Toward a Biophysical Theory of Organogenesis:
Birefringence Observations on Regenerating Leaves in the Succulent,
Graptopetalum paraguayense E. Walther

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Abstract. Polarity shifts occur during organogenesis. The histological criterion for polarity is the direction of cell division. The biophysical criterion is the orientation of reinforcing cellulose microfibrils which lie normal to the organ axis and which determine the preferred growth direction. Using cell pattern to deduce cell lineage, and polarized light to study cellulose alignment, both aspects of polarity were examined in the epidermis of regenerating G. paraguayense. In this system new leaves and a stem arise from parallel cell files on a mature leaf. Large (90°) shifts in polarity occur in regions of the epidermis to give the new organs radial symmetry in the surface plane (files radiating from a pole). Study of the shifts in the epidermis showed that, during certain stages, shifts in the division direction are accompanied by shifts in the cellulose deposition direction, as expected. The new cellulose orientation is parallel to the new cross wall. During normal organ extension, however, shifts in division direction do not bring on changes in cellulose pattern. Thus the coupling between the two kinds of polarity is facultative. This variable relation is used in a biophysical model which can account for the reorganization of cell file pattern and cellulose reinforcement pattern into the radial symmetry of the new organ.

Key words: Cellulose microfibrils – Graptopetalum – Leaf formation – Microtubules – Organogenesis (biophysical theory) – Polarity

Introduction

Most formative regions of plant organs such as roots, stems, and young leaves consist of arrays of parallel files of cells. Organogenesis takes place when a small region of such an organ produces a new array of parallel cell files oriented roughly at right angles to the original. The lateral organ has its own axially, or bipolarity. The new organ has distal and proximal ends so it also has polarity in the strict sense (i.e., a head and tail) superimposed on the axiality. This paper is concerned with the origin of the new axially and so the familiar word, polarity, will be used mainly in the sense of bipolarity (e.g., a mitotic spindle is bipolar).

Histological studies describe how a shift in the direction of cell division gives rise to new cell patterns in the interior of appendages such as leaves and lateral roots. This new orientation of cross walls, however, does not in itself bring on a new direction of growth. “Growth by cell division”, as against the simple subdivision of cells, can occur only if expansion takes place between the formation of new cross walls. Thus a second polarity shift, relating to the growth direction, must be involved in organogenesis.

The growth direction is believed to be based on the transverse reinforcement, by cellulose microfibrils, of the side walls of the cells of the organ (Frey-Wyssling 1976). Simply put, a growing hoop-reinforced cylindrical cell will resist increase in girth and will extend along its axis. If the young organ is viewed as an aggregate of such cells, its elongation is accounted for.

Roland and Vian (1979) have shown that many walls have a polylamellate structure. The walls to be dealt with here either have most of the lamellae showing transverse order, or have only a single major transverse alignment as in Roland and Vian’s “primordial wall” or in Frey-Wyssling’s (1976) “tube texture”. The oriented growth in question is based on directional surface expansion along the whole cell axis. It is not to be confused with tip-growth where extension is achieved by localized non-directional expansion.
In any theory of formation of lateral organs, two major polar structural features, division direction and reinforcement direction, must shift. The present study was undertaken to follow these polarities in the same tissue to see how and when they shifted direction. The simplest possibility, that the two polarities are rigidly coupled and hence automatically shift together, was not found. Rather, there was a facultative association between the two phenomena: only a certain kind of cell division leads to a shift in the reinforcement polarity of the daughter cells. The new reinforcement polarity, involving cellulose direction, also involves a shift in the direction of the cortical microtubular array (Hardham et al. 1980). This shift is thus assumed to involve a comprehensive change in cell polarity.

To relate cellular polarity shifts to organogenesis, it is appropriate to ask how many major shifts are needed to produce a new organ. It will develop that a) established nomenclature needs modification to deal with the two kinds of polarity shift and b) a major but unrecognized polarity shift, occurring in the epidermis, is especially suitable for study.

The issue of symmetry change appears to have been overly simply conceived in the histological literature. While no fully explicit theory of organogenesis has been proposed, most accounts imply that a single polarity shift (of division direction) in a single tissue suffices to generate a new organ. The generally accepted view is paraphrased in many texts. For example, “A leaf is initiated by periclinal divisions in a small group of cells in the peripheral zone of an apical meristem” (Esau 1965, p. 104). This single polarity shift in the interior cells is generally thought to be the key event. The epidermal layer “enlarges by numerous anticlinal divisions of its cells and so becomes adapted to the growth of the primordium” (Fahn 1967, p. 224). This apparently straightforward account, shown in the top row in Fig. 1, leads to certain terminological difficulties.

The first difficulty is that the terms anti- and periclinal are suitable for static images, but are awkward for phrasing developmental mechanisms. The first periclinal divisions, cross-walls with a dot in Fig. 1A, produce progeny whose cross-walls, a in Fig. 1A, are actually anticlinal (normal to the nearest surface), despite the developmental concept that there is continued periclinal division. The second difficulty is that the first periclinal divisions (shown with a dot in the cross wall) alter polarity. Later divisions (no dot) merely extend the new polarity. Developmentally there are two kinds of periclinal divisions: polarity-altering and polarity-conserving. These ambiguities are avoided in the dual notation used in the lower rows where the apparent direction of cellulose reinforcement has been drawn in.

The first term in the notation for a division in a cell file reveals whether the orientation of the new cross wall is novel relative to the previous reinforcement polarity. Usually cross walls lie in the plane of cellulose reinforcement, the transverse plane. If the division is usual, it is termed "E". The middle bar of the E may be considered as a section of the new wall, being formed parallel to previous cross walls. Fig. 1A-C. Views of organogenesis. A Traditional assumed sequence, in longitudinal section. Periclinal divisions give rise to the interior of the lateral; anticlinal divisions extend the epidermis. Note that later “periclinal” divisions are actually anticlinal (a). Note that some periclinal divisions (dot on cross wall) change cell polarity while others (no dot) extend the previous polarity. These ambiguities are avoided below. B Same assumed sequence but with cell polarity, perpendicular to cellulose reinforcement, drawn in. A few periclinal polarity-changing divisions, H(A), repolarize the interior. Later polarity-conserving divisions, E(C), extend the new polarity. Epidermal polarity is simply extended. C Observed sequence in Graptopetalum. Interior appears to behave as in B. In the epidermis some polarity-shifting divisions occur in the other novel plane (longi-anticlinal), T(A). Descendants of these cells undergo E(C) divisions to produce files outside the plane of section; this adds radial symmetry to the new organ. Line x-x shows the lateral as an aggregate of hoop reinforced cells.