Supporting Information

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SI Materials and Methods

Generation and Identification of *myoG* Null Mutants. A *myoG* disruption plasmid was created by first PCR cloning 1.4 kb of the 5′ region of the *myoG* gene (Dictybase Gene ID DDB_G0276363; nts 2711–4093 of genomic sequence), and then inserting a blasticidin resistance cassette (1) into the internal Cla I site. The resulting pDTx4 plasmid was linearized and electrotransformed into Ax2 cells (2). Colonies selected for growth in blasticidin were screened for homologous recombination at the *myoG* locus by Southern analysis of genomic DNA digested with Apa LI using the Roche DIG system (Roche). A drug-resistant nonhomologous recombinant (2-1; nonhomologous recombinants) that behaved identically to the parental Ax2 parental strain was isolated from the same transformation as HTD36-2 and used as a control for the experiments reported here.

RNA Isolation and RT-PCR. Bacterially grown early log-phase cells (i.e., 0.8–1.0 × 10^6 cells/mL) were washed into MMC, shaken at 150 rpm for 1 h and the pulsed to 100 nM cAMP for several hours while shaking. Total RNA was isolated from samples using TRIZOL (Invitrogen) at each time point. Oligo dT-primed cDNA was prepared using MuLV reverse transcriptase (New England Biolabs) and the following primer sets used for subsequent PCR:

H7: H7-S: 5′-ACGTTCAAACTAAATACGGAGCTGGT-3′
H7-AS: 5′-TTTGAGTGGTTTGCCAATTTCTTTT-3′
carA: carA-1S: 5′-GGTTGTATGGCAGTGTTGATTGG-3′
carA-2AS: 5′-CAAATCTTTCTGGTTCTGGTTCTC-3′;
acaA: aca-3S: 5′-ATGGCGGTTTCAGGTTTAGATGG-3′
aca-4AS: 5′-TGAGCCAATTTCACCCAAGAGCG-3′.
myoG: myx57: 5′-CAAAGAATGGGATGGCGTTGAAAG-3
myx58: 5′-CCTCAGAGATGATTGGAGTGTC-3′

The *H7* gene was used as an internal control for cDNA synthesis in the RT-PCR experiments as it is expressed at a constant level throughout development (3). No RT control reactions were performed to verify the absence of genomic DNA in each sample. The levels of cDNA used in each reaction was normalized by first performing PCR using *H7* primers and determining the volume of cDNA to obtain a similar level of product in each reaction. The numbers of cycles were varied for each primer pair to determine the number necessary to obtain detectable amounts of product without saturating the reaction.

Localization of cAR1-GFP and CRAC-GFP. Control or *myoG* mutant transformants expressing cAR1-GFP (4) or CRAC (cytosolic regulator of adenylate cyclase)-GFP (5) were maintained in HL5 supplemented with 10 μg/mL G418 (Fisher Chemical). Aggregation competent CRAC-GFP-expressing cells were allowed to adhere to coverslips and treated with latrunculinA for 20 min before cAMP pulsing (5). cAMP was administered to a final concentration of 100 nM during the second frame of each movie. Movies were made for 45 s at 20 frames per minute. Images were obtained with a Zeiss Axiovert microscope and 63× Plan-Apo, 1.4 NA oil-immersion lens.

1. Sutoh K (1993) A transformation vector for *Dictyostelium discoideum* with a new selectable marker bsr. *Plasmid* 30:150–154.
2. Lee E, Shelden EA, Knecht DA (1998) Formation of F-actin aggregates in cells treated with actin stabilizing drugs. *Cell Motil Cytoskeleton* 39:122–133.
3. de Chassey B, Dubois A, Lefkir Y, Letourneur F (2001) Identification of clathrin-adaptor medium chains in *Dictyostelium discoideum*: differential expression during development. *Gene* 262:115–122.
4. Xiao Z, Zhang N, Murphy DB, Devreotes PN (1997) Dynamic distribution of chemoattractant receptors in living cells during chemotaxis and persistent stimulation. *J Cell Biol* 139: 365–374.
5. Parent CA, Blacklock BJ, Froehlich WM, Murphy DB, Devreotes PN (1998) G protein signaling events are activated at the leading edge of chemotactic cells. *Cell* 95:81–91.

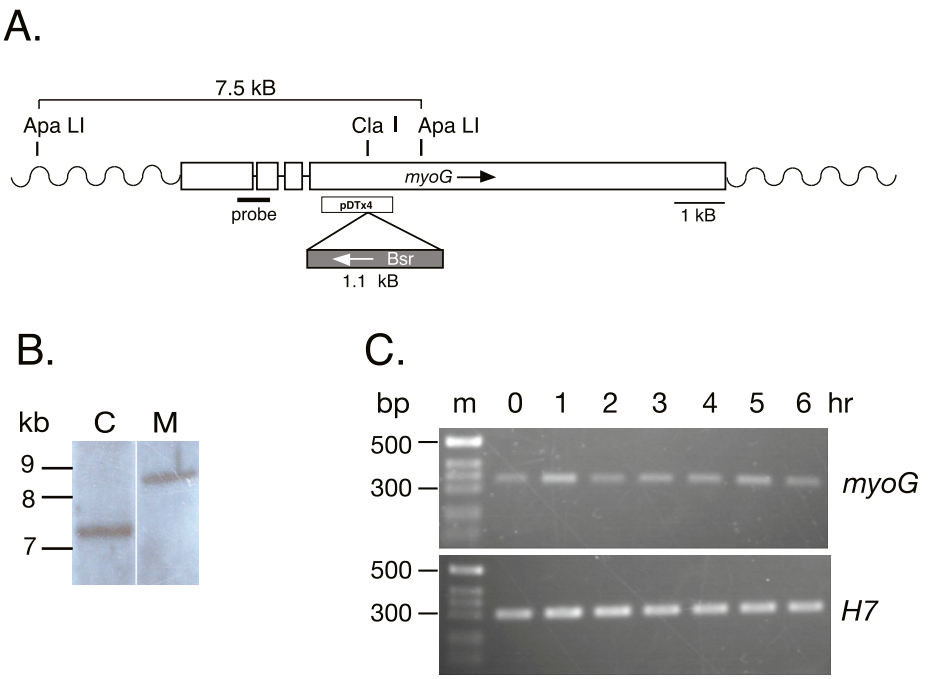

Fig. S1. Disruption of *myoG* and analysis of *myoG* expression during early development. (*A*) Schematic illustration of the *myoG* locus indicating the positions of introns (straight line), exons (open boxes), and noncoding 5′ and 3′ regions (wavy lines). Arrows indicate the direction of transcription. The region of the gene used to generate the gene disruption cassette (pDTx4) and Cla I site where the blasticidin resistance cassette (Bsr) was inserted are illustrated. The location of the Southern probe and the two Apa LI sites used for Southern analysis are also shown. (*B*) Southern analysis of control (C) and 2–13 *myoG* mutant (M) genomic DNA digested with Apa LI. (*C*) Analysis of *myoG* expression during early development. Total RNA isolated from control *Dictyostelium* following the onset of starvation (0–6 h) was used for RT-PCR using primers specific for the *H7* and *myoG* genes.

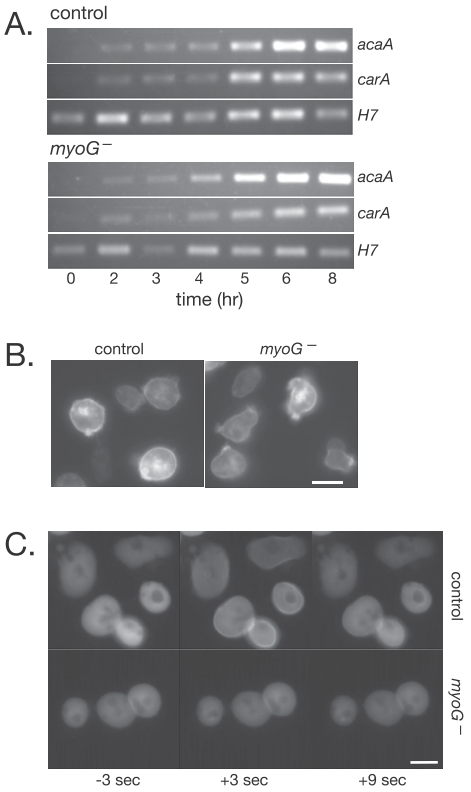

Fig. S2. Early gene expression and cAR1 localization are normal in *myoG* null mutant cells. (*A*) RT-PCR analysis of early gene expression in control and *myoG* mutant cells. Cells were washed into starvation buffer and then pulsed with cAMP and total RNA isolated at the indicated time points. Oligo-dT primed cDNA was synthesized and used as a template for PCR using primers specific for the *acaA* (471-bp product), *carA* (277-bp product), and *H7* (294-bp product) genes. (*B*) Localization of cAR1-GFP in vegetative control and *myoG*- cells. (Scale bar, 10 μm.) (*C*) Analysis of CRAC-GFP recruitment to the cortex in response to chemotactic stimulation. Aggregation competent control and *myoG* mutant cells expressing the CRAC-GFP reporter were treated with latrunculin A and then pulsed with 100 nM cAMP. Shown are images from 3 s before stimulation (–3 s) and +3 and +9 s following stimulation. (Scale bar, 10 μm.)