Analysis of the Signal Transduction Dynamics Regulating mTOR with Mathematical Modeling, Petri Nets and Dynamic Graphs

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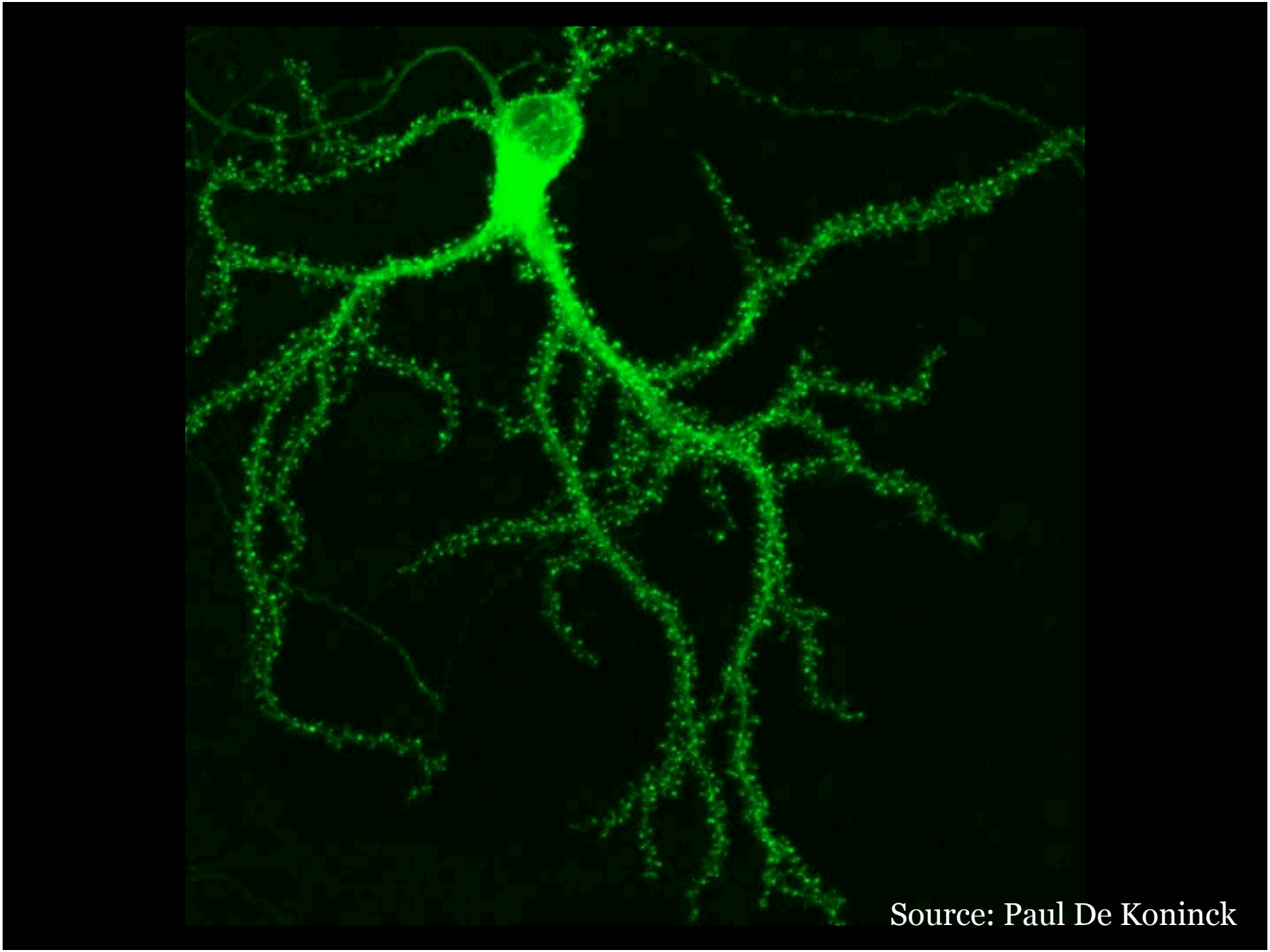
Simon V. Hardy and Mathieu Pagé Fortin

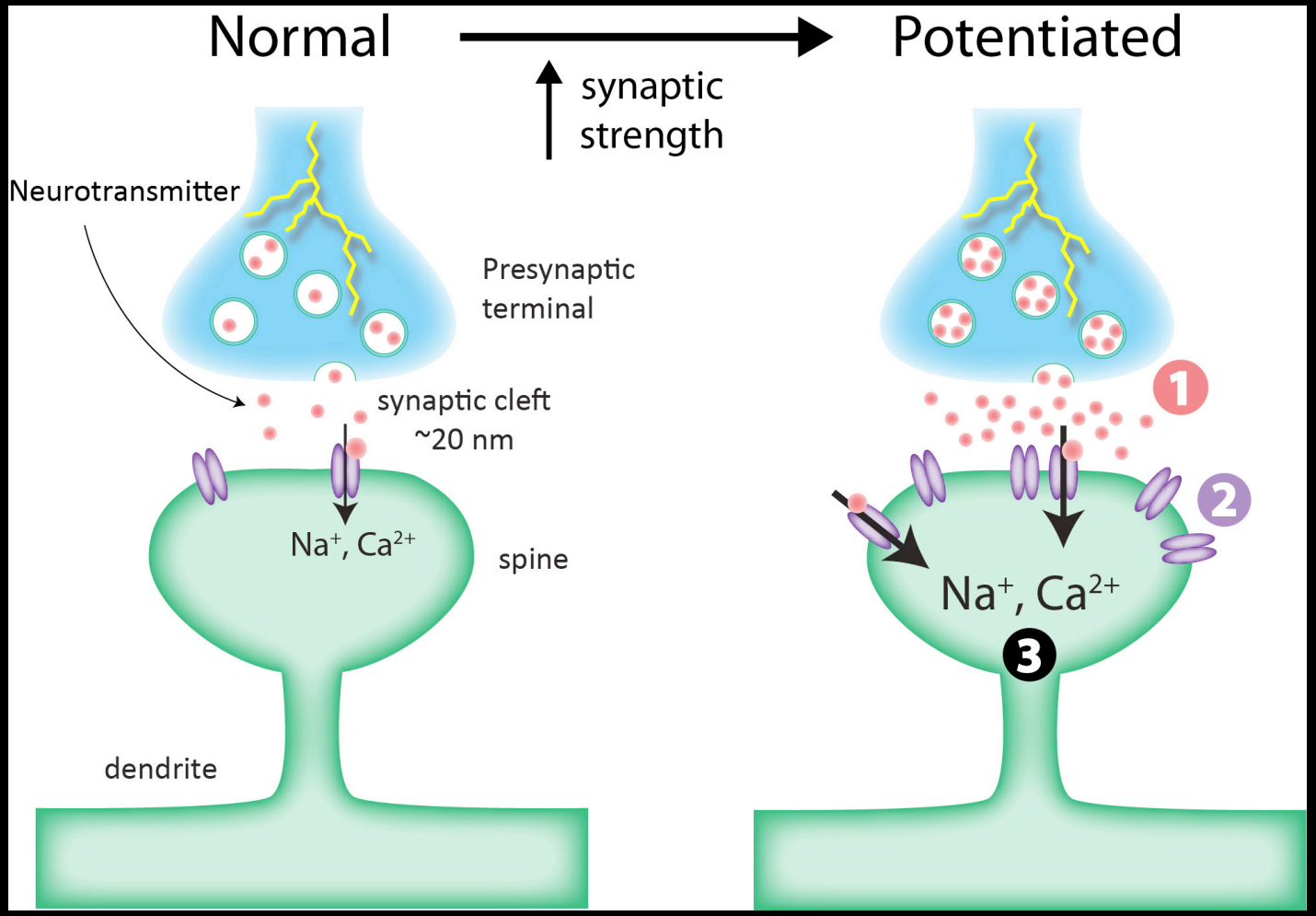
UNIVERSITÉ LAVAL

Outline

- The biology behind this project
- Modeling and analysis methodology
- Models of the regulation network of mTOR
  - Iteration 1
  - Iteration 2
- Results

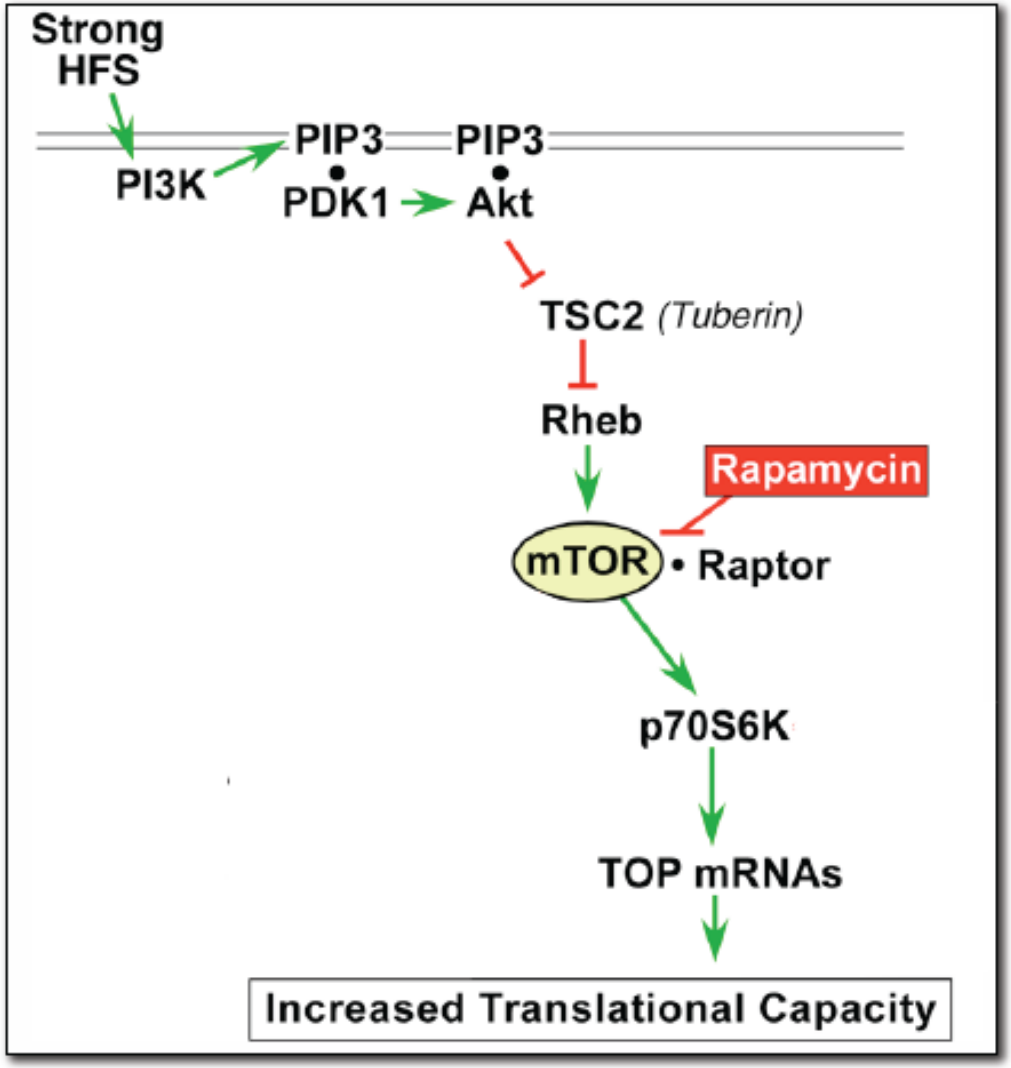

Source: Paul De Koninck

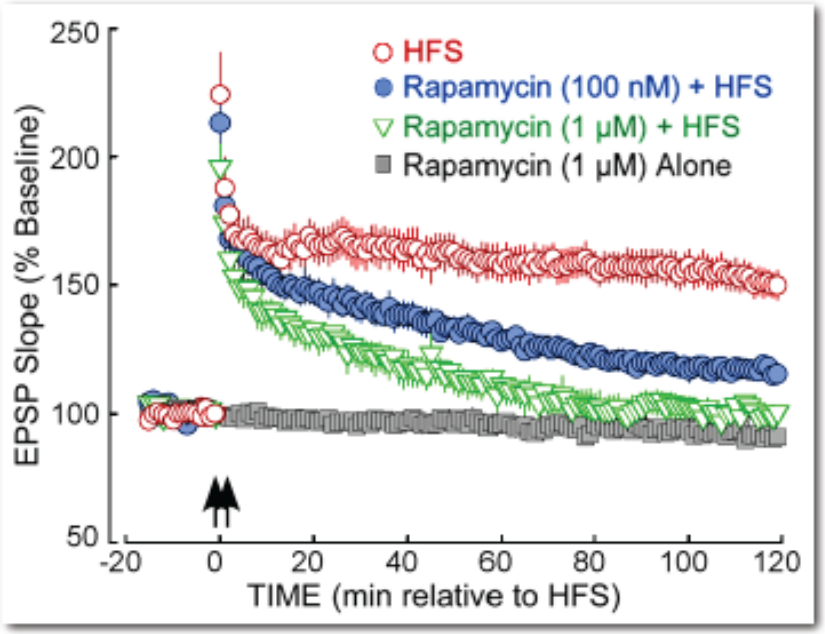
What is synaptic plasticity?



(Image: Alan Woodruff / QBI)

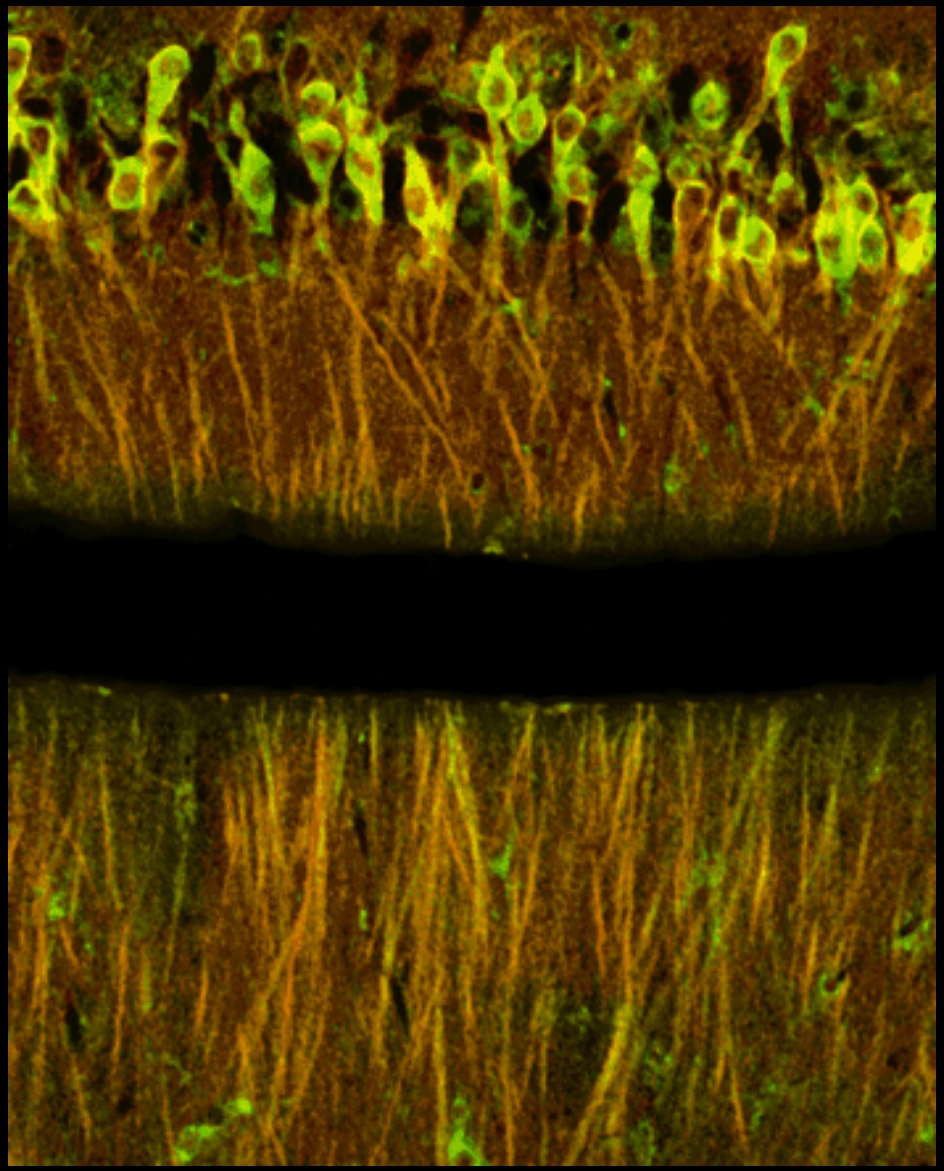
Translation control by the mTOR pathway and its role in late long term potentiation (LTP)





TOP mRNA-encoded proteins include:

- All translation elongation factors
- All ribosomal proteins
- Poly-A binding protein (PABP)



eEF1A and p70S6K staining in dendrites of CA1 neurons

Synaptic stimulation, through the mTOR and p70S6K pathway, can increase the **local synthesis** of proteins in CA1 neurons and contributes to the maintenance phase of LTP.

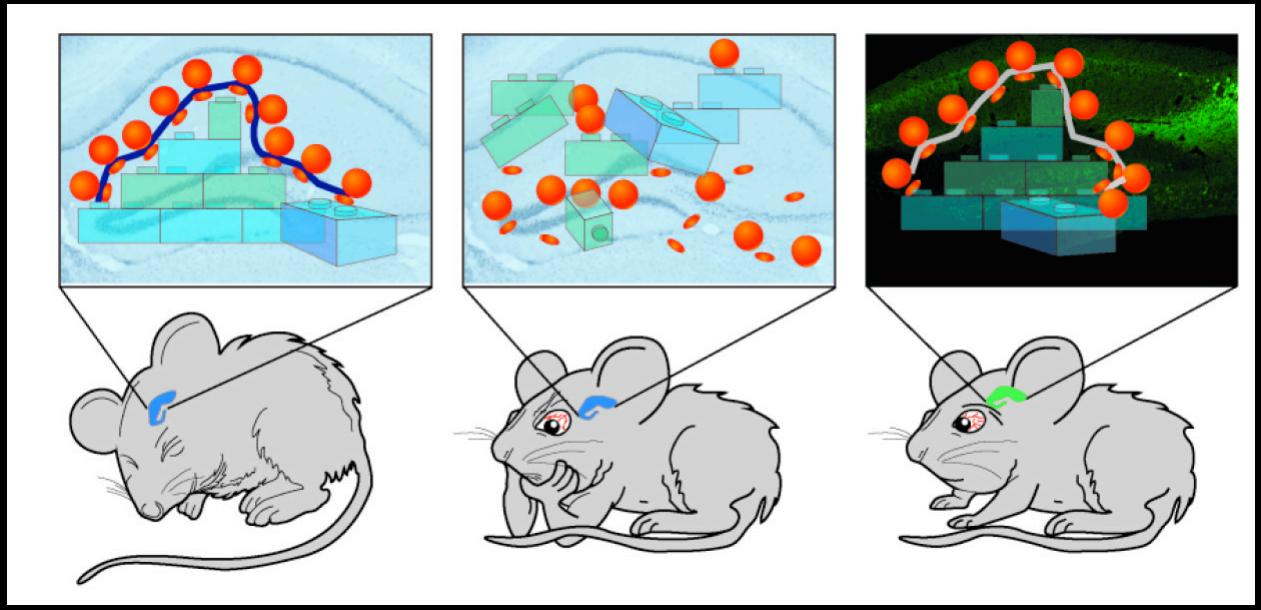
Source : Tsokas et al, *J Neuroscience* 25, 2005

mTOR, memory and sleep deprivation

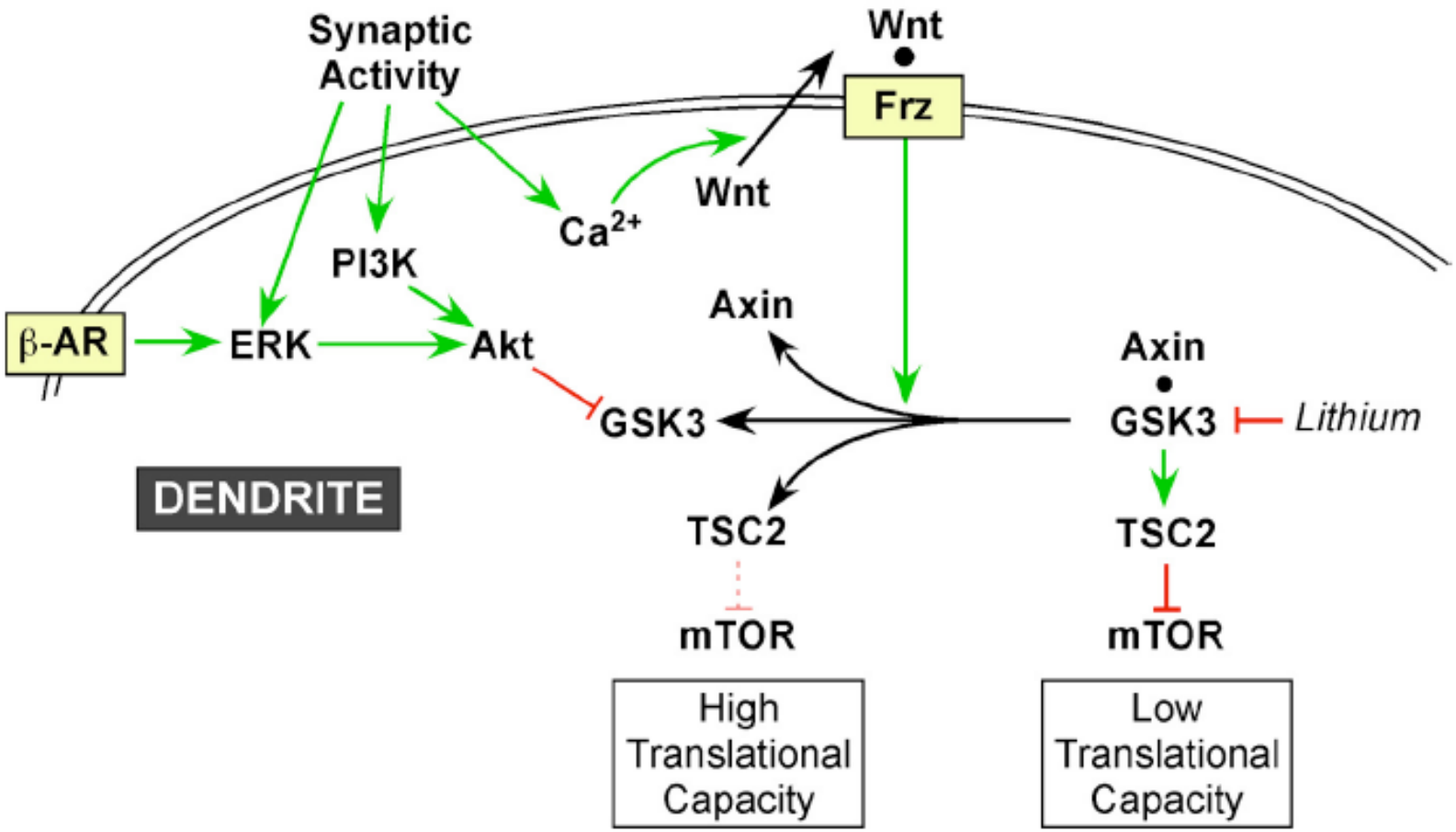
RESEARCH ARTICLE

NEUROSCIENCE

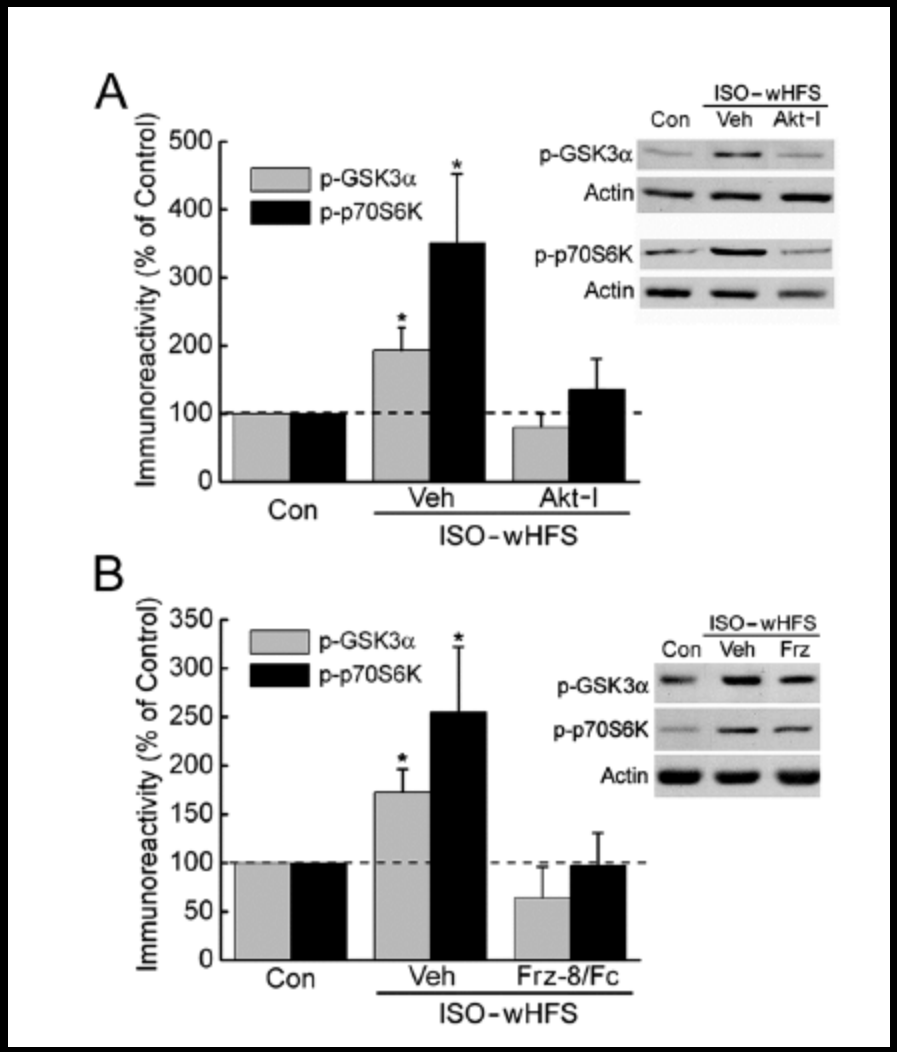
Sleep deprivation impairs memory by attenuating mTORC1-dependent protein synthesis



The Wnt pathway contributes to the activation of mTOR during LTP

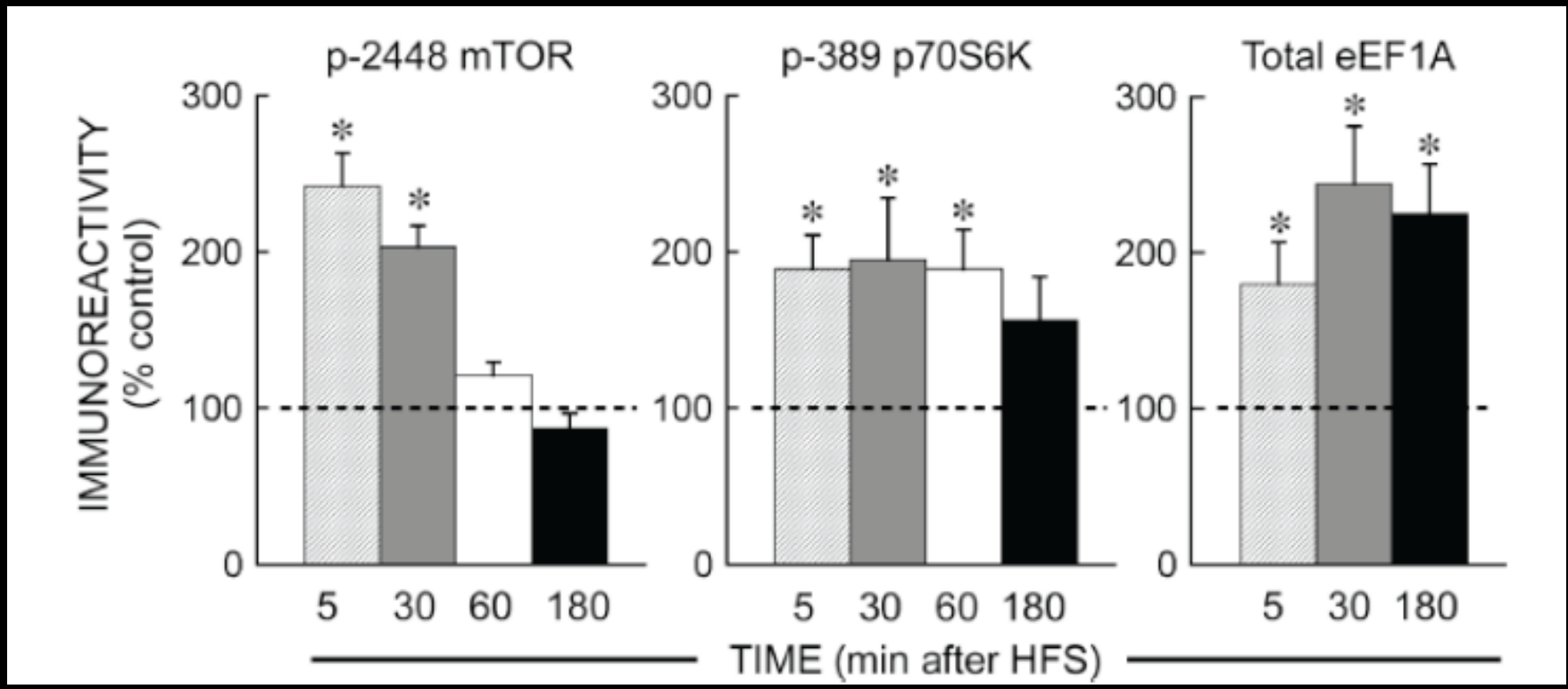


Akt and Wnt mediate the phosphorylation of p70S6K (target of mTOR)



Source: Ma *et al.*, *J Neuroscience* 48, 2011

Prolonged activation of mTOR and p70S6K following the induction of LTP



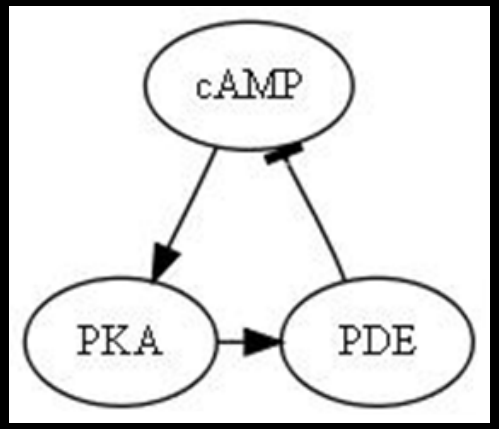
Source : Tsokas et al, *J Neuroscience* 25, 2005

Questions

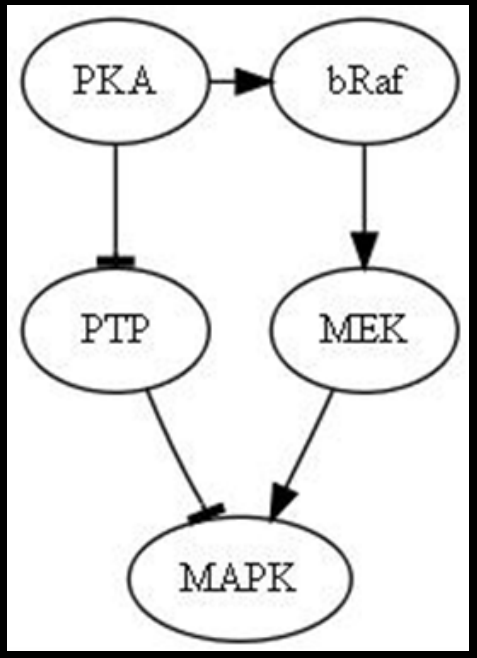
- What is the contribution of the Akt and Wnt signaling pathways to the activity of mTOR?
- What signaling mechanisms (i.e. network of regulatory motifs) can prolong the activity of mTOR and p70S6K and thus create the temporal window for synaptic capture?
- Can modeling and simulation suggest some answers?

Methodology

The dynamic behavior of biological systems and their processes depends largely on the interaction of regulatory mechanisms.



Negative feedback loop



Feedforward motif

Methodology

- Modeling

$$A + B \leftrightarrow AB$$

$$v_1 = -k_f[A][B] + k_b[AB]$$

$$C \xrightarrow{AB} D$$

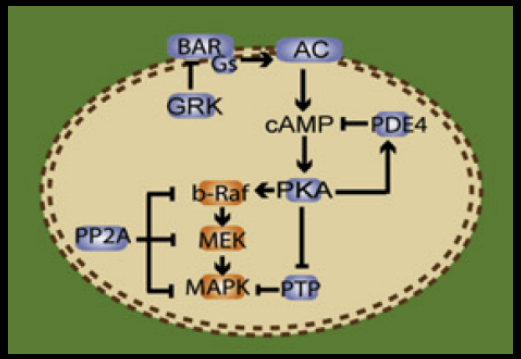
$$v_2 = -\frac{k_{cat}[AB][C]}{k_M + [C]}$$

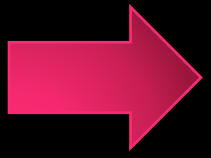
- Simulation

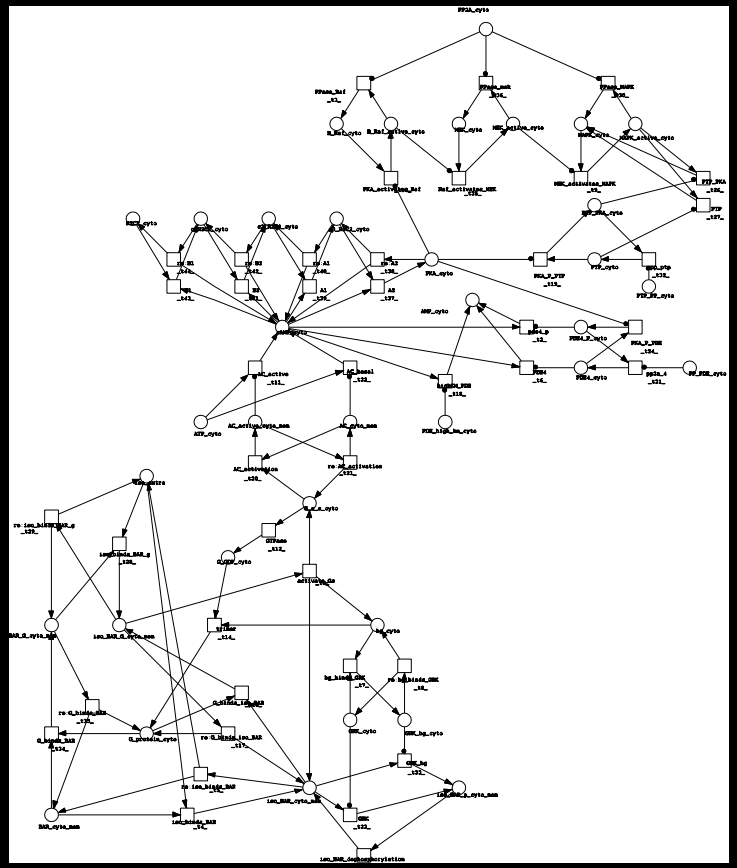
- Systems analysis with dynamics graphs

Dynamic graphs

1. Transform ODEs into a Petri net.







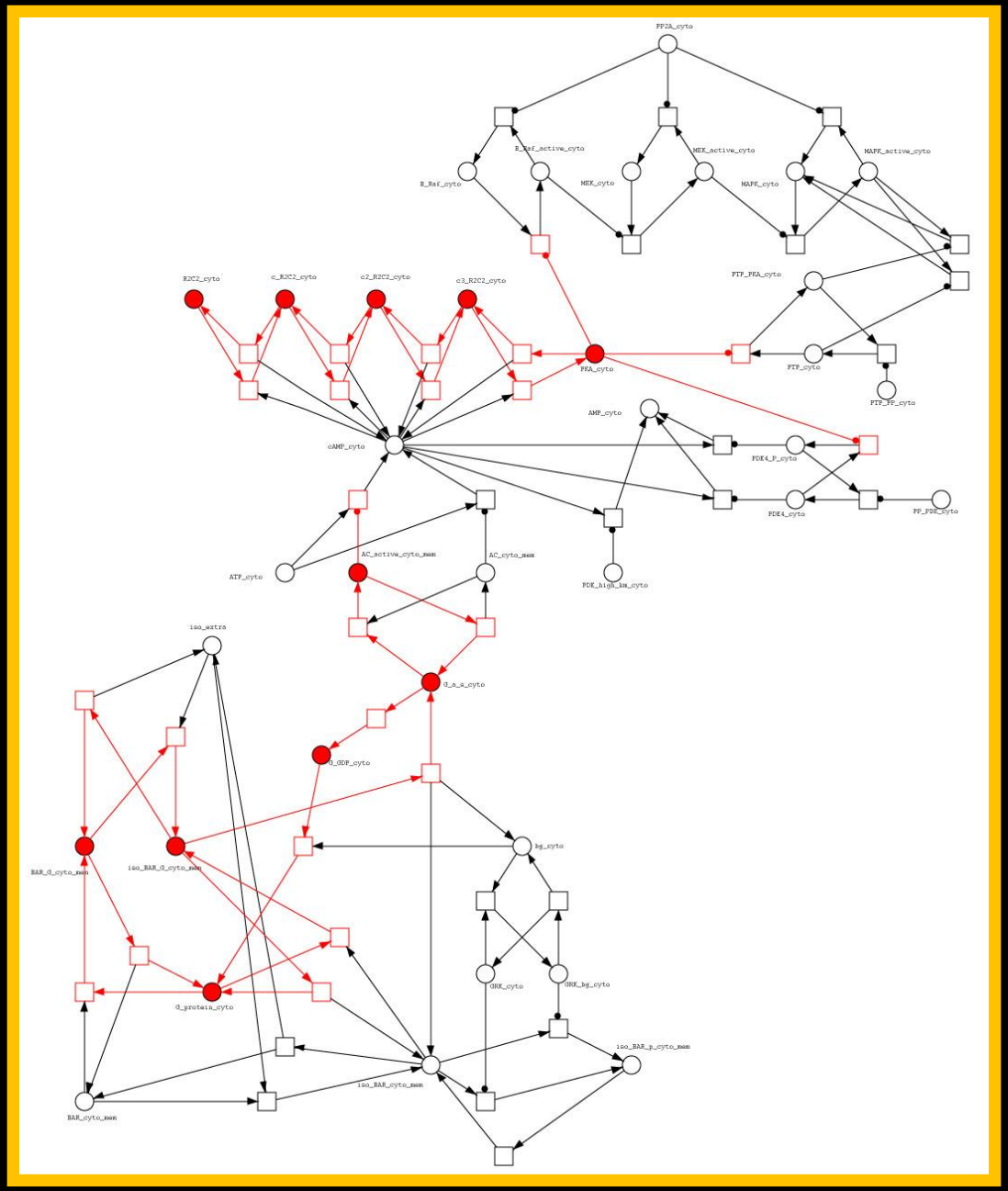
Component	Compartment	Concentration	Units	Diffusion ($\mu m^2/s$)	References and notes
BAR	membrane	94	molecules/um2		(S15)
G_protein	cytoplasmic	3.6	µM	6.0	(S15)
ATP	cytoplasmic	5000	µM	300	(S2)
AC	membrane	300	molecules/um2	-	(S15)
PKA (R2C2)	cytoplasmic	0.2	µM	3.0	(S16)
PDE4	cytoplasmic	0.4	µM	5.0	(S2)
PP2A	cytoplasmic	0.1	µM	-	(S2)
MEK	cytoplasmic	0.18	µM	7.0	(S17)
B_Raf	cytoplasmic	0.2	µM	6.0	(S16)
AMP	cytoplasmic	1000	µM	300.0	(S2)
Iso	extracellular	1	µM	300.0	Saturating concentration
MAPK	cytoplasmic	0.36	µM	7.0	(S19)
PTP	cytoplasmic	0.2	µM	6.0	Estimated*
PDE_high_km	cytoplasmic	0.5	µM	5.0	Estimated*
GRK	cytoplasmic	0.001	µM	6.0	Estimated*
PPase_PDE	cytoplasmic	0.2	µM	-	Estimated*
PP1	cytoplasmic	0.1	µM	-	Estimated*

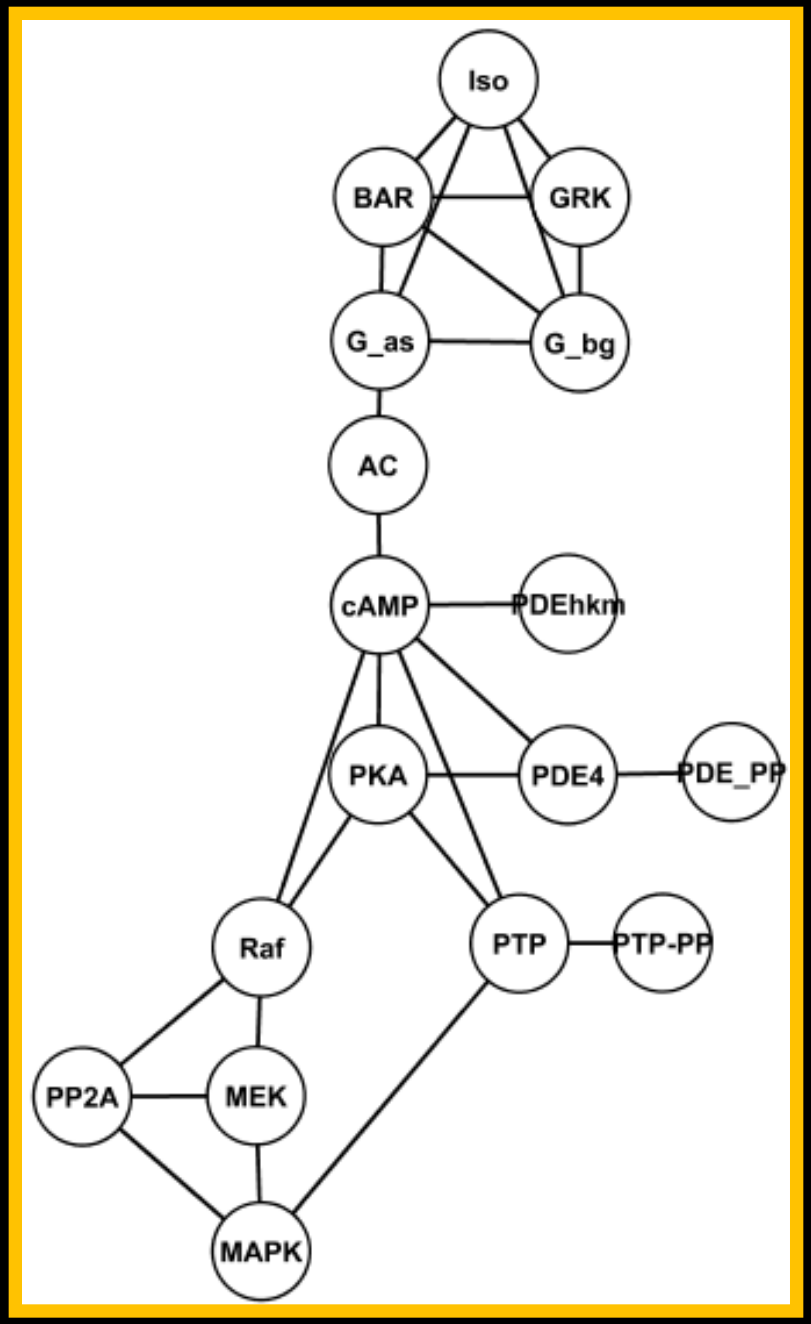
	Reaction		Parameters		References and Notes
membrane reactions					
1	Iso + BAR <=> Iso_BAR	Kf	1.0000	µM-1.s-1	(S20)
		Kb	0.2000	s-1	
2	G_protein + BAR <=> G_BAR	Kf	0.3000	µM-1.s-1	(S21)
		Kb	0.1000	s-1	
3	Iso + G_BAR <=> Iso_G_BAR	Kf	1.0000	µM-1.s-1	(S20)
		Kb	0.0620	s-1	(S22)
4	Iso_BAR + G_protein <=> Iso_G_BAR	Kf	10.0000	µM-1.s-1	(S21)
		Kb	0.1000	s-1	
5	Iso_G_BAR <=> Iso_BAR + G_GTP + bg	Kf	0.0250	s-1	(S23)
		Kb	0.0000		
6	**AC** + ATP -> cAMP	Km	1030.0000	µM	(S24)
		Kcat	0.2000	s-1	
7	AC + Gs_GTP <=> AC_Gs	Km	500.0000	µM-1.s-1	(S25)
		Kcat	1.0000	s-1	
8	ATP + **AC_Gs** -> cAMP	Km	3.15	µM	(S24)
		Kcat	8.5000	s-1	
cytoplasmic reactions					
9	G_GTP <=> G_GDP	Kf	0.0670	µM-1.s-1	(S23)
		Kb	0.0000	s-1	
10	G_GDP + bg <=> G_protein	Kf	6.0000	µM-1.s-1	(S2)
		Kb	0.0000	s-1	
11	cAMP + R2C2 <=> cAMP_R2C2	Kf	0.0059	µM-1.s-1	(S26, S27)
		Kb	0.0003	s-1	
12	cAMP + cAMP_R2C2 <=> cAMP2_R2C2	Kf	0.0059	µM-1.s-1	(S26, S27)
		Kb	0.0003	s-1	
13	cAMP + cAMP2_R2C2 <=> cAMP3_R2C2	Kf	8.3500	µM-1.s-1	(S26, S27)
		Kb	0.0167	s-1	
14	cAMP + cAMP3_R2C2 <=> PKA	Kf	8.3500	µM-1.s-1	
		Kb	0.0167	s-1	(S26, S27)
15	cAMP + **PDE4** -> AMP	Km	1.3000	µM	(S28, S29, S30)
		Kcat	8.0000	s-1	
16	cAMP + **highKm_PDE** -> AMP	Km	15.0000	µM	Estimated*
		Kcat	8.0000	s-1	
17	PDE4 + **PKA** -> PDE4_P	Km	0.5000	µM	(S31)
		Kcat	10.0000	s-1	
18	cAMP + **PDE4_P** -> AMP	Km	1.3000	µM	(S31, S32)
		Kcat	20.0000	s-1	
19	PDE4_P + **ppase_PDE** -> PDE4	Km	8.0000	µM	Estimated*
		Kcat	5.0000	s-1	
20	bRaf + **PKA** -> bRaf_active	Km	0.5000	µM	Estimated*
		Kcat	10.000	s-1	
21	bRaf_active + **PP2A** -> bRaf	Km	15.7000	µM	(S2)
		Kcat	5.0000	s-1	
22	MEK + **bRAF_active** -> MEK_active	Km	0.1591	µM	(S33)
		Kcat	0.1050	s-1	

Dynamic graphs

1. Transform ODEs into a Petri net.
2. Find P-invariants and compute an interaction graph.
   - Verify mass conservation condition and identify marking invariants (= different configurations of the same molecular component)
   - Determine connectivity between invariants.

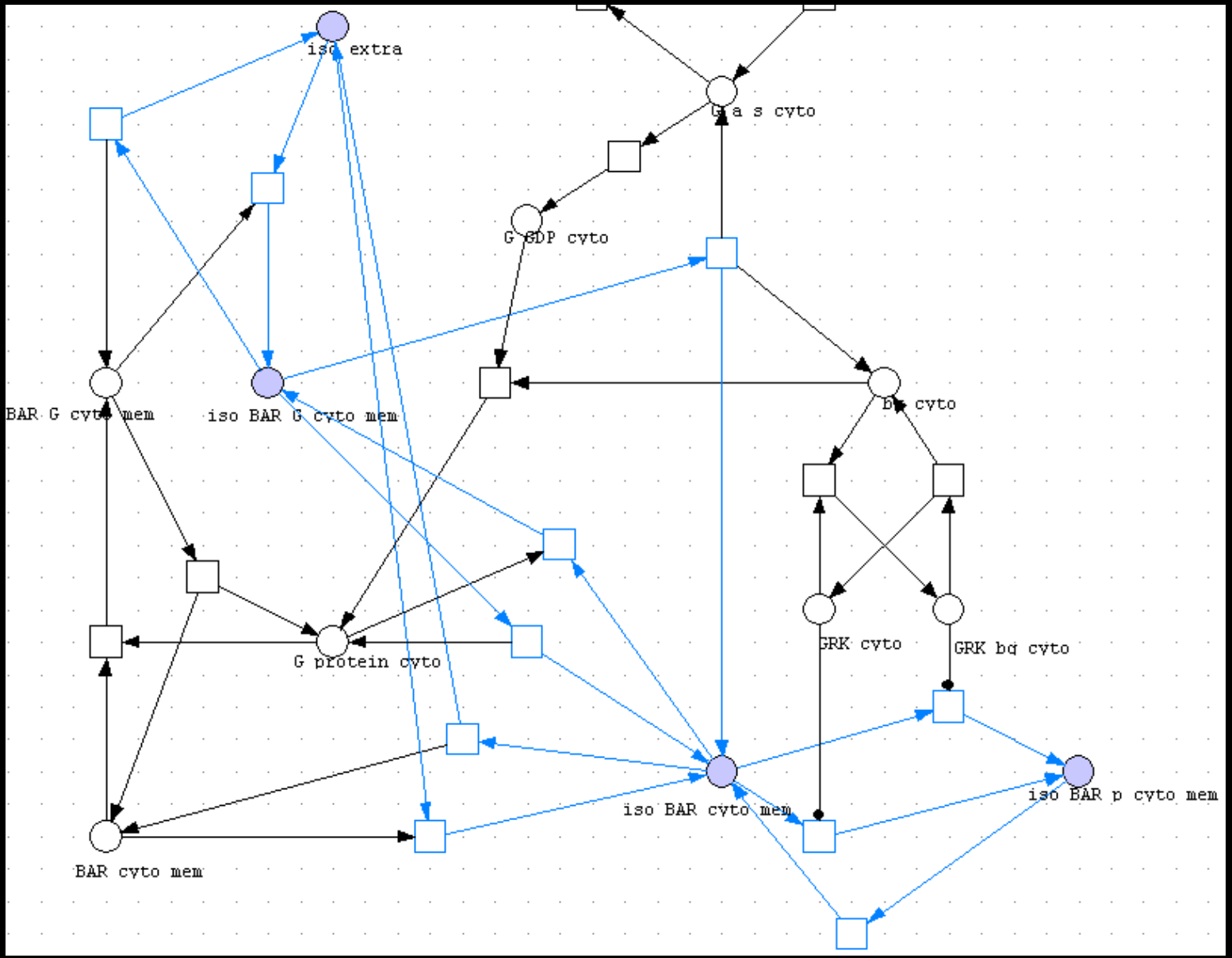
1. $P'_x \cap P'_y \neq \emptyset$; the P-invariants P'_x and P'_y have at least one place in common, or
2. $\exists t : (W(p_1, t) > 0 \wedge W(t, p_2) > 0) \vee (W(p_2, t) > 0 \wedge W(t, p_1) > 0)$, where $p_1 \in P'_x$, $p_2 \in P'_y$, $t \in T$ and $W : ((P \times T) \cup (T \times P) \rightarrow \mathbb{N})$; the places p_1 and p_2 from the P-invariants P'_x and P'_y are connected through transition t from the set of transitions T. W is the multiset of arcs of the Petri net.

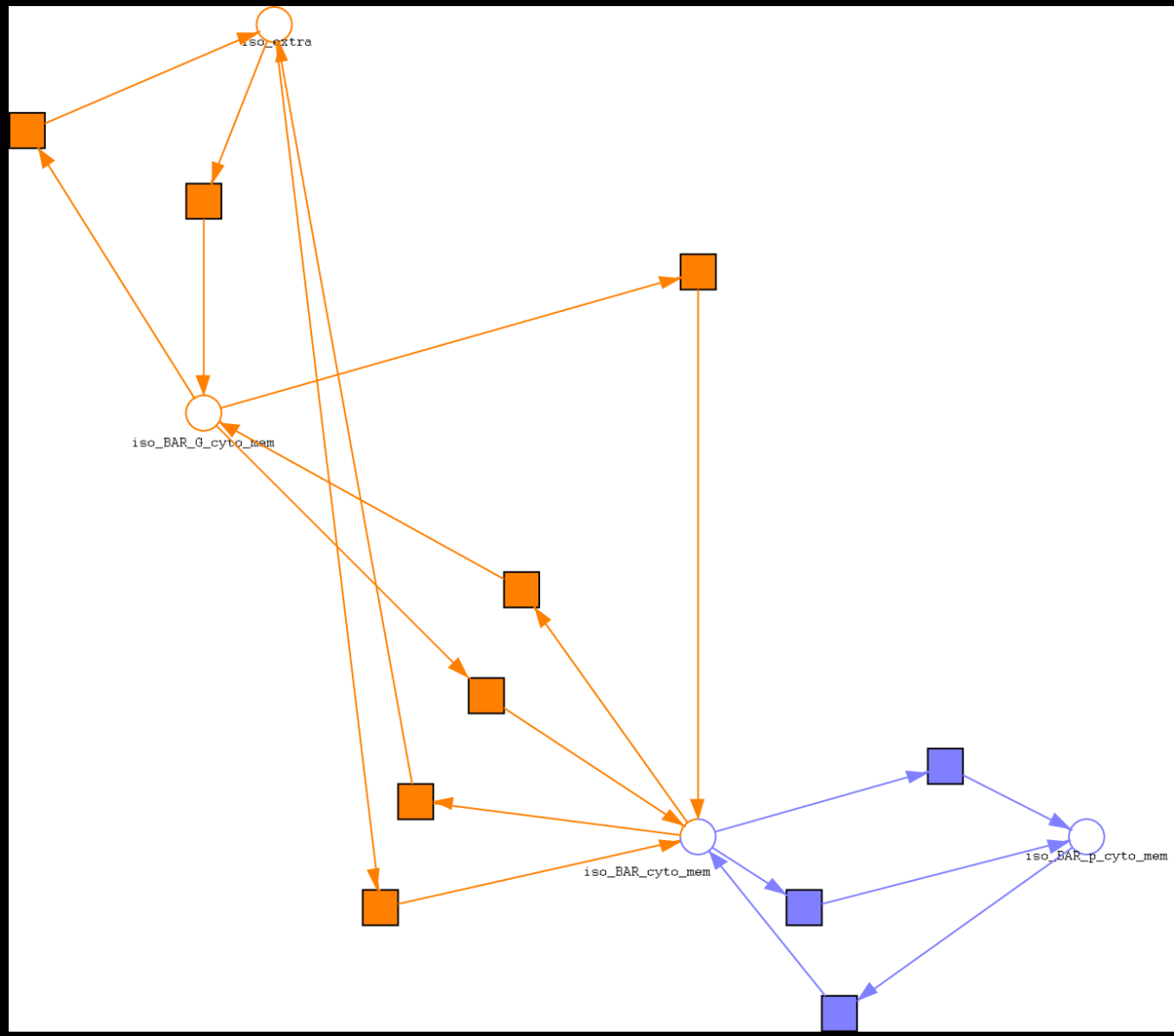

PP2A_cyto
B_Raf_active_cyto
MEK_active_cyto
MAPK_active_cyto
B_Raf_cyto
MEK_cyto
MAPK_cyto
R2C2_cyto
c_R2C2_cyto
c2_R2C2_cyto
c3_R2C2_cyto
PKA_cyto
PTP_PKA_cyto
PTP_cyto
PTP_PP_cyto
AMP_cyto
cAMP_cyto
PDE4_P_cyto
PDE4_cyto
PP_PDE_cyto
ATP_cyto
AC_active_cyto_mem
AC_cyto_mem
PDE_high_Km_cyto
iso_extra
G_a_s_cyto
G_GDP_cyto
bg_cyto
BAR_G_cyto_mem
iso_BAR_G_cyto_mem
GRK_cyto
GRK_bg_cyto
G_protein_cyto
iso_BAR_p_cyto_mem
iso_BAR_cyto_mem
BAR_cyto_mem


Iso
BAR
GRK
G_as
G_bg
AC
cAMP
PDEhkm
PKA
PDE4
PDE_PP
Raf
PTP
PTP-PP
PP2A
MEK
MAPK

Dynamic graphs

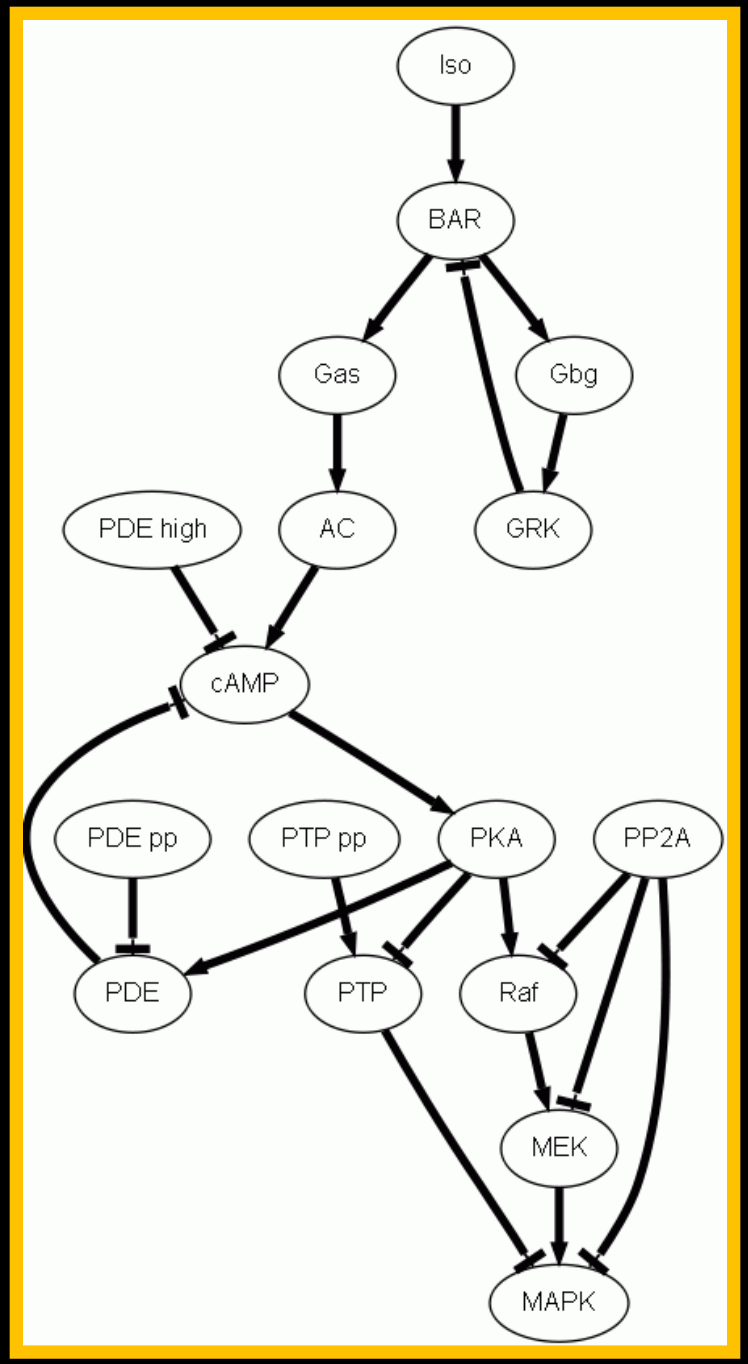
1. Transform ODEs into a Petri net.
2. Find P-invariants and compute an interaction graph.
3. Find T-invariants within P-invariant subnets and perform union operation of invariants sharing transitions.


iso extra
G a s cyto
G GDP cyto
BAR G cyto mem
iso BAR G cyto mem
b g cyto
G protein cyto
GRK cyto
GRK bg cyto
iso BAR cyto mem
iso BAR p cyto mem
BAR cyto mem


iso_extra
iso_BAR_G_cyto_mem
iso_BAR_cyto_mem
iso_BAR_p_cyto_mem

Dynamic graphs

1. Transform ODEs into a Petri net.
2. Find P-invariants and compute an interaction graph.
3. Find T-invariants within P-invariant subnets and perform union operation of invariants sharing transitions.
4. Compute influence graph (*algorithm still in development to accomodate models with complex regulation*).

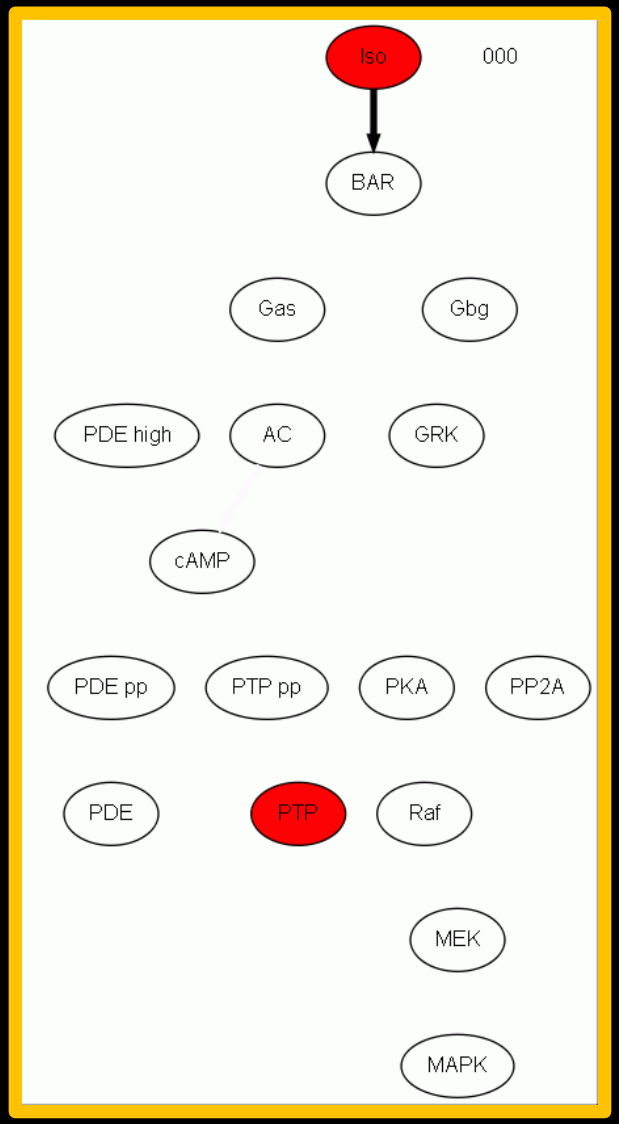


For a signaling network, the influence graph shows the activation and inhibition interactions between nodes (molecules) as the signal propagates from a source.

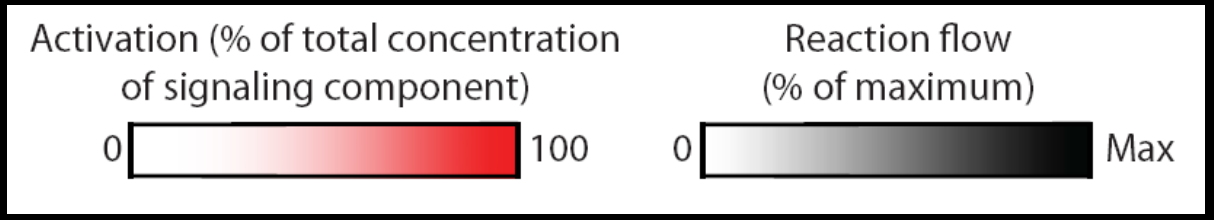
Most nodes correspond to more than one variable (place).

Most edges correspond to more than one flux (transition).

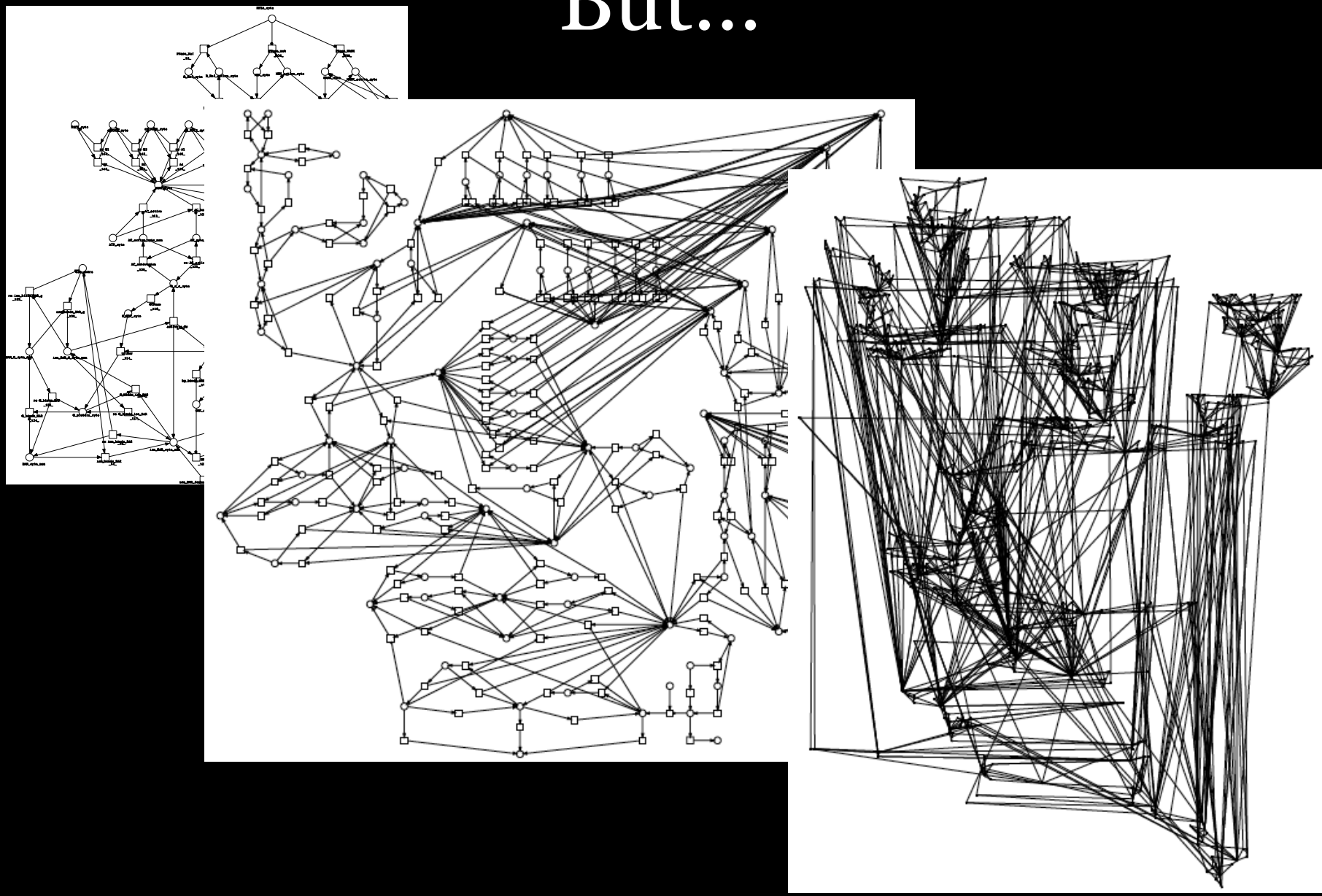
Dynamic graph



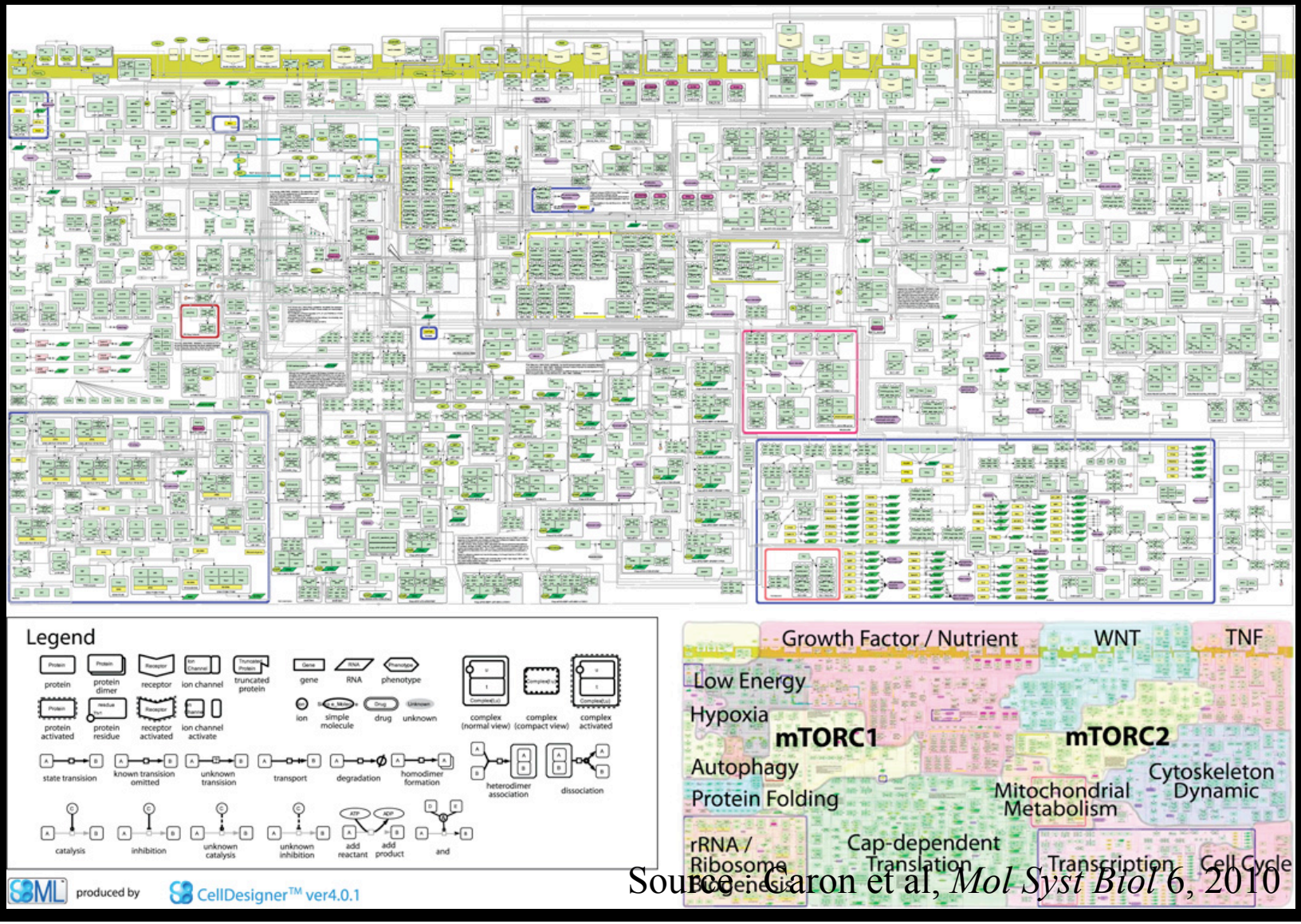
The simulation data from the mathematical model that is numerically solved can then be color coded onto the influence graph to create a dynamic representation of the model. A systems view.



But...



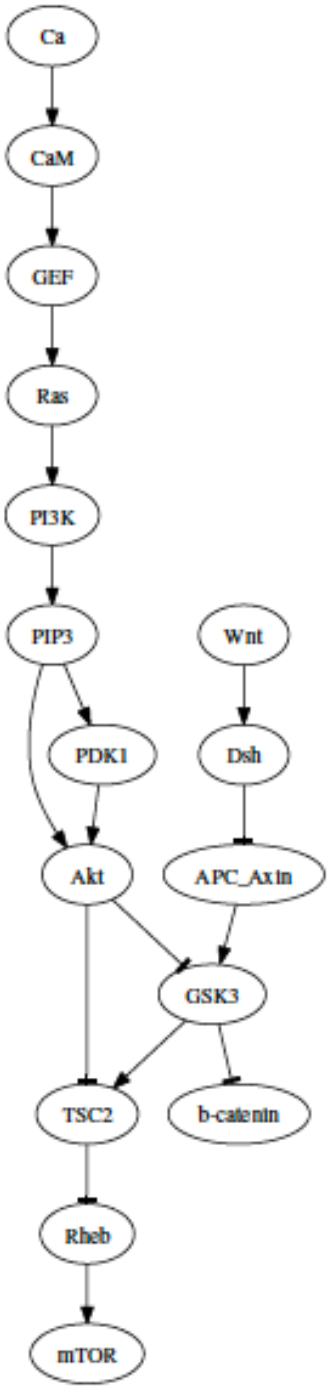
Building a model of a regulatory network

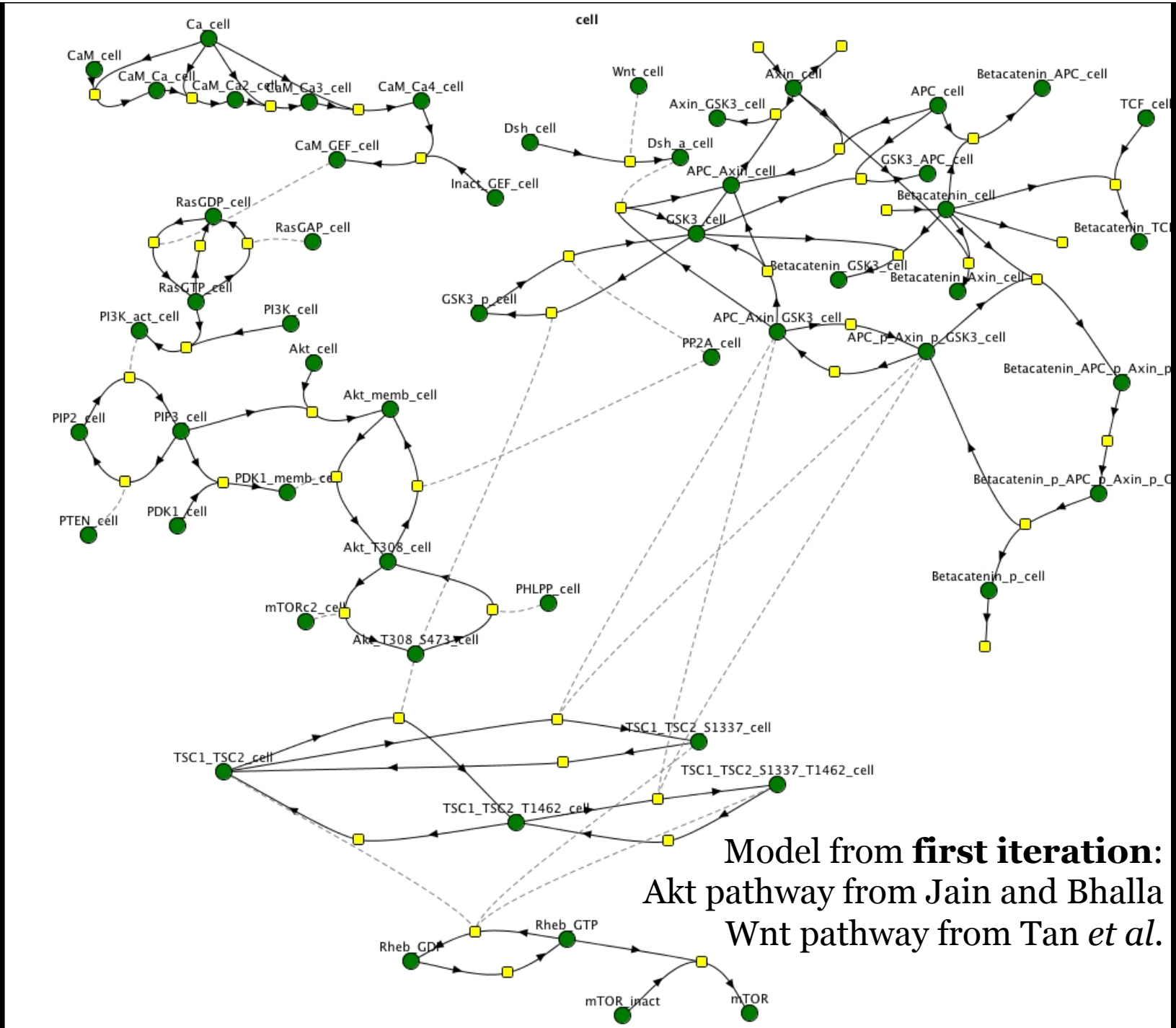


Source: Caron et al, *Mol Syst Biol* 6, 2010

Signaling patways of the model

1. Electrical stimulation causes calcium influx.
2. Calcium activates the Akt pathway.
3. Calcium causes Wnt exocytosis.
4. Akt and Wnt interact in the regulation of GSK3 and TSC2.

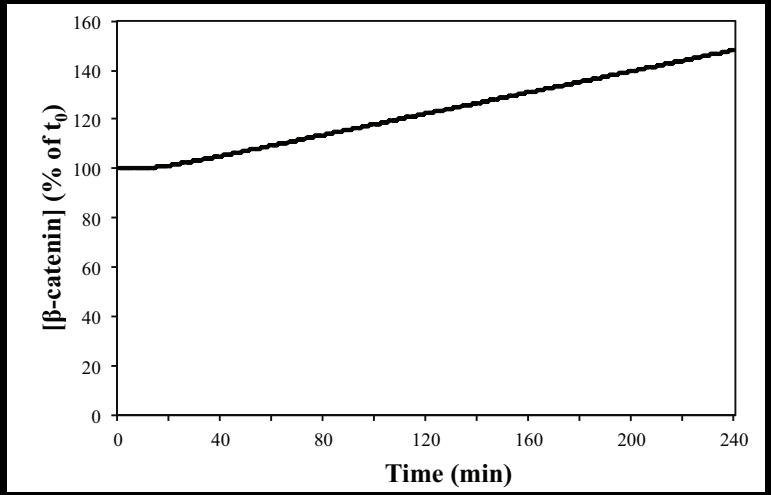




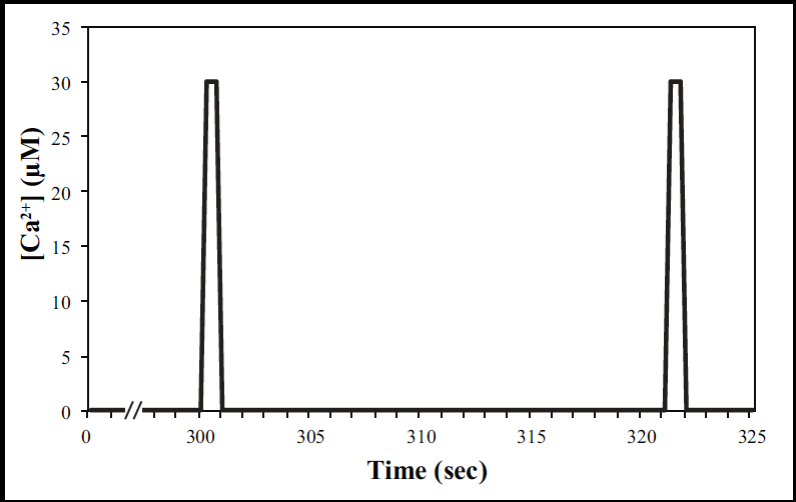
Model from **first iteration**:
Akt pathway from Jain and Bhalla
Wnt pathway from Tan *et al.*

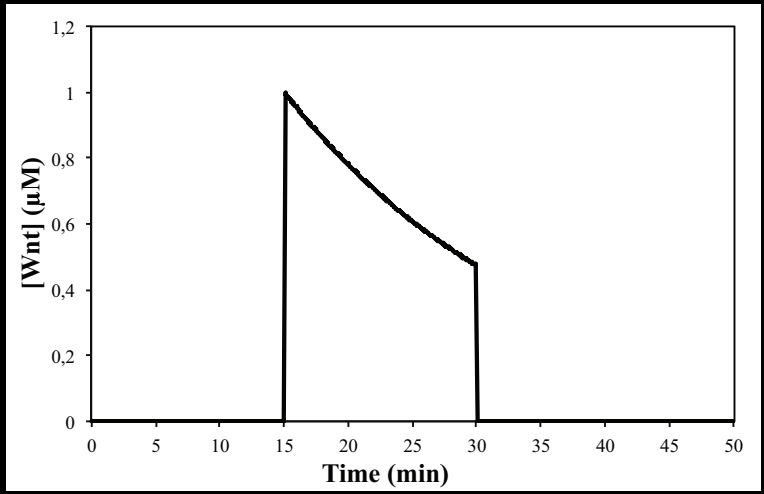
Model 1: Simulation results

Model validation

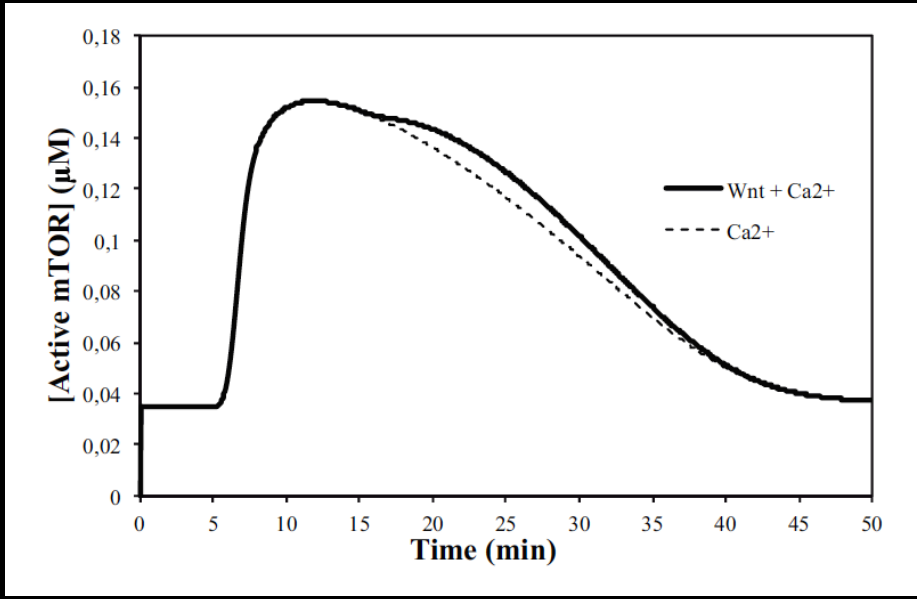


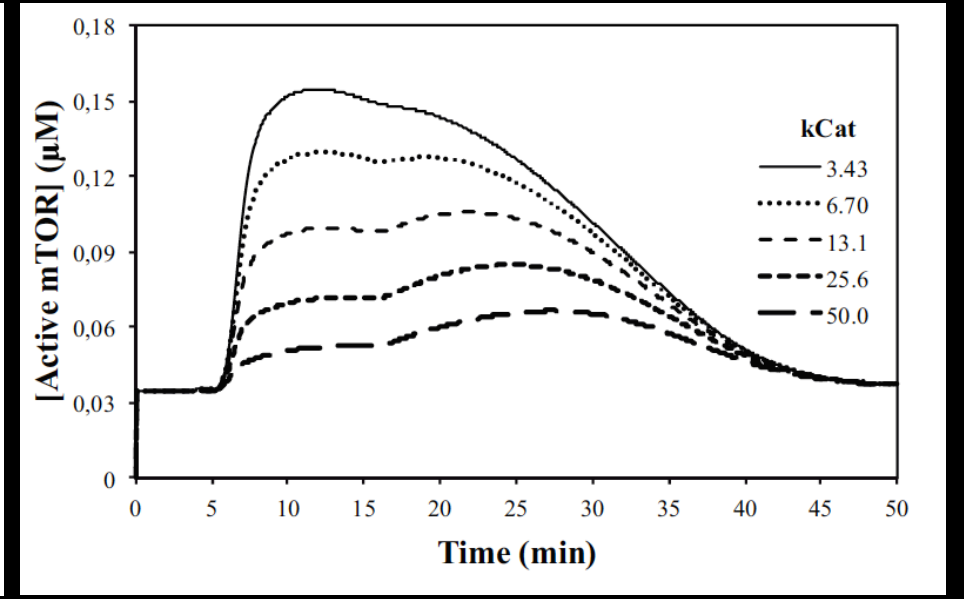
HFS-like stimulation inputs: Ca and Wnt





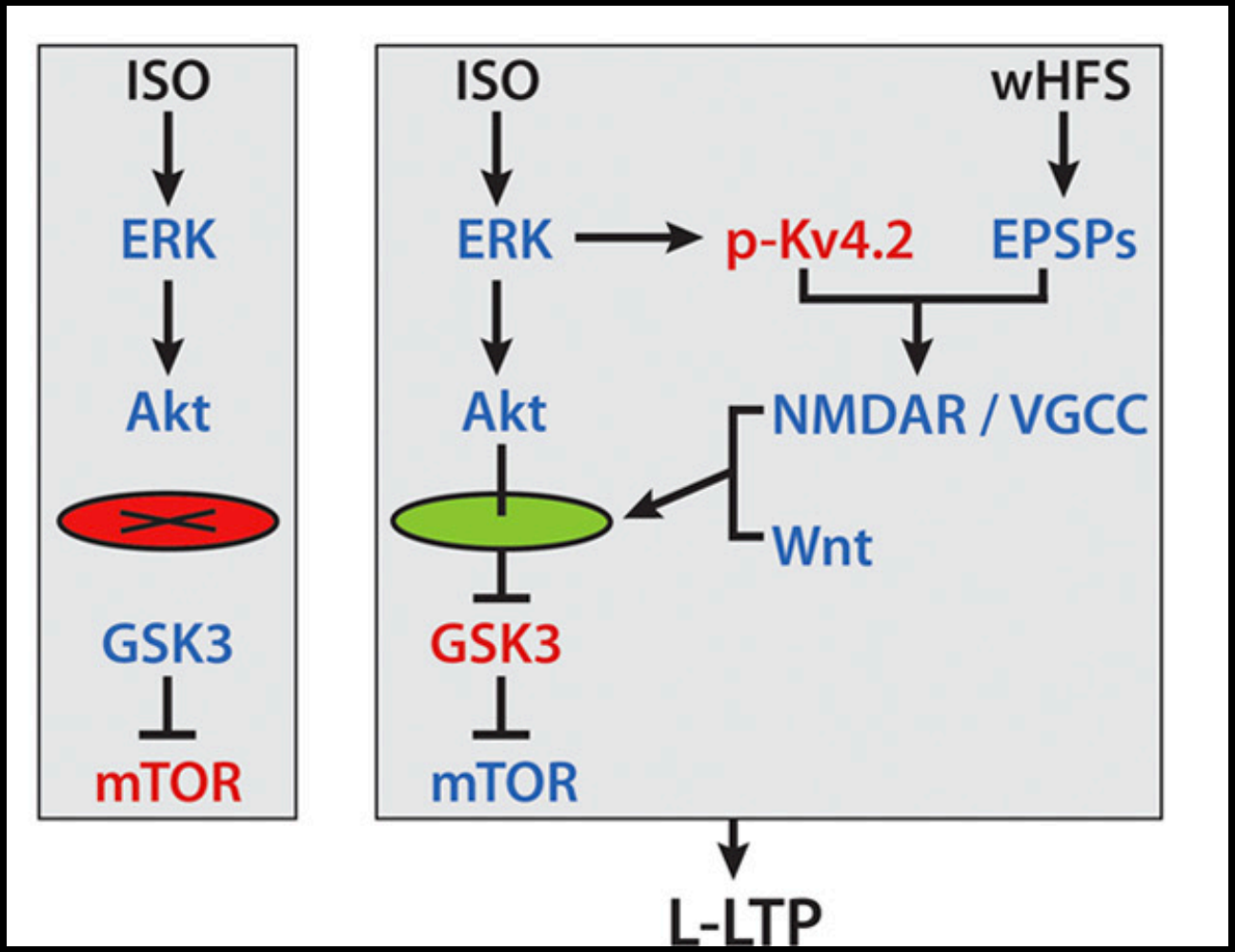
Model 1: Simulation results





Different values of the kcat parameter of the phosphorylation of TSC2 by GSK3 modifies the balance between the Akt and Wnt inputs on the activity of mTOR.

Model 1 failed to reproduce experimental data



THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 8, Issue of February 21, pp. 5947–5951, 2003
Printed in U.S.A.

The Tuberin-Hamartin Complex Negatively Regulates β-Catenin Signaling Activity*

Received for publication, August 20, 2002, and in revised form, December 10, 2002

Published April 24, 2006

JCB: ARTICLE

Activity of TSC2 is inhibited by AKT-mediated phosphorylation and membrane partitioning

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 20, Issue of May 18, pp. 17479–17483, 2001
Printed in U.S.A.

Akt Participation in the Wnt Signaling Pathway through Dishevelled*

Received for publication, December 14, 2000, and in revised form, February 28, 2001
Published, JBC Papers in Press, March 9, 2001, DOI 10.1074/jbc.C000880200

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 6, pp. 3823–3832, February 3, 2012

Adenomatous Polyposis Coli (APC) Regulates Multiple Signaling Pathways by Enhancing Glycogen Synthase Kinase-3 (GSK-3) Activity*[S]

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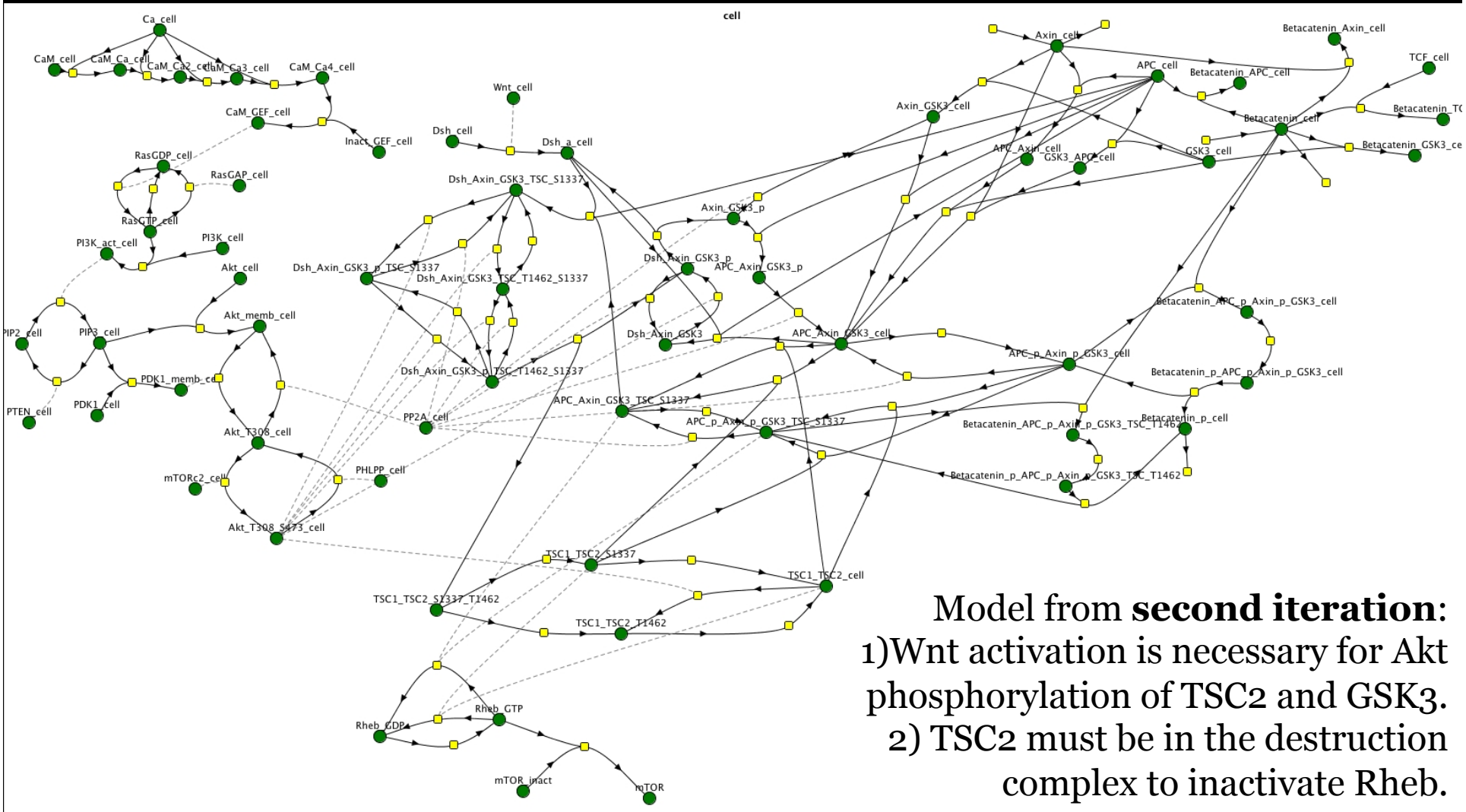
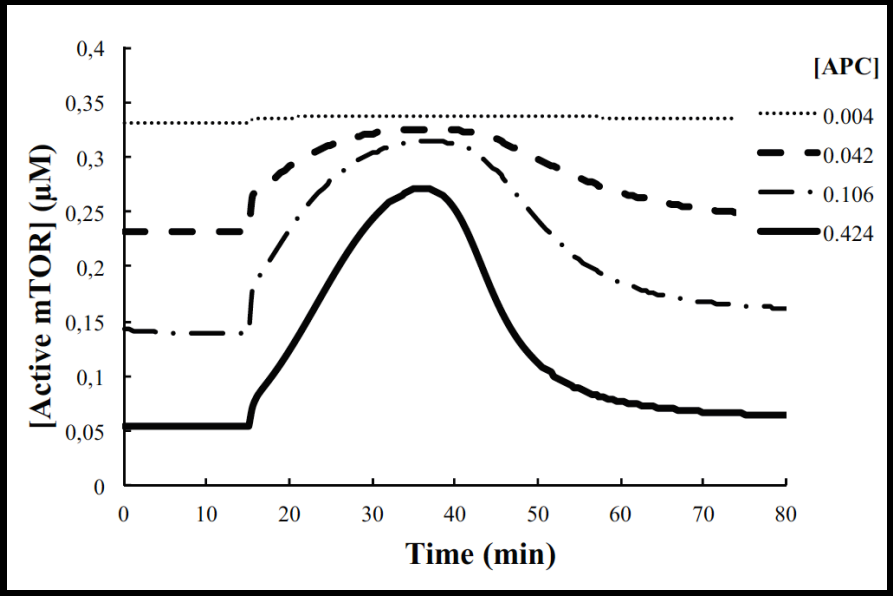
Alexander J. Valvezan[‡1], Fang Zhang[§¶], J. Alan Diehl[¶], and Peter S. Klein[‡§‖2]

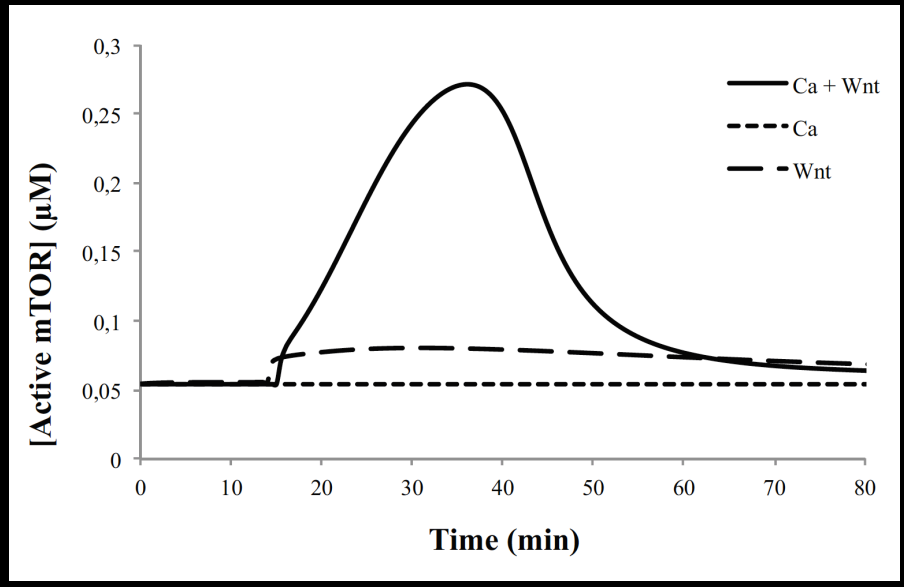
From the [‡]Cell and Molecular Biology Graduate Group, [§]Pharmacology Graduate Group, [¶]Department of Cancer Biology, The Leonard and Madlyn Abramson Family Cancer Research Institute and Cancer Center, and the [‖]Department of Medicine (Hematology-Oncology Division), University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104



Model from **second iteration**:
1)Wnt activation is necessary for Akt phosphorylation of TSC2 and GSK3.
2) TSC2 must be in the destruction complex to inactivate Rheb.

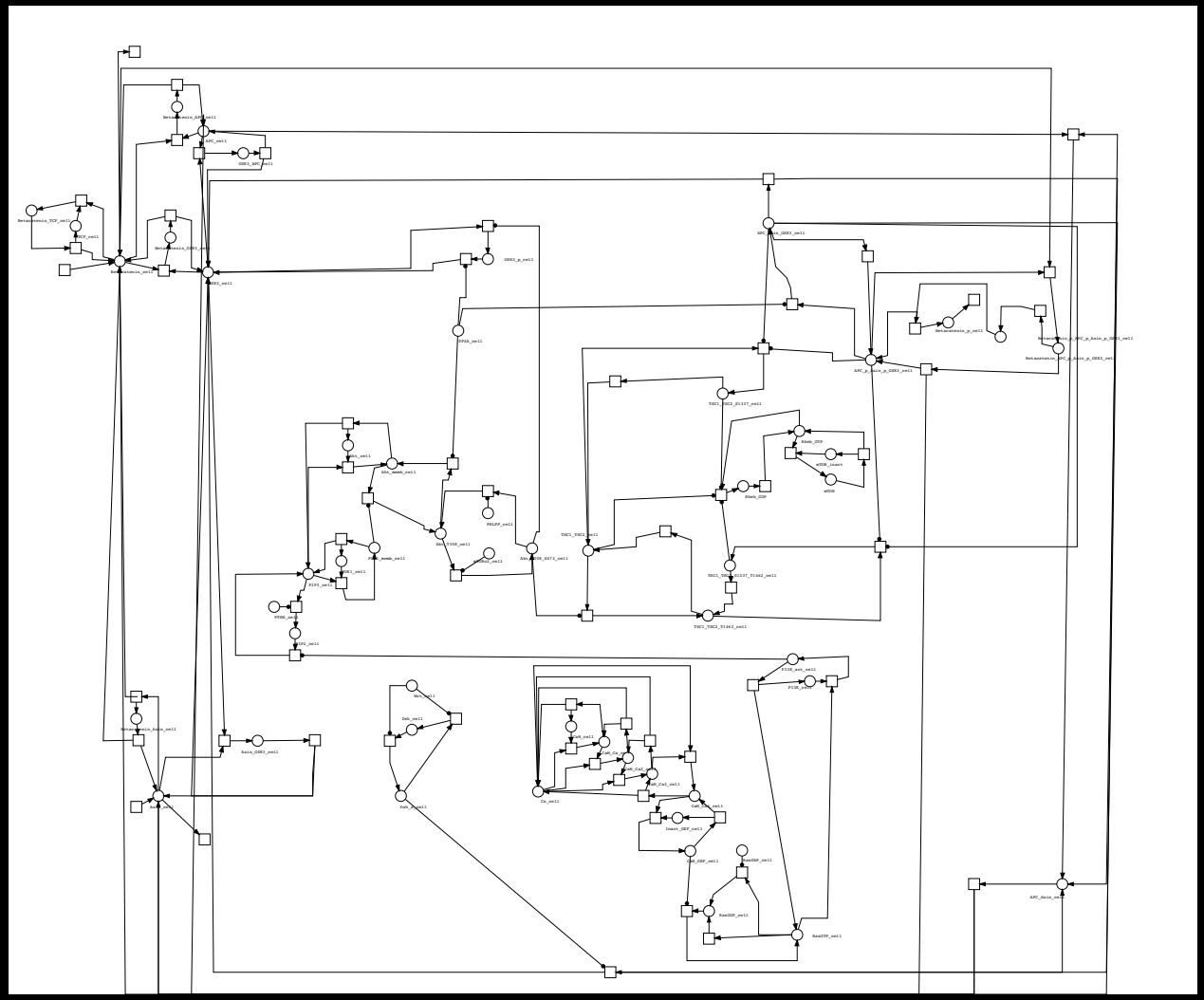
Model 2: Simulation results



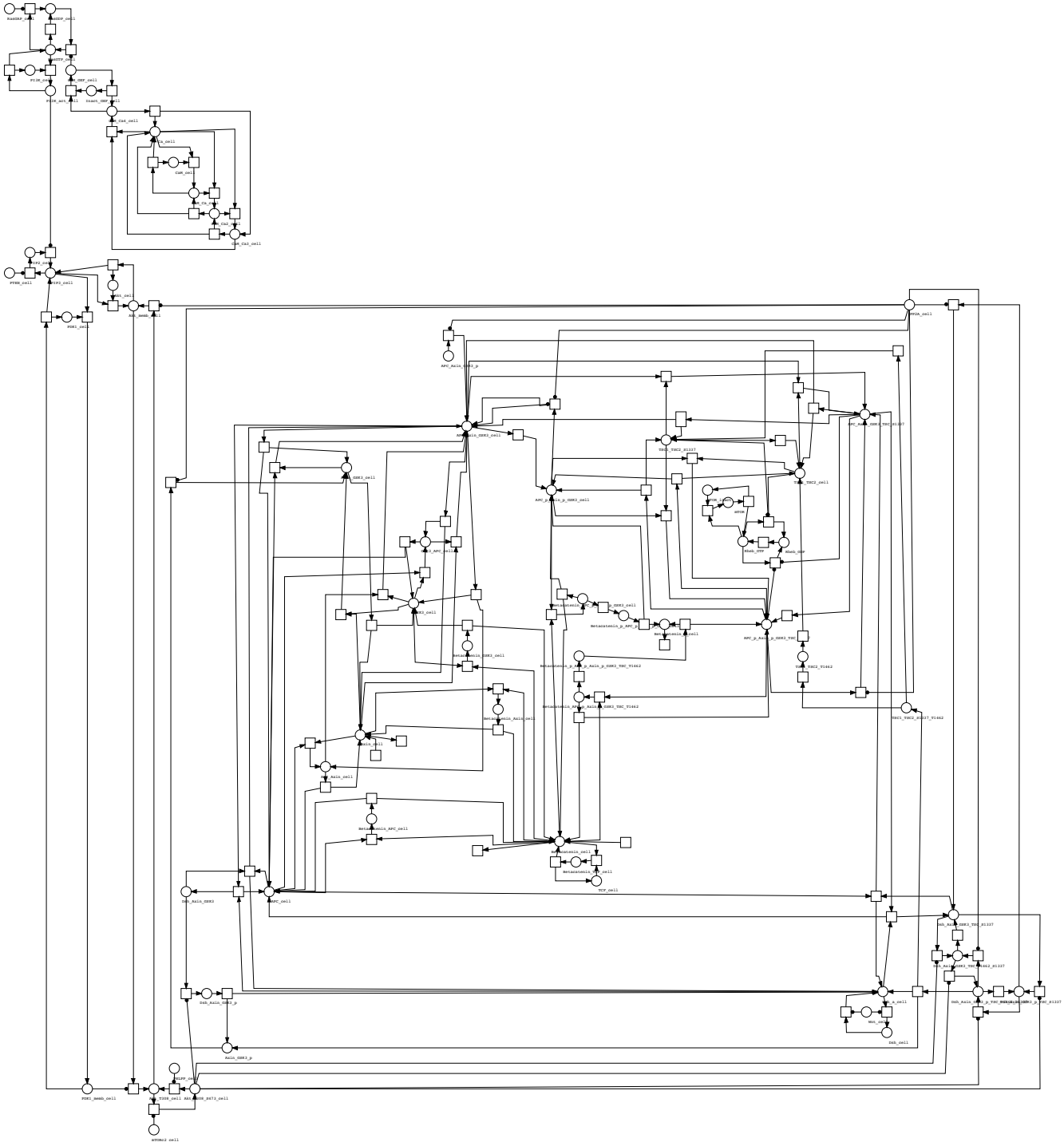


With the modifications, the model is now in good agreement with experimental data.

Dynamics of the models of the mTOR regulatory models



Model 1

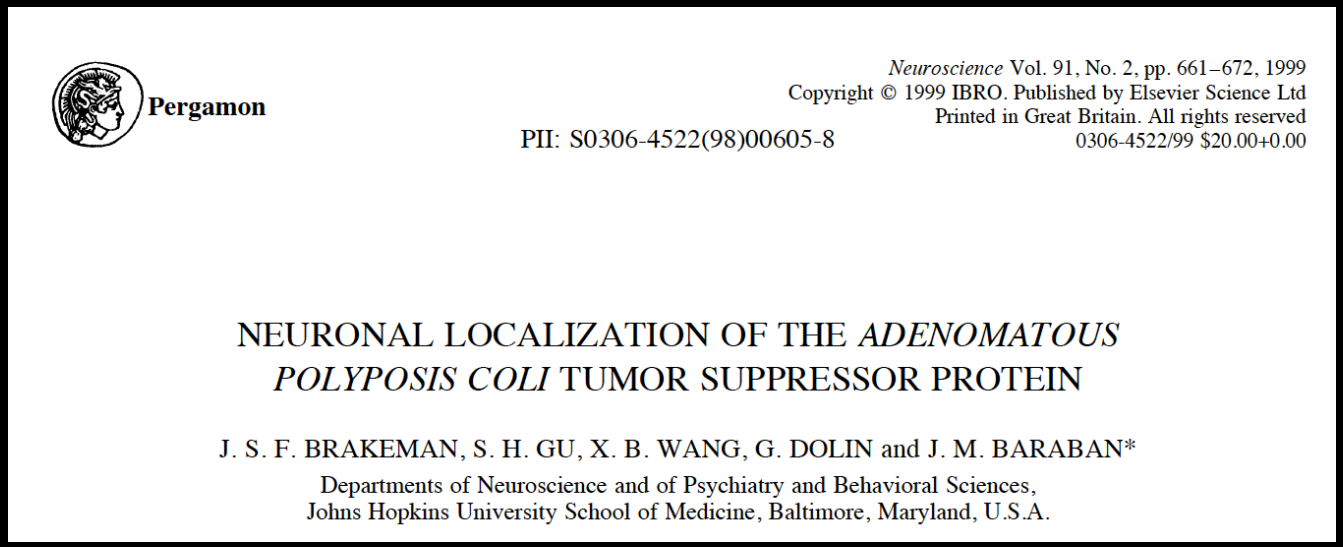


Model 2

Conclusion

- There are still controversial issues around the Wnt pathway because of conflicting experimental results. A tighter integration than expected of the two pathways in the model was needed to reproduce experimental data.
- The Akt-GSK3-TSC2 feedforward motif can behave as a coincidence detector (an AND logical gate) only under certain conditions.
  - Abundance of APC

APC in neurons



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NEURONAL LOCALIZATION OF THE *ADENOMATOUS POLYPOSIS COLI* TUMOR SUPPRESSOR PROTEIN

J. S. F. BRAKEMAN, S. H. GU, X. B. WANG, G. DOLIN and J. M. BARABAN*
Departments of Neuroscience and of Psychiatry and Behavioral Sciences,
Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.

"Our finding that APC is expressed at high levels in neuronal cell bodies, dendrites and axons raise the possibility that this tumor suppressor protein may help regulate b-catenin signalling in multiple neuronal compartments."

Conclusion

- There are still controversial issues around the Wnt pathway because of conflicting experimental results. A tighter integration than expected of the two pathways in the model was needed to reproduce experimental data.
- The Akt-GSK3-TSC2 feedforward motif can behave as a coincidence detector (an AND logical gate) only under certain conditions.
  - Abundance of APC
- A better understanding of the regulatory network with modeling and simulation can lead to better experiments.
- Future work: complete the dynamic graph algorithm, adding the ERK pathway and the target of mTOR, p70S6K.

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