Electron Microscopic Evidence for Plasmodesmata in Dicotyledonous Guard Cells

Abstract. *In* Nicotianna tobaccum *and* Vicia faba *leaves, plasmodesmata were observed by electron microscopy in walls between sister guard cells and walls between guard and epidermal cells. The latter were found primarily in pit fields of anticlinal walls and showed considerable complexity as evidenced by branching. Cytologically, the plasmodesmata appear functional in operative guard cells and should be considered in the mechanism of stomatal movements.*

Almost a century ago Tangle (*1*) discovered that protoplasmic strands exist between plant cells. These strands were named plasmodesmata (*2*). Until recently (*3*), the preparations used to demonstrate their presence under light microscopy were severely criticized as introducing artifacts.

It remained for the more sophisticated techniques associated with electron microscopy to prove that there was continuity of protoplasts between cells including their plasma membranes via plasmodesmata (*4*). Although they are now considered a common occurrence in plant cells (*5*), it is generally accepted that guard cells usually do not contain them, or if they do there are few (*6*). Because of their probable importance in transmission of stimuli (*7*) and translocation of materials symplastically in the plant (*8*), a systematic search for their presence in guard and neighboring cells of *Nicotianna tobaccum* and *Vicia faba* was made.

Small leaf sections from both young and expanded leaves were obtained from greenhouse-grown plants. The sections were fixed in a mixture of 2 percent glutaraldehyde and 2 percent depolymerized paraformaldehyde with 0.06*M* sucrose (in 0.05*M* collidine buffer, *p*H 7.3) for 2 hours at 0° to 4°C, followed by postosmication (1 percent OsO_4 in collidine buffer, *p*H 7.3) overnight in the refrigerator. A subsequent stain of 0.5 percent aqueous uranyl acetate was used. Tissues were washed in distilled water, dehydrated in an acetone series, and embedded in a Spurr epoxy resin mixture (*9*). After being cut, sections were further stained with alkaline lead citrate and observed with a Philips EM 200 electron microscope.

Plasmodesmata were observed in ventral walls between sister guard cells and, most important, between guard and epidermal cells (Fig. 1) in both *Vicia* and tobacco. Their presence was not at first easily detected between the guard and neighboring cells. They were found in large numbers (15 to 20/μm^2) and almost exclusively in pit fields of end walls of functioning guard cells. Contrary to reports on most other cells (*10*), no restriction of thickening in the primary wall was observed in such pit fields. The significance of the osmiophilic core (see arrow) in what would normally be middle lamellae is unknown. However, in transection such material appears to encompass individual plasmodesma (Fig. 2). The plasmodesmata in the fields are complex with frequent multiple branching. This has been noted only for cells very active metabolically, such as between sieve tubes and their companion cells and around the secretory cells of the pitcher of Nepenthes (*11*). No structural continuity of a plasmodesma with endoplasmic reticulum was noted. The plug may be a condensation of cytoplasmic ground substance, possibly containing a high proportion of lipids. The trilaminar structure of the outer limiting membrane of a plasmodesma, as seen in cross section, had the same dimension and structure as the plasma membrane.

These observations should dispel the

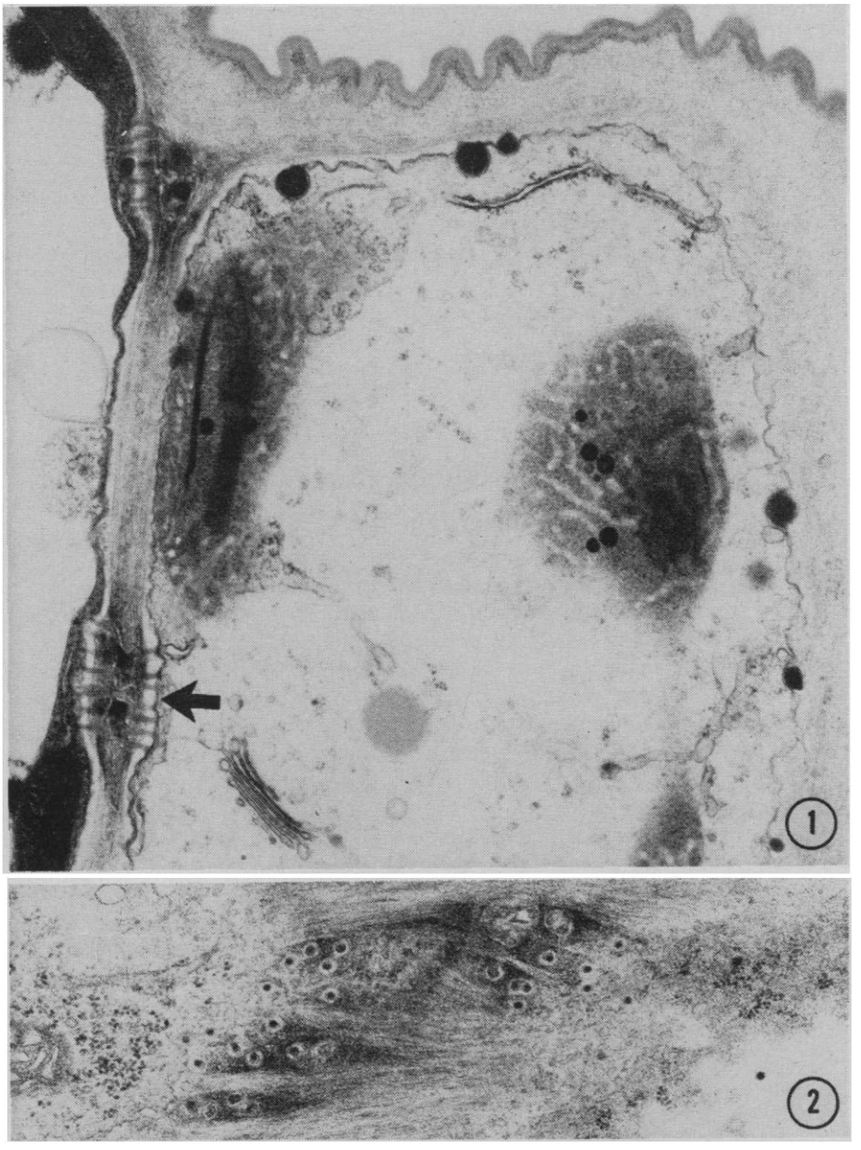

Fig. 1. Multibranched plasmodesmata in a pit field (see arrow) traversing the common wall of *Vicia faba* between an epidermal (left) and a guard cell (right). The leaf surface is identified by the wrinkled wall covered with cuticle at the top of the micrograph (× 27,700). Fig. 2. Parallel section of wall between guard and epidermal cells of tobacco leaf showing cross sections of plasmodesmata (× 33,100).

notion that plasmodesmata of guard cells are missing or of little importance. Cytologically, the plasmodesmata appear functional and probably play a major role in the movement of substances in and out of the guard cell or the transmission of stimuli. These observations confirm reports, based on light microscopy (*12*), that plasmodesmata exist between neighboring cells and guard cells of Dicotyledonous species and complement recent electron microscopic evidence for their existence in a monocotyledonous species (*13*). Certain other aspects of *Vicia faba* and *Nicotianna tobaccum* guard cell ultrastructure have been described (*14*).

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White-Noise Analysis of a Neuron Chain: An Application of the Wiener Theory

Abstract. *The Wiener theory of nonlinear system identification was applied to a three-stage neuron chain in the catfish retina in order to determine the functional relationship between the artificial polarization of the horizontal cell membrane potential and the resulting discharge of the ganglion cell. A mathematical model was obtained that can predict quantitatively, with reasonable accuracy, the nonlinear, dynamic behavior of the neuron chain. The applicability of the method is discussed. We conclude that this is a very powerful method in the analysis of information transfer in the central nervous system.*

Analysis techniques for linear systems have been used extensively in the study of biological systems, often by using "small signals" or by making certain assumptions about the behavior of the system. However, a biological system seldom behaves linearly even under "small signal" conditions (*1*, *2*). In fact, from the functional point of view, nonlinearities in biological systems appear to be a necessity as is, for example, the logarithmic transformation of the sensory input in order to accommodate large ranges.

Wiener (*3*) postulated that a nonlinear system can be identified by its response to white Gaussian noise, since with such an input there is a nonzero probability that any given time function over a finite interval of time will be closely represented by some sample of this noise, and therefore the system will effectively be tested with all possible inputs (*4*).

Considering a nonlinear system S, which is subjected to an input white noise $x(t)$ and whose output is $y(t)$, Wiener has shown that $y(t)$ can be represented by the expansion

$$y(t) = \sum_{n=0}^{\infty} G_n [h_n, x(t)]$$

where t is time, $\{G_n\}$ is a complete set of orthogonal functionals, and $\{h_n\}$ is the set of "Wiener kernels" of S. The set $\{h_n\}$ completely characterizes a particular system. The power density spectrum of the input white noise is $\phi_{xx}(f) = P$, where P is the power spectrum level and f is the frequency. Lee and Schetzen (*5*) have shown that h_n is given by the expression

$$h_n(\tau_1, \tau_2, \ldots, \tau_n) = \frac{1}{n!\,P^n} \cdot \frac{\{y(t) - \sum_{m=0}^{n-1} G_m [h_m, x(t)]\} \cdot}{x(t-\tau_1)x(t-\tau_2)\ldots x(t-\tau_n)}$$

which is simply a cross-correlation between functions of the input white noise and the response of the system (*6*); τ is in seconds.

In this report we apply the Wiener theory to a three-stage neuron chain formed by the horizontal cell, bipolar cell, and ganglion cell in the catfish *Ictalurus punctatus* retina (*7*). The input to this chain is an extrinsic current injected into a horizontal cell that forms part of a laminar structure extending across the entire retina. This extrinsic current gives rise to a potential change in the bipolar cells, which, in turn, evokes spike discharges from the ganglion cells, the output element in our study (we assume that potential change inside the horizontal cell is proportional to the magnitude of the extrinsic current).

A preliminary analysis for the system under study showed that (i) the series can be truncated after the second order term with small error, (ii) the kernels should be computed for values of their arguments up to 300 msec, (iii) the white-noise bandwidth should be flat from essentially 0 up to 25 hz, and (iv) the temporal length of the identifying experiment should exceed 30 seconds in order to expect less than 5 percent error in the statistical estimates of the cross-correlations (kernels).

A white-noise signal (35 seconds long) was stored on analog magnetic tape and was concatenated with itself ten times to form the stimulus record (350 seconds long). An electrical circuit was designed so that the magnitude of the current passed through the electrode in the horizontal cell was proportional to this white-noise signal. The ganglion responses in ten experiments with identical white noise were superimposed and represented by histo-