

## MOLECULE PAGE

# Galpha12/13

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Gene symbols: [GNA12](#), [GNA13](#)

## 1. General Function

G $\alpha_{12/13}$  are the unique  $\alpha$  subunits of a class of heterotrimeric G proteins along with G $\alpha_s$ , G $\alpha_{i/o}$ , and G $\alpha_q$ .  $\alpha_{12}$  and  $\alpha_{13}$  were initially cloned from a mouse brain cDNA library by PCR and show 67% amino acid identity with each other but only 35-40% with other G $\alpha$  subunits (32). These  $\alpha$  subunits are expressed in most tissues (34) and are activated by over 25 receptors mostly of the 7TM class but also by some receptor tyrosine kinases (18,27). A *Drosophila* homolog known as  $\alpha_{cta}$  is 55% identical at the amino acid level. The G $\alpha_{12/13}$  polypeptides have a Mol Wt of 43,000 and are not ribosylated by Pertussis or Cholera toxin. They are palmitoylated at a cysteine residue near the N-terminal which is believed important for plasma membrane targeting (1); G $\alpha_{12}$  but not G $\alpha_{13}$  is located predominately in lipid rafts (37). This targeting also depends upon interaction with HSP90. In addition, the biochemical properties of G $\alpha_{12}$  can be modified by phosphorylation and PKC phosphorylates purified  $\alpha_{12}$  (18). Not surprisingly because of their divergence in sequence,  $\alpha_{12}$  and  $\alpha_{13}$  do not always show the

same effect (2,13) and are not always activated by the same agonist (24). This difference is mediated by a short N terminal sequence where homology is only 16% (39). In addition to these differences, in several cell types G $\alpha_{12}$  and G $\alpha_{13}$  have shown different subcellular localization with G $\alpha_{12}$  localized to the plasma membrane while G $\alpha_{13}$  localizes to the cytosol and upon stimulation translocates to the plasma membrane (40). A striking difference is that G $\alpha_{12}$  deficient mice are viable with no obvious phenotype while G $\alpha_{13}$  deficient mice die in mid-gestation with defects in angiogenesis (26). This defect is due to an essential role for G $\alpha_{13}$  in endothelial cells (28).

As with other G proteins, G $_{12/13}$  undergoes a cycle where receptor induced activation involves binding of GTP which is latter hydrolyzed to GDP returning the protein to the inactive state. These changes are mediated by GEFs and GAPs with the receptor acting as a GEF when liganded. Several RGS proteins particularly RGS1 and -16 may also regulate G $_{12/13}$  (13). Constitutively active forms (G $\alpha_{12}$  Q229L or G $\alpha_{13}$  Q226L) can induce transformation of fibroblasts and tumorigenesis in animal models. G $\alpha_{12}$  has a role in cell-cell

interactions, invasion and differentiation (14). In addition to stimulating DNA synthesis, active  $G\alpha_{12/13}$  promotes stress fibers and cell adhesion, inhibits cadherin-induced aggregation, activates or inhibits  $Na^+ - H^+$  exchange, stimulates smooth muscle contraction, and affects secretion (11,13,22). Tissue specific knockout studies have shown the requirement for  $G\alpha_{12/13}$  for developmental cell migration in the brain (21).  $G\alpha_{12/13}$  also plays a role in platelet activation, cardiovascular function and immune function (25,38).

Use of constitutively active mutants of  $\alpha_{12}$  and  $\alpha_{13}$  has generally shown that these G proteins do not regulate adenylate cyclase or phospholipase C ( but see Ref 10 for an exception). The most well studied action of  $G\alpha_{12/13}$  is to activate the small G protein Rho in response to a GPCR and through RhoA and its downstream effectors affect the actin cytoskeleton, cell migration and invasion, phospholipase D activation, protein kinase D activation,  $Na^+ - H^+$  exchange, JNK activation and serum response factor (SRF) production (4,7,13,15,22,27,30,33). All these actions are sensitive to *C. botulinum* C3 exotoxin which inactivates Rho. More recently, Rho activation has been directly measured through pull down assays. The primary mechanism for  $G\alpha_{12/13}$  to activate Rho involves Rho GEFs which contain a RGS like domain that binds to active  $\alpha_{12}$  or  $\alpha_{13}$  (31). Three Rho GEFs have been identified with RGS like domains near the amino terminus, p115 RhoGEF, PDZ-RhoGEF and leukemia associated RhoGEF (LARG) (6,35). Another RhoGEF, Lbc-RhoGEF has a RGS like domain of lower homology in the carboxyl terminus and is activated selectively by  $G\alpha_{12}$  (5). All of these RhoGEFs are potent RhoA activators while the isolated RGS like domain from p115 RhoGEF when overexpressed acts as a specific inhibitor of  $G_{12/13}$  signaling through Rho. In some cases for full RhoGEF activation the GEF also has to be phosphorylated by a nonreceptor tyrosine kinase.  $G\alpha_{13}$  also activates a RhoGEF without a RGS like

domain, proto-Dbl which translocates to the plasma membrane where it interacts with ezrin (36). In addition to these RhoGEFs, activated  $G\alpha_{12/13}$  can also bind to certain cadherins, the protein radixin of the ERM family, some nonreceptor tyrosine kinases (Bruton's tyrosine kinase or BTK and Tec) , some AKAPS, zonula occludens proteins, protein phosphatase type 5 and JNK-interacting protein (JIP) (12,13,17). Thus in some cell types active  $G\alpha_{12}$  or  $G\alpha_{13}$  can activate signaling independent of Rho. These include activation of JNK, ERKs, Pyk2, and phospholipase A2. For example, in a thyroid cell line,  $G\alpha_{13}$  but not  $G\alpha_{12}$  activated ERK and subsequent induction of c-Fos independent of Rho (3).

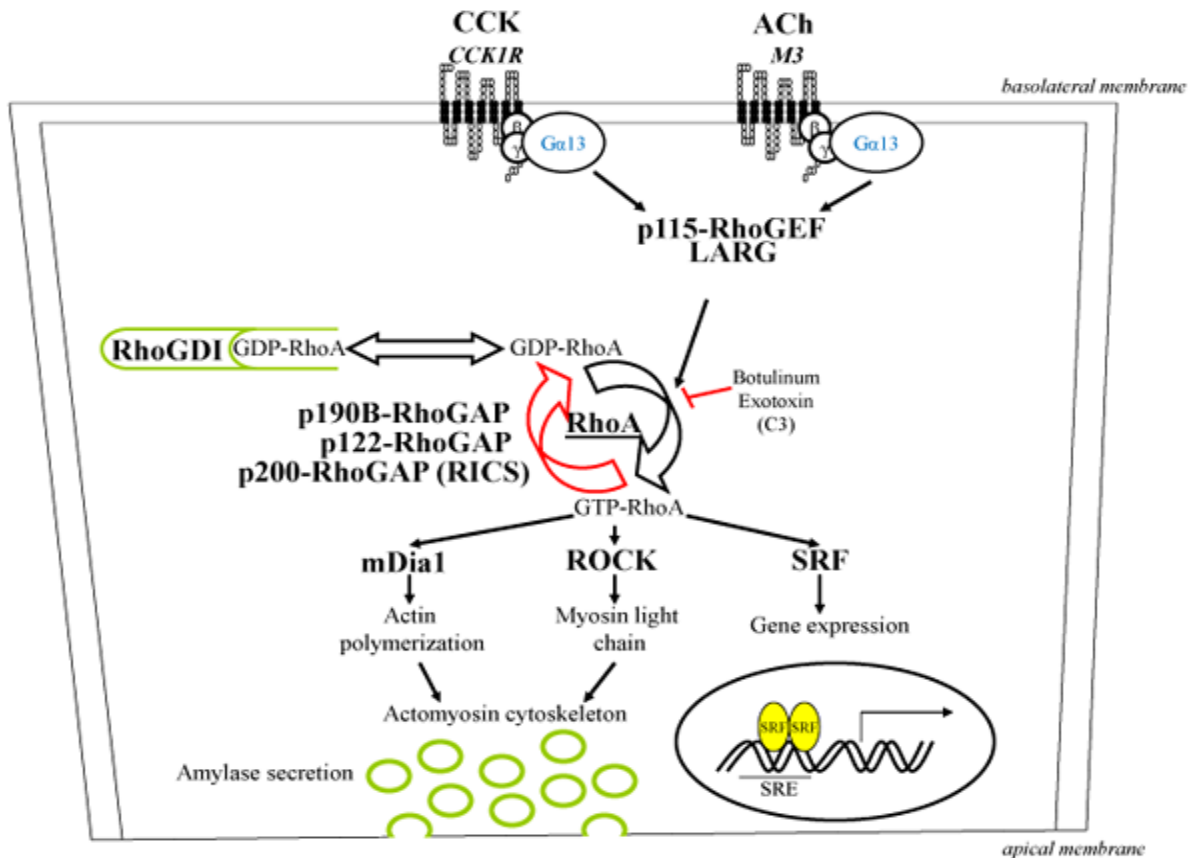
## 2. Specific Function in the Pancreas

Only a few studies have addressed the role of  $G\alpha_{12/13}$  in pancreatic cells. Both  $\alpha_{12}$  and  $\alpha_{13}$  were reported to be present in rat pancreatic acini as shown by Western blotting (16). In this study CCK was shown to rapidly increase the expression of both  $\alpha_{12}$  and  $\alpha_{13}$  as well as increasing the association of RhoA and Vav2 with  $G\alpha_{13}$  but not  $G\alpha_{12}$ . In mouse pancreas and pancreatic acini both PCR and Western blotting revealed the presence of  $G\alpha_{13}$  but not  $G\alpha_{12}$  (29). In accord with previous studies (1),  $G\alpha_{13}$  was associated with a membrane fraction in both control and stimulated acini.

Constitutively active  $G\alpha_{13}$  (Q226L) delivered by adenoviral vector was shown to activate RhoA similar to CCK in mouse acini and to alter the actin cytoskeleton leading to bleb formation (29). In this study, expression of a p115 RhoGEF RGS like domain (p115-RGS) abolished RhoA activation in response to CCK suggesting that the action of CCK receptors to activate RhoA was mediated by  $G\alpha_{12/13}$ . Similar results had been reported earlier in intestinal smooth muscle cells expressing  $G\alpha_{13}$  (23) and in NIH 3T3 cells stably transfected with CCKA receptors (19). A

mutant form of p115-RGS (E29K) failed to modify CCK-induced RhoA activation (29). The effect of p115-RGS expression was shown to be specific for  $G_{12/13}$  signaling as it had no effect on  $Ca^{2+}$  mobilization or cAMP formation. Expression of p115-RGS inhibited both basal and CCK-stimulated amylase release. Prior studies had shown that inhibition of RhoA activation by C3 exotoxin or dominant negative RhoA also inhibited amylase release (2). Thus these results suggest that CCK-induced activation of  $G_{\alpha_{13}}$  in addition to CCK-induced activation of  $G_{\alpha_{q/11}}$  is responsible for induction of amylase secretion. Whereas activation of  $G_{\alpha_q}$  stimulates PLC and calcium mobilization, activation of  $G_{\alpha_{13}}$  induces activation

of RhoA and reorganization of the actin cytoskeleton. At present the nature of the specific RhoGEF activated in acinar cells by  $G_{\alpha_{13}}$  is unknown. Both p115 RhoGEF and LARG have been identified by PCR in acinar cells (unpublished data). Whether  $G_{\alpha_{12/13}}$  will have other actions in acinar cells or plays a role in other pancreatic cells such as pancreatic stellate cells remains to be determined. The position of  $G_{\alpha_{13}}$  in the pathway of Rho activation in mouse acinar cells is shown in the schema below. For further details see  $G_{\alpha_{12/13}} - RhoA$  in the Pathways section.



### 3. Tools to Study $G\alpha_{12/13}$

#### **a. cDNA Clones**

Multiple clones for  $G\alpha_{12/13}$  are available from Missouri Science and Technology cDNA resource. A number of investigators have prepared or used plasmids for  $G\alpha_{12/13}$  and their constitutively active mutants,  $G\alpha_{12}$  Q229L and  $G\alpha_{13}$  Q226L. Dominant negative mutants have also been prepared,  $G\alpha_{12}$  G228A and  $G\alpha_{13}$  G225A and shown to block stress fiber formation (8).

#### **b. Antibodies**

We have used antibodies from Santa Cruz to  $G\alpha_{12}$  (sc-409) and  $G\alpha_{13}$  (sc-410) and a rabbit polyclonal to  $G\alpha_{12}$  from Abcam (ab35016) for Western blotting in our studies of mouse pancreas (29).

Other commercial antibodies to  $G\alpha_{12}$  are listed in Ref (18). A series of antibodies against peptide sequences and validation of their specificity was also carried out by the group of G. Schultz (34).

#### **c. Viral Vectors**

Constitutively active  $G\alpha_{13}$  Q226L in a adenoviral vector has been prepared and used by us in mouse pancreatic acini (29).

#### **d. Mouse lines**

Gene deletion has been carried out for  $Gna13$  where mice died around embryonic day 10 (26) and for  $Gna12$  where mice develop normally (9). Mice with floxed  $Gna13$  have been generated and used for tissue specific deletion with Cre alone or combined with  $Gna12$  deletion (20,21).

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