Experimental and genetic analysis of root development in *Arabidopsis thaliana*

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Abstract

The cellular organisation of the *Arabidopsis thaliana* root is remarkably regular. A fate map of the primary root and root meristem that predicts the developmental destinies of cells within the embryonic root primordium has been constructed. Nevertheless, laser ablation experiments demonstrate that root meristem cells develop according to position and not according to lineage. Mutational analysis has identified genes required for cell specification in the radial as well as in the apical-basal dimension. The corresponding gene functions appear to be necessary during embryogenesis for the formation of a correctly patterned primary root.

Introduction

Roots have been studied in earlier decades by researchers interested in plant development, who noticed the ordered patterns of cell division and the resulting regularity of cell files. Early workers focused on understanding division patterns within root meristems. Histological studies provided evidence for the existence of a few “initial cells” from which all root tissues arose (e.g. Von Guttenberg, 1955). Thymidine labelling demonstrated the existence of a “quiescent centre” in root apices. Surgical experiments indicated the presence of multiple initials, and substantiated the concept of the “promeristem” in root apices: a quiescent centre surrounded by initials for all cell types (Clowes, 1961).

A question that could not be addressed at that time was whether the initial cells were specifically programmed for the formation of only one or few related tissues. The idea that regularly arranged meristem cells were programmed to form specific tissues was designated the “histogen” concept (Hanstein, 1870). A variety of cell division patterns in roots of different species have been described. Some division patterns provided clear evidence for separate initial cells for particular cell types. In other cases a single founder cell appeared to give rise to progeny of all cell types (cf. Steeves and Sussex, 1989). Yet other root apices displayed irregular cell divisions, in line with clonal analyses on shoot development that emphasised flexibility and the role of cell position rather than lineage in plant development (reviewed by Poethig, 1987). The underlying mechanisms of flexible shoot meristem development are yet to be elucidated. Whether cell specification in some, or all, root meristems operated via similar flexible mechanisms could not be answered.

At present, the methodology developed by animal developmental biologists has an increasing impact on developmental studies in plants (e.g. Jürgens, 1995; Weigel and Meyerowitz, 1994). This approach can take a new advantage of the regularity of root development to probe mechanisms of cell programming within meristems. In this chapter we present experimental and genetic analyses, carried out in the model plant *Arabidopsis thaliana*. The *Arabidopsis* root is very regular and excellently suited to exploit fully the available experimental and genetic tools. We have combined clonal analysis with cell ablation studies to study the flexibility of meristem initial cells, and the source of their developmental information. Furthermore, we
present an analysis of mutations affecting the embryonic formation of the root meristem. These mutations point to a pivotal role of daughters from one embryonic cell, the hypophyseal cell, in establishing the primary root meristem. Taken together, these studies suggest how root meristem cells learn their fate and provide a genetic entry into how the meristem itself is organised during ontogeny.

The *Arabidopsis* root promeristem has a fixed clonal origin

The *Arabidopsis* primary root meristem contains a surprisingly constant number of cell files of each cell type that terminate in the initial cells. A small set of initials for all tissues surround four quiescent cells (Dolan et al., 1993). This quiescent centre contacts all the initials, an observation that suggests regulatory functions. Quiescent centre and initials together are termed the promeristem, the minimal construction centre of the root (Clowes, 1953). All cells within the promeristem are laid down during embryogenesis, and exhibit the division pattern typical for the root meristem from the heart stage of embryogenesis onward.

Two sets of initials within the promeristem can give rise to more than one differentiated cell type. The epidermal initial gives rise to both epidermis and lateral root cap, while the cortical initial forms endodermis and a cortical cell layer. These relations have been demonstrated by anatomical as well as sector analysis (Dolan et al., 1993, 1994). The rigidity of these relations superficially suggests that both types of initial cells and programmed by lineage to give rise to two alternative cell types.

We have performed clonal analysis to study how the primary root meristem is laid down during embryo development. We analysed embryonic sectors which arose by transposon excision from the *uidA (GUS)* marker gene in transgenic plants. The end points and width of these sectors allowed us to deduce a complete fate map for the *Arabidopsis* root (Figure 1A; Scheres et al., 1994). The root promeristem arises from two distinct groups of cells that are separated at the first zygotic division: the quiescent centre and columella root cap arise from the hypophyseal cell that is, in turn, derived from the basal cell, while the proximal initials arise from the apical cell (Figure 2). Apparently, the daughters of the hypophyseal cell come to cooperate with the proximal initials to give rise to the functionally integrated root meristem. The separation

Figure 1. Fate map of wild type and hypophyseal cell group early heart stage embryo. Corresponding regions in embryo and seedling are connected by lines. Broadening of the connecting lines visualises the variability in the location of embryonic cell division planes in the seedling axis. Components of the seedling axis below the cotyledons from top to bottom: cotyledon shoulder, hypocotyl (ending basally just above the uppermost root hairs), embryonic root and meristemaic root. (A) Wildtype embryo and seedling with all tissue types. (B) "Hypophyseal cell" mutant embryo and seedling lack cells with the anatomical features of quiescent centre and columella, and display no or limited proximal meristem activity.

Figure 2. 16-cell embryo. P: protoderm, h: hypophyseal cell, 1: plane of first zygotic division.