Signal Interpretation

• Static and dynamic responses
• Decision making
• Feedback and feedforward loops
What are the cells sensing?

1995, Marshall – the duration of signaling can determine response:

- Long signal – differentiation/Short signal – proliferation
- Long signal – response/ Transient signal – none
Mitotic Cell Cycle

life of a cell from its origin in cell division until it divides in two
S phase (synthesis)

- **DNA replication**

Each chromosome has 2 sister chromatids
Persistent ERK signaling induces S-phase entry.

Transient signaling does not.

How do cells sense the duration of signaling?

Feedforward loop – mechanism for sensing the duration of signaling

p.E187-8, 2002
compare

**a**

- c-Fos
- P T325
- ERK1
- ERK2
- P ERK1/2
- ERK1
- ERK2
- MAPK

EGF: 0, 5, 45, 60, 90, 120, 300 (min)

**b**

- c-Fos
- P T325
- ERK1
- ERK2
- P ERK1/2
- ERK1
- ERK2
- MAPK

PDGF: 0, 5, 45, 60, 90, 120, 300 (min)
### Table 1. DEF domains identified in immediate early gene products

<table>
<thead>
<tr>
<th>Protein</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Fos</td>
<td>GPMVTELEPLCTP-VVTCTPSCTTYSFVFTYPEEADS</td>
</tr>
<tr>
<td>Fra-1</td>
<td>G-P-VLEPEALHTPTLMT-TPSLTPFTPSLVTYPSTPEP</td>
</tr>
<tr>
<td>Fra-2</td>
<td>GGFYGB-EPLHTP-IVVTSTPAITPGTSNLVFTYPSVLEQ</td>
</tr>
<tr>
<td>FosB</td>
<td>HSEVQV--LGDPPVY--S--YTSSFVLTCEPVSAF</td>
</tr>
<tr>
<td>JunD</td>
<td>LLASPDLLKLKASPELRLIIQS-NGLVTTPPTST-QFLYPKV</td>
</tr>
<tr>
<td>JunB</td>
<td>QGQGDSGTGASLKLASSELERLIVPNSSNGVTITPTPQPQYYPYPRG</td>
</tr>
<tr>
<td>c-Jun</td>
<td>LLTSPDVLKLLKLASPELRLIIQSSNGHTTPPTQPLCPKN</td>
</tr>
<tr>
<td>c-Myc</td>
<td>LPPTPPLSPPRSSGCSPSYV//LTA--AASECIDPSVFPYPLYND</td>
</tr>
<tr>
<td>N-Myc</td>
<td>QSPGAGAAASPAGRHGGAGA//AHPAECEVDPAVVFPFPVNK</td>
</tr>
<tr>
<td>Egr-1</td>
<td>QSPPLSACVSNDSSPIYSAAPTFPTPNTD///PMIPDYLFPPQQ</td>
</tr>
<tr>
<td>mPer1</td>
<td>PRGQPPQLPPAPTSVPPAAFPAPLVTPMVALPNLYFPSPSY</td>
</tr>
</tbody>
</table>

DEF domains are in bold and numbers indicate amino acid position. Sequences are from rat (c-Fos, Fra-1 and Fra-2), mouse (FosB, JunD, c-Jun, c-Myc and Egr-1) or human (JunB, N-Myc and mPer1).

Prediction of a functional role of the DEF domain
Pharmacological proof of the feedback

- Egr-1
- Fra-2
- c-Jun
- JunB
- ERK1
- ERK2

<table>
<thead>
<tr>
<th>PDGF (min)</th>
<th>0</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
<th>210</th>
<th>DMSO</th>
<th>UO126</th>
</tr>
</thead>
</table>

Arrows indicate DMSO and UO126 treatments.
Detection of signal duration
Detection of signal duration

\[
\frac{dY}{dt} = F(X, T_y) - aY \\
\frac{dZ}{dt} = F(X, T_y)F(Y, T_z) - aZ
\]

\[a = 1, \ T_z = T_y = 0.5, \ F(u, T) = \begin{cases} 
0, & u < T \\
1, & u \geq T 
\end{cases}\]

\[X(t) = \begin{cases} 
1, & t < t_w \\
0, & t \geq t_w 
\end{cases}\]

Q: What is the minimum pulse width that switches on Z?

- Solve analytically
- Check/illustrate numerically
Fig. 1. Schematic view of the MAPK cascade. Activation of MAPK depends upon the phosphorylation of two conserved sites [Thr-183 and Tyr-185 in rat p42 MAPK/Erk2 (4, 5)]. Full activation of MAPKK also requires phosphorylation of two sites [Ser-218 and Ser-222 in mouse Mek-1/MKK1 (6–10)]. Detailed mechanisms for the activation of various MAPKKKs (e.g., Raf-1, B-Raf, Mos) are not yet established; here we assume that MAPKKKs are activated and inactivated by enzymes we denote E1 and E2. MAPKK* denotes activated MAPKK. MAPKK-P and MAPKK-PP denote singly and doubly phosphorylated MAPKK, respectively. MAPK-P and MAPK-PP denote singly and doubly phosphorylated MAPK. P’ase denotes phosphatase.
Fig. 2. Predicted stimulus/response curves for MAPK cascade components calculated by numerical solution of the rate equations for the MAP kinase cascade. (A) Predicted responses (solid lines) on a linear plot. The input stimulus is expressed in multiples of the EC$_{50}$, the concentration of E$_{1_{tot}}$ that produces a 50% maximal response. The dashed lines are Hill equation curves whose steepness (the ratio of their EC$_{90}$ to EC$_{10}$) is the same as the steepness of the calculated curves. (B) A semi-logarithmic plot of the predicted responses. Here the input stimulus (E$_{1_{tot}}$) is expressed in absolute, rather than relative, terms.
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>1.0 1.7 4.9</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. MAPKK $\rightarrow$ MAPKK-P</td>
<td>60–1500 nM</td>
<td>1.0 1.3–2.3 4.0–5.1</td>
</tr>
<tr>
<td>4. MAPKK-P $\rightarrow$ MAPKK</td>
<td>60–1500 nM</td>
<td>1.0 1.5–1.9 3.6–6.7</td>
</tr>
<tr>
<td>5. MAPKK-P $\rightarrow$ MAPKK-PP</td>
<td>60–1500 nM</td>
<td>1.0 1.3–2.4 3.8–5.2</td>
</tr>
<tr>
<td>6. MAPKK-PP $\rightarrow$ MAPKK-P</td>
<td>60–1500 nM</td>
<td>1.0 1.7–1.8 4.1–6.4</td>
</tr>
<tr>
<td>7. MAPK $\rightarrow$ MAPK-P</td>
<td>60–1500 nM (300 nM†)</td>
<td>1.0 1.7 3.7–6.2</td>
</tr>
<tr>
<td>8. MAPK-P $\rightarrow$ MAPK</td>
<td>60–1500 nM</td>
<td>1.0 1.7 4.3–5.2</td>
</tr>
<tr>
<td>9. MAPK-P $\rightarrow$ MAPK-PP</td>
<td>60–1500 nM</td>
<td>1.0 1.7 3.4–6.1</td>
</tr>
<tr>
<td>10. MAPK-PP $\rightarrow$ MAPK-P</td>
<td>60–1500 nM</td>
<td>1.0 1.7 4.7–5.1</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 MAPK 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;</td>
<td>MAPKKKK</td>
</tr>
<tr>
<td>One-step phosphorylation for MAPK activation</td>
<td>1.0</td>
</tr>
<tr>
<td>One-step phosphorylation for MAPKK activation;</td>
<td>1.0</td>
</tr>
<tr>
<td>Two-step phosphorylation for MAPKK activation;</td>
<td>1.0</td>
</tr>
<tr>
<td>Two-step phosphorylation for MAPKK activation;</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Biochemical experiment: purified enzymes, cellular extracts

Is the effect observed in cells?
Xenopus laevis oocyte maturation
Good model for studying cell cycle regulation

MPF purified using “maturation assay”
Control of Xenopus oocyte maturation
• Population average – no cooperativity
• Single cells – Hill coefficient is MUCH larger than expected
Many bistable networks can be activated by the same stimulus.

A different kinase cascade: JNK - SAPK

Many bistable networks can be activated by the same stimulus.
A different input – osmotic stress

Strong cooperativity – even at the population average level
Memory based on bistability
Irreversibility of responses
Simple models

1. No feedback
2. Linear Feedback
3. Nonlinear Feedback
Simple model of bistability

\[
\frac{dA^*}{dt} = k_1 S (A_T - A^*) + k_2 (A_T - A^*) \frac{A^{*n}}{\Gamma + A^{*n}} - k_{-1} IA^*
\]

\[
\alpha \equiv \frac{k_1 S}{k_{-1} I} \quad \text{strength of input}
\]

\[
\beta \equiv \frac{k_2}{k_{-1} I} \quad \text{strength of feedback}
\]

\[
\gamma \equiv \frac{\Gamma}{A^n} \quad \text{cooperativity of feedback}
\]

\[
\alpha (1 - a) + \beta (1 - a) \frac{a^n}{\gamma + a^n} - a = 0 \quad \text{Steady states}
\]
effect of feedback strength

\[ \frac{[A^*]}{[A_{tot}]} \]

stimulus, \( k_1[S]/k_{-1} \)

all @\( n=3 \)

- \( \beta=1 \) (black)
- \( \beta=1.5 \) (blue)
- \( \beta=2 \) (green)
- \( \beta=3 \) (red)
\[ n=3; \quad g=0.1; \]
\[ a=\text{linspace}(0.001, 0.999, 3000); \]
\[ \beta = \frac{(g+a.\,^n)^2}{g*n*(a.\,^{(n-1)})*((1-a)^2)}; \]
\[ \alpha = \frac{a}{(1-a)} - \beta*(a.\,^n)/(g+a.\,^n); \]
\[ \text{plot}(\alpha, \beta, \text{'k'}); \]
Feedforward and Feedback Loops

(transcriptional autoregulation and postranslational stabilization)

Multiple roles of ERK signaling
• Induction of transcription
• Protein stabilization
• Protein-protein interaction

Again, the network can be activated only by persistent signals