Ultrasensitivity in the mitogen-activated protein kinase cascade

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ABSTRACT The mitogen-activated protein kinase (MAPK) cascade is a highly conserved series of three protein kinases implicated in diverse biological processes. Here we demonstrate that the cascade arrangement has unexpected consequences for the dynamics of MAPK signaling. We solved the rate equations for the cascade numerically and found that MAPK is predicted to behave like a highly cooperative enzyme, even though it was not assumed that any of the enzymes in the cascade were regulated cooperatively. Measurements of MAPK activation in Xenopus oocyte extracts confirmed this prediction. The stimulus/response curve of the MAPK was found to be as steep as that of a cooperative enzyme with a Hill coefficient of 4-5, well in excess of that of the classical allosteric protein hemoglobin. The shape of the MAPK stimulus/response curve may make the cascade particularly appropriate for mediating processes like mitogenesis, cell fate induction, and oocyte maturation, where a cell switches from one discrete state to another.

Although the biological responses associated with mitogen-activated protein kinase (MAPK) signaling are highly varied, the basic structure of the MAPK cascade is well conserved (1-3). The cascade always consists of a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK), and a MAPK. MAPKKKs activate MAPKKs by phosphorylation at two conserved serine residues and MAPKKs activate MAPKs by phosphorylation at conserved threonine and tyrosine residues (Fig. 1). The cascade relays signals from the plasma membrane to targets in the cytoplasm and nucleus.

A number of other membrane-to-nucleus signaling pathways, such as the Jak/Stat pathways and the cAMP/protein kinase A pathway, employ just a single protein kinase. Why does the MAPK cascade invariably use three kinases instead of one? The possibility that the three kinase arrangement has evolved to allow signal amplification or amplification is attractive but, as yet, not well supported by genetic or biochemical evidence.

We have explored the possibility that the cascade arrangement has important consequences for the dynamics of MAPK signaling. Here we shall focus on the steady-state responses of enzymes at each level in the cascade to varying input stimuli. The stimulus/response curve of a typical Michaelis-Menten enzyme is hyperbolic, and the enzyme responds in a graded fashion to increasing stimuli. An 81-fold increase in stimulus is needed to drive the enzyme from 10% to 90% maximal response (see for example, the MAPKK curves in Fig. 2). However, some enzymes exhibit stimulus/response curves that are steeper or less steep than the Michaelis-Menten curve. Goldbeter and Koshland have termed these responses “ultrasensitivity” and “subsensitivity,” respectively (11-13). An ultrasensitive enzyme requires less than an 81-fold stimulus to drive it from 10% to 90% maximal response. However, cooperativity is not the only mechanism through which ultrasensitive responses can be generated. Ultrasensitivity also arises when enzyme cycles operate near saturation (“zero-order ultrasensitivity” (11)) and when stimuli impinge upon multiple steps of an enzyme cascade (“multistep ultrasensitivity” (12-14)).

We have investigated whether an ultrasensitive, switch-like response would be expected of the vertebrate Erk1/Erk2 MAPK cascade, given what is known about the abundances of the members of the cascade and their affinities for each other. We solved the rate equations for the cascade numerically, and found that the dose/response curves for MAPK and MAPKK are predicted to be sigmoidal, with the MAPK curve predicted to be as steep as that of a cooperative enzyme with a Hill coefficient of nearly 5. We then carried out detailed measurements of the stimulus/response curves for one MAPKK (Mek-1) and one MAPK (p42 MAPK/Erk2) in a highly

Abbreviations: MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPK kinase kinase.
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manipulable system, *Xenopus* oocyte extracts. We found that p42 MAPK does exhibit a steep sigmoidal stimulus/response curve, as predicted. Thus, the MAPK cascade can convert a graded input into a switch-like output.

**MATERIALS AND METHODS**

**Calculations.** Eqs. 1-10 represent the reactions of the MAPK cascade, which are shown schematically in Fig. 1. We have used Goldbeter and Koshland's nomenclature for the rate constants—the letter a denotes association, d denotes dissociation without catalysis, and k denotes product formation (11). KKK denotes MAPKKK; KK denotes MAPKK; and K denotes MAPK.

\[
\frac{d}{dt}[KKK] = -a_1[KKK][E1] + d_1[KKK-E1] + k_{3}[KKK* \cdot E2] \quad [11]
\]

\[
\frac{d}{dt}[KKK-E1] = a_1[KKK][E1] - (d_1 + k_1)[KKK-E1] \quad [12]
\]

\[
\frac{d}{dt}[KKK*] = -a_2[KKK*][E2] + d_2[KKK*-E2] + k_1[KKK \cdot E1] + (k_3 + d_3)[KK \cdot KKK*] - a_3[KKK][KKK*] + (k_5 + d_5)[KK \cdot P \cdot KKK*] - a_5[KK \cdot P \cdot KKK*] \quad [13]
\]

\[
\frac{d}{dt}[KKK*-E2] = a_2[KKK*][E2] - (d_2 + k_2)[KKK*-E2] \quad [14]
\]

\[
\frac{d}{dt}[KK] = -a_3[KK][KKK*] + d_3[KK \cdot KKK*] + k_4[KK \cdot P \cdot KK P'ase] \quad [15]
\]

\[
\frac{d}{dt}[KKK*] = a_3[KK][KKK*] - (d_3 + k_3)[KK \cdot KKK*] \quad [16]
\]

\[
\frac{d}{dt}[KK-P] = -a_4[KK-P][KK P'ase] + d_4[KK-P \cdot KK P'ase] + k_5[KK \cdot KKK*] + k_6[KK \cdot P \cdot KK P'ase] + a_5[KK \cdot P \cdot KK P'ase] \quad [17]
\]

\[
\frac{d}{dt}[KK-P\cdotKK P'ase] = a_5[KK-P][KK P'ase] - (d_4 + k_3)[KK-P \cdot KK P'ase] \quad [18]
\]

\[
\frac{d}{dt}[KK-P \cdot KKK*] = a_5[KK-P][KKK*] - (d_5 + k_5)[KK-P \cdot KKK*] \quad [19]
\]

\[
\frac{d}{dt}[KK-P\cdotKK P'ase] = a_5[KK-P][KK P'ase] + d_4[KK-P \cdot KK P'ase] + k_7[KK \cdot PP \cdot KK P'ase] - a_8[KK \cdot PP][KK P'ase] + (d_7 + k_7)[KK \cdot PP] + (d_8 + k_8)[KK \cdot PP] \quad [20]
\]

\[
\frac{d}{dt}[KK-P\cdotKK P'ase] = a_5[KK-P][KK P'ase] - (d_6 + k_6)[KK-P \cdot KK P'ase] \quad [21]
\]

\[
\frac{d}{dt}[K] = -a_9[K][KK-P] + d_9[K][KK-P] + k_9[K \cdot P \cdot KK P'ase] \quad [22]
\]

\[
\frac{d}{dt}[K \cdot KK-P] = a_9[K][KK-P] - (d_7 + k_7)[K \cdot KK-P] \quad [23]
\]

We assumed that other reactants (Mg$^{2+}$ ATP, water) are present at constant concentration and so can be included in the rate constants.

The 10 reactions described above give rise to 18 rate equations.
the input stimulus (E1 tot) is expressed in absolute, rather than relative, curves. (The concentration of E1 tot that produces a 50% maximal response. The dashed lines are Hill equation curves whose steepness (the ratio of their EC50 to EC10) is the same as the steepness of the calculated curves. (B) A semi-logarithmic plot of the predicted responses. Here the input stimulus (E1 tot) is expressed in absolute, rather than relative, terms.

\[
\frac{d}{dt}[K-P] = k_9 [K-KK-PP] - a_6 [K-P][K P'ase]
+ d_8 [K-P \cdot K P'ase] - a_4 [K-P][KK-PP]
+ d_4 [K-P \cdot KK-PP] + k_{10} [KK-PP \cdot K P'ase]
\]

[24]

\[
\frac{d}{dt}[K-P K P'ase] = a_6 [K-P][K P'ase]
- (d_8 + k_8) [K-P \cdot K P'ase]
\]

[25]

\[
\frac{d}{dt}[K-P \cdot KK-PP] = a_9 [K-P][KK-PP]
- (d_9 + k_9) [K-P \cdot KK-PP]
\]

[26]

\[
\frac{d}{dt}[K-P] = -a_10 [K-PP][K P'ase]
+ d_{10} [K-PP \cdot K P'ase] + k_8 [K-P \cdot KK-PP]
\]

[27]

\[
\frac{d}{dt}[K-PP K P'ase] = a_{10} [K-PP][K P'ase]
- (d_{10} + k_{10}) [K-PP \cdot K P'ase]
\]

[28]

In addition, there are seven conservation equations (Eqs. 29-35).

\[
[KKK_{tot}] = [KKK] + [KKK^*] + [KKK-E1]
+ [KKK^* \cdot E2]
+ [KKK^* \cdot K] + [KKK^* \cdot K-P]
\]

[29]

These equations were solved numerically using the Runge–Kutta-based NDSolve algorithm in Mathematica (Wolfram Research, Champaign, IL). An annotated copy of the Mathematica code for the MAPK cascade rate equations can be obtained from J.E.F.

The equations written above assume that the dual phosphorylations of MAPK and MAPKK occur by two-step, distributive mechanisms. MAPK collides with its activator (MAPKKK-P), undergoes the first phosphorylation, and is released by its activator; then the monophosphorylated MAPK-P collides with MAPKKK-P, undergoes the second phosphorylation, and is released as active MAPK-P (and likewise for the double phosphorylation of MAPKK by MAPKKK). We also set up and solved the equations for cascades where MAPK is activated in a two-step process but MAPKK is activated by a one-collision, processive mechanism; where MAPK is activated by a one-collision mechanism and MAPKK is activated by a two-collision mechanism; or where both enzymes are activated by one-collision, processive mechanisms, as described below.

Assumed Concentrations and K_m Values. We initially assumed the total concentrations of MAPKKK, MAPKK, and MAPK to be 3 nM, 1.2 μM, and 1.2 μM, respectively, based on estimates for the concentrations of Mos (a MAPKKK), Mek-1 (a MAPKK), and p42 MAPK, in mature Xenopus oocytes (17–19). We initially assumed that E2 tot is 0.3 nM (10-fold less abundant than its substrate Mos); that MAPKK P'ase tot is 0.3 nM (so that the maximal concentration of activated MAPKKKK is 10-fold higher than that of this opposing phosphatase); and that MAPKK P'ase tot is 120 nM (so that the maximal concentration of activated MAPKKK is 10-fold higher than that of this opposing phosphatase). E1 tot was taken to represent the level of input stimulus to the cascade, and was varied over a wide range.

For the purposes of calculating steady-state levels of the enzyme species, it is the K_m values [K_m = (d_i + k_i)/a_i] rather than the individual rate constants that are pertinent. We initially assumed the K_m value for phosphorylation of MAPK by MAPKKKK to be 300 nM, based on a value measured for the phosphorylation of mammalian p42 MAPK/Erk2 by active MKK-1 (N. Ahn, personal communication), and arbitrarily took all of the other K_m values to be 300 nM as well. We subsequently varied all of these K_m values and concentrations over a 25-fold range.

In the numerical studies, the input stimulus to the cascade was varied to be the concentration of E1 tot, whereas in the experimental studies, what was varied was the concentration of Mos tot (the relevant MAPKKK). However, the calculated
curves for MAPK or MAPKK activation versus [MAPKKK]<sub>tot</sub> are similar in steepness to those for MAPK or MAPKK activation versus [E<sub>1tot</sub>]. For example, if [E<sub>1tot</sub>] is fixed at 0.01 nM and [Mos<sub>tot</sub>] is varied, the predicted Hill coefficients for MAPK and MAPKK are 5.0 and 1.7, respectively.

**Experimental Studies.** Concentrated *Xenopus* oocyte extracts were prepared as described (20–22). Briefly, oocytes were defolliculated, washed twice with extract buffer (0.25 M sucrose/0.1 M NaCl, 2.5 mM MgCl<sub>2</sub>/20 mM Heps, pH 7.2/10 μg leupeptin per ml/10 μg pepstatin per ml/10 μg chymostatin per ml/10 μg aprotinin per ml/1 mM phenylmethylsulfonyl fluoride), and twice with extract buffer supplemented with 100 μg cytochalasin B per ml. The washed, defolliculated oocytes were centrifuged at low speeds to remove excess buffer, and crushed by centrifugation. The cytoplasm was collected, clarified by centrifugation, and stored at –80°C.

Thawed extracts (7 volumes) were mixed with an ATP regeneration system (20 mM ATP/20 mM MgCl<sub>2</sub>/200 mM creatine phosphate/1 mg creatine kinase per ml; 1 volume); various concentrations of bacterially-expressed malE-Mos (18) (2 volumes in extract buffer) were added, and the reactions were incubated at room temperature for 100 min. This length of time was sufficient to allow Mek-1 and p42 MAPK to reach steady-state activity levels (data not shown).

The steady-state activity of Mek-1 was assessed by a linked immune complex kinase assay, and the activity of p42 MAPK by a myelin basic protein kinase assay (23). Care was taken to ensure that the Mek-1 and p42 MAPK activity assays were linear with respect to time and enzyme concentration (data not shown). The phosphorylation state of p42 MAPK was also assessed by immunoblotting; phosphorylated forms of p42 MAPK exhibit a retarded electrophoretic mobility (24).

## Results

**Predicted Stimulus/Response Relationships.** We set up and numerically solved the rate equations for the reactions shown in Fig. 1. We assumed that the MAPK and MAPKK phosphorylation reactions occurred in two steps, as indicated in Fig. 1, and that neither step was markedly more rapid than the other. We initially fixed the enzyme concentrations and [E<sub>2</sub>]<sub>initially assumed</sub> and 20-fold above the measured K<sub>m</sub> value for the phosphorylation of MAPK by active MAPKK (300 nM), the Hill coefficient predicted for MAPK would be 9.1 (Table 1). Similarly, if all of the reactions in which MAPKK participates (Eqs. 3–7 and 9) had K<sub>m</sub> values of 60 nM rather than the initially-assumed 300 nM, the predicted Hill coefficient for MAPK would be 8.5 (not shown); if only the reactions that convert MAPKK among its various phosphorylation states had K<sub>m</sub> values of 60 nM (Eqs. 3–6), the predicted Hill coefficient for MAPK would be 9.4 (not shown). Thus, the concentration of MAPKK relative to the K<sub>m</sub> values for the reactions that activate and inactivate is important in determining the MAPK.

### Table 1. Predicted Hill coefficients for MAPK cascade components: Varying the assumed enzyme concentrations

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Range of assumed concentrations</th>
<th>MAPKKK</th>
<th>MAPKK</th>
<th>MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPKK</td>
<td>0.6–15 nM (3 nM&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>0.9–1.0</td>
<td>1.6–1.7</td>
<td>3.8–5.1</td>
</tr>
<tr>
<td>MAPKK</td>
<td>0.24–6 μM (1.2 μM&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>1.0</td>
<td>1.4–1.9</td>
<td>2.4–9.1</td>
</tr>
<tr>
<td>MAPKK</td>
<td>24–600 nM</td>
<td>1.0</td>
<td>1.6–1.7</td>
<td>2.5–5.1</td>
</tr>
</tbody>
</table>

The assumed concentrations of each enzyme were individually varied over the ranges shown, with the assumed concentrations of the other five enzymes held constant. The effective Hill coefficients were calculated from the steepness of the predicted stimulus/response curves, as described in the text.

The numbers shown in parentheses are estimated values for the concentrations of Mos (a MAPKKK), Mek-1 (a MAPKK), and p42 MAPK (a MAPK) in *Xenopus* oocytes. We initially assumed [E2] to be 0.3 nM, [MAPKKK P′ase] to be 0.3 nM, and [MAPK P′ase] to be 120 nM. See text for details.
response; zero-order ultrasensitivity contributes to the overall response of the cascade.\(^3\)

The Role of Dual Phosphorylation. As shown in Table 3, the high degree of ultrasensitivity was found to depend critically upon the assumption that the dual phosphorylation of MAPKK and MAPK occurred by two-collision mechanisms. Assuming a single-collision, processive mechanism for phosphorylation of both kinases reduced the predicted Hill coefficient for MAPK from 1.7 to 1.3 and reduced the predicted Hill coefficient for MAPK from 4.9 to 1.5. Both of the two-collision mechanisms were found to contribute to the steepness of the MAPK stimulus/response curve, whereas the steepness of the MAPKK stimulus/response curve depended only on the assumption that MAPKK was activated by a two-collision mechanism (Table 1). A single-collision mechanism for both MAPKK and MAPK activation would permit high degrees of ultrasensitivity only if the concentration of MAPKK present in cells were substantially higher than what has been measured for Mek-1.

The connection between a two-collision mechanism for kinase activation and a sigmoidal, ultrasensitive response can be rationalized by considering the expected stimulus/response curves for the first and the second phosphorylation of one of the enzymes (e.g., MAPKK) for the special case of a small input stimulus. The first phosphorylation is driven by a linearly increasing input stimulus (MAPKK\(^*\)) and a constant concentration of its substrate (MAPKK). The result is that the rate and equilibrium level of phosphorylation of the substrate increase linearly with the input stimulus. The second phosphorylation is driven by a linearly increasing input stimulus (MAPKK\(^*\)) and a linearly increasing substrate concentration (singly phosphorylated MAPKK), so the rate and equilibrium of the second phosphorylation increase as the square of the input stimulus. Thus, the stimulus/response relationship initially curves upward. The response must approach a limit as the stimulus is increased to high levels, and the overall result is a sigmoidal stimulus/response relationship.

Summary of Calculations. The MAPK cascade is predicted to exhibit ultrasensitivity, with the degree of ultrasensitivity increasing as the cascade is descended. This behavior is robustly predicted for a wide range of assumed concentrations and \(K_m\) values for the cascade enzymes and reactions, although the exact extent of the predicted ultrasensitivity varies as the assumed values are varied.

Experimental Studies of MAPK and MAPKK Activation in Xenopus Oocyte Extracts. We chose to test the predicted ultrasensitivity in a highly manipulable experimental system, Xenopus oocyte extracts (20, 28, 29). These extracts possess a MAPK (p42 MAPK/Erk2), a MAPKK (Mek-1/MKK-1), and Mos activating enzymes, but no Mos (the relevant MAPKK). We added various concentrations of recombinant malE-Mos to the extracts and monitored the responses of Mek-1 and p42 MAPK.

### Table 2. Predicted Hill coefficients for MAP kinase cascade components: Varying the assumed \(K_m\) values

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Range of assumed (K_m) values</th>
<th>Range of effective Hill coefficients (nH) predicted for</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MAPKKK (\rightarrow) MAPKKK(^*)</td>
<td>60–1500 nM</td>
<td>MAPKKK MAPKK MAPK</td>
</tr>
<tr>
<td>2. MAPKKK(^*) (\rightarrow) MAPKKK</td>
<td>60–1500 nM</td>
<td>1.0 1.7 4.9</td>
</tr>
<tr>
<td>3. MAPKKK (\rightarrow) MAPKK-P</td>
<td>60–1500 nM</td>
<td>1.0 1.7 4.9</td>
</tr>
<tr>
<td>4. MAPKK-P (\rightarrow) MAPK</td>
<td>60–1500 nM</td>
<td>1.0 1.5–3.6</td>
</tr>
<tr>
<td>5. MAPKK-P (\rightarrow) MAPKK-PP</td>
<td>60–1500 nM</td>
<td>1.0 2.3–3.8</td>
</tr>
<tr>
<td>6. MAPK-P (\rightarrow) MAPK-P</td>
<td>60–1500 nM</td>
<td>1.0 2.4–4.1</td>
</tr>
<tr>
<td>7. MAPK (\rightarrow) MAPK-P</td>
<td>60–1500 nM (300 nM)</td>
<td>1.0 2.6–3.7</td>
</tr>
<tr>
<td>8. MAPK (\rightarrow) MAPK</td>
<td>60–1500 nM</td>
<td>1.0 2.6–3.7</td>
</tr>
<tr>
<td>9. MAPK (\rightarrow) MAPK-PP</td>
<td>60–1500 nM</td>
<td>1.0 2.6–3.7</td>
</tr>
<tr>
<td>10. MAPK-PP (\rightarrow) MAPK-P</td>
<td>60–1500 nM</td>
<td>1.0 2.6–3.7</td>
</tr>
</tbody>
</table>

The assumed \(K_m\) values for each reaction were individually varied over the ranges shown, with the assumed \(K_m\) values for the other nine reactions held constant. The effective Hill coefficients were calculated from the steepness of the predicted stimulus/response curves, as described in the text.

\(^{†}\)The \(K_m\) value for reaction 7 has been measured to be 300 nM for the phosphorylation of a mammalian MAPKK by a MAPKK (N. Ahn, personal communication). All of the other \(K_m\) values were initially assumed to be 300 nM as well.

### Table 3. Predicted Hill coefficients for MAPK cascade components assuming one-step (processive) or two-step (distributive) models for the phosphorylation of MAPK and MAPKK

<table>
<thead>
<tr>
<th>Model</th>
<th>Effective Hill coefficient (nH) predicted for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-step phosphorylation for MAPKK activation; One-step phosphorylation for MAPK activation</td>
<td>MAPKKK MAPKK MAPK</td>
</tr>
<tr>
<td>Two-step phosphorylation for MAPKK activation; Two-step phosphorylation for MAPK activation</td>
<td>1.0 1.3 1.5</td>
</tr>
<tr>
<td>Two-step phosphorylation for MAPKK activation; Two-step phosphorylation for MAPK activation</td>
<td>1.0 1.3 2.0</td>
</tr>
<tr>
<td>Two-step phosphorylation for MAPKK activation; Two-step phosphorylation for MAPK activation</td>
<td>1.0 1.7 3.7</td>
</tr>
<tr>
<td>Two-step phosphorylation for MAPK activation</td>
<td>1.0 1.7 4.9</td>
</tr>
</tbody>
</table>
enzymes can be accounted for by the known reactions of the MAPK cascade, if it is assumed that MAPK and MAPKK are activated by two-collision mechanisms and that the reactions that activate and inactive MAPK are partially saturated. Clearly it will be of interest to test the validity of these assumptions, to determine whether the scheme shown in Fig. 1 is incomplete in some important way.

We suspect that ultrasensitivity is important for the biological function of the MAPK cascade. The cascade should be able to filter out noise—respond less to small stimuli than a single Michaelis–Menten enzyme would—and then flip from off to on over a narrow range of input stimuli. This sort of behavior would be particularly appropriate for a signaling system that mediates processes like mitogenesis, cell fate induction, and oocyte maturation, where cells switch rapidly between discrete states without assuming stable intermediate positions.

**Note Added in Proof.** We have recently determined that MAPK is phosphorylated by MAPKK through a two-collision, distributive mechanism *in vitro*, and that the MAPK kinase phosphate activity in an oocyte extract is partially saturated under physiological conditions (apparent $K_m \approx 300 \mu M$). These findings validate key assumptions that underpinned the prediction of a highly ultrasensitive response for MAPK.

We thank B. Osgood and R. Pattis for aiming us toward Mathematics; G. Vande Woude for providing malE-Mos plasmids; M. Cobb, J. Cooper, T. Geppert, and J. Posada for providing MAPK plasmids; N. Ahn and J. Thorner for communicating unpublished results; and members of the Ferrell laboratory for helpful comments on this manuscript. This work was supported by National Institutes of Health Grant GM46383 and by a Faculty Development Award from the Pharmaceutical Research and Manufacturers of America Foundation.

As shown in Fig. 3 A and B, the activation of p42 MAPK was found to be a steep sigmoidal function of the amount of malE-Mos added to the extract, as predicted. The steepness of the response was evident both from the myelin basic protein kinase assay data (Fig. 3A) and from the mobility shift data (Fig. 3B). Mek-1 activation was found to be a less steeply sigmoidal function of the amount of malE-Mos added to the extract (Fig. 3C). The experimental data are in excellent agreement with the predicted $n_H = 4.9$ (p42 MAPK) and $n_H = 1.7$ (Mek-1) curves (Fig. 3 A and C). The amount of malE-Mos needed to half-maximally activate p42 MAPK was lower than the amount needed to half-maximally activate Mek-1 (Fig. 3 A and C), again as predicted.

**DISCUSSION**

Here we have demonstrated that the MAPK cascade converts graded inputs into switch-like outputs. The stimulus/response curves of the enzymes in the cascade become progressively steeper—more highly ultrasensitive—as the cascade is descended. The shapes of the stimulus/response curves of the enzymes can be accounted for by the known reactions of the MAPK cascade, if it is assumed that MAPK and MAPKK are activated by two-collision mechanisms and that the reactions that activate and inactive MAPKK are partially saturated. Clearly it will be of interest to test the validity of these assumptions, to determine whether the scheme shown in Fig. 1 is incomplete in some important way.