a patch, it is advantageous for her to lay male-destined eggs if she has only a few eggs to lay and there are many female-destined eggs present because her male offspring would then experience less competition for mates. However, if she has many eggs to lay, it would be disadvantageous if she laid too many male-destined eggs because they would be competing over fewer females. Given the greater likelihood that small red spider mite eggs will remain unfertilized then females in poor condition would be predicted to produce mainly males, thereby exposing a greater number of haploid genotypes to selection, illustrating the responsiveness of this mechanism to local conditions.

Study of the red spider mite provides an invaluable contribution to our rapidly advancing understanding of the mechanisms of sex determination. Adjustment of egg size production in response to local conditions is suggested to fine tune the sex allocation of offspring. This new level of knowledge means this model system can provide further tests of sex allocation theory, and its constraints, as well as wider evolutionary hypotheses.

References

1. Fisher, R.A. (1958). The Genetical Theory of Natural Selection (New York: Dover).
2. Hamilton, W.D. (1967). Extraordinary sex ratios. Science 156, 477–488.
3. West, S.A., and Sheldon, B.C. (2002). Constraints in the evolution of sex ratio adjustment. Science 295, 1685–1688.
4. West, S.A. (2009). Sex Allocation (Princeton, N.J: Princeton University Press).
5. Macke, E., Magalhaes, S., Khan, H.D., Luciano, A., Frantz, A., Facon, B., and Olivieri, I. (2010). Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite Tetranychus urticae Koch. Proc. R. Soc. Lond. B, in press.
6. Komdeur, J., Magrath, M.J.L., and Krackow, S. (2002). Pre-ovulation control of hatchling sex ratio in the Seychelles warbler. Proc. R. Soc. Lond. B 269, 1067–1072.
7. Ferguson, M.W.J., and Joanen, T. (1982). Temperature of egg incubation determines sex in Alligator mississippiensis. Nature 296, 850–853.
8. Warner, D.A., and Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. Nature 451, 566–568.
9. Ueno, T. (1999). Host-size-dependent sex ratio in a parasitoid wasp. Res. Popul. Ecol. 41, 47–57.
10. Clutton-Brock, T.H., Albon, S.D., and Guinness, F.E. (1984). Maternal dominance, breeding success and birth sex ratios in red deer. Nature 308, 358–360.
11. Saino, N., Romano, M., Caprioli, M., Ambrosini, R., Rubolini, D., and Fasola, M. (2010). Sex allocation in yellow-legged gulls (Larus michahellis) depends on nutritional constraints on production of last eggs. Proc. R. Soc. Lond. B 277, 1203–1208.
12. Fox, C.W., and Czesak, M.E. (2000). Evolutionary ecology of progeny size in arthropods. Annu. Rev. Entomol. 45, 341–369.
13. Radder, R.S., Pike, D.A., Quinn, A.E., and Shine, R. (2009). Offspring sex in a lizard depends on egg size. Curr. Biol. 19, 1102–1105.
14. Roeder, C. (1992). Sex ratio response of the two-spotted spider mite Tetranychus urticae Koch to changes in density under local mate competition. Can. J. Zool. 70, 1965–1967.
15. Trivers, R.L., and Willard, D.E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. Science 179, 90–92.

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Fruit Development: New Directions for an Old Pathway

A recent study investigating the molecular mechanisms of seed pod shattering has shown that the basic helix-loop-helix (bHLH) proteins INDEHISCENT and ALCATRAZ appear to regulate fruit patterning through gibberellic acid (GA)–DELLA signalling, revealing a central role for bHLH family members in GA response specificity.

Colin N. Moran and Karen J. Halliday

Crop yield can be dramatically reduced by seed pod shattering prior to harvest. Oil seed rape farmers loose 11–25% of their crop each year due to asynchronous seed opening and seeds falling to the ground [1]. A new study by Arnaud et al. [2] in Genes and Development defines the molecular circuitry and identifies a pivotal role for the plant hormone gibberellic acid (GA) in Arabidopsis seed dispersal. These findings generate formal links between the GA pathway and fruit developmental patterning while providing a framework to understand the versatility of GA signalling [3]. This improved resolution of the molecular events that underpin seed dispersal expands the application

routes for novel crop varieties with reduced pod shatter.

Like other Brassicaceae, such as oil seed rape, Arabidopsis fruit have a simple structure. Seeds are encased in two elongated compartments separated by a central replum [4]. The seeds are covered by valves, the margins of which meet at the replum. The valve margins comprise two narrow strips of cells, a lignification layer (LL) and a separation layer (SL), that facilitate fruit opening and the efficient release of the seeds [5]. As variations in valve margin structure influence pod shatter rate, the molecular control pathways are an obvious target for crop yield enhancement [6].

In Arabidopsis, a number of genes have been shown to be required

for valve margin development. SHATTERPROOF (SHP1/2) and INDEHISCENT (IND) are valve margin identity factors that promote differentiation of both the lignification layer and separation layer [6,7]. ALCATRAZ (ALC), another identity factor, specifies the SL only [8]. The expression of these genes is restricted to the valve margin layers by the action of FRUITFULL (FUL) and REPLUMLESS (RPL), which are expressed in the valves and replum, respectively [9,10]. Although IND and ALC have been known for some time, their precise method of action in margin identity has remained elusive.

GA has previously been shown to promote cell elongation during Arabidopsis fruit development [11,12]. Here its role appears to be similar to that observed in other organs where GA acts as a prominent growth regulator. A body of work supports the central dogma that GA operates by destabilising and degrading DELLAs, potent repressors of cell expansion and division [13]. The molecular events that underlie this process are initiated by the docking of GA at its receptor GIBBERELLIN-INSENSITIVE DWARF 1 (GID1) [14]. GA–GID1 then binds to DELLA proteins, promoting their
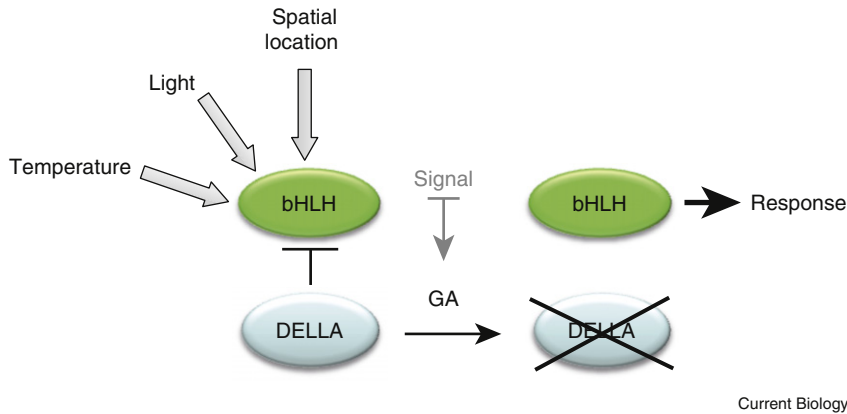
Figure 1. Basic helix-loop-helix (bHLH) transcription factors confer response specificity to the GA–GID1–DELLA pathway.

When GA levels are low, DELLAs bind to and inactivate bHLH transcription factors (*e.g.*, ALC, PIF3, PIF4). A rise in GA levels (triggered by INDEHISCENT promotion of *GA3ox1* in the *Arabidopsis* fruit) leads to DELLA degradation and the release of the bHLH transcription factor that can then resume activity.

ubiquitination by the E3 ligase SCF$^{SLY1/GID2}$ complex and subsequent degradation by the 26S proteasome [15]. Thus, GA drives growth by relieving DELLA-imposed restraint on this process.

The recent study by Arnaud and co-workers [2] demonstrated that in the fruit GA does not simply control elongation growth; it also regulates developmental patterning of this organ. This work established that the classical GA–GID1–DELLA pathway is central to the activity of IND and ALC, basic-helix-loop-helix (bHLH) transcription factors that control valve margin identity. A primary step in the process appears to be the stimulation of GA production by IND. Transcript and chromatin immunoprecipitation (ChIP) analyses provided evidence that IND directly targets *GIBBERELLIN 3-OXIDASE* (*GA3ox1*), which encodes an enzyme that catalyses the final step in the biosynthesis of bioactive gibberellins GA1 and GA4 [16]. In support of this proposal, *GA3ox1 TC-GUS* (*GA3ox1* promoter fused to the beta-glucuronidase reporter gene) expression was observed in the valve margins, where IND is located, while IND loss severely attenuated *GA3ox1 TC-GUS* expression. Furthermore, local GA depletion led to valve margin defects and increased shatter-resistance that resembled, though were less severe than, the *ind-1* mutant. Transmission electron microscopy analysis revealed the source of this milder phenotype. In contrast to *ind-1*, which lacks both

SL and LL, the valve margins of *ga4-1*, a *GA3ox1* mutant, lacked only SL. An SL-specific phenotype had been previously noted in *alc-1*, and complementation analysis supported the notion that ALC and GA3ox1 act in the same pathway. Collectively, these results suggest that ALC and GA3ox1 reside in a branch in the IND pathway.

Although both IND and ALC are bHLH transcription factors, ALC is more closely related to the subgroup that contains the PHYTOCHROME INTERACTING FACTORS (PIFs). PIF3 and PIF4 are well known repressors of light signalling that have recently been shown to bind to DELLA growth repressors through the conserved bHLH region. The rationale is as follows. When GA levels are low, DELLAs accumulate, bind to PIFs and prevent target gene activation. Elevated GA triggers DELLA degradation, relieving this repression [17,18] (Figure 1). Yeast-two-hybrid and bimolecular fluorescence complementation assays conducted by Arnaud and co-workers [2] provided evidence that ALC can interact with the DELLA proteins GAI, RGA and RGL2. Furthermore, genetic evidence was provided that supported a role for GAI, RGA and RGL2 in valve margin formation. The recent observation that other PIF-like proteins (SPT, PIL2 and PIL5) are similarly able to bind to DELLAs in *in vitro* assays collectively suggests that this family of transcription factors may be integral to GA signalling [19].

The study by Arnaud and co-workers [2] has uncovered an integral role for GA signalling in the regulation of fruit valve margin formation and pod shatter. GA appears to have a specific role in the specification of the separation layer in the valve margin. IND is proposed to boost GA levels by directly activating the expression of *GA3ox1*. Arnaud *et al.* postulate that prior to SL specification, DELLA proteins are bound to ALC, restricting its activity. The availability of GA following IND activation degrades DELLAs, liberating ALC, which can now modulate the expression of target genes that lead to SL differentiation (Figure 1). This work and other recent studies highlight the connectivity between PIF-like bHLH transcription factors and GA signalling [17,18]. PIF-like transcription factors are known to control distinct molecular and cellular events [20]. Thus, PIFs appear to provide the specificity that enables GA to control quite disparate responses, such as hypocotyl growth and fruit development. PIFs are also known to integrate external signals such as light quality and temperature; therefore, they may also serve as molecular links between GA and the exterior [17,18]. Connections of this type are vital in nature as the reproductive success of a plant depends on its ability to adapt to constraints imposed by a changing environment.

References
1. Price, J.S., Hobson, R.N., Neale, M.A., and Bruce, D.M. (1996). Seed losses in commercial harvesting of oilseed rape. J. Agr. Eng. Res. *65*, 183–191.
2. Arnaud, N., Girin, T., Sorefan, K., Fuentes, S., Wood, T.A., Lawrenson, T., Sablowski, R., and Ostergaard, L. (2010). Gibberellins control fruit patterning in Arabidopsis thaliana. Genes Dev. *24*, 2127–2132.
3. Harberd, N.P., Belfield, E., and Yasumura, Y. (2009). The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell *21*, 1328–1339.
4. Ostergaard, L. (2009). Don't 'leaf' now. The making of a fruit. Curr. Opin. Plant Biol. *12*, 36–41.
5. Mitsuda, N., and Ohme-Takagi, M. (2008). NAC transcription factors NST1 and NST3 regulate pod shattering in a partially redundant manner by promoting secondary wall formation after the establishment of tissue identity. Plant J. *56*, 768–778.
6. Liljegren, S.J., Ditta, G.S., Eshed, Y., Savidge, B., Bowman, J.L., and Yanofsky, M.F. (2000). SHATTERPROOF MADS-box genes control seed dispersal in Arabidopsis. Nature *404*, 766–770.
7. Liljegren, S.J., Roeder, A.H., Kempin, S.A., Gremski, K., Ostergaard, L., Guimil, S., Reyes, D.K., and Yanofsky, M.F. (2004). Control of fruit patterning in Arabidopsis by INDEHISCENT. Cell *116*, 843–853.

8. Rajani, S., and Sundaresan, V. (2001). The Arabidopsis myc/bHLH gene ALCATRAZ enables cell separation in fruit dehiscence. Curr. Biol. 11, 1914–1922.

9. Ferrandiz, C., Liljegren, S.J., and Yanofsky, M.F. (2000). Negative regulation of the SHATTERPROOF genes by FRUITFULL during Arabidopsis fruit development. Science 289, 436–438.

10. Roeder, A.H., Ferrandiz, C., and Yanofsky, M.F. (2003). The role of the REPLUMLESS homeodomain protein in patterning the Arabidopsis fruit. Curr. Biol. 13, 1630–1635.

11. Chiang, H.H., Hwang, I., and Goodman, H.M. (1995). Isolation of the Arabidopsis GA4 locus. Plant Cell 7, 195–201.

12. Koornneef, M., and Vanderveen, J.H. (1980). Induction and analysis of gibberellin sensitive mutants in Arabidopsis-thaliana (L) Heynh. Theor. Appl. Genet. 58, 257–263.

13. Schwechheimer, C. (2008). Understanding gibberellic acid signaling–are we there yet? Curr. Opin. Plant Biol. 11, 9–15.

14. Murase, K., Hirano, Y., Sun, T.P., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456, 459–463.

15. Dill, A., Thomas, S.G., Hu, J., Steber, C.M., and Sun, T.P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell 16, 1392–1405.

16. Talon, M., Koornneef, M., and Zeevaart, J.A. (1990). Endogenous gibberellins in Arabidopsis thaliana and possible steps blocked in the biosynthetic pathways of the semidwarf ga4 and ga5 mutants. Proc. Natl. Acad. Sci. USA 87, 7983–7987.

17. de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blazquez, M.A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature 451, 480–484.

18. Feng, S., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J., Wang, F., Chen, L., Yu, L., Iglesias-Pedraz, J.M., Kircher, S., et al. (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature 451, 475–479.

19. Gallego-Bartolome, J., Minguet, E.G., Marin, J.A., Prat, S., Blazquez, M.A., and Alabadi, D. (2010). Transcriptional diversification and functional conservation between DELLA proteins in Arabidopsis. Mol. Biol. Evol. 27, 1247–1256.

20. Leivar, P., and Quail, P.H. (2010). PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci. Article in Press, 10.1016/j.tplants.2010.08.003.

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Kinetochores: NDC80 Toes the Line

Kinetochore-associated NDC80 complexes serve as the primary binding site for the plus-ends of spindle microtubules in mitosis. A recent study proposes a novel mechanism for regulating kinetochore-microtubule binding involving NDC80 complex oligomerization, which could be mediated by Aurora B kinase.

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To properly segregate chromosomes during mitotic cell division, kinetochores on each sister chromatid must stably attach to the plus-ends of spindle microtubules. Researchers have long sought to understand how kinetochores not only generate, but also regulate attachments to microtubule plus-ends. Early in mitosis, errors in kinetochore-microtubule attachment are frequent, and kinetochores must continually release attached microtubules to prevent error accumulation. As mitosis proceeds, however, kinetochore-microtubule attachments must eventually be stabilized so that forces can be generated for directed chromosome movements and to satisfy the spindle assembly checkpoint.

A large body of work has demonstrated that the evolutionarily conserved NDC80 complex of proteins (Ndc80/Hec1, Nuf2, Spc24, and Spc25) is a core component of the kinetochore-microtubule attachment site. Perturbation of NDC80 complex proteins prevents the formation of stable kinetochore-microtubule attachments in many cell types [1], and the complex binds directly to microtubules in vitro via the Hec1 and Nuf2 proteins [2]. Portions of the amino termini of both Hec1 and Nuf2 fold into calponin homology (CH) domains [3,4], which are known microtubule-binding motifs [5], and point mutations within the CH domains of either Hec1 or Nuf2 significantly decrease the affinity of NDC80 complexes for microtubules in vitro [4]. Preceding the CH domain in Hec1 is a positively-charged 'tail' (80 amino acids in the human complex) that is required for both high affinity microtubule binding in vitro [3,4] and stable kinetochore-microtubule attachments in vivo [6,7], perhaps through direct binding to the acidic caboxy-terminal 'E-hook' domains of tubulin [6]. The Hec1 tail, for which there are no structural data due to its tendency towards disorder, is phosphorylated by the Aurora B protein, a kinase whose function is to induce kinetochore-microtubule turnover to facilitate attachment error correction in mitosis [8]. Despite intensive in vitro and in vivo study, how the NDC80 complex contributes to the generation and regulation of kinetochore-microtubule attachments remains highly debated. A recent study by Alushin et al. [9], published in Nature, provides a new perspective on the subject, and the authors propose a novel mode of kinetochore-microtubule attachment regulation that is based on oligomerization of the NDC80 complex, mediated by the Hec1 tail.

Alushin et al. [9] used cryo-electron microscopy techniques to build a reconstruction of NDC80 complexes bound to microtubules in vitro. By docking published crystal structures of NDC80Bonsai complexes [4] and tubulin [10] onto their reconstruction, they generated a high-resolution model of the NDC80-complex–microtubule interface (Figure 1A). Their data reveal that NDC80 complexes bind to each tubulin monomer, confirming earlier findings from a study carried out in the Milligan lab using similar techniques [11]. Alushin et al. mapped the point of microtubule contact in the NDC80 complex to a small region they refer to as the 'toe' within the CH domain of Hec1 (Figure 1A). On the microtubule, the toe domain binds a region between tubulin monomers at both the inter- and intra-dimer interfaces. As expected, amino acids in the Hec1 CH domain that were found in an earlier study to be required for high-affinity microtubule binding in vitro [4] were resident within or near the Hec1 toe domain (Figure 1A). A surprising detail revealed in both this and the previous reconstruction study [11] is that the Nuf2 CH domain does not interface with the microtubule lattice (Figure 1A). This is somewhat surprising since in vitro studies demonstrated that mutating even single amino acids within the Nuf2 CH domain severely