inorganic carbon in the deep ocean, this is where 90% of the cosmic ray–produced ^{14}C decays. In contrast, the atmosphere is one of the smallest reservoirs of radiocarbon and is thus very susceptible to changes in the fluxes between them. If all deep-water formation were shut off, then the ^{14}C production rate would remain unchanged but the accessible carbon reservoir would be much smaller. This would cause atmospheric $\Delta^{14}C$ to rise at a rate of ~2‰/year. Changes of this type have indeed been observed in the atmospheric record.

Because the atmosphere's $\Delta^{14}C$ value is the basis for all radiocarbon ages, the most recent literature is periodically summarized in a calibration paper in which radiocarbon ages are compared with true "calendar" ages (*4*). The bulk of the data comes from tree rings and layered sediments, and the curve is well constrained back to about 14,600 years ago. Spot measurements of uranium-rich shallow water corals extend the record back to about 24,000 years (*5, 6*), and a recent stalagmite record extends all the way back to the limit of ^{14}C dating at ~40,000 years (*7*). These records demonstrate variations in atmospheric $\Delta^{14}C$ with large amplitudes at many time scales.

The variations cannot all be explained by changes in the ^{14}C production rate and must therefore reflect changes in carbon fluxes and hence climate (*8*). It is these climate changes that give rise to variations in the surface radiocarbon reservoir ages measured by Siani *et al.* (*1*). Together with the recent work of Waelbroeck *et al.* (*9*), the results allows us to paint a consistent picture of the timing between changes in North Atlantic sea surface temperature and the air over Greenland.

Waelbroeck *et al.* estimated the reservoir age by assuming that North Atlantic sea surface temperatures and air temperatures over Greenland had to be in phase. Using the difference between the ice sheet model and their sediment radiocarbon ages, they calculated reservoir ages for their cores. Siani *et al.* measured the ice versus sea surface temperature phase independently by using charcoal dates to constrain the past atmosphere. The two papers agree that the Younger Dryas, a ~1500-year-long return to cold climate during the last deglaciation, saw a twofold increase of the high-latitude reservoir age of the North Atlantic (*10*) and that this signal did not make it as far south as the Straits of Gibraltar.

However, they disagree about Heinrich event 1 (H1), when a massive discharge of icebergs into the North Atlantic about 16,000 years ago caused the polar frontal zone, the region between cold high-latitude waters and warmer subtropical waters, to shift far to the south. Waelbroeck *et al.* (*9*) assume that their core at 38°N (SU-81-18) did not have a change in reservoir age during H1. But Siani *et al.* measure a twofold change in this value in the Mediterranean, several degrees farther to the south.

The studies can be reconciled if we allow the reservoir age in Waelbroeck's core to increase by about 400 years during H1. This increase leads to a better fit between the start of the sea surface temperature increase in the core and the Greenland air temperature increase at the end of H1. In Waelbroeck *et al.*'s analysis (*9*), the air temperature increase occurs at the midpoint of the sea surface temperature rise.

Why do these shifts in the radiocarbon age of surface waters occur? A first answer is that the polar front moves past the core site (see figure caption). But that just moves the questions to why the polar waters themselves are older. Variability in both sea ice cover and radiocarbon ages of intermediate waters (the explanation favored by Siani *et al.*) is the most likely explanation to this question. However, a change in the exchange rate across the low-latitude thermocline will also change the radiocarbon age and could have profound implications for the heat budget of the past ocean. Measuring the radiocarbon ages of intermediate waters of the past would be a very useful constraint to this problem.

References and Notes

1. G. Siani *et al.*, *Science* **294**, 1917 (2001).
2. On this scale, the preindustrial, prenuclear atmosphere is defined as 0‰, and any material that has lost all of its ^{14}C is −1000‰.
3. The thermocline separates the warm, well-mixed surface layer of the ocean from the cold, deep waters and is usually found at depths between 50 and 500 m.
4. M. Stuiver *et al.*, *Radiocarbon* **40**, 1041 (1998).
5. E. Bard *et al.*, *Radiocarbon* **35**, 191 (1993).
6. R. L. Edwards, *Science* **260**, 962 (1993).
7. W. J. Beck *et al.*, *Science* **292**, 2453 (2001).
8. T. Goslar *et al.*, *Nature* **377**, 414 (1995).
9. C. Waelbroeck *et al.*, *Nature* **412**, 724 (2001).
10. E. Bard *et al.*, *Earth Planet. Sci. Lett.* **126**, 275 (1994).

PERSPECTIVES: SIGNAL TRANSDUCTION

A New Thread in an Intricate Web

Mark von Zastrow and Keith Mostov

Most neurotransmitters, hormones, and growth factors activate cellular signaling pathways by binding to specific membrane receptors. These signaling pathways subsequently modulate biochemical networks comprising various cytoplasmic kinases, phosphatases, and guanosine triphosphate (GTP)–binding proteins. Information flow through these complex signaling networks requires a precise spatiotemporal organization of the appropriate signaling partners, many of which interact promiscuously when isolated from their native cellular environment. Thus, a critical aspect of cellular signaling is a matter of molecular choreography: getting the right proteins to the right place at the right time. It is not surprising, then, that membrane-trafficking pathways—which determine the structure and biochemical composition of the specialized membrane compartments in eukaryotic cells—have important effects on cellular signal transduction and, conversely, that signaling events can modulate membrane trafficking. Indeed, there appear to be many functional interactions between the otherwise distinct processes of signaling and membrane trafficking. This realization, which emerged over the last decade (*1*), has motivated a convergence between traditionally separate fields of cell biology. From this convergence emanates the question: How are signaling and membrane trafficking related at the molecular level? On page 1939 of this issue, Zheng *et al.* (*2*) describe a protein, RGS-PX1, that may be a new molecular thread in the intricate web that links signaling and membrane trafficking events.

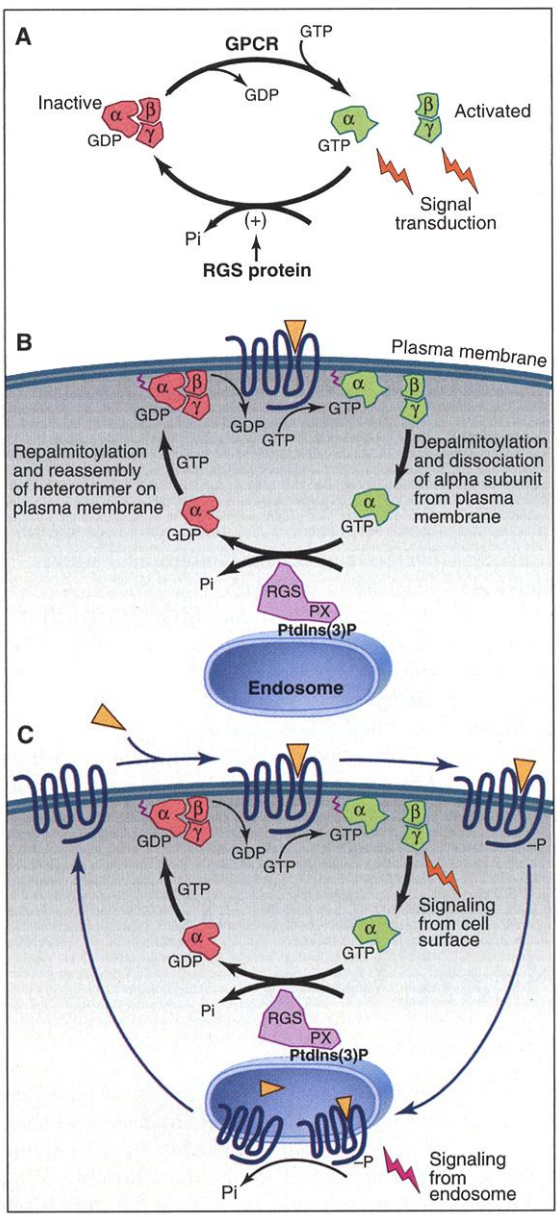
Zheng and colleagues mined sequence databases for candidate proteins containing RGS (regulators of G protein signaling) domains. RGS domains are conserved in diverse organisms and have profound effects on cellular signal transduction triggered by seven-transmembrane G protein–coupled receptors (GPCRs). GPCRs trigger signaling by prompting guanine nucleotide exchange on the α subunit of heterotrimeric G proteins. This results in conversion of the "inactive" guanosine diphosphate (GDP)–bound α subunit to the "activated" GTP-bound form. RGS proteins are crucial for accelerating the conversion of the activated G

The authors are in the Program in Cell Biology, Departments of Psychiatry, Cellular and Molecular Pharmacology, Anatomy, and Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA. E-mail: zastrow@itsa.ucsf.edu

protein back to its inactive GDP-bound form by potentiating an intrinsic GTPase activity present in the α subunit (*3*) (see the figure). All known RGS proteins regulate G_i- or G_q-type heterotrimeric G proteins, whereas no RGS proteins characterized so far act on G_s. This is important because G_s mediates a spectrum of effects on cellular signaling (such as stimulating various isoforms of adenylyl cyclase) that are different from the effects of G_i- and G_q-type heterotrimeric G proteins. Zheng *et al.* demonstrate that the RGS domain in RGS-PX1 selectively promotes GTP hydrolysis on purified G_s and modulates G_s activity in intact cells, thus establishing RGS-PX1 as a member of the RGS protein family that regulates signaling through this heterotrimeric G protein.

In addition to identifying the unique specificity of the RGS domain in RGS-PX1, Zheng *et al.* note sequences outside of the RGS domain that are homologous to another family of cytoplasmic proteins called sorting nexins. Sorting nexins contain PX domains, a conserved protein module that binds to phosphatidylinositol 3-phosphate. This phospholipid is highly concentrated in endosomal membranes, and the interaction of PX domains with phosphatidylinositol 3-phosphate modulates the association of sorting nexins with endosomes (*4*). Some sorting nexins interact directly with endocytosed receptors, such as receptor tyrosine kinases activated by epidermal growth factor (EGF) (*5*), but probably they also have more general (although poorly defined) effects on endosomal traffic. Perhaps the best understood sorting nexin is the yeast protein Vps5p, which, together with Vps17p, Vps26p, Vps29p, and Vps35p, forms the "retromer," a complex that retrieves proteins from the prevacuolar compartment (a type of endosome in yeast) and shuttles them back to the trans-Golgi network. Vps5p does not itself interact with specific membrane cargo, although other proteins in the retromer complex (such as Vps35p) do so (*6*).

Zheng *et al.* demonstrate that overexpression of RGS-PX1 in mammalian cells inhibits ligand-induced proteolysis of coexpressed EGF receptors. This finding suggests that RGS-PX1 inhibits the endocytic sorting of this receptor tyrosine kinase to lysosomes. Thus, RGS-PX1 may have an opposite effect to that of SNX1, a sorting nexin that enhances endocytic trafficking to lysosomes (*5*). Nevertheless, RGS-PX1 is associated with endosomal membranes in transfected cells, supporting the idea that this protein is a sorting nexin. Although RGS-PX1 has sequence homology with both sorting nexins (hence a previous designation as SNX13) and RGS domains (*4*), Zheng *et al.* make the critical observation that both domains in this protein are functional. They provide compelling evidence that these domains mediate, in intact cells, distinct functional effects on the signaling activity of a heterotrimeric G protein and the endocytic membrane trafficking of a receptor tyrosine kinase.



Cycles within cycles. (**A**) GPCRs promote binding of GTP to the α subunit of heterotrimeric G proteins. This leads to dissociation of the heterotrimer into the corresponding "activated" α subunit and βγ subcomplex. RGS proteins potentiate an intrinsic GTPase activity present in the α subunit that terminates the signaling activity of this subunit and promotes reassembly of the (inactive) heterotrimer. (**B**) The endosomal localization of RGS-PX1—which is dependent on binding of its PX domain to phosphatidylinositol 3-phosphate, PtdIns(3)P, in the endosome membrane—suggests that the biochemical cycle of G_s activation and inactivation may be linked to a physical cycle of α-subunit translocation between the plasma membrane and endosomes. The physical translocation of G_s is linked to a cycle of regulated depalmitoylation and repalmitoylation of the α subunit. (**C**) The proposed cycling of G_s α subunits between the plasma membrane and RGS-PX1–associated endosomes parallels a cycle of ligand-regulated endocytosis and recycling of many GPCRs. This suggests that endosomes may link the activities of the receptor and the G protein. One possible consequence of this linkage would be to physically organize distinct components of signal transduction triggered by activated GPCRs between a "peripheral" component (mediated by activated G_s) and a "deeper" component (dependent on protein kinases associated with phosphorylated receptors in endosome-associated signaling complexes). Alternatively, linkage could promote reassembly of receptor-G_s complexes in the plasma membrane after termination of signaling, thereby helping to restore responsiveness to a subsequent round of receptor activation.

The idea of linking signaling and trafficking activities in the same protein may be a general theme in cell biology. For example, β-arrestins (nonvisual arrestins), a family of cytoplasmic proteins that interact with a variety of GPCRs after ligand-induced activation, are involved in both signaling and membrane trafficking (*7*). First, β-arrestins attenuate signal transduction by preventing coupling of receptors to heterotrimeric G proteins within seconds to minutes after initial receptor activation, a process often called rapid desensitization. Second, β-arrestins regulate membrane trafficking by promoting endocytosis (also called sequestration) of receptors via clathrin-coated pits. Third, sometimes β-arrestins appear to participate in a distinct signaling pathway activated by receptors that have been endocytosed; components of this signaling pathway include endosome-associated protein tyrosine kinases or mitogen-activated protein kinase cascades. The diverse biochemical effects of the same β-arrestin can be understood in terms of its capacity for coordinating the complex functional itinerary of the same GPCR. The effects of RGS-PX1 are more difficult to rationalize because this protein modulates pathways that do not appear to be closely linked in function. The existence of such a linkage between apparently disparate signaling and trafficking events is

exciting because it challenges us to explore previously unanticipated possibilities.

A possible rationale for linking a G_s-specific RGS domain to a sorting nexin derives from the unusual biology of G_s. Inactive GDP-bound G_s is associated with the inner leaflet of the plasma membrane by a covalently attached palmitoyl moiety and by binding to membrane-anchored β and γ subunits. Upon activation by an appropriate GPCR, the GTP-bound α subunit of G_s dissociates from the βγ subcomplex; a fraction of the activated α subunit then becomes depalmitoylated and moves away from the plasma membrane and into the peripheral cytoplasm. In contrast, α subunits from other heterotrimeric G proteins contain a stably attached myristoyl modification and remain attached to membranes after activation (*8*). The association of a G_s-selective RGS protein with endosomal membranes could provide a way to limit the range (or duration) of signaling induced by liberated G_s α subunits to a cytoplasmic region delineated by RGS-PX1–associated endosomes (see the figure). By superimposing on this model of G protein cycling the itinerary of certain G_s-coupled GPCRs (such as the β_2 adrenergic receptor), which cycle through endosomes after ligand-induced activation (*9*), one can envisage that localizing RGS activity could physically separate distinct signals. Thus, signals initiated by receptors at the plasma membrane and mediated by activation of G_s could be separated from signals emanating from internalized receptors and mediated by endosome-associated kinases (*7*). It is also conceivable that localized inactivation of α subunits near endosomes could facilitate regeneration of G_s heterotrimers in the vicinity of recycling GPCRs. This would help in the reassembly of receptor-G_s complexes in the plasma membrane (or perhaps on endosomes that later recycle to the plasma membrane), thereby rendering the receptor-G_s signaling system competent to be retriggered by a subsequent round of ligand-induced activation at the cell surface (see the figure).

Of course, these and other hypotheses about RGS-PX1 remain to be tested, and many questions relating to the structure and function of this G_s-selective RGS protein remain to be addressed. The Zheng *et al.* study should stimulate efforts to define additional molecular threads in the complex web linking signaling and membrane-trafficking events, and to investigate the functional significance of the connections thus revealed.

References

1. M. Bomsel, K. Mostov, *Mol. Biol. Cell* **3**, 1317 (1992).
2. B. Zheng *et al.*, *Science* **294**, 1939 (2001).
3. D. M. Berman, A. G. Gilman, *J. Biol. Chem.* **273**, 1269 (1998).
4. Y. Xu *et al.*, *Nature Cell Biol.* **3**, 658 (2001).
5. R. C. Kurten, D. L. Cadena, G. N. Gill, *Science* **272**, 1008 (1996).
6. S. R. Pfeffer, *Curr. Biol.* **11**, R109 (2001).
7. W. E. Miller, R. J. Lefkowitz, *Curr. Opin. Cell Biol.* **13**, 139 (2001).
8. P. B. Wedegaertner, *Biol. Signals Recept.* **7**, 125 (1998).
9. P. I. Tsao, M. von Zastrow, *Pharmacol. Ther.* **89**, 139 (2001).

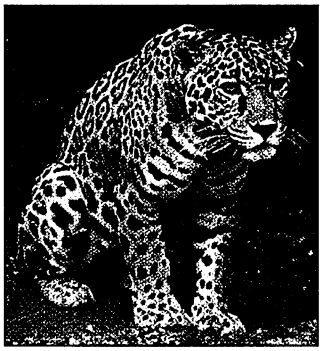
PERSPECTIVES: ECOLOGY

Dammed Experiments!

Jared Diamond

Fragmentation of large expanses of habitat into many smaller patches is a problem of both scientific and practical interest. From a scientific perspective, habitat fragmentation is the way in which much of the modern world was formed. At the end of the last Ice Age 13,000 years ago, melting glaciers raised sea levels, drowned low-lying land bridges, and carved off edges of continents into islands such as Britain and Japan. But fragmentation is not just a feature of the last Ice Age, it is also the fate befalling most natural habitats today—hence the practical interest. In either case, fragmentation causes loss of animal and plant populations by a process termed faunal relaxation. How fast does relaxation occur, and which species are most likely to survive?

A report by Terborgh, Rao, and their colleagues (*1*) on page 1923 of this issue describes a grand natural experiment in habitat fragmentation. In 1986, construction of a dam in Venezuela created the 4300-km^2 Lake Guri, flooding valleys and turning hundreds of former hilltops into islands ranging in area from less than 0.1 ha to 150 ha. Barely 4 years later, surveys of many groups of plants and animals began (*2*–*5*). The Lake Guri study reminds us of two other famous fragmentation studies: the surveys of Barro Colorado Island and other islands in Panama's Lake Gatun, created by damming in 1913 during construction of the Panama Canal (*6*); and the Minimum Area Project in the Brazilian Amazon near Manaus, involving forest tracts isolated from each other by forest clearance since around 1980 (*7*). These three studies complement each other. For instance, relaxation has been proceeding much longer in Lake Gatun (88 years) than in Lake Guri (15 years), but detailed faunal surveys began much sooner in Lake Guri than in Lake Gatun.



Many generalizations emerge about the biotas of Lake Guri islands of different sizes. The smallest islands (<1 ha) quickly lost 75% of their original species, and most species that persisted fell into just three ecological groups: canopy herbivores (howler monkeys, an iguana, and leaf-cutter ants); ground-dwelling insectivores (birds, lizards, frogs, and tarantulas); and seed consumers (small rodents and parrots). Medium-sized islands (4 to 12 ha) possessed in addition armadillos, agoutis, and phorid fly parasitoids of ants. Large islands (150 ha) retained most of their original species, including army ants, big frugivorous birds, anteaters, and big herbivorous and omnivorous mammals (deer, peccaries, tapir, and monkeys). But,

The author is in the Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90095, USA. E-mail: jdiamond@mednet.ucla.edu