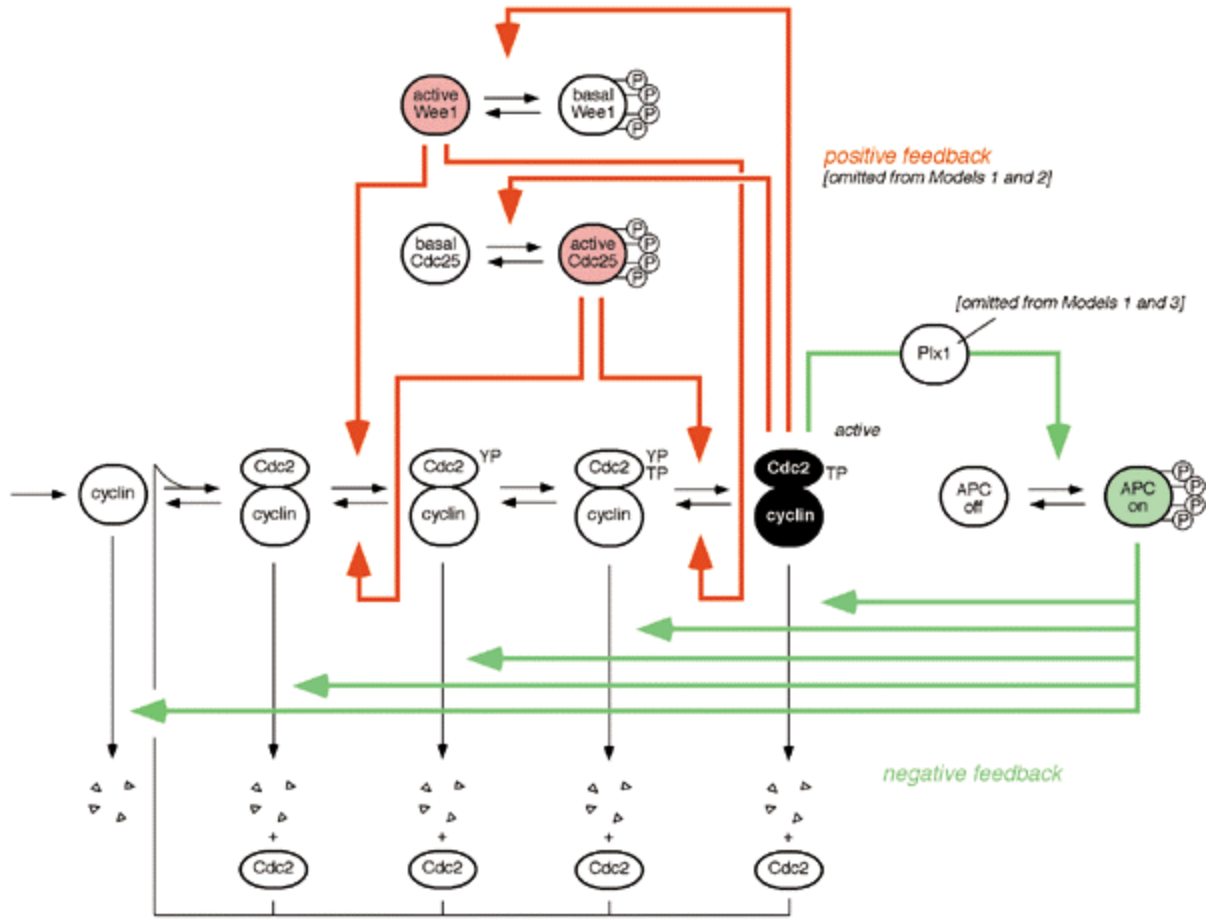


Pomerening, Sontag, and Ferrell  
Supplementary Information  
Figure 1



Pomerening, Sontag, and Ferrell  
 Supplementary Materials  
 Figure 3

### Supplementary Materials, Part III. Modeling the Cdc2/APC system

Here we present three simplified models for the Cdc2/APC system. In the first, Cdc2 activates the APC and the APC inactivates Cdc2, but there is no positive feedback. This model produces a stable steady state for any choice of parameters. In the second, Cdc2 activates the APC through the intermediacy of Plx1 (or some other protein), and the combination of this longer negative feedback loop and some assumed ultrasensitivity within the loop allows the model to yield limit cycle oscillations for some choices of parameters. The third model is like the first, but includes positive feedback in the activation of Cdc2 by cyclin. For some choices of parameters, this model produces the explosive limit cycle oscillations of a relaxation oscillator.

In each case, there must be some mechanism through which cyclin accumulation triggers the switch from an interphase-like state to an M-phase-like state. Here we assumed that the accumulation of cyclin drives the formation of a small amount of active Cdc2 even in interphase, because the basal activity of Cdc25 is non-zero. Alternatively, we could have assumed that the activity of the “inactive” Tyr-phosphorylated Cdc2-cyclin complex is non-zero.

A schematic view of the model system is shown in Figure 3, and Mathematica 2.2.2 code for each of the three models is appended below. For simplicity we have omitted some aspects of Cdc2 activation that would make the models more complicated without fundamentally altering their behavior (translational regulation of cyclin B1<sup>1</sup>; phosphorylation and dephosphorylation of Cdc2 at Thr 14<sup>2, 3</sup>; localization<sup>4</sup>).

### Model 1: The Cdc2/APC system as a simple negative feedback loop.

```
( *   First we define the model's parameters.
      Concentration units are nM; time units are min.
      ksynth defines the constant rate of cyclin
      synthesis, and kdest defines the rate constant for
      cyclin destruction. *)

      ksynth=1.2;
      kdest=0.005;

( *   ka is the association constant for Cdc2+cyclin.
      kd is the dissociation constant. kd/ka should be
      less than 1 nM *)

      ka=0.1;
      kd=0.001;

( *   kweel and kcdc25 are the rate constants for the
      inactivation and activation of Cdc2/cyclin by Wee1
      and Cdc25.  If Km = 1 uM and kcat = 1
      reaction/min, then kcat/Km would be 1 uM-1 min-1
      or 0.001 nM-1 min-1

      The bigger the value for 'factor', the stronger
      the feedback.  If factor = 1 then the mitotic and
      interphase forms of Cdc25 and Wee1 do not differ
      in activity, and so there is no feedback from Cdc2
      to Cdc25 and Wee1.  If it's 10, the feedback is
      strong enough to give sustained,
      realistic-looking oscillations.

      *)

      factor=1;
      kweel=0.1;
      kweelbasal=kweel/factor;
      kcdc25=0.1;
      kcdc25basal=kcdc25/factor;

( *   cdc2tot, cdc25tot, weeltot, and apctot are total
      concentrations in nM *)

      cdc2tot=100;
      cdc25tot=15;
      weeltot=15;
      apctot=50;

( *   We'll assume that the dependence of Cdc25 and Wee1
      on Cdc2 activity is described by Hill functions
```

with Hill coefficients of nweel and ncdc25 and EC50's (in nM) of ec50weel and ec50cdc25. \*)

```
nweel=4;
ncdc25=4;
napc=5;
```

```
ec50weel=25;
ec50cdc25=25;
ec50apc=30;
```

(\* Define the rate constants for turning Cdc25 on (by Cdc2) and off (by ?) and for turning Weel off (by Cdc2) and on (by ?) \*)

```
kcdc25on=0.8;
kcdc25off=0.08;
```

```
kapcon=0.8;
kapcoff=0.08;
```

```
kweelon=0.8;
kweeloff=0.08;
```

(\* And define CAK activity and PP2C activity in nM-1 min-1 \*)

```
kcak=0.8;
kpp2c=0.008;
```

(\* Then write the differential equations. Note there are five forms of Cdc2:

```
cdc2 (monomer) = cdctot - all the complexes
cdc2cyclin (inactive, non-phos complex)
cdc2cyclinyp (inactive, Y15-phosphorylated)
cdc2cyclinyptp (inactive, Y15- and T161-
phosphorylated)
cdc2cyclintp (active, T161-phosphorylated)
```

Five forms of cyclin:

```
cyclin (monomer)
cdc2cyclin (inactive, non-phos complex)
cdc2cyclinyp (inactive, Y15-phosphorylated)
cdc2cyclinyptp (inactive, Y15- and T161-
phosphorylated)
cdc2cyclintp (active, T161-phosphorylated)
```

Two forms of Cdc25:

```
cdc25act
cdc25tot
```

Two forms of Weel:

```
weelact
weeltot
```

\*)

(\* And finally, define the time range over which we want to watch the system, in min \*)

```
maxtime=400;
```

```
sol =
NDSolve[{

cyclin'[t] ==
ksynth-kdest*apcstar[t]*cyclin[t]-
ka*cdc2[t]*cyclin[t]+kd*cdc2cyclin[t],

cdc2cyclin'[t] ==
ka*cdc2[t]*cyclin[t]-kd*cdc2cyclin[t]-
kdest*apcstar[t]*cdc2cyclin[t]-
kweel*weelact[t]*cdc2cyclin[t]-kweelbasal*(weeltot-
weelact[t])*cdc2cyclin[t]+kcdc25*cdc25act[t]*cdc2cycliny
p[t]+kcdc25basal*(cdc25tot-
cdc25act[t])*cdc2cycliny[p],

cdc2cycliny[p]'[t] ==
kweel*weelact[t]*cdc2cyclin[t]+kweelbasal*(weeltot-
weelact[t])*cdc2cyclin[t]-
kcdc25*cdc25act[t]*cdc2cycliny[p]-
kcdc25basal*(cdc25tot-cdc25act[t])*cdc2cycliny[p]-
kcak*cdc2cycliny[p]+kpp2c*cdc2cycliny[ptp]-
kdest*apcstar[t]*cdc2cycliny[p],

cdc2cycliny[ptp]'[t] == kcak*cdc2cycliny[p]-
kpp2c*cdc2cycliny[ptp]-
kcdc25*cdc25act[t]*cdc2cycliny[ptp]-
kcdc25basal*(cdc25tot-
cdc25act[t])*cdc2cycliny[ptp]+kweel*weelact[t]*cdc2cyc
lintp[t]+kweelbasal*(weeltot-
weelact[t])*cdc2cyclintp[t]-
kdest*apcstar[t]*cdc2cycliny[ptp],

cdc2cyclintp'[t] ==
kcdc25*cdc25act[t]*cdc2cycliny[ptp]+kcdc25basal*(cdc25
tot-cdc25act[t])*cdc2cycliny[ptp]-
kweel*weelact[t]*cdc2cyclintp[t]-kweelbasal*(weeltot-
weelact[t])*cdc2cyclintp[t]-
kdest*apcstar[t]*cdc2cyclintp[t],

cdc2'[t] ==
kdest*apcstar[t]*(cdc2cyclin[t]+cdc2cycliny[p]+cdc2cyc
```

```

linyptp[t]+cdc2cyclintp[t])+kd*cdc2cyclin[t]-
ka*cdc2[t]*cyclin[t],

cdc25act'[t] ==
kcdc25on*(cdc2cyclintp[t]^ncdc25/(ec50cdc25^ncdc25+cdc2
cyclintp[t]^ncdc25))*(cdc25tot-cdc25act[t])-
kcdc25off*cdc25act[t],

weelact'[t] == -
kweeloff*(cdc2cyclintp[t]^nweel/(ec50weel^nweel+cdc2cyc
lintp[t]^nweel))*(weelact[t])+kweelon*(weeltot-
weelact[t]),

apcstar'[t] ==
kapcon*(cdc2cyclintp[t]^napc/(ec50apc^napc+cdc2cyclintp
[t]^napc))*(apctot-apcstar[t])-kapcoff*apcstar[t],

apc0'[t] == -
kapcon*(cdc2cyclintp[t]^napc/(ec50apc^napc+cdc2cyclintp
[t]^napc))*(apctot-apcstar[t])+ kapcoff*apcstar[t],

apcstar[t] == apctot-apc0[t],

apc0[0] == 50,
apcstar[0] == 0,

cdc2[t] == cdc2tot-
(cdc2cyclin[t]+cdc2cyclinyptp[t]+cdc2cyclinyptp[t]+cdc2cyc
clintp[t]),

cyclin[0] == 0,
cdc2cyclin[0] == 0,
cdc2cyclinyptp[0] == 0,
cdc2cyclinyptp[0] == 0,
cdc2cyclintp[0] == 0,
cdc2[0] == 100,
cdc25act[0] == 0,
weelact[0] == 0

},

{cyclin,cdc2cyclin,cdc2cyclinyptp,cdc2cyclinyptp,cdc2cycl
intp,cdc2,cdc25act,weelact,apc0,apcstar},
{t,0,maxtime},MaxSteps->30000];

Plot[{cdc2cyclintp[t] /. sol},
{t,0,maxtime},PlotRange->{0,40}]

```

**Model 2: The Cdc2/APC system as a three-component negative feedback loop with ultrasensitivity.** The only changes to the model (highlighted below in red) are the

inclusion of Plx1, which is assumed to be activated by Cdc2 in an ultrasensitive fashion, and to then activate the APC. The role of Plx1 in activating Cdc25 (and other possible important roles of Plx1) are neglected for simplicity.

```
(* First we define the model's parameters.
Concentration units are nM; time units are min.
ksynth defines the constant rate of cyclin
synthesis, and kdest defines the rate constant for
cyclin destruction. *)

ksynth=1.2;
kdest=0.005;

(* ka is the association constant for Cdc2+cyclin.
kd is the dissociation constant. kd/ka should be
less than 1 nM *)

ka=0.1;
kd=0.001;

(* kweel and kcdc25 are the rate constants for the
inactivation and activation of Cdc2/cyclin by Wee1
and Cdc25. If Km = 1 uM and kcat = 1
reaction/min, then kcat/Km would be 1 uM-1 min-1
or 0.001 nM-1 min-1

The bigger the value for 'factor', the stronger
the feedback. If factor = 1 then the mitotic and
interphase forms of Cdc25 and Wee1 do not differ
in activity, and so there is no feedback from Cdc2
to Cdc25 and Wee1. If it's 10, the feedback is
strong enough to give sustained,
realistic-looking oscillations.

*)

factor=1;
kweel=0.1;
kweelbasal=kweel/factor;
kcdc25=0.1;
kcdc25basal=kcdc25/factor;

(* cdc2tot, cdc25tot, weeltot, and apctot are total
concentrations in nM *)

cdc2tot=100;
cdc25tot=15;
weeltot=15;
apctot=50;
plxtot=50;
```

```

(* We'll assume that the dependence of Cdc25, Wee1,
and Plx1 on Cdc2 activity is described by Hill
functions with Hill coefficients of nweel, ncdc25,
and nplx and EC50's (in nM) of ec50weel,
ec50cdc25, and ec50plx. *)

nweel=4;
ncdc25=4;
nadc=5;
nplx=5;

ec50weel=25;
ec50cdc25=25;
ec50adc=30;
ec50plx=30;

(* Define the rate constants for turning Cdc25 and
Plx1 on (by Cdc2) and off (by ?) and for turning
Wee1 off (by Cdc2) and on (by ?) *)

kcdc25on=0.8;
kcdc25off=0.08;

kapcon=0.8;
kapcoff=0.08;

kplxon=0.8;
kplxoff=0.08;

kweelon=0.8;
kweeloff=0.08;

(* And define CAK activity and PP2C activity in nM-1
min-1 *)

kcak=0.8;
kpp2c=0.008;

(* Then write the differential equations. Note there
are five forms of Cdc2:

cdc2 (monomer) = cdctot - all the complexes
cdc2cyclin (inactive, non-phos complex)
cdc2cyclinyp (inactive, Y15-phosphorylated)
cdc2cyclinyp (inactive, Y15- and T161-
phosphorylated)
cdc2cyclintp (active, T161-phosphorylated)

Five forms of cyclin:

cyclin (monomer)
cdc2cyclin (inactive, non-phos complex)
cdc2cyclinyp (inactive, Y15-phosphorylated)

```

```
cdc2cyclinyptp (inactive, Y15- and T161-
phosphorylated)
cdc2cyclintp (active, T161-phosphorylated)
```

Two forms of Cdc25:

```
cdc25act
cdc25tot
```

Two forms of Wee1:

```
weelact
weeltot
```

And two forms of Plx1:

```
plxact
plxtot
```

\*)

(\* And finally, define the time range over which we want to watch the system, in min \*)

```
maxtime=400;
```

```
sol =
NDSolve[{

cyclin'[t] ==
ksynth-kdest*apcstar[t]*cyclin[t]-
ka*cdc2[t]*cyclin[t]+kd*cdc2cyclin[t],

cdc2cyclin'[t] ==
ka*cdc2[t]*cyclin[t]-kd*cdc2cyclin[t]-
kdest*apcstar[t]*cdc2cyclin[t]-
kweel*weelact[t]*cdc2cyclin[t]-kweelbasal*(weeltot-
weelact[t])*cdc2cyclin[t]+kcdc25*cdc25act[t]*cdc2cycliny
p[t]+kcdc25basal*(cdc25tot-
cdc25act[t])*cdc2cyclinyp[t],

cdc2cyclinyp'[t] ==
kweel*weelact[t]*cdc2cyclin[t]+kweelbasal*(weeltot-
weelact[t])*cdc2cyclin[t]-
kcdc25*cdc25act[t]*cdc2cyclinyp[t]-
kcdc25basal*(cdc25tot-cdc25act[t])*cdc2cyclinyp[t]-
kcak*cdc2cyclinyp[t]+kpp2c*cdc2cyclinyptp[t]-
kdest*apcstar[t]*cdc2cyclinyp[t],

cdc2cyclinyptp'[t] == kcak*cdc2cyclinyp[t]-
kpp2c*cdc2cyclinyptp[t]-
kcdc25*cdc25act[t]*cdc2cyclinyptp[t]-
kcdc25basal*(cdc25tot-
```

```

cdc25act[t])*cdc2cyclinyptp[t]+kweel*weelact[t]*cdc2cyc
lintp[t]+kweelbasal*(weeltot-
weelact[t])*cdc2cyclintp[t]-
kdest*apcstar[t]*cdc2cyclinyptp[t],

cdc2cyclintp'[t] ==
kcdc25*cdc25act[t]*cdc2cyclinyptp[t]+kcdc25basal*(cdc25
tot-cdc25act[t])*cdc2cyclinyptp[t]-
kweel*weelact[t]*cdc2cyclintp[t]-kweelbasal*(weeltot-
weelact[t])*cdc2cyclintp[t]-
kdest*apcstar[t]*cdc2cyclintp[t],

cdc2'[t] ==
kdest*apcstar[t]*(cdc2cyclin[t]+cdc2cyclinyp[t]+cdc2cyc
linyptp[t]+cdc2cyclintp[t])+kd*cdc2cyclin[t]-
ka*cdc2[t]*cyclin[t],

cdc25act'[t] ==
kcdc25on*(cdc2cyclintp[t]^ncdc25/(ec50cdc25^ncdc25+cdc2
cyclintp[t]^ncdc25))*(cdc25tot-cdc25act[t])-
kcdc25off*cdc25act[t],

weelact'[t] == -
kweeloff*(cdc2cyclintp[t]^nweel/(ec50weel^nweel+cdc2cyc
lintp[t]^nweel))*(weelact[t]) + kweelon*(weeltot-
weelact[t]),

plxact'[t] ==
kplxon*(cdc2cyclintp[t]^nplx/(ec50plx^nplx+cdc2cyclintp[t]^nplx))
*(plxtot-plxact[t])-kplxoff*plxact[t],

apcstar'[t] ==
kapcon*(plxact[t]^napc/(ec50apc^napc+plxact[t]^napc))*(
apctot-apcstar[t])-kapcoff*apcstar[t],

apc0'[t] == -
kapcon*(plxact[t]^napc/(ec50apc^napc+plxact[t]^napc))*(
apctot-apcstar[t])+kapcoff*apcstar[t],

apcstar[t] == apctot-apc0[t],

apc0[0] == 50,
apcstar[0] == 0,

cdc2[t] == cdc2tot-
(cdc2cyclin[t]+cdc2cyclinyp[t]+cdc2cyclinyptp[t]+cdc2cyc
clintp[t]),

cyclin[0] == 0,
cdc2cyclin[0] == 0,
cdc2cyclinyp[0] == 0,
cdc2cyclinyptp[0] == 0,
cdc2cyclintp[0] == 0,
cdc2[0] == 100,

```

```

cdc25act[0] == 0,
plxact[0] == 0,
weelact[0] == 0

},

{cyclin,cdc2cyclin,cdc2cyclinyp,cdc2cyclinyptp,cdc2cycl
intp,cdc2,cdc25act,weelact,apc0,apcstar, plxact},
{t,0,maxtime},MaxSteps->30000];

Plot[{cdc2cyclintp[t] /. sol},
{t,0,maxtime},PlotRange->{0,40}]

```

**Model 3: The Cdc2/APC system as a negative feedback loop with a bistable (positive feedback) trigger.** The Mathematica code for this model is the same as that for Model 1, except that the parameter “factor”, which effectively determines the strength of the Cdc2 -> Wee1 and Cdc2 -> Cdc25 positive feedback loops, is taken to be 20.

## References

1. Groisman, I., Jung, M.-Y., Sarkissian, M., Cao, Q. & Richter, J.D. *Cell* **109**, 473-483 (2002).
2. Mueller, P.R., Coleman, T.R., Kumagai, A. & Dunphy, W.G. *Science* **270**, 86-90 (1995).
3. Liu, F., Stanton, J.J., Wu, Z. & Piwnica-Worms, H. *Mol. Cell. Biol.* **17**, 571-583 (1997).
4. Jin, P., Hardy, S. & Morgan, D.O. *J. Cell Biol.* **141**, 875-885 (1998).