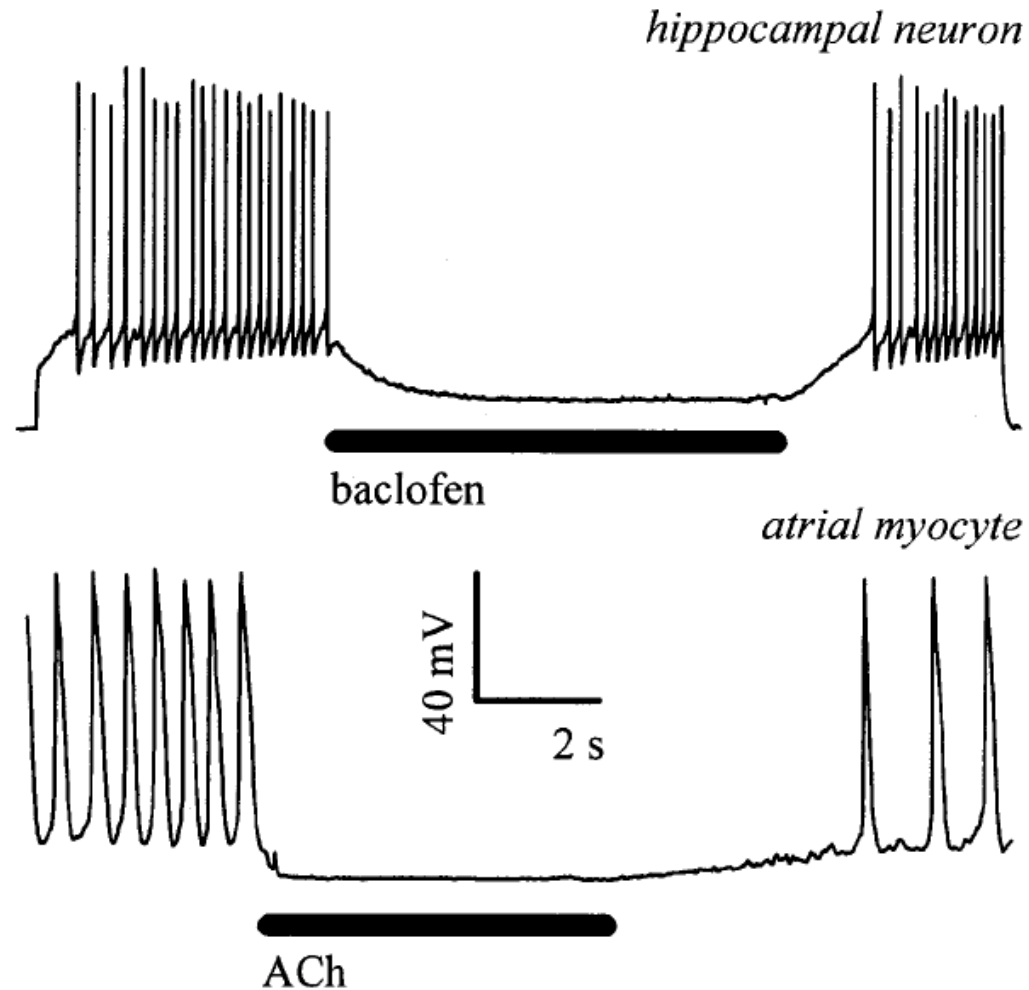


Modulation of Neuronal Ion channels by ‘Regulators of G protein Signaling’

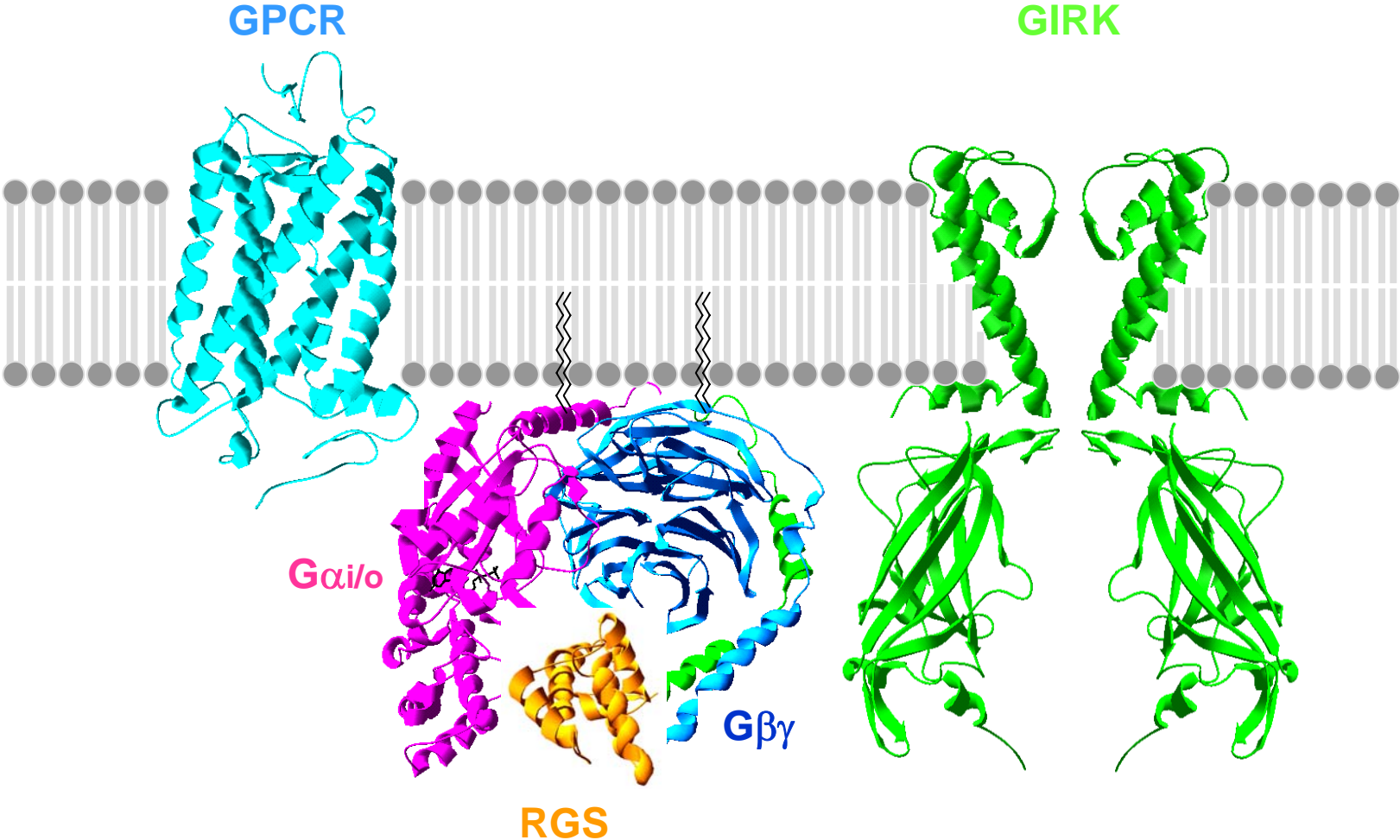
Craig A. Doupnik, Ph.D.

Department of Molecular Pharmacology & Physiology
University of South Florida College of Medicine
Tampa, FL

GPCR-activated GIRK channels inhibit membrane excitability

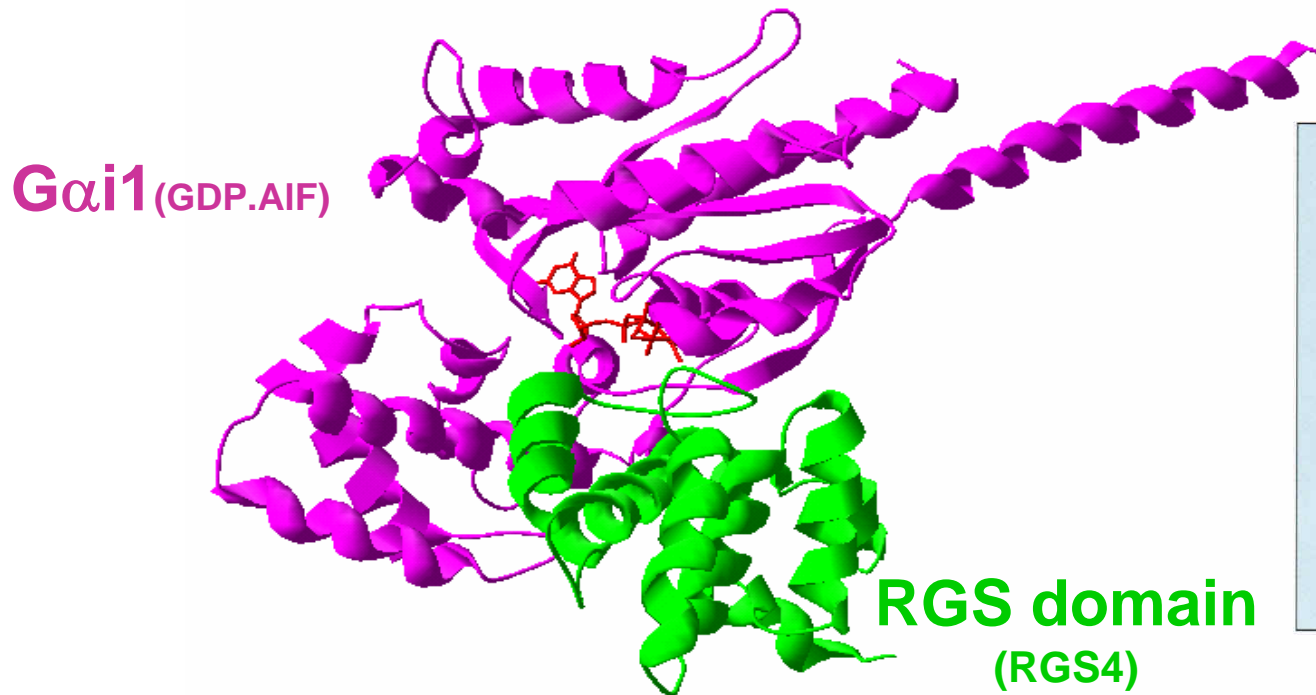


Key Components of the GPCR-GIRK channel signaling complex (2005)

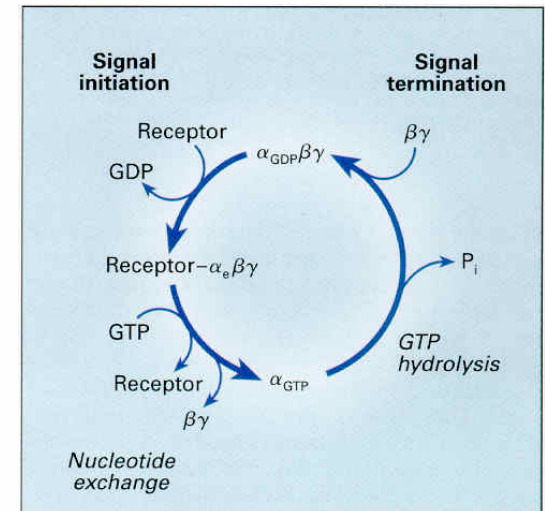


RGS proteins are GTPase-activating proteins (GAP's) via direct interactions with G α subunits

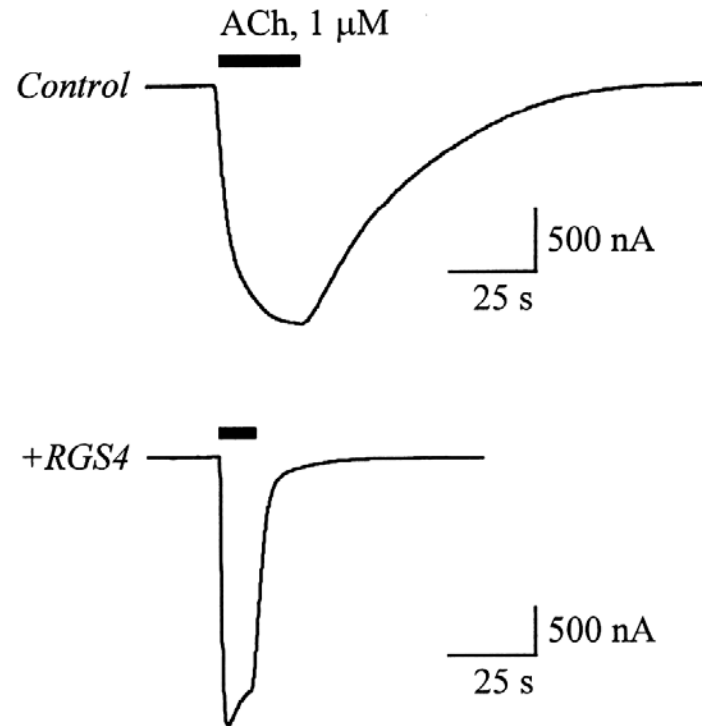
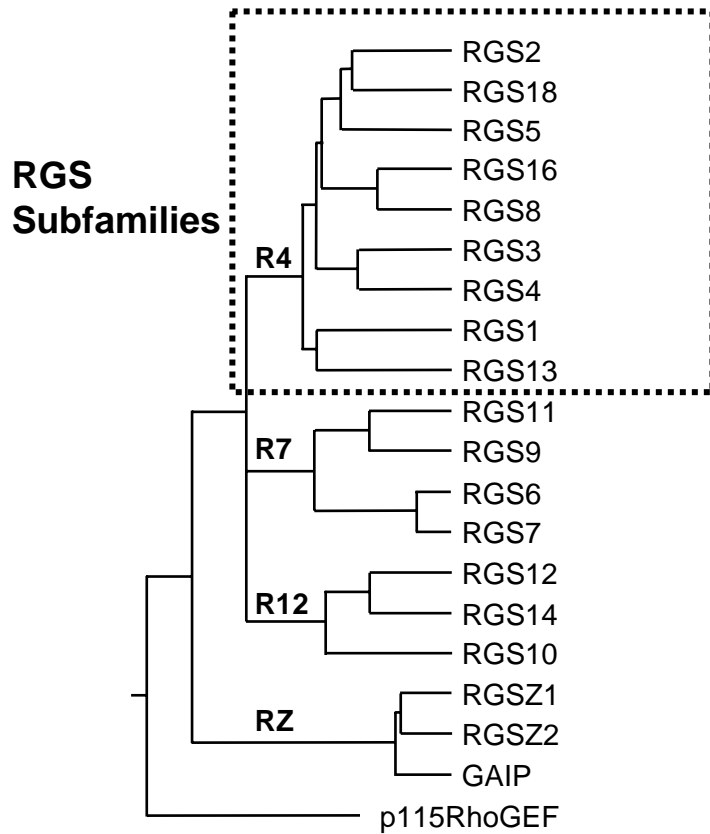
(Tesmer et al., 1997)



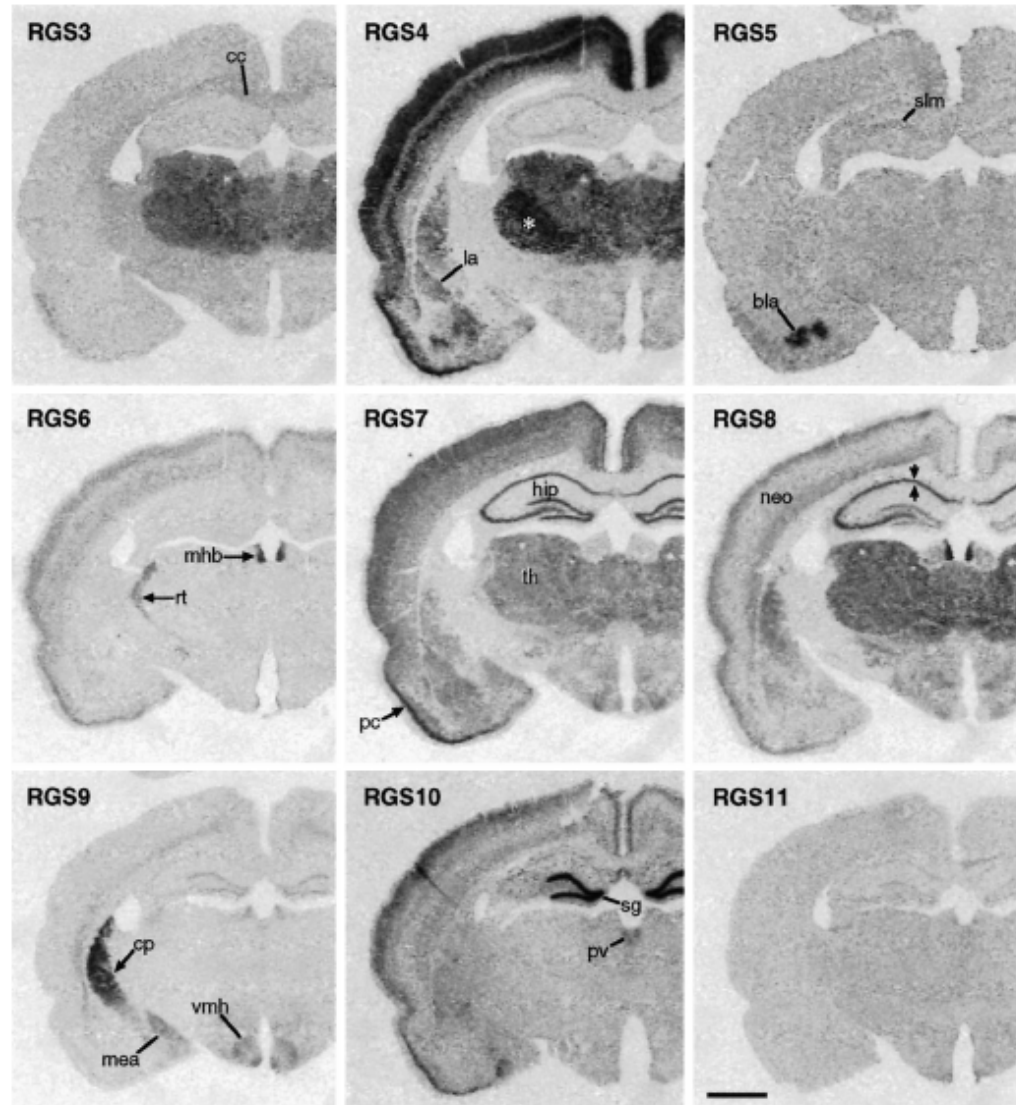
G protein cycle



RGS4 accelerates the kinetics of GPCR-activated GIRK channels



RGS4 is highly expressed in the thalamus and neocortex





ORIGINAL RESEARCH ARTICLE

Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia

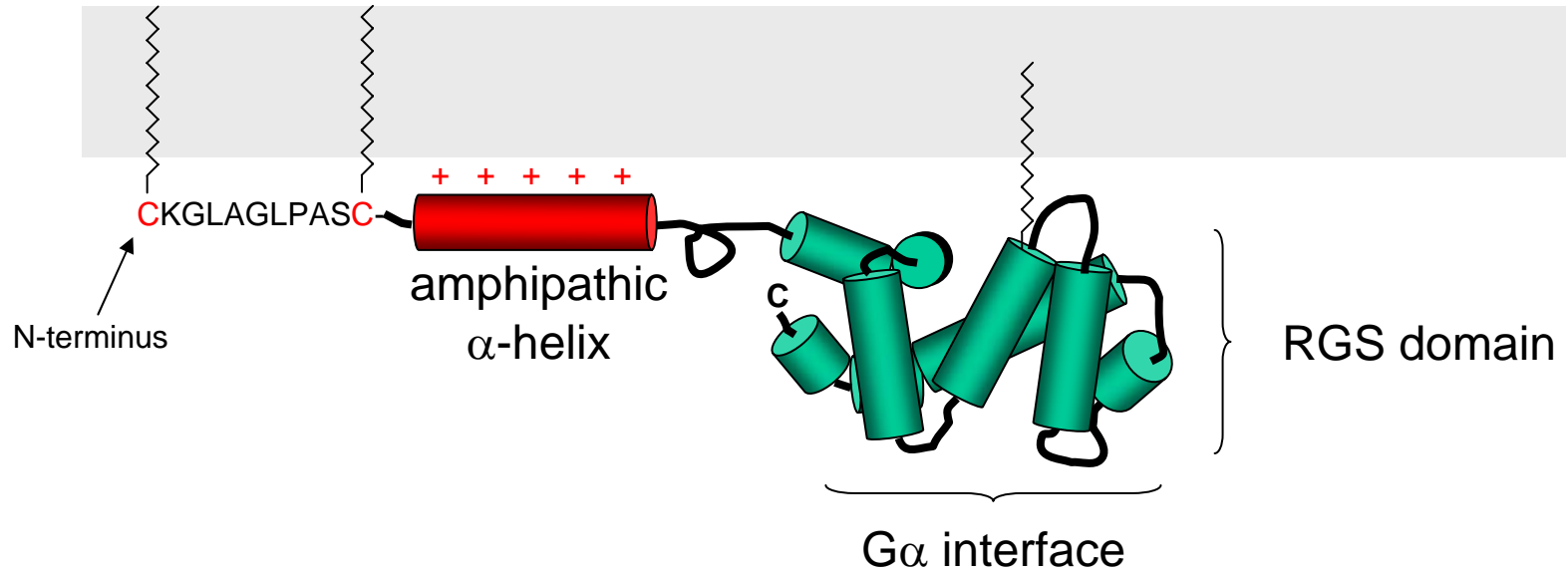
K Mirnics^{1,2,4}, FA Middleton¹, GD Stanwood¹, DA Lewis^{2,3} and P Levitt^{1,4}

Departments of ¹Neurobiology; ²Psychiatry; ³Neuroscience; ⁴PittArray University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

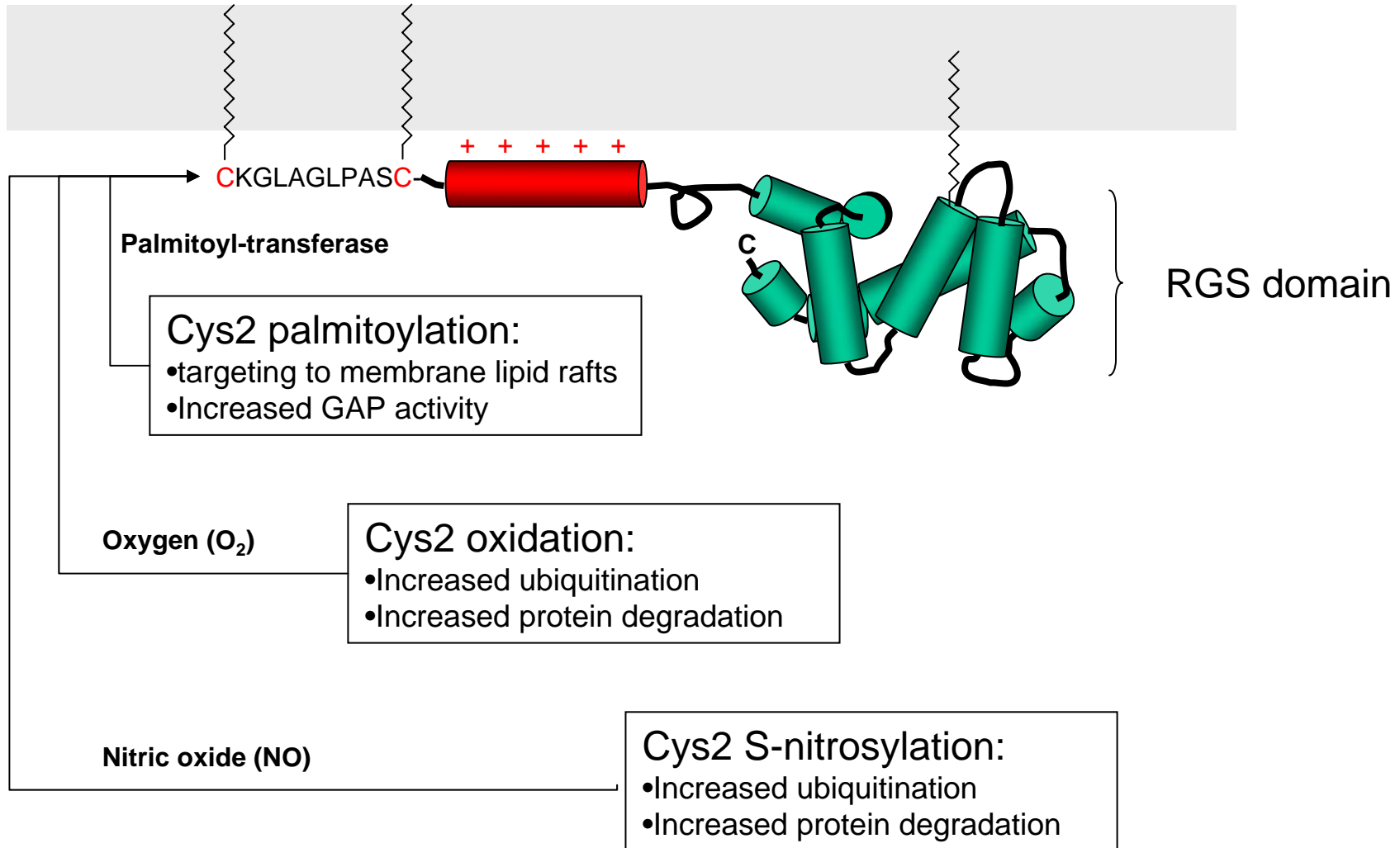
Complex defects in neuronal signaling may underlie the dysfunctions that characterize schizophrenia. Using cDNA microarrays, we discovered that the transcript encoding regulator of G-protein signaling 4 (RGS4) was the most consistently and significantly decreased in the prefrontal cortex of all schizophrenic subjects examined. The expression levels of ten other RGS family members represented on the microarrays were unchanged and hierarchical data analysis revealed that as a group, 274 genes associated with G-protein signaling were unchanged. Quantitative *in situ* hybridization verified the microarray RGS4 data, and demonstrated highly correlated decreases in RGS4 expression across three cortical areas of ten subjects with schizophrenia. RGS4 expression was not altered in the prefrontal cortex of subjects with major depressive disorder or in monkeys treated chronically with haloperidol. Interestingly, targets for 70 genes mapped to the major schizophrenia susceptibility locus 1q21–22 were present on the microarrays, of which only RGS4 gene expression was consistently altered. The combined data indicate that a decrease in RGS4 expression may be a common and specific feature of schizophrenia, which could be due either to genetic factors or a disease-specific adaptation, both of which could affect neuronal signaling. *Molecular Psychiatry* (2001) 6, 293–301.

Keywords: schizophrenia; major depression; antipsychotic; haloperidol; gene expression; regulator of G-protein signaling; RGS4; microarray; prefrontal; cerebral cortex; susceptibility gene; 1q21–22

Structural features of RGS4



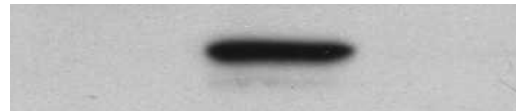
Cys2 in RGS4 is a site of convergent modification: Palmitoylation, Oxidation, and S-nitrosylation



RGS4 is rapidly degraded when expressed in CHO-K1 cells and is 'rescued' by a C2V mutation

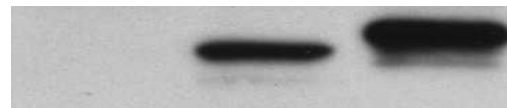
A.

HA-m2R	+	+	+
Kir3.1-MYC / Kir3.2a	+	+	+
RGS3s-FLAG	-	+	-
RGS4-FLAG	-	-	+



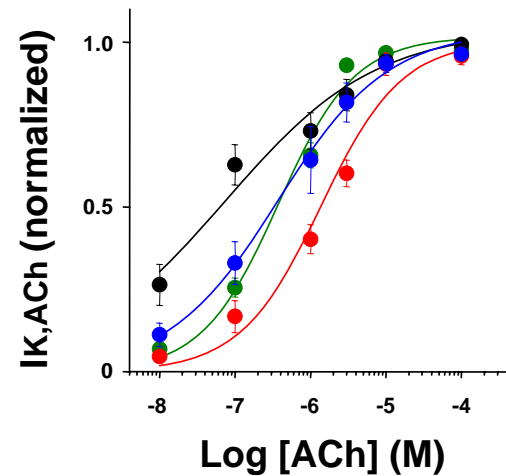
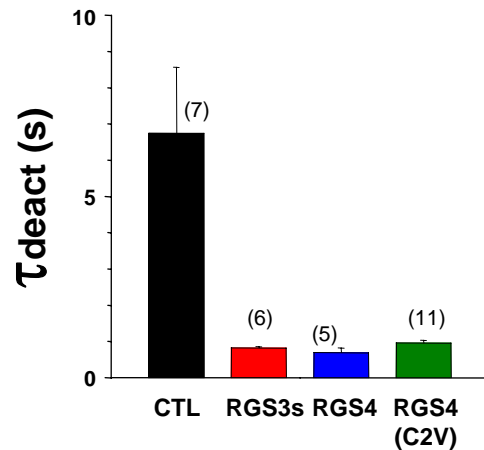
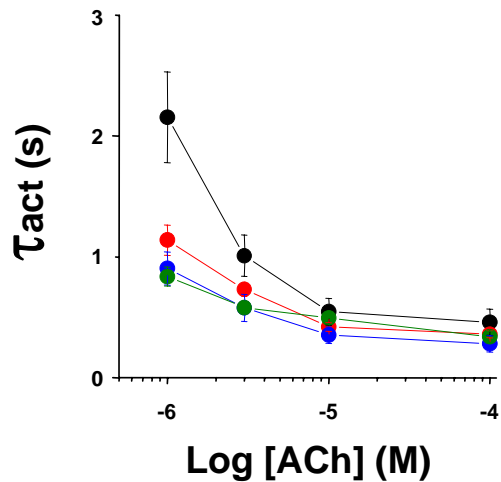
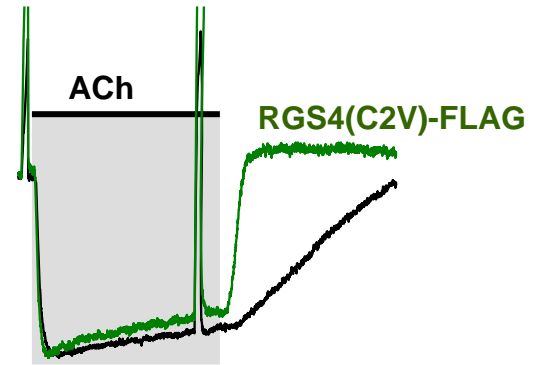
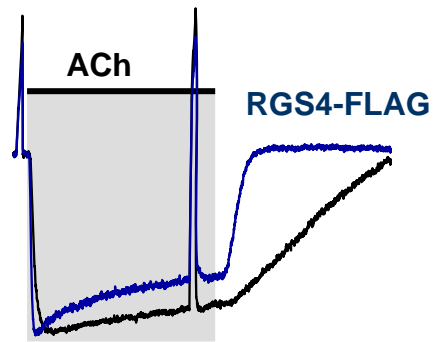
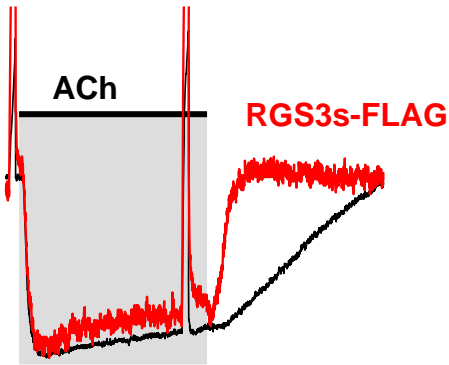
B.

HA-m2R	+	+	+
Kir3.1-MYC / Kir3.2a	+	+	+
RGS3s-FLAG	-	+	-
RGS4(C2V)-FLAG	-	-	+

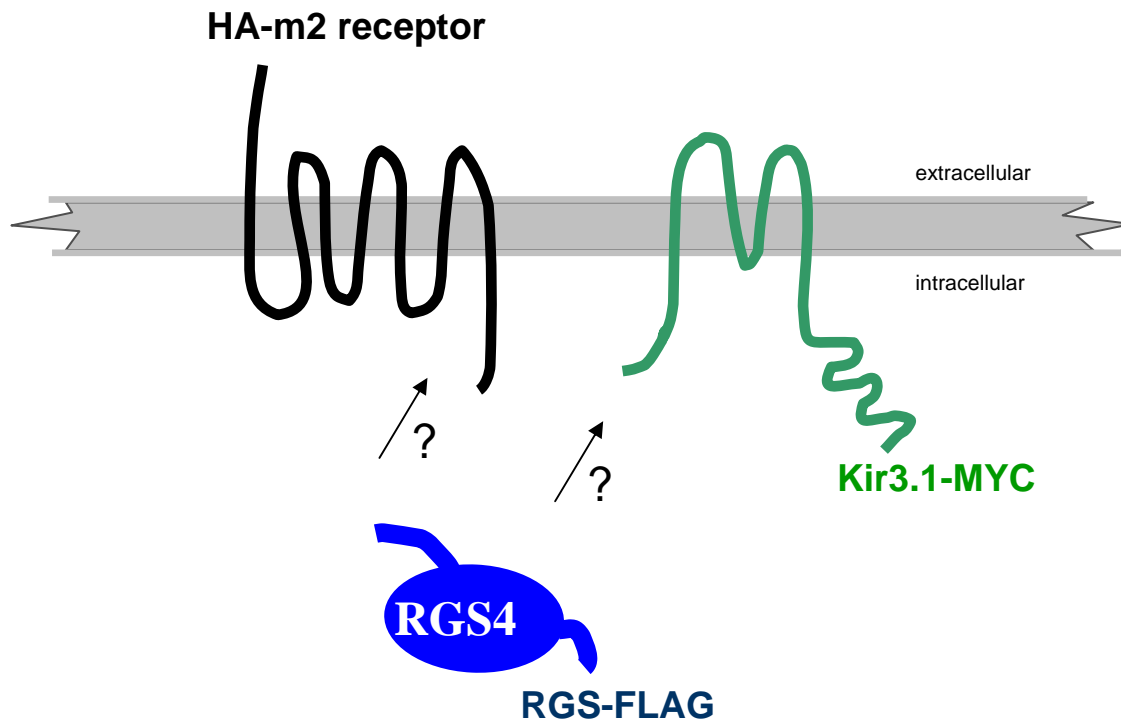


Electrophysiological Recordings from transfected CHO-K1 cells:

RGS4 and RGS4(C2V) both accelerate GIRK channel kinetics

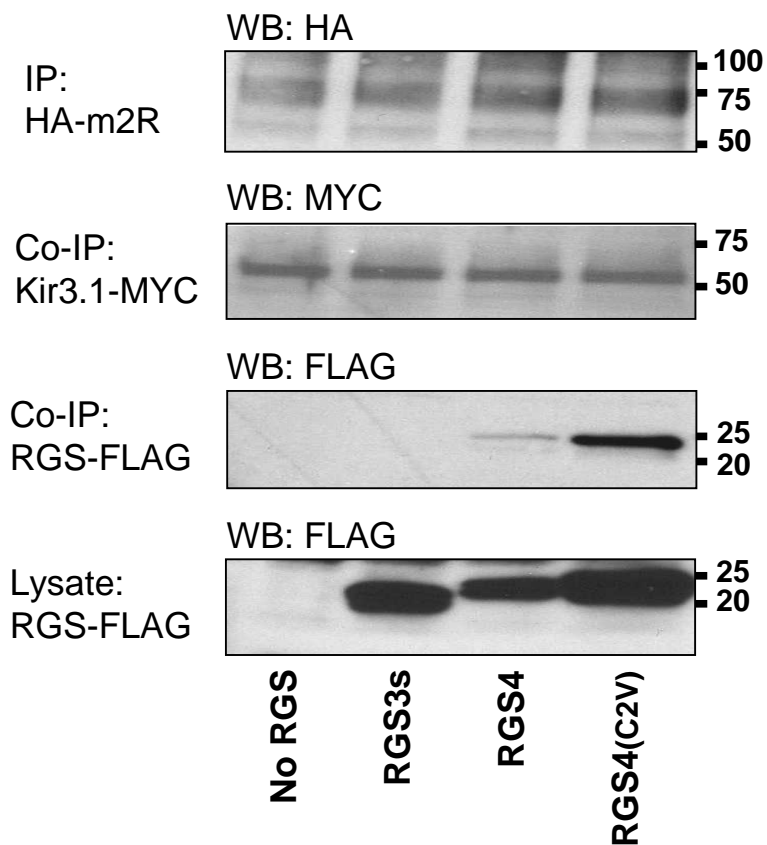


Does RGS4 directly associate with GPCR-GIRK channel complexes?



Immunoprecipitation of GPCR-GIRK complexes include RGS4

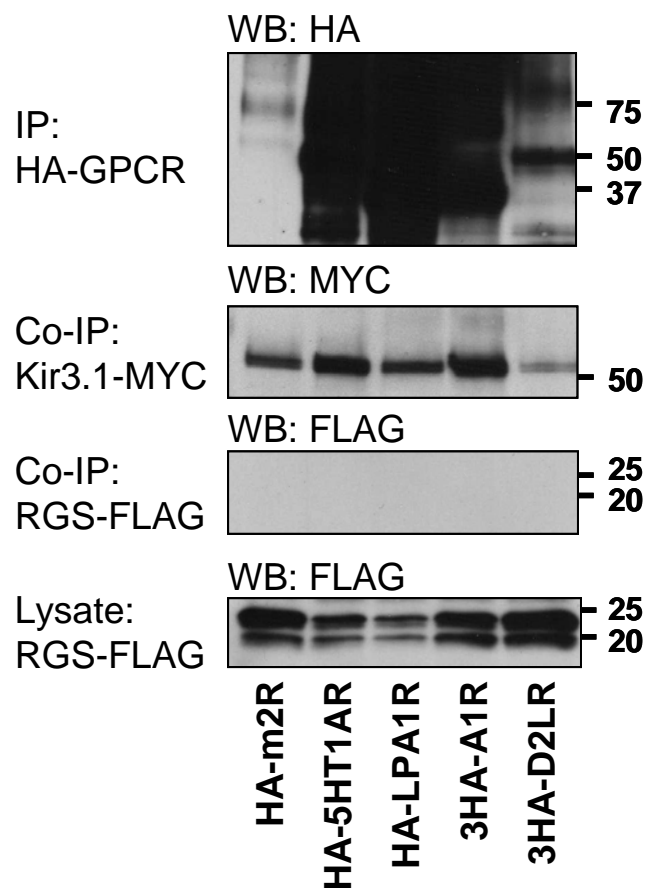
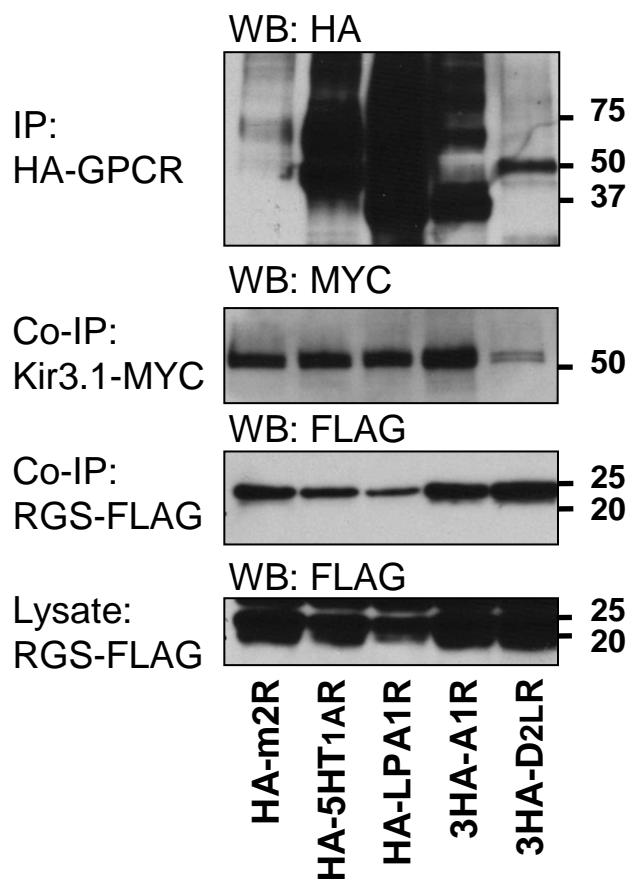
HA-m2R	+	+	+	+
Kir3.1-MYC / Kir3.2a	+	+	+	+
RGS-FLAG	-	+	+	+
Gαi2	+	+	+	+



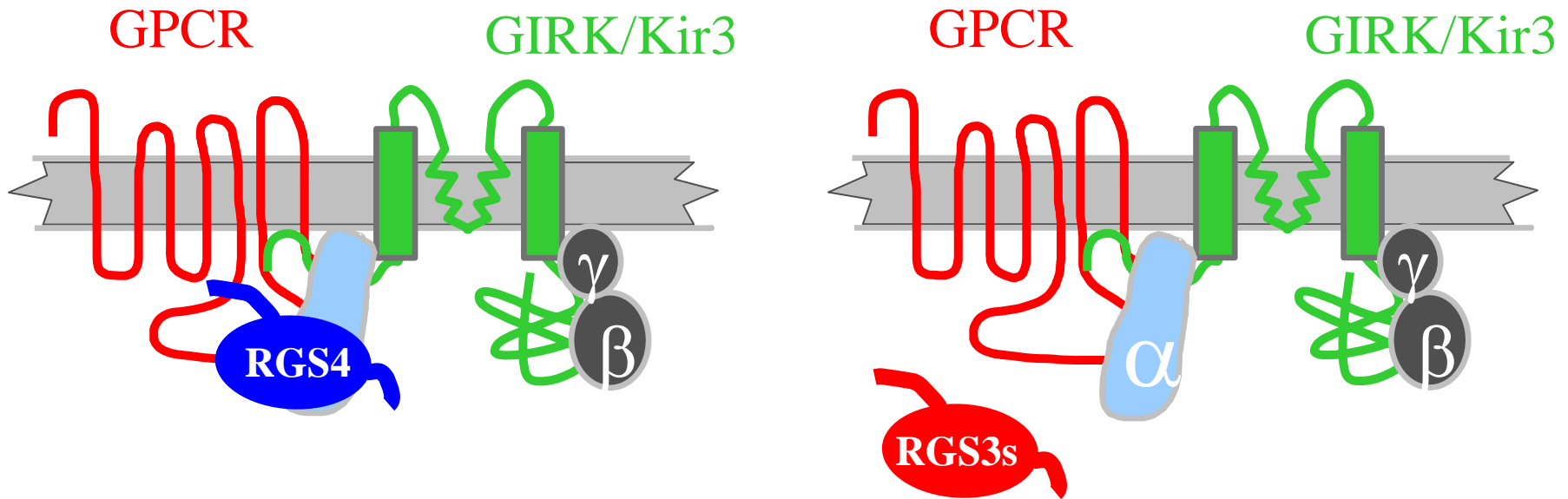
Multiple neuronal GPCR's complex with GIRK channels and RGS4(C2V)

HA-GPCR	+	+	+	+	+
Kir3.1-MYC / Kir3.2a	+	+	+	+	+
RGS4(C2V)-FLAG	+	+	+	+	+
GαoA	+	+	+	+	+

HA-GPCR	+	+	+	+	+
Kir3.1-MYC / Kir3.2a	+	+	+	+	+
RGS3s-FLAG	+	+	+	+	+
GαoA	+	+	+	+	+



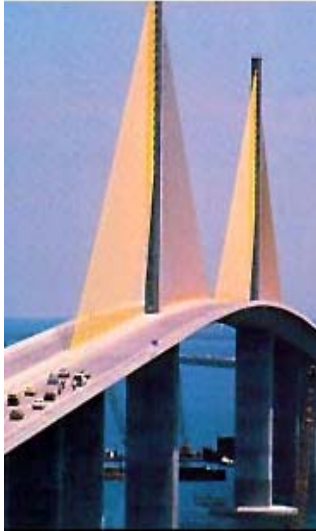
RGS4 and RGS3s differential couple to GPCR-GIRK channel signaling complexes



Summary

- RGS4 is a highly expressed G protein regulator in the brain and severely down regulated in schizophrenia.
- RGS4 accelerates the temporal kinetics of GPCR signaling and GIRK channel gating.
- RGS4 directly associates with several GPCR-GIRK channel macromolecular signaling complexes.

Acknowledgements



USF – Tampa

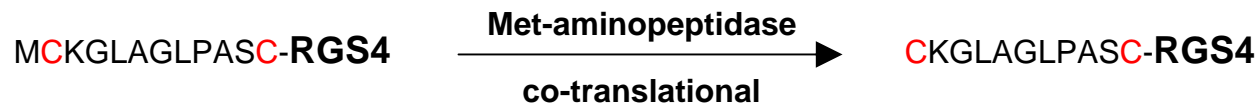
- **Cristina Jaén**

The N-end Rule pathway for RGS4 degradation

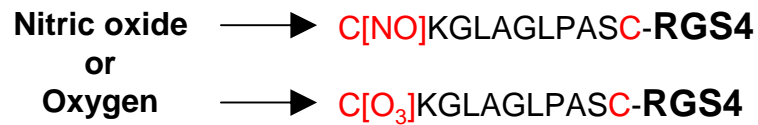
(also applies for RGS5 and RGS16)

The N-end rule relates the in vivo half-life of a protein to the identity of its N-terminal residue.

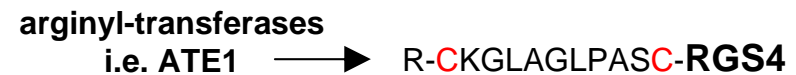
Step 1. Amino terminal methionine excision



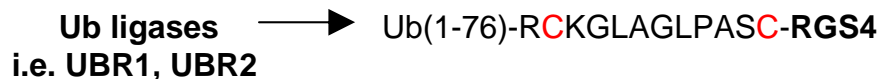
Step 2. Oxidation or S-nitrosylation of Cys2



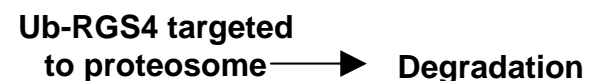
Step 3. Arginylation of reactive Cys2



Step 4. Ubiquitylation at N-terminal arginine



Step 5. Proteosomal degradation



RGS4 half-life is <1 hr (~10 min)