

A stress-free walk from Arabidopsis to crops

Yin Hoon Chew¹ and Karen J Halliday^{1,2}

Global concerns such as food security and climate change have highlighted an urgent need for improved crop yield. Breakthroughs in Arabidopsis research provide fresh application routes to achieve novel crop varieties that can withstand or avoid stresses imposed by a changing growth environment. This review features advances in CBF-stress signalling that expand opportunities to produce super hardy crops that can withstand multiple abiotic stresses. It examines molecular external coincidence mechanisms that avoid abiotic stresses by confining plant growth and reproduction to favourable times of the year. The potential value of mathematical modelling approaches is discussed in relation to improving crop-stress resistance or avoidance, and forecasting crop performance.

Addresses

¹ Institute of Structural and Molecular Biology, School of Biological Sciences, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, UK

² Centre for Systems Biology at Edinburgh, C. H. Waddington Building, King's Buildings, Edinburgh EH9 3JR, UK

Corresponding author: Halliday, Karen J (Karen.Halliday@ed.ac.uk)

Current Opinion in Biotechnology 2011, 22:281–286

This review comes from a themed issue on
Plant biotechnology
Edited by Adi Avni and Miguel Blazquez

Available online 17th December 2010

0958-1669/\$ – see front matter
Published by Elsevier Ltd.

DOI [10.1016/j.copbio.2010.11.011](https://doi.org/10.1016/j.copbio.2010.11.011)

Introduction

International concerns regarding food security and a competing demand to produce biofuels have highlighted the requirement for renewed efforts to increase plant yield and productivity [1]. The improvements in grain yield achieved over the last 50 years have now reached a plateau at a time when yields are threatened by global warming and increasing CO₂ levels [2]. To ensure food supplies keep pace with a growing population we need to develop and exploit new crops, and adopt new approaches to improving popular grain crops. Recent advances in our understanding of abiotic stress tolerance and avoidance in Arabidopsis offer considerable potential for crop improvement in the future [2].

Abiotic stresses such as cold, heat, drought and salinity can have a strongly detrimental effect on crop biomass and yield [3]. The production of crops with improved responses to wide-ranging environmental conditions experienced in

nature offers obvious benefits for both the farmer and wider society. Two general approaches have been applied to this problem. First, the development of robust stress-tolerant cultivars with improved resistance to abiotic stress. Second, the production of stress-avoiding varieties that can adjust their growing habit to favourable times of the year. The substantial expansion in our knowledge of abiotic stress tolerance and avoidance strategies in Arabidopsis provides increased potential for exploitation in crops. Judicious use of Arabidopsis alongside model crop plants such as rice and wheat can generate an iterative cycle that expedites understanding. This review highlights the exemplar CBF stress response pathway as a means to improve tolerance of stresses that are highly correlated in nature, and examines the molecular networks that underpin stress avoidance. A brief overview is provided on how modelling approaches can be employed to predict responses to changing environmental conditions.

Coping with stress

In the natural environment, plants have to cope with temperature extremes. Temperature can change dramatically through a season, and even during a daily photo-cycle, 20–30 °C fluctuations are not unusual [4••]. Environmental temperature has a direct impact on biochemical reaction rates, water and nutrient uptake, membrane fluidity, protein and nucleic acid conformation whereas freezing leads to the formation of ice crystals that can inflict physical damage within the cell [5,6]. Exposure to cool temperatures induces the transcription of a suite of genes that offer protection and adjust the plants' metabolic and regulatory pathways to the new operational temperature range. As dehydration and osmotic stress often accompany temperature stress, the molecular response elicited by temperature includes dehydrins, a subgroup of cold responsive genes that offer protection from dehydration and osmotic stress. Principal components in the stress response network are C-REPEAT BINDING FACTORS (CBFs), transcription factors that improve the robustness of crops to both the direct and indirect effects of temperature.

The CBF regulon: a case study

Periods of cold can severely restrict growth, diminish plant survival rates and reduce crop productivity. However, many plants from temperate climates are not only able to withstand cold spells, they can acquire freezing tolerance following exposure to chilling temperatures, through a process known as cold acclimation [5]. Central to this process is the CBF regulon: CBF genes and their transcriptional targets that trigger a suite of pathways that collectively protect the plant from the harmful effects of cold [7].

Studies in *Arabidopsis* have defined important elements in the CBF pathway and provided a molecular platform for extended study and application in crops [8]. CBFs, also known as DREBs, are transcription factors that recognize the cold-responsive and dehydration-responsive DNA regulatory element designated the *C-REPEAT/DEHYDRATION-RESPONSIVE ELEMENT* (CRT/DRE). Exposure to low temperatures induces the expression of *CBFs* that regulate the transcription of *COLD-RESPONSIVE/LATE EMBRYOGENESIS-ABUNDANT* (*COR/LEA*) genes [9]. CBF-induced molecular events trigger a number of biochemical changes such as the accumulation of simple sugars and the amino acid proline that are thought to protect the plant against cold and subsequent exposure to sub-zero temperatures [7]. A key controller of the CBF regulon is INDUCER OF CBF EXPRESSION 1 (*ICE1*), an MYC-type basic helix–loop–helix (bHLH) transcription factor that enhances the expression of *CBFs* in a temperature-dependent manner [5,10]. Constitutive expression of the *CBF* genes in transgenic *Arabidopsis* plants results in the induction of *COR* gene expression and an increase in freezing tolerance without a low temperature stimulus [6,7,11].

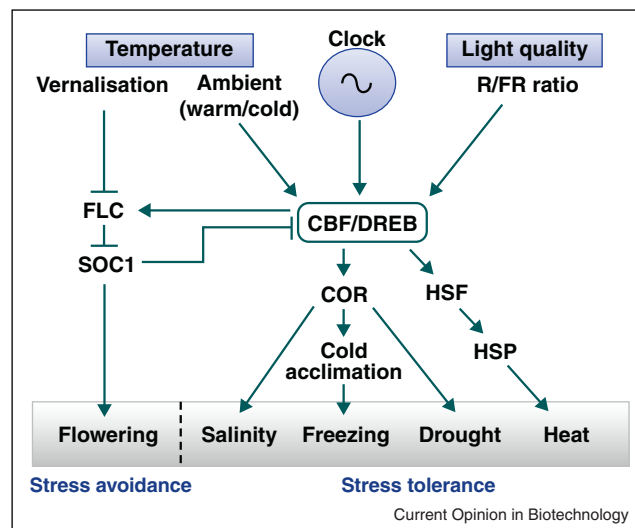
Improving crop stress resistance

The CBF pathway is highly conserved in flowering plants. Components are present in both temperate cereals (e.g. barley, rye and wheat) and species that suffer damage at chilling temperatures and that are completely unable to tolerate freezing, such as tomato, maize and rice [12,13]. Targeted manipulation of the CBF pathway therefore offers real scope to improve survival rates and yield in winter crop varieties and extend the geographical range of crops that cannot currently withstand cold spells or sub-zero temperatures. As the CBF regulon also enhances resistance to drought and salinity, this pathway is a logical target for boosting crop protection from abiotic stresses that are highly correlated in nature (Figure 1).

A number of recent studies have started to map the cold regulated transcriptome in economically important crops such as rice and wheat [14,15,16,17]. These and related studies have significantly expanded our knowledge of the CBF pathway in these species, which we now know comprises multiple CBF subgroups [6]. Studies in wheat have identified CBFs that are constitutively elevated and cold-inducible in winter but not spring cultivars [16,18]. These differences are thought to contribute to the superior cold tolerance of winter cultivars.

Conservation of CBF pathways in higher plants permits application of the *Arabidopsis* tool-kit to component testing and delineating molecular mechanisms that operate in crops. *TaICE41* and *TaICE87*, *ICE1*-like genes from wheat were recently shown to bind the MYC elements in the promoters of wheat *CBFs* [19]. Overexpression of *TaICE41* or *TaICE87* in transgenic *Arabi-*

Figure 1



CBFs are pathway integrators that improve tolerance to multiple abiotic stresses. Temporal regulation of *C-REPEAT BINDING FACTORS* (*CBFs*) by temperature and light is 'gated' by the circadian clock. *CBFs* activate *COLD-RESPONSIVE/LATE EMBRYOGENESIS-ABUNDANT* (*COR/LEA*) genes improving tolerance to freezing, salinity and drought. Extreme heat induces *DREB2A* which improves heat stress tolerance through the HEAT SHOCK TRANSCRIPTION FACTOR–HEAT SHOCK PROTEIN (*HSF-HSP*) pathway. Activities of the CBF and vernalisation pathways are coordinated. Before vernalisation, CBF activation by cold suppresses flowering by elevating *FLOWERING LOCUS C* (*FLC*) levels. Following the transition to reproductive development, the floral gene *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) feeds back to negatively regulate *CBFs*. This cross regulation ensures that flowering is suppressed during cold snaps, and that the *CBFs* are restrained following the switch to reproductive growth.

dopsis resulted in enhanced freezing tolerance following cold acclimation. Here, *TaICE41* and *TaICE87* were shown to operate through the classical CBF regulon, as *AtCBF2*, *AtCBF3* and other known cold-regulated genes were expressed at higher levels in these plants. Other studies have shown that improved abiotic stress tolerance can be achieved by manipulating CBF levels. Constitutive expression of wheat *wCBF2* in tobacco led to significant improvements in freezing tolerance [20], while elevated expression of *TaDREB2* or *TaDREB3*, *CBFs* from wheat, greatly increased drought and frost tolerance in transgenic wheat and barley [21].

A number of studies have also been conducted in rice. A comparative study demonstrated that rice *OsDREB1A* and *OsDREB1B* had high functional overlap with the *Arabidopsis* orthologues *CBF3/DREB1A*, *CBF1/DREB1B* and *DREB1C*. Like their *Arabidopsis* counterparts, *OsDREB1A* and *OsDREB1B* genes were shown to be induced by cold stress [22]. Overexpression of these genes led to improved stress tolerance to drought, high salt and cold in transgenic rice plants. Transgenic overexpression of barley *HvCBF4* in rice also resulted in

elevated tolerance to drought, high-salinity and low-temperature stresses [23]. Recently, the MYB transcription factor *MYBS3* was shown to enhance cold tolerance in rice [24]. In contrast to *DREB1/CBF* that is known to respond rapidly and transiently to cold stress, the *MYBS3* response was gradual. This suggested *MYBS3* may be important for long-term adaptation to cold stress in rice.

In tomato the CBF pathway has been shown to be important for the induction of chilling tolerance in the mature fruit. Ethylene, which is important for fruit ripening, together with cold, has been shown to be required for the induction of *LeCBF1* [25[•]]. These findings indicate that CBF regulon activation in tomato protects mature tomatoes from damage imposed by cold storage. This finding offers a clear application route aimed at improving the quality and shelf-life of commercial tomato varieties.

Unwanted side-effects

While elevating CBF levels can significantly improve stress tolerance, an unwelcome consequence of high CBF1 or CBF3 levels can be growth retardation and reduced yield [7,11,12]. Interestingly a recent study illustrated that use of the drought-inducible maize *Rab17* promoter rather than a constitutive promoter, reduced the deleterious effects of *TaDREB2* and *TaDREB3* on development [21]. Growth retardation can also be avoided by using alternative CBFs, for example, *wCBF2* expression in tobacco and *HvCBF4* expression in rice improved stress tolerance without stunting growth [20,23].

CBFs prevent over-heating

Thermo-tolerance in plants and other organisms relies heavily on the accumulation of HEAT SHOCK PROTEINS (HSPs) that are thought to act as molecular chaperones in protein quality control [26]. Heat stress transcription factors (HSFs) operate as central regulators of HSP expression. Recent work has shown that Arabidopsis *DREB2A* and *DREB2C*, not only improve resistance to drought and salinity stress, but also provide protection from severe heat [27[•],28]. This appears to be achieved by activating the heat shock pathway. Heat stress boosts *DREB2B* transcript levels, which induces *HSPA3* transcription and downstream *HSP* targets. These studies illustrate that individual CBF/DREBs have different thermal dependencies with CBFs specialising in either cold or warm protection.

Light modulates CBF expression

Many molecular, cellular and physiological responses are subject to regulation by the plants' internal circadian oscillator and the CBF regulon is no exception. The induction of *CBF2* and *CBF3* transcription by low-temperature abundance is gated by the circadian clock [29^{••}]. *CBF* expression has also been shown to be highly dependent on light quality in Arabidopsis [30^{••}]. Lowering the red to far-red ratio (R/FR), which stimulates vegetation

shade, increases the amplitude of circadian-gated *CBF* gene expression. This light quality-dependent increase in *CBF* expression raises the temperature at which freezing tolerance is induced. Thus light appears to moderate the temperature threshold of cold acclimation. Interestingly the phytochrome light receptors have been implicated in the suppression of flowering at warm ambient temperatures [31]. The light pathways may therefore have an explicit role in buffering the impact of temperature on environment-driven plant responses. The link between light and the CBF regulon provides additional routes for pathway modification extending molecular options for crop improvement. These studies in Arabidopsis also reinforce the notion that the CBF regulon has a central role in the signalling network, integrating internal clock-driven and varied environmental cues (Figure 1).

Stress avoidance

In addition to strategies dealing with the direct consequences of environmental stress, plants have developed sophisticated stress-avoidance mechanisms. An effective way to improve survival rate and yield is to restrict vegetative growth to the season that offers the most favourable conditions [32,33[•]]. To do this the plant must detect and interpret seasonal cues and measure the passage of time. Central to this process is the circadian clock that allows accurate day length measurement and crucially, anticipation of the coming season [34]. Much of our understanding of the plant circadian clockwork has come from detailed studies in Arabidopsis, however, similar clock structures are thought to operate in a range of crop species [35]. We now understand the basic principles that underlie the 'external coincidence' mechanism that integrates light and clock signals in day length recognition. Key components that operate at this junction are the growth promoters PHYTOCHROME INTERACTING FACTOR 4 and 5 (PIF4 and PIF5) and the flowering time regulator CONSTANS (CO) [36,37]. Dual action of the clock that drives rhythmic expression, and light that controls protein stability, underlies PIF4/5-mediated growth and CO-controlled flowering time by day length.

As well as day length, temperature can also deliver strong seasonal cues. In winter annuals that germinate in the fall, flowering is blocked until the plants have experienced a prolonged period of cold [38]. This protective vernalisation pathway ensures that flowering is arrested until the spring and the onset of more favourable conditions. Intensive study of this pathway in Arabidopsis has unraveled the complex epigenetic mechanism that underlies this control. Central to this process is the FLOWERING LOCUS C (FLC), a MADs box protein that prevents flowering by repressing transcription of floral activators such as *FLOWERING LOCUS T (FT)*, *SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1)* and *LEAFY (LFY)*. FLC is subject to epigenetic regulation that either activates or suppresses its transcription. In winter annuals

FLC is highly expressed and vernalisation results in histone modifications that prevent *FLC* transcription [39]. Studies have shown that although elements of this pathway differ, vernalisation is also achieved through epigenetic modification in cereals [40,41]. Here, *VERNALIZATION 1* and *2* (*VRN1* and *VRN2*) are key vernalisation targets. Cold-induced epigenetic changes promote *VRN1* expression, which is accompanied by transcriptional repression of *VRN2* [42]. These changes promote transition of the shoot apex from the vegetative to reproductive state. Interestingly in both *Arabidopsis* and cereals the vernalisation and cold acclimation pathways are linked [43^{••},44]. In *Arabidopsis*, brief cold spells before vernalisation lead to CBF-activated *FLC* expression, which delays flowering and improves freezing tolerance. Post-vernalisation, the CBF pathway is suppressed by the floral activator SOC1. In wheat *VRN-1* has been shown to negatively regulate the cold acclimation pathway suggesting similar pathway connections in *Arabidopsis* and cereals. This cross-pathway regulation could be important to restrain flowering during cold periods experienced during the summer or fall. SOC1 negative feedback appears to suppress the cold response in plants that have switched from vegetative to reproductive growth (Figure 1).

Models that have predictive power

Modelling strategies can be used to identify and interpret molecular events that result from complex circuit behaviour. Recently a modelling approach has been used to provide a fuller understanding of the photoperiodic regulation of flowering time [45^{••}]. Model prediction and experimental verification identified a novel function for FLAVIN-BINDING, KELCH REPEAT, F BOX 1 (FKF1), an important regulator of photoperiodic flowering. The formal integration of complex molecular events and the potential predictive power of models have obvious applications in crop improvement. Advancements in our understanding of the circadian clock network and the molecular links that determine flowering time in *Arabidopsis* have aided the development of an eco-physiological flowering in pea [46]. This approach led to the prediction and experimental validation of the hypothesis that the flowering gene *HIGH RESPONSE TO PHOTOPERIOD* (HR), interacted with the photoperiod pathway.

An *Arabidopsis* phenology model [4^{••}] has been developed that considers the environmental effects of photoperiod, vernalisation and ambient temperature fluctuations on flowering time. The model was developed using field data from a range of *Arabidopsis* flowering pathway mutants situated at five sites across Europe in different seasons. This allowed the relative contributions of different signalling pathways to be assessed across environments. Interestingly, this study suggested that the previous notion of discrete classes of winter-annual

and rapid-cycling *Arabidopsis* ecotypes may be inaccurate, rather most ecotypes are capable of both life histories. The model could predict the effects of novel environments on flowering time across a range of genotypes.

Sophisticated model-assisted strategies have been used to identify traits that are involved in drought tolerance. One approach utilises a phenotyping platform that measures the contribution of stress-QTLs to simple heritable traits under differing environmental conditions [47]. These traits and the underlying QTLs are linked to output yield allowing the identification of traits that are beneficial in complex stress conditions.

Modelling approaches are applicable to crop research at different levels. Models that integrate genetic, molecular and biochemical information facilitate understanding of complex behaviour, such as pathway integration whereas models that cross scales from the molecular, cellular to whole plant or field level have enormous potential in crop improvement and crop yield forecasting.

Concluding remarks

The convergence of research effort on *Arabidopsis* has generated a knowledge base and variety of valuable tools that are amenable to crop research. The *Arabidopsis* system has proved to be a useful and time-saving testing ground for components discovered in crops. Interchangeable use of *Arabidopsis* and crops, or 'system-swapping' has advanced our understanding of stress tolerance and stress avoidance strategies in crops. As improving stress avoidance strategies or a broader tolerance of environmental stresses can dramatically enhance crop survival, these are obvious exploitation routes to enhance crop yield. Targeting the CBF pathway, that integrates environmental and internal signals, increases the potential to protect crops from stresses that are highly correlated in nature (Figure 1). With the global demand for food projected to increase 70–100% by 2050, innovative approaches to enhance crop robustness and yield will play an increasingly important role in meeting the nutritional demands of a growing population [1,48–50].

Acknowledgements

YHC is the recipient of a Darwin Trust PhD studentship. KJH is supported by Biotechnology and Biological Sciences Research Council (grant BBF005237/1) and by the Scottish Universities Life Sciences Alliance.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Borlaug N: **Feeding a hungry world.** *Science* 2007, **318**:359-1359.
2. Long SP, Ort DR: **More than taking the heat: crops and global change.** *Curr Opin Plant Biol* 2010, **13**:241-248.

3. Mittler R, Blumwald E: **Genetic engineering for modern agriculture: challenges and perspectives.** *Annu Rev Plant Biol* 2010, **61**:443-462.
4. Wilczek AM, Roe JL, Knapp MC, Cooper MD, Lopez-Gallego C, Martin LJ, Muir CD, Sim S, Walker A, Anderson J et al.: **Effects of genetic perturbation on seasonal life history plasticity.** *Science* 2009, **323**:930-934.
- This is a good example of genetically informed phenology model that can accurately predict Arabidopsis flowering time based on genotype and the climatic conditions experienced through a season.
5. Chinnusamy V, Zhu J, Zhu JK: **Cold stress regulation of gene expression in plants.** *Trends Plant Sci* 2007, **12**:444-451.
6. Winfield MO, Lu CG, Wilson ID, Coghill JA, Edwards KJ: **Plant responses to cold: transcriptome analysis of wheat.** *Plant Biotechnol J* 2010, **8**:749-771.
7. Gilmour SJ, Sebolt AM, Salazar MP, Everard JD, Thomashow MF: **Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation.** *Plant Physiol* 2000, **124**:1854-1865.
8. Bartels D, Phillips J: **Drought stress tolerance.** In *Genetic Modification of Plants. Biotechnology in Agriculture and Forestry*, vol. 64. Edited by Kempken F, Jung C. Berlin Heidelberg: Springer-Verlag; 2010:139-157.
9. Thomashow MF: **PLANT COLD ACCLIMATION: freezing tolerance genes and regulatory mechanisms.** *Annu Rev Plant Phys* 1999, **50**:571-599.
10. Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK: **ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis.** *Gene Dev* 2003, **17**:1043-1054.
11. Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF: **Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance.** *Science* 1998, **280**:104-106.
12. Jaglo KR, Kleff S, Amundsen KL, Zhang X, Haake V, Zhang JZ, Deits T, Thomashow MF: **Components of the Arabidopsis C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in Brassica napus and other plant species.** *Plant Physiol* 2001, **127**:910-917.
13. Nakashima K, Yamaguchi-Shinozaki K: **Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants.** *Physiol Plantarum* 2006, **126**:62-71.
14. Skinner DZ: **Phospholipase A(2) activity during cold acclimation of wheat.** *Crop Sci* 2009, **49**:323-328.
15. Winfield MO, Lu CG, Wilson ID, Coghill JA, Edwards KJ: **Cold- and light-induced changes in the transcriptome of wheat leading to phase transition from vegetative to reproductive growth.** *BMC Plant Biol* 2009, **9**:55.
- The authors used a microarray approach to survey floral pathway gene regulation in response to a gradual decline in temperature, day-length and light intensity, conditions that mimic a British winter. Novel genes involved in floral transition were identified as well as new roles for previously defined pathways.
16. Sutton F, Chen DG, Ge XJ, Kenefick D: **Cbf genes of the Fr-A2 allele are differentially regulated between long-term cold acclimated crown tissue of freeze-resistant and—susceptible, winter wheat mutant lines.** *BMC Plant Biol* 2009, **9**:34.
17. Yun KY, Park MR, Mohanty B, Herath V, Xu FY, Mauleon R, Wijaya E, Bajic VB, Bruskiewich R, de los Reyes BG: **Transcriptional regulatory network triggered by oxidative signals configures the early response mechanisms of japonica rice to chilling stress.** *BMC Plant Biol* 2010, **10**:16.
18. Badawi M, Danyluk J, Boucho B, Houde M, Sarhan F: **The CBF gene family in hexaploid wheat and its relationship to the phylogenetic complexity of cereal CBFs.** *Mol Genet Genomics* 2007, **277**:533-554.
19. Badawi M, Reddy YV, Agharbaoui Z, Tominaga Y, Danyluk J, Sarhan F, Houde M: **Structure and functional analysis of wheat ICE (inducer of CBF expression) genes.** *Plant Cell Physiol* 2008, **49**:1237-1249.
20. Takumi S, Shimamura C, Kobayashi F: **Increased freezing tolerance through up-regulation of downstream genes via the wheat CBF gene in transgenic tobacco.** *Plant Physiol Biochem* 2008, **46**:205-211.
21. Morran S, Eini O, Pyvovarenko T, Parent B, Singh R, Ismagul A, Eliby S, Shirley N, Langridge P, Lopato S: **Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors.** *Plant Biotechnol J* 2010:1-20.
22. Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K: **OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression.** *Plant J* 2003, **33**:751-763.
23. Oh SJ, Kwon CW, Choi DW, Song SI, Kim JK: **Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice.** *Plant Biotechnol J* 2007, **5**:646-656.
24. Su CF, Wang YC, Hsieh TH, Lu CA, Tseng TH, Yu SM: **A novel MYBS3-dependent pathway confers cold tolerance in rice.** *Plant Physiol* 2010, **153**:145-158.
25. Zhao DY, Shen L, Fan B, Yu MM, Zheng Y, Lv SN, Sheng JP: **Ethylene and cold participate in the regulation of LeCBF1 gene expression in postharvest tomato fruits.** *FEBS Lett* 2009, **583**:3329-3334.
- Ethylene was shown to enhance CBF expression and reduce the degree of chilling-induced tissue damage in postharvest tomato fruit.
26. Kotak S, Larkindale J, Lee U, von Koskull-Doring P, Vierling E, Scharf KD: **Complexity of the heat stress response in plants.** *Curr Opin Plant Biol* 2007, **10**:310-316.
27. Schramm F, Larkindale J, Kiehlmann E, Ganguli A, Englich G, Vierling E, von Koskull-Doring P: **A cascade of transcription factor DREB2A and heat stress transcription factor HsfA3 regulates the heat stress response of Arabidopsis.** *Plant J* 2008, **53**:264-274.
- This study demonstrates that DREB2A, a member of the DREB/CBF family involved in plant responses to cold, drought, and salt stress, can deliver thermo-tolerance. During heat shock DREB2A induces the expression of HEAT SHOCK PROTEINS (HSPs) via the HEAT SHOCK TRANSCRIPTION FACTOR A3 (HSFA3).
28. Chen H, Hwang JE, Lim CJ, Kim DY, Lee SY, Lim CO: **Arabidopsis DREB2C functions as a transcriptional activator of HsfA3 during the heat stress response.** *Biochem Biophys Res Commun* 2010, **401**:238-244.
29. Fowler SG, Cook D, Thomashow ME: **Low temperature induction of Arabidopsis CBF1, 2, and 3 is gated by the circadian clock.** *Plant Physiol* 2005, **137**:961-968.
- This work illustrates that cold-induced CBF gene expression is gated by the circadian oscillator.
30. Franklin KA, Whitelam GC: **Light-quality regulation of freezing tolerance in Arabidopsis thaliana.** *Nat Genet* 2007, **39**:1410-1413.
- This study reveals coupling between the light signalling and CBF pathways. Light quality alters the amplitude of clock-gated CBF gene expression and the induction threshold for freezing tolerance.
31. Halliday KJ, Salter MG, Thingnaes E, Whitelam GC: **Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT.** *Plant J* 2003, **33**:875-885.
32. Jung C, Muller AE: **Flowering time control and applications in plant breeding.** *Trends Plant Sci* 2009, **14**:563-573.
33. Dalchau N, Hubbard KE, Robertson FC, Hotta CT, Briggs HM, Stan GB, Goncalves JM, Webb AA: **Correct biological timing in Arabidopsis requires multiple light-signaling pathways.** *Proc Natl Acad Sci USA* 2010, **107**:13171-13176.
- The authors used reverse engineering with linear time-invariant (LTI) models and identified the importance of rapid light signalling pathways in circadian regulation.
34. Yanovsky MJ, Kay SA: **Molecular basis of seasonal time measurement in Arabidopsis.** *Nature* 2002, **419**:308-312.
35. McClung CR: **A modern circadian clock in the common angiosperm ancestor of monocots and eudicots.** *BMC Biol* 2010, **8**:55.

36. Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G: **Photoreceptor regulation of CONSTANS protein in photoperiodic flowering.** *Science* 2004, **303**:1003-1006.
 37. Nozue K, Covington MF, Duek PD, Lorrain S, Fankhauser C, Harmer SL, Maloof JN: **Rhythmic growth explained by coincidence between internal and external cues.** *Nature* 2007, **448**:358-361.
 38. Amasino R: **Seasonal and developmental timing of flowering.** *Plant J* 2010, **61**:1001-1013.
 39. He Y: **Control of the transition to flowering by chromatin modifications.** *Mol Plant* 2009, **2**:554-564.
 40. Dennis ES, Peacock WJ: **Vernalization in cereals.** *J Biol* 2009, **8**:57.
 41. Greenup A, Peacock WJ, Dennis ES, Trevaskis B: **The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals.** *Ann Bot* 2009, **103**:1165-1172.
 42. Trevaskis B, Hemming MN, Dennis ES, Peacock WJ: **The molecular basis of vernalization-induced flowering in cereals.** *Trends Plant Sci* 2007, **12**:352-357.
 43. Seo E, Lee H, Jeon J, Park H, Kim J, Noh YS, Lee I: **Crosstalk between cold response and flowering in Arabidopsis is mediated through the flowering-time gene SOC1 and its upstream negative regulator FLC.** *Plant Cell* 2009, **21**:3185-3197.
- Results suggest the presence of a feedback loop between cold response and flowering pathways in Arabidopsis. These cross-pathway connections allow flowering to be delayed by cold spells and suppress the cold response following floral induction.
44. Dhillon T, Pearce SP, Stockinger EJ, Distelfeld A, Li C, Knox AK, Vashegyi I, Vagujfalvi A, Galiba G, Dubcovsky J: **Regulation of freezing tolerance and flowering in temperate cereals: the VRN-1 connection.** *Plant Physiol* 2010, **153**:1846-1858.
 45. Salazar JD, Saithong T, Brown PE, Foreman J, Locke JCW, Halliday KJ, Carre IA, Rand DA, Millar AJ: **Prediction of photoperiodic regulators from quantitative gene circuit models.** *Cell* 2009, **139**:1170-1179.
- The combination of modelling and experimental approaches demonstrated here provides insights into the complexity of external coincidence in flowering time regulation. Quantitative predictions combined with molecular testing revealed a novel function for FLAVIN-BINDING, KELCH REPEAT, F BOX 1 (FKF1) as a major regulator of photoperiodic flowering time operating independently of CONSTANS (CO).
46. Wenden B, Dun EA, Hanan J, Andrieu B, Weller JL, Beveridge CA, Rameau C: **Computational analysis of flowering in pea (*Pisum sativum*).** *New Phytol* 2009, **184**:153-167.
 47. Tardieu F, Tuberosa R: **Dissection and modelling of abiotic stress tolerance in plants.** *Curr Opin Plant Biol* 2010, **13**:206-212.
 48. Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C: **Food security: the challenge of feeding 9 billion people.** *Science* 2010, **327**:812-818.
 49. Parry MA, Hawkesford MJ: **Food security: increasing yield and improving resource use efficiency.** *Proc Nutr Soc* 2010, **69**:592-600.
 50. Tester M, Langridge P: **Breeding technologies to increase crop production in a changing world.** *Science* 2010, **327**:818-822.