quence of events leading to platelet aggregation could account for the half-hour delay in onset of thrombocytopenia following injection of toxin-LR.

Toxin-LR elicits hepatomegaly due to pooling of blood in and around liver sinusoids. The sharp rise in Spearman's rank correlation between platelet count and liver weight that occurred 30 minutes after injection (Table 2), when changes in these parameters were observed in some animals, indicates that thrombocytopenia and hepatomegaly were almost concurrent. We postulate that the hepatomegaly was due to pulmonary vascular occlusion, possibly by platelet thrombi, with secondary hypoxemia, heart failure, and shock.

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Cell Polarity: Endogenous Ion Currents Precede and Predict Branching in the Water Mold *Achyla*

Abstract. *The hyphae of the water mold* Achyla bisexualis *generate electrical currents that enter the growing tips and leave farther back. An inward-moving current also precedes branching and predicts the site of branch emergence; during the branching process, the current at the original tip declines or even reverses transiently without any change in growth rate. The inward current probably acts as an early signal during branch differentiation. The flow of specific ions rather than the flow of electrical charge probably serves to localize growth.*

Fungi exemplify to an extreme degree the eukaryotic habit of polarized growth. The mechanisms by which cell polarity is established and maintained are not well understood. Much interest therefore was generated by the discovery of Jaffe and his co-workers that many growing and developing systems drive electrical currents through themselves. In two instances, the developing zygote of the brown alga *Pelvetia* and the germinating lily pollen grain, the site of current entry predicts the site of future outgrowth (*1*). We report here that growing hyphae of the water mold *Achyla bisexualis* also generate endogenous ion currents that appear to play an important role in the genesis and maintenance of polarized growth.

Transcellular ion currents were measured with a vibrating probe constructed as described by Jaffe and Nuccitelli (*2*). The sensing element of this instrument consists of a microelectrode tipped with a ball of platinum black (approximately 20 μm in diameter). The electrode vibrates over an amplitude of 30 μm and measures the small potential differences between the extremes of its sweep (as little as 20 nV). The potential differences are converted to current densities by use of Ohm's law and the known resistivity of the medium. The probe measures only the net flow of electrical current, which is a summation of the fluxes of individual ionic species; by convention, current refers to the flow of positive charge.

Figure 1 shows the current densities measured along the length of a hypha of *A. bisexualis* growing in DMA medium (*3*). The probe was vibrating perpendicular to the hyphal axis, and the ordinate therefore gives the magnitude of the current vector entering or leaving the membrane. The inward-flowing current extended 350 μm behind the tip, beyond which the current turned outward (*4*).

Hyphae growing in this medium branched frequently, converting a nongrowing region of the hyphal trunk into a new tip that elongated at the same rate as the original tip (5 μm/min). Branches never emerged closer than 150 μm to the old tip but appeared at random farther back. Branching had a marked effect on the current pattern, especially if the branch emerged more than 300 μm behind the tip. In the hyphae that we studied every new branch emerged from a region of inward current, and in nearly every case (25 out of 27) the branch was associated with a peak of inward current. Remarkably, current entry into the site of the future branch was routinely detected 20 minutes or more before there was any visible sign of branch emergence, and the intensity of inward current was maximal before the new branch could be observed. Figure 2 shows an example of this phenomenon; the arrows mark the positions at which branches were seen 20 minutes after this current pattern was recorded.

What effect does the emergence of a branch have on current flow into the original tip? Current into the old tip

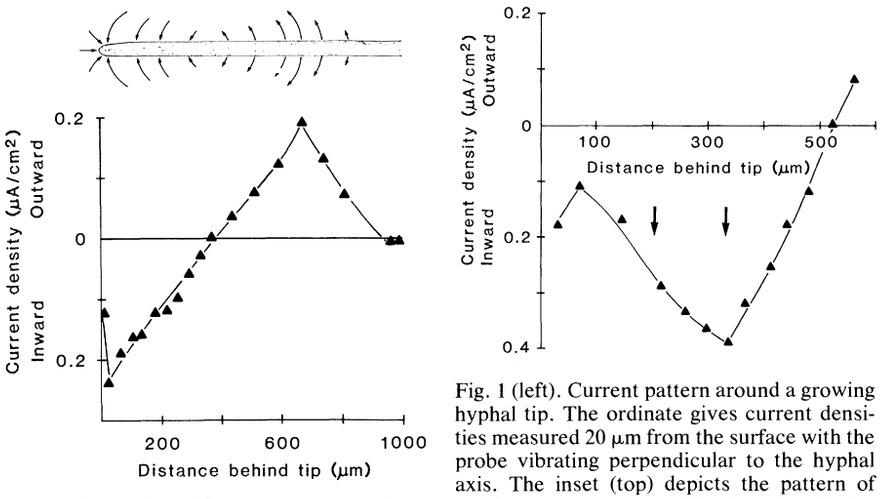

Fig. 1 (left). Current pattern around a growing hyphal tip. The ordinate gives current densities measured 20 μm from the surface with the probe vibrating perpendicular to the hyphal axis. The inset (top) depicts the pattern of current flow inferred from a more extensive series of measurements made both perpendicular and parallel to the hyphal axis. The inset is not to scale. Fig. 2 (right). Current map recorded from a hypha during a 5-minute period from 16 to 21 minutes before two branches appeared at the site marked by arrows. The current at the old tip is inward but small, whereas the peak inward current accurately predicts the location of a future branch site.

declined (Fig. 2) or even reversed, depending on the position of the new branch. A striking example of current reversal is shown in Fig. 3. In this hypha a single branch emerged 350 μm behind the original tip. Figure 3A depicts the current pattern when the branch was first noted. It shows a strong peak of inward current associated with the new branch, while the direction of current flow at the old tip had turned outward. Figure 3B shows the course of events at the old tip as a function of time (*5*). At the beginning of the observation period, the current was strongly inward. Over the next 2 hours the current declined, turned outward, and then returned to inward, while the new branch emerged at 70 minutes (arrow). Throughout this time, the original tip continued to grow at a steady rate of 4.2 μm/min.

The details of the current pattern vary from one hypha to another, depending on the position of the emerging branch, but the following features were constant: (i) every growing tip that we examined (over 200) generated an electrical current; (ii) a new branch was always preceded by inward current and usually emerged at, or close to, the point of peak inward current; and (iii) current into the old tip declined and sometimes reversed transiently, but the old tip continued to grow at a constant rate regardless of the magnitude or direction of the current.

The significance of these ion currents becomes apparent from a consideration of branching. Inward current precedes any visible signs of branching by 20 minutes or more. The current cannot, therefore, be merely a consequence of growth (for example, cannot be due to vesicle fusion with the membrane or to cell wall biosynthesis). It either must be a causal element in the localization of the branch or must be closely linked to the localizing mechanisms (*6*). What role might the current play in the localization of growth? Jaffe *et al.* have proposed that the electric field generated by the current drives cytoplasmic vesicles and membrane proteins toward the point of peak inward current (*7*). According to a second hypothesis, the flow of specific ions rather than the flow of charge could polarize growth by establishing regional differences in the ionic composition of the cytoplasm (*8*). (These two mechanisms are not mutually exclusive.)

Let us now examine these mechanisms in light of our observations. In an established tip the direction of flow, and with it the polarity of the electric field, can reverse without altering the growth rate. Therefore, unless other factors affect the cytoplasmic field, the self-electrophoresis mechanism cannot account for the maintenance of polarity during hyphal growth (*9*). Instead, we propose that the inward current creates a permissive ionic environment for growth. We have evidence (*10*) suggesting that the inward current is carried by an influx of protons, and we speculate that this flow of protons into the tip directs tip growth, perhaps by lowering the *p*H of the apical cytoplasm, thereby affecting the differential assembly of cytoskeletal elements (*11*). According to this explanation, the flux of a particular ion is a signal that guides growth rather than a driving force for electrophoresis.

If this explanation is correct, we must account for continued tip growth when the electrical current turns outward (Fig. 3). We suspect that the outward electrical current results from a transient flux of some other ion, possibly K^+ efflux, that masks continued influx of protons. However, it is possible that, once the branch has emerged, its cytoskeleton provides a fixed axis such that polarized growth no longer depends on the direction of ion flow (*12*).

With regard to the localization of branch emergence, both electrophoresis and signal remain viable hypotheses. The new tip always emerges in a region of inward current. The current, therefore, could serve either as a signal con-

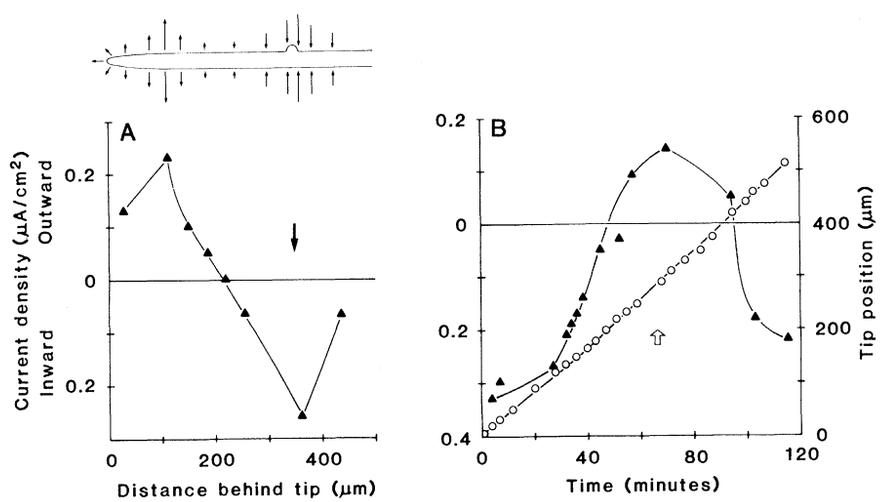

Fig. 3. (A) Current map surrounding a branching hypha. These measurements were recorded a few minutes before, and immediately after, the time at which a branch emerged at the position of the arrow. The current at the old tip was outward, and the inward current peak resided at the new branch (inset). (B) The current flow (▲) at the old tip mapped in (A) monitored at a point 30 μm behind the tip (*5*). The direction of current flow reversed transiently while growth (○) continued. Current reversal at a tip was always correlated with branching. The arrow indicates both the time at which the pattern in (A) was recorded and the time at which the branch was first observed.

veying spatial information or as a driving force for the electrophoresis of material to the future branch site.

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4. The pattern of current flow at the very tip of the hypha may be complicated by the conical shape of the tip. Current flow into a hyphal tip has also been observed in *Achlya debaryana* by B. Armbruster and M. H. Weisenseel [*Protoplasma* **11**, 65 (1983)].
5. Since the tip grew at 5 μm/min, the probe was moved in order to follow a position 30 μm behind the tip.
6. Branches never emerged closer than 150 μm to the original tip. Therefore, the maximum time from the initiation of branch differentiation to its visible emergence cannot be longer than 30 minutes (the time it takes the old tip to grow 150 μm at 5 μm/min). We can detect inward current at the future branch site at least 20 minutes, and sometimes as early as 40 minutes, before branching; this result suggests that the current acts early in the branching process.
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Sex Pheromone Biosynthesis in *Trichoplusia ni*: Key Steps Involve Delta-11 Desaturation and Chain-Shortening

Abstract. *In addition to the primary pheromone component (Z)-7-dodecenyl acetate, the sex pheromone gland of* Trichoplusia ni *contains the immediate fatty acyl precursor (Z)-7-dodecenoate and a large quantity of (Z)-11-hexadecenoate. Labeling experiments showed that (Z)-11-hexadecenoate is chain-shortened to (Z)-9-tetradecenoate, and that this in turn is chain-shortened to (Z)-7-dodecenoate. The same mechanism appears to explain the sex pheromone compositions of many other moth species.*

The sex pheromones of more than a hundred species of moths, totaling more than 200 compounds, have been chemically identified (*1*). Most of these compounds are straight-chain acetates, alcohols, or aldehydes, with chain lengths of 10, 12, 14, 16, or 18 carbon atoms, and with one or two double bonds. One of the most puzzling aspects of these studies has been that different double bond positions seem to predominate in different chain lengths. For example, in the Noctuidae, the family of moths for which the greatest number of sex attractants has been characterized, (*Z*)-5-decenyl, (*Z*)-7-dodecenyl, (*Z*)-9-tetradecenyl, and (*Z*)-11-hexadecenyl moieties comprise 80 percent of the known sex pheromone components (*1, 2*). In the course of work with the cabbage looper moth, *Tricho-*

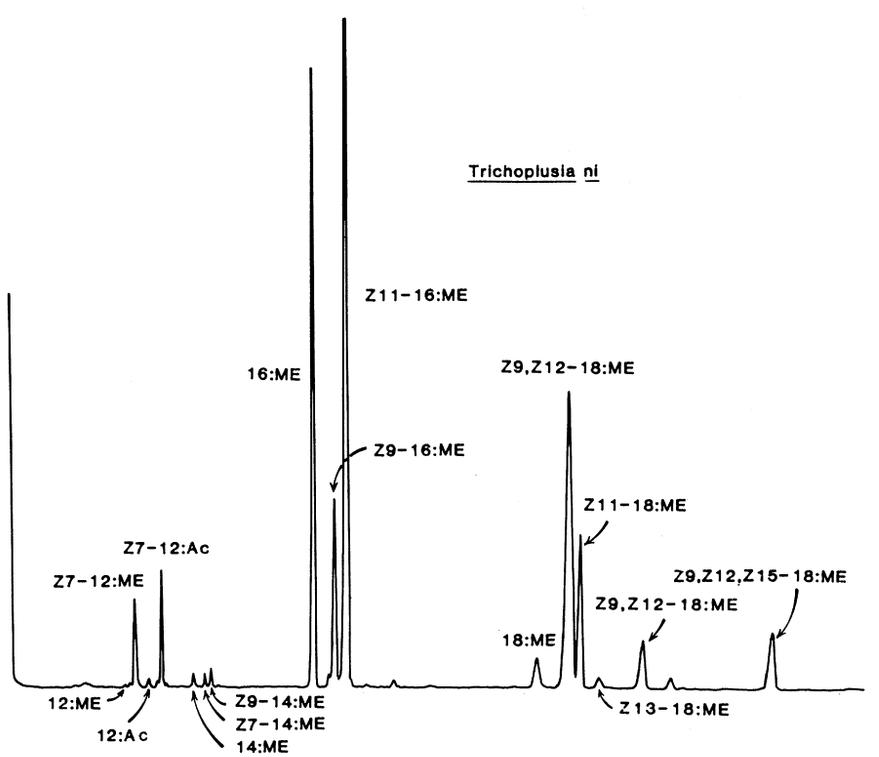

Fig. 1. Capillary GLC trace of sex pheromone components and total complement of fatty acyl moieties in pheromone glands of *Trichoplusia ni*, after methanolysis and acetylation. Abbreviations: *Z7-12:Ac*, (*Z*)-7-dodecenyl acetate; *Z7-12:ME*, (*Z*)-7-dodecenoate; and so on.