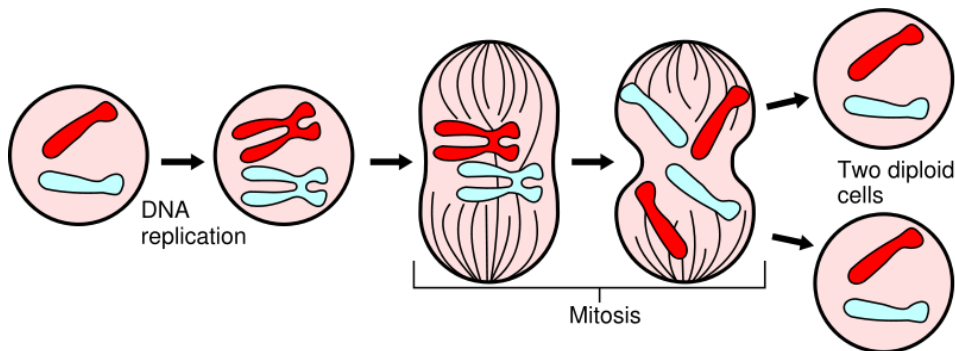


# V4 Cell Cycle

The cell cycle, or cell-division cycle, is the series of events that takes place in a cell leading to its division and duplication (replication).

In cells without a nucleus (prokaryotes), the cell cycle occurs via a process termed **binary fission**.



In cells with a nucleus (eukaryotes), the cell cycle can be divided in 2 brief periods:

**interphase**—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and

the **mitosis** (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells".

# Phases of the eukaryotic cell cycle

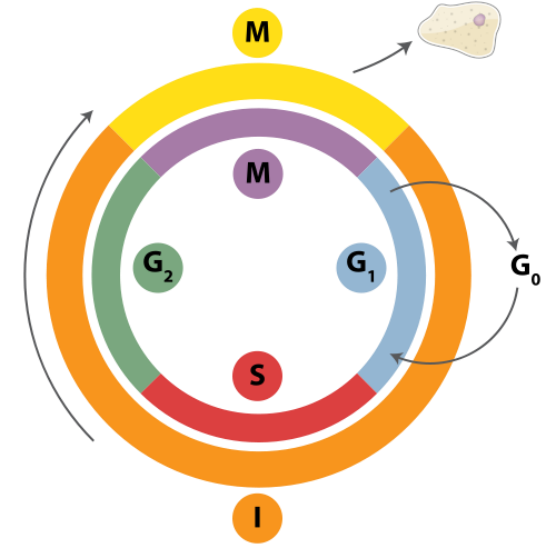
The cell cycle consists of **4 distinct phases**:

- G<sub>1</sub> phase,
- S phase (synthesis),
- G<sub>2</sub> phase
- and M phase (mitosis).

Interphase: combines G<sub>1</sub>, S, and G<sub>2</sub>

Activation of each phase is dependent on the proper progression and completion of the previous one.

Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called **G<sub>0</sub> phase**.



Schematic of the cell cycle.

Outer ring:

I = Interphase, M = Mitosis;

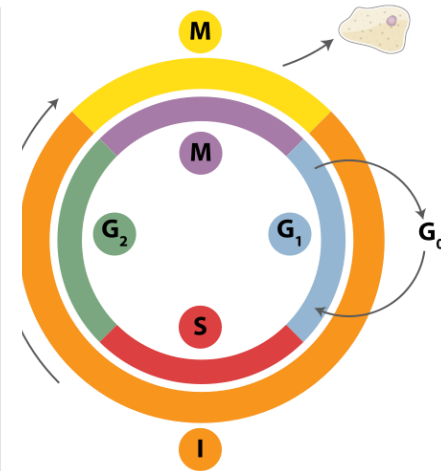
Inner ring:

M = Mitosis, G<sub>1</sub> = Gap 1, G<sub>2</sub> = Gap 2, S = Synthesis.

[www.wikipedia.org](http://www.wikipedia.org)

# Activity during 4 phases

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	$G_0$	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	$G_1$	Cells increase in size in Gap 1. The $G_1$ checkpoint control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	S	DNA replication occurs during this phase.
	Gap 2	$G_2$	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The $G_2$ checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	M	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis ( <i>Metaphase Checkpoint</i> ) ensures that the cell is ready to complete cell division.



**M phase** itself is composed of 2 tightly coupled processes:

- **mitosis**, in which the cell's chromosomes are divided between the two daughter cells, and
- **cytokinesis**, in which the cell's cytoplasm divides in half forming distinct cells.

[www.wikipedia.org](http://www.wikipedia.org)

# Accidental discovery of cyclins

Cell, Vol. 33, 389-396, June 1983, Copyright © 1983 by MIT

## Cyclin: A Protein Specified by Maternal mRNA in Sea Urchin Eggs That Is Destroyed at Each Cleavage Division

Tom Evans,\* Eric T. Rosenthal,<sup>†</sup>  
Jim Youngblom,<sup>‡</sup> Dan Distel,<sup>§</sup> and  
Tim Hunt<sup>||</sup>

Marine Biological Laboratory  
Woods Hole, Massachusetts 02543

*It is difficult to believe that the behavior of the cyclins is not connected with processes involved in cell division, but at this stage we have no direct evidence that it is. ...*

*Unfortunately, we have no direct evidence as to the physiological role of cyclin, but one of its more plausible roles is promoting either directly or indirectly the breakdown of the nuclear envelope ...*

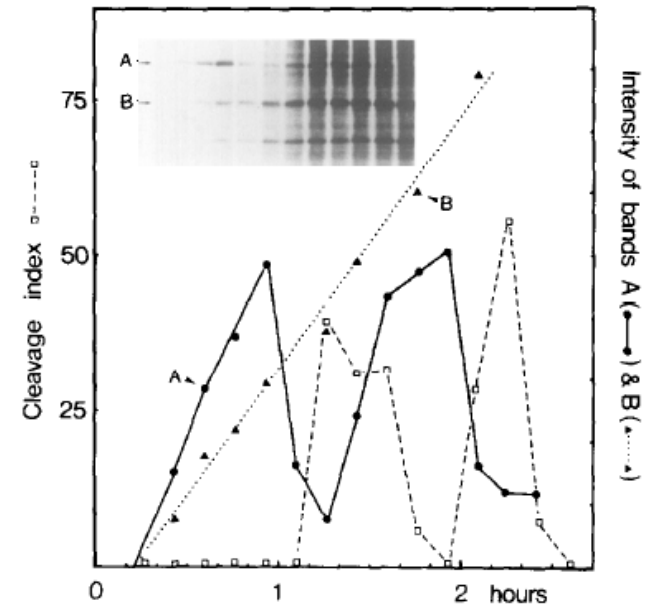


Figure 2. Correlation of the Level of Cyclin with the Cell Division Cycle  
A suspension of eggs was fertilized, and after 6 min, <sup>35</sup>S-methionine was added to a final concentration of 25  $\mu$ Ci/ml. Samples were taken for analysis on gels at 10 min intervals, starting at 16 min after fertilization. Samples were taken 20-30 sec later into 1% glutaraldehyde in calcium-free artificial seawater for later microscopic examination; the cleavage index is shown thus: □---□. The autoradiograph shown as an inset was scanned to yield the data plotted thus: cyclin, ●—●; protein B, ▲····▲.

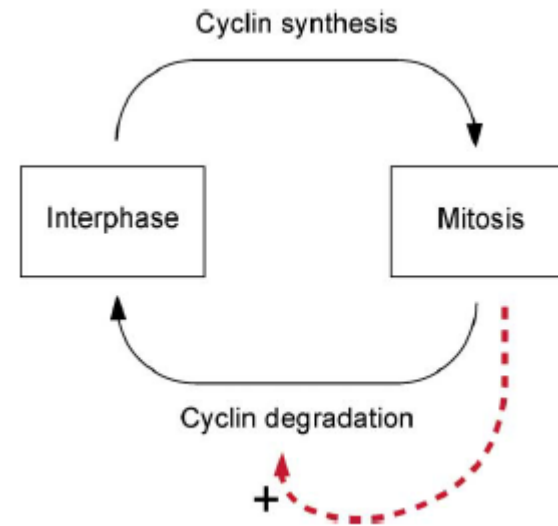
# Simplest model for cell cycle

Cell, Vol. 116, 221–234, January 23, 2004, Copyright ©2004 by Cell Press

## Recycling the Cell Cycle: Cyclins Revisited

**Andrew W. Murray\***

Department of Molecular and Cellular Biology  
Biological Laboratories  
Harvard University  
Cambridge, Massachusetts 02138



Cyclin's discovery led to a model of the autonomous oscillator that drove the cell cycle of early embryonic cells.

Murray AW, Cell 116, 221-234 (2004)

# Who regulates the cell cycle?

The discovery of cyclin was one of 3 strands of work that came together to produce the first working model of the cell cycle oscillator.

Nurse *et al.* identified a network of genes that controlled entry into mitosis. Its key component is the protein kinase **Cdk1**.

Masui and Smith identified **maturation-promoting factor** (MPF), a biochemical activity that induces meiosis and mitosis.

Lohka purified MPF. Its two subunits turned out to be Cdk1 and cyclin B.

Later work showed that

- different cyclin-Cdk complexes are activated at different points in the cell cycle,
- cyclins must be destroyed before cells can escape from mitosis, and that
- mitotic cyclins were destroyed by ubiquitin-mediated proteolysis

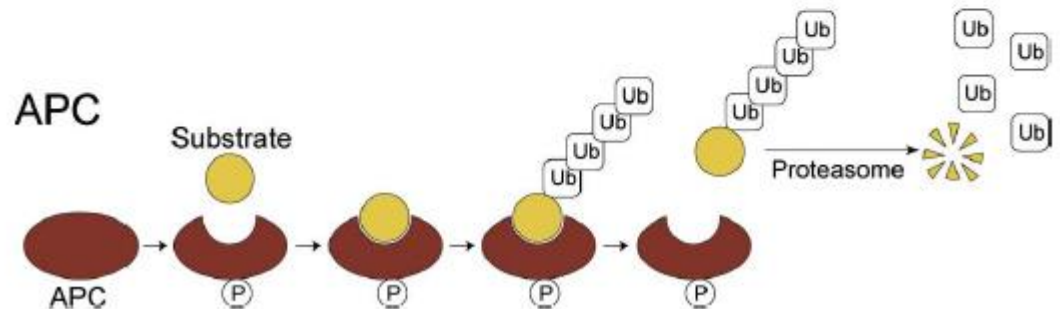
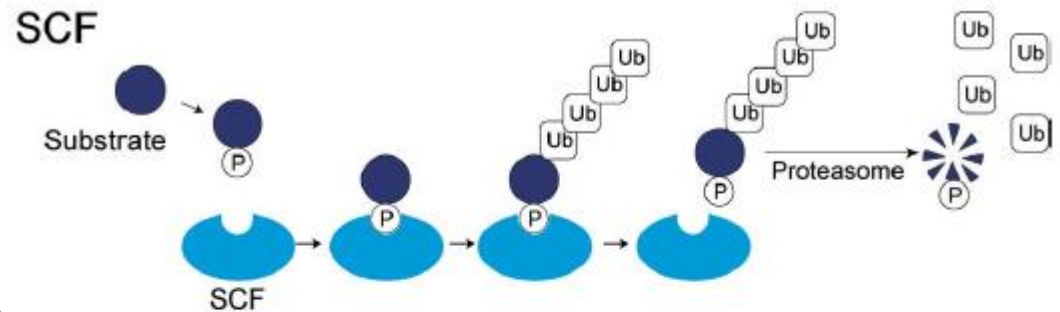
Murray AW, Cell 116, 221-234 (2004)

# How do cyclins die?

The obvious questions for cyclin were  
how is it degraded, by whom, and  
how is its degradation regulated?

All known cyclins are targeted to the  
proteasome by the addition of a  
chain of ubiquitins.

G1 cyclins are ubiquitinated by the  
**SCF complex**,  
whereas mitotic cyclins are  
ubiquitinated by the **anaphase-  
promoting complex (APC)**.



Murray AW, Cell 116, 221-234 (2004)

# Regulation of the eukaryotic cell cycle

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division.

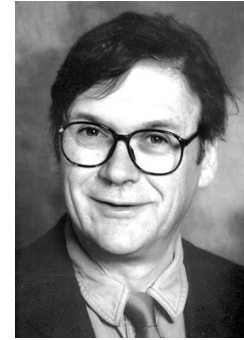
The molecular events that control the cell cycle are ordered and directional.

Each process occurs in a sequential fashion.

It is impossible to "reverse" the cycle.



Leland Hartwell



Tim Hunt



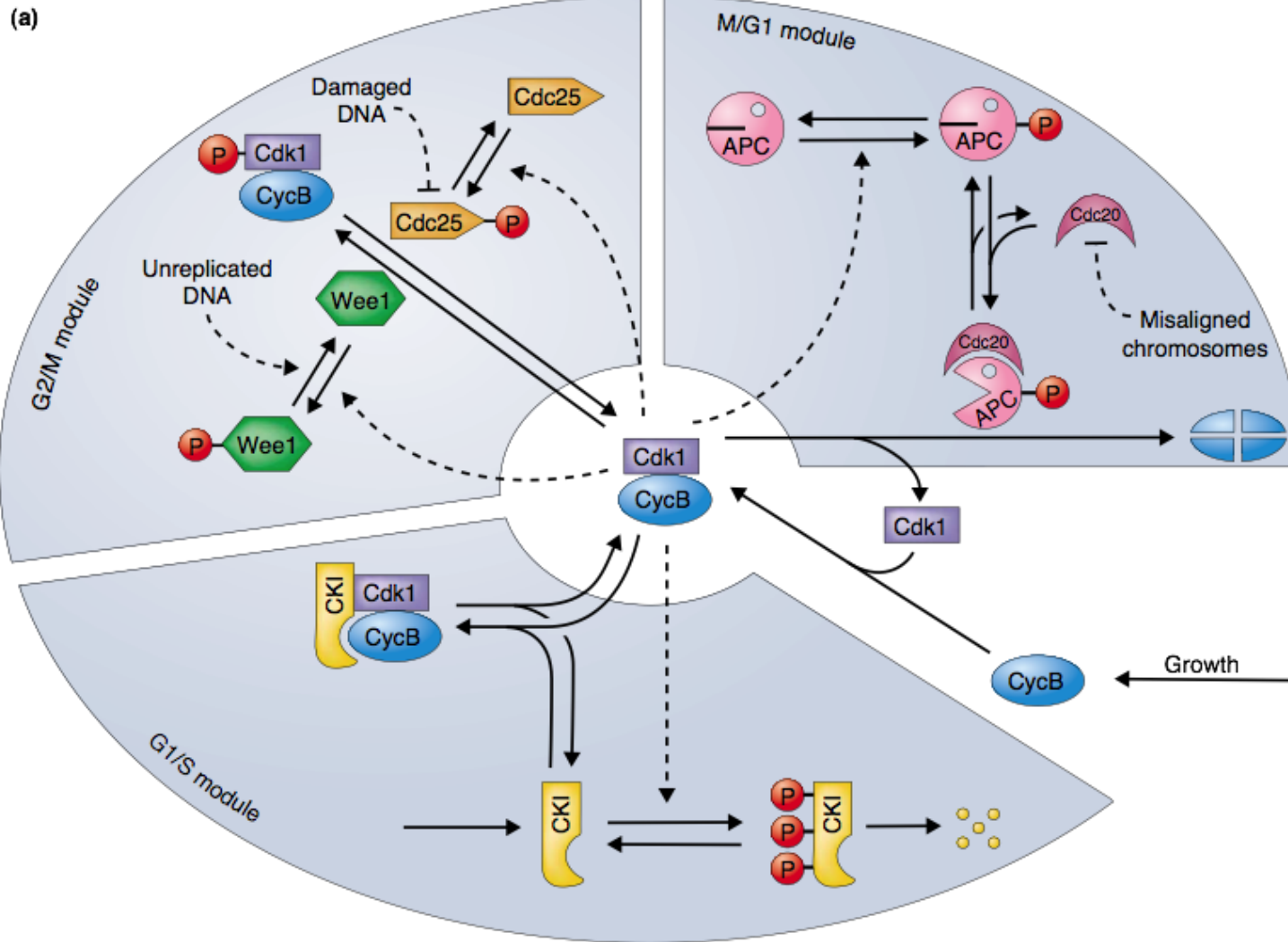
Paul Nurse

*Noble Prize in Physiology/Medicine 2001  
„for their discoveries of key regulators of  
the cell cycle“*

Two key classes of regulatory molecules, **cyclins** and **cyclin-dependent kinases (CDKs)**, determine a cell's progress through the cell cycle.



# Cell cycle control model

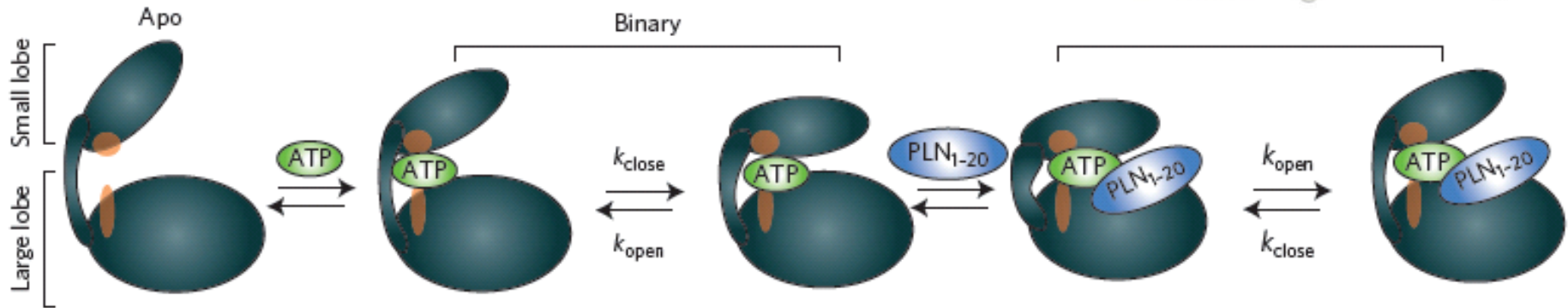
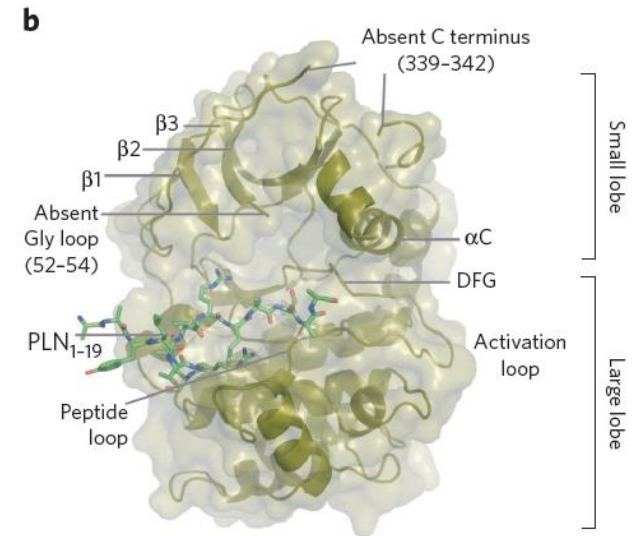
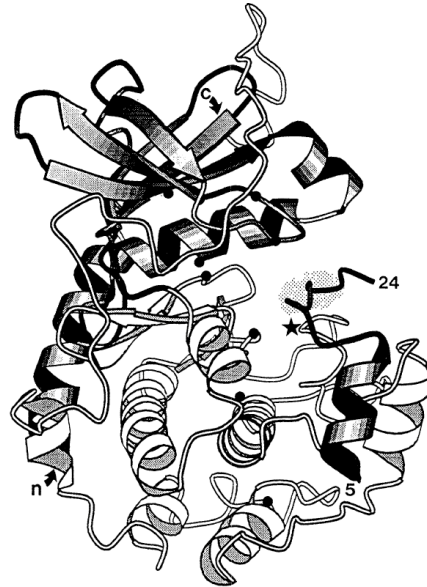


Tyson et al, *Curr. Op. Cell Biol.* **15** (2003) 221

# protein kinase A: a model system for phosphate transfer



Susan S. Taylor  
UC San Diego

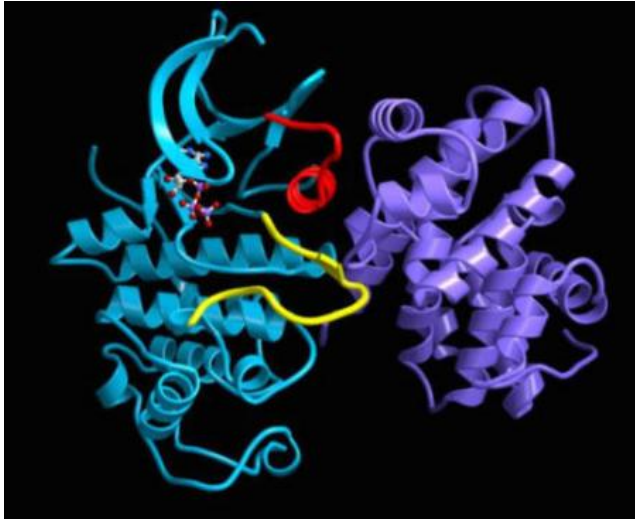


**Figure 5 | Model for the mechanism of the formation of a catalytically competent ternary complex.** The apo form contains the C-spine residues (red), which are disengaged from the two lobes. Nucleotide binding completes the C-spine architecture and induces the conformational changes throughout the enzyme. The conformational fluctuations (opening and closing) present in the ternary complex limit the rate of catalysis.

Masterson et al. *Nat Chem Biol.* 6, 825 (2010)

Taylor et al. *Phil Trans R.Soc. B* (1993)10

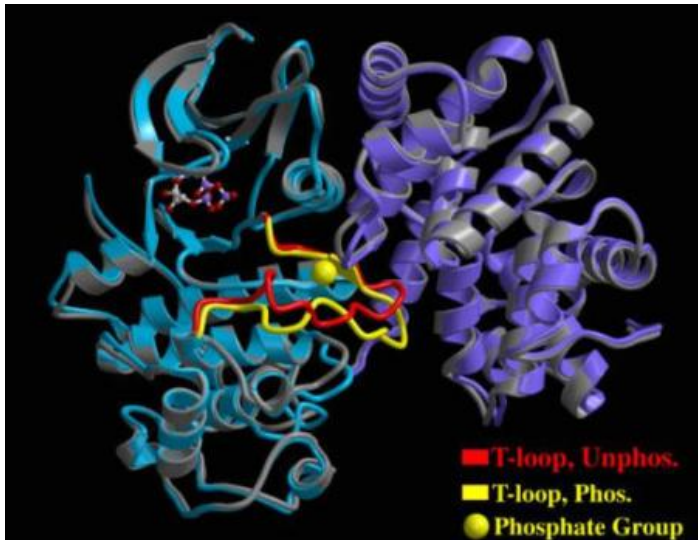
# Cyclin – cdk2 complex crystal structure



Cyclin A – cdk 2  
complex  
red: PSTAIRE motif  
yellow: activation loop



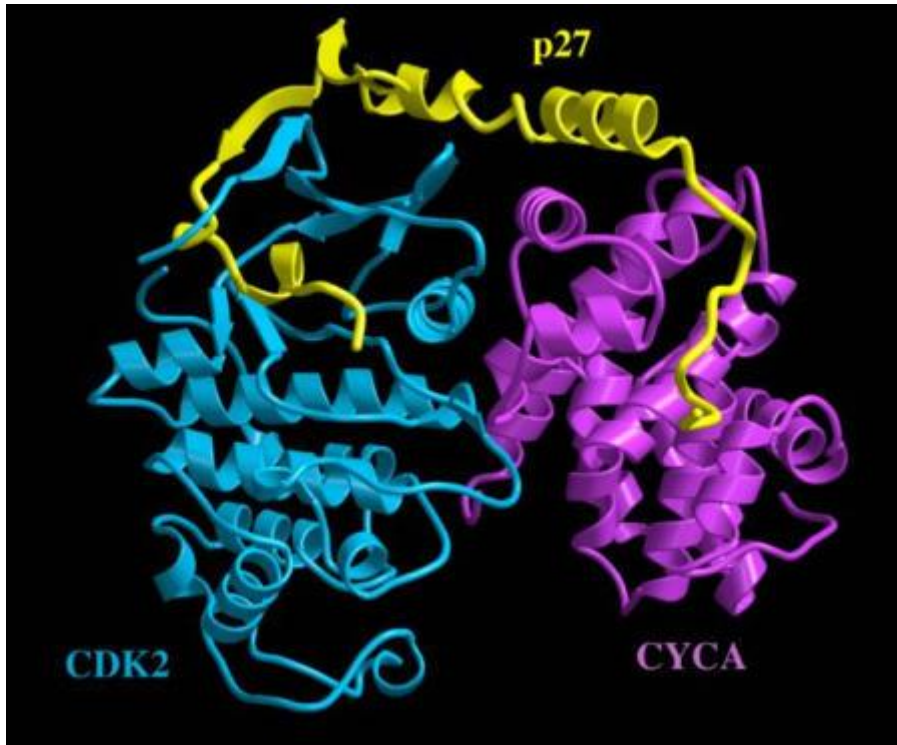
Nikola Pavletich  
Memorial Sloan-Kettering  
Cancer Center



Cyclin A – cdk2 phosphorylated  
at Thr160

[www.wikipedia.org](http://www.wikipedia.org)

# Crystal structure



p27 (Kip1) is shown bound to the CyclinA-Cdk2 complex, provoking profound changes in the kinase active site and rendering it inactive.

p27(Kip1)-CyclinA-Cdk2 Complex

p27 also interacts with the secondary substrate recognition site on the cyclin.

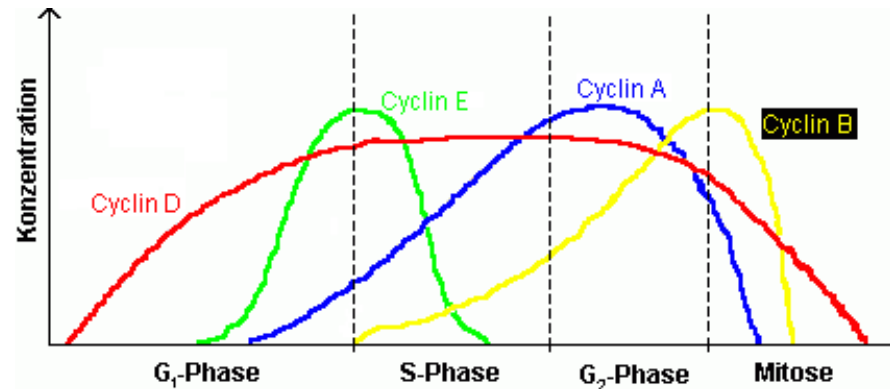
[www.wikipedia.org](http://www.wikipedia.org)

# Cdk1-phosphorylation sites

Cdk1 substrates frequently contain multiple phosphorylation sites that are clustered in regions of intrinsic disorder.

Their exact position in the protein is often poorly conserved in evolution, indicating that precise positioning of phosphorylation is not required for regulation of the substrate.

Human Cdk1 interacts with 9 different cyclins throughout the cell cycle.



Expression of human cyclins through the cell cycle.

Enserink and Kolodner  
*Cell Division* 2010 **5**:11

[www.wikipedia.org](http://www.wikipedia.org)



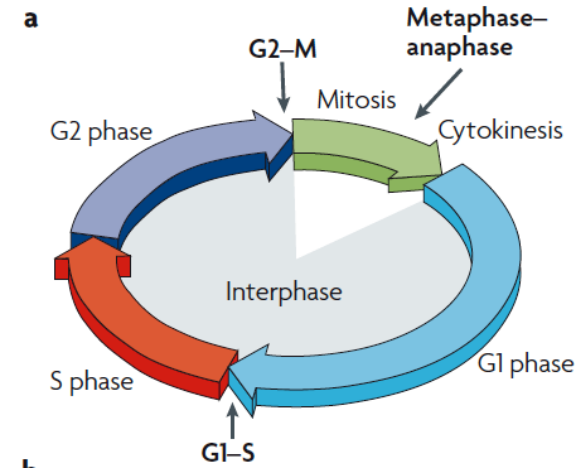
# The classical model of cell-cycle control

## OPINION

### Cyclin-dependent kinases and cell-cycle transitions: does one fit all?

Helfrid Hochegger, Shunichi Takeda and Tim Hunt

*Nature Reviews Molecular Cell Biology* 9, 910-916 (2008)



Cyclin-dependent kinases (cDKs) trigger the transition from  $G_1$  to S phase and from  $G_2$  to M phase by phosphorylating distinct sets of substrates.

The metaphase-to-anaphase transition requires the ubiquitylation and proteasome-mediated degradation of mitotic B-type cyclins and various other proteins, and is triggered by the anaphase-promoting complex/cyclosome (APc/c) e3 ubiquitin ligase

# Cell cycle checkpoints

Cell cycle **checkpoints** are control mechanisms that ensure the fidelity of cell division in eukaryotic cells.

These checkpoints verify whether the processes at each phase of the cell cycle have been accurately completed before progression into the next phase.

An important function of many checkpoints is to **assess DNA damage**, which is detected by sensor mechanisms.

When damage is found, the checkpoint uses a signal mechanism either to stall the cell cycle until **repairs** are made or, if repairs cannot be made, to target the cell for destruction via **apoptosis** (effector mechanism).

All the checkpoints that assess DNA damage appear to utilize the same sensor-signal-effector mechanism.

# Is the cyclin-CDK oscillator essential?

The cyclin–CDK oscillator governs the major events of the cell cycle.

In embryonic systems this oscillator functions in the absence of transcription, relying only on maternal stockpiles of messenger RNAs and proteins.

CDKs are also thought to act as the central oscillator in somatic cells and yeast.

Orlando et al., Nature 453, 944-947 (2008)



# What happens in cyclin-mutant cells?

However, by correlating genome-wide transcription data with global TF binding data, models have been constructed in which periodic transcription is an **emergent property of a TF network**.

In these networks, TFs expressed in one cell-cycle phase bind to the promoters of genes encoding TFs that function in a subsequent phase.

Thus, the temporal program of transcription could be controlled by sequential waves of TF expression, even in the absence of extrinsic control by cyclin–CDK complexes

Orlando et al., Nature 453, 944-947 (2008)

# What happens in cyclin-mutant cells?

-> investigate the dynamics of genome-wide transcription in budding yeast cells that are disrupted for all S-phase and mitotic cyclins (clb1,2,3,4,5,6).

These cyclin-mutant cells are unable to replicate DNA, to separate spindle pole bodies, to undergo isotropic bud growth or to complete nuclear division.

-> indicates that mutant cells are devoid of functional Clb–CDK complexes.

So, by conventional cell-cycle measures, clb1,2,3,4,5,6 cells arrest at the G1/S border.

Expectation:

if Clb–CDK activities are essential for triggering the transcriptional program, then periodic expression of S-phase-specific and G2/M-specific genes should not be observed.

Orlando et al., Nature 453, 944-947 (2008)

# Periodic transcripts in wt and cyclin-mutant cells

Aim: Identify periodically expressed genes.

For each gene,  $i$ , a Fourier score,  $F_i$ , was computed as

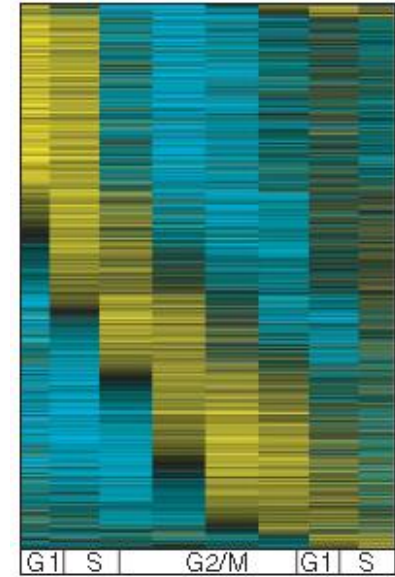
$$F_i = \sqrt{\left(\sum_t \sin(\omega t) \cdot x_i(t)\right)^2 + \left(\sum_t \cos(\omega t) \cdot x_i(t)\right)^2}$$

where  $\omega = 2\pi/T$  and  $T$  is the interdivision time.

Similarly, scores were calculated for 1 000 000 artificial profiles constructed by random shuffling of the data points within the expression profile of the gene in question.

The  $P$ -value for periodicity was calculated as the fraction of artificial profiles with Fourier scores equal to or larger than that observed for the real expression profile.

Orlando et al., Nature 453, 944-947 (2008)



Heat maps depicting mRNA levels of 1271 periodic genes for wild-type cells.

Each row represents data for one gene.

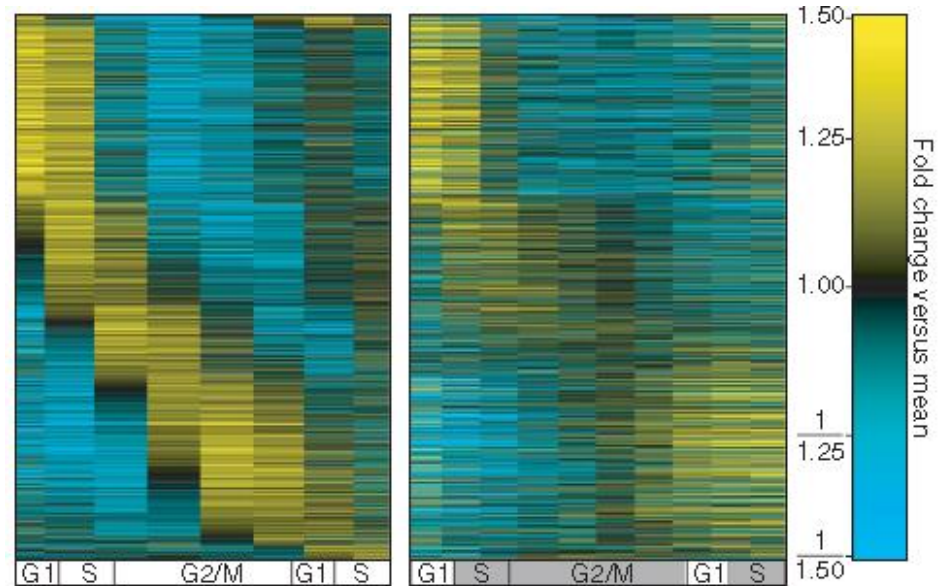
# Periodic transcripts in wt and cyclin-mutant cells

mRNA levels of periodic genes for wild-type (a) and cyclin-mutant (b) cells.

Each row in a and b represents data for the same gene.

The S and G2/M phases of the cyclin-mutant timeline are shaded.

By conventional definitions, cyclin-mutant cells arrest at the G1/S-phase border.



## Observations

- (1) Expression of 883 genes is altered in the mutant (so that they are likely regulated by B-cyclin CDK,
- (2) However, although mutant cells are arrested at G1/S border, gene regulation program seems to continue ...

Orlando et al., Nature 453, 944-947 (2008)

# Transcriptional dynamics of cyclin-CDK regulated genes

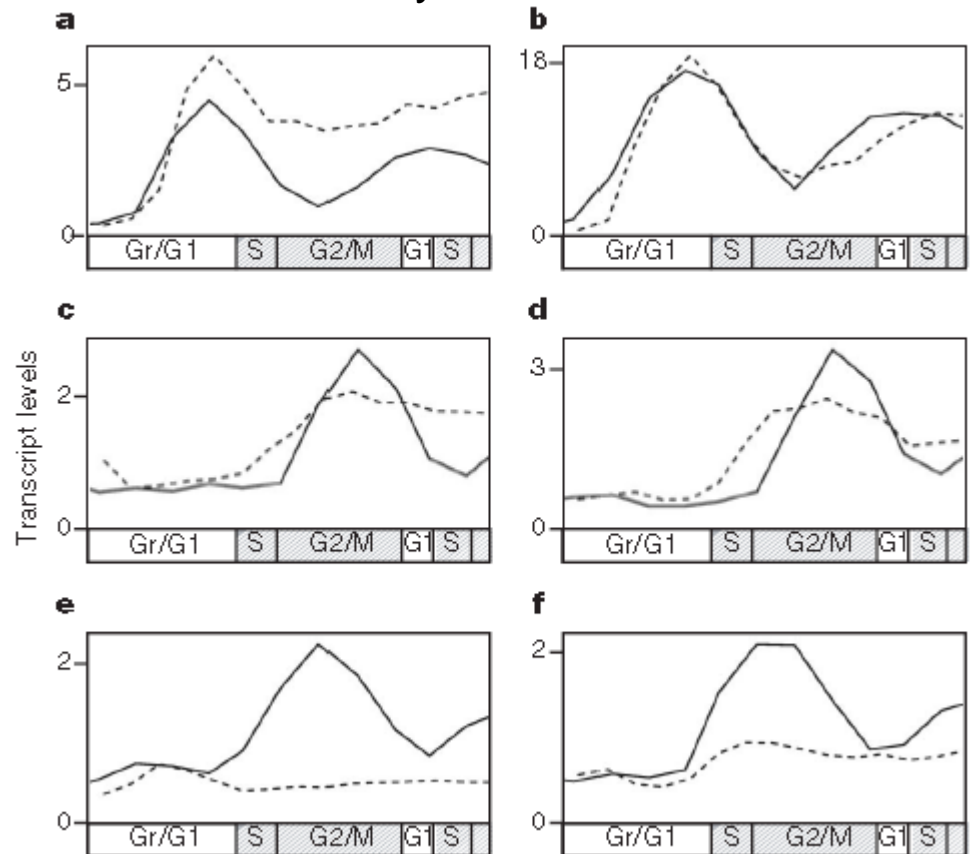
(a) Gene CLN2 (that is regulated by late G1-transcript SBF) is not fully repressed in mutant. ok

(b) Gene RNR1 (that is regulated by MBF) is not affected.

Genes SIC1 (c) and NIS1 (d) are regulated by Ace2/Swi5. These TFs are usually excluded from the nucleus by CDK phosphorylation until late meiosis. In cyclin-mutant cells, nuclear exclusion of Swi5 and Ace2 is probably lost -> Early onset observed in the mutant.

The Clb2-cluster genes CDC20 (e) and ACE2 (f) are strongly down-regulated.

Solid lines, wild-type cells;  
dashed lines, cyclin-mutant cells.



Orlando et al., Nature 453, 944-947 (2008)

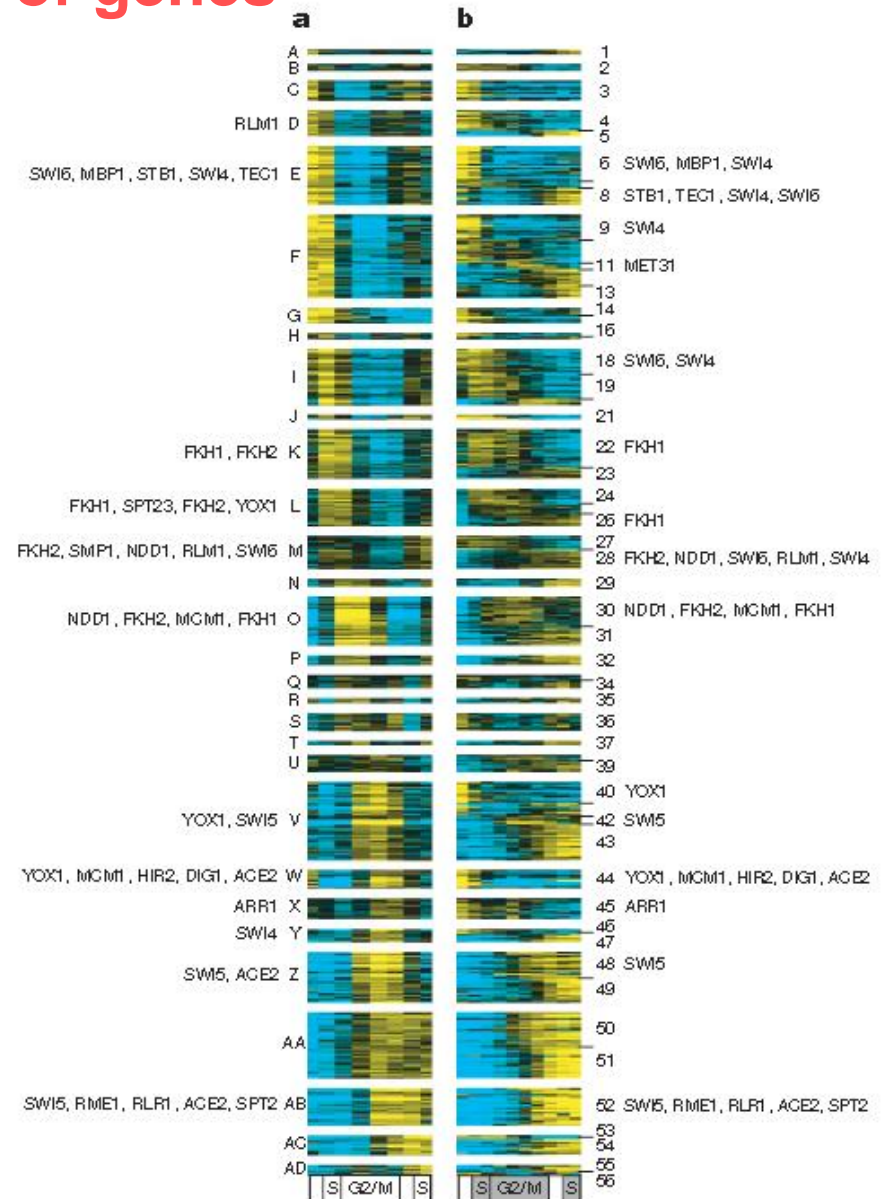
# Clustering of genes

Cluster genes showing altered behaviors in cyclin-mutant cells.

a, Clusters of genes with similar expression patterns in wild-type cells.

b, Subclusters of genes with similarly altered expression patterns in cyclin-mutant cells.

Associate each cluster with up to 5 over-represented TFs (hypergeometric test).



Orlando et al., Nature 453, 944-947 (2008)



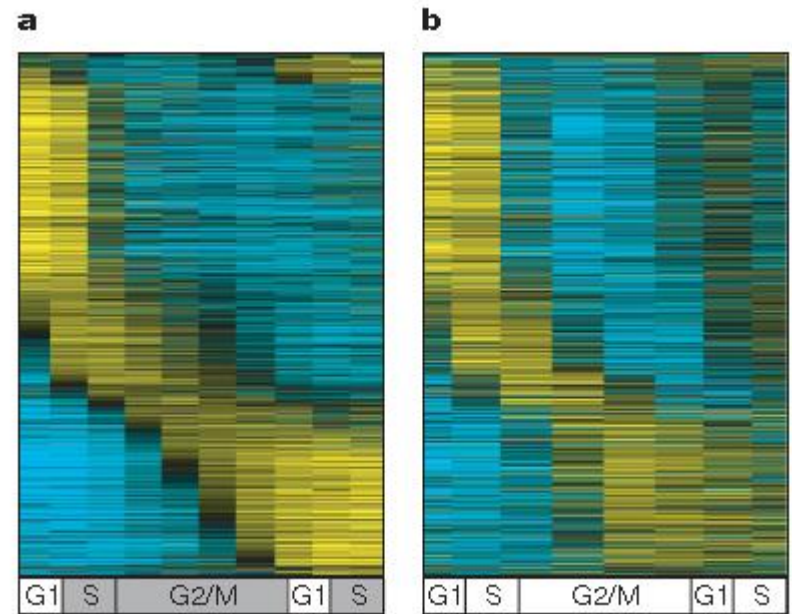
# Independent transcriptional program

The periodic transcription program is largely intact in cyclin mutant cells that arrest at the G1/S border.

a, b, Genes maintaining periodic expression in cyclin-mutant cells (a) show similar dynamics in wildtype cells (b).

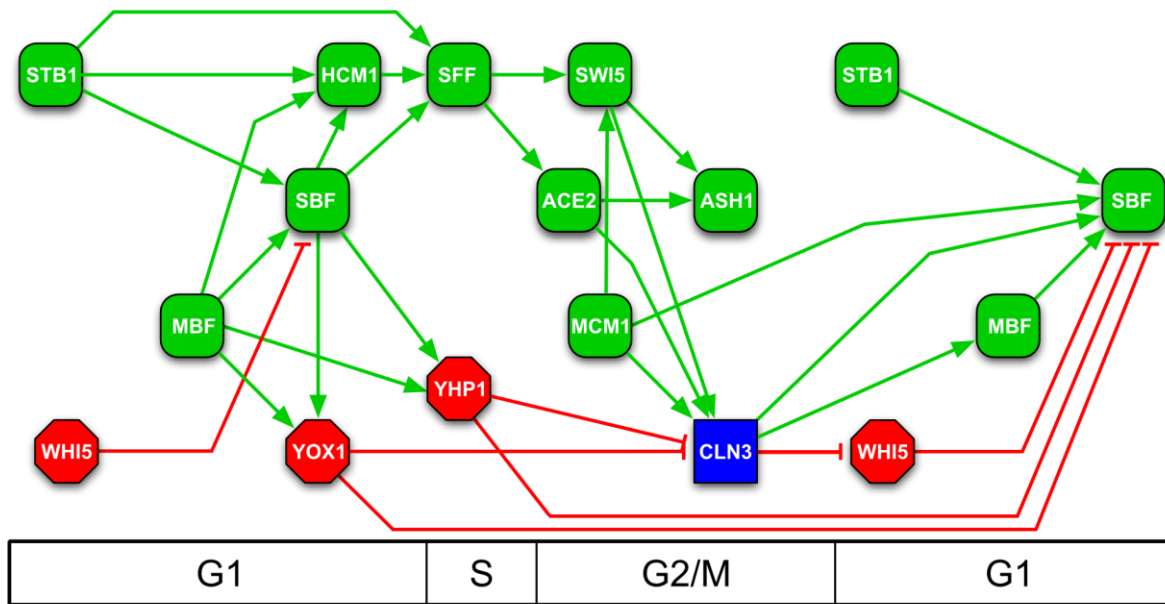
On the other hand, nearly 70% of the genes identified as periodic in wild-type cells are still expressed on schedule in cyclin-mutant cells.

This demonstrates the existence of a cyclin–CDK-independent mechanism that regulates temporal transcription dynamics during the cell cycle.



Orlando et al.,  
Nature 453, 944-947 (2008)

# Generate TF networks for wt and cyclin-mutant cells



Transcriptional activators are depicted in green, repressors in red, and the cyclin Cln3 in blue.

Periodically expressed TFs are placed on the cell-cycle timeline on the basis of the time of peak transcript levels.

Arrows indicate a documented interaction between a TF and promoter elements upstream of a gene encoding another TF.

Orlando et al., Nature 453, 944-947 (2008)



# Summary

The cyclin–CDK oscillator governs the major events of the cell cycle.

Simple Boolean networks or ODE-models can generate oscillatory behavior.

There exists an independent TF network in yeast (in all higher eukaryotes?) that drives periodic expression of many genes throughout cell cycle.

**Paper #4 (to be presented on: Tuesday, Dec. 1 and subject of assignment #2)**

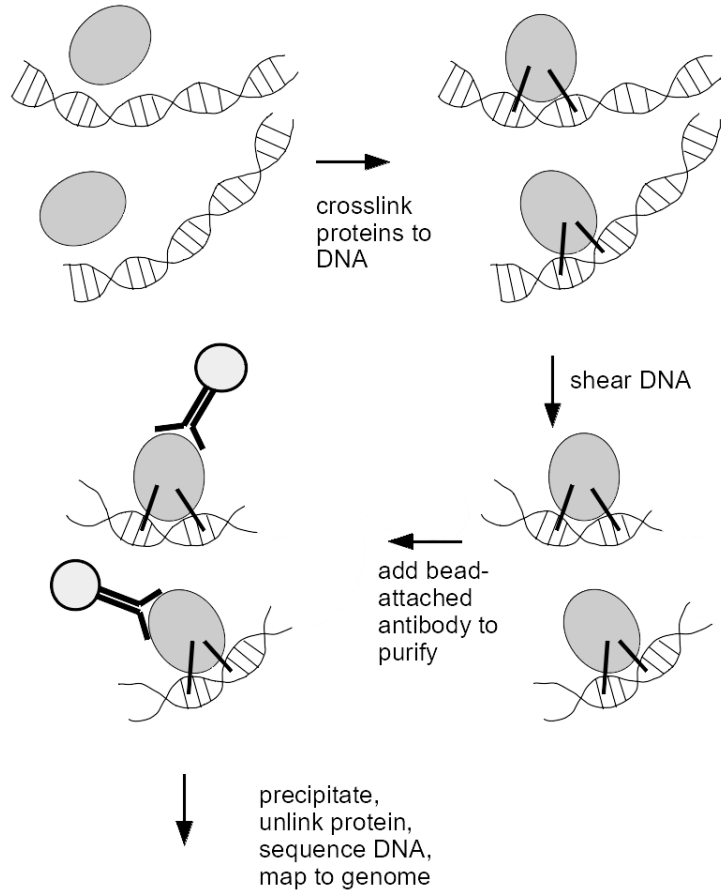
## Transcriptional landscape of the human cell cycle

Yin Liu, et al. PNAS 114, 3473-3478 (2017)

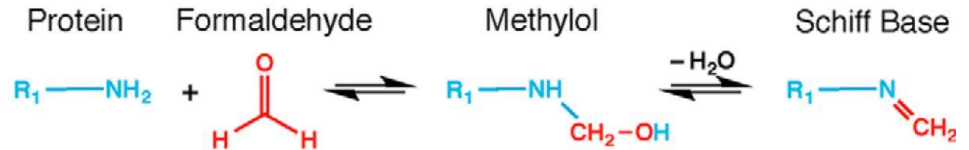
*Results part starts with* “To investigate dynamic transcriptional and epigenetic gene regulation across the cell cycle, we performed GRO-seq, RNA-seq, and ChIP-seq of H3K27ac and H3K4me2, two histone modifications that mark promoters and enhancers, in the synchronized human breast cancer cell line MCF-7. The cells were synchronized to G0/G1 with hormone starvation, to G1/S with double thymidine treatment, and to early M phase with thymidine-nocodazole treatment ...

# ChIP-seq protocol

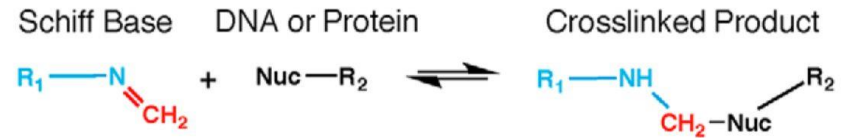
Main experimental steps of the **ChIP-seq** protocol.



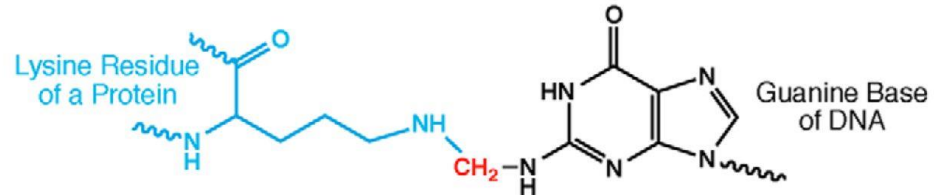
## Step 1



## Step 2



## Example Protein-DNA Crosslink



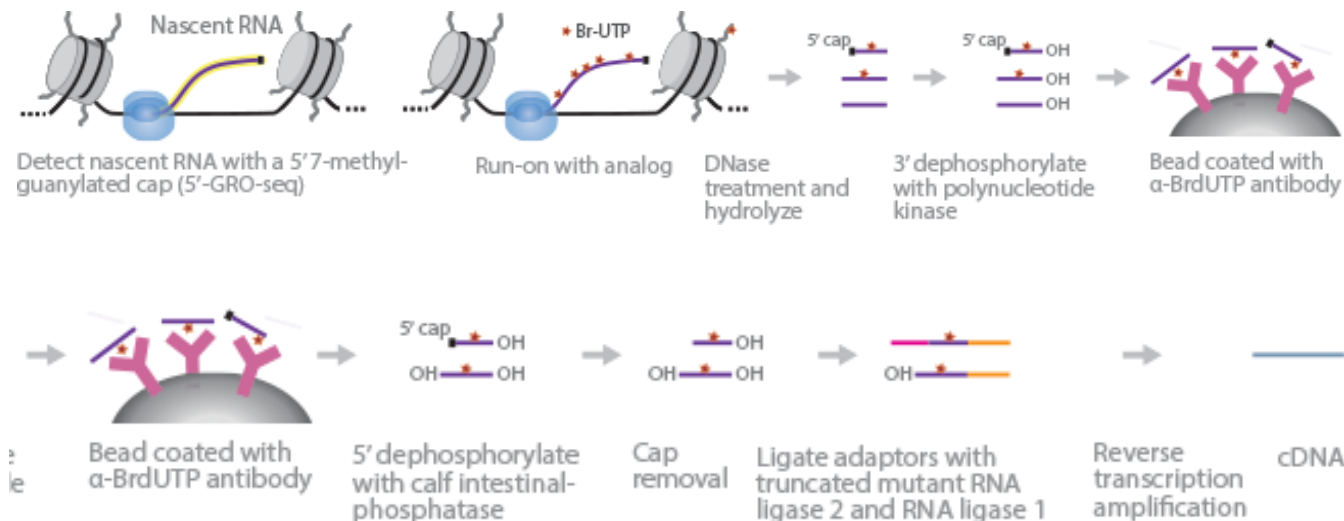
Park J, Nature Reviews Genetics, 10, 669 (2009)  
Thomas et al. Brief Bioinform. 18: 441–450 (2017) .

Hoffman et al, J Biol Chem 290, 26404 (2015)

# What is GRO-seq?

GRO-seq method first presented in:  
Nascent RNA Sequencing Reveals  
Widespread Pausing and Divergent  
Initiation at Human Promoters  
Leighton J. Core, Joshua J. Waterfall,  
John T. Lis, Science 322, 1845-1848  
(2008)

GRO-seq (global run-on sequencing) maps  
the position, amount, and orientation of  
transcriptionally engaged RNA  
polymerases genome-wide.  
Peaks of promoter-proximal polymerase  
reside on ~30% of human genes ...



## Pros:

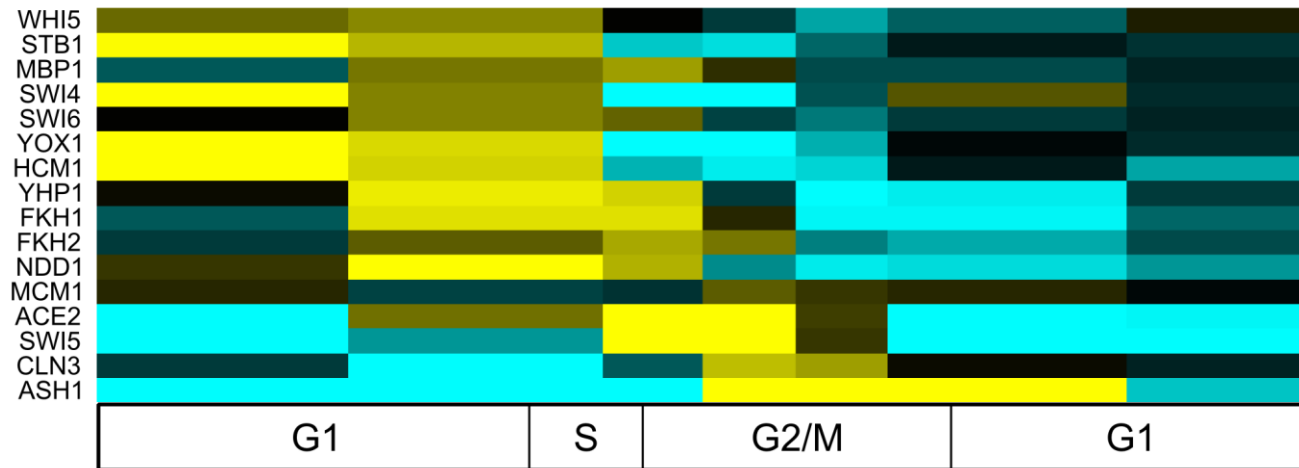
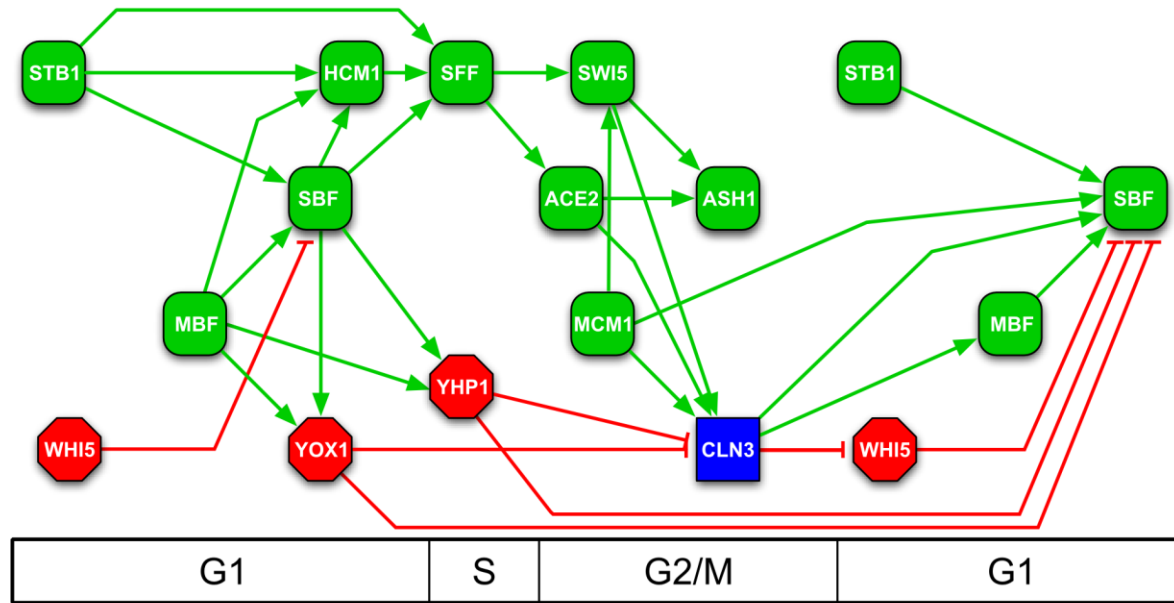
- Maps nascent capped 5' RNA sequence at any given time
- Determines activity of transcription sites
- No prior knowledge of transcription sites needed

## Cons:

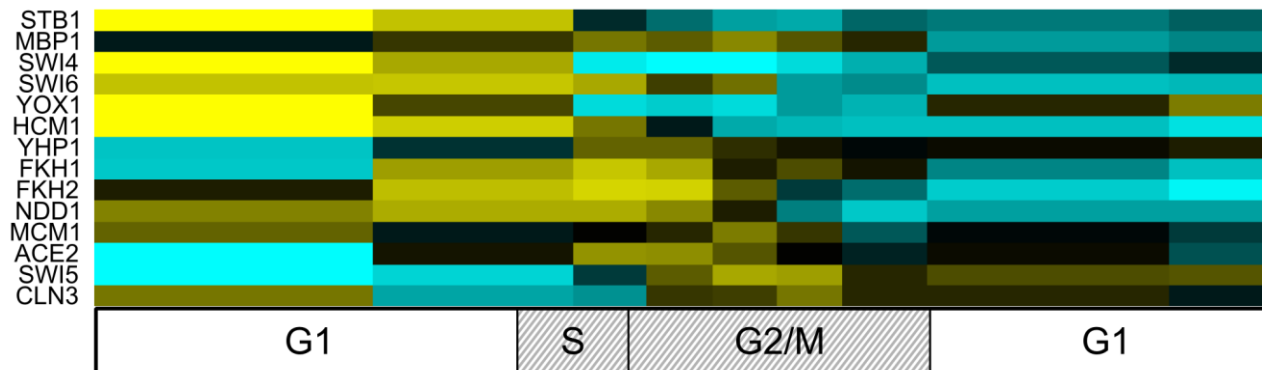
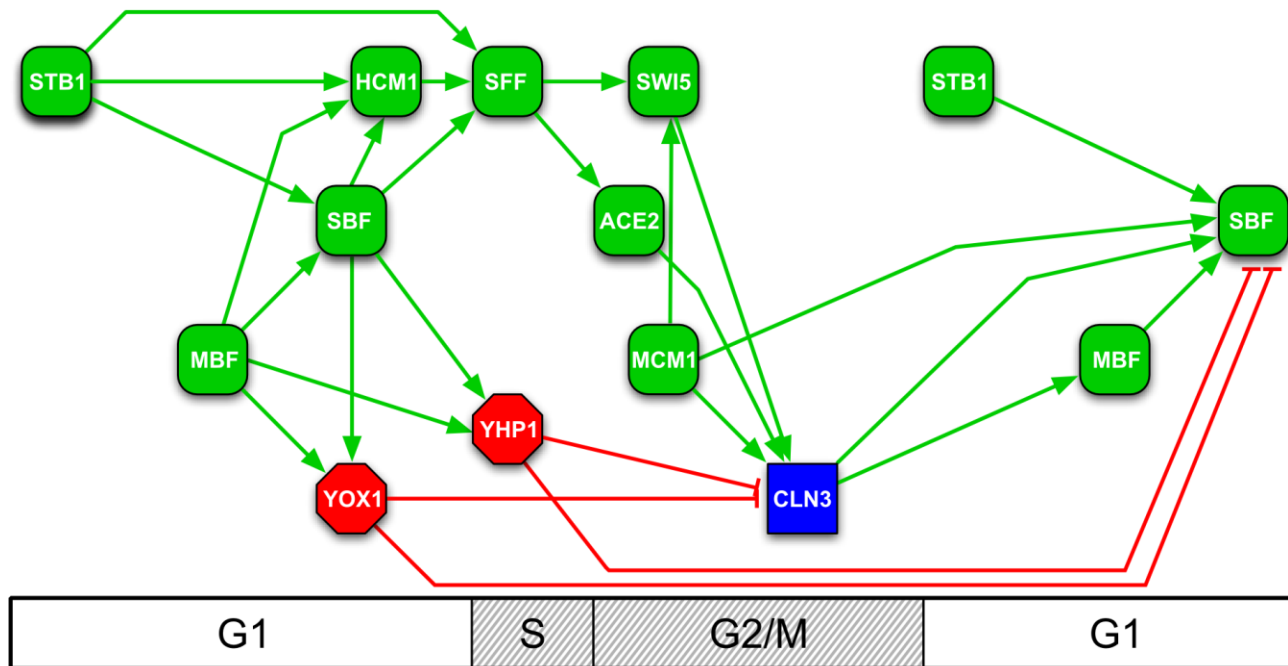
- Limited to cell cultures and other artificial systems due to incubation with labelled nucleotides

<https://www.illumina.com/science/sequencing-method-explorer/kits-and-arrays/5--gro-seq.html>

**Slides not used**



Orlando et al., Nature 453, 944-947 (2008)



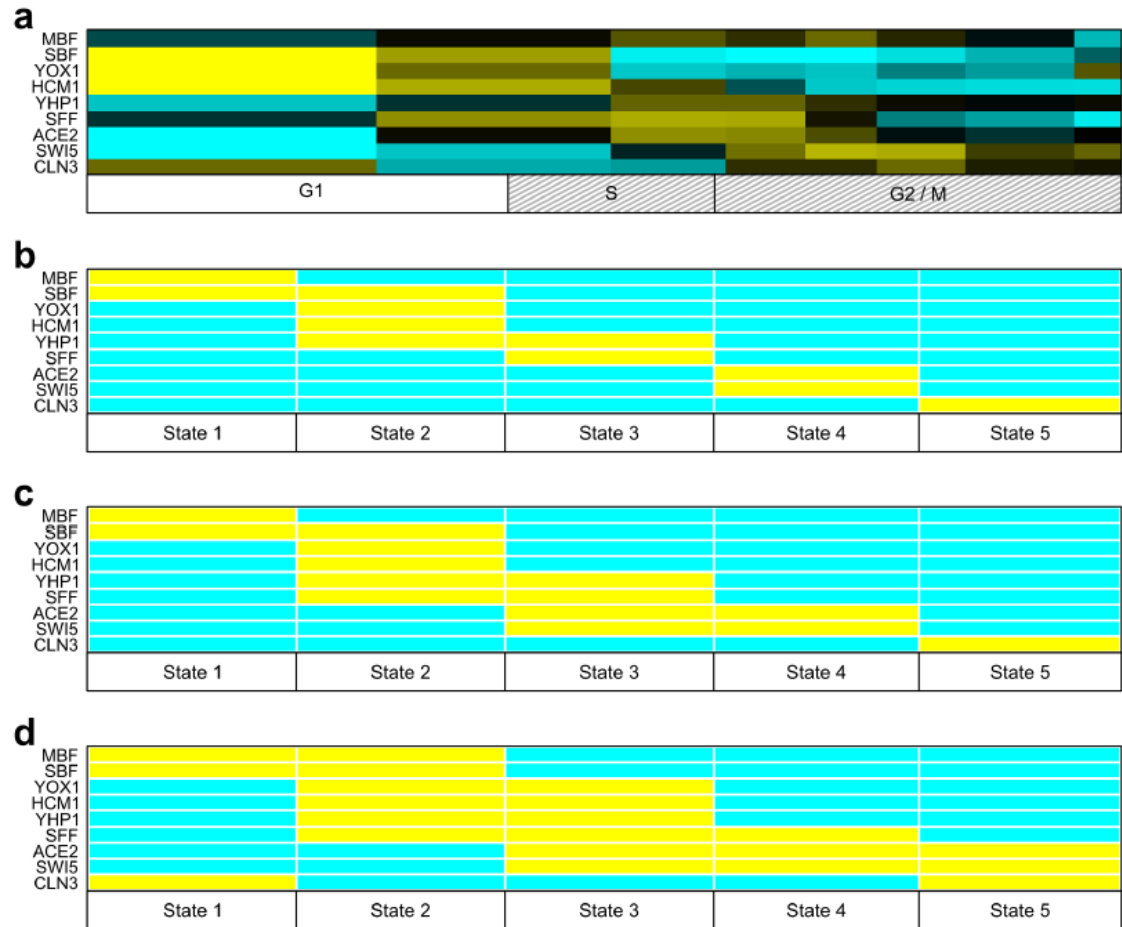
Orlando et al., Nature 453, 944-947 (2008)

# Boolean model

A synchronously updating Boolean model can reproduce the sequential order of TF expression.

**a**, The actual expression of the variables in Fig. 4c compared to the on/off (yellow/cyan) states of those variables in

**b**, Cycle 1,  
**c**, Cycle 2, and  
**d**, Cycle 3.



Orlando et al., Nature 453, 944-947 (2008)

# Sschematic of the computational analysis pipeline.

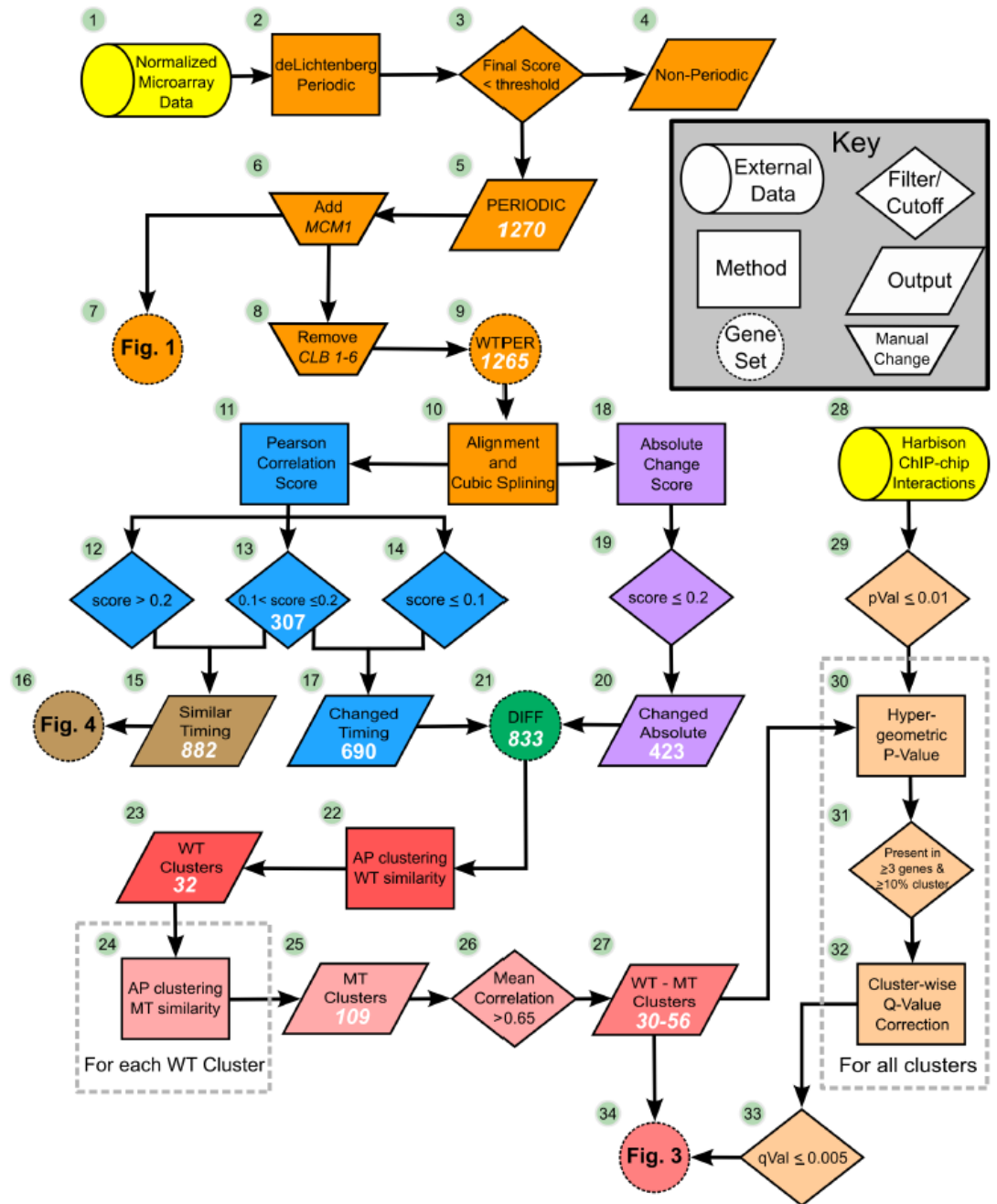
Colors correspond to different analysis topics (e.g., yellow represents identification of periodic genes).

Shapes correspond to particular types of procedures or data (e.g., diamonds are filters and cylinders are external data).

White numbers within a shape indicate the size of the corresponding gene set (e.g., the 882 in Item 15 indicates that 882 genes maintain their periodic expression in cyclin-mutant cells)

Orlando et al.,

Nature 453, 944-947 (2008)





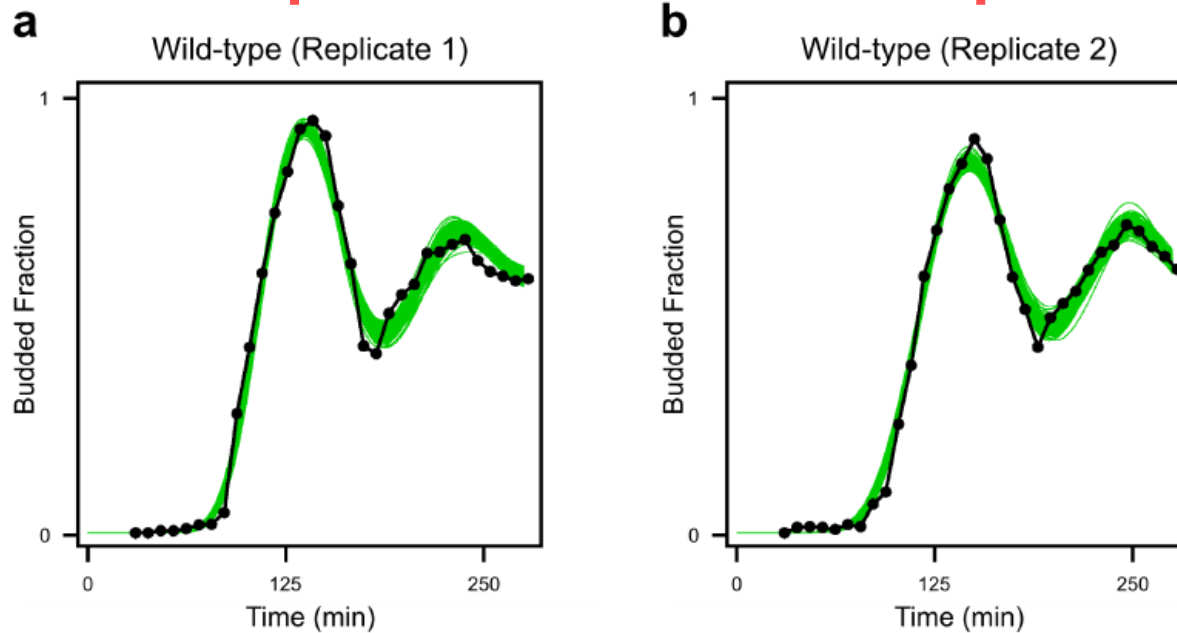
# Logic of Boolean network

## **a** Initial Regulatory Logic Choice

TF	Activation Rule
MBF	CLN3
SBF	$(\text{CLN3} \vee \text{MBF}) \wedge \neg(\text{YOX1} \wedge \text{YHP1})$
YOX1	$\text{MBF} \wedge \text{SBF}$
HCM1	$\text{MBF} \wedge \text{SBF}$
YHP1	$\text{MBF} \vee \text{SBF}$
SFF	$\text{SBF} \wedge \text{HCM1}$
ACE2	SFF
SWI5	SFF
CLN3	$(\text{SWI5} \wedge \text{ACE2}) \wedge \neg(\text{YOX1} \wedge \text{YHP1})$

Orlando et al., Nature 453, 944-947 (2008)

## Model parameters fitted to exp. data



### CLOCCS model fits: synchronizes exp. data from different measurements

100 random realizations from the Markov chain used to fit each experiment were used as parameterizations for the model, and the resulting predicted budding curves for one bud (green lines) and two or more buds (red lines) are shown.

Orlando et al., Nature 453, 944-947 (2008)

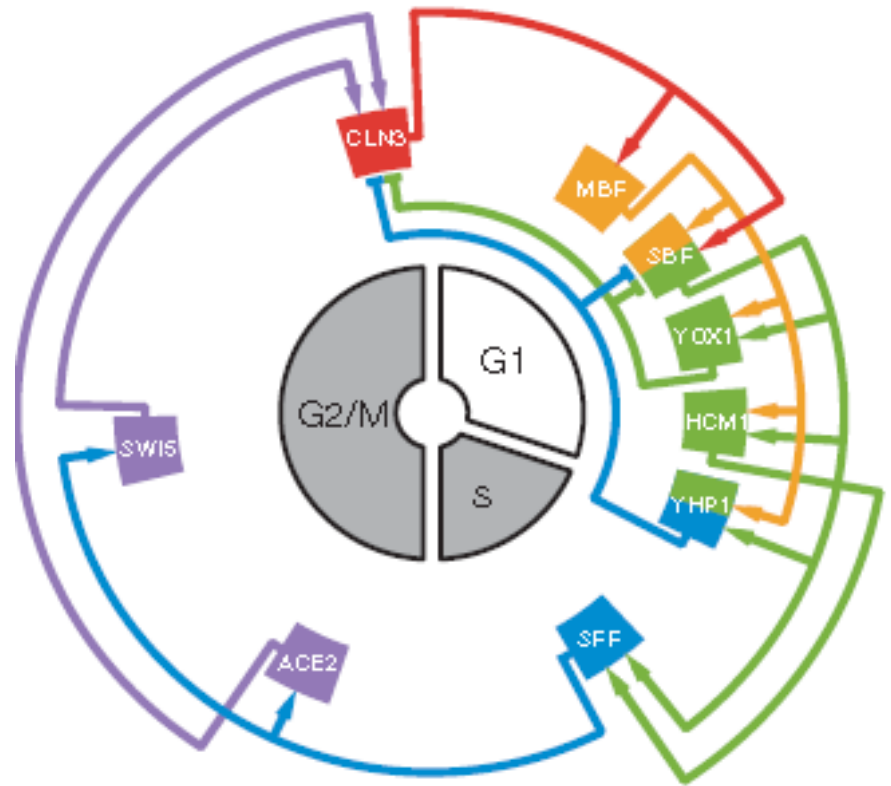
# Boolean network model

c, Synchronously updating boolean network model.

TFs are arranged on the basis of the time of peak transcript levels in cyclin-mutant cells.

Arrows indicate TF/promoter interaction. Activating interactions, outer rings; repressive interactions, inner rings.

Colouring indicates activity in one of five successive states; SBF and YHP1 are active in two states.



Orlando et al.,

Nature 453, 944-947 (2008)

# Boolean attractors

## b Attractor Distributions

Repressor	SFF	CLN3	All Off	Cycle 1	Cycle 2	Cycle 3
(YOX1 $\wedge$ YHP1)	SBF $\wedge$ HCM1	SWI5 $\wedge$ ACE2	19.7%	80.3%	0%	0%
(YOX1 $\wedge$ YHP1)	SBF $\wedge$ HCM1	SWI5 $\vee$ ACE2	13.9%	86.1%	0%	0%
(YOX1 $\wedge$ YHP1)	SBF $\vee$ HCM1	SWI5 $\wedge$ ACE2	3.3%	0%	0%	96.7%
(YOX1 $\wedge$ YHP1)	SBF $\vee$ HCM1	SWI5 $\vee$ ACE2	2.1%	0%	0%	97.9%
(YOX1 $\vee$ YHP1)	SBF $\wedge$ HCM1	SWI5 $\wedge$ ACE2	76.6%	23.4%	0%	0%
(YOX1 $\vee$ YHP1)	SBF $\wedge$ HCM1	SWI5 $\vee$ ACE2	79.7%	20.3%	0%	0%
(YOX1 $\vee$ YHP1)	SBF $\vee$ HCM1	SWI5 $\wedge$ ACE2	34.8%	0%	45.3%	19.9%
(YOX1 $\vee$ YHP1)	SBF $\vee$ HCM1	SWI5 $\vee$ ACE2	38.7%	0%	45.3%	16.0%

## c Cycle 1 (used to color Fig. 4c)

	S1	S2	S3	S4	S5
MBF	1	0	0	0	0
SBF	1	1	0	0	0
YOX1	0	1	0	0	0
HCM1	0	1	0	0	0
YHP1	0	1	1	0	0
SFF	0	0	1	0	0
ACE2	0	0	0	1	0
SWI5	0	0	0	1	0
CLN3	0	0	0	0	1

## d Cycle 2

	S1	S2	S3	S4	S5
MBF	1	0	0	0	0
SBF	1	1	0	0	0
YOX1	0	1	0	0	0
HCM1	0	1	0	0	0
YHP1	0	1	1	0	0
SFF	0	1	1	0	0
ACE2	0	0	1	1	0
SWI5	0	0	1	1	0
CLN3	0	0	0	0	1

## e Cycle 3

	S1	S2	S3	S4	S5
MBF	1	1	0	0	0
SBF	1	1	0	0	0
YOX1	0	1	1	0	0
HCM1	0	1	1	0	0
YHP1	0	1	1	0	0
SFF	0	1	1	1	0
ACE2	0	0	1	1	1
SWI5	0	0	1	1	1
CLN3	1	0	0	0	1

80.3% of all the 512 possible starting states enter a cycle containing five states (Cycle 1).

Cycle 2 and Cycle 3 are qualitatively similar to Cycle 1. They maintain the same temporal order of expression as Cycle 1, and differ only in the duration of expression of certain TFs

Orlando et al., Nature 453, 944-947 (2008)