7.1.2 Shoot apical meristems

Shoot apical meristems are minute yet complex structures that are ensheathed within new developing leaves or bracts. A vegetative meristem gives rise to leaves or other organs, for example thorns, tendrils, axillary buds and internodes (Figure 7.7a). Axillary buds are themselves complete shoot meristems from which branches are produced (cf. lateral roots described above). In angiosperms, when a plant shifts from vegetative to reproductive growth some meristems undergo a transition to the reproductive state and give rise either to multiple flowers in an inflorescence, as in mango (Figure 7.7b), or to a single terminal flower, for example a poppy or waterlily (Figure 7.7c). All axial growth from meristems, be they vegetative or floral, is continuous or indeterminate until topped by the formation of a flower. When this occurs, floral organ primordia arise in whorls from the shoot meristem and differentiate into the familiar sepals, petals, stamens and carpels. Sometimes, however, the indeterminate inflorescence meristem reverts back instead to a vegetative status. Think of a bottlebrush or a pineapple with a leafy axis extending beyond the flower or fruit (Figure 7.8a). What determines whether a meristem is vegetative or floral? In many species, environmental signals cause the switching between vegetative and floral states (Section 8.3) and a picture is now emerging of the genes and molecular mechanisms responsible for defining structures that are generated by meristems (see below).
Although meristems function as generic sources of cells for differentiation into organs, each type of meristem is programmed to produce only certain structures. Across all species, there is a small, finite range of these structures, yet we observe an amazingly diverse array of final vegetative and floral morphologies. A leaf is always recognizable as a leaf but consider the vast structural differences between a pine needle, a waterlily pad and a tree fern frond. The generation and spatial patterning of plant organs are determined by early events within the vegetative meristem. This precise positioning of organs around the shoot meristem is called phyllotaxis. Later in development, a dramatic meristematic switch will give rise to a terminal inflorescence, often with an abrupt change in patterning of organs. Phyllotaxis also applies to floral structures, for example spiral patterns of scales of a pine cone or flowers of Grevillea (Figure 7.8b), or vertical rows on a Banksia inflorescence (Figure 7.8c). Organ spacing is a terminal determinant of shoot appearance. For example, leaves forming a rosette as in Arabidopsis are separated by short internodes compared with longer internodes intervening between whorls of leaves of a blue gum seedling. The resulting morphologies are strikingly different. The question of what determines phyllotaxis and internode length is discussed later.

(a) Shoot meristem anatomy
Shoot meristems are small, with a dome typically of 100–300 µm in diameter consisting of no more than a few hundred cells. In the 1970s, Williams (1974) published elegant reconstructions of shoot meristems derived from serial sections (Figure 7.9) which revealed the extent of variability in the shape and dimensions of shoot apices. However, the overriding organisation is of a central dome with groups of cells partitioned off from its periphery to form either determinate organ primordia or secondary meristems (axillary buds). Some cells in between are not destined to become primordia and will instead later become the internodes of the axis. Different models have been proposed to describe the regions of shoot meristems. In a functional sense, vegetative meristems have three main components: the central zone, peripheral zone and the file meristem zone, all of which tend to disappear or become indistinct in inflorescence meristems (Figure 7.10).

Figure 7.9 Three-dimensional reconstruction of vegetative shoot apex of lupin showing central dome and spirally arranged leaf primordia on flanks.

(Based on Williams 1974; reproduced with the premission of Cambridge University Press)

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Figure 7.10 The structure and zonation of shoot apices can be described in two main ways. Zonation in vegetative apices (top) can be based on relative cell division rates and differential staining: central zone (cz, slow division rate), peripheral zone (pz) and file meristem zone (fmz). Layers of cells are usually also visible. L1 and L2 are often referred to as the tunica and L3 as the corpus. The layering remains visible during early floral development (bottom), but the central zone disappears in determinate inflorescences.

(Based on Huala and Sussex 1993)

<table>
<thead>
<tr>
<th>Cell layer</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Epidermis of shoot organs, stem, leaves</td>
</tr>
<tr>
<td>L2</td>
<td>Mesophyll cells of leaf</td>
</tr>
<tr>
<td>L3</td>
<td>Vascular tissue of leaf and stem</td>
</tr>
<tr>
<td></td>
<td>Pith and cortex of stem</td>
</tr>
</tbody>
</table>
Superimposed on this functional zonation are usually three distinct cell layers which give rise to separate cell lineages (Kerstetter and Hake 1997). These cell layers, designated L1, L2 and L3, are distinguishable by their positions in the meristem and their pattern of cell divisions, and are evident in both vegetative and reproductive meristems. Surface cells of L1 divide anticlinally while within the meristem and during subsequent differentiation of the organs. Not surprisingly, they form the epidermis. Within the apical dome, the plane of cell division within L2 is also purely anticlinal, but later on during organ formation divisions occur in other planes. In contrast, cells of the deepest layer, L3, divide in all planes. The two inner layers, L2 and L3, contribute cells to form the body of the plant with the proportion of cells derived from each layer varying in different organ types. Although the cell lineages produced by each layer usually contribute to distinct regions within each organ (Table 7.3), invasion of cell derivatives of one layer into another has been observed.

Invading cells differentiate in accordance with their new position, which we interpret to mean that developmental fate of cells appears to be governed more by position than by cell lineage. However, meristematic cells may already be functionally distinct as evidenced by patterns of gene expression which reflect the layered cellular organisation (Figure 7.11 and see Section 10.3.3).

Table 7.3

<table>
<thead>
<tr>
<th>(a)</th>
<th>Vegetative</th>
<th>Inflorescence</th>
<th>Floral</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tobacco</em> Δ3 cDNA</td>
<td>L1</td>
<td>L2</td>
<td>L3</td>
</tr>
<tr>
<td><em>Sinapis</em> pSFD5.44 cDNA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Maize</em> Krl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td>Modified</td>
<td>ND</td>
</tr>
<tr>
<td><em>Arabidopsis</em> CDC2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tomato</em> PDF</td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td><em>Tomato</em> dUTPase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td></td>
<td>Floral stage-specific</td>
<td></td>
</tr>
<tr>
<td><em>Tobacco</em> F22 cDNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arabidopsis</em> Florencia and Squamosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arabidopsis</em> Leafy and Apetala 1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 7.11 Complexities of meristem functioning are revealed by specific staining (in situ mRNA hybridisation and antibody techniques) for expression of different genes. Dark shading represents protein patterns, light shading represents mRNA expression. Many of these patterns match the zonations in Figure 7.10.

(Based on Meeks-Wagner 1993)
In order to maintain the very precise organisation of vegetative meristems over long periods, or to accommodate rapid changes during flower formation, some signalling process must exist to coordinate division between the cell layers. Evidence for such signalling has been established through the development of chimeras, where genetically different cell types exist together in a single apex, yet still achieve normal developmental patterns (Figure 7.12).

(b) Phyllotaxis and internode length
We previously raised the question of what determines phyllotaxis and internode length. Organs derived from the shoot meristem can arise in whorls (two or more organs simultaneously at one node), alternately (two ?les displaced by 180° with a single organ at each node) or in spirals (each organ displaced from the previous one by approximately 137° with a single organ at each node). These organs may be separated by very short or long internodes. Phyllotaxis patterns are usually stable, but often change abruptly with floral induction or when seedlings undergo transition to their mature morphology. In many species of Eucalyptus, this 'phase change' from juvenile to adult is very striking and is accompanied by a change from whorled to alternate or spiral phyllotaxis (Figure 7.2b). How is the change in phyllotaxis effected? We gain some insight from experiments on chrysanthemum meristems where application of the inhibitor of polar auxin transport TIBA (tri-iodo benzoic acid; see Section 9.1.3) induced changes in inter-node length and displacement angle between leaf primordia. The data are consistent with a change from 137° spiral (control) to alternate (50 ppm TIBA) phyllotaxis and are presumed to result from increased concentrations of auxin in the meristem resulting from inhibition of transport away from the existing primordia. Meristems are deduced to be sites of auxin synthesis (Schwabe and Clewer 1984). One interpretation is that each primordium acts as a ?eld with a de?ned radius of inhibition preventing other primordia from initiating too close to it. Another option is mechanical control by pressure and tension gradients within the meristem (Green et al. 1996).

Supporting evidence comes from experiments on cells in tissue culture in which applied pressure altered their morphogenetic patterns. At a whole-plant scale, tension and compression wood in trees are further examples of speci?c developmental responses to physical forces.

(c)??Determination of shoot meristem and organ identity

Genes controlling meristem identity

What determines whether a meristem is vegetative or re-productive has long been a vexing question. Now, by using molecular technology and studying the transition of meristems from vegetative to reproductive in species amenable to analysis of single gene mutations, for example Arabidopsis and Antirrhinum, some of the mystery is being unravelled. Considering the obvious morphological and functional dif-ferences between a vegetative shoot and an inflorescence, we can reasonably assume that a number of genes will be expressed sequentially during determination of ?rst the inflorescence, second the flowers and ?nally the sets of floral organs. Identifi?cation of at least six groups of genes in Arabidopsis has con?rmed that this is indeed so. Three groups are involved in establishing the identity of organs of the flower (e.g. sepals, petals; see below). Expression of the other genes influences identity of the meristem as an indeterminate structure. Two genes, Leafy (Lfy) and Apetala 1 (Ap1) in Arabidopsis and floricaula (Flo) and Squamosa (Squa) in Antirrhinum, have been shown, by in situ hybridisation (Chapter 10) of their RNA to thin slices of the meristem, to be expressed in bract and floral bud tissue in inflorescences (Figure 7.11). If these genes are not expressed, inflorescence development is incomplete with some primordia remaining vegetative and some becoming partially floral. Note from Figure 7.11 that these genes do not appear to be expressed in the apical dome, which is consistent with the inflorescence initially remaining indeterminate. This pattern of gene expression has been shown to depend upon the expression of a third gene, Terminal flower (Tfl) in Arabidopsis and Centroradialis (Cen) in Antirrhinum. In tfl or cen mutant plants, a terminal flower develops and prevents further growth of the inflorescence. The story is complex and incomplete. Although different names have been assigned to the genes identi?ed for each genus studied, a common and elegant pattern of gene regulation during transition to a reproductive meristem is emerging. Other examples of sequential gene expression during development are described in Section 10.3.3.
Organ determination

Studies of gene regulation during the transition from vegetative to reproductive meristems have established that differential expression of suites of genes in specific regions of the meristem is responsible for the organ identity of each primordium derived from the periphery of the inflorescence or floral meristem. Some of these genes are described below. The determination of primordia derived from the vegetative meristem, for example as leaves, thorns or lateral axes, will also be regulated through gene expression, but the identity of the genes involved is unknown. However, information about the timing of determination of leaves has been obtained by excising primordia from the shoot meristem and culturing them on agar. Data for fern primordia (Table 7.4) indicate that young primordia (P1–6) remain undetermined and can differentiate into either leaves or shoots (lateral axes), but as primordia grow older, determination as leaves becomes more fixed (P7, 8). In fern meristems, time between appearance of successive primordia may be several days, so determination appears to be a surprisingly protracted process. However, similar experiments on angio-sperm shoot meristems (e.g. tobacco) demonstrate that determination occurs much earlier, at P1–2. Clearly, much remains to be discovered about the timing of these processes and of the nature of communication between cells that allows coordinated formation of organs.

Genes that define primordium identity

<table>
<thead>
<tr>
<th>Primordium</th>
<th>Leaves</th>
<th>Shoots</th>
<th>Doubtful or no growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>10</td>
<td>35</td>
<td>55</td>
</tr>
<tr>
<td>P2</td>
<td>10</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>P3</td>
<td>20</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>P4</td>
<td>20</td>
<td>55</td>
<td>25</td>
</tr>
<tr>
<td>P5</td>
<td>40</td>
<td>55</td>
<td>5</td>
</tr>
<tr>
<td>P6</td>
<td>60</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>P7</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>P8</td>
<td>89</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>P9</td>
<td>95</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>P10</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(Data from Steeves 1966)

Table 7.4
As mentioned above, since the late 1980s we have seen major advances in our understanding of the molecular genetics of control of flower development, and this includes several mutants with altered organ identity in Arabidopsis and snapdragon (Antirrhinum). These mutants are termed homeotic because normal organs may develop in abnormal positions. The simplest transformations are (1) sepals to carpels and petals to stamens, (2) petals to sepals and stamens to carpels, and (3) stamens to petals and carpels to sepals (see summary in Meyerowitz 1994). On this basis, it appears that wild-type floral development depends on the activities of three gene classes (A, B and C) with each function being active in two adjacent whorls (Figure 7.13). This means that A is active in sepal and petal whorls, B is active in petals and stamens, and C is active in stamens and carpels. Activity of A alone leads to sepals, activity of A and B together lead to petals, B and C together lead to stamens, and activity of C alone leads to carpels. Activity of A is also involved earlier, during meristem identity determination (e.g. Apetala1 described above) before any organ determination has occurred. More than one gene is involved with each of the three functions so we have yet to comprehend fully aspects of this model, such as gene hierarchy and overlap of functions.

The DNA sequence for many of these genes suggests that all are putative transcription factors involved in regulation of gene expression (see Section 10.3). Most of the genes are members of the so-called ‘MADS box’ class. This is an acronym from the founding members of the class:

MCM1, a cell division gene from yeast

Agamous, from Arabidopsis

De?ciens, from Antirrhinum

Serum Response Factor for humans

Although most dicotyledonous flowers have four floral whorls, there are species differences in organ number per whorl (e.g. 4:4:6:2 in Arabidopsis versus 5:5:5:2 in Antirrhinum), in the level of fusion of organs and in floral symmetry. Various ‘master’ genes may control some of these differences, although frequently there is intraspecies and even intraplant variation in organ number, for example 4, 5 or 6 petals. The latter observation tells us that some of the control is not at the gene level.

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