smaller branching pocilloporid corals, which preceded elkhorn and staghorn corals in the same environments, persisted alongside the latter for nearly 2 million years before finally going extinct about 120 thousand years ago (*19*). Thus, modern Caribbean reef communities did not develop until at least 1 million years after the origin of the crucial species involved.

Why do such profound delays occur in the origin of novel communities? One possibility is that gradual evolution due to some process such as "neighborhood selection" slowly facilitates positive interactions among coexisting species that alter community structure (*20*). Alternatively, dominance by a few species that share a particular suite of characteristics may emerge as an epiphenomenon of local threshold effects (*21*) and regional metapopulation dynamics (*22*) that "lock" species associations into a limited number of states, once abundances somehow rise above certain critical levels. For example, staghorn and elkhorn corals grow up to 10 times faster than other Caribbean corals (*8*), which may have greatly increased their success relative to other branching species when glacial cycles and sea-level fluctuations intensified substantially about 1.0 to 1.4 million years ago (*23*). In the latter case, the success of these newly dominant corals is an accidental side effect of characters selected for other reasons rather than an adaptation to their present circumstances. The apparently punctuated evolution of most marine species (*24*) argues for the latter interpretation, except that life history traits and behaviors rarely fossilize, so it may be impossible to tell.

Paleoecologists need to pay more attention to the relative abundance of species if we are to resolve the issue of how much community structure is more than just the sum of the component species parts. But whatever the outcome, paleontology continues to contribute fundamentally to ecological and evolutionary theory, be it through the discovery of punctuated evolution of species or synchronous turnover of entire biotas, or the demonstration of the broadly open structure of marine communities. Paleontology still provides the only empirical test of the history of life and models of global change.

### References and Notes


1. C. Elton, *The Ecology of Animals* (Sidgwick & Jackson, London, 1927; reprinted by Science Paperbacks, Metheun, London, 1966).
2. H. Gleason, *Bull. Torrey Bot. Club* **53**, 1 (1926).
3. P. M. Kareiva, J. G. Kingsolver, R. B. Huey, *Biotic Interactions and Global Climate Change* (Sinauer, Sunderland, MA, 1993).
4. J. Roughgarden, in *Perspectives in Ecological Theory*, J. Roughgarden, R. M. May, S. A. Levin, Eds. (Princeton Univ. Press, Princeton, NJ, 1989).
5. M. A. Buzas and S. J. Culver, *Science* **264**, 1439 (1994).
6. J. H. Lawton, in *Ecological Communities: Conceptual Issues and the Evidence*, D. R. Strong, D. Simberloff, L. G. Abele, A. B. Thistle, Eds. (Princeton Univ. Press, Princeton, NJ, 1984); D. R. Strong, J. H. Lawton, R. Southwood, *Insects on Plants* (Harvard Univ. Press, Cambridge, MA, 1984).
7. J. B. C. Jackson, in *Biology and Systematics of Colonial Organisms*, G. P. Larwood and B. R. Rosen, Eds. (Academic Press, London, 1979); G. H. Orians and R. T. Paine, in *Coevolution*, D. J. Futuyma and M. Slatkin, Eds. (Sinauer, Sunderland, MA, 1983); J. H. Connell and M. J. Keough, in *The Ecology of Natural Disturbance and Patch Dynamics*, S. T. A. Pickett and P. S. White, Eds. (Academic Press, Orlando, FL, 1985).
8. N. Knowlton, J. C. Lang, B. D. Keller, *Smithson. Contrib. Mar. Sci.* **31**, 1 (1990); J. B. C. Jackson, *BioScience* **41**, 475 (1991); C. S. Rogers, *Coral Reefs* **12**, 127 (1993).
9. R. T. Paine and S. A. Levin, *Ecol. Monogr.* **51**, 145 (1981).
10. J. L. Wulff and L. W. Buss, *Nature* **281**, 474 (1979); J. B. C. Jackson, in *Population Biology and Evolution of Clonal Organisms*, J. B. C. Jackson, L. W. Buss, R. E. Cook, Eds. (Yale Univ. Press, New Haven, CT, 1985).
11. J. W. Valentine and D. Jablonski, in *Species Diversity in Ecological Communities*, R. E. Ricklefs and D. Schluter, Eds. (Univ. of Chicago Press, Chicago, IL, 1993).
12. J. T. Carlton, *Bull. Mar. Sci.* **41**, 452 (1987); J. T. Carlton and J. B. Geller, *Science* **261**, 78 (1993).
13. J. T. Carlton, *Am. Zool.* **33**, 499 (1993).
14. J. B. C. Jackson, *ibid.* **32**, 719 (1992).
15. T. Webb III and P. J. Bartlein, *Annu. Rev. Ecol. Syst.* **23**, 141 (1992).
16. J. D. Taylor, *Palaeontology* **21**, 1 (1978); G. Paulay, *Paleobiology* **16**, 415 (1990).
17. T. J. Crowley and G. R. North, *Science* **240**, 996 (1988); J. P. Kennett and L. D. Stott, *Nature* **353**, 225 (1991); D. R. Prothero and W. A. Berggren, Eds., *Eocene-Oligocene Climatic and Biotic Evolution* (Princeton Univ. Press, Princeton, NJ, 1992).
18. S. M. Stanley, *Palaios* **1**, 17 (1986); J. B. C. Jackson, *Philos. Trans. R. Soc. London Ser. B*, in press; S. M. Stanley and L. D. Campbell, *Nature* **293**, 457 (1981).
19. A. F. Budd, T. A. Stemann, K. G. Johnson, *J. Paleontol.*, in press; A. F. Budd and C. M. Kievman, *Final Draft Report: Bahamas Drilling Project* (Rosentiel School Marine and Atmospheric Science, University of Miami, Miami, FL, 1994).
20. D. S. Wilson, *The Natural Selection of Populations and Communities* (Benjamin/Cummings, Menlo Park, CA, 1980); E. G. Leigh Jr., *Trends Ecol. Evol.* **6**, 257 (1991).
21. N. Knowlton, *Am. Zool.* **32**, 674 (1992).
22. S. Nee and R. M. May, *J. Anim. Ecol.* **51**, 37 (1992).
23. N. J. Shackleton *et al.*, *Nature* **307**, 620 (1984); E. Jansen and J. Sjoholm, *ibid.* **349**, 600 (1991).
24. S. J. Gould and N. Eldredge, *ibid.* **366**, 223 (1993).


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# A Biochemical Function for Ras—At Last


Alan Hall


Ras, a guanosine triphosphatase (GTPase), is a molecular switch for signal transduction pathways that control growth and differentiation. Its critical importance in growth control was known since the early 1980s when activated *ras* oncogenes were identified in certain human cancers (*1*). More recently, elegant genetic experiments in yeast, *Caenorhabditis elegans*, and *Drosophila* have established a universal function for Ras in controlling a cell's decision to grow or to differentiate (*2*). Tremendous effort has gone into characterizing the mechanism of action of this critical molecule. Like all GTP-binding proteins, Ras cycles between an inactive [guanosine diphosphate (GDP)–bound] and an active (GTP-bound) conformation, and a wide variety of extracellular signals can stimulate the formation of active Ras:GTP (*3*). The downstream function of Ras is to regulate a protein kinase cascade (*4*); two reports this week, one in *Science* from Hancock's group and another in *Nature* from Marshall's group, have finally pinned down exactly how Ras does this (*5*, *6*).


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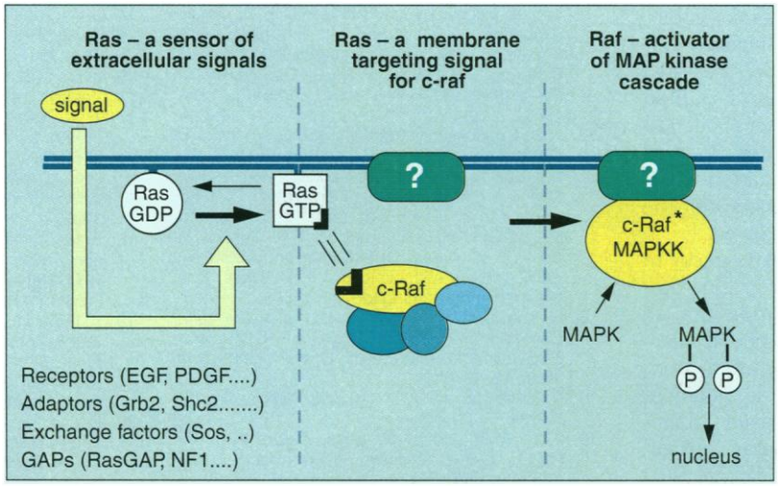
In all eukaryotic cells so far examined (except *Saccharomyces cerevisiae*), Ras controls a mitogen activated protein (MAP) kinase cascade (*4*). After many false candidates came and went, it was eventually realized that stimulation of Ras invariably leads to an increase in the activity of two cytoplasmic serine-threonine MAP kinases, Erk-1 and Erk-2, which subsequently translocate to the nucleus where they phosphorylate key transcription factors such as elk (*7*). Unraveling the sequence of events that connects Erk-1 and -2 to Ras has, up to a point, been a relatively straightforward problem in protein biochemistry. MAP kinase activity depends on concomitant phosphorylation of a threonine and a tyrosine residue by a dual specificity kinase, MAP kinase kinase (MAPKK). MAPKK is itself activated by phosphorylation, and a number of MAPKK kinase activities have been detected in cell extracts.

One protein that clearly functions as a MAPKK kinase is Raf (*8*). This serine-threonine kinase was first characterized by Ulf Rapp's lab as the product of the *v-raf* retroviral oncogene, and in 1986 some elegant microinjection experiments by Stacey's group showed that transformation of cells by v-Raf is independent of Ras (*9*).

Stacey speculated then that Raf might be downstream of Ras in a signal transduction pathway. It turns out he was right.

Closer inspection of v-Raf reveals that it is derived from the *c-raf* proto-oncogene by deletion of amino-terminal sequences (*10*). It is likely, therefore, that the activity of the c-Raf kinase domain (located at the carboxyl terminus) is regulated by sequences in the amino-terminal domain. Last summer, it seemed that five groups had simultaneously found the final piece of the puzzle when they reported that Ras physically interacts with the amino-terminal domain of c-Raf in a GTP-dependent manner (*11*). The excitement was slightly tempered when attempts to activate recombinant c-Raf with Ras in vitro failed—something critical was still missing from the pathway. We still do not know what that something is, but the papers from the Hancock and Marshall labs have at least now identified how Ras interacts with Raf in vivo (*5*, *6*).




**Ras is a membrane targeting signal for c-Raf.** Extracellular signals activate Ras, and in this GTP-bound form, the effector region of Ras interacts with the amino-terminal regulatory domain of c-Raf, localizing this MAPKK kinase to the plasma membrane. c-Raf then dissociates from Ras and stays at the membrane in a detergent-insoluble complex that may be associated with the cytoskeleton and includes chaperones and perhaps MAPKK.

Ras must be at the plasma membrane to function, and a series of posttranslational events, first clarified in detail by Hancock when he was a student in Marshall's lab, are required to target Ras to the membrane (*12*). For Ki-ras, a carboxyl-terminal CAAX box (a signal for farnesylation) and a neighboring polybasic domain of six lysine residues are necessary and sufficient for plasma membrane targeting. In the new work, the Hancock and Marshall groups have now added this targeting signal to the carboxyl terminus of c-Raf and as expected, when expressed in cells, the chimeric protein RafCAAX localizes exclusively to the plasma membrane. Both groups show that membrane-localized RafCAAX kinase is constitutively active, but most important of all, its activity is now completely independent of Ras. The simplest interpretation of this result is clear-cut: The only role of Ras:GTP in the MAP kinase cascade is to localize c-Raf to the plasma membrane. The Ras GTPase is a plasma membrane targeting signal for c-Raf.

This result comes as a surprise; it has been generally assumed that Ras, in combination with some other signal, acts as an allosteric regulator of the c-Raf kinase. This is not the case—once Ras has done its job in localizing c-Raf to the plasma membrane, it is no longer required.

Why is targeting c-Raf to the membrane controlled by a GTPase rather than a kinase? Kinases are effective on-off switches, but a GTPase such as Ras may be a more sensitive sensor of the extracellular milieu—effectively functioning as a rheostat. Indeed, in PC12 cells activation of Ras by epidermal growth factor causes proliferation, but activation of Ras by nerve growth factor induces differentiation (*13*). *Quantitative* differences in Ras-mediated MAP kinase activation are thought to be responsible for these different decisions made by the cell. Such effects are likely to be particularly important during development, where a gradient of an extracellular factor can induce different responses in apparently identical cells. A GTPase switch could provide a way to fine tune signal transduction pathways.

So is everything cut-and-dried? As far as Ras and Raf are concerned, it seems so. There are two questions still to be answered, however, before Ras enters the biochemistry texts as a fait accompli. The first is whether this is the only function of Ras. v-Raf, like activated Ras, will transform NIH 3T3 fibroblasts to a fully malignant phenotype and, on the face of it, there appear to be no biological differences between the two phenotypes. I suspect that this could be because no one has looked carefully enough: Do activated versions of Ras and Raf induce similar changes in cell morphology and motility, in addition to both providing a deregulated proliferative signal? Additional roles for Ras are also suggested by the existence of other proteins that interact with Ras (*14*), and there is still the unresolved question of whether the two Ras GTPase activating proteins, RasGAP and neurofibromin, have an effector function (*3*).

The other unresolved problem is how the c-Raf kinase is activated after translocation to the membrane; an observation in the Hancock paper may be relevant. These authors find that neither RafCAAX nor c-Raf that has been induced to translocate to the plasma membrane by activated Ras can be solubilized with the detergent 1% NP-40. This result indicates that, after moving to the membrane, c-Raf (but not Ras) becomes tightly associated with the cytoskeleton. Both cytosolic and membrane-bound c-Raf are found in a large (300- to 500-kilodalton) complex that includes two chaperones, hsp90 and p50, and possibly MAPKK (*15*). I am sure there will now be a concerted effort to identify proteins that interact with c-Raf at the plasma membrane, and this may provide the final piece in the biochemical puzzle that links growth factors to the MAP kinase cascade.

### References and Notes


1. M. Barbacid, *Annu. Rev. Biochem.* **56**, 779 (1987).
2. B. Dickson and E. Hafen, *Curr. Opin. Genet. Dev.* **4**, 64 (1994).
3. M. S. Boguski and F. McCormick, *Nature* **366**, 643 (1993); F. McCormick, *Curr. Opin. Genet. Dev.* **4**. 71 (1994).
4. E. Nishida and Y. Gotoh, *Trends Biochem. Sci.* **18**, 128 (1993); C. J. Marshall, *Curr. Opin. Genet. Dev.* **4**, 82 (1994).
5. D. Stokoe, S. G. Macdonald, K. Cadwallader, M. Symons, J. F. Hancock, *Science* **264**, 1463 (1994).
6. S. J. Leevers, H. F. Paterson, C. J. Marshall, *Nature* **369**, 411 (1994).
7. R. Treisman, *Curr. Opin. Genet. Dev.* **4**, 96 (1994).
8. J. Kyriakis *et al.*, *Nature* **358**, 417 (1992); L. R. Howe *et al.*, *Cell* **71**, 335 (1992); P. Dent *et al.*, *Science* **257**, 1404 (1992).
9. M. Smith, S. DeGudicibus, D. W. Stacey, *Nature* **320**, 540 (1986).
10. T. I. Bonner *et al.*, *Mol. Cell. Biol.* **5**, 1400 (1985); V. P. Stanton and G. M. Cooper, *ibid.* **7**, 1171 (1987).
11. S. A. Moodie, B. M. Willumsen, M. J. Weber, A. Wolfman, *Science* **260**, 1658 (1993); L. Van Aelst, M. Barr, S. Marcus, P. Polverino, M. Wigler, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6213 (1993); A. B. Vojtek, S. M. Gollenberg, J. A. Cooper, *Cell* **74**, 205 (1993); X. F. Zhang *et al.*, *Nature* **364**, 308 (1993); P. H. Warne, P. R. Viciana, J. Downward, *ibid.*, p. 352.
12. J. F. Hancock, A. I. Magee, J. E. Childs, C. J. Marshall, *Cell* **57**, 1167 (1989); J. F. Hancock, H. Paterson, C. J. Marshall, *ibid.* **63**, 133 (1990).
13. Q. Meng-Sheng and S. H. Green, *Neuron* **7**, 937 (1991).
14. A. B. Vojtek, S. M. Gollenberg, J. A. Cooper, *Cell* **74**, 205 (1993).
15. M. Wartmann and R. J. Davis, *J. Biol. Chem.* **269**, 6695 (1994).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