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Heterotrimeric G protein signaling and RGSs in *Aspergillus nidulans*

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Heterotrimeric G proteins (G proteins) are conserved in all eukaryotes and are crucial components sensing and relaying external cues into the cells to elicit appropriate physiological and biochemical responses. Basic units of the heterotrimeric G protein signaling system include a G protein-coupled receptor (GPCR), a G protein composed of α, β, and γ subunits, and variety of effectors. Sequential sensitization and activation of these G protein elements translates external signals into gene expression changes, resulting in appropriate cellular behaviors. Regulators of G protein signaling (RGSs) constitute a crucial element of appropriate control of the intensity and duration of G protein signaling. For the past decade, G protein signaling and its regulation have been intensively studied in a number of model and/or pathogenic fungi and outcomes of the studies provided better understanding on the upstream regulation of vegetative growth, mating, development, virulence/pathogenicity establishment, and biosynthesis of secondary metabolites in fungi. This review focuses on the characteristics of the basic upstream G protein components and RGS proteins, and their roles controlling various aspects of biological processes in the model filamentous ascomycete fungus *Aspergillus nidulans*. In particular, their functions in controlling hyphal proliferation, asexual spore formation, sexual fruiting, and the mycotoxin sterigmatocystin production are discussed.

Keywords: fungi, aspergillus, heterotrimeric G protein, RGS, growth and development, mycotoxin

All cells have the capacity to sense and respond to various external signals including nutrients, hormones, physical/chemical stimuli, and environmental stress. In this signal transduction, the heterotrimeric G protein (G protein) system composed of a seven-transmembrane-domain G protein coupled receptor (GPCR), the canonical heterotrimeric G protein consisting of α, β and γ subunits, and an effector plays a pivotal role (reviewed in Morris and Malbon, 1999; Neves *et al.*, 2002; McCudden *et al.*, 2005). In fungi, G proteins play integral roles for cell growth/division, mating, cell-cell fusion, morphogenesis, chemotaxis, virulence establishment, pathogenic development and secondary metabolite production (reviewed in Bölker, 1998; Lengeler *et al.*, 2000; Lee *et al.*, 2003; Feldbrügge *et al.*, 2004; Yu and Keller, 2005).

Upon binding of ligands, GPCRs are sensitized, and physically interact with heterotrimeric G proteins. Physical interaction of inactive heterotrimeric Gαβγ with GPCRs causes GDP-GTP exchange of Gα, which results in the dissociation of GTP-Gα from the Gβγ heterodimer. Once dissociated, GTP-Gα, Gβγ or both can amplify and propagate signals by modulating activities of a number of effector proteins. In fungi, G protein mediated signaling is transmitted via one or more of the following pathways: 1) adenylyl cyclase - cAMP-dependent protein kinase (PKA); 2) Mitogen-Activated Protein (MAP) kinase pathways; and 3) IP_3-[Ca^{++}]-DAG (diacyl-glycerol)-dependent protein kinase C (PKC; see Fig. 1, for general review see Morris and Malbon, 1999; Feldbrügge *et al.*, 2004; McCudden *et al.*, 2005). Many of the components of the G-protein and cAMP-signaling pathways have been identified in the model filamentous fungus *Aspergillus nidulans* through the use of genetic screens, analyses of expressed sequence tags (ESTs), or partial examination of the genome. Due to space limitations, this review will not cover downstream signaling elements.

Proper control of the specificity and intensity of G protein signaling is essential for the accurate translation of signals into an apposite cellular response.

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The signal is turned off when GTP is hydrolyzed to GDP by the intrinsic GTPase activity of Gα, resulting in the formation of the inactive heterotrimer GDP-Gαβγ. Thus, the rates of GTP hydrolysis of the Gα subunit determine the intensity of the signal (reviewed in McCudden *et al.*, 2005). Among many regulatory mechanisms, regulators of G protein signaling (RGS proteins) play a key role in tight control of GPCR-G protein-mediated signaling. This review describes the characteristics and the roles of G protein components and RGS proteins in vegetative growth, developmental control and toxin biosynthesis in *A. nidulans*.

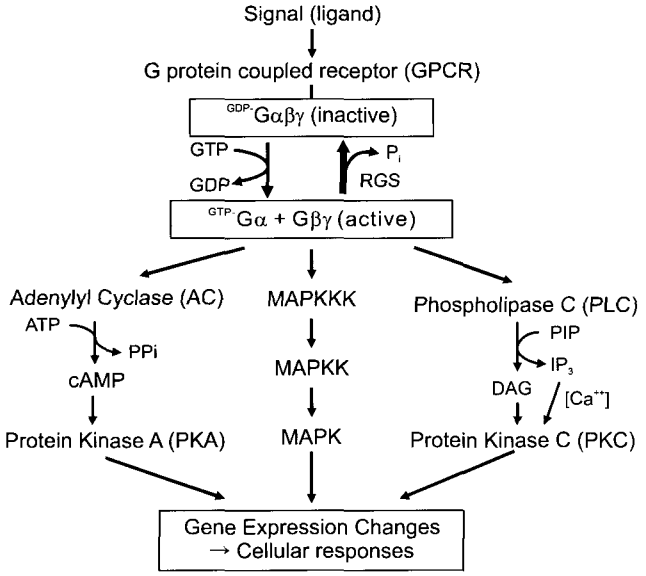

Fig. 1. Schematic presentation of the three major signal transduction pathways. Rapid inactivation of G protein signaling by RGS proteins by increasing intrinsic GTPase activity of GTP-bound Gαsubunits is indicated.

Heterotrimeric G protein components

G protein coupled receptors (GPCRs)

The G-protein-coupled receptor (GPCR) family represents the largest and most diverse group of membrane-bound proteins. At least 800 potential GPCRs have been identified in the human genome (reviewed in Hill, 2006). While GPCRs respond to a vast array of ligands, a typical GPCR contains a conserved structure of seven transmembrane (7-TM) spanning (or hepta-helical) domains. This characteristic enabled Han *et al.* (2004a) to identify nine GPCRs (GprA-I) in the *A. nidulans* genome (Fig. 2), which are divided into five classes: classes I and II include GprA (PreB) and GprB (PreA) that are similar to the yeast pheromone receptors, and function in self-fertilized sexual development in *A. nidulans* (Seo *et al.*, 2004); class III includes GprC, GprD and GprE receptors that might be involved in carbon-source sensing on the basis of their high similarity to the *Saccharomyces cerevisiae* Gpr1 receptor (Xue *et al.*, 1998; Kraakman *et al.*, 1999); class IV includes GprF and GprG that are similar to the *Schizosaccharomyces pombe* Stm1 receptor (Chung *et al.*, 2001); class V

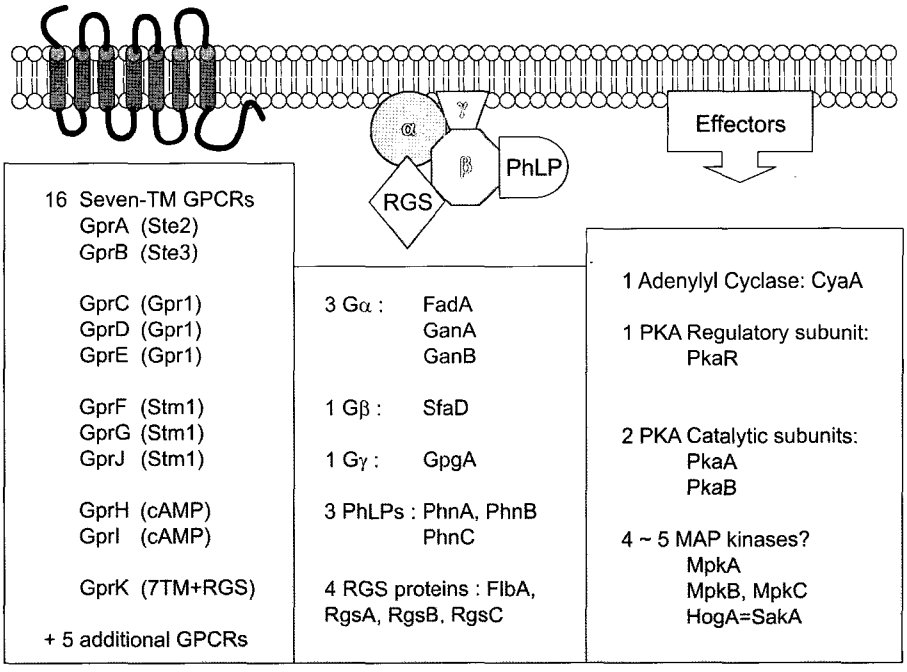

Fig. 2. Basic components of heterotrimeric G protein signaling in *A. nidulans*.

includes GprH and GprI that are similar to the *Dictyostelium discoideum* cAMP receptor cAR1 and as such have been proposed to play a role in cAMP sensing (Galagan *et al.*, 2003). Later, Lafon *et al* (2006) carried out exhaustive comparative analyses of the genomes of the three aspergilli, *A. nidulans*, *A. fumigatus* and *A. oryzae*, and identified seven additional GPCRs in *A. nidulans*: GprJ (class IV), GprK (class VI), GprM and GprN (class VII), GprO and GprP (class VIII), and NopA (class IX), totaling 16 potential GPCRs classified into nine classes (Lafon *et al.*, 2006; Fig 2).

Among these, functions of GprA, GprB and GprD were further studied. Unlike many aspergilli, *A. nidulans* can reproduce by asexual and sexual means. Sexual fruiting bodies (cleistothecia) can be formed in both homothallic (self) and heterothallic (outcross) conditions. Deletion of *gprD* caused restricted hyphal growth, delayed conidial germination and uncontrolled activation of sexual development resulting in a small colony covered by cleistothecia (Han *et al.*, 2004a). Han *et al* further found that GprD might not signal through the FadA (Gα)-protein kinase A (PKA) pathway (see below), and that genetic or environmental alterations resulting in the blockage of sexual development rescued both growth and developmental abnormalities caused by Δ*gprD*. These observations led to the hypothesis that the primary role of GprD is to negatively regulate sexual development, which is required for proper vegetative growth of *A. nidulans* (Fig. 3).

Later, Seo *et al.* (2004) characterized the *gprA* and *gprB* genes encoding putative GPCRs similar to the yeast pheromone receptors Ste2p and Ste3p, respectively. Deletion of *gprA* or *gprB* resulted in the production of reduced number/size of cleistothecia carrying a few ascospores (sexual spores), whereas the Δ*gprA* Δ*gprB* double deletion caused the absence of cleistothecia formation in homothallic conditions. However, Seo *et al* found that no *gprA* and/or *gprB* mutations affected vegetative growth, asexual sporulation, Hülle cell (specialized cell for supporting the production of cleistothecia) formation, or even cleistothecia formation in outcross. These results led to the conclusion that GprA and GprB are specifically required for self-fertilization in homothallic conditions. Transcripts of *gprA* and *gprB* accumulate during sexual development particularly at 48 h post sexual-developmental induction, suggesting potential developmental stage specific signaling for sexual fruiting. Upregulation of *nsdD* encoding a key transcription factor required for sexual development (Han *et al.*, 2001) resulted in the production of infertile cleistothecia in the Δ*gprA* Δ*gprB* mutant. These results suggest that NsdD only partially rescues the developmental defects caused by the absence of GPCR functions and that GprA/B-mediated signaling may activate other genes necessary for the completion of cleistothecia and ascospore formation (Fig. 3). Moreover, reduced (or lack of) sexual development caused by deletion of *gprA* and/or

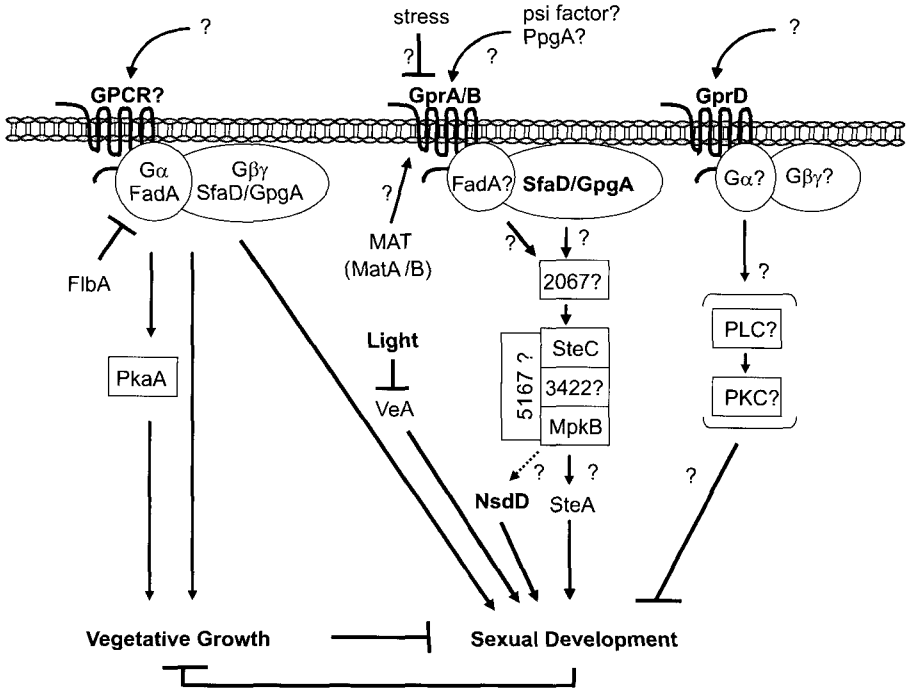

Fig. 3. *The three proposed signaling pathways controlling vegetative growth and sexual development (adapted and modified from Seo et al., 2004).*

gprB suppressed growth defects of the Δ*gprD* mutant. These results further corroborate that the primary role of GprD is to negatively control sexual development, and that GprA/B function downstream of GprD. A current model depicting GPCR-mediated signaling in *A. nidulans* is presented in Fig. 3. Functional characterization of the rest of the GPCRs is in progress.

G protein α subunits: FadA, GanB and GanA

FadA: The heterotrimeric G-protein α subunit functions as the on-off switch that controls the duration of signal transduction by GPCRs. The first Gα subunit studied in *A. nidulans* is FadA (fluffy autolytic dominant, maps to chromosome VIII), which was identified by investigating a dominant activating mutation (d+: G42R) that caused uncontrolled vegetative growth followed by autolysis, i.e., the "fluffy autolytic" phenotype (Yu *et al.*, 1996a). Constitutively active dominant FadA mutant alleles are predicted to have reduced (or absent) intrinsic GTPase activity, resulting in the prolonged activated state of FadA-GTP. Other FadA^{d+} mutant alleles include R178L, G183S, R178C and Q204L (Wieser *et al.*, 1997; Yu *et al.*, 1999). All FadA^{d+} mutants exhibited the fluffy-autolytic phenotype and the lack of the mycotoxin sterigmatocystin (ST) production (Yu *et al.*, 1996a; Hicks *et al.*, 1997; Wieser *et al.*, 1997; Yu *et al.*, 1999). Conversely, the dominant interfering (d-) FadA G203R mutant allele caused reduced vegetative growth, hyper-active asexual sporulation and precocious ST production (Yu *et al.*, 1996a; Hicks *et al.*, 1997).

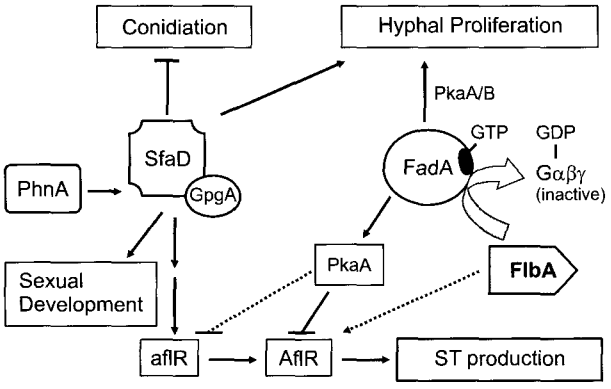

Fig. 4. The roles of FadA, SfaD, GpgA and PhnA in controlling growth, development and ST production (adapted and modified from Seo and Yu, 2006). FadA-mediated vegetative growth signaling is transduced in part by PkaA (the primary PKA; Shimizu and Keller, 2001). PkaB is the secondary (backup) PKA catalytic subunit, playing a role in hyphal growth and germination (Ni *et al.*, 2005). The FadA-PkaA signaling pathway is primarily responsible for the repression of ST biosynthesis. Seo and Yu (2006) further proposed that the results of SfaD::GpgA signaling include transcriptional activation of *aflR* and subsequent ST production.

These observations led to the conclusion that activated GTP-FadA (Gα) mediates signaling that promotes vegetative growth, which in turn inhibits both asexual and sexual development as well as toxin production (Fig. 4).

GanA and GanB: Two other Gα subunits (GanA and GanB) in *A. nidulans* were identified via either heterologous hybridization or PCR-amplification with degenerative primers. GAN stands for **G** protein **a**lpha subunit in *A. nidulans*. The *ganA* and *ganB* genes map to chromosome VI and VIII, respectively. At present, only GanB has been functionally characterized (Chang *et al.*, 2004). The *ganB* deletion or dominant interfering (G207R) mutants exhibited conidiophore development in submerged cultures, indicating that GanB plays a role in inhibition of asexual development. Perhaps somewhat unexpectedly, constitutively active GanB mutant alleles (Q208L and R182L) caused a reduction in hyphal growth and a severe defect in asexual sporulation. Moreover, while loss of function or dominant negative GanB mutants exhibited reduced germination rate, the GanB^{Q208L} mutation resulted in not only precocious conidial germination but also germination of conidia in the absence of an external carbon source. Based on these observations, Chang *et al* (2004) proposed that GanB negatively regulates asexual sporulation and plays a positive role in germination of conidia, possibly through sensing external carbon sources. The role of GanB and cAMP signaling in carbon sensing and conidial germination was further investigated by Lafon *et al* (2005), and it was shown that GanB mediates a rapid and transient activation of cAMP synthesis in response to glucose during the early period of germination. Moreover, Lafon *et al* (2005) showed that GanB and SfaD::GpgA (Gβγ subunit see below) constitutes a functional heterotrimer and controls cAMP/PKA signaling in response to glucose as well as conidial germination, where GanB is a primary signaling element and SfaD::GpgA functions in proper activation of GanB signaling. Function of GanA is yet to be uncovered.

G protein β subunit: SfaD

The Gβ subunit (SfaD) of *A. nidulans* was identified via both forward and reverse genetic methods. Due to the semi-dominant nature of the *sfaD* loss-of-function mutations (likely caused by a dosage effect), a complementation-based gene cloning approach was unfruitful. Thus, Rosén *et al* (1999) attempted to clone the *sfaD* gene by PCR-amplification of a highly conserved region using degenerative primers followed by library screening. This approach resulted in the isolation of SfaD composed of 352 amino acids that shares 60% identity with mammalian Gβ subunits.

SfaD has a conserved Trp-Asp sequence that referred to as the "WD-40" motif (Rosén *et al.*, 1999). Deletion of *sfaD* caused hyper-active sporulation and severely reduced vegetative growth, indicating that SfaD is required for normal hyphal growth and proper down-regulation of asexual sporulation (Rosén *et al.*, 1999). However, deletion of *sfaD* could not suppress uncontrolled activation of vegetative growth caused by the $FadA^{d+}$ (R178C and Q204L) alleles, indicating that constitutive activation of FadA-GTP signaling alone is sufficient to give rise to the fluffy-autolytic phenotype in the absence of Gβγ. Later, Seo *et al* (2005) further speculated that FadA might be the primary component responsible for hyphal proliferation. Elimination of FadA or SfaD could not bypass the need for FluG (an early developmental activator: Lee and Adams, 1994b) in asexual development, suggesting that these two pathways are separate and independent. Furthermore, SfaD is found to be essential for sexual fruiting body formation and ST production (Rosén *et al.*, 1999; Seo and Yu, 2006, see below).

G protein γ subunit: GpgA

As has been found all eukaryotes (reviewed in McCudden *et al.*, 2005), it has been presumed that SfaD functions as a heterodimer with the cognate Gγ subunit in *A. nidulans*. Seo *et al.*, (2005) analyzed the *A. nidulans* genome and identified a single gene named *gpgA* encoding a putative Gγ subunit. GpgA consists of 90 amino acids and exhibits 72% similarity with the yeast Ste18p. GpgA contains a typical coiled-coil domain at the N-terminal region, which is necessary for the interaction of a Gγ with the cognate Gβ to form a heterodimer (Seo *et al.*, 2005). The *gpgA* null mutant displayed restricted vegetative growth, and reduced (delayed) conidiation. Moreover, similar to Δ*sfaD*, deletion of *gpgA* resulted in the absence of cleistothecia formation in self-fertilization and caused a severe impairment in sexual development in outcrosses. These observations led Seo *et al* (2005) to hypothesize that the SfaD::GpgA heterodimer is the primary signaling component for sexual development in *A. nidulans*. Like Δ*sfaD* or Δ*fadA*, deletion of *gpgA* was not sufficient to bypass the need for FluG in asexual development. GpgA is also found to be required for the production of ST (Seo and Yu, 2006). Seo *et al* (2005) concluded that only one each of Gβ- and Gγ-subunit exists in the *A. nidulans* genome.

Phosducin-like proteins (PhLPs)

Phosducin or phosducin-like proteins (PhLPs) are a group of evolutionarily conserved positive regulators of Gβγ signaling. They act as molecular chaperones for Gβγ assembly and are necessary for normal levels of Gβ and Gγ subunits (Kasahara *et al.*, 2000; Lukov *et al.*, 2005; Knol *et al.*, 2005). The *A. nidulans* genome contains three potential PhLPs (PhnA, PhnB and PhnC; Seo and Yu, 2006). Because PhnA (a 281 aa protein) is most similar to Bdm-1, a proven fungal Gβγ activator (Kasahara *et al.*, 2000), Seo and Yu investigated the functions of *phnA* first. Interestingly, *phnA* is located on chromosome VIII tightly linked to *sfaD* (1.4 kb apart).

Seo and Yu (2006) found that the *phnA* deletion mutant exhibited a phenotype almost identical to that of the Δ*sfaD* mutant, i.e., reduced biomass and hyper-active conidiation, but different from that of the Δ*gpgA* mutant. These results support the idea that PhnA is an essential element for SfaD functionality and that, in addition to functioning as a heterodimer, SfaD and GpgA may have distinct signaling roles (Seo *et al.*, 2005). As mentioned, SfaD and GpgA are required for sexual fruiting body formation in a rather dominant manner (Rosén *et al.*, 1999; Seo *et al.*, 2005). Likewise, the *phnA* deletion mutant was severely impaired in sexual reproduction even in outcrosses (Seo and Yu, 2006), further supporting the hypothesis that the SfaD::GpgA heterodimer is the primary signaler for sexual development. Seo and Yu (2006) also demonstrated that the requirement for PhnA in cleistothecia development is not due to the altered expression of *nsdD* (Han *et al.*, 2001). This result is consistent with the previous proposal that GPCR-G protein (yet to be identified) and NsdD might function in separate regulatory branches (Seo *et al.*, 2004).

Importantly, Seo and Yu (2006) found that SfaD, GpgA, and PhnA are necessary for the biosynthesis of ST. The requirement of SfaD for ST production was through the expression of *aflR*, encoding a Gla4-type transcription factor carrying the $Zn(II)_2Cys_6$ binuclear cluster DNA binding motif (Brown *et al.*, 1996; Yu *et al.*, 1996b). Seo and Yu (2006) found that overexpression of *aflR* under the control of the inducible promoter *alcA*(p) could restore ST production in the absence of SfaD function. These results indicate that individual G protein components may play differential (or opposite) roles in controlling ST production and the end results of SfaD::GpgA signaling may include transcriptional activation of *aflR* (Fig. 4).

Regulators of G protein signaling (RGSs)

RGS proteins are a group of proteins containing a conserved ~ 130 amino acid RGS box that interacts with an activated GTP-Gα subunit and increases its intrinsic GTPase activity, thereby rapidly turning-off the GPCR-mediated signaling pathways (reviewed in Chidiac and Roy, 2003; McCudden *et al.*, 2005). Via activities of various RGS proteins, cells can translate

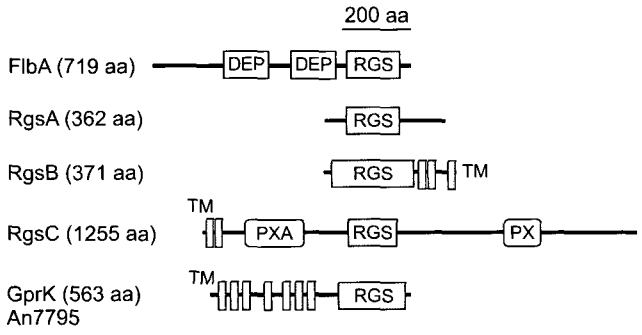

Fig. 5. Five RGS proteins in *A. nidulans*.

and fine-tune diverse incoming signals into appropriate cellular responses. Moreover, in addition to modulating G protein signals, RGS proteins can enhance G protein activation, serve as effector antagonists, and act as scaffold proteins to congregate receptors, G proteins, effectors as well as other regulatory molecules (Zhong and Neubig, 2001). To date, more than 30 members of the mammalian RGS protein family have been reported (reviewed in Chidiac and Roy, 2003). RGS proteins play pivotal roles in upstream regulation of fundamental biological processes in filamentous fungi including vegetative growth, sporulation, mycotoxin/pigment production, pathogenicity and mating. Five distinct RGS proteins are found in the *A. nidulans* genome (Fig. 5).

FlbA

The first *A. nidulans* RGS protein FlbA was identified by studying a fluffy-autolytic mutant, and is similar to *S. cerevisiae* Sst2p (Lee and Adams, 1994a), carrying one RGS and two DEP (dishevelled, Egl-10, pleckstrin) domains (Fig. 5). The existence of repeated DEP is apparently fungal specific (Han *et al.*, 2004b). The DEP domain might be associated with targeting RGS proteins to the Golgi and plasma membranes (Burchett, 2000) as well as inducing the expression of a group of genes containing stress response elements (STRE) in the promoter regions (Burchett *et al.*, 2002).

The FlbA and FadA pair is the first identified RGS-Gα set in filamentous fungi, and is responsible for upstream regulation of hyphal proliferation, development and biosynthesis of secondary metabolites (Yu *et al.*, 1996a; Hicks *et al.*, 1997; Tag *et al.*, 2000; reviewed in Yu and Keller, 2005). As mentioned, FadA and SfaD::GpgA stimulate vegetative growth in part through PKA, and FlbA is a specific RGS protein controlling FadA-mediated signaling, likely by enhancing the intrinsic GTPase activity of FadA (Yu *et al.*, 1996a, 1999; Rosén *et al.*, 1999; Shimizu and Keller, 2001). Loss of *flbA* function results in the fluffy-autolytic phenotype similar to that caused by $FadA^{d+}$ mutant alleles (Lee and Adams, 1994b; Yu *et al.*, 1996a, 1999; Wieser *et al.*, 1997). As if FadA is the primary target of FlbA function, the deletion (Δ) or dominant negative (G203R) FadA mutations suppress the fluffy-autolytic phenotype caused by Δ*flbA* and restore asexual development and ST production (Yu *et al.*, 1996a; Hicks *et al.*, 1997). Similarly, mutational inactivation of *sfaD*, *gpgA* or *phnA* bypasses the need for FlbA in asexual development (Rosén *et al.*, 1999; Seo *et al.*, 2005; Seo and Yu, 2006), indicating that that FadA, SfaD, and GpgA constitute the major heterotrimer for vegetative growth signaling and the primary role of FlbA is to attenuate this signaling (Fig. 6).

RgsA

As shown in Fig. 5, RgsA contains an RGS domain at the N-terminus (Han *et al.*, 2004b). It shows 28% identity and 43% similarity to *S. cerevisiae* Rgs2p (Versele *et al.*, 1999). Unlike *flbA* (constitutive expression; Lee and Adams, 1994a), *rgsA* mRNA (~2.0 kb) levels are quite high during early vegetative growth phase, relatively low in asexual and sexual development, and high in ascospores, indicating *rgsA* expression is subjected to complex transcriptional control.

Han *et al* (2004b) demonstrated that RgsA is a specific RGS protein that negatively regulates GanB signaling, which activates stress responses and inhibits asexual sporulation. As deletion of *rgsA* would result in prolonged activation of GTP-bound GanB, the *rgsA* deletion mutant exhibited a phenotype highly similar to that of the $GanB^{d+}$ (Q208L) mutant (Chang *et al.*, 2004), i.e. reduced colony size, elevated germination without external carbon sources and accumulation of dark brown pigments. Conversely, among the three *A. nidulans* Gα subunits, only *ganB* deletion suppressed morphological, physiological and metabolic alterations caused by Δ*rgsA*. Furthermore, Han *et al* (2004b) showed that overexpression of *rgsA* caused elaboration of conidiophores in liquid submerged culture as observed in the Δ*ganB* or $GanB^{G207R}$ mutants (Chang *et al.*, 2004). Later, Lafon *et al* (2005) demonstrated that RgsA is involved in regulation of the cAMP/PKA pathway and germination via attenuation of GanB signaling, and concluded that all controls exerted by GanB-SfaD-GpgA on conidial germination are mediated through the cAMP/PKA pathway.

Importantly, identification and characterization of RgsA revealed upstream regulation of the rather under-exploited stress response mechanisms in *A. nidulans*. Deletion of *rgsA* caused elevated pigmentation levels in both hyphae and conidia, and increased oxidative- and thermo-tolerance (Han *et al.*, 2004b). These results suggest that GanB signaling is associated with activation of stress response and

RgsA is required to negatively control this potentially energy-costing process. Collectively, it can be summarized that GanB activates a PKA signaling pathway, which in turn induces various stress responses in *A. nidulans*, and RgsA is required for downregulation of this GanB-PKA pathway (Fig 6). It is important to note that this model is opposite to the yeast stress response mechanism. In *S. cerevisiae*, deletion of Rgs2p increased sensitivity to thermal stress, whereas overexpression of Rgs2p caused significant elevation in thermo-tolerance (Versele *et al.*, 1999). Moreover, PKA activity antagonizes induction of the general stress response as well as glycogen accumulation in yeast (Smith *et al.*, 1998). Therefore, it needs to be emphasized that, while two fungi utilize the same machinery for signal transduction, their roles and cellular/physiological outcomes can be quite different.

RgsB, RgsC and GprK:

Characterization of these RGS proteins is in progress, thus, only limited information is available. RgsB is similar to the yeast Rax1 protein, which is associated with bipolar budding in yeast, and has three putative transmembrane domains at the C-terminus (Lafuente and Gancedo, 1999). The *rgsB* gene encodes a 2.5 kb transcript, which is present at relatively constant levels throughout the life cycle of *A. nidulans* (Han *et al.*, 2004b). Unlike FlbA and RgsC, both RgsA- and RgsB-type RGS proteins are highly fungal specific, and there are apparently no mammalian counterparts.

RgsC has the RGS domain at the central region and PXA and PX domains at the N- and C-termini (Fig. 5). The PX domain might act as a sorting signal to make proteins reach their appropriate location by binding to phosphoinositides (Sato *et al.*, 2001). RgsC-type fungal RGS proteins are similar to those belonging to the mammalian subfamily F, which contains PhoX (PX), PX-associated (PXA) and RGS domains. RGS-PX1 is known to play a bifunctional role as a GTPase-activating protein for Gαs and a sorting nexin protein (Zheng *et al.*, 2001). The yeast Mdm1p protein is known to be required for trans-

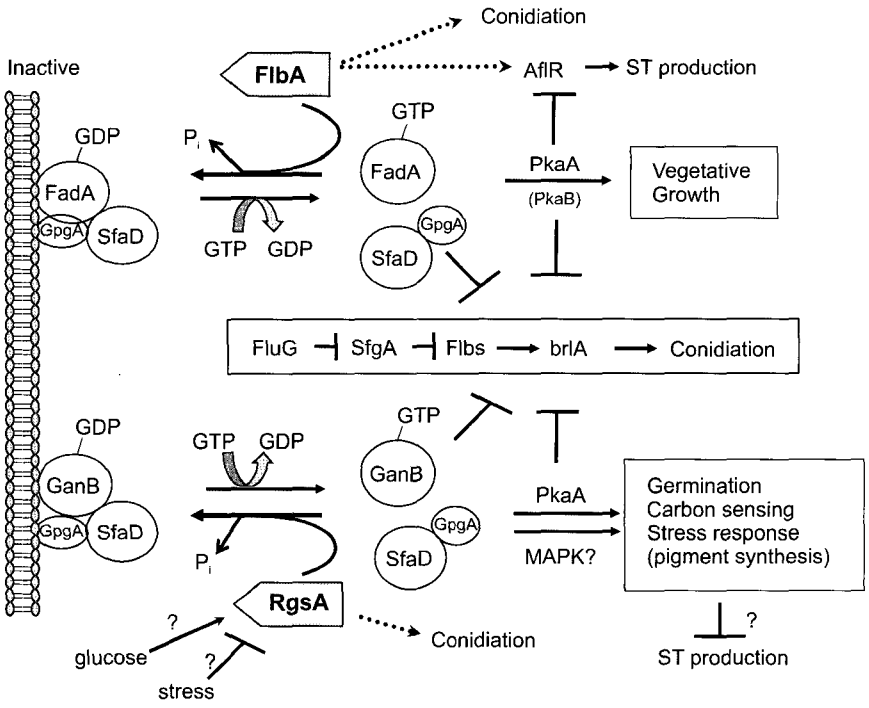

Fig. 6. G protein-RGS mediated regulation of development and ST production in *A. nidulans* (adapted from Han *et al.*, 2004b). Two independent Gα-RGS signaling pathways coordinately control various cellular responses to external cues. FlbA-FadA primarily governs vegetative growth vs. development and ST production, and RgsA-GanB controls stress response (pigmentation), carbon sensing and germination (Han *et al.*, 2004b; Lafon *et al.*, 2005). Asexual development occurs through activation of *brlA*, encoding a key transcription factor required for conidiophore development (Adams *et al.*, 1988), which requires multiple upstream genes including *fluG*, *flbE*, *flbD*, *flbD* and *flbC* (reviewed in Adams *et al.*, 1998). Recently, Seo *et al* reported that FluG-dependent conidiation occurs via removing repressive effects imposed by the potential transcription factor SfgA with the $Zn(II)_2Cys_6$ motif (Seo *et al.*, 2003, 2006). Both GanB and SfaD::GpgA have been proposed to function in inhibition of conidiation (Rosén *et al.*, 1999; Chang *et al.*, 2004; Han *et al.*, 2004b). Potential direct roles of FlbA and RgsA in conidiation (and ST production) are presented as dotted arrows. Because a MAPK (HogA/SakA) has been shown to function in stress response in *A. nidulans* (Han and Prade, 2002; Kawasaki *et al.*, 2002), a potential involvement of MAPK in GanB-mediated signaling is indicated.

mission of nuclei and mitochondria to daughter cells (Fisk and Yaffe, 1997). Based on this information, Han *et al* (2004b) speculated that RgsC might function in coordinating heterotrimeric G-protein signaling, hyphal extension, nuclear positioning and organelle transport (vesicular trafficking). The *rgsC* gene encodes a 4.7 kb transcript, and levels of *rgsC* mRNA appear to be high during asexual development (Han *et al*., 2004b).

GprK is unique in that it contains both 7-TM and RGS domains, thus is named as GprK rather than RgsD (Lafon *et al*., 2006). It is similar to the *Arabidopsis thaliana* RGS protein AtRGS1, which has been shown to negatively regulate the Gpa1 Gα subunit, and is associated with cellular proliferation in *A. thaliana* (Chen *et al*., 2003). The presence of GprK-like proteins (class VI GPCRs) in filamentous fungi suggests that the dual function signaling GPCRs may play crucial roles in eukaryotes other than plants.

Conclusions and perspectives

Cells are constantly exposed to various signals and must elicit appropriate behaviors in response to external cues. While much remains to be investigated, our knowledge of the molecular mechanisms of signal transduction and its regulation in the model fungus *A. nidulans* has soared in the last decade. The near complete identification and characterization of both positive (GPCRs, G proteins, PhLPs and effectors) and negative (RGS proteins) controllers of G protein signaling in *A. nidulans* will provide us with insights into understanding the mechanisms underlying morphogenesis, pathogenicity and toxigenesis in less genetically tractable but otherwise medically/agriculturally important fungi. Moreover, as many human diseases are associated with deleterious G protein-mediated signals, understanding the molecular events resulting from dysfunctional regulation of G protein signaling in *A. nidulans* may illuminate the nature of certain human diseases.

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References

Adams, T.H., J.K. Wieser, and J-H Yu, 1998. Asexual sporulation in *Aspergillus nidulans*. *Microbiol. Mol. Biol. Rev.* 12, 3827-3833.

Bölker, M. 1998. Sex and crime: heterotrimeric G proteins in fungal mating and pathogenesis. *Fungal Genet. Biol.*, 25, 143-156.

Brown, D.W., J.H. Yu, H.S. Kelkar, M. Fernandes, T.C. Nesbitt, N.P. Keller, T.H. Adams, T.H., and T.J. Leonard. 1996. Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. USA* 93, 1418-1422.

Burchett, S.A. 2000. Regulators of G protein signaling: a bestiary of modular protein binding domains. *J. Neurochem.* 75, 1335-1351.

Burchett, S.A., P. Flanary, C. Aston, L. Jiang, K.H. Young, P. Uetz, *et al.*, 2002. Regulation of stress response signaling by the N-terminal dishevelled/EGL-10/pleckstrin domain of Sst2, a regulator of G protein signaling in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 277, 22156-22167.

Chang, M.H., K.S. Chae, D.M. Han, and K.Y. Jahng. 2004. The GanB Gα-protein negatively regulates asexual sporulation and plays a positive role in conidial germination in *Aspergillus nidulans*. *Genetics* 167, 1305-1315.

Chen, J.G., F.S. Willard, J. Huang, J. Liang, S.A. Chasse, A.M. Jones, and D.P. Siderovski. 2003. A seven-transmembrane RGS protein that modulates plant cell proliferation. *Science* 301, 1728-1731.

Chidiac, P. and A.A. Roy. 2003. Activity, regulation, and intracellular localization of RGS proteins. *Receptors Channels* 9, 135-147.

Chung, K.S., M. Won, S.B. Lee, Y.J. Jang, K.L. Hoe, D.U. Kim, *et al.*, 2001. Isolation of a novel gene from *Schizosaccharomyces pombe*: stm1^{+} encoding a seven-transmembrane loop protein that may couple with the heterotrimeric Galpha 2 protein, Gpa2. *J. Biol. Chem.* 276, 40190-40201.

Feldbrügge, M., J. Kämper, S. Gero, and R. Kahmann. 2004. Regulation of mating and pathogenic development in *Ustilago maydis*. *Curr. Opin. Microbiol.* 7, 666-672

Fisk, H.A. and M.P. Yaffe. 1997. Mutational analysis of Mdm1p function in nuclear and mitochondrial inheritance. *J. Cell. Biol.* 138, 485-494.

Galagan, J.E., S.E. Calvo, K.A. Borkovich, E.U. Selker, N.D. Read, D. Jaffe, *et al.*, 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* 422, 859-868.

Han, K.H., K.Y. Han, J.H. Yu, K.S. Chae, K.Y. Jahng, and D.M. Han. 2001 The *nsdD* gene encodes a putative GATA-type transcription factor necessary for sexual development of *Aspergillus nidulans*. *Mol. Microbiol.* 41, 299-309.

Han, K.H. and R.A. Prade. 2002. Osmotic stress-coupled maintenance of polar growth in *Aspergillus nidulans*. *Mol. Microbiol.* 43, 1065-1078.

Han, K.H., J.A. Seo, and J.H. Yu. 2004a. A putative G protein -coupled receptor negatively controls sexual development in *Aspergillus nidulans*. *Mol. Microbiol.* 51, 1333-1345.

Han, K.H., J.A. Seo, and J.H Yu. 2004b. Regulators of G-protein signaling in *Aspergillus nidulans*: RgsA down-regulates stress response and stimulate asexual sporulation through attenuation of GanB (Gα) signaling. *Mol.*

Microbiol. 53, 529-540.

Hicks, J.K., J.H. Yu, N.P. Keller, and T.H. Adams. 1997. *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA Gα protein-dependent signaling pathway. *EMBO J.* 16, 4916-4923.

Hill, S.J. 2006. G-protein-coupled receptors: past, present and future. *Br. J. Pharmacol.* 147 Suppl 1, S27-37.

Kasahara, S., P. Wang, and D.L. Nuss. 2000. Identification of *bdm-1*, a gene involved in G protein β-subunit function and α-subunit accumulation. *Proc. Natl. Acad. Sci. USA* 97, 412-417.

Kawasaki, L., O. Sanchez, K. Shiozaki, and J. Aguirre. 2002. SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. *Mol. Microbiol.* 45, 1153-1163.

Knol, J.C., R. Engel, M. Blaauw, A.J. Visser, and P.J. van Haastert. 2005. The phosducin-like protein PhLP1 is essential for Gβγ dimer formation in *Dictyostelium discoideum*. *Mol. Cell. Biol.* 25, 8393-8400.

Kraakman, L., K. Lemaire, P. Ma, A.W. Teunissen, M.C. Donaton, P. Van Dijck, *et al.*, 1999. A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. *Mol. Microbiol.* 32, 1002-1012.

Lafon, A, J.A. Seo, K.H. Han, J.H. Yu, and C. d'Enfert. 2005. The heterotrimeric G-protein GanB(α)-SfaD(β)-GpgA(γ) is a carbon source sensor involved in early cAMP-dependent germination in *Aspergillus nidulans*. *Genetics* 171, 71-80.

Lafon, A., K.H. Han, J.A. Seo, J.H. Yu, and C. d'Enfert. 2006. G protein and cAMP-mediated signaling in aspergilli: a genomic perspective. *Fungal Genet. Biol.* In press.

Lafuente, M.J. and C. Gancedo. 1999. Disruption and basic functional analysis of six novel ORFs of chromosome XV from *Saccharomyces cerevisiae*. *Yeast* 15, 935-943.

Lee, B.N. and T.H. Adams. 1994a. Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation leads to activation of *brlA* and premature initiation of development. *Mol. Microbiol.* 14, 323-334.

Lee, B.N. and T.H. Adams. 1994b. The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal. *Genes Dev.* 8, 641-651.

Lee, N., C.A. D'Souza, and J.W. Kronstad. 2003. Of smuts, blasts, mildews, and blights: cAMP signaling in phytopathogenic fungi. *Annu. Rev. Phytopathol.* 41, 399-427.

Lengeler, K.B., R.C. Davidson, C. D'souza, T. Harashima, W.C. Shen, P. Wang, X. Pan, M. Waugh, and J. Heitman. 2000. Signal transduction cascades regulating fungal development and virulence. *Microbiol. Mol. Biol. Rev.* 64, 746-785.

Lukov, G.L., T. Hu, J.N. Mclaughlin, H.E. Hamm, and B.M. Willardson. 2005. Phosducin-like protein acts as a molecular chaperone for G protein βγ dimer assembly. *EMBO J.* 24, 1965-1975.

McCudden, C.R., M.D. Hains, R.J. Kimple, D.P. Siderovski, and F.S. Willard. 2005. G-protein signaling: back to the future. *Cell. Mol. Life Sci.* 62, 551-577.

Morris, A.J. and C.C. Malbon. 1999. Physiological regulation of G protein-linked signaling. *Physiol. Rev.* 79, 1373-1430.

Neves, S.R., P.T. Ram, and R. Iyengar. 2002. G protein pathways. *Science* 296, 1636-1639.

Ni, M., S. Rierson, J.-A. Seo, and J.-H. Yu. 2005. The *pkaB* gene encoding the secondary PKA catalytic subunit has a synthetic lethal interaction with *pkaA* and plays overlapping and opposite roles in *Aspergillus nidulans*. *Eukaryot. Cell* 4, 1465-1476.

Rosén, S., J.H. Yu, and T.H. Adams. 1999. The *Aspergillus nidulans sfaD* gene encodes a G protein βsubunit that is required for normal growth and repression of sporulation. *EMBO J.* 18, 5592-5600.

Sato, T.K., M. Overduin, and S.D. Emr. 2001. Location, location, location: membrane targeting directed by PX domains. *Science* 294, 1881-1885.

Seo, J.A., Y. Guan, and J.H. Yu. 2003. Suppressor mutations bypass the requirement of *fluG* for asexual sporulation and sterigmatocystin production in *Aspergillus nidulans*. *Genetics* 165, 1083-1093.

Seo, J.A., K.H. Han, and J.H. Yu. 2004. The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. *Mol. Microbiol.* 53, 1611-1623.

Seo, J.A., K.H. Han, and J.H. Yu. 2005. Multiple roles of a heterotrimeric G protein γ subunit in governing growth and development of *Aspergillus nidulans*. *Genetics* 171, 81-89.

Seo, J.A. and J.H. Yu. 2006. The phosducin-like protein PhnA is required for Gβγ-mediated signaling for vegetative growth, developmental control and toxin biosynthesis in *Aspergillus nidulans*. *Eukaryot. Cell* 5, 400-410.

Seo, J.A., Y. Guan, and J.H. Yu. 2006. FluG-dependent asexual development in *Aspergillus nidulans* occurs via derepression. *Genetics* 172, 1535-1544

Shimizu, K. and N.P. Keller. 2001. Genetic involvement of a cAMP-dependent protein kinase in a G protein signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans*. *Genetics* 157, 591-600.

Smith, A., M.P. Ward, and S. Garrett. 1998. Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation. *EMBO J.* 17, 3556-3564.

Tag, A., J. Hicks, G. Garifullina, C. Ake Jr, T.D. Phillips, M. Beremand, and N. Keller. 2000. G-protein signalling mediates differential production of toxic secondary metabolites. *Mol. Microbiol.* 38, 658-665.

Versele, M., J.H. de Winde, and J.M.Thevelein. 1999. A novel regulator of G protein signalling in yeast, Rgs2, downregulates glucose-activation of the cAMP pathway through direct inhibition of Gpa2. *EMBO J.* 18, 5577-5591.

Wieser J., J.H. Yu, and T.H. Adams. 1997. Dominant mutations affecting both sporulation and sterigmatocystin biosynthesis in *Aspergillus nidulans*. *Curr. Genet.* 32, 218-224.

Xue, Y., M. Batlle, and J.P. Hirsch. 1998. *GPR1* encodes a putative G protein-coupled receptor that associates with the Gpa2p Gα subunit and functions in a ras-independent pathway. *EMBO J.* 17, 1996-2007.

Yu, J.H., J. Wieser, and T.H. Adams. 1996a. The *Aspergillus* FlbA RGS domain protein antagonizes G-protein signaling to block proliferation and allow development. *EMBO J.*

15, 5184-5190.

Yu, J.H., R.A.E. Butchko, M. Fernandes, N.P. Keller, T.J. Leonard, and T.H. Adams. 1996b. Conservation of structure and function of the aflatoxin regulatory gene *aflR* from *Aspergillus nidulans* and *A. flavus*. *Curr. Genet.* 29, 549-555.

Yu, JH., S. Rosèn, and T.H. Adams. 1999. Extragenic suppressors of loss-of-function mutations in the *Aspergillus* FlbA regulator of G-protein signaling domain protein. *Genetics* 151, 97-105.

Yu, J.H. and N.P. Keller. 2005. Regulation of secondary metabolism in filamentous fungi. *Annu. Rev. Phytopathol.* 43, 437-458.

Zheng, B., Y.C. Ma, R.S. Ostrom, C. Lavoie, G.N. Gill, P.A. Insel, *et al*,. 2001. RGS-PX1, a GAP for Gαs and sorting nexin in vesicular trafficking. *Science* 294, 1939-1942.

Zhong, H. and R.R. Neubig. 2001. Regulator of G protein signaling proteins: novel multifunctional drug targets. *J. Pharmacol. Exp. Ther.* 297, 837-845.