

Control of Cell Axis

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Abstract Cell movement constitutes a basic mechanism in animal development, for instance during gastrulation or during the development of neural systems. Plant cells with their rigid cell walls cannot move and therefore had to evolve alternative mechanisms to organize their *Bauplan*. In plants, morphogenesis is controlled by the initiation of a cell axis during cell division and by the expression of this axis during subsequent cell expansion. Axiality of both division and expansion is intimately linked with specific microtubular arrays such as the radial array of endoplasmic microtubules, the preprophase band, the phragmoplast, and the cortical cytoskeleton. This chapter will review the role of microtubules in the control of cell axis, and attempt a synthesis of classical research with recent developments in the field. During the last few years, our understanding of two central enigmas of plant microtubule organization has been advanced substantially.

It had been observed for a long time that the spatial configuration of the phragmoplast was guided by events that take place prior to mitosis. However, the premitotic microtubular arrays disappear at the time when the spindle appears. It was therefore unclear how they could define the formation of a phragmoplast. The deposition of an endosomic belt adjacent to the phragmoplast, in combination with highly dynamic exploratory microtubules nucleated at the spindle poles, provides a conceptual framework for understanding these key events of cell axiality.

The microtubule–microfibril concept, which is central to understanding the axiality of cell expansion, has been enriched by molecular candidates and elaborate feedback controls between the cell wall and cytoskeleton. Special attention is paid to the impact of signalling to cortical microtubules, and to the mechanisms of microtubule reorientation. By means of live-cell imaging it has become possible to follow the behaviour of individual microtubules and thus to assess the roles of treadmilling and mutual sliding in the organization of microtubular arrays. Direction-dependent microtubule lifetimes, spatial patterns of post-translational modifications, and new mutants with deviating orientation of microtubules shed light on a complexity that is still far from being understood, but reveals a network of highly dynamic, nonlinear interactions that are endowed with pattern-generating properties. The chapter concludes with potential approaches to manipulation of the cell axis either through cell division or through cell expansion.

1

Cell Axis and Plant Development

During the growth of any organism, volume increases with the third power of the radius. Surface extension, however, increases only with the second power and thus progressively lags behind. In order to balance these two processes, the surface has to be enlarged substantially, either by internal or external exten-

sions. Due to their photosynthetic lifestyle, plants must increase their surface in an outward direction. As a consequence, plant architecture must be able to cope with a considerable degree of mechanical load. In aquatic plants, this is partially relieved by buoyancy, allowing considerable body sizes even on the base of fairly simple architectures. The transition to terrestrial habitats, however, required the development of a flexible and simultaneously robust mechanical lattice, the vessel system. The evolutionary importance of the vessel is emphasized by a large body of evidence. For instance, the so-called telome theory (Zimmermann 1965) had been quite successfully employed to describe the evolution of higher land plants in terms of a modular complexity based on load-bearing elements (the telomes) that are organized around such vessels.

The architectural response of plant evolution to the challenges of mechanical load had a second consequence, namely, a completely sessile lifestyle. This immobility, in turn, determined plant development with respect to its dependence on the environment. During animal development, body shape is mostly independent of the environment. In contrast, plants have to tune their *Bauplan* to a large degree to the conditions of their habitat. Morphogenetic plasticity thus has been the major evolutionary strategy of plants to cope with environmental changes, and fitness seems to be intimately linked to plant shape (Fig. 1).

Mechanical load shapes plant architecture, reaching down to the cellular level. Plant cells are endowed with a rigid cell wall and this affects plant development very specifically and fundamentally. The morphogenetic plasticity of a plant is therefore mirrored by a plastic response of both cell division and cell expansion with respect to axiality. In this response, cell division has to be placed upstream of cell expansion because it defines the original axis of a cell and thus the framework in which expansion can proceed. The deposition of the new cell plate determines the patterns of mechanical strain that, during subsequent cell expansion, will guide the complex interplay between protoplast expansion. This is mainly driven by the swelling vacuole, with the



Fig. 1 Adaptive response of morphogenesis in a tendril of *Vicia faba*. In response to the mechanical stimulus, upon contact with the support, cell elongation becomes arrested in the flank facing the support, whereas it continues at the opposite flank. The resulting growth differential causes a bending response towards the support and will, eventually, result in spiral growth of the tendril around the support. The time-course of the figure covers 24 h

cell wall as a limiting and guiding counterforce. It is even possible to describe the shape of individual cells in a plant tissue as a manifestation of minimal mechanical tension (Thompson 1959), emphasizing the strong influence of mechanical load on plant development.

When plants are challenged by mechanical load, they respond by changes in architecture that will allocate load-bearing elements (vessels and fibres on the organ level, cellulose microfibrils and lignin incrustations on the cellular level) in such a way that mechanical strains are balanced in an optimal fashion at minimal investment of energy and biomatter. This response of architecture is fundamental and involves changes on different levels of organization, from the spatial arrangement of macromolecules up to the allocation of biomatter to different organs.

Mechanical load affects architecture and the composition of the cell wall during cell elongation and subsequent cell differentiation. For instance, mechanical compression leads to a suppression of certain layers of the cell wall (the so-called S_3 -layer) in conifer tracheids (Timell 1986; Yoshizawa 1987). Conversely, mechanical tension causes a shift in orientation of cellulose in the gelatinous layer of the challenged wood fibres in such a way that the mechanical strain is optimally buffered (Prodhan et al. 1995).

However, the effect of mechanical load by far exceeds these responses on the subcellular level. Plant cells can respond to a mechanical challenge by acute changes of cell axially. It is even possible to demonstrate this directly: When protoplasts are embedded into agarose and the agarose block is subsequently subjected to controlled mechanical load (Lynch and Lintilhac 1997), the division planes of the embedded cells will then be aligned either perpendicular or parallel to the principle stress tensors (Fig. 2).

On the level of whole-plant physiology, mechanical stress can cause so-called thigmomorphogenesis, i.e. alterations of growth that result in adaptive changes of shape. For instance, unidirectional stem flexure of young pines (as produced, for instance, by exposure to wind) induced a larger biomass allocation to the roots parallel to the plane of flexing, which in turn resulted in an increased mechanical resistance within the plane of bending stress (Mickovski and Ennon 2003). In other words, the mechanical stimulus altered root architecture in an adaptive way to ensure optimal resistance to the triggering mechanical stress. The losses in yield that are caused by wind are conspicuous – estimates range between 20 and 50% for Graminean crops and reach up to 80% for certain apple varieties (Grace 1977). In addition to the allocation of lateral roots, it is the angle between the primary root and the branch roots that defines the uprooting resistance of a root system to wind stress (Stokes et al. 1996).

The economic impact of thigmomorphogenesis is tremendous, but very often overlooked. Repetitive mechanical stimulation, e.g. by wind, will cause a redistribution of growth towards lateral expansion. Again, this thigmomorphogenetic response is clearly of adaptive quality. The resistance of a plant to

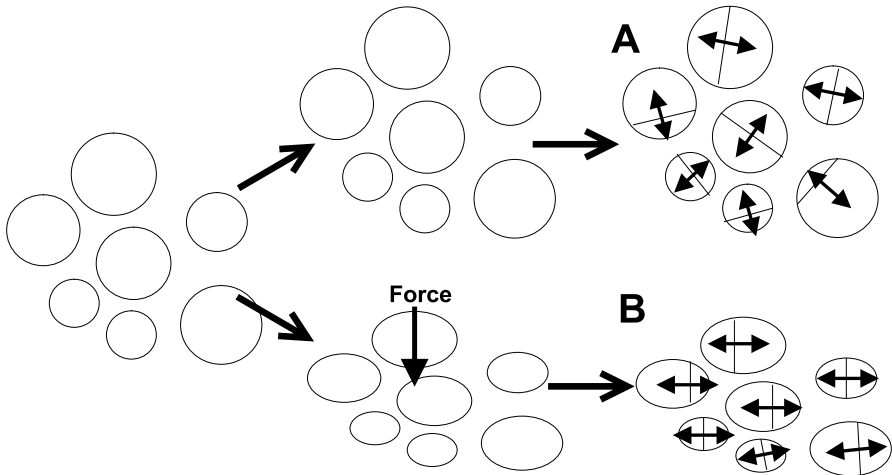


Fig. 2 Alignment of cell division in response to mechanical tension. Protoplasts that are embedded into agarose will divide randomly upon regeneration of the cell wall (A). However, when they subjected to mechanical tension, the direction of the subsequent division will be aligned (B)

windbreak and lodging is inversely related to plant height (Oda et al. 1966):

$$L_R = \frac{W \cdot M}{L^2 w},$$

with W = fresh weight, M = bending momentum at breaking, L = shoot length and w = dry weight of the shoot. Thus, lodging resistance will increase parabolically with decreasing plant weight, and a repartitioning of growth from elongation to thickening is a very efficient strategy for increasing lodging resistance, because fresh weight W is kept constant, while the reduction of the shoot length by a given factor will contribute with the second power of this factor.

Lodging is of enormous importance for agriculture and accounts for yield losses up to 10–50% in wheat (Laude and Pauli 1956; Weibel and Pendleton 1961), up to 60% in barley (Schott and Lang 1977; Knittel et al. 1983) and 20–40% in rice (Basak 1962; Kwon and Yim 1986; Nishiyama 1986). The increase of lodging resistance therefore has been a traditional target for agricultural technology over several decades, especially in Graminean crops. This includes genetic approaches, where dwarfing genes are introduced into high-yield cultivars (Borner et al. 1996; Makela et al. 1996; Mcleod and Payne 1996), as well as the application of growth regulators such as chlormequat chloride or ethephone (Schott and Lang 1977; Schreiner and Reed 1908; Tolbert 1960).

The success of these strategies is limited by the specific environment generated by modern agriculture, such as high nutrient influx and high canopy densities. These conditions stimulate internode elongation and thus increase

the susceptibility of the crops to lodging and windbreak (Luib and Schott 1990). Most crop plants are typical sun plants, i.e. they exhibit a pronounced shade-avoidance response when grown in dense canopies (Smith 1981). They are able to sense their neighbours through subtle changes in the ratio between red and far-red light utilizing the photoreversible plant photoreceptor phytochrome. They respond to this change in red/far-red ratio by enhanced stem and petiole elongation. The shade-avoidance response is supposed to protect these plants against overgrowth by neighbouring plants. Indeed, this has been confirmed in field trials, where photoreceptor mutants of *Arabidopsis thaliana* that were not able to trigger shade avoidance were monitored under field conditions and found to be less competitive as compared to the respective wild type (Ballaré and Scopel 1997). As useful as this response may be for the survival of a weed like thale cress in a canopy, it is undesired for a crop plant. In the dense canopy of a wheat field, for example, shade avoidance will increase the risk of lodging. In fact, field trials with tobacco plants that over-express phytochrome and are thus incapable of sensing the reflected light from their neighbours demonstrated that the suppression of shade avoidance allows for increased yield (Robson et al. 1996).

A classical example of thigmomorphogenesis is the barrier response of young seedlings. Upon contact with a mechanical barrier, the major axis of growth tilts from elongation towards stem thickening. This barrier response is triggered by the ethylene that is constantly released from growing stems and accumulates in front of physical obstacles (Nee et al. 1978). The increase in diameter improves the mechanical properties of the seedling, for instance the flexural rigidity, and thus allows the seedling to remove the barrier.

These examples may suffice to illustrate the impact of cell axis on growth, architecture and eventually on the performance of the plant under challenge by the environment. There are basically two mechanisms that define and contribute to the axis of a plant cell: first, the basic geometry of a cell is defined by the axis of cell division; and second, the manifestation of this geometry depends on the axis of subsequent cell expansion. The next two sections will therefore survey the mechanisms that control the axiality of division and expansion.

2

Control of Cell Division

The spatial control of cell division employs specialized populations of microtubules that are unique to plant cells: cortical microtubules, preprophase band (PPB) and phragmoplast (Fig. 3). The cortical microtubules prevailing in interphase cells are usually arranged in parallel bundles perpendicular to the main axis of cell expansion (Fig. 3a). They are involved in the directional control of cellulose deposition and thus in the axiality of cell growth and will