Introduction to Systems Biology
Lecture 12 Part B-1 Iyengar

From Model Predictions to Experiments

How can the predicted behavior in the simulations be tested experimentally ?

Use NIH -3T3 fibroblast cells
It is stimulated by a growth factor – plated derived growth factor (PDGF)

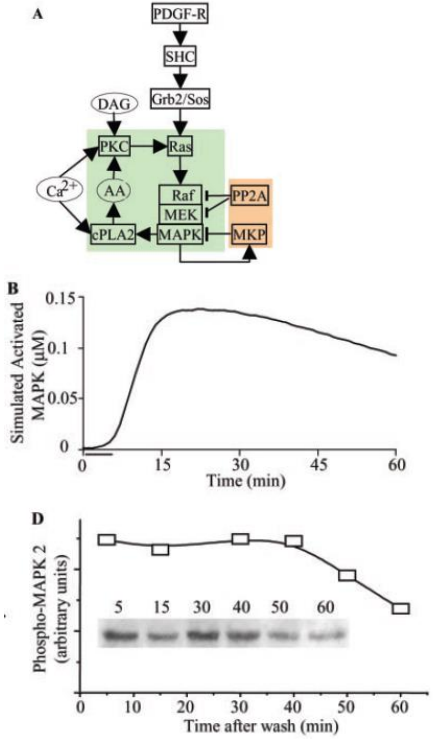
PDGF activates MAPK using the same pathway as EGF

B - Simulation

D - Experiment

Apply PDGF for a brief (5min) period
Wash cells to remove PDGF
Follow MAP-kinase activity

MAP-kinase activity is measured by immunoblotting
Using antibodies the recognize the activated form



Bhalla, Ram Iyengar (2002) Science 297 :1018

Introduction to Systems Biology
Lecture 12 Part B-2

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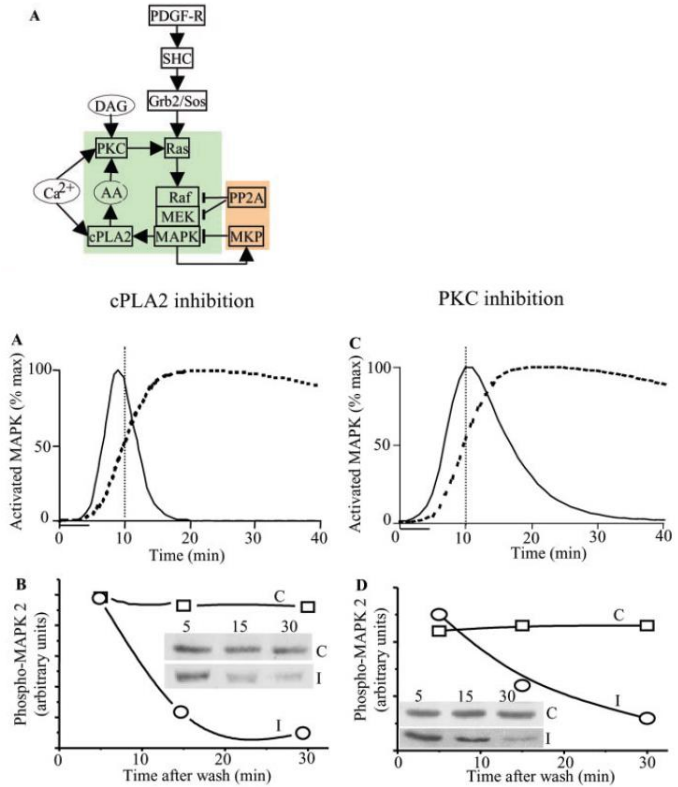
Testing the simulations by experiments to verify subnetwork topology

A and C – Simulations
Shows response for brief application of PDGF without (dotted line) and with Solid lines inhibition of other components

Experiments (B and D) in NIH-3T3 cells that show

That MAPK activity is transient with brief 5 min stimulation with PDGF if PKC or cPLA2 inhibitors are present.



Bhalla, Ram Iyengar (2002) Science 297 :1018

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Lecture 12 Part B-3

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Positive Feedback Loops as Flexible Systems

Can be both Analog and Digital?

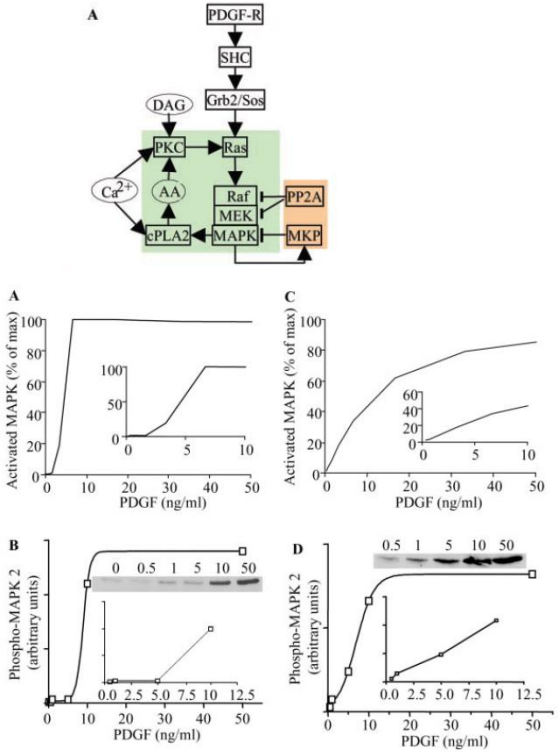
A and C are simulations:

A - basal MKP (~ 2nM) C- 10 nM MKP

B and D are experiments

Note A & B are switch like response to increasing PDGF while C and D are graded

At high MKP, although the loop is operative there is no switching



Bhalla, Ram Iyengar (2002) Science 297 :1018

Introduction to Systems Biology
Lecture 12 Part B-4

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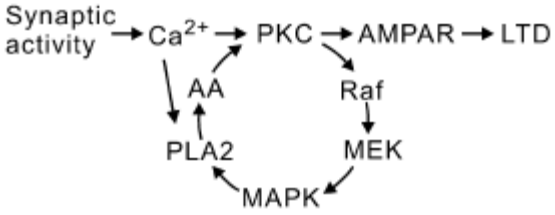
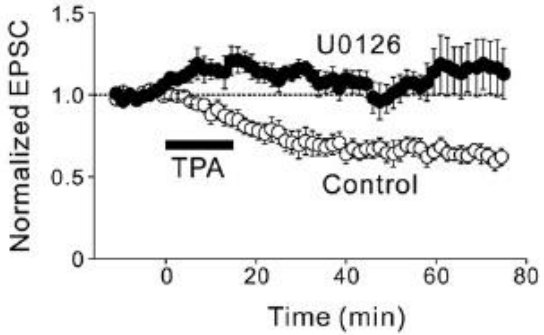
Positive Feedback Loop in Cerebellar Long-Term Depression

Synaptic plasticity in cerebellar Purkinje neurons involve Long-term depression (LTD) of synaptic responses (EPSC)

The electrical response can be evoked by activation of PKC with the chemical TPA

PKC evoked LTD is blocked by inhibition of MAPK by the compound U-0126





Tanaka & Augustine (2008) Neuron 59: 608-620

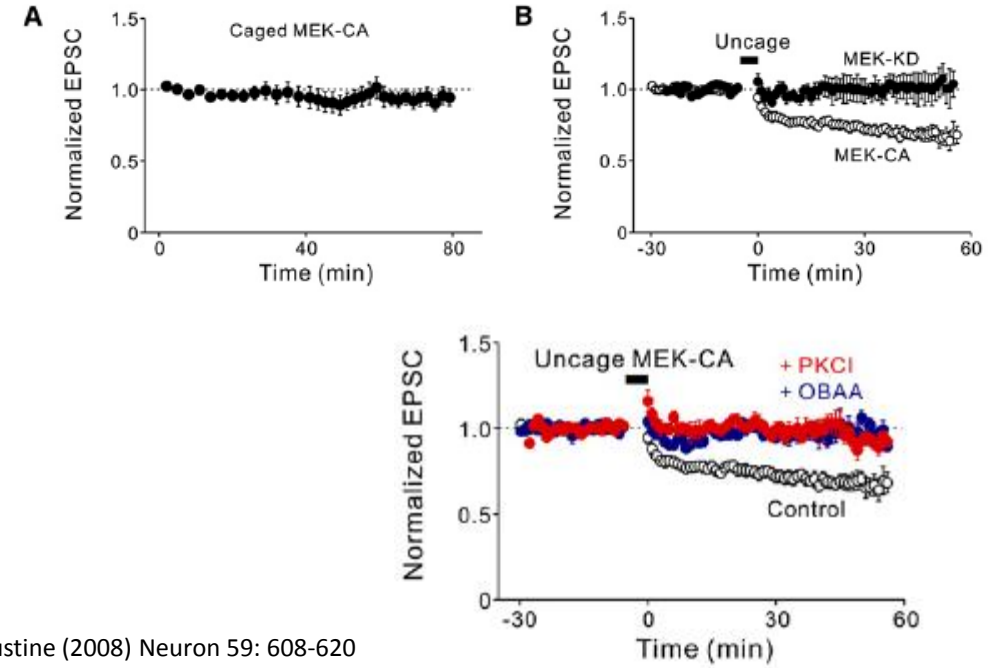
MAPK induced LTD is blocked by PKC inhibitor

MAPK in cerebellar neurons

A light activated form of MEK (Caged MEK-CA) is introduced Into neurons

Upon light activation (uncaging) LTD is obtained

Blocked by PKC inhibitor (PKCI) or PLA2 inhibitor - OBAA



Tanaka & Augustine (2008) Neuron 59: 608-620

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Lecture 12 Part B-6

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Conversion of short duration signal into long-lasting effects

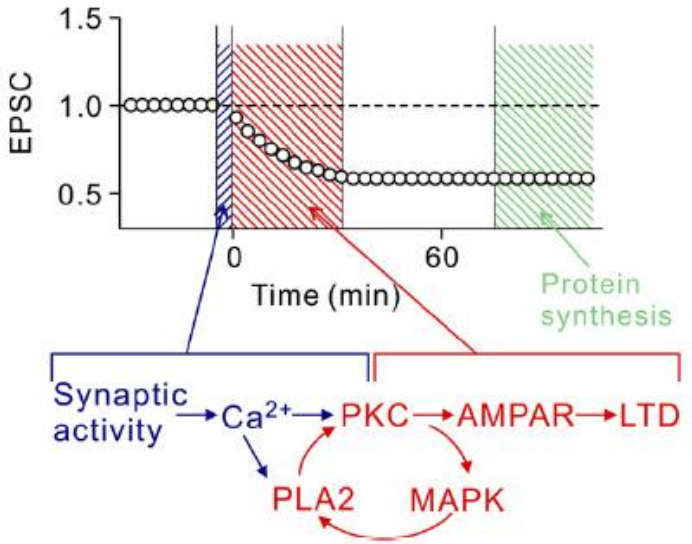
Very brief synaptic (electrical) activity (msec)

leads to brief (sec to min) Ca^{2+} elevation

PKC-MAPK- PLA2 positive feedback loop
Is engaged

Long duration regulation of AMPA channels

Manifestation of long-term depression



Tanaka & Augustine (2008) Neuron 59: 608-620

Introduction to Systems Biology
Lecture 12 Part B-7

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Hunger regulation in mice through bistability

Sternson and colleagues study the neuronal circuitry that controls hunger in mice

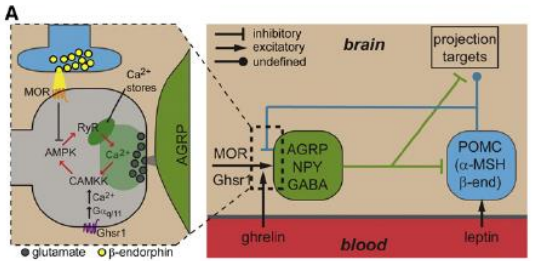
A class of neurons called AGRP neurons are activated by food deprivation. Excitatory input to AGRP neurons are activated

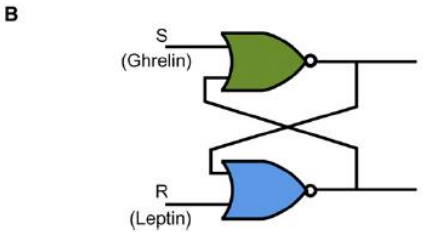
Grehlin – a hormone from gut is elevated in blood upon food deprivation

Grehlin activates a AMP-kinase dependent positive feedback loop in the presynaptic neuron
Transient grehlin- long duration activation of the synapse
Bistable synapse

5 min induction gives 3 -5 hr activation of synapse at AGRP neurons

Bistability enables the ability to remember that you are hungry!





Yang Y et al (2011) Cell 146: 992-1003

Lecture 12 Take Home Points

- Emergent properties such as bistability are systems level behaviors that arise from interactions between components of the system
- Coupled biochemical reactions in positive feedback loop can behave as bistable systems
- Bistable systems can be triggered by external stimuli – leading to stimulus dependent change in cell state
- Bistable systems function as memory storage devices
- Bistability is found in a range of biological systems in simple and complex organisms