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Activators of G-protein Signaling in the kidney

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4. List of non-standard abbreviations:

AGS = Activators of G-protein Signaling

AKI = acute kidney injury

AMPK = AMP-activated protein kinase

APE = Akt phosphorylation enhancer

CX-43 = connexin-43

Dexras1 = Ras dexamethasone induced 1

FLCN = folliculin

FNDC1 = fibronectin type III containing I

GDI = guanine nucleotide dissociation inhibitor

GEF = guanine nucleotide exchange factor

Girdin = Girders of actin filaments

GPCR = G-protein coupled receptor

GPR = G-protein regulatory

GPSM1 = G-protein signaling modulator 1

GPSM2 = G-protein signaling modulator 2

GTPase = guanosine triphosphatase

LGN = Leu-Gly-Asn repeat-enriched protein

LKB1 = liver kinase B1

MitF = microphthalmia-associated transcription factor

mTOR = mammalian target of rapamycin

mPCH2 = mouse pachytene checkpoint 2

PAN = puromycin aminonucleoside

PI3K = phosphoinositide 3 kinase

PKD = polycystic kidney disease

Psmd1 = proteasome (prosome, macropain) 26S subunit, non-ATPase, 4

RGS = Regulators of G-protein Signaling

Rhe2 = Ras homolog enriched in striatum

Ric-8 = resistance to inhibitors of cholinesterase 8

Rpn10 = Regulatory particle non-ATPases 1

RT-PCR = reverse transcription-polymerase chain reaction

TEM2 = tumor endothelial marker 2

TFE3 = transcription factor E3

TFEB = transcription factor B

Trip13 = thyroid receptor interacting protein 13

UACR = urinary albumin-to-creatinine ratio

UPS = Ubiquitin-proteosome system

VEGFR2 = vascular endothelial growth factor receptor 2

Abstract

Heterotrimeric G-proteins play a crucial role in regulating signal processing to maintain normal cellular homeostasis, and subtle perturbations in its activity can potentially lead to the pathogenesis of renal disorders or diseases. Cell surface receptors and accessory proteins, which normally modify and organize the coupling of individual G-protein subunits, contribute to the regulation of heterotrimeric G-protein activity and their convergence and/or divergence of downstream signaling by effector systems. Activators of G-protein Signaling (AGS) are a family of accessory proteins that intervene at multiple distinct points during the activation-inactivation cycle of G-proteins, even in the absence of receptor stimulation. Perturbations in the expression of individual AGS proteins have been reported to modulate signal transduction pathways in a wide array of diseases and disorders within the brain, heart, immune system, and more recently, the kidney. This review will provide an overview of the expression profile, localization and putative biological role of the AGS family in the context of normal and diseased states of the kidney.

Introduction

Heterotrimeric G-protein signaling system enables the kidney to readily adapt to an ever changing environment by controlling cellular responses to various hormones and physical stimuli to maintain normal homeostasis of kidney function. The diversity of the heterotrimeric G-proteins allows these distinct subunits to perform unique modes of action within the cell, including the mobilization of specific signaling molecules to intracellular organelles or microdomains, receptor phosphorylation and trafficking, and integration of signaling pathways to optimize signal specificity and/or intensity (Wettschureck and Offermanns, 2005). Historically, canonical G-protein signaling involves the stimulation of cell surface G-protein coupled receptors (GPCR), which function as guanine nucleotide exchange factors (GEFs) to facilitate Gα subunits to release GDP and promote GTP binding. As a consequence of the central role of cell surface receptors in mediating G-protein signaling, there has been enormous interest in the development of drugs to control the signaling output from this GPCR-G-protein complex (Wettschureck and Offermanns, 2005; Brogi et al., 2014).

Over the past 20 years, however, the regulation of G-protein signaling has become increasingly more diverse through the identification of an increasing number of accessory proteins. Initially, numerous groups discovered negative regulators of GPCR signaling, known as Regulators of G-protein Signaling (RGS) (De Vries et al., 1995; Berman et al., 1996; Druey et al., 1996; Hunt et al., 1996; Koelle and Horvitz, 1996; Siderovski et al., 1996; Watson et al., 1996), which functions as GTPase-activating protein (GAP) by accelerating the intrinsic GTP catalysis in G-protein α subunits. Subsequently, Cismowski *et al.* (Cismowski et al., 1999) identified a novel family of distinct G-receptor coupled receptor (GPCR) independent regulators, known as Activator of G-protein Signaling (AGS), using a pheromone receptor-deficient yeast screen. The AGS family members are a compilation of previously unrelated proteins to be grouped together based on protein structure and/or biological function, i.e., modulate G-protein signaling.

Unlike RGS proteins, AGS proteins regulate G-proteins with a broader array of mechanisms by forming complexes with Gα or Gβγ independent of the typical heterotrimeric Gαβγ, and are categorized into four separate groups: 1) guanine nucleotide exchange factors

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(GEF); 2) guanine nucleotide dissociation inhibitors (GDIs); 3) Gβγ binding proteins; and 4) $G\alpha_{16}$ binding proteins. Although these modes of action by the AGS proteins have been extensively reviewed elsewhere (Sato et al., 2006a; Blumer et al., 2007; Blumer and Lanier, 2014), the biological function in the kidney remains to be fully determined.

In the normal kidney, AGS proteins appear to be fairly quiescent and play a minimal role in the maintenance of renal function. However, there is emerging and compelling evidence that some of the AGS proteins can play a pivotal role during renal pathologies due to biological or genetic stress (Nadella et al., 2010; Zheng et al., 2010; Regner et al., 2011; Kwon et al., 2012; Lenarczyk et al., 2014; Potla et al., 2014; Wang et al., 2014a). These functional changes in the renal phenotype are likely associated with the ability of AGS proteins to intervene at alternate sites during the typical activation/inactivation cycle of the heterotrimeric G-protein complex, as well as the distinct distribution pattern of AGS proteins and their associated heterotrimeric G-protein subunits within the nephron, glomerulus, and vasculature.

In this mini-review, we will summarize the expression and spatial localization of G-proteins and the AGS protein family in the normal and pathological diseased kidneys, and provide some biological insight into their functional roles in the context of the kidney.

G-protein subunit localization in the kidney. Currently, there are 4 distinct isoforms of Gα, specifically $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$, 5 β, and 12 γ subunits (Wettschureck and Offermanns, 2005), and their localization can be segment specific within the nephron and renal vasculature. A summary of this section on G-protein subunit localization is shown in Table 1.

G-protein α_s subunit. Biodistribution of Gα subunits in human tissues using Northern blot analysis demonstrated minimal to moderate mRNA expression of $G\alpha_s$ compared to $G\alpha_i$ (Brann et al., 1987), which was consistent with the expression of $G\alpha_s$ protein in the whole renal cortex and inner medulla from mice (Yu et al., 1998). $G\alpha_s$ mRNA is widely expressed throughout the renal cells in the nephron and blood vessels. $G\alpha_s$ mRNA was detected in isolated rat nephron segments, including medullary thick ascending limbs and collecting ducts using RT-PCR (Senkfor et al., 1993), and mouse glomeruli and proximal tubules using *in situ* hybridization (Williamson et al., 1996; Yu et al., 1998). At the protein level, $G\alpha_s$ was detected in the glomeruli (Brunskill et al., 1991; Yanagisawa et al., 1993; Nitta et al., 1994; Yanagisawa et

al., 1994), proximal convoluted tubules (Brunskill et al., 1991; Stow et al., 1991), thick ascending limbs of Henle (Stow et al., 1991), collecting ducts (Stow et al., 1991), and pre-glomerular vessels from rats (Ruan et al., 1999).

G-protein $\alpha_{i/o}$ subunits. Brann *et al*. (Brann et al., 1987) demonstrated $G\alpha_{i2}$ mRNA expression, but $G\alpha_{i1}$ mRNA was undetectable in rat kidneys using Northern blot analysis. However, Hansen *et al.* (Hansen et al., 2003) detected all three isoforms of $G\alpha_i$ mRNA in the renal cortex using RT-PCR. To obtain more discrete information on the localization of $G\alpha_i$ subunits within the kidney, RT-PCR analysis was performed on isolated nephron and blood vessel segments from rats (Senkfor et al., 1993; Hansen et al., 2003). $G\alpha_{i2}$ was highly identified as the predominant subunit in the medullary thick ascending limb and collecting ducts (Senkfor et al., 1993). $G\alpha_{i1}$, $G\alpha_{i3}$, and $G\alpha_o$ were undetectable in the nephron segments (Senkfor et al., 1993), but $G\alpha_{i1}$ was robustly expressed in pre-glomerular vessels (Hansen et al., 2003).

Localization of $G\alpha_i$ subunits at the protein level was fairly consistent with spatial expression detected with mRNA assays. $G\alpha_{i2/i3}$ was detected in the rat glomeruli using immunohistochemistry (Brunskill et al., 1991; Yanagisawa et al., 1993; Yanagisawa et al., 1994) and all $G_{i1/i2/i3}$ isoforms were detected in isolated glomerular membranes (Nitta et al., 1994) and pre-glomerular blood vessels from rats using immunoblot analysis (Ruan et al., 1999). Using immunohistochemistry with rat kidneys, $G\alpha_i$ subunits were segment-specific within the nephron where $G\alpha_{i1}$ was localized to the thick ascending limb, including the macula densa, and papillary epithelial cells, $G\alpha_{i2}$ found in the collecting duct cells, and $G\alpha_{i3}$ was in the S1 segment proximal tubule cells and macula densa cells in the thick ascending limb of Henle (Stow et al., 1991). Any differences between mRNA and protein localization of the $G\alpha_i$ subunits may be attributed to the relative non-selectivity of the antibodies compared to the nucleotide probes/primers used in the previous studies. In most cases, however, the mRNA and protein results provided confirmatory evidence that one, if not, all of the isoforms exist within specific cell types in the kidney.

$G\alpha_o$ mRNA expression was undetectable in rodent kidneys using Northern blot analysis (Brann et al., 1987; Strathmann et al., 1990) and RT-PCR analyses (Hansen et al., 2003), although RT-PCR analysis showed a specific $G\alpha_{oA}$ mRNA isoform could be minimally detected in

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the mouse kidney (Strathmann et al., 1990). $G\alpha_o$ protein expression was minimally expressed in isolated glomerular membranes from humans (Nitta et al., 1994).

G-protein α_q subunits. The G_q class of α subunits consists of $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_{15}$ (or $G\alpha_{16}$ in humans). $G\alpha_q$, $G\alpha_{11}$ and $G\alpha_{14}$ mRNA were highly expressed (Strathmann et al., 1989; Wilkie et al., 1991), but minimally expressed for $G\alpha_{15}$ mRNA using Northern blot analysis in mouse kidney tissue (Wilkie et al., 1991). Antibodies targeted to $G\alpha_q$ and $G\alpha_{11}$ subunits confirmed their expression in isolated whole rat kidney membranes (Gutowski et al., 1991). In human tissue, $G\alpha_q$ presented variable renal mRNA levels from fetus to adult, whereas $G\alpha_{14}$ mRNA was only detected in fetal human kidney tissue (Rubio et al., 1999).

Using RT-PCR in isolated rat nephron segments, $G\alpha_{11}$ and $G\alpha_{14}$ were minimally detected in the outer medullary and upper portion of the inner medullary collecting ducts, respectively (Senkfor et al., 1993). Immunoblot analyses demonstrated $G\alpha_{q/11}$ protein in pre-glomerular vessels (Ruan et al., 1999) and glomeruli from rats (Brunskill et al., 1991; Yanagisawa et al., 1993; Yanagisawa et al., 1994).

G-protein $\alpha_{12/13}$ subunits. $G\alpha_{12}$ was robustly detected in the brush border membrane of the proximal tubule, with moderate staining in the thick ascending limb of Henle, cortical collecting ducts, and renal blood vessels in rats (Zheng et al., 2003). In mice, $G\alpha_{12}$ was detected uniformly throughout the nephron and the podocytes of the glomeruli (Boucher et al., 2012). $G\alpha_{13}$ was similarly expressed in high abundance in the proximal tubules, juxtaglomerular cells, and renal artery with lesser staining in the distal convoluted tubules, medullary collecting ducts, and renal veins (Zheng et al., 2003).

Gβγ subunits. The molecular expression of all 5 Gβ mRNA isoforms was detected in either mouse, chicken, cow or human kidneys using RNA detection methods. Bovine $G\beta_1$ (Fong et al., 1987), chicken $G\beta_3$ mRNA (Tummala et al., 2011), and human $G\beta_5$ mRNA (Snow et al., 1999) was detected in the kidneys using Northern blot methods. Mouse $G\beta_4$ (von Weizsacker et al., 1992) or bovine $G\beta_2$ mRNA (Fong et al., 1987) was not detected in the kidney using Northern blot analysis, but a more sensitive RT-PCR assay followed by Southern blotting of the PCR bands enabled the detection of mouse kidney $G\beta_1$, $G\beta_2$ and $G\beta_4$ mRNA (von Weizsacker et al., 1992). These contrasting results are likely attributed to the sensitivity of the RNA detection methods,

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and the lack of bovine $G\beta_2$ mRNA expression in the kidney may be attributed to differential species-specific gene expression regulation.

Out of the 12 Gγ isoforms, only 6 were detected in the kidney. Northern blot analysis was used to detect the mRNA for human $G\gamma_4$ (Ray et al., 1995), bovine $G\gamma_5$ (Cali et al., 1992), bovine $G\gamma_7$ (Cali et al., 1992), human $G\gamma_{10}$ (Ray et al., 1995), and human $G\gamma_{11}$ (Ray et al., 1995). $G\gamma_{12}$ protein was detected in the rat kidneys using Western blot analysis (Asano et al., 1998).

The spatial localization of each distinct β or γ isoforms within distinct cell types in the kidney remains to be determined. However, the diversity of each distinct Gα subunit within specific cell types within the kidney may be more expansive than described using molecular techniques, particularly for $G\alpha_q$. Many published studies investigating GPCR activity have described Gα function to specific vascular, tubular or interstitial cells within the kidney, without actual evidence for the presence of those G-protein subunits being expressed in those segments (Zhang et al., 2011; Zhuo and Li, 2011; Horita et al., 2013). As an example, angiotensin II is well known to regulate proximal tubular sodium reabsorption through activation of the AT_{1A} receptor and its association with $G\alpha_q$, but there is no definitive data showing the presence of this subunit in that particular segment of the kidney other than interaction studies unrelated to the kidney (Sano et al., 1997; Li et al., 2011b).

Regardless, these molecular studies describing the expression of Gα, β and γ subunits in the kidney provides some potential insight into the types of interactions that may occur with accessory proteins in each respective renal cell type, but further studies are needed to confirm the identity of particular G-protein subunits within specific segments of the kidney.

Activators of G-protein Signaling (AGS). Since the initial findings by Cismowski *et al.* (Cismowski et al., 1999) using the genetically modified yeast system, there are currently 13 identified AGS proteins using cDNA libraries from multiple organ systems, which have been grouped into 4 distinct groups based on their mode of action (Sato et al., 2006a; Blumer et al., 2007).

Group I AGS proteins. There are currently four AGS proteins that are categorized in this group: 1) Activator of G-protein Signaling 1 (AGS1)/RasD1/Dexras1; 2) RasD2/Ras homolog enriched in striatum (Rhes)/tumor endothelial marker 2 (TEM2); 3) GIV/Girdin (Girders of actin

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filaments)/APE (Akt phosphorylation enhancer); and 4) resistance to inhibitors of cholinesterase 8 (Ric-8). The latter three accessory proteins were not identified from the yeast screen, but have similar protein structure and/or mechanism of action so they have been included as part of the Group I AGS proteins.

AGS1/Dexras1/RasD1. AGS1 was the initial AGS protein that was characterized by Cismowski *et al*. (Cismowski et al., 1999), and demonstrated homology with a dexamethasone-inducible Ras protein, known as Dexras1 or RasD1 (Cismowski et al., 2000). Ras proteins are a family of small (typically 20-25 kDa) monomeric G-proteins, which can alternate the binding of guanine nucleotides (GDP and GTP) to control the signaling output within the cell. AGS1 demonstrates selective interaction with $G\alpha_i$, and not $G\alpha_s$, $G\alpha_{16}$ or $G\beta\gamma$ subunits (Cismowski et al., 1999; Cismowski et al., 2000; Cismowski et al., 2001). In yeast, over-expression of AGS1 with either mutant $G\alpha_{i2}$ (Cismowski et al., 1999; Cismowski et al., 2000) or RGS4 (Cismowski et al., 1999), a GTPase-activating protein (GAP) for G_i/G_o proteins, prevented the functional signaling output mediated by AGS1. These studies demonstrate that the biological function of AGS1/Dexras1 requires the GTP-bound forms of $G\alpha_i$ subunits to activate signaling pathways (Tu and Wu, 1999).

The renal expression of AGS1/Dexras1 mRNA was developmentally regulated where higher levels were detected in the adult versus fetus (Kemppainen et al., 2003). In terms of tissue biodistribution, AGS1/Dexras1 mRNA was minimally expressed in adult mice (Kemppainen and Behrend, 1998) or human kidneys (Tu and Wu, 1999; Kemppainen et al., 2003) compared to other sites, including the liver and brain. AGS1/Dexras1 mRNA expression was induced by dexamethasone administration (Kemppainen and Behrend, 1998; Tu and Wu, 1999; Brogan et al., 2001; Vaidyanathan et al., 2004), including in the kidney (Kemppainen and Behrend, 1998), or by biological stress applied to the kidney (Lenarczyk et al., 2014). Coincidentally, dexamethasone has been shown to exhibit a protective role in the kidney following renal ischemia-reperfusion injury (Rusai et al., 2013). Considering that AGS1/Dexras1 protein was predominantly localized to the cortical and outer medullary proximal tubular epithelial cells in normal mouse kidneys (Lenarczyk et al., 2014), which are nephron sites that are highly sensitive to ischemia (Devarajan, 2006; Bonventre and Yang, 2011), there may be a

role for AGS1/Dexras1 to modulate the extent of renal tubular epithelial cell injury through its ability to activate downstream signaling pathways (Tu and Wu, 1999), which are involved in cell growth, differentiation and transformation (Kemppainen and Behrend, 1998).

There may be additional roles for AGS1/Dexras1 in the kidney to control cardiovascular function. AGS1/Dexras1 can control renin transcription (Tan et al., 2011) or nitric oxide synthase activity (Fang et al., 2000), which are well established to play a crucial role in long-term sodium reabsorption and blood pressure regulation by controlling regional blood flow distribution and fluid and electrolyte balance in the kidney (O'Connor and Cowley, 2010; Aksu et al., 2011; Toda and Okamura, 2011). In cell culture, AGS1/Dexras1 modulated cAMP production by adenylyl cyclase II (AC2) through the actions of the dopamine D2 receptor (Nguyen and Watts, 2006). This may implicate another alternate role for AGS1/Dexras1 in the kidney by regulating Na^+-K^+ ATPase activity in a cAMP-dependent mechanism through the dopamine D2 receptor (Bertorello and Aperia, 1990). The functional roles of AGS1/Dexras1 and its impact on renal function require further investigation.

RasD2/Rhes/TEM2. RasD2/Rhes/TEM2 has moderate sequence homology (~60%) to AGS1/RasD1. Rhes/RasD2 was highly expressed in the striatum with minimal detection in the kidney using RNA blot analysis (Spano et al., 2004). Unlike AGS1/Dexras1, Rhes/RasD2 has yet to be confirmed as a GEF, so further evaluation is necessary to confirm whether it fulfills the criteria to be categorized as a legitimate AGS protein (Falk et al., 1999; Vargiu et al., 2004; Thapliyal et al., 2008; Harrison and He, 2011).

GIV/Girdin/APE. GIV/Girdin/APE was initially shown to regulate actin organization and cell motility by acting as a substrate for Akt phosphorylation. Subsequently, studies have demonstrated that the C-terminus of GIV/Girdin can directly interact with receptor tyrosine kinases, G-protein subunits, and Akt to control processes, including cell migration (Ghosh et al., 2008; Ghosh et al., 2010), autophagy (Garcia-Marcos et al., 2011) and metastasis (Ghosh et al., 2010). Initial biodistribution studies by Anai *et al.* (Anai et al., 2005) were unable to reliably detect the expression of GIV/Girdin/APE mRNA and protein in the kidney using Northern blot and immunoblot analysis following immunoprecipitation of tissue lysates, respectively. More recently, the expression of GIV/Girdin was highly induced in glomerular podocytes following

administration of puromycin aminonucleoside (PAN) in rats (Wang et al., 2014a), which has been previously shown to be an animal model of reversible podocyte injury (Pippin et al., 2009). In this study, GIV/Girdin promoted the formation of a signaling complex with $G\alpha_i$ and vascular endothelial growth factor receptor 2 (VEGFR2) leading to elevated PI3K/Akt survival signaling and protection from damage by PAN (Wang et al., 2014a).

Ric-8. In mammals, there are two isoforms, Ric-8A and Ric-8B, which function as guanine exchange factors (GEF) for the Gα subunits (Tall et al., 2003; Chan et al., 2011). Ric-8A promoted the intrinsic nucleotide exchange rate for $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$, but Ric-8B interacts only with $G\alpha_s$ subunits. Ric-8A functions as a chaperone protein to direct Gα subunit association to the plasma membrane. In the absence of Ric-8A/B, the steady state levels of Gα are appreciably reduced suggesting a role for Ric-8 to stabilize the Gα subunit from degradation during the biosynthetic process (Gabay et al., 2011). To date, the role of Ric-8 in the kidney remains largely unexplored since Ric-8A was exclusively detected in the nervous system of lower invertebrates and mice (Miller et al., 2000; Tonissoo et al., 2003; Tonissoo et al., 2006; Maldonado-Agurto et al., 2011). Using a more sensitive RT-PCR assay, however, adult frogs presented a more widespread expression pattern for Ric-8 mRNA, including modest expression in the kidney, but these levels may not be sufficient to alter renal function.

Group II AGS proteins. Group II AGS proteins are categorized by virtue of one or more protein sequences known as GoLoco (Siderovski et al., 1999) or G-protein regulatory (GPR) domains (Peterson et al., 2000; Bernard et al., 2001). Depending on the protein structure, three distinct types of Group II AGS proteins have been identified.

AGS3/GPSM1. AGS3 is also known as G-protein signaling modulator 1 (GPSM1), and was first described by Takesono *et al*. (Takesono et al., 1999) as the initial member of the guanine nucleotide dissociation inhibitors (GDIs). AGS3/GPSM1 has a protein structure containing N-terminal tetratricopeptide repeats, a linker region, and 4 C-terminal GPR/GoLoco motifs, which can bind one or more $G\alpha_{i/o}$ subunits and regulate heterotrimeric G-protein activity (12–14).

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Under basal conditions, full-length and truncated forms of AGS3/GPSM1 mRNA (De Vries et al., 2000a; Pizzinat et al., 2001) and protein (De Vries et al., 2000a; Pizzinat et al., 2001; Blumer et al., 2002; Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012) were expressed at minimal to undetectable levels in mouse or rat kidneys. This was likely attributed to the selective localization of AGS3/GPSM1 to the distal portion of the nephron (Nadella et al., 2010; Regner et al., 2011). No expression was detected in other nephron segments of the renal cortex or medulla, and no expression was detected in the vasculature or glomerulus.

Under conditions of acute or chronic kidney injury to the renal tubular epithelia, the extent of AGS3/GPSM1 protein induction was dependent upon the site of epithelial cell injury or disruption (Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012). Ischemia-reperfusion injury, a common form of acute kidney injury, resulted in a dramatic temporal induction of AGS3/GPSM1 expression during the reparative phase of recovery in the outer medullary proximal tubular epithelial cells (Regner et al., 2011; Lenarczyk et al., 2014), which was a nephron segment normally absent in AGS3/GPSM1 expression. In multiple genetic models of polycystic kidney disease (PKD) from mice, rats and humans (Nadella et al., 2010; Kwon et al., 2012), AGS3/GPSM1 expression was abnormally elevated in the cystic tubular epithelial cells derived from the collecting ducts (Nadella et al., 2010; Kwon et al., 2012).

However, AGS3/GPSM1 does not appear to play a role during compensatory hypertrophy, which is an alternate mechanism to produce increased cell size (Lenarczyk et al., 2014). During hypertrophy, AGS3/GPSM1 protein expression was not induced (Lenarczyk et al., 2014). Moreover, unilateral nephrectomy in the *Gpsm1*-deficient mice did not affect the ability of the remaining kidney to increase its size compared to wild-type mice (Lenarczyk et al., 2014).

From these studies, AGS3/GPSM1 appeared to act as a stress-response protein to promote tubular epithelial cell repair in an attempt to restore normal kidney function following renal injury or in genetic diseases involving cellular hyperplasia.

Mechanistically, AGS3/GPSM1 sequesters $G\alpha_i$ subunits to prevent the inactivation of Gβγ signaling (Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012). Gβγ activity has been implicated in mitotic spindle orientation (Sanada and Tsai, 2005), ion channel activity (Kwon et al., 2012), cAMP production (Fan et al., 2009), or modulation of GPCR-dependent

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(Sato et al., 2004; Oner et al., 2010; Oner et al., 2013) and -independent signaling pathways (Smrcka, 2008; Smrcka et al., 2008; Lin and Smrcka, 2011). Over-expression of AGS3/GPSM1 facilitated an increase of polycystin-1/polycystin-2 ion channel activity, which could be blocked by a scavenger of Gβγ dimers (Kwon et al., 2012). Alternatively, there was evidence to suggest that AGS3/GPSM1 through its interaction with $G\alpha_i$ subunits could regulate the autophagic process (Pattingre et al., 2003; Pattingre et al., 2004; Ghosh et al., 2010; Groves et al., 2010), but this may be cell-type dependent (Vural et al., 2013). AGS3/GPSM1 may also influence signal transduction pathways through other protein-protein interactions, such as LKB1 (Blumer et al., 2003), which is a serine/threonine kinase known to regulate AMPK signaling during states of energetic stress (Alexander and Walker, 2011). These studies demonstrate the potentially diverse pathways that may be controlled by AGS3/GPSM1 during normal or pathological states.

AGS5/GPSM2/LGN. AGS5 is also known as LGN (Leu-Gly-Asn repeat-enriched protein) or G-protein signaling modulator 2 (GPSM2), and functions as a GDI. AGS5/LGN exhibits a similar protein structure as AGS3/GPSM1, and is considered a mammalian homolog of AGS3/GPSM1. Unlike AGS3/GPSM1, AGS5/LGN was ubiquitously expressed in mammalian organs, including the kidney (Blumer et al., 2002). Our group has detected AGS5/LGN in mouse, rat and human tubular epithelial cell lines *in vitro* and whole kidneys *in vivo* (Regner et al., 2011; Kwon et al., 2012; Lenarczyk et al., 2014). In the normal rat kidneys, AGS5/LGN was detected in the distal tubules, including thick ascending limb and collecting ducts (Lenarczyk et al., 2014). Unlike AGS3/GPSM1, there was no observable change in the AGS5/LGN protein levels or localization pattern following acute kidney injury (Regner et al., 2011; Lenarczyk et al., 2014) or in genetically mutant cystic kidneys (Nadella et al., 2010).

AGS5/LGN and AGS3/GPSM1 are also considered as mammalian homologs of the Drosophila Partner of Inscuteable (Pins) protein. Functionally, AGS5/LGN has been well characterized as a regulator of mitotic spindle orientation, which is a process that participates in asymmetric cell division of epithelial cells (Fuja et al., 2004; Lechler and Fuchs, 2005; Culurgioni et al., 2011; Zhu et al., 2011). Consistent with these findings, AGS5/LGN was determined to play a key role in cyst formation by controlling mitotic spindle orientation in a renal epithelial cell system *in vitro* (Zheng et al., 2010; Xiao et al., 2012), but the LGN-$G\alpha_i$

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complex may not be involved in asymmetric daughter cell formation (Xiao et al., 2012). These findings may suggest that AGS5/LGN could compensate for the loss of its homolog, AGS3/GPSM1, as observed in a recent study investigating acute or chronic kidney injury (Regner et al., 2011; Kwon et al., 2012). Alternatively, there may be other biological roles for AGS5/LGN in tubular epithelial cells in the kidney by virtue of its interaction with other proteins, such as soluble guanylate cyclase (Chauhan et al., 2012), which is an important regulator of fluid and electrolytes balance through the actions of hormones, such as nitric oxide (O'Connor and Cowley, 2010; Aksu et al., 2011; Toda and Okamura, 2011). To date, the loss of AGS5/LGN in the kidney and its effects on renal function remains to be determined.

The second type of Group II AGS proteins, including AGS6, Regulator of G-protein Signaling 14 (RGS14) and Rap1GAP, were identified by the presence of a single GPR motif and other protein regulatory domains that function to accelerate Gα-GTP hydrolysis (Blumer and Lanier, 2014).

AGS6/RGS12. AGS6 has sequence identity with Regulator of G-protein Signaling 12 (RGS12), which is the largest member of the RGS protein family. AGS6/RGS12 has a multi-domain protein structure in which it contains the RGS domain, a single GoLoco/GPR motif, and other protein–binding domains. AGS6/RGS12 inactivated G-protein signaling by accelerating the intrinsic GTPase activity for $G\alpha_{i/o}$ subunits through the N-terminal RGS domain (De Vries et al., 2000b), while the C-terminal GPR/GoLoco motif interacted with all isoforms of the $G\alpha_i$ subunits (Kimple et al., 2001). The expression profile for AGS6/RGS12 in the kidney may be species- or isoform-dependent, since multiple RGS12 mRNA isoforms were expressed in the human (Snow et al., 1998), but not in mouse kidneys (Yang and Li, 2007). At the protein level, however, we have observed low uniform staining throughout the rat nephron and the endothelial cells of the blood vessels in the renal cortex (Lenarczyk et al., 2014).

Functionally, there is a paucity of data regarding the role of AGS6/RGS12 in the kidney, but there are number of studies demonstrating that AGS6/RGS12 played a critical role in the regulation of calcium channels, including N-type (Schiff et al., 2000; Yang and Li, 2007) and $Ca_{V2.2}$ calcium channels (Anantharam and Diverse-Pierluissi, 2002; Richman and Diverse-

Pierluissi, 2004; Richman et al., 2005). At this time, the role of the GPR domain in RGS12 and how its regulate G-protein signaling compared to its RGS effects remains to be determined.

RGS14. RGS14 is an accessory protein initially cloned by Snow *et al.* (Snow et al., 1997), and contains a single GPR/GoLoco motif and a RGS domain. RGS14 interacted with $G\alpha_{i/o}$ subunits in the RGS box (Traver et al., 2000; Kimple et al., 2001) whereas the GPR/GoLoco motif selectively bound to the $G\alpha_{i1}$ and $G\alpha_{i3}$ (Mittal and Linder, 2004) with weaker interaction with $G\alpha_{i2}$ (Kimple et al., 2001). RGS14 mRNA distribution was relatively exclusive to the spleen, lung and select regions in the rat brain (Snow et al., 1997; Reif and Cyster, 2000) with no apparent expression in the kidney (Snow et al., 1997).

Rap1GAP. Rap1GAP has limited expression distribution in mammalian organs under normal conditions (Rubinfeld et al., 1991). Recent studies, however, have shown that the regulation of Rap1GAP expression was dependent upon the disease stimulus in the kidney. Rap1GAP expression was induced in renal glomerular podocytes from HIV-1 transgenic mice and in human kidney biopsies of focal and segmental glomerulosclerosis (FSGS) (Potla et al., 2014), which appeared to contribute to the podocyte dysfunction after injury by altering β1-integrin mediated adhesion. In renal carcinoma, the Rap1GAP protein levels were reduced leading to increased cellular invasion (Kim et al., 2012). The importance of these observations in terms of renal function needs further elucidation.

The third type of group II AGS proteins consists of 3 proteins, a truncated form of AGS3 (AGS3-SHORT) (Pizzinat et al., 2001), AGS4/GPSM3/G18.1b (Cao et al., 2004), and Pcp-2/L7/GPSM4 (Nordquist et al., 1988), which has multiple C-terminal GPR domains, but no other defined regulatory protein binding sites. Minimal to no expression of AGS3-SHORT (Pizzinat et al., 2001), AGS4/GPSM3/G18.1b (Cao et al., 2004; Zhao et al., 2010), or Pcp-2/L7/GPSM4 (Nordquist et al., 1988; Saito et al., 2005) have been detected in the kidneys.

Group III AGS proteins. Group III AGS proteins include AGS2/TcTex1 (DiBella et al., 2001), AGS7/Trip13 (Li and Schimenti, 2007; Roig et al., 2010), AGS8/FNDC1 (Nielsen et al., 1993), AGS9/Rpn10/S5a/Psmd4 (Deveraux et al., 1995), and AGS10/GNAO (Neer et al., 1984; Sternweis and Robishaw, 1984). The biological function of group III AGS proteins remains

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largely undefined in the kidney, but each of these proteins interact directly with Gβγ subunits complexed with or without the Gα subunits.

AGS2/Tctex1. AGS2 was initially characterized by Takesono *et al*. (Takesono et al., 1999), and was homologous to Tctex1. Tctex1 is a cytoplasmic dynein light chain essential for dynein assembly and participates in specific motor cargo interactions (DiBella et al., 2001). Previous studies have detected Tctex1 mRNA (DiBella et al., 2001) and protein (King et al., 1998) in fetal and adult mouse kidneys (King et al., 1998; DiBella et al., 2001). Human kidneys express higher levels of Tctex1 mRNA compared to the other organs analyzed (DiBella et al., 2001). Recent studies using neural progenitor cells have shown that AGS2/Tctex1 is recruited to the ciliary transition zone prior to the DNA synthesis phase of the cell cycle (Li et al., 2011a), and is modulated by interaction with AGS3/GPSM1 (Yeh et al., 2013). For this reason, AGS2/Tctex1 may have clinical relevance with renal pathologies associated with ciliary defects, such as polycystic kidney disease.

AGS7/Trip13/mPCH2. AGS7 was isolated from a yeast screen investigating receptor-independent G-protein regulators using a prostate leiosarcoma cDNA library (Sato et al., 2006b). AGS7 demonstrated sequence identity with the C-terminal portion of the thyroid receptor interacting protein 13 (Trip13) (Cismowski and Lanier, 2005), which was originally identified amongst a group of other proteins that interacted with the thyroid hormone receptor in a yeast two-hybrid screen (Lee et al., 1995). Neither the role of AGS7/Trip13 as a regulator of the thyroid hormone receptor nor G-protein signaling functions have been elucidated in the kidney *in vivo*. Instead, most of the work involving AGS7/Trip13, which is also a homolog to mouse pachytene checkpoint 2 (mPCH2), has focused on its AAA-ATPase activity with emphasis on the mechanistic control of DNA damage repair (Bolcun-Filas et al., 2014), cell cycle checkpoint, kinetochore control (Tipton et al., 2012; Wang et al., 2014b), and meiotic recombination (Li and Schimenti, 2007; Roig et al., 2010). These initial studies led to recent high impact findings regarding AGS7/Trip13 in reproductive cell biology (Li and Schimenti, 2007; Roig et al., 2010) and cancer (Banerjee et al., 2014).

In the kidney, the expression of Trip13 mRNA was minimally detected in the kidney using qualitative RT-PCR (Li and Schimenti, 2007). This was consistent with the Trip13 protein

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localization found only in the principal cells of the collecting ducts in the normal rat kidney (Lenarcyzk et al., 2014). The site of expression in the nephron may implicates AGS7/Trip13 as a potential regulator of Na^+, water or urea reabsorption, but the biological importance of AGS7/Trip13 during normal and pathological conditions in the kidney needs further investigation.

AGS8/FNDC1/KIAA1866. AGS8 was identified using cDNA library from the ischemic injured mouse hearts (Nielsen et al., 1993), and was homologous to Fibronectin type III containing I (FNDC1) (Gao et al., 2003) and KIAA1866 (GenBank Accession number XM_217792.2). Transcript profiling by RT-PCR analysis showed that the basal expression of AGS8 mRNA was most abundant in the skeletal muscle and the kidney (Sato et al., 2006b). AGS8/FNDC1 localization was detected exclusively in distal tubular epithelial cells using immunohistochemistry of Sprague Dawley rat kidneys (Lenarcyzk et al., 2014).

Little is known about the function of AGS8/FNDC1 in the kidney. A recent genome wide association study suggested a potential link between AGS8/FNDC1 and urinary albumin-to-creatinine ratio (UACR) in African Americans from the CARe Renal Consortium (Liu et al., 2011). However, morpholino knockdown of AGS8/FNDC1 in zebrafish did not support a role for this protein during renal development or dysfunction (Liu et al., 2011).

In terms of G-protein regulation, epistasis experiments demonstrated that AGS8 interacted selectively with Gβγ, but not $G\alpha_{i1/2/3/0}$ or $G\alpha_s$. AGS8 has been shown to interact with Gβγ activates connexin-43, which can regulate gap junction function (Sato et al., 2009). Considering that CX-43 is abundantly expressed in the glomerulus and inner medullary collecting duct epithelial cells (Guo et al., 1998), which is a primary site of AGS8 expression, the role of AGS8 with connexins, including CX-43, may need further exploration in the kidney. Alternatively, AGS8/*Fndc1* may play an important role in ischemia-reperfusion injury. In the heart, AGS8/Fndc1 gene expression could be robustly induced in cardiomyocytes during ischemia (Sato et al., 2006b; Sato et al., 2009; Sato et al., 2014). In the kidney, however, our lab detected a marked reduction in AGS8/Fndc1 mRNA after 24 hours following ischemia-reperfusion injury, which slowly returned back toward normal levels after 168 hrs (Lenarcyzk et al., 2014). It is not presently known whether the reduction in renal AGS8/*Fndc1* mRNA is

attributed directly to negative transcriptional regulation or whether damaged nephron segments were unable to continue expressing AGS8/*Fndc1* due to the biological insult.

AGS9/PSMD4/Rpn10/S5a. AGS9 has sequence identity to a protein known as S5a, which was encoded by the gene for the multiubiquitin-chain-binding protein (Mcb1) in rodents and humans (Ferrell et al., 1996; Pusch et al., 1998). S5a has multiple other nomenclatures, including Rpn10 and Psmd4. S5a/Rpn10 was ubiquitously expressed, but it has multiple splice variants, Rpn10a, b, c, d and e. The expression pattern was dependent on the stage of development (Hamazaki et al., 2007), and the kidneys were one of the major organs that had the highest level of S5a/Rpn10 expression using Northern blot analysis (Pusch et al., 1998).

S5a is part of a large multimeric protein complex involved in protein degradation through the ubiquitin-proteosome system (UPS). In the UPS, protein substrates that were destined for degradation were poly-ubiquitinated for subsequent catabolism through a non-lysosomal pathway (Ciechanover, 1993), which involved the 26S proteasome (Tanaka, 1995). The binding of the poly-ubiquitinated protein substrate to the 26S proteasome was facilitated by the S5a subunit, and was one of the major docking sites in the proteosome (Verma et al., 2004). Genetic loss of the S5a/Rpn10 expression in mice resulted in embryonic lethality (Hamazaki et al., 2007), and this would suggest that S5a/Rpn10-mediated degradation of ubiquitinated proteins was an indispensable cellular function essential to maintain mammalian life. To date, it remains to be determined by which heterotrimeric G-protein subunits, namely Gβγ, interact with S5a/Rpn10 to control renal function.

AGS10/GNAO. The more commonly known name for AGS10 is GNAO, which is a G-protein α subunit that was initially isolated as a 39 kD polypeptide from rat brain and liver, and bovine heart (Neer et al., 1984; Sternweis and Robishaw, 1984). AGS10/GNAO expression appears to be more broadly detected in many other cell types, including pancreatic cells (El-Mansoury and Morgan, 1998), but there is minimal, if any, expression within the kidney (Brann et al., 1987; Senkfor et al., 1993). Due to a lack of GNAO expression in the kidney as well as the lethality of GNAO knockout mice, there is minimal knowledge regarding any role for GNAO in the kidney.

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Group IV AGS proteins. The most recent AGS proteins were identified by their interaction with a specific subunit in the $G\alpha_q$ family, $G\alpha_{16}$ (Sato et al., 2011). Sequence analysis of the $G\alpha_{16}$-specific cDNAs were identified as the microphthalmia (MiT) family of basic helix-loop-helix-leucine zipper transcription factors (Sato et al., 2011), specifically transcription factor E3 (TFE3), transcription factor EB (TFEB), and MiTF. Numerically, TFE3, TFEB and MiTF were designated as AGS11, AGS12, and AGS13, respectively (Sato et al., 2011).

In normal kidneys, the mRNA expression profile of AGS11/TFE3, AGS12/TFEB and AGS13/MiTF is generally quite low (Kuiper et al., 2003; Kuiper et al., 2004), and the protein localization is either observed in predominantly in the cytoplasm or weakly in the nucleus (Hong et al., 2010). There is some evidence that the basal role of TFE3 in proximal tubules is to control inorganic phosphate reabsorption (Miyamoto and Itho, 2001). However, MitF/TFE transcription factors appears to play a fundamentally important role during translocation renal cell carcinomas from children and young adults (Armah and Parwani, 2010; Hong et al., 2010), in which there is modest to robust nuclear localization of AGS11/TFE3 and to a lesser extent, AGS12/TFEB. Because of this shift in subcellular location to the nucleus, the MiTF/TFE transcription factors are being used as diagnostic biomarkers for specific subtypes of renal cell carcinoma. In these subtypes of renal cancer, the cellular transformation is believed to be attributed to abnormal chromosomal translocation or inversion of genomic DNA containing either TFE3 (Meloni et al., 1993; Weterman et al., 1996a; Weterman et al., 1996b) or TFEB (Kuiper et al., 2003) into other genes (Weterman et al., 2000; Weterman et al., 2001; Mathur and Samuels, 2007). To date, the nephron segment responsible for the renal cell carcinoma remains to be determined, but there is evidence for TFEB translocation originating from distal tubules (Rao et al., 2012).

MiTF has also been linked as an oncogene for renal cell carcinoma, but its function has been described in greater detail for melanoma (Granter et al., 2002; Bertolotto et al., 2011). There is evidence that MiTF/TFE transcription factors have an inter-relationship between each other through redundant oncogenic function whereby TFE3 knockdown decreased viability of TFE3-translocated papillary renal carcinoma cells, but could be rescued by expression of MiTF (Davis et al., 2006).

In addition to renal oncogenesis, there is increasing evidence that TFE3 plays a role in the pathogenesis of renal cystic disease. Increased chimeric TFE3 protein led to dedifferentiation of renal proximal tubule cells as determined by a loss of cilia formation and key functional transporter proteins (Mathur and Samuels, 2007). Other studies have shown that folliculin (FLCN), a crucial negative regulator of TFE3 nuclear translocation, promoted renal cell carcinoma and produced the phenotypic appearance of polycystic kidney disease (Chen et al., 2008; Hong et al., 2010). In FLCN-deficient mice, renal tubular epithelial cells transformed into a cystic phenotype that increased nuclear localization of TFE3 compared to the non-cystic kidneys (Chen et al., 2008).

At present, however, there remains a paucity of information regarding the mode of action by which MitF/TFE regulates the process of oncogenesis and cystogenesis, particular its role in modulating G-protein signaling in the kidney. $G\alpha_{16}$ was normally localized to the cytoplasm and plasma membrane (Sato et al., 2011). Co-expression of $G\alpha_{16}$ with TFE3 led to nuclear accumulation, which induced the expression of claudin 14, a cell junction protein, in cardiomyocytes (Sato et al., 2011). Claudins are widely expressed in the kidney and could implicate these transcription factors as regulators of paracellular ion transport to control fluid and electrolyte balance (Yu, 2015). On the other hand, MitF has been shown to be a transcriptional regulator of hypoxia inducible factor 1α (Busca et al., 2005), which was shown to be abundantly expressed in various renal cell types (Haase, 2006).

There is definitely a need to further elucidate the functional role of these transcription factors with respect to G-protein regulation. Since the expression of $G\alpha_{15/16}$ is much lower compared to other isoforms of the $G\alpha_q$ isoforms in the kidney (Wilkie et al., 1991), it remains to be determined whether this is a signaling mechanism by which AGS11-13 promotes carcinogenesis in the kidney.

Summary and Perspective. AGS exhibit a distinct distribution pattern within the nephron, glomerulus, and vasculature, which helps in producing a diverse control mechanism on G-protein signaling evoked by receptor-dependent or -independent stimuli. Although none of the currently known AGS proteins were initially discovered using a normal or diseased kidney-specific cDNA library, several have shown biological importance in the kidney, including

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AGS3 during acute and chronic kidney injury (Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012) and AGS11-13 in different forms of renal cell carcinoma (Granter et al., 2002; Armah and Parwani, 2010; Hong et al., 2010; Bertolotto et al., 2011). Further progress regarding the importance of AGS proteins in the kidney is needed under various stressful conditions, and more importantly, determine whether the mode of regulation is through the perturbation of G-protein signaling. Some of the identified AGS proteins, such as AGS2, AGS7, AGS9, and AGS11-13, were not previously known to interact with G-protein α or βγ subunits, and have only begun to be studied in greater detail.

Identification of additional AGS proteins, if they even exist, using kidney-specific cDNA libraries in the yeast screen may also offer new avenues of research in normal, and possibly diseased kidneys. Even without the identification of new AGS family members, there will be continual accumulation of knowledge regarding the expression profile, spatial localization and biological function of the AGS proteins in various renal pathologies. This will warrant the development of therapeutic interventions targeting individual AGS proteins, particularly in disease states with an active state of growth or hyperplasia. As an example, Group II AGS proteins contain one or more of the unique GPR motifs that could be targeted using peptide sequences to manipulate the binding of $G\alpha_i$ subunits in the renal epithelial cells (Blumer et al., 2003; Webb et al., 2005). Considering that the induction of the AGS proteins is generally cell-type specific, the pathologically defective or actively recovering renal cells may be more permissive for uptake of small molecular reagents (eg. peptide or nucleic acid sequences) or chemical molecules to manipulate AGS function.

Modulating the protein function of intracellular versus cell surface receptors in the kidney is obviously more challenging for a variety of factors, largely due to the complex architecture of the kidney with its numerous cell types, irregular blood blow distribution, solute gradients, and wide pH ranges. Each of these factors can complicate the development of an effective therapeutic to modulate AGS. However, the molecular and cellular technologies continue to evolve leading to new discoveries focusing on effective drug delivery systems. Small molecules are being identified to target specific proteins (Jonasch et al., 2012) or cell types (Raj et al., 2011), which may be a viable approach to develop therapeutics targeting

Group IV AGS proteins to combat renal cell carcinoma. Alternatively, there is emerging new viral vector systems, such as replication-defective lentiviral vectors (Kim et al., 2010; Park et al., 2010) or adeno-associated virus type 9 (Bostick et al., 2007), which has shown promise for renal cell transduction. These vector systems could be designed to either over-express small sequences to block $G\alpha_i$ binding or inhibit $G\beta\gamma$ activity, or possibly knockdown the expression levels of AGS genes to control their effect on G-protein signaling. Although there has yet to be a successful method to correct renal diseases using these approaches to date, the continued study into the biology of AGS proteins will hopefully elucidate pathways that are crucial to G-protein regulation during various renal pathologies.

In summary, the field of AGS protein biology is poised to make significant advances in the kidney over the next few years. With the inevitable convergence between the fields of medicinal chemistry, gene therapy, and G-protein biology, new therapies targeting AGS proteins should be considered as potential clinical interventions for acute and chronic kidney diseases.

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REFERENCES

Aksu U, Demirci C and Ince C (2011) The pathogenesis of acute kidney injury and the toxic triangle of oxygen, reactive oxygen species and nitric oxide. *Contributions to nephrology* **174**:119-128.

Alexander A and Walker CL (2011) The role of LKB1 and AMPK in cellular responses to stress and damage. *FEBS letters* **585**:952-957.

Anai M, Shojima N, Katagiri H, Ogihara T, Sakoda H, Onishi Y, Ono H, Fujishiro M, Fukushima Y, Horike N, Viana A, Kikuchi M, Noguchi N, Takahashi S, Takata K, Oka Y, Uchijima Y, Kurihara H and Asano T (2005) A novel protein kinase B (PKB)/AKT-binding protein enhances PKB kinase activity and regulates DNA synthesis. *The Journal of biological chemistry* **280**:18525-18535.

Anantharam A and Diverse-Pierluissi MA (2002) Biochemical approaches to study interaction of calcium channels with RGS12 in primary neuronal cultures. *Methods in enzymology* **345**:60-70.

Armah HB and Parwani AV (2010) Xp11.2 translocation renal cell carcinoma. *Archives of pathology & laboratory medicine* **134**:124-129.

Asano T, Morishita R, Ueda H, Asano M and Kato K (1998) GTP-binding protein gamma12 subunit phosphorylation by protein kinase C--identification of the phosphorylation site and factors involved in cultured cells and rat tissues in vivo. *European journal of biochemistry / FEBS* **251**:314-319.

Banerjee R, Russo N, Liu M, Basrur V, Bellile E, Palanisamy N, Scanlon CS, van Tubergen E, Inglehart RC, Metwally T, Mani RS, Yocum A, Nyati MK, Castilho RM, Varambally S, Chinnaiyan AM and D'Silva NJ (2014) TRIP13 promotes error-prone nonhomologous end joining and induces chemoresistance in head and neck cancer. *Nature communications* **5**:4527.

Berman DM, Wilkie TM and Gilman AG (1996) GAIP and RGS4 are GTPase-activating proteins for the Gi subfamily of G protein alpha subunits. *Cell* **86**:445-452.

Bernard ML, Peterson YK, Chung P, Jourdan J and Lanier SM (2001) Selective interaction of AGS3 with G-proteins and the influence of AGS3 on the activation state of G-proteins. *The Journal of biological chemistry* **276**:1585-1593.

Bertolotto C, Lesueur F, Giuliano S, Strub T, de Lichy M, Bille K, Dessen P, d'Hayer B, Mohamdi H, Remenieras A, Maubec E, de la Fouchardiere A, Molinie V, Vabres P, Dalle S, Poulalhon N, Martin-Denavit T, Thomas L, Andry-Benzaquen P, Dupin N, Boitier F, Rossi A, Perrot JL, Labeille B, Robert C, Escudier B, Caron O, Brugieres L, Saule S, Gardie B, Gad S, Richard S, Couturier J, Teh BT, Ghiorzo P, Pastorino L, Puig S, Badenas C, Olsson H, Ingvar C, Rouleau E, Lidereau R, Bahadoran P, Vielh P, Corda E, Blanche H, Zelenika D, Galan P, French Familial Melanoma Study G, Aubin F, Bachollet B, Becuwe C, Berthet P, Bignon YJ, Bonadona V, Bonafe JL, Bonnet-Dupeyron MN, Cambazard F, Chevrant-Breton J, Coupier I, Dalac S, Demange L, d'Incan M, Dugast C, Faivre L, Vincent-Fetita L, Gauthier-Villars M, Gilbert B, Grange F, Grob JJ, Humbert P, Janin N, Joly P, Kerob D, Lasset C, Leroux D, Levang J, Limacher JM, Livideanu C, Longy M, Lortholary A, Stoppa-Lyonnet D, Mansard S, Mansuy L, Marrou K, Mateus C, Maugard C, Meyer N, Nogues C, Souteyrand P, Venat-Bouvet L, Zattara H, Chaudru V, Lenoir GM, Lathrop M, Davidson I, Avril MF, Demenais F, Ballotti R and Bressac-de Paillerets B (2011) A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature* **480**:94-98.

Bertorello A and Aperia A (1990) Inhibition of proximal tubule Na(+)-K(+)-ATPase activity requires simultaneous activation of DA1 and DA2 receptors. *The American journal of physiology* **259**:F924-928.

Blumer JB, Bernard ML, Peterson YK, Nezu J, Chung P, Dunican DJ, Knoblich JA and Lanier SM (2003) Interaction of activator of G-protein signaling 3 (AGS3) with LKB1, a serine/threonine kinase involved in cell polarity and cell cycle progression: phosphorylation of the G-protein regulatory

(GPR) motif as a regulatory mechanism for the interaction of GPR motifs with Gi alpha. *The Journal of biological chemistry* **278**:23217-23220.

Blumer JB, Chandler LJ and Lanier SM (2002) Expression analysis and subcellular distribution of the two G-protein regulators AGS3 and LGN indicate distinct functionality. Localization of LGN to the midbody during cytokinesis. *The Journal of biological chemistry* **277**:15897-15903.

Blumer JB and Lanier SM (2014) Activators of G protein signaling exhibit broad functionality and define a distinct core signaling triad. *Molecular pharmacology* **85**:388-396.

Blumer JB, Smrcka AV and Lanier SM (2007) Mechanistic pathways and biological roles for receptor-independent activators of G-protein signaling. *Pharmacology & therapeutics* **113**:488-506.

Bolcun-Filas E, Rinaldi VD, White ME and Schimenti JC (2014) Reversal of female infertility by Chk2 ablation reveals the oocyte DNA damage checkpoint pathway. *Science* **343**:533-536.

Bonventre JV and Yang L (2011) Cellular pathophysiology of ischemic acute kidney injury. *The Journal of clinical investigation* **121**:4210-4221.

Bostick B, Ghosh A, Yue Y, Long C and Duan D (2007) Systemic AAV-9 transduction in mice is influenced by animal age but not by the route of administration. *Gene therapy* **14**:1605-1609.

Boucher I, Yu W, Beaudry S, Negoro H, Tran M, Pollak MR, Henderson JM and Denker BM (2012) Galpha12 activation in podocytes leads to cumulative changes in glomerular collagen expression, proteinuria and glomerulosclerosis. *Laboratory investigation; a journal of technical methods and pathology* **92**:662-675.

Brann MR, Collins RM and Spiegel A (1987) Localization of mRNAs encoding the alpha-subunits of signal-transducing G-proteins within rat brain and among peripheral tissues. *FEBS letters* **222**:191-198.

Brogan MD, Behrend EN and Kemppainen RJ (2001) Regulation of Dexras1 expression by endogenous steroids. *Neuroendocrinology* **74**:244-250.

Brogi S, Tafi A, Desaubry L and Nebigil CG (2014) Discovery of GPCR ligands for probing signal transduction pathways. *Frontiers in pharmacology* **5**:255.

Brunskill N, Bastani B, Hayes C, Morrissey J and Klahr S (1991) Localization and polar distribution of several G-protein subunits along nephron segments. *Kidney international* **40**:997-1006.

Busca R, Berra E, Gaggioli C, Khaled M, Bille K, Marchetti B, Thyss R, Fitsialos G, Larribere L, Bertolotto C, Virolle T, Barbry P, Pouyssegur J, Ponzio G and Ballotti R (2005) Hypoxia-inducible factor 1{alpha} is a new target of microphthalmia-associated transcription factor (MITF) in melanoma cells. *The Journal of cell biology* **170**:49-59.

Cali JJ, Balcueva EA, Rybalkin I and Robishaw JD (1992) Selective tissue distribution of G protein gamma subunits, including a new form of the gamma subunits identified by cDNA cloning. *The Journal of biological chemistry* **267**:24023-24027.

Cao X, Cismowski MJ, Sato M, Blumer JB and Lanier SM (2004) Identification and characterization of AGS4: a protein containing three G-protein regulatory motifs that regulate the activation state of Gialpha. *The Journal of biological chemistry* **279**:27567-27574.

Chan P, Gabay M, Wright FA and Tall GG (2011) Ric-8B is a GTP-dependent G protein alphas guanine nucleotide exchange factor. *The Journal of biological chemistry* **286**:19932-19942.

Chauhan S, Jelen F, Sharina I and Martin E (2012) The G-protein regulator LGN modulates the activity of the NO receptor soluble guanylate cyclase. *The Biochemical journal* **446**:445-453.

Chen J, Futami K, Petillo D, Peng J, Wang P, Knol J, Li Y, Khoo SK, Huang D, Qian CN, Zhao P, Dykema K, Zhang R, Cao B, Yang XJ, Furge K, Williams BO and Teh BT (2008) Deficiency of FLCN in mouse kidney led to development of polycystic kidneys and renal neoplasia. *PloS one* **3**:e3581.

Ciechanover A (1993) The ubiquitin-mediated proteolytic pathway. *Brain pathology* **3**:67-75.

Cismowski MJ and Lanier SM (2005) Activation of heterotrimeric G-proteins independent of a G-protein coupled receptor and the implications for signal processing. *Reviews of physiology, biochemistry and pharmacology* **155**:57-80.

Cismowski MJ, Ma C, Ribas C, Xie X, Spruyt M, Lizano JS, Lanier SM and Duzic E (2000) Activation of heterotrimeric G-protein signaling by a ras-related protein. Implications for signal integration. *The Journal of biological chemistry* **275**:23421-23424.

Cismowski MJ, Takesono A, Bernard ML, Duzic E and Lanier SM (2001) Receptor-independent activators of heterotrimeric G-proteins. *Life sciences* **68**:2301-2308.

Cismowski MJ, Takesono A, Ma C, Lizano JS, Xie X, Fuernkranz H, Lanier SM and Duzic E (1999) Genetic screens in yeast to identify mammalian nonreceptor modulators of G-protein signaling. *Nature biotechnology* **17**:878-883.

Culurgioni S, Alfieri A, Pendolino V, Laddomada F and Mapelli M (2011) Inscuteable and NuMA proteins bind competitively to Leu-Gly-Asn repeat-enriched protein (LGN) during asymmetric cell divisions. *Proceedings of the National Academy of Sciences of the United States of America* **108**:20998-21003.

Davis IJ, Kim JJ, Ozsolak F, Widlund HR, Rozenblatt-Rosen O, Granter SR, Du J, Fletcher JA, Denny CT, Lessnick SL, Linehan WM, Kung AL and Fisher DE (2006) Oncogenic MITF dysregulation in clear cell sarcoma: defining the MiT family of human cancers. *Cancer cell* **9**:473-484.

De Vries L, Fischer T, Tronchere H, Brothers GM, Strockbine B, Siderovski DP and Farquhar MG (2000a) Activator of G protein signaling 3 is a guanine dissociation inhibitor for Galpha i subunits. *Proceedings of the National Academy of Sciences of the United States of America* **97**:14364-14369.

De Vries L, Mousli M, Wurmser A and Farquhar MG (1995) GAIP, a protein that specifically interacts with the trimeric G protein G alpha i3, is a member of a protein family with a highly conserved core domain. *Proceedings of the National Academy of Sciences of the United States of America* **92**:11916-11920.

De Vries L, Zheng B, Fischer T, Elenko E and Farquhar MG (2000b) The regulator of G protein signaling family. *Annu Rev Pharmacol Toxicol* **40**:235-271.

Devarajan P (2006) Update on mechanisms of ischemic acute kidney injury. *Journal of the American Society of Nephrology : JASN* **17**:1503-1520.

Deveraux Q, Jensen C and Rechsteiner M (1995) Molecular cloning and expression of a 26 S protease subunit enriched in dileucine repeats. *The Journal of biological chemistry* **270**:23726-23729.

DiBella LM, Benashski SE, Tedford HW, Harrison A, Patel-King RS and King SM (2001) The Tctex1/Tctex2 class of dynein light chains. Dimerization, differential expression, and interaction with the LC8 protein family. *The Journal of biological chemistry* **276**:14366-14373.

Druey KM, Blumer KJ, Kang VH and Kehrl JH (1996) Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. *Nature* **379**:742-746.

El-Mansoury AM and Morgan NG (1998) Activation of protein kinase C modulates alpha2-adrenergic signalling in rat pancreatic islets. *Cellular signalling* **10**:637-643.

Falk JD, Vargiu P, Foye PE, Usui H, Perez J, Danielson PE, Lerner DL, Bernal J and Sutcliffe JG (1999) Rhes: A striatal-specific Ras homolog related to Dexras1. *Journal of neuroscience research* **57**:782-788.

Fan P, Jiang Z, Diamond I and Yao L (2009) Up-regulation of AGS3 during morphine withdrawal promotes cAMP superactivation via adenylyl cyclase 5 and 7 in rat nucleus accumbens/striatal neurons. *Molecular pharmacology* **76**:526-533.

Fang M, Jaffrey SR, Sawa A, Ye K, Luo X and Snyder SH (2000) Dexras1: a G protein specifically coupled to neuronal nitric oxide synthase via CAPON. *Neuron* **28**:183-193.

Ferrell K, Deveraux Q, van Nocker S and Rechsteiner M (1996) Molecular cloning and expression of a multiubiquitin chain binding subunit of the human 26S protease. *FEBS letters* **381**:143-148.

Fong HK, Amatruda TT, 3rd, Birren BW and Simon MI (1987) Distinct forms of the beta subunit of GTP-binding regulatory proteins identified by molecular cloning. *Proceedings of the National Academy of Sciences of the United States of America* **84**:3792-3796.

Fuja TJ, Schwartz PH, Darcy D and Bryant PJ (2004) Asymmetric localization of LGN but not AGS3, two homologs of Drosophila pins, in dividing human neural progenitor cells. *Journal of neuroscience research* **75**:782-793.

Gabay M, Pinter ME, Wright FA, Chan P, Murphy AJ, Valenzuela DM, Yancopoulos GD and Tall GG (2011) Ric-8 proteins are molecular chaperones that direct nascent G protein alpha subunit membrane association. *Science signaling* **4**:ra79.

Gao M, Craig D, Lequin O, Campbell ID, Vogel V and Schulten K (2003) Structure and functional significance of mechanically unfolded fibronectin type III1 intermediates. *Proceedings of the National Academy of Sciences of the United States of America* **100**:14784-14789.

Garcia-Marcos M, Ear J, Farquhar MG and Ghosh P (2011) A GDI (AGS3) and a GEF (GIV) regulate autophagy by balancing G protein activity and growth factor signals. *Molecular biology of the cell* **22**:673-686.

Ghosh P, Beas AO, Bornheimer SJ, Garcia-Marcos M, Forry EP, Johannson C, Ear J, Jung BH, Cabrera B, Carethers JM and Farquhar MG (2010) A G{alpha}i-GIV molecular complex binds epidermal growth factor receptor and determines whether cells migrate or proliferate. *Molecular biology of the cell* **21**:2338-2354.

Ghosh P, Garcia-Marcos M, Bornheimer SJ and Farquhar MG (2008) Activation of Galphai3 triggers cell migration via regulation of GIV. *The Journal of cell biology* **182**:381-393.

Granter SR, Weilbaecher KN, Quigley C and Fisher DE (2002) Role for microphthalmia transcription factor in the diagnosis of metastatic malignant melanoma. *Applied immunohistochemistry & molecular morphology : AIMM / official publication of the Society for Applied Immunohistochemistry* **10**:47-51.

Groves B, Abrahamsen H, Clingan H, Frantz M, Mavor L, Bailey J and Ma D (2010) An inhibitory role of the G-protein regulator AGS3 in mTOR-dependent macroautophagy. *PloS one* **5**:e8877.

Guo R, Liu L and Barajas L (1998) RT-PCR study of the distribution of connexin 43 mRNA in the glomerulus and renal tubular segments. *The American journal of physiology* **275**:R439-447.

Gutowski S, Smrcka A, Nowak L, Wu DG, Simon M and Sternweis PC (1991) Antibodies to the alpha q subfamily of guanine nucleotide-binding regulatory protein alpha subunits attenuate activation of phosphatidylinositol 4,5-bisphosphate hydrolysis by hormones. *The Journal of biological chemistry* **266**:20519-20524.

Haase VH (2006) Hypoxia-inducible factors in the kidney. *American journal of physiology Renal physiology* **291**:F271-281.

Hamazaki J, Sasaki K, Kawahara H, Hisanaga S, Tanaka K and Murata S (2007) Rpn10-mediated degradation of ubiquitinated proteins is essential for mouse development. *Molecular and cellular biology* **27**:6629-6638.

Hansen PB, Castrop H, Briggs J and Schnermann J (2003) Adenosine induces vasoconstriction through Gi-dependent activation of phospholipase C in isolated perfused afferent arterioles of mice. *Journal of the American Society of Nephrology : JASN* **14**:2457-2465.

Harrison LM and He Y (2011) Rhes and AGS1/Dexras1 affect signaling by dopamine D1 receptors through adenylyl cyclase. *Journal of neuroscience research* **89**:874-882.

Hong SB, Oh H, Valera VA, Baba M, Schmidt LS and Linehan WM (2010) Inactivation of the FLCN tumor suppressor gene induces TFE3 transcriptional activity by increasing its nuclear localization. *PloS one* **5**:e15793.

Horita S, Seki G, Yamada H, Suzuki M, Koike K and Fujita T (2013) Roles of renal proximal tubule transport in the pathogenesis of hypertension. *Current hypertension reviews* **9**:148-155.

Hunt TW, Fields TA, Casey PJ and Peralta EG (1996) RGS10 is a selective activator of G alpha i GTPase activity. *Nature* **383**:175-177.

Jonasch E, Futreal PA, Davis IJ, Bailey ST, Kim WY, Brugarolas J, Giaccia AJ, Kurban G, Pause A, Frydman J, Zurita AJ, Rini BI, Sharma P, Atkins MB, Walker CL and Rathmell WK (2012) State of the science: an update on renal cell carcinoma. *Molecular cancer research : MCR* **10**:859-880.

Kemppainen RJ and Behrend EN (1998) Dexamethasone rapidly induces a novel ras superfamily member-related gene in AtT-20 cells. *The Journal of biological chemistry* **273**:3129-3131.

Kemppainen RJ, Cox E, Behrend EN, Brogan MD and Ammons JM (2003) Identification of a glucocorticoid response element in the 3'-flanking region of the human Dexras1 gene. *Biochimica et biophysica acta* **1627**:85-89.

Kim M, Park SW, Kim M, Chen SW, Gerthoffer WT, D'Agati VD and Lee HT (2010) Selective renal overexpression of human heat shock protein 27 reduces renal ischemia-reperfusion injury in mice. *American journal of physiology Renal physiology* **299**:F347-358.

Kim WJ, Gersey Z and Daaka Y (2012) Rap1GAP regulates renal cell carcinoma invasion. *Cancer letters* **320**:65-71.

Kimple RJ, De Vries L, Tronchere H, Behe CI, Morris RA, Gist Farquhar M and Siderovski DP (2001) RGS12 and RGS14 GoLoco motifs are G alpha(i) interaction sites with guanine nucleotide dissociation inhibitor Activity. *The Journal of biological chemistry* **276**:29275-29281.

King SM, Barbarese E, Dillman JF, 3rd, Benashski SE, Do KT, Patel-King RS and Pfister KK (1998) Cytoplasmic dynein contains a family of differentially expressed light chains. *Biochemistry* **37**:15033-15041.

Koelle MR and Horvitz HR (1996) EGL-10 regulates G protein signaling in the C. elegans nervous system and shares a conserved domain with many mammalian proteins. *Cell* **84**:115-125.

Kuiper RP, Schepens M, Thijssen J, Schoenmakers EF and van Kessel AG (2004) Regulation of the MiTF/TFE bHLH-LZ transcription factors through restricted spatial expression and alternative splicing of functional domains. *Nucleic acids research* **32**:2315-2322.

Kuiper RP, Schepens M, Thijssen J, van Asseldonk M, van den Berg E, Bridge J, Schuuring E, Schoenmakers EF and van Kessel AG (2003) Upregulation of the transcription factor TFEB in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. *Human molecular genetics* **12**:1661-1669.

Kwon M, Pavlov TS, Nozu K, Rasmussen SA, Ilatovskaya DV, Lerch-Gaggl A, North LM, Kim H, Qian F, Sweeney WE, Jr., Avner ED, Blumer JB, Staruschenko A and Park F (2012) G-protein signaling modulator 1 deficiency accelerates cystic disease in an orthologous mouse model of autosomal dominant polycystic kidney disease. *Proceedings of the National Academy of Sciences of the United States of America* **109**:21462-21467.

Lechler T and Fuchs E (2005) Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437**:275-280.

Lee JW, Choi HS, Gyuris J, Brent R and Moore DD (1995) Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. *Molecular endocrinology* **9**:243-254.

Lenarcyzk M, Regner KR and Park F (2014) Localization and expression profile of Activators of G-protein Signaling in the kidney, in *The FASEB Journal* p 690.696.

Lenarczyk M, Pressly JD, Arnett J, Regner KR and Park F (2014) Localization and expression profile of Group I and II AGS proteins in the kidney. *J Mol Histology*.

Li A, Saito M, Chuang JZ, Tseng YY, Dedesma C, Tomizawa K, Kaitsuka T and Sung CH (2011a) Ciliary transition zone activation of phosphorylated Tctex-1 controls ciliary resorption, S-phase entry and fate of neural progenitors. *Nature cell biology* **13**:402-411.

Li H, Weatherford ET, Davis DR, Keen HL, Grobe JL, Daugherty A, Cassis LA, Allen AM and Sigmund CD (2011b) Renal proximal tubule angiotensin AT1A receptors regulate blood pressure. *American journal of physiology Regulatory, integrative and comparative physiology* **301**:R1067-1077.

Li XC and Schimenti JC (2007) Mouse pachytene checkpoint 2 (trip13) is required for completing meiotic recombination but not synapsis. *PLoS genetics* **3**:e130.

Lin Y and Smrcka AV (2011) Understanding molecular recognition by G protein betagamma subunits on the path to pharmacological targeting. *Molecular pharmacology* **80**:551-557.

Liu CT, Garnaas MK, Tin A, Kottgen A, Franceschini N, Peralta CA, de Boer IH, Lu X, Atkinson E, Ding J, Nalls M, Shriner D, Coresh J, Kutlar A, Bibbins-Domingo K, Siscovick D, Akylbekova E, Wyatt S, Astor B, Mychaleckjy J, Li M, Reilly MP, Townsend RR, Adeyemo A, Zonderman AB, de Andrade M, Turner ST, Mosley TH, Harris TB, Consortium CK, Rotimi CN, Liu Y, Kardia SL, Evans MK, Shlipak MG, Kramer H, Flessner MF, Dreisbach AW, Goessling W, Cupples LA, Kao WL and Fox CS (2011) Genetic association for renal traits among participants of African ancestry reveals new loci for renal function. *PLoS genetics* **7**:e1002264.

Maldonado-Agurto R, Toro G, Fuentealba J, Arriagada C, Campos T, Albistur M, Henriquez JP, Olate J, Hinrichs MV and Torrejon M (2011) Cloning and spatiotemporal expression of RIC-8 in Xenopus embryogenesis. *Gene expression patterns : GEP* **11**:401-408.

Mathur M and Samuels HH (2007) Role of PSF-TFE3 oncoprotein in the development of papillary renal cell carcinomas. *Oncogene* **26**:277-283.

Meloni AM, Dobbs RM, Pontes JE and Sandberg AA (1993) Translocation (X;1) in papillary renal cell carcinoma. A new cytogenetic subtype. *Cancer genetics and cytogenetics* **65**:1-6.

Miller KG, Emerson MD, McManus JR and Rand JB (2000) RIC-8 (Synembryn): a novel conserved protein that is required for G(q)alpha signaling in the C. elegans nervous system. *Neuron* **27**:289-299.

Mittal V and Linder ME (2004) The RGS14 GoLoco domain discriminates among Galphai isoforms. *The Journal of biological chemistry* **279**:46772-46778.

Miyamoto KI and Itho M (2001) Transcriptional regulation of the NPT2 gene by dietary phosphate. *Kidney international* **60**:412-415.

Nadella R, Blumer JB, Jia G, Kwon M, Akbulut T, Qian F, Sedlic F, Wakatsuki T, Sweeney WE, Jr., Wilson PD, Lanier SM and Park F (2010) Activator of G protein signaling 3 promotes epithelial cell proliferation in PKD. *Journal of the American Society of Nephrology : JASN* **21**:1275-1280.

Neer EJ, Lok JM and Wolf LG (1984) Purification and properties of the inhibitory guanine nucleotide regulatory unit of brain adenylate cyclase. *The Journal of biological chemistry* **259**:14222-14229.

Nguyen CH and Watts VJ (2006) Dexamethasone-induced Ras protein 1 negatively regulates protein kinase C delta: implications for adenylyl cyclase 2 signaling. *Molecular pharmacology* **69**:1763-1771.

Nielsen S, DiGiovanni SR, Christensen EI, Knepper MA and Harris HW (1993) Cellular and subcellular immunolocalization of vasopressin-regulated water channel in rat kidney. *Proceedings of the National Academy of Sciences of the United States of America* **90**:11663-11667.

Nitta K, Uchida K, Kawashima A, Tsutsui T, Ozu H, Naito T, Yumura W and Nihei H (1994) Identification of GTP-binding proteins in human glomeruli. *Nihon Jinzo Gakkai shi* **36**:9-12.

Nordquist DT, Kozak CA and Orr HT (1988) cDNA cloning and characterization of three genes uniquely expressed in cerebellum by Purkinje neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **8**:4780-4789.

O'Connor PM and Cowley AW, Jr. (2010) Modulation of pressure-natriuresis by renal medullary reactive oxygen species and nitric oxide. *Current hypertension reports* **12**:86-92.

Oner SS, An N, Vural A, Breton B, Bouvier M, Blumer JB and Lanier SM (2010) Regulation of the AGS3.G{alpha}i signaling complex by a seven-transmembrane span receptor. *The Journal of biological chemistry* **285**:33949-33958.

Oner SS, Vural A and Lanier SM (2013) Translocation of activator of G-protein signaling 3 to the Golgi apparatus in response to receptor activation and its effect on the trans-Golgi network. *The Journal of biological chemistry* **288**:24091-24103.

Park SW, Chen SW, Kim M, D'Agati VD and Lee HT (2010) Selective intrarenal human A1 adenosine receptor overexpression reduces acute liver and kidney injury after hepatic ischemia reperfusion in mice. *Laboratory investigation; a journal of technical methods and pathology* **90**:476-495.

Pattingre S, De Vries L, Bauvy C, Chantret I, Cluzeaud F, Ogier-Denis E, Vandewalle A and Codogno P (2003) The G-protein regulator AGS3 controls an early event during macroautophagy in human intestinal HT-29 cells. *The Journal of biological chemistry* **278**:20995-21002.

Pattingre S, Petiot A and Codogno P (2004) Analyses of Galpha-interacting protein and activator of G-protein-signaling-3 functions in macroautophagy. *Methods in enzymology* **390**:17-31.

Peterson YK, Bernard ML, Ma H, Hazard S, 3rd, Graber SG and Lanier SM (2000) Stabilization of the GDP-bound conformation of Gialpha by a peptide derived from the G-protein regulatory motif of AGS3. *The Journal of biological chemistry* **275**:33193-33196.

Pippin JW, Brinkkoetter PT, Cormack-Aboud FC, Durvasula RV, Hauser PV, Kowalewska J, Krofft RD, Logar CM, Marshall CB, Ohse T and Shankland SJ (2009) Inducible rodent models of acquired podocyte diseases. *American journal of physiology Renal physiology* **296**:F213-229.

Pizzinat N, Takesono A and Lanier SM (2001) Identification of a truncated form of the G-protein regulator AGS3 in heart that lacks the tetratricopeptide repeat domains. *The Journal of biological chemistry* **276**:16601-16610.

Potla U, Ni J, Vadaparampil J, Yang G, Leventhal JS, Campbell KN, Chuang PY, Morozov A, He JC, D'Agati VD, Klotman PE and Kaufman L (2014) Podocyte-specific RAP1GAP expression contributes to focal segmental glomerulosclerosis-associated glomerular injury. *The Journal of clinical investigation* **124**:1757-1769.

Pusch W, Jahner D and Ivell R (1998) Molecular cloning and testicular expression of the gene transcripts encoding the murine multiubiquitin-chain-binding protein (Mcb1). *Gene* **207**:19-24.

Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X, Tolliday NJ, Golub TR, Carr SA, Shamji AF, Stern AM, Mandinova A, Schreiber SL and Lee SW (2011) Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature* **475**:231-234.

Rao Q, Liu B, Cheng L, Zhu Y, Shi QL, Wu B, Jiang SJ, Wang Y, Wang X, Yu B, Zhang RS, Ma HH, Lu ZF, Tu P, Wang JD and Zhou XJ (2012) Renal cell carcinomas with t(6;11)(p21;q12): A clinicopathologic study emphasizing unusual morphology, novel alpha-TFEB gene fusion point, immunobiomarkers, and ultrastructural features, as well as detection of the gene fusion by fluorescence in situ hybridization. *The American journal of surgical pathology* **36**:1327-1338.

Ray K, Kunsch C, Bonner LM and Robishaw JD (1995) Isolation of cDNA clones encoding eight different human G protein gamma subunits, including three novel forms designated the gamma 4, gamma 10, and gamma 11 subunits. *The Journal of biological chemistry* **270**:21765-21771.

Regner KR, Nozu K, Lanier SM, Blumer JB, Avner ED, Sweeney WE, Jr. and Park F (2011) Loss of activator of G-protein signaling 3 impairs renal tubular regeneration following acute kidney injury in rodents. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **25**:1844-1855.

Reif K and Cyster JG (2000) RGS molecule expression in murine B lymphocytes and ability to down-regulate chemotaxis to lymphoid chemokines. *Journal of immunology* **164**:4720-4729.

Richman RW and Diverse-Pierluissi MA (2004) Mapping of RGS12-Cav2.2 channel interaction. *Methods in enzymology* **390**:224-239.

Richman RW, Strock J, Hains MD, Cabanilla NJ, Lau KK, Siderovski DP and Diverse-Pierluissi M (2005) RGS12 interacts with the SNARE-binding region of the Cav2.2 calcium channel. *The Journal of biological chemistry* **280**:1521-1528.

Roig I, Dowdle JA, Toth A, de Rooij DG, Jasin M and Keeney S (2010) Mouse TRIP13/PCH2 is required for recombination and normal higher-order chromosome structure during meiosis. *PLoS genetics* **6**.

Ruan X, Chatziantoniou C and Arendshorst WJ (1999) Impaired prostaglandin E(2)/prostaglandin I(2) receptor-G(s) protein interactions in isolated renal resistance arterioles of spontaneously hypertensive rats. *Hypertension* **34**:1134-1140.

Rubinfeld B, Munemitsu S, Clark R, Conroy L, Watt K, Crosier WJ, McCormick F and Polakis P (1991) Molecular cloning of a GTPase activating protein specific for the Krev-1 protein p21rap1. *Cell* **65**:1033-1042.

Rubio JP, Levy ER, Dobson-Stone C and Monaco AP (1999) Genomic organization of the human galpha14 and Galphaq genes and mutation analysis in chorea-acanthocytosis (CHAC). *Genomics* **57**:84-93.

Rusai K, Prokai A, Juanxing C, Meszaros K, Szalay B, Pasti K, Muller V, Heemann U, Lutz J, Tulassay T and Szabo AJ (2013) Dexamethasone protects from renal ischemia/reperfusion injury: a possible association with SGK-1. *Acta physiologica Hungarica* **100**:173-185.

Saito H, Tsumura H, Otake S, Nishida A, Furukawa T and Suzuki N (2005) L7/Pcp-2-specific expression of Cre recombinase using knock-in approach. *Biochemical and biophysical research communications* **331**:1216-1221.

Sanada K and Tsai LH (2005) G protein betagamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. *Cell* **122**:119-131.

Sano T, Ohyama K, Yamano Y, Nakagomi Y, Nakazawa S, Kikyo M, Shirai H, Blank JS, Exton JH and Inagami T (1997) A domain for G protein coupling in carboxyl-terminal tail of rat angiotensin II receptor type 1A. *The Journal of biological chemistry* **272**:23631-23636.

Sato M, Blumer JB, Simon V and Lanier SM (2006a) Accessory proteins for G proteins: partners in signaling. *Annu Rev Pharmacol Toxicol* **46**:151-187.

Sato M, Cismowski MJ, Toyota E, Smrcka AV, Lucchesi PA, Chilian WM and Lanier SM (2006b) Identification of a receptor-independent activator of G protein signaling (AGS8) in ischemic heart and its interaction with Gbetagamma. *Proceedings of the National Academy of Sciences of the United States of America* **103**:797-802.

Sato M, Gettys TW and Lanier SM (2004) AGS3 and signal integration by Galpha(s)- and Galpha(i)-coupled receptors: AGS3 blocks the sensitization of adenylyl cyclase following prolonged stimulation of a Galpha(i)-coupled receptor by influencing processing of Galpha(i). *The Journal of biological chemistry* **279**:13375-13382.

Sato M, Hiraoka M, Suzuki H, Bai Y, Kurotani R, Yokoyama U, Okumura S, Cismowski MJ, Lanier SM and Ishikawa Y (2011) Identification of transcription factor E3 (TFE3) as a receptor-independent activator of Galpha16: gene regulation by nuclear Galpha subunit and its activator. *The Journal of biological chemistry* **286**:17766-17776.

Sato M, Hiraoka M, Suzuki H, Sakima M, Mamun AA, Yamane Y, Fujita T, Yokoyama U, Okumura S and Ishikawa Y (2014) Protection of cardiomyocytes from the hypoxia-mediated injury by a peptide targeting the activator of G-protein signaling 8. *PloS one* **9**:e91980.

Sato M, Jiao Q, Honda T, Kurotani R, Toyota E, Okumura S, Takeya T, Minamisawa S, Lanier SM and Ishikawa Y (2009) Activator of G protein signaling 8 (AGS8) is required for hypoxia-induced apoptosis of cardiomyocytes: role of G betagamma and connexin 43 (CX43). *The Journal of biological chemistry* **284**:31431-31440.

Schiff ML, Siderovski DP, Jordan JD, Brothers G, Snow B, De Vries L, Ortiz DF and Diverse-Pierluissi M (2000) Tyrosine-kinase-dependent recruitment of RGS12 to the N-type calcium channel. *Nature* **408**:723-727.

Senkfor SI, Johnson GL and Berl T (1993) A molecular map of G protein alpha chains in microdissected rat nephron segments. *The Journal of clinical investigation* **92**:786-790.

Siderovski DP, Diverse-Pierluissi M and De Vries L (1999) The GoLoco motif: a Galphai/o binding motif and potential guanine-nucleotide exchange factor. *Trends in biochemical sciences* **24**:340-341.

Siderovski DP, Hessel A, Chung S, Mak TW and Tyers M (1996) A new family of regulators of G-protein-coupled receptors? *Current biology : CB* **6**:211-212.

Smrcka AV (2008) G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. *Cellular and molecular life sciences : CMLS* **65**:2191-2214.

Smrcka AV, Lehmann DM and Dessal AL (2008) G protein betagamma subunits as targets for small molecule therapeutic development. *Combinatorial chemistry & high throughput screening* **11**:382-395.

Snow BE, Antonio L, Suggs S, Gutstein HB and Siderovski DP (1997) Molecular cloning and expression analysis of rat Rgs12 and Rgs14. *Biochemical and biophysical research communications* **233**:770-777.

Snow BE, Betts L, Mangion J, Sondek J and Siderovski DP (1999) Fidelity of G protein beta-subunit association by the G protein gamma-subunit-like domains of RGS6, RGS7, and RGS11. *Proceedings of the National Academy of Sciences of the United States of America* **96**:6489-6494.

Snow BE, Hall RA, Krumins AM, Brothers GM, Bouchard D, Brothers CA, Chung S, Mangion J, Gilman AG, Lefkowitz RJ and Siderovski DP (1998) GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. *The Journal of biological chemistry* **273**:17749-17755.

Spano D, Branchi I, Rosica A, Pirro MT, Riccio A, Mithbaokar P, Affuso A, Arra C, Campolongo P, Terracciano D, Macchia V, Bernal J, Alleva E and Di Lauro R (2004) Rhes is involved in striatal function. *Molecular and cellular biology* **24**:5788-5796.

Sternweis PC and Robishaw JD (1984) Isolation of two proteins with high affinity for guanine nucleotides from membranes of bovine brain. *The Journal of biological chemistry* **259**:13806-13813.

Stow JL, Sabolic I and Brown D (1991) Heterogeneous localization of G protein alpha-subunits in rat kidney. *The American journal of physiology* **261**:F831-840.

Strathmann M, Wilkie TM and Simon MI (1989) Diversity of the G-protein family: sequences from five additional alpha subunits in the mouse. *Proceedings of the National Academy of Sciences of the United States of America* **86**:7407-7409.

Strathmann M, Wilkie TM and Simon MI (1990) Alternative splicing produces transcripts encoding two forms of the alpha subunit of GTP-binding protein Go. *Proceedings of the National Academy of Sciences of the United States of America* **87**:6477-6481.

Takesono A, Cismowski MJ, Ribas C, Bernard M, Chung P, Hazard S, 3rd, Duzic E and Lanier SM (1999) Receptor-independent activators of heterotrimeric G-protein signaling pathways. *The Journal of biological chemistry* **274**:33202-33205.

Tall GG, Krumins AM and Gilman AG (2003) Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor. *The Journal of biological chemistry* **278**:8356-8362.

Tan JJ, Ong SA and Chen KS (2011) Rasd1 interacts with Ear2 (Nr2f6) to regulate renin transcription. *BMC molecular biology* **12**:4.

Tanaka K (1995) Molecular biology of proteasomes. *Molecular biology reports* **21**:21-26.

Thapliyal A, Bannister RA, Hanks C and Adams BA (2008) The monomeric G proteins AGS1 and Rhes selectively influence Galphai-dependent signaling to modulate N-type (CaV2.2) calcium channels. *American journal of physiology Cell physiology* **295**:C1417-1426.

Tipton AR, Wang K, Oladimeji P, Sufi S, Gu Z and Liu ST (2012) Identification of novel mitosis regulators through data mining with human centromere/kinetochore proteins as group queries. *BMC cell biology* **13**:15.

Toda N and Okamura T (2011) Modulation of renal blood flow and vascular tone by neuronal nitric oxide synthase-derived nitric oxide. *Journal of vascular research* **48**:1-10.

Tonissoo T, Koks S, Meier R, Raud S, Plaas M, Vasar E and Karis A (2006) Heterozygous mice with Ric-8 mutation exhibit impaired spatial memory and decreased anxiety. *Behavioural brain research* **167**:42-48.

Tonissoo T, Meier R, Talts K, Plaas M and Karis A (2003) Expression of ric-8 (synembryn) gene in the nervous system of developing and adult mouse. *Gene expression patterns : GEP* **3**:591-594.

Traver S, Bidot C, Spassky N, Baltauss T, De Tand MF, Thomas JL, Zalc B, Janoueix-Lerosey I and Gunzburg JD (2000) RGS14 is a novel Rap effector that preferentially regulates the GTPase activity of galphao. *The Biochemical journal* **350 Pt 1**:19-29.

Tu Y and Wu C (1999) Cloning, expression and characterization of a novel human Ras-related protein that is regulated by glucocorticoid hormone. *Biochimica et biophysica acta* **1489**:452-456.

Tummala H, Fleming S, Hocking PM, Wehner D, Naseem Z, Ali M, Inglehearn CF, Zhelev N and Lester DH (2011) The D153del mutation in GNB3 gene causes tissue specific signalling patterns and an abnormal renal morphology in Rge chickens. *PloS one* **6**:e21156.

Vaidyanathan G, Cismowski MJ, Wang G, Vincent TS, Brown KD and Lanier SM (2004) The Ras-related protein AGS1/RASD1 suppresses cell growth. *Oncogene* **23**:5858-5863.

Vargiu P, De Abajo R, Garcia-Ranea JA, Valencia A, Santisteban P, Crespo P and Bernal J (2004) The small GTP-binding protein, Rhes, regulates signal transduction from G protein-coupled receptors. *Oncogene* **23**:559-568.

Verma R, Oania R, Graumann J and Deshaies RJ (2004) Multiubiquitin chain receptors define a layer of substrate selectivity in the ubiquitin-proteasome system. *Cell* **118**:99-110.

von Weizsacker E, Strathmann MP and Simon MI (1992) Diversity among the beta subunits of heterotrimeric GTP-binding proteins: characterization of a novel beta-subunit cDNA. *Biochemical and biophysical research communications* **183**:350-356.

Vural A, McQuiston TJ, Blumer JB, Park C, Hwang IY, Williams-Bey Y, Shi CS, Ma DZ and Kehrl JH (2013) Normal autophagic activity in macrophages from mice lacking Galphai3, AGS3, or RGS19. *PloS one* **8**:e81886.

Wang H, Misaki T, Taupin V, Eguchi A, Ghosh P and Farquhar MG (2014a) GIV/Girdin Links Vascular Endothelial Growth Factor Signaling to Akt Survival Signaling in Podocytes Independent of Nephrin. *Journal of the American Society of Nephrology : JASN*.

Wang K, Sturt-Gillespie B, Hittle JC, Macdonald D, Chan GK, Yen TJ and Liu ST (2014b) Thyroid hormone receptor interacting protein 13 (TRIP13) AAA-ATPase is a novel mitotic checkpoint-silencing protein. *The Journal of biological chemistry* **289**:23928-23937.

Watson N, Linder ME, Druey KM, Kehrl JH and Blumer KJ (1996) RGS family members: GTPase-activating proteins for heterotrimeric G-protein alpha-subunits. *Nature* **383**:172-175.

Webb CK, McCudden CR, Willard FS, Kimple RJ, Siderovski DP and Oxford GS (2005) D2 dopamine receptor activation of potassium channels is selectively decoupled by Galpha-specific GoLoco motif peptides. *Journal of neurochemistry* **92**:1408-1418.

Weterman MA, van Groningen JJ, den Hartog A and Geurts van Kessel A (2001) Transformation capacities of the papillary renal cell carcinoma-associated PRCCTFE3 and TFE3PRCC fusion genes. *Oncogene* **20**:1414-1424.

Weterman MA, Wilbrink M and Geurts van Kessel A (1996a) Fusion of the transcription factor TFE3 gene to a novel gene, PRCC, in t(X;1)(p11;q21)-positive papillary renal cell carcinomas. *Proceedings of the National Academy of Sciences of the United States of America* **93**:15294-15298.

Weterman MA, Wilbrink M, Janssen I, Janssen HA, van den Berg E, Fisher SE, Craig I and Geurts van Kessel A (1996b) Molecular cloning of the papillary renal cell carcinoma-associated translocation (X;1)(p11;q21) breakpoint. *Cytogenetics and cell genetics* **75**:2-6.

Weterman MJ, van Groningen JJ, Jansen A and van Kessel AG (2000) Nuclear localization and transactivating capacities of the papillary renal cell carcinoma-associated TFE3 and PRCC (fusion) proteins. *Oncogene* **19**:69-74.

Wettschureck N and Offermanns S (2005) Mammalian G proteins and their cell type specific functions. *Physiological reviews* **85**:1159-1204.

Wilkie TM, Scherle PA, Strathmann MP, Slepak VZ and Simon MI (1991) Characterization of G-protein alpha subunits in the Gq class: expression in murine tissues and in stromal and hematopoietic cell lines. *Proceedings of the National Academy of Sciences of the United States of America* **88**:10049-10053.

Williamson CM, Schofield J, Dutton ER, Seymour A, Beechey CV, Edwards YH and Peters J (1996) Glomerular-specific imprinting of the mouse gsalpha gene: how does this relate to hormone resistance in albright hereditary osteodystrophy? *Genomics* **36**:280-287.

Xiao Z, Wan Q, Du Q and Zheng Z (2012) Galpha/LGN-mediated asymmetric spindle positioning does not lead to unequal cleavage of the mother cell in 3-D cultured MDCK cells. *Biochemical and biophysical research communications* **420**:888-894.

Yanagisawa H, Kurihara N, Klahr S, Morrissey J and Wada O (1994) Regional characterization of G-protein subunits in glomeruli, cortices and medullas of the rat kidney. *Nephron* **66**:447-452.

Yanagisawa H, Morrissey J and Klahr S (1993) Bilateral ureteral obstruction alters levels of the G-protein subunits G alpha s and G alpha q/11. *Kidney international* **43**:865-871.

Yang S and Li YP (2007) RGS12 is essential for RANKL-evoked signaling for terminal differentiation of osteoclasts in vitro. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **22**:45-54.

Yeh C, Li A, Chuang JZ, Saito M, Caceres A and Sung CH (2013) IGF-1 activates a cilium-localized noncanonical Gbetagamma signaling pathway that regulates cell-cycle progression. *Developmental cell* **26**:358-368.

Yu AS (2015) Claudins and the kidney. *Journal of the American Society of Nephrology : JASN* **26**:11-19.

Yu S, Yu D, Lee E, Eckhaus M, Lee R, Corria Z, Accili D, Westphal H and Weinstein LS (1998) Variable and tissue-specific hormone resistance in heterotrimeric Gs protein alpha-subunit (Gsalpha) knockout mice is due to tissue-specific imprinting of the gsalpha gene. *Proceedings of the National Academy of Sciences of the United States of America* **95**:8715-8720.

Zhang Y, Jose PA and Zeng C (2011) Regulation of sodium transport in the proximal tubule by endothelin. *Contributions to nephrology* **172**:63-75.

Zhao P, Nguyen CH and Chidiac P (2010) The proline-rich N-terminal domain of G18 exhibits a novel G protein regulatory function. *The Journal of biological chemistry* **285**:9008-9017.

Zheng S, Yu P, Zeng C, Wang Z, Yang Z, Andrews PM, Felder RA and Jose PA (2003) Galpha12- and Galpha13-protein subunit linkage of D5 dopamine receptors in the nephron. *Hypertension* **41**:604-610.

Zheng Z, Zhu H, Wan Q, Liu J, Xiao Z, Siderovski DP and Du Q (2010) LGN regulates mitotic spindle orientation during epithelial morphogenesis. *The Journal of cell biology* **189**:275-288.

Zhu J, Wen W, Zheng Z, Shang Y, Wei Z, Xiao Z, Pan Z, Du Q, Wang W and Zhang M (2011) LGN/mInsc and LGN/NuMA complex structures suggest distinct functions in asymmetric cell division for the Par3/mInsc/LGN and Galphai/LGN/NuMA pathways. *Molecular cell* **43**:418-431.

Zhuo JL and Li XC (2011) New insights and perspectives on intrarenal renin-angiotensin system: focus on intracrine/intracellular angiotensin II. *Peptides* **32**:1551-1565.

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Footnotes

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Table 1: G-protein localization in the kidney

Gα subunits	Glm	PCT	TAL	CD	BV
G_s	$+^{A, D}$	$+^{A, E}$	$+^{C, E}$	$+^{C, E}$	$+^{D}$
G_{i1}	$+^{D}$	$-^{E}$	$-^{C}; +^{E}$	$-^{C}$	$+^{C, D}$
G_{i2}	$+^{D, E}$	$-^{E}$	$+^{C}; -^{E}$	$+^{C, E}$	$+^{D}$
G_{i3}	$+^{D, E}$	$+^{E}$	$-^{C}; +^{E}$	$-^{C}$	$+^{D}$
G_o	$+^{D}$	N/D	$-^{C}$	$-^{C}$	N/D
G_q	$+^{D}$	- *	$-^{C}$	$-^{C}$	$+^{D}$
G_{11}	$+^{D}$	N/D	$-^{C}$	$+^{C}$	$+^{D}$
G_{14}	N/D	N/D	$-^{C}$	$+^{C}$	N/D
$G_{15/16}$	N/D	N/D	N/D	N/D	N/D
G_{12}	$+^{E}$	$+^{E}$	$+^{E}$	$+^{E}$	$+^{E}$
G_{13}	$-^{E}$	$+^{E}$	$-^{E}$	$+^{E}$	$+^{E}$

Table 1. G-protein localization in the kidney. Summary of the vascular and nephron segments that have been identified for Gα subunits in the kidney. Glm = glomerulus; PCT = proximal convoluted tubule; TAL = thick ascending limb; CD = collecting duct; BV = pre-glomerular blood vessels. Techniques used to determine the presence of the Gα subunits are shown as: A = in situ hybridization; B = Northern blot analysis; C = microdissection of renal tubular segments followed by RT-PCR; D = Western blot analysis of isolated segments; and E = immunohistochemistry. + = positive; - = negative; N/D = not determined. * = positive through its interaction with GPCR

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Table 2. Localization of AGS proteins in the kidney

	Alternate names	Glm	PCT	TAL	CD	BV
AGS1	RasD1/Dexras1	-	+	-	-	-
AGS2	Tctex1	N/D	N/D	N/D	N/D	N/D
AGS3	GPSM1	-	- *	-	+	-
AGS4	GPSM3/G18.1b	N/D	N/D	N/D	N/D	N/D
AGS5	GPSM2/LGN	-	-	+	+	-
AGS6	RGS12	+	+	+	+	+
AGS7	Trip13/PCH2/16E1-BP	-	-	-	+	-
AGS8	FNDC1/KIAA1866	-	-	+	+	-
AGS9	Rpn10/Psmd4	N/D	N/D	N/D	N/D	N/D
AGS10	GNAO	+	N/D	N/D	N/D	N/D
AGS11	TFE3	-	$-^{N/D}$	$-^{N/D}$	$-^{N/D}$	-
AGS12	TFEB	-	$-^{N/D}$	$-^{N/D}$	$-^{N/D}$	-
AGS13	MitF	N/D	N/D	N/D	N/D	N/D

Table 2. Localization of AGS proteins in the kidney. This provides a summary of the localization of AGS family members within distinct segments of the normal kidney using immunohistochemistry. - = low to absent expression; + = detectable protein expression; * detectable protein expression following ischemia-reperfusion injury N/D = not specifically determined. $-^{N/D}$ = low to undetectable levels for TFE3 and TFEB in the cytoplasm of renal tubules. In studies with low staining, the cell types could not be determined.