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15. A downfield displacement of 22.6 ppm in the ^{15}N chemical shift of $N^{\delta 1}$ has been reported for subtilisin from *B. lentus* complexed with Z-LLF-CF3 (*11*). Although this effect was attributed to LBHB formation with ~30% proton transfer, such a fraction is inconsistent with the $^1J_{NH}$ value of 81 Hz reported by the same authors, which would indicate only ~14 ± 8% delocalization with respect to the average imidazole value of 94 ± 4 Hz. The inconsistency may be attributable to incomplete characterization of the system, including ^{15}N shifts for both $N^{\delta 1}$ and $N^{\varepsilon 2}$ in the complex and resting enzyme.
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25. Uniformly ^{15}N-labeled (>96%) α-lytic protease (E.C. 3.4.21.12) was expressed by fermentation of wild-type *Lysobacter enzymogenes* in Celtone-N (Martek Biosciences), a medium containing ^{15}N-enriched peptides and amino acids, which was supplemented with glucose and various salts. Purification of α-lytic protease and assay of enzymatic activity was accomplished as described (*18*).
26. Expression of ^{15}N histidine–labeled subtilisin BPN′97 was accomplished by fermentation of a histidine auxotrophic strain of *Bacillus subtilis* DB104 in a minimal medium containing histidine labeled with ^{15}N at both ring nitrogens (*18*). Purification of labeled subtilisin BPN′97 was accomplished by dialysis into a 10 mM tris solution (pH 6.0) and subsequent passage over a CM52 cellulose ion-exchange column, with elution of the protein in 0.01 M MES and 0.1 M KCl. Enzyme activity was measured by a colorimetric assay with the substrate succinyl-L-Ala-L-Ala-L-Pro-L-Phe-paranitroanilide (*18*).
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Inhibition of Brain G_z GAP and Other RGS Proteins by Palmitoylation of G Protein α Subunits

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Palmitoylation of the α subunit of the guanine nucleotide-binding protein G_z inhibited by more than 90 percent its response to the guanosine triphosphatase (GTPase)–accelerating activity of G_z GAP, a G_z-selective member of the regulators of G-protein signaling (RGS) protein family of GTPase-activating proteins (GAPs). Palmitoylation both decreased the affinity of G_z GAP for the GTP-bound form of $G\alpha_z$ by at least 90 percent and decreased the maximum rate of GTP hydrolysis. Inhibition was reversed by removal of the palmitoyl group by dithiothreitol. Palmitoylation of $G\alpha_z$ also inhibited its response to the GAP activity of Gα-interacting protein (GAIP), another RGS protein, and palmitoylation of $G\alpha_{i1}$ inhibited its response to RGS4. The extent of inhibition of G_z GAP, GAIP, RGS4, and RGS10 correlated roughly with their intrinsic GAP activities for the Gα target used in the assay. Reversible palmitoylation is thus a major determinant of G_z deactivation after its stimulation by receptors, and may be a general mechanism for prolonging or potentiating G-protein signaling.

The α subunits of most heterotrimeric G proteins are modified by irreversible lipid amidation of the NH_2-terminus and by addition of a palmitoyl thioester at a nearby, conserved cysteine residue (*1, 2*). Unlike myristoylation, palmitoylation of Gα subunits is reversible, and bound palmitate turns over rapidly in cells. Although virtually nothing is known of the enzymes that catalyze addition and removal of palmitate, palmitate turnover on G-protein α subunits appears to be regulated coordinately with their activation and deactivation. In the case of $G\alpha_s$ (*3, 4*) and $G\alpha_q$ (*5*), substantial depalmitoylation occurs upon receptor-promoted activation, and repalmitoylation of $G\alpha_s$ coincides at least roughly with deactivation (*3*). Treatment with cholera toxin, which prolongs activation of G_s by blocking hydrolysis of bound GTP, also promotes turnover of bound palmitate (*6*). Conversely, palmitate turnover on $G\alpha_i$ and $G\alpha_s$ is decreased by coexpression of excess Gβγ, which inhibits activation (*6, 7*).

Palmitoylation is involved in anchoring Gα subunits to the membrane or specifying their membrane localization, or both (*1–4, 7–9*), by increasing their intrinsic hydrophobicity and, at least for $G\alpha_s$, by increasing affinity for Gβγ (*7*). Mutation of the palmitoylated cysteine of $G\alpha_z$ to alanine also potentiated inhibition of adenylyl cyclase in transfected cells (*9*). Palmitoylation has not yet been linked to alteration of a specific G-protein signaling function, however. It is not required for interaction of Gα subunits with receptors or effectors in vitro (*10*), and no effect of palmitoylation on the binding or hydrolysis of guanine nucleotides has been reported. Mutation of the palmitoylatable cysteine residue in $G\alpha_q$ or $G\alpha_s$ inhibited signaling (*11*), but signaling was potentiated by the same mutation in $G\alpha_z$ or Gpa1p, the major Gα subunit in *Saccharomyces cerevisiae* (*9, 12*). Although palmitoylation may be responsible for such variable effects on different Gα subunits, these results may also arise from effects of mutating the cysteine residue that are unrelated to palmitoylation (*10*).

We describe the inhibition of the effects of the major G_z GTPase activating protein, G_z GAP, by palmitoylation of $G\alpha_z$. G_z is a relatively rare member of the G_i family that is found in brain, platelets and adrenal medulla, and is therefore suspected to be involved in regulation of secretion (*13*). Isolated $G\alpha_z$ hydrolyzes bound GTP slowly,

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such that the half-life of the active, GTP-bound species is about 7 min at 30°C (*14, 15*). G_z GAP, which we recently purified from bovine brain, accelerates the hydrolysis of G_z-bound GTP over 200-fold (*15*). G_z GAP is a novel member of the RGS family (*16*), whose members attenuate G-protein signaling at least in part through their GAP activity (*17*). G_z GAP is most abundant in tissues that also express G_z. It thus appears to be the major determinant of G_z deactivation and, therefore, of the amplitude and duration of G_z-mediated signals.

To examine the effect of palmitoylation of $G\alpha_z$ on its deactivation, we palmitoylated purified $G\alpha_z$ in vitro (*18, 19*) and measured the rate of hydrolysis of bound [γ-^{32}P]GTP in the presence and absence of G_z GAP (*15, 20*). Fractional autopalmitoylation of purified $G\alpha_z$ in vitro was nearly complete, 80 ± 10% according to total protein or 120 ± 15% according to the number of GTP-γ-S binding sites (n = 6) (Fig. 1) (*19*). Treatment with either neutral hydroxylamine or dithiothreitol (DTT) removed the palmitate (Fig. 1), consistent with its addition through a thioester bond. [^{3}H]Palmitate could also be completely removed from $G\alpha_z$ by tryptic proteolysis after protection with GTP-γ-S or Al^{3+}/F^- (Fig. 1). Because Cys^3 is the only cysteine residue before the Arg^{29} tryptic cleavage site (*21*), palmitoylation of Cys^3 is unique and nearly quantitative under the conditions used here. There was no difference in the rate of autopalmitoylation of $G\alpha_z$ when it was bound to either GDP or GTP-γ-S (*22*).

Palmitoylation of $G\alpha_z$ blocked the action of bovine brain G_z GAP by nearly 90% (87 ± 3.5%, n = 6, in matched experiments) (Fig. 2 and Table 1) (*20*). Because palmitoylation of $G\alpha_z$ may be incomplete and because GTP bound to residual nonpalmitoylated $G\alpha_z$ will be disproportionately hydrolyzed during a brief GAP assay (Fig. 2A), inhibition of the GAP by palmitoylation of $G\alpha_z$ is underestimated and exceeds 90%. Palmitoylation of $G\alpha_z$ had no effect on the rate at which it hydrolyzed bound GTP in the absence of GAP (Table 1 and multiple control experiments) or on the rate of binding of GTP-γ-S (*22*).

Palmitoylation of $G\alpha_z$ inhibited its response to G_z GAP by decreasing both its affinity for the GAP and the maximal rate of hydrolysis of the GAP–$G\alpha_z$-GTP complex. The dependence of GAP activity on the concentration of palmitoylated $G\alpha_z$ was biphasic (Fig. 2A). The first phase indicates the presence of about 10% residual nonpalmitoylated $G\alpha_z$-GTP, which displays unaltered substrate kinetics. The second phase reveals that palmitoylated $G\alpha_z$-GTP has both a 7- to 15-fold increase in Michaelis constant (K_m) combined with a 50 to 80% decrease in maximum V (V_{max}). Essentially identical results were obtained in two other experiments with separately palmitoylated batches of $G\alpha_z$. The precision of the fit for the palmitoylated component is limited because its concentration could not be increased above the high apparent K_m. To confirm the effect of palmitoylation on the affinity of $G\alpha_z$ for the GAP, we measured the ability of palmitoyl–$G\alpha_z$–GTP-γ-S to compete with nonpalmitoylated $G\alpha_z$-GTP in a standard GAP assay (Fig. 2B). The data were again biphasic. They indicate that palmitoylation of $G\alpha_z$ was 92% complete and that palmitoyl–$G\alpha_z$–GTP-γ-S bound to the GAP with an inhibition constant (K_i) of about 75 nM, about 30-fold greater than that of the nonpalmitoylated protein.

Treatment of palmitoylated $G\alpha_z$ with 15 mM DTT restored G_z GAP activity to more

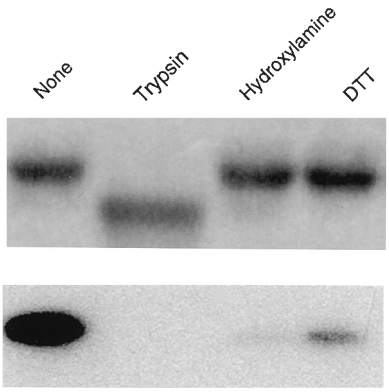

Fig. 1. Autopalmitoylation of $G\alpha_z$. GTP-γ-S-activated $G\alpha_z$ (4 μM), purified from Sf9 cells (*35*), was incubated with 50 μM [^{3}H]Pal-CoA (450 cpm/pmol) for 2 hours at 30°C (*19*). The extent of palmitoylation in this experiment was 74%, based on total protein. Samples were then incubated for 45 min at 30°C either with no addition, with 0.45 μM trypsin, 0.5 M hydroxylamine, or 15 mM DTT. Samples were analyzed by SDS-PAGE. The gel was stained with Coomassie brilliant blue (top panel) and then subjected to fluorography (bottom panel). The trypsin lane contains threefold more total sample than the other lanes, because 70% of the initial $G\alpha_z$ was totally proteolyzed even after GTP-γ-S protection.

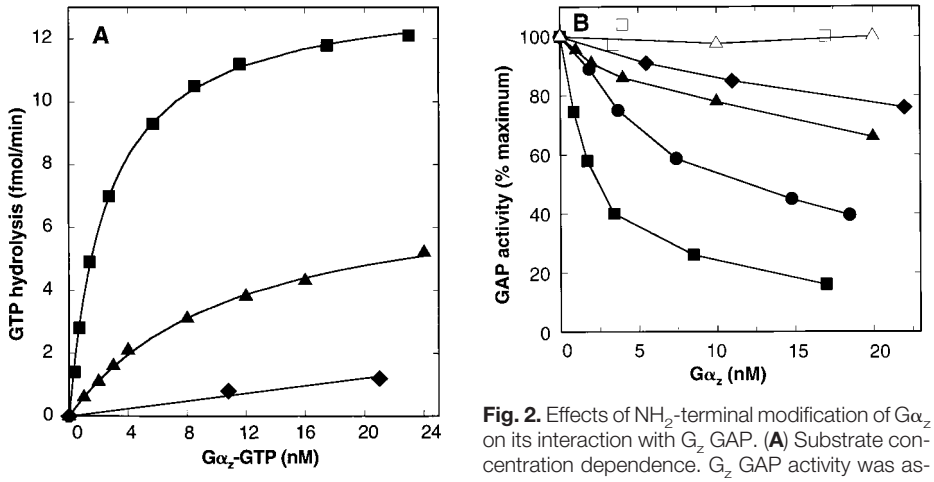

Fig. 2. Effects of NH_2-terminal modification of $G\alpha_z$ on its interaction with G_z GAP. (**A**) Substrate concentration dependence. G_z GAP activity was assayed as described (*15*) at the concentrations of $G\alpha_z$-[γ-^{32}P]GTP shown on the abscissa. The $G\alpha_z$, purified from Sf9 cells (*35*), was either untreated (■), palmitoylated to 0.8 mol/mol based on total protein (▲), or treated with trypsin in the presence of Al^{3+} and F^- before binding to [γ-^{32}P]GTP (◆). The concentration of each substrate was determined according to [γ-^{32}P]GTP binding (*15*). The concentration of G_z GAP was 50 pM. After subtraction of unstimulated hydrolysis, data for $G\alpha_z$-GTP were fit to the Michaelis-Menten equation (K_m = 2.4 nM, V_{max} = 13.0 fmol/min, k_{cat} = 3.3 min^{-1}). GAP-stimulated hydrolysis for palmitoylated $G\alpha_z$ was fit to a two-component Michaelis-Menten equation using the Marquardt-Levenberg algorithm in the SigmaPlot (Jandel Scientific Software, San Rafael, California) program package. The nonpalmitoylated component, with unchanged K_m and V_{max}, reflected 7 to 10% of the total protein-bound [γ-^{32}P]GTP. Because the highest substrate concentration was still below the K_m for palmitoyl-$G\alpha_z$-GTP, the fitted value of K_m varied from 15 to 30 nM and V_{max} varied from 2 to 7 fmol/min depending on initial conditions used in the fit. The drawn line shows simulated values for 90% palmitoylation, K_m = 19 nM and V_{max} = 2.6 fmol/min. (If the K_m for the palmitoylated $G\alpha_z$ were set equal to 75 nM, the K_i from (B), then the V_{max} would be 10 fmol/min) (**B**) Competitive inhibition of G_z GAP activity by different preparations of $G\alpha_z$. GAP activity was measured using 2 nM $G\alpha_z$-[γ-^{32}P]GTP. The substrate $G\alpha_z$ was purified from Sf9 cells. Competing $G\alpha_z$ was bound either to GTP-γ-S (solid symbols) or GDP (open symbols) and added at the concentrations shown. ■, □: $G\alpha_z$ purified from Sf9 cells, untreated; ●: $G\alpha_z$ purified from *E. coli* (*14*), untreated; ▲, △: palmitoylated $G\alpha_z$ (*19*); ◆: $G\alpha_z$ from Sf9 cells treated with trypsin as described for Fig. 1. The extrapolated K_i for palmitoyl–$G\alpha_z$–GTP-γ-S, corrected for substrate concentration, was 76 nM, and the K_i for trypsin-treated $G\alpha_z$–GTP-γ-S was >110 nM. Concentrations of each $G\alpha_z$–GTP-γ-S species were measured by direct binding assays using trace amounts of [^{35}S]GTP-γ-S. GAP activity without inhibitor was 40 mU.

than 85% of that displayed with $G\alpha_z$ that had not been palmitoylated (Table 1). Restoration of activity is consistent with the removal of more than 90% of the palmitate from $G\alpha_z$ by the identical treatment (Fig. 1). Treatment with DTT also restored the affinity of $G\alpha_z$ for the GAP (*22*). Both these effects of DTT on palmitoylated $G\alpha_z$ required prolonged incubation at 30°C and were not observed if DTT was simply added to the GAP assay. DTT treatment had no effect on the basal rate of hydrolysis of $G\alpha_z$-bound GTP with or without palmitate, but usually slightly enhanced GAP-stimulated hydrolysis by nonpalmitoylated $G\alpha_z$ (<10%; Table 1) (*23*).

G_z GAP is a member of the RGS protein family (*16*), many of which have GAP activity toward members of the G_i and G_q families (*17, 24, 25*). Palmitoylation of other Gα subunits also inhibited their responses to the GAP activities of RGS proteins (Table 2). The extent of inhibition depended on which Gα was used in the assay. RGS proteins are selective among individual members of the $G\alpha_i$ and $G\alpha_q$ families, including $G\alpha_z$ (*24, 25*), and fractional blockade of GAP activity by Gα palmitoylation was generally greatest when a GAP was assayed with a good Gα-GTP substrate. For example, GAIP (*26*) displays somewhat lower GAP activity toward $G\alpha_z$ than does brain G_z GAP, and palmitoylation of $G\alpha_z$ inhibited the GAP activity of GAIP by about 45% (corrected for substoichiometric palmitoylation). RGS4 (*27*) and RGS10 (*28*) are much less active on $G\alpha_z$, and their GAP activities were inhibited by only about 20%. When RGS4 was assayed with $G\alpha_{i1}$ as substrate, however, its activity was inhibited by 65 to 70%. For each RGS protein and Gα substrate, inhibition was reversed by removal of palmitate by DTT.

Table 1. Inhibition of G_z GAP activity after palmitoylation of $G\alpha_z$. Hydrolysis of [γ-^{32}P]GTP bound to $G\alpha_z$ (from Sf9 cells, 1.8 nM) was measured over 2 min in the presence or absence of 400 pM G_z GAP (100% is 246 mU) (*15, 20*). Before binding to [γ-^{32}P]GTP, the $G\alpha_z$ was treated in the order shown with Pal-CoA (*19*), with 15 mM DTT at 30°C for 40 min, or with trypsin after Al^{3+}/F^- protection (*21*). Al^{3+} and F^- were removed by gel filtration after binding of [γ-^{32}P]GTP (*15*).

$G\alpha_z$	k_{app} (min^{-1})		GAP activity (%)
	Control	+GAP	
Untreated	0.0134	0.259	100
Pal-CoA	0.0135	0.059	18
DTT	0.0134	0.270	104
Pal-CoA, DTT	0.0137	0.235	90
Trypsin	0.0310	0.040	4
Pal-CoA, trypsin	0.0270	0.033	2

These data suggest that palmitoylation is a general mechanism for protecting GTP-activated G-proteins against GAP-accelerated deactivation.

Inhibition of the response to GAPs by palmitoylation is apparently highly specific, not simply the result of increased NH_2-terminal hydrophobicity of the Gα substrate (*29*). In contrast to palmitoylation, myristoylation of the NH_2-terminal amine of $G\alpha_z$ enhanced G_z GAP activity. Although myristoylation had no effect on the basal rate of hydrolysis of G_z-bound GTP (*22*), the response to G_z GAP of 2.2 nM nonmyristoylated $G\alpha_z$-GTP, purified from *Escherichia coli*, was only 31% that of myristoylated $G\alpha_z$. This difference primarily reflects the lower affinity of nonmyristoylated $G\alpha_z$ for G_z GAP, reflected in an increase in K_d of three- to fivefold relative to myristoylated $G\alpha_z$ (Fig. 2B). A similar but smaller difference was obtained with RGS4; the GAP accelerated rate for unmodified $G\alpha_z$ was 76% that of myristoyl-$G\alpha_z$. Myristoylation of $G\alpha_{i1}$ and $G\alpha_o$ also increased their affinities for G_z GAP (*15*). Because autopalmitoylation of α subunits requires prior NH_2-terminal myristoylation (*18*), we were unable to determine the effect of palmitoylation of nonmyristoylated $G\alpha_z$ on its activity as a GAP substrate. Regardless, although palmitoylation of Cys^3 markedly inhibits the binding of $G\alpha_z$ to G_z GAP, myristoylation enhances affinity for the GAP.

Table 2. Inhibited response to GAIP and RGS4 after palmitoylation of Gα subunits. GAP activity was assayed as described (*15, 20*) with either 2.2 nM $G\alpha_z$–[γ-^{32}P]GTP or 5 nM $G\alpha_{i1}$-[γ-^{32}P]GTP as substrate, either palmitoylated or not. The data show percent inhibition by palmitoylation relative to parallel assays with a nonpalmitoylated Gα control at the same concentration. For assays using $G\alpha_z$ substrate, the specific activity of each RGS protein is given in standard units (*15*), with molar amounts of each GAP calculated according to total protein. The maximum specific activity of purified RGS10 was somewhat higher than that of the preparation used for these experiments. For RGS4 and $G\alpha_{i1}$, the assay underestimates GAP activity for kinetic reasons (*15, 25*), and inhibition of RGS4 by palmitoylation of the $G\alpha_{i1}$-GTP substrate is therefore also underestimated. Assays contained 0.15 nM G_z GAP or GAIP, 6 nM RGS10 or either 1.5 nM or 1.0 nM RGS4 (for $G\alpha_z$ or $G\alpha_{i1}$, respectively). GAIP (*26*), RGS4 (*27*), and RGS10 (*28*), all His_6-tagged, were purified from *E. coli* (*25*). Preliminary data indicate that GAIP expressed in *E. coli*, Sf9 cells, or HEK 293 cells displays similar enzymatic properties. Data for $G\alpha_{i1}$ are corrected for substoichiometric palmitoylation: 70 and 75% in the experiments from which data were averaged. Data for $G\alpha_z$ are not corrected, but palmitoylation of $G\alpha_z$ is usually ≥90%. Data are averages of at least two experiments, with duplicate determinations.

GAP	Gα-GTP	Inhibition (%)	Specific activity (U/pmol)
G_z GAP	$G\alpha_z$	87	7.8
GAIP	$G\alpha_z$	37	5.4
RGS4	$G\alpha_z$	22	0.75
RGS4	$G\alpha_{i1}$	69	—
RGS10	$G\alpha_z$	20	0.14

Because both palmitoylation and myristoylation occur near the NH_2-terminus, we examined the interaction of G_z GAP with $G\alpha_z$ from which the NH_2-terminal α helix was removed by tryptic cleavage at Arg^{29} (Fig. 1). NH_2-terminal truncation of $G\alpha_z$ essentially abolished GAP activity (Table 1 and Fig. 2). Remaining activity was largely accounted for by incomplete proteolysis, because incubation of trypsin-treated $G\alpha_z$ with palmitoyl–coenzyme A (Pal-CoA) further inhibited the low residual GAP activity by about 50% (Table 1). NH_2-terminal proteolysis of $G\alpha_{i1}$ also inhibited its response to the GAP activity of RGS4 (*22*). As was the case for palmitoyl-$G\alpha_z$, the insensitivity of proteolyzed $G\alpha_z$ to G_z GAP reflected a grossly diminished affinity (K_d >100 nM; Fig. 2A). Proteolysis reproducibly increased the intrinsic rate at which $G\alpha_z$ hydrolyzed bound GTP by two- to threefold (Table 1).

Because three different NH_2-terminal modifications of $G\alpha_z$ and $G\alpha_{i1}$—palmitoylation, myristoylation, and proteolysis—all modulate their responses to the GAP activities of several RGS proteins, this region of Gα subunits is apparently crucial for RGS protein recognition. However, no contact between RGS4 and the NH_2-terminus of its $G\alpha_{i1}$ substrate was observed in a crystal structure of the RGS4-$G\alpha_{i1}$ complex (*30*). The NH_2-terminus did contact an adjacent RGS4 molecule, but this was judged to be an artifact of crystal packing and it is unlikely that relevant contact could take place even if the NH_2-terminus were freed of packing constraints when in solution. The Gα NH_2-terminus may regulate sensitivity to GAPs but not lie at the protein interface. Alternatively, because only the central portion of RGS4 was defined in the crystallographic structure of the complex (*30*), it is also possible that the Gα NH_2-terminus binds to the RGS protein outside of its central, conserved domain (*17*). Such an interaction is consistent with the idea that unconserved regions of RGS proteins are important for the specificity of their interactions with G proteins and is also consistent with our finding that palmitoylation inhibits most strongly when a GAP is assayed with a preferred Gα target.

Because inhibition of the action of RGS proteins by palmitoylation of their Gα substrates correlates roughly with GAP activity and can be virtually complete for a specific RGS-Gα pair, palmitoylation has the capac-

ity to totally inhibit the GAP activity of RGS proteins for their correct cellular targets. Thus, the palmitoylation-depalmitoylation cycle may control both the signal amplitude and the temporal response in G-protein pathways. Palmitoylation can amplify G protein–mediated signals or, alternatively, regulated depalmitoylation could serve as either an off-switch or signal attenuator. Such controls may be G protein–specific, and their complete elucidation awaits better understanding of the control of palmitate addition and removal. Regardless, any of these mechanisms would be compatible with the enhanced binding of palmitoylated Gα to Gβγ (*7*), which would serve to lower background signaling in the absence of stimulation. Regulation of GAP activity may be a major function of Gα palmitoylation.

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19. $G\alpha_z$ expressed in Sf9 cells is both myristoylated and palmitoylated (*2*), but palmitate is largely removed during purification. $G\alpha_z$ (4 μM) purified from Sf9 cells or myristoylated $G_{\alpha i1}$ from *E. coli* (*32*) was palmitoylated by incubation for 2 to 3 hours at 30°C with 50 μM Pal-CoA, either unlabeled or ^{3}H-labeled at 1000 cpm/pmol, in 50 mM NaHepes (pH 7.8), 2 mM EDTA, and 7.5 mM CHAPS detergent (*18*). [^{3}H]Palmitoylation was measured by liquid scintillation counting after precipitation with 10% trichloroacetic acid (*18*) or SDS–polyacrylamide gel electrophoresis (PAGE). The concentration of active $G\alpha_z$ was assayed according to the binding of 10 μM [^{35}S]GTP-γ-S (*15, 33*) except that the binding reaction mixture was incubated for 60 min in the presence of 25 mM $(NH_4)_2SO_4$, which increases the rate of nucleotide exchange on G-protein α subunits (*34*). Maximal observed binding of [^{35}S]GTP-γ-S was 65 to 70% of that predicted from assay of total protein.
20. Hydrolysis of $G\alpha_z$-bound [γ-^{32}P]GTP and G_z GAP activity were measured at 15°C as described (*15*). GAP activity is expressed either as the amount of bound GTP hydrolyzed above background at early times (quasi-linear time course) or as an apparent first-order rate constant (k_{app}). A unit of GAP activity is defined as an increment in k_{app} of 1 min^{-1} (*15*). Basal and stimulated hydrolysis of GTP bound to $G\alpha_{i1}$ was measured with a single-turnover assay (*15*) that included centrifugal gel filtration to remove unbound [γ-^{32}P]GTP and [^{32}P]Pi formed during the binding reaction.
21. To proteolyze $G\alpha_z$ near its NH_2-terminus, $G\alpha_z$ was first incubated with either 50 μM GTP-γ-S (30°C for 90 min) or 50 μM guanosine diphosphate (GDP), 30 μM $AlCl_3$, 10 mM NaF, and 10 mM $MgCl_2$ (15°C for 20 min) in 25 mM NaHepes (pH 7.5), 1 mM EDTA, 0.5 mM Mg^{2+}, 1 mM DTT, and 0.1% Triton X-100. The mixture was further incubated with trypsin (0.05 milligrams per milligram of $G\alpha_z$) at 30°C for 30 min. Phenylmethylsulfonylfluoride (1 mM) and tosyl-lysyl-chloromethylketone (0.2 mM) were added and digestion was checked by SDS-PAGE. Trypsin cleaved activated $G\alpha_z$ after Arg^{29}, according to automated Edman sequencing of the large tryptic fragment (*22*).
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Areal Segregation of Face-Processing Neurons in Prefrontal Cortex

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A central issue in cognitive neuroscience concerns the functional architecture of the prefrontal cortex and the degree to which it is organized by sensory domain. To examine this issue, multiple areas of the macaque monkey prefrontal cortex were mapped for selective responses to visual stimuli that are prototypical of the brain's object vision pathway—pictorial representations of faces. Prefrontal neurons not only selectively process information related to the identity of faces but, importantly, such neurons are localized to a remarkably restricted area. These findings suggest that the prefrontal cortex is functionally compartmentalized with respect to the nature of its inputs.

A major advance in understanding cortical organization has been the partitioning of large territories of cortex into regions on the basis of sensory modalities and submodalities (*1*, *2*). This is particularly striking in the visual system, where processes related to central and peripheral vision can be traced from the retina to the highest levels of visual association cortex in the inferior temporal cortex (IT) and the posterior parietal cortex. The situation is less clear for prefrontal cortex. Because of its status as the archetypal association cortex, the functional architecture of prefrontal cortex has theoretical implications for the issue of whether association cortex has a modular functional organization like that of the sensory regions or is instead relatively undifferentiated. Evidence from the study of lesions (*3*),

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