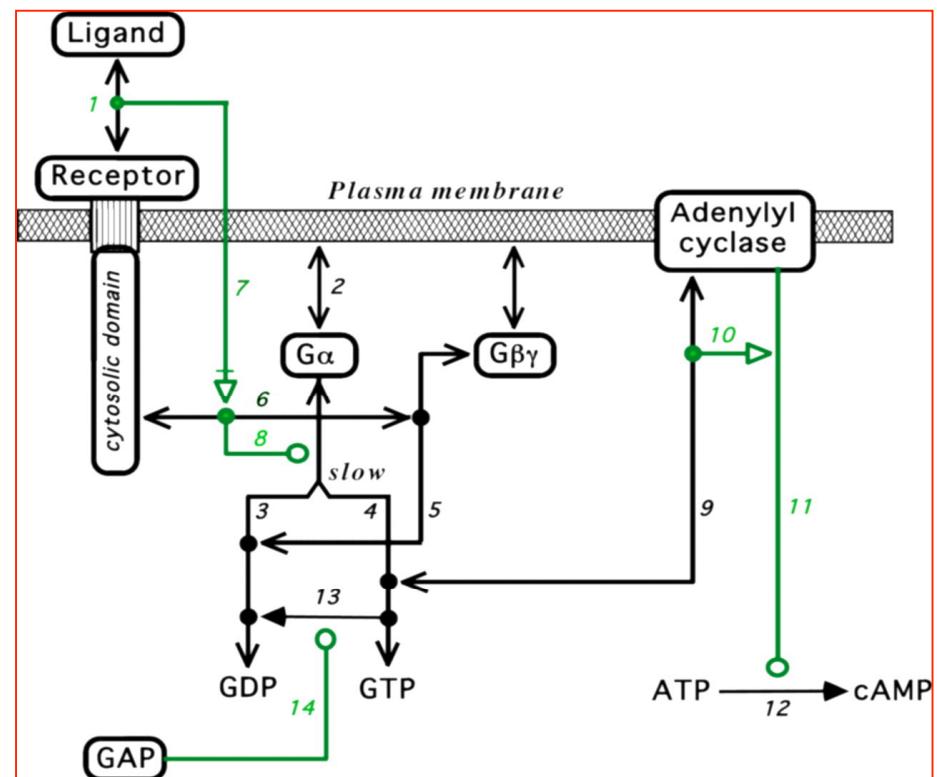


Mirit I. Aladjem

Laboratory of Molecular Pharmacology, NCI

# Molecular Interaction Maps: Circuit Diagrams for Bioregulatory Networks



## The Challenge: Depicting Interaction Networks

*Organize information about complex networks in a concise graphical manner while presenting sufficient detail to describe models for simulation.*

*There are several different types of possible interactions*

*Interactions may involve distinct intracellular domains*

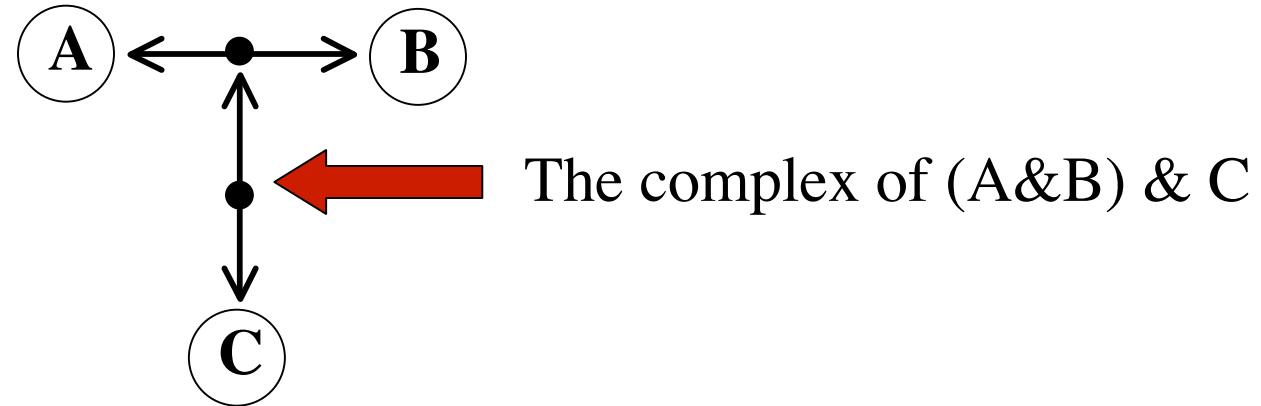
*Each component of the network may interact with several other components*

*Interactions may affect the ability to form other interactions*

*All the interactions involving each component should be traceable on the diagrams*

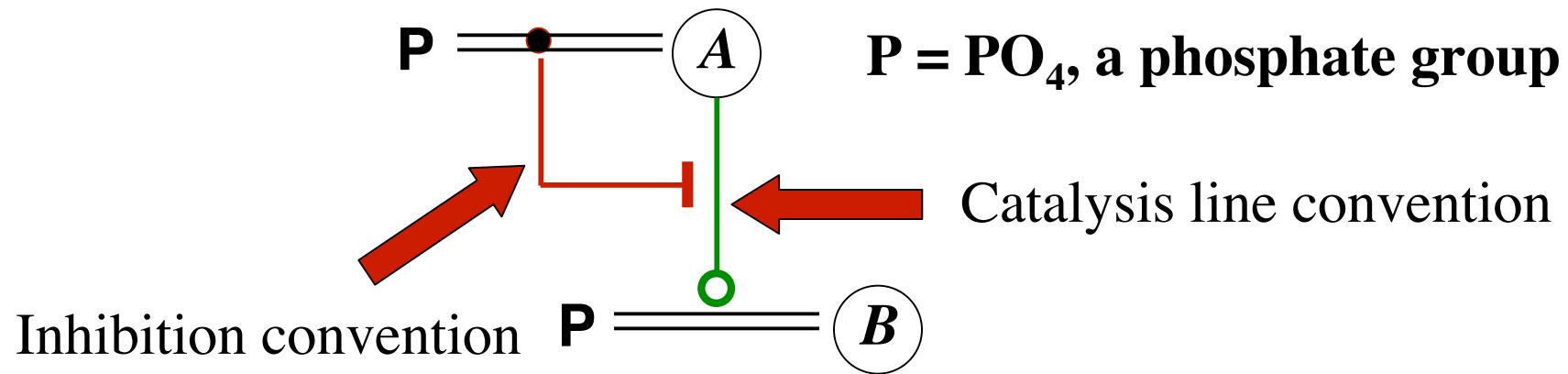
***Molecular Interaction Maps depict bioregulatory interactions unambiguously in diagram form using specific lines and nodes.***

## Multi-protein complexes



Each molecule appears only once per diagram.  
Interaction outcomes - complexes or modified molecules - are depicted as nodes on the interaction lines.

## Covalent modification (e.g., protein phosphorylation)

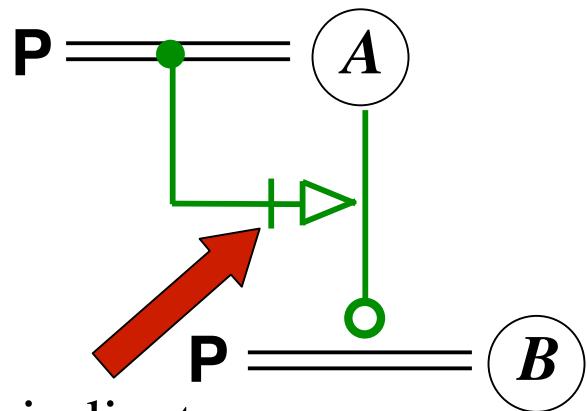


Inhibitory phosphorylation:

Phosphorylation of A blocks the kinase activity of A.

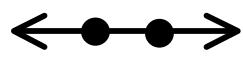
Activating phosphorylation:

The phosphorylated form of kinase *A* is the active form, phosphorylates *B*

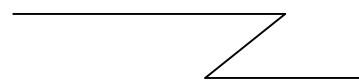


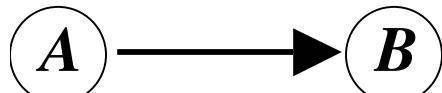
Bar added to indicate  
obligatory requirement

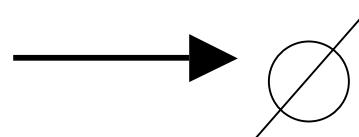
## *Reactions*

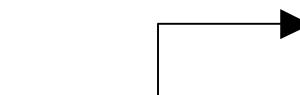
 Binding (non-covalent)

 Covalent Modification  
(e.g. phosphorylation)

 Bond cleavage  
(e.g. Phosphatase)

 Stoichiometric  
Conversion (A to B)

 Degradation

 Transcription/translation

 Dimerization

## *Contingencies*

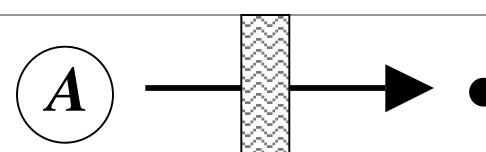
 Catalysis

 Stimulation

 Stimulation  
required

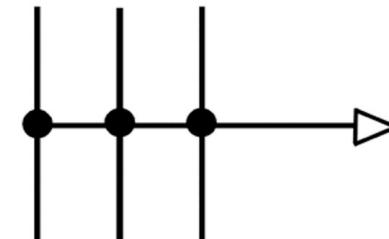
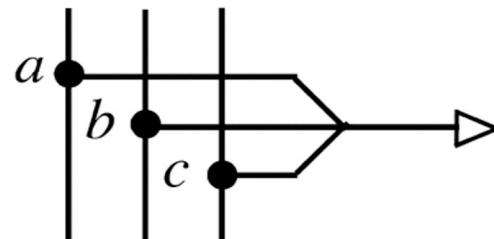
 Inhibition

*Reactions operate on molecular species; contingencies operate on reactions, or on other contingencies; reaction outcomes (nodes) are treated as molecular species.*

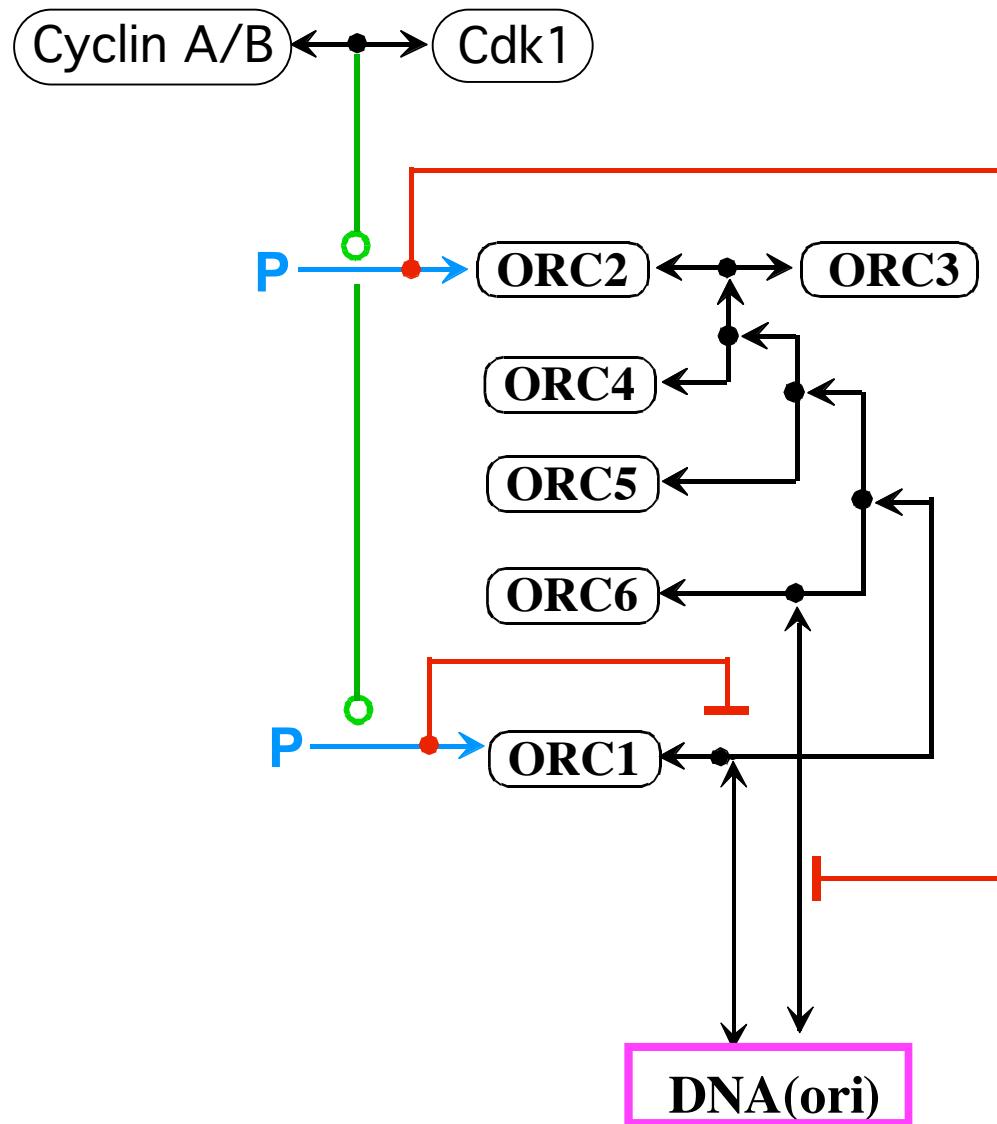
 Transport

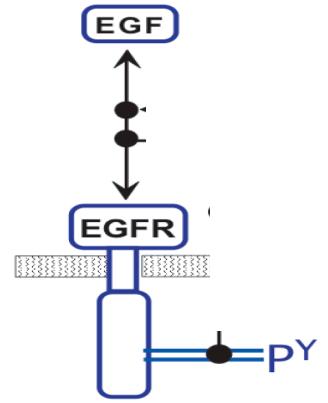
## *Boolean Functions in MIMs:*

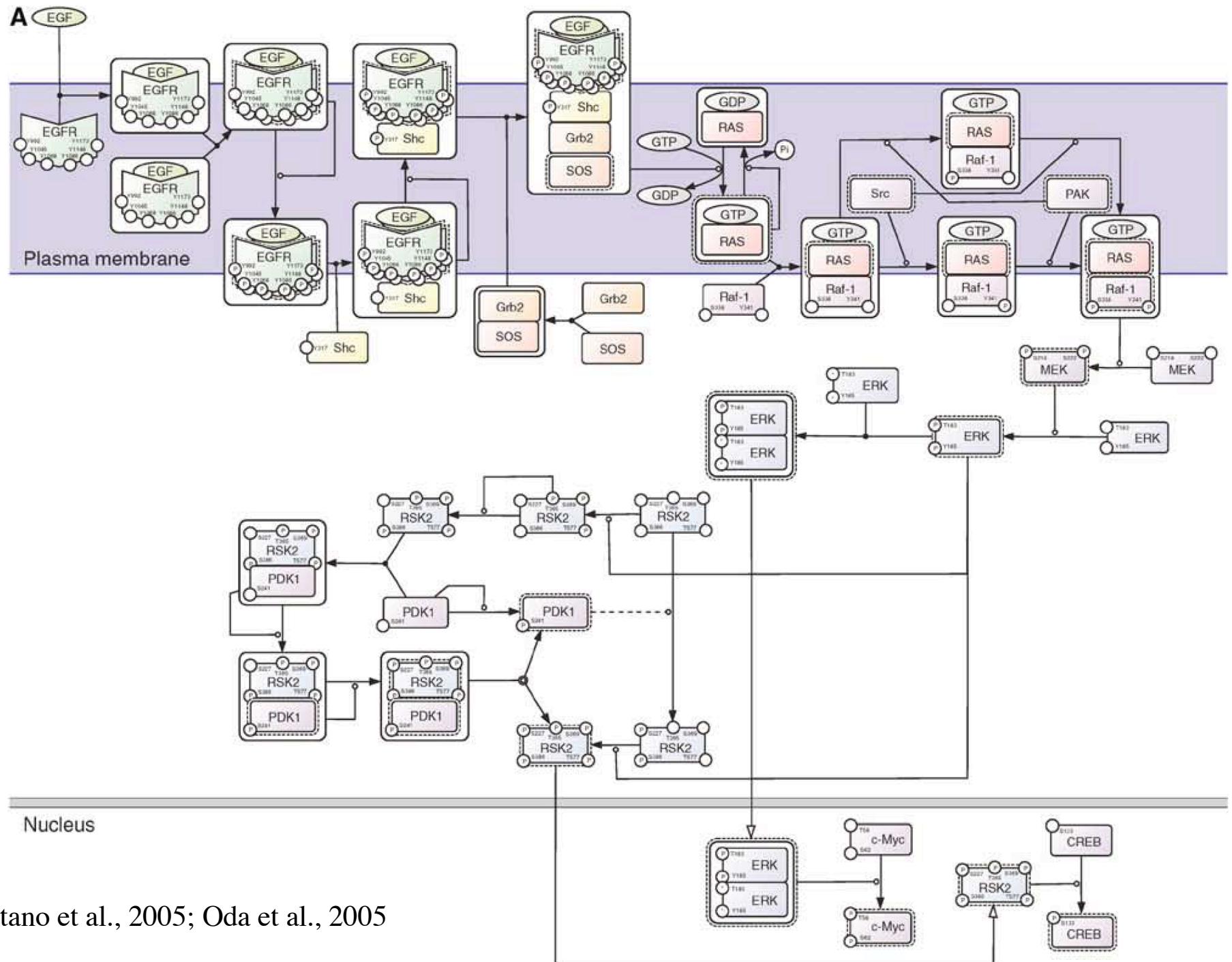
*a AND b AND c required for stimulation*



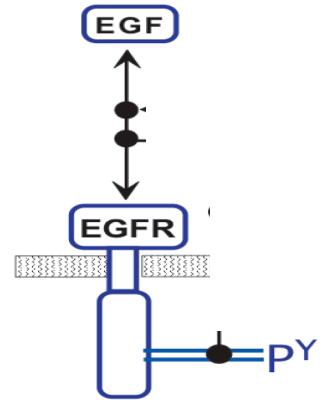
# Assembly of a multimolecular complex: ORC, the origin recognition complex (involved in cell cycle regulation)

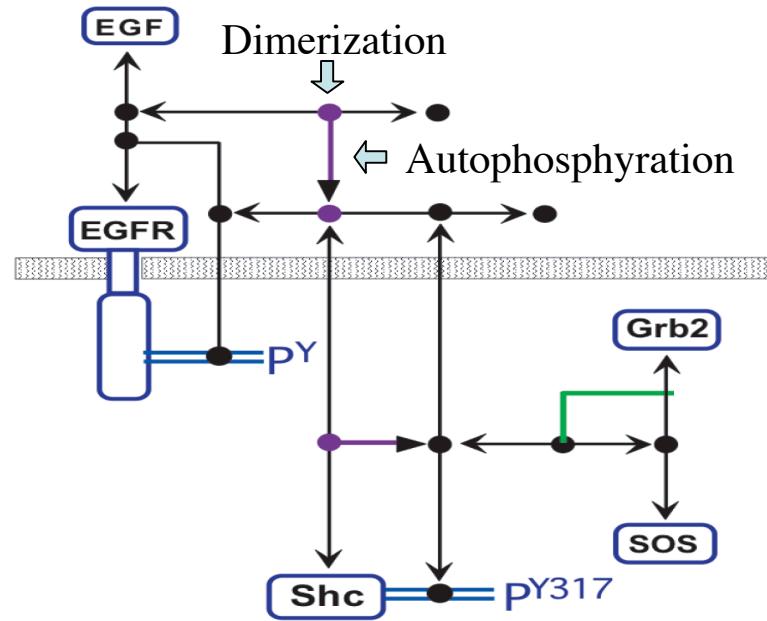


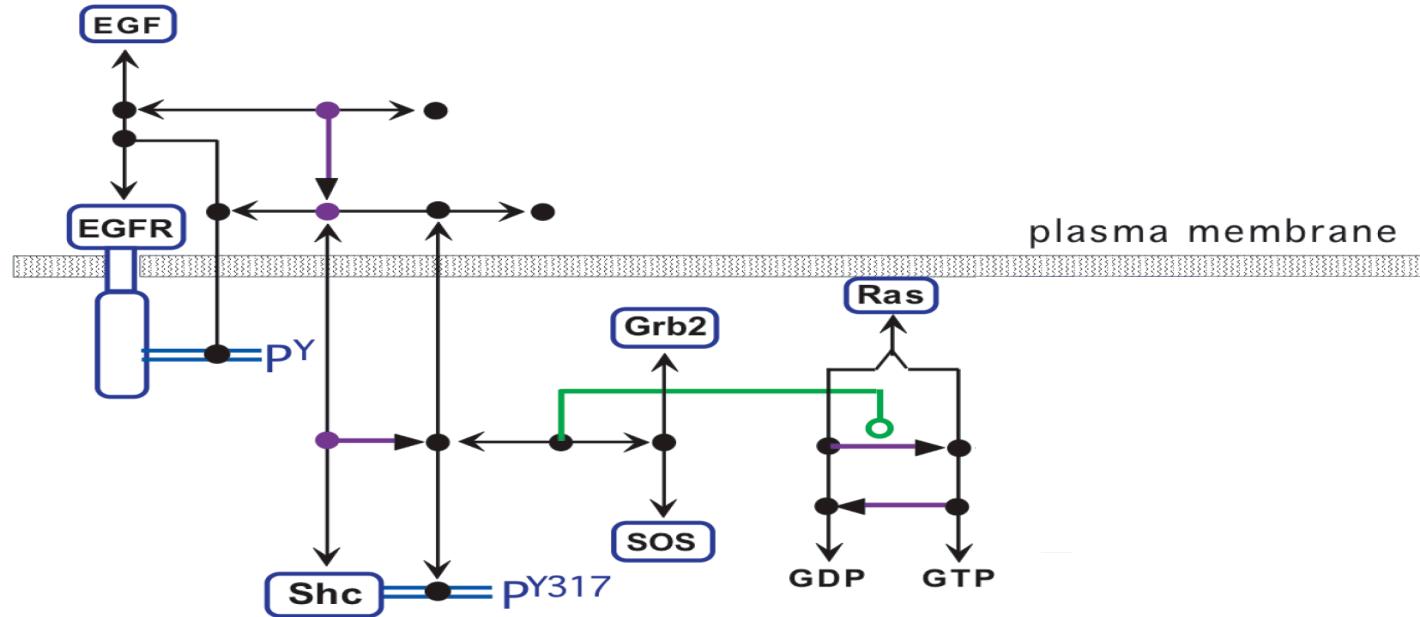


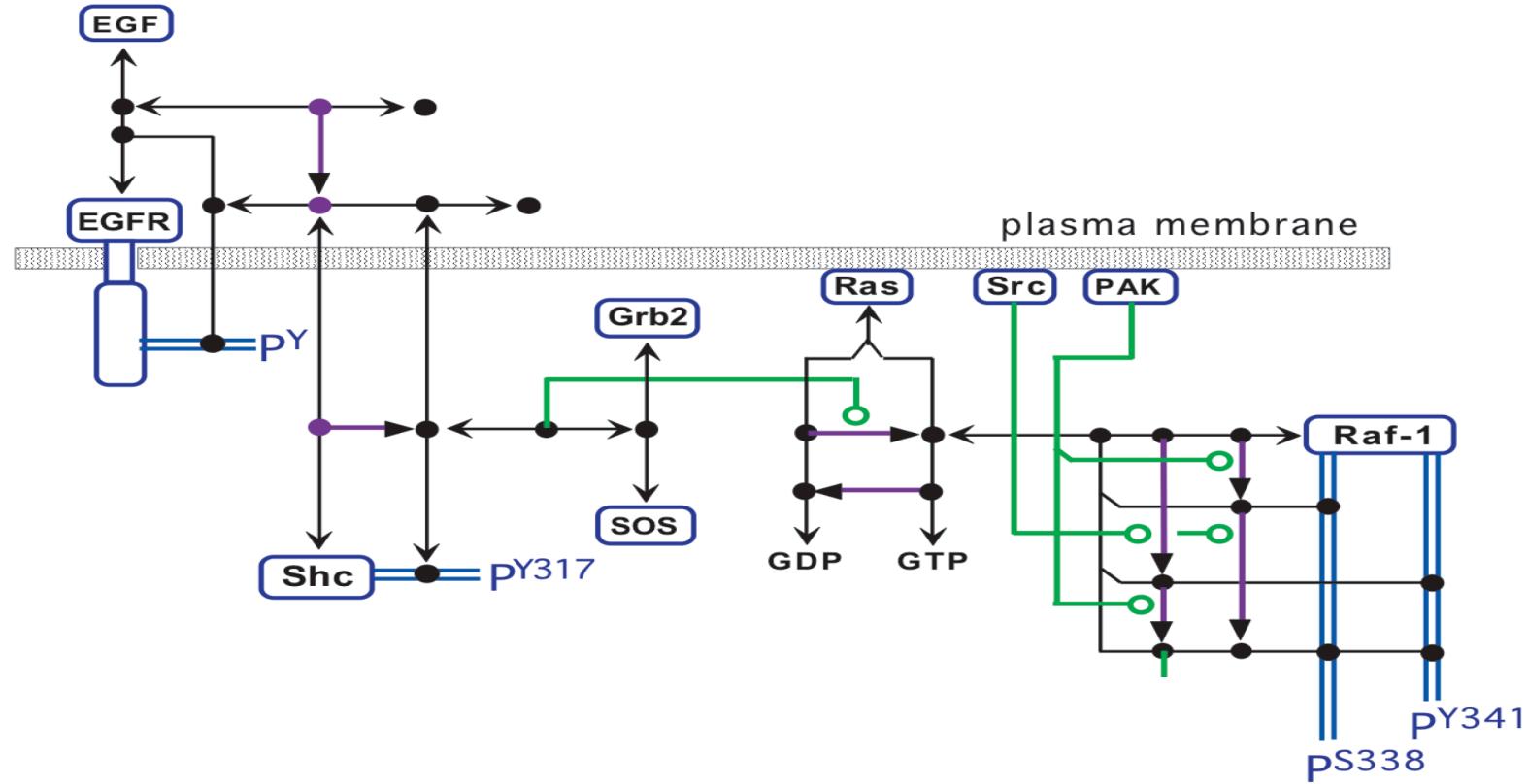


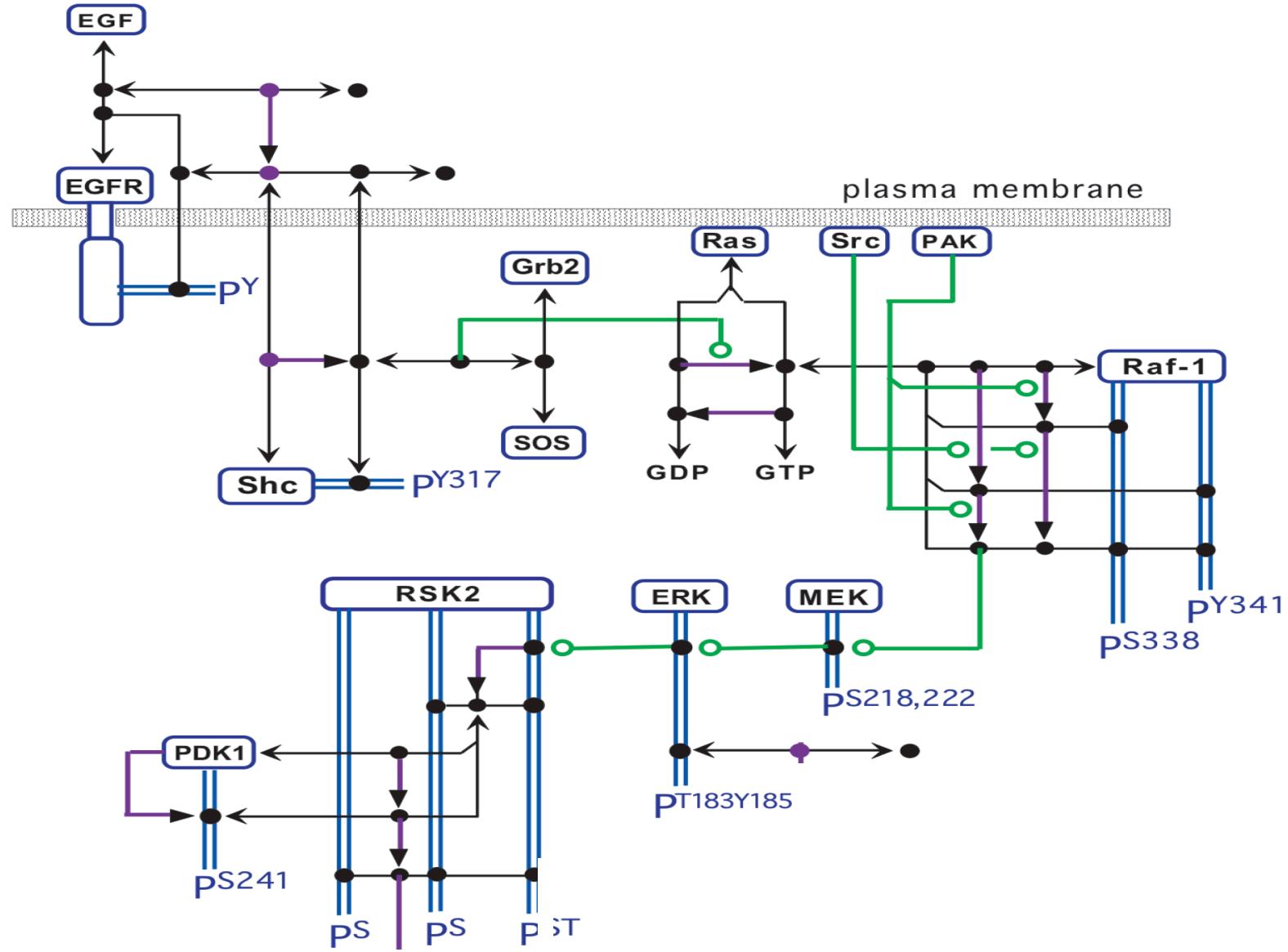
Kitano et al., 2005; Oda et al., 2005

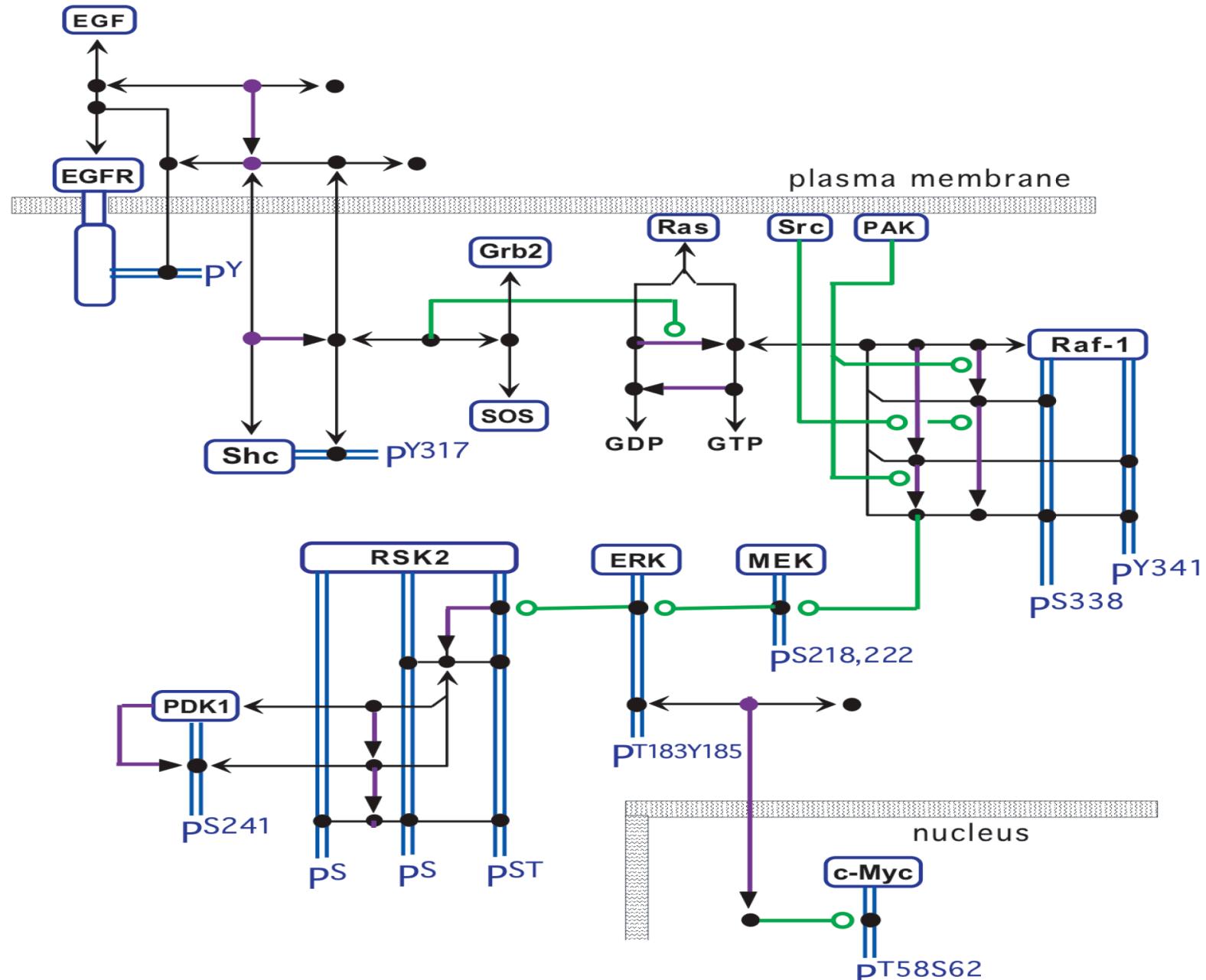


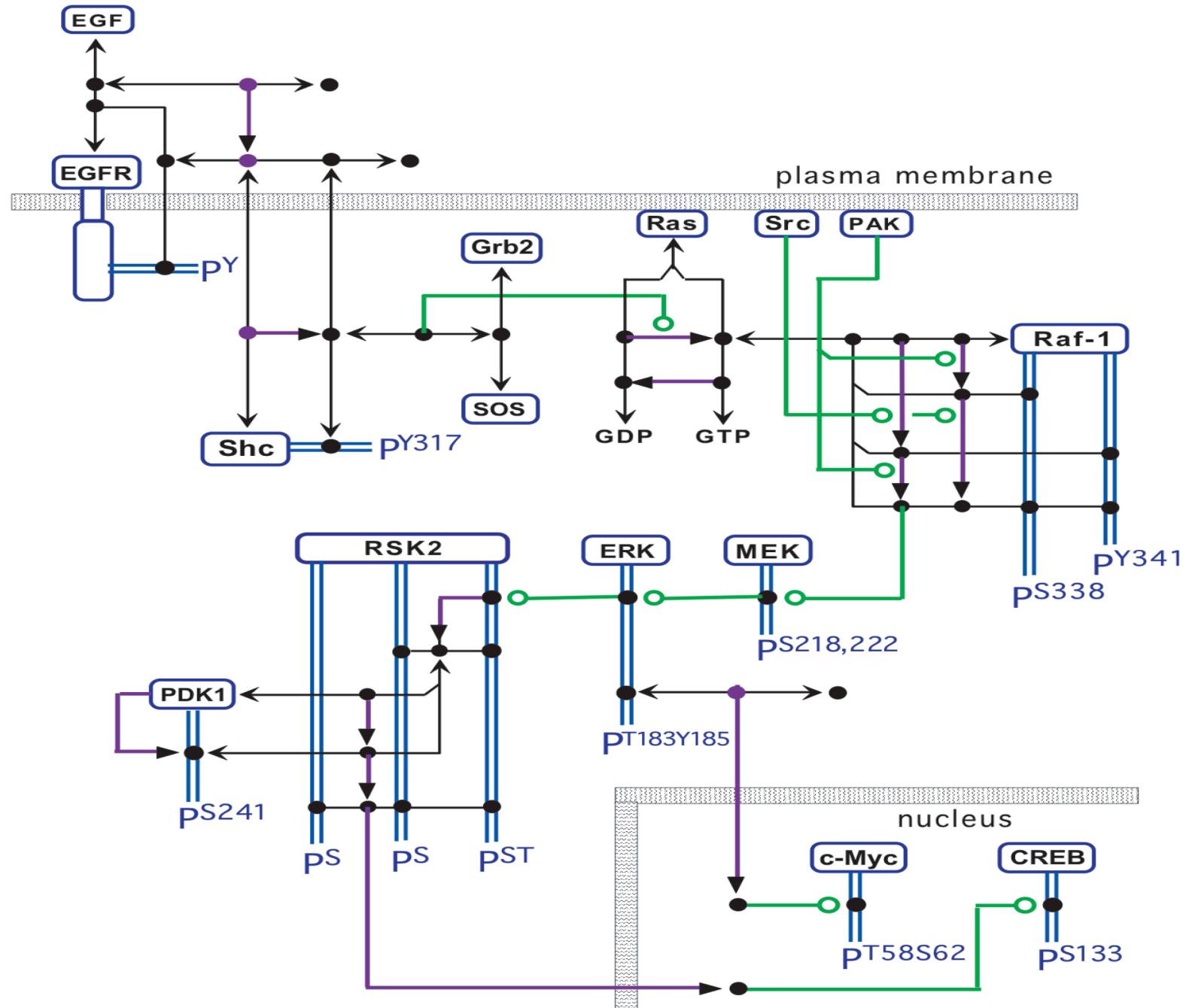




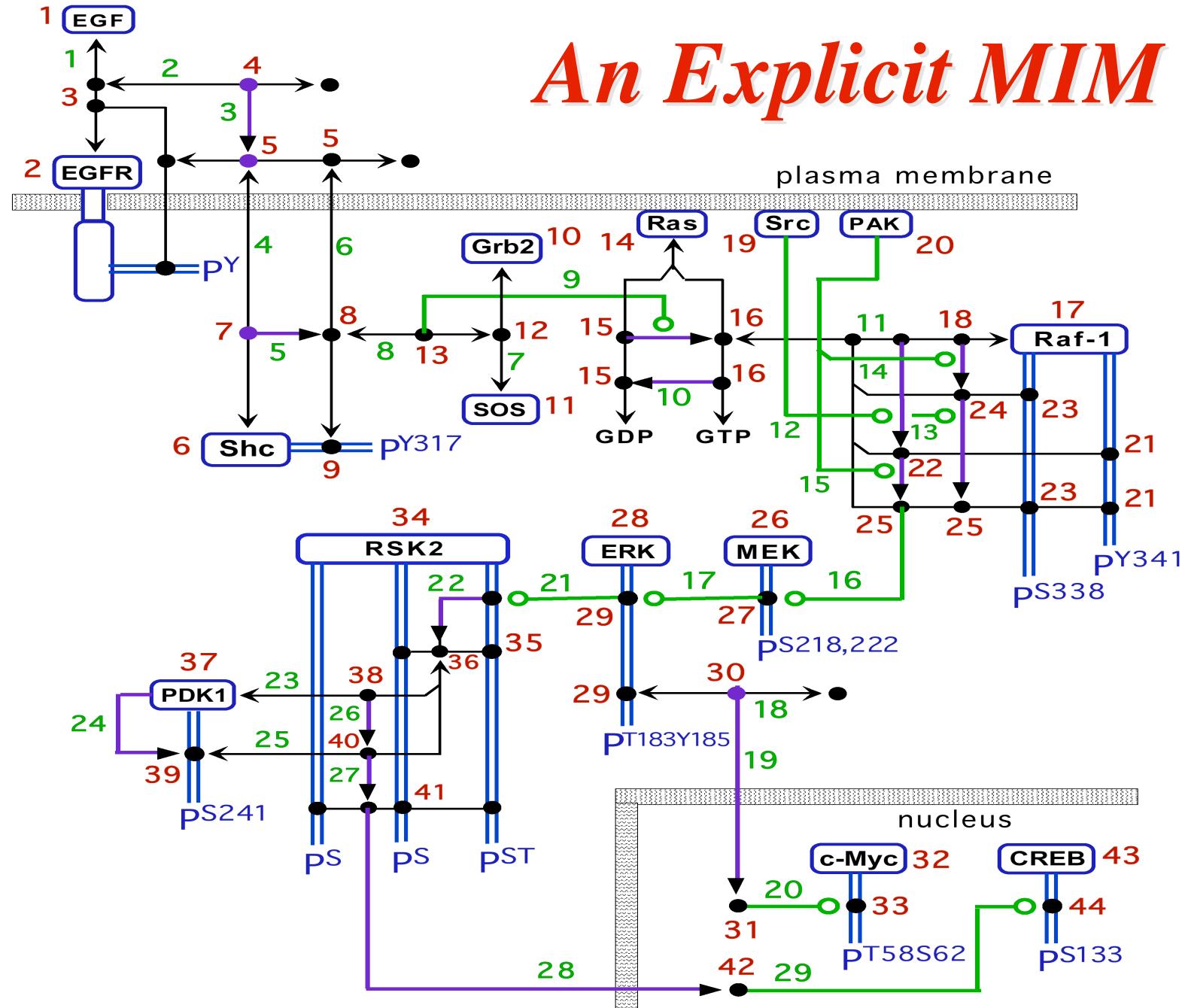








# An Explicit MIM



# *Translation of the Explicit MIM: a Reaction Table and ODEs Based on Mass Action Laws*

Rxn	reactants		products		Rxn	reactants		products	
1a	1	2		3	16a	25	26		26a
1b	3			1 2	16b	26a		25 26	
2a	3	3		4	16c	26a		25 27	
2b	4			3 3	17a	27	28		28a
3	4			5	17b	28a		27 28	
4a	5	6		7	17c	28a		27 29	
4b	7			5 6	18a	29	29		30
5	7			8	18b	30		29 29	
6a	5	9		8	19	30			31
:					:				

$$x_1' = x_2' = -k_{1a}x_1x_2 + k_{1b}x_3$$

$$x_3' = k_{1a}x_1x_2 - k_{1b}x_3 - 2k_{2a}x_3x_3 + 2k_{2b}x_4$$

$$x_4' = k_{2a}x_3x_3 - (k_{2b} + k_3)x_4$$

$$x_5' = k_3x_4 - k_{4a}x_5x_6 + k_{4b}x_7 - k_{6a}x_5x_9 + k_{6b}x_8$$

$$x_6' = -k_{4a}x_5x_6 + k_{4b}x_7$$

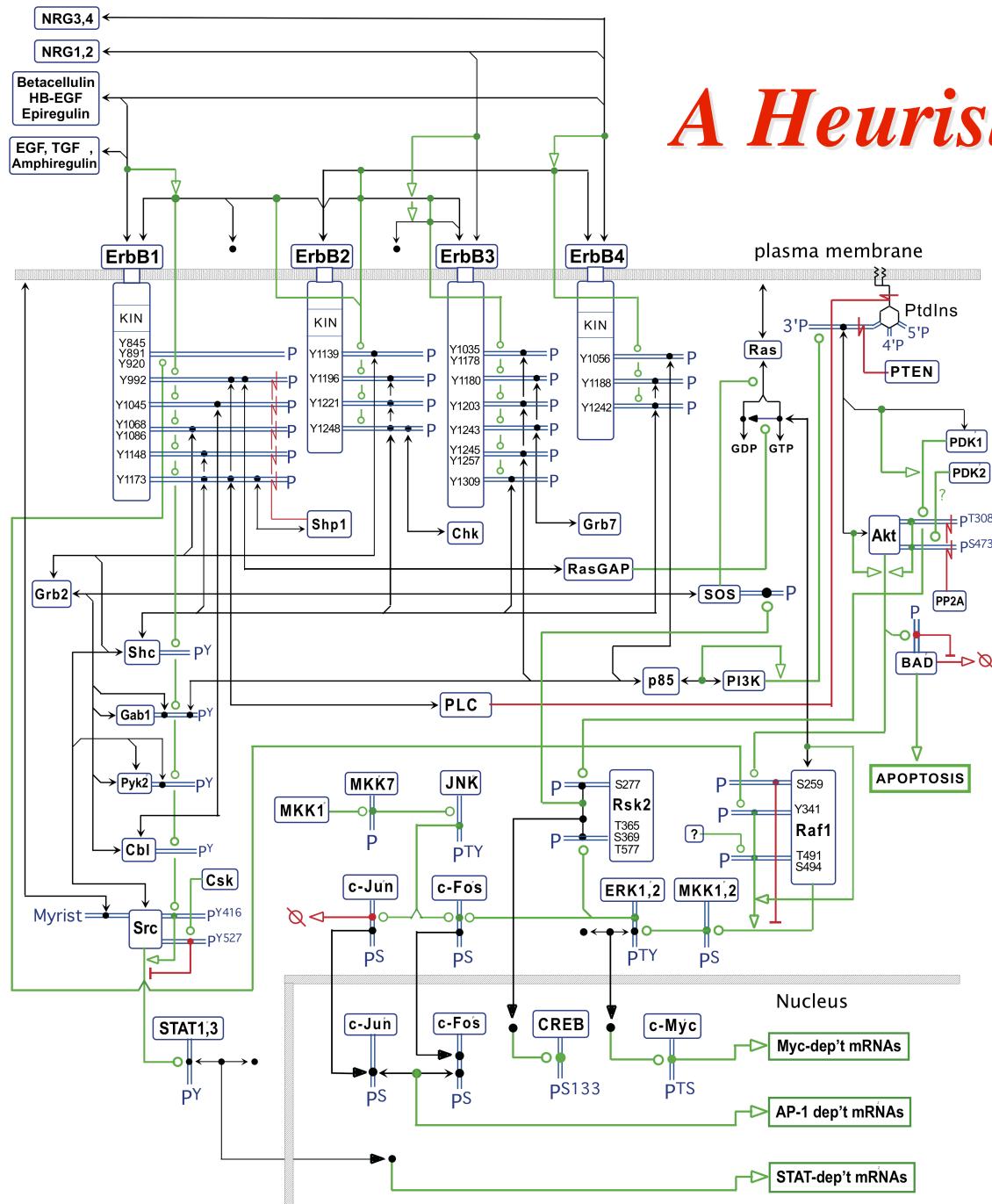
$$x_7' = k_{4a}x_5x_6 - (k_{4b} + k_5)x_7$$

$$x_8' = k_5x_7 + k_{6a}x_5x_9 - k_{6b}x_8 - k_{8a}x_8x_{12} + k_{8b}x_{13}$$

:

An Explicit MIM Depicts  
Molecular Interactions With  
Sufficient Detail Required for  
Simulation

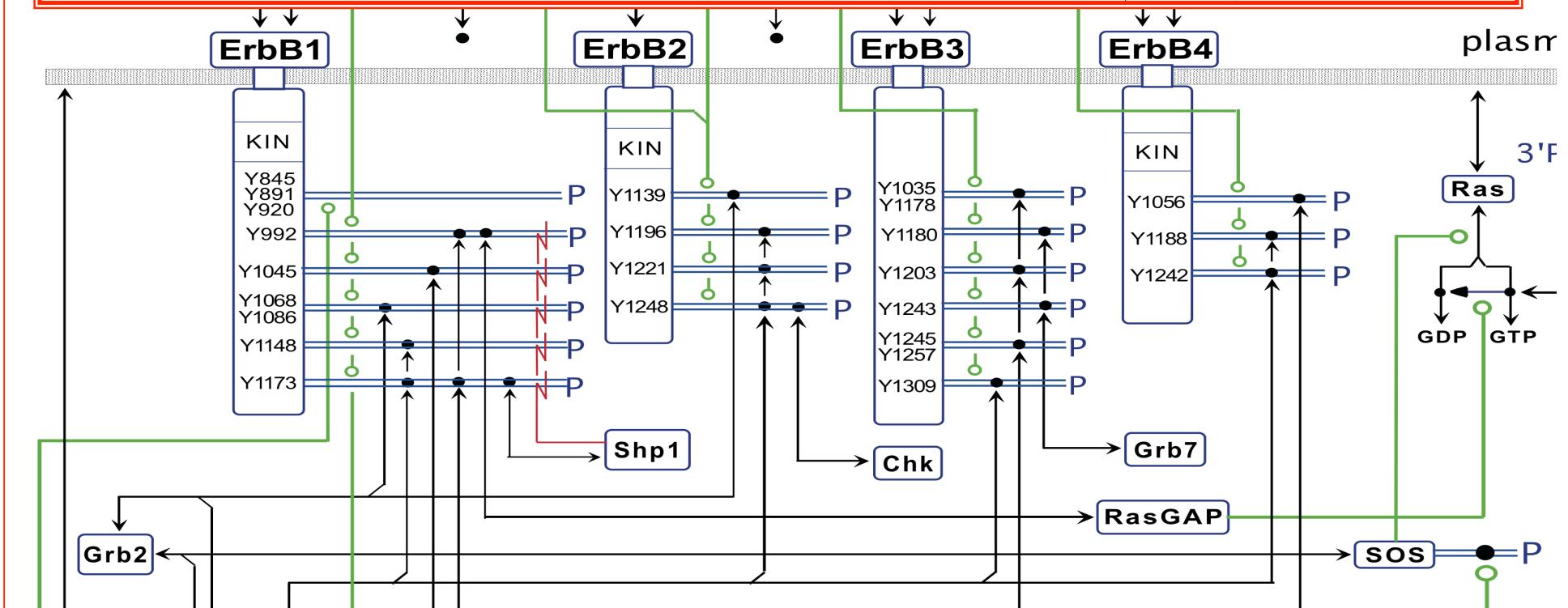
# A Heuristic MIM



Heuristic MIMs concisely depict  
complex networks of molecular  
interactions

# Combinatorial Explosion

48 Receptor-ligand-dimer combinations;  
96 phosphorylation reactions involving a single tyrosine residue (Y1068);  
144 phosphorylation reactions involving two tyrosines (Y1148/73)  
A simple model for SOS recruitment by EGFR enumerates 3749  
individual reactions. (Blinov et al., 2005)



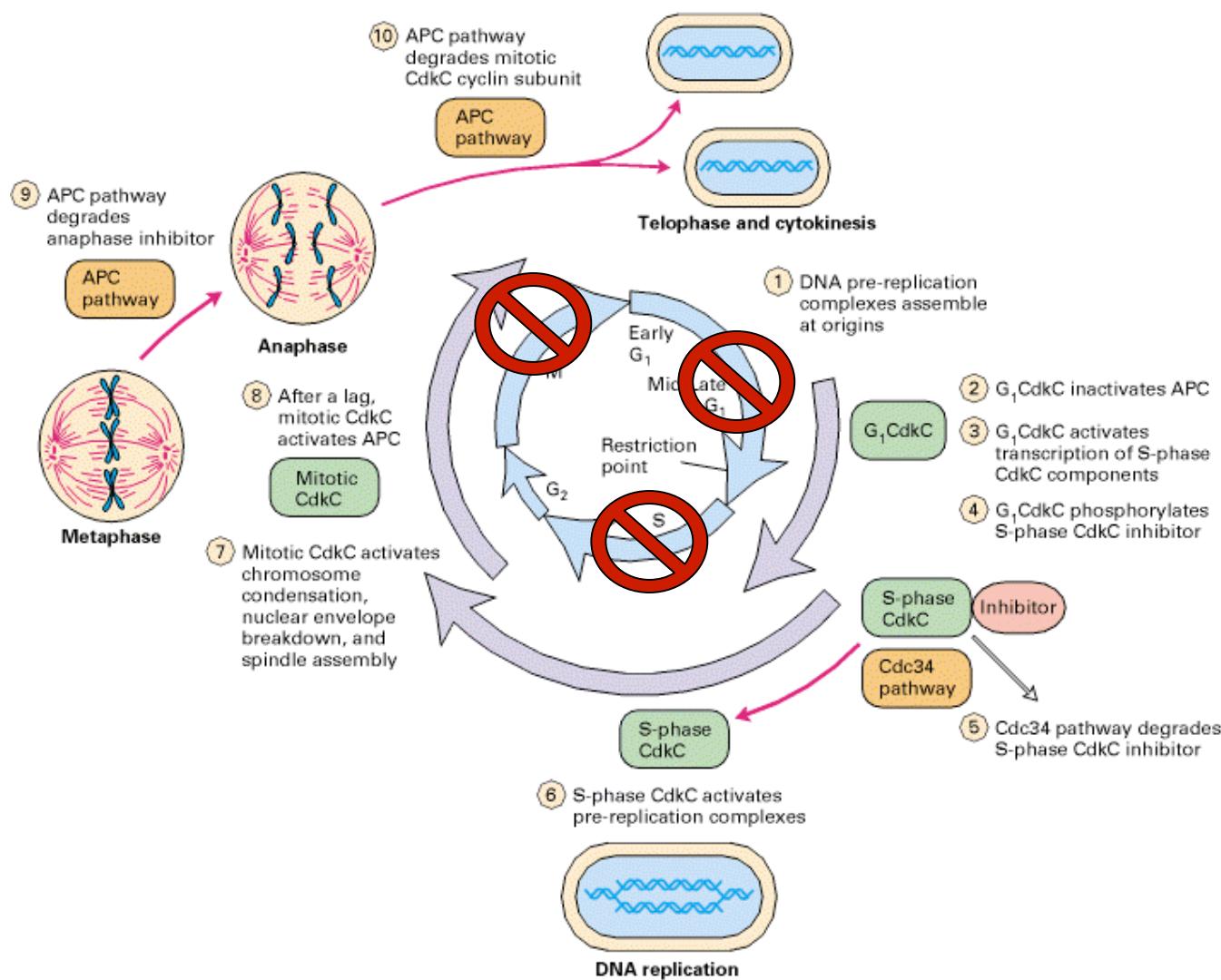
MIMs depict what molecules “see”

- potential interactions between depicted molecular species.

Heuristic MIMs can describe complex networks of potential interactions without encountering “combinatorial explosion”.

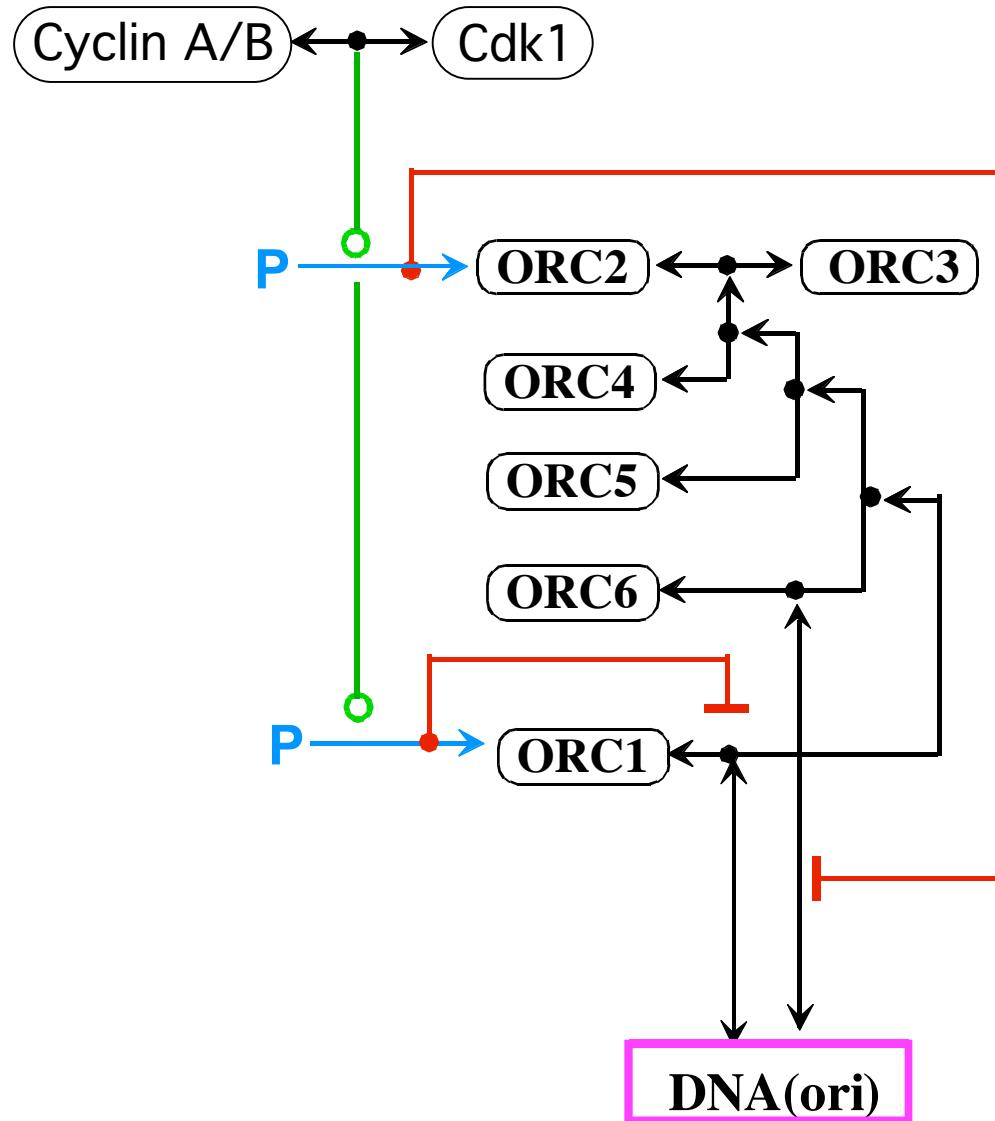
*Can biological processes be  
inferred from the potential  
interactions described in  
heuristic MIMs?*

# MIMs depicting key cell cycle events

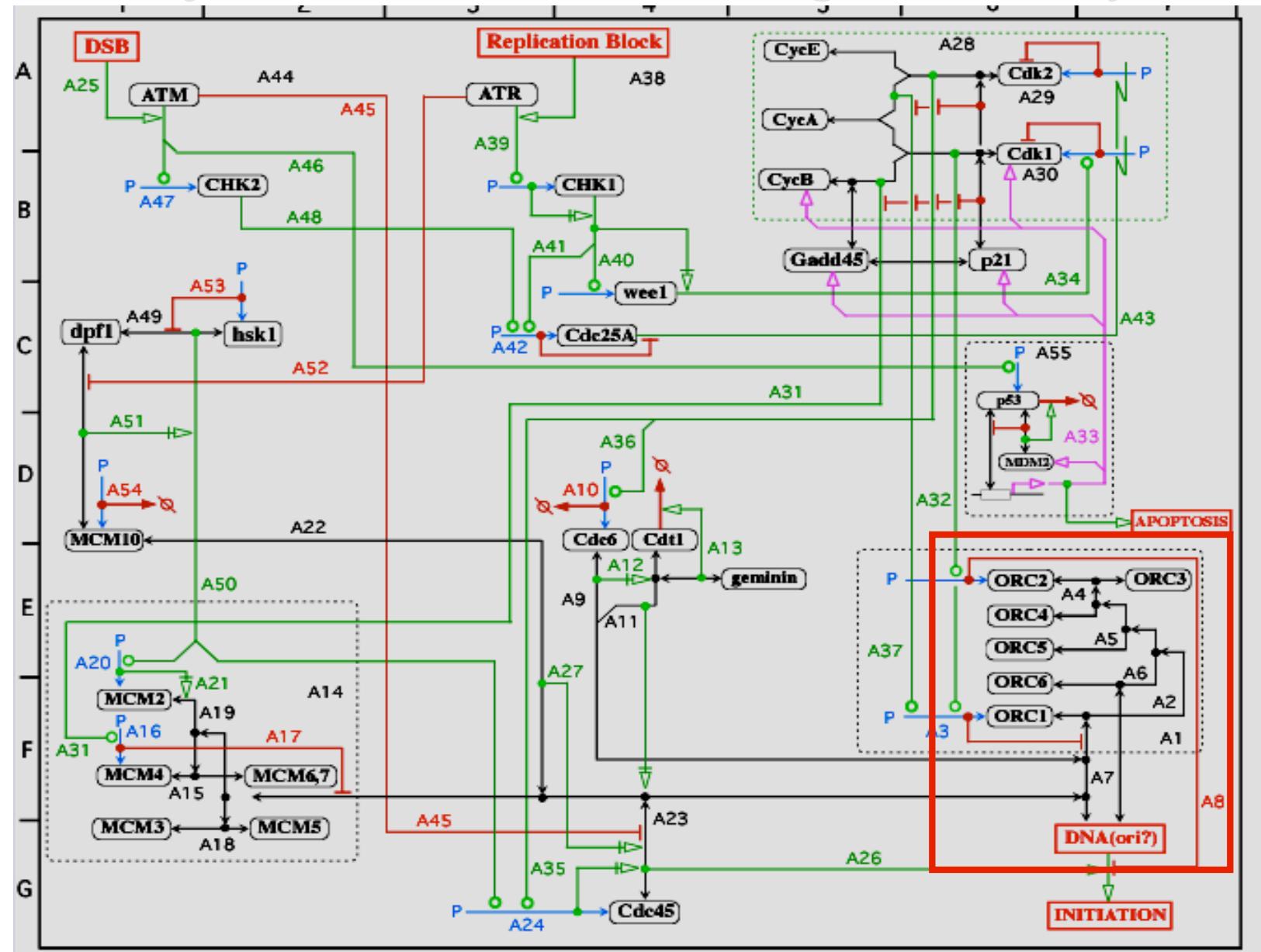


- G<sub>0</sub>: Resting**
- G<sub>1</sub>: Cell Growth**
- S: DNA Replication**
- G<sub>2</sub>: Preparation for Division**
- M phase: Cell Division**

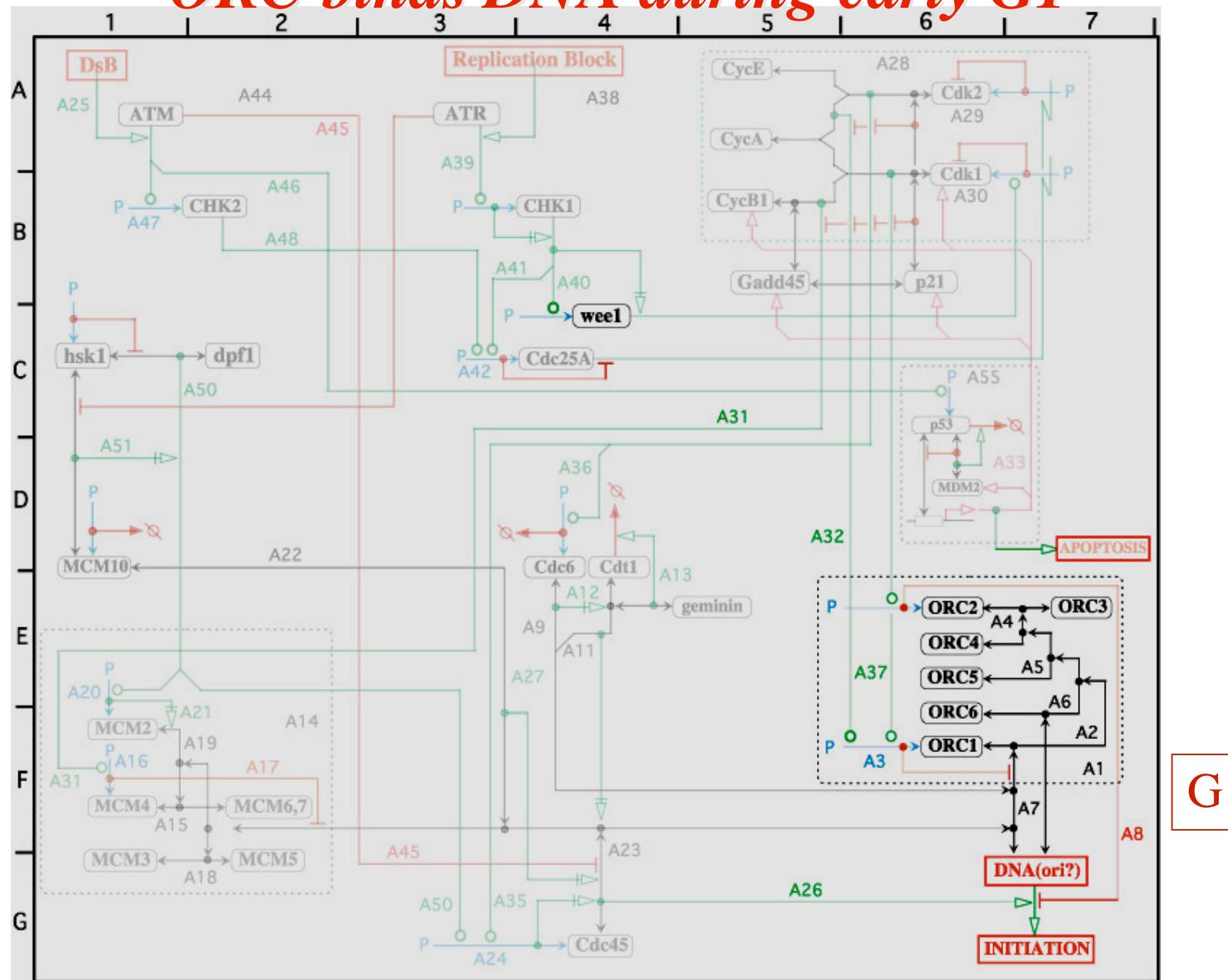
# *What does the ORC complex do?*



# Role of ORC in events leading to DNA synthesis

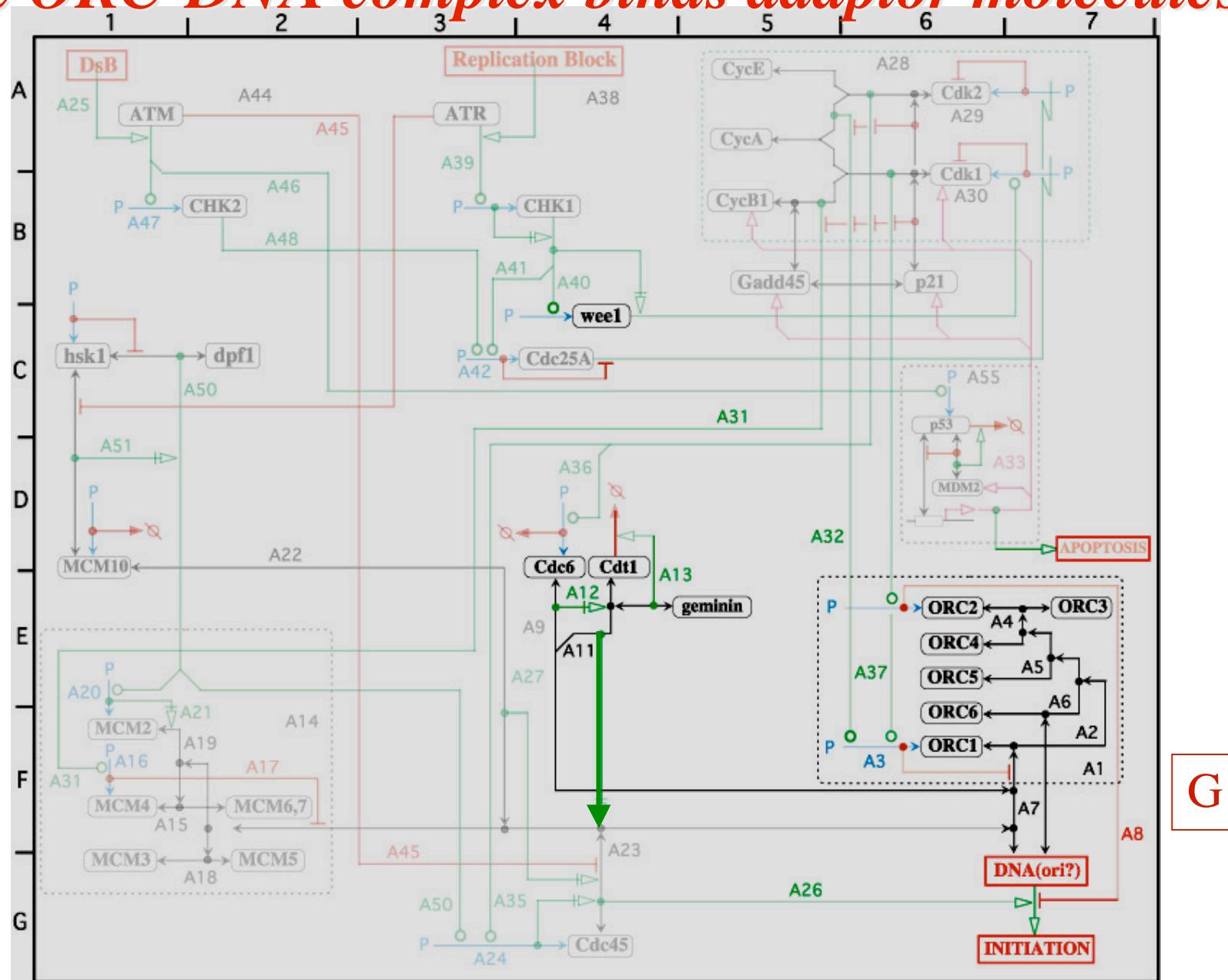


# *ORC binds DNA during early G1*

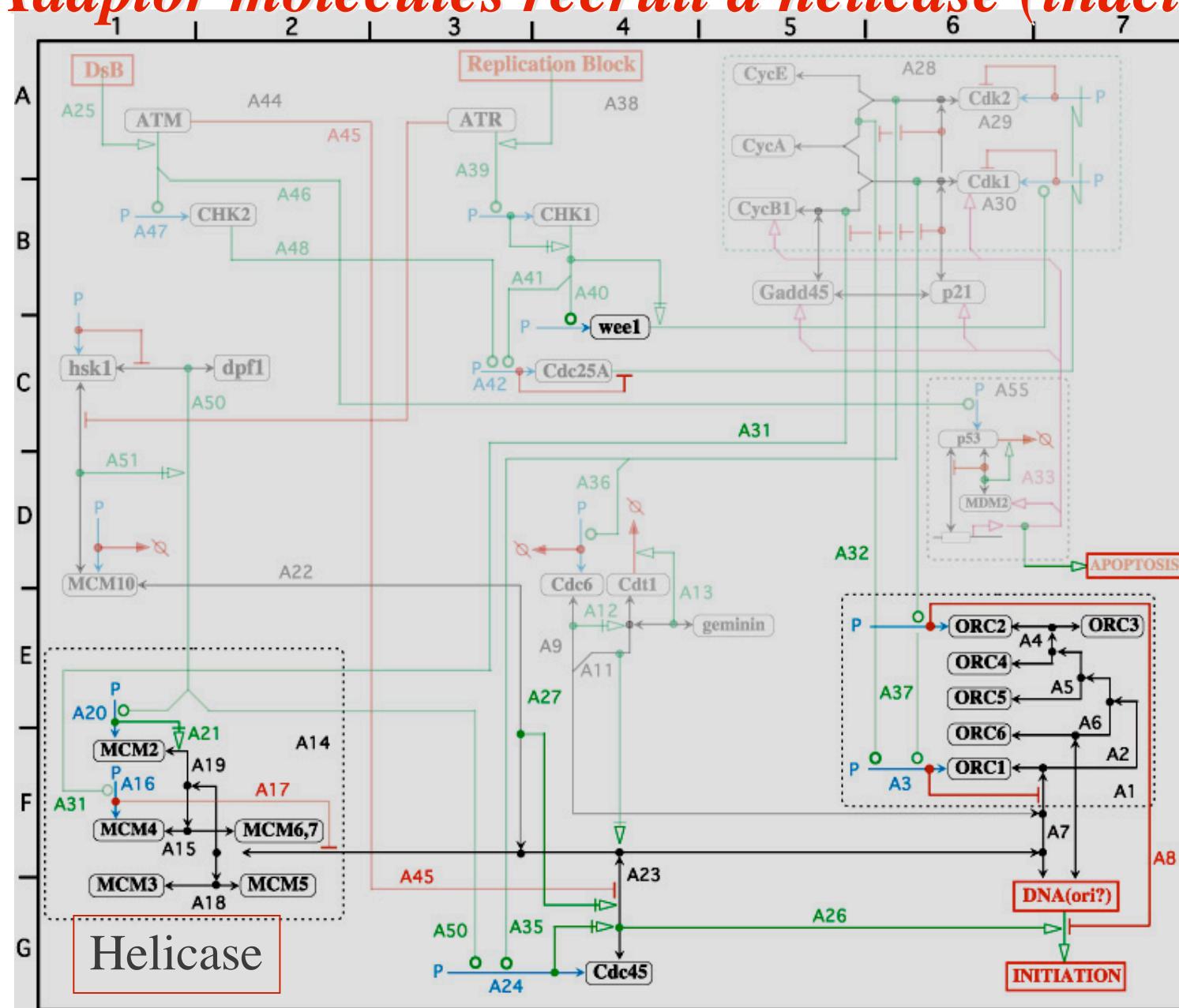


G1

# The ORC-DNA complex binds adaptor molecules

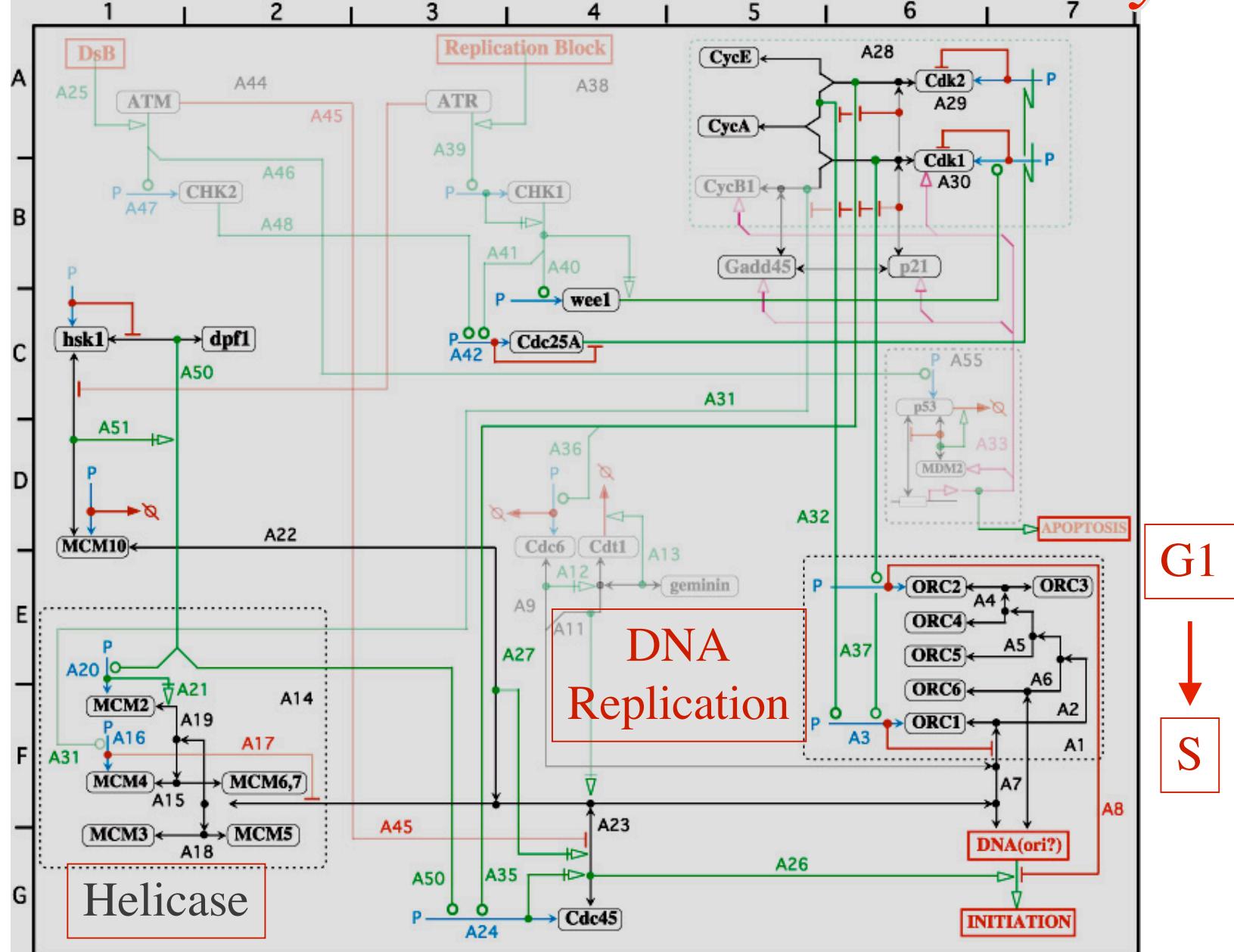


# Adaptor molecules recruit a helicase (inactive)

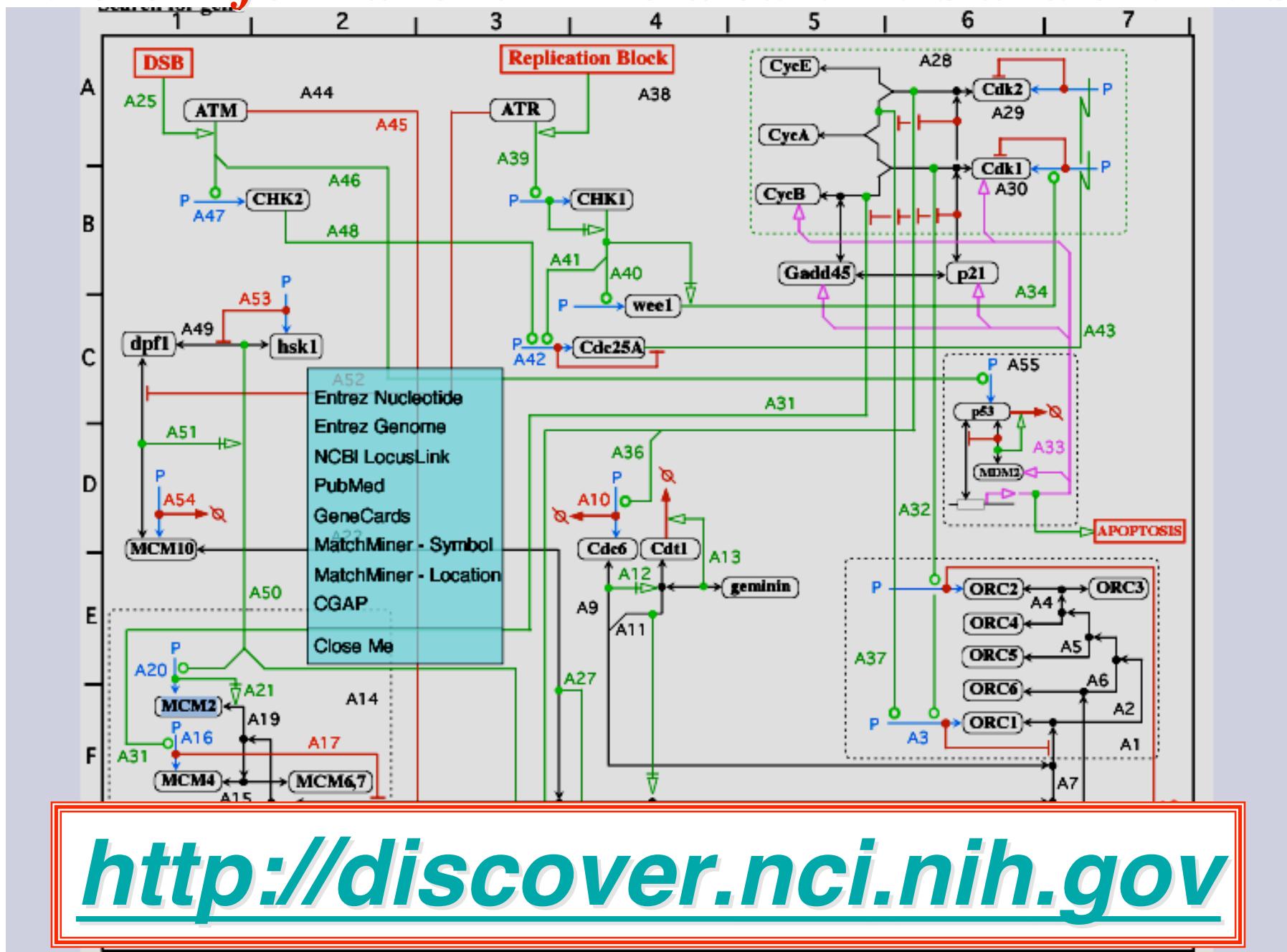


G1

# A switch to an active helicase: kinase activity



## More Information: Annotated MIMs and e-MIMs

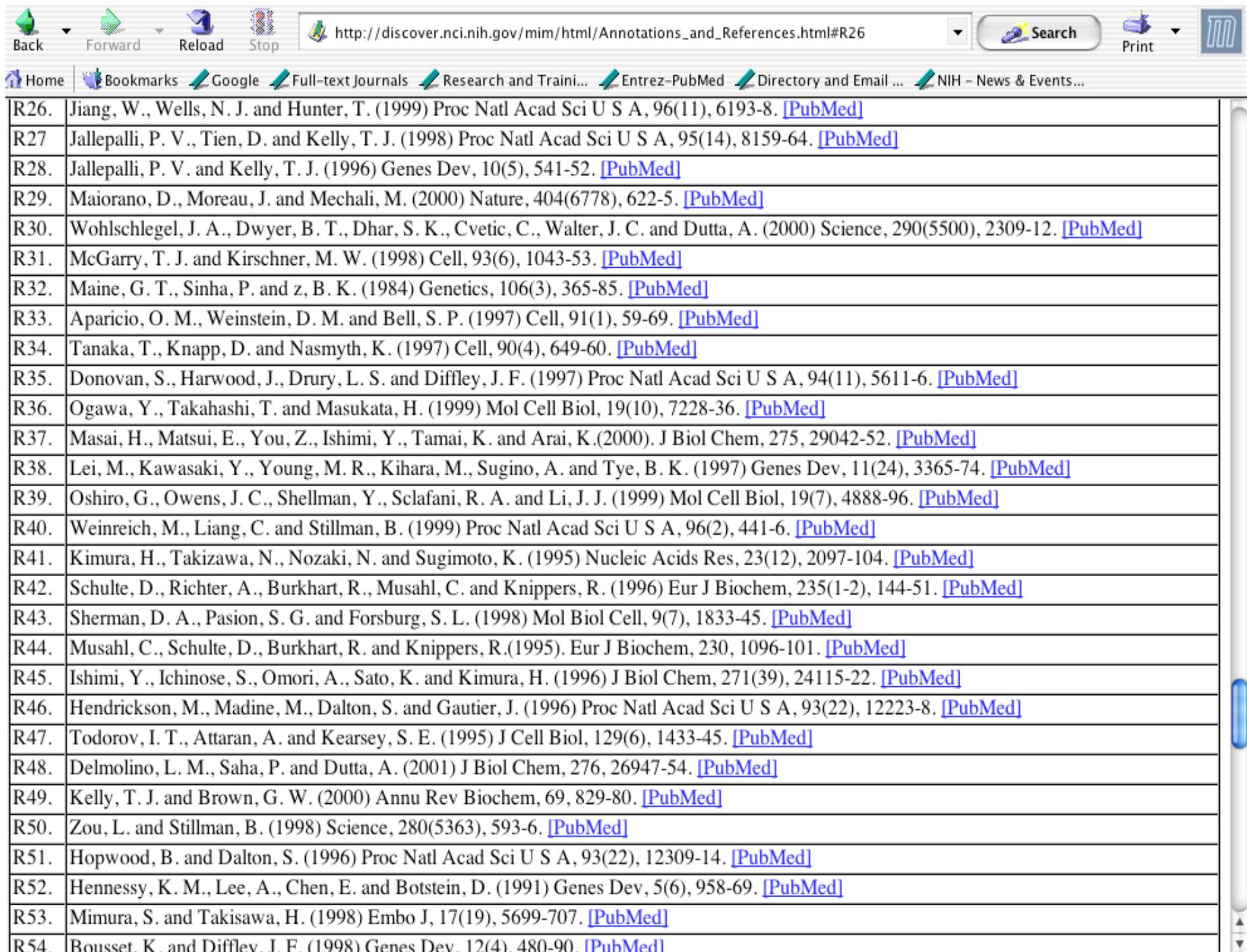


Annotations for the replication interaction maps: – Mozilla

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A20	1E	MCM2 is a target of phosphorylation by a kinase family that includes cdc7 in yeast and hsk1 in humans ( <a href="#">R26</a> ) <a href="#">(R37)</a> <a href="#">(R38)</a> <a href="#">(R39)</a> <a href="#">(R40)</a> . Phosphorylation facilitates binding of MCM2 to other MCM subunits (see interaction 21).
A21	1E-F	MCM2 phosphorylation by cdc7 type kinases (Cdc7:Dbf4 in yeast, hsk/dpf4 in humans) facilitates binding of MCM2 to other MCM subunits and initiation of DNA replication ( <a href="#">R37</a> ).
A22	2D-3E	MCM10 associates with MCM2-7 and chromatin in humans ( <a href="#">R75</a> ). MCM10 is required for assembly of the pre-replication complex in <i>S. cerevisiae</i> ( <a href="#">R79</a> ) Xenopus ( <a href="#">R99</a> ) <a href="#">(R100)</a> and human cells. Binding of MCM10 to chromatin seems to occur throughout the cell cycle ( <a href="#">R103</a> ) <a href="#">(R104)</a> .
A23	4F-G	Cdc45 incorporates into the pre-replication complex ( <a href="#">R50</a> ) and is essential for initiation of DNA replication in yeast. Cdc45 co-immunoprecipitates with MCMs ( <a href="#">R51</a> ). In yeast, Cdc45 interacts with MCMs ( <a href="#">R52</a> ) and ORC2 ( <a href="#">R33</a> ) ( <a href="#">R50</a> ) and its association with DNA depends on Cdc6 and MCM ( <a href="#">R50</a> ) <a href="#">(R33)</a> . Cdc45 binding to chromatin, but not Cdc45 transcription, requires an active E2F in Drosophila ( <a href="#">R97</a> ). Chromatin binding is inhibited by the DNA damage checkpoint ( <a href="#">R94</a> ).
A24	3-4G	Binding of Cdc45 to the ORC:MCM complex on chromatin necessitates two kinase complexes: S-phase Cyclin:Cdk, especially Cyclin E:Cdk2, and homologs of yeast Cdc7:Dbf4 (DDK - hsk1 in humans). The yeast homologue of Cyclin E:Cdk2 was shown to be required for Cdc45 binding to chromatin in yeast ( <a href="#">R50</a> ); Cdc7:Dbf4 was shown to be also necessary ( <a href="#">R101</a> ) <a href="#">(R102)</a> . Cdc7:Dbf4 was shown directly to phosphorylate Cdc45.
A25	1A	Double stranded breaks inhibit the addition of Cdc45 to the pre-initiation complex (ORC:MCM:Cdc7) in Xenopus extracts. This inhibition depends on the activity of the ATM kinase ( <a href="#">R94</a> ). ATM dependent activation of Mre1:nbs:Rad50 complex can also inhibit progression through S-phase, but this activation does not inhibit binding of Cdc45 to chromatin ( <a href="#">R98</a> ).
A26	5-6G	Cdc45 immunoprecipitates with origin DNA before initiation, but binds non-specifically to DNA after initiation of DNA replication ( <a href="#">R33</a> ). Cdc45 interacts with DNA polymerase alpha ( <a href="#">R53</a> ) and hsCdc45 associates with ORC2 ( <a href="#">R5</a> ).
A27	4F-G	MCM10 binds to the chromatin-bound MCM complex and this binding is essential for recruitment of Cdc45 to the pre-initiation complex ( <a href="#">R104</a> ).





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1: Proc Natl Acad Sci U S A. 1999 May 25;96(11):6193-8.

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## Multistep regulation of DNA replication by Cdk phosphorylation of HsCdc6.

Jiang W, Wells NJ, Hunter T.

Molecular Biology and Virology Laboratory, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037,  
USA. [wjiang@salk.edu](mailto:wjiang@salk.edu)

We have characterized HsCdc6, a human protein homologous to the budding yeast Cdc6p that is essential for DNA replication. We show that, unlike Cdc6p, the levels of HsCdc6 protein remain constant throughout the cell cycle in human cells. However, phosphorylation of HsCdc6 is regulated during the cell cycle. HsCdc6 is an excellent substrate for Cdk2 *in vitro* and is phosphorylated *in vivo* at three sites (Ser-54, Ser-74, and Ser-106) that are phosphorylated by Cdk2 *in vitro*, strongly suggesting that HsCdc6 is an *in vivo* Cdk substrate. HsCdc6 is nuclear in G1, but translocates to the cytoplasm at the start of S phase via Crm1-dependent export. An HsCdc6A1A2A3 mutant, which mimics unphosphorylated HsCdc6, is exclusively nuclear, and its expression inhibits initiation of DNA replication. An HsCdc6E1E2E3 mutant, which mimics phosphorylated HsCdc6, is exclusively cytoplasmic and is not associated with the chromatin/nuclear matrix fraction. Based on these results, we propose that phosphorylation of HsCdc6 by Cdks regulates DNA replication of at least two steps: first, by promoting initiation of DNA replication and, second, through nuclear exclusion preventing DNA rereplication.

PMID: 10339564 [PubMed - indexed for MEDLINE]

# Deducing Regulatory Pathways from MIMs

[MIM home](#)

[STKE Paper](#)

[Introduction](#)

[Replication Initiation Maps](#)

- Complete map
- Radiation
- Inhibitors
- Mitosis

[Map Symbols](#)

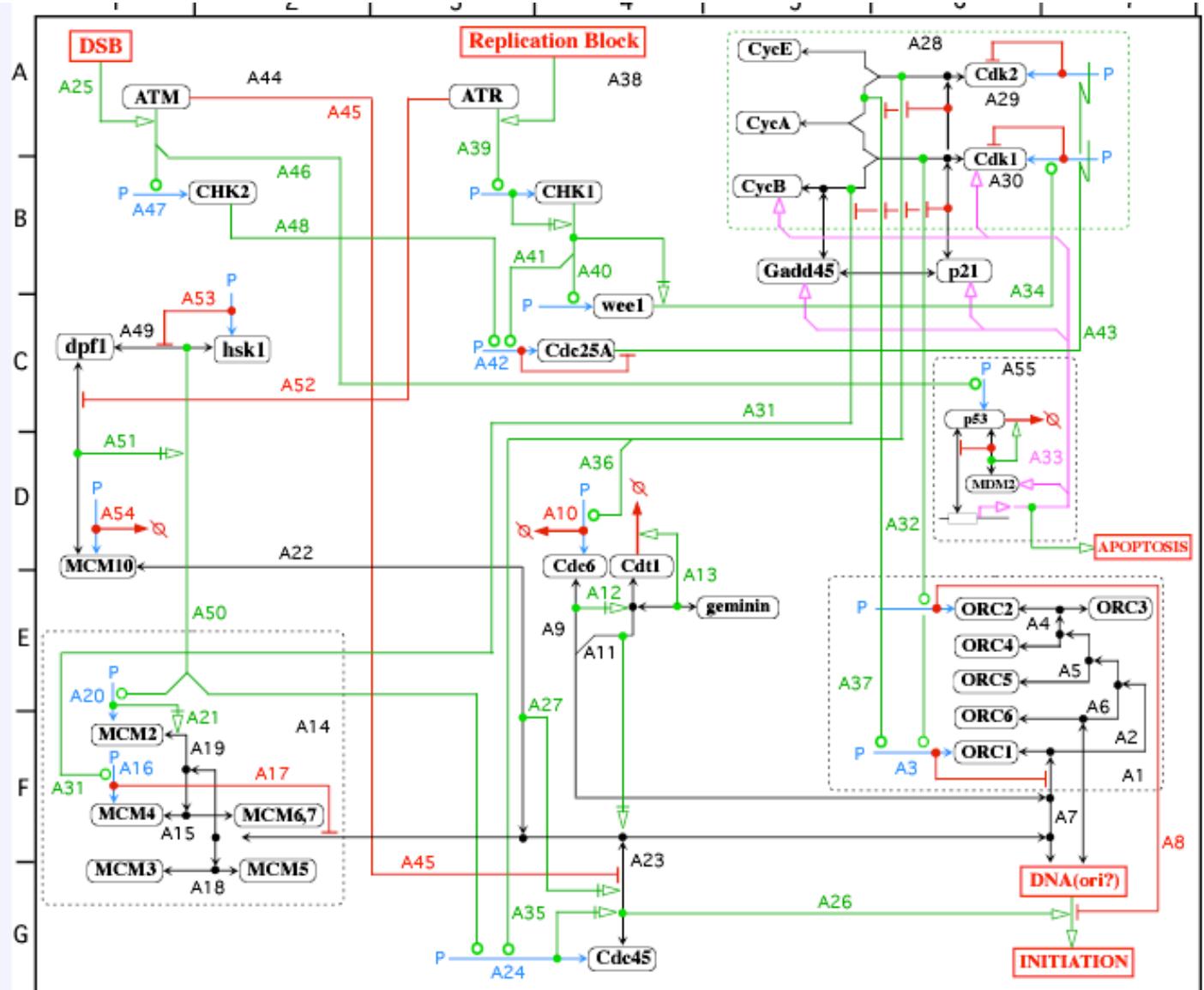
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[Annotations](#)

[References](#)

[System Requirements](#)

[Credits](#)



# How Do Cells Prevent Replication During Mitosis?

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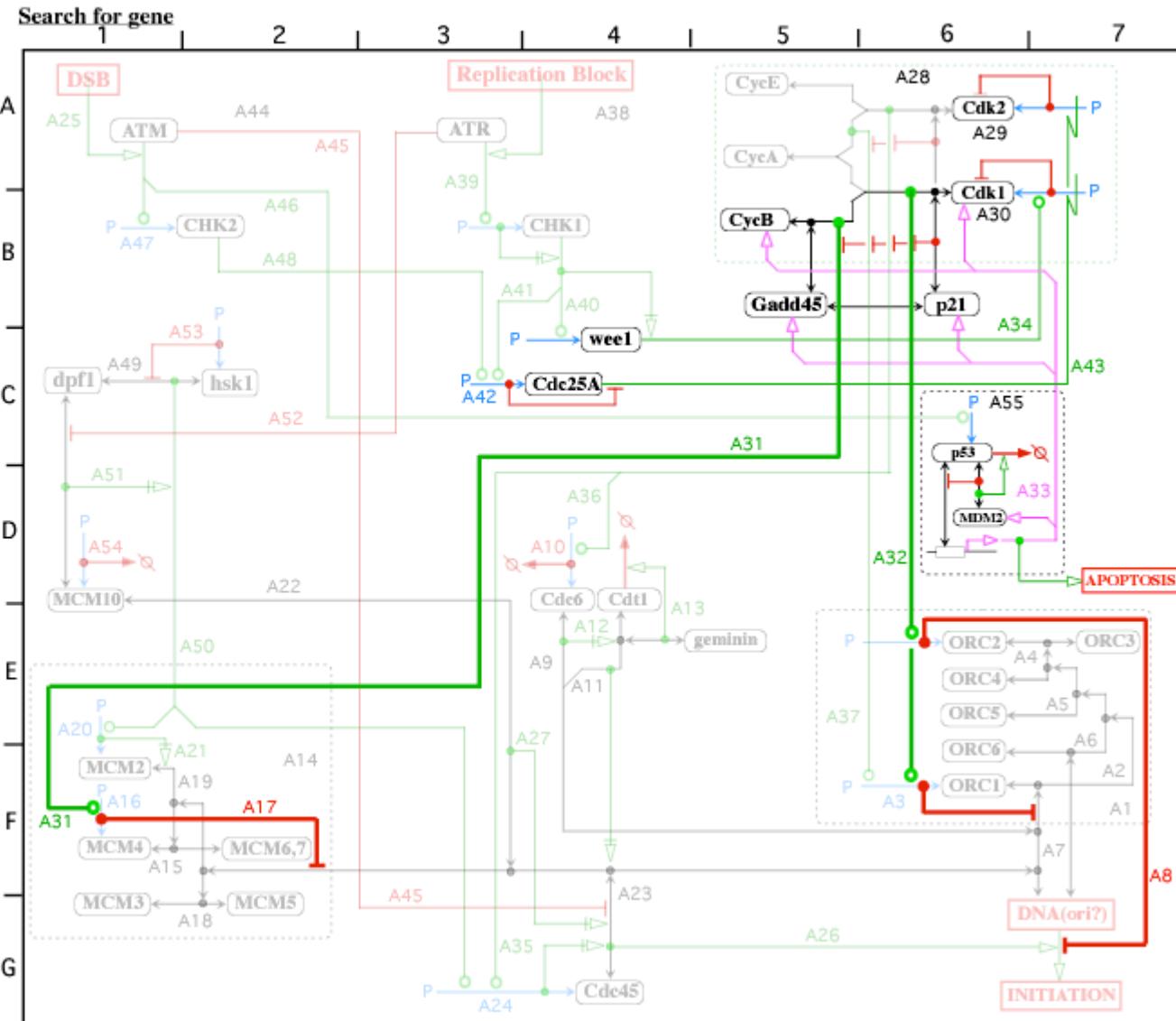
[Annotations](#)

[References](#)

[System Requirements](#)

[Credits](#)

Map description    Interactive Map requires Adobe SVG viewer (see System Requirements)    Static Map Image



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# How do cells respond to a replication block?

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[Introduction](#)

[Replication Initiation Maps](#)

- Complete map
- Radiation
- Inhibitors
- Mitosis

[Map Symbols](#)

[Map Navigation](#)

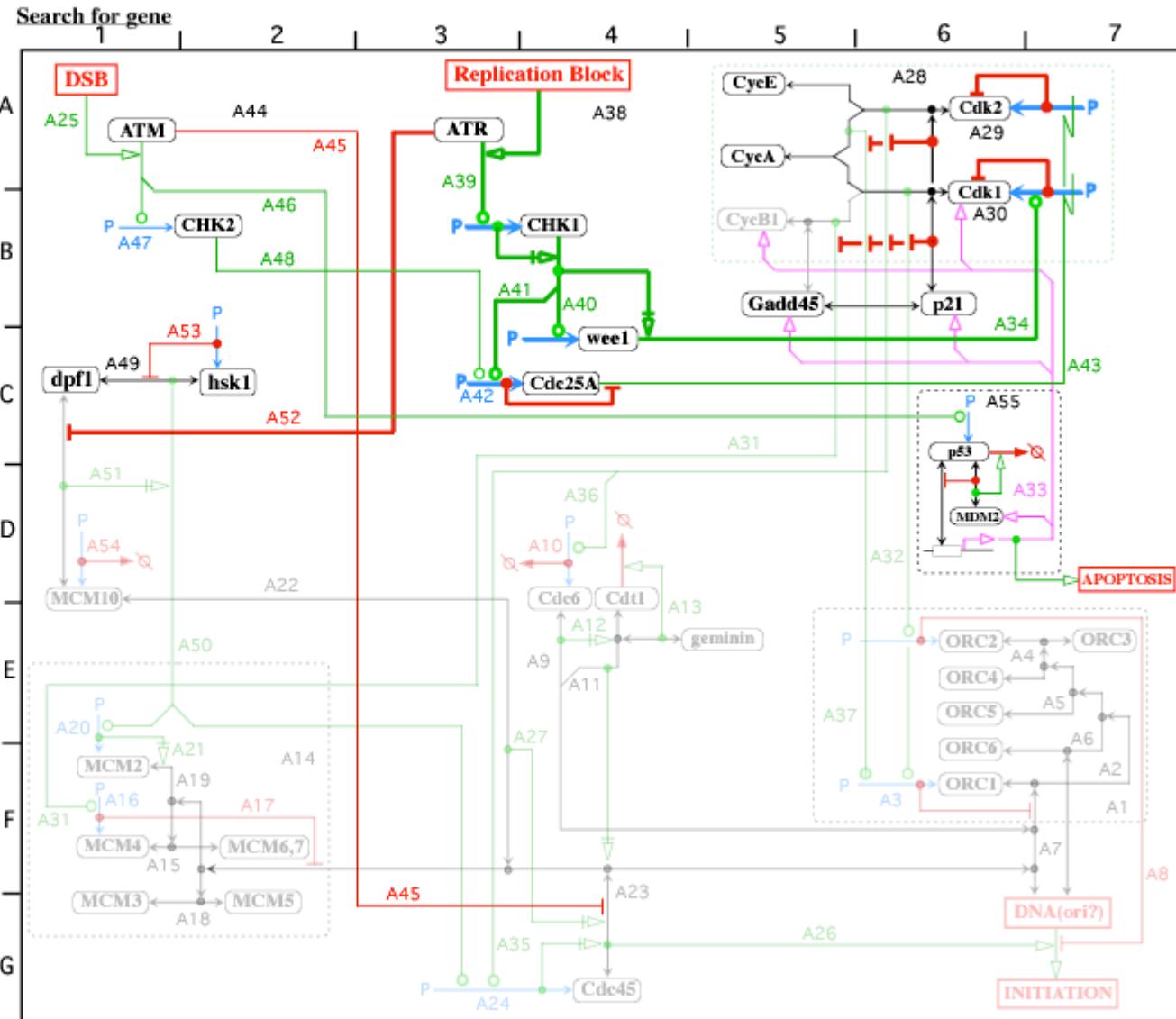
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[System Requirements](#)

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[Map description](#)   [Interactive Map requires Adobe SVG viewer \(see System Requirements\)](#)   [Static Map Image](#)

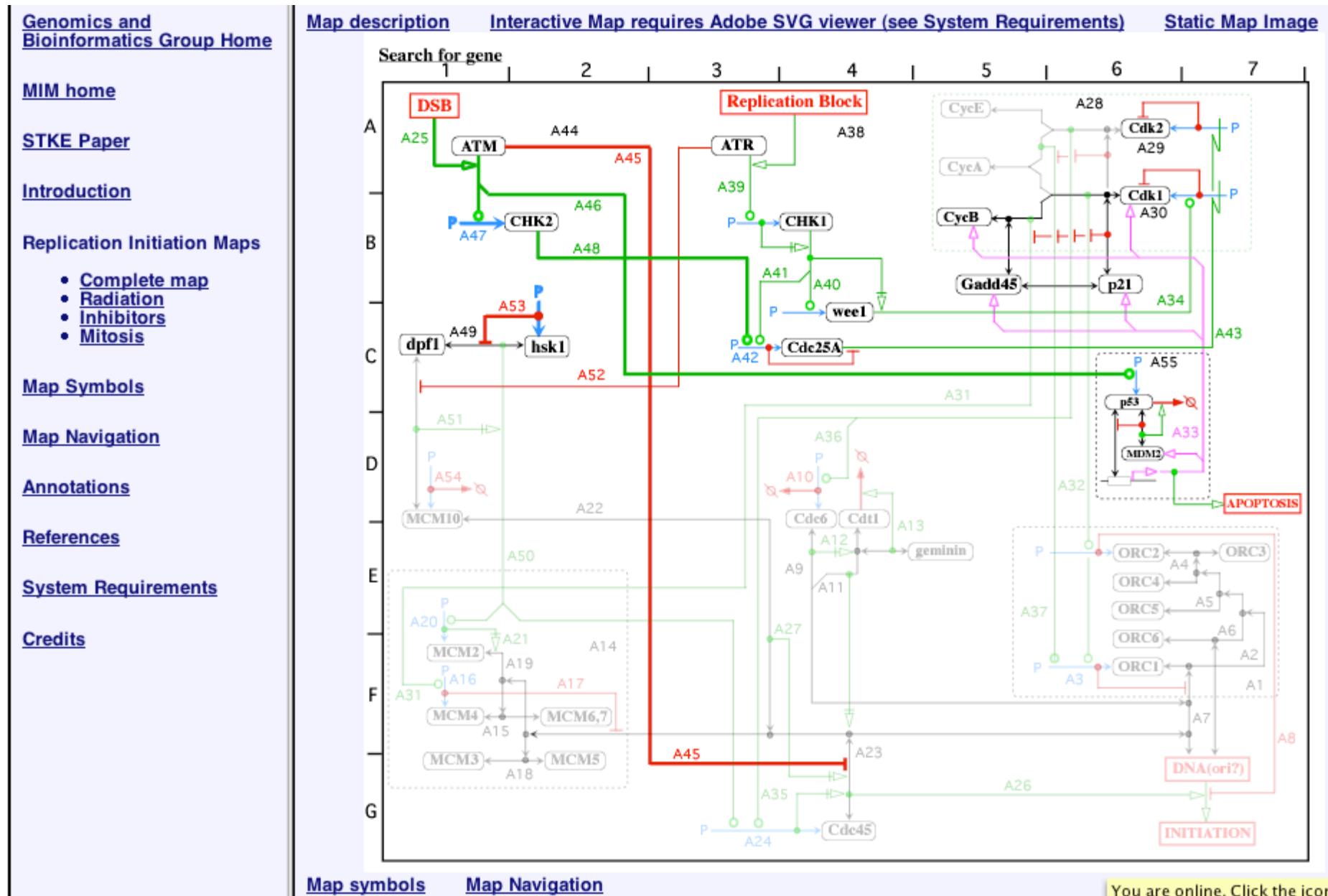


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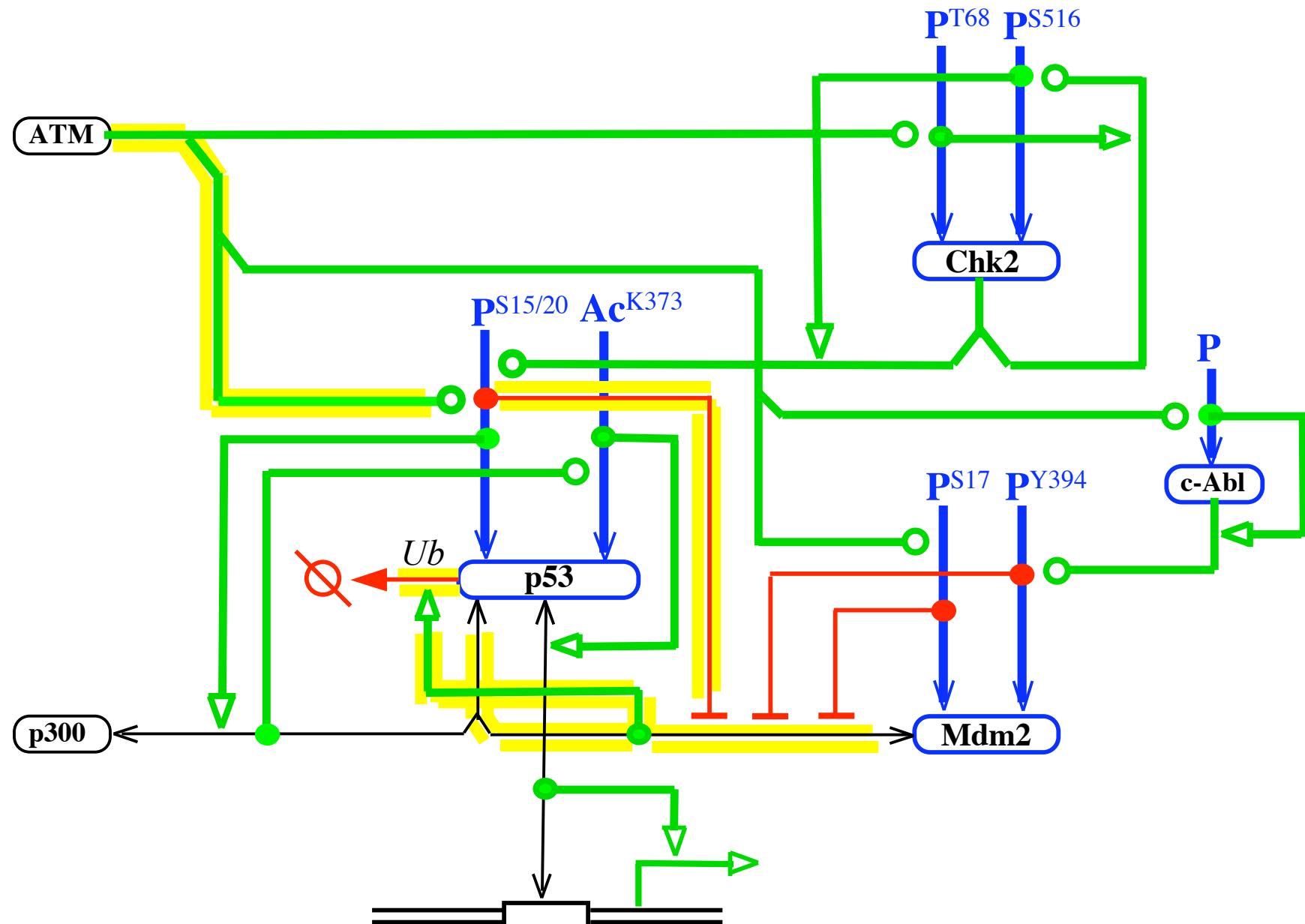
# How do cells respond to a DNA break?



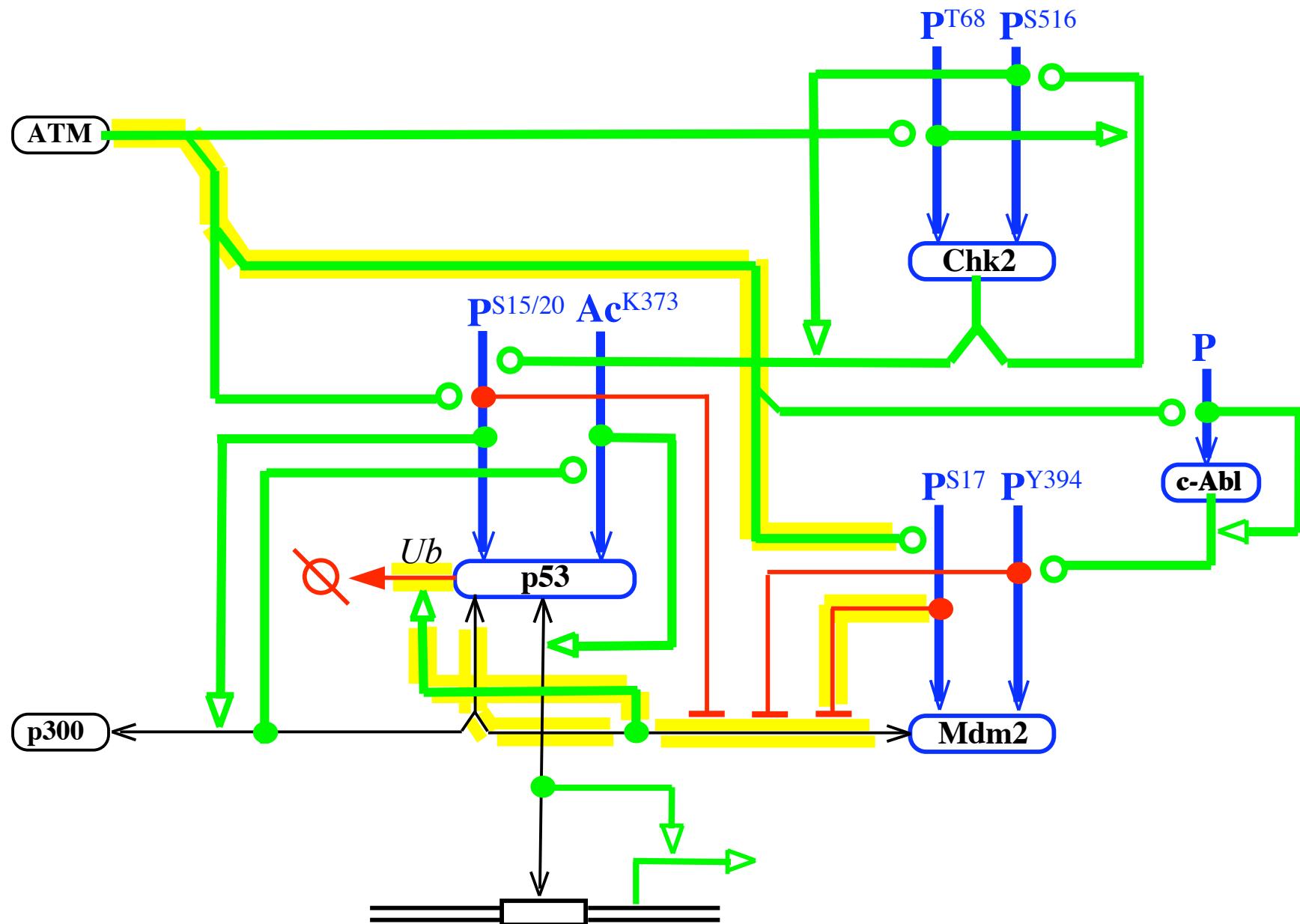
**Biological processes can be  
inferred from the interactions  
described in heuristic MIMs**

*Process control principles  
inferred from heuristic MIMs:  
severe DNA damage activates a  
tumor suppressor protein, p53,  
via several avenues that exhibit  
remarkable coherence.*

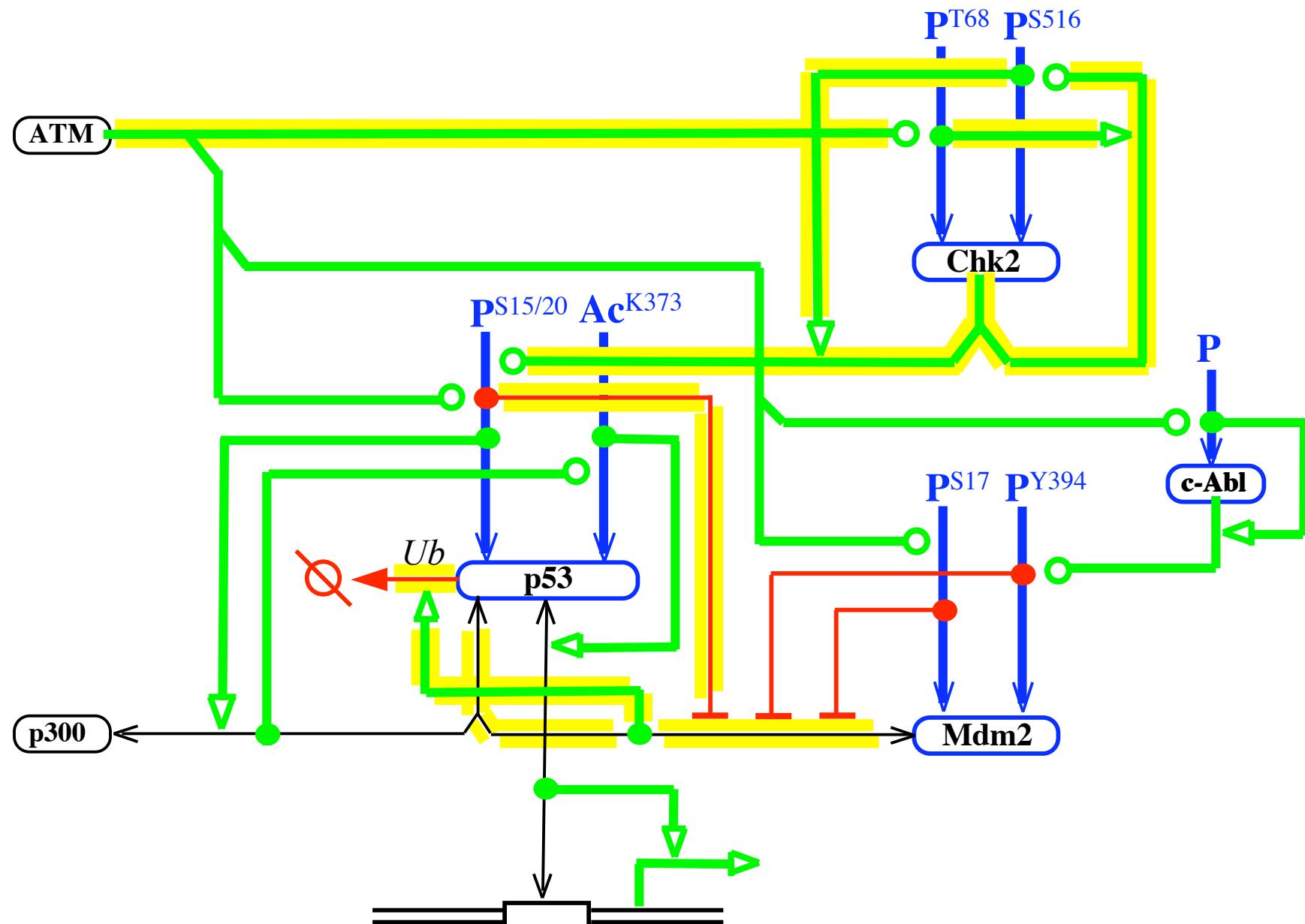
ATM &(S15P) --| p53 :>Mdm2 &(Ub) >% p53 [4+]



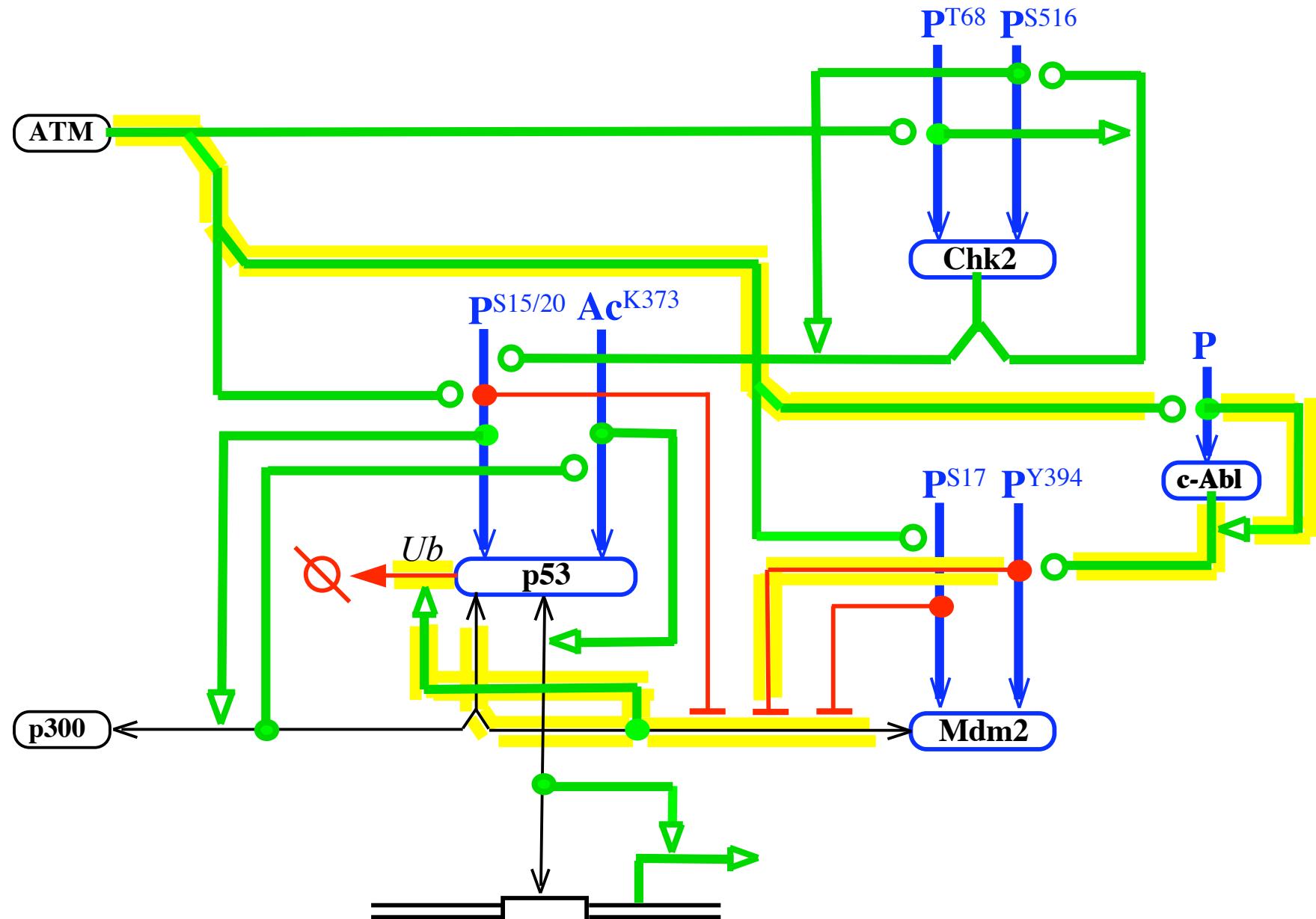
ATM &(S17P) --| Mdm2 :> p53 &(Ub)>% p53 [4+]



ATM &(T68P) >Chk2 &(S516P) >Chk2 &(S20P) --| p53 : >Mdm2 &(Ub) >%p53 [6+]



ATM &(P) > c-Abl &Mdm2(*Y394P*) --| Mdm2 :> p53 &p53(*Ub*) >% p53 [5+]



*Depiction of intramolecular  
interactions in MIMs:  
SRC Activation By Receptor  
Tyrosine Kinases*

Src has the following domains:

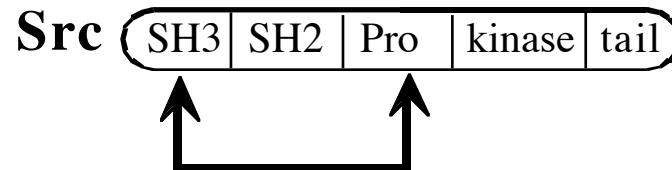
SH3: binds to proline-rich domains

SH2: binds to phosphotyrosine motifs

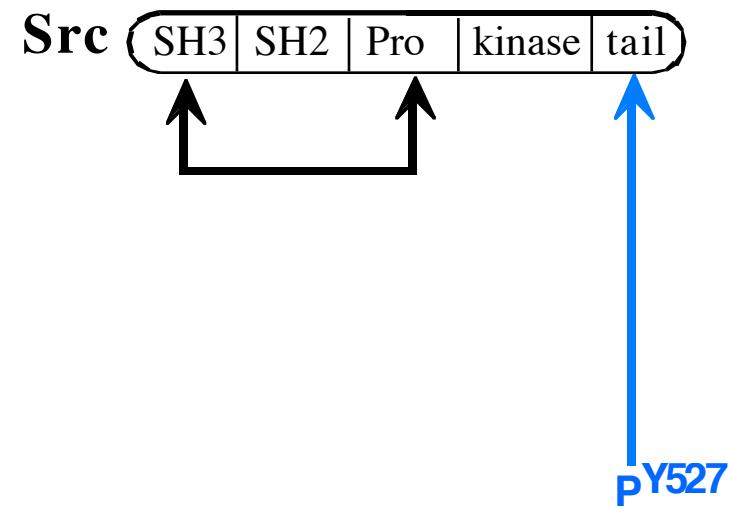
Pro: proline-rich domain

kinase: tyrosine kinase domain

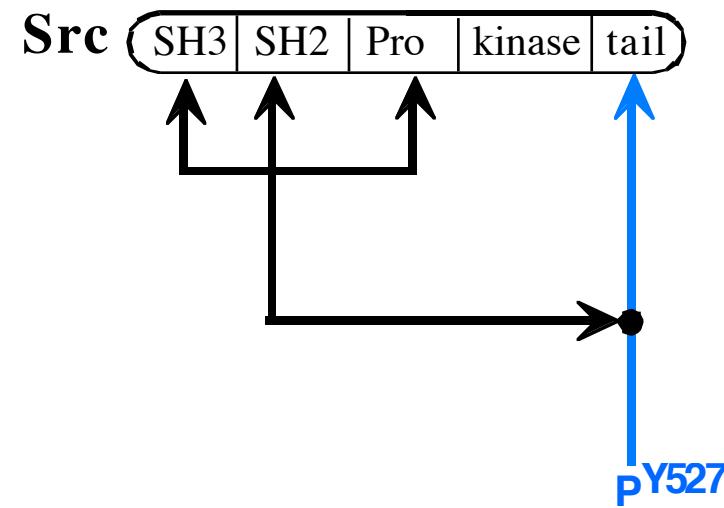
The SH3 domain binds the Pro domain.



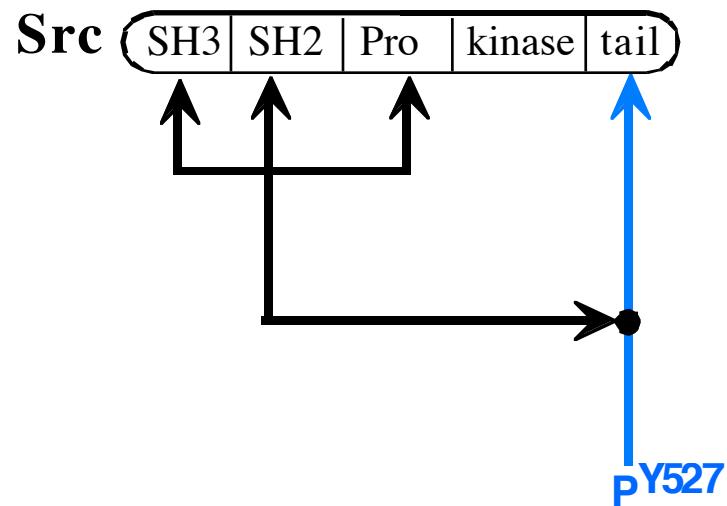
The Src tail region can be phosphorylated at Tyr 527.



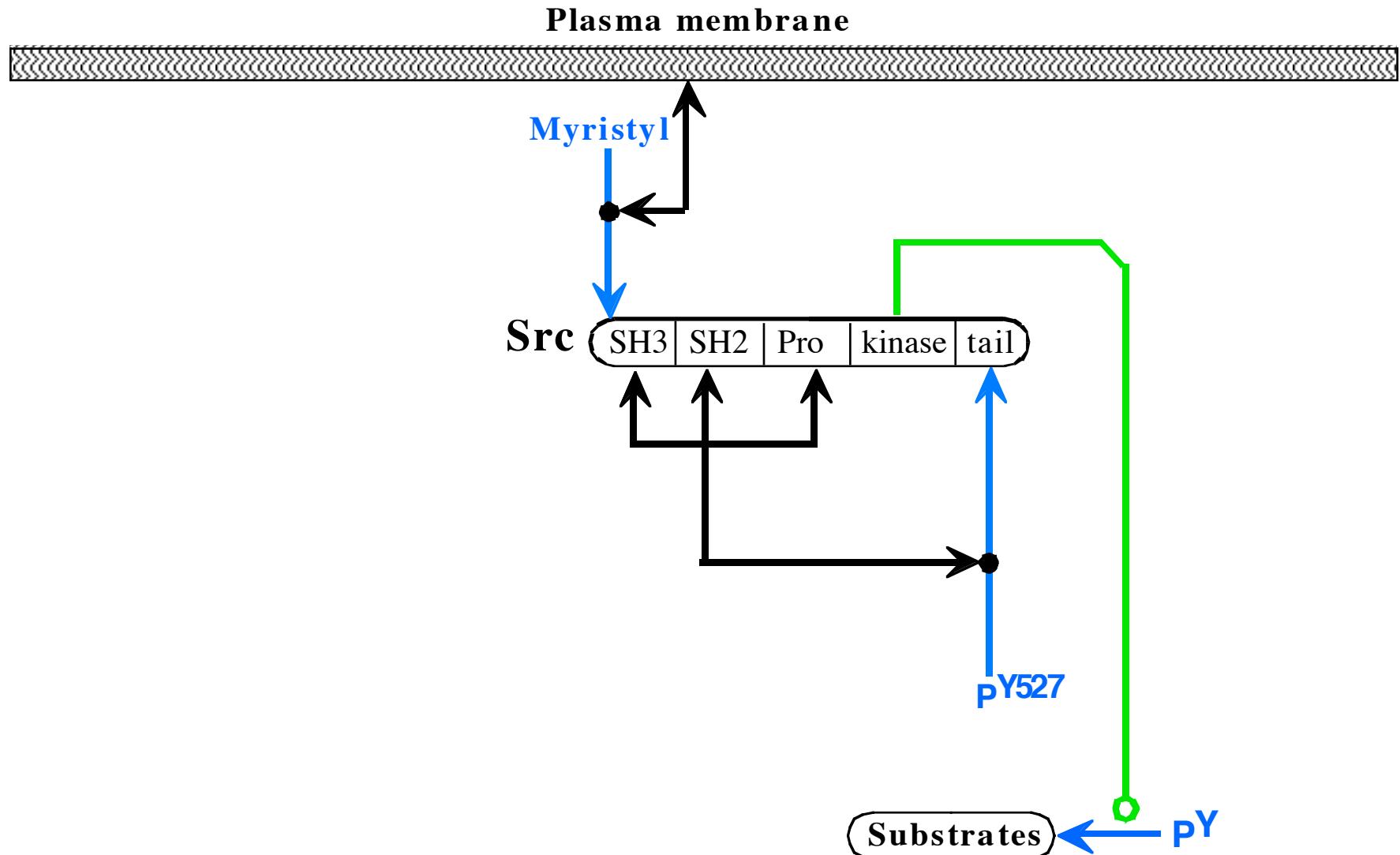
The SH2 domain binds to the tyrosine-phosphorylated tail.



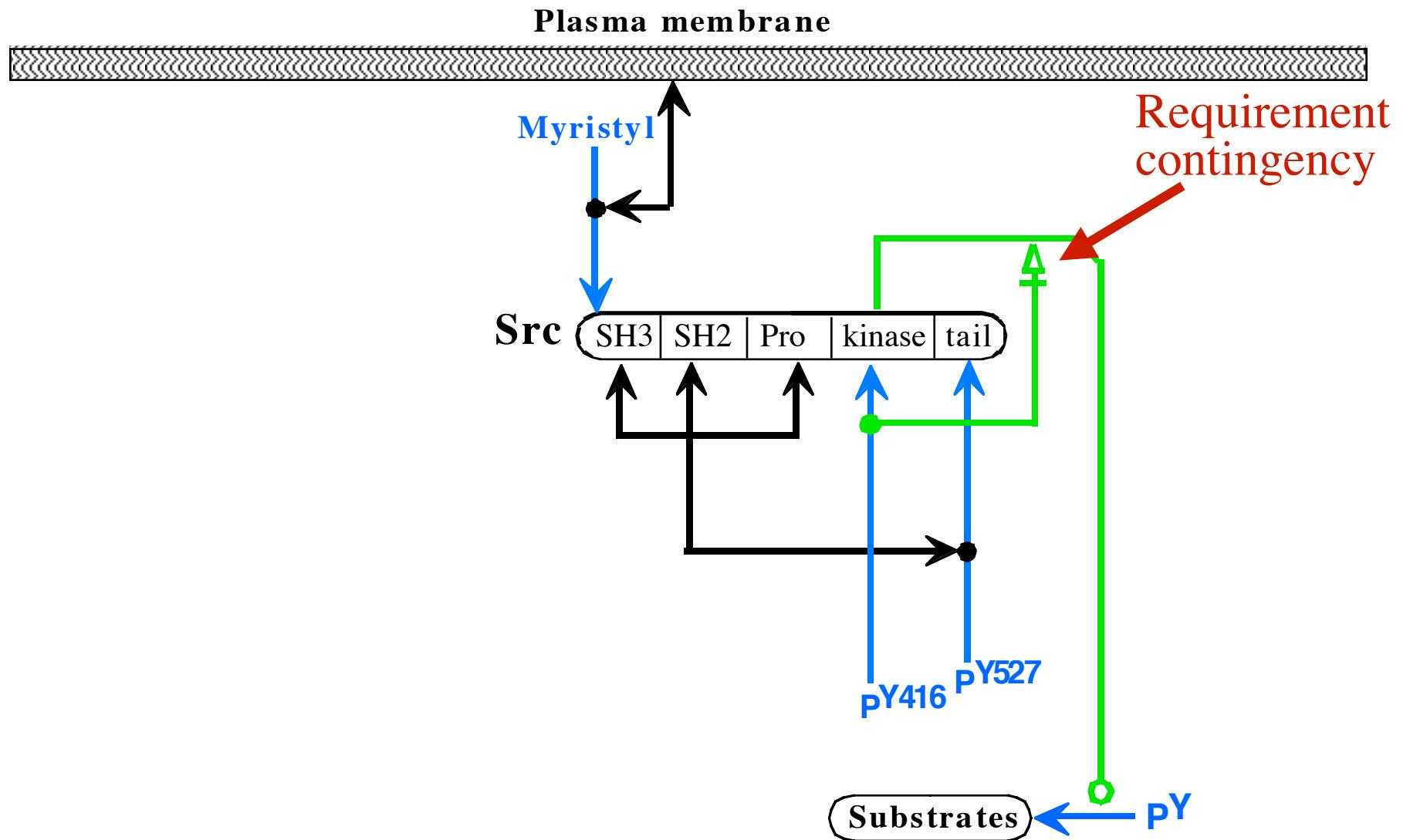
The 2 intra-molecular bonds form cooperatively, and fold the Src molecule, hiding the kinase domain and keeping Src in an inactive configuration.



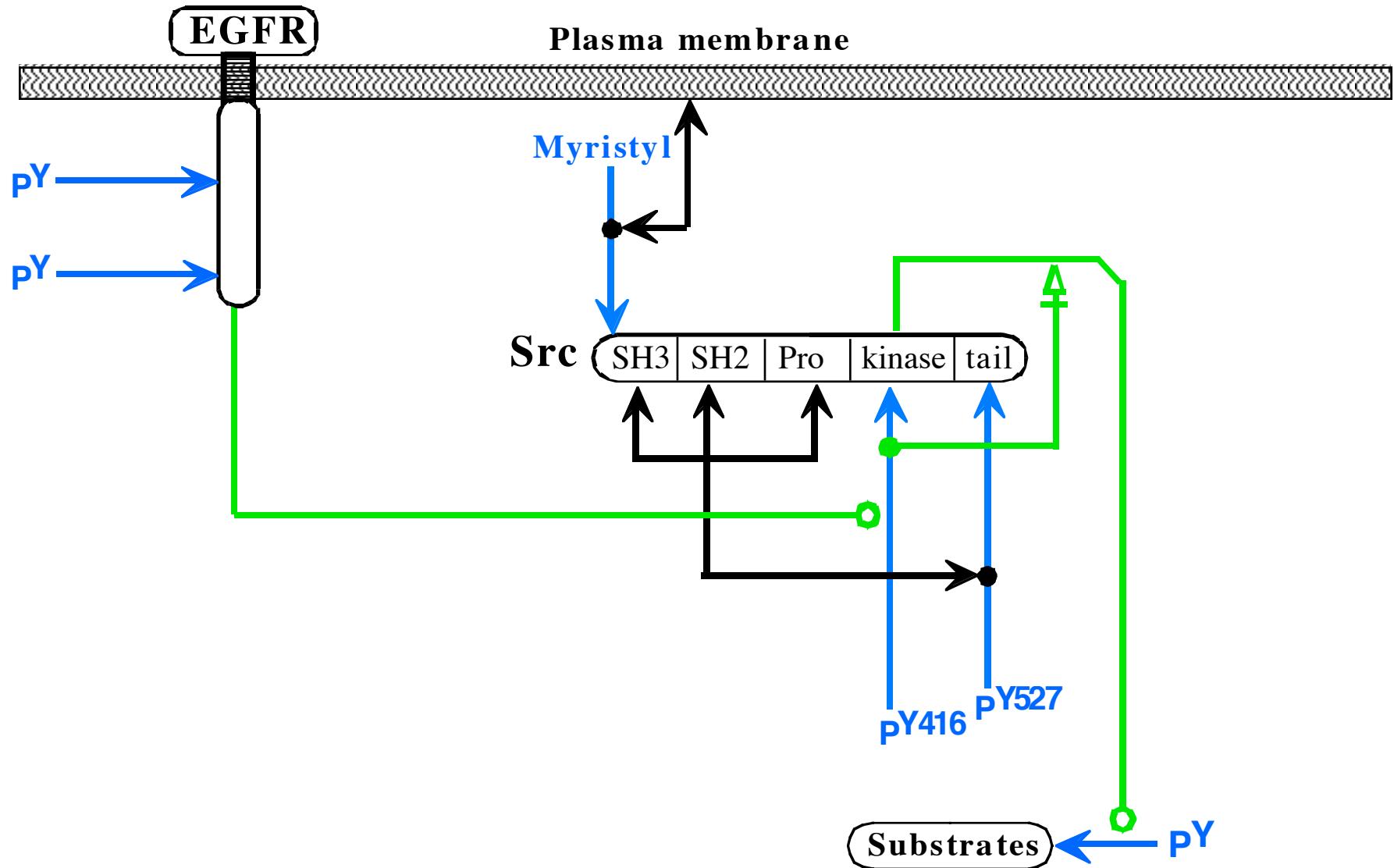
Src's tyrosine kinase domain could phosphorylate various substrates



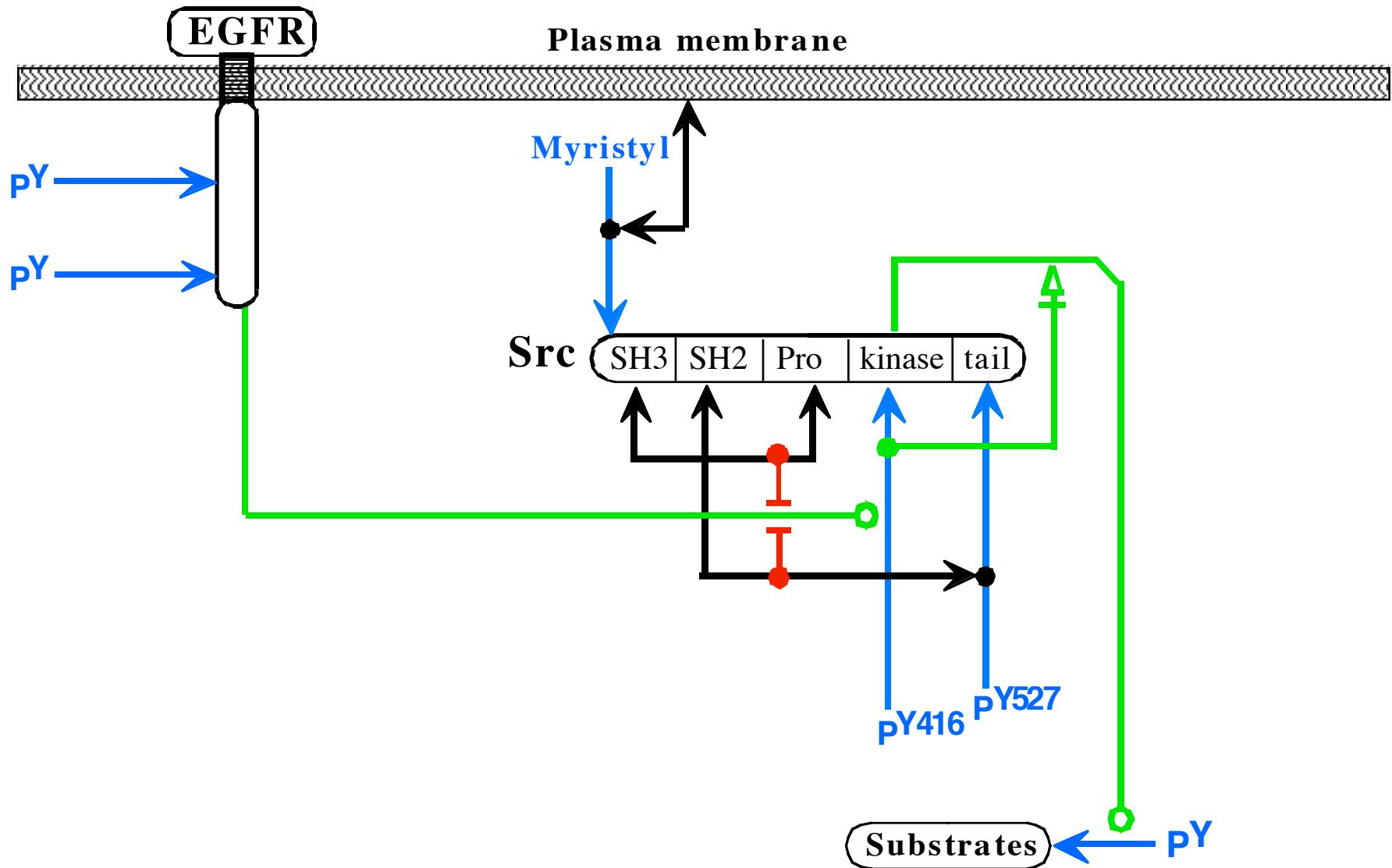
Phosphorylation of Tyr416 is required for the kinase to be active.



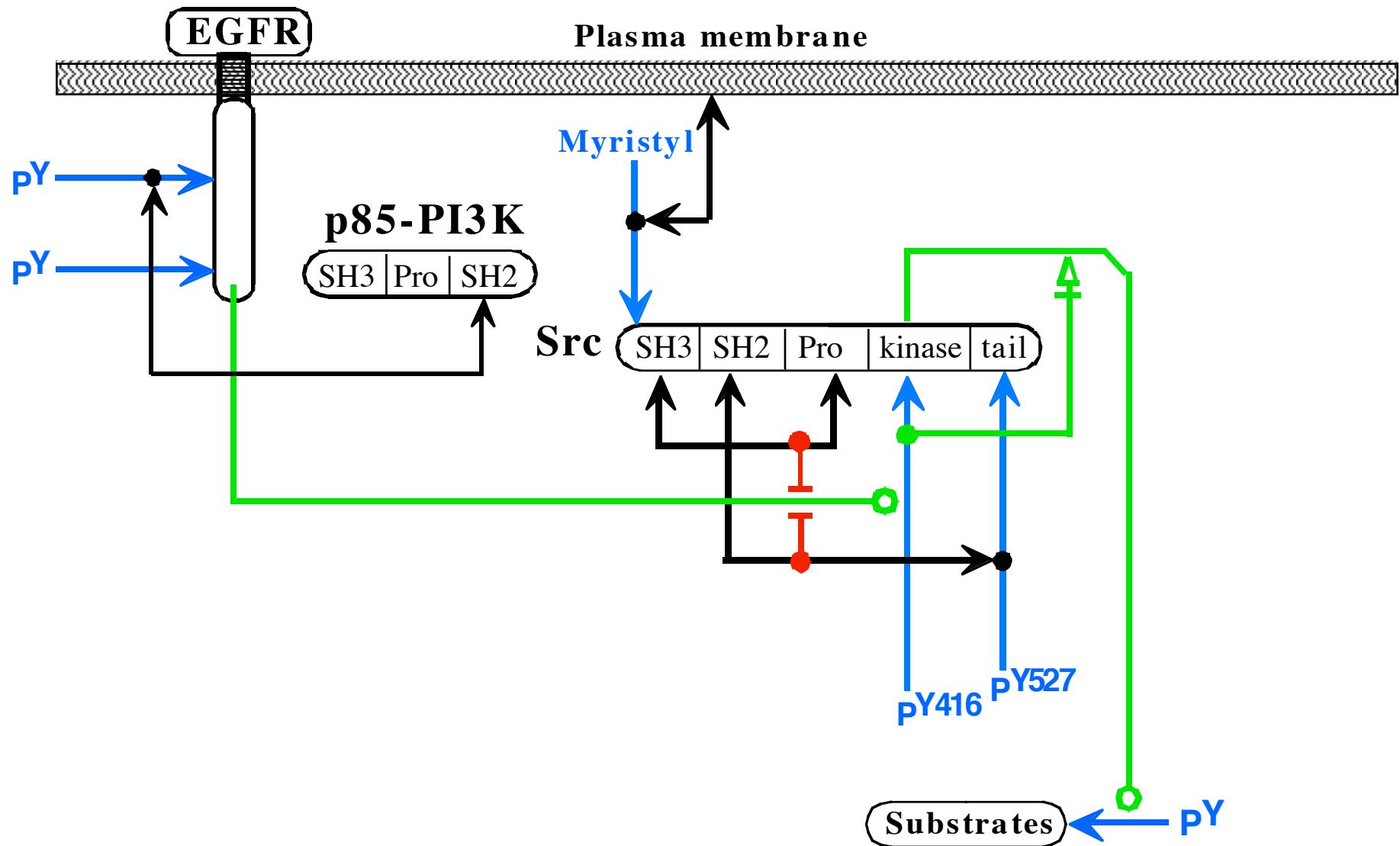
Activated (phosphorylated) EGFR could phosphorylate Tyr416



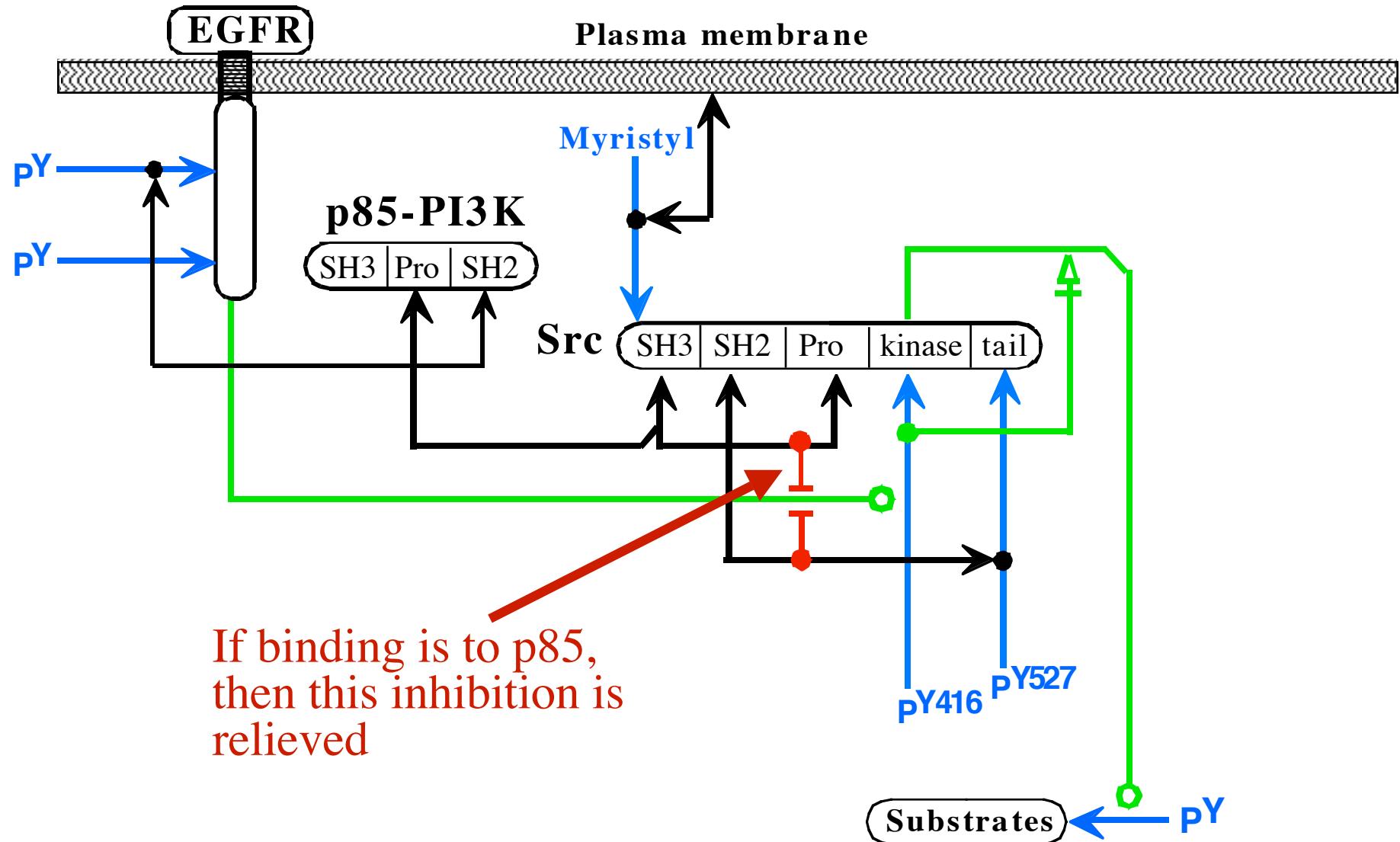
However, access to Tyr416 is blocked by the intra-molecular folding.



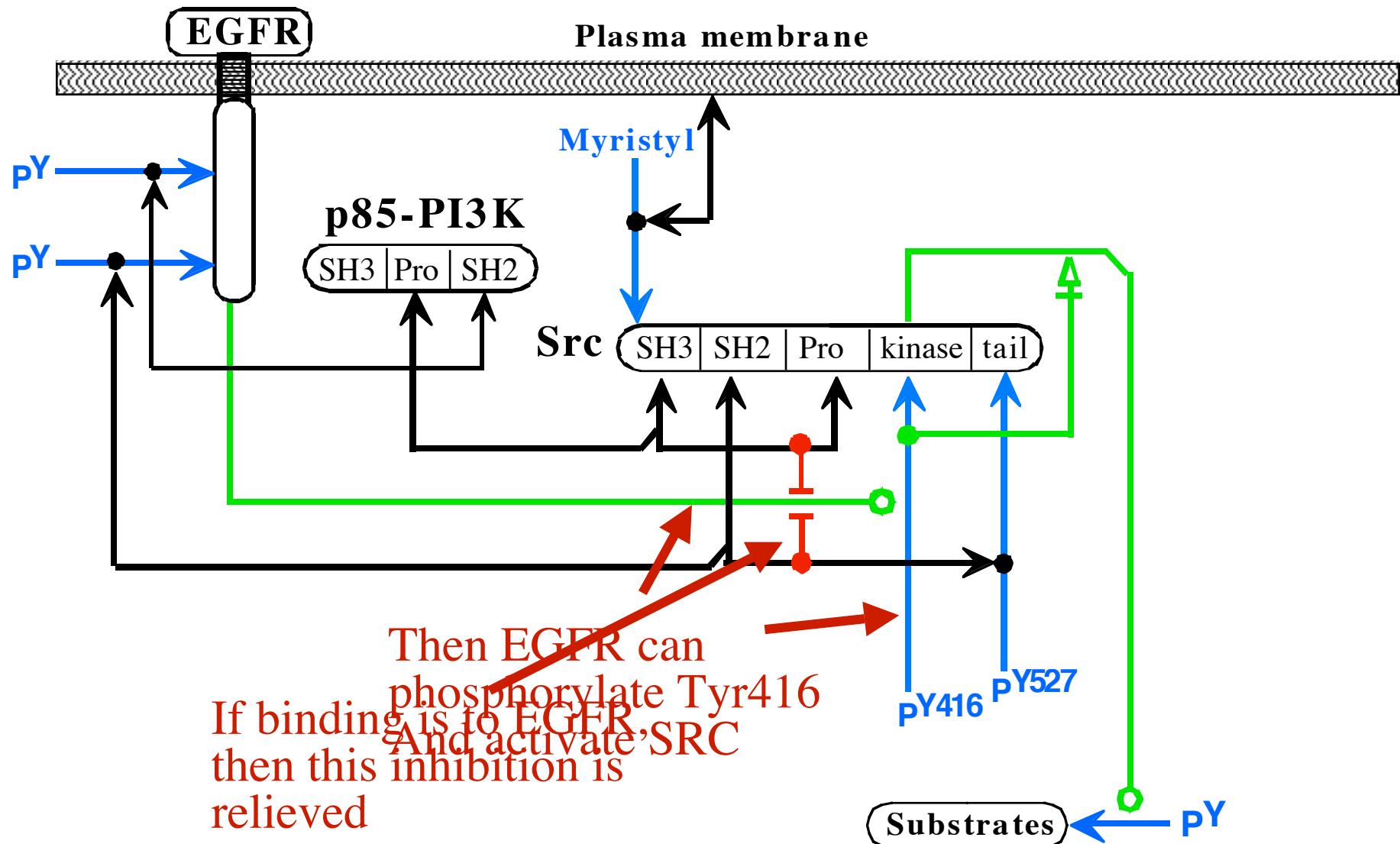
SRC activation:  
Through one of its phosphotyrosines, activated EGFR recruits p85.



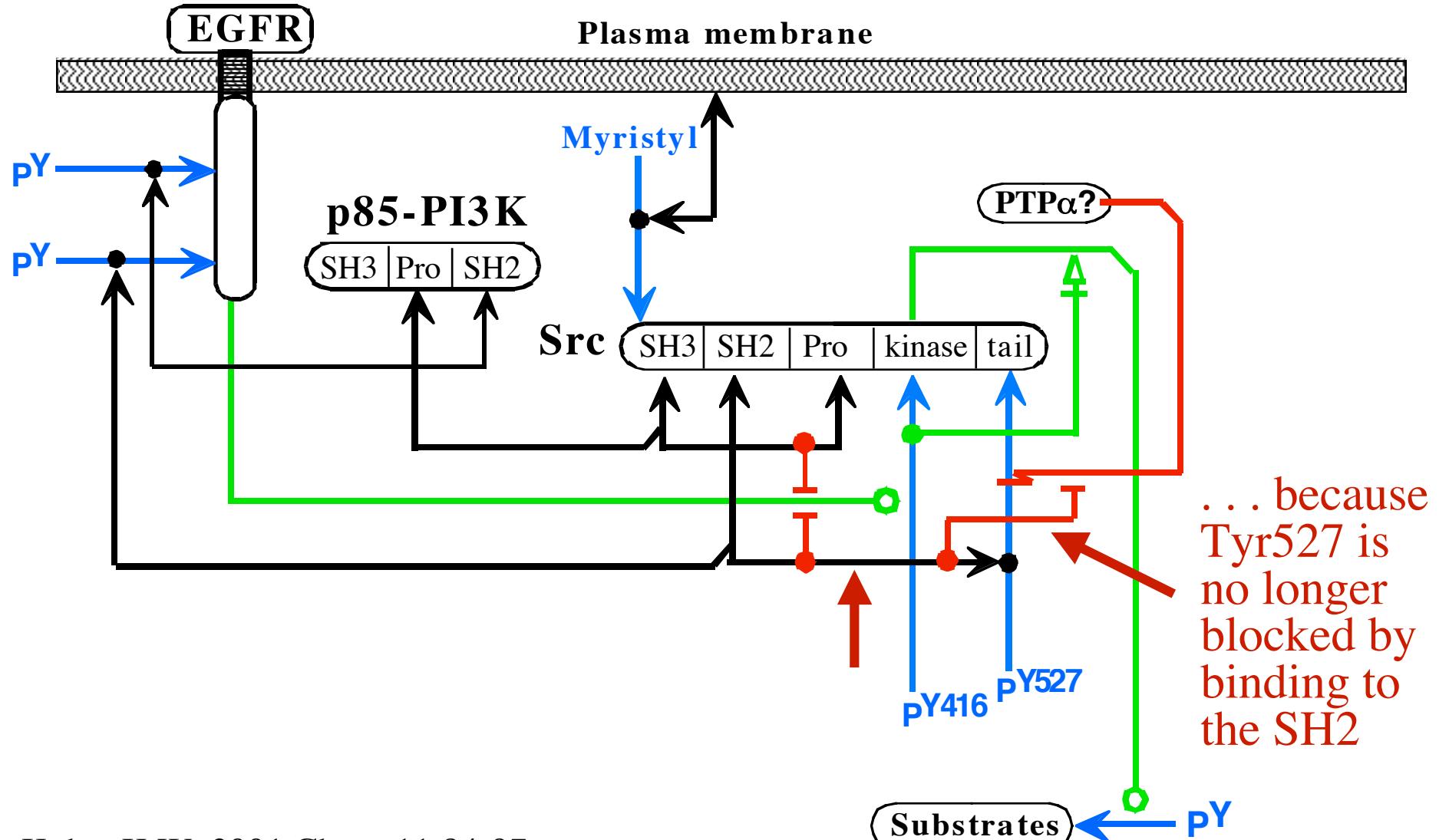
Pro domain of p85 competes with Pro of Src for binding to SH3 of Src



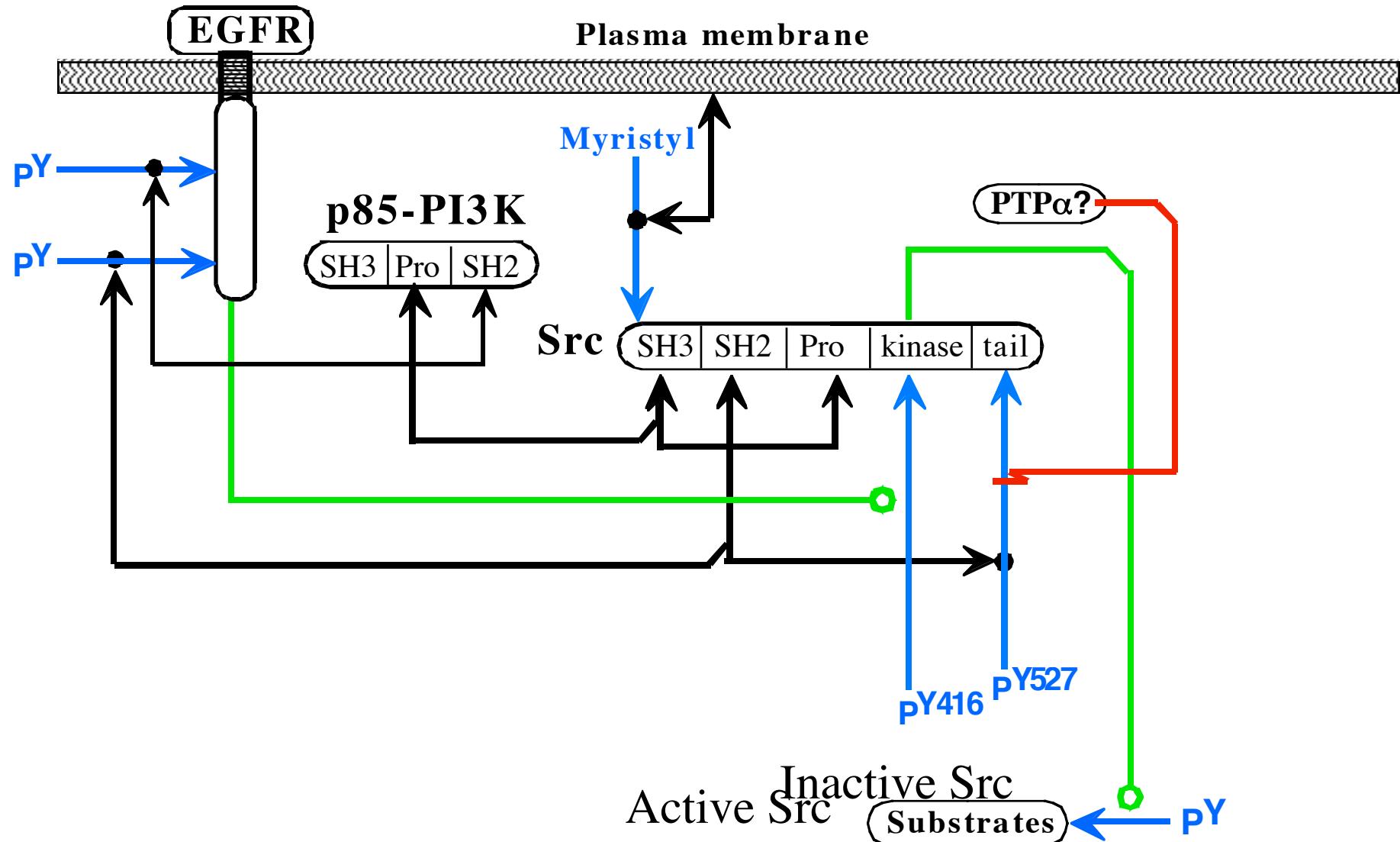
Phosphotyrosine of EGFR competes with P-Tyr527 of Src  
for binding to SH2 of Src

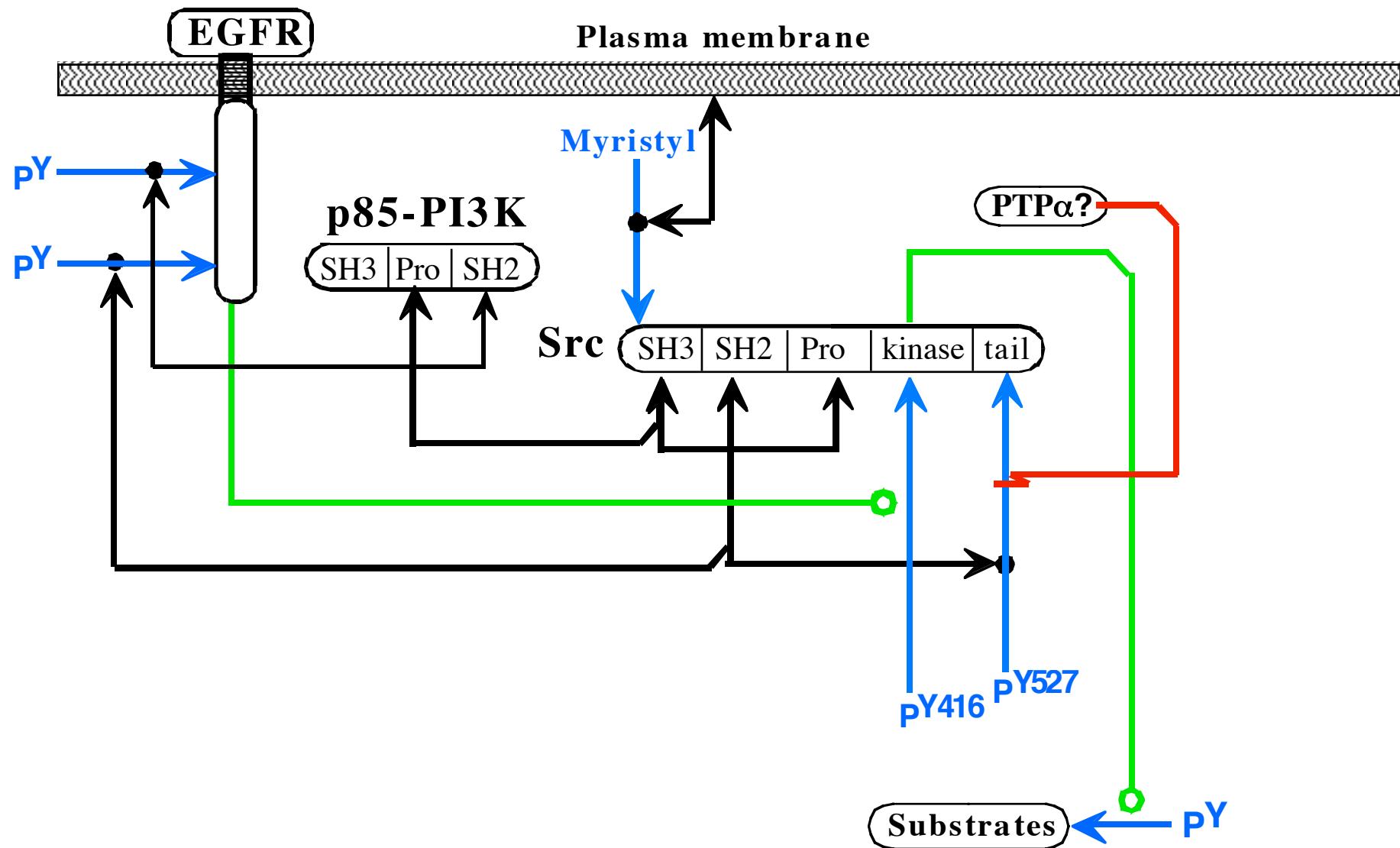


With Tyr527 gone, Src cannot refold and remains active even if it dissociates from the EGFR:p85 complex.  
 Then a phosphatase can remove Tyr527...  
 Thus multiple Src's can be activated by a single active EGFR -- *i.e.*, an amplification step.



# A dynamic animated map of Src activation by EGFR





## *Do MIMs Meet the Challenge?*

*Using specific lines and nodes, MIMs can depict the different types of reactions common in bioregulatory networks, and contingencies affecting such reactions.*

*MIMs can unambiguously and concisely represent intramolecular interactions.*

*Explicit MIMs contain sufficient detail to describe models suitable for simulation of biological networks*

*Heuristic MIMs summarize large sets of data about molecular interactions with different levels of detail; eMIMs can provide links to pertinent external information*

*MIMs are useful to represent the combinatorial complexity of biological networks.*



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