The kinetics of $I_{K(Ca)}$ provide insight into the manner of its activation. At 40 mV, calcium accumulation is rapid, but potassium is driven by a smaller EMF than it is at 80 mV. The result at 40 mV is (i) a fast rise in $I_{K(Ca)}$, consistent with rapid activation of $g_{K(Ca)}$ by rapid accumulation of Ca^{2+} near the inner surface of the membrane, and (ii) a smaller outward-current maximum than at 80 mV. In going from the peak of the $[Ca]_i$-voltage plot toward E_{Ca} (40 to 140 mV) the EMF acting on potassium increases while that acting on calcium decreases. Over this range the rate of entry and accumulation of Ca^{2+} progressively declines, as does the rate of activation of $I_{K(Ca)}$. Thus, although g_{Ca} and $V_m - E_K$ are high, and presumably g_{Ca} (and hence I_{Ca}) is activated rapidly at 120 and 140 mV, $g_{K(Ca)}$ is activated very slowly, more or less in parallel with the slow and weak rise in $[Ca]_i$ inferred from the aequorin signal at those potentials (Fig. 1A).

The observed agreement between the voltage dependence of a component of outward current and the voltage relations of Ca^{2+} entry that we detected with the aid of injected aequorin provides independent evidence in support of the hypothesis (*1*–*4*) that Ca^{2+} entry during depolarization leads to the activation of a calcium-dependent potassium current. The similarities between the activation kinetics of this current and the kinetics of intracellular free Ca^{2+} accumulation indicate that activation of $I_{K(Ca)}$ during depolarization is causally related to the intracellular concentration of free Ca^{2+} rather than to the passage of Ca^{2+} through the membrane (*23*)

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21. We recorded no signals during 200- or 300-msec pulses going to − 10 mV or less, and usually not below 0 mV, even though Ca^{2+} currents have been detected at less positive potentials electrophysiologically in snail neurons (*12*, *13*), and optically in *Aplysia* by the arsenazo method (*10*). The small amount of Ca^{2+} that enters under small depolarizations may be rapidly bound to anionic sites, producing little buildup of $[Ca]_i$. Since the aequorin reacts only with free Ca^{2+}, rapidly bound Ca^{2+} would produce little light emission. This may also explain the latency between onset of depolarization and onset of the aequorin signal (Fig. 1A). Because of the power relation between Ca^{2+} concentration and aequorin emission (*19*) the aequorin method should be most sensitive to $[Ca]_i$ transients near the inner surface of the membrane, where Ca^{2+} is most concentrated during its entry before becoming dissipated by diffusion and removed by sequestering mechanisms.
22. It should not be overlooked that the net current trajectory must depart somewhat from the trajectory of potassium outward current, because a slow inward current sums algebraically with the late outward current [H. D. Lux and R. Eckert, *Nature (London)* **250**, 574 (1974)].
23. The intracellular accumulation of calcium in some neurons leads to a secondary effect, namely the desensitization or depression of $g_{K(Ca)}$ during subsequent depolarization (*5*).
24. We thank E. B. Ridgway for advice and help with the aequorin technique and for the use of essential items of equipment. We are grateful to E. B. Ridgway, O. Shimomura, and F. H. Johnson for the gift of purified aequorin, to T. Eckert for collecting specimens, and to the director and staff of Friday Harbor Laboratories of the University of Washington for providing space and facilities. Supported by PHS grants NS 8364, S07 RR07009, and GM 7191 and by NSF grant BMS 19464.

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Induction of Stalk and Spore Cell Differentiation by Cyclic AMP in Slugs of *Dictyostelium discoideum*

Abstract. *Multicellular masses of the cellular slime mold* Dictyostelium discoideum, *under conditions which ordinarily suppress cell differentiation, develop clusters of stalk cells and spore cells when implanted with Sephadex particles that had been soaked in* 5×10^{-3} M *cyclic adenosine monophosphate (AMP). A possible relation exists between oxygen gradients, cyclic AMP gradients, and the pattern of morphogenesis and cell differentiation during fruiting.*

The developmental program of *Dictyostelium discoideum*, and that of most other members of the group Acrasiales (cellular slime molds or social amoebas), includes the differentiation of two distinct cell types: thick-walled, vacuolated stalk cells and elliptical, encapsulated spore cells (*1*). Adenosine 3′,5′-monophosphate (cyclic AMP) has been identified as the chemotactic agent during the aggregation phase of the *D. discoideum* life cycle (*2*). This substance is also present in the migrating multicellular mass, the slug or pseudoplasmodium, in which prespore and prestalk regions are established. The concentration of bound cyclic AMP is highest at the anterior end of the slug, which is the region in which prestalk cells are localized (*3*). During fruiting body formation, these prestalk cells differentiate into mature stalk cells. A rise in extracellular cyclic AMP, with no increase in the level of intracellular cyclic AMP, has been reported at the time of fruiting (*4*). Bonner (*5*) demonstrated that exposure to $10^{-3}M$ cyclic AMP can cause isolated postvegetative amoebas or groups of amoebas to differentiate into stalk cells without involvement in fruiting body formation. A mutant strain (P-4) of *D. discoideum* undergoes up to 100 percent stalk cell differentiation when exposed to $10^{-4}M$ or $10^{-5}M$ cyclic AMP in 1 percent Bonner's salt solution (*6*). Thus, it is reasonable to hypothesize that cyclic AMP may be the normal control agent in stalk cell differentiation (*5*) and may in some way be involved in spore cell differentiation as well. Such a hypothesis asserts a "first messenger" role for cyclic AMP in the control of differentiation comparable to its role during the earlier aggregation stage.

As one test of this hypothesis, we decided to suppress fruiting and then implant sources of cyclic AMP into the suppressed slugs. We took advantage of the fact that slugs are prevented from fruiting when submerged in water (*7*). *Dictyostelium discoideum* NC-4 was maintained in two-membered culture with *Escherichia coli* B/r (*8*) and slugs were obtained on moist 2 percent nonnutrient agar surfaces by seeding mounds of *E. coli* with *D. discoideum* spores. After about 48 hours in darkness at 21°C, slugs migrated away from the mounds. Particles of Sephadex (G-50, fine, Pharmacia Fine Chemicals) were soaked in $5 \times 10^{-3}M$ cyclic AMP, collected by centrifugation, and dried overnight at room temperature in a hood. For each implantation, a particle was picked up on the

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20-μm tip of a micropipette that had been pulled on a micropipette puller (1754-6, Narashige Scientific Instrument Laboratory, Tokyo). The particle was then implanted in the anterior region of the slug, by a micromanipulator (Sobotka, Farmingdale, New York). Control slugs were implanted with particles that had been soaked in deionized water (or, in five cases, in $5 \times 10^{-3}M$ 5′-AMP) and dried. A glass ring (6705-R24, Thomas, Philadelphia) was then positioned around the slug and filled with Bonner's salt solution (*6*), and a cover slip was placed on the ring in such a way as to avoid air bubbles. Implanted slugs were incubated at 18°C in the dark for 40 to 68 hours. At this point, either a squash preparation was prepared and studied immediately or, in some cases, the slugs were fixed for histological examination (*9*).

Implantation of $5 \times 10^{-3}M$ cyclic AMP resulted in the production of clusters of stalk cells in more than two-thirds of the cases (37 out of 52 cases), while 22 control implantations uniformly showed a lack of such stalk cells. In 8 of 27 cases (29.9 percent), clusters of spore cells were also produced; none of the 22 control implantations produced spore cells.

Serial histological sections (Fig. 1, a to e) showed that the clusters of stalk and spore cells extended into the interior of the slug, but in all cases had at least some contact with the surface. No definite relation of the position of these masses to that of the particle was observed, but this result is inconclusive since the particles appear to be displaced during the histological procedure (Fig. 1, f to h). In several slugs, a sheath appeared to be present around the stalk cells, creating the appearance of a portion of an actual stalk (Fig. 1i).

The fact that implanted cyclic AMP can induce stalk cells in slugs, together with evidence that cyclic AMP is especially concentrated at the tip of the slug (*3*) and that extracellular cyclic AMP concentration rises at the time of fruiting (*4*), supports the hypothesis that cyclic AMP is the normal control agent for stalk cell differentiation. The formation of spore cells in almost one-third of the implanted slugs also suggests that cyclic AMP may be involved directly or indirectly in spore cell differentiation.

Clumps of *D. discoideum* cells suspended in oxygenated salt solution in roller tubes have been reported to undergo stalk and spore cell differentiation, with stalk cell differentiation beginning at the periphery and proceeding inward (*10*). It is possible that a decrease in respiration under unaerated submerged conditions causes a decrease in the concentration of adenosine triphosphate (ATP). Since ATP serves as a precursor of cyclic AMP, reduced cyclic AMP levels may be responsible for the suppression of differentiation under such conditions. This would explain our discovery

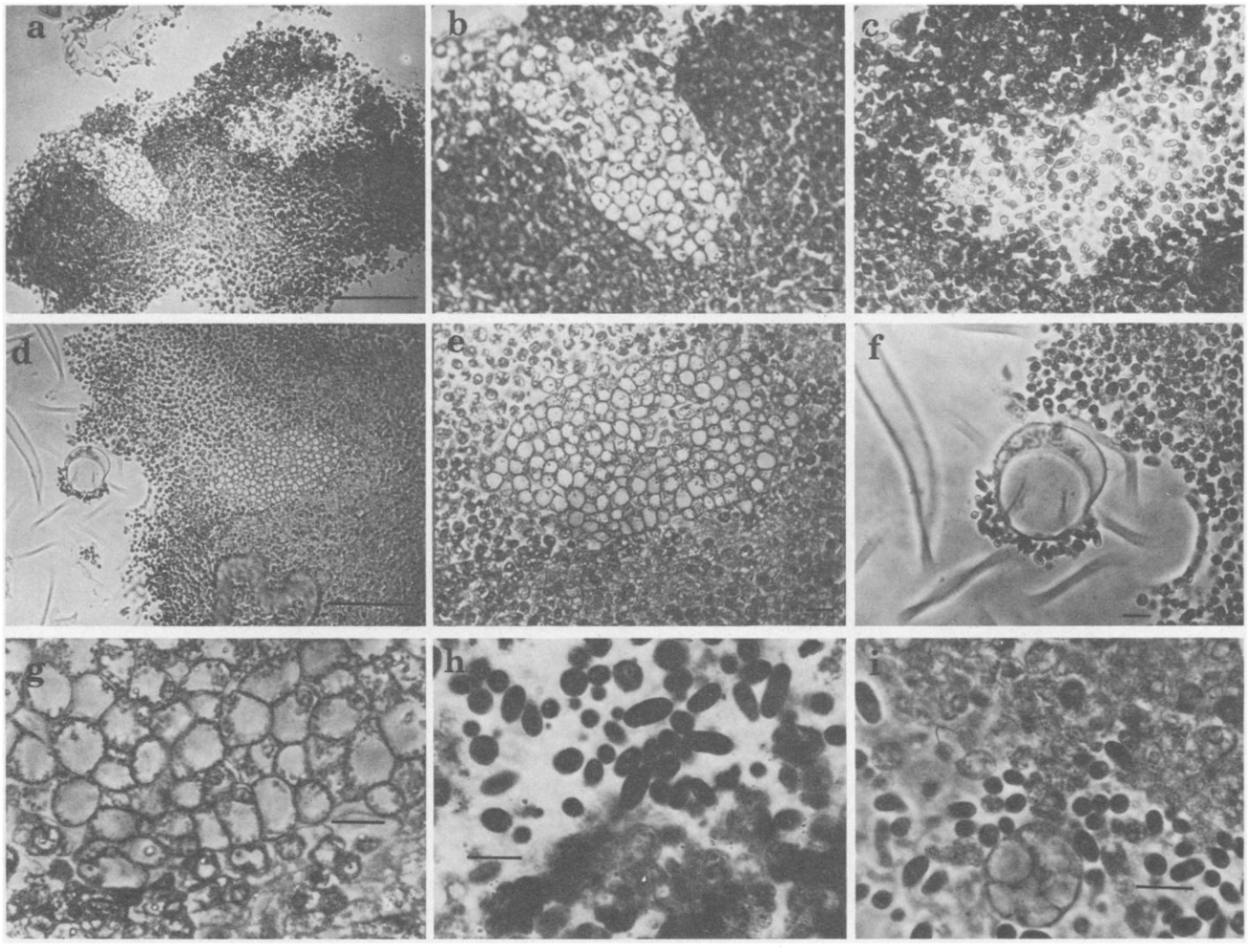

Fig. 1. Sections (3 μm) of slugs embedded in Epon-araldite and stained with 1 percent toluidine blue (*9*). (a) Slug implanted with cyclic AMP (Sephadex particle not visible at this level); (b) cluster of stalk cells in (a); (c) cluster of spore cells in (a); (d) slug implanted with cyclic AMP (displaced Sephadex particle visible to left of slug); (e) cluster of stalk cells in (d); (f) cluster of spore cells in (d); (g) cyclic AMP–induced stalk cells; (h) cyclic AMP–induced spore cells; (i) cyclic AMP–induced spore cells and cross section of induced "stalk" with sheath surrounding stalk cells. [Scale bars, 100 μm in (a) and (d), and 10 μm in (b) and (c) and (e) to (i)].

that implantation with cyclic AMP induces such differentiation. Measurements of ATP and extracellular cyclic AMP levels under aerated and unaerated conditions would be of interest in this regard.

It is possible that these considerations are relevant to the normal control of morphogenesis and differentiation. No measurements of oxygen concentration in different regions of the slug are available. If, however, increased oxygen permeability through the more fluid sheath at the slug tip created an increased rate of respiration in this region, heightened ATP and cyclic AMP levels could explain the differentiation of pre-stalk and stalk cells and the occurrence of stalk morphogenesis in this part of the slug. The question of the involvement of metabolic gradients in the establishment of polarity and pattern is of general interest in the formulation of developmental models.

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Temporary Queens in *Metapolybia* Wasps: Nonreproductive Helpers Without Altruism?

Abstract. *In* Metapolybia aztecoides *some mated females produce only workers, losing in competition with other similar egg-layers before producing either males or queens. Worker production by these ultimately nonreproductive females may incidentally benefit others without lowering individual fitness (without "altruism"). It could be a by-product of mutualism rather than of kin selection or parental manipulation.*

The social insects are of special interest to evolutionary theorists because they live in groups containing nonreproductive individuals (workers) that help rear the offspring of others (queens). Young colonies of the neotropical social wasp *Metapolybia aztecoides* (*1*) observed near Cali, Colombia (4°N, 915 m elevation), contain an additional kind of helper—mated egg-layers that produce workers but which are eventually forced to leave the colony or to become workers before producing males or queens. These temporary queens resemble workers in that they make no direct contribution to the genetic composition of future generations and they help (by worker production) maintain a colony used by other females to produce sexual brood. In this report, I describe the circumstances giving rise to temporary worker-producing queens in *Metapolybia* and discuss their possible evolutionary basis.

The colony cycle and behavior at the nest in *M. aztecoides* was observed in an undisturbed colony and in two of its offspring colonies during the 18-month period (23 February 1974 to 1 September 1975) encompassing their development from initiation to abandonment. Brood development was monitored by periodically recording cell contents on maps of the combs; 905 females were marked for individual identification with quick-drying enamel. These three colonies were observed for 260 hours with special attention to the functional roles of the marked individuals. The representativeness of the data was checked by observations and dissections of 20 additional colonies of the same species.

There are no dependable morphological means of distinguishing the castes of living females in *M. aztecoides*, although an old queen's abdomen is usually swollen by ovarial eggs, and (even in young egg-layers) extended rather than inclined ventrally during locomotion (see cover). However, egg-layers are recognizable because (i) they perform a characteristic aggressive "bending display" (cover); (ii) they usually cluster at one edge of the comb; and (iii) workers perform a distinctive shaking "dance" toward them as they move about on the comb. These behavior patterns unequivocally distinguish queens (egg-layers) from workers (nonegg-laying females seen building or foraging).

Young colonies of *M. aztecoides* alternate between multiple queen (polygynous) and single queen (monogynous) phases (*2*) (Fig. 1A). As in most other tropical social wasps, nests are founded by swarms containing numerous workers and usually several egg-layers. The number of egg-layers then gradually declines: some queens leave with swarms; some cease laying eggs and become workers when workers are scarce; and some are forced off the nest by aggressive workers and queens (*2*), and fail to return. Sometimes there is only one egg-layer for several months (Fig. 1A). If such a lone reproductive disappears, a number of young females immediately (within a few hours) begin to elicit the workers' dance. This cycle of alternating polygyny and monogyny can apparently be repeated indefinitely until, in large colonies producing males as well as females, polygyny becomes permanent (*3*). This report concerns only small, temporarily polygynous or monogynous colonies, in which male production was not observed.

The initial functional caste of a young *M. aztecoides* female depends largely on conditions in the colony soon after her emergence as an adult; if a queen or group of queens is present, she is likely to become a worker; if not, she is likely to mate and, at least temporarily, become a queen. Of the 840 female offspring marked on long-term observation nests, only 82 were known to lay eggs, and all of these were marked between 17 February and 30 March—they were newly emerged when the lone queen of the preceding 6 months disappeared on 18 March (Fig. 1). Of 96 newly emerged females marked during this critical period, 78 (81 percent) were behaviorally recognized as queens. In contrast, none of the more than 700 female offspring marked at other times on these nests were ever seen to lay eggs or evoke dancing on ei-

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