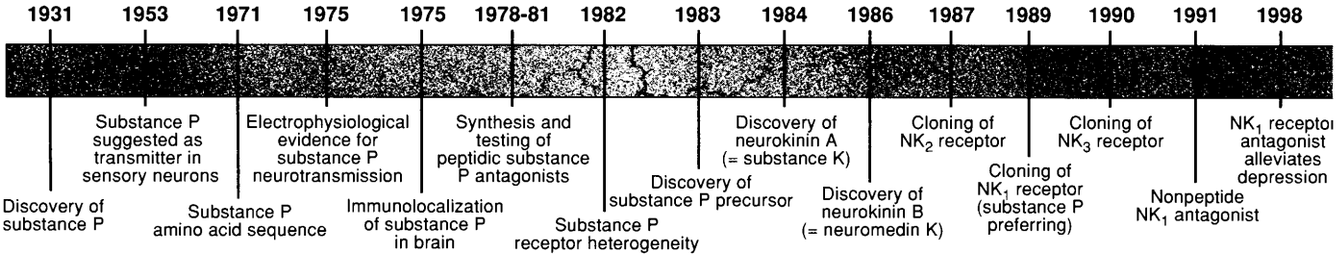

Substance P and related neuropeptides. A few landmarks from discovery to potential therapeutic relevance [(*2*, *3*, *5*, *8*)].

serotonin or norepinephrine (the two neurotransmitter systems that are implicated in the actions of known antidepressants) in animals, nor do these clinically effective antidepressants block NK_1 receptors in vitro. However, additional data on the effective clinical dose will be needed because the 300-mg dose of MK-869 (which likely produces micromolar plasma levels) is arguably high, especially in view of the fact that NK_1 receptor blockade is seen at subnanomolar concentrations in vitro. As a result, there will be some speculation as to whether NK_1 receptor blockade is the only molecular site of MK-869 action in humans with MDD. Moreover, the bulk of the animal data presented in (*5*) relates to acute doses, yet the clinical efficacy of MK-869 was expressed after 2 to 3 weeks, which does not appear to distinguish this compound from known antidepressants and may suggest a "final common pathway" mechanism.

An enticing possibility from the present study (*5*) is that a NK_1 antagonist might also be useful clinically as an anxiolytic agent; this warrants further investigation. On the basis of animal studies, substance P was already known to be present within neuronal circuits (amygdala, hypothalamus) that mediate central stress responses, and it was suggested that a NK_1 antagonist might thus be useful to treat stress and anxiety (*6*, *7*). In fact, another NK_1 antagonist, GCP-49823, was discontinued in a phase I trial aimed toward treatment of anxiety. Interestingly, the clinical data with differential time course of antidepressant and anxiolytic actions of MK-869 [see (*5*)] do not exclude the possibility that the antidepressant effects of the NK_1 antagonist are independent of its anxiolytic effects.

What lessons can be learned for the future discovery and development of pharmaceutical agents? In the field of psychiatric drug discovery, with its shortage of reliable animal models, the argument of a tight link between animal and human data (*5*) will be well received. However, there will be continued debate regarding which findings concerning neurotransmitter actions in animals have a clinical correlate. Only a fraction of the observations made in animals can be extended to costly clinical trials, no matter how spectacular the animal data may be and how pharmacokinetically attractive the compounds at hand. Phenomenological observations during the 1980s (changes in the levels of neuropeptides and their receptors after drug or other treatment in animals; changes in levels of neuropeptides or neuropeptide receptors during human disease) and the 1990s (comparison of a knockout mouse phenotype to human pathophysiology; the influences of genomic polymorphisms on function) are generally an insufficient basis for making rational decisions.

Finally, with respect to NK_1 antagonists, the pharmaceutical industry must be commended for its persistence. However, if this class of compounds indeed finds a role in the pharmacotherapy of depression, then the total expenditures must still be assessed on the basis of the many years of research and failed clinical trials that pursued other (and arguably more intuitive) directions.

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PERSPECTIVES: SIGNAL TRANSDUCTION

Routing MAP Kinase Cascades

Elaine A. Elion

Cells are constantly bombarded by external signals that regulate their growth, differentiation, and stress level. To respond properly to these signals, eukaryotic cells assemble cascades of highly conserved protein kinases (mitogen-activated protein kinases, MAPKs, and their activator kinases), which form the central elements of signal transduction pathways that lead to and activate transcription factors in the nucleus and other effectors throughout the cell (*1*). Cells contain multiple MAPK cascades that can use subsets of the same kinases yet activate different effector proteins, depending on the stimulus. This sharing of kinases makes it critical that the cell properly route the various signals to prevent cross talk between pathways. Yeast cells seem to have solved this problem with the use of scaffolding proteins like Ste5, by forming multienzyme complexes with kinases that are used by more than one pathway and are therefore shared (*2*). On pages 1671and 1668 of this issue, Whitmarsh *et al.* (*3*) and Schaeffer *et al.* (*4*) extend this mechanism to mammalian cells by identifying two proteins, JIP-1 and MP1, that help route two different MAPK cascades. Their findings point to the universal function of scaffolding/adapter proteins in the assembly of information highways inside cells.

The core elements of a MAPK module are three sequentially activated protein kinases, named after the last kinase in the series (see the figure) (*1*). A module can be activated by multiple stimuli and more than one receptor. MAPKs and their activators MAPK kinases are quite homologous within their respective subgroups, while MAP kinase kinases include at least four subtypes—Raf, MEKK, mixed lineage kinases (or MLKs), and Mos. The six MAPKs, seven MAPK kinases, and seven MAPK kinase kinases thus far defined in mammalian cells, set the stage for potential cross-regu-

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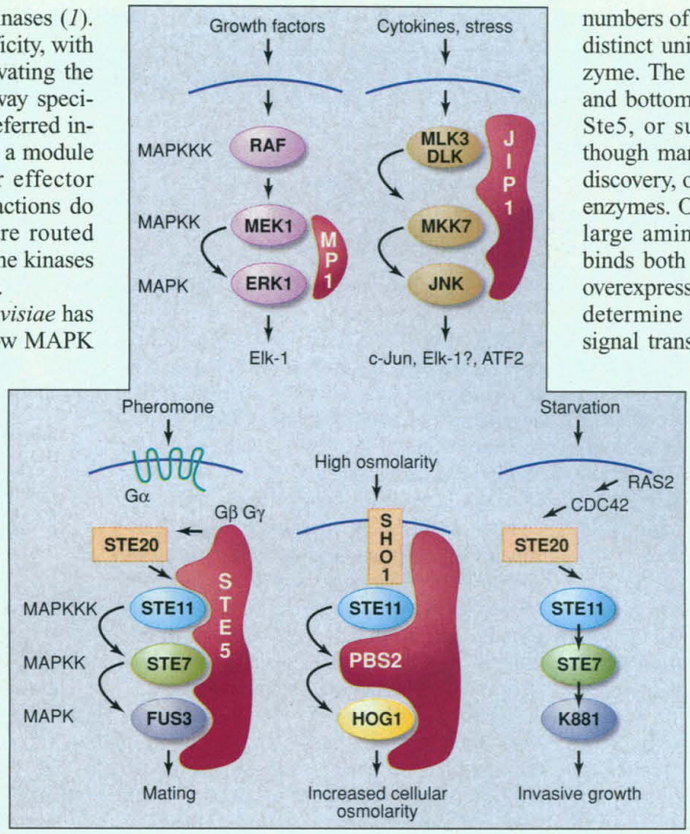
lation between different sets of kinases (*1*). Yet cells maintain exquisite specificity, with extracellular signals reliably activating the proper target. Some of this pathway specificity can be accounted for by preferred interactions between kinases within a module and between MAPKs and their effector substrates (*1, 5*). But these interactions do not fully explain how signals are routed through only one pathway when the kinases can function in multiple pathways.

The yeast *Saccharomyces cerevisiae* has provided important clues as to how MAPK cascades with shared components may be segregated. Yeast cells use the MEKK Ste11 in their response to high osmolarity, and in mating and invasive-growth pathways, with the latter two pathways also sharing PAK Ste20 and MAPK Ste7 (see the figure). Functional analysis suggests that these pathways are highly specific, despite the sharing of kinases (*5*). Ste5 is thought to regulate mating pathway specificity by simultaneously binding Ste11, Ste7 and the MAPK Fus3 and enhancing Fus3 activity (*6*). Moreover, Ste5 binds the mating pathway G protein (*7*) and this interaction channels the pheromone signal through Ste11 to Fus3 (*8*). The MAPK kinase Pbs2 may perform an analogous function for the osmolarity pathway (*9*), for it associates with Ste11, the MAPK Hog1, and Sho1, the possible sensor of the signal. Still missing is proof that Ste5 and Pbs2 selectively activate a MAPK module by selective binding.

The new work identifies two proteins that seem to act in a similar manner in mammalian cells, selectively enhancing activation of some MAPK cascade components to the exclusion of others. These proteins, JIP-1 (JNK interacting protein–1) and MP1 (MEK partner 1), fall into two classes. JIP-1 is similar to Ste5, whereas MP1 defines a novel class of adapter. Both molecules were identified by two-hybrid screening for protein-protein interactions that permits identification of proteins without enzymatic activity (*4, 10*). JIP-1—first characterized as a cytoplasmic inhibitor of JNK pathway responses when overexpressed—binds nuclear localized JNK1 and JNK2 and retains them in the cytoplasm (*10*). Whitmarsh *et al.* (*3*) now show that JIP-1 is likely to be a scaffold that channels signals through a specific set of kinases that activate JNKs (see figure). In addition to the JNKs, JIP-1 also binds the MAPK kinase MKK7 and the MAPK kinase kinases MLK3 and DLK, and enhances in vivo activation of JNK1 by MKK7 and MLK3 when overexpressed. Remarkably, JIP-1 is selective and does not bind to or enhance the activity of a variety of other MAPK cascade enzymes. Like Ste5, JIP-1 may channel the signal from farther up in the pathway, because it also associates with HPK1, a potential kinase activator of the MLKs.



Kinase road map. Custom-designed scaffolding/adapter proteins route MAPK modules in mammals (**top**) and yeast (**bottom**).

The second adapter, MP1, may not route an entire pathway, but only predispose a final destination. In vitro, MP1 enhances activation of MEK1 by B-Raf and ERK1 by MEK1. In vivo, MP1 selectively associates with MEK1 and ERK1, but not with MEK2 or ERK2. When overexpressed, MP1 increases the number of MEK1-ERK1 complexes and the amount of MEK1-ERK1–dependent activation of Elk-1. Thus, MP1 may link MEK1 with ERK1 and prime both for activation. MP1 is small (126 residues), so it may bind MEK1 and ERK1 through a common site and dimerize, like 14-3-3 proteins (*11*). MP1 binds MEK1 through a proline-rich domain shared by MEK2, implying that additional factors may dictate preferred binding in vivo. MP1 has a human homolog, so its function is likely conserved.

Collectively, these findings suggest that we will find more custom-designed MAPK cascade regulatory proteins, with variable numbers of enzyme-binding sites, present as distinct units or attached to a pathway enzyme. The regulators may connect the top and bottom components of a pathway, like Ste5, or subsets of them, like MP1. Although many regulators undoubtedly await discovery, others may lurk as already known enzymes. One candidate is MEKK1, whose large amino-terminal regulatory domain binds both JNKs and activates them when overexpressed in vivo (*12*). Future work will determine how these regulators facilitate signal transduction, and whether they control the amplification of the initial signal (*13*). Studies on Ste5 (*14*), JIP-1 (*3, 10*), and MP1 (*4*) underscore the importance of their stoichiometry in regulating a cellular response. For example, removal of Ste5 from yeast cells liberates Ste7 to function in an alternative MAPK cascade (*14*) while overexpression of JIP-1 inhibits JNK pathway responses, possibly by inappropriately sequestering JNKs from nuclear targets (*3*).

The wide use of adapter proteins in eukaryotic signal transduction pathways (*11*) contrasts sharply with their absence in prokaryotes, where a basic two-component signaling unit has been reiterated more than 50 times (*15*). Perhaps signal transduction pathways in more complex eukaryotes have evolved by modification of the adapter/scaffolding proteins to allow use of limited sets of enzymes in highly specialized ways. The human analogs of these cellular scaffolds will provide useful targets for the design of inhibitors of specific responses.

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