RGS Proteins: The Early Days

HENRIK G. DOHLMAN
Department of Biochemistry and
Biophysics, University of North Carolina,
Chapel Hill, North Carolina 27599-7260

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This chapter describes events that transpired in the mid-1990s during the pioneering days of RGS protein research. This period began with early studies of Sst2 in yeast, EGL-10 in nematodes, and the discovery of an expansive family of similar proteins in animals. Within a period of only 2 years, the RGS gene family was first identified, functionally characterized in diverse organisms, and their mechanism of action firmly established. Long considered a three-component signaling system (comprised of a receptor, G protein, and effector enzyme), the discovery of RGS proteins revealed a critical new component of the signaling apparatus, and resolved a long-recognized discrepancy in the rates of G protein inactivation measured in vitro and in vivo.

I. Reflection

This chapter describes events that transpired in 1995 and 1996, a period during which the term “RGS proteins” first emerged in the literature. My objective is not to be comprehensive, as there are many excellent reviews that have already achieved that goal. Recent advances are also summarized in the chapters that follow. My objective is instead to highlight a few early experiments and discuss how they influenced the field. I will begin by describing what was known before 1995, focusing on the yeast RGS protein Sst2. Sst2 was first characterized genetically and physiologically in 1982, and the gene was cloned and sequenced in 1987. Yet, it was not until a decade later that the significance of Sst2's discovery became broadly appreciated. This chapter is written with a decidedly personal perspective. I describe my own experience...
working on Sst2 in the early 1990s, and I provide a timeline for a handful of papers published during those “early days” of RGS proteins. I apologize to the many respected colleagues who have made major contributions to the field, both before and after, whose work I have not detailed.

My own interest in Sst2 stems from my experience as a graduate student with Robert Lefkowitz in the mid-1980s. During that period, I witnessed pioneering efforts to purify, clone, sequence, and characterize G protein-coupled receptors (GPCRs), GPCR kinases (GRKs), and β-arrestins. Parallel studies in other laboratories led to the identification of visual opsins, G proteins, effector enzymes, visual arrestin, and second-messenger-stimulated protein kinases. There was, at the time, ample evidence that receptor phosphorylation triggered events resulting in GPCR desensitization. Phosphorylation, arrestin binding, and desensitization were already known to be temporally correlated, and it was postulated that phosphorylation and arrestin binding promoted uncoupling of the receptor from the G protein. But the question remained; is phosphorylation the cause or the consequence of desensitization? In today’s world, we would approach the problem by engineering receptors that cannot be phosphorylated, or by constructing cells or animals that lack the relevant kinase or receptor-binding partner. The technologies needed to knock in, knock out, or knock down expression of, for example, GRKs and arrestins were not widely available until later.

In contrast to mammalian cells, gene disruption and gene replacement were already routine in the yeast *Saccharomyces cerevisiae* (Baker’s or Brewer’s yeast). Even earlier, during the 1970s, Lee Harwell had identified mutant strains deficient in pheromone-induced mating responses (exhibiting a sterile or ste phenotype). When these genes were cloned and sequenced during the 1980s, they were found to encode a putative GPCR, a heterotrimeric G protein, and components of a MAP kinase cascade. Further, it was later demonstrated that yeast become desensitized to prolonged pheromone stimulation, and a number of desensitization mutants had been identified; these included most prominently sst1, also known as bar1, and sst2. Sst2 we now recognize as the founding member of the RGS protein family.

My own interest in Sst2 emerged from failed attempts to find additional desensitization factors in yeast. After receiving my PhD, I moved to a postdoctoral fellowship position with Jeremy Thorner at the University of California, Berkeley. The San Francisco Bay area was (and remains) a nucleus of yeast research. Moreover, Thorner had been trained as a biochemist but turned to yeast late in life, after joining the faculty at UC Berkeley. There he employed a combination of genetic, biochemical, and pharmacological approaches to study pheromone peptide maturation, receptor binding, receptor phosphorylation, and desensitization. Such biochemical and pharmacological approaches were unusual in a field populated largely by microbiologists and geneticists.
My first project in the laboratory was to isolate and clone yeast arrestin and yeast GRK, disrupt the genes, and establish their contribution to desensitization \textit{in vivo}. In essence, I hoped to do in yeast what was not yet possible in animals. The popular strategy at the time was to identify homologous genes through low stringency hybridization and PCR amplification using degenerate oligonucleotides, based on regions of sequence conservation within the mammalian arrestins and GRKs. The approach failed for reasons that became abundantly clear once the yeast genome was sequenced in 1996\textsuperscript{8}: arrestin and GRK do not exist in yeast.

\section*{II. Identification}

However, it was during that time that I first became aware of \textit{SST2}, and realized that \textit{sst2} mutants bore many of the functional properties expected for a yeast arrestin or GRK knock out. \textit{SST2} had been identified by Russell Chan and Carol Otte, who screened mutagenized yeast cells and identified those that failed to grow on agar dishes containing $\alpha$-factor pheromone. To this end they chose a dose that was sufficient to arrest growth of another (previously identified) pheromone-supersensitivity mutant, \textit{sst1}, but not of wild-type cells.\textsuperscript{9,10} An \textit{sst1} mutant had been obtained fortuitously in a screen for cell cycle mutants carried out by Lee Hartwell’s group, and later demonstrated to encode a protease that degrades $\alpha$-factor pheromone.\textsuperscript{9,10} In the Chan and Otte screen, two distinct complementation groups were identified: one corresponding to \textit{sst1} and a second designated \textit{sst2}.\textsuperscript{5–7} Further characterization revealed that mutations in the \textit{SST2} gene had no effect on cell viability, but allowed cells to respond to much lower doses of pheromone (by some measures up to 200-fold lower than in wild-type cells) and further prevented recovery from pheromone-induced growth arrest even if the ligand was removed.\textsuperscript{6} Thus, \textit{sst2} mutants exhibited increased sensitivity to pheromone stimulation and a defect in recovery from pheromone-induced cell division arrest. The authors concluded that \textit{Sst2} was “intrinsic” to the cell, and acted at some step after the initial interaction of the cell with pheromone. It is important to realize that at the time, the signaling apparatus in yeast was unknown.

The \textit{SST2} gene was cloned several years later by Christine Dietzel and Janet Kurjan,\textsuperscript{11} and an ortholog in \textit{Aspergillus nidulans} was similarly identified by Thomas Adams’ group in 1994.\textsuperscript{12} The strategy was simple and elegant. Mutant \textit{sst2} cells were transformed with a gene library and plated on doses of pheromone sufficient to impose G1 arrest in the mutant but not in wild-type cells. Thus, \textit{SST2} was cloned through its ability to rescue the pheromone-supersensitive phenotype. The sequence of \textit{SST2} revealed a protein of 698
amino acids, but otherwise lacked any clue about its function, target, or mechanism of action. At the time, it was not even known that pheromone signaling required a G protein; although the same authors soon after published a paper describing the identification of the first G protein α subunit in yeast, which they called Sgc1 (more broadly known today as Gpa1). Indeed the investigators isolated Gpa1 and Sst2 in the same screen for high-copy suppressors of the sst2 mutant. Working independently, Courchesne and Thorner later isolated another high-copy suppressor of sst2, called KSS1 (for kinase suppressor of Sst2), representing the first MAP kinase to be cloned in any organism. By this time the pheromone receptors (Ste2 and Ste3) had been cloned and sequenced. These receptors were demonstrated to bind to α-factor pheromone as well as to undergo phosphorylation and internalization. Moreover, removal of the C-terminal cytoplasmic domain of the receptor abrogated phosphorylation and internalization, but had no effect on ligand-binding activity. Thus, the desensitization function of the pheromone receptors mirrored that of mammalian adrenergic- and photo-receptors (e.g., rhodopsin). Moreover, by this time arrestins had been identified and documented to uncouple phosphorylated rhodopsin from its cognate G protein Gαt (transducin). As noted by Dietzel and Kurjan, the sst2 mutant exhibited precisely the phenotype expected of a receptor-kinase or arrestin. Although the Sst2 sequence did not resemble a protein kinase, the visual- and β-arrestins had not yet been cloned and so the authors logically speculated that Sst2 might represent a yeast arrestin. There were several problems with the model, however. Visual arrestins were known to bind to phosphorylated receptors and promote uncoupling from G proteins. Truncated forms of the pheromone receptor (missing the C-terminal phosphorylation sites) were supersensitive to pheromone, and deletion of SST2 further increased pheromone sensitivity. Those findings implied that Sst2 regulates events downstream of the receptor, independent of receptor phosphorylation. Conversely, receptor mutations that conferred diminished pheromone signaling (or more precisely, enhanced recovery from pheromone-induced growth arrest) were also dependent on SST2 expression, again suggesting that Sst2 operates downstream of the receptor. Thus, it seemed unlikely that Sst2 binds to receptors, in the manner of an arrestin. (Ironically we know now that Sst2 does bind to Ste2 as well as to Gpa1). Nevertheless, there remained many potential targets downstream of the receptor. By this time, most of the core components of the pheromone pathway had been cloned and sequenced, and the order of events was largely established. There was a cell surface receptor (Ste2), a G protein heterotrimer (Gpa1, Ste4, Ste18), a MAP kinase cascade (Ste20, Ste11, Ste7, and Fus3 or Kss1), and a transcription factor (Ste12). However, there was, during this period, only occasional mention of Sst2 in the literature and the question
remained, what does Sst2 do? In principle, Sst2 could act at any step of the pathway, and modulate any aspect of protein expression, localization, or catalytic activity.

III. Function

It was during this time that I started my own laboratory, and set out to establish where in the pathway Sst2 operates. To this end, we employed several genetic strategies. First, we undertook a series of large-scale mutagenesis experiments, hoping to find additional desensitization factors. Given that sst2 mutants exhibited pheromone-supersensitivity, it might be possible to identify a mutation in some Sst2-targeted protein with the same supersensitive phenotype. If the two affected proteins work together, the double mutant should be no more supersensitive than either mutant alone. There was also the exciting possibility of finding yet another signaling component, for example, a protein that served the function of an arrestin or receptor-kinase, or perhaps something entirely new. A particular advantage of the genetic approach was that it was unbiased. A well-designed screen for desensitization mutants would only reveal factors truly necessary for desensitization, without regard for any particular mechanism or target. Initially, these efforts met with failure, since we never found an arrestin or GRK, and found Sst2 mutants repeatedly. However, we also found mutations in Gpa1, the G protein \( \alpha \) subunit, and those mutants mimicked the loss of SST2. Given that the same genetic screen repeatedly yielded both Sst2 and Gpa1, perhaps they had some common function? Several years later, we would return to investigating the RGS-insensitive G protein mutants, as detailed below.

Thus, we set out to characterize SST2 in more detail and, more specifically, to determine if Sst2 might act specifically on the G protein. To this end we used another genetic strategy, one aimed at isolating “dominant sterile” alleles of SST2. Given that loss-of-function mutants amplify the pheromone response, we thought it might be possible to isolate gain-of-function mutants that block signaling altogether, making the cells unresponsive to pheromone stimulation. If Sst2 acted directly on the G protein, gain-of-function mutations should block signaling initiated by the receptor but not by the G protein. After extensive screening, we succeeded in isolating a variant of SST2 that blocked pheromone-dependent growth arrest and gene transcription. We then examined if the SST2 mutant could suppress signaling when the receptor was bypassed, using constitutively active forms of the G protein or an effector kinase. For example, deletion of the Gz gene, or overexpression of the G\( \beta \gamma \) subunits, results in sustained signaling (G\( \beta \gamma \) is primarily responsible for transmitting the signal in this case). If Sst2 functioned downstream of the G protein,
the signal-promoting effects of these G protein mutants would be abrogated by the Sst2 gain-of-function mutant. If Sst2 functioned at the receptor level, the gain-of-function mutant would make no difference in the response. The result was striking. Whereas, the SST2 mutant blocked pheromone-dependent growth arrest, it was ineffective in cells activated by disruption of GPA1, or by overexpression of Gβγ, or by expression of a Gβ mutant that cannot bind Gz/Gpa1. These results pointed to Gpa1 as the most likely target of Sst2 action.

IV. Family

Our first paper on Sst2 was published in August of 1995. Meanwhile at least a dozen other groups were investigating Sst2-like proteins in other systems. There were also active efforts to characterize a GTPase-accelerating activity detected in retinas (later shown to be RGS9), which was being conducted in the laboratories of Theodore Wensel, Derek Bownds, and others. Laboratories investigating RGS proteins included those of Thomas Wilkie and Alfred Gilman at the University of Texas Southwestern, Kendall Blumer and Maurine Linder at Washington University, John Kehrl at NIH, Patrick Casey at Duke, Ernest Peralta at Harvard, Marilyn Farquhar at University of California, San Diego, Michael Tyers and Tak Mak in Toronto, as well as Robert Horvitz at MIT. There were also a number of students and postdocs who I regard as true pioneers in the RGS field, and who continue to make substantial contributions to the field. These include David Siderovski (Tak Mak), Michael Koelle (Robert Horvitz), and Kirk Druey (John Kehrl). While these are the individuals who (in my view) “discovered” RGS proteins, others have made major contributions to the field and have also contributed chapters to this volume.

The advances generated by these laboratories occurred at a dizzying pace. Within a year a dozen RGS proteins had been identified. Several were documented to accelerate G protein GTPase activity. Within 2 years there was a crystal structure of an RGS-Gz protein complex, and the mechanism of RGS action was firmly established. Specifically, by accelerating Gz GTPase activity, RGS proteins decreased the lifetime of the active GTP-bound species and dampened the cellular response. Long considered a three-component signaling system (comprised of a receptor, G protein, and effector) was now a four-component system.

In August 1995, Siderovski et al. published an analysis of G0S8 transcription; this was a gene shown previously to be expressed in acute leukemias. In this paper, the authors noted for the first time sequence similarities between Sst2, the A. nidulans gene FlbA, the Caenorhabditis elegans gene C05B5.7 (now rgs-1), human BL34/1R20 (now RGS1), and G0S8 (now RGS2). BL34/1R20 had also been cloned earlier as a gene upregulated in activated B
lymphocytes. Thus, there was a sizeable family of Sst2-related proteins present in a wide range of organisms, ranging from yeast to nematodes to humans.

In December, DeVries, Mousli, Wurmser, and Farquhar reported the cloning of a novel protein, dubbed GAIP (G alpha interacting protein, now RGS19). The Farquhar lab had long been interested in the possible trafficking function of heterotrimeric G proteins, and set out to identify potential binding partners (receptors or effectors) of G proteins located at intracellular membranes. To this end, they conducted a yeast two-hybrid screen using as bait Gz13, a G protein isoform that had been found earlier to be concentrated at the Golgi compartment in many cell lines. Whereas GAIP bound well to Gz13, it bound only nominally to Gz2 and not at all to Gz4. They then corroborated the interaction by coprecipitation of recombinant GAIP and Gz13. These investigators also noted sequence similarities between GAIP and BL34/1R20 (RGS1), G0S8 (RGS2), FlbA, Sst2, as well as with another C. elegans gene (C29H12.3, now rgs-3). This work was a critical advance because it demonstrated for the first time a physical association of an RGS protein with a Gz subunit.

In the following month, Michael Koelle and H. Robert Horvitz published a tour-de-force article in Cell characterizing RGS proteins in the nematode C. elegans (in fact this was the first paper to use the term “RGS proteins”). C. elegans is one of the simplest organisms with a nervous system, and every one of its 302 neurons had been mapped. Further, the neural mechanisms responsible for various behaviors such as chemotaxis, egg laying, and locomotion had been characterized genetically. Here, Koelle and Horvitz undertook an analysis of previously identified mutants deficient in egg laying behavior, a process that involves serotonergic motor neurons and a G protein G subunit (GOA-1). Whereas loss-of-function alleles of the goa-1 gene increased foraging, locomotion, and egg laying frequency, mutants lacking a functional egl-10 exhibited the opposite behavior. Further genetic analysis revealed that EGL-10 acts as a negative regulator of GOA-1/Gz, in the manner of Sst2 and Gpa1 in yeast. For example, goa-1; egl-10 double mutants are phenotypically indistinguishable from goa-1 single mutants, indicating that EGL-10 functions upstream or parallel to the G protein. In addition, they showed that EGL-10 was homologous to Sst2 and to at least 16 mammalian proteins, including GAIP (later renamed RGS19), BL34/1R20 (RGS1), G0S8 (RGS2), as well as to 13 additional mammalian cDNAs. These included three partial clones identified previously (through random sequencing of cDNA libraries) and nine new members of the family identified using degenerate PCR. Thus, the paper highlighted the fact that this would be a very large gene family. Most significantly, it demonstrated striking functional similarities between EGL-10 and Sst2 despite modest sequence similarity between these two proteins. On the
other hand, the sequence of EGL-10 was unmistakably similar to that of
the mammalian RGS proteins, which had not yet been characterized function-
ally. Thus, there was a clear functional (but not structural) relationship between
the yeast and nematode proteins, and a clear structural (but not functional)
relationship between the nematode and mammalian proteins. There was now
little doubt that the yeast, nematode, and human proteins were truly part of the
same gene family.

Two articles published in February of 1996 cemented the functional rela-
tionship of Sst2 and the mammalian RGS proteins. In the first, Siderovski,
Hessel, Chung, Mak, and Tyers showed that RGS2 expressed in yeast could
dampen pheromone-induced growth arrest in an sst2 mutant.\(^{42}\) In the second
paper, Druey, Blumer, Kang, and Kehrl\(^ {43}\) reported the identification and
functional characterization of several new members of the RGS gene family.
An RGS3 cDNA was identified by its ability to anneal to RGS1. RGS4 was
identified by screening a rat brain cDNA library for genes that, when expressed
in yeast, could dampen pheromone-induced growth arrest response. Further
tests revealed that RGS1, RGS2, and RGS3 could likewise complement the
pheromone supersensitivity exhibited by the sst2 mutant. Moreover, RGS4
failed to dampen signaling resulting from the absence of Gpa1, suggesting that
RGS4 and Sst2 acted at the same early point in the pathway. Finally, these
investigators showed that overexpression of the mammalian RGS proteins
could dampen ERK1 kinase activation resulting from interleukin-8 or platelet
activating factor stimulation (both acting on G protein-coupled receptors).

These papers by Siderovski \textit{et al.} and Druey \textit{et al.} represented the first
demonstration of a functional interchangeability among the mammalian and
yeast RGS proteins. The Druey paper was especially significant because it
demonstrated the ability of RGS proteins to dampen G protein-mediated
signaling in cultured mammalian cells, in the manner of Sst2 in yeast.

\textbf{V. Mechanism}

It was by this time clearly established that RGS proteins constituted a large
and diverse family. Clear homologues existed in yeast, worms, and animal cells.
And in each case, deletion or overexpression of RGS proteins had dramatic
effects on G protein-mediated signaling responses. RGS1, RGS2, RGS3, and
RGS4 shared the ability to functionally “rescue” an sst2 mutant strain. At least
one family member (GAIP) was capable of binding directly to a G protein
\(\alpha\) subunit. The remaining issue was to establish mechanism. A role of Sst2 in
receptor phosphorylation had been ruled out,\(^ {22,44}\) but several alternative
mechanisms had been postulated; for example, RGS proteins might regulate
transcription,\(^ {37}\) promote G protein degradation,\(^ {45}\) uncouple receptor-G
protein interactions (in the manner of arrestins), degrade second messengers, or accelerate G protein GTPase activity. This last suggestion was particularly prescient, given that RGS proteins do not share sequence similarity with other GTPase accelerating proteins (e.g., Ras-GAP) or with Phospholipase C-β1, an effector enzyme previously shown to accelerate the GTPase activity of Gz1. Then, in the Summer and Fall of 1996, three papers were published in rapid succession, each demonstrating that RGS proteins indeed accelerate G protein GTPase activity.

The first was published in August of 1996. In that work Berman, Wilkie, and Gilman demonstrated GTPase accelerating activity for two RGS family members, RGS4 and GAIP. These proteins were active for all members of the Gz1 subfamily, but not for Gzs. In a follow up publication Berman, Kozasa, and Gilman showed that RGS4 bound most tightly to Gz1 in association with GDP-AlF4−, which is presumed to impose the transition state conformation for GTP hydrolysis. The next two papers were published back to back in Nature in September of 2006. Watson et al. showed that purified RGS1, RGS4, and GAIP accelerated the GTPase activity of Gz1, Gz2, Gz3, Gzo, Gzi but not Gzs. Hunt, Fields, Casey, and Peralta conducted similar experiments to show that RGS10 accelerated the GTPase activity of Gz3, Gzo, Gzx but not Gzs. RGS10 had been isolated in a two-hybrid screen using an activated (GTPase deficient) mutant form of Gz3 (Gz3-Q204L) as the bait protein. Coprecipitation experiments revealed that RGS10 bound to Gz3-Q204L in preference to the wild-type (GDP-bound) form of the protein. In comparison, the Watson paper showed that RGS1, RGS4, and GAIP interacted with Gz–GDP–AlF4−, in preference to either the GDP or GTPγS bound forms of the G protein. Given that GDP–AlF4− is thought to mimic the transition state and promotes dissociation of Gβγ, these binding properties suggested that RGS1 acts by lowering the energy of activation for the reaction.

Thus, in rapid succession, at least four RGS proteins had been tested and documented to promote the catalytic activity of six different Gz isoforms. Similar experiments were used later to show similar activities for Sst2 and EGL-10. It is worth emphasizing that these are challenging experiments to perform. At steady state, GTP hydrolysis is limited by the slow dissociation rate of the GDP product. One way to accelerate GDP release is to reconstitute the G protein with lipid vesicles and an agonist-occupied receptor, itself a tedious and difficult process. The alternative is to preload the G protein with [γ32P]GTP under conditions where the nucleotide cannot be hydrolyzed. This is generally done by omitting magnesium during the binding step. Magnesium is required because it coordinates the β and γ phosphates of GTP, stabilizing the transition state for hydrolysis. Once the G protein is equilibrated with GTP, the RGS protein and magnesium are added
simultaneously to start the reaction, and the release of $^{32}\text{P}$ is followed over time. Hydrolysis of $[^32\text{P}]\text{GTP}$ is limited to a single cycle by the addition of unlabeled GTP. Because only one cycle of hydrolysis is measured, the method requires large amounts of G protein and RGS protein.

### VI. Structure

In April of 1997, only 15 months after the term “RGS protein” first appeared in the literature, there was a crystal structure. Tesmer, Berman, Gilman, and Sprang described the three-dimensional structure of a high-affinity complex between RGS4 and $G_{z1}$–GDP–AlF$_4^-$. Given that RGS proteins bind preferentially to the GDP–AlF$_4^-$ complex, and this is presumed to mimic the transition state, the authors surmised that RGS proteins act by lowering the activation energy barrier for hydrolysis of GTP. Accordingly, RGS4 recognized the switch regions of $G_a$ in the activated conformation, but did not contribute any catalytic residues to the active site of the enzyme. This is in marked contrast to Ras–GAP, which was proposed to act catalytically by introducing a conserved arginine residue into the active site of Ras. That “arginine finger” is thought to stabilize the $\gamma$-phosphate in GTP in the transition state and is therefore critical for efficient catalysis. In the case of Gz, the arginine is provided in cis at residue 178. As noted by the authors, the arginine was dramatically reoriented in the GDP–AlF$_4^-$–bound structure of Gz, even as compared to the GTP$_{\gamma}$S–bound structure.

### VII. Perspective

During the past decade RGS proteins have been the topic of nearly 1400 publications. In hindsight, we (or at least I) should not have been surprised to learn of their existence. RGS stimulation of GTPase activity explains a discrepancy between the rate of Gz GTPase activity and, for example, the much faster rate of potassium channel deactivation after removal of acetylcholine from muscarinic receptors. A similar discrepancy was apparent for the G protein in the visual system and the rate of deactivation following a light pulse.

While we have gained much knowledge over the past decade, much remains to be learned. It is well established that RGS proteins act by accelerating G-protein GTP hydrolysis; however, it is also evident that they act through a variety of other protein–protein (or protein–lipid) interactions mediated by a diverse collection of interaction domains, many of which remain to be fully characterized. Moreover, their potential as drug targets remains untapped. Drugs found to modify the activity of RGS proteins could potentially
be of value in the modulation of signaling by endogenous hormones or neurotransmitters, in the manner of monoamine oxidase inhibitors and serotonin-specific reuptake inhibitors. Such inhibitors have proved extremely useful in the treatment of depression.

Another challenge is the functional redundancy of RGS proteins. One tool that may prove useful is the RGS-insensitive mutant identified in the original genetic screens for desensitization mutants. Analysis of these mutants corroborated the notion that RGS proteins act principally through Gα, and that the GTPase accelerating activity is primarily responsible for their regulatory activity in cells. Analogous mutations in mammalian Gα proteins proved resistant to RGS action in transfected cells as well as in transgenic animals, and will be useful for distinguishing RGS-mediated regulation from other modes of signal regulation. Indeed, they have provided “proof of principle” that RGS inhibition would result in profound functional changes in whole cells and animals.

RGS-inhibitors do not necessarily need to interfere with GAP activity to be effective, however. There are numerous functional domains outside the RGS box that could be targeted. Further specificity could come from tissue-specificity in RGS protein expression. In one early analysis, Gold et al. showed by in situ hybridization that nine representative RGS proteins are expressed in striking region-specific patterns within the brain.

Beyond the practical issue of drug discovery, the biology of RGS proteins remains a fascinating topic. All signaling pathways are subject to some sort of negative feedback regulation. For instance, persistent stimulation by light or odors results in desensitization of the affected sensory pathways. Desensitization is likewise thought to result in a diminished response to a variety of chemical stimulants and narcotics, including opioids, cannabinoids, caffeine, and drugs that inhibit serotonin or dopamine uptake.

In closing, I believe the major legacy of Sst2 is not any advances in sensory biology or even molecular pharmacology, but rather the dialog that emerged between scientific disciplines. A class of proteins first identified in yeast is now being studied by physiologists and pharmacologists, in academia and industry. It is no longer surprising to find a pharmacology department—long home to mice, rats, and dogs—that employs individuals working on signal transduction in zebrafish, flies, nematodes, and yeast. We have learned much from these “simple” organisms, and there is undoubtedly much more to come.

References


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