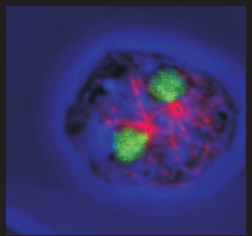
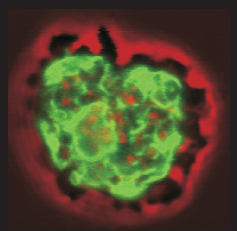
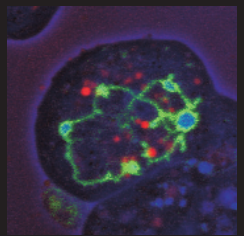
GFP Fusions Labeling Organelles and Cytoskeletal Proteins



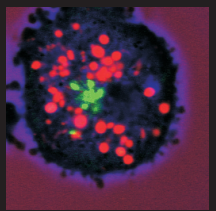
Nuclei + Microtubules



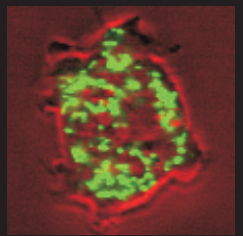
ER



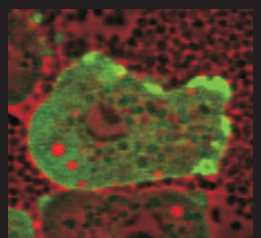
Contractile Vacuoles



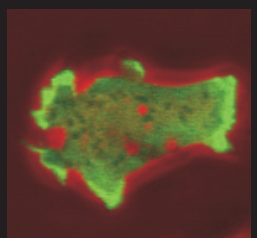
Golgi + Endosomes



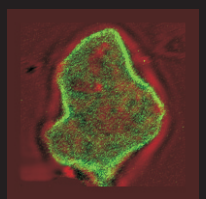
Mitochondria



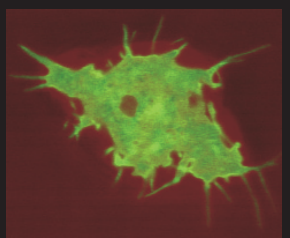
Actin



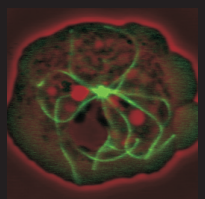
Coronin



Cortexillin



Rac 1A



Tubulin

10 μm

Plate 1 The compartments of a living *Dictyostelium* cell can be visualized. The upper panels show organelles in *D. discoideum* cells visualized by GFP fusion proteins. From left to right: nuclei labeled with histone 2 (and anti-tubulin antibody showing microtubules in red); membranes of the endoplasmic reticulum labeled with calnexin; the contractile vacuole network labeled with dajumin; the Golgi apparatus labeled with golvesin, and endosomes filled with a fluid-phase marker in red; mitochondria loaded with a construct that carries a targeted signal. In all panels except for the Golgi apparatus, fluorescence images are superimposed to phase-contrast images. Lower panels, GFP constructs representing dynamic cytoskeletal structures: actin and the actin-binding protein coronin accumulating at motile protrusions of the cells; cortexillin decorating the entire cell cortex; overexpressed Rac1 inducing filopods; microtubules and the centrosome visualized by α-tubulin. (Courtesy of Professor Günther Gerisch, with contributions by Mary Ecke, Jan Faix, Daniela Gabriel, Annette Huettig, Jana Koehler, Natalie Schneider, and Monika Westphal.)

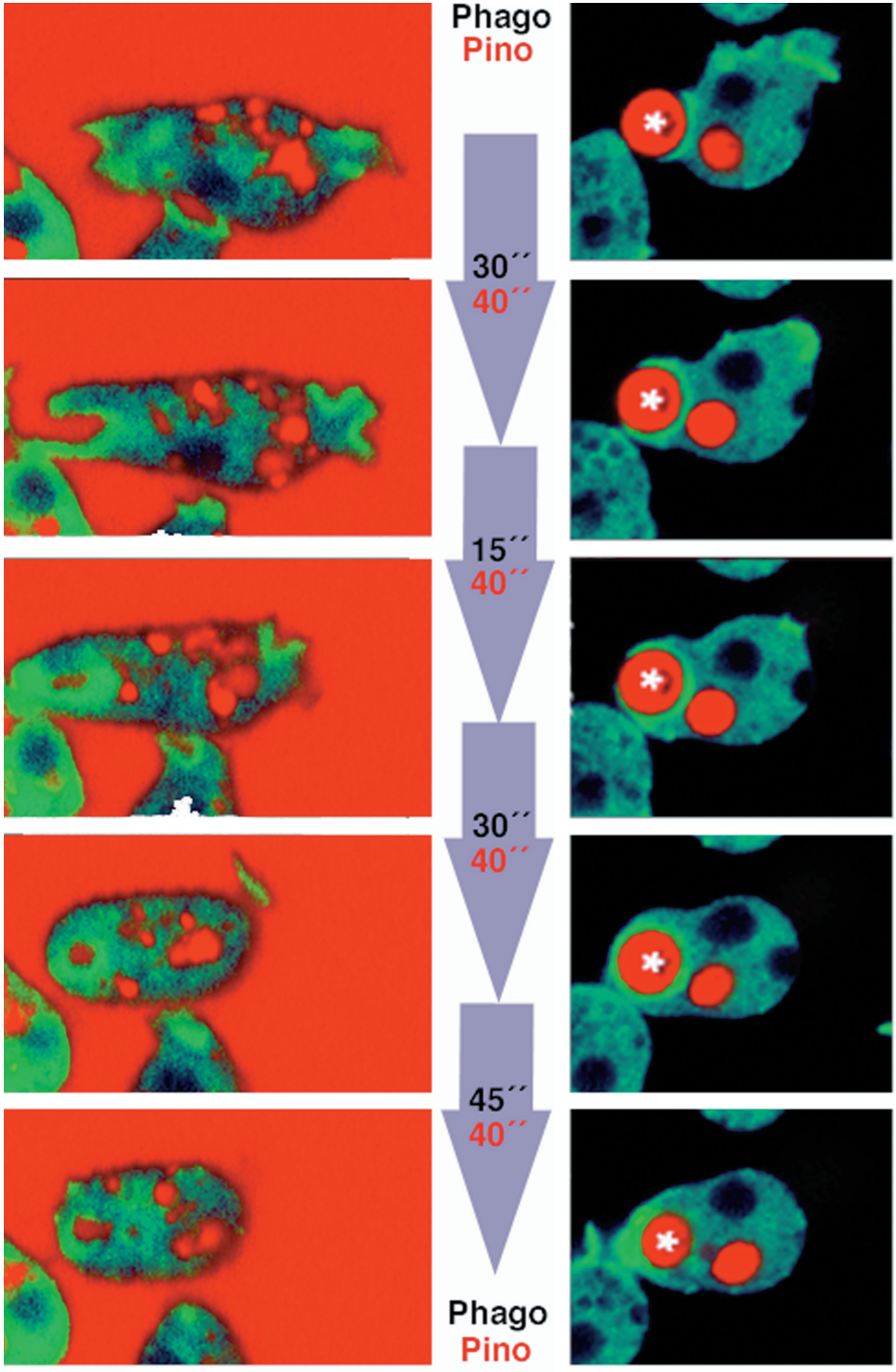

Phago
Pino
30´´
40´´
15´´
40´´
30´´
40´´
45´´
40´´
Phago
Pino

Plate 2 Comparison of cytoskeletal dynamics during macropinocytosis and phagocytosis *in vivo*. Fluid-phase uptake (left) is visualized with a red-fluorescent tracer added to the medium. For particle uptake (right) covalently labeled yeast cells (asterisk) were used. The actin cytoskeleton is revealed by a fusion of coronin to the green fluorescent protein (GFP). Single optical sections were taken at intervals using a confocal laser scanning microscope. The times between the consecutive frames are given in seconds. For each endocytic process, the following stages are shown (from top to bottom): (1) Accumulation of the cytoskeleton at the periphery of the cell. (2) Membrane protrusion toward the left of the cell. (3) Late stage just prior to engulfment of the liquid droplet (left) or yeast particle (right). (4) Endocytic vesicle surrounded by cytoskeletal coat. (5) Dissociation of the coat and release of the endosome into the cytoplasm. (Courtesy of Markus Maniak, University of Kassel.)

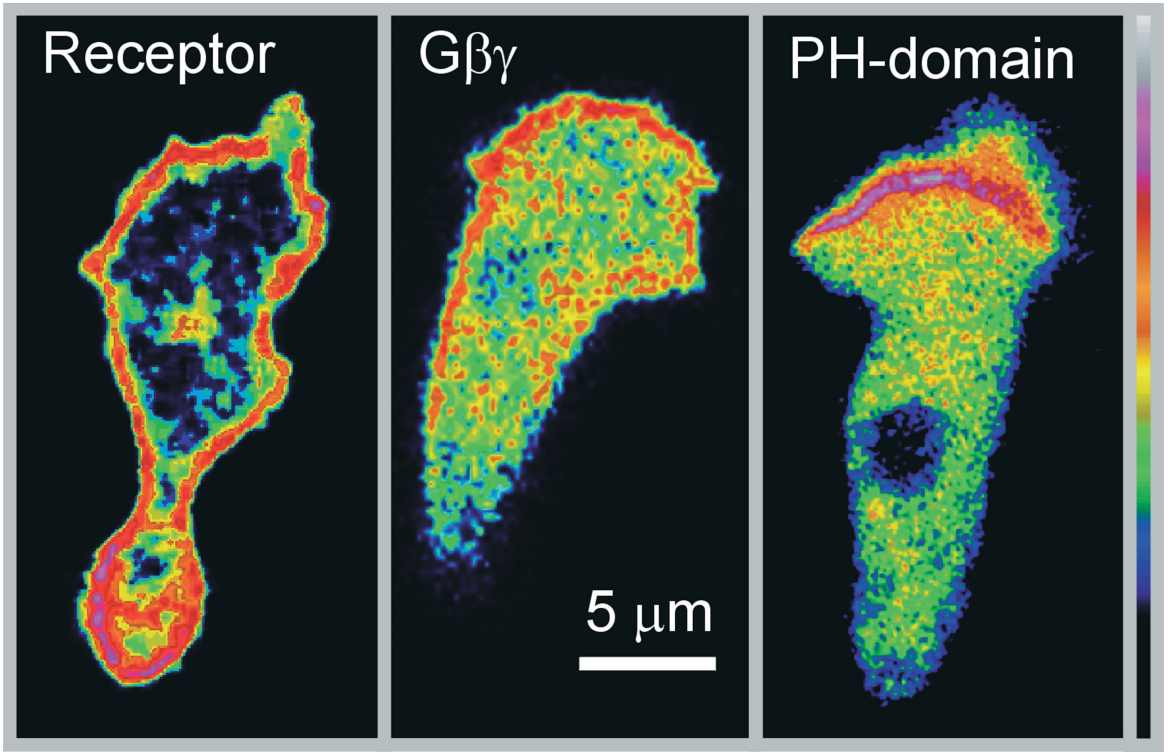


Plate 3 Orientation of proteins during chemotaxis. Left: Confocal image of GFP-tagged chemoattractant receptors in living amoebae undergoing chemotaxis. The chemoattractant gradient is established using a micropipet located just below the frame. (From Z. Xiao and P.N. Devreotes, Johns Hopkins Medical Institutions.) Center: Confocal image of GFP-tagged Gb-subunits in living amoebae undergoing chemotaxis. The chemoattractant gradient is established using a micropipet located just above the frames. (From T. Jin, N. Zhang, and P.N. Devreotes, Johns Hopkins Medical Institutions.) Right: Confocal image of GFP-tagged CRAC in living amoebae undergoing chemotaxis. CRAC-GFP specifically translocates to the leading edge of newly elicited pseudopods. The chemoattractant gradient is established using a micropipet located near the upper right corner of the frame. (From C.A. Parent and P.N. Devreotes, Johns Hopkins Medical Institutions.) See Tian Jin, Ning Zhang, Yu Long, Carole A. Parent, and Peter Devreotes, *Science* **287**, 1034–1036, 2000.

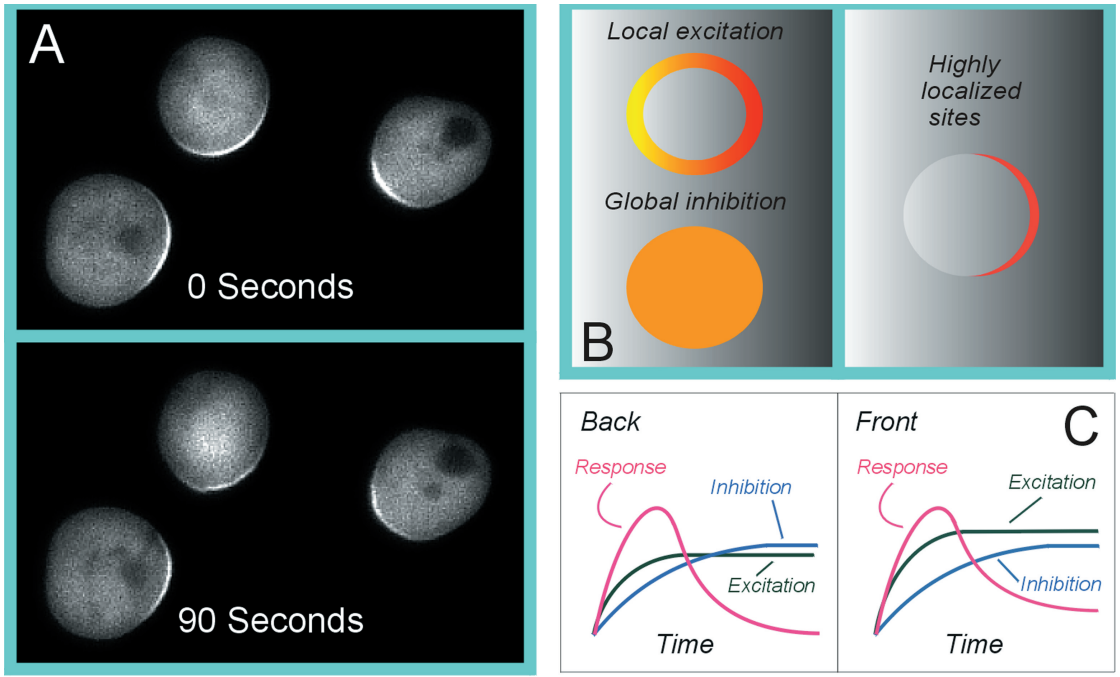


Plate 4 A model to explain directional sensing. Panel A shows that directional sensing does not require cell movement or the actin cytoskeleton. Amoebae expressing CRAC-GFP were treated with latrunculin for 20 minutes to disrupt the actin cytoskeleton. Note the round shape of the cells. A pipet containing cAMP was placed to the lower right center of the frames. The CRAC-GFP label accumulates on the side of the cells facing the source of cAMP and persists. Panels B and C show an illustration of a hybrid spatial-temporal model for directional sensing. The gradient of chemoattractant is depicted as a gradation in the gray background crossing the field from right to left. (Left) Excitation occurs locally near the membrane and is slightly graded (from yellow to red). Inhibition occurs throughout the cell and is uniform at an intermediate level (orange). The response, the difference between excitation and inhibition, is sharply localized on the right side of the cell (red). The steady-state situation, several minutes after the cells have been placed in a stable spatial gradient, is illustrated. When the cell is initially placed into the gradient, both sides will experience an increment in stimulus and respond. However, as steady state is reached, inhibition exceeds excitation at the back of the cell and excitation exceeds inhibition at the front, as shown in panel C. See text for further explanation and a website for on-line dynamic illustrations. (From Parent and Devreotes (1999), reprinted with permission. Courtesy of Carole A. Parent and Peter N. Devreotes.)

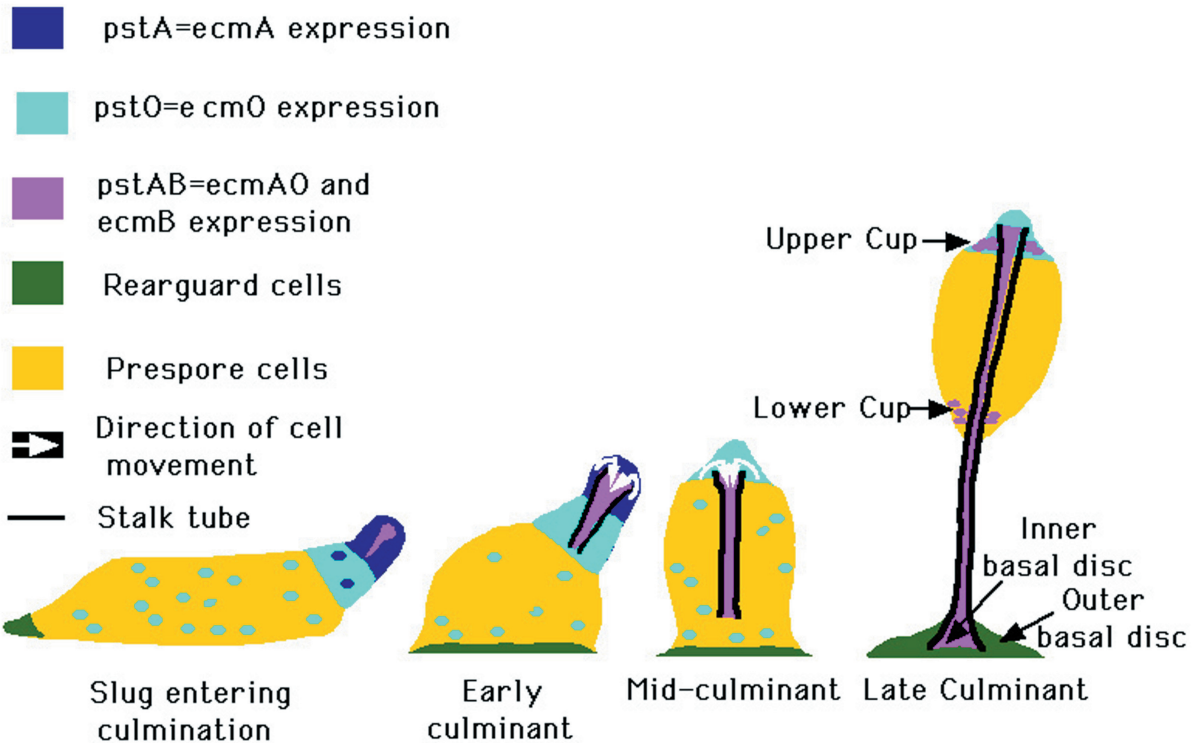


Plate 5 The movement of cells during culmination. This is a diagrammatic representation of culmination where, for the sake of clarity, the band of pstB cells that will form the outer basal disc (see Plate 6) is not shown. The *ecmA* promoter can be divided into two parts, a proximal part (the *ecmA* region) that directs expression predominantly in cells within the tip (i.e., in the pstA cells) and a distal part (the *ecmO* region) that directs expression in cells in the back of the prestalk region (the pstO cells) and in a subset of the anterior-like cells. The latter population has been termed the pstO:ALC (Abe *et al*., 1995). The whole *ecmA* promoter (the *ecmAO* promoter) directs expression in all these cell subtypes, which collectively are called the pstAO population. (Courtesy of Jeffrey Williams, University of Dundee. Reprinted with permission.)

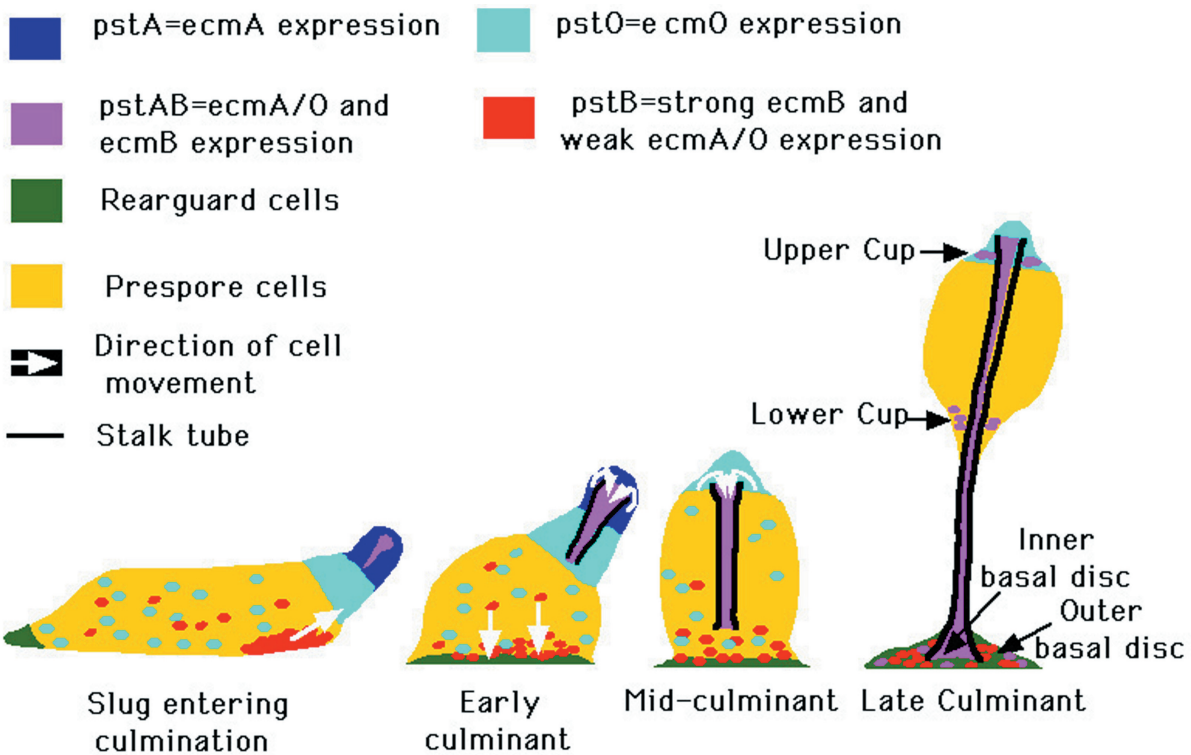


Plate 6 The movement of pstB cells at culmination. The pstB cells are defined by selective staining with neutral red and because they express the *ecmB* gene at a high level relative to the *ecmA* gene (Jermyn *et al*., 1996; Dormann *et al*., 1996). They have a complex movement pattern during slug migration (Dormann *et al*., 1996). For the sake of simplicity, separate pstA and pstO populations are not shown, rather the behavior of the entire pstAO population is represented. (Courtesy of Jeffrey Williams, University of Dundee. Reprinted with permission.)