Scaffold-mediated interaction between cAMP and the Raf/MEK/ERK pathway

Oana ANDREI

joint work with Muffy Calder, Walter Kolch, George Baillie, Kim Brown

DCS, University of Glasgow
May 26, 2009
Why formal methods?

Lab experiments

Results/Analysis

Model

suggest new hypotheses

simulate

biological knowledge, observations
Why formal methods?

Lab experiments

- to understand
- to predict
- to control

suggest new hypotheses

Results/Analysis

simulate

Model
Cell signalling

* communication between cells
* cellular processes: proliferation, cell growth, programmed cell death...
* malfunctions: cancer, diabetes, autoimmune diseases...
Cell signalling

- communication between cells
- cellular processes: proliferation, cell growth, programmed cell death...
- malfunctions: cancer, diabetes, autoimmune diseases...
Cell signalling

- **communication** between cells
- **cellular processes:** proliferation, cell growth, programmed cell death...
- **malfunctions:** cancer, diabetes, autoimmune diseases...
Cell signalling

- communication between cells
- cellular processes: proliferation, cell growth, programmed cell death...
- malfunctions: cancer, diabetes, autoimmune diseases...
Cell signalling

- communication between cells
- cellular processes: proliferation, cell growth, programmed cell death...
**Cell signalling**

- **communication** between cells
- **cellular processes**: proliferation, cell growth, programmed cell death...
- **malfunctions**: cancer, diabetes, autoimmune diseases...
Cell signalling

- Communication between cells
- Cellular processes: proliferation, cell growth, programmed cell death...
- Malfunctions: cancer, diabetes, autoimmune diseases...

Need of good, predictive models for guiding experimentation and drug development.
Scaffold proteins

A

B

C
Scaffold proteins
Scaffold proteins

A

B

C
Scaffold proteins
Scaffold proteins
Scaffold proteins
Scaffold proteins

* organisational role rather than a signalling role

  ▶ anchoring function (binding proteins)

  ▶ catalytic function (increasing/decreasing the output of a signalling cascade) under some conditions
Scaffold proteins

- organisational role rather than a signalling role
  - anchoring function (binding proteins)
  - catalytic function (increasing/decreasing the output of a signalling cascade) under some conditions

Do scaffolds make signals stronger, or faster, or do they just localize them?
AKAP
(A-kinase anchoring protein)
AKAP
(A-kinase anchoring protein)
AKAP
(A-kinase anchoring protein)
AKAP
(A-kinase anchoring protein)

AKAP

PKA

Raf-1

cAMP

PP

S338

S259
AKAP
(A-kinase anchoring protein)

PKA

Raf-1

cAMP

PDE8A

S259

S338

PP
AKAP
(A-kinase anchoring protein)

AKAP
PKA
Raf-1
PP

cAMP
PDE8A

S259
S338

+ -

+ -
AKAP
(A-kinase anchoring protein)
AKAP
(A-kinase anchoring protein)
AKAP
(A-kinase anchoring protein)
AKAP
(A-kinase anchoring protein)

AKAP

PKA

PDE8A

Raf-1

PP

cAMP

PDE8A

S259

S338
Formal model

* stochastic process algebra
* continuous time Markov Chains
* PRISM model checker
PRISM module

\begin{verbatim}
endmodule

module Raf
    RAF : [0..N] init raf_init;
    RAF_P : [0..N] init raf_p_init;

    [ dephospho_Raf ] (RAF < raf_max) & (RAF_P > 0) ->
        (raf_activate) : (RAF_P' = RAF_P-1) & (RAF' = RAF+1);
    [ phospho_Raf ] (RAF_P < raf_p_max) & (RAF > 0) ->
        (raf_deactivate) : (RAF_P' = RAF_P+1) & (RAF' = RAF-1);
endmodule

module PP
    PP : [0..N] init pp_init;
\end{verbatim}

* 3 abstract levels of concentrations: low (0), medium (1), high (N=2)
Markov chain

\[ s_0 \xrightarrow{r_1} s_1 \xrightarrow{r_2} s_4 \xrightarrow{r_4} s_5 \xrightarrow{r_6} \ldots \]

\[ \text{"init"} \xrightarrow{r_3} s_2 \xrightarrow{r_5} s_3 \xrightarrow{\ldots} \ldots \]
Our models have:

- $10^4$-$10^6$ states
- $10^5$-$10^7$ transitions
Quantitative analysis

- use of rewards (or costs) in CSL
  - real values assigned to states or transitions
  - to track variable values in states
  - to compute the expected value of a variable at a given time
**PRISM experiment: with scaffold**

- cAMP is diffused every 5 rounds from 10 with rate 1.0
- PKA phosphorylates PDE8A and S259 with the same rate

![Graph showing expected amounts over time](image-url)
PKA phosphorylates a very small amount of PDE8A compared to S259: PDE8A is not on the scaffold.

**PRISM experiment: without scaffold**

\[ \uparrow p\text{PDE8A} \rightarrow \downarrow \text{cAMP} \rightarrow \downarrow \text{PKA}^+ \rightarrow \uparrow \text{phosphorylated S259} \rightarrow \downarrow \text{Raf activity} \]
Temporal queries in CSL

* reward-based analysis
* temporal properties

- “cAMP goes below a certain level $k$ only if $PDE8$ goes above a level $k'$”

- use of derivatives to keep track of decreasing or increasing variable values
Necessary preceded

➡ requirement / necessary preceded pattern: a state $\phi$ is reachable and is necessary preceded all the time by a state $\psi$

$\phi = \downarrow \text{cAMP} \land \downarrow \text{PKA}^+$

$\psi = \uparrow \text{pPDE8A}$
Necessary preceded

$$\phi = \downarrow \text{cAMP} \land \downarrow \text{PKA}^+ \quad \psi = \uparrow \text{pPDE8A}$$

CTL:

$$\mathbf{EF} \phi \land \mathbf{AG}(\neg \psi) \Rightarrow \mathbf{AG}(\neg \phi)$$

CSL:

$$P_{>0}[\mathbf{F}\phi] \land P_{\leq 0}[\mathbf{F}(\neg(\neg \psi) \Rightarrow P_{\geq 1}[\mathbf{F}(\neg \phi)])]$$
Oscillations

\[ \phi = \uparrow pPDE8A \land \downarrow cAMP \land \downarrow PKA^+ \]

\[ \psi = \downarrow pPDE8A \land \uparrow cAMP \land \uparrow PKA^+ \]

**CTL:** \( AG ((\phi \Rightarrow EF \psi) \land (\psi \Rightarrow EF \phi)) \)

**CSL:**

\[ P_{\leq 0} EF (-(\phi \Rightarrow P_{>0}[F \psi])) \lor -(\psi \Rightarrow P_{>0}[F \phi])) \]
New hypothesis

- we introduce an inhibitor for PDE8
- either Dipyridamole (a drug causing vasodilation)
- or dominant negative PDE8
New hypothesis

- we introduce an inhibitor for PDE8
- either Dipyridamole (a drug causing vasodilation)
- or dominant negative PDE8

does the level of pS259 increase?
cAMP is diffused every 5 consecutive rounds every 10 rounds

pPDE8A degrades 5 times as much cAMP as PDE8A does

PKA equally phosphorylates PDE8A and Raf-S259

---

**PRISM experiment**

- Dipyridamole binds to PDE8A and inhibits it
Conclusions

- formal model of a biological process
- the biologists validated our results
- refine the model with more experimental data
- find new questions on the model and express them using a temporal logic