MOLECULE PAGE

Galpha s

John A. Williams, MD, PhD

Department of Integrated and Molecular Physiology, University of Michigan,
Ann Arbor, Michigan 48109-0622

e-mail: jawillms@umich.edu


Gene symbols: GNAS/Gnas

1. General Information

Gαs is the catalytic subunit of one of the first heterotrimeric G proteins indentified and is among
the best characterized. It becomes activated upon binding GTP and then activates adenyyl
cyclase (AC) leading to the production of cyclic AMP (2,5). Because of the widespread use of
cAMP as a signaling molecule the activating pathway plays a role in many functions including
development, muscle contraction, learning and memory and endocrine and exocrine secretion.
Gαs is expressed in almost all cells and is activated by beta adrenergic, dopamine, H2,
secretin, VIP, TSH, LH and GLP-1 receptors among others. These receptors are all of the 7
transmembrane domain family and when liganded act as a guanine nucleotide exchange factor
(gef) for Gαs and accelerate the release of GDP. The αs subunit can then bind GTP, dissociate
from its associated βγ complex and activate all known isoforms of AC. Gαs has an intrinsic rate
of deactivation by hydrolyzing the bound GTP. Whether this can be accelerated by its effector
acting as a GTPase activating protein (GAP) is unclear. The GDP bound form can also be
activated by aluminum fluoride (5).

Gαs is coded in humans and mice by a gene with
13 exons that can generate multiple gene products through 4 alternative promoters and first
exons (21). Two of these are Gαs which is broadly expressed and an N terminal extended
version XLαs expressed primarily in neuroendocrine tissues. The gene is imprinted in
a tissue specific manner with Gαs being expressed primarily from the maternal allele. The
protein Gαs is a 45 KDa protein which is postranslationally modified by N-terminal palmitoylation which targets it to the plasma
membrane. Gαs is alternatively spliced in exon 3
to produce a long and short form differing by 14
amino acids (2) which function similarly. Gαs is
also located on intracellular membranes and may
play a role in membrane trafficking. This may
involve additional effectors beyond AC.

The structure of Gαs has been solved both while
binding GTPyS or GDP plus AlF and in
combination with its effector AC (17,18). Like
other Gα subunits, Gαs is made up of a ~220
amino acid Ras like GTPase domain which
includes the sites for GTP binding and effector interaction and a ~120 amino acid alpha helical domain that helps form a pocket for guanine nucleotides. Binding of GTP leads to structural movement in several switch regions. Mutations in specific amino acids in these regions can lead to permanent activation or inactivation. Cholera toxin (CT) catalyzes the ADP ribosylation of Arg 201 leading to constitutive activation of Gαs by blocking the GTPase turn off mechanism. An activating mutation (GαsQ227L) exists similar to Q to L mutations in other G proteins.

Mutations in Gαs have been linked to a number of diseases (8,12). Inactivating mutations in Gαs are associated with the inherited disorder, Albrights Hereditary Osteodystrophy or pseudohyperparathyroidism with the resulting syndrome affected by whether the mutation is on the maternal or paternal allele. Activating mutations are associated with pituitary or thyroid adenomas and the McCune-Albright syndrome. Some of these mutations have been reproduced with mouse models (21). Whole body knockout of Gαs is embryonic lethal and heterozygotes show reduced viability. Floxed Gαs mice have been generated and used in a tissue specific manner by the Weinstein group to delete Gαs.

2. Specific Function in the Pancreas

Gαs has been observed by immunohistochemistry to be expressed at high levels in mouse islet beta cells and at lower levels in surrounding acini (22). Within acini, Gαs is localized to the plasma membrane and to a lesser extent intracellularly in the Golgi region (3,9). Isolated rat zymogen granule membranes were reported to contain Gαs by Western blotting in one study (10) but not to be present in another study (9). ADP ribosylation studies have labeled multiple forms of the protein in response to CT in acinar cell membranes (14) and in AR42J cells (7). It has also been identified in rat parotid gland membranes (1,20). We are not aware of similar studies on pancreatic ductal epithelium or in stellate cells.

Functional studies of Gαs have mainly used CT to activate the G protein. CT increases AC activity in pancreatic membranes (6,19), increases cAMP in dissociated acini (4,11,15) and slightly increases in amylase secretion (4,11,15). In the perfused cat and rat pancreas, CT stimulates bicarbonate rich fluid secretion (6,16).

Activation of Gαs can also be carried out by overexpression of Gαs(Q227L) mutant (the long form) either by plasmid or adenoviral vector (13) which increases cyclic AMP in acini. This overexpression of active Gαs did not affect the activation of RhoA or Rac1 and did not affect acinar morphology.

The importance of Gαs can also be assessed by tissue specific knockout studies. Xie et al used a Pdx1-Cre to delete Gαs throughout the pancreas (23). Most of the findings were due to effects on the islets similar to earlier study with Beta cell deletion using Rat insulin-Cre (22) but in addition the pancreas weight was larger than normal and exocrine histology was stated to be altered. More definitive analysis will require deleting in acinar or duct cells independent of the islets.

3. Tools to Study Gαs

a. cDNA clones
cDNA clones for human GNAS are available from the Missouri S & T cDNA Resource Center for both the short and long forms of human GNAS including wild type, Q to L activating mutations, and an internal Glu-Glu epitope tagged version.

b. Antibodies
Biocompare lists 45 commercially available Gαs antibodies. We are unable to provide a recommendation of which ones work.
c. Viral Vectors
A constitutively active Ga_s Q227L mutant has been prepared and used by us in mouse pancreatic acini (13).

d. Mouse lines
Whole body gene deletion is embryonic lethal. A Ga_s with floxed exon 1 has been constructed by the laboratory of Lee Weinstein and used to delete Ga_s in osteoblasts, liver, kidney, and islets (21).

4. References

2. Berlot C. G protein alpha s. UCSD-Nature Molecule Pages Published online: 26 June 2004. doi:10.1038/mp.a000002.01.

