AGS3 and AGS4 in G-protein Signaling

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Signal regulator (Accessory protein)

Plasma membrane

stimulus

extracellular

G-protein

effectors

Expanded functional roles for G-proteins

G-protein

G-protein

G-protein

G-protein

G-protein

G-protein

G-protein

G-protein

G-protein
Activators of G-protein Signaling

**GROUP I**

(GEF) (activate $G_{\alpha i}$ but not $G_{\alpha s}$, $G_{\alpha 16}$ in yeast functional assay)

AGS1 - (RASD1, DexRas1) - ras-related protein

**GROUP II**

(GDI) (activate $G_{\alpha i}$ but not $G_{\alpha s}$, $G_{\alpha 16}$ in yeast functional assay)

AGS3 - (Gpsm1) four GPR motifs
AGS4 - (Gpsm3, G18.1b) - three GPR motifs
AGS5 - (Gpsm2, LGN, mPINS) - four GPR motifs
AGS6 - (RGS12) - one GPR motif

**GROUP III**

Bind $G_{\beta\gamma}$

AGS2 - (tctex-1) - light chain of cytoplasmic dynein
AGS7 - (Trip13)
AGS8 - KIAA1866
AGS9 - (Rpn10) - proteosome component
AGS10 - (Go$\alpha$)

*Group II*

AGS3

AGS4

AGS5
GDP + GDP

GTP + GTP

GDI

(GPR/GoLoco motif)

GPR

\( \alpha_{GDP} \) + \( \beta \gamma \)

\( \alpha_{GDP} \) + \( \beta \gamma \)

GAP/RGS

\( P_i \)

GDP

GTP

GPCR*

Effectors

Effectors
AGS3 is widely distributed in the brain and is developmentally regulated.

AGS3 is widely distributed in the brain and is developmentally regulated. AGS3

OFB--olfactory bulb
CB--cerebellum
HP--hippocampus
MED--medulla
VTA--ventral tegmental area
SN--substantia nigra
NA--nucleus accumbens
ST--striatum
PFC--prefrontal cortex
CC--cerebral cortex
THAL--thalamus
HYPO--hypothalamus
AMY--amygdala

Exploring the *in vivo* role of AGS proteins: Generation of a conditional AGS3 (Gpsm1) null mouse

**A**

**Gpsm1 Targeting Vector**

<table>
<thead>
<tr>
<th>Exon</th>
<th>WT</th>
<th>Gpsm1 null</th>
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**B**

**Western Blot Analysis**

<table>
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<tr>
<th>Protein</th>
<th>Rat Brain</th>
<th>Mouse Brain</th>
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</thead>
<tbody>
<tr>
<td>AGS3</td>
<td>(+/+)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>Giα3</td>
<td>(+/-)</td>
<td>(-/-)</td>
</tr>
<tr>
<td>AGS5 (LGN)</td>
<td>(+/-)</td>
<td>(-/-)</td>
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</table>

WT | Gpsm1

Cerebellum

Cerebral cortex

Nissl stained sagittal brain sections
8 week male littermates
Motor coordination and learning appear unaffected in AGS3 (Gpsm1) null mice

![Accelerating Rotarod Performance Test](image)
Spatial learning and memory appear unaffected in AGS3 (Gpsm1) null mice

Radial arm water maze
Reductions in AGS3 expression in PFC or NAc blocks reinstatement of drug-seeking behavior in rats.
Cocaine-induced locomotor sensitization in WT vs AGS3 (Gpsm1) null mice
Summary: Behavioral studies of AGS3 (Gpsm1) null mice

- AGS3 knockout mice are viable and fertile
- No obvious alterations in brain cellularity or morphology
- Initial behavioral testing revealed no obvious differences in motor coordination or in spatial learning and working memory
- Locomotor sensitization to cocaine is similar to wild-type mice
- Phase II phenotyping: Targeted disruption of AGS3 by tissue-specific or inducible Cre expression
How is this regulated??

GDI
(GPR/GoLoco motif)

GPR

$\alpha_{\text{GDP}}$

$\beta\gamma$

$\alpha_{\text{GDP}}$

$\beta\gamma$

GTP

GDP

GAP/
RGS

$P_i$

$\alpha_{\text{GTP}}$

$\beta\gamma$

Effectors

Effectors

GPCR*
AGS3 (650 aa) - TPR TPR TPR TPR TPR TPR TPR - GPR GPR GPR GPR

AGS4 (160 aa) - GPR GPR GPR

Bioluminescence Resonance Energy Transfer (BRET): A tool to measure AGS-Gαi interaction dynamics

AGS4-RLuc + Giα1 + YFP


AGS4 – Goi BRET signals are specific, saturable and inhibited by excess Gβγ
Agonist-induced GPCR activation decreases AGS4 – Goi interaction
Agonist-induced decreases AGS4 – Giα interaction are blocked by antagonist and pertussis toxin.
Gαi redistributes AGS4 to the plasma membrane

AGS4-GFP

AGS4-GFP + Gαi3-WT

AGS4-GFP + Gαi3-Q204L

AGS4-Q/A-GFP

AGS4-Q/A-GFP + Gαi3-WT
Agonist-induced translocation of AGS4 from membrane to cytosol

**A**

<table>
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<tr>
<td>$G_{\alpha_1}$-YFP</td>
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<td>+</td>
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<tr>
<td>$\alpha_{2A}$-AR</td>
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<td>+</td>
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<td>UK14304</td>
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**B**

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Summary: GPCR regulation of AGS4 – Gαi BRET

- AGS4 – Gαi BRET signals are decreased by Gαi-linked GPCR activation
- This effect is blocked by antagonist and pertussis toxin
- Receptor activation may result in either dissociation or conformational rearrangement of a pre-formed AGS4 – Gαi complex
  - Agonist-induced translocation of AGS4 from membrane to cytosol suggests AGS4 – Gαi dissociation
- Is the receptor directly interfacing with the AGS4 – Gαi complex?
AGS4 – $G\alpha_\text{i}$ complexes are proximal to GPCRs and agonist-sensitive
AGS4 interacts with Gαi-linked GPCRs in a Gαi-dependent manner
Summary and Perspective

- The interaction of AGS4 with $G\alpha_i$ as measured by BRET is robust, saturable and specific.
- Receptor activation results in a decrease in AGS4 – $G\alpha_i$ BRET that is blocked by antagonist and PTX.
- AGS4 – $\alpha_2^A$-AR BRET signals are $G\alpha_i$-dependent and reduced by receptor activation; this effect is blocked by antagonist and PTX.
- The data suggest that the interaction of AGS4 with $G\alpha_i$ enhances the presence of AGS4 at the cell surface whereupon receptor activation then dissociates AGS4 from $G\alpha_i$ and the cell cortex and into the cytosol.
- The possibility that proteins like AGS4 with multiple GPR motifs may “seed” $G\alpha$ complexes that interface with receptor provides an interesting alternative mechanism for signal processing through a GPCR.
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Membrane targeting AGS4 is not sufficient to observe BRET with $\text{G}\alpha_1$-YFP.

myr-AGS4-Rluc + $\text{G}\alpha_1$ + $\alpha_2\text{A}-\text{AR}$

$\text{G}\alpha_1$-N149I + $\alpha_2\text{A}-\text{AR}$

**Graph:**
- myr-AGS4 + $\text{G}\alpha_1$YFP + $\alpha_2\text{A}-\text{AR}$
- myr-AGS4 + $\text{G}\alpha_1$-YFP + $\alpha_2\text{A}-\text{AR}$ + UK
- myr-AGS4 + $\text{G}\alpha_1$-N149I + $\alpha_2\text{A}-\text{AR}$
- myr-AGS4 + $\text{G}\alpha_1$-N149I + $\alpha_2\text{A}-\text{AR}$ + UK

**Axes:**
- Y-axis: net BRET
- X-axis: Acceptor/Donor
Membrane targeting AGS4 is not sufficient to observe BRET with Gαi1-YFP

myr-AGS4-Rluc + Gαi1-YFP + α2A-AR

myr-AGS4 + Gαi1YFP + α2A-AR

myr-AGS4-Q/A + Gαi1-YFP + α2A-AR + UK

myr-AGS4-Q/A + Gαi1-YFP + α2A-AR + UK
AGS4-Rluc shows reduced BRET with RGS-insensitive Gαi1-YFP
AGS4 – G\(\alpha\)i BRET signals are reduced in G\(\alpha\)i-YFP mutants but are unaltered by PTX.
AGS4 – $\alpha_{2A}$-AR BRET requires wild-type $G\alpha_i$

![Graph showing net BRET values for different conditions](image)

- **AGS4-Rluc**
- **$\alpha_{2A}$-AR-Venus**

**Values:**
- Control: 0.00
- $G\alpha_i$: 0.04
- $G\alpha_i$ Q204L: 0.02
- $G\alpha_i$ G202T: 0.06
- $G\alpha_s$: 0.01

**Significance:**
- * indicates statistically significant difference.
AGS4 interacts with Gαi-linked GPCRs in a Gαi-dependent manner
Agonist Regulation of the AGS4 – Gαi complex: Dose Response & Timecourse
AGS4 – Gαi complexes are proximal to GPCRs and agonist-sensitive
Agonist-regulated AGS4 – GPCR BRET signals are PTX sensitive

AGS4-Rluc + $\alpha_{2A}$-AR-Venus

![Diagram of AGS4-Rluc and $\alpha_{2A}$-AR-Venus](image)

![Bar graph showing net BRET](image)

- Control
- UK14304
- PTX
- UK14304 + PTX

Significant changes indicated by * and **.
Classical paradigm of G protein signaling

Unexpected Roles for G protein Signaling

Accessory Proteins

Expanded functional roles for G-protein signaling
Phase I phenotyping: Behavioral Studies

- Brain Histology/Morphology
- Accelerating Rotarod Performance
- Radial Arm Water Maze
- Cocaine-induced Locomotor Sensitization