

# Introduction

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In the Introduction of the old Chinese text, *Da Xue* (*Great Learning*), there is a passage that is reproduced on the left side of this page. In a free translation, this passage says: “One who destroys his roots will never be able to keep order in his branches.” This metaphor can be understood as the requirement to maintain good foundations in order to provide proper future development. It also may hint on the establishment of Bonsai trees. The Bonsai practice of obtaining miniature trees was started in Japan about 1000 years ago but it was based on an older Chinese practice to trim, continuously, the roots of young trees and by that, to obtain dwarf trees.

## Remarks on the Study of Plant Architecture

With respect to architecture of plants, there are two points that deserve some discussion. Some attention to these points, but from a different point of view, was already presented in my previous book (Galun, 2007). The first point is the relevance of “tools” in the history of the study of pattern formation. In a way, the history of understanding plant patterning is parallel to the history of emerging “tools” that enabled progress in this field of endeavor.

Looking back, the first decisive “tool,” that was of benefit to developmental biology, was the optical lens. The use of the optical lens is well known in the history of astronomy: lenses provided Galileo

Galilei (1564–1642) with a much sharper vision of celestial bodies than looking at these bodies with naked eyes. Galilei was a member of the first post-Renaissance academy that was founded in Italy in 1603 by Federico Cesi: the Lincean Academy (see Freedberg, 2002). Other members of this academy used optical lenses to probe into the morphology and the anatomy of various organisms, including plants. Such probings were then carried out also by investigators in other countries, such as England (e.g. Robert Hooke, 1635–1703) and the Netherlands (e.g. Antony van Leeuwenhoek, 1632–1723). Not much later (in 1759), Caspar Friedrich Wolff submitted his doctorate thesis (“Theoria Generationis”) to the University of Halle in Germany, in which one part out of three was devoted to the development of plants. In his thesis, Wolff stated that all the parts of plants (angiosperms), but roots and shoots (i.e. including flowers), are modified leaves. This statement was made several years earlier than a similar statement on the metamorphosis of leaves made by J.W. von Goethe, although Goethe’s statement was commonly cited in the botanical literature. Since about 150 years ago, several novel “tools” were developed, such as chemistry, biochemistry, genetics, electron microscopy, genetic transformation, system biology, etc.

An important “tool” for development biology investigations is the *organism* that serves these investigations. In animals, it was the fly *Drosophila melanogaster* and its related species that was employed since the early years of the 20th century by T.H. Morgan (1866–1945) and associates, and subsequently by many other geneticists and developmental biologists. *Drosophila* flies kept their place in the “Hall of Fame” till present, hence for about 100 years. In plant research, the favorite model plant is *Arabidopsis thaliana* (Arabidopsis). Arabidopsis has many features that render it a model plant of choice for genetic and developmental biology studies. Arabidopsis has an impressive “history” that was reviewed in detail by Meyerowitz (2001). The first botanical information on Arabidopsis is attributed to Johannes Thal (1542–1583), a physician from Thüringen, Germany. *Arabidopsis thaliana* changed names. Linnaeus termed it *Arabis thaliana*. It was later given the name *Stenophragma Thalianum*. When the German botanist/geneticist Friedrich Laibach (1885–1967)

found (in 1907) that this plant has only five pairs of chromosomes, he still used the latter name. But in a later publication, in 1943, where Laibach recommended this plant for genetic studies, he used the name that is used presently: *Arabidopsis thaliana*. The species name, *thaliana*, was given in the honor of Johannes Thal, mentioned above. The torch of *Arabidopsis* investigations in Germany was passed over to Röbbelen, in Göttingen, who started to publish studies on this plant in 1956. The gospel of *Arabidopsis* as a model plant for genetic studies probably came to the USA from Hungary, when due to the Hungarian revolt in 1956, George P. Redei immigrated from Hungary to the USA. He brought the *Arabidopsis* to the University of Missouri in Columbia. In Germany, Andreas Müller of the Gatersleben Institute (then in the DDR) as well as other investigators performed *Arabidopsis* studies. As indicated above, Redei planted the “seeds” of *Arabidopsis* in the USA. Already in 1965, when the first International Symposium on *Arabidopsis* was held in Göttingen, in honor of Laibach’s 80th birthday, that the US investigators attended. A few years after his arrival in Columbia, Redei began to publish a long list of papers on the genetics of *Arabidopsis*. The yield of the early years of Redei’s work was published in two reviews (Redei, 1970, 1975). The advantages of *Arabidopsis* as a model plant appealed to several US investigators, such as Chris Somerville, then in East Lansing, Michigan (e.g. Somerville and Ogren, 1979) and Elliot Meyerowitz, at the California Institute of Technology. The latter, who came from the *Drosophila* world, set up a very prolific *Arabidopsis* laboratory. In his historical review, Meyerowitz (2001) detailed the advantages of *Arabidopsis* as a “tool” in genetic and development studies. He mentioned the ease of mutagenesis and the screening of mutants, the small genome that is convenient for gene cloning and the ease of genetic transformation. To that, one should add, now, one of the greatest advantages of *Arabidopsis* as a model plant: its genome is completely sequenced and the respective information can be accessed readily by every investigator. It can be claimed that other plants, such as tomato, petunia, pea, rice and maize, were successfully used in studies of plant pattern formation, but *Arabidopsis* had a central role as a “tool” in these studies.

With each additional “tool” that was added to the arsenal of investigations, the understanding of patterning was elevated to a higher level. The history of the evolution of knowledge of plant architecture was provided in Galun (2007), where several books on this subject were recommended (e.g. Sachs, 1991; Wolpert, 1998; Coen, 1999) as general references for studies on the development of patterns in plants.

The second point that I wish to bring up is about genes that are controlling a three-dimensional structure. The novice may infer that there are genes that are well defined (i.e. having known DNA sequences) that are leading to certain three-dimensional structures in plants, such as trichomes. This is obviously not so. Known genes may *modify* the three-dimensional structures (as reducing or increasing the number of cells in trichomes or affecting trichome branching), but we lack knowledge on genes that are causing the realization of the *idea* of the three-dimensional structure. In other words, in no case we know, in a cellular organism, how a linear information (the DNA sequence) is causing the formation of a three-dimensional form. We have to go “down” the phylogenetic ladder and reach the bacteriophages in order to find a system in which we know how such DNA sequences cause the final shape of a mature T4 bacteriophage. In T4, we are very close to a situation in which we can add, into a “soup”, defined DNA sequences that cause the synthesis of specific proteins and by ordered sequence of these syntheses, will obtain viral “heads”, viral base plates, viral “tails,” etc. and then ultimately, mature and functional bacteriophages. What is known in T4 is not known even in bacteria, let alone in multicellular organisms.

There is another “unknown” in patterning: for correct patterning of a cell (or rather the genome in a given cell) it should receive and sense information on its *position*. Both the spatial and the temporal positions are vital. This means where in the space of a given organism and even the organ of an organism, the cell is located. Also vital is the information on the developmental stage of the organ or the organism, in which the cell is harbored. This spatial/temporal information, together with the influx of hormones, will then regulate the expression of specific genes required for correct patterning in the

given cell. Only in very rare cases do we know what are the cues for position information. One example is the zygote of the brown alga *Fucus*; when illuminated by an unidirectional light beam, the embryo will initiate apical hairs towards the source of the light and rhizoids at the opposite side of the zygote (Galun and Torrey, 1969; Torrey and Galun, 1970). Another example is root epidermis cells that are destined to become root hairs (in *Arabidopsis*). In this case, if the epidermal cells are in contact with *two* cortex cells, genes that trigger the formation of root hairs will be activated in the epidermal cell, but if only *one* cortex cell contacts the epidermal cell, no root hair will be formed. We should keep in mind that the positioning of a cell in a plant is more complicated than positioning a driver with the aid of a GPS instrument, in a specific location, because in the latter case, the localization is on a two-dimensional grid while in the former case, the localization should be in a three-dimensional grid. Nevertheless, there is already some progress in understanding positioning in angiosperms. This knowledge concerns the phenomenon known as “shade avoidance.” It appears that plants have an efficient (but rather complicated) way in which they avoid the shade of another plant and the shoot then escapes the shade of another shoot. This set of plant responses was termed SAS for shade avoidance syndrome. The presence of a nearby plant or shoot causes a reduction of the ratio of red (R) to far-red (FR) light. The relative increase of FR light is perceived by the phytochromes of the leaves and in a cascade of regulatory processes (most of them not yet fully understood, but probably involving the phytohormone auxin), causes the SAS. More details on SAS and on light-regulated transcriptional networks in plants can be obtained from a recent research publication (Roig-Villanova *et al.*, 2007) and a review (Jiao *et al.*, 2007).

There is a further note on the establishment of specific architectures in plants that may be termed “The Pillars of the Mosque of Acre.” I eluded on this metaphor in my previous book (Galun, 2007) but here is the essence of its meaning. The Mosque of Acre was built by the Turkish ruler of Lebanon and Syria, Achmad (al-Jazar — the beheader) Pecha. Achmad Pecha built the mosque in 1781 with an

impressive court full of marble pillars. When one looks closely at the pillars, it is clear that the pillars differ considerably from each other. They are Doric, Ionic, Corinthian or Composite. No wonder: Achmad Pecha collected marble pillars from numerous Greek and Roman ruined temples of ancient cities along the Eastern Mediterranean shore. The pillars were modified to fit their setup in the court of the mosque. Well, nature acts in a way similar to Achmad Pecha: when a novel regulation of patterning is required, it does not invent completely *novel* regulatory components, but rather amends *existing* components for servicing the required novel regulations. This point should be remembered when we deal with the regulation of patterning. The regulatory systems frequently appear to us as excessively complicated; why were they not composed in a simpler way? However, we should recall that the various complex systems were formed from already available components (some of them already complex) that were further modified as required.

## Ubiquitination (Ubiquitylation)

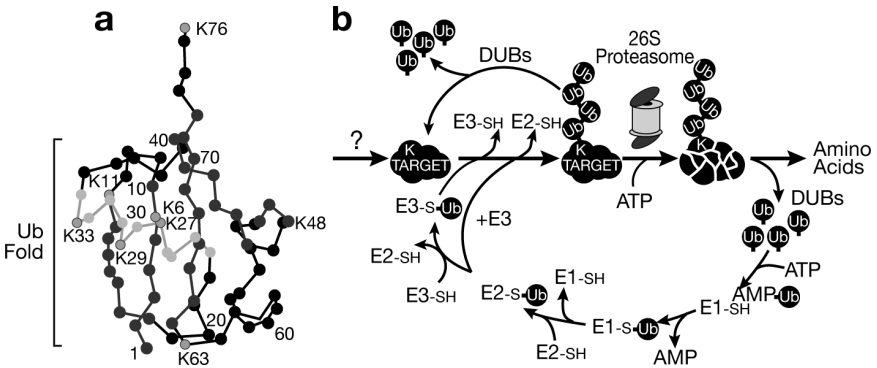
The ubiquitin-proteasome system (UPS) is a widespread process that causes the regulated protein degradation in eukaryotes. It was revealed only a little over 10 years ago by Aaron Ciechanover, Avram Hershko and Irwin Rose (Hershko and Ciechanover, 1998), but since then it was found to have a major role in many processes. We shall see in Part I below that the activity of several phytohormones is involved with a UPS. Thus, a short description of the UPSs in plants will precede the handling of the individual phytohormones. In *Arabidopsis*, more than 1400 genes encode components of the ubiquitin-proteasome protein degradation. While the UPS has several variants, there is a similar overall strategy of the UPS that assures that the degradation will affect very specific proteins and will take place only in the presence of specific signals. For our deliberations, ubiquitination is of great interest because it is involved with phytohormones and plant patterning. Albeit, even if we focus on plants, the UPS is rather elaborated. To get an idea of how complicated it is, I shall note that in their review, Dreher and Callis (2007) had to list over 200

abbreviations that were used in their text! The UPS will therefore be described here only schematically and additional information will be provided in Part I, where the various phytohormones are handled. Readers interested in more details on UPS of plants are referred to reviews on this subject (e.g. Smalle and Vierstra, 2004; Moon *et al.*, 2004; Schwechheimer and Schwager, 2004; Dreher and Callis, 2007).

The main components of UPS are the ubiquitin (Ub), the proteasome and three E proteins: Ub activator (E1), Ub conjugating enzyme (E2) and Ub ligase (E3). In most eukaryotes, there is a small number of E1 proteins, more E2 proteins and a much greater number of E3 proteins. The latter many E3 ligases provide the final specificity: rendering a substrate to be degraded in the proteasome. The UPS is schematically shown in Fig. 1 and a scheme that includes the proteasome is shown in Fig. 2.

The Ub is a 76-amino acid protein and it is attached to the targeted substrate by a series of steps that involve the E proteins, until a “tail” of poly-Ub (commonly a chain of four Ub units connected by lysyl linkages) is attached to it. Then the poly-ubiquitylated target can enter the proteasome. The 26S proteasome is a 2 mD proteinous complex. The Ub is very similar in all eukaryotes. Plant Ubs differ from yeast Ub by only two amino acids and from animal Ub by three amino acids. Ubs are identical in all plants. The bulk of the Ub protein has a compact globulus shape. A flexible C-terminal extension protrudes from the Ub that includes an essential glycine. The glycine can interact with the E1, E2 and in some cases, with E3. Hence, this glycine serves to connect the Ub with its target protein that is destined for degradation. Interestingly, although Arabidopsis has 28 Ub-coding genes, they all encode the same Ub protein.

The modification of the Ub starts with the formation of an acyl phosphoanhydride bond between AMP and the above-mentioned glycine of the Ub, mediated by ATP. Then a thiol-ester is formed between the glycine of the Ub and a cysteine of E1. The activated Ub is then transferred to a cysteine in an E2 by trans-esterification. Finally, the Ub-E2 intermediate can bind to the substrate by E3. Commonly, more Ub units are added so that the target protein is



**Fig. 1.** Ub and the Ub/26S proteasome pathway. (a) The three-dimensional structure of plant Ub. The Ub fold is indicated with its mixed  $\beta$  sheets and  $\alpha$  helix. The lysines (K) at positions 6, 11, 27, 29, 48 and 63 that can participate in forming polyUb chains and the C-terminal glycine that forms the isopeptide bonds with targets. (b) Diagram of the Ub-26S proteasome pathway. The pathway begins with the adenosine triphosphate (ATP)-dependent activation of ubiquitin (Ub) by Ub-activating enzyme (E1), followed by the transfer of the Ub to a Ub conjugating enzyme (E2) and finally ends with the attachment of the Ub to a lysine in the target protein with the help of a Ub ligase (E3). Once the Ub-protein conjugate is formed bearing a poly-Ub chain, it is either recognized by the 26S proteasome and degraded in an ATP-dependent process with the concomitant release of Ub monomers, or the conjugate is disassembled by deubiquitinating enzymes (DUBs) to regenerate both the target protein and Ub intact. (From Smalle and Vierstra, 2004).

attached to a poly-Ub chain. In plants, the Ubs are usually linked to each other via their lysine48. The activating enzyme E1 is a relatively large protein (of about 1100 amino acids) but it is a very efficient enzyme; even a low concentration of it is sufficient for the UPS. The binding of E1 to Ub does not lead to specificity of target decomposition. The Arabidopsis genome encodes only two E1s. The Ub-conjugation enzyme E2 already leads to some specificity. Thus, there are many different E2s encoded by the genome of each organism. Arabidopsis produces at least 37 E2, and eight additional genes encode putative UEV (ubiquitin-conjugating E2 enzyme variants). The E3s are the members of the UPS that bestow specificity.



target, causing conjugation without forming an E3-Ub intermediate. The vast number of the F-box variants cause the target specificity. Although the E3 proteins seem already rather complicated, there are several accessory factors that are associated with SCF E3. The details are beyond the scope of this book but can be found in the above-mentioned reviews (e.g. Smalle and Vierstra, 2004). We should keep in mind that several of the subunits of SCF have numerous variants; the combination of these variants could generate an “infinite” array of distinct SCF ligases, each with its specificity to a target (substrate) protein that will enter the proteasome. While the RING/U-box types of E3 ligases are linked covalently with an E2 that carries a Ub, there is a different linking with the HECT domain E3 ligases. In the latter, Ub polypeptides form a covalent thioester linkage with a cysteinyl sulfydryl group on HECT protein before being transferred to a lysine on the substrate (target protein). Again, as noted above, one may wonder why was the “palace” of the UPS built with such complicated building stones? The answer is that nature acts as Achmad Pecha (the al-Jazar of Acre): amending available building stones rather than using stones from the virgin rock.

A rough scheme of the 26S proteasome is shown in Fig. 2. It is an ATP-dependent proteolytic complex that degrades Ub-conjugates. Plants have several isoforms of proteasomes. The proteasomes are rather elaborate proteinous organelles. Again, the details of the structure of proteasomes will not be provided in this book. They were revealed mainly in yeast and animals. Albeit there is also some information on proteasomes of plants, based particularly on studies with *Arabidopsis* and rice. Here, only the main features of plant proteasomes will be given. The 26S proteasome contains about 30 principal subunits that are located in two subcomplexes: the 20S core protein (CP) and the 19S regulatory particle (RP). The CP has a wide range of proteases; it is a barrel-shaped organelle that has four “rings”, each of which is composed of seven subunits. On both ends of this “barrel” are the  $\alpha$ -subunit rings and the middle two rings are composed of  $\beta$ -subunits. In the middle of the “barrel” is an active-site threonine. The “barrel” has trypsin-like and chemotrypsin-like activities, rendering the CP capable to clear most, if not all, peptide bonds. The “barrel” allows only unfolded proteins to enter the protease chamber.

The RPs associate with one or both ends of the CP and cause ATP-dependence as well as specificity to Lys48-linked poly-Ub chains to enter the proteasome. The RP has two subcomplexes: the “Lid” and the “Base.” The RP assists in recognizing and unfolding the poly-Ub-tagged target proteins. The Ubs are removed, by the activity of the deubiquitylating enzyme (DUB), and released while the unfolded target protein then enters the proteolytic chamber of the CP. One may claim that the proteasome is an “environment-friendly” garbage disposal device — it separates the Ub particles from their complex with the target protein, digests the target protein into amino acids and allows the Ub particles to be reused as well as the amino acids to be polymerized into new proteins.

One final remark before we leave this general introduction to ubiquitylation and turn, in Part I, to UPSs related to specific hormones. The general strategy in many UPSs may be exemplified by the involvement of UPS with auxin (e.g. IAA) responsive genes (RG). The gene expression is regulated in a dual manner: there is a transcription activator (TA) that activates the expression of the auxin responsive gene (ARG); but commonly, this does not happen because the activity of TA is inhibited by another protein, the transcriptional repressor (TR) that binds to the TA. Only when the TR is eliminated, will the TA function and the ARG will be expressed. The elimination of the TR is mediated by the UPS. Before this happens, the TR has to undergo an elaborate process that includes the involvement of E1, E2 and E3. The E3 provides the final specificity; it is a multi-subunit complex and in the presence of IAA, will “mark” (attaching Ub) the TR for future degradation in the proteasome. For all this to happen correctly, a large number of genes are involved. Mutations in any of these may severely affect the UPS process. We are thus facing a situation that is problematic to men and plants — men are bewildered by the complexity of the process and plants have to cope with a very elaborate system that must flow without any faultiness.

As said above, the roles of UPS will be handled again, in the chapters dealing with the various types of phytohormones (e.g. auxins, gibberellins, abscisic acid, brassinosteroids, ethylene, jasmonates). A list of identified UPS components of Arabidopsis is updated on a website dedicated to this subject (<http://plantsubq.genomics.purdue.edu>).