V8 Cell cycle - summary
Phases of the eukaryotic cell cycle

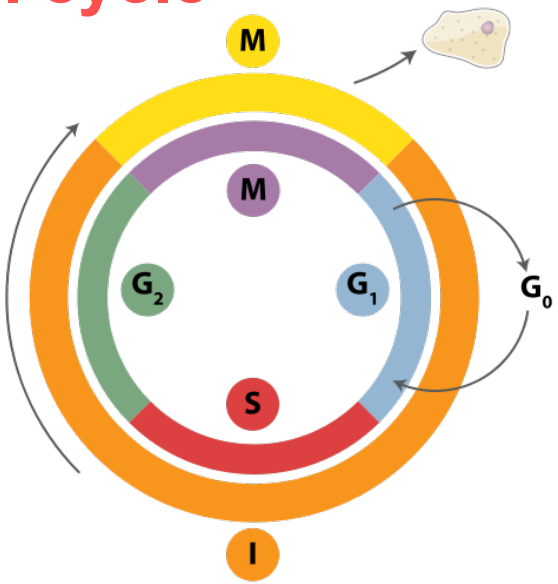
The cell cycle consists of **4 distinct phases**:

- G_1 phase,
- S phase (synthesis),
- G_2 phase
- and M phase (mitosis).

Interphase: combines G_1, S, and G_2

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_0 phase**.



Schematic of the cell cycle.
Outer ring:
I = Interphase, M = Mitosis;
Inner ring:
M = Mitosis, G1 = Gap 1, G2 = Gap 2, S = Synthesis.

www.wikipedia.org

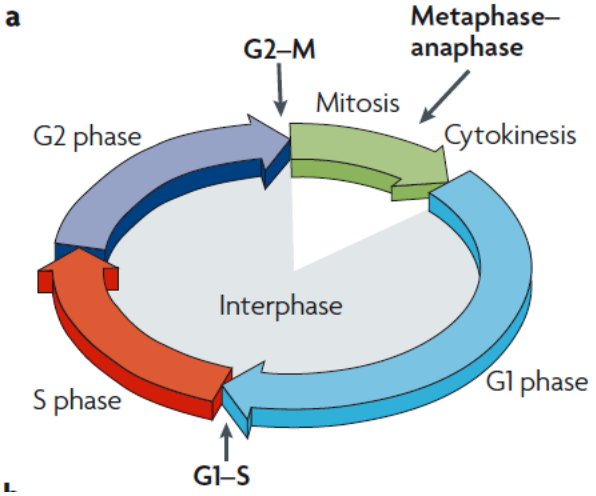
The classical model of cell-cycle control

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.

www.wikipedia.org

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.

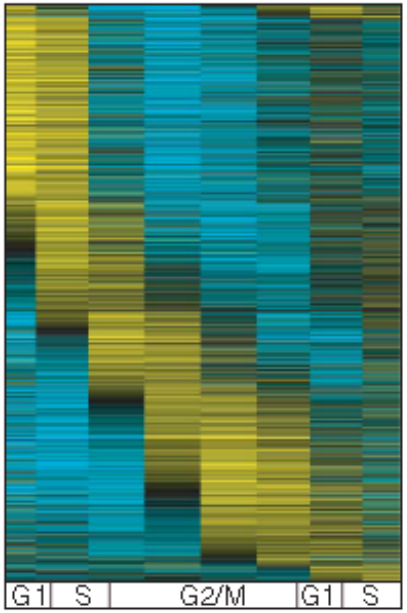
For each gene, *i*, a Fourier score, F_i, was computed as

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

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.

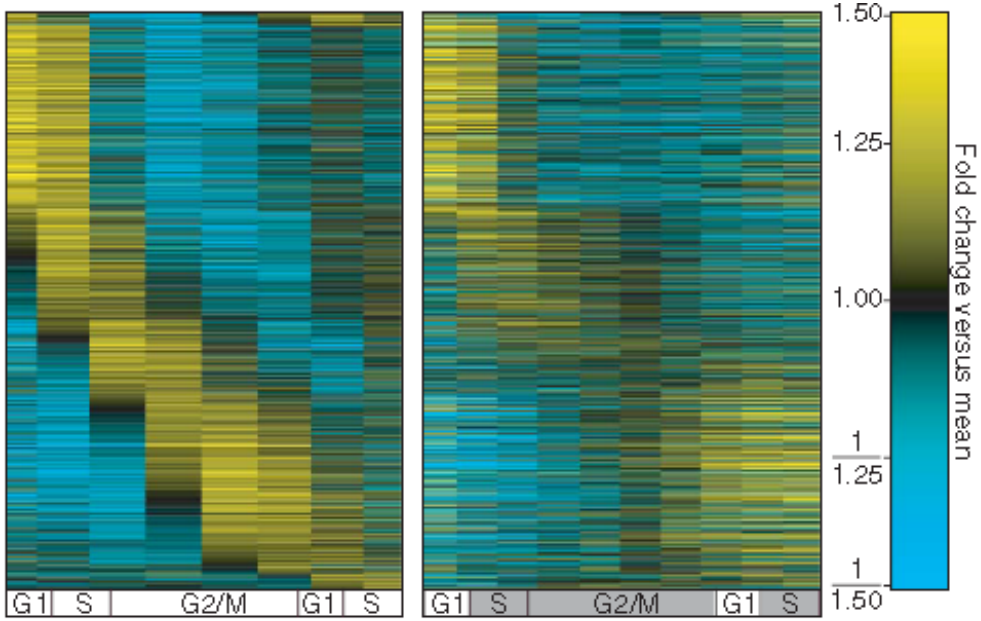
Periodic transcripts in wt and cyclin-mutant cells

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)

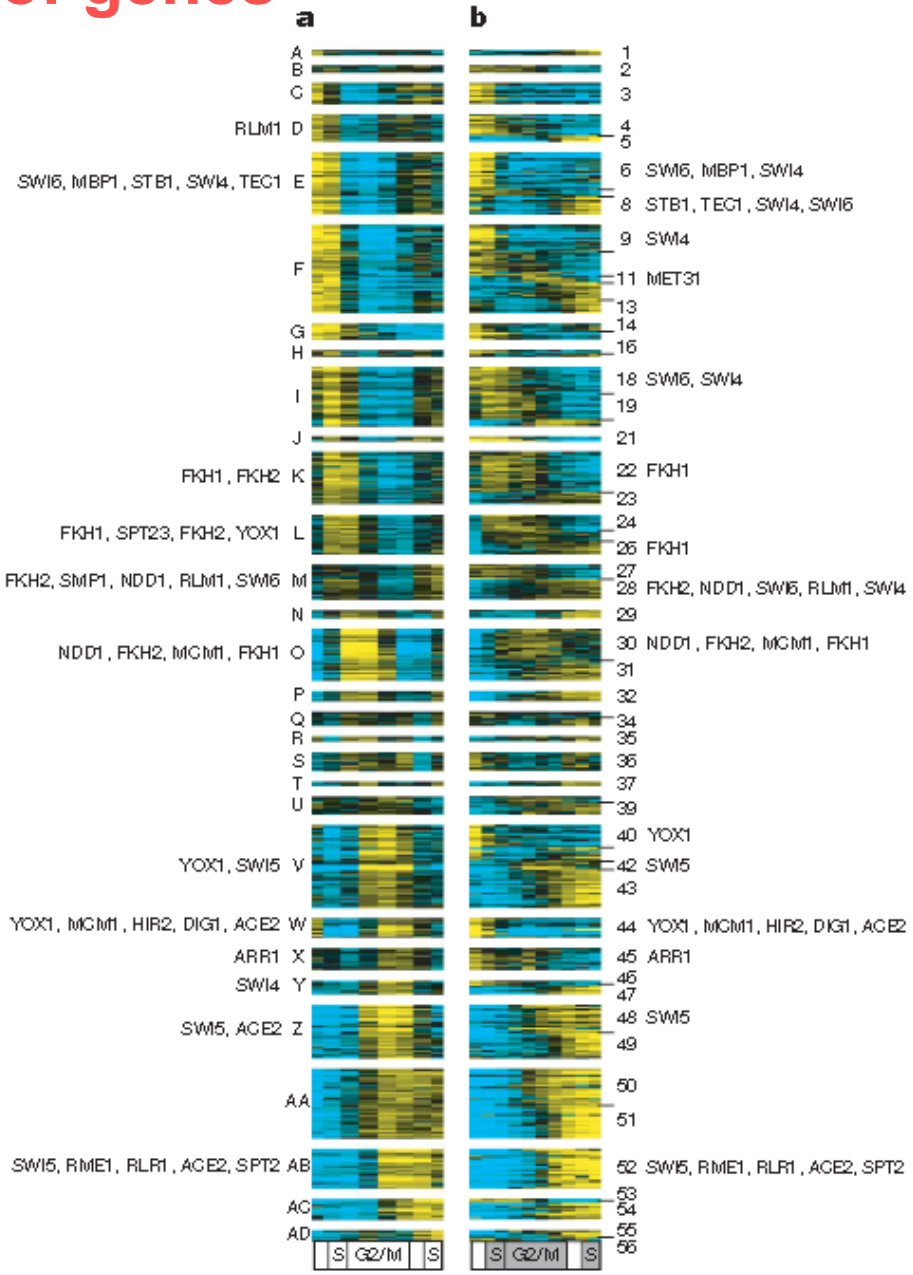
Clustering of genes

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)

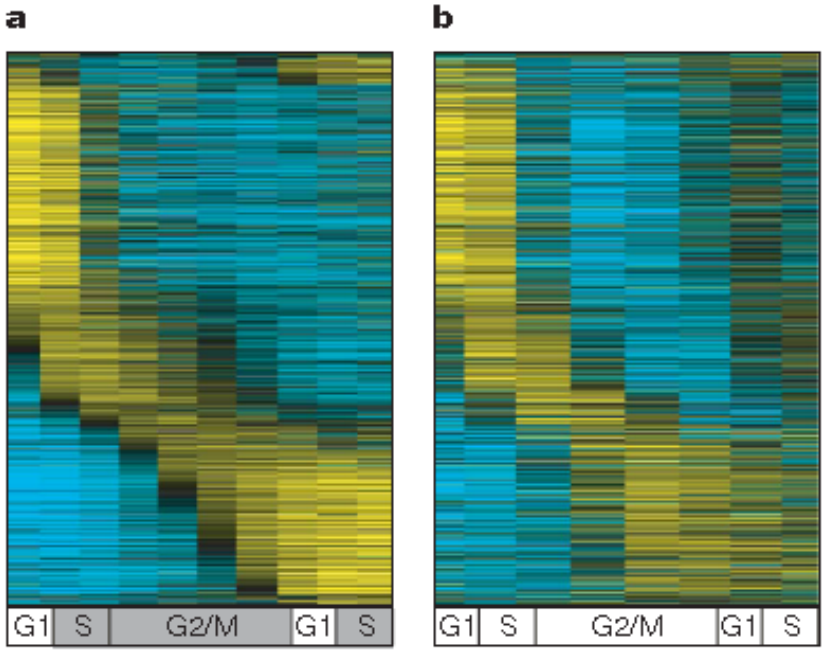
Independent transcriptional program

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.

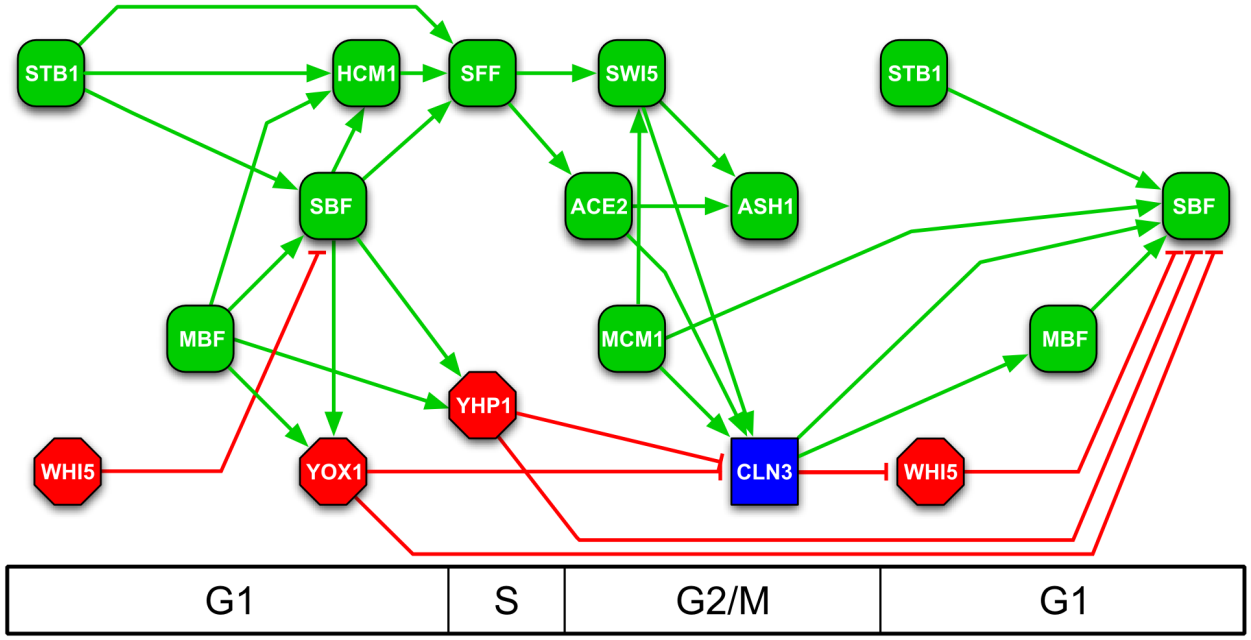
This demonstrates the existence of a cyclin–CDK-independent mechanism that regulates temporal transcription dynamics during the cell cycle.



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. (see assignment 1)

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

Subject of Minitest #2 is also

Paper #4 (presented on: Monday, Nov. 27):

Patterns of organelle ontogeny through a cell cycle revealed by whole-cell reconstructions using 3D electron microscopy

Louise Hughes, Samantha Borrett, Katie Towers, Tobias Starborg and Sue Vaughan, Journal of Cell Science (2017) 130, 637-647 doi:10.1242/jcs.198887

Abstract starts with "The major mammalian bloodstream form of the African sleeping sickness parasite Trypanosoma brucei multiplies rapidly, and it is important to understand how these cells divide."

V6: Protein phosphorylation during cell cycle

Protein **phosphorylation** and **dephosphorylation** are highly controlled biochemical processes that respond to various intracellular and extracellular stimuli.

Phosphorylation status modulates protein activity by
- influencing the tertiary and quaternary **structure** of a protein,
- controlling its **subcellular distribution**, and
- regulating its **interactions** with other proteins.

Regulatory protein phosphorylation is a **transient modification** that is often of low occupancy or “stoichiometry”

This means that only a fraction of a particular protein may be phosphorylated on a given site at any particular time, and that occurs on regulatory proteins of low abundance, such as protein kinases and transcription factors.

Olsen Science Signaling 3 (2010)

Cell Cycle and the Phosphoproteome

CELL CYCLE

Quantitative Phosphoproteomics Reveals Widespread Full Phosphorylation Site Occupancy During Mitosis

Jesper V. Olsen,[1,2*] Michiel Vermeulen,[1,3*] Anna Santamaria,[4*] Chanchal Kumar,[1,5*] Martin L. Miller,[2,6] Lars J. Jensen,[2] Florian Gnad,[1] Jürgen Cox,[1] Thomas S. Jensen,[7] Erich A. Nigg,[4] Søren Brunak,[2,7] Matthias Mann[1,2†]

(Published 12 January 2010; Volume 3 Issue 104 ra3)

www.SCIENCESIGNALING.org 12 January 2010 Vol 3 Issue 104 ra3

Aim: Analyze all proteins that are modified by phosphorylation during different stages of the cell cycle of human HeLa cells.

Ion-exchange chromatography + HPLC + MS + sequencing led to the identification of 6695 phosphorylated proteins („the phospho-proteome“). From this, 6027 quantitative cell cycle profiles were obtained.

A total of 24,714 phosphorylation events were identified.
20,443 of them were assigned to a specific residue with high confidence.

Finding: about **70%** of all proteins get phosphorylated.

Review: protein quantification by SILAC

ARTICLE

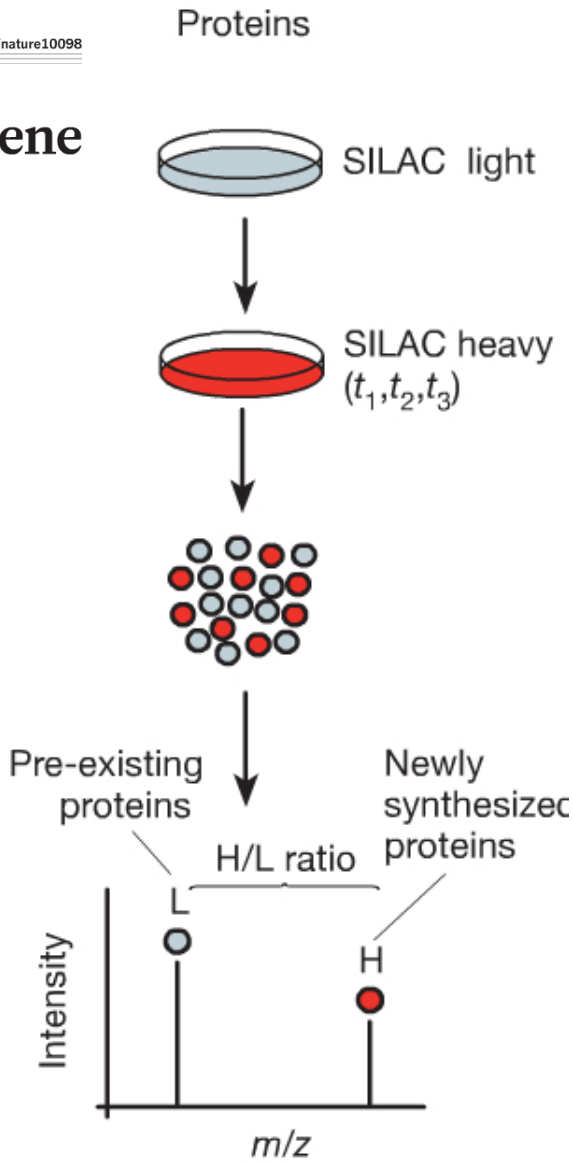
doi:10.1038/nature10098

Global quantification of mammalian gene expression control

Björn Schwanhäusser[1], Dorothea Busse[1], Na Li[1], Gunnar Dittmar[1], Johannes Schuchhardt[2], Jana Wolf[1], Wei Chen[1] & Matthias Selbach[1]

SILAC: „**s**table **i**sotope **l**abelling by **a**mino acids in **c**ell culture“ means that cells are cultivated in a medium containing heavy stable-isotope versions of essential amino acids.

When non-labelled (i.e. light) cells are transferred to heavy SILAC growth medium, newly synthesized proteins incorporate the heavy label while pre-existing proteins remain in the light form.



Protein turnover is quantified by mass spectrometry and next-generation sequencing, respectively.

Schwanhäuser et al. Nature 473, 337 (2011)

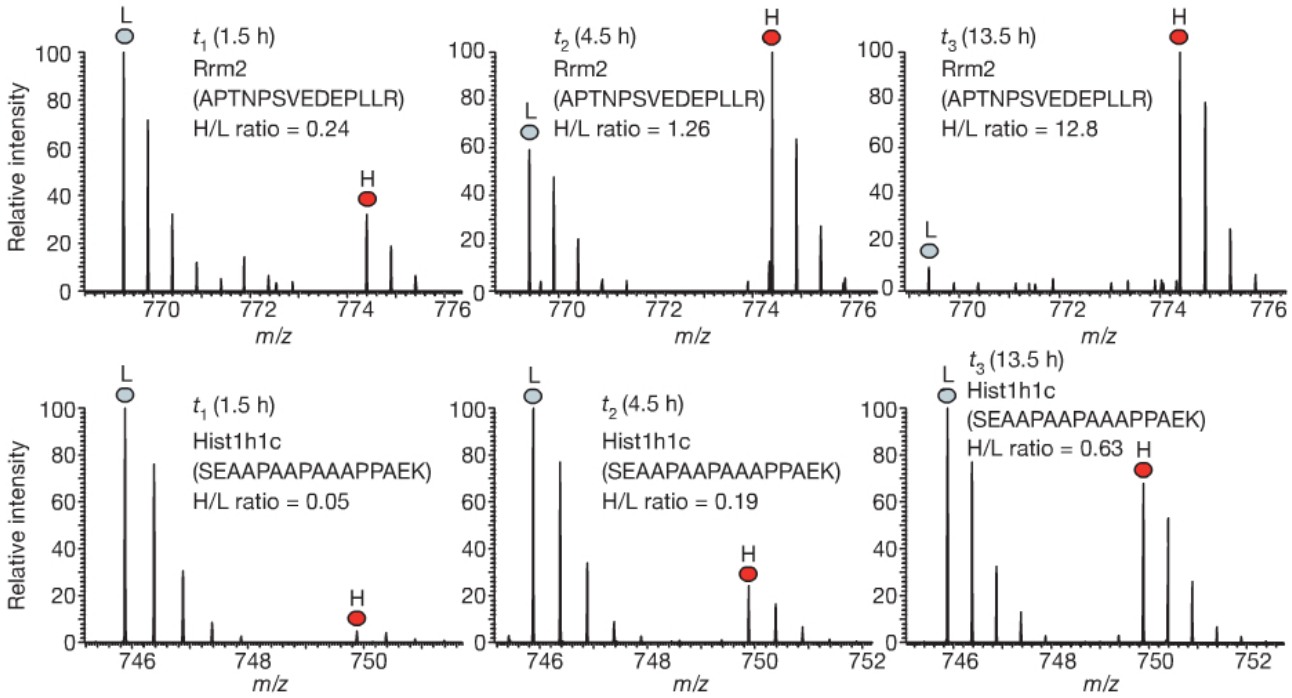
H/L ratios of individual proteins

Mass spectra of peptides for two proteins.

Top: **high-turnover protein**
Bottom: **low-turnover protein**.

Over time, the heavy to light (H/L) ratios increase.

H-concentration of high-turnover protein saturates.
That of low-turnover protein still increases.

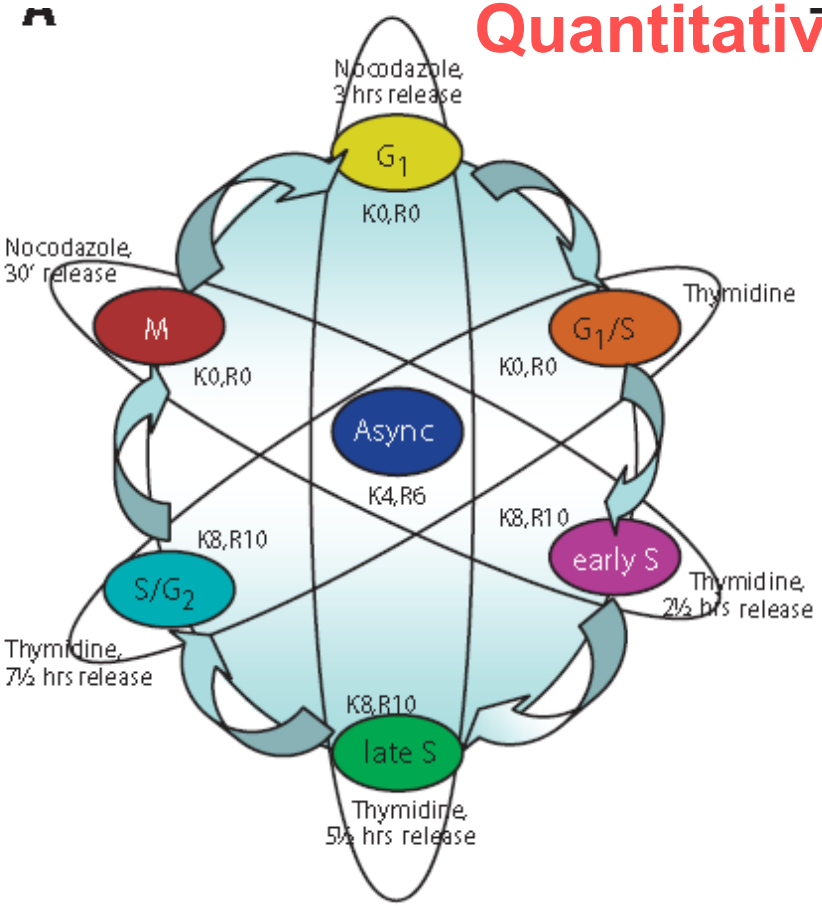


This example illustrates the principles of SILAC and mass spectroscopy signals (peaks).
m/z: mass over charge ratio of a peptide fragment

In the Olson et al. study, the authors used H and L forms to label different stages of the cell cycle.

Schwanhäuser et al. Nature 473, 337 (2011)

Quantitative proteomic analysis



Center: asynchronously growing cell population as internal standard to allow normalization between experiments.

HeLa S3 cells were SILAC-labeled with 3 different isotopic forms (light – medium – heavy) of arginine and lysine.

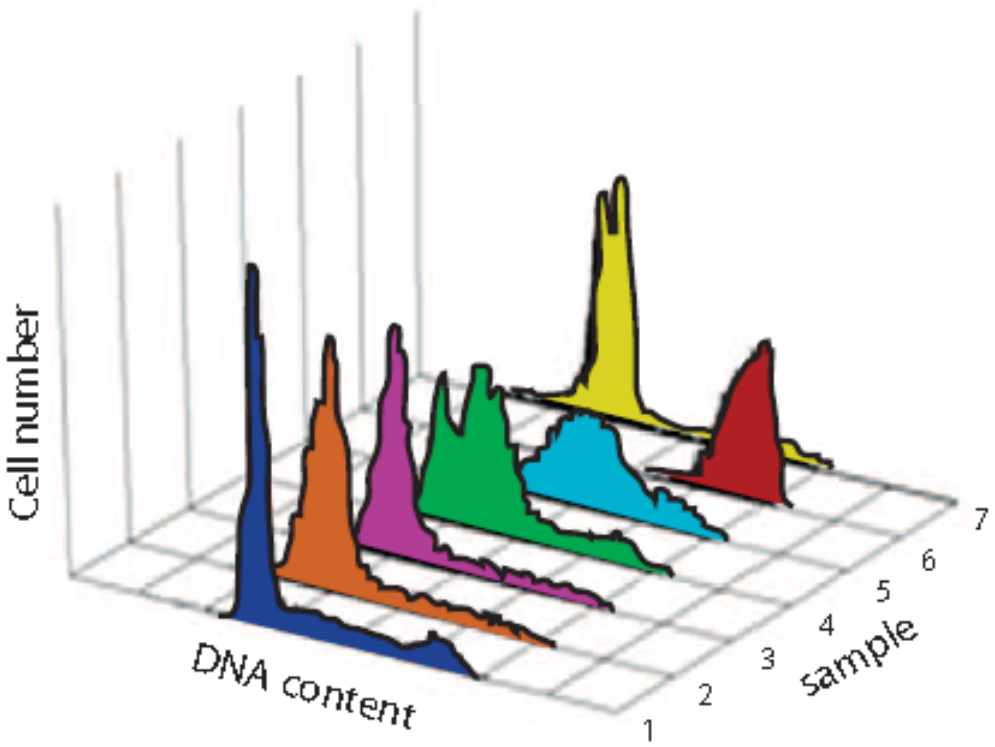
3 individual populations of heavy and light SILAC cells were synchronized with a **thymidine** block (analog of thymine, blocks entry into S phase).

Cells were then collected at 6 different time points across the cell cycle after release from the **thymidine arrest**.

Out of this, 2 samples were collected after a further **cell cycle arrest** with **nocodazole** and release. (Nocodazole interferes with polymerization of microtubules.)

FACS profiles of individual HeLa populations

	% Cells		
	G_1	S	G_2/M
1. Asynchronous	64	27	9
2. Thymidine block	50	46	4
3. Thymidine block + release 2½ h	36	60	4
4. Thymidine block + release 5½ h	23	70	7
5. Thymidine block + release 7½ h	15	70	15
6. Nocodazole block + release ½ h	1	11	88
7. Nocodazole block + release 3 h	82	12	6



Cells were fixed and collected by centrifugation.

Then the **DNA content** of the cells was determined with propidium iodide.

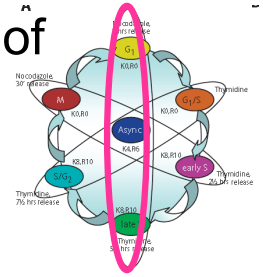
The DNA content is the basis for classifying the **state** along the cell cycle.

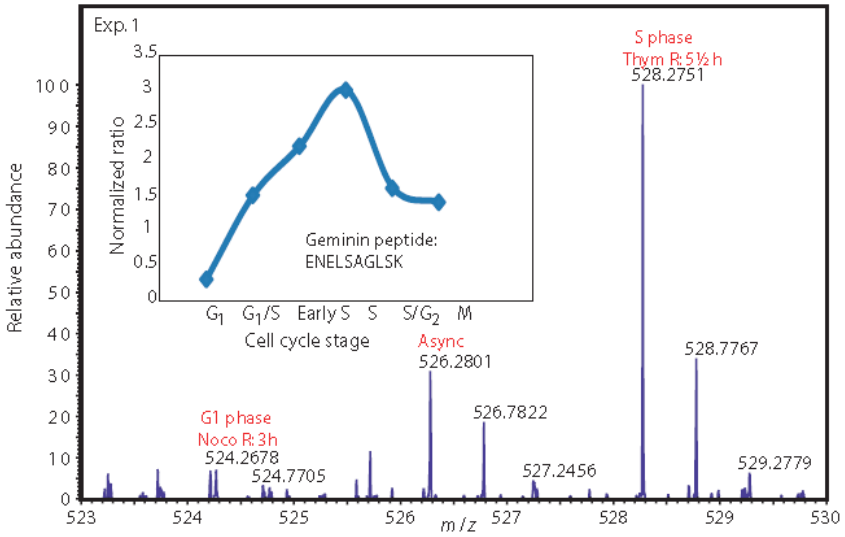
→ Samples 1 – 5 are not pure states, but **mixtures**.
Nocodazole block is quite efficient in synchronizing cells (samples 6 and 7).

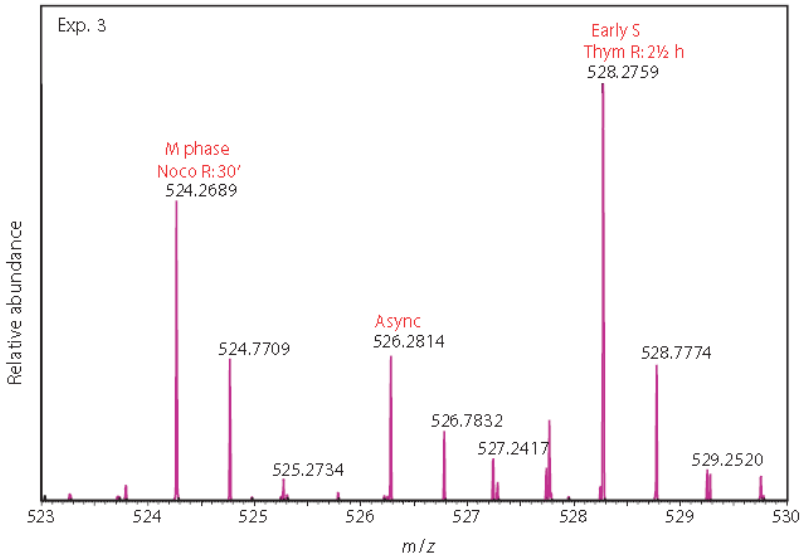
Olsen Science Signaling 3 (2010)

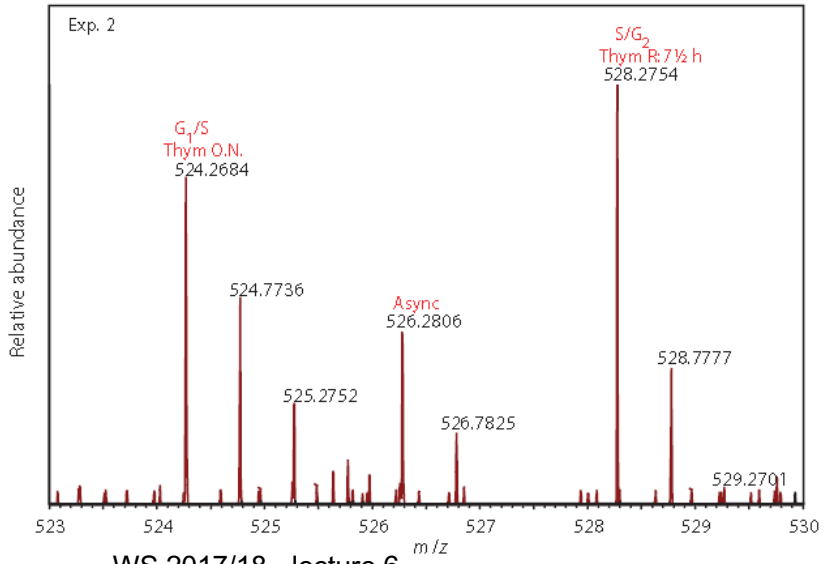
Monitor protein abundance by MS

Experiment 1: mixture of
L = G1 phase
M = Async
H = S phase









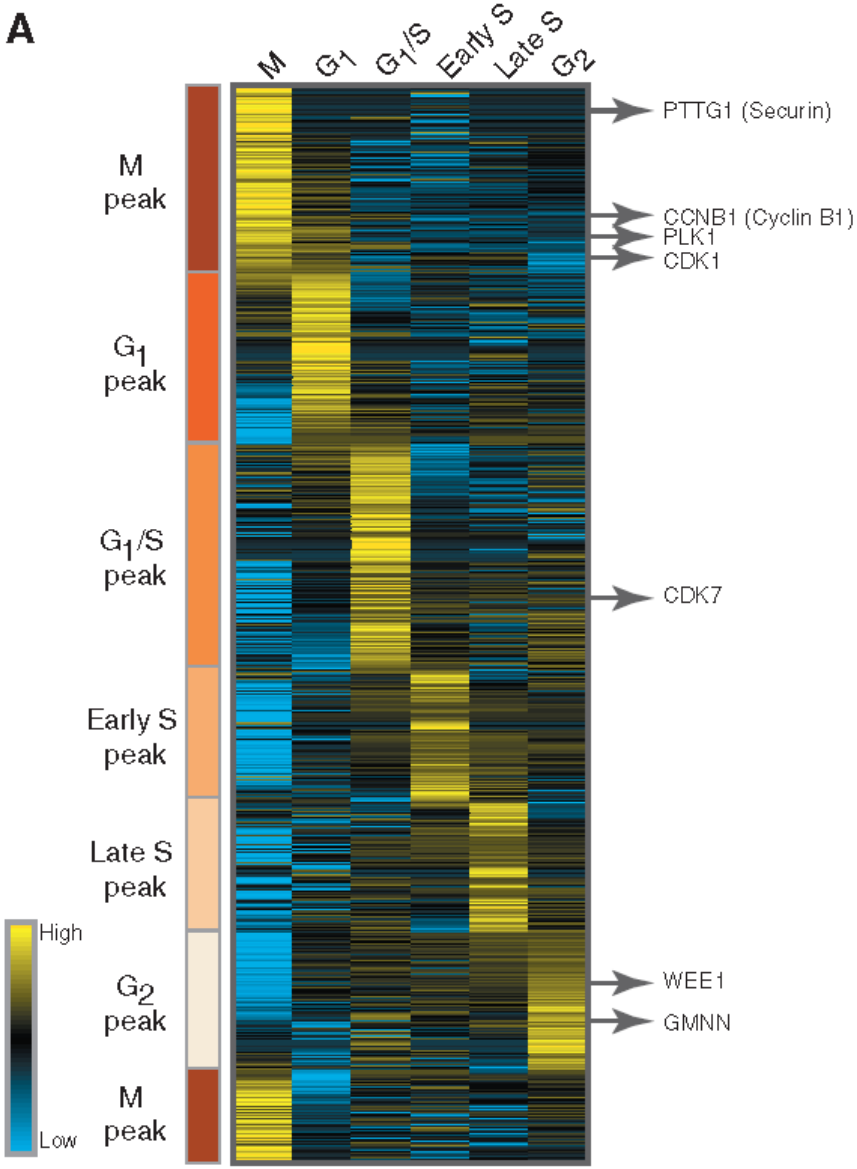
Representative MS data showing how the abundance of the proteins was monitored in 3 experiments to obtain information from the 6 stages of the cell cycle.

The data show the MS analysis of a tryptic SILAC peptide triplet derived from the cell cycle marker protein **Geminin**.

Relative peptide abundance changes were normalized to the medium SILAC peptide derived from the asynchronously grown cells in all three experiments.
The inset of Exp. 1 shows the combined six-time profile of Geminin over the cell cycle.

Olsen Science Signaling 3 (2010)

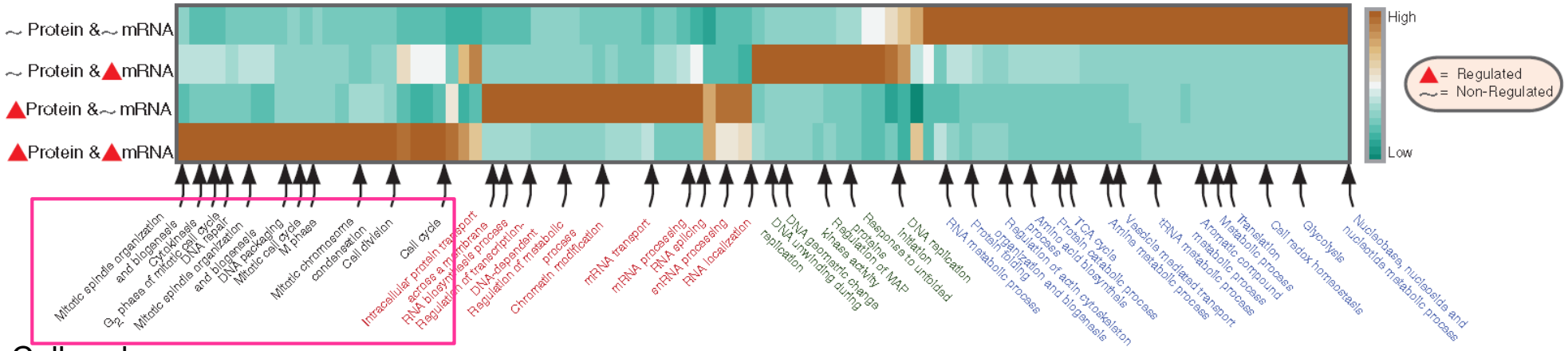
Dynamics of the proteome during the cell cycle



Proteins whose abundance changed at least fourfold during the cell cycle were clustered in all cell cycle stages by calculating a time peak index by weighted mean of the ratio of maximal abundance.

For each cell cycle stage, there are clear patterns of up- and down-regulation.

Comparison of mRNA and protein dynamics



Cell cycle processes

Comparison of mRNA and protein dynamics during the cell cycle.
Measured protein dynamics were correlated to published mRNA data.

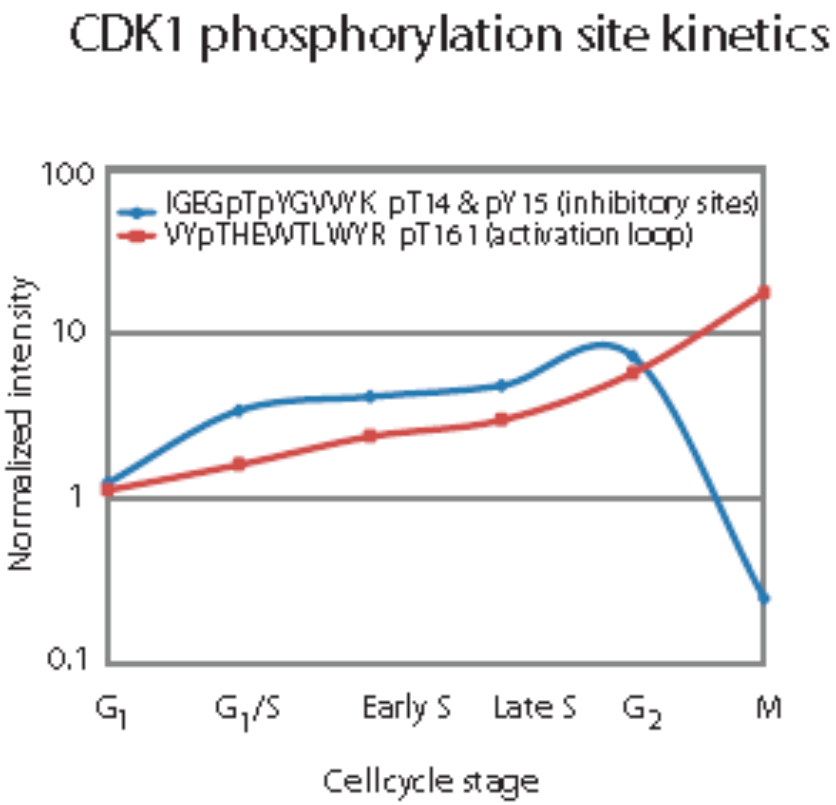
Proteins were grouped on the y axis in 4 categories from top to bottom:

- unchanging mRNA and protein
- changing mRNA and unchanging protein
- unchanging mRNA and changing protein
- and changing mRNA and changing protein.

The x axis shows clustered gene ontology (GO) biological process terms enriched in at least one of the above 4 categories.

High and **low** represent statistical over- or underrepresentation, respectively.

Example: Dynamic phosphorylation of CDK1

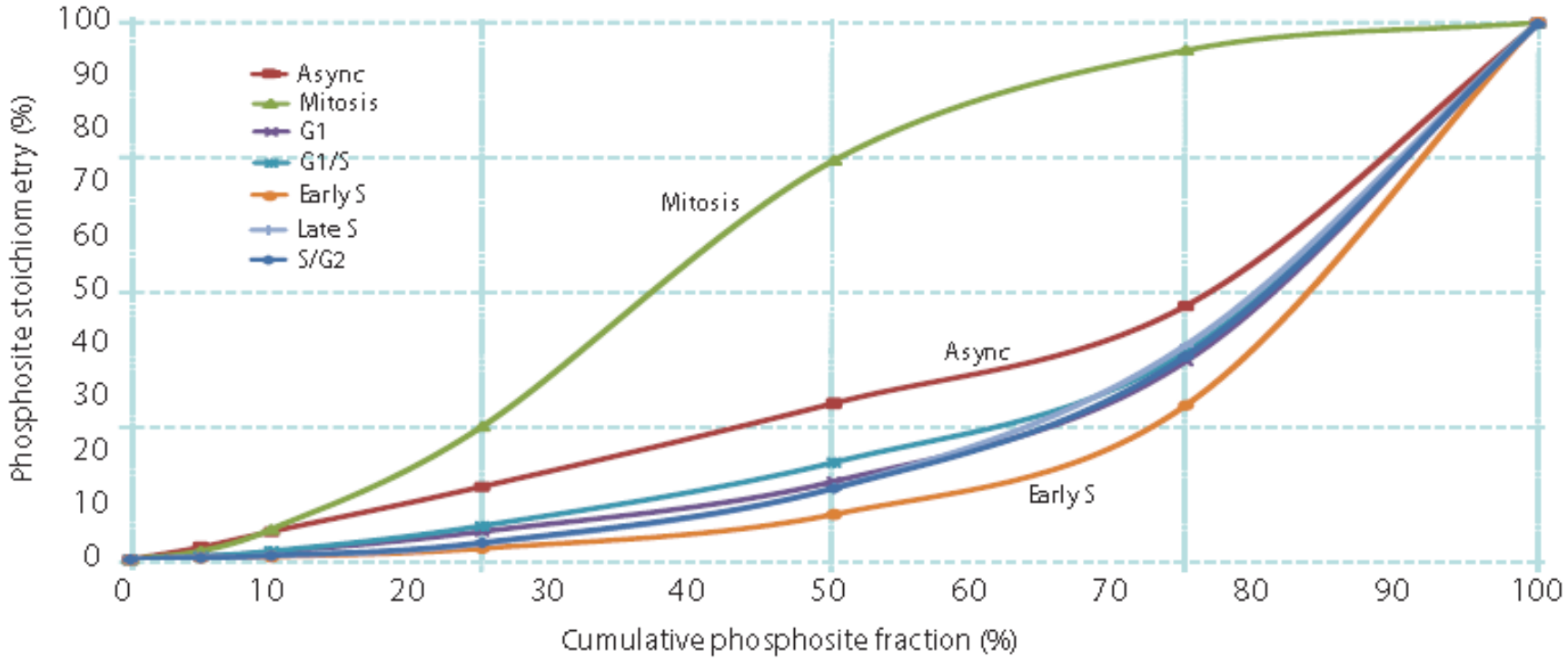


Dynamic profile of two CDK1 phosphopeptides during the cell cycle.

The activating site Thr161 (red) peaks in mitosis, whereas phosphorylation of the inhibitory sites Thr14 and Tyr15 (blue) is decreased in mitosis

Olsen Science Signaling 3 (2010)

Total phosphosite occupancy in different stages of cell cycle



Fifty percent of all mitotic phosphorylation sites have occupancy of 75% or more.

Olsen Science Signaling 3 (2010)

Summary

Phosphorylation of protein residues is an important mechanism to regulate protein structure, protein activity, protein localization, and protein interactions.

About 70% of all cellular proteins are phosphorylated to some extent.

Phosphorylation is a dynamic state variable during the cell cycle.

Phosphorylation levels are controlled by the ca. 518 different human kinases as well as by phosphatases.

-> these are important potential drug targets (problem is achieving specificity)

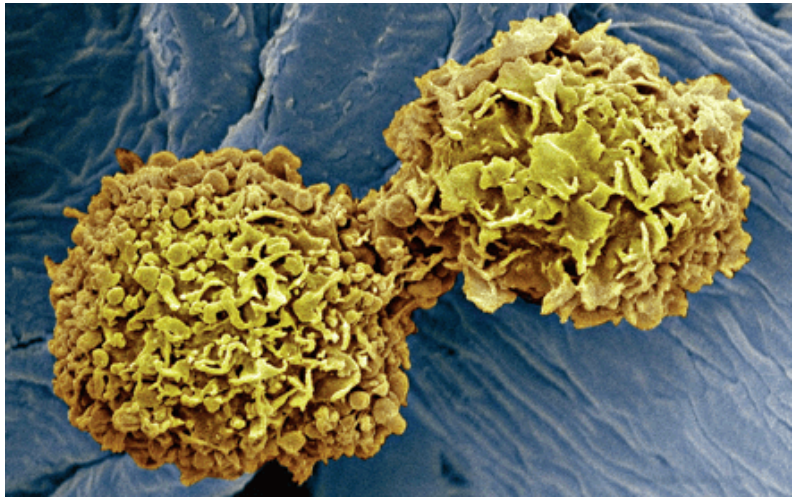
Subject of Minitest #2 is also
Paper #5:
CDK Substrate Phosphorylation and Ordering the Cell Cycle
Swaffer, Matthew P. … Nurse, Paul
Cell , Volume 167 , Issue 7 , 1750 - 1761 (2016)

V7: CDK inhibitors

Cancer is characterized by **aberrant cell cycle activity**.

This occurs either as result of **mutations** in **upstream signaling pathways** or by **genetic lesions** within genes encoding cell cycle proteins.

Aberrant activation of CDKs, which is frequently seen in human cancers, provided a rationale for designing synthetic inhibitors of CDKs as anticancer drugs.



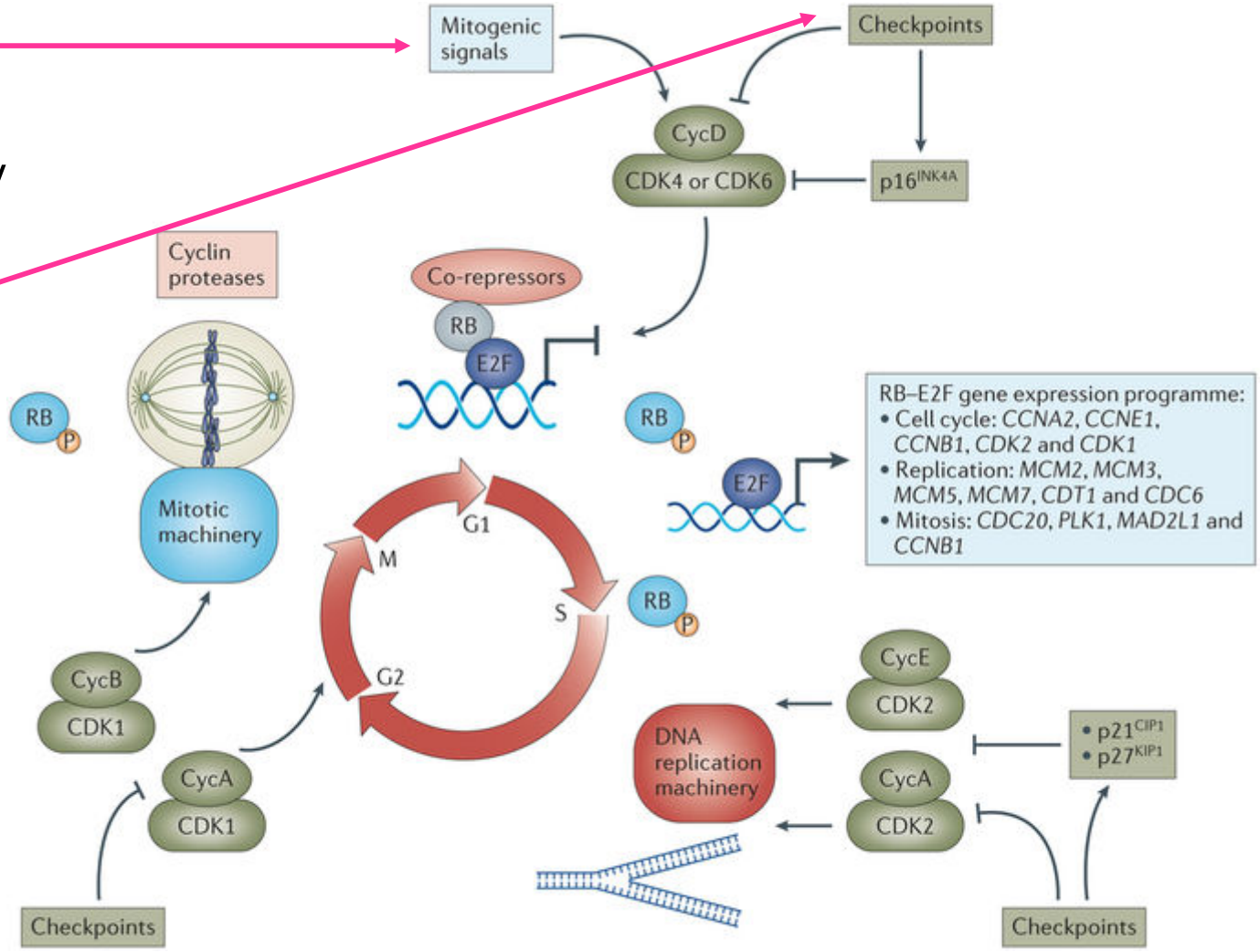
A dividing cancer cell.

http://www.nature.com/articles/nrd4504 (2015)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5345933/
http://science.sciencemag.org/content/345/6199/865.full
Mol Cancer Ther **15** 2273-2281 (2016)

Review: Progression of the human cell cycle driven by CDKs

Mitogenic signals stimulate CDK4 and CDK6 and promote entry into the cell cycle.

In contrast, **antiproliferative checkpoints** inhibit CDK4 and CDK6 activity or induce the expression of the CDK4 and CDK6 inhibitor **p16**^{INK4A} (compare lecture V5, p.12).



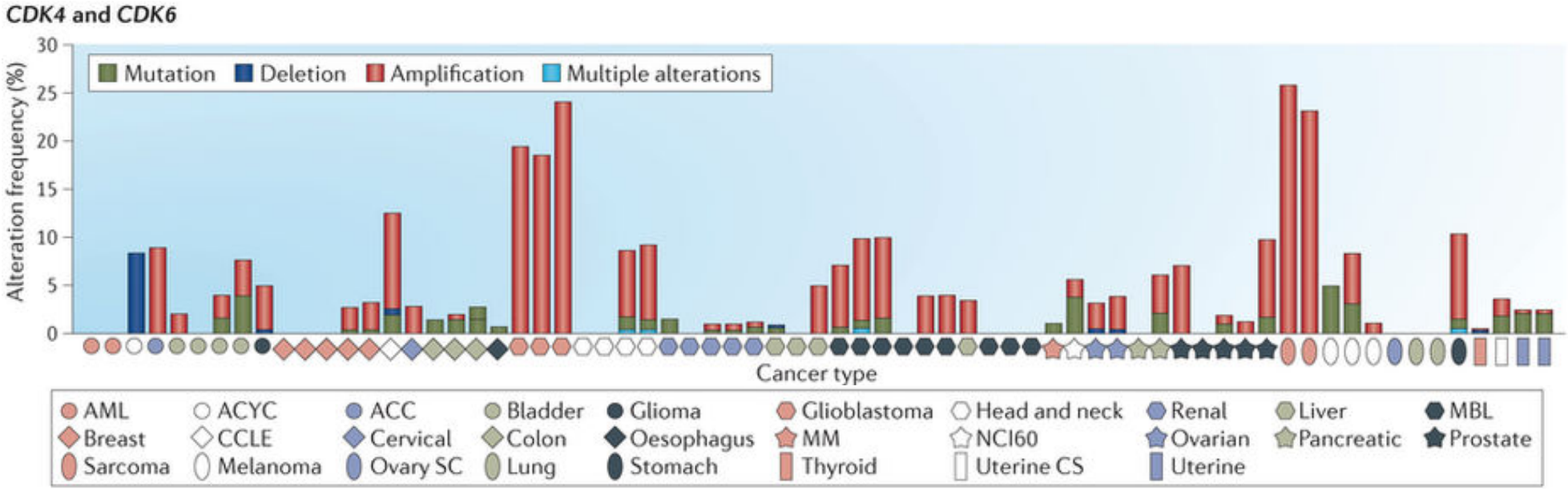
http://www.nature.com/articles/nrd4504

Cdk phosphorylation events in Rb

Sites	Domain	Structural Effect	Biochemical Output
S249/T252	RbN	Unknown	Inhibits protein interactions with RbN
T356	RbIDL	C-terminal helix of RbN becomes disordered	Unknown
T373	RbIDL	Nucleates N-terminal pocket helix to induce RbN-pocket association	Inhibits $E2F^{TD}$ and LxCxE binding to pocket domain
S608/S612	RbL	RbL binds pocket	Inhibits $E2F^{TD}$ binding
S780	Pocket	Unknown	Unknown
S788/S795	RbC	Unknown	Inhibits RbC-$E2F1^{MB}$-UP^{MB} binding
S807/S811	RbC	Unknown	Might prime phosphorylation at other sites
T821/T826	RbC	Induces RbC binding to the pocket domain	Inhibits RbC-$E2F1^{MB}$-DP^{MB} binding and inhibits LxCxE binding to pocket domain.

Trends Biochem Sci. 2013 Jan; 38(1): 12–19.

Deregulation of CDK regulatory genes in cancer.



Frequencies of genetic amplification of *CDK4* and *CDK6* across multiple disease sites.

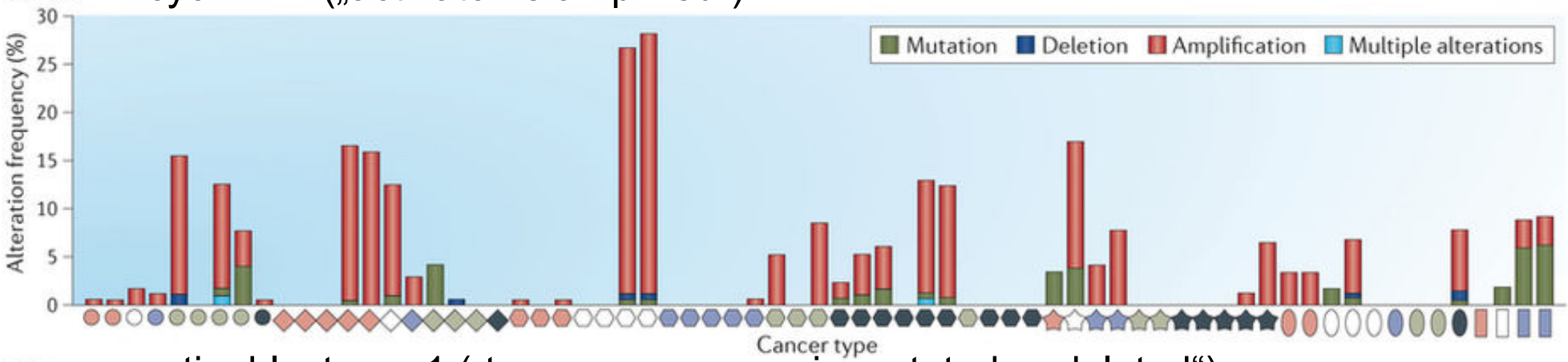
The frequencies of mutation (green), amplification (red) and homozygous deletion (dark blue) were determined using genetic data from >2,000 cancer cases.

Different types of cancer exhibit distinct predominant mechanisms of genetic alterations in cell cycle control.

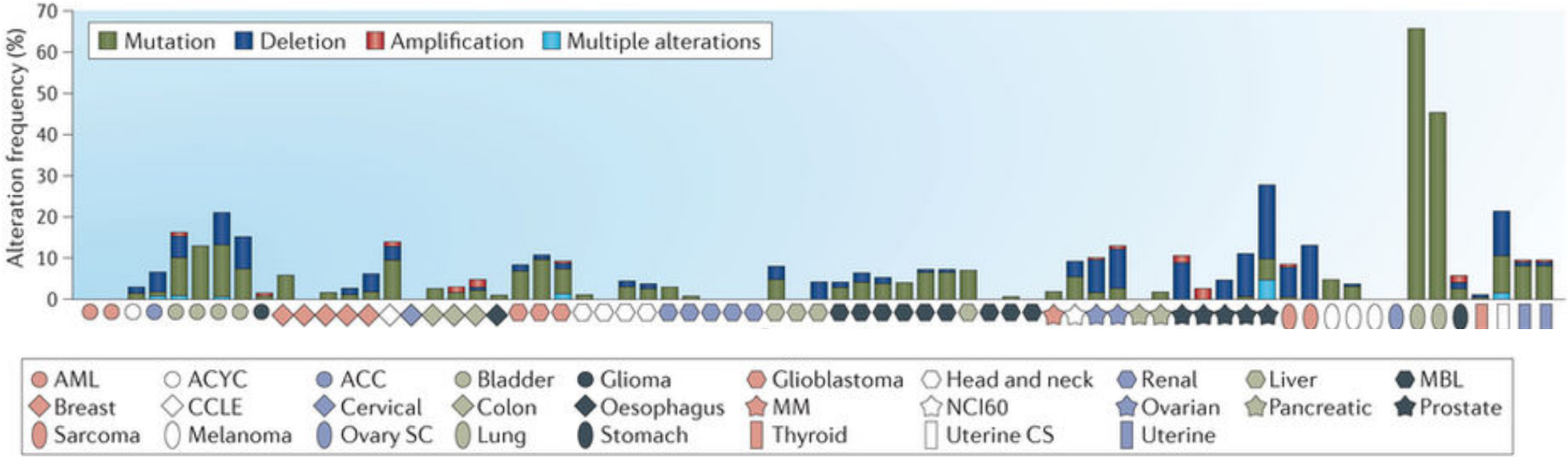
http://www.nature.com/articles/nrd4504

Deregulation of CDK regulatory genes in cancer.

CCND1 cyclin D1 („activator is amplified“)



RB1 retinoblastoma 1 („tumor suppressor is mutated or deleted“)



http://www.nature.com/articles/nrd4504

First generation of CDK inhibitors

Over the past 20 years, several small molecule inhibitors of CDKs have been developed as potential cancer therapeutics and tested in numerous trials and in several tumor types.

The first-generation CDK inhibitors developed were relatively nonspecific and may therefore be referred to as **'pan- CDK' inhibitors**.

Of these inhibitors, **flavopiridol** is the most extensively investigated CDK inhibitor so far, with >60 clinical trials carried out between 1998 and 2014.

Although flavopiridol can induce **cell cycle arrest** in G1 and G2 phases, in certain contexts it also induces a **cytotoxic response**.

Flavopiridol did not meet the initial high expectations. Low levels of clinical activity were seen in Phase II studies in several solid tumor types

Despite extensive investment, no Phase III studies have emerged and drug development of flavopiridol was discontinued in 2012.

http://www.nature.com/articles/nrd4504

Second generation inhibitors of multiple CDKs

Other CDK inhibitors were developed with the aim of increasing selectivity for CDK1 and CDK2 and/or increasing overall potency.

Again, numerous CDK inhibitors seemed to be particularly promising in preclinical studies, but only a few progressed past Phase I clinical trials.

http://www.nature.com/articles/nrd4504

Reasons for failure of broad-specificity CDK inhibitors

The general failure of non-selective CDK inhibitors in the clinic can be partly explained by at least 3 key underlying principles.

(1) There was a lack of clear understanding of the **mechanism of action**. For many of the CDK inhibitors with low specificity, there remains a lack of clarity with regard to which CDKs are actually being inhibited *in vivo* and therefore the corresponding mechanism that could underlie the therapeutic effect.

(2) There was a lack of **appropriate patient selection**. The vast majority of studies conducted with CDK inhibitors with low specificity were in unstratified patient cohorts. This is because there are essentially no **biomarkers** that may select for sensitive subpopulations for this class of inhibitors.

(3) There is a lack of a **therapeutic window**. Many of these CDK inhibitors target several proteins that are critical to the **proliferation** (e.g. CDK1) and **survival** (e.g. CDK9) of **normal cells**. This limits the ability to achieve therapeutic levels of these drugs because of their intrinsic inability to discriminate between cancerous and healthy tissues.

http://www.nature.com/articles/nrd4504

The Palbociclib story

In 2017, palbociclib was approved by the Food and Drug Administration (*FDA*) as a first-in-class cyclin-dependent kinase (CDK) 4/6 inhibitor,

Palbociblib has traveled a long and tortuous road. It is the product of a project started in 1995 by researchers at Parke-Davis, a now-vanished drug company.

Palbociclib blocks key enzymes driving the cell cycle.

Mounting scientific evidence suggested its potential in breast cancer.

Yet Pfizer, where the compound was ultimately synthesized by the Parke-Davis team after Pfizer acquired their company, later shelved the then-unique drug for much of a decade.

In the end, it took several dedicated outside researchers to demonstrate the worth of this drug.

Garber K. The cancer drug that almost wasn't. Science. 2014;345:865–7.

Paper #6 is not subject of minitest 2