (E1-E2-E4) is higher in the *fpa-7* mutant plants than in wild type (Col-0) plants (A-C). Upon thermal induction the difference between the active isoform ratios is more pronounced. We found changes in the level of alternatively spliced *FLM* variants in other *fpa* backgrounds. In *fpa-1* mutant plants the active isoform of *FLM* shows a higher accumulation level at the same temperature comparing to wild type (Ler) plants (D) similarly to the effect in *fpa-7* background.



Our results further demonstrate the essential role of *FPA* in normal flower development.

Conclusions

•We have found that the ratio of mutually exclusive splicing of *FLM* is dependent on ambient temperature and genotype. Working with the Col-0 accession, we find a shift towards the incorporation of exon 3 at elevated ambient temperatures.
• We showed that *FPA* affects the ratio of *FLM* mutually exclusive splicing. This effect is most pronounced at elevated ambient temperatures, revealing that *FPA* is necessary for the normal flowering response to ambient temperature.
• We have also found that the mutually exclusive splicing of *FLM* is conserved between the Col-0 and Ler accessions cultivated in identical conditions. However, the relative ratios of exon incorporation are very different, raising the possibility that variation in alternative splicing could underpin adaptation to environment through flowering time control.

References

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Acknowledgment

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