and after eliminating these, they also claim a null result with similar sensitivity. Meanwhile Lyons *et al.* (*8*) have further refined their apparatus and found yet other techniques to eliminate spurious errors. They continue to find, as originally reported, a signal that is zero on average but fluctuates from point to point on the sample. They interpret this as being the result of domains whose T signal fluctuates in sign.

There are possible practical loopholes in Wen and Zee's argument associated with the difficulty of actually computing for optical photon energies the [probably very small (9)] magnitude of T-symmetry breaking signal which presumably involves very low energy scales (on the order of $T_c \sim 10^{-2}$ eV). There is also the possibility of partial or complete cancellation of the effect in adjacent planes owing to a kind of antiferromagnetic ordering (*10*). Although the experimental situation remains somewhat controversial, the simplest interpretation at present is that sensitive optical and other (*11*, *12*) measurements have failed to unambiguously detect signatures of anyons. It is now quite clear from theoretical work that anyons superconduct, but it is by no means clear from experiment that cuprates are superconductors because of anyons.

REFERENCES AND NOTES

1. R. B. Laughlin, *Science* **242**, 525 (1988).
2. J. M. Leinaas and J. Myrheim, *Nuovo Cimento* **37B**, 1 (1977); F. Wilczek, *Sci. Am.* **264**, 24 (May 1991); G. S. Canright and S. M. Girvin, *Science* **247**, 1197 (1990).
3. X. G. Wen and A. Zee, *Phys. Rev. Lett.* **62**, 2873 (1989).
4. K. B. Lyons *et al.*, *ibid.* **64**, 2949 (1990).
5. H. J. Weber *et al.*, *Solid State Commun.* **76**, 511 (1990); H. J. Weber, *Int. J. Mod. Phys. B* 5, 1539 (1991).
6. S. Spielman *et al.*, *Phys. Rev. Lett.* **65**, 123 (1990); *ibid.* **68**, 3472 (1992).
7. T. W. Lawrence, A. Szöke, R. B. Laughlin, unpublished results.
8. K. B. Lyons *et al.*, unpublished results.
9. S. Yip and J. A. Sauls, *Bull. Am. Phys. Soc.* **37**, 171 (1992).
10. A. G. Rojo and G. S. Canright, *Phys. Rev. Lett.* **66**, 949 (1991); *ibid.* **68**, 1601 (1992); I. E. Dyzaloshinski, *Phys. Lett. A* 155, 62 (1991).
11. R. F. Kiefl *et al.*, *Phys. Rev. Lett.* **64**, 2082 (1990); N. Nishida and H. Miyatake, *Hyperfine Interactions* **63**, 183 (1991); R. F. Kiefl *et al.*, *ibid.*, p. 139.
12. P. L. Gammel, P. A. Polakos, C. E. Rice, L. R. Harriott, D. J. Bishop, *Phys. Rev. B* **41**, 2593 (1990).

MAP Kinases: Charting the Regulatory Pathways

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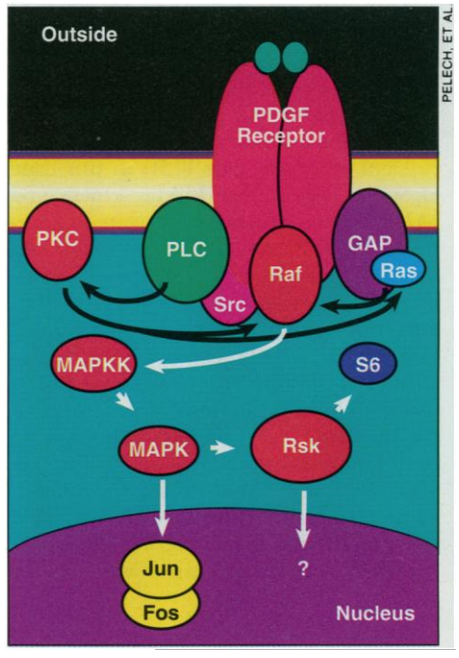
The intracellular transmission of growth factor signals is presumed to be mediated by sequentially activated protein kinases integrated into an elaborate network (*1*). Despite the identification of hundreds of distinct protein kinases, few interconnections between these kinases have been firmly established. Now at last, one of the major mitogenic signaling routes is finally coming into focus. The emerging picture is far more complex than what might have been anticipated even a few years ago (see figure).

It has long been suspected that protein kinase cascades emanating from growth factor receptors are responsible for the phosphorylation of such targets as ribosomal protein S6. Many extracellular mitogens, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and nerve growth factor (NGF), induce autophosphorylation of their respective receptors on tyrosine residues by activation of intrinsic catalytic kinase domains. This is ultimately translated into phosphorylation of serine and threonine residues in proteins throughout the cell. To chart the major routes for mitogenic signaling, the S6 protein has been employed as a convenient starting point for the elucidation of upstream regulatory events. Diverse stimuli can activate S6 protein kinases, in particular the 70-kD S6 kinase and a 90-kD kinase encoded by the *rsk* gene family (*1*). These S6 kinases are, in turn, activated by phosphorylation on serine and threonine residues. Multisite phosphorylation of $p90^{rsk}$ by the mitogen-activated protein (MAP) kinases, or extracellular signal–regulated protein kinases (ERKs) as they are occasionally called, is sufficient to markedly stimulate its S6 phosphotransferase activity (*2*). MAP kinases are probably the physiological activators of $p90^{rsk}$, since both are activated simultaneously in response to the same stimuli (*1*).

The MAP kinase family is composed of 40- to 46-kD isoforms that will phosphorylate myelin basic protein in vitro on a threonine residue (*3*). At least two of these isozymes, $p42^{mapk}$ and $p44^{erk1}$, require both threonine and tyrosine phosphorylation for maximal activation (*4*). The regulatory phosphoacceptor sites in murine $p42^{mapk}$ have been identified as Thr^{183} and Tyr^{185} (*5*), and these residues are conserved in $p44^{erk1}$. Both $p42^{mapk}$ and $p44^{erk1}$ can undergo autophosphorylation on tyrosine and become slightly activated (*6*). However, the kinetics of autophosphorylation and autoactivation are much too slow to account for the stimulation of these MAP kinases in vivo.

Rapid stoichiometric threonine and tyrosine phosphorylation of $p42^{mapk}$ and $p44^{erk1}$ and stimulation of their ability to phosphorylate myelin basic protein can be achieved by at least two "activating factors." These were first detected in cytosolic extracts from EGF-stimulated Swiss mouse 3T3 cells and subsequently in NGF-treated rat PC12 cells and maturing *Xenopus* oocytes (*7*). There is an excellent correlation between the stimulation of these activators and increases in activity of MAP kinases in these diverse systems. These activators do not simply accelerate MAP kinase autophosphorylation, but are bona fide MAP kinase (MAPK) kinases, since they induce the tyrosine and threonine phosphorylation of mutant $p42^{mapk}$ in which autophosphorylation has been almost completely eliminated by mutagenesis (*8*, *9*).

Two human A431 cell MAPK kinases (*10*), their murine and rabbit cognates (*11*), and the *Xenopus* MAPK kinase (*7*)



Representation of mitogenic signal transduction from the PDGF receptor.

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appear to correspond to ~45-kD proteins by SDS–polyacrylamide gel analysis. Antiserum developed to the *Xenopus* MAPK kinase cross-reacts with a 45-kD MAPK kinase that is activated in rat 3Y1 cells treated with EGF, PDGF, insulin, and phorbol ester tumor promoters and in PC12 cells exposed to NGF (*12*). Protein microsequencing analyses of the 45-kD protein in mammalian (*11*) and *Xenopus* (*12*) preparations of MAPK kinase have confirmed its identity as a protein kinase and revealed strong homology to the yeast protein kinases encoded by the *Schizosaccharomyces pombe byr1* and *Saccharomyces cerevisiae ste7* genes (*13*). These findings are consistent with genetic studies of *S. cerevisiae* mating, in which $p55^{ste7}$ acts as an intermediary in the α pheromone–induced activation of the kinase $p40^{fus3}$, a MAP kinase homolog (*13*). Many other yeast kinases are highly related to $p38^{byr1}$ and $p55^{ste7}$, such as those encoded by the *pbs2*, *wis1*, *ste11*, *byr2*, and *slk* genes (*14*). This raises the tantalizing prospect that an extended family of kinases may impinge upon MAP kinases and closely related protein kinases.

The identification and purification of the 45-kD MAPK kinases have facilitated studies of their direct regulation. Initial hopes that they might be tyrosine phosphorylated and activated by receptor tyrosine kinases have been dashed by the resistance of MAPK kinases to inactivation by tyrosine-specific protein phosphatases (*7*). However, the sensitivity of MAPK kinases to inhibition by the serine-threonine–specific protein phosphatase 2A implicates at least one other intermediary protein kinase that acts proximal to receptor tyrosine kinases (*7, 10*). The 74-kD serine-threonine kinase encoded by the proto-oncogene *raf1* has now emerged as a very promising candidate for such a MAPK kinase kinase.

Dent and colleagues (*15*), in this issue of *Science*, show that MAPK kinase and $p42^{mapk}$ are constitutively activated in murine NIH 3T3 cells transformed with oncogenic forms of truncated $p74^{raf1}$. Furthermore, viral *raf1* protein that has been immunoprecipitated from transformed cells or bacterially expressed as a glutathione fusion protein catalyzes the activation of MAPK kinase from skeletal muscle in vitro. Very similar findings have been reported by Kyriakis and colleagues (*16*), who also demonstrated that immunoprecipitates of constitutively activated *raf1* protein can reactivate MAPK kinase that had been previously inactivated by phosphatase 2A. Evidence for direct phosphorylation of MAPK kinase by the *raf1* protein is limited in both studies, but Kyriakis and co-workers (*16*) have observed that the *raf1* protein phosphorylates an appropriately sized 50-kD polypeptide on both serine and threonine residues in their preparations of MAPK kinase.

The direct activator of $p74^{raf1}$ is still elusive. Although MAP kinase phosphorylates $p74^{raf1}$ in vitro at a site that is modified in vivo in response to insulin, this phosphorylation is insufficient for $p74^{raf1}$ activation (*17*). $p74^{raf1}$ is also subject to phosphorylation at two additional sites that are not phosphorylated by MAP kinases but could be required for $p74^{raf1}$ activation (*16*). Like protein kinase C, $p74^{raf1}$ features a zinc finger motif in its amino-terminal regulatory domain that could serve as a binding site for a regulatory molecule (*18*).

There is strong evidence for the placement of the guanine nucleotide–binding protein $p21^{ras}$ upstream of $p74^{raf1}$ in a growth factor activation pathway (*19–22*). $p21^{ras}$ mediates signaling from several inputs including the phorbol-ester receptor protein kinase C, the tyrosine kinase $p60^{src}$, and the guanosine triphosphatase– activating protein (GAP) that inactivates $p21^{ras}$ function (*23, 24*). Other lines of evidence imply protein kinase C may indirectly stimulate MAP kinase independently of $p21^{ras}$ (*20, 25*).

The very strong recruitment of MAP kinases by a powerful mitogen such as PDGF may reflect the multiple signaling systems that are elicited upon engagement of PDGF with its receptor. Although there is evidence for physical association of activated $p60^{src}$ and $p74^{raf1}$ with the ligand-occupied PDGF receptor, other signal-transducing proteins that interact with the autophosphorylated receptor via SH2 domains contribute to MAP kinase activation (*26*). These receptor-associated proteins, which are also tyrosine-phosphorylated by the PDGF receptor, include phospholipase Cγ, GAP, and phosphatidyl inositol (PI) 3-kinase. Although the function of PI 3-kinase has yet to be elucidated, phospholipase Cγ and GAP may facilitate MAP kinase activation: phospholipase Cγ by its production of second messengers for the mobilization of protein kinase C and GAP through its action on $p21^{ras}$. It should not be too long before the remaining intermediary components that mediate protein kinase C, $p60^{src}$, and $p21^{ras}$ activation of $p74^{raf1}$ also come to light.

REFERENCES AND NOTES

1. S. L. Pelech, J. S. Sanghera, M. Daya-Makin, *Biochem. Cell Biol.* **68**, 1297 (1990).
2. T. W. Sturgill, L. B. Ray, E. Erikson, J. L. Maller, *Nature* **334**, 715 (1988); J. Chung, S. L. Pelech, J. Blenis, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4981 (1991).
3. M. H. Cobb, T. G. Boulton, D. J. Robbins, *Cell Regul.* **2**, 965 (1991), S. L. Pelech and J. S. Sanghera, *Trends Biochem. Sci.* 17, 233 (1992).
4. N. G. Anderson, J. L. Maller, N. K. Tonks, T. W. Sturgill, *Nature* **343**, 651 (1990).
5. D. M. Payne *et al.*, *EMBO J.* 10, 885 (1991).
6. R. Seger *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6142 (1991); C. M. Crews, A. A. Alessandrini, R. L. Erikson, *ibid.*, p 8845.
7. N. G. Ahn *et al.*, *J. Biol. Chem.* **266**, 4220 (1991); N. Gomez and P. Cohen, *Nature* **353**, 170 (1991); Y. Gotoh *et al.*, *EMBO J.* 10, 2661 (1991); S. Matsuda *et al.*, *ibid.* 11, 973 (1992).
8. J. Posada and J. A. Cooper, *Science* **255**, 212 (1992).
9. S. Nakielny, P. Cohen, J. Wu, T. Sturgill, *EMBO J.* 11, 2123 (1992), A. Rossomando, J. Wu, M. J Weber, T. W. Sturgill, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5221 (1992).
10. R. Seger *et al.*, *J. Biol. Chem.* **267**, 14373 (1992).
11. C. Crew and R. L. Erikson, *Proc. Natl. Acad. Sci. U.S.A.*, in press; J. Wu *et al*, *Biochem. J.* **285**, 701 (1992).
12. H. Kosako, Y. Gotoh, S. Matsuda, M. Ishikawa, E. Nishida, *EMBO J.* 11, 2309 (1992).
13. S. A. Nadin-Davis and A. Nasim, *ibid.* **4**, 985 (1988); M. A. Teague, D. T. Chaleff, B. Errede, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7371 (1986); A. Gartner, K. Nasmyth, G. Ammerer, *Genes Dev.* **6**, 1280 (1992).
14. E. Warbrick and P. A. Fantes, *EMBO J* 10, 4291 (1991); Y. Wang, H.-P. Xu, M. Riggs, L. Rogers, M. Wigler, *Mol. Cell. Biol.* 11, 3554 (1991); C. Costigan, S. Gehrung, M. Synder, *ibid.* **12**, 1162 (1992); N. Rhodes, L. Connell, B. Errede, *Genes Dev.* **4**, 1862 (1990).
15. P. Dent *et al.*, *Science* **257**, 1404 (1992).
16. J. M. Kyriakis *et al.*, *Nature* **358**, 417 (1992).
17. N. G. Anderson *et al.*, *Biochem. J.* **277**, 573 (1991); R. Lee, M. H. Cobb, P. J. Blackshear, *J. Biol. Chem.* **267**, 1088 (1992).
18. U. R. Rapp, *Oncogene* **6**, 495 (1991), P. Li, K. Wood, H. Mamon, W. Haser, T. Roberts, *Cell* **64**, 479 (1991).
19. K. W. Wood, C. Sarnecki, T. M Roberts, J. Blenis, *Cell* **68**, 1041 (1992), S. M. Thomas *et al.*, *ibid.* p. 1031.
20. A. M. M. de Vries-Smits *et al.*, *Nature* **357**, 602 (1992).
21. S. Hattori *et al.*, *J. Biol. Chem.*, in press.
22. S. L. Leevers and C. J. Marshall, *EMBO J.* **11**, 569 (1992).
23. J. Downward, J. D. Graves, P. H. Warne, S. Rayter, D. A Cantrell, *Nature* **346**, 719 (1990).
24. M. Nori, G. L'Allemain, M. J. Weber, *Mol. Cell. Biol.* **12**, 936 (1992).
25. S. K. Gupta, C. Gallego, G. L Johnson, L. E. Heasley, *J. Biol. Chem.* **267**, 7987 (1992); C. Gallego, S. K Gupta, L E. Heasley, N.-X. Qian, G. L. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7355 (1992).
26. A. Ullrich and J. Schlessinger, *Cell* **61**, 203 (1990); R. M. Kypta, Y. Goldberg, E. T. Ulug, S. A. Courtneidge, *ibid* **62**, 481 (1990).

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