Chemotaxis is composed of motility, directional sensing and polarity. In the presence of a chemoattractant (or chemorepellent) gradient, cells move toward (or away from) higher concentrations.

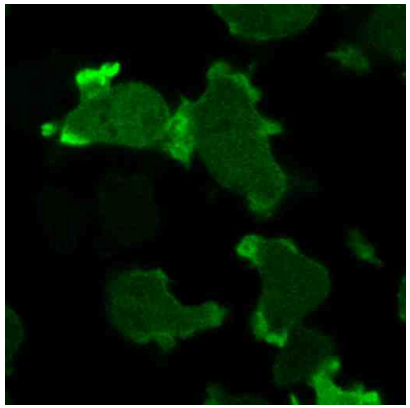
Free amoeboid cells rhythmically extend pseudopodia and move in random directions.

Spatial sensing, a means of directional sensing, can be demonstrated by the gradient-mediated relocalization of proteins in cells immobilized by actin inhibitors.

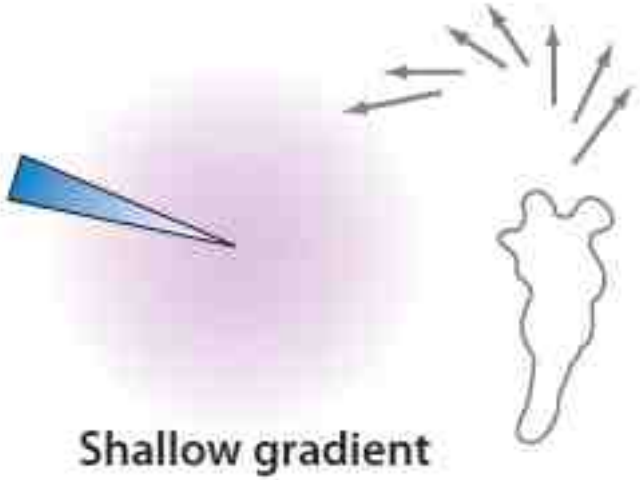
Chemotactic cells are often polarized, with a stable leading edge from which pseudopodia are extended.

16_bcma_2011

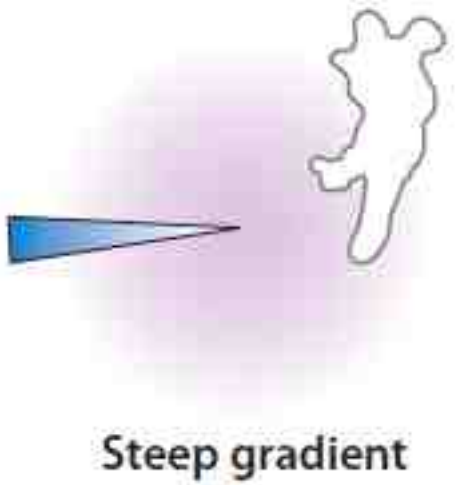
Cite this article as *Cold Spring Harb Perspect Biol* 2009;1:a002980



Vedi filmati su moodle



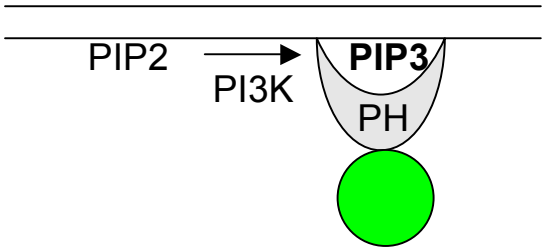
In a shallow gradient, polarized cells display biased patterns of pseudopodia extension at the leading edge that cause cells to turn gradually toward higher concentrations of chemoattractant.

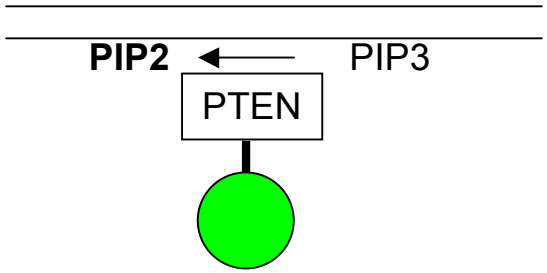


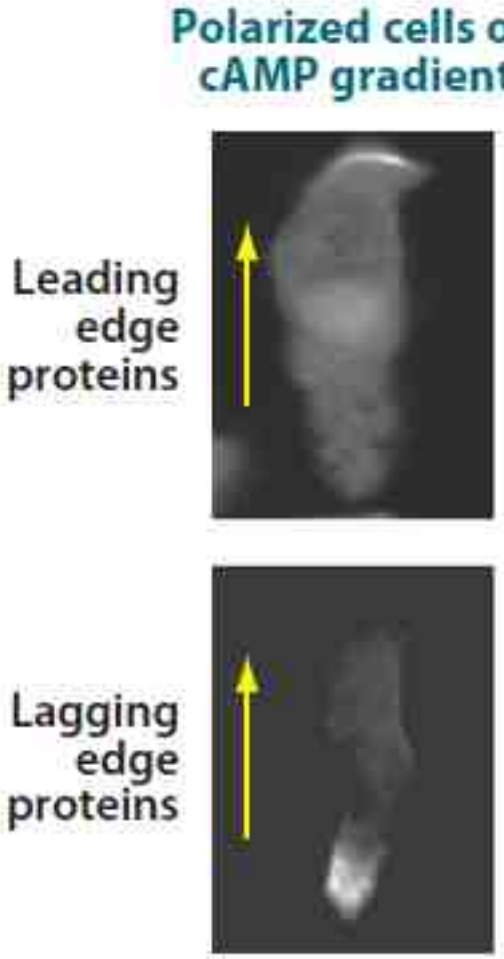
Sufficiently steep gradients can trigger new projections anywhere along the cell periphery.

Localization of signaling components to the leading or lagging edge.

In polarized or chemotaxing cells, many proteins are recruited to the leading or lagging edge. Arrows reflect the direction of migration.





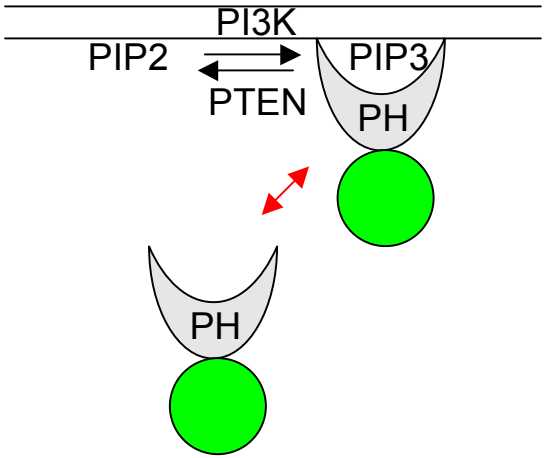


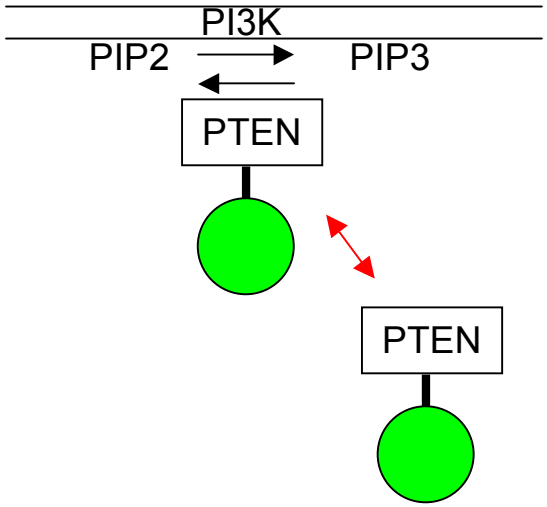
The distributions of leading edge proteins are represented by a PIP3-specific PH domain tagged with GFP

The distributions of lagging edge proteins are represented by PTEN-GFP.

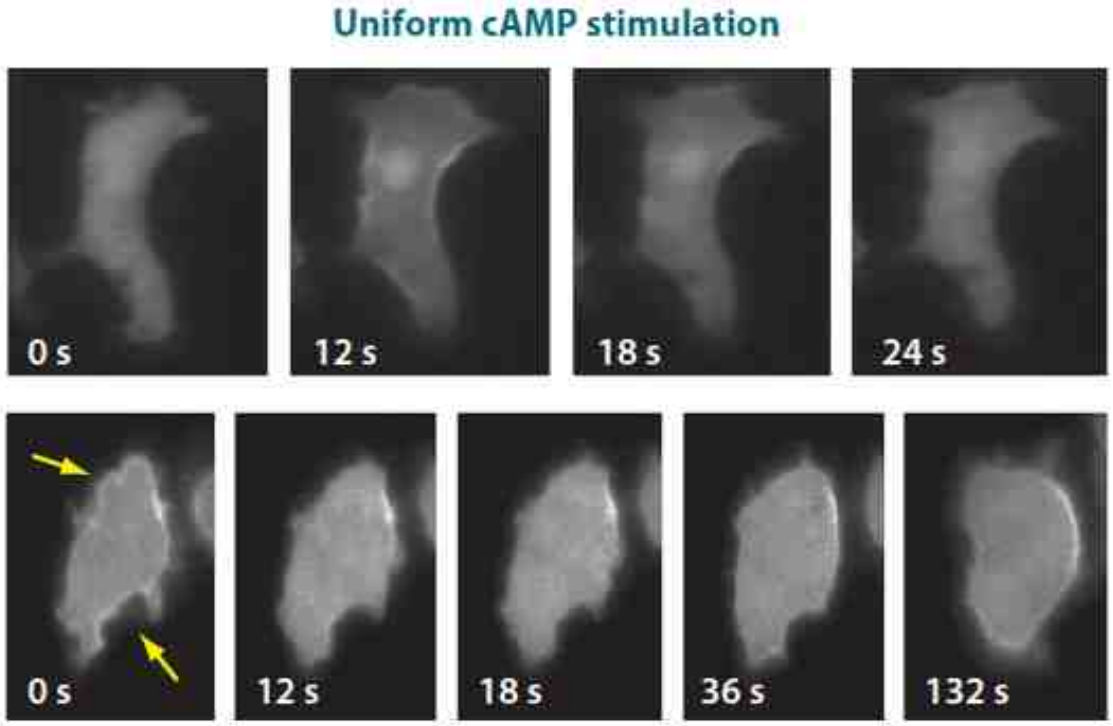
Abbreviations:
PIP3, phosphatidylinositol 3,4,5-trisphosphate,
PTEN, phosphatase and Tensin homolog on chromosome ten.

When unpolarized cells are stimulated globally with cAMP:





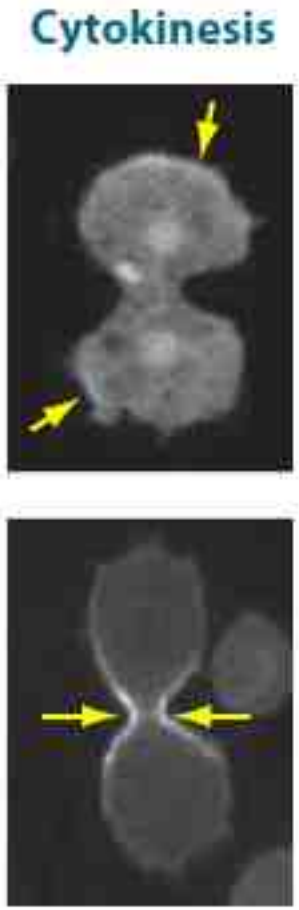
"leading edge" proteins, such as PI3Ks and several actin-associated proteins, translocate uniformly to the plasma membrane or cortex and then return to the cytosol.



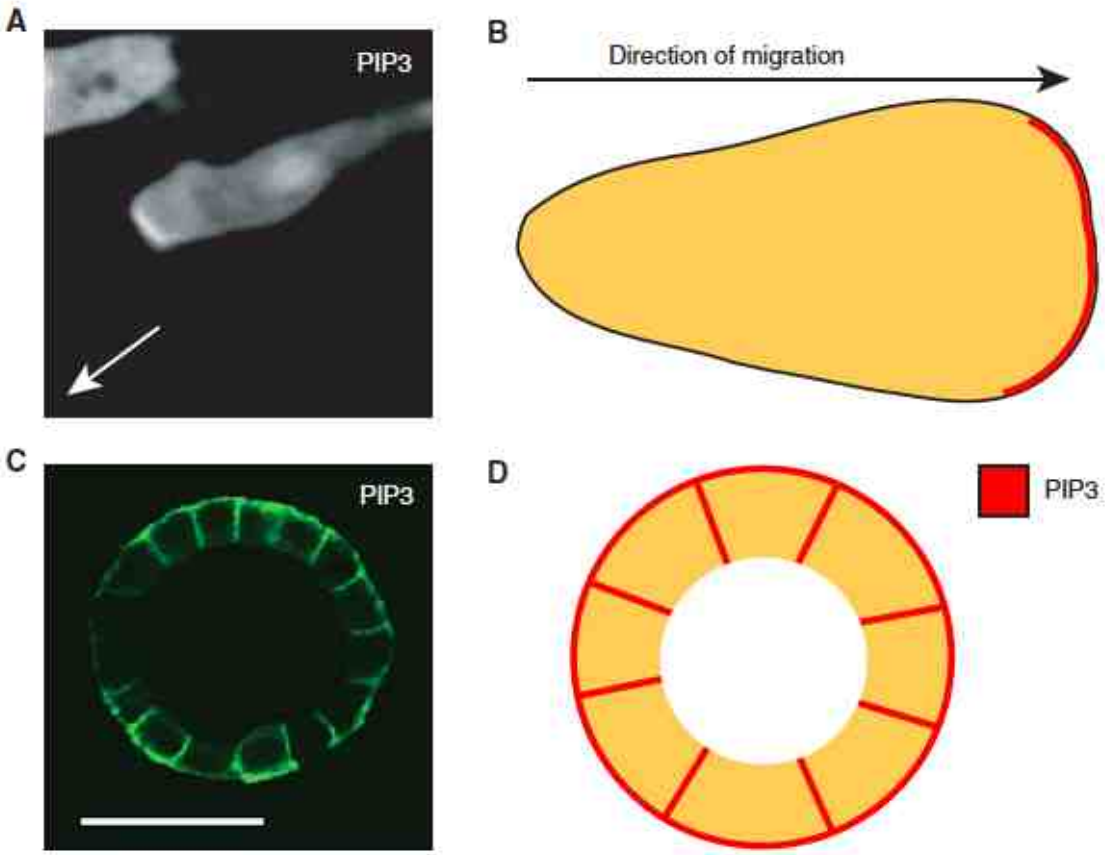
"lagging edge" proteins, such as PTEN or Myosin II, transiently fall off the membrane or cortex (*arrows*) and then return to the periphery. Time in seconds after the addition of chemoattractant is indicated for each frame.

16_bcma_2011

During cytokinesis, “leading edge” proteins localize to the poles, whereas “lagging edge” proteins are targeted to the cleavage furrow (*arrows*).



PI(3,4,5)P3 localization in polarized cells.



(A-B) PI(3,4,5)P3 localization in migrating Dictyostelium. PHCrac-GFP is a PH domain-containing protein that is a known probe for PI(3,4,5)P3. PHCrac-GFP is localized to the leading edge in migrating Dictyostelium cells (Funamoto et al. 2002). The arrow represents direction of migration toward a cAMP-filled pipet tip in the lower left corner. The average length of a migrating Dictyostelium is 20 μm.

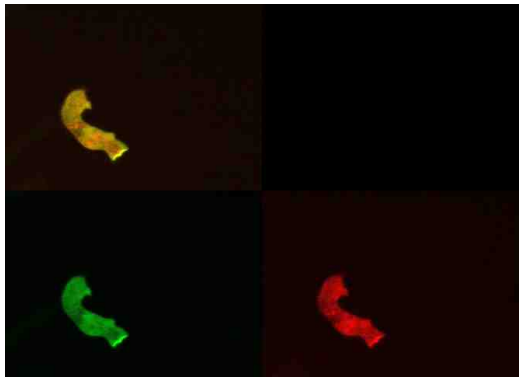
(C-D) PI(3,4,5)P3 localization in polarized Madin-Darby canine kidney (MDCK) epithelial cyst. GFP-PH-Akt is also a probe for PI(3,4,5)P3, and is localized to the basal and lateral domains of MDCK cells in cysts (Martin-Belmonte et al. 2007). Scale bar represents 50 mm (Courtesy of K. Mostov and W. Yu, UCSF). (D) Schematic showing the localization of PI(3,4,5)P2 in an MDCK cell cyst.

16 bcma 2011

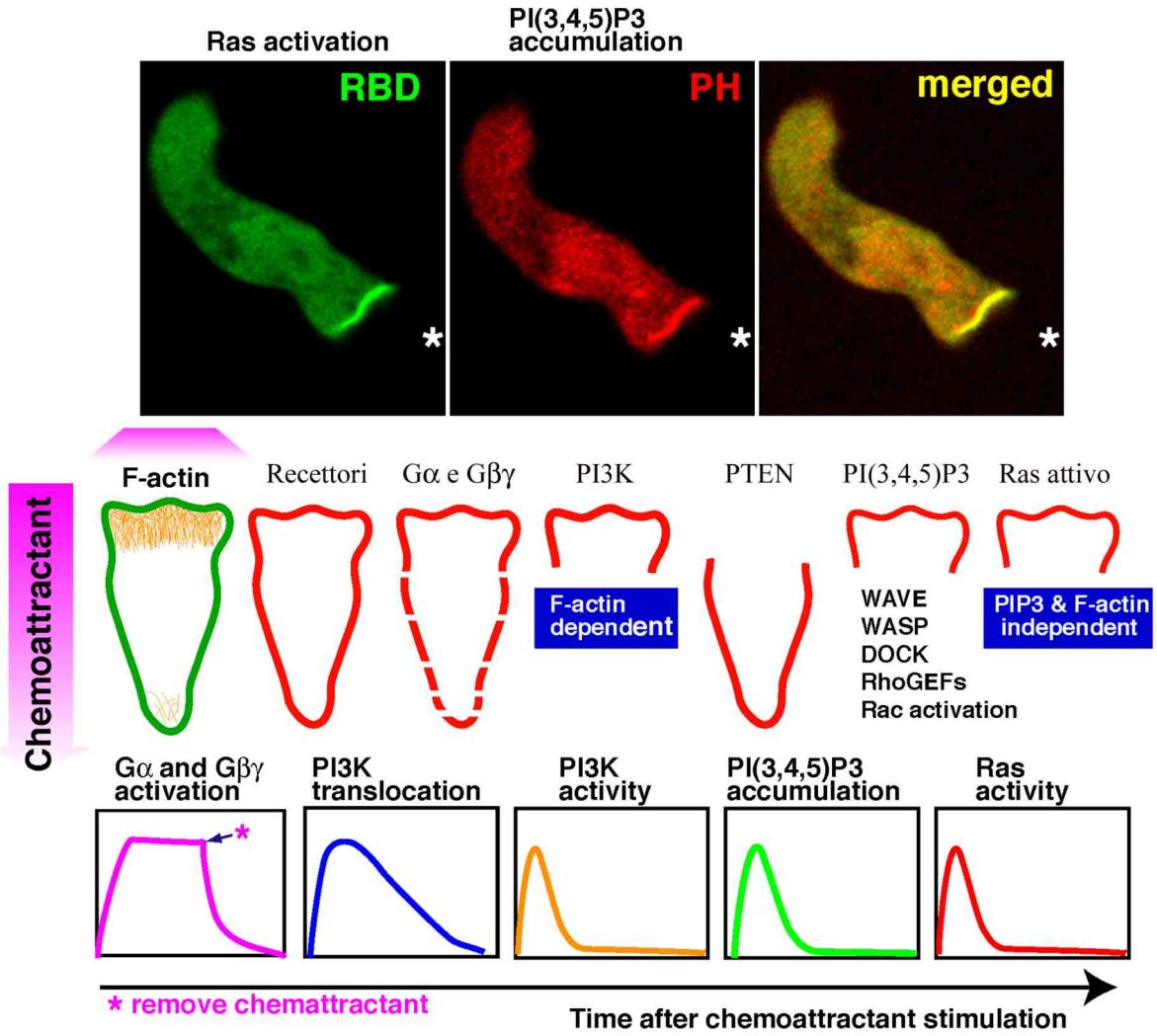
Cite this article as *Cold Spring Harb Perspect Biol* 2009;1:a001321

Co-localized activation of Ras and PI3K at the leading edge.

The movie shows the spatial and temporal co-localization of activated Ras (Ras-GTP) and PI3K at the leading edge of wild-type chemotaxing cells. Cells are co-expressing a reporter for activated Ras (GFP-RBD, Ras binding domain) and one (RFP-for PI(3,4,5)P3, the product of PI3K. Green- GFP-RBD; Red, RFP-PHcrac; Yellow, merge of the two images. Contemporaneous image collection was achieved using a beam splitter(Firtel lab).



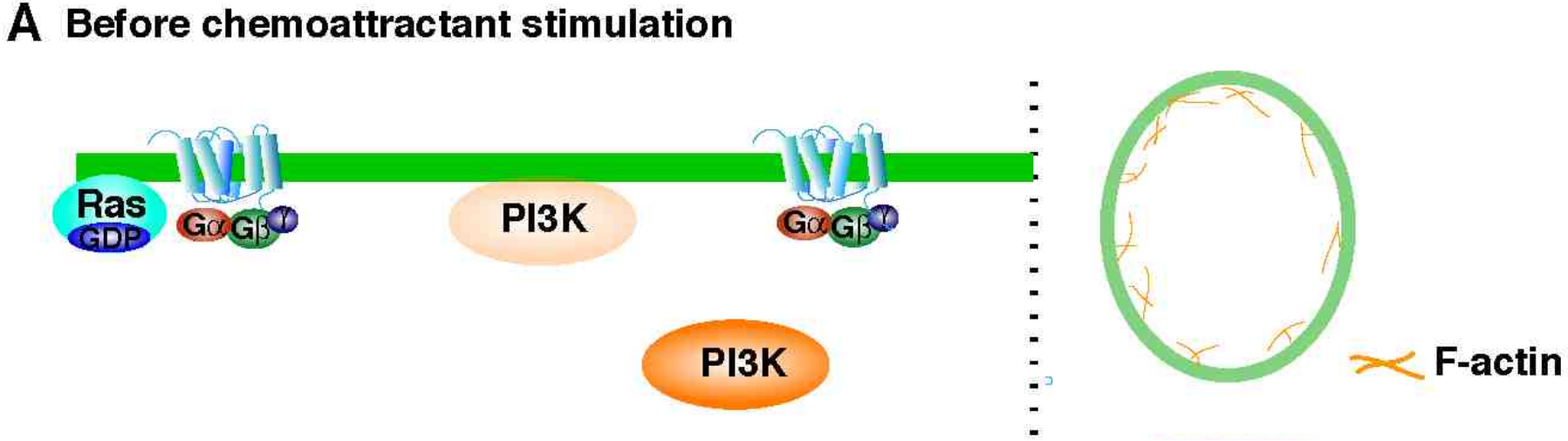
Early signalling upon chemotactic stimulation

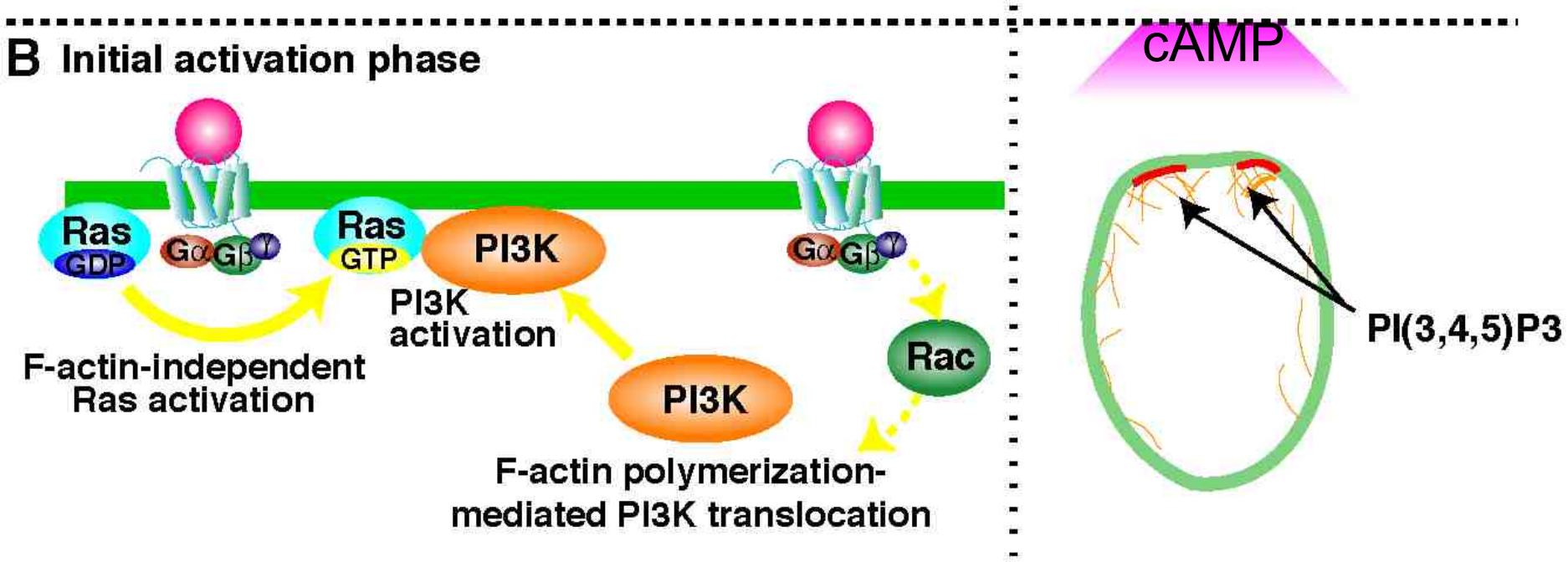


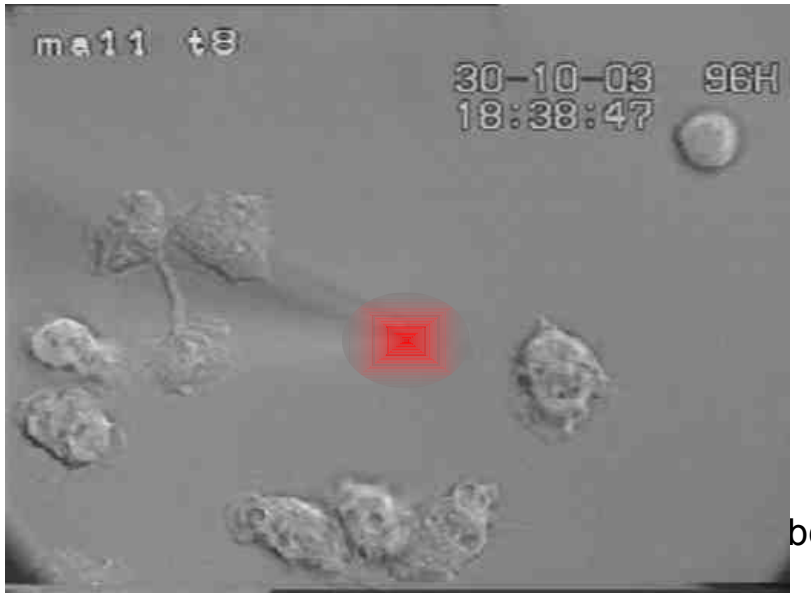
R. Firtel

SUPPLEMENTAL TABLE 2. Signaling components that are localized to the leading or lagging edge of polarized cells

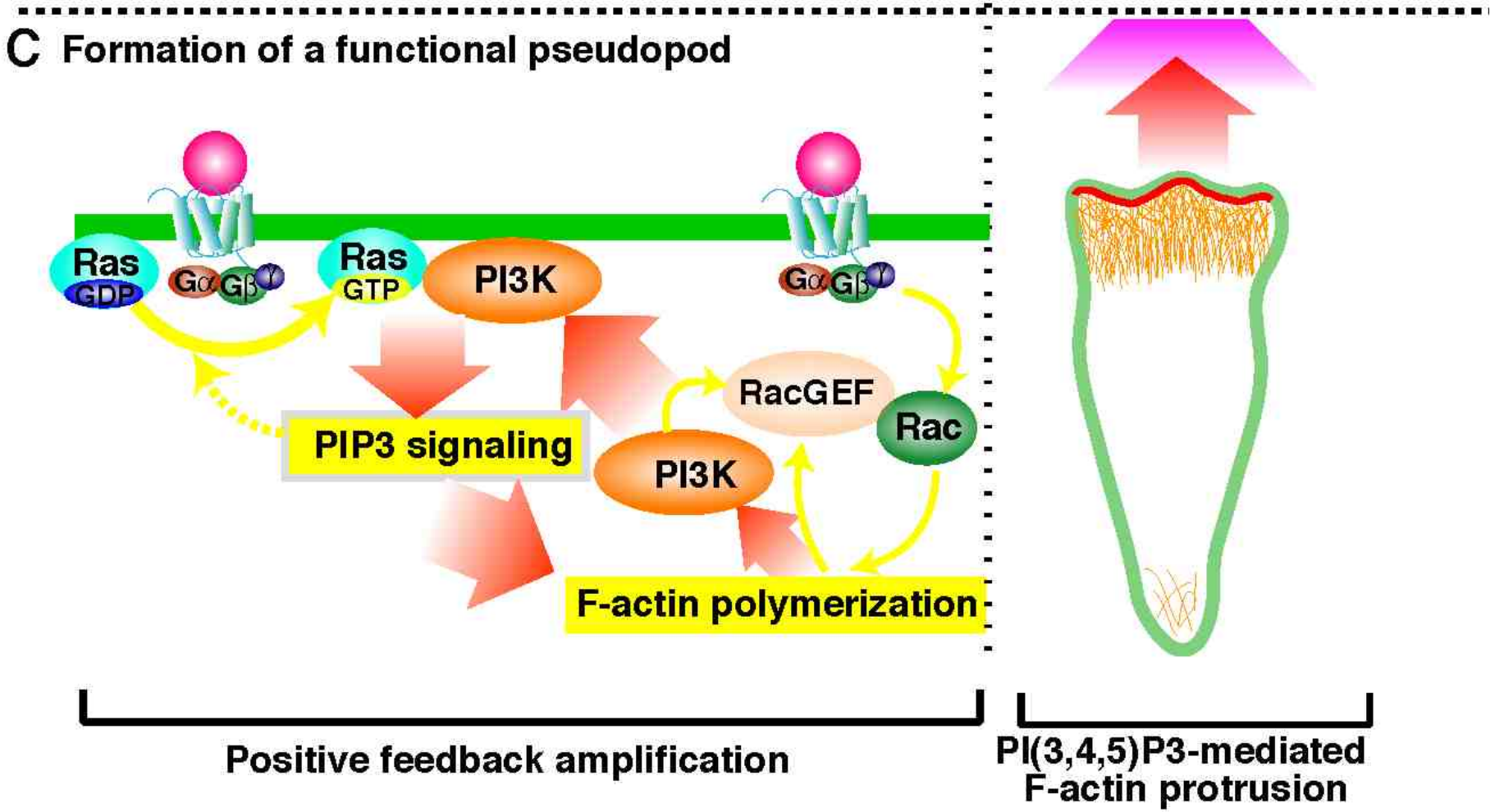
Lagging edge		Leading edge	
Protein	**Reference(s)**	**Protein**	**Reference(s)**
ACA	(24)	α-Actinin	(3, 43)
Cortexillin I	(8)	Actin polymerization	(7, 13, 37, 45, 46)
MHCKC	(25)	Activated PKBR1	(21)
Myosin II	(27, 46)	Activated Ras proteins	(17, 38)
PakA	(4, 29)	Activated TorC2	(21)
PTEN	(10, 14)	Arp3	(15)
		Cofilin	(1, 2)
		Coronin	(5, 12)
		Crac	(31)
		DockD	(30)
		Dynacortin	(20)
		Fimbrin	(36)
		LimC, D, E	(22, 35)
		Mek1	(40)
		MHCKA	(41)
		MyoB, D	(9, 19, 28)
		Nhe1	(33)
		PakB	(6)
		PhdA	(11)
		Phg2	(17)
		PI3K	(10)
		PKBA	(26)
		RacGEF1	(32)
		RapGAP1, 3	(16, 18)
		SAHH	(39)
		SBDS	(44)
		Scar	(34)
		sGC	(42)
		XacA	(23)


A Before chemoattractant stimulation
Ras
GDP
Gα
Gβ
γ
PI3K
PI3K
F-actin





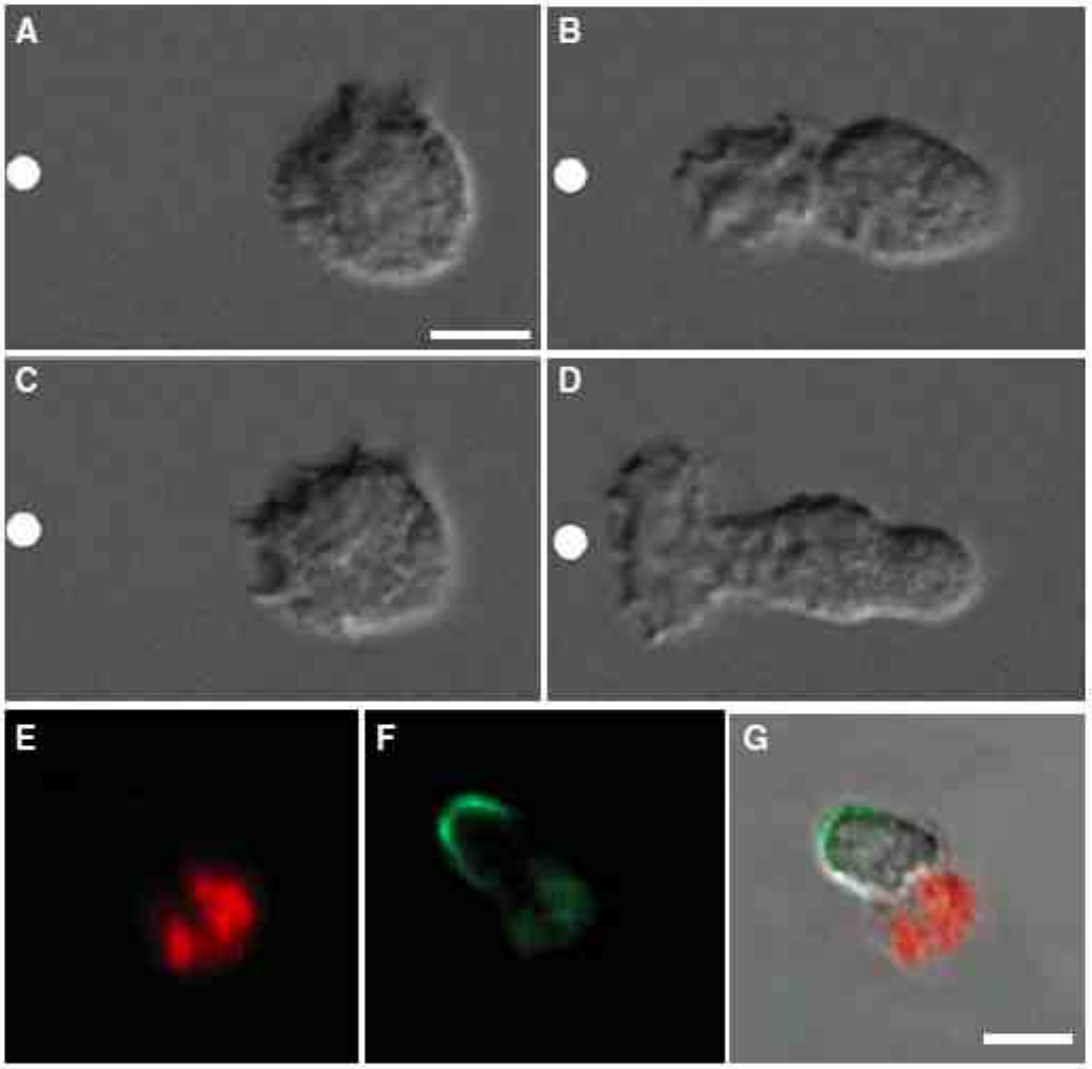
rasGEFM null: no polarity


C Formation of a functional pseudopod
Ras
GDP
Gα Gβ γ
Ras
GTP
PI3K
Gα Gβ γ
PIP3 signaling
RacGEF
Rac
PI3K
F-actin polymerization
Positive feedback amplification
PI(3,4,5)P3-mediated
F-actin protrusion

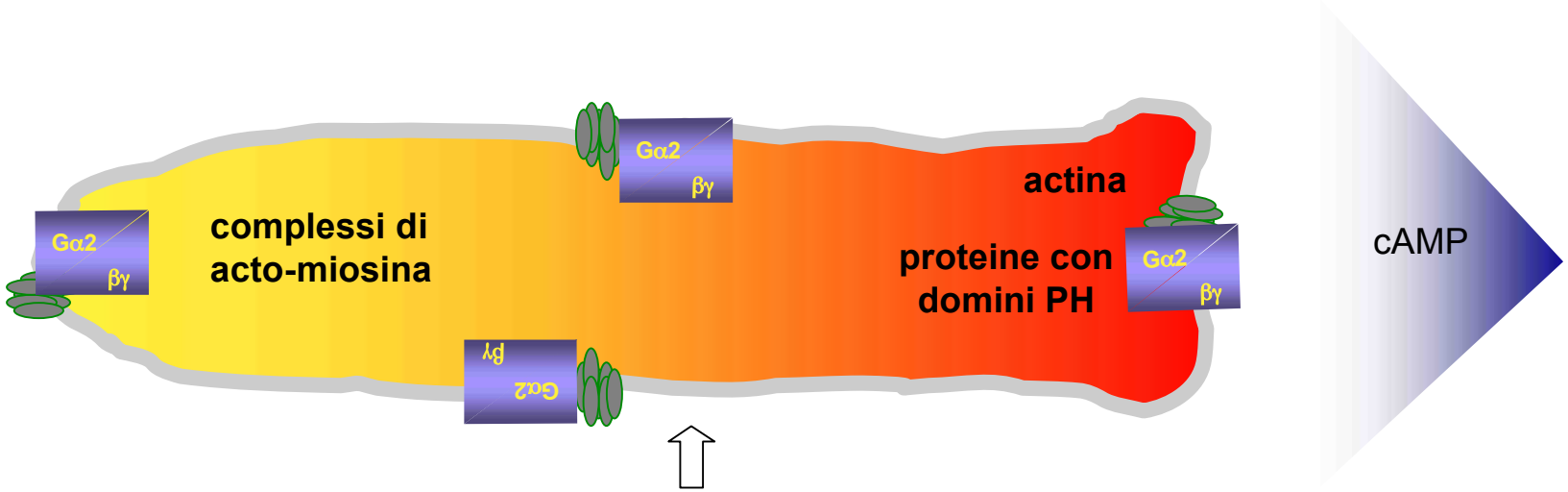
Polarization of a neutrophil in response to gradient of chemoattractant.

unpolarized neutrophil responding to a micropipette containing the chemoattractant fMLP (white circle) at (A) 5 s, (B) 30 s, (C) 81 s, and (D) 129 s of stimulation

Fixed cells were stained for F-actin with rhodamine-phalloidin (E, red) and an antibody raised against activated myosin II (phosphorylated specifically at Ser19, p[19]-MLC) (F, green).

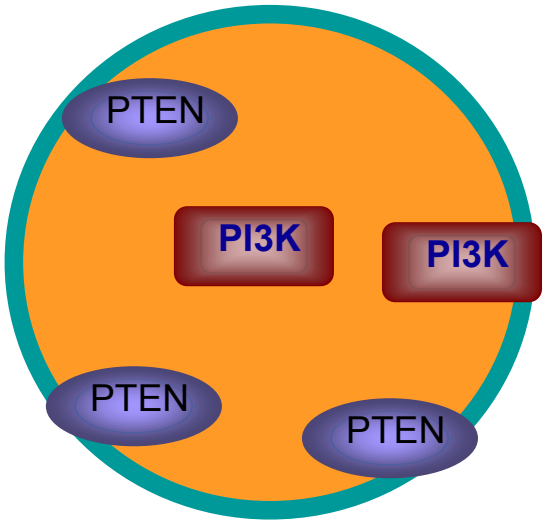


Formazione del fronte-guida e della polarità cellulare



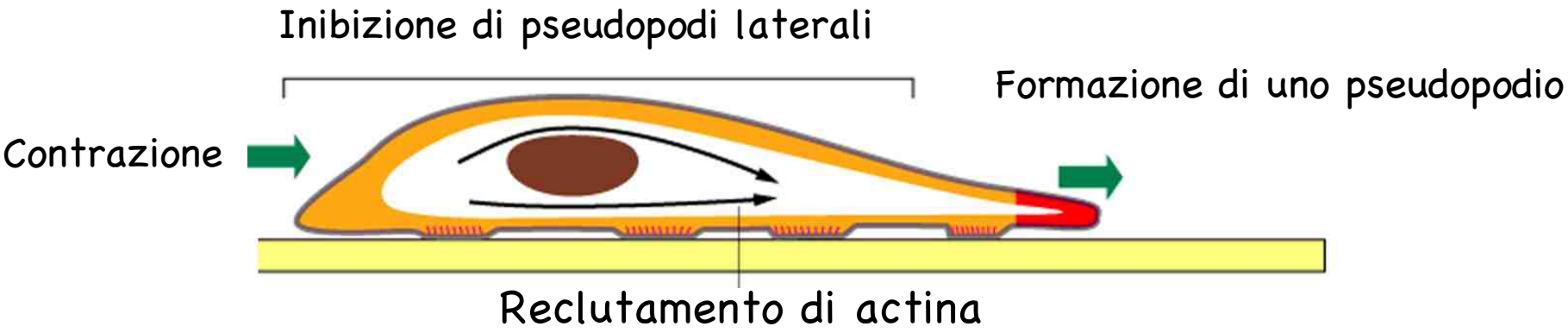
Proteine con domini PH:

(1) CRAC
(2) ABP 120
(3) CAP
(4) Akt/PKB
(5) Scar/Wave
(6) Wasp
(7) Profilina



$$PI(4,5)P \underset{PTEN}{\overset{PI3K}{\rightleftharpoons}} PI(3,4,5)P$$

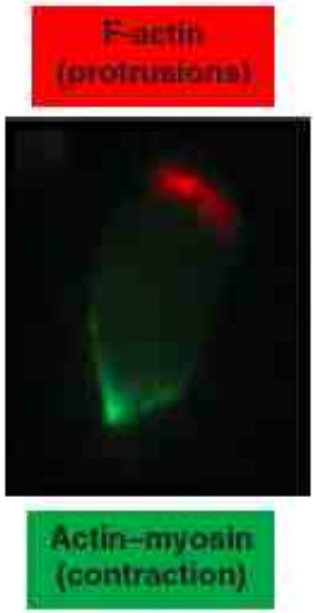
Polarizzazione e motilità chemiotattica (coordinamento del citoscheletro di actina e acto-miosina)

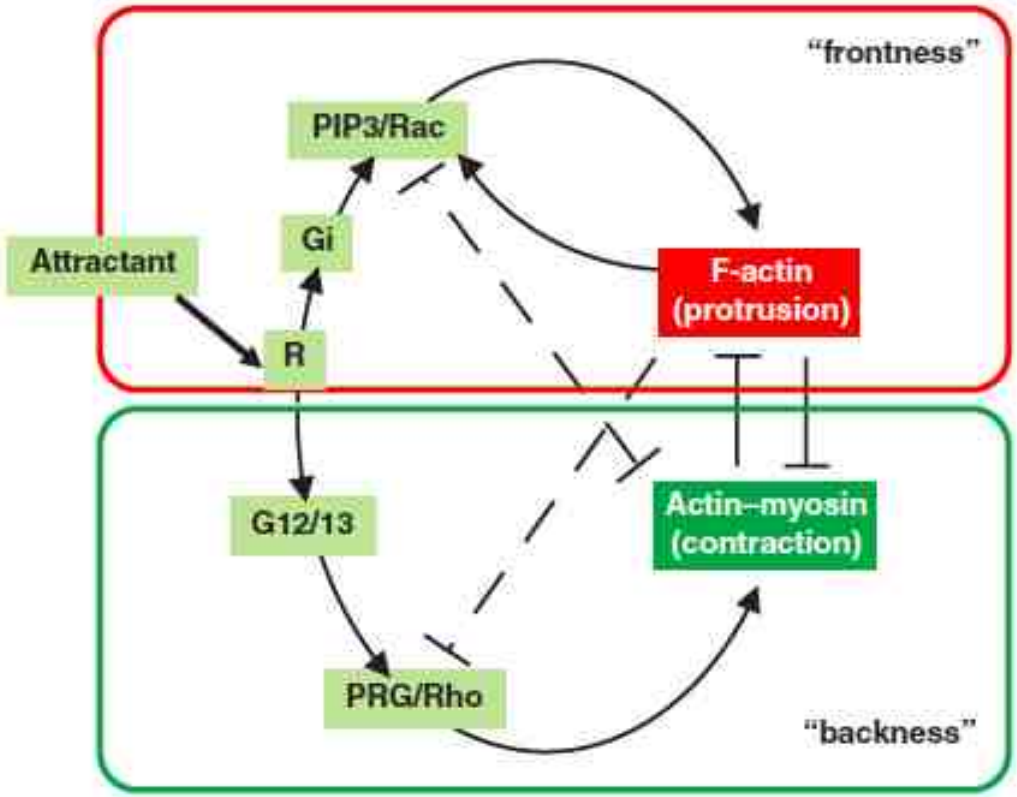


- L'actina è reclutata nella parte anteriore (fronte guida) e polimerizza in filamenti reticolati dando origine allo pseudopodio
- Lateralmente l'actina corticale forma fibre da stress con la miosina, rendendo rigido il cortex e impedendo la formazione di pseudopodi laterali
- Posteriormente actina e miosina formano miofibrille contrattili che permettono la retrazione della parte posteriore della cellula
- Come sono regolati e coordinati questi 3 processi?

The Signaling Mechanisms Underlying Cell Polarity and Chemotaxis

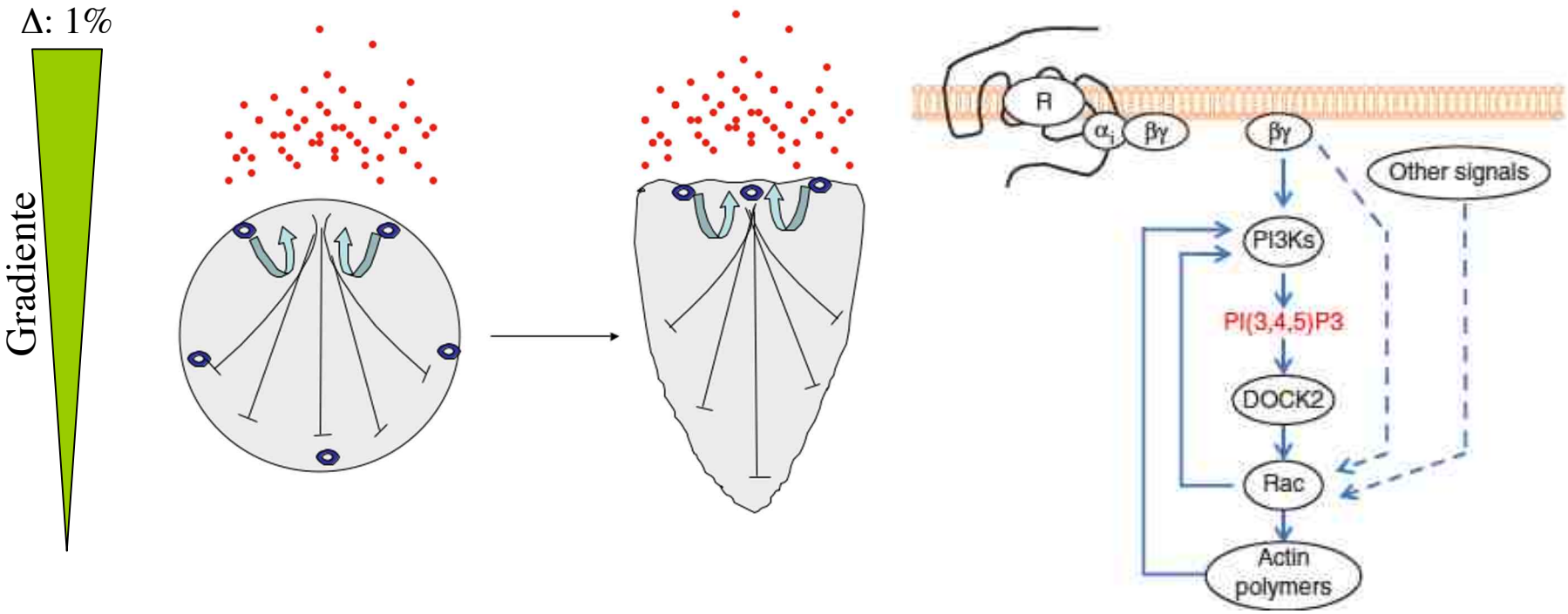
Distinct actin assemblies modulate sensitivity to attractant and self-organizing polarity of neutrophils.





Chemoattractant binds to a GPCR (R), which in turn activates different trimeric G proteins to generate two divergent, opposing signaling pathways, which promote actin polymerization (frontness) and actin–myosin contraction (backness), respectively. Localized mechanochemical incompatibility of the two cytoskeletal responses, combined with the ability of each to damp signals that promote the other (dashed inhibitor lines), then gradually drive them to separate into distinct domains of the membrane. As a result, a morphologically distinct pseudopod, which is highly sensitive to attractant, demarcates itself from relatively insensitive membrane, enriched with myosin, at the back and sides.

Modello di amplificazione del segnale: attivazione locale e inibizione globale



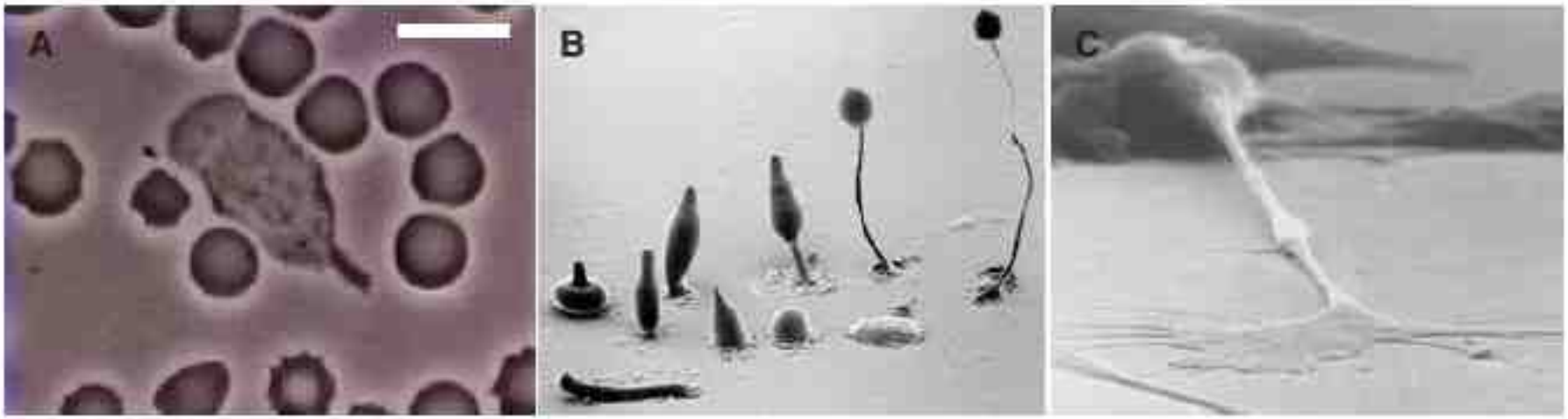
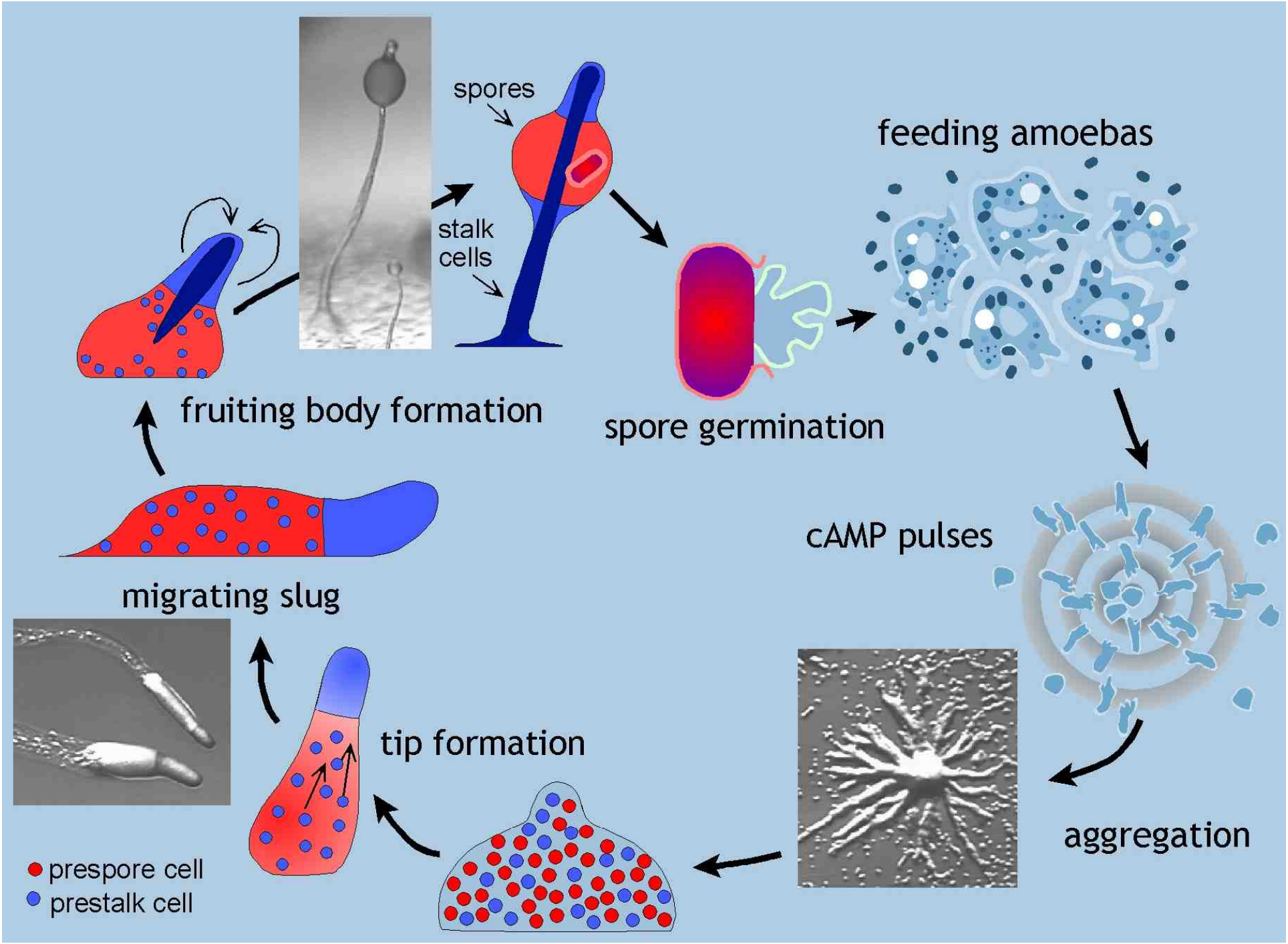
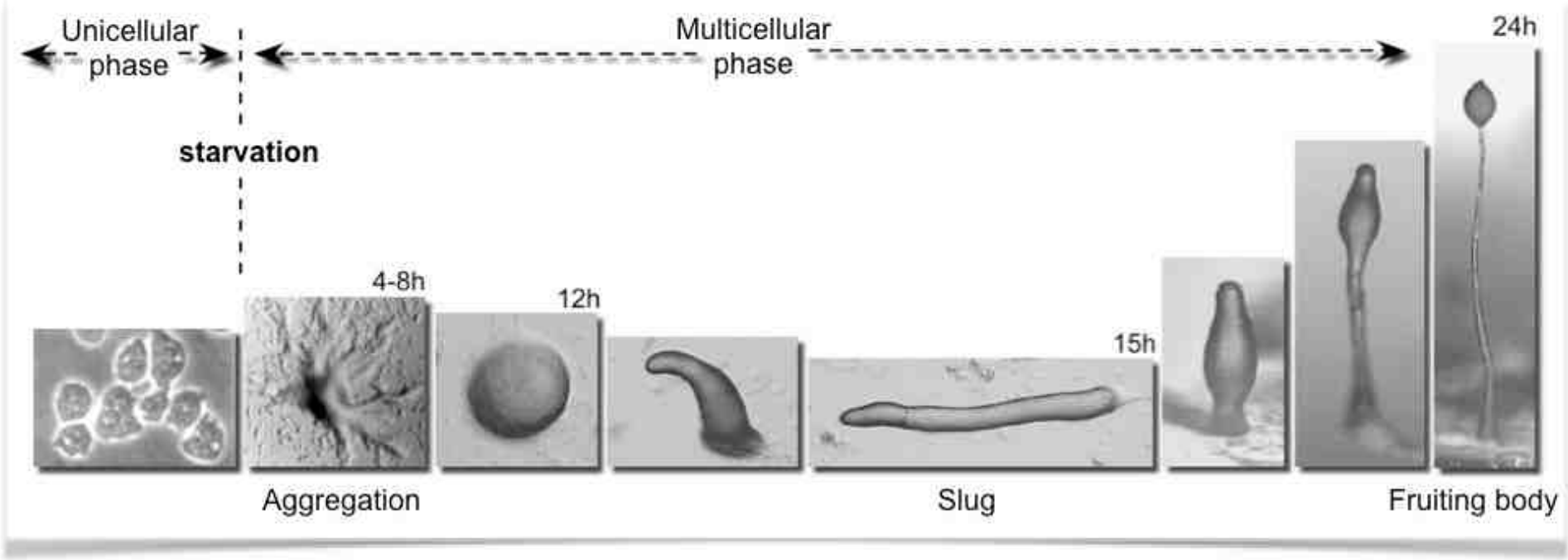
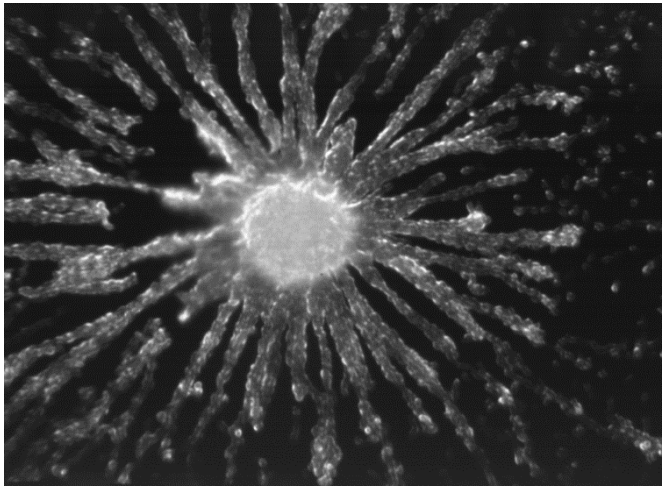


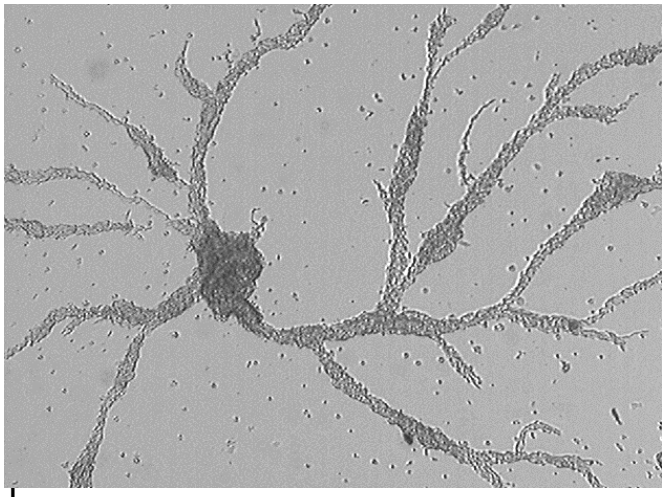
Figure 1. Examples of chemotaxis. (*A*) A human neutrophil chasing a *Staphylococcus aureus* microorganism on a blood film among red blood cells, notable for their dark color and principally spherical shape (imaged by David Rogers, courtesy of Thomas P. Stossel). Bar, 10 μm. Chemotaxis is also necessary for (*B*) *D. discoideum* to form multicellular aggregates during development (courtesy of M.J. Grimson and R.L. Blanton, Texas Tech University), and (*C*) for axons to find their way in the developing nervous system. Photo provided by Kathryn Tosney, University of Miami.


spores
stalk cells
feeding amoebas
fruiting body formation
spore germination
cAMP pulses
migrating slug
tip formation
aggregation
prespore cell
prestalk cell

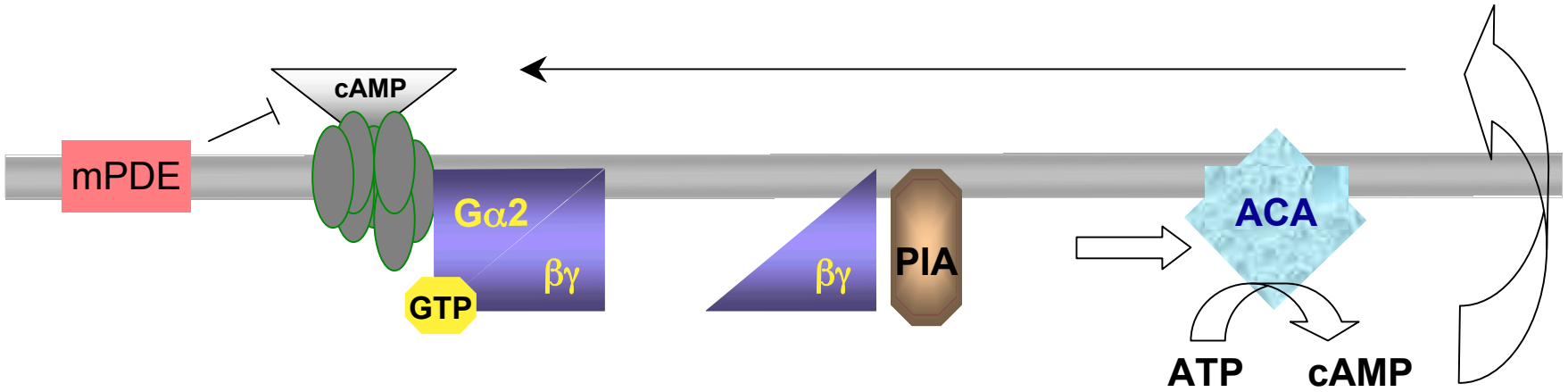

Unicellular phase
Multicellular phase
24h
starvation
4-8h
12h
15h
Aggregation
Slug
Fruiting body

Propagazione del segnale chemiotattico (signal relay)

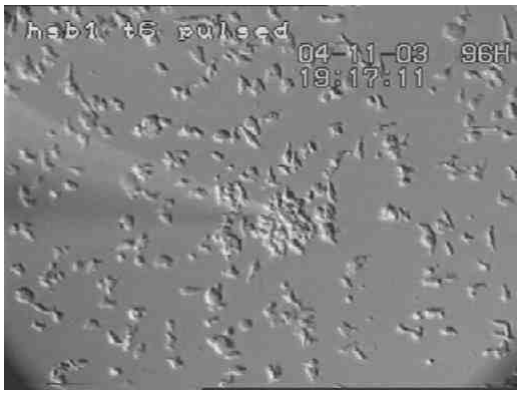




Regolazione del relay di cAMP (I)



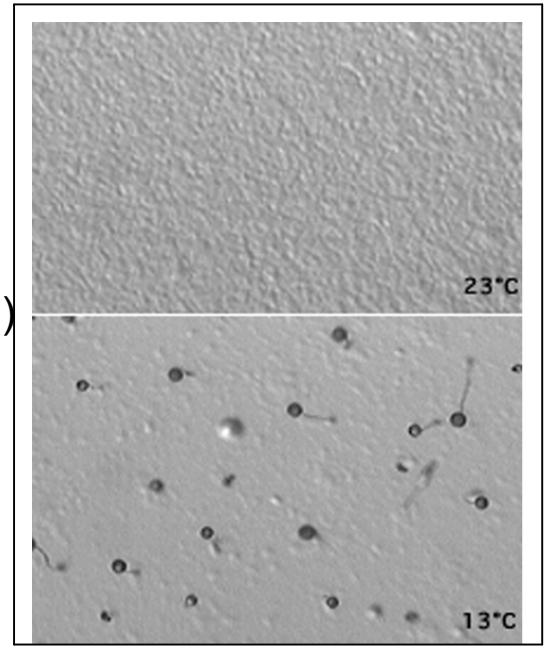
10 recettori diversi per cAMP
8 Gα, 1 Gβ e 1 Gg

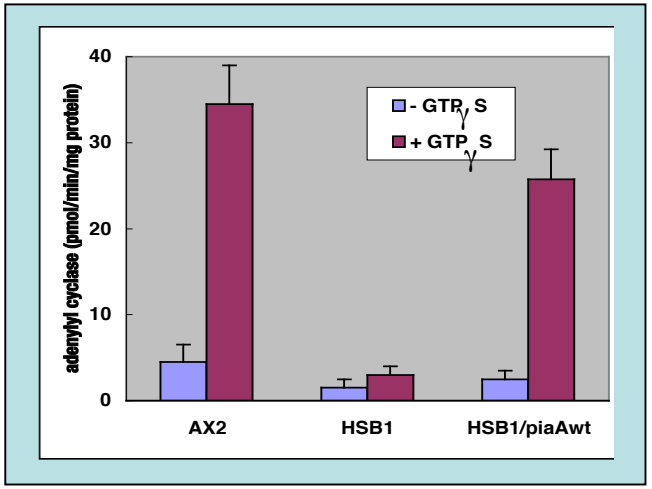


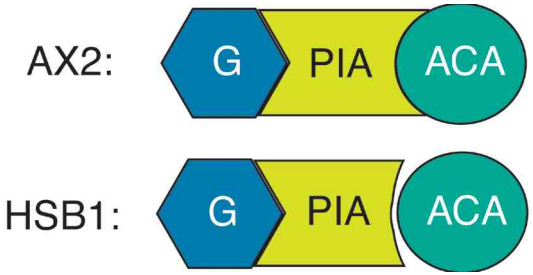
pia null

Pia è essenziale per l'attivazione G protein-dipendente dell'AC

HSB1
(*PIA*:G^{917}D)







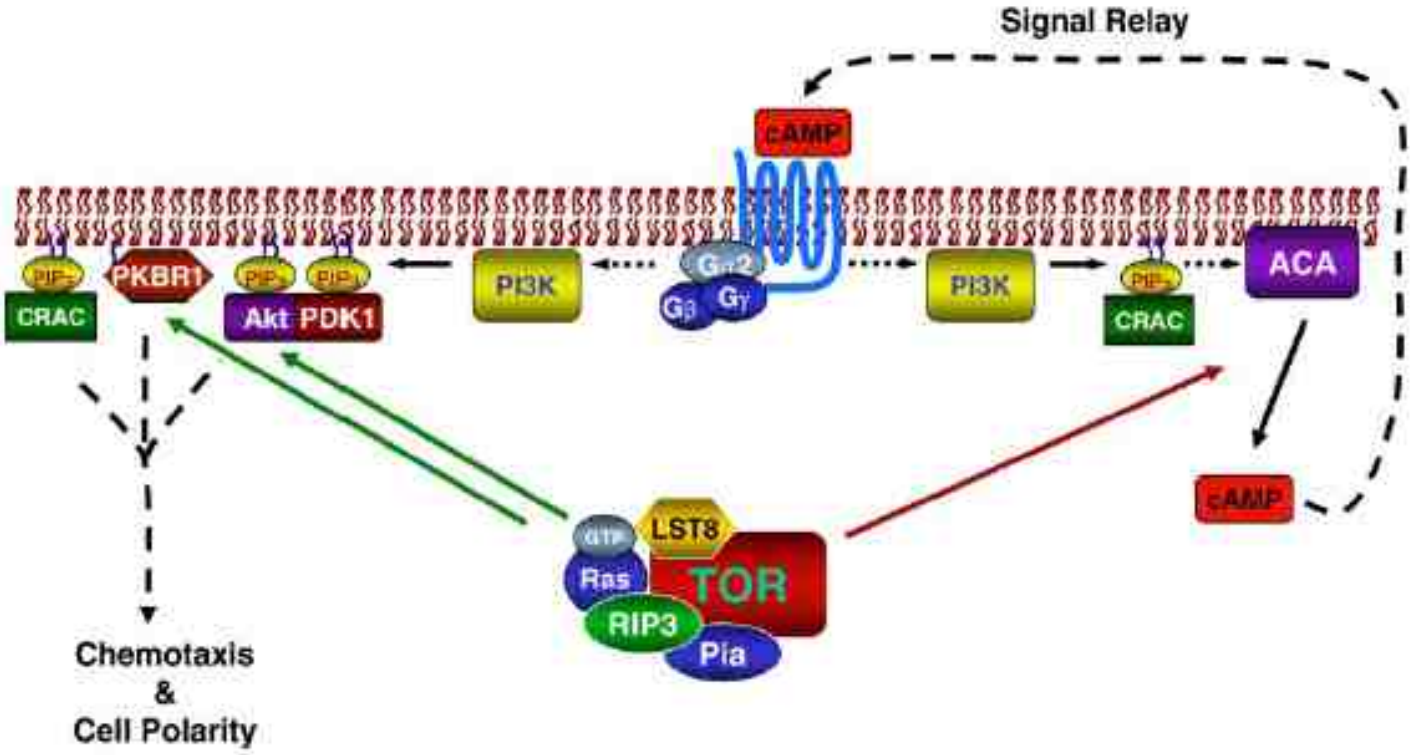
Pia = omologo di Rictor e Avo3

Pergolizzi et al. (2002) Dev. Biol. **251**, 18-26
Bozzaro et al. (1987) Dev.Biol. **123,** 540-548

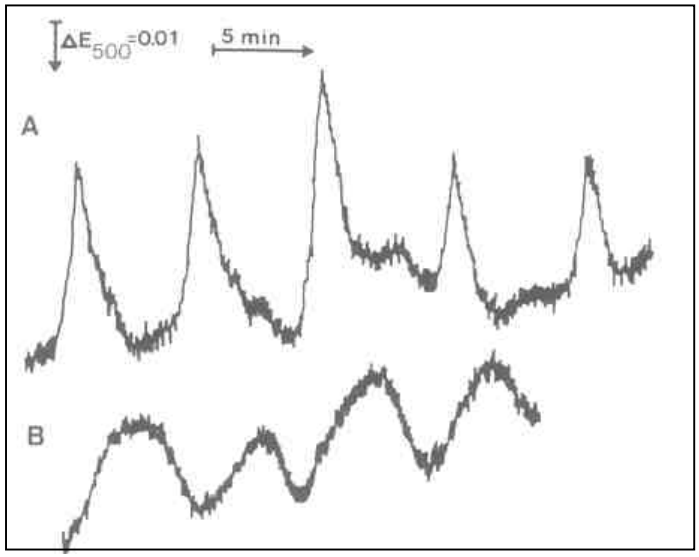
Molecular Biology of the Cell
Vol. 16, 4572–4583, October 2005

TOR Complex 2 Integrates Cell Movement during Chemotaxis and Signal Relay in *Dictyostelium*

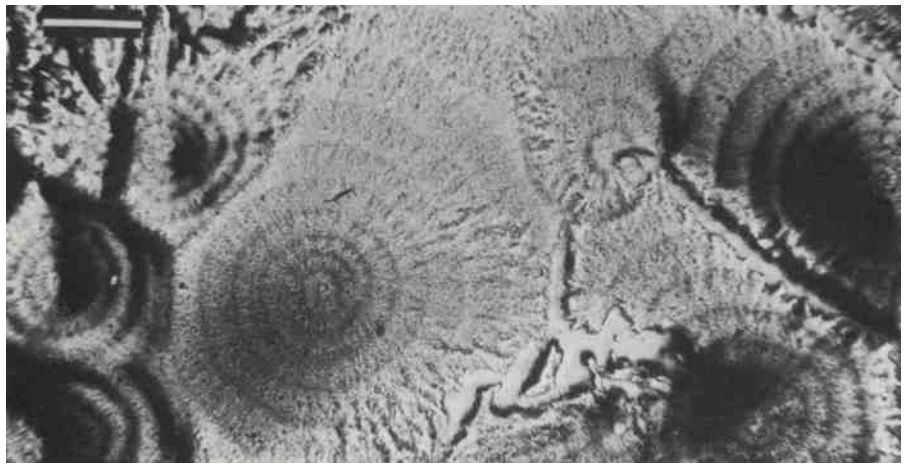
Susan Lee,* Frank I. Comer,† Atsuo Sasaki,* Ian X. McLeod,‡ Yung Duong,*
Koichi Okumura,§ John R. Yates III,‡ Carole A. Parent,† and Richard A. Firtel*



Proprietà oscillatorie della risposta chemiotattica



Oscillazioni di “light scattering” in una sospensione cellulare



Propagazione di “onde di light scattering” in una popolazione cellulare su agar (Gerisch, 1961)

Oscillatori biochimici durante il light scattering:

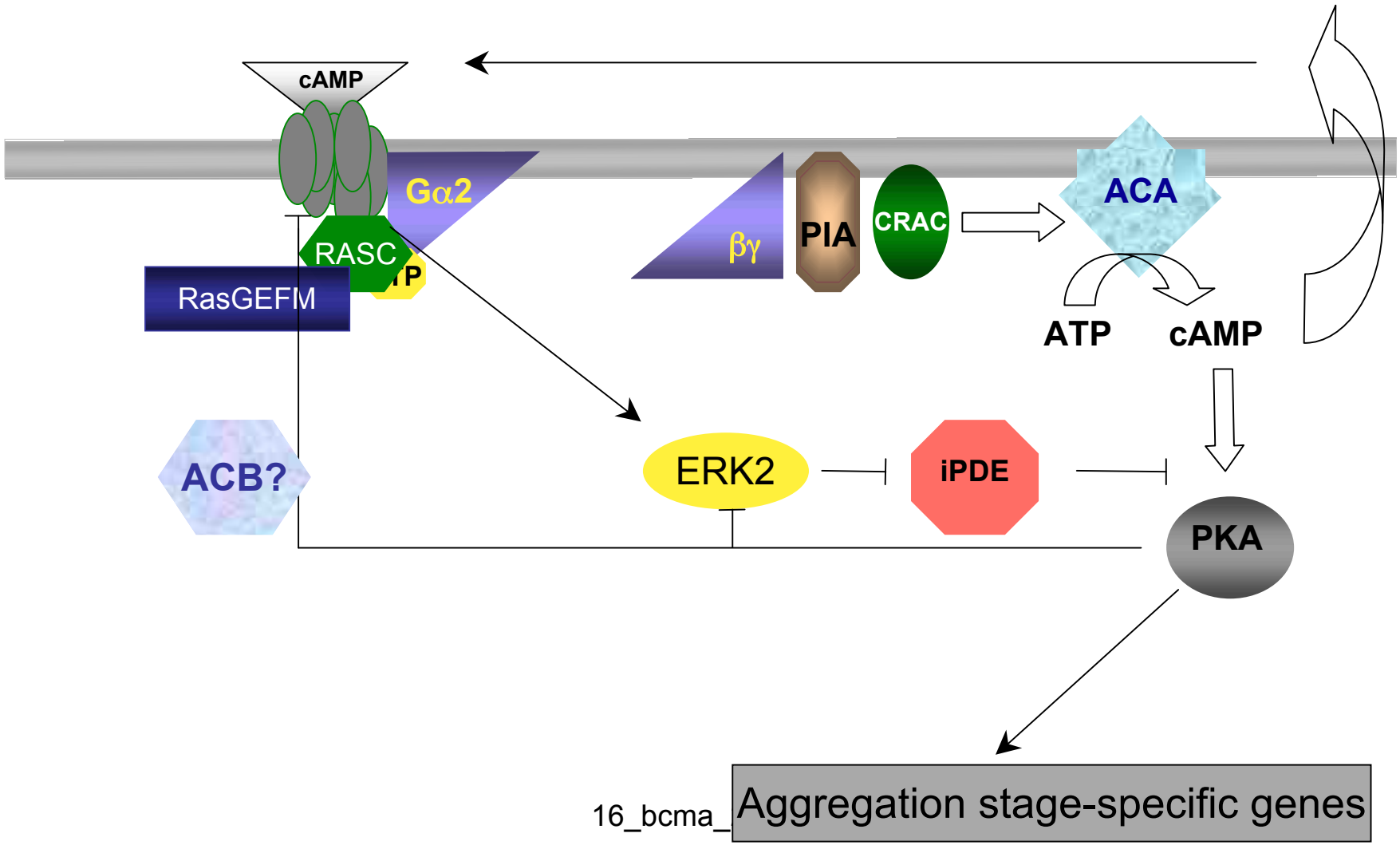
Guanilato ciclasi — flusso di Ca

Adenilato ciclasi — PLC

PI3K

Le cellule vanno incontro a stadi successivi di sensibilità e refrattarietà al segnale, dando origine a onde oscillatorie di cAMP, che si propagano dal centro alla periferia di un campo di aggregazione

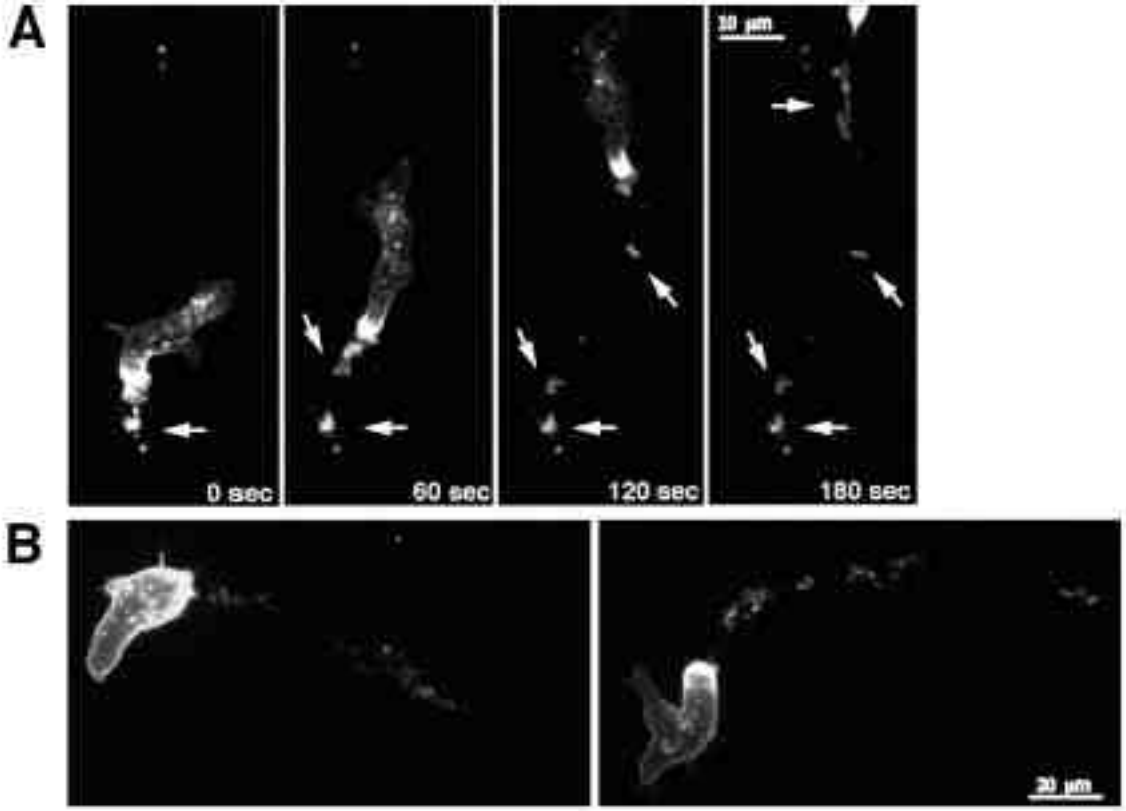
cAMP in Dictyostelium: perchè 2 AC?



Collective cell migration requires vesicular trafficking for chemoattractant delivery at the trailing edge

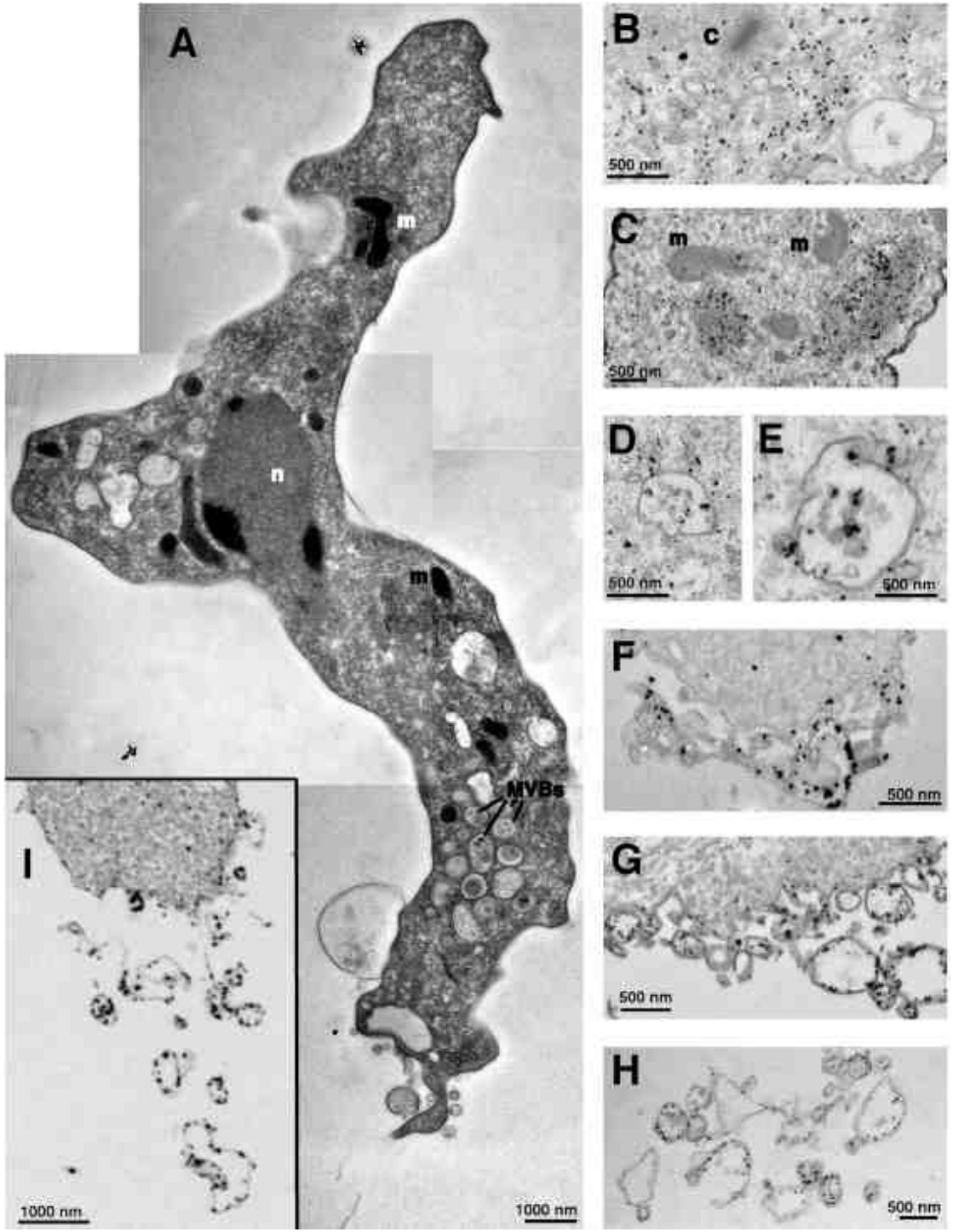
Paul W. Kriebel,[1] Valarie A. Barr,[1] Erin C. Rericha,[1,3] Guofeng Zhang,[2] and Carole A. Parent[1]

Migrating cells leave behind ACA-containing vesicles.



J. Cell Biol. Vol. 183 No. 5 949–961
www.jcb.org/cgi/doi/10.1083/jcb.200808105

16_bcma_2011

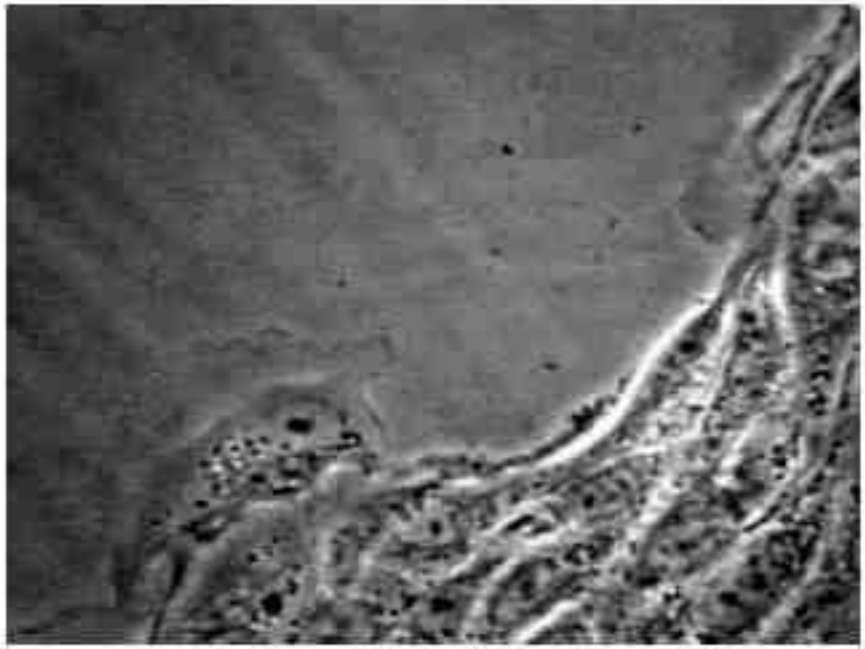

A
n
m
MVBs
1000 nm
I
1000 nm
B
c
500 nm
C
m
m
500 nm
D
500 nm
E
500 nm
F
500 nm
G
500 nm
H
500 nm

Cell Migration

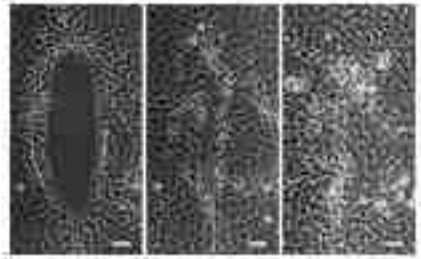
Single cell migration

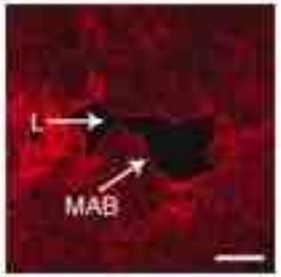
Collective cell migration

Migration of an Epithelial Cell Sheet



Leading edge of a scratch-wounded epithelial cell sheet





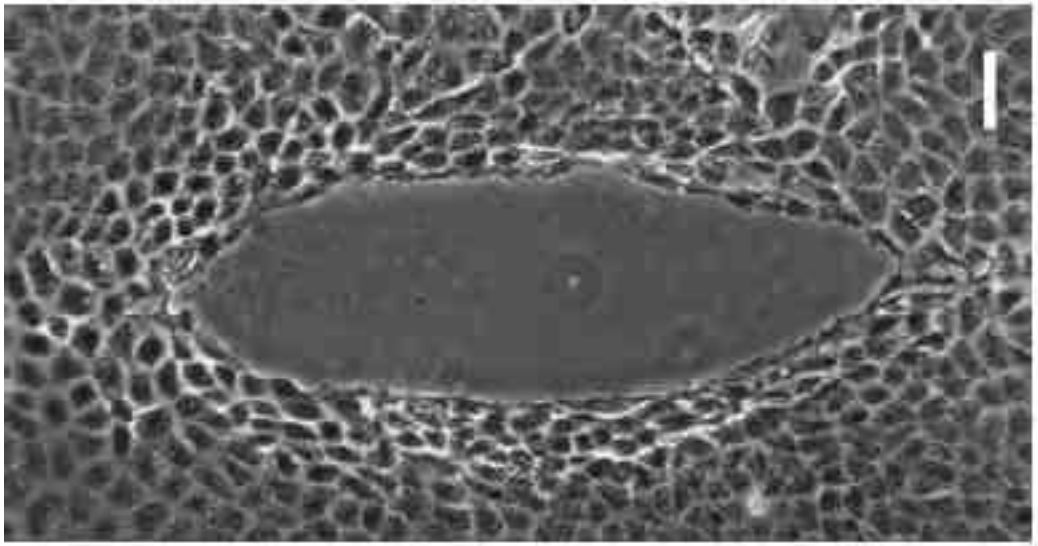
Collective cell migration - similar mechanism to single cell migration.

Fenteany, G., Janmey, P.A., Stossel, T.P. *Curr. Biol.* 2000, *10*, 831;
Altan, Z.M., Fenteany, G. *Biochem. Biophys. Res. Commun.* 2004, *322*, 56;
Farooqui, R., Fenteany, G. *J. Cell Sci.* 2005, *118*, 51.

Investigators have proposed two distinct mechanisms to account for wound closure in epithelial cell sheets:

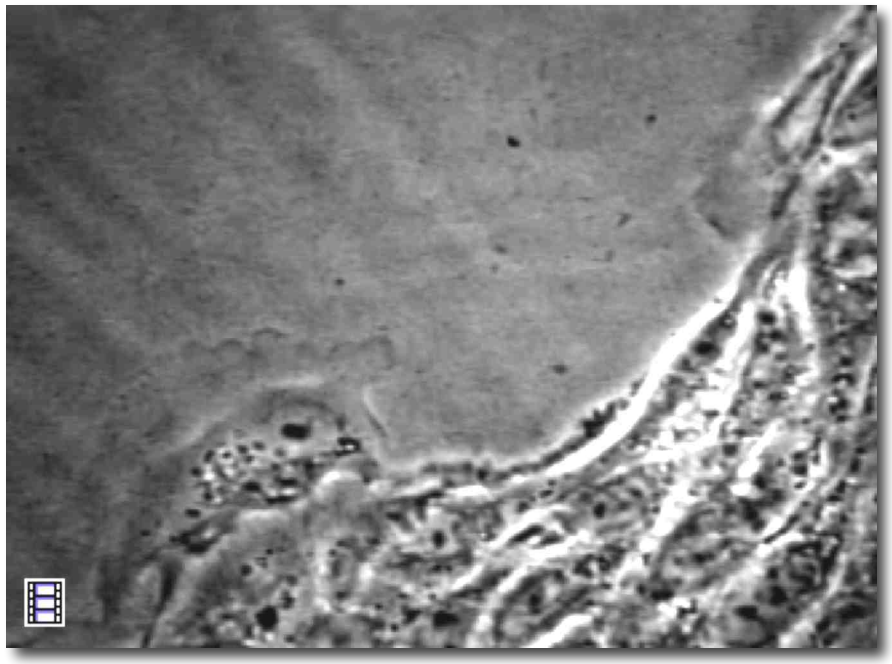
Lamellipodia and purse-string structures

When wounds close by a purse-string mechanism, the obvious driving force is the contraction of actomyosin complexes.
For wound closure by crawling, the mechanics are less clear. Do the cells at the wound edge pull the sheet in centripetally, do cells behind the margin (submarginal cells) push, or do both processes occur?

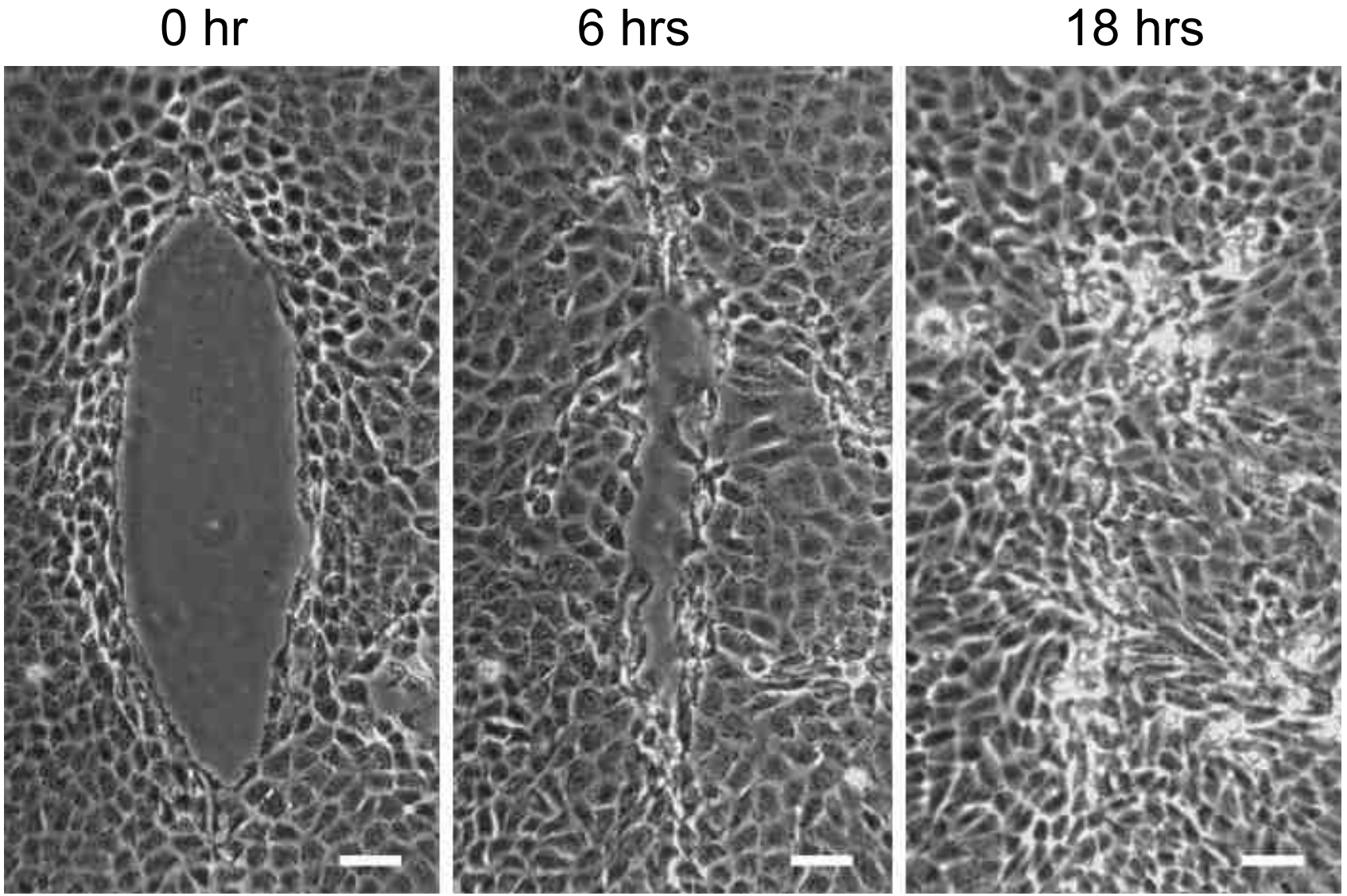


Wounding induces migration of the remaining intact MDCK cell sheet into the gap. As previously observed, cell **proliferation does not contribute** to the filling of small wounds in this system. After the wound is covered and the cells cease to migrate, however, cell division in the former wound area occurs.

Cells at the **wound margin** in MDCK cell monolayers **extend lamellipodia** in the direction of movement into the denuded area, followed by tandem movement of the submarginal cells. During this process, the **cell sheet maintains its coherence**, yet **displacement of cells** relative to one another occurs in **both wounded and unwounded monolayers** over a time scale of hours.



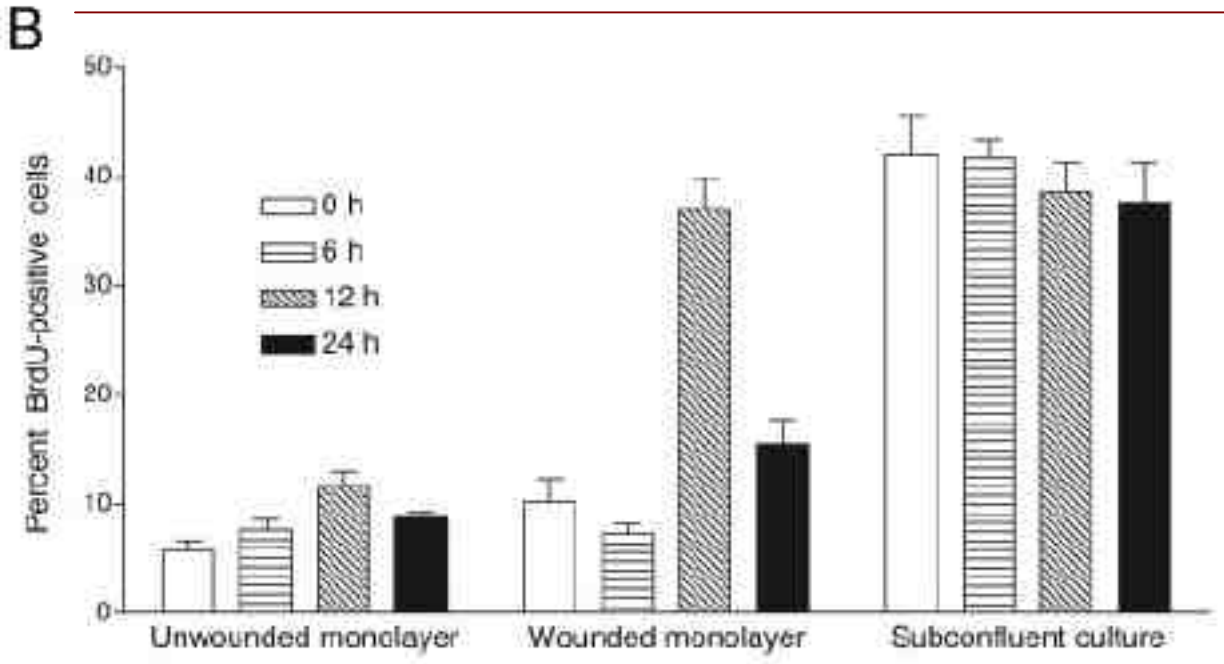
control

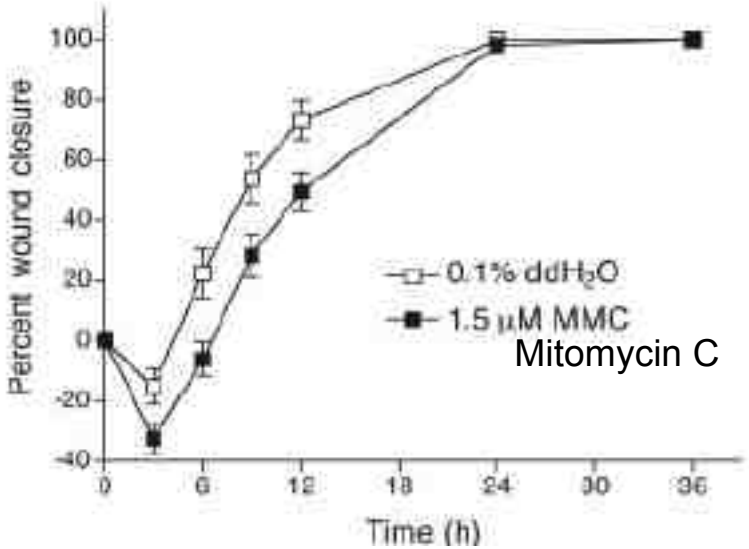


16_bcma_2011

Current Biology 2000, 10:831–838

Wounding triggers delayed cell proliferation but inhibition of proliferation does not affect wound closure

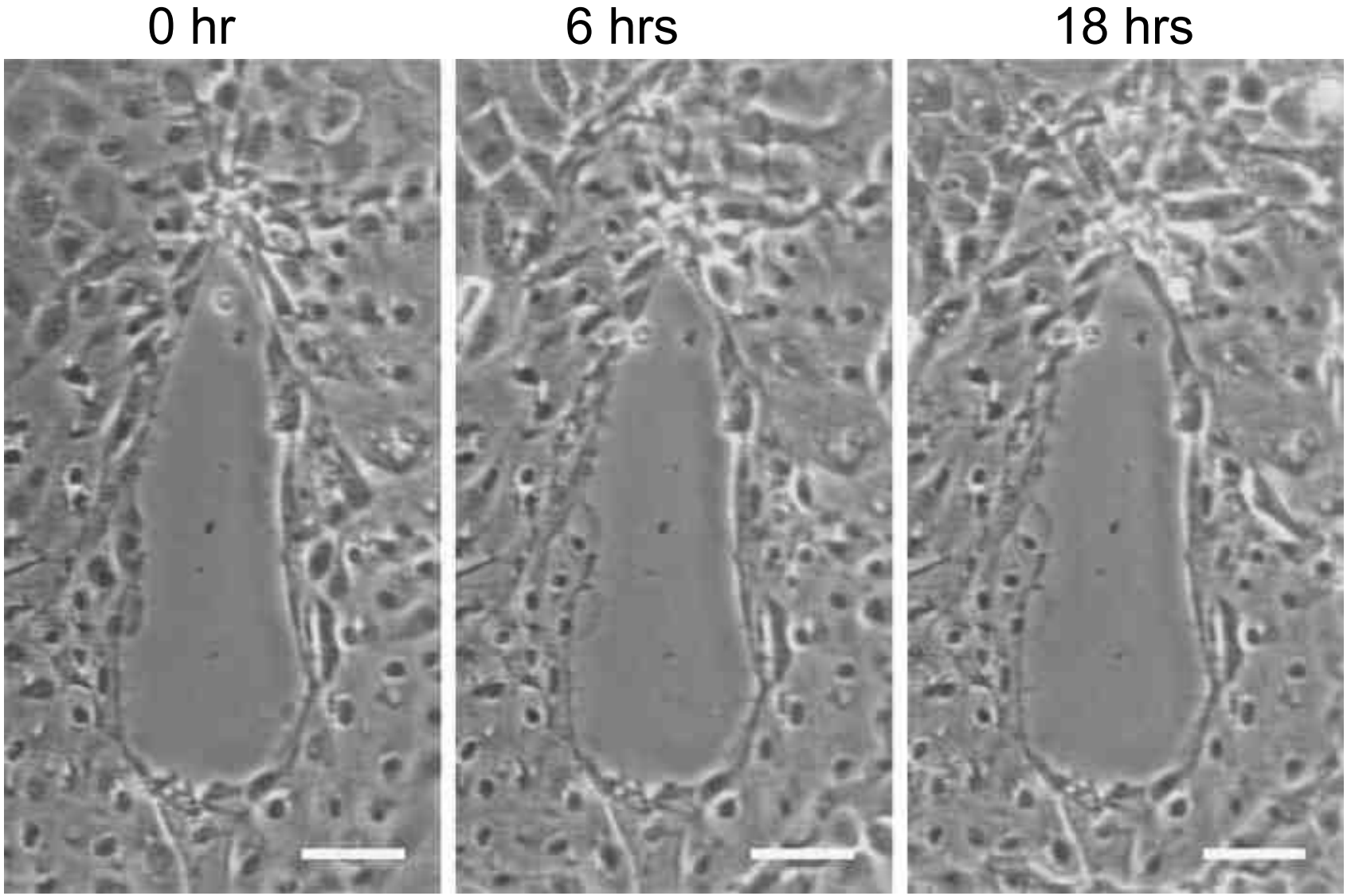




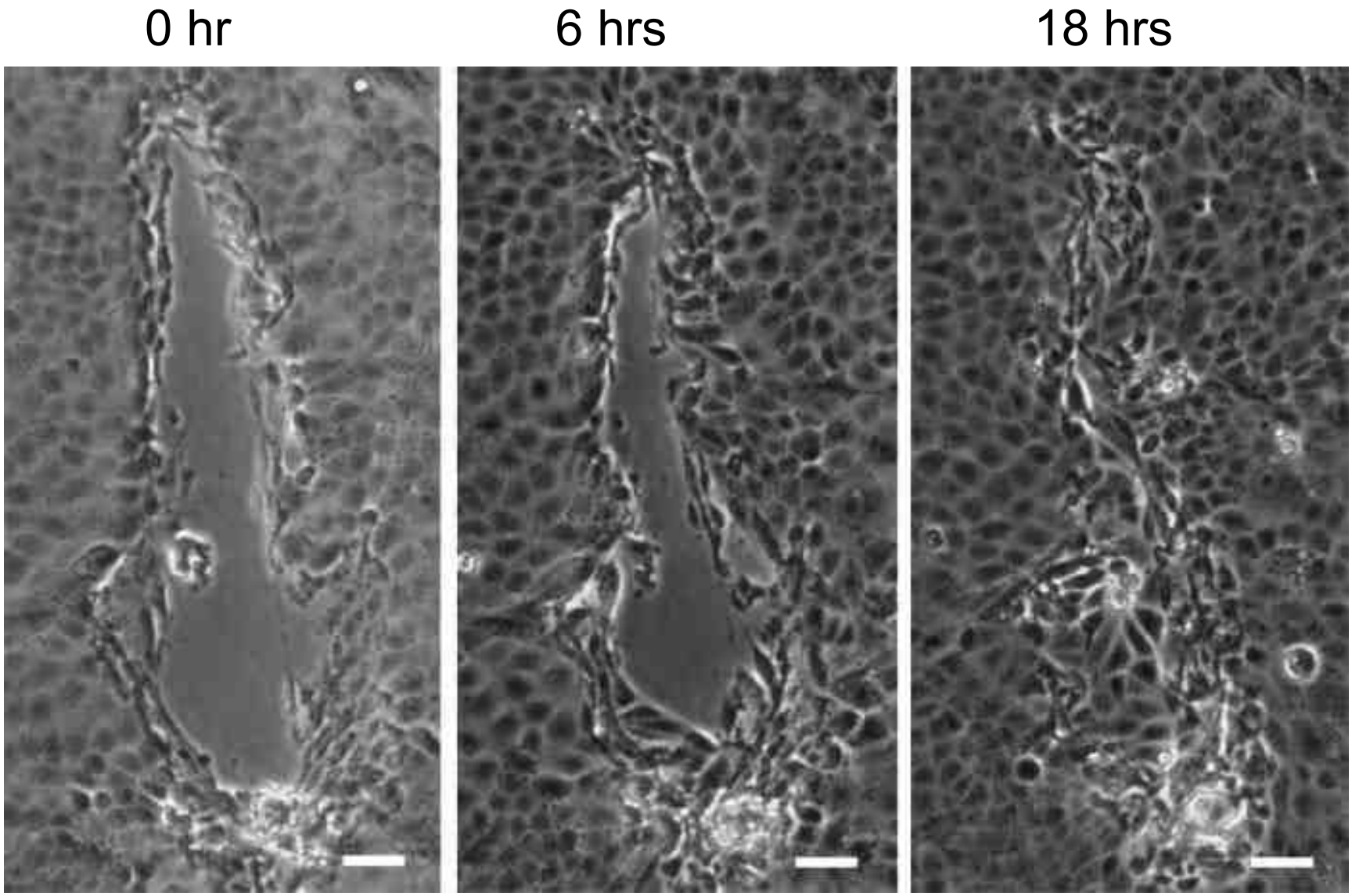
16_bcma_2011

Cells at the wound margin extend Rac- and PI-dependent lamellipodia

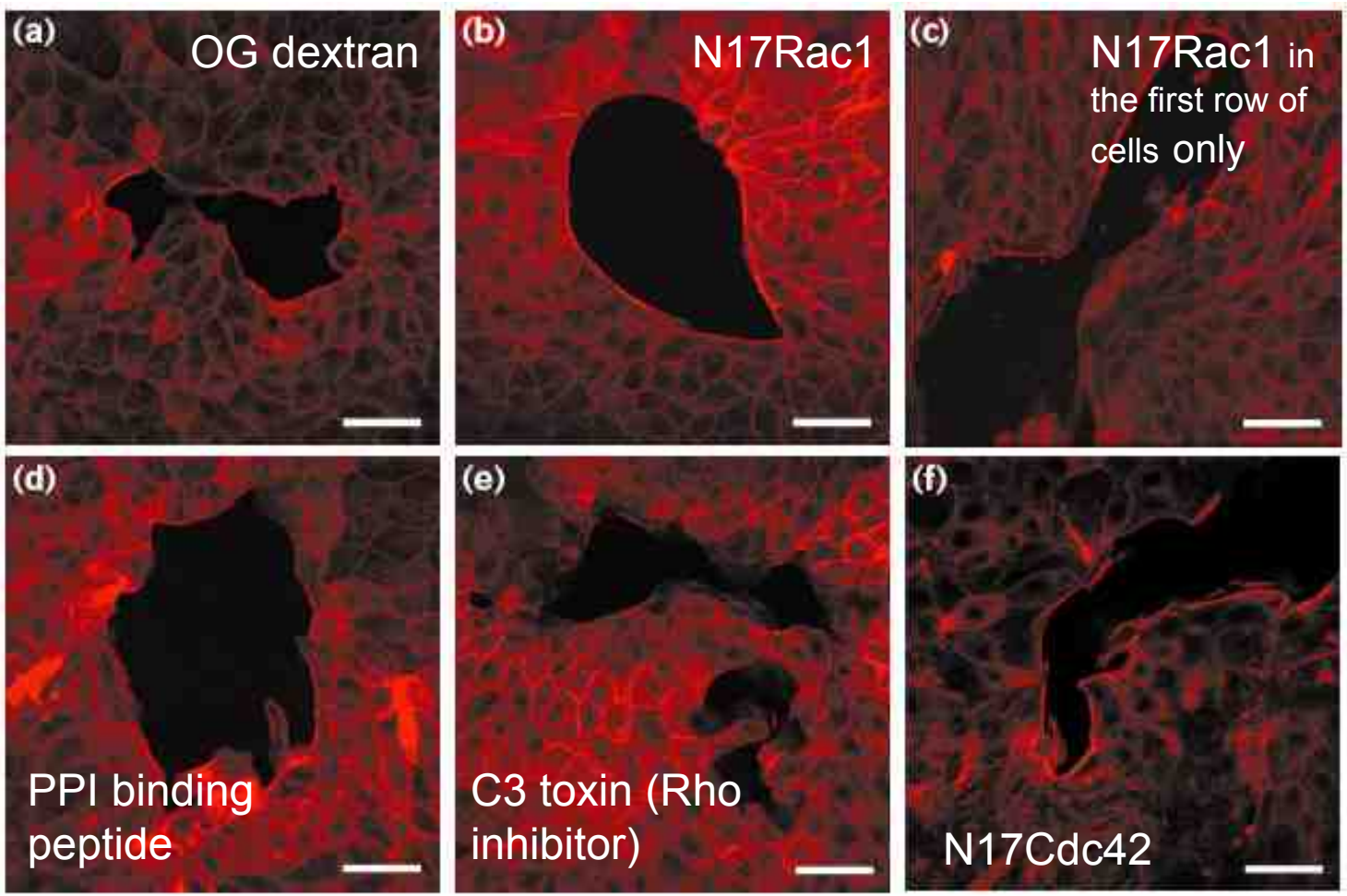
Microinjection of dominant-negative Rac1 protein (N17Rac1) into all the cells in the **first three rows** at the wound margin completely abolishes wound closure



microinjection of N17Rac1 into the **first row of cells** only does not inhibit wound closure



Microinjection of a gelsolin-derived peptide that binds and titrates polyphosphoinositides (PPIs) into the first three rows of cells also completely inhibits wound closure. In contrast, lamellipodium formation and wound closure are not inhibited by microinjection of C3 exoenzyme (an inactivator of RhoA, RhoB and RhoC) or dominant-negative Cdc42 (N17Cdc42).



inhibition

No inhibition

No inhibition

Regularity of wound closure for each treatment.

Treatment	Percentage change in (wound perimeter length/remaining bare area)
Control	30.2 ± 8.1
N17Rac1 (three rows)	9.7 ± 9.1*
N17Rac1 (first row)	21.4 ± 12.6
PPI-binding peptide (three rows)	3.4 ± 7.9*
C3 toxin (three rows)	49.4 ± 15.1*
N17Cdc42 (three rows)	51.5 ± 8.8*

The regularity of wound closure was measured as a percentage change in the ratio of wound perimeter length over the remaining bare area from 0 to 6 h. Numbers are given as mean ± SD with $n = 9$ in each case. Asterisks indicate statistically significant differences ($p < 0.05$) from control values as determined by Student's t test.

Cells behind the wound margin can drive wound closure

Mechanically killing the first few rows of cells by puncturing them with a wide-tip glass needle also does not inhibit wound closure. The damaged cells remain intact but do not prevent undamaged submarginal cells from pushing in and closing the wound

Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement

Rizwan Farooqui and Gabriel Fenteany*

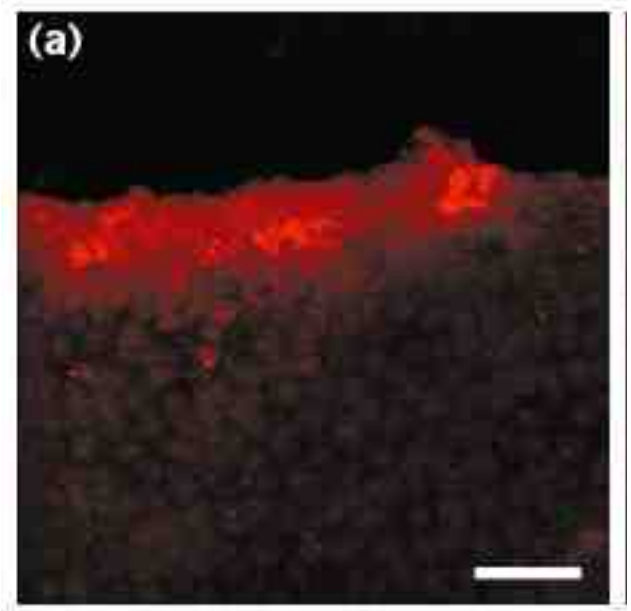
Department of Chemistry, University of Illinois at Chicago, 845 West Taylor Street, Chicago, IL 60607, USA

*Author for correspondence (e-mail: fenteany@uic.edu)

Accepted 4 October 2004
Journal of Cell Science 118, 51-63 Published by The Company of Biologists 2005
doi:10.1242/jcs.01577

We report here for the first time that cells behind the margin of wounded MDCK cell monolayers, even hundreds of microns from the edge, extend 'cryptic' lamellipodia against the substratum beneath cells in front of them, toward the wound.

Actin filament assembly is detectable in cells several rows from the wound margin

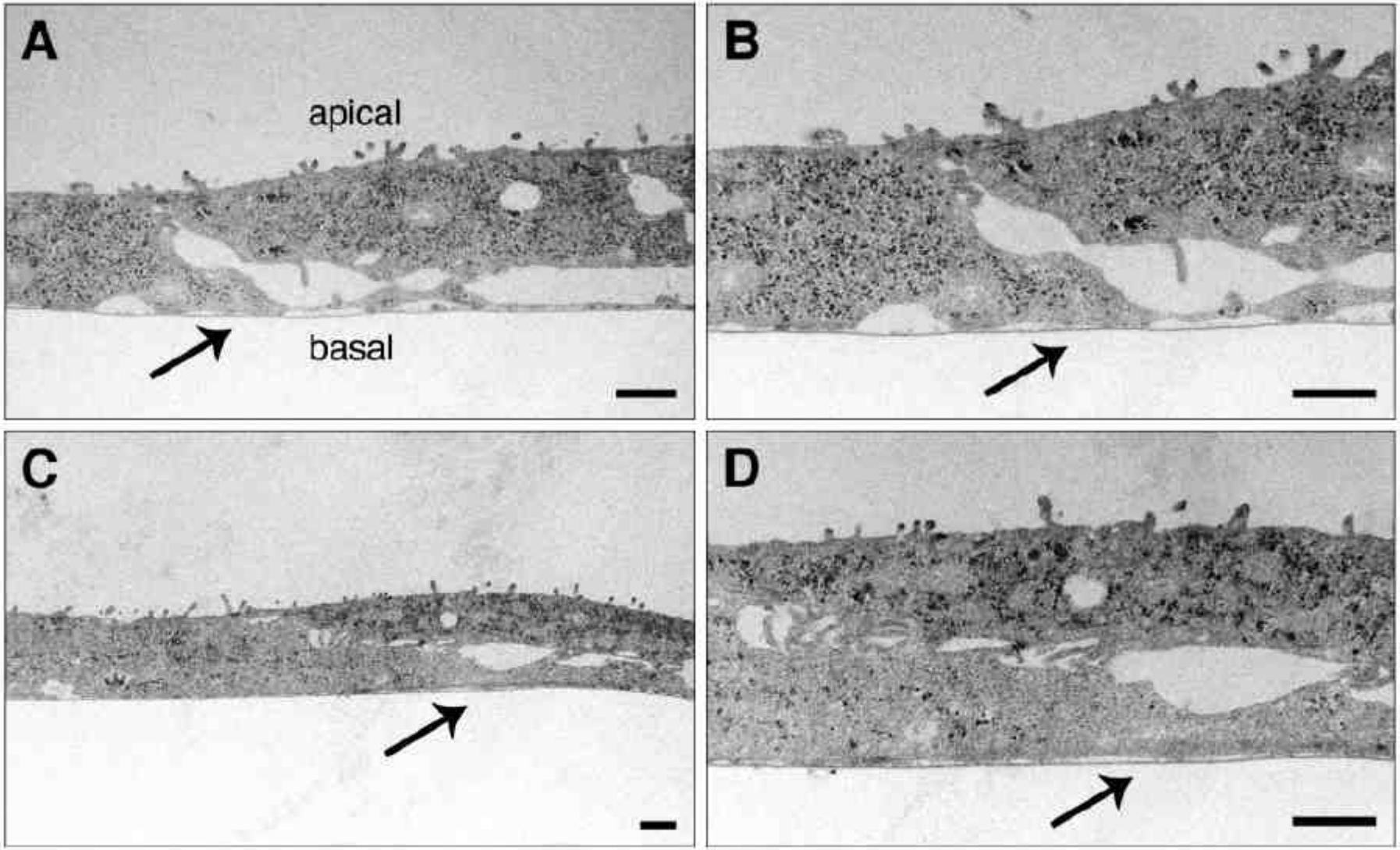


Rac-dependent actin assembly in multiple rows of cells from the wound margin. Confocal micrographs of MDCK cells showing rhodamine-conjugated actin incorporation into the insoluble cytoskeleton (incorporation started 6 h after wounding).

These so-called **submarginal cells nevertheless strictly maintain their more apical cell-cell contacts** when they migrate as part of a coherent cell sheet, hiding their basal protrusions from conventional microscopy. **The submarginal protrusions display the hallmarks of traditional lamellipodia based on morphology and dynamics**.

Cells behind the margin therefore actively crawl, instead of just moving passively when cells at the margin pull on them. The rate of migration is inversely proportional to the distance from the margin, and cells move coordinately, yet still in part autonomously, toward the wound area.

TEM Sections of Wounded MDCK Cell Monolayers



Farooqui, R., Fenteany, G. *J. Cell Sci.* 2005, *118*, 51.