# Genetic analysis of the role of G protein–coupled receptor signaling in electrotaxis

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Cells display chemotaxis and electrotaxis by migrating directionally in gradients of specific chemicals or electrical potential. Chemotaxis in *Dictyostelium discoideum* is mediated by G protein–coupled receptors. The unique Gβ is essential for all chemotactic responses, although different chemoattractants use different receptors and Gα subunits. *Dictyostelium* amoebae show striking electrotaxis in an applied direct current electric field. Perhaps electrotaxis and chemotaxis share similar signaling mechanisms? Null mutation of Gβ and cAMP receptor 1 and Gα2 did not abolish electrotaxis, although Gβ-null mutations showed suppressed electrotaxis. By contrast, G protein signaling plays an essential role in chemotaxis. G protein–coupled receptor signaling was monitored with PHcrac–green fluorescent protein, which translocates to inositol phospholipids at the leading edge of cells during chemotaxis. There was no intracellular gradient of this protein during electrotaxis. However, F-actin was polymerized at the leading edge of cells during electrotaxis. We conclude that reception and transduction of the electrotaxis signal are largely independent of G protein–coupled receptor signaling and that the pathways driving chemotaxis and electrotaxis intersect downstream of heterotrimeric G proteins to invoke cytoskeletal elements.

## Introduction

Directional cell migration is fundamental in development and pathology. Recent work indicates that asymmetric signaling underlies directional sensing in chemotaxis, where cells move up a chemical gradient. Chemoattractants bind to specific receptors and activate signal transduction cascades locally with leading edge actin polymerization and oriented membrane protrusion determining directionality. At the leading edge of migrating *Dictyostelium* and mammalian neutrophils, there is localized formation of inositol phospholipid binding sites for pleckstrin homology (PH)* domain–containing proteins (Parent et al., 1998; Meili et al., 1999; Parent and Devreotes, 1999; Jin et al., 2000; Servant et al., 2000).

Motile cells also detect physiological gradients in electrical potential and show directional migration (electrotaxis or galvanotaxis) or growth (galvanotropism) (Robinson, 1985). This includes bacteria (Rajnicek et al., 1994), fungi (Gow, 1994), amoeba (Korohoda et al., 2000), amphibian neuronal growth cones (Hinkle et al., 1981), fish and human keratinocytes (Cooper and Schliwa, 1986; Nishimura et al., 1996), and bovine and human corneal epithelial cells (Zhao et al., 1996). There is some subtlety in their responsiveness (McCaig et al., 2002). Most cells migrate cathodally, but corneal fibroblasts and lens epithelial cells migrate anodally (Soong et al., 1990; Wang et al., 2000). Neuronal growth cones are attracted cathodally and repelled anodally, but this is reversed when grown on positively charged polylysine (Rajnicek et al., 1998). In hippocampal neurones, dendrites are attracted cathodally, but axons are not (Davenport and McCaig, 1993). Corneal epithelial cells migrate cathodally, and this is enhanced on fibronectin or laminin (Zhao et al., 1999), but lens epithelial cells are attracted or repelled cathodally at electric field (EF) strengths that vary only twofold (E. Wang, personal communication; unpublished data). Polarized signaling of the EGFR–MAP kinase pathway underpins directional migration of corneal epithelial cells in a physiological electric fields (Zhao et al., 2002).

Direct current (DC) EFs similar to those inducing these effects have been measured in areas where cells migrate developmentally and during wound healing (Jaffe and Stern, 1979; Chiang et al., 1992; Nuccitelli, 1992; Shi and Borgens, 1995). These arise because of spatial and temporal
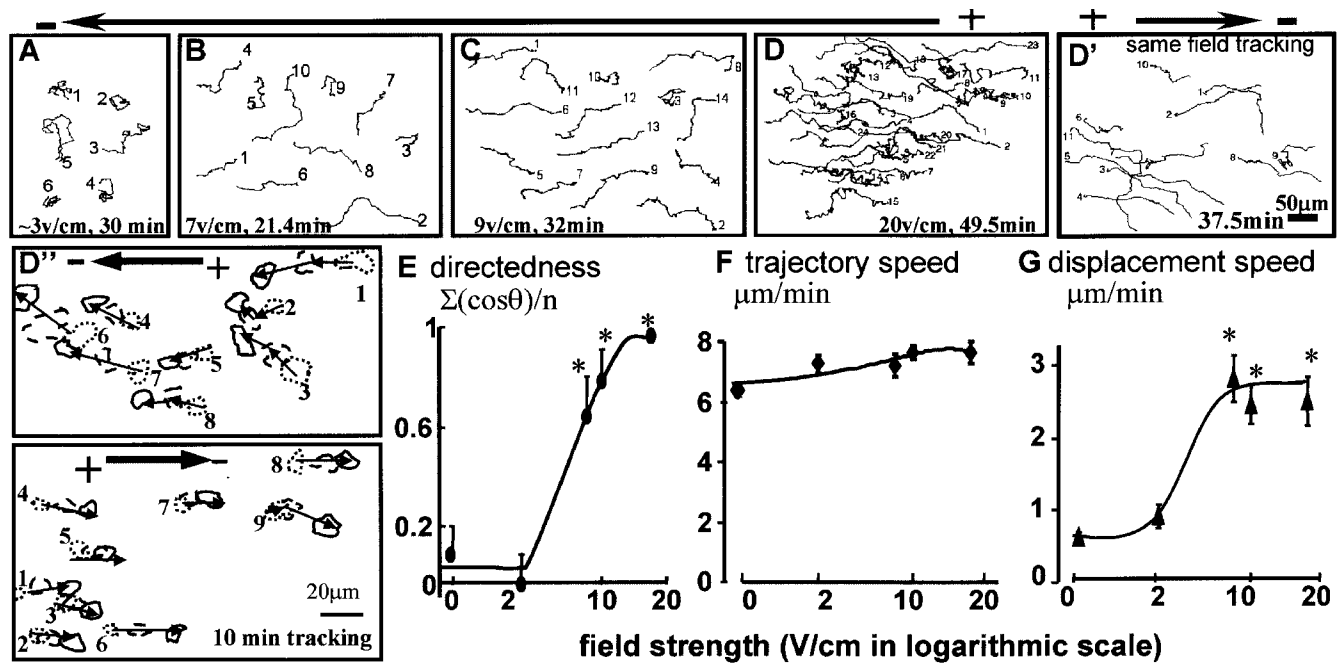
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Figure 1.   **Wild-type *Dictyostelium*.** Cells migrate cathodally (left) in a DC EF as shown by trajectories mapping the cell centers starting from the numbered ends. (B, C, and D). Electrotaxis depended on field strength (A–E and G). Reversal of field polarity reversed migration direction (D, D′, and D″). D′ is the same field tracking of D. D″ shows cell movements during 10 min field application pointing to the left and 10 min after reversing the field polarity. (E) Voltage dependence of electrotaxis (for directedness; as described in Materials and methods). Trajectory speed was similar between no field control and at different voltages (F), but movement in an EF was more persistent in one direction (G). n = ~36–46 from at least three independent experiments. *P < 0.01 compared with no field control. See also video 1 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1.

variations in epithelial transport or spatial variations in the tightness (electrical resistance) of epithelial sheets (Robinson and Messerli, 1996). Experimental disruption of such endogenous EFs indicates their physiological importance, since this both impairs wound healing and results in gross developmental abnormalities of embryonic nervous and skeletal systems. In each case, these defects could be the consequence of impaired directional cell migration (Hotary and Robinson, 1992; Sta Iglesia and Vanable, 1998). One interesting question is whether cells use similar directional sensing mechanisms to detect an EF and a chemical gradient.

*Dictyostelium discoideum* is a well-used model in which the pathway driving chemotaxis has been characterized. *Dictyostelium* also show robust electrotaxis. Although these amoebae would not naturally encounter DC EFs, they offer a powerful system in which to make molecular and genetic analyses of the mechanisms underpinning electrotaxis. In chemotaxis, G protein–coupled receptors sense chemoattractants and regulate pseudopod extension at the leading edge (Parent and Devreotes, 1999; Chung et al., 2001). We have investigated whether electrotaxis and chemotaxis share signaling mechanisms through G protein–coupled receptors. We show that the early stages of signal reception and transduction are not shared but that the respective signaling strategies converge somewhere upstream of directed actin polymerization.

## Results and discussion

### *D. discoideum* shows robust electrotaxis

*Dictyostelium* cells migrated cathodally in an applied EF (Fig. 1); control cells with no EF moved randomly. Cells ex-

tended cathodally directed pseudopodia within seconds of switching the EF on and began directed migration. Morphological polarization of cells toward the cathode was more evident at later stages of development. However, cells at early developmental stages, although they lacked obvious elongation and intrinsic polarization, still showed equally robust EF-directed migration. Reversal of the EF polarity caused rapid reversal of directed migration (within seconds) (Fig. 1). In control experiments, which exclude the build up of EF-generated chemical gradients by continuous perfusion of developing buffer between either end of the electrotaxis chamber, cells moved cathodally to a similar extent (0.74 ± 0.06, n = 38). Cells in 2 mM caffeine, which blocks adenylate cyclase activation and therefore excludes involvement of cell to cell cAMP signaling, also maintained directional migration (directedness 0.78 ± 0.10, n = 15). In addition, mutant cells that are incapable of producing or sensing a chemical gradient such as the Gβ null (see below); nonetheless, they did detect an EF and showed strong electrotaxis. Therefore, the directional cue presented by the EF is unlikely to be mediated by a standing chemical gradient created in the culture medium.

Electrotaxis of *Dictyostelium* was voltage dependent. The threshold voltage inducing directional migration was between 3 and 7 V/cm (Fig. 1, A, B, and E). Directedness increased with increasing field strength reaching 0.96, almost perfect directional migration toward the cathode. At ~15–20 V/cm, 100% of cells moved directly toward the cathode with a directedness close to 1 (Fig. 1, D and E). Trajectory speed was the same for control (no EF) and EF-stimulated cells (up to 20 V/cm) (Fig. 1 F). However, displacement
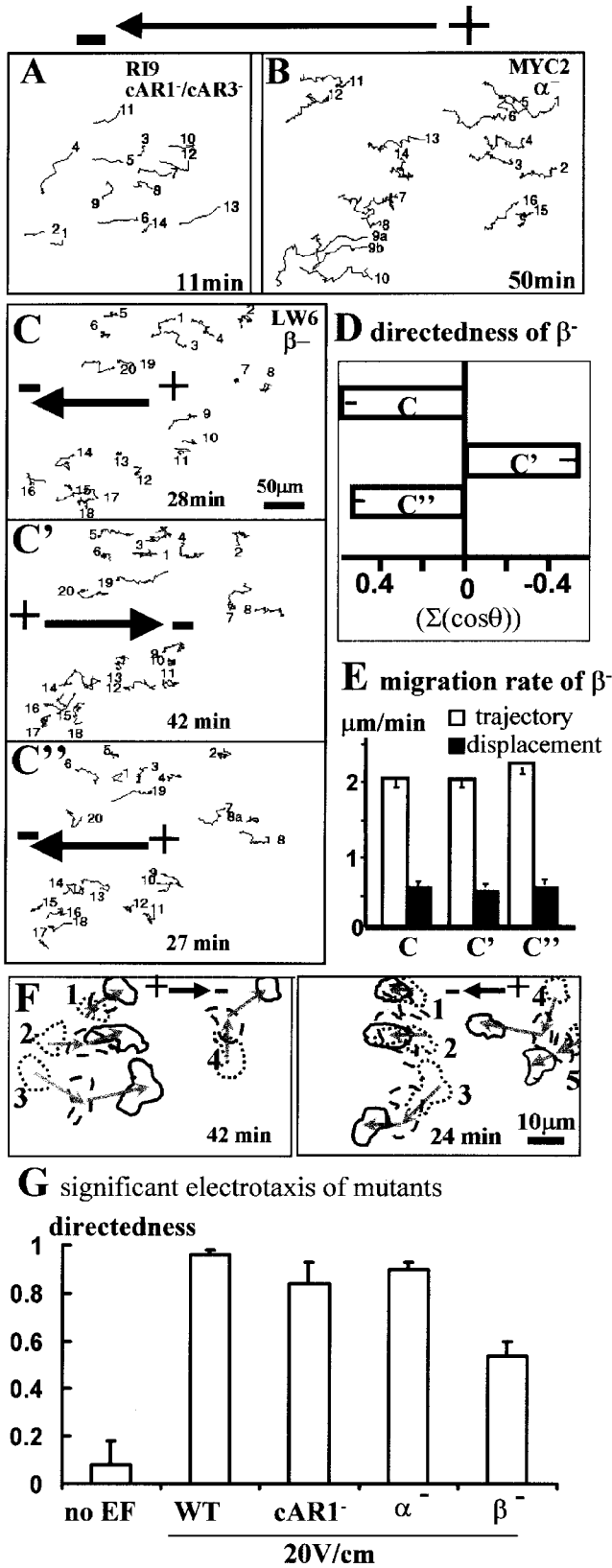
**Figure 2.** **cAMP receptor and g protein mutant cells migrate cathodally.** cAR1⁻/cAR3⁻, Gα2⁻, Gβ⁻ mutants migrated cathodally in an applied EF (A, B, C). Trajectories plotting the movement of the cell centers. Electrotaxis of Gβ⁻ cells was confirmed by reversing EF polarity twice (C', C''). (F) Directedness values and trajectory and displacement speed of β⁻ cells remained virtually the same (D and E).

Directedness indicates that wild-type (WT), cAR1⁻/cAR3⁻ (cAR1⁻), Gα2⁻ (α⁻), and Gβ⁻ (β⁻) all showed electrotaxis (G). 36–46 cells were tracked for directedness and migration rate, and the results were confirmed from two more independent experiments. See also videos 2, 3, and 4 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1.

speed, an index showing how efficiently cells moved in a certain direction, increased gradually with the applied voltage, indicating an increased efficiency or persistence in cathodal directedness (Fig. 1 G). The threshold for *Dictyostelium* electrotaxis was ~4–10-fold of that in mammalian cells. At 7 V/cm, directedness was ~0.6, similar to that of mammalian epithelial cells at 1–2 V/cm (Nishimura et al., 1996; Zhao et al., 1996). To achieve optimal electrotaxis, we used a field strength ~7–10-fold that for mammalian cells. Except for straightening migration trajectories and increasing the percentage (100%) of cells moving toward the cathode, no differences in general cell morphology, behavior, and trajectory speed were observed between low and high EF strengths (Fig. 1 and video 1 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1). Therefore, we consider that the directed electrotaxis shown by *Dictyostelium* cells to high EFs is similar to that shown by mammalian cells to lower EF strengths.

No clear drop in response was found during several hours of exposure to an EF. Successive directedness values for the same cells were 0.96 ± 0.02 and 0.88 ± 0.04 for 1 and 2 h, respectively (20 V/cm). Trajectory and displacement speeds also were identical (7.6 ± 0.2, 7.6 ± 0.2 μm/min for trajectory speed and 2.5 ± 0.3, 2.5 ± 0.3 μm/min for displacement speed at both 1 and 2 h; $n = 11–24$).

Electrotaxis has been reported in cells from humans to amoeba, and many experience endogenous EFs in vivo (see Introduction). The importance of studying the effects of DC EFs on cell migration includes (a) using EFs as a tool to understand direction sensing, (b) determining the involvement of endogenous EFs in directing cells, and (c) shaping tissues in vivo and using EFs to spatially control the engineering of cells and tissues. Because chemotaxis in *Dictyostelium* is well understood, this is an excellent model to study the molecular and genetic basis of electrotaxis and to compare and contrast the signaling strategies underpinning electrotaxis and chemotaxis.
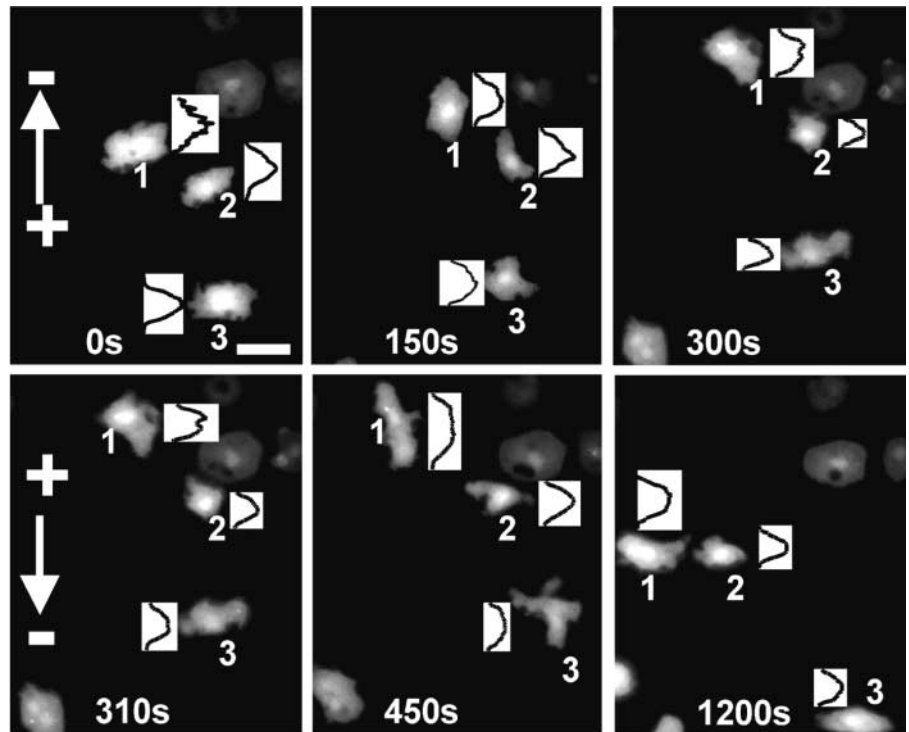
## cAR1⁻/cAR3⁻, Gα2⁻, Gβ⁻ cells maintained directional migration toward the cathode

*Dictyostelium* cells that enter the development stage use G protein–coupled receptor signaling to direct chemotactic migration to a source of cAMP. The most important receptor for this is cAR1. To test whether cAMP receptors were involved in electrotaxis, cAR1⁻/cAR3⁻ cells (RI9) were used. These cells, which show no response to cAMP, maintained significant directional migration toward the cathode when stimulated with DC EFs (Fig. 2, A and G). The displacement speed and the directedness were comparable to wild-type cells (2.7 ± 0.2 μm/min, 0.8 ± 0.1, respectively; 20 V/cm, $n = 36$).

The Gα2 subunit together with the Gβγ complex couple with cAR1 and transduce cAMP binding into various

Figure 3. **PHcrac-GFP is not redistributed during electrotaxis.** The top panel shows a complete sequence of electrotaxis; the bottom panel shows the same cells after EF reversal. The EF did not influence PHcrac-GFP distribution. The line charts show fluorescent intensity along a line drawn across the cells vertically and show even distribution of PH-crac-GFP. The same observation was repeated in three independent experiments. Polarity is as shown. Bar, 10 μm. See also video 5 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1.
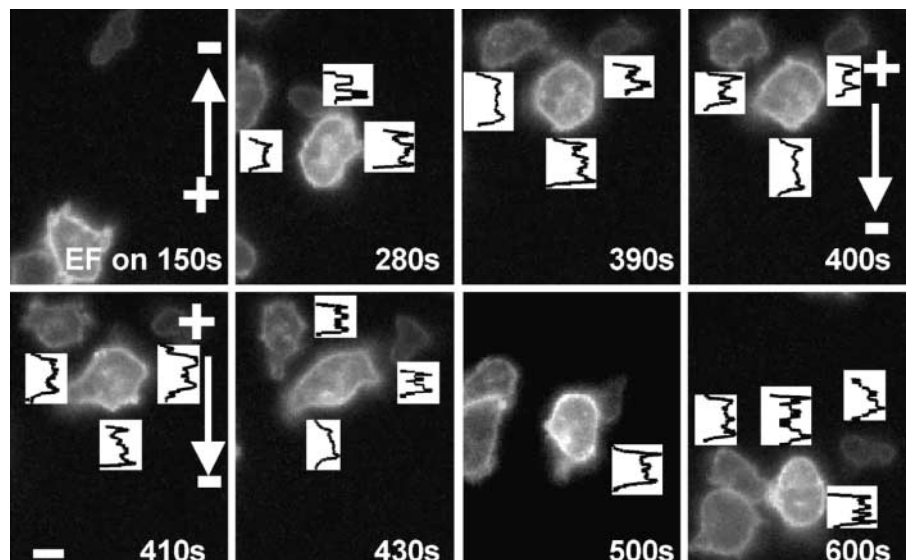


responses. Like cAR1/cAR3-null mutants, Gα2⁻ cells (MYC2) also maintained directional migration in EFs (Fig. 2 B). The directedness was comparable to wild-type cells (0.9 ± 0.2, 20 V/cm) (Fig. 2 G), but trajectory speed and displacement speed were lower (2.6 ± 0.2 μm/min, 1.1 ± 0.1 μm/min, respectively; $n = 37$). In general Gα2⁻ cells migrated less rapidly.

The Gβ subunit is essential for chemotaxis to all chemoattractants (Wu et al., 1995; Jin et al., 1998). However, although markedly suppressed, significant directional migration remained in Gβ⁻ cells in an EF (Figs. 2, C, D, F, and G). These data are robust. Gβ⁻ cells were processed exactly as wild-type cells (starved for 1 h, cAMP pulsed for an additional 1–2 h). The same cells were subjected to an EF of 20 V/cm. EF polarity was reversed twice (Fig. 2 C, C′, and C″). Significant directional migration is seen in the composite trajectories of the cells. Cells moved to the left (cathode) at the beginning (Fig. 2 C), then to the right on reversing the polarity (Fig. 2 C′), and left again with a further polarity reversal (Fig. 2 C″). In each case, there was significant directedness toward the cathode (Fig. 2 D), and trajectory and displacement speeds remained virtually the same (Fig. 2 E). At 7 V/cm, Gβ⁻ cells maintained a directional migration of 0.25 ± 0.07 ($n = 69$; four experiments, significantly different from no field control), which was only half that of wild-type cells. Compared with wild-type, cAR1⁻/cAR3⁻ and Gα2⁻, Gβ⁻ cells had significantly lower trajectory and displacement speed ($P < 0.01$) (Fig. 2 E compared with Fig. 1, F and G).

Figure 4. **cAR1-GFP is not redistributed during electrotaxis.** The top panel shows a complete sequence of electrotaxis; the bottom panel shows the same cells after EF polarity reversal. The EF did not influence cAR1-GFP distribution. The line charts show fluorescent intensity along a line drawn across the cells vertically, and they show even distribution of the cAR1-GFP. The same observation was repeated in three independent experiments. Polarity is as shown. Bar, 5 μm. See also video 6 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1.
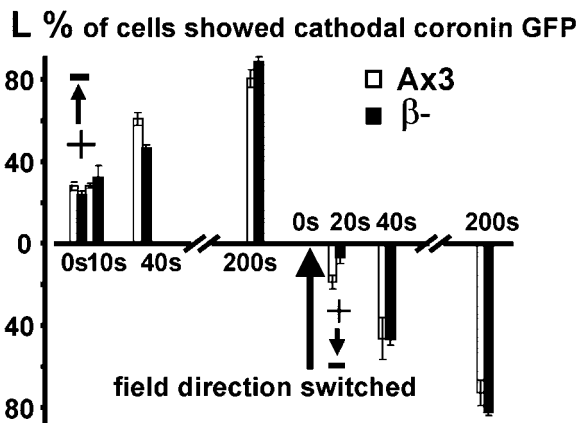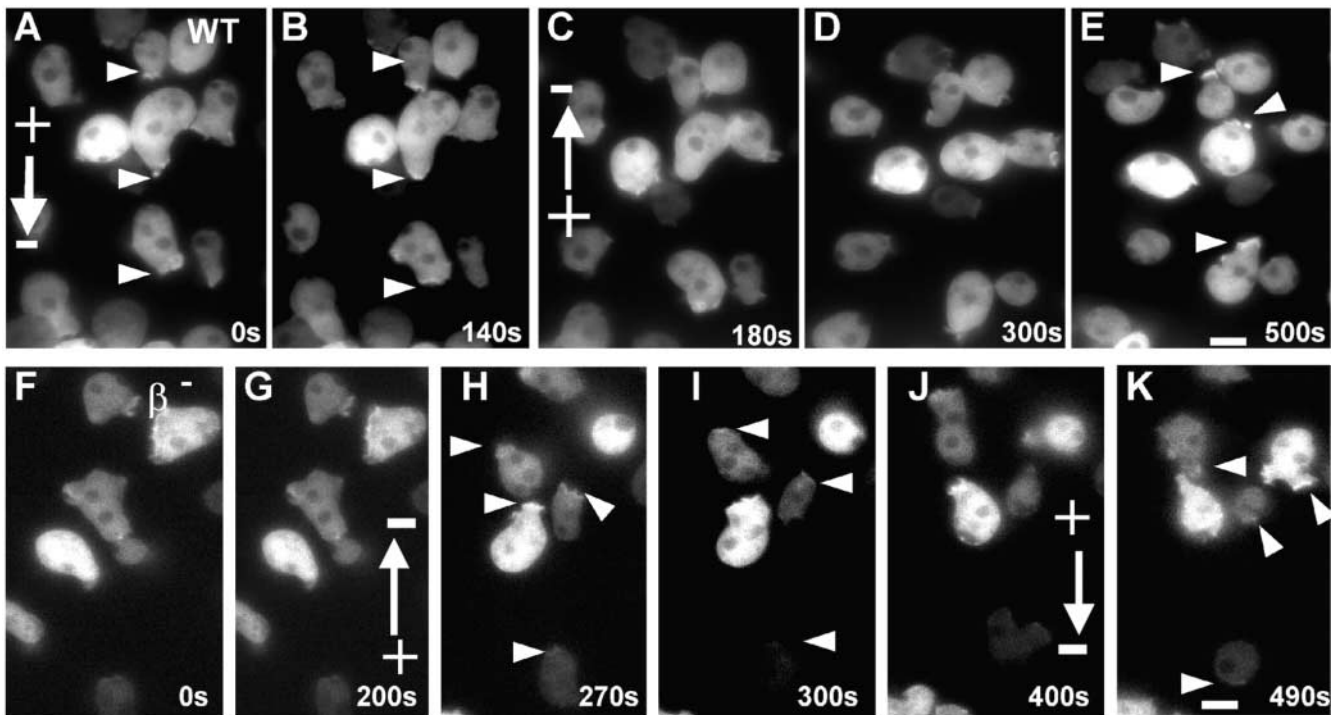
**L % of cells showed cathodal coronin GFP**

Figure 5. **Coronin-GFP redistributes to the leading edge during electrotaxis.** The top (A–E, wild type) and bottom panel (I–K, β⁻) show strong coronin-GFP accumulation at the cathode facing front of the cells (arrowhead). Reversal of the EF polarity redistributed coronin-GFP to the new cathode facing side. L represents the percentage of cells exhibiting this redistribution along with the time course. The same observation was repeated in three independent experiments. Polarity is as shown. Bar, 10 μm. See also videos 7 and 8 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1.

Heterotrimeric G proteins have crucial functions in *Dictyostelium* development. There are twelve Gα, one Gβ, and one Gγ subunits (Devreotes, 1994; Brzostowski and Kimmel, 2001; Zhang et al., 2001). Gα subunits are expressed at different stages, whereas Gβ and Gγ are expressed throughout development. The Gβ-null mutants are unable to form heterotrimeric G proteins, and their development is blocked at an early stage because of defects in gene induction (Wu et al., 1995; Jin et al., 1998). The Gα2 deletion mutants are impaired in cAMP-mediated responses in the aggregation stage. Gβ⁻ cells display a slower speed in random movements compared with wild-type, cAR1⁻/cAR3⁻ or Gα2⁻ cells, which may account for their lower trajectory and displacement speeds in an EF.

## Electrotaxis does not engage G protein–coupled receptor signaling

Membrane recruitment of the PH domain–containing protein CRAC (cytosolic regulator of adenylate cyclase, PH-crac) is an indicator of G protein signaling in cells. Using

PHcrac fused with green fluorescent protein (GFP) (PH-crac-GFP) has provided significant insight in understanding the molecular mechanisms of chemotaxis. Asymmetry in signaling drives polarized actin changes needed for lamellipodium or pseudopodium extension and motility (Zigmond, 1996; Parent and Devreotes, 1999; Chung et al., 2001). Using GFP fusion constructs in *Dictyostelium* (PHcrac-GFP) and neutrophils (PHAkt-GFP) has shown that phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol-3,4-trisphosphate generated upon activation of the G protein–coupled receptors are polarized toward the chemoattractant source (Parent et al., 1998; Meili et al., 1999; Jin et al., 2000; Servant et al., 2000). Spatial regulation of PI 3-kinase and phosphatase activities therefore are crucial for directional sensing during G protein–mediated chemotaxis (Rickert et al., 2000; Chung et al., 2001).

In wild-type cells expressing PHcrac-GFP, there was no redistribution of PHcrac-GFP during electrotaxis or after polarity reversal (Fig. 3; Table S1 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1 for quantitative re-

sults), indicating that the EF did not act upon the G protein subunits or their immediate effectors to direct movement.

G protein–coupled receptor signaling underlies chemotaxis in *Dictyostelium* amoebae and neutrophils. cAR1⁻/ cAR3⁻, Gα2⁻ cells had virtually no defect in directional migration in EF when compared with the wild-type (Fig. 2). Although the directedness of Gβ ⁻ cells was significantly lower than that of the wild-type, cAR1⁻/cAR3⁻ and Gα2⁻ cells, Gβ ⁻ cells still migrated cathodally (Fig. 2, C, D, F, and G). Since the receptor, Gα2, and Gβ-null mutants did respond to the EF, these G proteins cannot be essential to detect the electrical gradient. Nonetheless, there was some weak involvement of Gβ in electrotaxis in *Dictyostelium*. This contrasts remarkably with the essential role of the Gβ subunit in all chemotactic responses. In further support of the conclusion that the EF is not transduced simply via the activation of the cAR1 receptor and G proteins, we demonstrated that PHcrac-GFP did not translocate to the leading edge of cells undergoing electrotaxis.

### cAR1 receptor did not redistribute during steady state electrotaxis and reversal of EF

Membrane receptor redistribution may be involved in cell responses to EFs and has been demonstrated in several cell types (Poo and Robinson, 1977; Brown and Loew, 1994; McCaig and Zhao, 1997; Fang et al., 1999; Zhao et al., 1999, 2002). Cathodally directed migration of corneal epithelial cells involved induced asymmetry of membrane lipids and associated EGF receptors and asymmetric activation of MAP kinase signaling shown by leading edge asymmetry of dual phosphorylated extracellular signal–regulated kinase (Zhao et al., 2002). Using cAR1-GFP–expressing cells, we monitored the dynamic distribution of receptors during electrotaxis in *Dictyostelium*. Neither obvious redistribution nor accumulation at the leading edge was observed during electrotaxis or during field polarity reversal (Fig. 4; Table S2 available at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1).  Therefore, EF-induced receptor asymmetry is a selective event, perhaps depending in part on the balance between the charge carried by the receptor and that on the membrane surface.

### Actin was polymerized at the leading edge in electrotaxing cells

Coronin is an actin binding protein important for actin reorganization in *Dictyostelium* (Gerisch et al., 1995), and coronin-GFP marks regions of intense actin polymerization. We monitored the dynamic distribution of this construct in both wild-type and β⁻ cells. Coronin-GFP accumulated at the leading edge in both cell types and reversed to the other end when the EF polarity was reversed (Fig. 5). The percentage of cells showing cathodal redistribution of coronin-GFP was assessed before the EF was switched on and at different time points after EF application and polarity reversal. Similar proportions of cells showed cathodal redistribution of coronin-GFP in both AX3 and β⁻ cells (Fig. 5 L). This suggests that although the Gβ subunit may contribute to electrotaxis (directionality data), when this was nullified, substantial asymmetry of F-actin still developed to drive electrotaxis. How the EF directs actin polymerization remains to be elucidated.

In conclusion, *Dictyostelium* cells migrated cathodally. This response remained robust in cells with null mutations of the cAR1 receptor and the Gα2 and Gβ subunits. Most importantly, the ability of null mutations in Gβ to sense and respond to an EF gradient, although compromised, contrasts markedly with the essential role of this subunit in chemotaxis. There was no asymmetry of PHcrac-GFP or of receptor cAR1-GFP in cells undergoing electrotaxis; however, actin polymerization was polarized to the leading edge as indicated by coronin-GFP in the cells migrating directionally in an EF. Therefore, electrotaxis in *Dictyostelium* does not use the signaling elements which underpin chemotaxis with the exception of partial dependency on the Gβ subunit. Thus, we have largely excluded one of the most important pathways in chemotaxis for *Dictyostelium* electrotaxis. The mechanism of transduction of the EF signal in *Dictyostelium* nonetheless remains elusive.

## Materials and methods

cAR1⁻/cAR3⁻, Gα2⁻, Gβ⁻ cells and cAR1-GFP, PHcrac-GFP, and coronin-GFP–expressing cells were as described before. Cells after starvation and cAMP pulsation for 2–6 h were seeded on coverglass within a trough. Nonadherent cells were washed off (15 min), and a roof of coverglass was applied to the chamber and sealed with silicone grease (Zhao et al., 1996).

Cell migration was analyzed with a frame interval of 30 s using Meta-Morph (Universal Imaging Corp.). Trajectories of cells were pooled to make composite graphs. The directedness of migration was assessed as cosine θ (Zhao et al., 1996), where θ is the angle between the EF vector and a straight line connecting start and end positions of a cell. A cell moving directly to the left (cathodally) would have a directedness of 1; a cell moving directly to the right (anodally) would have a directedness of –1. A value close to 0 represents random cell movement. Therefore, the average directedness of a population of cells gives an objective quantification of how directionally cells have moved. The trajectory speed is the total length traveled by the cells divided by time, and the displacement speed is the straight line distance between the start and end positions of a cell, divided by time.

Fluorescence microscopy on live cells was performed as described previously (Parent et al., 1998). Statistical analysis was performed using Student's $t$ tests. Data were mean ± SEM.

### Online supplemental material

Details of cells used are available online at http://www.jcb.org/cgi/content/full/jcb.200112070/DC1. Video 1 shows wild-type cell electrotaxis. Videos 2, 3, and 4 show electrotactic responses of cAR1⁻/cAR3⁻, Gα2⁻, and Gβ⁻ cells, respectively. Videos 5 and 6 show PHcrac-GFP and cAR1-GFP distribution in wild-type *Dictyostelium* undergoing electrotaxis. Videos 7 and 8 show coronin-GFP distribution in wild-type and Gβ⁻ cells responding to DC EF.

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