Morphogenetic Factors Controlling Differentiation and Dedifferentiation of Epidermal Cells in the Gynoecium of *Catharanthus roseus*

I. The Role of Pressure and Cell Confinement

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Abstract. To test the morphogenetic role of equilateral stress and physiological confinement in dedifferentiation of epidermal cells, impermeable barriers of gold-metal foil were placed between the postgenitally fusing epidermal surfaces in the gynoecium of *Catharanthus roseus* (L.) G. Don. Cells contacting the foil barrier were firmly compressed and air spaces were almost eliminated, thus mimicking an internal cellular environment. However, the cells adjacent to the barriers retained epidermal characteristics and did not dedifferentiate. In contrast, epidermal cells that could establish a direct cell-to-cell contact along the margins of the barriers rapidly dedifferentiated as in normal development. The results indicate that neither physical stress on the cell surfaces nor modifications in gradients of gases or volatile products around the cells control the process of epidermal dedifferentiation. The morphogenetic stimulus in this system requires direct cell contact, and may thus consist of a diffusible messenger molecule or some kind of cell surface interaction. Aspects of this cell interaction and of epidermal cell differentiation generally are discussed.

Key words: *Catharanthus* — Cell confinement — Differentiation and dedifferentiation — Epidermis — Physical pressure — Postgenital fusion.

Introduction

The morphogenetic factors (cell interactions) responsible for controlling differentiation between adjacent or nearby plant cells and tissues have been little studied and largely remain obscure (for a recent review, see Lang, 1974). Systems for study are required that lend themselves well to experimental approaches, exhibit a rapid and well-defined response, and eliminate as many of the complex variables as possible. The dedifferentiation of epidermal cells that occurs during certain postgenital tissue fusions in plants fulfills these criteria and is a favorable system for studying cellular morphogenesis.

Postgenital tissue fusion is the union (wall adherence) of free surfaces to form a compound tissue and is a relatively common event during floral ontogeny (see discussions in Baum, 1948; Cusick, 1966). Studies on the mechanism of postgenital carpel fusion in *Catharanthus roseus* (Apocynaceae) (Walker, 1975a, b, c, 1978) indicate that this system is especially suitable for studies of cell differentiation because experimental approaches used demand little or no surgical manipulation which results in traumatization of the tissue and thus introduces a complex variable. During gynoecial ontogeny in *C. roseus*, the free epidermal surfaces of the two carpel primordia come together. This is followed by rapid dedifferentiation of the contacting epidermal layers and (subsequent) redifferentiation into stigmatic transmitting tissue. Thus a rapid and well-defined type of cell differentiation is stimulated externally (by cell contact) and can be experimentally manipulated by modifying the cell contact without wounding the cells.

Because of its position, the epidermis is subject to influence both from adjoining cells (to the sides and interior) and from immediate physical factors (direct association with the atmosphere and lack of confinement of the outer tangential wall). It is possible that directions of stress or gradients of humidity, gases or volatile products create microenvironments that somehow induce specific pathways of cell differentiation in epidermal tissue.

The experiments reported in this paper test the role of physical confinement on epidermal differentia-
tion-dedifferentiation in the gynoecium of *C. roseus*. Physical barriers impermeable to movement of soluble compounds and preventing direct cell-to-cell contact were placed between the epidermal surfaces that would otherwise fuse. Thin chips of gold-metal foil were used because gold is biologically inert and is pliable enough to be cut readily. The reactions of epidermal cells pressed against the foil barrier during normal carpel expansion were compared to the reactions of epidermal cells experiencing normal cell-to-cell contact. The primary question was, does physical confinement alone trigger dedifferentiation in the epidermal cells?

**Material and Methods**

A population of *Catharanthus roseus* (L.) G. Don (*Vinca rosea* L.) maintained in a growth chamber was employed for the study. Plants were grown in clay pots in a porous soil mixture. Temperature was 32°C during the 12 h light period and 21°C during the 12 h dark period. A mixture of incandescent and fluorescent lights provided illumination of about 20,000 lx.

### Preparation and Insertion of Gold-foil Barriers

Rectangular chips of gold-metal foil ca. 150 µm x 200 µm x 15 µm were prepared by flattening a segment of a gold leaf (obtained from Arthur H. Thomas Co., Philadelphia, Pa., USA). A region of the leaf was pressed on to the bottom of a glass Petri dish using a small tool with a smooth, curved surface. The foil rectangles were cut from the thinly pressed area using razor-blade fragments. Two de Fonbrune pneumatic micromanipulators (Beaudouin Co., Paris, France) positioned around a Wild M7A stereomicroscope were used to handle and insert the gold-foil barriers.

### Scanning Electron Microscopy

Following removal from the parent plant, each floral bud was dissected to remove the sepals, petals and stamens. The resulting buds with exposed gynoecia were fixed for a least 48 h in 4% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0), post-fixed at least 48 h in 2% osmium tetroxide buffered as above, dehydrated in a graded ethanol series, and critical-point-dried with CO2. Mounted specimens were gold coated and examined with a Cambridge Stereoscan Scanning Electron Microscope at 10 kV.

### Light Microscopy

Material was fixed in 4% buffered glutaraldehyde, dehydrated in a graded ethanol series, and embedded in Spurr's Epoxy resin (Spurr, 1969). Alternatively, material previously examined by scanning electron microscopy was embedded in Spurr's Epoxy resin following treatment for several days in propylene oxide as a transition fluid. Sections 1–1.5 µm thick were obtained with glass knives and stained with toluidine blue 0 (Allied Chemical, Morristown, N.J., USA). Although the gold-foil did not infiltrate with epoxy, when sectioned it usually adhered in place.

### Results

Two unfused carpel primordia, each ca. 250 µm high, with a gold-foil barrier inserted between them are shown in Figure 1. The space between the two unfused carpels at this stage normally is about 15 µm wide, and insertion of the foil barriers usually required forcing the primordia slightly apart (Fig. 1).

With continuing gynoecial development, the gold-foil barriers progressively became engulfed between the expanding and fusing carpel tips (Figs. 2, 3). The fused carpel tips in *C. roseus* normally form the united stigma and style of the gynoecium (Figs. 2, 3), and the barriers ultimately became trapped within the differentiating stigma, either completely or partially (Figs. 3, 4). The epidermal cells near the margin of a protruding barrier did not expand to fill the fissure (Fig. 4), unlike the epidermal cells during normal carpel fusion (Fig. 5, arrows).

The differences in behavior of the epidermal cells between cell-to-foil versus cell-to-cell contact were more dramatically apparent in sectioned material (Figs. 6–10). A transverse section of a normal stigma is shown in Figure 6 at a comparable stage to that shown in Figure 5. Whereas the cells along the outer organ surface continued epidermal development, characterized by their rectangular shape in sectional view, lack of periclinal cell divisions, and little vacuolation of the cytoplasm, the (previously epidermal) cells along the line of fusion (Fig. 6, internal to arrow) de- and redifferentiated to form stigmatic transmitting tissue, characterized by both anticlinal and periclinal cell divisions, irregular cell expansion, and extensive vacuolation of the cytoplasm.

The appearance of the epidermal cells at a cell-to-foil interface is shown at an early developmental stage in Figures 7 and 8, a stage comparable to that of Figure 2. The cells adjacent to the gold-foil barrier (GF) were tightly compressed against the barrier (Figs. 7, 8) such that the pressure flattened the outer tangential cell wall (Fig. 8). In Figure 8 (arrow) one cell extended slightly into an indentation in the foil barrier. The compression of the outer wall largely