baseline for a major component of the global climate system, Anderson *et al.* have taken a large step toward this goal.

References

1. J. T. Overpeck *et al.*, *Science* **278**, 1251 (1997).
2. M. E. Mann, R. S. Bradley, M. K. Hughes, *Geophys. Res. Let.* **26**, 759 (1999).
3. J. E. Cole, R. B. Dunbar, T. R. McClanahan, N. A. Muthiga, *Science* **287**, 617 (2000).
4. A. C. Clement, R. Seager, M. A. Cane, *Paleoceanography* **15**, 731 (2000).
5. J. W. Hurrell, *Science* **269**, 676 (1995).
6. P. D. Jones, T. J. Osborn, K. R. Briffa, *Science* **292**, 662 (2001).
7. D. M. Anderson, J. T. Overpeck, A. K. Gupta, *Science* **297**, 596 (2002).
8. W. L. Prell, D. Murray, S. C. Clemens, D. M. Anderson, in *Synthesis of Results from Scientific Drilling in the Indian Ocean*, Geophysical Monograph 70, R. A. Duncan *et al.*, Eds. (American Geophysical Union, Washington, DC, 1992), pp. 447–469.
9. M. A. Altabet, R. Francoise, D. M. Murray, W. L. Prell, *Nature* **373**, 506 (1995).
10. S. C. Clemens, D. W. Murray, W. L. Prell, *Science* **274**, 943 (1996).
11. J. A. Eddy, *Science* **192**, 1189 (1976).
12. F. Sirocko, D. Garbe-Schomberg, A. McIntyre, B. Molfino, *Science* **272**, 526 (1996).
13. K. B. Liu, Z. J. Yao, L. G. Thompson, *Geology* **26**, 135 (1998).
14. D. A. Hodell, J. H. Curtis, M. Brenner, *Nature* **375**, 391 (1995).
15. R. S. Bradley, P. D. Jones, Eds., *Climate Since A.D. 1500* (Routledge, New York, 1995).
16. C. S. Ramage, *Monsoon Meteorology*, International Geophysical Series (Academic Press, New York, 1971), vol. 15.

PERSPECTIVES: CELL BIOLOGY

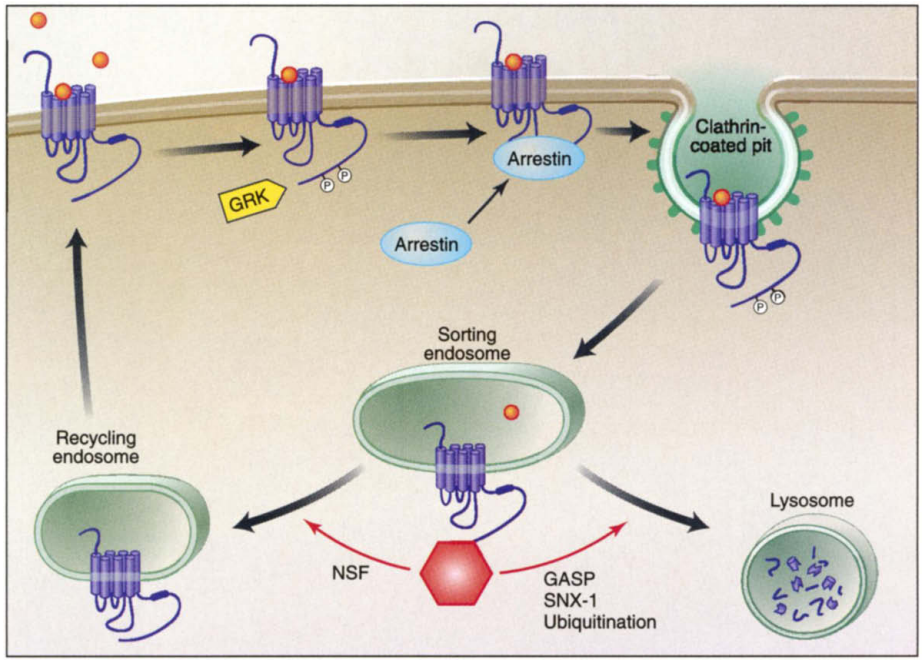
A Last GASP for GPCRs?

John A. Gray and Bryan L. Roth

One of the largest gene families in the human genome is that encoding the G protein–coupled receptors (GPCRs). These plasma membrane receptors, with their trademark seven-transmembrane helices, bind to and transduce signals for a huge variety of ligands including neurotransmitters, odorants, hormones, and other small molecules. GPCRs also mediate the actions of certain medications used to treat disorders as diverse as cardiovascular disease (*1*), drug dependency (*2*), and mental illness (*2*). Prolonged exposure of GPCRs to their endogenous (natural) or exogenous ligands (agonists) induces compensatory decrements in receptor sensitivity (desensitization) and receptor number (down-regulation). A prominent feature of the regulation of GPCR activity after ligand binding is the rapid internalization of these receptors and their sorting to intracellular endocytic compartments (*3*). Internalized GPCRs suffer one of two fates: Either they are rapidly recycled back to the plasma membrane (recycling pathway), or they are targeted to lysosomes for proteolysis (degradative pathway). Several recent studies, including a report on page 615 of this issue by Whistler *et al.* (*4*), identify GPCR-interacting proteins that specify the preferential sorting of GPCRs for either recycling or degradation (see the figure).

What structural motifs must interacting proteins recognize in order to determine the fate of internalized GPCRs? A number of recent studies have described sequences in the cytoplasmic domains of GPCRs, particularly in the carboxyl terminus, that are important for recognition by interacting proteins. For instance, swapping the carboxyl termini of protease-activated receptor–1 (PAR-1), a receptor that is targeted to lysosomes, and the substance P receptor, a GPCR that is recycled rapidly to the plasma membrane, results in a "swap" of the sorting pathways (*5*). Thus, PAR-1 with a substance P receptor carboxyl terminus is recycled to the plasma membrane, whereas the substance P GPCR with a PAR-1 carboxyl terminus undergoes degradation in lysosomes. Studies have also revealed the importance of amino acid residues at the distal carboxyl terminus of GPCRs for mediating receptor recycling, and have identified potential interacting proteins involved in this process. Most notably, interaction of the β_2 adrenergic receptor with NSF-1 (*N*-ethylmaleimide–sensitive factor)—a protein important for intracellular membrane trafficking and release of vesicles from the plasma membrane—regulates recycling of this GPCR (*6*).

New work, including that by Whistler *et al.* (*4*), reveals the identity of several interacting proteins that target GPCRs for lysosomal degradation. Whistler and colleagues have identified a protein they call GASP (GPCR-associated sorting protein) that turns out to be a key player in the lysosomal sorting of δ-opioid receptor (DOR) and probably of other GPCRs. They disclose that disrupting the interaction between GASP and DOR (a GPCR that is normally preferentially sorted to lysosomes) blocks lysosomal sorting and promotes recycling of internalized DORs to the cell surface. Importantly, GASP has a high affinity for the carboxyl terminus of GPCRs that are normally targeted to the degradative pathway, but a low affinity for GPCRs that prefer the recycling pathway. The authors also found that a dominant-negative form of GASP blocked the lysosomal targeting of DOR or of a mutant β_2 adrenergic receptor. Taken together, these findings identify nor-



Getting sorted. After activation by their ligands (orange), GPCRs (blue) become desensitized and are then internalized into endocytic compartments in the cell [see (*3*) for a review]. Within the endosomes, a sorting decision is made either to recycle the receptor to the plasma membrane (resensitization) or to transfer the receptor to lysosomes for degradation (down-regulation). New studies have identified interacting proteins (pink), such as GASP (*4*) and SNX-1 (*7*), that interact with the carboxyl terminus of GPCRs and contribute to this sorting decision. GRK, G protein–coupled receptor kinase.

CREDIT: KATHARINE SUTLIFF/*SCIENCE*

The authors are in the Department of Biochemistry, Case Western Reserve University Medical School, Cleveland, OH 44106–4936, USA. E-mail: roth@biocserver.bioc.cwru.edu

mal GASP as a protein that determines the degradative fate of some GPCRs.

Another recent study disclosed that a protein called sorting nexin–1 (SNX-1) is important for specifying the preferential targeting of PAR-1 to lysosomes (*7*). SNX-1 is a membrane-associated protein that is already known to promote lysosomal sorting and degradation of the epidermal growth factor receptor (*8*). Interestingly, Wang *et al.* (*7*) report that SNX-1 has a low affinity for GPCRs that prefer the recycling pathway. Thus, SNX-1 may be another candidate sorting protein involved in targeting GPCRs to the degradative pathway.

Some GPCRs can be modified by the covalent attachment of ubiquitin molecules (ubiquitination), which determines their sorting fate. Ubiquitination usually tags cellular proteins for degradation within intracellular organelles called proteasomes. However, recent work has demonstrated that ubiquitination of some GPCRs targets them for degradation in lysosomes instead of proteasomes (*9, 10*).

It appears, then, that the sorting fate of individual GPCRs is determined by a complex array of protein interactions that direct the receptors to opposing pathways (recycling versus degradation). Although recent studies have identified several interacting proteins, including GASP, SNX-1, and NSF-1, that target internalized GPCRs for recycling or degradation, there are probably plenty more interacting proteins and sorting motifs waiting to be discovered. Moreover, a number of important questions persist: Given that even recycled GPCRs are eventually degraded, how are these receptors ultimately targeted to the degradative pathway? How do GASP and SNX-1 interact with the sorting machinery to direct GPCRs to lysosomes? Are the mechanisms involving GASP and SNX-1 specific for some lysosomally sorted GPCRs, but not for others? How do the processes that regulate GPCR recycling and degradation modulate GPCR activity in vivo? The answer to this last question is likely to have tremendous implications for understanding the actions of drugs that target GPCRs and for designing new medications with fewer side effects and greater efficacy.

References

1. H. A. Rockman *et al.*, *Nature* **415**, 206 (2002).
2. B. L. Roth *et al.*, *Drug Alcohol Depend.* **51**, 73 (1998).
3. S. S. Ferguson, *Pharmacol. Rev.* **53**, 1 (2001).
4. J. L. Whistler *et al.*, *Science* **297**, 615 (2002).
5. J. Trejo, S. R. Coughlin, *J. Biol. Chem.* **274**, 2216 (1999).
6. M. Cong *et al.*, *J. Biol. Chem.* **276**, 45145 (2001).
7. Y. Wang *et al.*, *Mol. Biol. Cell* **13**, 1965 (2002).
8. R. C. Kurten *et al.*, *Science* **272**, 1008 (1996).
9. A. Marchese, J. L. Benovic, *J. Biol. Chem.* **276**, 45509 (2001).
10. S. K. Shenoy *et al.*, *Science* **294**, 1307 (2001).

Formins Set the Record Straight

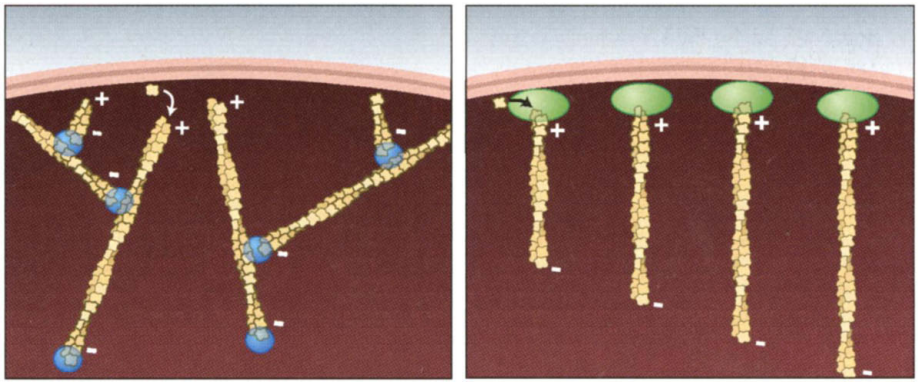
Fred Chang and Matthias Peter

Cell polarity, cell migration, cytokinesis, vesicle transport, and the formation of membrane protrusions are just some of the cellular processes that depend on actin filaments. Actin filaments are assembled by polymerization of monomers, and can be either branched or straight. For example, at the leading edge of motile cells the entire network of actin filaments is branched, whereas in microvilli, stereocilia, stress fibers, and contractile rings, actin is organized into bundles of linear filaments. Yeast contain linear structures called actin cables that enable directional transport of vesicles. A protein complex called Arp2/3 is the molecular machine that nucleates and drives actin monomers to polymerize into branched filaments (*1*). Now, two recent studies, including one on page 612 of this issue (*2*), identify the formins as a new class of actin nucleator that directs assembly of straight filaments (*2, 3*).

Nucleation of actin (the formation of a short filament by two or three actin monomers) is the critical first step in actin filament assembly. Actin monomers are poor initiators of new filament assembly, and thus actin nucleation is the rate-limiting step. Actin filaments have structurally distinct ends: the barbed end and the pointed end (see the figure). The barbed end is the faster growing end where most of new filament assembly takes place.

Much recent research has centered on the Arp2/3 complex, composed of at least seven proteins, which binds to the pointed ends and to the sides of existing actin filaments. Arp2/3 prefers to nucleate new filaments at a 70° angle to existing filaments, resulting in formation of a branched network (see the figure). In vivo, the Arp2/3 complex is required for cellular processes that use branched actin filaments, for example, the extension of membrane protrusions in animal cells, and the intracellular motility of the bacterium *Listeria monocytogenes*. Because accessory proteins such as tropomyosin promote Arp2/3 to form actin filaments with fewer branches (*4*), many researchers have presumed that Arp2/3 is the primary and perhaps sole actin nucleator in the cell.

Recent studies, however, suggest that there is another actin nucleator at work, one that may specifically direct formation of straight actin filaments. Formins are large multidomain proteins that are required for cytokinesis and maintenance of cell polarity (*5*). These cytoskeleton-organizing proteins direct assembly of actin structures, such as the contractile ring, actin cables, and stress fibers, and also regulate microtubule stability in eukaryotic cells. Formins become activated when they bind to Rho guanosine triphosphatases, such as Cdc42, and are known transducers of the Rho signaling



CREDIT: KATHARINE SUTLIFF/*SCIENCE*

Straight arrows and branched networks. The Arp2/3 complex and formins organize different actin structures. **(Left)** The Arp2/3 complex (blue) tends to nucleate new actin filaments at the sides of existing actin filaments, resulting in a branching filament network. **(Right)** In contrast, formins (green) nucleate the assembly of straight filaments. The Arp2/3 complex binds to the minus (pointed) end of the actin filament, whereas formins bind to the growing plus (barbed) end of the actin filament.

F. Chang is in the Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA. E-mail: fc99@columbia.edu M. Peter is at the Swiss Institute for Experimental Cancer Research (ISREC), 1066 Epalinges/VD, Switzerland, and the Institute of Biochemistry, CH-8032 Zurich, Switzerland.