Chemoattractant-induced Ras activation during *Dictyostelium* aggregation

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Ras proteins are highly conserved molecular switches that regulate cellular response to external stimuli. *Dictyostelium discoideum* contains an extensive family of Ras proteins that function in regulation of mitosis, cytoskeletal function and motility, and the onset of development. Little is known about the events that lead to the activation of Ras proteins in *Dictyostelium*, primarily owing to a lack of a biochemical assay to measure the levels of activated Ras. We have adapted an assay, used successfully to measure activated Ras in mammalian cells, to monitor activation of two *Dictyostelium* Ras proteins, RasC and RasG. We have found that the Ras-binding domain (RBD) of mammalian Raf1 was capable of binding to the activated form of RasG, but not to the activated form of RasC; however, the RBD of *Schizosaccharomyces pombe* Byr2 was capable of binding preferentially to the activated forms of both RasC and RasG. Using this assay, we discovered that RasC and RasG showed a rapid and transient activation when aggregation-competent cells were stimulated with the chemoattractant cAMP, and this activation did not occur in a number of cAMP signalling mutants. These data provide further evidence of a role for both RasC and RasG in the early development of *Dictyostelium*.

Keywords: *Dictyostelium*; Ras; cAMP

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INTRODUCTION

The Ras subfamily consists of small, monomeric GTPases that act as molecular switches in cellular signalling events. They perform their regulatory function by cycling between two conformations, a GDP-bound 'OFF' state and a GTP-bound 'ON' state. Extracellular signals stimulate guanine nucleotide exchange factors (GEFs) that catalyse the exchange of GDP for GTP, thereby activating the Ras protein, and the subsequent inactivation is due to an enhancement of the intrinsic GTPase activity, which is stimulated by the GTPase-activating proteins (GAPs) (Boguski & McCormick, 1993). Ras activation regulates a number of cellular events such as

proliferation, differentiation and cytoskeletal rearrangement, and the maintenance of proper Ras function is critical. For example, its uncontrolled activation contributes to anchorage-independent growth and tumorigenesis in mammalian cells (Tokumitsu *et al*, 1998).

Whereas biochemical analyses are clearly highly informative, the targeted generation of knockout strains in organisms such as *Dictyostelium* renders an alternative, complementary approach. Furthermore, the unique biology of *Dictyostelium* allows growth and differentiation to be studied as separate phenomena. Vegetative *Dictyostelium* are single-celled amoebae that feed on bacteria by phagocytosis, or in liquid media via fluid-phase endocytosis. Upon nutrient depletion, *Dictyostelium* undergoes a complex, tightly regulated development process, beginning with the secretion of, and movement towards, cAMP, resulting in an aggregation of $\sim 10^5$ cells. The aggregate then elongates to become a motile slug, and cells within the slug differentiate into spatially segregated prespore and prestalk cells. Upon culmination, the fully differentiated stalk cells hold up a sorus of viable spores, which germinate into free-living amoebae (reviewed in Kessin, 2001).

Six *Dictyostelium* Ras subfamily proteins have been previously described, with at least two additional members identified by the *Dictyostelium discoideum* genome sequencing projects (Weeks & Spiegelman, 2003). Whereas such a large number of related proteins imply functional redundancy, gene deletions or temporal gene expression profiles indicate that each Ras protein serves at least some unique functions. For example, although *rasB*, *rasG* and *rasS* all have maximal gene expression during vegetative growth, RasG regulates aspects of motility and the cytoskeleton (Tuxworth *et al*, 1997), RasS is involved in phagocytosis (Chubb *et al*, 2000) and RasB appears to be linked to the progression through the cell cycle (Sutherland *et al*, 2001). In contrast, RasC is important for early development, as *rasC*[−] cells do not aggregate upon starvation, primarily because they are unable to relay the cAMP signal, even though they are capable of responding to cAMP (Lim *et al*, 2001).

Stimulation of aggregation-competent *Dictyostelium* cells with cAMP initiates a plethora of cellular activities, including the activation of phosphatidylinositol-3-OH kinases (PI(3)Ks) (Huang *et al*, 2003). PI(3)K activity produces elevated levels of the lipid PtdIns(3,4,5)P₃, providing docking sites for PH domain-containing proteins, such as Akt/PKB (referred to as PKB henceforth), that facilitate their activation. Evidence that a Ras protein is involved in

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this process include the following: (1) deletion of the genes encoding RasC, RasG the *Dictyostelium* RasGEF AleA results in a reduced activation of PKB when stimulated with cAMP (Lim *et al*, 2001; C.J.L., unpublished observations); (2) *Dictyostelium* PI(3)K1 and PI(3)K2, which are related to the mammalian p110 α PI(3)Ks, contain Ras-binding domains (RBDs), and it has been demonstrated that these RBDs are capable of binding to activated Ras in a two-hybrid assay (Funamoto *et al*, 2002); and (3) *pi3k1*[−]/*pi3k2*[−] cells that express ectopic PI(3)K1 are able to restore cAMP-stimulated PKB activation, but cells expressing ectopic PI(3)K1 with a mutation in the RBD that abolishes Ras binding are unable to do so (Funamoto *et al*, 2002).

An important piece of evidence needed to corroborate the link between Ras activation and PI(3)K is to show that Ras is activated when cells are stimulated with cAMP, but this has yet to be experimentally demonstrated in *Dictyostelium*. Ras activation in mammalian cells has been measured by using the ability of the RBD of Raf1, a known downstream effector of Ras, to bind preferentially to the activated form of Ras (Taylor & Shalloway, 1996). In this report, we describe the development of an RBD binding assay for use in studying Ras activation in *Dictyostelium*, and we have used this assay to confirm that RasC is activated in response to cAMP stimulation. In the course of these studies, we found that RasG is also activated in response to cAMP.

RESULTS

An assay for activated Ras proteins in *Dictyostelium*

We initially tested the ability of the RBD of Raf1 to bind to activated RasC in lysates of *Dictyostelium* cells expressing activated RasC (G13T), the equivalent of the mammalian H-Ras (G12T)-activating mutation, but we detected no significant binding (Fig 1A). Similar results were obtained using the RBD of RalGDS (Fig 1A), an RBD that we previously had found to be effective for detecting activated *Dictyostelium* Rap1 (Kang *et al*, 2002). Despite their inability to bind activated RasC effectively, both RBDs were able to bind activated RasG (G12T) (Fig 1A).

In view of the possible link between RasC and PI(3)K, we tested the ability of the RBDs of PI(3)K1 and PI(3)K2 to bind to RasC (G13T). Although Fig 1A shows that the PI(3)K1 and PI(3)K2 RBDs were able to bind activated RasC, the small amounts of the fusion proteins expressed in bacteria (data not shown) rendered them insufficient for use in a practical assay. We also found that both the PI(3)K1 and PI(3)K2 RBDs were able to bind activated RasG (Fig 1A) even at low RBD input, and RasG showed a greater affinity for PI(3)K1 RBD than for PI(3)K2 RBD under the conditions of this assay, supporting previous yeast two-hybrid assay data (Funamoto *et al*, 2002).

Although RasC has very high overall identity to mammalian H-Ras, its effector domain, the region that interacts with the downstream effectors, contains a single amino-acid difference at position 39 (the equivalent of position 38 in H-Ras): asparagine instead of aspartate. A substitution (D38N) in the effector domain of H-Ras had been shown to abrogate binding to its effector proteins (Akasaka *et al*, 1996), and this may explain the inability of either Raf1 or RalGDS to bind to activated RasC. However, because the *Schizosaccharomyces pombe* Ras effector Byr2 had been shown to bind H-Ras containing the D38N substitution (Akasaka *et al*, 1996), we postulated that the Byr2 RBD might bind activated RasC. The data shown in Fig 1A indicate that this was

Fig 1 | RBD binding assay. (A) The RBDs of Raf1, RalGDS, Byr2, PI(3)K1 and PI(3)K2, as well as a GST-only control, were incubated with lysates from cells expressing either activated RasG (G12T) or RasC (G13T) and the bound Ras proteins were analysed by western blot using either RasG or RasC antibodies. (B) Wild-type cell lysates were incubated with either 1 mM GTP- γ S or 1 mM GDP for 1 h at 22 °C as indicated, and then incubated with the RBDs of Raf1, RalGDS or Byr2. Bound RasG was detected by western blot analysis, determined using the RasG antibody. (C) *rasC*[−]:*rasC*[−] cell lysates were incubated with either GTP- γ S or GDP as above, and the levels of bound RasC were determined using the RasC antibody. (D) Byr2 RBD was incubated with the indicated amounts of total protein from *rasC*[−]:*rasC*[−] cell lysates and the bound RasC was determined using the RasC antibody.

indeed true. In addition, Byr2 was also able to bind activated RasG in cell lysates (Fig 1A).

To determine whether the RBDs were specific for the GTP-bound forms of Ras, lysates from wild-type Ax2 cells were incubated with either GTP- γ S, a nonhydrolysable analogue of GTP, or GDP before incubating with the glutathione *S*-transferase (GST)–RBD. All of the tested RBDs were able to bind efficiently RasG from Ax2 lysates incubated with GTP- γ S, but only very low levels of RasG from lysates incubated with GDP (Fig 1B). However, although the Byr2 RBD bound overexpressed activated RasC (G13T), there was no binding to RasC when Ax2 lysates were incubated with GTP- γ S (data not shown), suggesting that there was insufficient RasC present in vegetative cell extracts to be detected

with the Byr2 RBD assay. To try to circumvent this problem, cells overexpressing RasC, expressed in a *rasC*[−] background (referred to as '*rasC*[−]::*rasC*' henceforth) were used. Byr2 bound RasC in lysates of *rasC*[−]::*rasC* cells incubated with GTP-γS, but not in lysates incubated with GDP (Fig 1C), indicating that in these cells there was sufficient RasC to be able to detect the activated form of the protein.

Finally, we loaded increasing amounts of lysate from *rasC*[−]::*rasC* cells with the Byr2 RBD to examine the dose response and reproducibility of the assay. Relatively small changes in activated RasC could be detected, and the same amounts of bound RasC were detected in replicate samples of the lysate (Fig 1D). Similar results were observed for RasG (data not shown). These studies indicate that the Byr2 RBD can be used reproducibly to measure alterations in the levels of both activated RasC and RasG.

Activation of RasC in response to cAMP

As the RBD binding assay was insufficiently sensitive to detect RasC-GTP in Ax2 cells (see above), the possible activation of RasC in response to cAMP was determined in *rasC*[−]::*rasC* cells. These cells were made aggregation-competent by pulsing with 50 nM cAMP for 5 h (referred to as 'pulsed' cells henceforth); they were then stimulated with 200 nM cAMP, and aliquots were taken at the time points indicated. An increase in activated RasC was observed at 5 s after stimulation, with a return to the basal level by 45 s (Fig 2A). The kinetics of activation were similar to that of cAMP-stimulated PI(3)K activation (Huang *et al*, 2003), consistent with the notion that RasC is involved in the regulation of PI(3)K activity. There was a relatively high basal level of RasC-GTP in unstimulated cells, which may be the result of RasC overexpression. Spot densitometry was performed on the blots from four independent experiments, and this revealed an average increase of 1.8 ± 0.3 -fold upon cAMP stimulation, ranging from a minimum value of 1.5-fold to a maximum value of 2.2-fold.

RasG is also activated in response to cAMP

As the RBD binding assay could also be used to measure RasG activation, the levels of activated RasG were determined in response to cAMP stimulation. Pulsed Ax2 cells were stimulated with 200 nM cAMP, and aliquots were taken at the time points

Fig 2 | cAMP-stimulated Ras activation. (A) Pulsed *rasC*[−]::*rasC* cells were stimulated with 200 nM cAMP, and the amount of RasC protein bound to Byr2 was determined at the time points indicated. The experiment is representative of four separate experiments. (B) Pulsed Ax2 cells were stimulated with 200 nM cAMP, and the amount of RasG protein bound to Byr2 was determined at the time points indicated. The experiment is representative of four separate experiments.

indicated. An increase of RasG-GTP was also detected within 5 s of stimulation, with levels returning to the prestimulation value by 90 s (Fig 2B). Although the initial kinetics of activation were similar to those for RasC, activated RasG was undetectable before cAMP stimulation, and the extent of RasG activation was therefore greater than that observed for RasC. In the experiment shown in Fig 2, there was an initial drop in the level of activated RasG after 5 s, followed by a second increase that reached a peak at 60 s. Although a second peak of RasG-GTP was consistently observed, the time at which the maximum level occurred varied from 45 to 60 s in different experiments.

Ras activation in G-protein signalling mutants

We measured the kinetics of Ras activation in three mutant strains deficient in cAMP signalling: *cAR1*[−]/*cAR3*[−], *gβ*[−] and *gx2*[−]. As these mutants are in an Ax3 background, we also studied RasC and RasG activation in Ax3. RasC was overexpressed in all four strains (designated *Ax3*::*rasC*, *cAR*[−]::*rasC*, *gβ*[−]::*rasC* and *gx2*[−]::*rasC*) to provide sufficient RasC protein to enable us to measure RasC activation. *Ax3*::*rasC* showed the same rapid activation of both RasC and RasG in response to cAMP as the Ax2-derived strain (Fig 3), except that the RasC activation was more pronounced in Ax3 than it was in Ax2. Importantly, there was no increase in activated RasC or RasG upon cAMP stimulation in the three cAMP signalling mutants. However, all three mutants exhibited a higher basal level of activated RasC and RasG (Fig 3), suggesting a negative regulatory role for the intact G-protein-coupled receptor complex in Ras activation.

Fig 3 | The Byr2 RBD binding assay was performed as described in pulsed *Ax3*::*rasC*, *cAR*[−]::*rasC*, *gβ*[−]::*rasC* and *gx2*[−]::*rasC* cells, and levels of RasC (A) and RasG (B) activation were monitored at the time points indicated. These data are representative of three separate experiments.

DISCUSSION

To develop an assay to detect activated Ras proteins in *Dictyostelium* cells, we initially tested the ability of the RBDs of the mammalian Ras effectors Raf1 and RalGDS to bind activated *Dictyostelium* Ras proteins. Although these RBDs were able to bind activated RasG (Fig 1) and RasB (data not shown) effectively, they were unable to bind activated RasC, presumably owing to an amino-acid difference in its effector domain, relative to H-Ras, RasG and RasB. We next tested the ability of the RBDs of *Dictyostelium* PI(3)K1 and PI(3)K2 to bind activated RasC, because a role for RasC in PI(3)K activation had been suggested by earlier studies (Lim *et al*, 2001). These RBDs bound activated RasC and activated RasG; however, the amounts of bacterially expressed PI(3)K1 (RBD) and PI(3)K2 (RBD) were too small to prove useful for use in an activation assay. In contrast, we found that the RBD of *S. pombe* Byr2 was extremely effective in binding activated RasC and RasG, providing an assay for the detection of both Ras proteins. Finally, we were unable to detect activated RasC in Ax2 cells, but were able to circumvent this problem by overexpressing RasC in *rasC* null cells (Fig 1C).

Using this assay, it was shown that RasC was activated in aggregation-competent cells in response to cAMP (Fig 2A). The rapid kinetics of this activation were similar to those for the cAMP-dependent activation of PI(3)K and PKB in aggregation-competent cells, consistent with the idea that RasC has a role in the activation of PI(3)K (Meili *et al*, 1999; Lim *et al*, 2001; Huang *et al*, 2003). However, it must be noted that there was a large amount of activated RasC in the unstimulated aggregation-competent cells (Fig 2A), and while the kinetics of the activation were very similar, the extent of the activation was therefore not as dramatic as that observed for PI(3)K and PKB. As the overexpression of RasC in the *rasC*[−]::*rasC* cells is able to rescue the aggregation defect of *rasC*[−] cells and the cells appear to be normal in all other respects (Lim *et al*, 2001), the presence of activated RasC in unstimulated cells does not appear to be deleterious, although the possibility that there may be signalling implications downstream of RasC must not be overlooked.

We have also demonstrated the activation of RasG in response to cAMP, and in this case both the kinetics and extent of activation were similar to the activation of PI(3)K and PKB in response to cAMP. Although *rasC*[−] cells are capable of aggregation, development is delayed (Tuxworth *et al*, 1997) and RasG may serve a function during early development. Furthermore, cAMP stimulation of PKB phosphorylation is reduced in *rasG*[−] cells (our unpublished observations) and the overexpression of activated RasG during growth blocks aggregation (Khosla *et al*, 1996).

There was no change in the levels of activated Ras following cAMP stimulation in mutant strains disrupted in the genes encoding the aggregation-stage cAMP receptors (*cAR1*[−]/*cAR3*[−]) and components of the associated heterotrimeric G-protein complex (*gβ*[−] and *gα2*[−]). However, there was an elevated level of both activated RasC and RasG before stimulation. This suggests that the cAMP receptor complex may serve to downregulate Ras activation.

As both *rasC*[−] and *rasG*[−] cells exhibit reduced cAMP-stimulated PKB activation, it is possible that both RasC and RasG are involved in stimulating PI(3)K activity. Although the presence of both Ras proteins is clearly required for optimal PKB activation, both null strains still show detectable activation of PKB (Lim *et al*,

2001; unpublished observations), suggesting that neither is completely necessary. There may be some degree of functional redundancy, with each protein compensating somewhat for the loss of the other and it is important to note that *rasG* null cells express elevated levels of RasC relative to wild type (C.J.L., unpublished observations).

AleA is the only RasGEF that has been thus far implicated in the aggregation process. *aleA*[−] cells are aggregation defective (Insall *et al*, 1996) and also show a dramatic reduction in cAMP-stimulated PKB activation (Lim *et al*, 2001), suggesting the possibility that AleA mediates activation of both RasC and RasG. However, although it is possible that AleA activates both RasC and RasG, there may be other GEFs that are involved in this process. With over 20 different GEFs identified by the *Dictyostelium* Sequencing Project (Wilkins & Insall, 2001), it is clear that the Ras activation pathways in *D. discoideum* are quite complex. We hope that the RBD binding assay will prove to be a powerful tool to allow the identification of the RasGEFs and other components involved in the Ras activation pathways in *D. discoideum*.

METHODS

Cell culture and development. *D. discoideum* Ax2 and Ax3 cells were grown in HL5 medium supplemented with 50 µg/ml streptomycin (Sigma). This medium was supplemented with either 10 µg/ml G418 (Invitrogen) or 5 µg/ml Blasticidin S (Calbiochem) for the growth of G418- and Blasticidin-resistant strains. RasG (G12T) was expressed behind a tetracycline-repressible promoter in Ax2 cells (Secko *et al*, 2004). Tetracycline (5 µg/ml; Sigma) was added to repress expression of RasG (G12T), and expression was induced by washing cells three times in KK₂ (20 mM potassium phosphate, pH 6.1) and resuspending in HL5 without tetracycline for 18 h. RasC and RasC (G13T) were expressed behind the endogenous *rasC* promoter in *rasC*[−] and Ax2 cells, respectively (Lim *et al*, 2001). Ax3, *cAR1*[−]/*cAR3*[−] (Kim *et al*, 1997), *gβ*[−] (Wu *et al*, 1995) and *gα2*[−] (Chen *et al*, 1994) were transformed with pJLW30 (Lim *et al*, 2001) to overexpress wild-type *rasC*.

Preparation of GST-RBD fusion proteins. pGEX-Raf1-RBD (amino acids (aa) 1–149) was obtained from David Shalloway. pGEX-RalGDS-RBD (aa 1–115) was obtained from Michael Gold. pGEX-PI(3)K1-RBD (aa 613–877) and pGEX-PI(3)K2-RBD (aa 753–977) were obtained from Richard A. Firtel. The RBD of Byr2 (aa 1–236) was cloned into pGEX 4T-1 by PCR to yield pGEX-Byr2-RBD. All fusion proteins were expressed in *Escherichia coli* BL21 DE3 (Invitrogen). Cells were cultured in 500 ml Luria broth (LB), and induced at OD₆₀₀ 0.5–0.6 with 0.1 mM isopropyl-β-D-thiogalactoside for 18 h at 22 °C. After induction, cells were harvested by centrifugation, resuspended in 10 ml STE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 150 mM NaCl), and frozen overnight at <−70 °C. Thawed cell suspensions were treated with lysozyme (10 µg/ml, 30 min on ice). In all, 100 µl 1 M dithiothreitol and 1.4 ml 10% Sarkosyl were added, and then cells were lysed by sonication. Lysates were cleared by centrifugation. A 4 ml portion of 10% Triton X-100 was added to the supernatant, and STE was added to a final volume of 20 ml. A 500 µl volume of STE buffer was added to 500 µl bed volume of glutathione-Sepharose beads (Pharmacia), and this mixture was added to 10 ml cell lysate and tumbled for 2 h at 4 °C. The beads were washed three times in 1 × HK-LB (10 mM sodium phosphate (pH 7.2), 1% Triton X-100, 0.05% SDS, 10% glycerol, 150 mM NaCl,

10 mM MgCl₂, 1 mM EDTA, 1 mM Na₃VO₄, 5 mM NaF, with one tablet of protease inhibitors (Roche complete) added per 50 ml buffer) and resuspended to a total volume of 1 ml (50% slurry).

RBD binding assay. Exponential-phase vegetative *Dictyostelium* cells were washed twice and resuspended at 5×10^7 cells/ml in KK₂. Aliquots (350 µl) were taken at selected time points and lysed in an equal volume of ice-cold 2 × HK-LB, and incubated on ice for 5 min. The lysates were cleared by centrifugation for 10 min and protein concentrations were determined using the DC Protein Assay (Bio-Rad). A 400 µg portion of protein lysates was incubated with 100 µg of GST-RBD and the mixture was incubated at 4 °C for 1 h. Beads were harvested by centrifugation and washed three times in 1 × HK-LB. A volume of 40 µl of 1 × SDS gel loading buffer was added to the pelleted beads and the mixture was boiled for 5 min. Samples were fractionated by SDS–polyacrylamide gel electrophoresis, blotted onto nitrocellulose, blocked with non-fat milk, and probed with either RasC (Lim *et al*, 2001) or RasG (Khosla *et al*, 1996) antibody. The amounts of bound RasC and RasG were determined by an enhanced chemiluminescence (Amersham) reaction.

Assay for activated Ras proteins in aggregation-competent cells. To prepare pulsed aggregation-competent cells, vegetative cells were washed three times, resuspended at 5×10^6 cells/ml in KK₂, shaken at 160 r.p.m. for 1 h, and then pulsed with 50 nM cAMP (final concentration) every 6 min. After pulsing for 5 h, cells were harvested by centrifugation, washed twice in KK₂, resuspended at 5×10^7 cells/ml, and stimulated with a final concentration of 200 nM cAMP. Before and after stimulation, 350 µl aliquots were lysed in 350 µl 2 × HK-LB, and subjected to the RBD binding assay.

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REFERENCES

- Akasaka K, Tamada M, Wanf F, Kariya K, Shima F, Kikuchi A, Yamamoto M, Shirouzu M, Yokoyama S, Kataoka T (1996) Differential structural requirements for interaction of Ras protein with its distinct downstream effectors. *J Biol Chem* **271**: 5353–5360
- Boguski MS, McCormick F (1993) Proteins regulating Ras and its relatives. *Nature* **366**: 643–654
- Chen MY, Devreotes PN, Gundersen RE (1994) Serine 113 is the site of receptor-mediated phosphorylation of the *Dictyostelium* G protein α -subunit G α 2. *J Biol Chem* **269**: 20925–20930
- Chubb JR, Wilkins A, Thomas GM, Insall RH (2000) The *Dictyostelium* Ras protein is required for macropinocytosis, phagocytosis and the control of cell movement. *J Cell Sci* **113**: 709–719
- Funamoto S, Meili R, Lee S, Parry L, Firtel RA (2002) Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109**: 611–623
- Huang YE, Iijima M, Parent CA, Funamoto S, Firtel RA, Devreotes PN (2003) Receptor-mediated regulation of PI3Ks confines PI(3,4,5)P₃ to the leading edge of chemotaxing cells. *Mol Biol Cell* **14**: 1913–1922
- Insall RH, Borleis J, Devreotes PN (1996) The *aimless* RasGEF is required for processing of chemotactic signals through G-protein-coupled receptors in *Dictyostelium*. *Curr Biol* **6**: 719–729
- Kang R, Kae H, Ip H, Spiegelman GB, Weeks G (2002) Evidence for a role for the *Dictyostelium* Rap1 in cell viability and the response to osmotic stress. *J Cell Sci* **115**: 3675–3682
- Kessin RH (2001) *Dictyostelium—Evolution, Cell Biology, and the Development of Multicellularity*. Cambridge, UK: Cambridge Univ. Press
- Khosla M, Spiegelman GB, Weeks G (1996) Overexpression of an activated *rasG* gene during growth blocks the initiation of *Dictyostelium* development. *Mol Cell Biol* **16**: 4156–4162
- Kim JY, Soede RDM, Schaap P, Valkema R, Borleis JA, Van Haastert PJM, Devreotes PN, Hereld D (1997) Phosphorylation of chemoattractant receptors is not essential for chemotaxis or termination of G-protein-mediated responses. *J Biol Chem* **272**: 27313–27318
- Lim CJ, Spiegelman GB, Weeks G (2001) RasC is required for optimal activation of adenylyl cyclase and Akt/PKB during aggregation. *EMBO J* **20**: 4490–4499
- Meili R, Ellsworth C, Lee S, Reddy TBK, Ma H, Firtel RA (1999) Chemoattractant-mediated transient activation and membrane localization of Akt/PKB is required for efficient chemotaxis to cAMP in *Dictyostelium*. *EMBO J* **18**: 2092–2105
- Secko DM, Insall R, Spiegelman GB, Weeks G (2004) The identification of *Dictyostelium* phosphoproteins in response to the activation of RasG. *Proteomics* (in press)
- Sutherland BW, Spiegelman GB, Weeks G (2001) A Ras subfamily GTPase shows cell cycle-dependent nuclear localization. *EMBO Rep* **2**: 1024–1028
- Taylor S, Shalloway D (1996) Cell cycle-dependent activation of Ras. *Curr Biol* **6**: 1621–1627
- Tokumitsu Y, Nakano S, Ueno H, Niho Y (1998) Ras signals to the cell cycle machinery via multiple pathways to induce anchorage-independent growth. *Mol Cell Biol* **18**: 2586–2595
- Tuxworth RI, Cheetham JL, Machesky LM, Spiegelman GB, Weeks G, Insall RH (1997) *Dictyostelium* RasG is required for normal motility and cytokinesis, but not growth. *J Cell Biol* **138**: 605–614
- Weeks G, Spiegelman GB (2003) Roles played by Ras subfamily proteins in the cell and developmental biology of microorganisms. *Cell Signal* **15**: 901–909
- Wilkins A, Insall R (2001) Small GTPases in *Dictyostelium*: lessons from a social amoeba. *Trends Genet* **17**: 41–48
- Wu L, Valkema R, Van Haastert PJM, Devreotes PN (1995) The G protein β -subunit is essential for multiple responses to chemoattractants in *Dictyostelium*. *J Cell Biol* **129**: 1667–1675