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Galpha s

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Gene symbols: GNAS/Gnas

1. General Information

$G\alpha_s$ 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 adenylyl 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. $G\alpha_s$ is expressed in almost all cells and is activated by beta adrenergic, dopamine, H_2, 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 $G\alpha_s$ and accelerate the release of GDP. The α_s subunit can then bind GTP, dissociate from its associated βγ complex and activate all known isoforms of AC. $G\alpha_s$ 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).

$G\alpha_s$ 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 $G\alpha_s$ which is broadly expressed and an N terminal extended version $XL\alpha_s$ expressed primarily in neuroendocrine tissues. The gene is imprinted in a tissue specific manner with $G\alpha_s$ being expressed primarily from the maternal allele. The protein $G\alpha_s$ is a 45 KDa protein which is postranslationally modified by N-terminal palmitoylation which targets it to the plasma membrane. $G\alpha_s$ is alternatively spliced in exon 3 to produce a long and short form differing by 14 amino acids (2) which function similarly. $G\alpha_s$ is also located on intracellular membranes and may play a role in membrane trafficking. This may involve additional effectors beyond AC.

The structure of $G\alpha_s$ has been solved both while binding GTPγS or GDP plus AlF and in combination with its effector AC (17,18). Like other Gα subunits, $G\alpha_s$ is made up of a ~220 amino acid Ras like GTPase domain which

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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 $G\alpha_s$ by blocking the GTPase turn off mechanism. An activating mutation (GαsQ227L) exists similar to Q to L mutations in other G proteins.

Mutations in $G\alpha_s$ have been linked to a number of diseases (8,12). Inactivating mutations in $G\alpha_s$ 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 $G\alpha_s$ is embryonic lethal and heterozygotes show reduced viability. Floxed $G\alpha_s$ mice have been generated and used in a tissue specific manner by the Weinstein group to delete $G\alpha_s$.

2. Specific Function in the Pancreas

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

The importance of $G\alpha_s$ can also be assessed by tissue specific knockout studies. Xie et al used a Pdx1-Cre to delete $G\alpha_s$ 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 $G\alpha_s$ antibodies. We are unable to provide a recommendation of which ones work.

c. Viral Vectors

A constitutively active $G\alpha_s$ 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 $G\alpha_s$ with floxed exon 1 has been constructed by the laboratory of Lee Weinstein and used to delete $G\alpha_s$ in osteoblasts, liver, kidney, and islets (21).

4. References

1. **Ali N, Agrawal DK, Cheung P.** Identification of G-proteins in rat parotid gland plasma membranes and granule membranes; presence of distinct components in granule membranes. *Mol Cell Biochem* 115: 155-162, 1992. PMID: 1280320
2. **Berlot C.** G protein alpha s. *UCSD-Nature Molecule Pages* Published online: 26 June 2004 l doi:10.1038/mp.a000002.01.
3. **Denker SP, McCaffery JM, Palade GE, Insel PA, Farquhar MG.** Differential distribution of α subunits and βγ subunits of heterotrimeric G proteins on golgi membranes of the exocrine pancreas. *J Cell Biol* 133: 1027-1040, 1996. PMID: 8655576
4. **Gardner JD, Rottman AJ.** Action of cholera toxin on dispersed acini from guinea pig pancreas. *Biochim Biophys Acta* 585: 250-265, 1979. PMID: 222350
5. **Gilman AG.** Nobel Lecture. G proteins and regulation of adenylyl cyclase. *Biosci Reports* 15:65-97, 1995. PMID: 7579036
6. **Kempen HJ, De Pont JJ, Bonting SL.** Rat pancreas adenylate cyclase. III. Its role in pancreatic secretion assessed by means of cholera toxin. *Biochim Biophys Acta* 392: 276-287, 1975. PMID: 1131364
7. **Lambert M, Diem Bui N, Christophe J.** Functional and molecular characterization of CCK receptors in the rat pancreatic acinar cell line AR4-2J. *Regulatory Peptides* 32: 151-167, 1991. PMID: 1709748
8. **Lania A, Mantovani G, Spada A.** G protein mutations in endocrine diseases. *Eur J Endocrinol* 145: 543-559, 2001. PMID: 11720871
9. **Ohnishi H, Ernst SA, Yule DI, Baker CW, Williams JA.** Heterotrimeric G-protein Gq/11 localized on pancreatic zymogen granules is involved in calcium-regulated amylase secretion. *J Biol Chem* 272: 16056-16061, 1997. PMID: 9188511
10. **Padfield PJ, Panesar N.** Identification of Goα. Gqα, and Gsα immunoreactivity associated with the rat pancreatic zymogen granule membrane. *Biochem Biophys Res Commun* 237: 235-238, 1997. PMID: 9268692
11. **Pan G-Z, Collen MJ, Gardner JD.** Action of cholera toxin on dispersed acini from rat pancreas post-receptor modulation involving cyclic AMP and calcium. *Biochim Biophys Acta* 720:338-345, 1982. PMID: 6180774
12. **Plagge A, Kelsey G, Germain-Lee EL.** Physiological functions of the imprinted Gnas locus and its protein variants Gαs and XLαs in human and mouse. *J Endocrinol* 196: 193-214, 2008. PMID: 18252944
13. **Sabbatini ME, Bi Y, Ji B, Ernst SA, Williams JA.** CCK activates RhoA and Rac1 differentially through Gα13 and Gαq in mouse pancreatic acini. *Am J Physiol Cell Physiol* 298: C592-C601, 2010. PMID: 19940064
14. **Schnefel S, Pröfrock A, Hinsch K-D, Schulz I.** Cholecystokinin activates Gi1-, Gi2-, Gi3- and several Gs-proteins in rat pancreatic acinar cells. *Biochem J* 269: 483-488, 1990. PMID: 2117441
15. **Singh M.** Role of cyclic adenosine monophosphate in amylase release from dissociated rat pancreatic acini. *J Physiol* 331: 547-555, 1982. PMID: 6185668
16. **Smith PA, Case RM.** Effects of cholera toxin on cyclic adenosine 3',5'-monophosphate concentration and secretory processes in the exocrine pancreas. *Biochim Biophys Acta* 399: 277-290, 1975. PMID: 169903
17. **Sprang SR, Chen Z, Du X.** Structural basis of effector regulation and signal termination in heterotrimeric Galpha proteins. *Adv Protein Chem* 74: 1-65, 2007. PMID: 17854654
18. **Sunahara RK, Tesmer JJG, Gilman AG, Sprang SR.** Crystal structure of the adenylyl cyclase activator Gsα. *Science* 278: 1943-1947, 1997. PMID: 9395396
19. **Svoboda M, Lambert M, Christophe J.** Distinct effects of the C-terminal octapeptide of cholecystokinin and of a cholera toxin pretreatment on the kinetics of rat pancreatic adenylate cyclase activity. *Biochim Biophys Acta* 675: 46-61, 1981. PMID: 6266495

20. **Watson EL, Di Julio D, Oda D, Izutsu KT.** Identification and localization of G proteins in exocrine glands. *Crit Rev Oral Biol Med* 4: 407-414, 1993. PMID: 8373995
21. **Weinstein LS, Xie T, Zhang Q-H, Chen M.** Studies of the regulation and function of the Gsα gene Gnas using gene targeting technology. *Pharmacology & Therapeutics* 115: 271-291, 2007. PMID: 17588669
22. **Xie,T, Chen M, Zhang Q-H, Ma Z, Weinstein LS**. B cell-specific deficiency of the stimulatory G protein α-subunit Gsα leads to reduced β cell mass and insulin-deficient diabetes. *Proc Natl Acad Sci USA* 104: 19601-19606, 2007. PMID: 18029451
23. **Xie, T, Chen M, Weinstein LS.** Pancreas-specific Gsα deficiency has divergent effects on pancreatic α- and β-cell proliferation. *J Endocrinol* 206: 261-269, 2010. PMID: 20543009