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The Protein Kinase YakA Regulates G-protein-linked Signaling Responses during Growth and Development of *Dictyostelium**

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A genetic screen for *Dictyostelium* mutants that phenotypically resemble cells lacking the G-protein β-subunit yielded the protein kinase YakA. Like *gβ*-null cells, *yakA*-null cells fail to enter development and display slow growth on bacterial lawns. We created a temperature-sensitive *yakA* mutant and showed that YakA activity is required not only at the onset but also during development. The *yakA*-null cells have strong defects in folic acid-induced responses, such as actin polymerization and cGMP accumulation, indicating that they play a role in G-protein-mediated signaling responses. We propose that YakA acts downstream of G-proteins, because cAMP receptors still couple to G-proteins in the *yakA* mutant. In addition, the previously observed growth arrest induced by overexpression of *YakA* also occurs in *gβ* mutants. We localized YakA-GFP to the cytosol suggesting that YakA may be a functional homolog of its mammalian counterparts Dyrk2 and Dyrk3, a subclass of dual-specificity Yak-related kinases (Dyrk) with unknown function.

Members of the novel family of Dyrk,[1] or dual specificity Yak-related kinases, have been identified in most eukaryotes and play roles in both growth and development. The Dyrk family is distinguished from other protein kinase families by its ability to phosphorylate both Ser/Thr and Tyr substrates (dual specificity) and the presence of several conserved sequence motifs, such as the Y*X*Y motif between subdomains VII and VIII, and a so-called Dyrk homology box just N-terminal of the catalytic domain (1–4). The cellular functions of the Dyrk kinases are unknown. Several lines of evidence suggest that the Dyrk homolog in *Saccharomyces cerevisiae*, Yak1, functions as a negative regulator of the cell cycle (5–7). Dyrk kinases also have a role in development, as in the *Drosophila minibrain* mutants, which have reduced numbers of neurons in the optic lobes and central hemispheres (8). A human homolog of *minibrain*, *Dyrk1A,* or *Mnb*, is expressed in the brain and localizes to the Downs Syndrome critical region on chromosome 21 (9, 10). Expression of this gene affects learning in mice (11). More recently, at least seven mammalian Dyrk proteins have been described that vary in their tissue expression patterns and subcellular localization, suggesting that members of the Dyrk family have a variety of cellular roles (12).

We have isolated a member of the Dyrk family from the amoeba *Dictyostelium discoideum* in a genetic screen for mutants that phenotypically resemble cells that lack functional heterotrimeric guanosine nucleotide-binding proteins (G-proteins). G-proteins consist of an α-subunit and a βγ-dimer, which dissociate upon ligand activation of heptahelical membrane receptors. Eleven Gα subunits, one Gβ subunit, and one Gγ subunit have been isolated from *D. discoideum* (13, 14).[2] Signaling through G-proteins is essential for chemotaxis toward the chemoattractant cAMP, and absence of functional G-proteins prevents aggregation into multicellular structures. Folic acid and cAMP receptors interact with G-proteins comprised of different Gα subunits. The pathways appear to converge at the level of the G-protein βγ subunits, since *gβ*-null mutants are unresponsive to both folic acid and cAMP (14–16). *Gβ* mutants also display reduced growth rates on bacterial substrates due to a failure to carry out chemotaxis toward bacterial secretion products such as folic acid as well as a reduced rate of phagocytosis (14, 17).

How are the signals transmitted to the different effector molecules downstream of the G-protein? Both Gα and Gβγ subunits are able to transduce signals from receptors to downstream effectors. Although evidence suggests that in *Dictyostelium* most of the signaling is mediated by Gβγ subunits (18) they may not directly interact with effector enzymes. In the case of adenylyl cyclase activation, an intermediate step involves the transient membrane localization of the PH-domain containing cytosolic regulator of adenylyl cyclase (Crac) (19). Similar intermediates may transduce signals from the G-protein to other effector enzymes or proteins involved in actin polymerization and guanylyl cyclase.

To isolate novel genes that mediate chemotactic responses downstream of Gβγ, we mutagenized amoebae using restriction enzyme-mediated integration (REMI) and isolated mutants with phenotypes similar to that of *gβ*-null cells. Large numbers of individual cells were plated clonally on bacterial lawns and analyzed for developmental phenotypes as well as plaque size, both of which are impaired in *gβ*-null cells. Mutants that formed small aggregation-minus plaques on bacterial lawns were selected and further screened for defects in chemoattractant-induced actin polymerization.

One of these mutants has an insertion in the *YakA* gene, a member of the Dyrk family. The mutant forms small plaques on bacteria and cells do not enter development. In addition to a failure to aggregate, the mutant expresses only low levels of

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[1] The abbreviations used are: Dyrk, dual specificity Yak-related kinases; PKA, cAMP-dependent protein kinase; REMI, restriction enzyme-mediated integration; kb, kilobase; GFP, green fluorescent protein; cAR, cAMP receptor.

[2] N. Zhang *et al.*, unpublished results.

aggregation-specific genes, such as cAMP receptor (cAR1), adenylyl cyclase (ACA), and cAMP-dependent protein kinase (PKA) (20). This indicates that YakA regulates the transition from growth to development. In addition, Souza *et al.* (20) previously isolated YakA in a different REMI screen, proposing that it plays a role in cell cycle control because overexpression of *YakA* causes cells to arrest in the G_1 phase of the cell cycle.

Our goal was to investigate the role of YakA during development and in particular in G-protein linked signaling pathways. The strong phenotypic resemblance between *yakA*-null and *gβ*-null mutants suggests that YakA and Gβγ operate in similar pathways. We created a temperature-sensitive *YakA* mutant and showed that YakA is not only required at the onset of development but also during aggregation and postaggregative development. To address its role in G-protein-mediated signaling, we focused on the role of YakA in folic acid signal transduction, which does not require development. Our results show that YakA is a cytosolic protein that functions downstream of the G-protein to activate a number of signal transduction responses including actin polymerization and cGMP accumulation.

EXPERIMENTAL PROCEDURES

Materials—cAMP and G418 were from Sigma. Blasticidin S was from CalBiochem. Monoclonal anti-GFP antibody was from Babco Covance (Berkeley).

D. discoideum Growth, Development, Clonal Selection, and Plaque Analysis on Bacterial Lawns—D. discoideum strains were grown in axenic medium (21) at 22 °C. 5 μg/ml Blasticidin S was added to yakA-null mutants. 20 μg/ml G418 was added to cell lines carrying YakA-GFP expression constructs. Cells were developed on Development Buffer-agar plates (10 mM $NaKPO_4$, 2 mM $MgSO_4$, 0.2 mM $CaCl_2$, 1.5% agar) at 1.5×10^6 cells/cm^2. Mutant clones were selected by plating 50–100 cells with 200 μl of an overnight culture of *Klebsiella aerogenes* on SM-agar plates (22). After 5 days at 22 °C, plaques were analyzed for mutant phenotypes and photographed.

REMI Mutagenesis and Plasmid Rescue—REMI mutagenesis was performed according to Adachi *et al.* (23), using plasmid pBSR3, carrying the blasticidin resistance cassette (gift of W. F. Loomis), linearized with *Bam*HI. 5 units of DpnII/ml were added during electroporation. The plasmid, along with the 4-kb flanking genomic sequence, was rescued from the yakA mutant as an 8-kb *Bgl*II fragment, which was then ligated and transformed to *Escherichia coli* (XL1Blue). Colonies carrying the rescued plasmid were selected on ampicillin plates. The genomic sequences flanking the pBSR3 construct were ~2 kb on each side.

cGMP and F-actin Measurements—For each assay cells were grown on SM plates in association with *K. aerogenes* (22). Cells were washed once in DB and 5×10^6 cells (cGMP response) or 2×10^6 cells (F-actin) were stimulated with 100 μM folic acid or 10% HL5 medium from an overnight 22 °C culture of *K. aerogenes*. cGMP levels were measured using a radioimmunoassay, [^{3}H]GMP detection kit (Amersham Pharmacia Biotech). F-actin measurements were performed as described (17).

Synthesis of tsYakA and YakA-GFP Constructs—The P375S mutation that created the tsYakA construct was made using a Morph site-directed mutagenesis kit (5′-3′) using a primer with the desired point mutation (TTATAGATCATCTGAAAATAT) using the Act6-*YakA* expression construct (20) as a template.

GTPγS Inhibition of cAMP Binding on Membranes—Cells were developed for 5 h in DB with 100 μM additions of 10^{-7} M cAMP pulses delivered every 6 min. Then cells were treated with 2.5 mM caffeine for 20 min, washed twice in cold DB and resuspended to 10^8 cells/ml in 40 mM Hepes, 250 mM sucrose, 0.5 mM EDTA, pH 7.7. Cells were lysed through 5 μm filters (Nucleopore) and centrifuged for 5 min at 14,000 × *g*. Pellets were resuspended in PB at a density of 10^8 cell equivalents/ml. Binding of 2 nm [^{3}H]cAMP in the presence or absence of 100 μM GTPγS was measured as described (23).

Fluorescence Microscopy on YakA-GFP-expressing Cells—Fluorescence microscopy on live cells was performed on a Zeiss microscope (Axiovert 135 TV as described in Ref. 24). Images were made using IP-lab software.

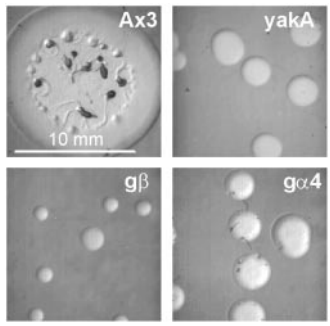


FIG. 1. **Plaque sizes and developmental phenotypes of *yakA*, *gβ*, and *gα4* mutants.** Cells were mixed with *K. aerogenes* and clonally seeded on agar plates. Photographs were taken after 5 days at 22 °C. Typical examples of each phenotype are shown.

RESULTS

Generation of Small Plaque Mutants by Restriction Enzyme-mediated Integration—To isolate novel mutants with defects in chemotaxis we used REMI to generate 25,000 transformants with random insertions in the genome. We clonally seeded ~100,000 cells on bacterial lawns and selected mutants that grew slowly under these conditions. We choose 65 "small plaque" mutants and tested each for defects in cAMP-induced actin polymerization. Five mutants were defective in this response and also failed to aggregate when plated on non-nutrient agar. We cloned a portion of the affected gene from one of these mutants and identified the sequence that flanked the insertion as the previously identified *YakA* gene (20). The insertion was in the protein kinase domain at amino acid 257. We further examined whether the newly identified small plaque phenotype, which was not reported previously, was caused by the *YakA* mutation. We re-created the phenotype through homologous recombination by transforming the linearized rescued plasmid, which contained the *YakA* flanking sequences, in wild-type cells. Fig. 1 shows that the fresh disruptant displayed the small plaque phenotype and the previously reported aggregation defect on bacterial lawns (20). The plaques of *yakA*$^-$ cells were slightly larger than those of *gβ*$^-$ cells. Expression of *YakA* from the *Act-6* promoter in the *yakA*$^-$ mutant restored the wild-type phenotype (data not shown), indicating that the phenotype was indeed caused by disruption of the *YakA* gene. In addition, mutants lacking the Gα subunit, Gα4, displayed plaque sizes that were similar to *yakA*$^-$ plaques, suggesting that mutants lacking responsiveness to folic acid display a small plaque phenotype.

YakA Activity Is Required Throughout Development—The phenotypic similarity between the *yakA*$^-$ and *gβ*$^-$ cells suggested that YakA has a role in G-protein-mediated signaling pathways. Since chemoattractant receptor signaling is required for development, such a defect would block development. Indeed, *yakA*$^-$ cells do not enter development when starved on non-nutrient agar and fail to express early developmental genes (20). We hypothesized that, like G*β*, YakA plays a role throughout development, not only during the transition from growth to development as was proposed earlier (20). *yakA*$^-$ cells express wild-type amounts of Gβ protein, so the *yakA*$^-$ phenotype cannot be explained by failure to express Gβ (data not shown).

In order to study the role of YakA during development, we made point mutations in the protein kinase domain that were previously shown to confer temperature sensitivity to homologous map kinase kinases (25, 26). One of these mutations, P375S, caused a temperature-sensitive phenotype when the gene was expressed in *yakA*$^-$ cells. Cells expressing this *tsYakA* gene failed to develop at restrictive temperature but

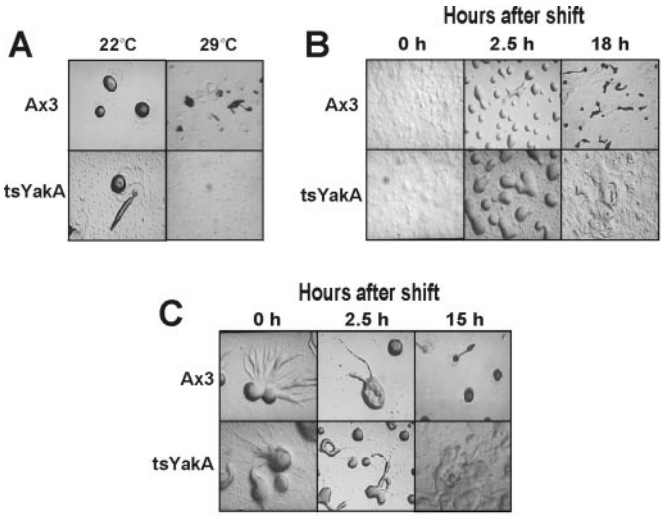


FIG. 2. **Developmental phenotype of *tsYakA* mutants at permissive and restrictive temperatures.** Ax3 and *tsyakA* cells were plated on DB agar at 5×10^5 cells/cm^2. Cells were allowed to develop at the permissive temperature for 0 h (*A*), 4 h (*B*), and 7 h (*C*). Then the temperature was shifted to 29 °C, and cells were photographed after 24 h (*A*) or at the indicated time points (*B* and *C*).

formed normal fruiting bodies at permissive temperature (Fig. 2*A*). Expression of *tsYakA* does not cause growth inhibition at the permissive temperature, as is the case for overexpression of wt*YakA* (data not shown). To study the role of YakA during development, *tsYakA*/*yakA*$^-$ cells were allowed to enter development at the permissive temperature for different periods of time and then shifted to the non-permissive temperature. When shifted to restrictive temperature at 4 h of development, the cells formed loose mounds but did not develop further, and eventually dispersed (Fig. 2*B*). When shifted at 7 h, cells formed tight mounds, then arrested, and the structures disintegrated (Fig. 2*C*). These results indicate that YakA protein kinase activity is required not only at the onset but also during development.

YakA Mutants Are Defective in Folic Acid-induced Signal Transduction Responses—Both folic acid and cAMP trigger similar chemotactic responses, but the responsiveness to each of these chemoattractants depends on the developmental stage. Vegetative cells respond with chemotaxis to folic acid but lose their responsiveness during the first few hours of development. As cells lose sensitivity to folic acid they acquire sensitivity to cAMP. The *yakA*$^-$ cells do not enter development and do not express cAMP receptors (20). In order to separate the effects of the *YakA* deletion on signal transduction from those on development, we measured responses to folic acid in growing *yakA*$^-$ cells.[3] In wild-type cells, folic acid stimulation triggers rapid accumulation of intracellular cGMP and transient polymerization of F-actin. These responses were essentially absent in several independent *yakA*$^-$ strains (Fig. 3).

We also tested bacterial-conditioned medium for its ability to trigger signal transduction responses in *yakA*$^-$ cells. This medium triggers robust F-actin and cGMP responses in growth stage wild-type cells and likely contains other chemoattractants besides folic acid. As shown in Fig. 3*B*, bacterial-conditioned medium triggers only weak cGMP responses in *yakA*$^-$, suggesting that the mutant has a general defect in transducing chemoattractant signals. Mutants lacking the Gα subunit Gα4 have been shown to be specifically defective in mediating the chemotactic response to folic acid (16). We found that these mutants displayed reduced growth rates on bacteria, resulting in plaque sizes similar to those of *yakA*$^-$ (Fig. 1). This suggests that the inability to respond to folic acid or other bacterial secretion products results in slow plaque expansion rates on bacteria.

YakA Functions Downstream of the Receptor-G-protein Complex—YakA might act downstream of G-proteins or regulate the coupling of G-proteins to receptors. In wild-type cells, *in vitro* activation of G-proteins with GTPγS results in a >70% loss of agonist binding, reflecting the shift of the receptor to a low affinity state (27). Since *yakA*$^-$ cells express low levels of cAMP receptors we transformed both *yakA*$^-$ and wild-type cells with an Act15-cAR1 construct and measured GTPγS inhibition of [^{3}H]cAMP binding in growth stage cells. Fig. 4 shows that the percentage inhibition of cAMP binding by GTPγS is the same for both wild-type and *yakA*$^-$ cells, indicating that YakA does not affect the coupling of receptors to G-proteins. Expression of *cAR1* did not restore development in *yakA*$^-$ cells (data not shown).

Previous studies have shown that, when overexpressed, YakA causes a growth arrest (20). In order to determine whether YakA is downstream of Gβ, we overexpressed the gene under an inducible (*Act-6*) promoter in *gβ*$^-$ cells. *YakA* overexpression caused a growth arrest in *gβ*$^-$ cells as was shown previously for wild-type cells. This observation suggests that YakA operates downstream of G$\beta\gamma$ (Fig. 4).

YakA Is a Cytosolic Protein—In order to determine the subcellular localization of YakA, we fused GFP to the C terminus of YakA, which was truncated by deleting the 190 C-terminal amino acids. Using fluorescence microscopy we showed that the GFP signal was entirely cytoplasmic and excluded from the nucleus (Fig. 5*A*). Expression of this construct restored development of *yakA*$^-$ cells, showing that, despite the truncation and GFP fusion, the protein was functional (Fig. 5*B*) This localization was independent of the developmental stage (data not shown). No relocalization of Yak-GFP was observed when appropriately differentiated cells were stimulated with cAMP even after longer periods of stimulation (data not shown). The strictly cytosolic localization of YakA and its absence from the nucleus favors a role of this kinase as a downstream regulator of G-proteins in a cytoplasmic signaling pathway.

DISCUSSION

Heterotrimeric G-proteins mediate a number of chemotactic responses in phagocytic cells such as amoebae and neutrophils. In *Dictyostelium*, chemotaxis is mediated by both cAMP and folic acid, which trigger a similar set of biochemical responses including polymerization of actin and the accumulation of cGMP. Interestingly, biochemical responses to folic acid and cAMP are transduced by separate Gα subunits, which share the unique Gβ subunit. This suggests that similar pathways mediated by different chemoattractants converge at the level of G$\beta\gamma$.

We have identified *YakA* in a screen for mutants that phenocopy the *gβ*-null cells. Both *yakA*$^-$ and *gβ*$^-$ cells form small plaques on bacterial lawns and fail to enter development. Besides their identical morphology, *gβ*-null and *yakA*-null cells show striking biochemical similarities: both mutants fail to express early developmental genes and show strongly reduced biochemical responses to folic acid or bacterial secretion products.

cAMP signaling is required for chemotaxis and postaggregative development, and YakA may regulate these responses. If YakA regulates G-protein-mediated signaling it is expected to have a role during development. Previous studies using a tem-

[3] We have used the *tsyakA* mutant to analyze cAMP-mediated signal transduction responses at permissive and restrictive temperatures. However, we were unable to draw conclusions from these experiments, because at the restrictive temperature the results varied greatly in wild-type and mutant strains.

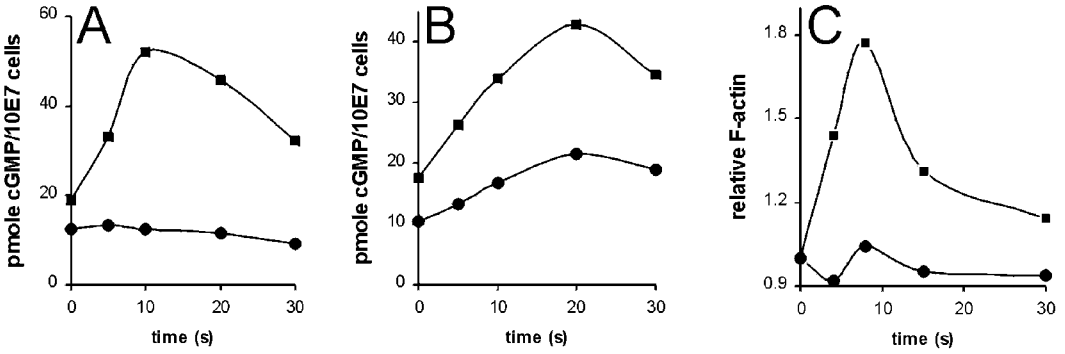
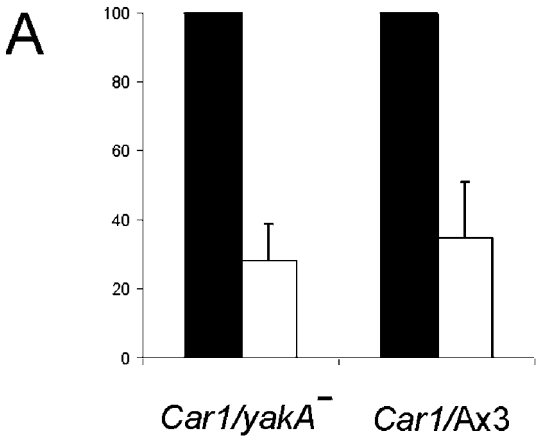


FIG. 3. **Biochemical responses toward folic acid and bacterial supernatants in *yakA*$^-$.** Cells were grown in association with bacteria and washed, and stimulated with 100 μM folic acid (*A* and *C*) or 10% bacterial-conditioned medium (*B*). *Circles, yakA*$^-$; *squares,* Ax3.



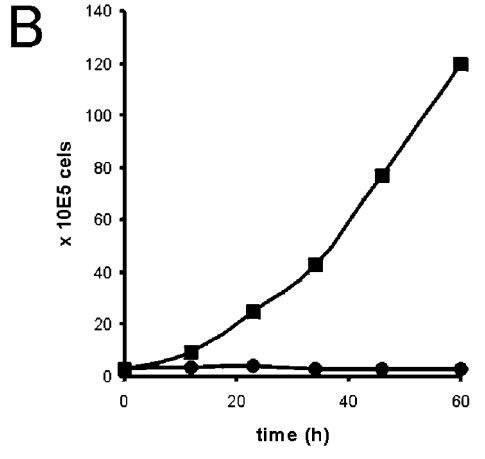


FIG. 4. *A,* GTPγS inhibition of cAMP binding in *cAR1/yak*$^-$ and *cAR1/Ax3* cells. Percent binding of 2 nM [^{3}H]cAMP in the absence (*black bars*) and presence (*white bars*) of GTPγS. *B,* growth curves of *g*β$^-$ and *YakA/g*β$^-$ cells. Cells were grown to confluency in HL5 medium, washed from the Petri dish and suspended in flasks at a density of 3×10^5 cells/ml. Cells were counted at the indicated time points. *Circles*, *YakA/g*β$^-$; *squares*, *g*β$^-$

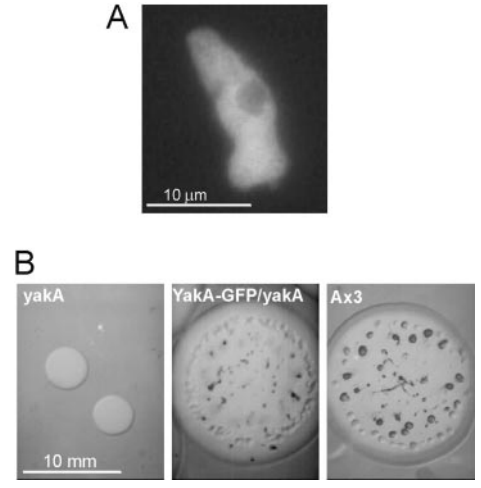


FIG. 5. *A*, localization of YakA-GFP. *B,* phenotypes of *yakA*$^-$, *YakA-GFP/yakA*$^-$, and Ax3. Cells were clonally seeded on *K. aerogenes* lawns, and plaques were photographed after 5 days.

perature-sensitive Gβ mutant indicate that functional G-proteins are essential during development (28). Our experiments using the temperature sensitive YakA mutant show that YakA activity is not only required at the onset of development, as was proposed previously by Souza *et al.* (20), but also during the aggregation process and postaggregative development. Further support for a role of YakA during development is the so-called rapid-development phenotype that cells acquire when YakA is overexpressed (20).

The small plaque phenotype and absence of development of *g*β-null and also *yakA*-null mutants can be explained by failure to signal through the G-protein subunits α4 and α2, respectively. Mutants that lack one of the Gα subunits, Gα4, are specifically defective in folic acid-mediated signaling and chemotaxis. Interestingly, these mutants do enter development, but like *yakA* and *g*β mutants, form small plaques on bacterial lawns. Unlike *g*β mutants (17), *yakA* and *g*α4 mutants display wild-type phagocytosis rates (data not shown), suggesting that a failure to respond with chemotaxis to bacterial secretion products leads to reduced growth rates on bacteria. The developmental defect in *g*β-null and *yakA*-null mutants can be explained by the failure to transduce external cAMP signals through Gα2. This α-subunit couples to the cAMP receptor and transduces chemotactic signals during chemotaxis and is therefore essential for the early developmental processes. In the *g*β mutant, none of the 11 known Gα subunits can form heterotrimers, but the absence of Gα4 and Gα2 is sufficient to explain the *g*β-null phenotype. Mutants, like *yakA*$^-$ that both form small plaques and fail to enter development are therefore good candidates for having general defects in G-protein signaling.

It has been suggested that YakA can regulate the activity or expression level of the cAMP-dependent protein kinase (PKA). PKA is only expressed at low levels in a *yakA*-null mutant and expression of the catalytic subunit of PKA (PKA-CAT) can restore development in *yakA*-null cells (20). The mechanism by which PKA restores development is unclear, but it has been shown that PKA is required for the expression of late developmental genes (29). In addition, expression of PKA-CAT has been shown to restore development in mutants that lack G-

protein signaling, such as mutants that lack cAMP receptors[4] or adenylyl cyclase A. Regulation of PKA activity is clearly not the only function of YakA, since the G1 arrest caused by overexpression of YakA also occurs in cells lacking the PKA catalytic subunit (20).

The homology between Dyrk, *minibrain,* and Yak proteins is restricted to the protein kinase and Dyrk homology domains, making it difficult to assign functional mammalian homologs of YakA. YakA localizes to the cytoplasm, suggesting that it is most homologous to the putative cytosolic members of the mammalian Dyrk family. Candidates are the Dyrk2 and Dyrk3 isoforms, which lack the putative nuclear localization signals that are present in *Drosophila minibrain* and Dyrk1 sequences. GFP fusion proteins of the N-terminal region of Dyrk2 remain cytoplasmic, whereas the N-terminal sequences of Dyrk1A targets to the nucleus (12). Dyrk1A and *Drosophila minibrain* have a role in brain development, but little is known about the role of Dyrk2 and Dyrk3. Dyrk2 and Dyrk3 are expressed in a variety of tissues, including brain, but their mRNAs are most abundant in testes (4). It remains to be investigated whether Dyrk2 and Dyrk3 have a role in G-protein-linked signaling pathways. The exact function of YakA in G-protein-linked signaling pathways is unknown. YakA may function as an effector of G$\beta\gamma$ to transduce the chemotactic signal to downstream effectors, for example by regulating their phosphorylation state. Tyrosine kinases have been implicated as effectors of G-proteins, such as members of the c-Src family. These tyrosine kinases have been shown to interact with mammalian Gα_s and Gα_i and are activated by these Gα subunits *in vitro* (30). Like c-Src, YakA may interact with Gα, but it is more likely that YakA interacts with, or is regulated by G$\beta\gamma$ subunits, because cAMP-induced responses are mediated by G$\beta\gamma$ rather than Gα subunits (18). The Gβ subunit is unique and thus far only one Gγ has been identified, so there may be less structural diversity between the G$\beta\gamma$ subunits than Gα subunits. This would make it more likely that G$\beta\gamma$-mediated responses are transmitted through a single effector pathway, which may involve YakA. We are currently investigating the binding partners of YakA to address these important questions.

[4] C. D. Reymond, personal communication.

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REFERENCES

1. Kentrup, H., Becker, W., Heukelbach, J., Wilmes, A., Schurmann, A., Huppertz, C., Kainulainen, H., and Joost, H. G. (1996) *J. Biol. Chem.* **271,** 3488–3495
2. Himpel, S., W. Tegge, R. Frank, S. Leder, H. G. Joost, and Becker, W. (2000) *J. Biol. Chem.* **275,** 2431–2438
3. Kassis, S., Melhuish, T., Annan, R. S., Chen, S. L., Lee, J. C., Livi, G. P., and Creasy, C. L. (2000) *Biochem. J.* **348,** 263–272
4. Becker, W., and Joost, H. G. (1999) *Prog. Nucleic Acids Res. Mol. Biol.* **62,** 1–17
5. Garrett, S., and Broach, J. (1989) *Genes Dev.* **9,** 1336–1348
6. Garrett, S., Menold, M. M., and Broach, J. R. (1991) *Mol. Cell. Biol.* **11,** 4045–4052
7. Hartley, A. D., Ward, M. P., and Garrett, S. (1994) *Genetics* **136,** 465–474
8. Tejedor, F., Zhu, X. R., Kaltenbach, E., Ackermann, A., Baumann, A., Canal, I., Heisenberg, M., Fischbach, K. F., and Pongs, O. (1995) *Neuron* **14,** 287–301
9. Shindoh, N., Kudoh, J., Maeda, H., Yamaki, A., Minoshima, S., Shimizu, Y., and Shimizu, N. (1996) *Biochem. Biophys. Res. Commun.* **225,** 92–99
10. Smith, D. J., Stevens, M. E., Sudanagunta, S. P., Bronson, R. T., Makhinson, M., Watabe, A. M., O'Dell, T. J., Fung, J., Weier, H. U., Cheng, J. F., and Rubin, E. M. (1997) *Nat. Genet.* **16,** 28–36
11. Smith, D. J., and Rubin, E. M. (1997) *Hum. Mol. Genet.* **6,** 1729–1733
12. Becker, W., Weber, Y., Wetzel, K., Eirmbter, K., Tejedor, F. J., and Joost, H. G. (1998) *J. Biol. Chem.* **273,** 25893–25902
13. van Es, S., and Devreotes, P. N. (1999) *Cell. Mol. Life Sci.* **55,** 1341–1351
14. Wu, L., Valkema, R., P. J. Van. Haastert, and Devreotes, P. N. (1995) *J. Cell Biol.* **129,** 1667–1675
15. Kumagai, A., Hadwiger, J. A., Pupillo, M., and Firtel, R. A. (1991) *J. Biol. Chem.* **266,** 1220–1228
16. Hadwiger, J. A., Lee, S., and Firtel, R. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 10566–10570
17. Peracino, B., Borleis, J., Jin, T., Westphal, M., Schwartz, J. M., Wu, L., Bracco, E., Gerisch, G., Devreotes, P., and Bozzaro, S. (1998) *J. Cell Biol.* **141,** 1529–1537
18. Lilly, P. J., and Devreotes, P. N. (1995) *J. Cell Biol.* **129,** 1659–1665
19. Insall, R., Kuspa, A., Lilly, P. J., Shaulsky, G., Levin, L. R., Loomis, W. F., and Devreotes, P. (1994) *J. Cell Biol.* **126,** 1537–1545
20. Souza, G. M., Lu S., and Kuspa, A. (1998) *Development* **125,** 2291–2302
21. Ashworth, J. M., and Watts, D. J. (1970) *Biochem. J.* **119,** 175–182
22. Sussman, M. (1987) *Molecular Biology in Dictyostelium: Tools and Applications. Methods in Cell Biology*, Vol. 28, pp. 67–100, Academic Press Inc.
23. Adachi, H., Hasebe, T., Yoshinaga, K., Ohta, T., and Sutoh, K. (1994) *Biochem. Biophys. Res. Commun.* **205,** 1808–1814
24. Parent, C. A., Blacklock, B. B., Froehlich, W. F., Murphy, D. B., and Devreotes, P. N. (1998) *Cell* **95,** 81–91
25. Ma, H., Gamper, M., Parent, C., and Firtel, R. A. (1997) *EMBO J.* **16,** 4317–4332
26. Hsu, J. C., and Perrimon, N. (1994) *Genes Dev.* **8,** 2176–2187
27. van Haastert, P. J. M. (1984) *Biochem. Biophys. Res. Commun.* **124,** 597–604
28. Jin, T., Soede, R. D., Liu, J., Kimmel A. R., Devreotes P. N., and Schaap P. (1998) *EMBO J.* **17,** 5076–5084
29. Williams J. G., Harwood A. J., Hopper N. A., Simon M. N., Bouzid S., and Veron, M. (1993) *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **340,** 305–313
30. Ma, Y., Huang, J., Ali, S., Lowry, W., and Huang, X. (2000) *Cell* **102,** 635–646