Genetic basis of the "sleeping leaves" revealed

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Like most animals, plants also sleep at night, or at least some of them. For instance, the flowers of many species, such as crocus, tulip, and morning glory, are open during the day or part of the day and close at night. Based on such observations, in 1751, the Swedish botanist Carl von Linné suggested combining several plant species in which the flowers open or close at specific and different times of the day to build a "*Horologium Florae*" (flower clock) that would accurately and colorfully predict time. Such daily movements of plants are not limited to flowers. In his book entitled *The Power of Movement in Plants*, Darwin (1880) described many examples of "sleep movements of leaves" and provided a "List of Genera, including species the leaves of which sleep" (1). Among these, he noted that the legume family "includes many more genera with sleeping species than all the other families put together." He also described a specialized organ, called a joint, cushion, or pulvinus responsible for such movement (1). In PNAS, Chen et al. (2) identify the genetic determinant for the formation of these pulvini in three legumes: *Pisum sativum* (pea), *Medicago truncatula* (barrel medic), and *Lotus japonicus*.

Contrary to intuition, plants are capable of moving in response to environmental stimuli. This movement is achieved either through irreversible differential growth or through reversible changes in turgor. An example of differential growth is the growth toward a light source (phototropism) observed in the majority of plant shoots. Tropisms are plant movements induced by directional stimuli, such as light or gravity. In addition, plants can move in response to nondirectional factors, such as humidity or contact. These movements are called nastic responses. Nyctinasty, the proper name for the "sleep movements of leaves" is a well-known example of a nastic response. In this case, plants close up their leaves and petals in response to the onset of darkness. Because it is a rather fast response, it does not involve differential growth but changes in cellular turgor.

The pulvinus is the organ responsible for the nyctinastic leaf movement. It is a specialized structure located at the base of the petiole of leaves or the petiolule of leaflets in the case of compound leaves (Fig. 1*A*). In the pulvinus (Fig. 1 *B* and *C*), the central vascular bundles and the supporting tissues (often, sclerenchyma) are surrounded by parenchyma. The outer cells of the parenchyma, called the motor cells, undergo water-driven volume changes and are the ultimate effectors of movement. Motor cells are distributed into two positionally and functionally opposed regions: extensor and flexor. Extensors cells are located in the upper side of the organ, whereas flexors are located in the lower side. During leaf opening, leaflets move downward by the simultaneous increase of turgor pressure in extensor and decrease in flexor cells. During closing, the inverse occurs, extensor cells shrink, and flexor cells swell, moving leaflets upward (3). These turgor changes in the motor cells are caused by ion movements followed by massive water flux across the plasma membrane. Swelling is caused by proton pump-driven accumulation in the cytoplasm of K^+ and Cl^-. This increase in solute concentration lowers water potential inside the cell, and thus drives the entrance of water in the cell. Shrinking is caused by a passive leaking of solutes that is accompanied by water loss. It is currently accepted that the osmotic volume changes of motor cells are analogous to those of stomatal guard cells (4, 5).

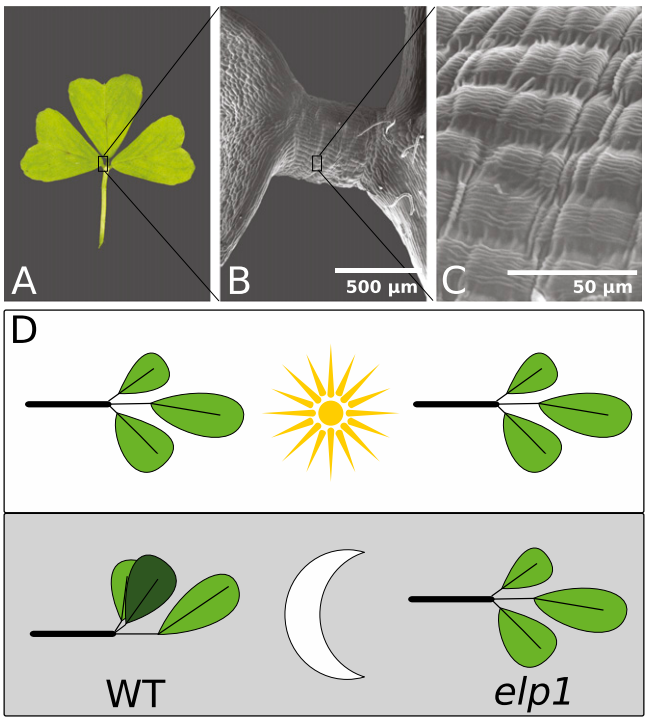

Fig. 1. Leaf of *M. arabica* (*A*) shows a detail of the pulvinus (*B*) and the extensor cells (*C*). (*D*) Schema depicts nyctinasty in *M. truncatula* WT and *elp1* mutant.

There is abundant literature describing the anatomy of the pulvinus and the physiology and biomechanics of nyctinastic movements in legumes (e.g., reviewed in 3). However, nothing was known about the development of this organ, probably because *Arabidopsis* lacks an equivalent structure. The report of Chen et al. (2) starts to fill this void through the identification of the genetic factor that determinates pulvinus formation in legumes. This collaborative work between teams of three different continents working on three different legume models nicely illustrates what can be achieved when the use of large, well-established collections of mutants meets the most advanced plant molecular genetic approaches.

The whole story started more than 50 y ago, when Stig Blixt identified a pea mutant he called *petiolulatus* in which foliar pulvini are replaced by petiolules (6).

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This mutant was later renamed *apulvinic* (*apu*), according to another mutant independently found in 1979 by D. M. Harvey (7). Although pea is a very nice model for performing genetic analyses, it is somehow more difficult to clone genes from this species, and this probably explains why no further progress toward this has been made. In 2003, Kawaguchi (8) identified from a population of chemically mutagenized *L. japonicus* a mutant that could not close its leaflets at night because of an absence of differentiated pulvini, which was therefore called *sleepless* (*slp*). Again, cloning the *SLP* gene through fine mapping was not an easy task. Finally, a similar mutant called *elongated petiolule1* (*elp1*) with pulvini replaced by longer petiolule-like structures was described in *M. truncatula*. In this mutant, leaves remain open at night (2) (Fig. 1*D*). The combination of fine genetic mapping of the *elp1* mutation with the identification of the flanking sequences of newly generated *elp1* alleles through the insertion of a retrotransposon allowed the identification of the *ELP1* gene. From this, the *APU* and *SLP* genes could be identified as *ELP1* orthologs bearing mutations in the respective pea and *L. japonicus* mutants. Therefore, the work of Chen et al. (2) reveals that the formation of the pulvinus in legumes is likely to be regulated by a conserved genetic network orchestrated by the *ELP1*/*APU*/*SLP1* genes. Identification of these genes also provides a straightforward way to test whether pulvinus formation in more distantly related species is controlled by the same genetic determinants.

What do the mutant phenotypes tell us about ELP1/APU/SLP functions? In the absence of these genes, the small, isodiametric, epidermal pulvinus cells with a highly convoluted surface are replaced by much larger and elongated petiole-like epidermal cells (2). This change in the cell type and expansion pattern may explain the elongated petiolule phenotype of the *elp1*/*apu*/*slp* mutants. Conversely, overexpression of ELP1 in *M. truncatula* leads to dwarf plants, with shorter petioles and leaf rachises, which correlates with a reduction in the size of the epidermal cells in the transgenic lines. Interestingly, in these plants, the small epidermal cells of the petiole and rachis show some convolution at their surface reminiscent of pulvinus cells, indicating that ELP1 may be sufficient to some degree for the acquisition of the pulvinus identity. Confirmation of this hypothesis awaits further identification of molecular markers of the pulvinus.

Formation of the pulvinus in legumes is likely to be regulated by a conserved genetic network orchestrated by the ELP1/APU/SLP1 genes.

ELP1/*APU*/*SLP1* codes for nuclear-localized proteins belonging to the plant-specific LATERAL ORGAN BOUNDARIES domain (LBD) transcription factor family (2). In the past years, LBD members have been shown to have essential regulatory functions for the development of plant lateral organs (9, 10). LBD proteins have a characteristic N-terminal lateral organ boundaries (LOB) domain that contains a putative DNA-binding domain consisting of four conserved cysteines in a CX2CX6CX3C motif and a 30-aa-long domain predicted to form a coiled coil reminiscent of a leucine zipper that may be involved in interaction with LBD or other proteins. The LBD family contains 43 members in either *Arabidopsis* or maize, and the LOB domain accounts for the specificity of the members of this family (11). Close homologs of the *ELP1*/*APU*/*SLP1* genes can been found in other species in which no similar pulvinus are observed, such as maize and *Arabidopsis*. Some of them, like LOB in *Arabidopsis*, have been implicated in the establishment of the frontiers between meristem and lateral organs (10). Similarly, *ELP1* is expressed very early on in the basal region of the leaflet, the region that will later differentiate into the pulvinus, in a fashion reminiscent of a frontier gene (2). Overexpression of ELP1, like overexpression of LOB, leads to dwarf plants (10), suggesting that one common function of these genes would be to control cell expansion. Therefore, ELP1 may share some functions with related LBD genes from other species, although retaining species- and even organ-specific roles, as shown by the differential response of *M. truncatula* rachis and petiolule to ELP1 overexpression (2). Further comparative analysis of the role of these genes between different species, including the elucidation of the downstream genetic network, will be necessary to understand how ELP1 triggers pulvinus differentiation. By identifying a key determinant of the formation of a specialized plant structure, the work of Chen et al. (2) provides a unique opportunity to understand better the genetic basis and evolution of the diversity observed in plants.

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