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Invited Review
Activation of G Proteins by GTP and the Mechanism of Gα-Catalyzed GTP Hydrolysis

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ABSTRACT:
This review addresses the regulatory consequences of the binding of GTP to the alpha subunits (Gα) of heterotrimeric G proteins, the reaction mechanism of GTP hydrolysis catalyzed by Gα and the means by which GTPase activating proteins (GAPs) stimulate the GTPase activity of Gα. The high energy of GTP binding is used to restrain and stabilize the conformation of the Gα switch segments, particularly switch II, to afford stable complementary to the surfaces of Gα effectors, while excluding interaction with Gβγ, the regulatory binding partner of GDP-bound Gα. Upon GTP hydrolysis, the energy of these conformational restraints is dissipated and the two switch segments, particularly switch II, become flexible and are able to adopt a conformation suitable for tight binding to Gβγ. Catalytic site pre-organization presents a significant activation energy barrier to Gα GTPase activity. The glutamine residue near the N-terminus of switch II (Gln_cat) must adopt a conformation in which it orients and stabilizes the γ phosphate and the water nucleophile for an in-line attack. The transition state is probably loose with dissociative character; phosphoryl transfer may be concerted. The catalytic arginine in switch I (Arg_cat), together with amide hydrogen bonds from the phosphate binding loop, stabilize charge at the β-γ bridge oxygen of the leaving group. GAPs that harbor “regulator of protein signaling” (RGS) domains, or structurally unrelated domains within G protein effectors that function as GAPs, accelerate catalysis by stabilizing the pre-transition state for Gα-catalyzed GTP hydrolysis, primarily by restraining Arg_cat and Gln_cat to their catalytic conformations. © 2016 Wiley Periodicals, Inc.

Keywords: Heterotrimeric G proteins; X-ray crystallography; Reaction mechanism; Signal Transduction; GTPase activating proteins

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INTRODUCTION
Heterotrimeric G protein alpha subunits (Gα), recognized first as regulatory GTPases activated by β adrenergic receptors and rhodopsin, were discovered over 40 years ago.1–6 Within 10 years the archetypal members of the family—Gαs, Gαl, and transducin (Gβγ) had been purified and enzymatically characterized.7–12 More than 20 years ago, the first three-dimensional structures of Gα subunits were described in...
Like all members of the Ras superfamily, GTP, GDP, and heterotrimeric states. Yet, only recently, with the advent of the crystal structure of Gz, a β2-adrenergic receptor:Gs complex have we begun to clearly understand how G protein-coupled receptors release GTP from the nucleotide binding site from Gz, leading to its activation.

Among guanine nucleotide binding proteins of the Ras superfamily, heterotrimeric G protein alpha subunits (Gz) constitute a distinct group. Gz are unique with respect to their tertiary and quaternary structure, mechanisms of activation and signal transduction, and in their kinetic properties. Like all members of the Ras superfamily, Gz subunits are composed of a six-stranded parallel β core in which most successive strands are connected by α helices (Figure 1). The guanine nucleotide binding sites of these proteins are similar in structure, and adopt a variety of conformations in several crystal structures, depending on crystal contacts and binding partners. The Helical domain is colored light brown and the Ras-like domain is rendered in gray. Switch segments involved in effector recognition and GTPase activity are labeled and colored cyan. The P-loop is colored green, and loop regions involved in recognition and binding of the guanine moiety of GDP and GDP are colored pink. GppNHp is shown as a stick figure, and the Mg2+ is represented by magenta sphere. Selected secondary structure elements in the Ras domain are labeled.

FIGURE 1 Tertiary structure of Gz. A model of a Gz subunit bound to GTP and Mg2+ is depicted as a ribbon drawing and is based on the crystal structure of Gz-GppNHp (PDB 1CIP). The N-terminal 31, and C-terminal 7 amino acid residues are disordered in this structure, and adopt a variety of conformations in several crystal structures, depending on crystal contacts and binding partners. The Helical domain is colored light brown and the Ras-like domain is rendered in gray. Switch segments involved in effector recognition and GTPase activity are labeled and colored cyan. The P-loop is colored green, and loop regions involved in recognition and binding of the guanine moiety of GDP and GDP are colored pink. GppNHp is shown as a stick figure, and the Mg2+ is represented by magenta sphere. Selected secondary structure elements in the Ras domain are labeled.

of the GTP γ phosphate (Figure 2, Table I). Two residues, a serine in the P-loop and a threonine in switch II coordinate Mg2+, which bridges the β and γ phosphates of GTP. In contrast to small G proteins of the Ras family, Mg2+ binds with nanomolar affinity to GTP-bound forms of Gz—it is present in all such complexes described in this review—but only weakly, with affinity in the millimolar range, to the GDP state. Both switch elements contain catalytic residues that participate in the mechanism of GTP hydrolysis. The switches themselves undergo conformational changes on conversion of GTP to GDP. Unique to Gz subunits is the insertion, within switch I, of a ~120 residue α-helical domain, and the presence of additional switch regions (III and IV) that participate in effector/regulator binding or show state-dependent conformations. The helical domain plays a regulatory role in the retention of guanine nucleotide, and contributes to Gz class-specific recognition of effectors and regulators. Roles of the helical domain in the regulatory and catalytic functions of Gz subunits continue to be discovered.

Gz regulatory activity is tightly integrated with that of heterodimers formed by G protein beta and gamma subunits (Gβγ). The canonical, nonsignaling state for both Gz and Gβγ exists in the form of a heterotrimer of GDP-bound Gz and Gβγ. As for Ras GTPases, Gz is "activated" for interaction with effectors when bound to GTP, and deactivated by its intrinsic GTPase activity—which, for most Gz proteins, is accelerated by GTPase activating proteins or protein domains (GAPs). Heterotrimeric G proteins are directly activated by integral membrane proteins (G protein-Coupled Receptors:GPCRs) that are stimulated by extracellular agonists. Cells that express heterotrimeric G proteins thereby monitor external stimuli to direct their metabolic, secretory and transcriptional programs, regulate electrical conductivity and control cellular motility. To a first approximation, the effector specificity and amino acid sequence identity of Gz subunits segregates the family into four distinct classes: s (activation of adenylyl cyclases), i (inhibition of certain adenylyl cyclase isoforms), q/11 (phospholipase β activation), and 12/13 (activation and plasma membrane localization of Rho guanine nucleotide exchange factors (GEFs). The catalog of effectors listed above exemplifies Gz class specificity but is by no means exhaustive. Gβγ heterodimers have their own regulatory targets (e.g., G protein-regulated inward rectifying potassium channels) and in some instances are co-regulators of Gz effectors (e.g., certain isoforms of adenylyl cyclase isoforms and phospholipase β). Most Gz subunits are reversibly localized at the membrane by palmitoylation at residues near their N-termini, and members of the Gz subfamily are also N-terminally myristoylated. Myristoylation increases GzI affinity for adenylyl cyclase, Gβγ subunits and the cytosolic

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In this review, I am generally concerned with activated, GTP bound Gz subunits: how the energy of GTP binding is utilized, and how its hydrolysis alters the regulatory capacity of Gz. In particular, I focus on the mechanism of Gz–catalyzed GTP hydrolysis, and the means by which the slow intrinsic GTPase activity of Gz is accelerated by GAPs. The forgoing introduction provides only a minimal foundation for our discussion of the role GTP binding and the mechanism of its hydrolysis. While no single review encompasses the complexity of G protein signaling, several provide starting points for more in-depth explorations.

Gz¢GTP IN EFFECTOR ACTIVATION

The energy of GTP binding is used to prevent Gz from interacting with Gβγ in a way that would prevent either species from expressing its regulatory functions (GTP does not, in all cases cause full dissociation of Gz from Gβγ). GTP also

FIGURE 2 Snapshots of the Gz catalytic site along the trajectory of GTP hydrolysis, derived from crystal structures. The coloring scheme is the same as that used in Figure 1. Nitrogen, oxygen, and phosphorus atoms are colored blue, red, and yellow, respectively. Sulfer atoms are colored yellow. The magenta and red spheres represent magnesium ion (Mg) and the water nucleophile (Wn), respectively. Residues of interest are labeled. The catalytic Gln and Arg residues are indicated with appropriate residue numbers and “cat” in parentheses. Hydrogen bonds (2.7–3.1 Å) and metal–ligand coordination bonds (1.9–2.2 Å) are shown as gray dashed lines. A, the structure of Gz bound to GTPγS and Mg2+ (PDB 1AZT, 2.3 Å resolution). Note that neither of the catalytic residues Gln 227 nor Arg 201 form direct contacts with the nucleotide; B, the complex of Gz1 with GppNHp and Mg2+ (PDB 1CIP, 1.5 Å resolution). Here Arg 178 (Argcat) is restrained in a hydrogen–bonded ionic interaction with the P-loop Glu 43. Gln 204 (Glncat) is a hydrogen bond donor to the water nucleophile, thereby orienting its lone pair electrons away from the γ phosphorus. This apparently stable ground-state conformation is expected to be anti-catalytic; C, Gz1 bound to GDP, Mg2+, and AlF4 (labeled ALF), a model of the preorganized or pre-transition state (PDB 1GFI, 2.2 Å resolution; the AlF4 moiety was not rigidly restrained to planarity during refinement). Argcat is within hydrogen bonding distance of the leaving group β-γ bridge oxygen and Gln was is a hydrogen bond donor to a fluorine (or O–) Al substituent and accepts a hydrogen bond from the presumptive water nucleophile. The hydrogen bond network (yellow dashed lines) involving Argcat, Gln, Wnuc, and the γ phosphate (modeled by ALF) orient Wnuc for nucleophilic attack and stabilize developing charge at the β-γ bridge leaving group oxygen (note also hydrogen bond to the latter from a P-loop amide, present also in GTP analog-bound structures); D, a model of the GDP, Pi ternary complex of Gz from the crystal structure of the G203A mutant of Gz1 (PDB 1GIT, 2.6 Å resolution). Note that switch II has reoriented and is refolded into an α helix at its N-terminus, forming an electropositive binding site for Pi. Both the β phosphate of GDP and Pi are retained in the catalytic site with multiple hydrogen bonds. The Mg2+ binding site is dismantled due to conformational changes in switches I and II.
Table I  Catalytic Residue Numbers in G Proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>P-Loop</th>
<th>Switch I(^a)</th>
<th>Switch II(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gz</td>
<td>GAGEGGKS</td>
<td>201 EVLT</td>
<td>225 GGGQ</td>
</tr>
<tr>
<td>Gz1</td>
<td>GAGEGGKS</td>
<td>178 EVKT</td>
<td>202 GGGQ</td>
</tr>
<tr>
<td>Gzt</td>
<td>GAGEGGKS</td>
<td>174 EVKT</td>
<td>198 GGGQ</td>
</tr>
<tr>
<td>Gzz</td>
<td>GTGGNNSGKS</td>
<td>179 EVKT</td>
<td>204 GGGQ</td>
</tr>
<tr>
<td>Gzq</td>
<td>GTGEGGKS</td>
<td>183 EVKT</td>
<td>207 GGGQ</td>
</tr>
<tr>
<td>Gz13</td>
<td>55 GAGEGGKS</td>
<td>200 EVRT</td>
<td>224 GGGQ</td>
</tr>
<tr>
<td>H-Ras</td>
<td>10 GAGGEGKKS</td>
<td>32 VPDT</td>
<td>59 AGGQ</td>
</tr>
</tbody>
</table>

\(^a\) Arg(cat) and Gln(cat) shown in italics and underlined.

stabilizes Gz for optimal interaction with effectors. Crystal structures reveal a variety of Gz-effector binding interfaces.\(^{34\text{-}38}\) Central to all, however, is a binding scaffold composed of switch II, an irregular helix, and z\(3\) (Figure 3). Parallel to each other and separated by 12–14Å, the two helices form a spacious groove into which structural elements of the effector penetrate. The stability of this binding surface depends on the conformation of switch II, an inherently dynamic structure, which is disordered in the crystal structures of GDP-bound states of Gz1\(^17\) and Gz12.\(^{50}\) Yet even in the GDP-bound complex of Gz, switch II can support a productive interaction with effectors. A catalytic domain construct of adenylyl cyclase is weakly activated by Gz-GDP,\(^{51}\) and PDZ-Rho-GEF forms a stable complex with Gz13-GDP, albeit with lower affinity than with Gz13 bound to the slowly hydrolyzing GTP analog, guanosine-5'-O-3-thiophosphate (GTP\(\gamma\)S).\(^{52}\) However, in the GTP-bound state, Switch II becomes more rigid, as is evident from several crystal structures.\(^{15\text{-}16,53}\) Even in the GDP-bound state, switch II is a dynamic structure; electron spin resonance studies of Gz1 harboring a spin-label near the middle of switch II show that it exhibits fast anisotropic motion in solution.\(^{54}\) Nevertheless, electrostatic and hydrogen bonding interactions between the \(\gamma\) phosphate of GTP and amide groups at its N-terminus tip the balance from global disorder to dynamic order in Switch II. These hydrogen bonds presumably pay the entropic cost of packing interactions between switch II and side-chains of the underlying \(\beta\)-sheet scaffold, which in turn affords stronger interactions with effectors.

THE MECHANISM OF Gz-CATALYZED GTP HYDROLYSIS

The kinetic properties of most Gz subunits were well established nearly thirty years ago.\(^{5,22,39}\) Single turnover rates at 30°C are in the range of 2–4 min\(^{-1}\) for most classes of Gz,\(^5\) but lower for Gzq (0.8 min\(^{-1}\)) and Gzz (0.1 min\(^{-1}\)).\(^{56}\) Yet, these sluggish GTPases are still remarkably efficient, with \(k_{\text{cat}}/K_{\text{m}}\) for some exceeding 10\(^5\), and comparable to the catalytic efficiencies of “average” enzymes.\(^{57}\) This surprising result is the consequence of the micromolar affinity of Gz for its substrate GTP, which is reduced to nanomolar affinity in the presence of the Mg\(^{2+}\) cofactor,\(^{10}\) far lower than the physiological concentration of either component.\(^{58}\) However, many of the physiological responses—particularly those related to ion channel regulation—require rapid signal termination that far exceeds the intrinsic rate of Gz GTPase activity.\(^{59}\) That the catalytic activity can be further stimulated by effectors (PLC-\(\beta\) on Gzq) and “regulators of G protein signaling” (RGS) domains, indicates that the catalytic potential of the Gz GTPase site is not fully realized within the architecture of the protein itself.

The catalytic sites of Ras superfamily proteins, including Gz, are well conserved\(^{23}\) (Table I) and hence the basic elements of the catalytic mechanism are likely to be the same for both families.\(^{25}\) Two amino acids were identified as essential to Gz GTPase activity\(^{22}\) (Figure 2). Near the amino terminus of switch II, a glutamine residue (at position 204 in Gz11 and 227 in Gzs) hereafter referred to as Glncat, is essential for catalytic activity. The conserved arginine residue in switch I (Arg 201 in Gzs, Arg 178 in Gz11), hereafter Argcat, is also a major determinant of catalytic activity. The catalytic roles of Glncat and Argcat were not fully appreciated until structures of the complexes of Gz11 and Gz with GDP, Mg\(^{2+}\), and AlF\(_3\) were determined.\(^{60,61}\) Fluoride ion had long been known to stimulate adenylyl cyclase activity,\(^{62}\) and possibly associated with GTP-dependent regulatory activity,\(^{46,61}\) but it was not until experiments were conducted with purified proteins that Gz, in the presence of Mg\(^{2+}\), was confirmed as the target of fluoride activation.\(^{62}\) A critical, but cryptic companionship of these ions was discovered by neutron activation analysis to be Al\(^{2+}\)—a contaminant in disposable borosilicate glass test tubes, and in preparations of ATP used in adenylyl cyclase assays. Together, these ions increase the intrinsic tryptophan fluorescence of GDP-bound Gz, a property characteristic of the Gz-AIF\(_3\)-activated state.\(^{64}\) The prescient hypothesis that the activating species is aluminum tetrafluoride, which functions as an analog of a \(\gamma\) phosphate moiety in Gz-GDP,\(^{65}\) was shown to be consistent with \(^{19}\)F NMR titration experiments. These indicated a stoichiometry of Mg\(^{2+}\) + AIF\(_n\) where \(n = 3\) or 4, the latter giving rise to the AIF\(_3\) anion and the former, the neutral trifluoride.\(^{66}\) Further studies of the pH and [F\(^-\)] dependence of activation suggested AIF\(_n\)(OH\(_x\)) to be the more likely species.\(^{67}\) For brevity, I shall henceforth refer to all relevant AIF\(_n\) species as AIF. The second row element Be, as BeF\(_3\) or BeF\(_2\)(OH), also activates Gz-GDP in the presence of Mg\(^{2+}\).\(^{65,67}\) At millimolar concentrations, Mg\(^{2+}\) and F\(^-\) are capable of inducing the activated state of Gz-GDP in the absence of AIF.\(^{68}\) Two Mg\(^{2+}\) ions and three to four F\(^-\) are required for activation. Although the structure of this complex has not been experimentally verified, it is likely...
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that, one Mg$_{2}^{2+}$, like Be$_{2}^{2+}$, forms a trifluoride ion—mimicking a γ phosphate—while the second reprises its role in G$_{a}•$GTP complexes, in this case bridging the β phosphate and a fluoride ligand of MgF$_{3}^{3}$

The crystal structures of GDP•Mg$_{2}^{2+}$•AlF$_{6}^{3-}$ (hereafter, GDP•MgAlF)-bound G$_{a}$ and G$_{z1}$ are illuminating. AlF$_{6}^{3-}$ appears to be a mimic, not of a γ-phosphate, but rather of a penta-coordinate transition state or intermediate for phosphorlyl transfer. In all instances, metal (Al$^{1+}$, Be$^{2+}$, or Mg$^{2+}$) fluoride complexes occupy the γ phosphate monoanion site.

It is important to point out at this stage of the discussion that the majority of experimental, and all of the computational studies of G protein-catalyzed GTP hydrolysis have focused on Ras, or the Ras:Ras-GAP complex. The latter is particularly relevant in the present context, in that it conserves all of the catalytic features found in the catalytic sites of G$_{a}$ subunits.

FIGURE 3  Structures of G$_{x}$ bound to effectors and effector-GAPs. In all panels, G$_{x}$ is rendered in gray except switch I and II, which are colored slate blue, and the α3 helix and switch III, which are rendered in turquoise. Effector domains are colored sage green and GAP domains are rendered in light brown. Ligands and nucleotides are rendered as stick models. (A), structure of the catalytic domains (C1 and C2) of adenylyl cyclase bound to the GTP$_{c}$S complex of G$_{z}$ (PDB 1AZS). Two helical segments of adenylyl cyclase and connecting loops engage the middle of switch II and trough between switch II and α3; B, the complex between cyclic GMP phosphodiesterase γ subunit (PDE$_{γ}$), the RGS domain of RGS9 and G$_{a}$i1(PDB 1FKQ). PDE$_{γ}$ binds at the switch II—α3 interface, while RGS9 occupies a distinct interface between the N-terminal half of switch II and switch I. PDE$_{γ}$ potentiates the GAP activity of RGS9 by stabilizing its interaction with G$_{x}$; C, complex of the rgRGS domain of p115RhoGEF with G$_{a}$i3/i1 (PDB 1SHZ). The RGS-like domain of p115RhoGEF occupies the effector binding region of G$_{x}$ at the switch II - α3 interface. The βN-αN hairpin domain that conveys GAP activity docks at the interface between the N-terminal half of switch II and switch I.

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quite similar to that of Gz bound to GDP-MgAlF (as the tetrafluoroaluminate).

At least three events must take place in the course of Gz-catalyzed GTP hydrolysis: 1, the catalytic site must undergo a preorganization step to support the transition state for phosphoryl transfer; 2, depending on the reaction mechanism, an intermediate or transition state for phosphoryl transfer develops; 3, a proton is transferred from the attacking water nucleophile to the γ phosphate leaving group. These steps can, in principle, be stepwise or concerted. A minimal catalytic scheme can be written as:

\[ G\cdot GTP \rightarrow G\cdot G\cdot \text{Pi} \rightarrow G\cdot G\cdot \text{GDP} + \text{Pi} \]

where G-GTP represents the Gz Michaelis complex with GTP and Mg\(^{2+}\). For Gz, catalytic site preorganization (transition to G* in the reaction scheme) encounters an activation energy barrier on the order of 3–4 Kcal/mol, as deduced from the rate enhancement provided by Gz GAPs of the Regulator of G protein Signaling (RGS) domain family and discussed in more detail below. In ground state structures of Gz crystallized with GTP\(^{2-}\) or guanosine-5′-(β,γ-imido)triphosphate (GppNHp) (Figures 2a and 2b), both Glncat and Argcat exhibit elevated thermal parameters, indicating that they undergo constrained dynamic motion. The average positions of these residues are not always the same in the GTP\(^{2-}\) complexes of different Gz proteins. \(^{15,16,53}\) In particular, the 1.5Å crystal structure of Gz1•GppNHp is unusual in that both Glncat and Argcat are highly constrained in conformations that would appear to impede their respective roles in catalysis (Figure 2b). \(^{72}\) In all of these structures, an ordered water molecule is located about 3.8Å from the γ phosphorus and distal to the β−γ bridging oxygen. Occupying a position consistent with its potential role as \(W_{nuc}\), this ordered water forms a hydrogen bond with a γ-phosphate oxygen, but is offset from the axis of in-line attack (Figure 2a). The structure of the pre-organized state for Gz•GTP is not known, but likely shares features with crystal structures of Gz•GDP-MgAlF, described in a preceding paragraph (Figure 2c). Warshel and coworkers have proposed that the catalytic role of Glncat in Ras is largely allosteric, aiding in the preorganization of the enzyme-substrate complex. \(^{73,74}\)

Indeed, the AlF-bound Gz structures clearly show that Glncat, together with the main chain carbonyl oxygen of Thr 181 (Gz1 numbering, and equivalent to Ras Thr 35) positions \(W_{nuc}\) for in-line attack, thus providing up to two orders of rate acceleration, even for a loose transition state. \(^{75}\) As discussed below, an important role of Gz GAPs is to maneuver Glncat into a catalytically functional orientation, as exemplified by the GDP-MgAlF complexes. More global allosteric effects of Glncat on the conformation of the enzyme active site itself appear to be subtle. Root mean square differences in the positions of main-chain P-loop atoms in the 1.5Å-resolution structure of Gz1•GppNHp relative to the corresponding 2.2Å-resolution structure of the GDP-MgAlF complex are less than 0.15Å. However, in the GDP-MgAlF complex of Gz1, switch II shifts slightly away from the nucleotide, such that the amide nitrogen of Gly 203 is displaced by 0.2Å from the γ phosphorus relative to its position in Gz1•GppNHp. This slight enlargement of the γ phosphate subsite appears to be a consequence of the rotation of the Glncat to its catalytically functional conformation in the AlF complex, and may preorganize the enzyme for orthophosphate formation.

The preponderance of evidence, both experimental and from Quantum Mechanics/Molecular Mechanics (QM/MM) and Electron Valence Bond (EVB) calculations, supports a mechanism in which G protein-catalyzed GTP hydrolysis proceeds through a loose transition state with dissociative character, as is typical for nucleophilic attack on phosphomonooesters. \(^{75,76}\) As such, a catalytic base to deprotonate the nucleophile would not promote catalysis (see Lassila et al. for a comprehensive discussion). \(^{77}\) Findings are based for the most part on studies of Ras:Ras-GAP, which, with some caution, may be applied to the intrinsic GTPase activity of Gz, in which the Argcat, that resides in switch I is a built-in component of the active site. Strong evidence for a loose transition state comes from the significant normal kinetic \(^{18}\)O isotope effect (\(V/K_{c} = 1.02\)) at the bridging β−γ oxygen atom of the leaving group, and secondary isotope effects in the non-bridging β phosphate oxygens. \(^{77,78}\) These are indicative of a redistribution of negative charge towards the \(β−γ\) bridge oxygen and to the non-bridging β phosphate oxygen atoms as well (with a concomitant reduction in their bond orders). The magnitude of this kinetic isotope effect (KIE) suggests a low forward commitment to the formation of this transition state and hence, that it is the rate-limiting step of the reaction. In contrast, KIEs at γ phosphate oxygen atoms are near unity, and hence inconsistent with an associative transition state or phosphoryl intermediate. Time-resolved Infrared and Raman spectroscopy of Ras-GAP catalyzed turnover of caged, \(^{18}\)O-labeled GTP after photoexcitation, similarly report accumulation of charge in the β phosphate oxygens, \(^{79–81}\) and further, vibrational decoupling of the phosphates resulting from their differential interactions with the Ras active site, QM/MM simulations based on the Ras-GAP complex with GDP-MgAlF indicate a metaphosphate (PO\(^{3-}\)) intermediate. \(^{82,83}\) The trend in KIE values with respect to the site of \(^{18}\)O labeling is correctly predicted by this model. \(^{84}\) Whether an actual metaphosphate intermediate forms is doubtful. Electron Valence Bond calculations and KIE effects appear to support a
more concerted reaction with dissociative character. In such a mechanism, to accelerate catalysis, the active site of Gα must draw electron density from the bond between the β-γ bridging oxygen and the leaving group, by stabilizing charge at that oxygen and delocalizing charge to the non-bridging oxygen atoms of the β-phosphate.

Part of the task of charge redistribution falls to amide groups of the P-loop. For Ras, Maegley et al. saw the amide group of Gly 13 (Glu 43 in Gz1) as a prime candidate for this role, in view of the short hydrogen bond that it forms to the β-γ bridging oxygen of GTP in several Ras structures. Indeed, a short, linear 2.7 Å hydrogen bond between the corresponding atoms is observed in the crystal structure of the RasRas-GAP complex. Accordingly, a normal isotope effect is observed for the β-γ bridging oxygen, as well as the Pro-3 β oxygen, which accepts hydrogen bonds from P-loop amides at residues 15 and 16, as well as the amine of lysine 16 (Lys 46 in Gz1). These hydrogen bonds are conserved in the GDP-MgAlF-bound structures of Gz1 and Gzt, as well as in the GTP•S or GppNHP ground states. Thus, there are ample hydrogen bond donors available to stabilize charge on the β-phosphate leaving group. Nevertheless, robust stabilization of charge on the β-γ bridging oxygen requires Argcat, whether it is supplied by an exogenous GAP, or, as in Gz subunits, is resident in switch I. KIE experiments suggest that the Argcat1Ala mutation considerably impairs the GAP activity of NFI by eliminating the Argcat contribution to charge stabilization at the pro-3 β-phosphate oxygen. In heterotrimeric G proteins, mutation of the endogenous Argcat results in loss of GTPase activity in Gz3. The same residue is the target of cholera toxin ADP ribosylation.

Argcat exerts its catalytic function at the transition state. As noted above, GTP-analog-bound structures of Gz subunits differ in the disposition of Argcat. In Gz-GTP•S, Argcat is hydrogen bonded to the β-γ bridging oxygen; in Gz-GTP•P•S and Gz-GTP•P•AlF, it is partially disordered, and in the “auto-inhibited” conformation observed in Gz1•GppNHP, Argcat is sequestered from the nucleotide by formation of an ion pair with the side chain of P-loop Glu 43. However, in the GDP-MgAlF complexes of Gz1, Gzo, Gzt, Gzq, Gz12, and Gz13 (some also bound to effector-GAPs), the conformation of Argcat is invariant, forming in all instances hydrogen bonds to both the bridging oxygen atom and a fluoride substituent of AlF. In these structures, Glncat accepts a hydrogen bond from Wnuc and donates to a fluoride substituent of AlF (Figure 2c).

Resolution of the loose dissociative transition state is achieved by scission of the bond between Pγ and the β-γ bridging oxygen of the leaving group and formation of a bond from the metaphosphate-like species to the attacking water. This step involves the transfer of a proton from the Wnuc to the γ-phosphate, yielding GDP and HPO4−. Whether concerted with the breakdown of the dissociative transition state or following it, direct transfer of the proton is energetically prohibitive. A “two-water” proton transfer trajectory, presumably along existing hydrogen bonds, would afford a lower energy route but would require shuttling the proton through an intermediary donor/acceptor. None of the crystal structures of Gz•GTP-MgAlF complexes reveal a water molecule optimally positioned to shuttle a proton from Wnuc to a γ phosphohydroxyl oxygen. Sondek et al. proposed such a role for Glncat, wherein the side chain Oδ abstracts a proton from Wnuc while the δ-amide donates a proton to the γ-phosphate. This highly unlikely tautomeric shift, in view of the pKas of the groups involved, could be driven by a highly reactive metaphosphate (PO3−2) intermediate. In any case, the absence, in Ras:Ras-GAP-catalyzed GTP hydrolysis, of a KIE on the γ-phosphate oxygen atoms suggests that proton transfer to the γ-phosphate is not rate limiting.

LESSONS FROM MUTANTS

Both site-directed mutagenesis and natural sequence variations have provided insight into Gz function. Some outcomes are expected, for example, that mutations of Glncat and Argcat result in constitutive activity, unregulated signaling and associations with pituitary and pancreatic cancer. The X-ray structures of Gz1•GTP•S harboring these mutations, Q204R and R178C, respectively, show no perturbations of the GTP binding site. The presumptive Wnuc is observed in the structures of both mutants, as in the structure of wild-type Gz1•GTP•S, some 3.8 Å from the γ-phosphate. Thus, neither residue participates significantly in substrate binding, as confirmed by the rates of nucleotide dissociation.

With a turnover rate less than 0.1 min−1 at 30°C, 100-fold slower than Gz1, Gz is an exceptionally sluggish GTPase. Gz harbors threonine and serine residues at positions 41 and 42 of the P-loop (Table I), whereas alanine and glycine are found in Gz subunits with typical levels of GTPase activity. Although a modeling experiment suggests that both sequence variations can be easily accommodated in the P-loop, it is possible that, together, they perturb the P-loop amide hydrogen bonds to the GTP β-phosphates, and impair the ability of the enzyme to stabilize negative charge at the transition state. Gzq also has a threonine at position 41 (but retains the canonical glycine at 42) and has moderately weak GTPase activity at 0.8 min−1. Thus, evolutionary forces are able to tune intrinsic Gz GTPase activities by P-loop mutations that do not appear to significantly perturb the stereochemistry of the catalytic site.

More surprising are mutations that alter Gz conformation or dynamics in a substrate-dependent manner. Seemingly
modest mutagenic perturbations of the P-loop and switch II can result in significant alterations in the GTP, Mg\(^{2+}\), or G\(\beta\gamma\) binding properties of G\(_{\alpha}\), and may be manifested in novel conformations that can be trapped in the solid state. In G\(_{\alpha}\), mutation of Gly 226 to alanine results in a serious signaling defect in which receptor engagement of heterotrimeric Gs fails to liberate G\(_{\alpha}\)•GTP and G\(\beta\gamma\).\(^{94,95}\) Gly 226 is located at the N-terminus of switch II, where its amide group forms a hydrogen bond with a \(\gamma\) phosphate oxygen atom (the equivalent residue in Ras is Gly 60). Because Gly 226 is in van der Waals contact with the P-loop residue Gly 49, substitution with alanine would be expected to introduce a steric clash in that region. Nevertheless, (G226A)/G\(_{\alpha}\) activates adenyl cyclase. It and its homolog (G203A)/G\(_{\alpha}\)I have nearly normal GTPase activity, but weaker-than-wild-type affinity for Mg\(^{2+}\) and GTP•S.\(^{95,96}\)

Surprisingly, an attempt to crystallize the GTP•S:Mg\(^{2+}\) complex of (G203A)/G\(_{\alpha}\)I instead yielded crystals of the Mg\(^{2+}\)-free complex of G\(_{\alpha}\) with inorganic phosphate (Pi): a model of the ternary product complex of GTP hydrolysis\(^{96}\) (Figure 2d). Presumably, at the low pH (\(\sim\)5.5) at which the complex was crystallized, the relatively unreactive GTP analog was hydrolyzed during the course of crystallization. The position occupied by GDP and its contacts with the P-loop in the G203A mutant are no different from those observed in wild-type G\(_{\alpha}\)I. The phosphate, most likely H\(\text{PO}_4\)^\(-\), is within hydrogen bonding distance of the \(\beta\) phosphate, stabilized by the P-loop lysine and switch II Arg\(_{\text{cat}}\). To accommodate Pi, switch II adopts a more regular helical structure at its N-terminus and becomes kinked near its mid-point, affording its movement away from the catalytic site. Meanwhile, the hydrogen bond between the 203 amide nitrogen and the erstwhile \(\gamma\) phosphate is maintained. Thus, due to a substantial change in the secondary structure of switch II, G\(_{\alpha}\)I adopts a conformation that is complementary to GDP•Pi. Yet, Pi binds very weakly to G\(_{\alpha}\)I-GDP—both to the wild-type and G203A and G42V (see below) mutants—with a Kd of at least 50 mM.\(^{98}\) Arguably, by inducing steric stress at the N-terminus of switch II, A203 stabilized a transitory conformational state, thus trapping the GDP•Pi product complex. Precisely the same GDP•Pi-bound state of the (A42V)/G\(_{\alpha}\)I mutant can be crystallized.\(^{98}\) Like its oncogenic G12A counterpart in Ras,\(^{99}\) (A42V)/G\(_{\alpha}\)I has weak GTP hydrolytic activity, with a turnover rate of 0.13 min\(^{-1}\) (Ref. 98). GTPase activity may be weakened in this mutant due to steric conflict between the Val 42 side chain and C\(\beta\) of Glu\(_{\text{cat}}\). Accordingly, the steric pressure that induces the reconfiguration of switch II, and affords crystallization of the GDP•Pi complex, originates from an increase in side-chain volume at position 42 in the P-loop.

As we have seen, mutations in switch I and switch II have the potential to drastically alter GTPase activity. Mutation of Gly 202 to alanine, perhaps because it forms a hydrophobic cage that restricts the mobility of W\(_{\text{nuc}}\), causes a 10-fold increase in the intrinsic GTPase rate of G\(_{\alpha}\)I.\(^{100}\) The reverse mutation in Ras, in which the corresponding wild-type residue at position 59 is alanine, results in the loss of GTPase activity.\(^{101}\) In the crystal structure of (A59G)/Ras•GppNHp, switch II adopts a conformation that is intermediate between the GDP and GDP-bound states. Unfortunately, it was not possible to crystallize (G202A)/G\(_{\alpha}\)I as a complex with a GTP analog.

Mutations that trap intermediate states have been found elsewhere near the catalytic site. The switch I residue located two positions N-terminal to Arg\(_{\text{cat}}\) is variable among the different G\(_{\alpha}\) classes. In Gi-class G\(_{\alpha}\) subunits, this position is occupied by lysine; in the q/11 G\(_{\alpha}\) class, proline is preferred. This substitution has no effect on the conformation of switch I, because the backbone \(\phi/\psi\) angles at that position are accessible to proline. However, substitution of lysine for proline resulted in an eightfold loss of GTPase activity,\(^{102}\) whereas a lysine-to-alanine mutation had no effect. However, the impact of this mutation on conformational change rates was startling.

Increase in intrinsic tryptophan fluorescence is a hallmark of G\(_{\alpha}\) activation\(^{64,103}\) that originates from changes in the solvent accessibility of a tryptophan in a switch II on exchange of GDP for GTP.\(^{104}\) Hence, a convenient way to follow GTP hydrolysis has been to monitor the rate at which tryptophan fluorescence is lost as hydrolysis proceeds.\(^{105}\) Indeed, the rate of the fluorescence transition is nearly identical to that at which GTP is hydrolyzed as measured by generation of radio-labeled Pi\(^{106}\) or fluorescence quenching of N-methyl-anthracyl guanine nucleotide derivatives (mGTP).\(^{107}\) For (K180P)/G\(_{\alpha}\)I, the rate of tryptophan fluorescence decay on addition of Mg\(^{2+}\) to GTP-bound protein exceeded by 60-fold that of Pi or mGDP production. Apparently, switch II began its conformational change before GTP was hydrolyzed, and thus the two events were decoupled. Attempts to crystallize the K180P mutant in the presence of Mg\(^{2+}\) and a hydrolysis-resistant GTP analog were not successful, so the structure of the intermediate from which hydrolysis proceeds remains unknown. The structure of the pretransition state GDP•MgAlF\(_3\) complex indicated destabilization of the Mg\(^{2+}\) binding site, with switch I constrained by the proline substitution to a conformation similar to the GDP-bound state. The behavior of this mutant suggests the possibility that catalytic preorganization may involve long-range structural changes that preserve the coupling between GTP hydrolysis and conformational changes in switch II.
CONVERGENT MECHANISMS OF Gα GTPASE ACTIVATING PROTEINS

In the mid-1990s, experiments with yeast and nematodes lead to the discovery of “Regulators of G protein Signaling” (RGS)\textsuperscript{108} which were ultimately found to function as GTPase activating proteins (GAPs) for Gα that act catalytically to increase the rate of Gα-catalyzed GTP hydrolysis by up to 100-fold \textit{in vitro}.\textsuperscript{109} GAP activity in these proteins is conveyed by a ~120 residue \(\alpha\)-helical domain. RGS proteins play complex, integrative roles in cell signaling, acting as “kinetic scaffolds” in conjunction with G protein heterotrimers and GPCRs to maintain high signaling throughput by coupling Gα activation to GTP hydrolysis.\textsuperscript{110–112} Much has been learned about the basis of Gα class specificity exhibited by members of the four major families of RGS GAPs, and their complex roles in cell signaling.\textsuperscript{40} Here, we focus on the mechanism by which they accelerate GTP hydrolysis and the remarkable functional convergence of RGS GAPs with certain G protein effectors, which also function as GAPs. Among these are isofoms of PLC-\(\beta\)\textsubscript{55} and p115RhoGEF, one of a family of Gα12/13-regulated guanine nucleotide exchange factors for Rho GTPases.\textsuperscript{113} When colocalized at the plasma membranes with GPCRs and Gbc, these effectors kinetically couple G-protein activation and deactivation, maintaining a high steady-state level of effector activation while agonists are present. This is possible because Gα GAPs and effectors occupy distinct and non-overlapping binding sites on Gα.\textsuperscript{25} In reconstituted vesicles containing Gα, Gβγ, a GPCR and the appropriate RGS protein or effector-GAP, GTP turnover rates can be 1000-fold higher than intrinsic GTPase rates.\textsuperscript{111} The maximal catalytic efficiency for Gα•GTP as a GAP substrate is ~10\(^8\) M\(^{-1}\) s\(^{-1}\) with \(K_M\) values ranging from ~2 to 600 nM for various RGS proteins, and in the nanomolar range for PLC-\(\beta\)\textsubscript{35,111} and p115RhoGEF.\textsuperscript{114} The effect of GAPs on the \(K_M\) for GTP at the catalytic site of Gα has not been determined, hence we do not know how the catalytic efficiency of Gα itself is affected.

RGS GAPs exhibit high affinity for the pre-transition state of Gα as modeled by the complex of Gα•GDP•MgAlF\textsubscript{3}.\textsuperscript{115} The crystal structure of Gαi1•GDP•MgAlF:RGS4 is the prototype

![Diagram of Gα GTPase interactions](https://example.com/diagram)

**FIGURE 4** Interactions between Gα•GDP•MgAlF active site and critical residues of RGS and effector-GAP domains. The coloring scheme in Figures 1–3 is used. A, contacts between switch I and switch II of Gαi1 and RGS4 (PDB 1AGR, 2.8 Å resolution) are shown. RGS residues Asn 128 and Glu163, respectively, constrain the conformation of Gαi1 Q204 (Gln, cat) to the pre-transition state conformation and stabilize switch I though a hydrogen bond to the backbone amide of Thr 181; B, Asn 260 from the loop between the EF 3 and EF 4 domains of PLC-\(\beta\)\textsubscript{3} form a network of hydrogen bonds with residues of switch II at the catalytic site of Gαq (PDB 3OHM, 2.7 Å resolution). Interactions between Asn 260 and Gαq mimic that of Asn 128 of RGS4 with Gαi1. The latter are strengthened by hydrogen bond network with residue Gln 212 and Thr 187 of switch I in Gαq. Not shown is the extensive interaction surface of PLC-\(\beta\)3 and the effector-binding surface of Gαq; C, The zN segment of the βN-zN hairpin of p115RhoGEF forms hydrogen bonds with switch I and switch II in Gα13/β1. Acidic residues Glu 27 and Glu 34 form ion pair contacts with Arg200 (Arg 200) and Lys 204, respectively; in switch I, stabilizing the interaction between Arg200 and the fluoroaluminate, and potentially, the β-γ bridge oxygen of GTP. Phe 31 sterically restrains the position of Gln26 as do Asn 128 and Asn 260 in RGS4 and PLC-\(\beta\)3.
for RGS domain-bound Gz complexes that have been subsequently determined and which currently represent three of the four subfamilies of RGS GAPs bound to α subunits of the i (Gzii, Gzii1, Gzii3) and q (Gqii) classes. As yet, no RGS GAP that recognizes Gzs has been discovered, and it appears that the $t_{1/2}$ for adenyl cyclase activation is similar to that of the intrinsic rate of Gzs-catalyzed GTP hydrolysis. In all of these complexes, we find that RGS, unlike small G protein GAPs that provide Argcat, do not contribute residues that appear to have a direct catalytic function. Rather, RGS sterically restrain the conformation of switch I and switch II, and in particular, Glncat and Argcat, to stabilize the pre-transition state conformation of Gz (Figure 4a). Accordingly, RGS domains form contacts with all three switch regions (I–III), and some engage the helical domain (viz. RGS2 and Gzq). Most RGS domains present a conserved asparagine as a hydrogen bonding partner for Glncat thereby stabilizing its conformation in the pre-transition state. However mutagenesis studies, reviewed by Ross and Wilkie, and structures that have been determined so far, suggest that considerable variation is tolerated at the RGS:Gz interface. Stabilization of Glncat is crucial. RGS4 cannot restore GTPase activity to (Q204L)Gz12 and Gz13, but can rescue the GTPase activity of the Argcat mutant R178C, although not to levels exhibited by wild-type Gz. The active site structure of the (R183C)GqiiGDP•MgAlF bound to RGS2 is virtually identical to that of RGS domains bound to wild-type Gz subunits. Thus, the incremental stabilization of charge at the β-γ leaving group oxygen is not essential if Glncat can be conformationally stabilized to effect catalysis.

The GAP activity of PLC-β results from the interaction of an extended loop between the third and fourth EF hand domains with the switch I and switch II regions of Gzq. At the contact site, PLC-β3 residue Asn 260 is juxtaposed to Glncat in much the same fashion as the essential Asn residue provided by RGS domains (Figure 4b). Here, too, it appears that PLC-β exerts GAP activity by stabilizing the pre-transition state of Glncat. As an effector, PLC-β3 also has high affinity for the GTP-bound forms of Gzq and accordingly, interactions between the two molecules involve an extensive interface that involve switch I and II as well as the trough between switch II and z3, which is typically reserved for effector binding.

Gz12/13-activated p115RhoGEF affords a 60-fold stimulation of the GTPase activity of Gz13, and a more modest sixfold acceleration of that for Gz12. Although p115RhoGEF and its homologs possess RGS-homology (RH or rRGS) domains, they are not involved in GAP activity. The structure of the complex between the rRGS domain and a Gz13/Gzii1 chimera revealed that the RGS-like domain binds to Gz13/I in the manner of an effector, with extensive contacts at the switch II—z3 interface, rather than as a GAP (Figure 3c). GAP activity was instead conferred by a 20-residue peptide segment (named βN-2N) directly N-terminal to the RGS-like domain (Figures 3c and 4c). The peptide is folded into an antiparallel β-z hairpin. The β segment contains a short hydrophobic sequence that docks against the helical domain of Gz13. A main-chain carbonyl oxygen within this sequence is engaged in a hydrogen bond with an arginine residue in switch III. The α-helical segment harbors a highly acidic sequence interrupted by a phenylalanine residue (DEDDEF). These residues are critical for GAP activity. The first glutamate residue in this acidic region stabilizes Argcat, and the phenylalanine side chain is positioned analogously to the conserved Asn residue of RGS domains, where it sterically restraints Glncat in its pre-transition state conformation (Figure 3c). Mutagenesis of either residue to alanine abolishes GAP activity. The related PDZRhoGEF retains the ability to bind GTP/γS-activated Gz12 and Gz13, but has no GAP activity, even though it has affinity for GDP-MgAlF-bound Gz13. The acidic motif of PDZRhoGEF contains a single deletion in the acidic motif and a tyrosine replaces the phenylalanine (EDDYD). Crystal structures show that the misalignment between the shortened acidic motif results in weakened interactions with switch I, reorientation of the tyrosyl residue relative to the position of phenylalanine at the corresponding site in p115RhoGEF, and a loss of order throughout the acidic region.

RGS GAPs, PLC-β, and p115RhoGEF, though disparate in structure and amino acid sequence, have converged on roughly the same mechanism for GAP activation. Each stabilizes the pre-transition state conformation of Gz by stabilizing catalytic conformations of Argcat and, particularly, Glncat. Overall binding energy derives from interactions with switches I–III, to differing extents with the Gz helical domain, and in the case of effector-GAPs, with Gz effector-binding regions that, at minimum, include switch II and z3. Unlike Ras-family GAPs, Gz GAPs do not participate in the chemistry of GTP hydrolysis. More generally, the structural and kinetic data obtained from Gz mutants suggest that protein dynamics may ultimately determine the rate of GTP hydrolysis by controlling the density of conformational states from which the active site of Gz can access the pre-transition state along a low activation energy pathway. Gz GAPs accelerate hydrolysis by constraining an otherwise mobile switch II, particularly Glncat, forcing it to orient the water nucleophile for in-line attack and stabilizing Argcat through a hydrogen bonding network that includes the γ phosphate. In this way Gz GAPs both promote the pre-organization of the catalytic site and indirectly assist in stabilizing charge at the βγ bridging oxygen – thus lowering the activation energy barrier to release of the leaving group.
FUNCTIONAL CONSEQUENCES OF GTP HYDROLYSIS

While GTP hydrolysis modestly diminishes the affinity of Gz for effectors, it markedly increases affinity for Gβγ.10 Gz1-GDP forms a high affinity, nanomolar KD complex with Gβγ that sequesters both signaling molecules in an inactive state at the plasma membrane. The release of interactions between the γ-phosphate and the N-terminus of switch II allows the latter to refold, affording new interactions at the Gβγ interface.18–20 In the heterotrimer, the β2 strand and the N-terminus of switch II—an extension of β3—are knit together as in a parallel, hydrogen-bonded network extending to Thr 181 in switch I and Ala 203 in switch II (residues 201–204 adopt an unusual 21 helical turn). In this configuration, strands β1 together with β3 and switch II form a platform for the Gβ subunit. Major switch II participants in this interaction are Lys 210 and Glncat, which now plays a structural rather than a catalytic role. Further along in Switch II, the side chain of Lys 210 is buried in the interface with Gβ. The importance of these residues to the affinity of the Gz:Gβ interaction has been noted in computational modeling studies.122

REACTIVATION OF Gz: EXCHANGE OF GDP FOR GTP

It is remarkable that a single phosphate moiety at the γ position of GTP is sufficient to effect major rearrangements in switch I and II that liberate both Gz and Gβγ to fulfill their respective roles in GPCR-actuated signaling. In cells, the preponderance of membrane-associated Gz-GDP is bound in a complex with Gβγ. The conformational changes within Switch I and II that are necessary to accommodate GTP cannot occur within the heterotrimer, which binds to GDP with 100-fold greater affinity than free Gz subunits,10 and within which GDP is inaccessible to solvent.19,20 Rather, GDP must first be released by engagement of the heterotrimer with an agonist-activated GPCR. The extensive conformational changes that result in the ejection of GDP are exemplified in the crystal structure of heterotrimeric Gs bound to the β2 adrenergic receptor.21 This structure, together with studies using structure-based mutagenesis,123–126 site-directed spin-labeling,127–129 molecular dynamics,130–132 and other computational approaches133 have arrived at a consistent picture of the receptor-induced conformational transitions that compel GDP release. These and seminal papers reviewed elsewhere (see Refs. 134 and 135) show that GPCRs engage the C-terminus of Gz,136,137 causing it to rotate slightly and translate with respect to the body of the Ras domain. This key perturbation induces conformational changes in the x5–β6 loop at the purine binding site, disrupts interactions with the α1 helix and succeeding P-loop, and destabilizes the nucleotide binding site and contacts between the Ras and helical domains, leading to their separation and facilitating egress of GDP. The cytosolic nonreceptor nucleotide exchange factor Ric-8A induces similar and possibly more extensive conformational changes in the structure of Gz1.138

While the interface between Gzs switch II and Gβγ is largely intact in the complex with the β2 receptor, this interaction is weakened with the disordering of switch I. The P-loop adopts an open conformation, ready to receive the β and γ phosphates of GTP and, with these moieties, to coordinate a magnesium ion.122 Awaiting further exploration are the coupled conformational pathways by which the P-loop, switch II and β5–x5 refold around GTP, and thus escape from the complex with Gβγ and the receptor. In aggregate, these rearrangements would eliminate the switch II interface with Gβ, and disrupt that between the receptor and x5.

CONCLUSIONS

In the presence of magnesium ion, GTP binds with nanomolar affinity to the x subunits of heterotrimeric G proteins. This extraordinarily high binding energy is used to restrain and stabilize the conformation of otherwise highly dynamic Gz switches I and II. The conformation in which these two structural elements are held is highly complementary to the surfaces of Gz effectors, but incompatible with the Gz binding site on Gβγ. On GTP hydrolysis, the energy of these conformational restraints is dissipated and the two switch segments, particularly switch II, become flexible. The GDP-bound state of Gz is easily remodeled for binding to Gβγ. Both signal transducers—Gz and Gβγ—are thereby locked into a nanomolar-affinity complex that can be released only by the catalytic action of agonist-activated G protein-coupled receptors, which allows GTP to disrupt the Gz:Gβγ interface and with that the receptor itself.

The mechanism by which Gz hydrolyzes GTP is likely the same as that used by Ras, with the important difference that Gz possesses a catalytic arginine residue that is absent in Ras, and must be supplied by an exogenous GAP. This provides Gz with about three orders of magnitude in rate enhancement relative to Ras with respect to intrinsic GTPase activity. The intrinsic GTPase rates of different classes of Gz range from ~0.1 min−1 to 4 min−1 at ~30°C. The differences are likely due in large part to the amino acid sequence of the P-loop, resulting in greater or lesser efficiency in stabilizing charge at the leaving group. Catalytic site pre-organization presents a significant barrier to catalysis due to the richness of non-catalytic states that are accessible to critical residues in the active site of Gz. Some of these states, exemplified by the
apparently “anticatalytic” conformation exhibited in the structure of Gxi1-GppNHp (Figure 2b), may actually impede catalytic action. Gx GAPs act by restricting the conformational freedom of Gx active site residues, particularly Glncat and Argcat and enforcing on them a conformation that is complementary to the transition state for GTP hydrolysis. Glncat in particular, appears to orient and stabilize the γ phosphate and the water nucleophile for an in-line attack. The transition state is probably loose with dissociative character, and phosphoryl transfer may be concerted. Experimental, structural and computational data suggest that electron density from the γ phosphate shifts to the β – γ bridge oxygen and is redistributed to the β non-bridging oxygens. Gx, and more effectively Gx:GAP, catalyzes GTP hydrolysis by promoting this charge redistribution. Along the reaction pathway, possibly in concert with the collapse of the loose transition state, a proton is shuttled from the water nucleophile to the γ phosphate, affording HPO₄⁻. An ordered water molecule would be an ideal candidate to serve as a shuttle, but it is also is possible that Glncat might act in this capacity. There is still a need for conclusive answers to several questions: does a metaphosphate intermediate occur in the reaction trajectory, or is the reaction concerted, with a loose-transition state? What is the mechanism of proton transfer to the γ phosphate? What, precisely is the role of Glncat? Importantly, why, given the relatively small structural differences between the Gx-GTP “Michaelis” complex and the pretransition state as modeled by the GDP•MgAlF complex, is the activation energy barrier to GTP hydrolysis so high?

It appears that Gx GAP function has arisen independently on several occasions during the evolution of Gx-regulated signal transduction networks. Incorporation of GAP activity into effectors affords exquisite regulation of GTPase kinetics and effector activation. Co-localization with GPRCs provides additional avenues for steady-state control of G protein signaling. RGS GAPs are structurally well conserved, but several have acquired signaling functions unrelated to GAP activity. For example, the RGS domains present in members of the RGS-RhoGEF family engage Gx in the manner of effectors, whereas GAP activity is conveyed by a short β-α peptide motif. Although they are structurally dissimilar, RGS GAPs and the GAP-domains of RGS-RhoGEFs and PLC-β converge on a common mechanism of action, which is to stabilize the pre-transition state for Gx-catalyzed GTP hydrolysis, acting primarily on the conformation of Argcat and Glncat.

Arguably, we have a fairly clear understanding of the reaction kinetics and structural transformations involving Gx subunits in the context of the canonical GTPase cycle of activation, effector regulation and signal termination. Considerably less well understood are Gx class-specific modes of signal integration—processes that may involve transient, often membrane-associated, multiprotein complexes that assemble at the plasma membrane and interact with other regulators. Remarkably, many of the proteins that support such signaling agendas harbor RGS domain modules, for example, RGS7, RGS14, and RGS-RhoGEFs. Unraveling the complex web of G protein regulatory interactions involving these and other signal transducers is our present task.

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