later stages of encapsulation. The spk^+ lamellocyte frequency was high at both early and late stages.

That the absence of melanotic tumors in *tu-Sz*^{ts} larvae at 18°C is due to normalization of the presumptive site of melanotic tumor formation and not to the inability of the lamellocytes to encapsulate at this temperature had been shown earlier by implanting tissues with modified surfaces and heterospecific tissues into *tu-Sz*^{ts} larvae maintained at 18°C (*4*). Such implants are encapsulated by lamellocytes and are usually melanized 24 to 48 hours later. To determine whether the presence of heterospecific tissue in the hemocoel elicits spk^+ lamellocytes, we implanted *D. virilis* imaginal disks into *tu-Sz*^{ts} larvae at 18°C and subsequently examined samples of the hosts' hemocytes. All of the hosts had a preponderance of spk^+ lamellocytes, whereas control larvae at 18°C had about 5 percent spk^+ lamelloctyes (Table 1).

Lamellocytes differentiate from plasmatocytes, which are spherical cells (*7*). Therefore, hemolymph samples with spk^+ lamellocytes should contain spk^+ plasmatocytes. This was indeed the case for the tumorous larvae. In *tu-Sz*^{ts} larvae, the percentage of spk^+ plasmatocytes at 26°C was significantly higher than the percentage of spk^+ plasmatocytes at 18°C. However, the percentage of spk^+ plasmatocytes at 26°C was much lower than the percentage of spk^+ lamellocytes at this temperature (Table 1). No spk^+ plasmatocytes were found in *Ore-R* samples that lacked spk^+ lamellocytes.

The increased frequency of spk^+ hemocytes in larvae in which tissues are being encapsulated suggests that the spk^+ sites on the hemocyte surfaces may play a role in encapsulation. One obvious function of these sites might be cell-to-cell binding. Therefore, we sought to determine whether spk^+ hemocytes are the sole participants in capsule formation and whether the tissue surfaces to which lamellocytes bind in capsule formation show a differential reaction to WGA.

We reported earlier (*3*) that the basement membrane of caudal fat bodies of *tu-Sz*^{ts} changes before the lamellocytes begin to accumulate at these tissue surfaces. The tissue surface changes are accompanied by a loss of WGA binding (Fig. 1d). Furthermore, in early stages of encapsulation, we find spk^+ lamellocytes adhering to affected adipose cell surfaces (Fig. 1e), and spk^+ and spk^- lamellocytes adhering to affected adipose cells that are coated with WGA material (Fig. 1f). Distinguishing between spk^+ and spk^- cells was difficult when lamellocytes were layered, but fully formed capsules showed mostly spk^- lamellocytes in their outermost layers (Fig. 1g).

To summarize the above observations and provide a mechanistic basis for capsule formation, we propose the following sequence of events:

1) The presence of an aberrant (nonself) surface in the hemocoel evokes a stress signal, a diffusible factor, that stimulates the differentiation of spk^+ hemocytes. This suggestion relies on the observation that spk^+ hemocytes circulate in the hemocoel and are not always found in direct contact with the aberrant surfaces. Whether a clone of spk^+ plasmatocytes gives rise to spk^+ lamellocytes is not known but is likely because both cell groups were found and we know from previous work (*5*) that plasmatocytes differentiate into lamellocytes.

2) The aberrant surfaces are coated with WGA material (vesicles), which we presume is released from the spk^+ plasmatocytes as they acquire the lamellocyte shape. This material, which has been described (3, 8), is seen only on nonself surfaces. We suggest that the binding of this material to the aberrant surfaces creates the recognition site for lamellocyte aggregation.

3) The spk^+ lamellocytes may bind directly to aberrant tissue surfaces, but spk^- lamellocytes bind to these surfaces via the coating material or spk^+ lamellocytes.

4) Lamellocyte-to-lamellocyte adhesion involves spk^+-to-spk^- surfaces as well as spk^--to-spk^- surfaces via the extruded materials that are the products of the competent cells (3).

5) Lamellocyte layering continues until the competent hemocyte population and its products in the hemolymph are exhausted and the stimulus for the stress signal has been obliterated by the formation of impenetrable capsular walls.

T. M. RIZKI
ROSE M. RIZKI
Division of Biological Sciences, University of Michigan, Ann Arbor 48109

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Plant Chimeras Used to Establish de novo Origin of Shoots

Abstract. *When African violet leaf explants are cultured in vitro, buds and shoots develop directly from the upper leaf surfaces. Three developmentally different African violet chimeras were cultured, and in each case adventitious shoots that developed into plants had the parent chimera pattern. A multicellular origin of the adventitious buds accounts for these results.*

Since 1937 when Naylor and Johnson presented data that have been popularly interpreted to suggest that a single epidermal cell is the source of adventitious shoots from African violet leaf tissue, the notion has pervaded the literature that adventitious shoot formation is a result of the division of a single epidermal cell (*1*). To the contrary, in our opinion the data of Naylor and Johnson suggest that adjacent epidermal and parenchyma cells also participate in the formation of adventitious shoots. Using chimeral cultivars of African violets, we show here that all layers of leaf tissue are involved in adventitious bud formation. Thus, adventitious shoots are of multicellular origin.

The ontogeny of de novo buds from leaf explants in tissue culture has enormous implications for basic and applied research. As scientific interest focuses on using plant tissue cultures for novel genetic engineering of crop plants, the understanding of the cellular origins of the plants from culture becomes a matter of paramount importance.

A plant chimera is an individual composed of two or more genotypically different tissues. The components of a chimera may differ with respect to their chromosome or plastid constitution. Cramer (*2*) added the important qualification that the genotypically different tissues in the chimera must be represented by their respective cell lines in the

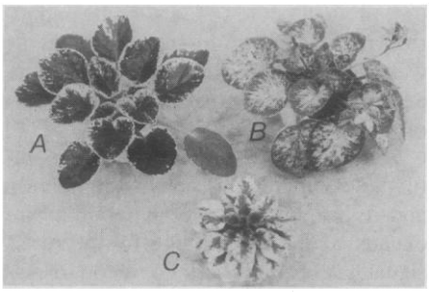

Fig. 1. Three African violet chimeras used in this study: (A) cv. Bold Dance; (B) cv. Marge Winters; and (C) cv. Calico Kitten.

growing point, the shoot apex. There are three independent germ layers of the dicotyledon apex (*3*). The outermost layer, the tunica, consists of an outer layer (LI) and a second layer (LII); the inner layer, the corpus, is LIII. The view that the layers in the shoot apex with this tunica-corpus organization are relatively independent is supported by observations on plant chimeras. Stewart and Burk (*4*) and others have shown that in the normal development of the dicotyledonous plant leaf LI gives rise to epidermis only; LII gives rise to the subepidermal layer of the leaf cells and to internal cells around the leaf edge. Tissue derived from LIII occupies the remaining internal portion of the leaf.

We utilized variegated leaf chimeras of *Saintpaulia* of various combinations of chimeral tissue to document the contributions of the various histogens to adventitious shoot formation (Fig. 1). Analysis by electron and light microscopy combined with inheritance data established the following LI, LII, and LIII periclinal chimera histogenic organizations: (i) cv. Bold Dance, GWG type; (ii) cv. Marge Winters, GGW type; (iii) cv. Calico Kitten, WWG type (for example, GGW = LI, green; LII, green, LIII, white). African violet chimeras have plastids in part of the leaf tissue that lack the capacity to produce either chlorophyll or thylakoid components necessary for chlorophyll stability; as a result, there are green and white areas in the leaf.

The following data unequivocally support the idea of the multilayer development of adventitious shoots from chimeral African violet leaf tissue:

1) Chimeral plants were regenerated in large numbers from the tissue culture of chimeral plants. Twenty initial cultures of cv. Marge Winters resulted in 792 plants; ten cultures of cv. Calico Kitten gave 350 plants; eight cultures of cv. Bold Dance gave 100 plants. Using another chimera hybrid, cv. Tommie Lou, we obtained over 3000 plants, all of which were identical to the original chimera. If the plants had a single-cell origin, one would expect all green or all white plants from leaf tissue. Figure 2 illustrates cultures of cv. Marge Winters and cv. Bold Dance that contain all chimeras exactly like the parent. More rarely, all white or all green plants were obtained.

2) Anatomical evidence also supports the multicellular origin of these adventitious shoots (Fig. 3). An early event of adventitious bud formation, as shown by microscopic study of upper-leaf cross sections, was an increased xylem vessel development in the spongy mesophyll in proximity to the differentiating regions above. The xylem proliferation in this region may suggest that the vascular system (probably derived from LIII) supplies the region with nutrients for organogenesis (*5*).

3) Three different periclinal chimeras (Fig. 1), each with different shoot apex organization (which is represented in different cell layers of the leaf), were uniformly regenerated. This could only have resulted from the participation of all three histogens in the development of adventitious buds from the leaf explant.

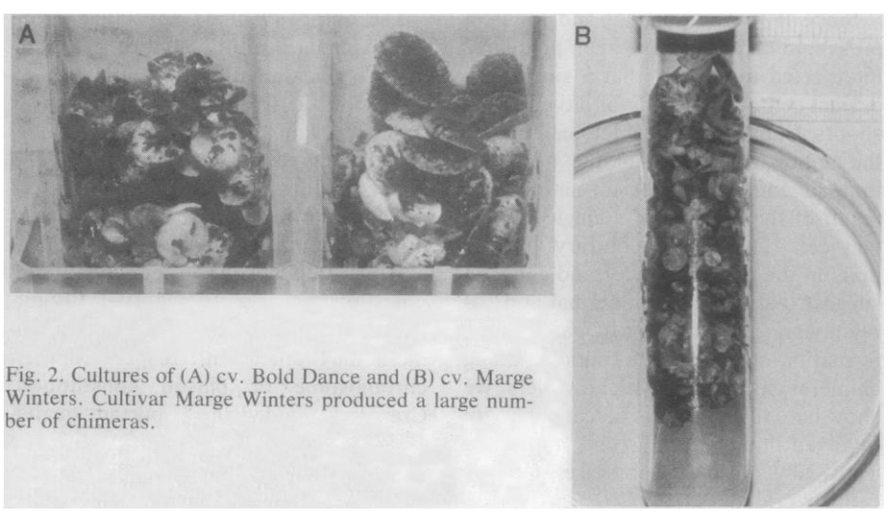

Fig. 2. Cultures of (A) cv. Bold Dance and (B) cv. Marge Winters. Cultivar Marge Winters produced a large number of chimeras.

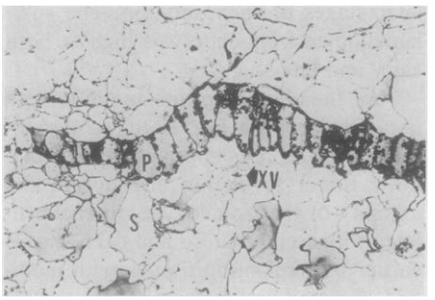

Fig. 3. Cross section (×250) of the upper leaf blade after 30 days in culture, showing palisade cells and periclinal cell divisions of the upper epidermal cells. Anticlinal divisions of epidermal cells produced noticeable protrusions from the upper leaf surface; *XV*, xylem vessels; *P*, palisade cells; *S*, spongy mesophyll cells.

This concept of the multicellular origin of adventitious shoots is important for a basic understanding of adventitious origin of shoots. It has application in mutation studies and, from an applied viewpoint, in the clonal propagation of important plant chimeras. Even though Naylor and Johnson are cited as having provided evidence for a single epidermal cell origin of adventitious shoots, their conclusions also are in agreement with our findings. Although Naylor and Johnson suggested that adjacent epidermal cells and parenchyma cells participate in the final formation of adventitious shoots, the extent of involvement of these cells was not clear at that time.

R. NORRIS
Post Office Box 3190, Conroe, Texas 77305

R. H. SMITH
Department of Plant Sciences, Texas A&M University, College Station 77843

K. C. VAUGHN
Southern Weed Science Laboratory, Department of Agriculture, Post Office Box 225, Stoneville, Mississippi 38776

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