The p21-Activated Kinase (PAK) Family Member PakD Is Required for Chemorepulsion and Proliferation Inhibition by Autocrine Signals in Dictyostelium discoideum

Jonathan E. Phillips, Richard H. Gomer*

Department of Biology, Texas A&M University, College Station, Texas, United States of America

Abstract

In Dictyostelium discoideum, the secreted proteins AprA and CfaD function as reporters of cell density and regulate cell number by inhibiting proliferation at high cell densities. AprA also functions to disperse groups of cells at high density by acting as a chemorepellent. However, the signal transduction pathways associated with AprA and CfaD are not clear, and little is known about how AprA affects the cytoskeleton to regulate cell movement. We found that the p21-activated kinase (PAK) family member PakD is required for both the proliferation-inhibiting activity of AprA and CfaD and the chemorepellent activity of AprA. Similar to cells lacking AprA or CfaD, cells lacking PakD proliferate to a higher cell density than wild-type cells. Recombinant AprA and CfaD inhibit the proliferation of wild-type cells but not cells lacking PakD. Like AprA and CfaD, PakD inhibits proliferation but does not significantly affect growth (the accumulation of mass) on a per-nucleus basis. In contrast to wild-type cells, cells lacking PakD are not repelled from a source of AprA, and colonies of cells lacking PakD expand at a slower rate than wild-type cells, indicating that PakD is required for AprA-mediated chemorepulsion. A PakD-GFP fusion protein localizes to an intracellular punctum that is not the nucleus or centrosome, and PakD-GFP is also occasionally observed at the rear cortex of moving cells. Vegetative cells lacking PakD show excessive proliferation and cell movement through partially overlapping actin-based filopodia-like structures, suggesting that PakD affects actin dynamics, consistent with previously characterized roles of PAK proteins in actin regulation. Together, our results implicate PakD in AprA/CfaD signaling and show that a PAK protein is required for proper chemorepulsive cell movement in Dictyostelium.

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* E-mail: rgomer@bio.tamu.edu

Introduction

Despite substantial progress, much remains to be understood about how the size of a tissue is established during development and then subsequently maintained [1]. One mechanism of tissue size regulation involves proliferation-inhibiting factors that are secreted by, and act on, cells within a specific tissue. As the tissue size increases, levels of the factors concomitantly increase, providing negative feedback on tissue size by inhibiting proliferation in a concentration-dependent manner [2]. Such tissue specific, proliferation-inhibiting signals have been termed “chalones” [3]. Bona fide examples of chalones include myostatin, which regulates skeletal muscle size [4,5], and GDF11, which regulates neuron number in the olfactory epithelium [6,7]. In addition, some tumors and their metastases secrete and respond to such factors, establishing a dormant state when a large tumor is established [8]. Understanding these factors and how they function might provide insight on ways to induce a dormant state in tumors and how to repress the proliferation of dormant metastases following surgical removal of a primary tumor.

We found that the secreted proteins AprA and CfaD function as chalones in the social amoeba Dictyostelium discoideum [9,10]. AprA, a protein with little similarity to known human proteins, and CfaD, a member of the conserved family of cathepsin L proteases [10] are secreted by proliferating Dictyostelium cells and inhibit their proliferation [9,10]. Cells lacking either AprA or CfaD proliferate more rapidly than wild-type cells and reach a higher stationary density. The addition of recombinant AprA (rAprA) or rCfaD slows the proliferation of cells. AprA shows saturable binding to cells [11], causes GTP uptake at the cell membrane [12], and requires the G proteins Gαq and Gβγ for activity [12], suggesting that AprA signals through a G protein-coupled receptor. AprA and CfaD require each other for activity [10], and also require the kinase QkgA [13], the phosphatase CnrN [14] and the putative transcription factor BzpN for activity [15]. In addition to its proliferation-inhibiting activity, AprA, but not CfaD, acts as an autocrine chemorepellent that functions to disperse groups of cells that are at high density [16]. This chemorepellent activity requires Gαq, QkgA, and CnrN but not BzpN, suggesting that AprA affects proliferation and cell movement through partially overlapping pathways.

p21-activated kinases (PAKs) are a conserved family of kinases that bind to and are activated by small GTPases such as Rac and...
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cdc42 [17]. PAKs function to regulate actin dynamics in processes such as bud growth in *Saccharomyces cerevisiae* [18], growth cone guidance in developing *Drosophila* neurons [19] and chemotaxis towards cAMP in *Dictyostelium* [20,21,22]. Pak1 induces the formation of filopodia and membrane ruffles in human fibroblasts [23], whereas *Drosophila* Pak3 inhibits lamellipodia formation in cell culture [24], indicating that PAKs can positively or negatively regulate actin-based structures. PAKs also regulate proliferation [17]. In COS-1 fibroblasts, Pak1 stimulates mitogenic MAP kinase signaling [25] and in human fibroblasts, Pak2 inhibits the tumor suppressor NF2 by phosphorylation, resulting in an increase in proliferation [26]. In contrast, *Xenopus* Pak1 acts to arrest cells at mitotic metaphase during embryogenesis [27], and *Xenopus* Pak3 arrests the cell cycle and promotes neuron differentiation during neurogenesis [28]. These results indicate that depending on the context, PAKs can promote or inhibit proliferation.

PakD is a putative *Dictyostelium* PAK kinase that is involved in the regulation of F-actin during development [22]. PakD is required for aggregation during development and is required for a normal actin polymerization response to the chemorepellant cAMP. In starved cells, PakD localizes to cell extensions and to subcellular punctum structures [22]. In this report, we show that PakD negatively regulates proliferation during vegetative growth. At low cell densities, pakD− cells proliferate at the same rate as wild-type cells, but pakD+ cells reach a higher maximum cell density than wild-type cells. PakD is required for the proliferation-inhibiting activity of both AprA and CfaD. Further, PakD is required for the chemorepellent effect of AprA, and pakD− cells show an increase in the size of filopodia, suggesting a role for PakD in the regulation of actin dynamics. Our data suggest that PakD is a regulator of proliferation and cell movement that functions downstream of AprA and CfaD.

**Materials and Methods**

The strains Ax2 (wild-type), pakD [22], pakD / act15::PakD-GFP, act15::PakD-GFP, and plc (DBS0236793, [29]) were grown in axenic shaking culture as described previously [16]. Proliferation curves, rAprA and rCfaD inhibition assays, measurement of mass, protein, and nuclei per cell, measurement of colony diameter on bacterial lawns, and measurement of proliferation on bacterial lawns were done as described previously [13]. Measurement of AprA and CfaD in conditioned media was done as described previously [13], except that conditioned media was collected from cells at a density of 1 × 10^7 cells/ml. Chemorepellent assays were done as previously described [16]. The data for wild-type response to the chemorepellent activity of rAprA is identical to that published previously [16], as the previously reported data and the data presented in this paper were generated concurrently.

To construct a PakD-GFP transgene, two partially overlapping fragments of the PakD open reading frame were amplified by PCR from vegetative stage *Dictyostelium* cDNA using the primer pairs GAGGTCTCATGAGTAGATTACAACCTCAAAAGAAG, GCACTTTTGAATCCCGCAACTTGC and GGAAGATCTATGAGTAGATTACAACCTCAACAAC. Both gene fragments contain an overlapping region of the PakD gene with a BglII site immediately following the codon encoding the terminal amino acid and thus replacing the stop codon. Respectively, these primer pairs generate a 5′ PakD gene fragment with a BglII site preceding the start codon, and a 3′ PakD gene fragment with a BglII site immediately following the codon encoding the terminal amino acid and thus replacing the stop codon. Both gene fragments contain an overlapping region of the

| Genotype | Doubling time, hours | Maximum observed cell density, 10^6 cells/ml |
|----------|----------------------|---------------------------------------------|
| Wild type | 13.6±0.3             | 24.3±0.7                                    |
| pakD−    | 12.0±0.6             | 43.6±2.7***                                |
| pakD−/act15::PakD-GFP | 13.7±0.6            | 29.3±1.3                                   |

Doubling times and stationary densities were calculated for the proliferation curves in Figure 1. Values are mean ± SEM from three independent experiments. ‘‘***’’ indicates that the difference between the value and the wild-type value is significant with p<0.001 (one-way ANOVA, Tukey’s test). The difference between the maximum observed density between pakD− and pakD/pakD-GFP is significant (p<0.01, Tukey’s test). doi:10.1371/journal.pone.0096633.g001

*Figure 1. Cells lacking PakD proliferate to a higher density than wild-type cells. (A) Cells were inoculated into axenic shaking culture at 1 × 10^6 cells/ml and counted daily. (B) The data from days 1–5 were plotted using a log scale. Values are mean ± SEM, n=3. The absence of error bars indicates that the SEM is smaller than the plot symbol. doi:10.1371/journal.pone.0096633.g001*
PakD gene that contains a SpeI site. These two fragments were independently cloned into pGEM-T Easy vectors (Promega, Madison, Wisconsin). The cloned gene fragments were then digested with BglII and SpeI, gel purified, and used in a three-part ligation with the extrachromosomal vector pDM323 [30] digested with BglII, resulting in a vector encoding a complete PakD gene with GFP fused to the C-terminus. Correct sequence and proper gene orientation in the vector were confirmed with DNA sequencing of the entire PakD gene and the restriction enzyme site junctions. This extrachromosomal vector was then transformed into Dictyostelium cells using standard electroporation protocols [31].

To image PakD-GFP localization by deconvolution microscopy, spots of actin15::PakD-GFP cells were grown in a 1.5 ml volume of HL5 in 2-well glass chamber slides (Nunc) overnight, and cells were subsequently fixed and stained with DAPI as described previously [15]. Cells were then imaged using an Olympus FV1000 microscope with a 100×1.2 NA objective, and image z-stacks were generated with a slice separation of 0.2 microns. Z-stacks were then processed using Autodeblur deconvolution software (Bitplane software, Zurich, Switzerland). To stain cells with Alexa Fluor 594 Phalloidin (Invitrogen, Carlsbad, CA), cells were fixed as described above and then stained with phalloidin as previously described [32]. To label the centrosome in cells expressing PakD-GFP, spots of actin15::PakD-GFP cells were grown in glass chamber slides overnight, and cells were then fixed for 30 minutes with 4% paraformaldehyde in PHEM buffer (30 mM Na-PIPES, 12.5 mM HEPES, 5 mM EGTA, 1 mM MgCl2, pH 6.9 [33]). Cells were washed three times in PBS and permeabilized in PBS with 0.1% NP-40 for 10 minutes. Cells were then stained with anti-DdCP224 antibodies as previously described [34]. Cells were then mounted in Vectashield mounting media with DAPI (Vector, Burlingame, CA) and imaged as described above. To image PakD-GFP in live cells, spots of pakD–/act15::PakD-GFP cells were grown in 2-well glass chamber slides (Nunc) overnight in FM media (Formedium, Norwich, UK). Cells were then imaged using an Olympus FV1000 confocal microscope with a 100× objective by time-lapse microscopy. All statistical analyses were done with Prism (GraphPad Software, San Diego, CA). Significance was defined as a p value of <0.05.

Results

PakD negatively regulates cell proliferation
Kinases of the p21-activated kinase (Pak) family are involved in signal transduction pathways regulating processes such as cell motility [24] and proliferation [27]. PakD is a 190 kDa PAK protein in Dictyostelium with putative kinase, diacylglycerol-binding, Cdc42/Rac interactive binding (CRIB), and calponin-homology.
Table 2. The effect of PakD on the mass, protein, and nuclei content of cells.

| Genotype          | Mass (mg) | Protein (mg) | Nuclei/100 cells | Per 10^7 cells | % cells with nuclei |
|-------------------|-----------|--------------|------------------|----------------|---------------------|
| Wild type         | 6.1       | 0.3          | 1                | 1              | 2                   |
| pakD−/act15::PakD-GFP | 5.6       | 0.6          | 3                | 3              | 5                   |
| pakD−/act15::PakD-GFP | 5.3       | 0.5          | 2                | 2              | 3                   |
| pakD−/act15::PakD-GFP | 5.6       | 0.6          | 3                | 3              | 5                   |

Mass and protein content were determined as described in the Materials and Methods. Values are the mean ± SEM from three or more independent experiments. Values for pakD−/act15::PakD-GFP were not significantly different from wild-type values or each other for any parameter shown (p > 0.05, one-way ANOVA, Tukey’s test).

 PakD Regulates Chemorepulsion and Proliferation

As AprA and CfaD are extracellular signals that slow proliferation and increase in concentration at high cell density, one explanation for an increased proliferation phenotype is a lack of extracellular AprA or CfaD. To test this possibility, we examined the levels of AprA and CfaD in high-density conditioned media from pakD− cells by Western blots. pakD− and pakD+/act15::PakD-GFP cells showed extracellular accumulation of AprA and CfaD similar to or higher than that of wild type (Figure 2). These results strongly suggest that the high cell density of pakD− cells is not due to a lack of AprA or CfaD.

Recombinant AprA (rAprA) and CfaD (rCfaD) slow the proliferation of wild-type cells [9,10]. To determine whether PakD plays a role in AprA- and/or CfaD-mediated inhibition of proliferation, we examined the effects of rAprA and rCfaD on pakD− cells. As observed previously, wild-type cells showed reduced proliferation in response to rAprA and rCfaD (Figure 3A). In contrast, pakD− cells showed no significant reduction in proliferation in the presence of either rAprA or rCfaD. AprA and CfaD slowed the proliferation of pakD+/act15::PakD-GFP cells, showing that the insensitivity of the pakD− cells is due specifically to the absence of the pakD gene. As PakD has a predicted diacylglycerol (DAG)-binding domain [36], and phospholipase C (PLC) enzymatically generates DAG, we tested whether PLC might signal through PakD to inhibit proliferation. The proliferation of plc− cells was inhibited by rAprA and rCfaD to the same degree as wild-type cells [Figure 3B], indicating that AprA and CfaD do not signal through PLC to affect PakD. Together, these results indicate that PakD is a negative regulator of proliferation and that PakD is necessary for proliferation inhibition by AprA and CfaD.

pakD− cells show normal nuclei, mass and protein content per cell during logarithmic growth

aprA− and cfaD− cells have more nuclei per cell than wild-type cells during vegetative growth, which may be due to an increased mitotic rate [9,10]. We examined the nuclei content of log-phase pakD− cells by DAPI staining, and found that there was no significant difference as compared to wild type (Table 2). These results indicate that PakD does not affect the multinuclearity of cells, and suggest that AprA and CfaD affect cellular nuclei content in a manner independent of PakD.

Cell proliferation (the increase in cell number) and cell growth (the accumulation of mass) can be regulated independently [37]. AprA and CfaD regulate cell proliferation, but not growth on a per nucleus basis [9,10]. To determine whether PakD affects cell mass
or cell growth, we first measured the mass and protein content of cells. During exponential growth, wild-type cells showed mass and protein values like those seen previously (Table 2; [13]). pakD− cells and pakD−/act15::PakD-GFP showed mass and protein content per cell and per nucleus that were not significantly different than wild-type values, indicating that PakD does not affect mass or protein content. We then estimated growth by dividing the mass and protein values by the measured doubling times during exponential growth to calculate the mass and protein accumulation per hour. Wild-type values for mass and protein accumulation were similar to those seen previously [13], and the values for mass and protein accumulation were not significantly different between wild-type, pakD−, and pakD−/act15::PakD-GFP strains on either a per cell or per nucleus basis (Table 3). These results indicate that PakD does not affect the mass or protein accumulation of cells undergoing exponential growth.

### PakD localizes to a punctum within cells

We examined the subcellular localization of PakD by imaging vegetative cells expressing a PakD-GFP fusion protein. PakD-GFP localized to a punctum within the cell (Figure 4), and there tended to be one structure per cell, although occasionally more than one PakD spot per cell was observed. A similar localization of the endogenous PakD protein in vegetative cells has been seen by immunofluorescence with an antibody raised against PakD (Derrick Brazil, unpublished observations), although in starved cells PakD also localizes to cell protrusions [22]. When examining PakD-GFP in live, motile cells, a similar punctum was seen, and occasionally localization at the rear cortex of the moving cell was observed (arrow, Figure 4B and Movie S1), suggesting a role in cell movement. PakD-GFP did not co-localize with the nucleus (Figure 4A), or with anti-DdCP224 staining, a marker for the centrosome (Figure 4C, [34]). No change in PakD-GFP localization was seen in cells at high density or in cells incubated with high-density conditioned media (data not shown), suggesting that PakD localization is not altered in response to high AprA or CfaD levels. These results indicate that PakD localizes to a punctum that is not the nucleus or centrosome, and that the localization of PakD may be polarized during cell movement.

### PakD affects the ability of colonies to spread and the response of cells to the chemorepellent AprA

AprA is an autocrine chemorepellent in vegetative Dictyostelium cells [16] that may facilitate the spreading out of dense groups of cells. Consistent with this model, colonies of cells lacking AprA show a reduced ability to spread on bacterial lawns [13] despite the fact that apa+ cells proliferate rapidly under these conditions [9]. To examine whether PakD may be involved in this process, we examined the size of pakD− colonies on bacterial lawns. Wild-type colonies showed a rate of expansion similar to what we observed previously [13], whereas pakD− cells showed a significantly reduced rate of expansion (Figure 5A), suggesting that PakD functions in the expansion of colonies. We then tested whether pakD− cells, like wild type cells, show directed movement away from areas of high cell density [16] by tracking cell movement at the edge of a cell colony. Under these conditions,

| Genotype                  | Per 10^7 cells per hour | Per 10^7 nuclei per hour |
|--------------------------|-------------------------|--------------------------|
|                          | Mass (mg) | Protein (μg) | Nuclei, \( \times 10^5 \) | Mass (mg) | Protein (μg) |
| Wild type                | 0.45±0.03 | 30±2         | 9.9±0.3                 | 0.33±0.02 | 22±2         |
| pakD−                    | 0.44±0.04 | 30±2         | 10.9±0.6                | 0.34±0.03 | 23±2         |
| pakD−/act15::PakD-GFP    | 0.41±0.06 | 31±3         | 8.9±0.6                 | 0.33±0.05 | 25±3         |

Table 3. The effect of PakD on the mass and protein accumulation of cells.

Mass and protein values from Table 2 were divided by the observed doubling time of the respective genotype. Doubling times were calculated as described in Materials and Methods. Values are the mean ± SEM from three or more independent experiments. Values for pakD− or pakD−/act15::PakD-GFP were not significantly different from wild-type values or each other for any parameter shown (p>0.05, one-way ANOVA, Tukey’s test). doi:10.1371/journal.pone.0096633.t003

Figure 4. PakD tends to localize to a single punctum or at the rear of motile cells. (A) act15::PakD-GFP cells were grown in glass chamber slides overnight, then fixed and stained with DAPI (blue), imaged by fluorescence microscopy using a 100× objective, and processed using Autodeblur deconvolution software (Bitplane software, Zurich, Switzerland). PakD-GFP signal (green) is shown superimposed on a transmitted light image. Bar is 10 μm. (B) Live pakD−/act15::PakD-GFP cells grown as a colony in glass chamber slides were imaged by fluorescence microscopy. The imaged cell is moving upwards, as seen by time-lapse microscopy. The arrow indicates PakD-GFP at the rear of the cell. A single punctum structure is also visible. The cell border is shown with a dashed line. Bar is 10 μm. (C) PakD-GFP (green) does not colocalize with either the cell nucleus (blue) or with staining for an antibody against DdCP224 (Red), a marker for the centrosome. Bar is 5 μm.

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Table 4. The effect of PakD on the movement of cells at the edge of colonies.

|                      | Cell speed (µm/minute) | Forward migration index |
|----------------------|------------------------|-------------------------|
| Wild type            | 6.7 ± 0.6              | 0.23 ± 0.03             |
| pakD−                | 6.3 ± 0.2              | 0.08 ± 0.03**           |
| pakD−/act15::PakD-GFP | 4.6 ± 0.2***          | 0.27 ± 0.03             |

Colonies of cells were established on glass chamber slides in HS5 media and, following a one-hour incubation at room temperature, cells at the edge of the colony were filmed. Videos were used to track cell movement, and the tracking data was used to calculate the given values. At least 10 randomly selected cells from each of 3 independent experiments were tracked. A positive forward migration index indicates directed movement away from the colony. "***" indicates p<0.001, and "**" indicates p<0.01 compared to wild type (One-way ANOVA, Tukey's test).

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cells have longer filopodia (Figure 7C) and more filopodia per cell (Figure 7D) than wild-type cells. These results suggest that PakD negatively regulates the formation of filopodia in vegetative cells.

Discussion

The mechanisms by which chalones act to slow proliferation and thus regulate tissue size are poorly understood. Our data indicate that the kinase PakD is a negative regulator of proliferation and is necessary for the activity of the chalones AprA and CfaD, indicating that PakD is involved in chalone signaling. Like aprA− and cfaD− cells, pakD− cells accumulate mass and protein on a per nucleus basis during exponential growth at a rate like that of wild type, indicating that PakD regulates proliferation but does not significantly affect cell growth. However, pakD− cells differ in some ways from aprA− or cfaD− cells. First, whereas aprA− and cfaD− cells show rapid proliferation even at lower densities [9,10], pakD− cells only show a difference in proliferation as compared to wild type at high densities. Second, aprA− and cfaD− cells are multinucleate, whereas pakD− cells are not. Therefore, PakD likely mediates some but not all of the affects of AprA and CfaD. As pakD− cells proliferate like wild type at lower

![Image](image1.png)

**Figure 6. PakD affects the proliferation of cells on bacterial lawns.** 1000 cells were spread with bacteria on SM/5 plates, and at the indicated times Dictyostelium cells were washed off the plate and counted. Values are mean ± SEM, with n = 3. *** indicates that p<0.01 (One-way ANOVA, Tukey’s test). At 72 hours, the difference between wild-type and pakD−/PakD-GFP is not significant.

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![Image](image2.png)

**Figure 7. pakD− cells show enlarged filopodia.** (A) Live vegetative cells in submerged culture were imaged using an inverted microscope with a 60× objective. Bar is 10 μm. (B) Cells were grown in chamber slides in HL5 media overnight and were then fixed and stained with Alexa Fluor 594 phalloidin. Expression of PakD-GFP in the shown pakD−/act15::PakD-GFP cell was confirmed by the presence of a punctum PakD-GFP signal (data not shown). Bar is 10 μm. (C) The filopodium length was measured for all filopodia on at least 30 cells per condition. *** indicates p<0.001 (t-test). (D) The average number of filopodia per cell visible in a single focal plane was measured by counting filopodia on at least 30 cells per condition. * indicates p<0.05 (t-test).

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densities, some separate branch of the signal transduction pathway must mediate the effects of AprA and CfaD for these lower density cells. This branch may involve the transcription factor BzpN, which is necessary for AprA/CfaD signaling and affects proliferation primarily at lower cell densities [15].

We found that PakD-GFP shows a punctum-like subcellular localization that tends to localize in a single spot, and that this structure is not the centrosome or part of the nucleus. This punctum structure is likely not an artifact of GFP fusion, as the native PakD protein shows a similar punctum localization in both starved and vegetative cells [22]. PakD thus may be part of the Golgi or an uncharacterized subcellular structure. Alternatively, PakD may form endogenous, functioning self-aggregates, analogous to proteins such as CPEB [39] or Orb2 [40]. In starved cells, some PakD appears to be associated with filopodia [22]. We did not observe PakD-GFP in filopodia in vegetative cells, suggesting that some of the PakD localization changes during differentiation.

PAPS are predominantly considered promoters and not inhibitors of cell growth and proliferation and are considered oncogenic in some circumstances [41]. However, PAKs function to inhibit proliferation during Xenopus development [27,28]. Our data suggests that PAKs also show antiproliferative activity in Dictyostelium, suggesting conservation of this function. It may therefore be useful to determine whether any human PAKs show a similar function, and whether Pak activity is affected by autocrine signaling. As PAKs are regulated by Rho/Rac/cdc42-type GTPases, it will be interesting in the future to test whether such proteins negatively regulate proliferation in vertebrates or in Dictyostelium, which has several Rho GTPase orthologs [42].

Apart from the proliferation-inhibiting activity of PakD, we also found that PakD is necessary for the chemorepellent activity of AprA, but does not affect the average speed of cells. Further, PakD is involved in the negative regulation of actin-based structures at the cell periphery. One appealing model for chemorepulsion consists of the recruitment of active PakD to subcellular areas of high AprA signaling due to an AprA gradient. This polarized PakD activity could potentially inhibit the development of actin-based structures in directions corresponding to high AprA levels, inhibiting movement up an AprA gradient, and thus potentiating movement down an AprA gradient. This model is supported by our observation of PakD-GFP at the rear of cells, but further studies are required to test this model more rigorously.

Much remains to be understood about how endogenous chemorepellent functions in eukaryotic cells. We have shown that PAKs, which have been found to play a role in Semaphorin-mediated chemorepulsion during axonal guidance in vertebrates [43], are also necessary for chemorepulsion in Dictyostelium. A better understanding of chemorepulsive processes could be useful for resolving inflammation [44] or for preventing the dispersion and metastasis of tumors [45]. The conservation of Pak function between Dictyostelium and metazoans suggests that further study of AprA-mediated chemorepulsion could reveal important, uncharacterized regulators of chemorepulsive processes.

Supporting Information

Movie S1 PakD-GFP localization in a motile cell. pakD / actin::GFP pakD-GFP cells were imaged as described in Figure 4B over a period of 5 minutes using time lapse microscopy.

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Author Contributions

Conceived and designed the experiments: JEP RHG. Performed the experiments: JEP. Analyzed the data: JEP RHG. Wrote the paper: JEP RHG.

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