Introduction to Systems Biology
Lecture 14 Part A-1

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Case Study : A Circadian Oscillator in Bacteria

Cyanobacterium *Synechococus elongatus*

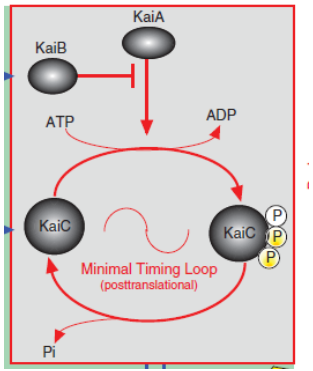
KaiC is the clock protein in this bacteria

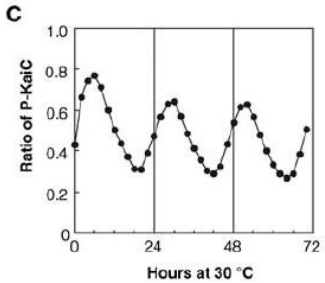
KaiC is a protein kinase and a protein phosphatase that autophosphorylates itself and also autodephosphrylates itself

KaiA stimulates KaiC autophosphorylation
KaiB inhibits the effect of KaiA

An in vitro reconstituted system consisting of just these three purified proteins is sufficient to produce a 24 hr cyclical phosphorylation of KaiC

Tomita J., Nakajima M., Kondo T. and Iwasaki H.(2005) Science 307: 251





Nakajima et al (2005) Science 308:414

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How this simple system of just three proteins produce these sustained circadian oscillations ?

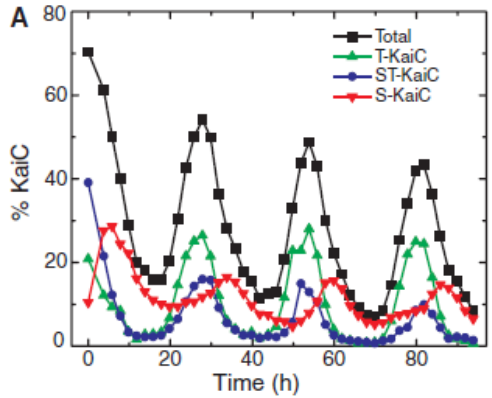
Total phosphorylation of KaiC cannot be the only dynamical variable - in a 24hr period the KaiC protein has the same level of phosphorylation twice, but going in opposite directions
So how does p-KaiC know which direction it should go in?

KaiC is phosphorylated at 2 sites:
Serine-431 and Threonine-432
The two states are phosphorylated with a 24 hr cycle but are phase separated Red vs. Green

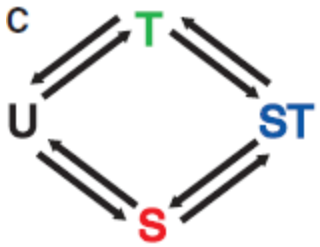
When KaiA is mixed with unphosphorylated KaiC
T432 – KaiC accumulates first then dually phosphorylated T432 , S431 KaiC and then S431- KaiC

When highly dually phosphorylated KaiC is incubated by itself it produces S431 KaiC

S431 KaiC - KaiA complex binds KaiB leading to inhibition of KaiA stimulation of the autokinase activity
The autophosphatase activity is unaffected by Kai B



Rust et al (2007) Science 318: 809



Fitting experimental data shows that a linear model of KaiC interconversion describes the process

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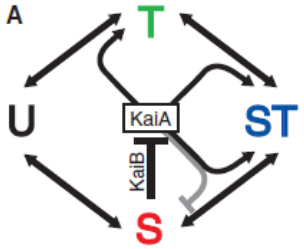
A computational model for the KaiC circadian oscillator

An ODE model with three phosphorylated states

S –Serine 431 T – Threonine-432 and
D is doubly phosphorylated

Kinetic parameters obtained from fitting of experimental phosphorylation data



$$k_{XY}(S) = k^0_{XY} + \frac{k^A_{XY} A(S)}{K_{1/2} + A(S)}$$

$$A(S) = \max\{0, [\text{KaiA}] - 2S\}$$

Rust et al (2007) Science 318: 809

" The key assumptions of this minimal model are :
(i) the concentrations of the three phosphorylated species are the only slow dynamical variables;

(ii) the interconversions between phosphoforms are first-order reactions with rates (table S2) that depend hyperbolically on the concentration of active KaiA (Fig. 4A and fig.S4); and

(iii) each S-KaiC monomer (together with KaiB) inactivates one KaiA dimer"

$$\frac{dT}{dt} = k_{UT}(S)\,U + k_{DT}(S)\,D - k_{TU}(S)\,T - k_{TD}(S)\,T \quad (1)$$

$$\frac{dD}{dt} = k_{TD}(S)\,T + k_{SD}(S)\,S - k_{DT}(S)\,D - k_{DS}(S)\,D \quad (2)$$

$$\frac{dS}{dt} = k_{US}(S)\,U + k_{DS}(S)\,D - k_{SU}(S)\,S - k_{SD}(S)\,S \quad (3)$$

$$A = \max\{0, [\text{KaiA}] - 2mS\} \quad (4)$$

$$k_{XY}(S) = k^0_{XY} + \frac{k^A_{XY} A(S)}{K_{1/2} + A(S)} \quad (5)$$

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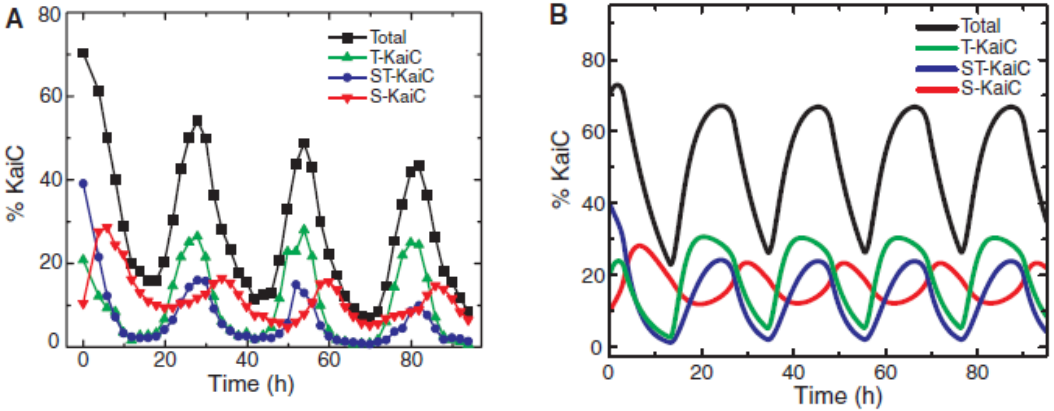
Rust et al (2007) Science 318: 809



Experiment

Simulation

The simple ODE model composed of a few reactions fully captures the observed circadian cycling of phosphorylated KaiC

Conclusions from the KaiC circadian cycle system

1. Coupled biochemical reactions can produce rhythmic oscillations even in the absence of any network motifs .

2. Appropriate relationships of interaction specificity such as only S431 – KaiC binding KaiB and appropriate rate constants are sufficient to produce complex behavior such as rhythmic oscillations

3. Although in this system the kinetic model is not used for predictions it is very useful in clearly proving that the empirically observed behavior arises only from the measured parameters and there are no hidden variables or mechanisms

   Model tells you that what you see is what you get !

Note- Mammalian circadian systems are much more complicated involving transcriptional processes

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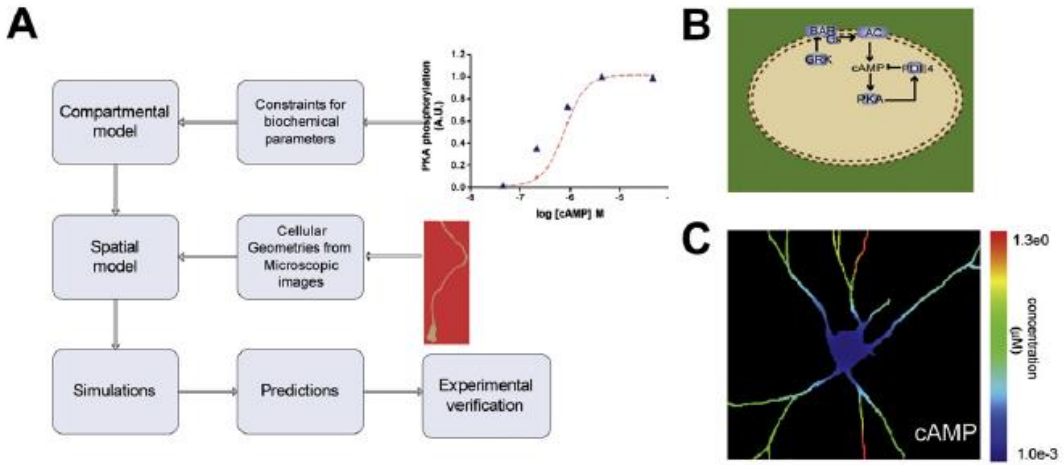
Case study: Understanding the dynamics of microdomains of signaling components within cells

When cells such as neurons are stimulated , activated signaling molecules transiently accumulate in small subcellular regions . These regions are called ***microdomains***

Questions :

How are microdomains formed ?

Can spatial information regarding microdomains be transmitted through signaling pathways?



Neves et al (2008) Cell 133, 666–680

To answer these questions we need partial differential equation (PDE) models

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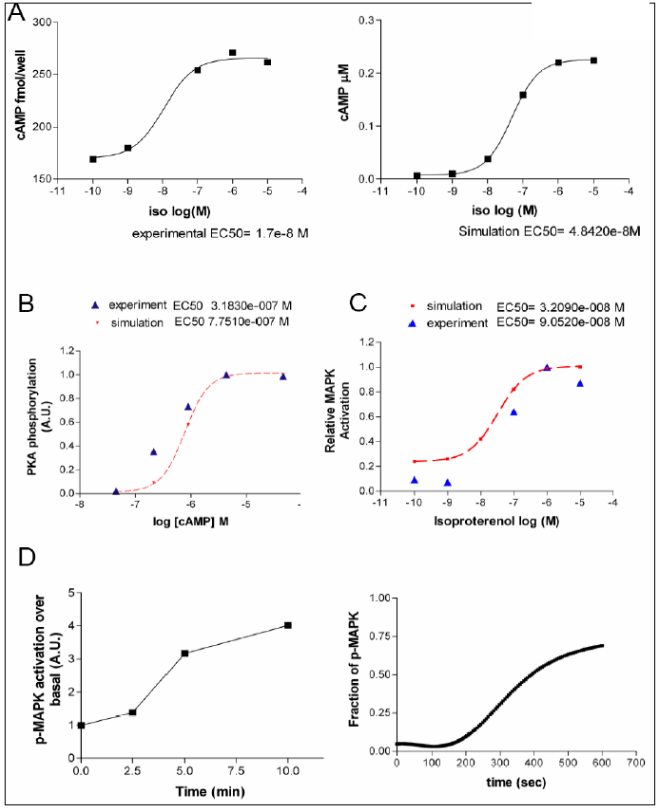
Model Development

To build PDE models
one can start with experimentally constrained ODE models

Examples of constraints for this model are shown here both for steady – state conditions and for time course

Such constrains of the core reactions ensure that model is a reasonable representation of the real system

There is still a limitation. These experiments (and hence constraints) are from tissue experiments and hence represent average behavior!



Neves et al (2008) Cell 133, 666–680

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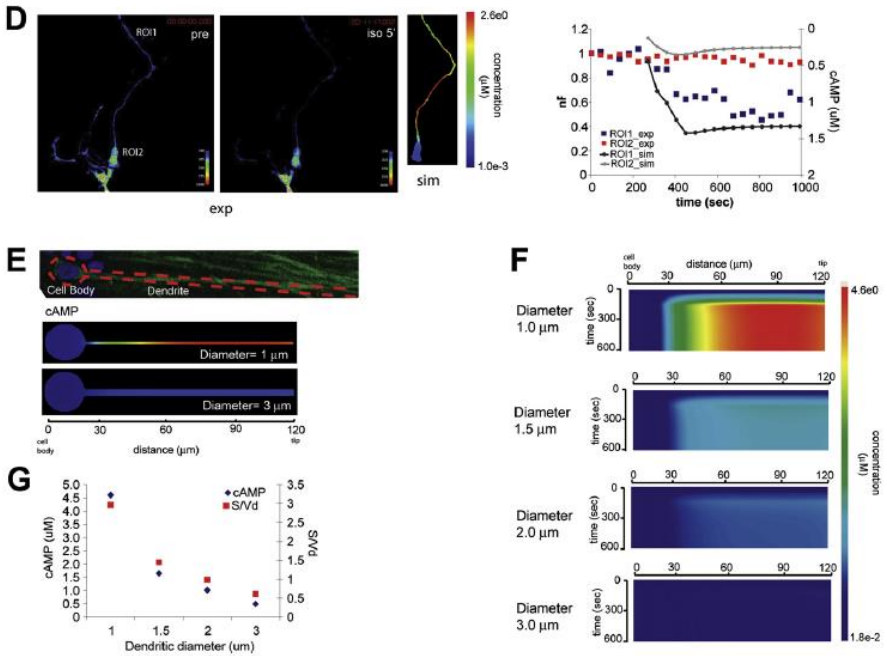
Matching simulations and experiments

Figure D shows how live cell imaging experiments (exp) showing cAMP accumulation can be matched to simulations (sim) in *Virtual Cell*

Simulations use a PDE model - : Trace cell shape into Virtual Cell – map the reactions onto the various regions. Impose a finite volume grid and compute

E, F, G - Simulations to show that the dendritic diameter is important for cAMP microdomains in the dendrite

S/V surface/volume phenomenon



Neves et al (2008) Cell 133, 666–680