

## Mutants, cell growth, and development

cAR1<sup>-</sup>/cAR3<sup>-</sup>, Gα2<sup>-</sup>, Gβ<sup>-</sup> cells and cAR1-GFP, PHcrac-GFP, and coronin-GFP-expressing cells were as described before (Sun, T.J., and P.N. Devreotes. 1991. *Genes Dev.* 5:572–582; Gerisch, G., R. Albrecht, C. Heizer, S. Hodgkinson, and M. Maniak. 1995. *Curr. Biol.* 5:1280–1285; Xiao, Z., N. Zhang, D.B. Murphy, and P.N. Devreotes. 1997. *J. Cell Biol.* 139:365–374; Jin, T., M. Amzel, P.N. Devreotes, and L. Wu. 1998a. *Mol. Biol. Cell.* 9:2949–2961; Jin, T., D.M. Soede, J. Liu, A.R. Kimmel, P.N. Devreotes, and P. Schaap. 1998b. *EMBO J.* 17:5056–5084; Parent, C.A., B.J. Blacklock, W.M. Froehlich, D.B. Murphy, and P.N. Devreotes. 1998. *Cell.* 95:81–91. Jin, T., N. Zhang, Y. Long, C.A. Parent, and P.N. Devreotes. 2000. *Science.* 287:1034–1036). Cells were grown in axenic medium (HL5) at 22°C. 20 μg/ml G418 was added where appropriate. Cells were washed twice with and developed in DB (10 mM Na/K PO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.2 mM CaCl<sub>2</sub>). After shaking 1 h (180 rpm), cells were pulsed with 100 nM cAMP at 6-min intervals.