current flow through Müller cells and demonstrates that spatial buffering by glial cells in situ occurs during the course of normal neuronal activity. Glial cells may function in a similar fashion to regulate $[K^+]_o$ throughout the brain.

REFERENCES AND NOTES

1. R. K. Orkand, J. G. Nicholls, S. W. Kuffler, *J. Neurophysiol.* **29**, 788 (1966).
2. B. R. Ransom and W. G. Carlini, *Astrocytes*, S. Fedoroff and A. Vernadakis, Eds. (Academic Press, Orlando, 1986), vol. 2, p. 1.
3. A. R. Gardner-Medwin and C. Nicholson, *J. Physiol. (London)* **335**, 375 (1983); I. Dietzel, U. Heinemann, G. Hofmeier, H. D. Lux, *Exp. Brain Res.* **40**, 432 (1980); A. R. Gardner-Medwin, J. A. Coles, M. Tsacopoulos, *Brain Res.* **209**, 452 (1981); J. A. Coles and R. K. Orkand, *J. Physiol. (London)* **340**, 157 (1983).
4. C. J. Karwoski, *Physiol. Bohemoslov.* **37**, 217 (1988).
5. E. A. Newman, D. A. Frambach, L. L. Odette, *Science* **225**, 1174 (1984).
6. E. A. Newman, *J. Neurosci.* **5**, 2225 (1985).
7. S. S. Miller and R. H. Steinberg, *J. Membr. Biol.* **36**, 337 (1977).
8. C. J. Karwoski, D. A. Frambach, L. M. Proenza, *J. Neurophysiol.* **54**, 1607 (1985).
9. W. Walz, M. Shargool, L. Hertz, *Neuroscience* **13**, 945 (1984).
10. B. Rudy, *ibid.* **25**, 729 (1988).
11. M. L. Åstion, J. A. Coles, R. K. Orkand, *Neurosci. Lett.* **76**, 47 (1987).
12. D. S. Henshel, P. L. Carras, P. A. Coleman, R. F. Miller, *Invest. Ophthalmol. Vis. Sci.* **26**, 116 (1985); E. A. Newman, *Nature* **317**, 809 (1985).
13. E. A. Newman, *J. Neurosci.* **7**, 2423 (1987).
14. J. D. Conner, P. B. Detwiler, P. V. Sarthy, *J. Physiol. (London)* **362**, 79 (1985).
15. C. J. Karwoski and L. M. Proenza, *J. Gen. Physiol.* **75**, 141 (1980).
16. ———, *J. Neurophysiol.* **40**, 244 (1977).
17. L. L. Odette and E. A. Newman, *Glia* **1**, 198 (1988).
18. Values of $[K^+]_o$ were calculated within the IPL (15 μm from the ganglion cell–IPL border) and 20 μm into the vitreous humor. Values of retinal layer thickness, extracellular volume fraction (α), and Müller cell conductance (g_{glia}) used in the simulation were as follows. [Format: retinal layer name, layer width (μm), α, g_{glia} (S cm^{-3})]: vitreous humor, 2750, 1.0, no glial cell; inner limiting membrane–endfoot, 2.5, 1.0, 330; nerve fiber layer, 17.5, 0.041, 6.72; ganglion cell layer, 25, 0.041, 0.504; IPL, 50, 0.182, 0.286; inner nuclear layer, 35, 0.048, 0.196; outer plexiform layer, 10, 0.182, 0.84; outer nuclear layer, 17.5, 0.048, 0.196; rod inner segment layer, 12.5, 0.198, 0.196; subretinal space, 60, 0.198, no glial cell; retinal pigment epithelium, 10, 0.0007, no glial cell. Volume fractions from (*8*), Müller cell conductance values from (*6*). Extracellular tortuosity factor, λ, 2.02; passive-uptake space volume fraction, 0.8; passive-uptake rate constant, 0.15 s^{-1}; active-uptake rate constant, 0.05 s^{-1}.
19. The simulated control K^+ response in the vitreous humor, although similar to the experimental response in time course, was smaller in amplitude (7% of the IPL response compared to 18% in experimental records; frog eyecups, diffuse flashes). This discrepancy may arise because we underestimated Müller cell K^+ conductance in the model or because we omitted (for simplicity) a light-evoked K^+ source in the ganglion cell layer.
20. C. J. Karwoski and E. A. Newman, *Vision Res.* **28**, 1095 (1988).

21. We thank B. Oakley II for suggesting the use of Ba^{2+} to test Müller cell function and L. L. Odette for assistance with the computer simulations. Supported by NIH grant EY 03526 to C.J.K. and grant EY 04077 to E.A.N.

13 January 1989; accepted 23 March 1989

Diffusible Factors Essential for Epidermal Cell Redifferentiation in *Catharanthus roseus*

BRUCE A. SIEGEL AND JUDITH A. VERBEKE*

During postgenital tissue fusions, some plant epidermal cells redifferentiate into parenchyma, a different cell type. Diffusible factors cause this response in the fusing gynoecium of the Madagascar periwinkle (*Catharanthus roseus*). Surgical manipulations of the gynoecium showed that epidermal cells from normally nonfusing surfaces could transmit and respond to the diffusible factors. Furthermore, the diffusible factors could be trapped in agar-impregnated barriers, as shown by the redifferentiation of carpel epidermal cells from nonfusing regions when the factor-loaded barriers were appressed to them.

EARLY IN ONTOGENY, CELLS AND TISSUES of common lineage diverge into contrasting paths of development as they respond to developmental signals (*1, 2*). This ability to develop in response to a specific signal is known as cellular competence (*3–6*) or cellular potentiality (*7*). As competent cells become committed to particular paths of differentiation, they are not readily diverted into other pathways; this restriction of the developmental potentiality of cells is referred to as determination (*8*).

In the absence of wounding (and usually in spite of wounding), virtually all plant epidermal cells are developmentally incompetent to redifferentiate (*9, 10*). This stable, differentiated state arises early in epidermal ontogeny (*11*). However, epidermal cells that take part in postgenital tissue fusions (*12*) are naturally occurring exceptions to this rule. These cells can redifferentiate. The postgenital tissue union that occurs in developing flowers of *Catharanthus roseus* L. (Apocynaceae) involves redifferentiation of epidermal cells in the young carpels, the structures in angiosperms that enclose the ovules

Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60680.

*To whom correspondence should be addressed.

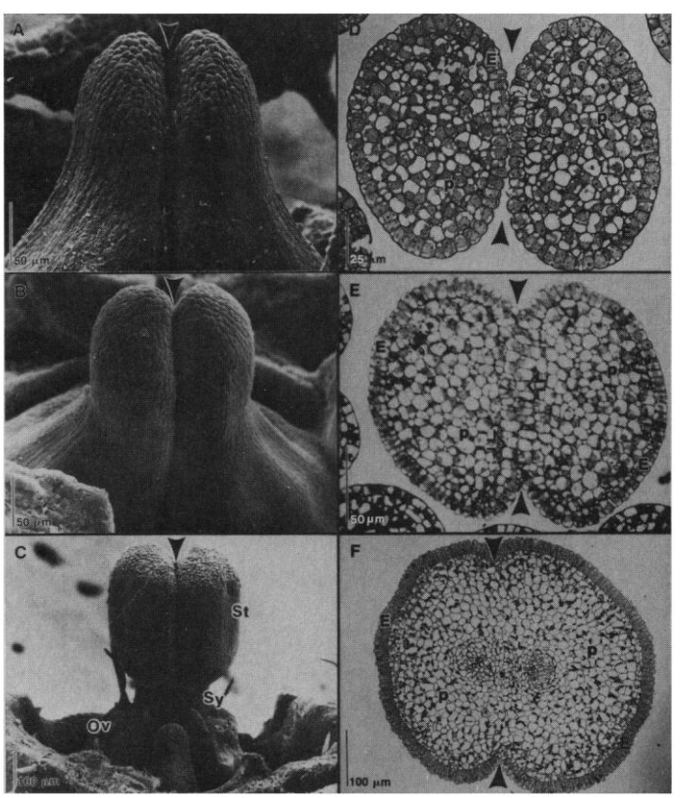

Fig. 1. Prefusion, fusing, and postfusion stages in *C. roseus* carpels. The large arrowheads indicate the plane of fusion. All tissue was fixed in 2% glutaraldehyde, postfixed in osmium tetroxide, and dehydrated in graded ethanol concentrations. Specimens for scanning electron microscopy (A to C) were dried to the critical point and gold-coated before observation. Light microscopy specimens (D to F) were embedded in Spurr's (*20*) epoxy resin; sections 0.90 μm thick were stained with 1% toluidine blue O. (**A**) Two prefusion carpels arise as separate primordia and grow toward each other until (**B**) the adaxial surfaces come into contact. (**C**) Cells in the fused region enlarge and develop into the stigma, style, and distal region of the ovaries. (**D**) Two separate carpels, each with an epidermal layer surrounding parenchyma. (**E**) As the adaxial surfaces of the carpels touch, epidermal redifferentiation (*13*) can be observed in some of the cells (open arrowheads). (**F**) All contacting adaxial epidermal cells subsequently redifferentiate and the carpels fuse. Abbreviations: St, stigma; Sy, style; Ov, ovaries; E, epidermal cells; and p, parenchyma.

(*13–15*) (Fig. 1). This change in cellular fate is complete within 9 hours (*16*) and results from cellular communication (*17–19*).

To demonstrate the presence and specificity of the morphogenetic signal and to explore the phenomena of cellular competence, differentiation, and determination, we performed the following experiments: (i) to determine whether the trigger for epidermal redifferentiation is specific to epidermal cells that are normally in contact with each other, we maneuvered the carpels so that normally nonfusing cells touched normally fusing cells, and we monitored their response; and (ii) to determine whether diffusible factors can change the developmental fate of other epidermal cells, we loaded agar-impregnated barriers (Fig. 2) with the factors and then applied the loaded barriers to other epidermal cells and monitored the response of the cells.

Throughout the development of the bud, the anthers and epidermal cells on distal surfaces of the carpels (abaxial cells) remained epidermal even when the carpels were tightly appressed to the anther (Fig. 3A). However, the abaxial cells of each carpel can redifferentiate. If one of the two prefusion carpels is excised at its base, rotated 180° on its vertical axis, and then grafted back to the receptacle so that normally nonfusing (abaxial) epidermal cells touch normally fusing (adaxial) epidermal cells of the intact carpel, the normally nonfusing cells redifferentiate (Fig. 3B). The original adaxial surface that no longer can touch the other carpel remains epidermal.

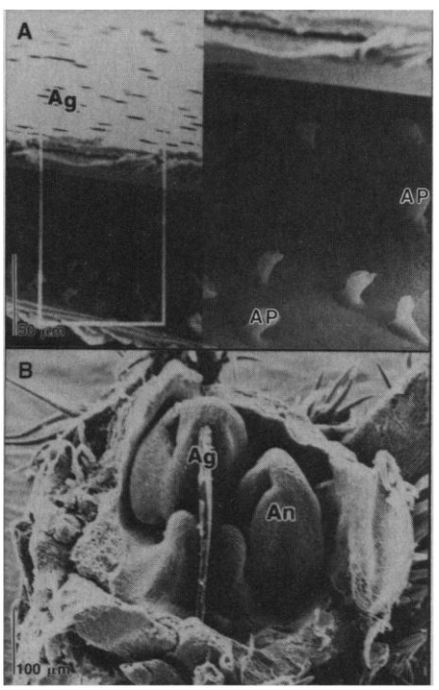

Fig. 2. Scanning electron microscopy showing agar-impregnated barriers. (**A**) Nuclepore polycarbonate barrier (pore diameter, 12.0 μm) impregnated with 1% agar. Agar layers can be seen above and below the barrier. The boxed area is enlarged ×2 at right, showing agar plugs that were pulled from the barrier pores during processing. (**B**) Bud with an agar-impregnated barrier between two prefusion carpels. Abbreviations: Ag, agar-impregnated barrier; AP, agar plugs; and An, anther.

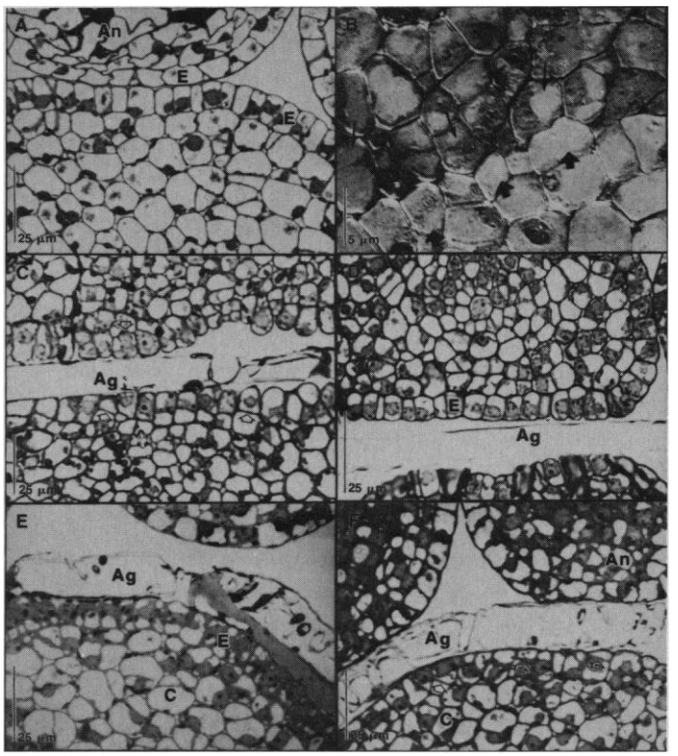

Fig. 3. Light microscopy of transverse sections of *C. roseus* gynoecium. (**A**) Normal bud with abaxial carpel epidermal cells appressed to anther epidermal cells. Neither the anther nor the carpel epidermal cells redifferentiate. (**B**) Bud with one carpel excised, rotated 180°, and grafted back to the receptacle. The formerly abaxial epidermal cells of the rotated carpel redifferentiate (large arrows) when placed next to the adaxial surface of the intact carpel (small arrows). (**C**) Carpels separated by an agar-impregnated polycarbonate barrier (pore diameter, 12.0 μm). Adaxial epidermal cells of each carpel redifferentiate. (**D**) Carpels separated by an agar-coated solid polycarbonate barrier. The adaxial epidermal cells do not redifferentiate. (**E**) Control agar barrier (not loaded) next to the abaxial surface of one carpel. These epidermal cells do not redifferentiate. (**F**) Factor-containing agar barrier between the abaxial surface of one carpel and an anther. Factors in the agar induce redifferentiation in the carpel epidermal cells but not in the anther epidermal cells. Abbreviations: E, epidermal cells; An, anther; Ag, agar-impregnated barriers; and C, carpel. Open arrowheads (C and F) show redifferentiated epidermal cells.

Porous barriers were impregnated with agar to trap, transport, and characterize the nature of the factors that induce redifferentiation (Fig. 2A). The agar barriers were placed between prefusion carpels (Fig. 2B), which were then allowed to develop to a normal postfusion stage and sectioned. If the barrier is water permeable (Fig. 3C), the cells redifferentiate. No redifferentiation occurs when the agar-impregnated barrier is impermeable to water (Fig. 3D). Use of the agar barrier permits tests of competence to be performed without a possible wound response from the grafting process. The loading of the agar barrier with factors is accomplished by placing the barrier between two prefusion carpels and leaving the barrier in place until both carpels have contacted the barrier. The loaded barrier is then removed and placed next to the abaxial surface of one of the two carpels. These loaded barriers induced redifferentiation of the abaxial epidermal cells (Fig. 3F) while the anther epidermal cells remained epidermal (Fig. 3F). When control agar barriers that had not been loaded with the factors were used (Fig. 3E), the cells did not redifferentiate. Anther cells did not respond to the stimulus. This condition may be constant for anther epidermal cells, or the period during which anther cells can redifferentiate may have passed because at this stage of floral development the anther epidermal cells have been differentiated for a much longer time than the carpel epidermal cells.

These results indicate that diffusible factors in *C. roseus* carpels trigger epidermal cell redifferentiation. Both adaxial and abaxial carpel epidermal cells can respond to the stimulus, but anther epidermal cells do not redifferentiate, at least at this stage of floral development. Factor-containing agar barriers can be used to investigate the specificity and timing of competence in epidermal cells of *C. roseus* and of other plant species.

REFERENCES AND NOTES

1. F. C. Steward, *Sci. Am.* **209**, 104 (October 1963).
2. P. F. Wareing, *Bot. Mag. (Tokyo)* **1**, 13 (1978).
3. R. Bloch, *Encycl. Plant Physiol.* **15**, 146 (1965).
4. M. L. Christianson and D. A. Warnick, *Dev. Biol.* **95**, 288 (1983).

5. C. N. McDaniel, in *Pattern Formation*, G. M. Malacinski and S. V. Bryant, Eds. (Macmillan, New York, 1984), p. 393.
6. F. Meins and A. N. Binns, *BioScience* **29**, 221 (1979).
7. D. J. Carr, in *Positional Controls in Plant Development*, P. W. Barlow and D. J. Carr, Eds. (Cambridge Univ. Press, Cambridge, 1984), p. 441.
8. P. F. Wareing and C. F. Graham, in *Developmental Control in Animals and Plants*, C. F. Graham and P. F. Wareing, Eds. (Blackwell, Palo Alto, CA, 1984), p. 73.
9. Intact epidermal cells do not redifferentiate or graft even after months of intimate contact with either wounded or nonwounded surfaces [D. B. Walker and D. K. Bruck, *Can. J. Bot.* **63**, 2129 (1985)].
10. R. Moore, *ibid.* **62**, 2476 (1984).
11. D. K. Bruck and D. B. Walker, *Bot. Gaz. (Chicago)* **146**, 188 (1985).
12. Postgenital fusions are tissue unions of previously free surfaces and occur by adhesion of the cell walls in the absence of protoplasmic union [F. Cusick, in *Trends in Plant Morphogenesis*, E. G. Cutter, Ed. (Longmans, Green, London, 1966), p. 170].
13. Epidermal redifferentiation into parenchyma is indicated by a change in shape, cytoplasmic density, and orientation of the plane of division. Epidermal cells are characteristically rectangular in shape and densely cytoplasmic, and they divide preferentially in the anticlinal plane. Parenchyma cells are isodiametric in shape and highly vacuolated, and they divide in both anticlinal and periclinal planes.
14. N. H. Boke, *Am. J. Bot.* **34**, 433 (1947); *ibid.* **35**, 413 (1948); *ibid.* **36**, 535 (1949).
15. D. B. Walker, *ibid.* **62**, 457 (1975); *Protoplasma* **86**, 29 (1975); *ibid.*, p. 43.
16. J. A. Verbeke and D. B. Walker, *Am. J. Bot.* **72**, 1314 (1985).
17. D. B. Walker, *ibid.* **65**, 119 (1978).
18. ———, *Planta* **142**, 181 (1978).
19. A wide variety of barrier pore sizes have been tested, ranging from a pore diameter of 12.0 μm to one of 0.1 μm. Dialysis membrane with molecular weight cutoffs ranging from 1,000,000 to 1,000 have been tested. Epidermal cell redifferentiation occurred regardless of barrier pore size [J. A. Verbeke and D. B. Walker, *Planta* **168**, 43 (1986)].
20. A. R. Spurr, *J. Ultrastruct. Res.* **23**, 31 (1969).
21. We thank R. Moore and R. Heupel for valuable discussions and advice. Supported by NSF grant DCB 86-15939 to J.A.V.

21 December 1988; accepted 3 March 1989

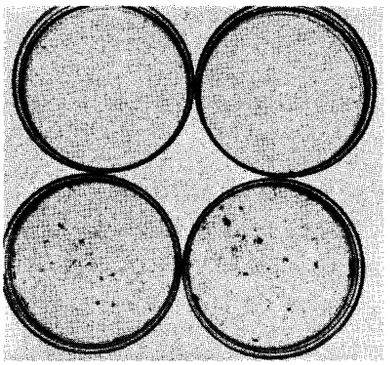

Fig. 1. Fat foci after transfection of DNA into 3T3-C2 cells. (**Top**) Transfection of uncut 3T3-C2 DNA in to 3T3-C2 cells. (**Bottom**) Transfection of uncut 3T3-F442A DNA into 3T3-C2 cells. Plated cells were treated with insulin (5 μg/ml) for 21 days and stained with oil red O.

Commitment of Mouse Fibroblasts to Adipocyte Differentiation by DNA Transfection

SUZIE CHEN, LUZ C. TEICHER, DEBORA KAZIM, ROBERT E. POLLACK, LEIGH S. WISE

Cells of the mouse cell line 3T3-F442A can be induced by various hormones to differentiate into adipocytes, whereas cells of 3T3-C2, a subclone of 3T3, cannot. However, transfection of DNA from uninduced 3T3-F422A cells into 3T3-C2 cells permits recovery of 3T3-C2 transfectants that differentiate into adipocytes in the presence of insulin. DNA isolated from human fat tissue, when transfected into 3T3-C2 mouse cells, also gives rise to mouse transfectants that are induced to differentiate into adipocytes by the addition of insulin. Apparently, transfection of a trans-regulatory gene (or genes) from 3T3-F442A or human fat cells into 3T3-C2 cells is sufficient to commit 3T3-C2 cells to adipocyte differentiation.

DIFFERENTIATION REQUIRES THE concerted expression of large numbers of structural and regulatory genes. Before entering a pathway of specific differentiation, a cell must be committed to that pathway. Commitment, or the capacity to become differentiated, can precede the differentiation itself by a considerable time. Committed cells require an inducer or inducers to initiate the cascade of biochemical events that result in a differentiation.

The capacity to introduce DNA from a committed but undifferentiated cell into an uncommitted recipient cell offers a straightforward strategy for detection and eventual isolation of a committing gene for that differentiation pathway. Lassar *et al.* (*1*) showed that DNA from 5-azacytidine–treated myoblasts can induce myoblast differentiation in a culture of mouse fibroblasts; the DNA of the cells of the donor must first be demethylated by 5-azacytidine for differentiation to occur on transfection. They identified a cDNA clone from committed myoblasts that, when transfected into fibroblast cells, is sufficient to convert them to stable myoblasts (*2*). Pinney *et al.* (*3*) passaged cosmid vectors containing the human genomic library and a selectable drug resistance gene through methylase-deficient bacteria to obtain hypomethylated and thus potentially active DNA; stable myogenic cell lines were isolated when this DNA was transfected into the multipotential C3H/10T½ mouse embryo cell line.

We studied the pathway of adipocyte differentiation. When the growth of 3T3 mouse fibroblasts is arrested in culture, a minority of susceptible fibroblasts express the adipocyte phenotype (*4, 5*). Expression is accelerated by long-term exposure of the cells to insulin or brief treatment of the cells at confluence with dexamethasone and methylisobutylxanthine (*4–7*). Induced and fully differentiated adipocytes derived from 3T3 cells show the biochemical characteristics and hormone responsiveness found in true fat tissue adipocytes (*8–19*). By cloning, Green and Kehinde (*4*) isolated several 3T3-derived preadipocyte cell lines, such as 3T3-L1 and 3T3-F442A, which convert to adipocytes with almost 100% efficiency in the presence of high levels of insulin. They also obtained sublines of 3T3, such as 3T3-C2, that have a small frequency of spontaneous fat conversion and cannot be induced to adipocyte differentiation even by high levels of insulin (*5*). Nixon and Green (*19*) reported the frequency of 3T3-C2 adipogenesis to be less than 1% of 3T3-F442A. In our experiments 3T3-C2 cells were not inducible: we detected no adipocytes in $>10^8$ insulin-treated 3T3-C2 cells.

We isolated high molecular weight genomic DNA from untreated 3T3-F442A cells and cotransfected it with a plasmid carrying a neomycin (G418) resistance gene into 3T3-C2 recipient cells. After about 2 weeks in selective G418 medium (400 μg/ml), many cells survived. Most did not form large colonies, but about ten visible G418-resistant colonies (~100 cells each) were found on each plate together with about 10^3 cells, separate and in small clusters. All survivor cells were pooled and replated onto a fresh plate. As replated survivors grew into a confluent monolayer, they were treated with insulin. The plates that survived the insulin treatment were then stained with oil red O (Fig. 1). Although the number of fat foci varied greatly from monolayer to monolayer, it is clear that 3T3-F442A DNA, but not 3T3-C2 DNA, conferred on 3T3-C2 cells the capacity to differentiate into adipocytes (Table 1).

Only cells that had taken up DNA sur-

S. Chen, L. C. Teicher, D. Kazim, R. E. Pollack, Department of Biological Sciences, Columbia University, New York, NY 10027.
L. S. Wise, Department of Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854.