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# Regulator of G-protein signalling 3 redirects prototypical $G_i$-coupled receptors from Rac1 to RhoA activation

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**Abstract**

The small GTPases, Rac1 and RhoA, are pivotal regulators of several essential, but distinct cellular processes. Numerous G-protein-coupled receptors signal to these GTPases, but with different specificities. Specifically, $G_i$-coupled receptors ($G_i$PCRs) are generally believed to activate Rac1, but not RhoA, a process involving Gβγ-dimers and phosphatidylinositol 3-kinase (PI3K). Here we show that, depending on the expression level of the 519 amino acid isoform of regulator of G-protein signalling 3 (RGS3L), prototypical $G_i$PCRs, like $M_2$ muscarinic, $A_1$ adenosine, and $\alpha_2$-adrenergic receptors, activate either Rac1 or RhoA in human embryonic kidney cells and neonatal rat cardiomyocyte-derived H10 cells. The switch from Rac1 to RhoA activation in H10 cells was controlled by fibroblast growth factor-2 (FGF-2), lowering the expression of RGS3L. Activation of both, Rac1 and RhoA, seen at low and high expression levels of RGS3L, respectively, was sensitive to pertussis toxin and the PI3K inhibitor LY294002 and mediated by Gβγ-dimers. We conclude that RGS3L functions as a molecular switch, redirecting $G_i$PCRs *via* Gβγ-dimers and PI3K from Rac1 to RhoA activation. Considering the essential roles of Rac1 and RhoA in many signalling pathways, this additional function of RGS3L indicates a specific role of this protein in cellular signalling networks.
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*Keywords:* $G_i$ proteins; Gβγ-dimers; RhoA; Rac1; FGF-2; RGS proteins; RGS3

## 1. Introduction

Members of the Rho GTPases family, like the best characterised family members RhoA, Rac1, and Cdc42, which has been first identified as regulators of actin cytoskeleton alterations, are now considered to be pivotal regulators of many signalling networks, participating in secretion, smooth muscle contraction, cell migration, membrane trafficking, gene transcription and cell cycle progression (for recent review see [1–3]). G-protein-coupled receptors (GPCRs) in numerous cell types have been shown to activate Rho GTPases. It is generally assumed that the activation of RhoA by GPCRs is mediated by Gα-subunits of the $G_{12}$ and/or $G_q$ subfamilies. By contrast, $G_i$-coupled receptors ($G_i$PCRs) are believed to activate Rac1, but not RhoA, *via* Gβγ-dimers and involvement of phosphoinositide 3-kinase (PI3K) [3,4]. Therefore, the distinct cellular responses induced by pertussis toxin (PTX)-sensitive $G_i$PCR signalling cascades and PTX-insensitive $G_{q/12}$ PCRs regulated pathways are often attributed to Rac1 and RhoA activation, respectively, and their different spectrum of downstream effectors [3].

RGS3, a member of the regulator of G-protein signalling (RGS) family, shares a high degree of homology within its RGS domain with the R4 subfamily and thus acts as GAP for the Gα-subunits of $G_i$ and $G_q$ family members [5–8]. Increases in cellular levels of RGS proteins apparently contribute to alterations in GPCR signalling [5,6] as for example seen in heart failure [9,10]. In contrast to other members of the R4 subfamily, RGS3 exists in several splice variants of which at least two exhibit an extended amino-terminal domain in

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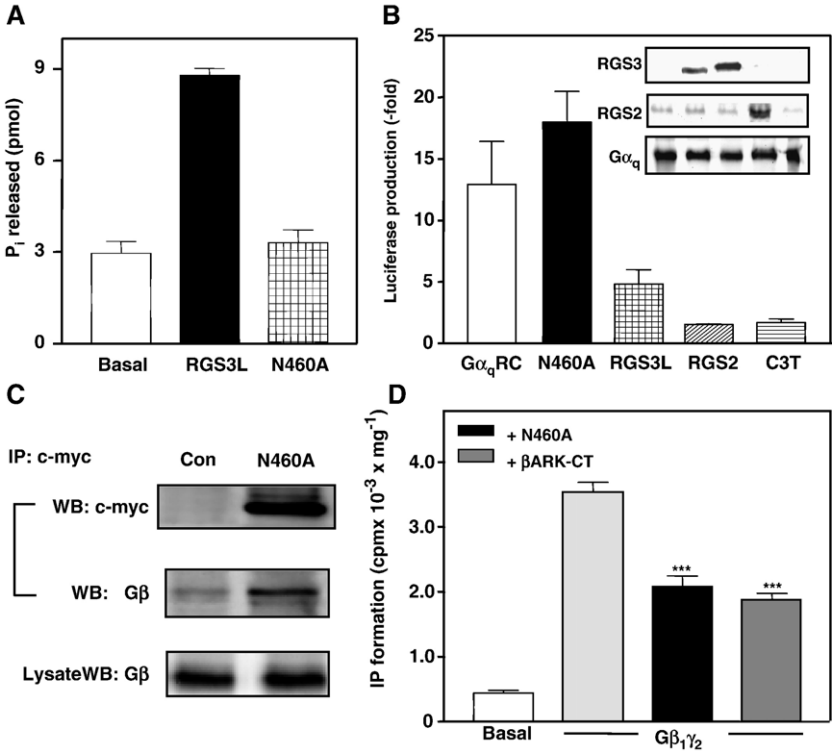


Fig. 1. The GAP deficient RGS3L mutant N460A acts as a Gβγ-scavenger (A) Single turnover GTPase activity of recombinant $G\alpha_{i1}$ measured in the absence (Basal) and presence of recombinant RGS3L or RGS3L N460A (N460A). (B) Luciferase production was measured in HEK-TsA201 cells transfected with the permanent active but GAP-sensitive $G\alpha_q$ mutant ($G\alpha_q$RC) (1 μg of DNA) alone and together with wild-type RGS3L, RGS3L N460A, RGS2 (2 μg of DNA each) or C3 transferase (C3T, 1 μg of DNA) as indicated. Mean±SEM; $n=8$–20. $G\alpha_q$ and RGS protein expression was monitored by immunoblotting (inset). (C) RGS3L N460A was immunoprecipitated from cell lysates with an anti-c-myc antibody and precipitated RGS3L N460A (upper panel) and Gβ (middle panel) were visualised by immunoblotting. Total content of Gβ in the cell lysates is shown as loading control (lower panel). (D) HEK-TsA201 cells were transfected with empty vector (Basal) and $G\beta_1$ plus $G\gamma_2$ (10 μg of DNA each) in the absence or presence of RGS3L N460A (25 μg of DNA) or βARK-CT (100 μg of DNA). (A) After 48 h, basal [$^3$H] inositol phosphate formation was measured. Data are means±SEM ($n=4$; ***, $p<0.001$ *vs.* $G\beta_1\gamma_2$). Means±SD of assay triplicates are given.

addition to the RGS domain [11,12]. It has been shown that the 519 amino acid isoform of RGS3 (RGS3L) binds Gβγ-dimers [13]. It thereby acts as a Gβγ-scavenger and inhibits the Gβγ-induced activation of phospholipase Cβ (PLCβ), Akt and mitogen-activated protein kinase independently of its GAP activity.

Herein we report that the expression level of RGS3L controls whether $G_i$PCRS activate Rac1 or RhoA in human embryonic kidney (HEK) and neonatal rat cardiac myocyte derived H10 cells via a Gβγ- and PI3K-mediated pathway.

## 2. Materials and methods

### 2.1. Plasmid construction

The cDNA encoding the 519 aa isoform of human RGS3 (a kind gift by Dr. J. H. Kehrl, Bethesda, MD, USA) was subcloned in the pCMV-Tag3b vector (Stratagene). Site-directed mutagenesis of RGS3L was performed using Quikchange (Stratagene) with the mutagenic primers RGS3N460Afw: GCATG-CAAGGAGGTAGCGCTGGACTCCTACACG, RGS3N460Abw: CGTGTAG-GAGTCCAGCGCTACCTCCTTGCATGC according to the manufacturer's protocol. The sequence of the mutant was verified by automated sequencing. The eukaryotic expression vectors encoding C3 transferase, $M_2$ muscarinic acetylcholine receptor ($M_2$ mAChR), $\alpha_{2A}$-adrenoceptor ($\alpha_{2A}$AR) and β-ARK-CT were kind gifts of Dr. A. Hall, London, UK, Dr. C. van Koppen, Essen, Germany, Dr. L. Hein, Freiburg, Germany and Dr. M. Lohse, Würzburg, Germany, respectively.

### 2.2. Cell culture and transfection

Culture of human embryonic kidney (HEK-TsA201) cells and transfection of the cells (3 μg of total DNA per well on a 12-well plate for SRF activation, and up to 250 μg of DNA per 145-mm culture dish for GTPase pull-down assays) were performed as described before [14]. Assays were performed 24 h or 48 h after transfection in serum-starved cells. H10 cells were cultured as reported before [15], and then treated for 24 h in serum-free medium without and with fibroblast growth factor-2 (FGF-2) (50 ng/ml) or PTX (100 ng/ml) as indicated in the figure legends. For transfection of H10 cells with siRNAs, the cells were cultured on 35-mm dishes or 6-well plates and transfected with 5 μl Lipofectamine 2000 in 500 μl OptiMEM and 50 pmol of each siRNA according to the manufacturer's instructions (Invitrogen). If vector DNA was co-transfected with siRNA, 800 ng of the individual vector DNA was used in a total amount of 2.5 μg. The specific siRNAs (Ambion) were si-RGS3 (1): 5′-GGAUAUGAAGAAUAAGCUGtt-3′, si-RGS3 (2): 5′-GGAAGGAAUC-CUUUUCAGGtt-3′. To evaluate transfection efficiency, fluorescence labelled siRNA (Alexa Fluor 488, Qiagen) was used. Scrambled siRNA served as control. The expression of surface $M_2$ mAChR was determined by [$^3$H] *N*-methylscopolamine ([$^3$H] NMS) binding as described before [16].

### 2.3. Assay of SRF activation

HEK-TsA201 cells seeded on 12-well plates were transfected with different expression plasmids together with the pSRE.L-luciferase reporter plasmid

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