Applications of Rewriting Logic in Biology
IV
Using the Pathway Logic Assistant

Carolyn Talcott
SRI International
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THE SMALL KB LIVE

A MODEL OF EGF STIMULATION

THE CANONICAL EGF-ERK PATHWAY

Egf ➔ EgfR ➔ Grb2 ➔ Sos1 ➔ a Ras family member ➔ Raf1 ➔ MEK1/2 ➔ ERK1/2

- Egf binds to the EGF receptor (EgfR) and stimulates its protein tyrosine kinase activity to cause autophosphorylation.
- A complex containing the adaptor protein Grb2 and the guanine nucleotide exchange factor Sos1 docks (binds) to the autophosphorylated (activated) EgfR.
- The Sos1-containing EgfR complex activates a Ras family GTPase,
- The activated Ras protein activates Raf1, a member of the RAF serine/threonine protein kinase family.
- Raf1 then activates the dual-specificity protein kinases Mek1 and/or Mek2 (MEK1/2), which activate Erk1 and/or Erk2 (ERK1/2).

A MODEL OF ASSEMBLED FROM THE PL KNOWLEDGE BASE

- The model is based on experimental results curated from the literature.
- It includes all events known to happen in response to Egf stimulation within ~ 15 minutes
- It shows that the series of events between activation of EgfR by Egf and activation of Erk2 may not be as simple as those described in the canonical pathway

DATA COLLECTION

174 papers were searched for appropriate experiments and the results were listed as 1373 evidence items. Below is an example of an item used as evidence for rule E19 which requires the presence of tyrosine phosphorylated Gab1 for the activation of Erk2 in response to Egf.

An Evidence Item

Source	PMID: 11323411 Type: data Figure : 2
Subject	Pathway Logic name: Erk2 Expressed: yes Identification method : expression tag antibody
State Change	Type: kinase activity Direction: increase Assay: IP Kinase assay, MBP as substrate
Cause	Stimulus: Egf Time: 5 minutes Concentration: 0.25 ng/ml
Requirement	Pathway Logic name: Gab1 Method: Y627F dominant-negative coexpression Method: Y659F dominant-negative coexpression
Environment	Cells: COS-7 State: serum starved for 20 hr

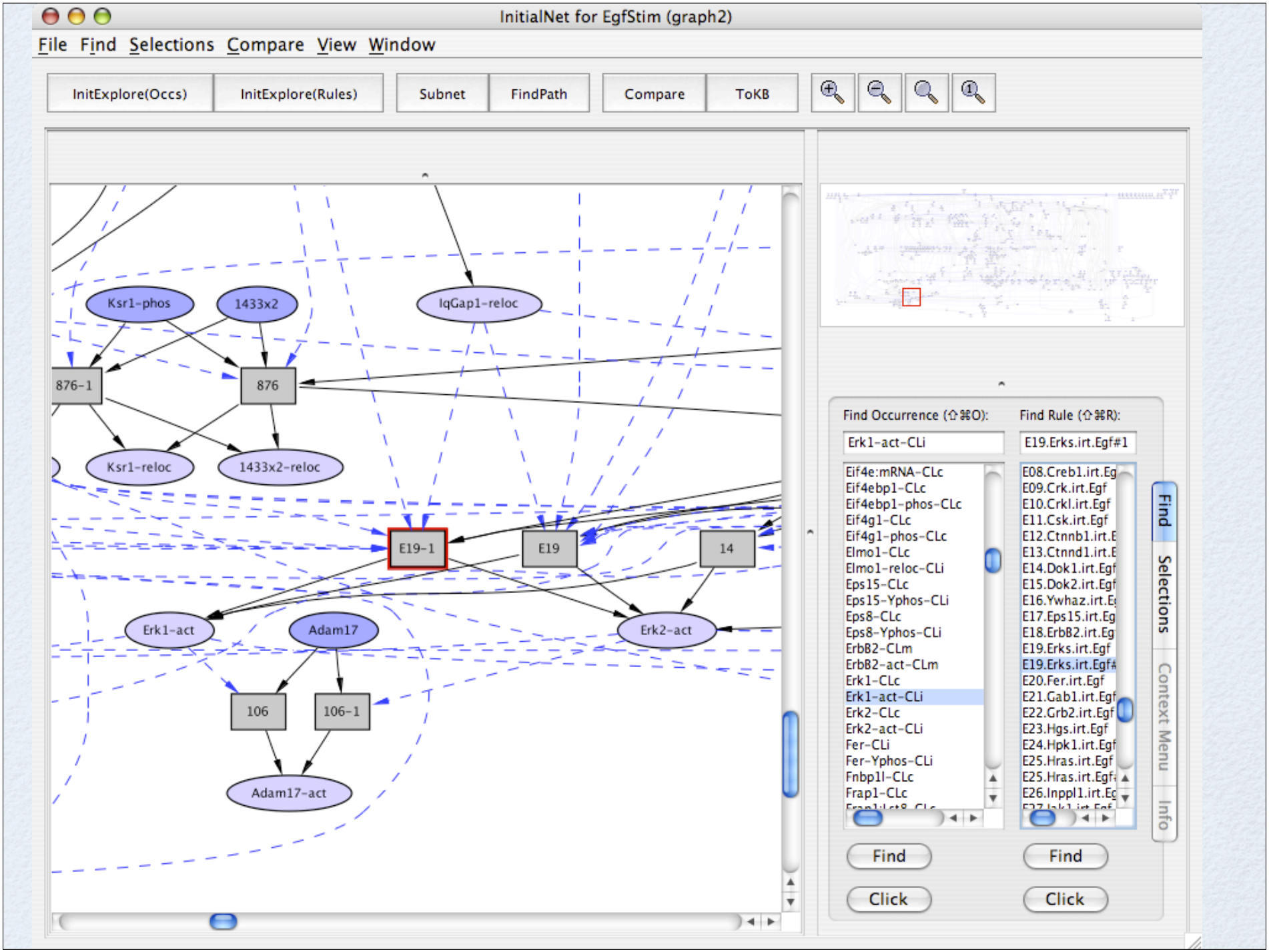
```
rl[E19.Erks.irt.Egf]:
  {CLm | clm ([EgfR - act] : Egf)                    }
  {CLi | cli  [Mlk3 - act] [mek:MekS - act]
       [Gab1 - Yphos][Src - act]
       [Ptk2b - act] [IqGap1 - reloc]                }
  {CLc | clc  Erk1 Erk2                              }
  =>
  {CLm | clm ([EgfR - act] : Egf)                    }
  {CLi | cli  [Mlk3 - act] [mek:MekS - act]
       [Gab1 - Yphos] [Src - act]
       [Ptk2b - act][IqGap1 - reloc]
       [Erk1 - act] [Erk2 - act]                     }
  {CLc | clc                                         } .
```

GENERATING THE MODEL

- Step 1: Generate a knowledge base from the curated rules and components. (It contains 1953 occurrences and 3440 transitions.)
- Step 2: Define the initial state (dish). It contains Egf in the supernatant, EgfR in the membrane and other proteins known to be expressed in epithelial-like cells.

```
PD(Egf[Cell | {CLo | ...} {CLm | EgfR ...}
              {CLi | ...}{CLc | ...} ...])
```

- Step 3: Generate the network relevant to this dish. (It contains 468 occurrences and 374 transitions. Complex!)


InitialNet for EgfStim (graph2)
File Find Selections Compare View Window
InitExplore(Occs)
InitExplore(Rules)
Subnet
FindPath
Compare
ToKB
Ksr1-phos
1433x2
IqGap1-reloc
876-1
876
Ksr1-reloc
1433x2-reloc
E19-1
E19
14
Erk1-act
Adam17
Erk2-act
106
106-1
Adam17-act
Find Occurrence (⇧⌘O):
Find Rule (⇧⌘R):
Erk1-act-CLi
E19.Erks.irt.Egf#1
Eif4e:mRNA-CLc
Eif4ebp1-CLc
Eif4ebp1-phos-CLc
Eif4g1-CLc
Eif4g1-phos-CLc
Elmo1-CLc
Elmo1-reloc-CLi
Eps15-CLc
Eps15-Yphos-CLi
Eps8-CLc
Eps8-Yphos-CLi
ErbB2-CLm
ErbB2-act-CLm
Erk1-CLc
Erk1-act-CLi
Erk2-CLc
Erk2-act-CLi
Fer-CLi
Fer-Yphos-CLi
Fnbp1l-CLc
Frap1-CLc
E08.Creb1.irt.Eg
E09.Crk.irt.Egf
E10.Crkl.irt.Egf
E11.Csk.irt.Egf
E12.Ctnnb1.irt.E
E13.Ctnnd1.irt.E
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E25.Hras.irt.Egf
E25.Hras.irt.Egf#
E26.Inppl1.irt.Eg
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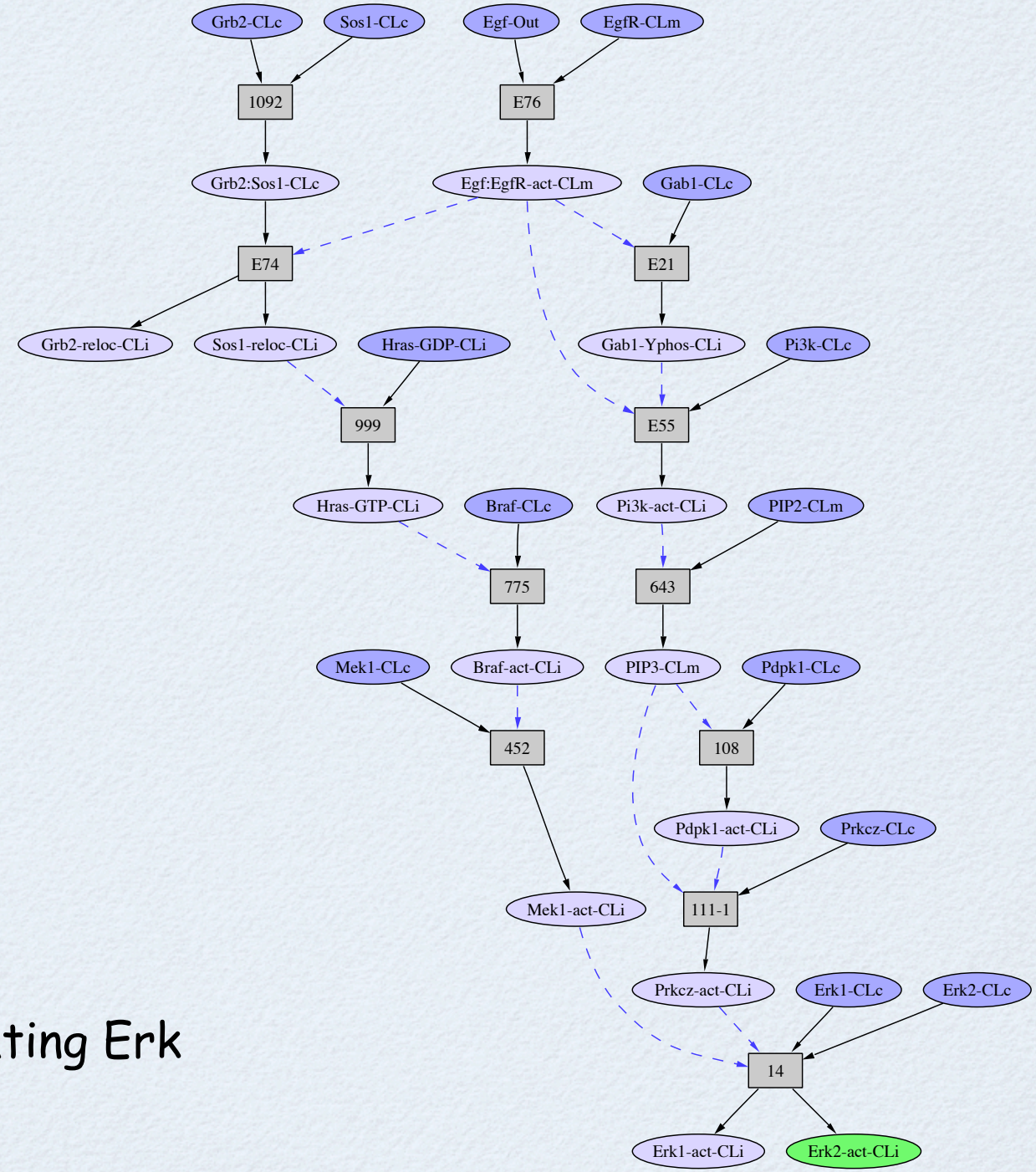
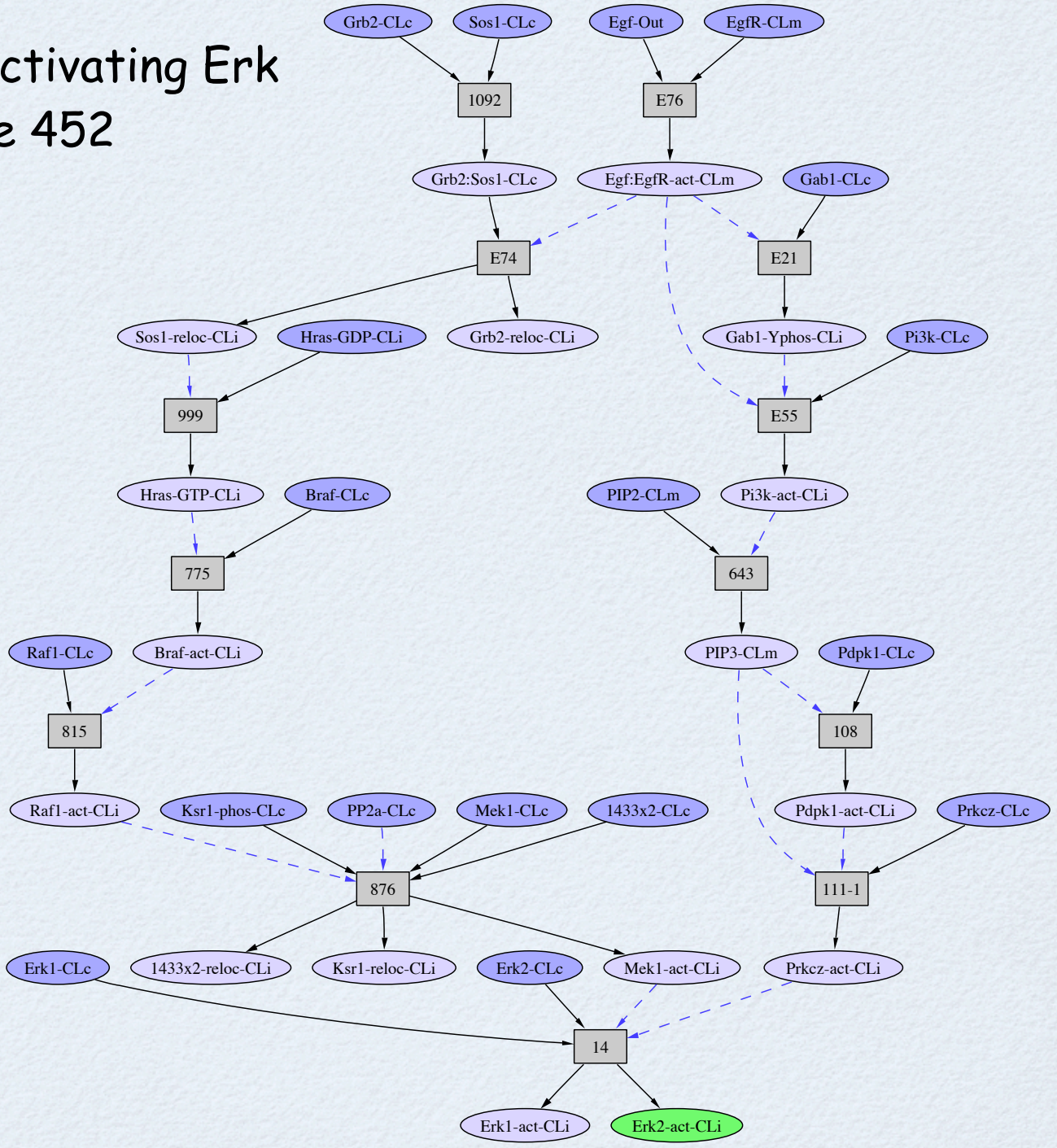
ACTIVATION OF ERKS

- Step 4: Select Erk2-act-CLi as a goal
  - Find a path in the dishnet
  - Hide a suspicious rule (452) and find another path
  - Generate the relevant subnet and find a path here
  - Compare the different paths



One pathway activating Erk

Another pathway activating Erk without rule 452

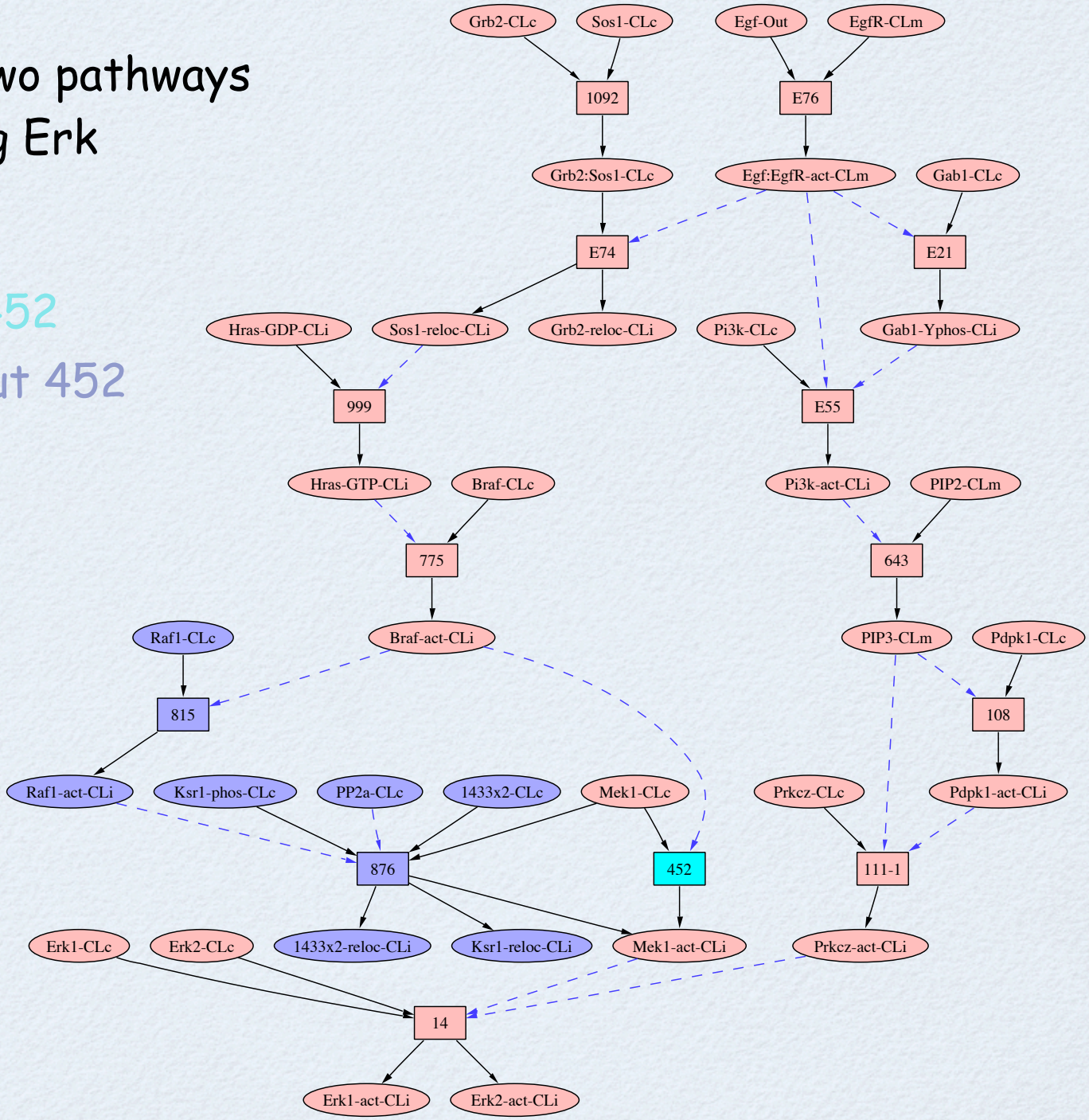


Comparing the two pathways activating Erk

Both pathways

Pathway with 452

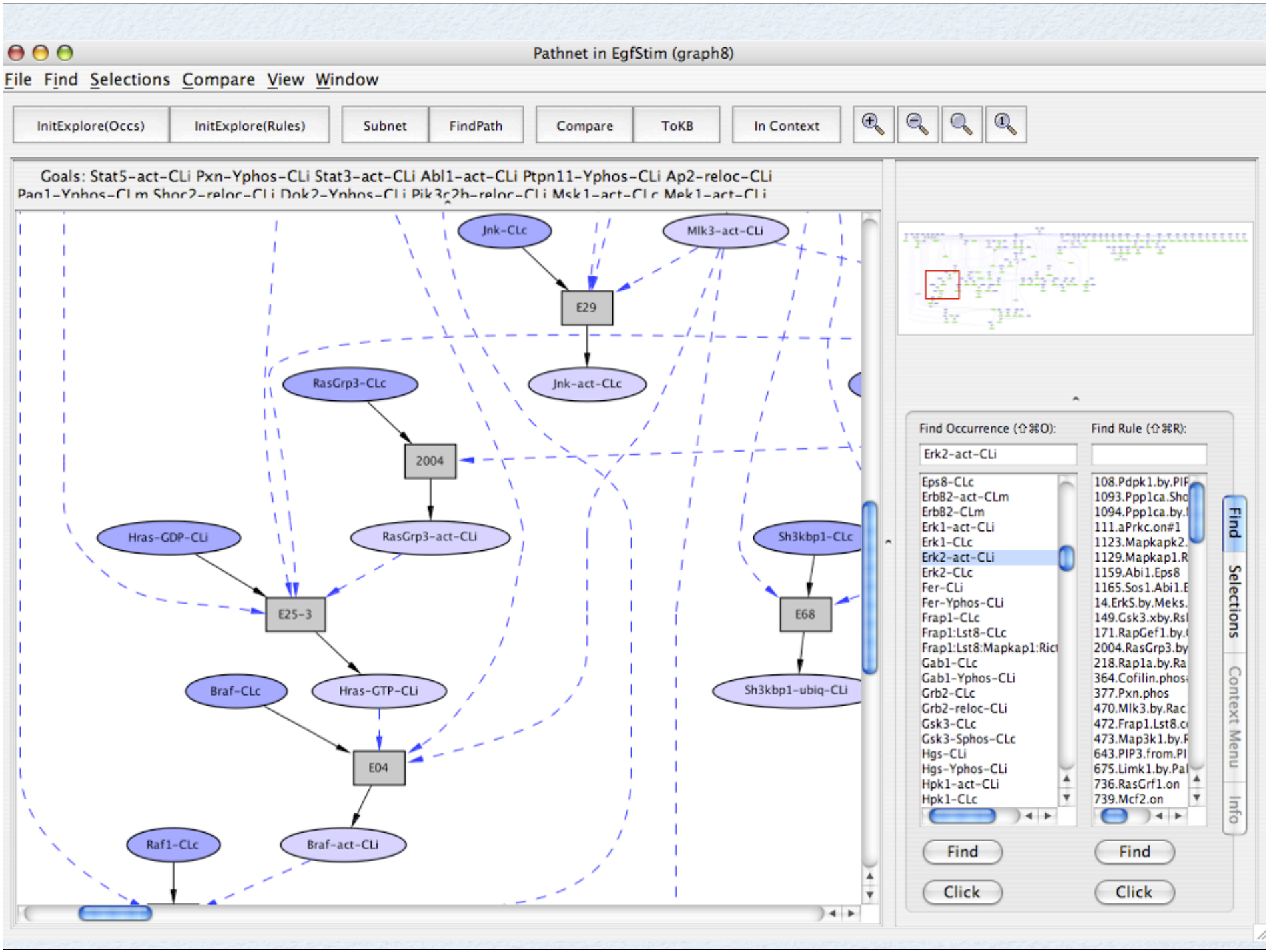
Pathway without 452



CONSTRAINING THE MODEL TO KNOWN OUTCOMES

THE CONSTRAINTS

- The model is constrained by giving precedence to events specifically found in response to Egf.
- The full model contains to types of rules: Common Rules formalizing local reactions, independent of the larger context, and Egf Rules formalizing requirements specific to Egf signaling that must be satisfied before they can fire.
- The Egf Rules are given precedence by hiding corresponding Common Rules.
- A list of 85 protein state changes demonstrated experimentally to occur in response to a short stimulus with Egf was collected as part of the curation process. These states are made goals.
- Finally a path is found satisfying these constraints.


Pathnet in EgfStim (graph8)
File Find Selections Compare View Window
InitExplore(Occs)
InitExplore(Rules)
Subnet
FindPath
Compare
ToKB
In Context
Goals: Stat5-act-CLi Pxn-Yphos-CLi Stat3-act-CLi Abl1-act-CLi Ptpn11-Yphos-CLi Ap2-reloc-CLi
Jnk-CLc
Mlk3-act-CLi
E29
Jnk-act-CLc
RasGrp3-CLc
2004
RasGrp3-act-CLi
Hras-GDP-CLi
E25-3
Braf-CLc
Hras-GTP-CLi
E04
Raf1-CLc
Braf-act-CLi
Sh3kbp1-CLc
E68
Sh3kbp1-ubiq-CLi
Find Occurrence (⇧⌘O):
Find Rule (⇧⌘R):
Erk2-act-CLi
Eps8-CLc
ErbB2-act-CLm
ErbB2-CLm
Erk1-act-CLi
Erk1-CLc
Erk2-act-CLi
Erk2-CLc
Fer-CLi
Fer-Yphos-CLi
Frap1-CLc
Frap1:Lst8-CLc
Gab1-CLc
Gab1-Yphos-CLi
Grb2-CLc
Grb2-reloc-CLi
Gsk3-CLc
Gsk3-Sphos-CLc
Hgs-CLi
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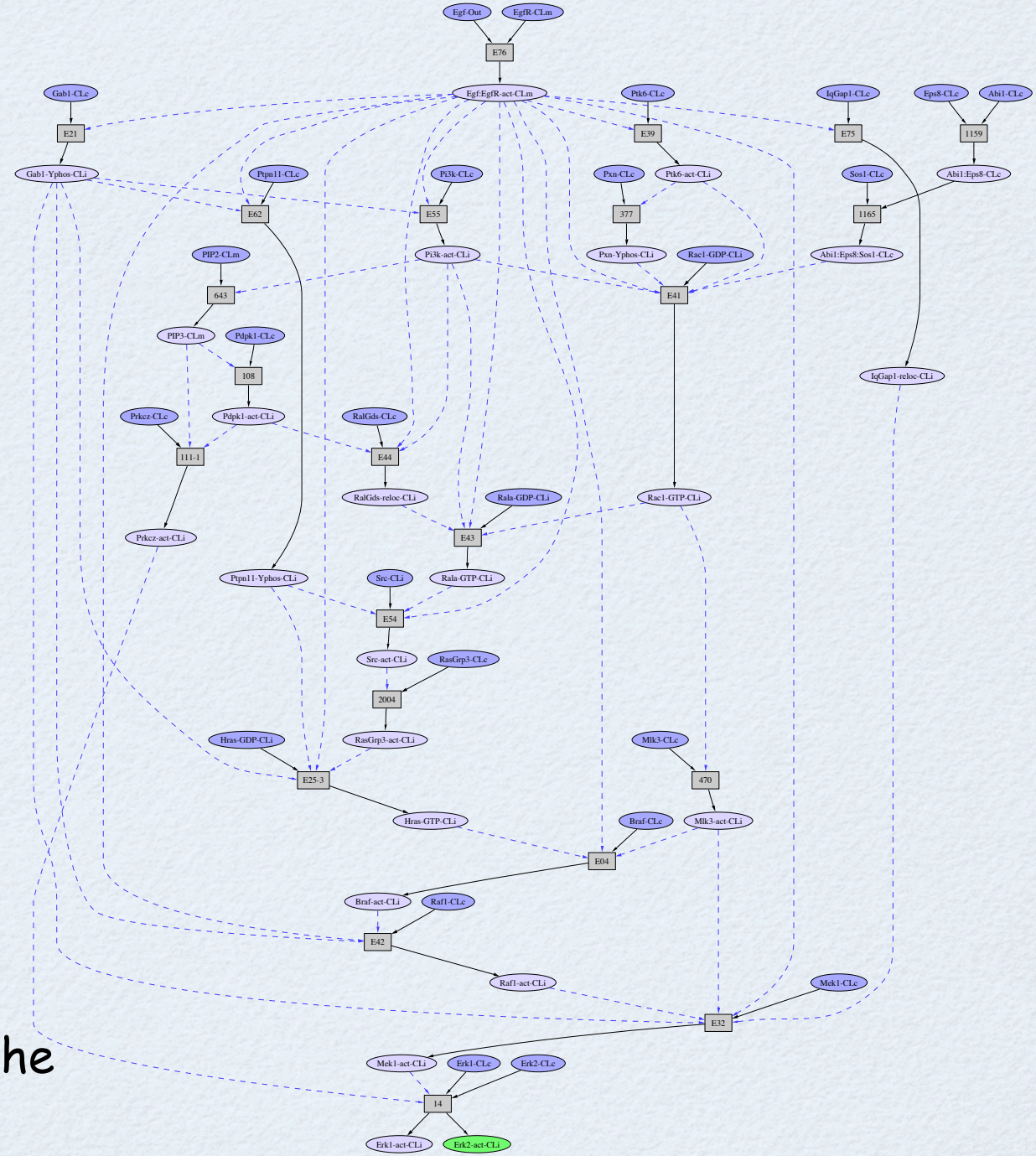


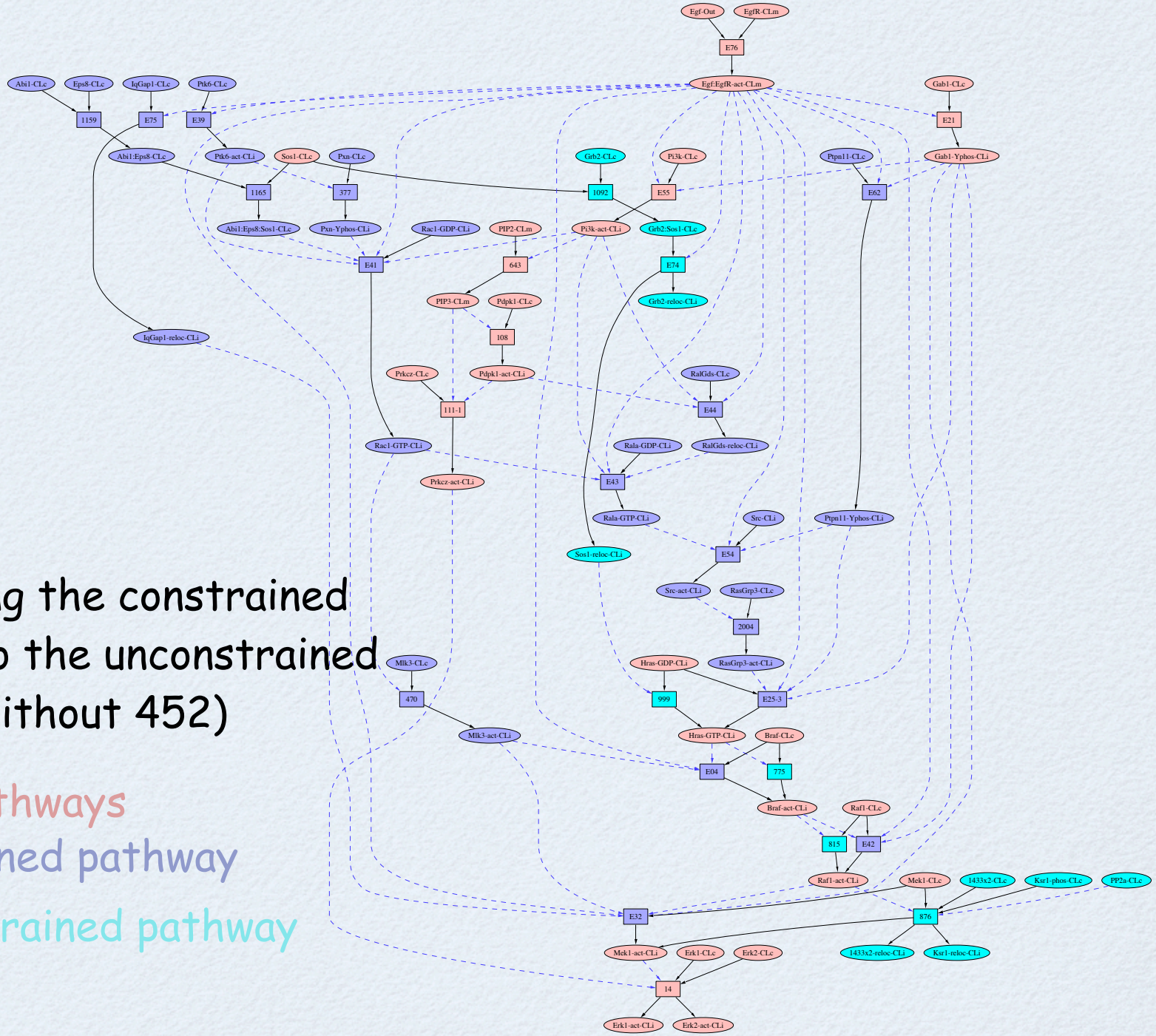
The Erk pathway in the constrained model

Comparing the constrained pathway to the unconstrained (without 452)

Both pathways

Constrained pathway

Unconstrained pathway



PATHWAY LOGIC TEAM

- Keith Laderoute
- Patrick Lincoln
- Carolyn Talcott

- Linda Briesemeister
- Steven Eker
- Merrill Knapp
- Ian Mason
- Andy Poggio
- Malabika Sarker

- Biology Computer Science Students