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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

Dexas1 = Ras dexamethasone induced 1

FLCN = folliculin

FNDC1 = fibronectin type III containing I

GDI = guanine nucleotide dissociation inhibitor

GEF = guanine nucleotide exchange factor

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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

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TFE3 = transcription factor E3

TFEB = transcription factor B

Trip13 = thyroid receptor interacting protein 13

UACR = urinary albumin-to-creatinine ratio

UPS = Ubiquitin-proteasome system

VEGFR2 = vascular endothelial growth factor receptor 2

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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.

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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 $G\alpha$ 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 $G\alpha$ or $G\beta\gamma$ independent of the typical heterotrimeric $G\alpha\beta\gamma$, and are categorized into four separate groups: 1) guanine nucleotide exchange factors

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(GEF); 2) guanine nucleotide dissociation inhibitors (GDIs); 3) G $\beta\gamma$ binding proteins; and 4) G α_{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 G α , specifically G α_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 G α subunits in human tissues using Northern blot analysis demonstrated minimal to moderate mRNA expression of G α_s compared to G α_i (Brann et al., 1987), which was consistent with the expression of G α_s protein in the whole renal cortex and inner medulla from mice (Yu et al., 1998). G α_s mRNA is widely expressed throughout the renal cells in the nephron and blood vessels. G α_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 α_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. $G\alpha_{i2/13}$ 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).

$G\beta\gamma$ subunits. The molecular expression of all 5 $G\beta$ 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 $G\gamma$ 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 $G\alpha$ 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 $G\alpha$ 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 $G\alpha$, 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/Dexas1; 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/Dexas1/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 Dexas1 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/Dexas1 requires the GTP-bound forms of $G\alpha_i$ subunits to activate signaling pathways (Tu and Wu, 1999).

The renal expression of AGS1/Dexas1 mRNA was developmentally regulated where higher levels were detected in the adult versus fetus (Kemppainen et al., 2003). In terms of tissue biodistribution, AGS1/Dexas1 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/Dexas1 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/Dexas1 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

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role for AGS1/Dexas1 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/Dexas1 in the kidney to control cardiovascular function. AGS1/Dexas1 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/Dexas1 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/Dexas1 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/Dexas1 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/Dexas1, 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

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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 $G\alpha$ 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 $G\alpha$ subunit association to the plasma membrane. In the absence of Ric-8A/B, the steady state levels of $G\alpha$ are appreciably reduced suggesting a role for Ric-8 to stabilize the $G\alpha$ 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 $G\beta\gamma$ signaling (Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012). $G\beta\gamma$ 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 β -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 $G\beta\gamma$ 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 $G\alpha$ -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-

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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 β 1-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 G β subunits complexed with or without the G α 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 leiomyosarcoma 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 (Lenarczyk et al., 2014). The site of expression in the nephron may implicate 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 (Lenarczyk 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 Gα_{i1/2/3/0} or Gα_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 (Lenarczyk et al., 2014). It is not presently known whether the reduction in renal AGS8/*Fndc1* mRNA is

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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-proteasome 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 proteasome (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 G $\beta\gamma$, 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.

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 1 α (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 $\beta\gamma$ 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 flow 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

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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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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

Table 2. Localization of AGS proteins in the kidney

	Alternate names	Glm	PCT	TAL	CD	BV
AGS1	RasD1/Dexas1	-	+	-	-	-
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	FND1/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.