Dynamic Topology of Biological Networks

Functional Consequences

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The CA1 hippocampal neuron as a network of interacting components

<table>
<thead>
<tr>
<th>Number of nodes (components)</th>
<th>546</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of links (connections)</td>
<td>1259</td>
</tr>
<tr>
<td>Number of references</td>
<td>1202</td>
</tr>
<tr>
<td>Activation links</td>
<td>690</td>
</tr>
<tr>
<td>Inhibitory links</td>
<td>306</td>
</tr>
<tr>
<td>Neutral links</td>
<td>263</td>
</tr>
<tr>
<td>Binding</td>
<td>793</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>277</td>
</tr>
<tr>
<td>Dephosphorylation</td>
<td>58</td>
</tr>
<tr>
<td>Enzymatic cleavage</td>
<td>44</td>
</tr>
<tr>
<td>Channels to Ions</td>
<td>19</td>
</tr>
<tr>
<td>GEF (small GTPase exchange)</td>
<td>28</td>
</tr>
<tr>
<td>GAP (GTPase activation)</td>
<td>18</td>
</tr>
<tr>
<td>Ubiquitination</td>
<td>9</td>
</tr>
<tr>
<td>Other enzymatic reactions</td>
<td>13</td>
</tr>
</tbody>
</table>

Central Signaling Network (312)

- Extracellular Ligands (33)
- Receptors and other membrane proteins (63)
- Ion Channels (16)
- Motility Machinery (25)
- Translation Machinery (37)
- Secretory Apparatus (27)
- Transcription Machinery (33)
The Cell as a Directed Graph

How do we identify regulatory patterns in such complex systems?

Network Sciences (graph theory)

Multiple levels of representation of cellular interactions as networks

We currently use directed graphs (c) for our studies
Methods to study small to large biochemical systems range from simple to complex approaches.

<table>
<thead>
<tr>
<th>Low</th>
<th>System complexity</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single gene</td>
<td>Small genetic circuit</td>
<td>Mid-size genetic network</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Great detail</th>
<th>Computer modeling</th>
<th>Less detail</th>
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</thead>
<tbody>
<tr>
<td>Stochastic molecular simulations</td>
<td>Differential equations</td>
<td>Discrete dynamics (connected switches)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single gene dynamics</th>
<th>Information</th>
<th>System dynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins over time</td>
<td>Gene activity over time</td>
<td>Flow pattern of network states</td>
</tr>
</tbody>
</table>

S. Bornholdt Science 310, 5747 (2005)
Recruitment of motifs within specified paths from receptor to effectors as connectivity propagates.

NE, which induces plasticity (state change) in CA1 neuron induces more +ve motifs as compared to –ve motifs as signal (functional connectivity) propagates through the network.

Preferential recruitments of positive motifs may trigger processes that lead to state change in cells.
Maps Defining Functional Locations of Motifs Between Ligands and Cellular Machines

\[
\text{MLI} = \frac{\sum_{i=1}^{n} \left( \frac{\text{CPLM}_i}{\text{CPLM}_i + \text{CPLL}_i} \right)}{n}
\]
Properties of Network Motifs

Feed-forward Motif
- Provides redundancy
- Leads to signal prolongation
- Coincidence detection

Feedback Loop
- Signal amplification
- Leads to signal prolongation
- Bistability and switching

Bifan Motif
- Noise filtering
- Coincidence detection
- Signal sorting

Three common motifs originally described by Uri Alon and colleagues

Bistable behavior of the positive feedback loop

Ordinary differential Eq based- Models

Stimulation of Proliferation

Upi Bhalla
Science (1999) 283:381
Bifan network motifs
Signal processing by the p38/JNK protein kinases cross regulating the transcription factors ATF2/Elk-1

Azi Lipshatz
Sudarshan Purshottaman
Avi Ma’ayan
Bifans are the most abundant network motifs in biological regulatory networks

Two protein kinases phosphorylate two transcription factors
Comparing OR and AND Gating by Bifans

OR gates are sharp and transient
AND gates prolong signals and are shallower
Bifans can Filter Noise

Smaller output from bifans when input signals are choppy
Extending the Bifan by Adding c-Jun

The context of the bifan affects the dynamics
Extending the circuit by adding transcriptional feedback

The bifan configuration can be used to control TFs homo and hetrodimer concentrations to tightly regulate gene expression.
Conclusions

- OR gate bifans produce transient with high amplitude output
  AND gate bifans prolongs signals and produce shallower output

- Bifans can filter noise

- The context of the bifan dramatically affects the signal output

Models of transcriptional feedback coupled with bifans show that the bifan configuration can be used to regulate homo and hetrodimer TFs concentrations
A feedforward motif and its functional significance

Modulation of Kidney Podocyte Differentiation by a Protein Tyrosine Phosphatase-SL-mediated Feedforward Motif from β-adrenergic receptors to CREB

Narat John Eungdamrong
John Cijiang He
Feed Forward Motifs

FFMs in Networks

Signaling System

Process Control

Social Network

Figure 1. Feedforward Control
Various types of FFMs

“coherent”

“incoherent”
Coherent FFM can yield prolonged outputs

- Coherent FFM with an OR gate (e.g. Z can be activated by either X or Y) can prolong output.

- This results from the time required for Y to deactivate.

- Difference in the kinetics of the “long” and “short” pathways does not significantly alter the motif behavior.
Coherent OR FFM can contribute to prolonged signaling in transcriptional networks

(Mol Syst Biol. 2005 Mar 29.)
Key Questions

• What is the regulatory role of FFMs in signaling networks?

• Can FFM sustain signaling in absence of a PFL?

• Does modulation of FFMs result in meaningful functional changes?
What is the regulatory role of FFMs in signaling networks?
Increasing pathlength increases length of output signal.
Effect of multisite phosphorylation on FFM behavior

input

kinase 1A

kinase 1B

kinase 1B-P

kinase 1B-PP

kinase 2B

kinase 2B-P

kinase 2B-PP

kinase 3B

kinase 3B-P

kinase 3B-PP

output
Noise Filtering by ultrasensitive FFM

stimulus

amplitude threshold

duration threshold
Conclusions

- Simple FFM can sustain signaling.
- Phosphatase activity sets the timescale for signaling.
- Motif behaviors are relatively robust to perturbation in [input] and changes in kinetic parameters.
- Increasing pathlength can prolong signal output.
- Incorporation of an ultrasensitive cascade allows detection of stimulus strength (amplitude & duration).
Can a feedforward motif prolong signal output in absence of a positive feedback loop?
CREB activation as an experimental model for studying FFM

- CREB, a key regulator of gene expression, is activated by phosphorylation on Ser-133.

- CREB is responsive to multiple intracellular signaling pathways (e.g. Ser133 is phosphorylated by PKA, and MSK)
A Feedforward Motif in CREB Activation in Kidney Podocytes

Diagram showing the pathway:
- β2AR
- GRK
- Gs
- AC
- cAMP
- PKA
- bRaf
- MEK
- MAPK
- Phosphatase
- CREB
- MSK
- PDE
- [cAMP (μM)]
- [nuclear PKA (μM)]
- [MSK (μM)]

Graphs showing changes over time:
- [cAMP (μM)] vs. t (min.)
- [nuclear PKA (μM)] vs. t (min.)
- [MSK (μM)] vs. t (min.)
MEK Inhibition Blocks Sustained CREB Activation

![Graph showing the effect of MEK inhibition on CREB activation](image)

- **Control**
- **U0126** (MEK inhibitor)

**Graph Details**
- **X-axis:** Time (min.)
- **Y-axis:** Normalized CREB* (AU)

**Experimental Conditions**
- **iso**
- **iso + U/PD**

**Time Points:** 0', 5', 10', 15', 30', 45'
PKA inhibition indicated a more complex network


PKA inhibition indicated a more complex network

Initial Conclusions

• FFM can prolong CREB signaling in kidney podocytes

• Sustained signaling requires both PKA and MAPK signaling ("coincidence detection")
  – Synergy between pathways, not simply additive

• Experiments indicate the following:
  – PKA-independent mechanism of MAPK activation
  – Potential interactions between PKA and MAPK pathways in prolonging output signals
MAPK Can be Activated via a PKA-independent Mechanism

EPAC Agonist Activates MAPK in Kidney Podocytes

8pMeOPT-cAMP  
EPAC specific agonist

6-Bnz-cAMP  
PKA specific agonist

MAPK1,2**
Total MAPK1,2
Modulation of MAPK activity via a Spatially Specific Feedback Loop

- PTP-SL contains a substrate recognition domain (KIM) in its noncatalytic region.

- Phosphorylation of the KIM of PTP-SL by PKA inhibits PTPSL's association with and the tyrosine dephosphorylation of ERK1/2 and p38.

- Nuclear translocation of ERK1/2 and p38, in the presence of PTP-SL, is favored upon activation of PKA.

- We hypothesize that Rp-cAMP treatment lifts PKA-dependent inhibition.

Thus, PTP-SL can retain MAPK in the cytoplasm and prevent its translocation to the nucleus.
PKA regulates nuclear localization of MAPK* by PTP-SL Inhibition
Nested Feedforward loops regulate the duration of CREB activation
Conclusions from 2\textsuperscript{nd} round models

- Nesting results in CREB control of CREB activation by time-dependent AND and OR gates.

- Iso stimulation activates MAPK through a PKA-independent mechanism (Epac/Rap1/BRaf).

- Rp-cAMP treatment did not strongly affect total cellular MAPK activity. However, persistent activation of CREB is strongly inhibited (due the spatial regulation).

- PKA modulates nuclear localization of MAPK* through inhibition of PTP-SL.

  *Immunofluorescence experiments indicate that PKA dynamically regulates the cellular location of MAPK signaling*

- Simulation of the nested FFM network qualitatively captures the salient features of CREB activation seen in the experiments.
What is the physiological function of FFM?
Modulation of Podocytes Differentiation by PTP-SL

Synaptopodin is a marker for the differentiated state of podocytes

as the name suggests synaptopodin is required for the formation of the foot processes
Primary Kidney podocytes

In the differentiated state, Synaptopodin colocalizes with the actin filaments. Inhibitors that block the FFL also block colocalization of synaptopodin with the actin bundles.
Conclusions
Functions of feedforward motifs

• FFM can prolong signal output in a mammalian signaling network

• Coincidence detection and synergy between short and long paths emerge as a result of spatially specific nesting (via PTP-SL)

• Nested FFM modulates the proliferation-differentiation switch in kidney podocytes, as measured by synaptopodin expression.
Overall Conclusions

Regulatory motifs possess considerable information processing capabilities

Spatial specification of motifs may be critical in understanding their functional capabilities

Combining motifs by stacking and nesting can lead to complex behaviors that underlie decisions regulating changes in cell state