Studying Steady States in Biochemical Reaction Systems by Time Petri Nets

Louchka Popova-Zeugmann[1]
Elisabeth Pelz[2]

[1] Department of Computer Science, Humboldt University, Berlin, Germany,
[2] LACL, University Paris Est Créteil, Fac de Sciences, Créteil, France

June, 20 2011

Outline

1. Biological Reaction Systems and their Models
2. Steady state in a Biochemical Reaction System and SPNs
3. Time Petri Nets
4. A Running Example
5. Conclusion

Biological Reaction Systems and their Models

Biological reaction systems are usually modeled with

- a system of ordinary differential equations (ODEs) (+ a graph),
- a Stochastic Petri Net (SPN),
- ...

Relationship between a System of ODEs and a SPN

$\Longrightarrow$

- Each system of ODEs defines a unique SPN.

$\Longleftarrow$

- In general:
  Different systems of ODEs can define the same SPN.
- In restricted classes:
  Each SPN defines a unique system of ODEs.

Steady state in a Biochemical Reaction System

yahoo: steady state, biochemical reaction system

$\Longrightarrow$ 3,290,000 results for ...

Steady state in a Biochemical Reaction System

yahoo: steady state, biochemical reaction system

$\Longrightarrow$ 3,290,000 results for ...

yahoo: steady-state, biochemical reaction system

$\Longrightarrow$ 762,000 results for ...

Steady state in a Biochemical Reaction System

"A condition in which the properties of any part of a system are constant during a process or reaction"

In: *Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 2006.*

Steady state in a Biochemical Reaction System and its SPN-Modeling

We consider the steady state:

- in the biochemical reaction system:

  The concentrations of the species do no longer change, i.e., the reaction rates stay constant.

- in the SPN model:

  The firing rates of the transitions are constant.

The SPN Model Behavior due to the steady state

BioRS	SPN
whole behavior (all possible situations)	reachability graph
steady state	subgraph of the reachability graph

The SPN Model Behavior due to the steady state

The steady state in the BioRS is modeled by a subset of

- reachable markings,
- firing of (some) transitions. Each of them has a constant rate and therefore a constant delay, as well.

The SPN Model Behavior due to the steady state

The steady state in the BioRS is modeled by a subset of

- reachable markings,
- firing of (some) transitions. Each of them has a constant rate and therefore a constant delay, as well.

delay of a transition = 1 / rate of a transition

The SPN Model Behavior due to the steady state

The observation of the SPN "in the steady state" yieldes

- A Petri Net,
- Each transitions has a constant delay,
- Firing mode: single transition.

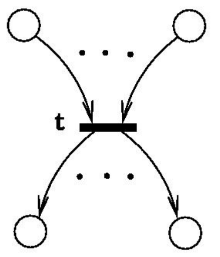
The SPN Model Behavior due to the steady state

The observation of the SPN "in the steady state"
yieldes

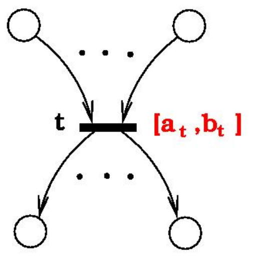
- A Petri Net,
- Each transitions has a constant delay,
- Firing mode: single transition.

This is a Time Petri Net (TPN), where for each transition t holds:
earliest firing time of t = latest firing time of t = delay of t.

Time Petri Nets

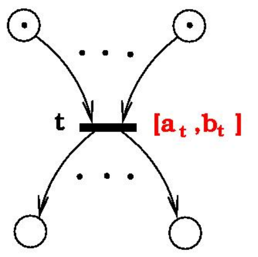


Time Petri Nets



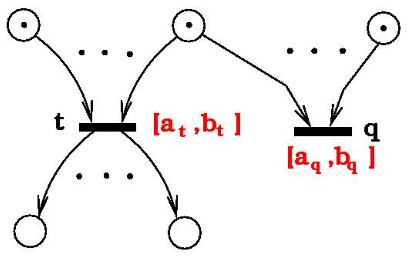
$$a_t = eft(t)$$
$$b_t = lft(t)$$

Time Petri Nets



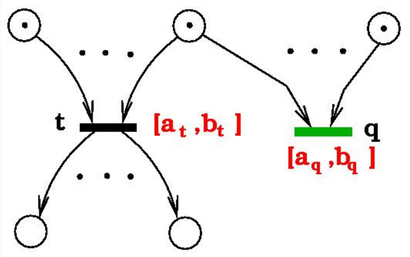
$$a_t = eft(t)$$
$$b_t = lft(t)$$

Time Petri Nets



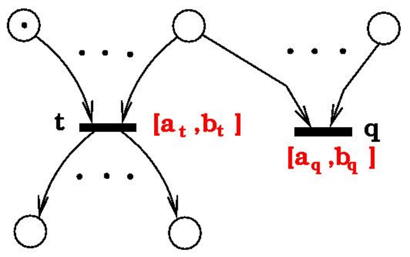
$$a_t = eft(t) \quad a_q = eft(q)$$
$$b_t = lft(t) \quad b_q = lft(q)$$

Time Petri Nets



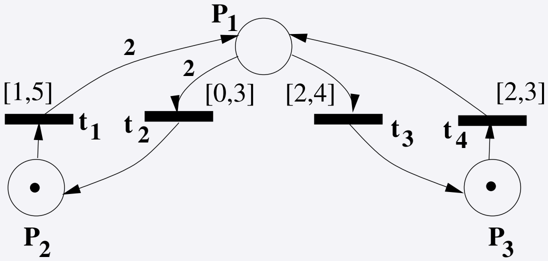
$$a_t = eft(t) \qquad a_q = eft(q)$$
$$b_t = lft(t) \qquad b_q = lft(q)$$

Time Petri Nets

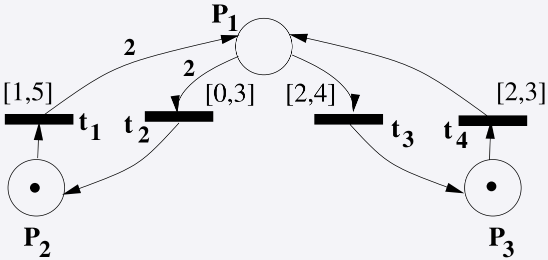


$$a_t = eft(t) \qquad a_q = eft(q)$$
$$b_t = lft(t) \qquad b_q = lft(q)$$

Time Petri Nets: Behavior

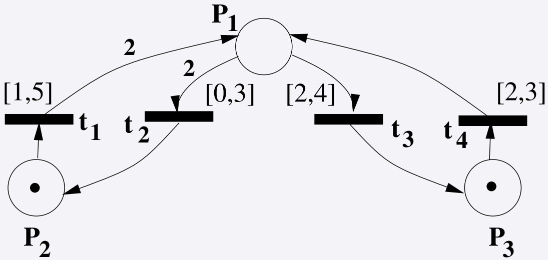


Time Petri Nets: Behavior



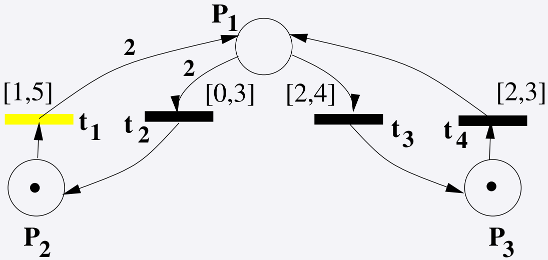
$$(m_0, \begin{pmatrix} 0 \\ \sharp \\ \sharp \\ 0 \end{pmatrix})$$

Time Petri Nets: Behavior



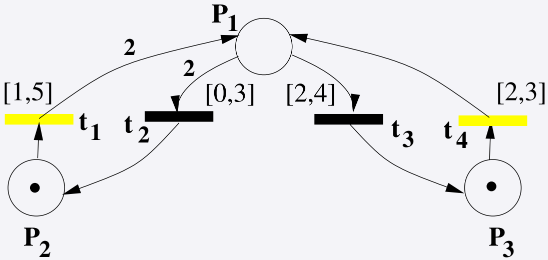
$$\text{initial state: } z_0 = \left(m_0, \begin{pmatrix} 0 \\ \sharp \\ \sharp \\ 0 \end{pmatrix}\right)$$

Time Petri Nets: Behavior



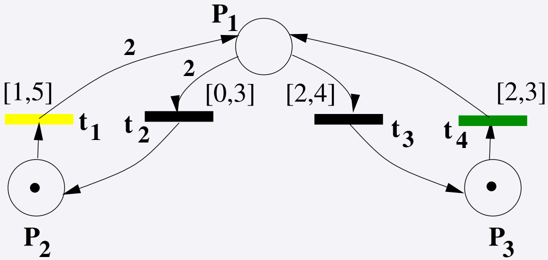
$$z_0 = (m_0, \begin{pmatrix} 0 \\ \sharp \\ \sharp \\ 0 \end{pmatrix}) \xrightarrow{1.3} (m_0, \begin{pmatrix} 1.3 \\ \sharp \\ \sharp \\ 1.3 \end{pmatrix})$$

Time Petri Nets: Behavior



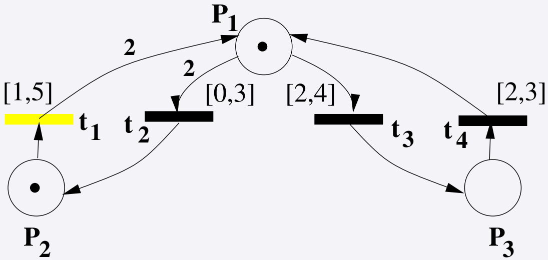
$$z_0 \xrightarrow{1.3} \left(m_0, \begin{pmatrix} 1.3 \\ \sharp \\ \sharp \\ 1.3 \end{pmatrix}\right) \xrightarrow{1.0} \left(m_0, \begin{pmatrix} 2.3 \\ \sharp \\ \sharp \\ 2.3 \end{pmatrix}\right)$$

Time Petri Nets: Behavior



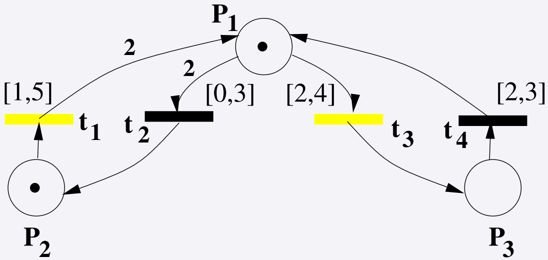
$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \left(m_0, \begin{pmatrix} 2.3 \\ \sharp \\ \sharp \\ 2.3 \end{pmatrix}\right) \xrightarrow{t_4}$$

Time Petri Nets: Behavior



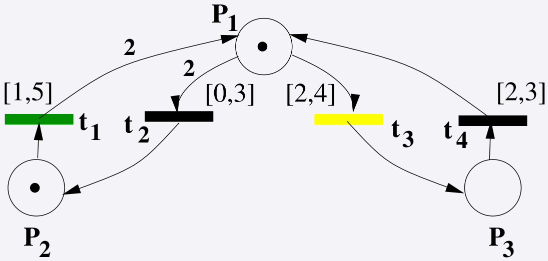
$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \left(m_0, \begin{pmatrix} 2.3 \\ \sharp \\ \sharp \\ 2.3 \end{pmatrix}\right) \xrightarrow{t_4} \left(m_1, \begin{pmatrix} 2.3 \\ \sharp \\ 0.0 \\ \sharp \end{pmatrix}\right)$$

Time Petri Nets: Behavior



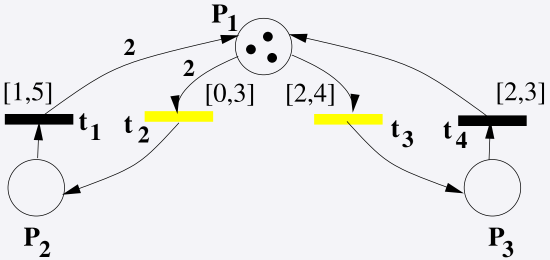
$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \xrightarrow{t_4} \left(m_1, \begin{pmatrix} 2.3 \\ \sharp \\ 0.0 \\ \sharp \end{pmatrix}\right) \xrightarrow{2.1} \left(m_1, \begin{pmatrix} 4.4 \\ \sharp \\ 2.1 \\ \sharp \end{pmatrix}\right)$$

Time Petri Nets: Behavior



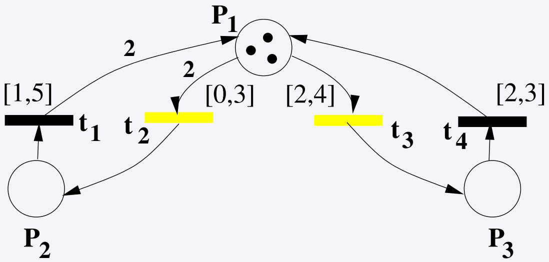
$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \xrightarrow{t_4} \xrightarrow{2.1} (m_1, \begin{pmatrix} 4.4 \\ \sharp \\ 2.1 \\ \sharp \end{pmatrix}) \xrightarrow{t_1}$$

Time Petri Nets: Behavior



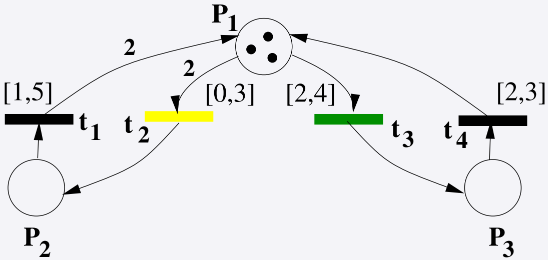
$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \xrightarrow{t_4} \xrightarrow{2.1} \left(m_1, \begin{pmatrix} 4.4 \\ \sharp \\ 2.1 \\ \sharp \end{pmatrix}\right) \xrightarrow{t_1} \left(m_2, \begin{pmatrix} \sharp \\ 0.0 \\ 2.1 \\ \sharp \end{pmatrix}\right)$$

Time Petri Nets: Behavior



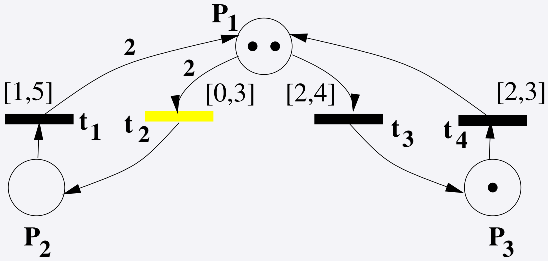
$$z_0 \xrightarrow{1.3}\xrightarrow{1.0}\xrightarrow{t_4}\xrightarrow{2.1}\xrightarrow{t_1} \left(m_2, \begin{pmatrix}\sharp\\ 0.0\\ 2.1\\ \sharp\end{pmatrix}\right) \xrightarrow{0.8} \left(m_2, \begin{pmatrix}\sharp\\ 0.8\\ 2.9\\ \sharp\end{pmatrix}\right)$$

Time Petri Nets: Behavior



$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \xrightarrow{t_4} \xrightarrow{2.1} \xrightarrow{t_1} \xrightarrow{0.8} \left(m_2, \begin{pmatrix} \sharp \\ 0.8 \\ 2.9 \\ \sharp \end{pmatrix}\right) \xrightarrow{t_3}$$

Time Petri Nets: Behavior



$$z_0 \xrightarrow{1.3} \xrightarrow{1.0} \xrightarrow{t_4} \xrightarrow{2.1} \xrightarrow{t_1} \xrightarrow{0.8} \left(m_2, \begin{pmatrix} \sharp \\ 0.8 \\ 2.9 \\ \sharp \end{pmatrix}\right) \xrightarrow{t_2} \left(m_3, \begin{pmatrix} \sharp \\ 0.8 \\ 0.0 \\ 0.0 \end{pmatrix}\right)$$

The RKIP Pathway: The influence of the Raf Kinase Inhibitor Protein (RKIP) on the Extracellular signal Regulated Kinase (ERK) signaling pathway

The Ras/Raf-1/MEK/ERK signaling pathway conveys mitogenic and differentiation signals from the cell membrane to the nucleus.

In:*K.-H. Cho, S.-Y. Shin, H.-W. Kim, O. Wolkenhauer, B. McFerran, and W. Kolch. Mathematical modeling of the influence of RKIP on the ERK signaling pathway, 2003.*

The RKIP Pathway: The influence of the Raf Kinase Inhibitor Protein (RKIP) on the Extracellular signal Regulated Kinase (ERK) signaling pathway

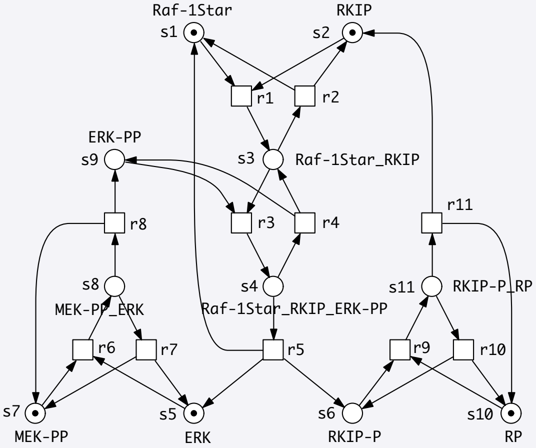
The Ras/Raf-1/MEK/ERK signaling pathway conveys mitogenic and differentiation signals from the cell membrane to the nucleus.

In:*K.-H. Cho, S.-Y. Shin, H.-W. Kim, O. Wolkenhauer, B. McFerran, and W. Kolch. Mathematical modeling of the influence of RKIP on the ERK signaling pathway, 2003.*

In:*D. Gilbert and M. Heiner. From Petri Nets to Differential Equations - an Integrative Approach for Biochemical Network Analysis, 2006.*

In:*M. Heiner, R. Donaldson, and D. Gilbert. Petri Nets for Systems Biology, 2010.*

The Core Model of the RKIP Pathway



In: *M. Heiner, R. Donaldson, and D. Gilbert. Petri Nets for Systems Biology, 2010.*

The rate functions and the rate constants

transition r_i	rate function v_i	rate constant c_i
$r1$	$c1 \cdot s1 \cdot s2$	0.053
$r2$	$c2 \cdot s3$	0.0072
$r3$	$c3 \cdot s3 \cdot s9$	0.625
$r4$	$c4 \cdot s4$	0.00245
$r5$	$c5 \cdot s4$	0.0315
$r6$	$c6 \cdot s5 \cdot s7$	0.8
$r7$	$c7 \cdot s8$	0.0075
$r8$	$c8 \cdot s8$	0.071
$r9$	$c9 \cdot s6 \cdot s10$	0.92
$r10$	$c10 \cdot s11$	0.00122
$r11$	$c11 \cdot s11$	0.87

In the rate functions each of the values $s1 \ldots s11$ is the mean concentration of the species $s1 \ldots s11$ in the simulated steady state in the SPN, cf. next table.

Mean steady state concentrations for all $s1 \ldots s11$

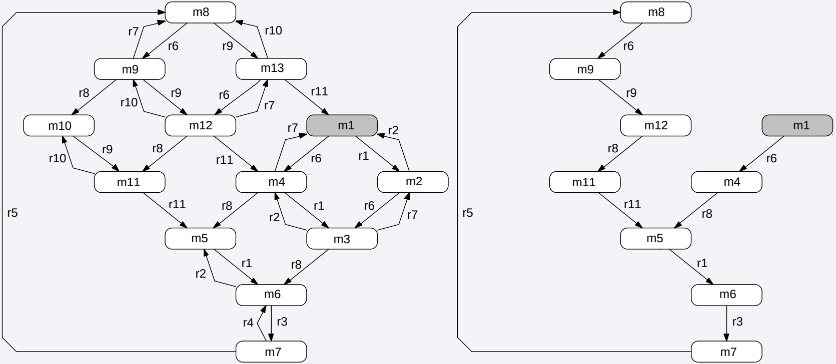
specie s_i	concentration
$s1$	0.2133
$s2$	0.1727
$s3$	0.2163
$s4$	0.5704
$s5$	0.0332
$s6$	0.0200
$s7$	0.7469
$s8$	0.2531
$s9$	0.1433
$s10$	0.9793
$s11$	0.0207

In: *D. Gilbert and M. Heiner. From Petri Nets to Differential Equations - an Integrative Approach for Biochemical Network Analysis, 2006.*

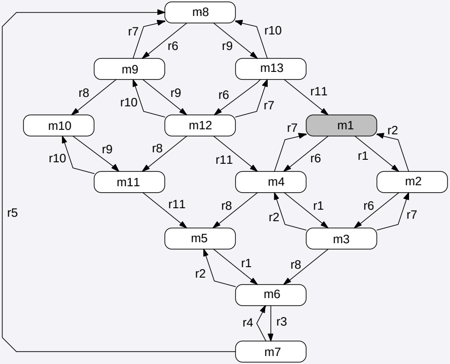
Rates in the mean steady state and delay times for $r1 \cdots r11$

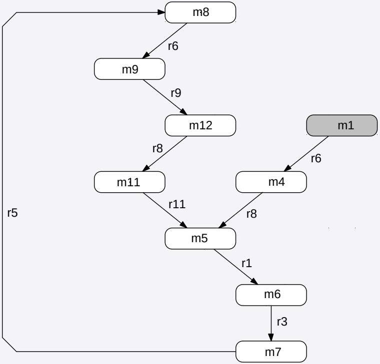
transition r_i	rate in the mean steady state v_i	delay time in the mean steady state τ_i (rounded)
$r1$	0.00195235623	512
$r2$	0.00155736	642
$r3$	0.019372369	52
$r4$	0.00139748	716
$r5$	0.0179676	56
$r6$	0.019837664	50
$r7$	0.00189825	527
$r8$	0.0179701	56
$r9$	0.01801912	55
$r10$	0.000025254	39598
$r11$	0.018009	56

The Reachability Graph for the SPN and the Reachable *p*-markings in the TPN



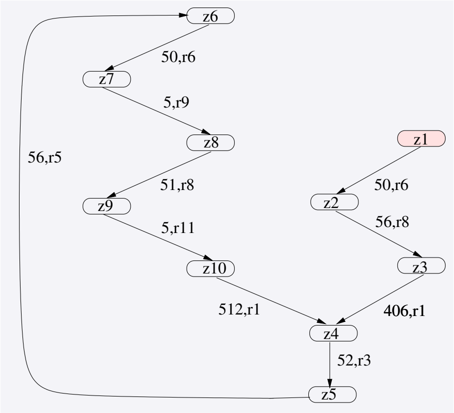
The Reachability Graph for the SPN and the Reachable *p*-markings in the TPN





This is not the reachability graph of the TPN.

The Reachability Graph for the TPN Model



Qualitative and Quantitative Analysis

Analysis this reduced reachability graph of the TPN shows that:

- The RG consists of the **cycle**
  $z4, r3, z5, r5, z6, r6, z7, r9, z8, r8, z9, r11, z10, r1$
  and an **initiation path** $z1, r6, z2, r8, z3, r1$.
- The time-length of the cycle (upper bount) is 731 time units.
- The minimal time-length of the cycle is 512 time units.
- The transitions $r2, r4, r7$ and $r10$ will never fire.

Conclusion

We introduced a method for

- extracting the part of a SPN model describing the steady state in a BioRS and its presentation as a TPN
- analysis of the TPN:
  - cycles
  - time-length of the cycles

Problem: The initial state for the TPN.

Thank You

"The Ras/Raf-1/MEK/ERK module is a ubiquitously expressed signaling pathway that conveys mitogenic and differentiation signals from the cell membrane to the nucleus. This kinase cascade appears to be spatially organized in a signaling complex nucleated by Ras proteins. The small G protein Ras is activated by many growth factor receptors and binds to the Raf-1 kinase with high affinity when activated. This induces the recruitment of Raf-1 from the cytosol to the cell membrane. Activated Raf-1 then phosphorylates and activate MAPK/ERK Kinase (MEK), a kinase that in turn phosphorylates and activates Extracellular signal Regulated Kinase (ERK), the prototypic Mitogen-Activated Protein Kinase (MAPK). Activated ERKs can translocate to the nucleus and regulate gene expression by the phosphorylation of transcription factors. This kinase cascade controls the proliferation and differentiation of different cell types. The specific biological effects are crucially dependent on the amplitude and kinetics of ERK activity.

"The Ras/Raf-1/MEK/ERK module is a ubiquitously expressed signaling pathway that conveys mitogenic and differentiation signals from the cell membrane to the nucleus. This kinase cascade appears to be spatially organized in a signaling complex nucleated by Ras proteins. The small G protein Ras is activated by many growth factor receptors and binds to the Raf-1 kinase with high affinity when activated. This induces the recruitment of Raf-1 from the cytosol to the cell membrane. Activated Raf-1 then phosphorylates and activate MAPK/ERK Kinase (MEK), a kinase that in turn phosphorylates and activates Extracellular signal Regulated Kinase (ERK), the prototypic Mitogen-Activated Protein Kinase (MAPK). Activated ERKs can translocate to the nucleus and regulate gene expression by the phosphorylation of transcription factors. This kinase cascade controls the proliferation and differentiation of different cell types. The specific biological effects are crucially dependent on the amplitude and kinetics of ERK activity.

membrane. Activated Raf-1 then phosphorylates and activate MAPK/ERK Kinase (MEK), a kinase that in turn phosphorylates and activates Extracellular signal Regulated Kinase (ERK), the prototypic Mitogen-Activated Protein Kinase (MAPK). Activated ERKs can translocate to the nucleus and regulate gene expression by the phosphorylation of transcription factors. This kinase cascade controls the proliferation and differentiation of different cell types. The specific biological effects are crucially dependent on the amplitude and kinetics of ERK activity. The adjustment of these parameters involves the regulation of protein interactions within this pathway. Here the Raf-1 kinase inhibitor protein (RKIP) plays a central role. RKIP binds to Raf-1 and MEK thereby disrupting the interaction between Raf-1 and MEK. As a consequence RKIP inhibits the phosphorylation and activation of MEK by Raf-1. RKIP overexpression interferes with the activation of MEK and ERK, induction of AP-1-dependent reporter genes and transformation elicited by an oncogenically activated Raf-1 kinase.

The adjustment of these parameters involves the regulation of protein interactions within this pathway. Here the Raf-1 kinase inhibitor protein (RKIP) plays a central role. RKIP binds to Raf-1 and MEK thereby disrupting the interaction between Raf-1 and MEK. As a consequence RKIP inhibits the phosphorylation and activation of MEK by Raf-1. RKIP overexpression interferes with the activation of MEK and ERK, induction of AP-1-dependent reporter genes and transformation elicited by an oncogenically activated Raf-1 kinase. Downregulation of endogenous RKIP by expression of antisense RNA or antibody microinjection induces the activation of MEK-, ERK-, AP-1-dependent transcription. Thus, RKIP represents a new class of protein-kinase-inhibitor protein that regulates the activity of the Raf- 1/MEK/ERK module."

Downregulation of endogenous RKIP by expression of antisense RNA or antibody microinjection induces the activation of MEK-, ERK-, AP-1-dependent transcription. Thus, RKIP represents a new class of protein-kinase-inhibitor protein that regulates the activity of the Raf- 1/MEK/ERK module."

In: K.-H. Cho, S.-Y. Shin, H.-W. Kim, O. Wolkenhauer, B. McFerran, and W. Kolch. Mathematical modeling of the influence of RKIP on the ERK signaling pathway, 2003.

Reached, if for each reachable *p*-marking *m* it holds:

$$m(p) \geq \sum_{t \in p^\bullet} V(p, t)$$