PATHWAY BASED MODELS TO UNDERSTAND THE ROLE OF BIOLOGICAL TIMING IN HEALTH AND DISEASE

Carolyn Talcott and Mary Ann Greco
SRI International
May, 2008

PLAN

- Big Picture
- Preliminary data and model
- SSB and PL [backup]

Vision

- System level model of biological timing using simple behaviors
  - Circadian rhythms
  - Sleep / wake behavior
  - Connected to activity, stress, disease
- Hypothesis: Biological timing is key to maintain health and for successful treatment of disease

SYMBOLIC SYSTEMS BIOLOGY

- Symbolic -- logical concepts and relations
- Systems -- mulitple aspects, views
- Requires integration of experiment and modeling
  - translate data into knowledge

INTER-ORGAN RELATIONSHIPS

- The human body is compartmentalized
  - Brain: regulation / corrordination of functions
  - Liver: acquisition / adaptation of ingested raw material for maintainence and growth
  - Heart: circulation of blood, delivery of oxygen
- Simple behavior -- baseline of how organs interact
  - Time of day (circadian) - effectiveness of chemotherapies
  - Sleep-wakefulness - affected by many factors

Biological Timing and Sleep

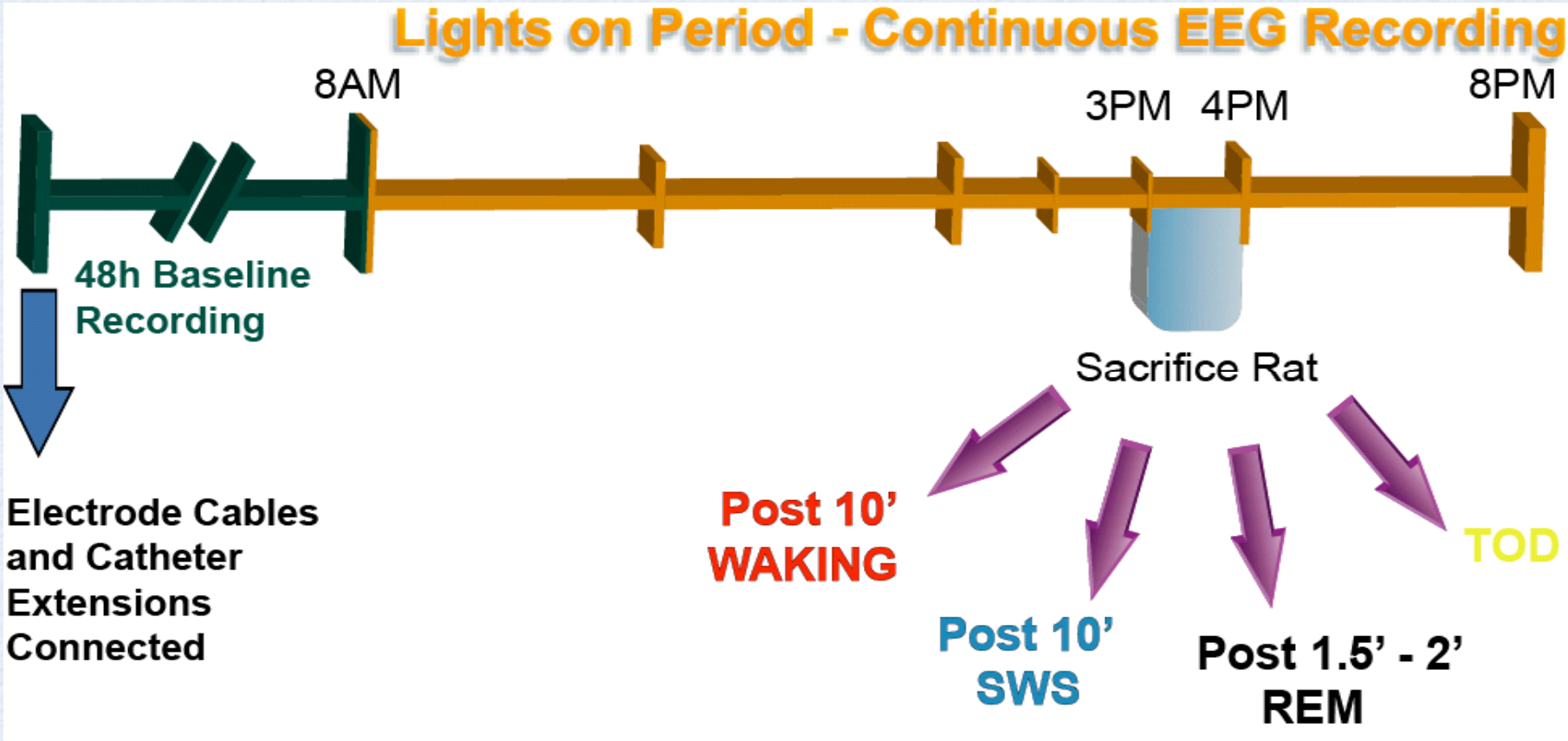
- Why sleep?
  - An essential behavior
  - Regulated by circadian and homeostatic influences
  - Accounts for about 1/3 of lifetime
    - the function is unknown
- Questions
  - What are your organs doing when you sleep?
  - When you are awake?
  - What is common across organs?
  - What is unique to an organ?

Biological Timing: Approach

Rat model

- Identify proteins in various organs differentially expressed across sleep-wake states
- Cellular functions extrapolated from protein IDs
- Pathway based modeling
  - Use known signal transduction pathways to create model for each organ
  - Identify common/unique pathways for each organ
- Verify results using other proteomics approaches (Western analysis, enzyme activity assays) in different behavioral paradigms (SD/RS)

NATURAL SLEEP PARADIGM



INTER-ORGAN MODELING OF SIMPLE BEHAVIOR

Hypothesis- Protein levels and activity in each of these organs are affected by sleep. Preliminary results indicate unique profiles underlie sleep-wake in each organ…

Total protein expression across behavioral state within organ

Whole Gel Analyses: BRAIN

717 total # of protein spots
516 protein spots **common to all states**
23 protein spots expressed only in **SWS**
14 protein spots expressed only in **Wake**
19 protein spots expressed only in **Time of Day**

Whole Gel Analyses: LIVER

816 total # of protein spots
519 protein spots **common to all states**
66 protein spots expressed only in **SWS**
231 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Whole Gel Analyses: HEART

1006 total # of protein spots
611 protein spots **common to all states**
261 protein spots expressed only in **SWS**
134 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Phosphoprotein expression across behavioral state within organ

Whole Gel Analyses: BRAIN

194 total # of phosphoprotein spots
60 protein spots **common to all states**
24 protein spots expressed only in **SWS**
5 protein spots expressed only in **Wake**
18 protein spots expressed only in **Time of Day**

Whole Gel Analyses: LIVER

51 total # of protein spots
19 protein spots **common to all states**
14 protein spots expressed only in **SWS**
18 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Whole Gel Analyses: HEART

517 total # of protein spots
250 protein spots **common to all states**
137 protein spots expressed only in **SWS**
130 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

…and a subset of these spots are present in all 3 organs

Comparison of total protein expression across Brain / Liver / Heart

Waking

1144 total # of protein spots

222 protein spots **common to all organs**

Proteins **unique** to each organ

93 protein spots expressed in **Brain**

123 protein spots expressed in **Liver**

205 protein spots expressed in **Heart**

Slow Wave Sleep

1212 total # of protein spots

195 protein spots **common to all organs**

Proteins **unique** to each organ

130 protein spots expressed in **Brain**

97 protein spots expressed in **Liver**

305 protein spots expressed in **Heart**

Comparison of phosphoprotein expression across Brain / Liver / Heart

Waking

432 total # of phosphoprotein spots

18 protein spots **common to all organs**

Proteins **unique** to each organ

39 protein spots expressed in **Brain**

8 protein spots expressed in **Liver**

330 protein spots expressed in **Heart**

Slow Wave Sleep

473 total # of phosphoprotein spots

11 protein spots **common to all organs**

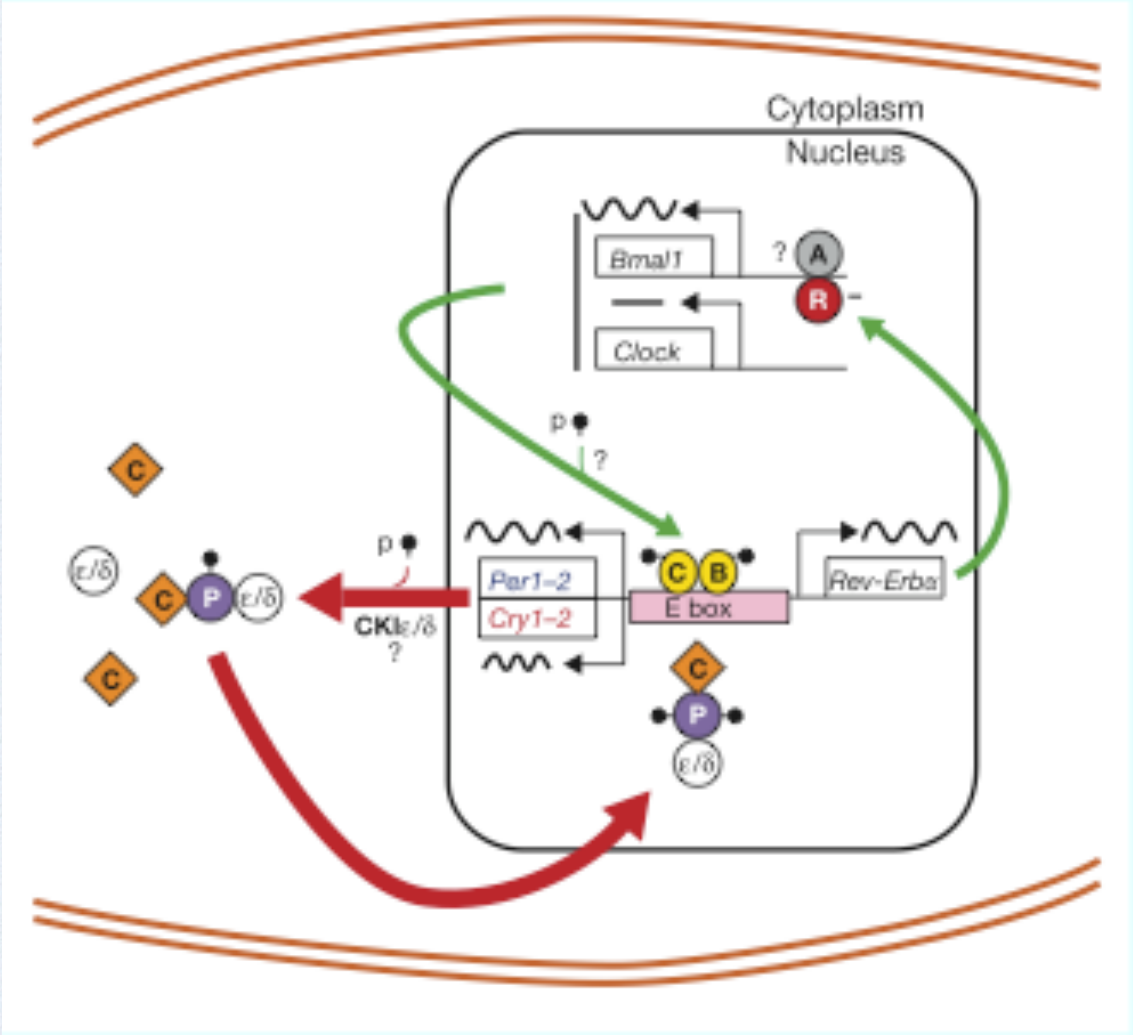
Proteints **unique** to each organ

77 protein spots expressed in **Brain**

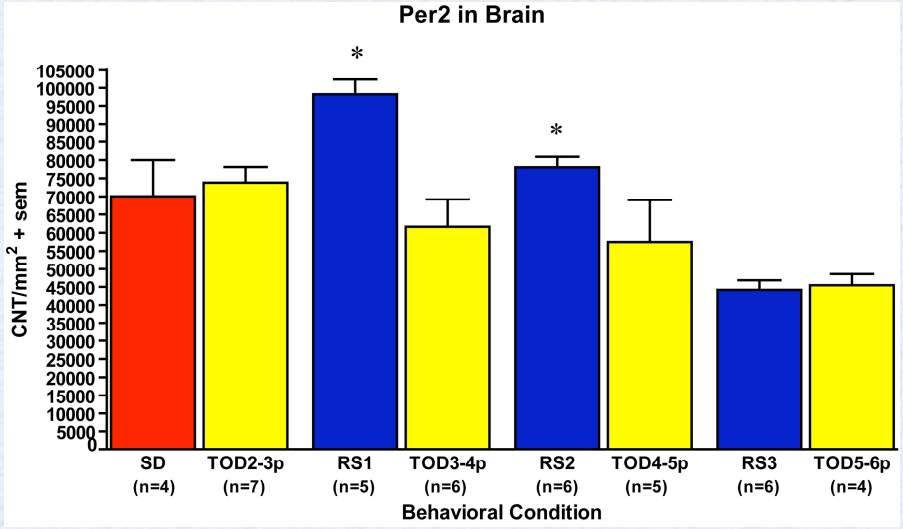
4 protein spots expressed in **Liver**

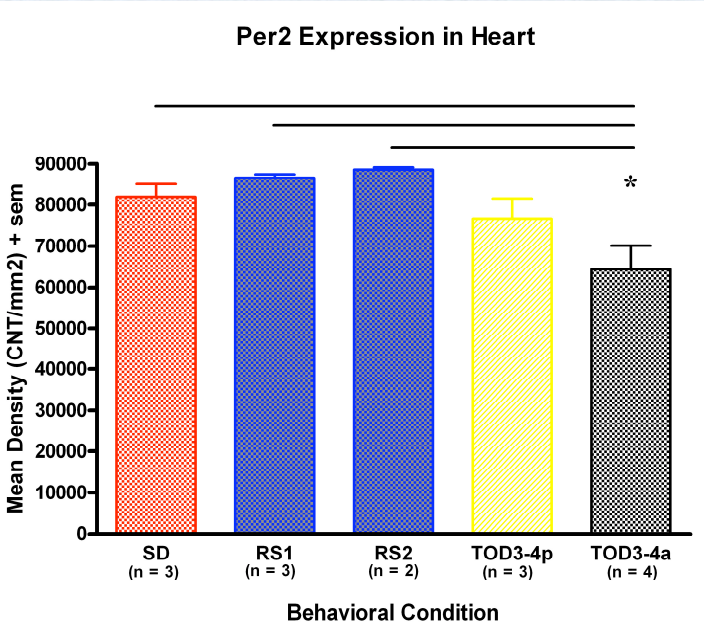
296 protein spots expressed in **Heart**

The time of day (circadian) influence on protein expression is well established.

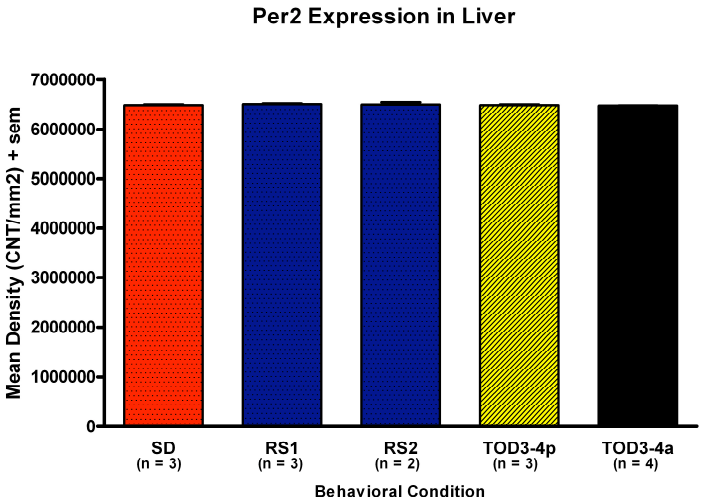


Per2 expression varies across SD/RS in these organs



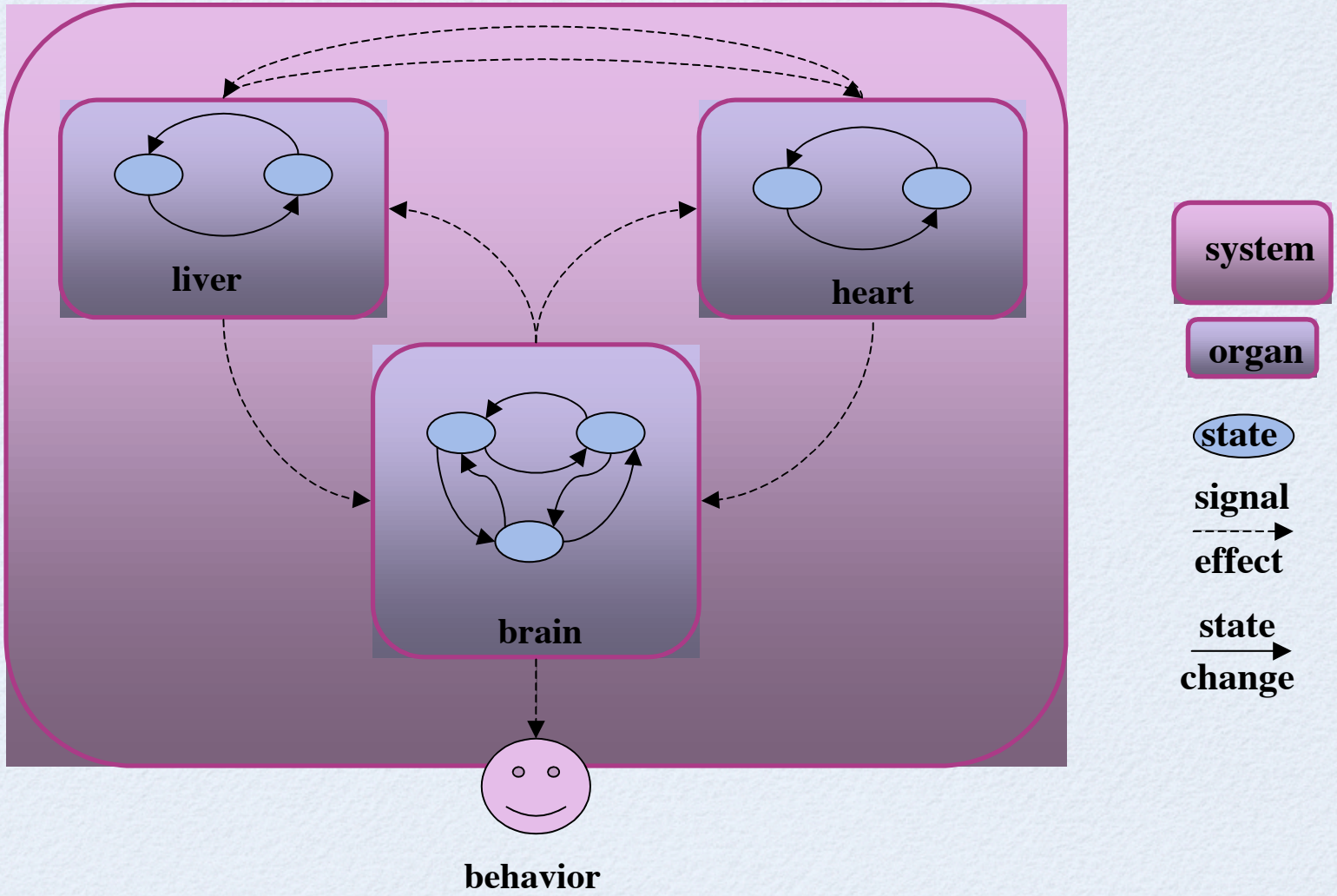


p<0.01 ANOVA followed by Fisher LSD post-hoc comparison



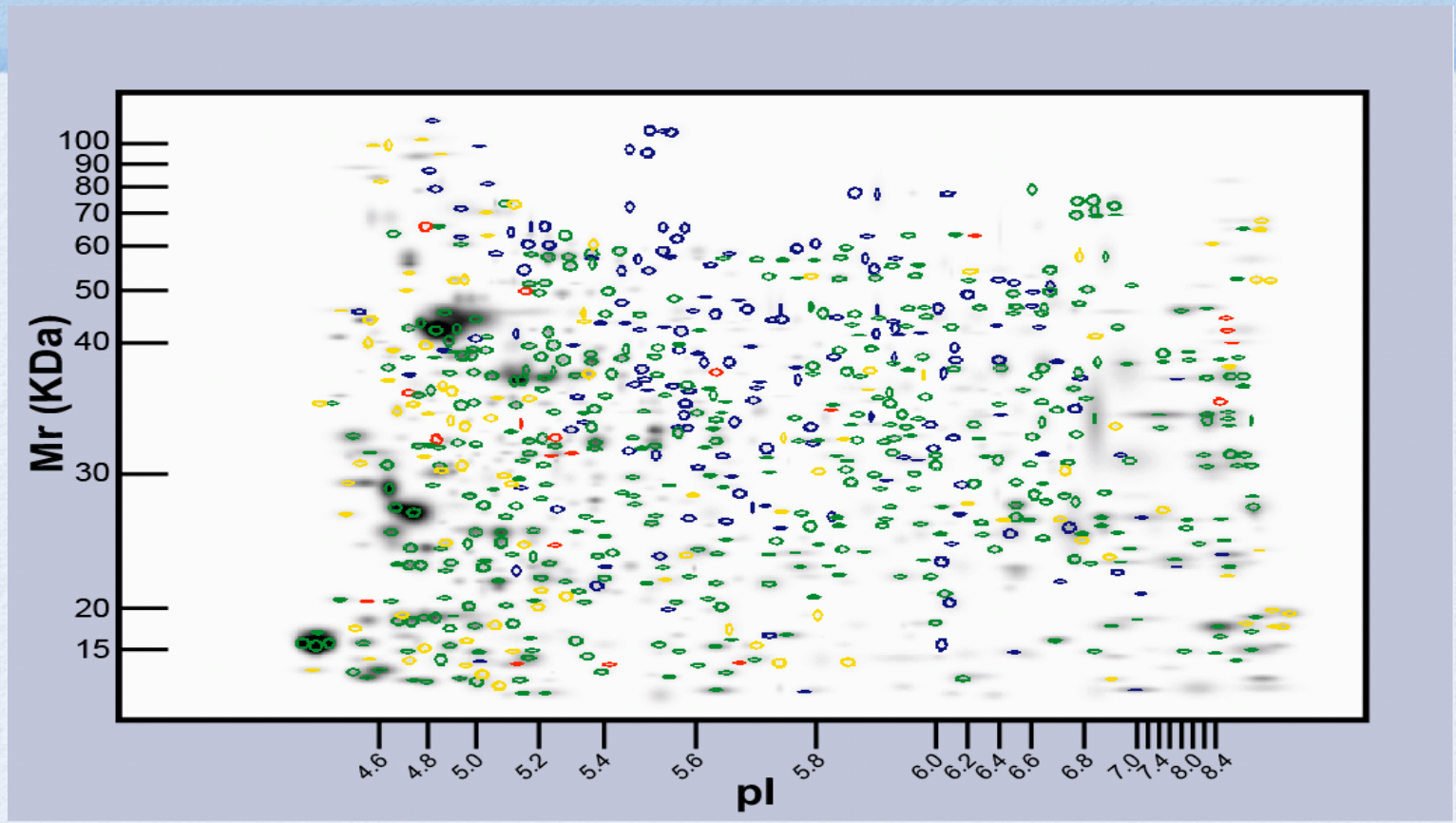
One-way ANOVA not signifcant (p=0.7721)

ARCHITECTURE OF A SYSTEMS MODEL



PRELIMINARY RESULTS FOR THE BRAIN

2D MASTER GEL -- FRONTAL CORTEX



Proteins unique to different states were identified

Those modeled in PL included Actin and Rhob

Use the PLA explorer to find signaling connections

SPOTS IDENTIFIED BY MS

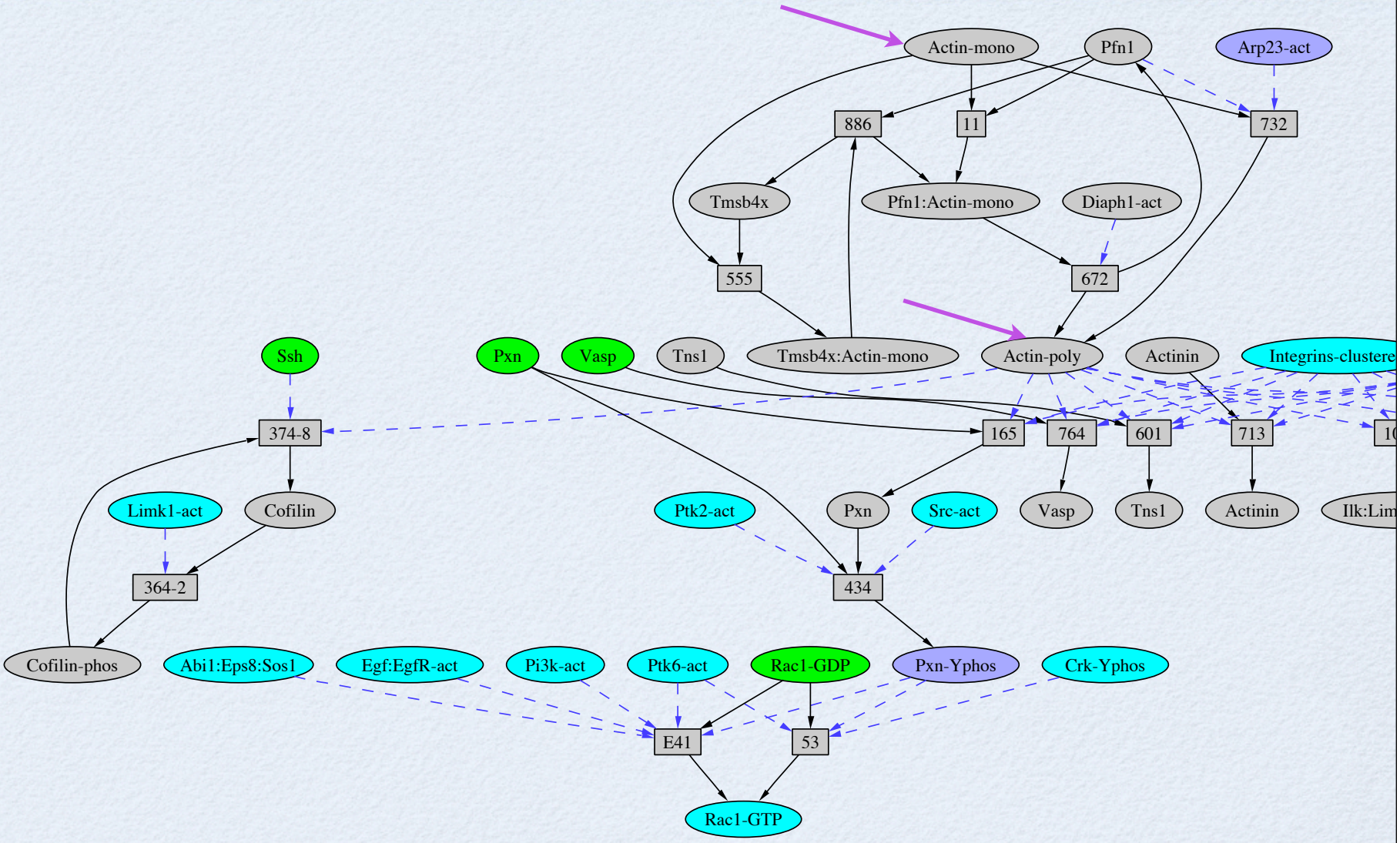
Spot ID #	Protein Identified	Mr	pI	NCBI Accession #	Associated Biological Function
15S, 20S, 22S	Actin, cytoplasmic 1 (beta-actin)	41.7	5.61	ATRTC	cytoskeletal structure and support; cell motility
21S, 20S	Creatine kinase, B chain	42.7	5.74	NP_036661	high energy fuel stores; cellular energy metabolism
10S	Glutathione S-transferase Yb-1	25.9	8.41	NP_058710	redox state; essential to olfactory processes
7S	Glyceraldehyde-3-phosphate dehydrogenase	35.8	8.45	CAA26150	ATP production; glycolysis
9S	Homolog of zebrafish ES1	28.2	9.13	AAH79380	mitochondrial precursor; cellular energy metabolism
8S	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	20.9	7.94	XP_213242	high energy fuel stores; cellular energy metabolism
14S	Vesicular-fusion protein NSF	82.6	7.22	P18708	vesicle-mediated transport in Golgi
24W	Creatine kinase, ubiquitous mitochondrial	47	8.36	AAH25976	high energy fuel stores; cellular energy metabolism
12W	Epidermal growth factor-receptor-binding protein GRB-3	26.0	5.17	A46243	intracellular signalling
23W	Glutamine synthetase	42.3	7.34	NP_058769	ATP production; cellular energy metabolism
12W	GTP-binding protein rab3D	24.4	5.04	A45384	protein transport; regulation of exocytosis
23W	Pyruvate dehydrogenase E1 alpha	43.3	8.22	CAA78146	cellular energy metabolism; glycolysis
12W	Rho GDP dissociation inhibitor (GDI) 1	22.9	5.39	BAC35881	protein signal transduction; mediates cell adhesion
12W	Rho-related GTP-binding protein RhoB	22.1	5.34	P62746	intracellular protein trafficking; mediates apoptosis
3N	Cofilin-1 (non-muscle isoform)	18.4	8.34	AAH86533	actin polymerization / depolymerization
13S, 19W	Mitochondrial aconitase	85.4	8.01	AAH61999	ATP production; cellular energy metabolism
11N, 15S	Phosphoglycerate kinase 1	44.5	8.11	NP_445743	ATP production; glycolysis
6N	Phosphoglycerate mutase 1	28.7	7.49	AAH02241	ATP production; glycolysis

Picking out the interesting bits (with some additional curation)

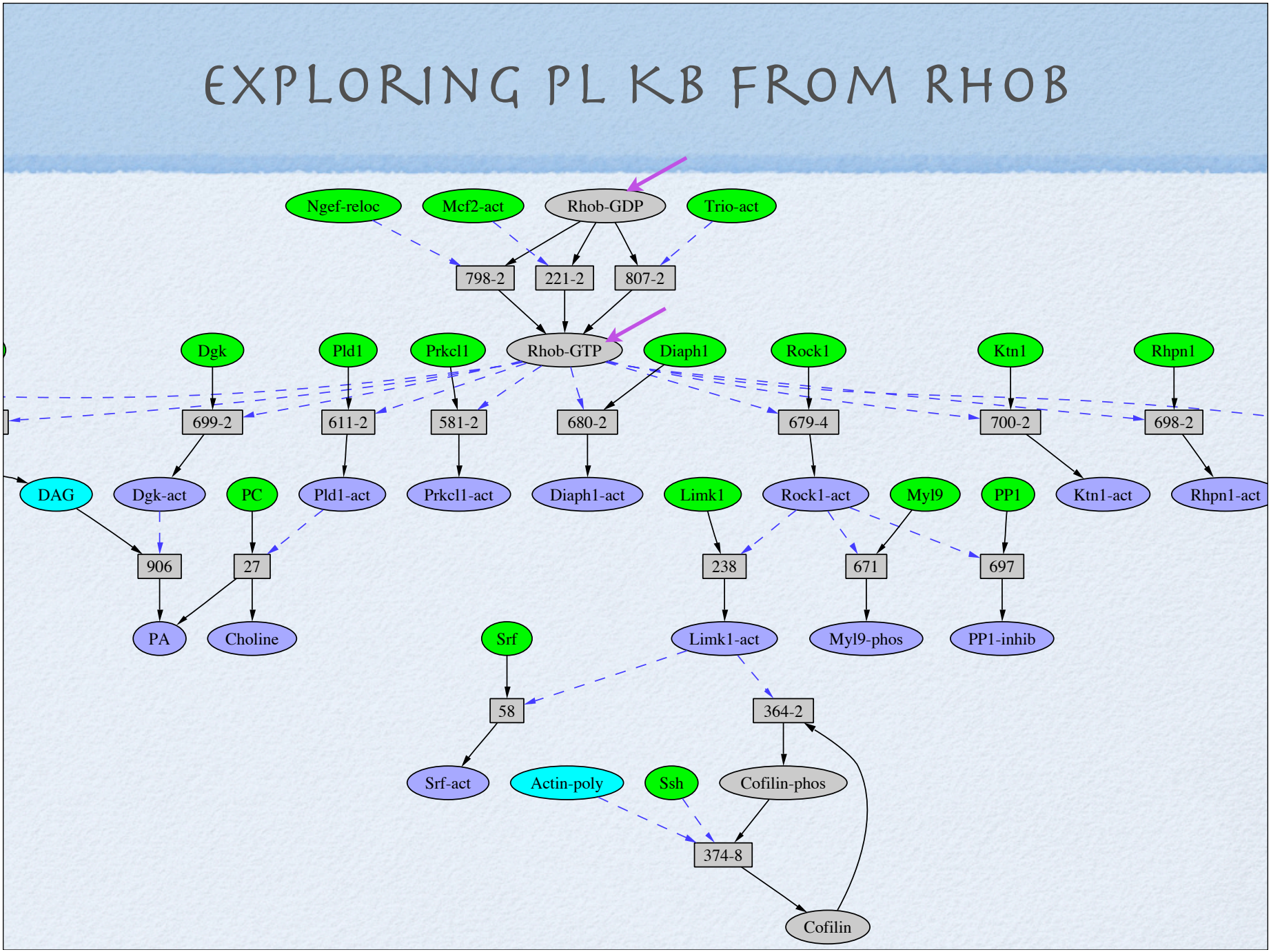
from 2D-Gels:

State	Protein	Data	Hypothetical Modification
Wake	RhoGdi	unique spot	Yphos
	RhoB	unique spot	GTP
	Grb3{1}	unique spot	upreg (splice variant)
	Cofillin	common	phos
	Actin	phosphorylated	polymerized
SWS	RhoGdi	unique spot gone	unphosed
	Rhob	unique spot gone	GDP
	Grb3	unique spot gone	downreg(splice variant)
	Cofillin	common	unphos
	Actin-phos	less phosphorylated	depolymerized{2}

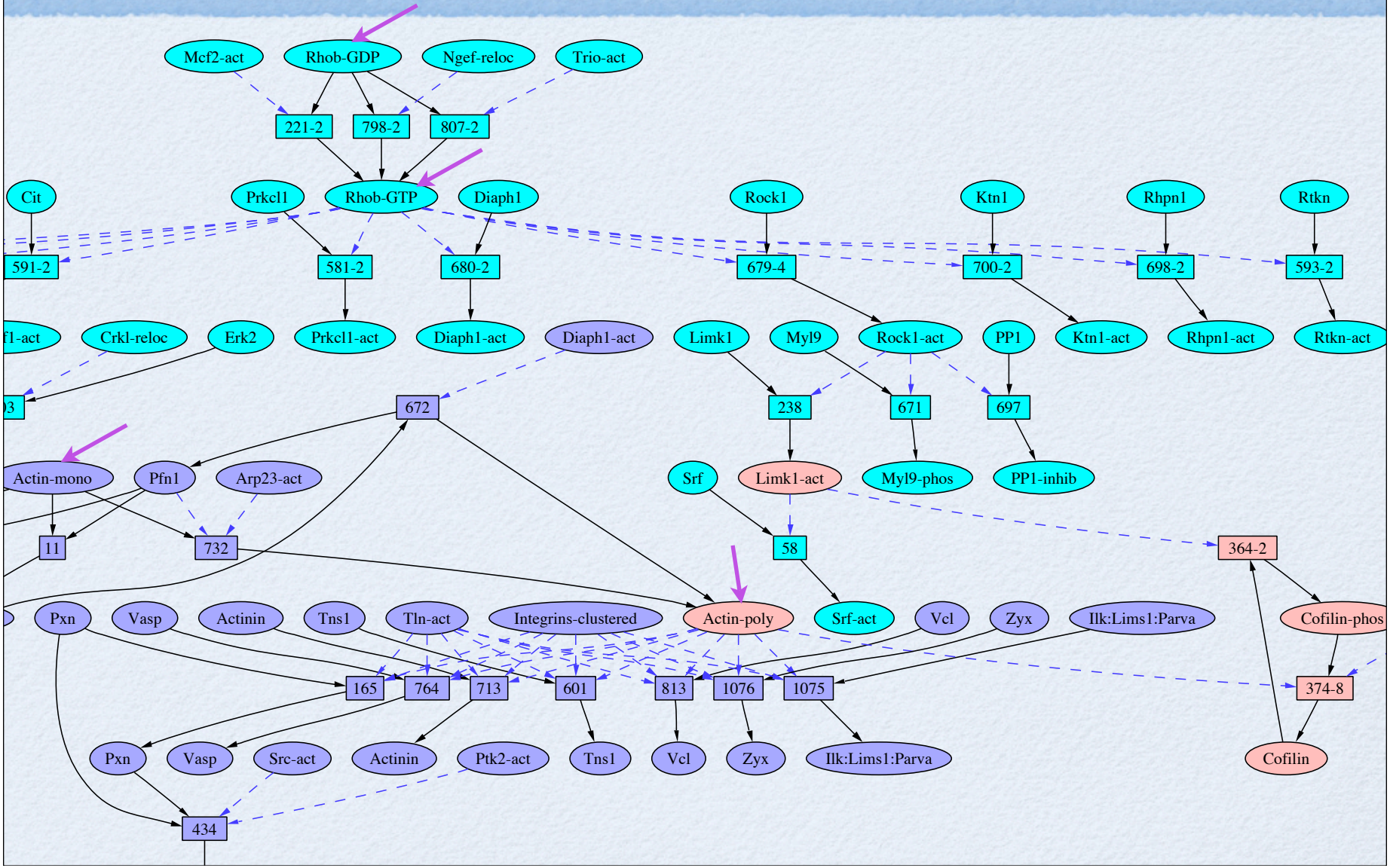
EXPLORING PL KB FROM ACTIN



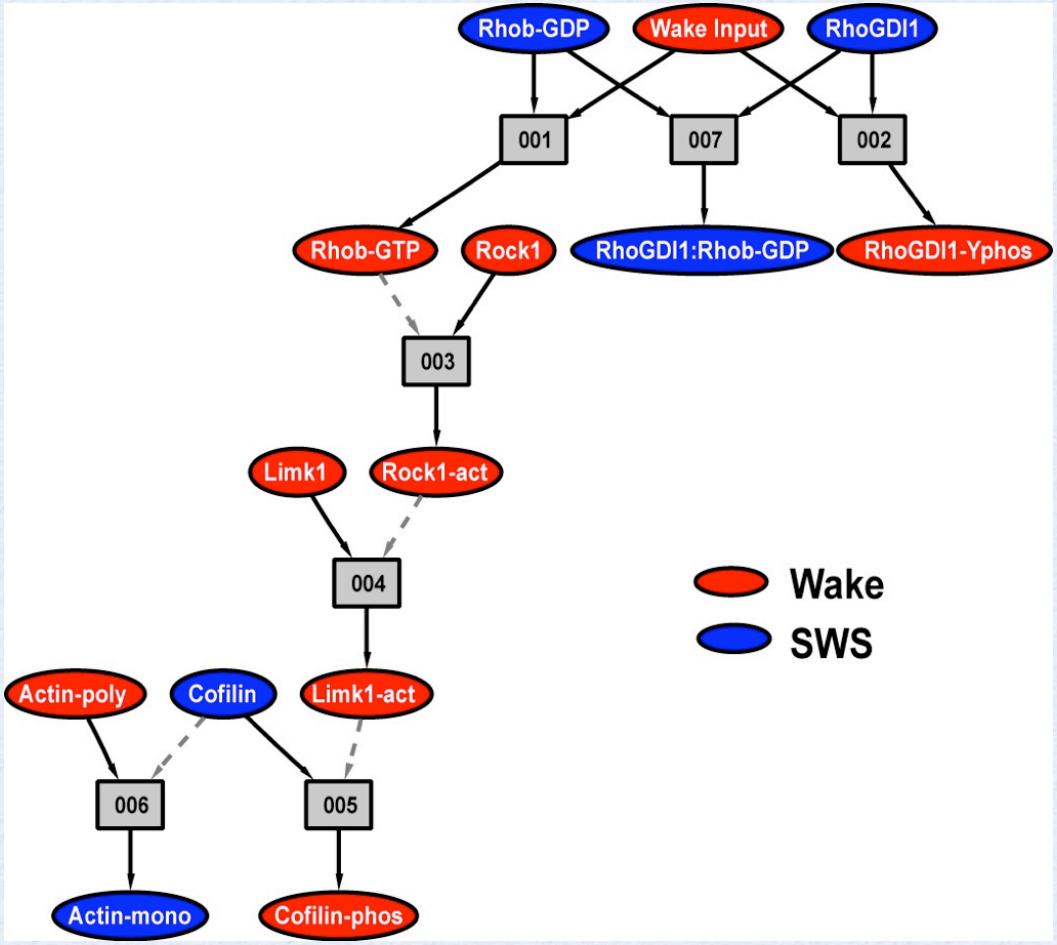
EXPLORING PL KB FROM RHOB



COMPARING THE EXPLORE NETS



A HYPOTHETICAL MODEL PATHWAY RELATING STATE AND SYNAPTIC PLASTICITY



Wake state:
unknown signal(s)
=> phosphorylation of Rock1
=> activation of Limk1
=> phosphorylation of cofilin
=> increase in polymerized actin
(Phosphorylated cofilin is unable to depolymerize actin)

SWS:
RhoDG11 binds Rhob-GDP
(is not phosphorylated)
=> Rock1, Limk1, and cofillin would not be phosphorylated and
=> actin depolymerization
=> decrease in synaptic weight

Next Steps

- Test pathway model
  - determine ratio of phosphorylated / non-phosphorylated proteins for spontaneous waking, sws, and recovery sleep following deprivation (Western analysis)
  - expect LimkP/Limk higher in wake than sws, and lower in sleep following deprivation
  - Cofilin should have opposite ratios
- Study effects of age
- Model behaviors / timing in liver and heart

SYMBOLIC SYSTEMS BIOLOGY AND PATHWAY LOGIC

SYMBOLIC SYSTEMS BIOLOGY

Symbolic systems biology is the qualitative and quantitative study of biological processes as integrated systems rather than as isolated parts.

Our initial goal for symbolic systems biology include:

- modeling causal networks of biomolecular interactions in a logical framework at multiple scales
- developing executable formal models that are as close as possible to domain expert's (biologists) mental models
- being able to compute with and analyze these complex networks
  - abstracting and refining the logical models
  - using simulation/deduction to compute/check postulated properties
  - making testable predictions about possible outcomes
  - using experimental results to update the models

Sampling of Symbolic/Executable Modeling Approaches

- Rewriting Logic + Temporal Logic (Pathway Logic!)
- Chemical Abstract Machine + Computation Tree Logic (BIOCHAM)
- Membrane calculi -- spatial process calculi / logics
  - BioAmbients / Brane calculus -- mobility of membranes
  - P Systems -- mobility of processes
- Statecharts + Live Sequence Charts
- Process Algebras + Stochastic Simulation & Probabilistic Model Checking
- Hybrid SAL -- hybrid (discrete + continuous) systems

ABOUT PATHWAY LOGIC

Pathway Logic (PL) uses rewriting logic to model biological processes as executable formal specifications with pathways as computations. Models can be queried

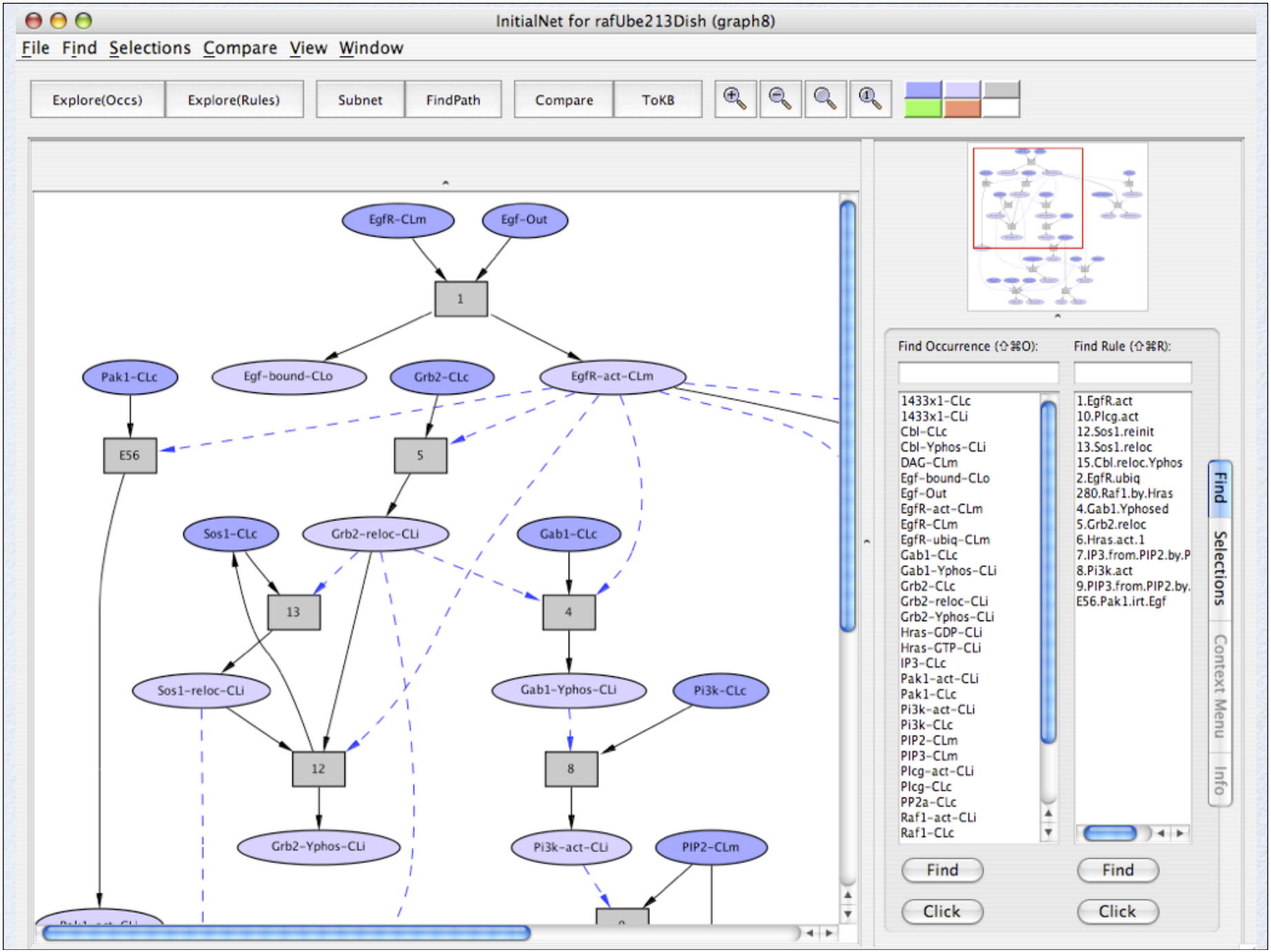
- using formal methods tools:
  - execute --- find some pathway
  - search --- find all reachable states satisfying a given property
  - model-check --- find a pathway satisfying a temporal formula
- using reflection
  - find all rules that use / produce X (for example, activated Rac)
  - find rules updown stream of a given rule or component
  - find the subnet relevant to a goal (desired state)

ESSENCE OF PATHWAY LOGIC

- Pathway Logic (PL) is an approach to modeling biological systems using facts and rules in a logical theory (symbolic systems).
- One aim is to directly represent the informal (mental) models that biologists use to understand experimental results.
- PL provides a way to organize information in a notation that has a well defined meaning and can be processed by a computer using powerful tools developed by the formal methods community.
- System components can be modeled at different levels of detail and at different scales.

The Pathway Logic Assistant (PLA)

- Provides a means to interact with a PL model
- Manages multiple representations
  - Maude module (logical representation)
  - PetriNet (process representation for efficient query)
  - Graph (for interactive visualization)
- Exports Representations to other tools
  - Lola (and SAL model checkers)
  - Dot -- graph layout
  - JLambda (interactive visualization, Java side)
  - SBML (xml based standard for model exchange)

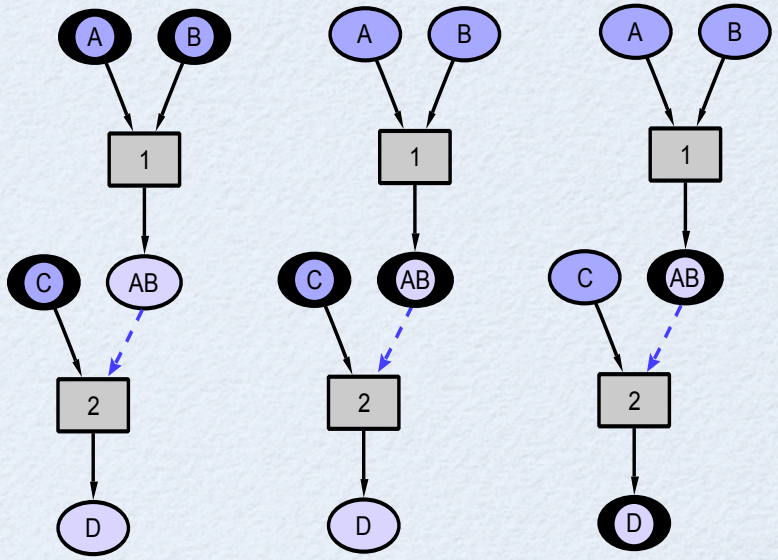

InitialNet for rafUbe213Dish (graph8)
File Find Selections Compare View Window
Explore(Occs)
Explore(Rules)
Subnet
FindPath
Compare
ToKB
EgfR-CLm
Egf-Out
1
Pak1-CLc
Egf-bound-CLo
Grb2-CLc
EgfR-act-CLm
E56
5
Sos1-CLc
Grb2-reloc-CLi
Gab1-CLc
13
4
Sos1-reloc-CLi
Gab1-Yphos-CLi
Pi3k-CLc
12
8
Grb2-Yphos-CLi
Pi3k-act-CLi
PIP2-CLm
Find Occurrence (⇧⌘O):
Find Rule (⇧⌘R):
1433x1-CLc
1433x1-CLi
Cbl-CLc
Cbl-Yphos-CLi
DAG-CLm
Egf-bound-CLo
Egf-Out
EgfR-act-CLm
EgfR-CLm
EgfR-ubiq-CLm
Gab1-CLc
Gab1-Yphos-CLi
Grb2-CLc
Grb2-reloc-CLi
Grb2-Yphos-CLi
Hras-GDP-CLi
Hras-GTP-CLi
IP3-CLc
Pak1-act-CLi
Pak1-CLc
Pi3k-act-CLi
Pi3k-CLc
PIP2-CLm
PIP3-CLm
Plcg-act-CLi
Plcg-CLc
PP2a-CLc
Raf1-act-CLi
Raf1-CLc
1.EgfR.act
10.Plcg.act
12.Sos1.reinit
13.Sos1.reloc
15.Cbl.reloc.Yphos
2.EgfR.ubiq
280.Raf1.by.Hras
4.Gab1.Yphosed
5.Grb2.reloc
6.Hras.act.1
7.IP3.from.PIP2.by.P
8.Pi3k.act
9.PIP3.from.PIP2.by.
E56.Pak1.irt.Egf
Find
Click
Find
Click
Find
Selections
Context Menu
Info

ABOUT PETRI NETS

A Petri net is represented as a graph with two kinds of nodes:

* transitions/rules (reactions--squares)
* places/occurrences (reactants, products, modifiers--ovals)

A Petri net process has tokens on some of its places. A rule can fire if all of its inputs have tokens. Firing a rule moves tokens from input to output.



An execution is a sequence of rule firings.
A pathway is represented as an execution subgraph.

INTER-ORGAN MODELING OF SIMPLE BEHAVIOR

Total protein expression across behavioral state within organ

Whole Gel Analyses: BRAIN

717 total # of protein spots
516 protein spots **common to all states**
23 protein spots expressed only in **SWS**
14 protein spots expressed only in **Wake**
19 protein spots expressed only in **Time of Day**

Whole Gel Analyses: LIVER

816 total # of protein spots
519 protein spots **common to all states**
66 protein spots expressed only in **SWS**
231 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Whole Gel Analyses: HEART

1006 total # of protein spots
611 protein spots **common to all states**
261 protein spots expressed only in **SWS**
134 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Phosphoprotein expression across behavioral state within organ

Whole Gel Analyses: BRAIN

194 total # of phosphoprotein spots
60 protein spots **common to all states**
24 protein spots expressed only in **SWS**
5 protein spots expressed only in **Wake**
18 protein spots expressed only in **Time of Day**

Whole Gel Analyses: LIVER

51 total # of protein spots
19 protein spots **common to all states**
14 protein spots expressed only in **SWS**
18 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Whole Gel Analyses: HEART

517 total # of protein spots
250 protein spots **common to all states**
137 protein spots expressed only in **SWS**
130 protein spots expressed only in **Wake**
? protein spots expressed in **Time of Day**

Proteins unique to different states were identified in all three organs. Phosphoprotein data suggests activities differ across states in these organs.