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## Expression of the G $\beta$ <sub>5</sub>/R7-RGS protein complex in pituitary and pancreatic islet cells

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### Introduction

Heterotrimeric G protein subunit G $\beta$ <sub>5</sub> is expressed primarily in the nervous system and retina and, among G $\beta$  isoforms, is unique in its ability to heterodimerize with regulator of G protein signaling (RGS) proteins of the R7 subfamily (R7-RGS) [see [1, 2] for recent reviews]. The R7-RGS subfamily consists of RGS proteins 6, 7, 9, and 11 which can form tight heterodimers with the G protein  $\beta$ <sub>5</sub> subunit through a G $\gamma$ -like (GGL) domain [1, 2]. R7-RGS subfamily members are unstable in the absence of G $\beta$ <sub>5</sub> [3]. Previous study from our laboratory showed that in addition to its expression in brain and neural cell lines, G $\beta$ <sub>5</sub> was also expressed in the  $\alpha$ T3-1 gonadotrophic pituitary cell line [4]. We show now that the G $\beta$ <sub>5</sub>/R7-RGS complex is expressed in corticotroph-derived pituitary AtT-20 cells, pituitary gland and purified pancreatic islets. The expression of G $\beta$ <sub>5</sub> in various endocrine cell lines and native tissues raises the possibility that hormone secretion, and not just neurotransmission and phototransduction, may be regulated by the G $\beta$ <sub>5</sub>/R7-RGS complex.

### Materials and methods

Antibodies employed in immunoblots and immunohistochemistry included normal rabbit IgG, rabbit anti-G $\beta$ <sub>5</sub> polyclonal N-terminal antibody ATDG [5], anti- $\beta$ -actin mouse monoclonal (Sigma, A-5315), mouse anti- $\alpha$ -tubulin (Calbiochem, CP06), mouse anti-ACTH monoclonal antibody (Abcam, ab8615), mouse anti-p84/N5 [5E10] monoclonal antibody (GeneTex, GTX70220), rabbit anti-RGS7 polyclonal antibody 7RC-1 (cross-reacts with RGS6) [6], and rabbit anti-RGS9-2 polyclonal antibody RGS9CT (raised against the synthetic peptide 31-mer H-CRSPRK PFASPSRFIRRPISIAICPSPSRVAL-NH<sub>2</sub>). Secondary antibodies included HRP-conjugated goat anti-mouse (no. 1858413) and anti-rabbit (no. 1858415) antibodies from Pierce. AtT-20 cells were grown in 75-cm<sup>2</sup> flasks at 37°C and 5% CO<sub>2</sub> containing DMEM supplemented with 10% bovine serum, 5% horse serum, 4 mM L-glutamine, and penicillin/streptomycin (Biofluids, Rockville, MD). MIN6 cells were cultured in DMEM medium containing 4.5 g/L glucose, 10% fetal bovine serum and penicillin/streptomycin. Fractions of cultured cells were prepared using NE-PER Nuclear

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and Cytoplasmic Extraction Reagents (Pierce, 78833) according to the manufacturer's instructions. For isolation of pancreatic islets the procedure of Saeki et al. [7] was followed with minor modification. For immunohistochemistry and immunofluorescence, pituitary glands were excised from the ventral portion of mouse brain and fixed overnight in 4% paraformaldehyde, and then incubated in 70% ethanol. The preserved pituitary was processed for paraffin embedding and microscopic sectioning (Histoserv, Inc., Germantown, MD). Slides were prepared for immunohistochemistry according to the manufacturer's recommendations (Bethyl Labs, Immunohistochemistry Accessory Kit, no. IHC-101). Confocal laser microscopy employed a Leica SP2-UV 405 confocal microscope (Leica Microsystems, Exton, PA, USA). Mice with a heterozygous deletion of exon 3 of *Gnb5* in the germline were kindly provided by Dr. Ching-Kang Jason Chen (Virginia Commonwealth University Medical Center, Richmond, VA). *Gnb5* KO pups were generated from heterozygotes as previously described [8].

## Results

We looked for expression of  $G\beta_5$  and R7-RGS protein in AtT-20 cells, a well-characterized mouse pituitary tumor-derived cell line that expresses adrenocorticotropic hormone (ACTH).  $G\beta_5$  and RGS7 were expressed in whole cell lysates of AtT-20 cells comparable to their expression in neuron-like PC12 cells (Fig. 1a). Subcellular fractionation showed that in AtT-20 cells, as in mouse brain and PC12 cells [9],  $G\beta_5$  and RGS7 are targeted to both the cytoplasm and the nucleus (Fig. 1b).

Because AtT-20 cells were derived from a pituitary tumor, we studied  $G\beta_5$  expression in native mouse pituitary gland. The expression of  $G\beta_5$  as well as the  $G\beta_5$ -dependent R7-RGS proteins RGS7 and RGS9-2 was evident in wild-type, but not *Gnb5* KO [3], pituitary lysate (Fig. 1c). To investigate the regional expression of  $G\beta_5$  in mouse pituitary, we performed dual immunofluorescence staining using anti- $G\beta_5$  and anti-ACTH antibodies (Fig. 1d).  $G\beta_5$  is widely expressed in both anterior and posterior pituitary lobes and it co-localizes with ACTH in the *pars intermedia* of the anterior murine pituitary gland (Fig. 1d).

Since  $G\beta_5$  was expressed widely throughout both the anterior and posterior mouse pituitary we wondered whether it might also be expressed in neuroendocrine islet cells of the pancreas. Immunoblots of insulinoma-derived MIN6 mouse pancreatic islet  $\beta$ -cells and a purified preparation of mouse pancreatic islets demonstrated robust expression of  $G\beta_5$  to a level comparable to that of neuronal controls (Fig. 1e, f).

## Discussion

The expression of  $G\beta_5$  and R7-RGS subfamily RGS proteins in brain and retina is well documented (reviewed in [1, 2]), yet previous study from our laboratory suggested that  $G\beta_5$  was also expressed in cells of neuroendocrine origin, including the  $\alpha$ T3-1 pituitary cell line [4]. In this study, besides documenting the expression of  $G\beta_5$  in corticotroph and islet  $\beta$ -cell derived cell lines, we demonstrate the unequivocal expression of  $G\beta_5$  in native endocrine tissue including cells of the pituitary and in pancreatic islet cells. The pancreatic islet cells derive from embryonic endodermal anlage, while the anterior and posterior pituitary develop from oral and neural ectoderm respectively. The expression of  $G\beta_5$  in tissues of such disparate embryologic origins implies an important role for the  $G\beta_5$ /R7-RGS complex in neuroendocrine cells and in the physiology of endocrine systems. Indeed, targeted disruption of the  $G\beta_5$  partner RGS7 in *Rgs7<sup>tm1Lex</sup>* mice results in a phenotype of impaired glucose tolerance (Mutant Mouse Regional Resource Center, strain 011655; see <http://www.mmrrc.org/strains/11655/011655.html>). Furthermore, while this manuscript was being completed, Slepak and co-workers published evidence suggesting a role for  $G\beta_5$  in the

regulation of energy metabolism at the organismal level and confirming the presence of the  $G\beta_5$  complex in cultured MIN6 cells and isolated pancreatic islets [10].

Even in the nervous system, few details of the function of the  $G\beta_5/R7$ -RGS complex at the cell biological level are understood. It is known that the  $G\beta_5/R7$ -RGS complex exhibits GTPase activating protein (GAP) activity for heterotrimeric  $G\alpha$  subunits and thus can function as a negative regulator of signaling to effectors from GPCRs. Studies in vitro have found that the GAP activity of  $G\beta_5/R7$ -RGS complexes is most efficacious against pertussis-sensitive  $G_o\alpha$  subunits [11]. The expression of  $G\beta_5$  in pituitary and islet cells makes it likely that modulation of such GPCR-coupled pertussis-sensitive pathways by the  $G\beta_5/R7$ -RGS complex may control hormone release and endocrine function in a physiologically important, and perhaps pharmacologically manipulable, fashion.

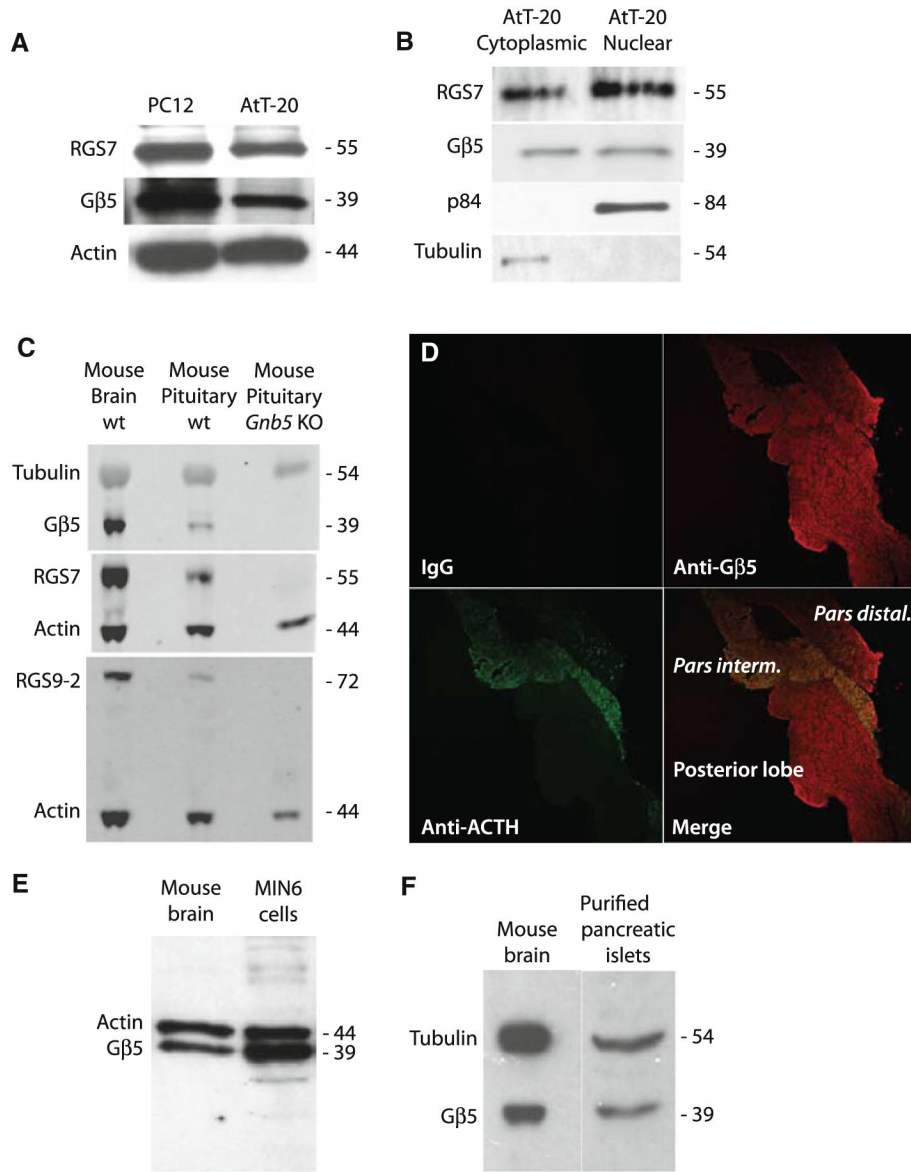
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**Fig. 1.** Expression of  $G\beta_5$  in AtT-20 cells, mouse pituitary, MIN6 insulinoma cells, and purified mouse pancreatic islets. **a** Whole cell lysates from AtT-20 and PC12 cells were prepared and analyzed for  $G\beta_5$  and RGS7 by SDS-PAGE followed by immunoblotting. The corresponding  $\beta$ -actin levels are shown as loading controls. **b** Subcellular distribution of  $G\beta_5$  and RGS7 in AtT-20 cells and PC12 cells are shown by immunoblotting using p84/N5 as marker for the nuclear fraction and  $\alpha$ -tubulin as a marker for the cytoplasmic fraction. **c** Whole lysates were prepared from mouse brain and wild-type or  $G\beta_5$ -knockout mouse pituitary gland and analyzed for  $G\beta_5$ , RGS7, or RGS9-2 by immunoblotting with the corresponding tubulin or  $\beta$ -actin levels shown as loading controls. **d** Slides of mouse pituitary were stained using control IgG, anti- $G\beta_5$ , or anti-ACTH antibodies as indicated. The primary antibodies were visualized with Cy3-conjugated secondary antibody (red) for  $G\beta_5$  and with FITC-conjugated secondary antibody (green) for ACTH. The slides were analyzed using confocal laser microscopy. Magnification is  $\times 40$ . **e** Whole cell lysates from MIN6 mouse insulinoma cells or mouse brain were prepared and analyzed for  $G\beta_5$  by

immunoblotting. The corresponding  $\beta$ -actin levels are shown as loading controls. **f** Whole lysates from mouse brain or a purified preparation of pancreatic islets were prepared and analyzed for  $G\beta_5$  by immunoblotting. The corresponding  $\alpha$ -tubulin levels are shown as loading controls