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The G alpha subunit Gα8 inhibits proliferation, promotes adhesion and regulates cell differentiation

Yuantai Wu [a], Chris Janetopoulos [a,b,*]

[a] Department of Biological Sciences, Vanderbilt University, Nashville, TN 37232, United States
[b] Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN 37232, United States

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ABSTRACT

Heterotrimeric G protein-mediated signal transduction plays a pivotal role in both vegetative and developmental stages in the eukaryote *Dictyostelium discoideum*. Here we describe novel functions of the G protein alpha subunit Gα8 during vegetative and development stages. Gα8 is expressed at low levels during vegetative growth. Loss of Gα8 promotes cell proliferation, whereas excess Gα8 expression dramatically inhibits growth and induces aberrant cytokinesis on substrates in a Gβ-dependent manner. Overexpression of Gα8 also leads to increased cell–cell cohesion and cell–substrate adhesion. We demonstrate that the increased cell–cell cohesion is mainly caused by induced CadA expression, and the induced cell–substrate adhesion is responsible for the cytokinesis defects. However, the expression of several putative constitutively active mutants of Gα8 does not augment the phenotypes caused by intact Gα8. Gα8 is strongly induced after starvation, and loss of Gα8 results in decreased expression of certain adhesion molecules including CsA and tgrC1. Interestingly, Gα8 is preferentially distributed in the upper and lower cup of the fruiting body. Lack of Gα8 decreases the expression of the specific marker of the anterior-like cells, suggesting that Gα8 is required for anterior-like cell differentiation.

Introduction

Heterotrimeric G proteins are central mediators in signal transduction pathways, with cells utilizing them to respond to the environment and communicate with each other. Heterotrimeric G protein consists of an α subunit and an obligate βγ dimer, and localize to the cytosolic face of the plasma membrane. G proteins typically transduce extracellular stimuli from G protein-coupled receptors (GPCRs) to downstream effectors. Ligand binding to the GPCR activates the G protein heterotrimer by facilitating GDP/GTP exchange on the Gα subunit which leads to the dissociation of the Gα and Gβγ dimer (Oldham and Hamm, 2008). The activated GTP bound Gα and free Gβγ interact with their downstream effectors, respectively, including adenylyl cyclases (Pierre et al., 2009), phospholipases (Mizuno and Itoh, 2009) and ion channels (Padgett and Slesinger, 2010). GPCR-mediated signaling has been implicated in numerous physiological and pathological processes and represent 50–60% of current drug targets (Overington et al., 2006).

The social amoeba *Dictyostelium discoideum* has been employed as a model system to study G protein signaling. The amoeba has a relatively short life cycle, a haploid genome and is amenable to numerous biochemical and genetic techniques (Schaap, 2011b). The *D. discoideum* genome contains 14Gα subunits, 2Gβ subunits and a single Gγ subunit (Eichinger et al., 2005; Heidel et al., 2011). The Gα2-mediated cAMP chemotaxis pathway has been intensively studied in this organism. The amoeba usually lives in the soil feeding on bacteria. Once the food source is depleted, cells start a developmental process that leads to the secretion of propagating waves of cAMP (Schaap, 2011a). Gradients of cAMP are formed and can be sensed by other cells through the cAMP receptor cAR1 (Klein et al., 1988). Binding of cAMP to cAR1 in turn activates Gα2 and leads to the dissociation of Gα2 from the Gβγ subunit (Elzie et al., 2009; Janetopoulos et al., 2001; Kesbeke et al., 1988; Kumagai et al., 1989). The activated Gα2 and Gβγ elicit a plethora of cellular responses which allow thousands of cells to stream toward the aggregation center, undergo morphological changes and finally form environmental-resistant spores (Franca-Koh et al., 2006). Another Gα subunit, Gα9, has been suggested as an inhibitor of the cAMP pathway (Brzostowski et al., 2002, 2004).

Vegetative *D. discoideum* cells can sense the bacterial metabolite folic acid to help track down bacteria. This process has also been shown to be G protein-mediated. Cells lacking the Gβ subunit form tiny plagues on bacterial lawn (Wu et al., 1995), and Gα4 likely couples to the folic acid receptor (Hadwiger et al., 1994), although the folic acid receptor itself has remained elusive and is still not identified. A recent study shows that several elements

* Corresponding author at: Vanderbilt University, Biological Sciences, 465 21st Ave South, U5215 MRB III, Nashville, TN 37232, United States. Fax: +1 16 153 436 707.
E-mail address: c.janetopoulos@vanderbilt.edu (C. Janetopoulos).

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thought to be required for cAMP chemotaxis are quite dispensable for folic acid chemotaxis (Srinivasan et al., 2012).

One of the Gα subunits, Gα8, has been investigated previously and no obvious function was revealed (Wu et al., 1994). Recently, Gα8 has been suggested to regulate the proliferation inhibition and chemorepellant activity of AprA (Bakthavatsalam et al., 2009; Phillips and Gomer, 2012). Here we generated *gα8*− cells in a new background and confirmed that the disruption of *gα8* leads to rapid proliferation. On the other hand, overexpression of *gα8* not only represses proliferation but also induces cytokinesis defects. We also found that overexpression of *gα8* promotes both cell–cell cohesion and cell–substrate adhesion, with the induced cell–substrate adhesion largely contributing to the cytokinesis deficiency. In addition, we present evidence showing that Gα8 modulates stalk cell fate determination and affects spore viability.

Materials and methods

Materials

Wild-type strains including Ax2, JH10, DH1, Ax3, KAx3, and the mutant strains summarized in Table 1 were obtained from dictyBase (*http://dictybase.org/*). Plasmids pLPBLP (Faix et al., 2004), pDM series (pDM304, pDM323, pDM326, and pDM358) (Veltman et al., 2009), GFP-Gβ (Jin et al., 2000), pDdGal-17 (Harwood and Drury, 1990), pVS (Zhang et al., 1999), pEcmAO-i-α-gal (Rafols et al., 2001), pEcmO-i-α-gal, pEcmB-i-α-gal, and pPsA-i-α-gal (Detterbeck et al., 1994) were also obtained from dictyBase. Polyclonal rabbit anti-Gα8 (Wu et al., 1994) and anti-Gα1 (Johnson et al., 1989) antisera were kindly provided by Dr. Peter Devreotes at John Hopkins University. Polyclonal rabbit anti-CadA antiserum (R851) (Knecht et al., 1987) and monoclonal mouse anti-CadA antibody (mLJ11) (Knecht et al., 1987) were kindly gifted by Dr. William Loomis at University of California San Diego. Rabbit anti-tgrC1 antiserum (Geltosky et al., 1979) was kindly provided by Dr. Charles Singleton at Vanderbilt University. Monoclonal mouse anti-CsA antibody (33-294-17) (Bertholdt et al., 1985) was obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. Monoclonal mouse anti-Actin antibody (MAB1501R) was purchased from Millipore. Monoclonal mouse anti-c-myc antibody (46-0603) was purchased from Invitrogen. Monoclonal mouse anti-GFP antibody (11814460001) was purchased from Roche.

Cell culture, growth and development

Cells were axenically maintained in HL-5 medium or grown with *Klebsiella aerogenes* bacteria on SM plates at 22 °C. 100 μg/ml thymidine was supplemented in HL-5 medium for JH10 cells. Wild-type background used in each experiment was indicated in the figure legends. For proliferation measurements of suspension cultures, axenic cells were harvested from plastic petri-dishes, diluted in 50 ml HL-5 medium to 5×10^4 cells/ml, and shaken at 175 rpm, 22 °C. Cell density was measured by a hemacytometer. To measure adherent cell proliferation, cells were spread on 35 mm petri-dishes at a density of 1×10^4 cells/cm^2. At indicated time points, cells were removed thoroughly from the dish bottom by repeatedly pipetting, and the cell number was determined by a hemacytometer. The cell density was defined as cell number divided by petri-dish bottom area. To examine the developmental process, cells were collected from dishes or suspension culture, washed twice with developmental buffer (DB: 5 mM Na_2HPO_4, 5 mM KH_2PO_4, 0.2 mM $CaCl_2$, 2 mM $MgSO_4$, pH 6.5), and then plated on 1.5% non-nutrient DB agar at a density of 5×10^5 cells/cm^2.

Generation of mutant and overexpression strains

All primers used for molecular cloning are listed in Table S1. To disrupt *gα8* in wild-type Ax2 cells, a 677 bp 5′ homologous region and a 726 bp 3′ homologous region were amplified from genomic DNA and directionally cloned into the vector pLPBLP. The resulting construct replaced a small region on exon 2 of *gα8* (genomic DNA fragment bp 690–721, beginning with the start codon ATG) with the Bsr cassette. The knockout construct was linearized by NotI and 2 μg linear DNA was then electroporated into 5×10^6 Ax2 cells. 20 h after transformation, cells were selected with 10 μg/ml Blasticidin S for 10 days. The clones were isolated, diluted and then clonally spread on a *K. aerogenes* lawn for 5 days. Successful gene disruption in plaques was confirmed by PCR of genomic DNA using one primer inside the Bsr cassette and one primer outside the homologous region on the genome (Charette and Cosson, 2004).

The coding region of *gα8* was amplified from the first strand cDNA prepared from Ax2 cells starved for 5 h and cloned into the pDM304, pDM358 and pDM326 expression vectors, respectively. To generate the Gα8-GFP fusion, a SpeI restriction site was first introduced after the amino acid 110 of Gα8 by PCR and then *gα8* was inserted back into pDM304. *gfp* flanked by three glycine codons encoding "-GGG-GFP-GGG-" was amplified from the pEGFP-C1 vector and inserted into the SpeI site of *gα8*. For the inducible expression of Gα8–GFP fusion, the *gα8–gfp* fragment was amplified and cloned into the pVS vector. The point mutations G41V, S46C and Q203L of Gα8 were introduced by PCR and the resulting *gα8* mutants were cloned into the pDM304 vector. The truncated Gα8$^{\Delta Tail}$ was generated by removing the 51 amino acids at the COOH-terminus through PCR and cloning into the pDM304 vector. The DNA fragment "gaacaaaaactcatttcagaagaagattta" encoding the c-myc epitope "EQKLISEEDL" was fused to the NH_2-terminus of the Gγ gene, and the fusion protein was cloned into the pDM358 vector. The coding region of *gα1* was also amplified from the cDNA and cloned into the pDM304 vector. Cells transformed with these expression plasmids were selected with 20 μg/ml G418 or 50 μg/ml Hygromycin B or 10 μg/ml Blasticidin S as required until single colonies emerged. To reduce the expression level of Gα8-GFP, *gα8*−cells carrying *gα8-gfp* driven by the *discoidin I* promoter were either supplemented with 1 mM folate in HL-5 medium or co-cultured with *K. aerogenes* bacteria.

Immunocytochemistry

Cells were grown on coverslips in HL-5 medium overnight, and then washed with phosphate buffered saline (PBS: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.4) twice. Cells

Table 1
Summary of mutants used in this study.

Strain	DictyBase ID	Background	Phenotype mentioned in this study	References
gα8–	DBS0236107	JH10	Rapid proliferation	Bakthavatsalam et al. (2009)
gβ–	DBS0236530	JH10	Rapid proliferation	Bakthavatsalam et al. (2009)
paxB–	DBS0236728	Ax2	Reduced cell–substrate adhesion	Bukharova et al. (2005)
sadA–	DBS0236921	Ax3	Abolished cell–substrate adhesion	Fey et al. (2002)
cadA–	DBS0237013	KAx3	Loss of Ca^{2+}-dependent cell–cell cohesion	Wong et al. (2002)