ment was ligated to Sal I– and Sph I–digested pKM1167 (which expressed *UBC2* from $P_{GAL1,10}$) (*7*), yielding pKM1308. The latter was cleaved with Pst I and Esp I [removing the adjacent *QCR9* gene (*7*)], treated with T4 DNA polymerase I, and self-ligated, yielding pKM1313. The plasmid pKM1319, which contained a frameshift mutation at codon 172 of *UBR1*, was produced from pKM1313 as described (*7*). *GPA1* (*13*) was isolated from *S. cerevisiae* genomic DNA by the polymerase chain reaction (PCR) [F. M. Ausubel *et al.*, *Current Protocols in Molecular Biology* (Wiley-Interscience, New York, 1992] with primers that allowed the excision of amplified *GPA1* as an ~1.4-kb Eco RI–Kpn I fragment. This fragment was ligated to the Eco RI– and Kpn I–cleaved high-copy plasmid pJDPREdha (provided by J. Dohmen) between its P_{CUP1} promoter and a sequence encoding two tandem HA epitopes (*7*, *15*) [E. S. Johnson, B. Bartel, W. Seufert, A. Varshavsky, *EMBO J.* **11**, 497 (1992)], yielding pKM1362-2. The plasmid pKM1363-2 was constructed similarly to pKM1362-2, except that an amplification primer was designed to convert codon 2 of *GPA1* into an Ala codon. The DNA encoding $G\alpha^{451}G\alpha^{472}$-HA was a by-product of the PCR reaction that yielded Gα-HA DNA. The PCR-derived Eco RI–Kpn I fragment encoding $G\alpha^{451}G\alpha^{472}$-HA was subcloned into pJDPREdha, yielding pKM1362-1. YEp105 (*18*) was provided by E. Johnson.

27. The *S. cerevisiae* strains were JD47-13C (*10*); JD55 (a *ubr1*Δ derivative of JD47-13C) (*7*); KMD60 [the product of a cross between JD47-13C and XS803-2C (*MAT*α *leu2-3,112 ura3-52 his1-7 can*^{r}) (Yeast Strain Collection, University of California, Berkeley)]; W303-1a (*MAT***a** *ade2-1 his3-11 leu2-2, 112 trp1-1 ura3-1 can1-100* [E. Leberer *et al.*, *Mol. Gen. Genet.* **241**, 241 (1993)]; YEL2, a *ste4*Δ*::URA3* derivative of W303-1a (provided by M. Whiteway and J. Dohmen); 29-6, an *sst2*Δ*::URA3* derivative of W303-1a (provided by J. Kurjan); and KMY901, a *Ura*$^-$ derivative of 29-6.
28. Cells were grown at 30°C to an optical density at 600 nm of ~1 in synthetic medium (*23*) containing 3% raffinose instead of glucose. Cultures were diluted by the addition of four volumes of rich (YEP) (*23*) medium containing raffinose or galactose and allowed to grow for 3 hours. Cells from 25-ml cultures were collected by centrifugation, washed with water, and resuspended in 0.4 ml of raffinose or galactose medium lacking methionine. [^{35}S]Methionine (0.2 mCi) (^{35}S-Translabel; ICN) was added, and the suspension was incubated for 5 min at 30°C. The cells were separated by centrifugation and resuspended in otherwise identical medium lacking ^{35}S and containing 10 mM L-methionine, 5 mM L-cysteine, and cycloheximide (0.5 mg/ml). Samples (0.1 ml) were removed either immediately or after 10, 30, or 60 min, added to microfuge tubes with 0.5 g of 0.5-mm glass beads and 0.8 ml of buffer A [1% Triton X-100, 0.15 M NaCl, 5 mM EDTA (Na^+ salt), 50 mM Hepes-NaOH (pH 7.5)] containing protease inhibitors (*6*), and frozen in liquid nitrogen. Thawed samples were agitated for 3 min, with intermittent cooling on ice. The lysates were centrifuged at 12,000*g* for 1 min. Portions of the supernatants containing equal amounts of ^{35}S were incubated with a monoclonal antibody to HA, and then with protein A–Sepharose (Pharmacia). Immunoprecipitated proteins were separated by SDS-PAGE (10% gel and labeled proteins were detected by fluorography (*7*, *17*).
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31. Each curve was determined at least twice (five times with cells overexpressing the N-end rule pathway components), in independent experiments, with the results differing by <15% (representative curves are shown in Fig. 3). Because of the non–first order kinetics of Gα degradation, especially in cells overexpressing the N-end rule pathway components, the cited half-lives of Gα were measured between 0 and 10 min after removal of cells from [^{35}S]methionine. Nonexponential degradation is also characteristic of the previously studied (engineered) N-end rule substrates (*1*, *2*, *17*). The effect detected by plotting the data as shown in Fig. 3F was not attributable to a decrease in the rate of Gα-HA synthesis on transfer of cells to galactose, because no such effect was observed with cells that overexpressed Ubc2 alone (*8*).
32. We thank E. Johnson, M. Whiteway, M. Ramezani-Rad, and J. Kurjan for their gifts of plasmids and strains; J. Dohmen for his advice and reagents; and members of our laboratory, especially G. Turner, N. Johnsson, F. Lévy, C. Byrd, M. Ghislain, and J. Johnston, for comments on the manuscript. Supported by grants to A.V. from NIH.

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Ras-Dependent Growth Factor Regulation of MEK Kinase in PC12 Cells

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Mitogen-activated protein kinases (MAPKs) are rapidly activated in response to stimulation of diverse receptor types. MAPKs are positively regulated by phosphorylation on threonine and tyrosine by MAP kinase or extracellular signal–regulated kinase (ERK) kinases (MEKs). MEK kinase (MEKK) is part of a family of serine-threonine protein kinases that phosphorylate and activate MEKs independently of Raf. MEKK was rapidly and persistently activated in response to stimulation of resting PC12 cells with epidermal growth factor (EGF). Nerve growth factor (NGF) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) also activated MEKK, although to a lesser degree than did EGF. Activation of MEKK and B-Raf in response to EGF was inhibited by expression of dominant negative N^{17}Ras. Expression of oncogenic Ras resulted in activation of MEKK. Stimulation of synthesis of cyclic adenosine 3′,5′-monophosphate abolished activation of MEKK and B-Raf by EGF, NGF, and TPA. Thus, Ras simultaneously controls the activation of members of the Raf and MEKK families of protein kinases.

The MAP kinase regulatory network is activated as a result of sequential phosphorylation reactions in response to stimulation of cells by growth factors, hormones, or neurotransmitters (*1*, *2*). MAPKs are activated by phosphorylation on specific tyrosine and threonine residues by MAPK (or ERK) kinases (MEKs) (*3*, *4*), of which at least three have been cloned from metazoan species (*5*–*7*). MEKs are in turn regulated by serine-threonine kinases of the Raf family, including c-Raf-1 (*8*, *9*) and B-Raf (*10*, *11*). The mammalian homolog of the yeast protein kinases Byr2 (*Schizosaccharomyces pombe*) and STE11 (*Saccharomyces cerevisiae*), termed MEK kinase (MEKK), also phosphorylates and activates MEKs independently of Raf (*12*). Raf-1 and MEKK phosphorylate the same sites on MEK-1 in vitro, and these sites are phosphorylated in vivo after growth factor stimulation of cells (*13*). Because there are multiple distinct but

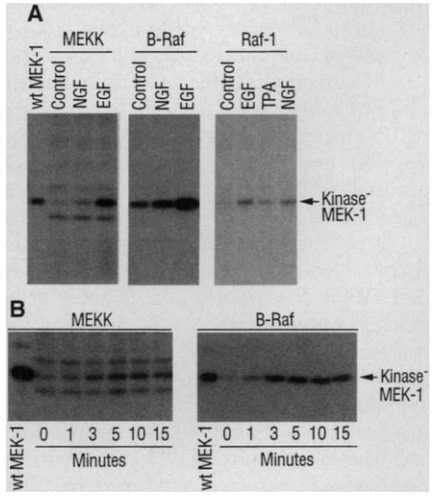

Fig. 1. Activation of MEKK, B-Raf, and Raf-1 by growth factors. (**A**) Phosphorylation of catalytically inactive MEK-1 by immunoprecipitated MEKK, B-Raf, or Raf-1. PC12 cells were incubated in starvation medium [Dulbecco's modified Eagle's medium (DMEM) and 0.1% bovine serum albumin (BSA)] for 18 to 20 hours and left untreated or treated with EGF (30 ng/ml), NGF (100 ng/ml), or TPA (200 nM) for 5 min. MEKK was immunoprecipitated from lysates containing equal amounts of protein with an anitserum to a fusion protein encoding the NH_2-terminal portion of MEKK (*32*). Immunoprecipitations and in vitro kinase assays were performed as described (*33*) with purified recombinant catalytically inactive MEK-1 (150 ng) and 40 μCi of [γ-^{32}P]ATP in a final volume of 20 μl for 25 min at 30°C. B-Raf and Raf-1 were immunoprecipitated from the same untreated and treated PC12 cell lysates described above. Antiserum to a COOH-terminal peptide of B-Raf and anitserum to the 12 COOH-terminal amino acids of Raf-1 (Santa Cruz Biotechnology) were used for the appropriate immunoprecipitations. Equal amounts of MEKK, B-Raf, or Raf-1 were immunoprecipitated from each lysate as demonstrated by immunoblotting (*17*). (**B**) Time course of EGF-stimulated MEKK and B-Raf activation. MEKK or B-Raf was immunoprecipitated from lysates of PC12 cells treated with EGF (30 ng/ml) for 0, 1, 3, 5, 10, 15, or 20 min and incubated with catalytically inactive MEK-1 (150 ng) and [γ-^{32}P]ATP as described for (A).

similar MAPK pathways in yeast, there may be a large family of MEKK-like isozymes in mammalian cells (*14, 15*). At least three other MEKKs encoded by distinct genes have been cloned (*16*).

In PC12 cells, B-Raf and Raf-1 are regulated in response to activation of growth factor receptor tyrosine kinases (*11*). MEKK mRNA is abundant in PC12 cells (*12*), suggesting that MEKKs may participate in growth factor regulation of the PC12 cell phenotype. MEKK activity was measured after immunoprecipitation of MEKK from lysates of growth factor–stimulated PC12 cells. Purified recombinant MEK that was catalytically inactive (*12*) was used as a substrate for in vitro kinase assays. Treatment with epidermal growth factor (EGF) of PC12 cells increased MEKK activity (Fig. 1A). Nerve growth factor (NGF) also stimulated MEKK activity, but to a lesser degree than did EGF. B-Raf activity was also stimulated by EGF and NGF; B-Raf from control cells consistently exhibited a high basal activity. Raf-1 was also activated in response to growth factors. PhosphorImager quantitation indicated that EGF stimulated MEKK 3.5- to 4-fold, B-Raf 4- to 5-fold, and Raf-1 2.5- to 3-fold over basal activity in the respective immunoprecipitates. A time course of EGF treatment indicated that MEKK activation was maximal after 5 min and persisted for at least 20 min (Fig. 1B). B-Raf exhibited a similar time course of activation.

Two MEKK species of approximately 98 and 82 kD present in PC12 cell lysates were recognized by a MEKK antiserum to a portion of the NH_2-terminus of MEKK (Fig. 2A). Visualization of both of these proteins was inhibited by incubation of the antibody with the purified recombinant NH_2-terminal fusion protein antigen (*17*). Only the 98-kD MEKK protein was immunoprecipitated with MEKK antiserum (Fig. 2A). The immunoprecipitated 98-kD MEKK comigrated on gels with the 98-kD MEKK protein detected by immunoblotting of cell lysates from PC12 cells, Rat1a, NIH 3T3, and Swiss 3T3 fibroblasts. The amount of the 98-kD MEKK expressed in PC12 cells is somewhat greater than that in fibroblast cell lines. The 98-kD MEKK is not recognized by antisera to a peptide encoding the extreme COOH-terminus of MEKK (*17*), suggesting that the 98-kD MEKK is either a splice variant of the originally cloned MEKK or a MEKK isoform with similar NH_2-terminal epitopes but a different COOH-terminus. Immunoblotting with antibodies to Raf-1 or B-Raf indicated that neither of these enzymes were present as contaminants of MEKK immunoprecipitates (Fig. 2B). Raf immunoblots of MEKK immunoprecipitates were overexposed to reveal possible trace amounts of each protein. The 98-kD MEKK in MEKK immunoprecipitates did not comigrate with Raf-1 or B-Raf in PC12 cell lysates, and no cross-reactivity between MEKK and Raf antibodies was observed.

Recombinant MEKK overexpressed in COS cells phosphorylates and activates MEK, leading to MAPK phosphorylation and activation (*12*). To determine whether the phosphorylation of MEK by immuno-

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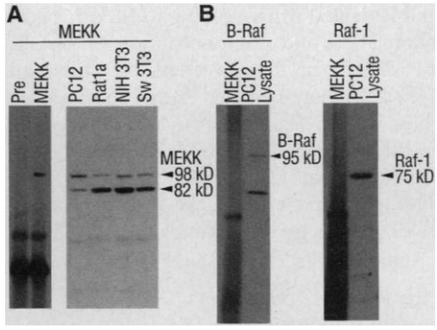

Fig. 2. Immunoblotting of MEKK in immunoprecipitates and cell lysates. (**A**) MEKK immunoblot showing presence of 98-kD MEKK in MEKK immunoprecipitates (MEKK) and expression of 82- and 98-kD forms of MEKK in rodent cell lines. Immunoprecipitated MEKK, a preimmune control immunoprecipitate (pre), and soluble cellular protein (125 μg) was loaded onto the gel for immunoblotting with affinity-purified antibody (1:500 dilution) to a fusion protein containing the NH_2-terminal portion of MEKK (*32*). (**B**) Immunoblotting of MEKK immunoprecipitates for the presence of B-Raf or Raf-1. Immunoprecipitated MEKK and PC12 cell soluble protein (100 μg, PC12 lysate) were loaded onto the gel for immunoblotting with either B-Raf antibody (1:100 dilution) or Raf-1 antibody (1:100 dilution).

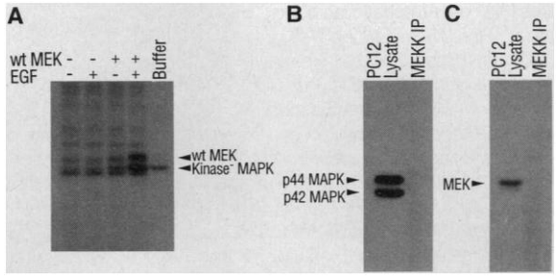

Fig. 3. Activation of MEK by 98-kD MEKK. (**A**) Phosphorylation of MAPK by activated MEK. MEKK was immunoprecipitated as described (Fig. 1) from untreated (−) or EGF-treated (+) PC12 cells and incubated in the presence (+) or absence (−) of purified recombinant wild-type (wt) MEK (150 ng) and in the presence of purified recombinant catalytically inactive MAPK (300 ng) and [γ-^{32}P]ATP as described (*33*). MAPK phosphorylation due to autoactivation of wild-type MEK (buffer) was less than 20% of the activity observed in the presence of activated MEKK. (**B**) Immunoblot of MAPK in PC12 cell lysates (100 μg of protein) and its absence in MEKK immunoprecipitates (MEKK IP). (**C**) Immunoblot of MEK in PC12 cell lysates (100 μg of protein) and its absence in MEKK immunoprecipitates.

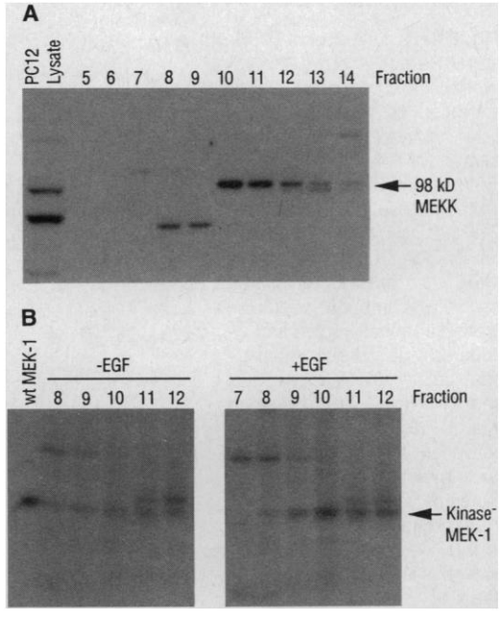

Fig. 4. MEKK activity in FPLC Mono Q ion-exchange column fractions of PC12 cell lysates. (**A**) Immunoblot showing MEKK in column fractions from lysates of EGF-stimulated PC12 cells. Portions (900 μl) of 1-ml column fractions (1 to 525 mM NaCl gradient) were concentrated by precipitation with trichloroacetic acid as described (*34*) and immunoblotted with MEKK antibody. The peak of MEKK immunoreactivity eluted in fractions 10 to 12. The peak of B-Raf immunoreactivity eluted in fraction 14, whereas Raf-1 was not detected in the collected eluates from the column (*17*). (**B**) Phosphorylation of MEK-1 by activated endogenous MEKK. Portions (30 μl) of each fraction from lysates of unstimulated control PC12 cells or EGF-treated PC12 cells were assayed as described (*12*) in buffer containing purified recombinant MEK-1 (150 ng) as a substrate. The peak of MEKK activity eluted in fractions 10 to 12.

precipitated 98-kD MEKK resulted in activation of MEK, we used purified recombinant wild-type MEK and catalytically inactive MAPK in a coupled assay system (Fig. 3A). Immunoprecipitated MEKK from EGF-treated PC12 cells was assayed for its ability to activate added wild-type MEK such that it would phosphorylate catalytically inactive recombinant MAPK in the presence of [γ-^{32}P]ATP. Immunoprecipitated MEKK from EGF-stimulated cells phosphorylated and activated MEK, leading to MAPK phosphorylation. No phosphorylation of MAPK occurred in the absence of added recombinant MEK. Immunoblotting demonstrated that there was no contaminating MAPK (Fig. 3B) or MEK (Fig. 3C) in the MEKK immunoprecipitates from EGF-stimulated PC12 cells. Thus, phosphorylation and activation of MEK is due to EGF stimulation of MEKK activity measured in the immunoprecipitates.

Lysates from control and EGF-stimulated PC12 cells were fractionated by fast protein liquid chromatography (FPLC) on a Mono Q column (Fig. 4). Immunoblotting of column fractions demonstrated that the 98-kD MEKK predominantly eluted in fractions 10 to 12 (Fig. 4A). To confirm that the 98-kD MEKK contained in fractions 10 to 12 was activated by EGF, we used catalytically inactive MEK as a substrate in column fractions from unstimulated and EGF-stimulated PC12 cells (Fig. 4B). Fractions 10 to 12 from EGF-stimulated PC12 cells phosphorylated MEK, whereas little MEK phosphorylation occurred in fractions from unstimulated cells. The peak of MEK phosphorylation was present in fraction 10 and co-eluted with the peak of MEKK immunoreactivity (Fig. 4A). EGF reproducibly stimulated an approximately two- to threefold increase in MEKK activity as quantitated by a PhosphorImager. This increased stimulation was similar in magnitude to the EGF-stimulated MEKK activation observed in MEKK immunoprecipitates (Fig. 1). Thus, the phosphorylation of MEK correlated with the presence of the activated 98-kD MEKK in both column fractions and immunoprecipitates.

Expression of a dominant negative mutant form of Ras, N^{17}Ras (*18*), inhibits growth factor–mediated activation of MAPK in PC12 cells (*19*). Raf-1 is an effector for Ras, and these proteins physically interact in cells (*20–22*). To test whether the 98-kD MEKK and B-Raf require functional Ras proteins for growth factor–mediated signalling, we induced expression of N^{17}Ras from a dexamethasone-sensitive promoter in PC12 cells and assayed EGF-stimulated activation of MEKK and B-Raf in immunoprecipitates with catalytically inactive MEK as a substrate (Fig. 5A). Expression of N^{17}Ras inhibited the activation of MEKK by EGF. EGF-mediated activation of B-Raf was also reduced in PC12 cells expressing N^{17}Ras. Addition of dexamethasone to wild-type PC12 cells had no effect on the magnitude of the activation of MEKK or B-Raf by EGF (Fig. 5B). These results indicate that functional Ras is required for growth factor–stimulated activation of both B-Raf and MEKK in PC12 cells. To confirm that MEKK is regulated by Ras, we transiently expressed activated oncogenic Ras in PC12 cells (Fig. 5C). Expression of oncogenic Ras resulted in strong MEKK activation; Raf-1 was also activated by oncogenic Ras. Therefore, Ras exerts effects on cell growth and differentiation through the activation of multiple protein kinases from the Raf and MEKK families.

Stimulation of cells with agents that raise intracellular concentrations of adenosine 3′,5′-monophosphate (cAMP), and thereby activate cAMP-dependent protein kinase A (PKA), inhibit activation of Raf-1 and B-Raf in response to growth factors in several systems (*11, 23–26*). Growth factor–stimulated activity of MEK-1 and MAPK is not inhibited by PKA activation in PC12 cells (*11*), suggesting that other growth factor–regulated MEKs or MEKKs may exist that are not negatively regulated by PKA. To determine whether the growth factor–mediated activation of 98-kD MEKK in PC12 cells was inhibited by PKA, we used forskolin to increase the intracellular concentration of cAMP and activate PKA (*17*). PC12 cells were treated with forskolin for 3 min and then with EGF, NGF, or TPA, and MEKK activity was assayed in immunoprecipitates. B-Raf activity was also assayed from the same cell lysates to test whether its regulation differed from that of MEKK. Treatment of cells with forskolin

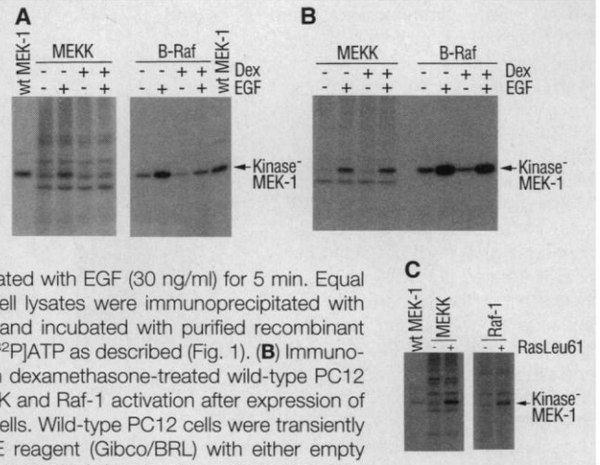

Fig. 5. Ras dependence of growth factor–stimulated MEKK and B-Raf activation. (**A**) Inhibition of MEKK and B-Raf activation by dominant negative N^{17}Ras expression. PC12 cells stably expressing dexamethasone-inducible N^{17}Ras (*18*) were deprived of serum for 18 to 20 hours in medium containing 0.1% BSA with or without 1 μM dexamethasone (Dex) and then left untreated or treated with EGF (30 ng/ml) for 5 min. Equal amounts of soluble protein from cell lysates were immunoprecipitated with antisera to either MEKK or B-Raf and incubated with purified recombinant catalytically inactive MEK-1 and [γ-^{32}P]ATP as described (Fig. 1). (**B**) Immunoprecipitated MEKK and B-Raf from dexamethasone-treated wild-type PC12 cells stimulated with EGF. (**C**) MEKK and Raf-1 activation after expression of oncogenic Ras in wild-type PC12 cells. Wild-type PC12 cells were transiently transfected with the lipofectAMINE reagent (Gibco/BRL) with either empty expression vector (−) or activated RasLeu61 (+) under the control of the SRα promoter. Forty-eight hours after transfection, MEKK or Raf-1 was immunoprecipitated from cell lysates containing equal amounts of protein and subjected to in vitro kinase assay as described (Fig. 1A). The autoradiogram depicting Raf-1 activation was exposed to film four times longer than that for MEKK.

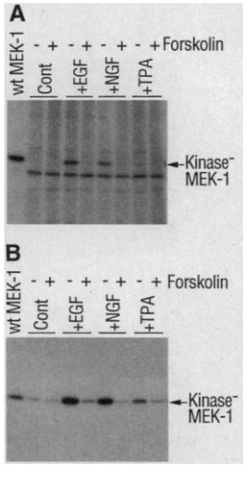

Fig. 6 (left). Inhibition of MEKK and B-Raf activation by PKA activation. (**A**) Inhibition of MEKK activation by forskolin. PC12 cells deprived of serum were treated with or without forskolin (50 μM) for 3 min to activate protein kinase A and then with EGF (30 ng/ml), NGF (100 ng/ml), or TPA (200 nM) for 5 min. MEKK was immunoprecipitated from equal amounts of soluble protein from cell lysates and incubated with purified recombinant catalytically inactive MEK and [γ-^{32}P]ATP as described (Fig. 1). (**B**) Inhibition of B-Raf activation by forskolin. B-Raf was immunoprecipitated from the same cell lysates described in (A) and assayed for its ability to phosphorylate MEK as described (Fig. 1). Cont, control.

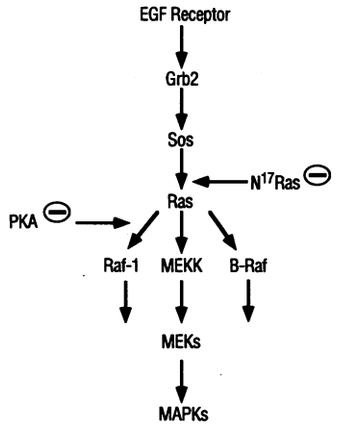

Fig. 7 (right). Ras-dependent regulation of growth factor–stimulated MEKK and Raf family members. Ras activation is a necessary event for MEKK activation; the interaction between Ras and MEKK may not be direct.

abolished the activation of both MEKK and B-Raf by EGF, NGF, and TPA (Fig. 6). These results demonstrate that PKA activation inhibits growth factor stimulation of the 98-kD MEKK, suggesting the existence of a common regulatory control point for PKA action that lies between or downstream of Ras and upstream or at the level of each of the three kinases.

Our results show that MEKK is regulated by growth factors in a Ras-dependent manner in PC12 cells. Thus, Ras serves as a common control point for the regulation of both MEKK and Raf protein kinases (Fig. 7). Insulin-stimulated activation of MAPK and 90-kD ribosomal protein S6 kinase (RSK) is blocked by expression of N^{17}Ras, but not by expression of dominant negative Raf mutants in 3T3 L1 cells (*27*), suggesting that Raf-independent pathways exist downstream of Ras. Treatment of adipocytes with insulin results in rapid transient activation of a MEKK of approximately 56 kD that is distinct from Raf-1 (*28*). These data support a role for Ras-dependent MEKKs in growth factor– and PKC-mediated signalling pathways. Unlike overexpression of Raf, overexpression of wild-type or constitutively active MEKK mutants is not transforming in fibroblasts, and stable expression appears to be growth inhibitory or lethal (*17*). This indicates that MEKK function must diverge from that of the Raf family members.

Parallel MAPK pathways regulate diverse cellular functions including mating, osmotic regulation, and cell wall biosynthesis in yeast (*14*, *15*). These MAPK pathways function largely independently of one another, suggesting that there is little crossover in the regulation of substrates by the respective kinases in each pathway within the cell. Similar parallel pathways may exist in mammalian cells. Although members of the Raf family have been detected only in higher eukaryotes and are not present in yeast, MEKKs are expressed in both yeast and metazoan organisms (*14*, *15*). Researchers are identifying a growing number of MAPKs (*29*, *30*), MEKs (*5–7*, *31*), and MEKKs (*12*, *16*) that are expressed in mammalian cells. When Raf kinases and MEKKs are properly assembled in cells with their constituent MEKs and MAPKs, they will predictably regulate different cellular functions. A key to defining these different functions will involve determining which MEKs are preferentially regulated by Raf kinases compared with MEKKs in different cell types and tissues.

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Genetics of a Pheromonal Difference Contributing to Reproductive Isolation in *Drosophila*

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Although sexual isolation is one of the most important causes of speciation, its genetic basis is largely unknown. Here evidence is presented that suggests that sexual isolation between two closely related species of *Drosophila* is largely caused by differences in female cuticular hydrocarbons. This difference maps to only one of the three major chromosomes, implying that reproductive isolation might have a fairly simple genetic basis. The effect of the hydrocarbons on courtship may help explain the ubiquitous asymmetry of sexual isolation between many pairs of *Drosophila* species.

One of the major unsolved questions of evolution is the genetic basis of speciation. Determination of the number, location, and effects of genes producing reproductive isolation bears critically on different theories of speciation and on the controversial idea that important evolutionary change may involve relatively few genes (*1–3*). Moreover, the mapping and localization of such loci may help reconstruct the origin of species on a gene-by-gene basis, and the subsequent molecular isolation of such genes might help determine their normal function within species. A recent interest in the genetics of postzygotic reproductive isolation (hybrid sterility and inviability) has yielded several theories of speciation (*4*). However, prezygotic isolation, especially mating discrimination, may be the primary cause of speciation in many animal taxa (*5*). There have been relatively few genetic analyses of this trait, although examples exist of both major gene and polygenic determination (*1*). This rarity reflects the difficulty of behavioral genetic work in many species, as well as the notorious lability of sexual behavior in the laboratory. Here we present evidence that an easily measured morphological character, the cuticular hydrocarbon profile, is a

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