V8: Cell cycle – summary

(1) Course evaluation

(2) Content of minitest #2:

- Lecture V5 (slides 15, 16, 18-20),
- V6 (slides 1-5,8,18)
- V7 (slides 1-2,18-20)

- Specified content from Papers 4 to 6:
  methods, results and discussion section related to the indicated figures.

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-deletion mutants?

Plan: investigate the dynamics of genome-wide transcription in budding yeast cells that are **disrupted** for all S-phase and mitotic **cyclins** (Δclb1,2,3,4,5,6).

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, Δclb1,2,3,4,5,6 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.

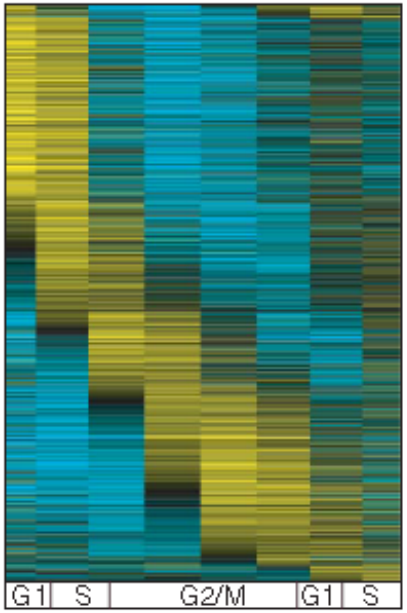
For each gene, *i*, a Fourier score, F_i, was computed as

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

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.

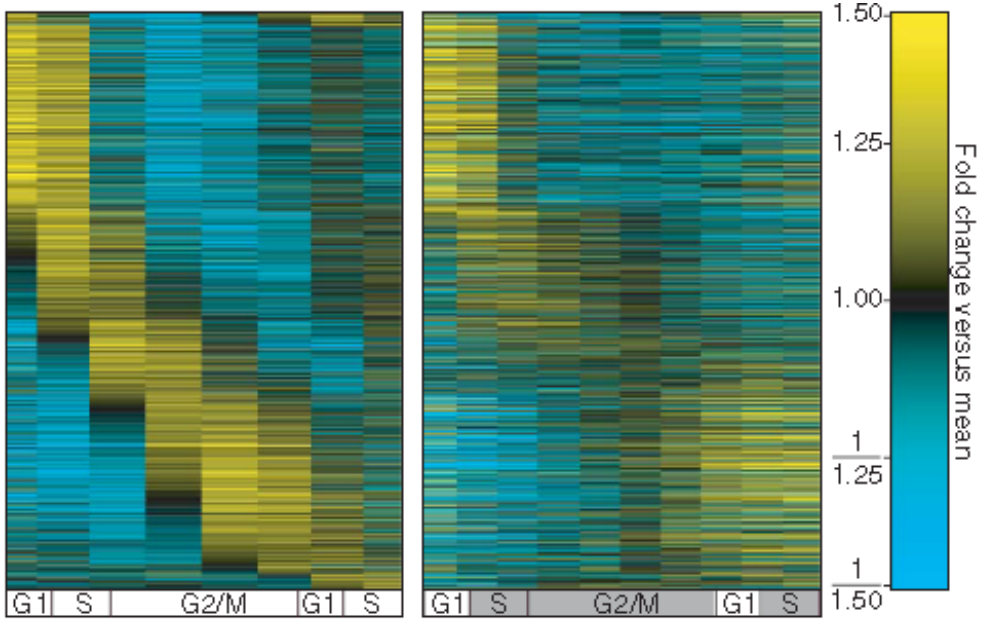
Periodic transcripts in wt and cyclin-mutant cells

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)

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

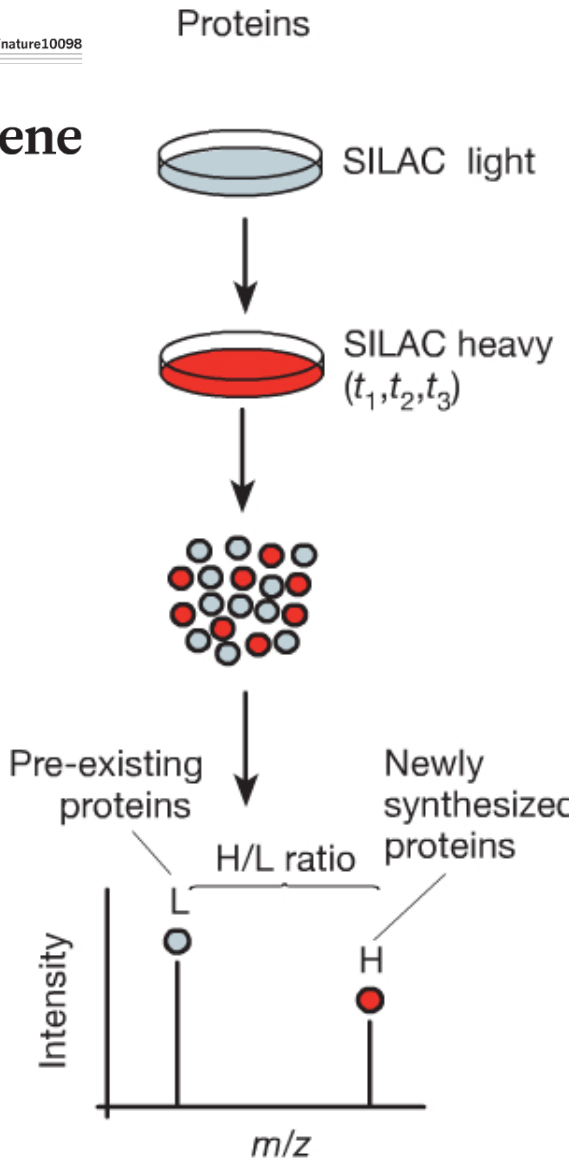
doi:10.1038/nature10098

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)

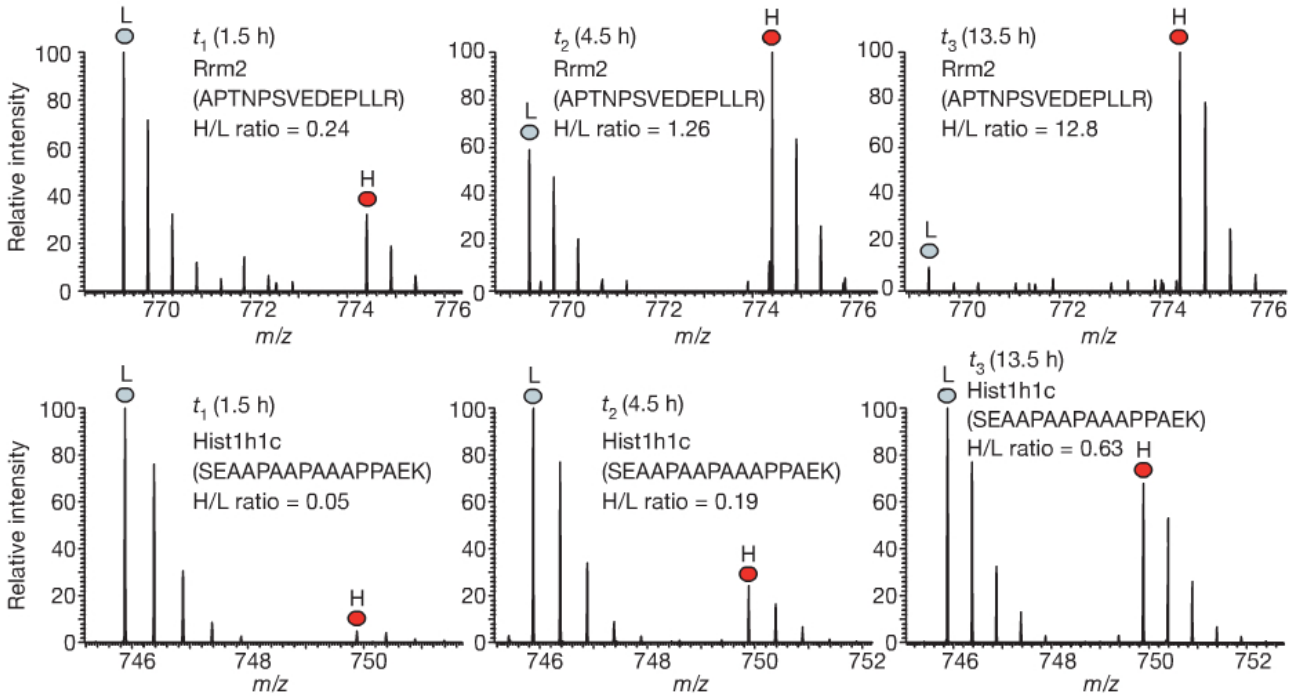
H/L ratios of individual proteins

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.

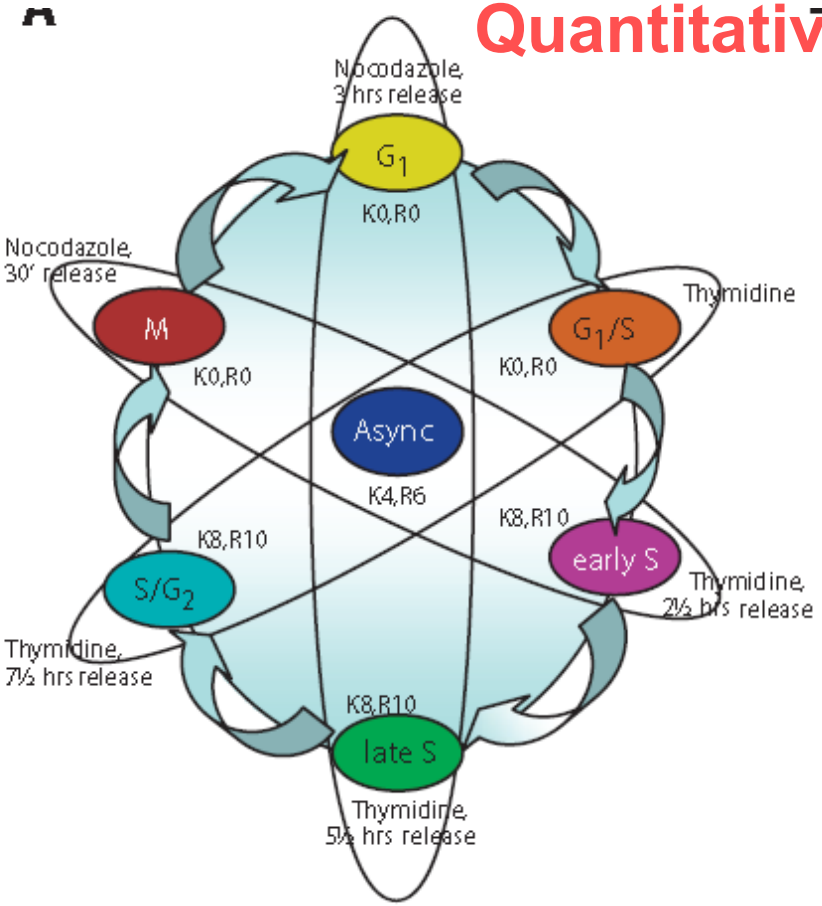


This example illustrates the principles of SILAC and mass spectroscopy signals (peaks).
m/z: mass over charge ratio of a peptide fragment

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.

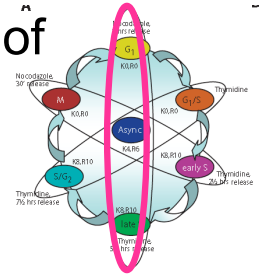
3 individual populations of heavy and light SILAC cells were synchronized with a **thymidine** block (analog of thymine, blocks entry into S phase).

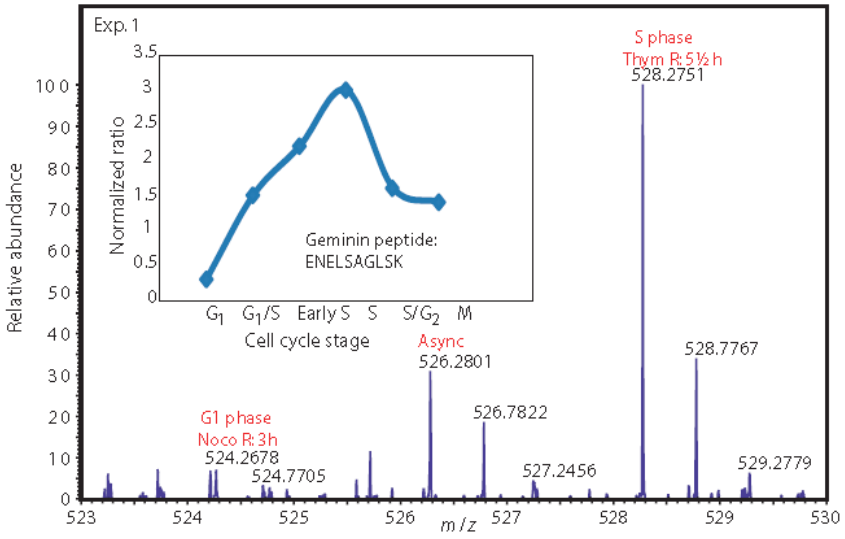
Cells were then collected at 6 different time points across the cell cycle after release from the **thymidine arrest**.

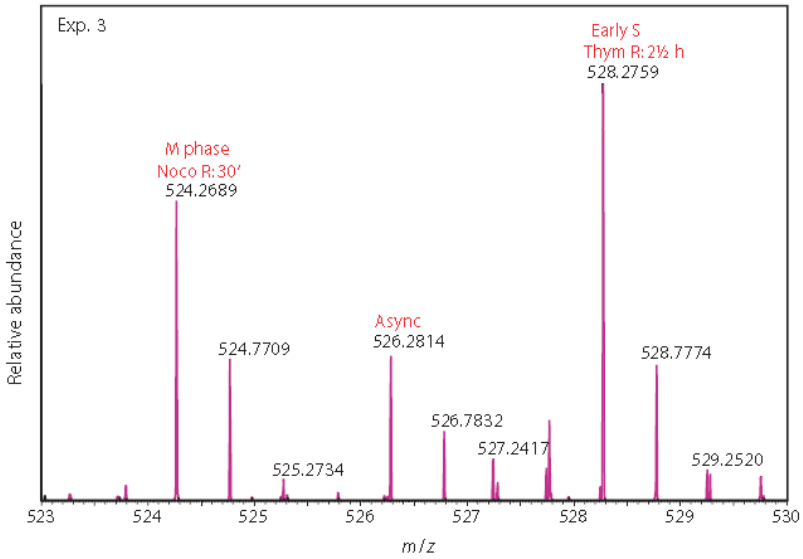
Out of this, 2 samples were collected after a further **cell cycle arrest** with **nocodazole** and release. (Nocodazole interferes with polymerization of microtubules.)

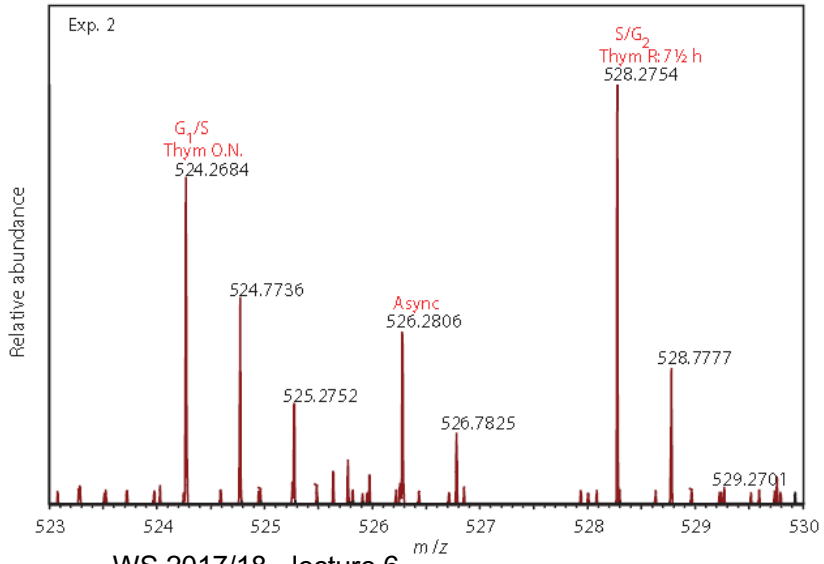
Monitor protein abundance by MS

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









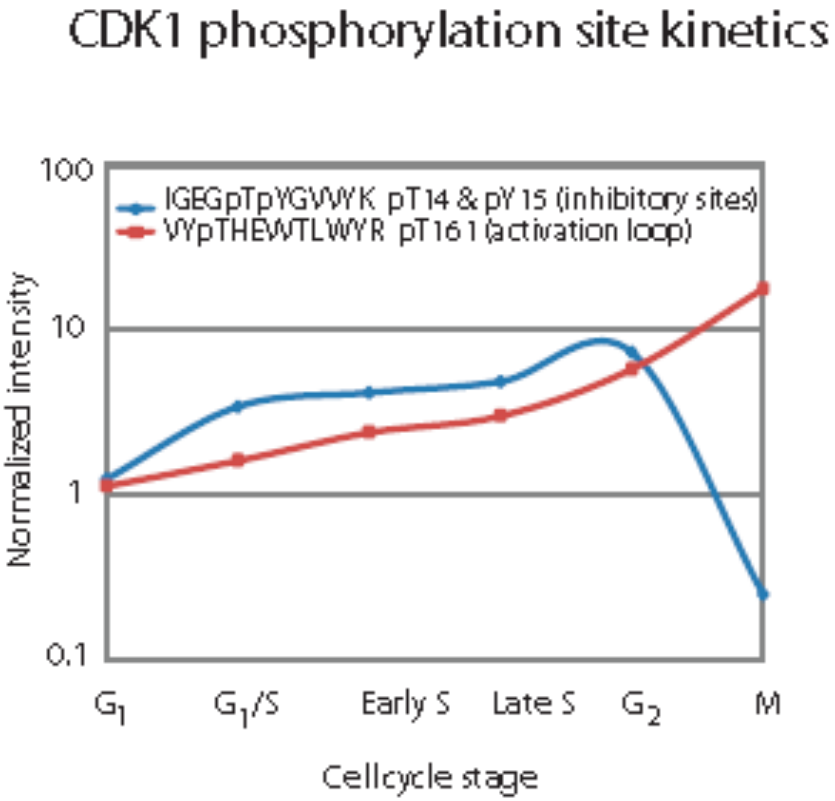
Representative MS data showing how the abundance of the proteins was monitored in 3 experiments to obtain information from the 6 stages of the cell cycle.

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)

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

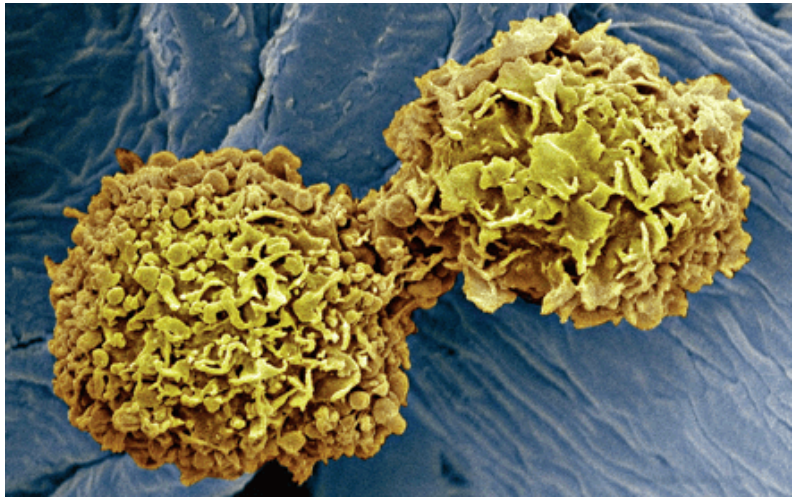
Olsen Science Signaling 3 (2010)

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.



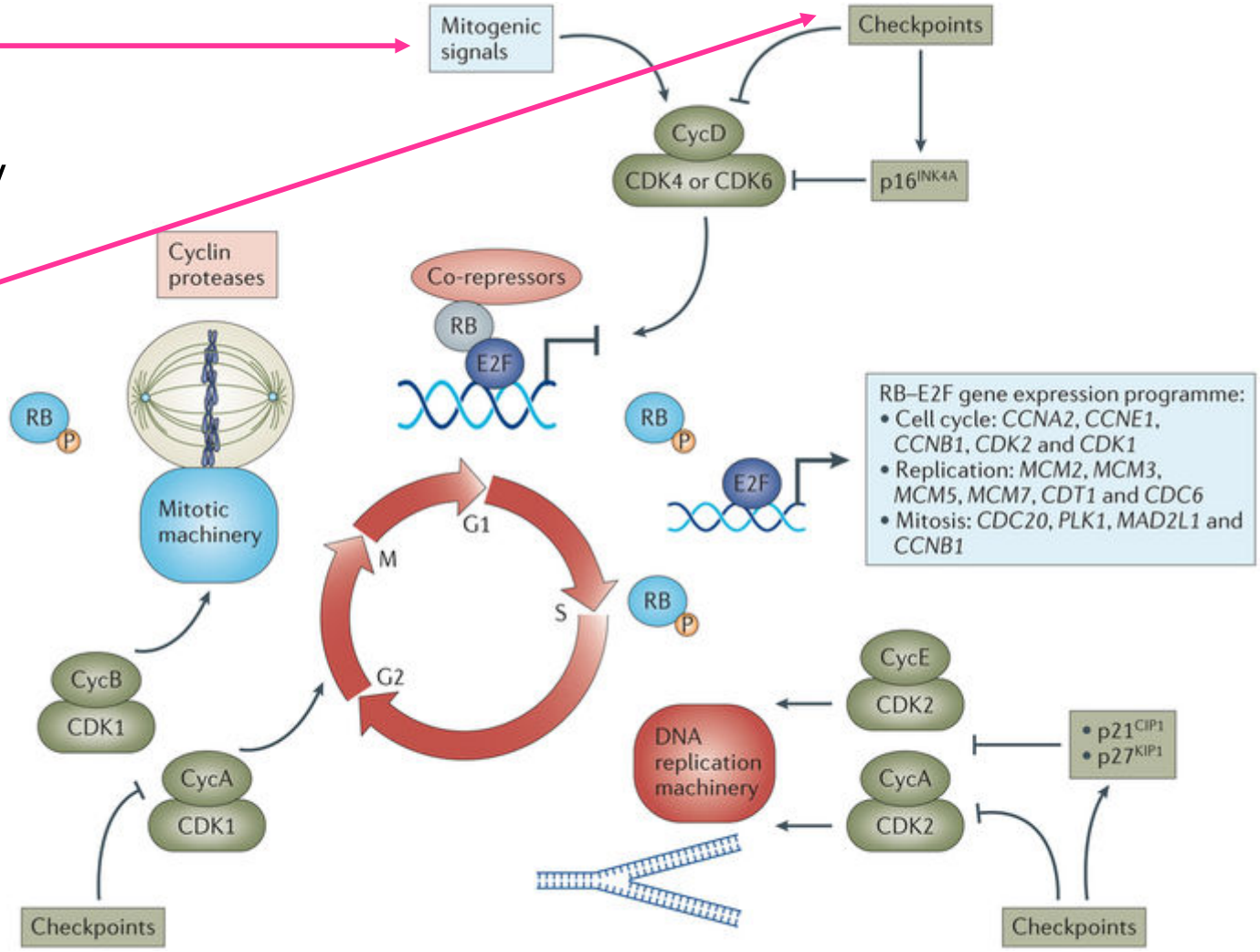
A dividing cancer cell.

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).



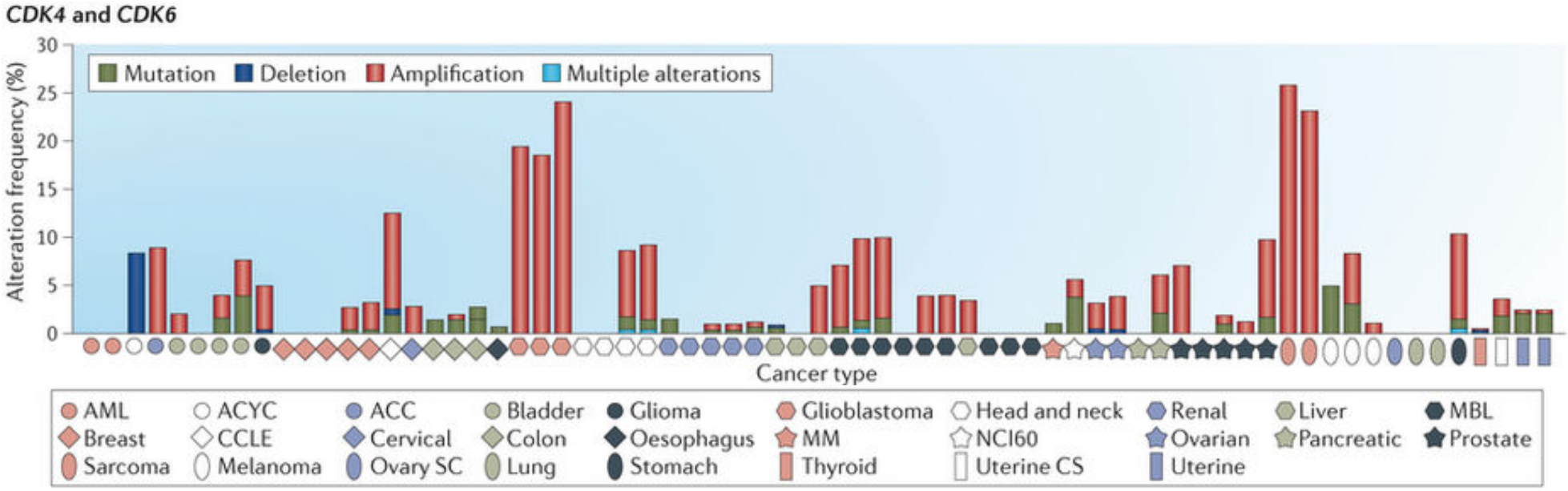
http://www.nature.com/articles/nrd4504

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.

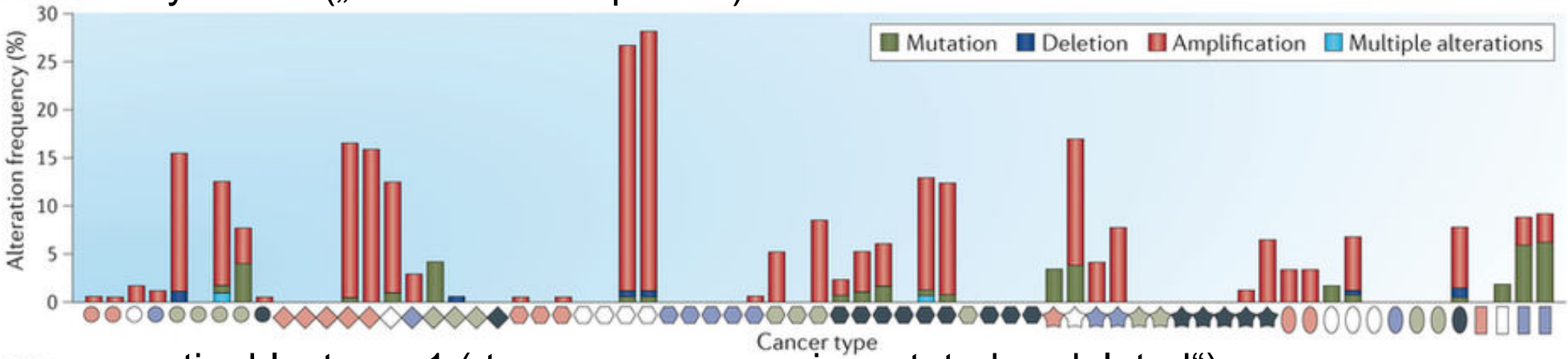
The frequencies of mutation (green), amplification (red) and homozygous deletion (dark blue) were determined using genetic data from >2,000 cancer cases.

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

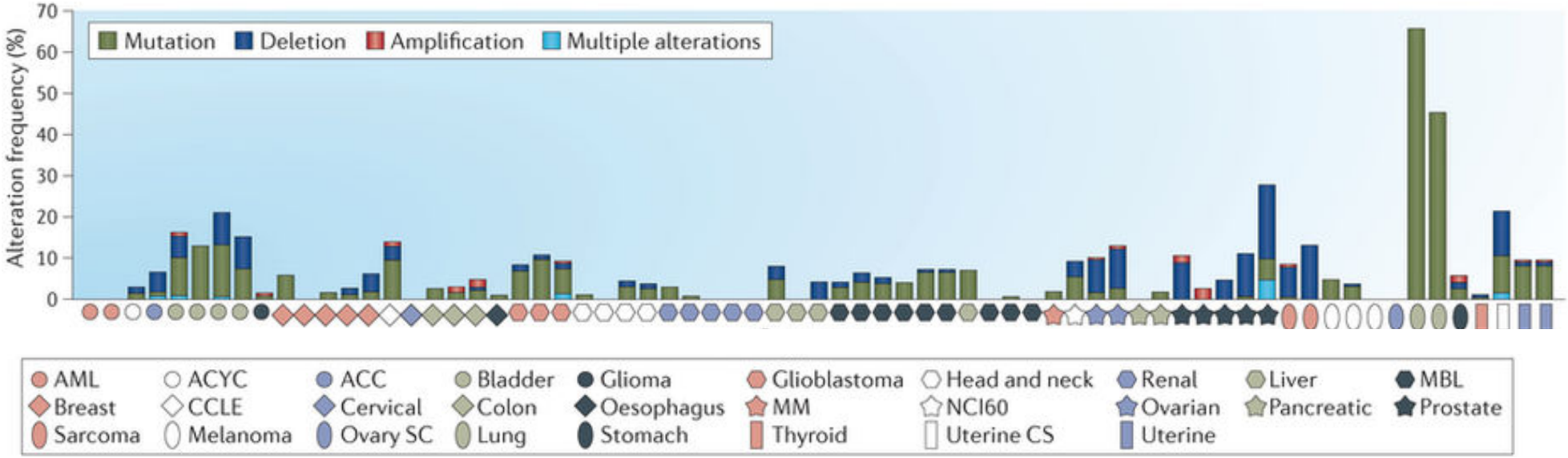
http://www.nature.com/articles/nrd4504

Deregulation of CDK regulatory genes in cancer.

CCND1 cyclin D1 („activator is amplified“)



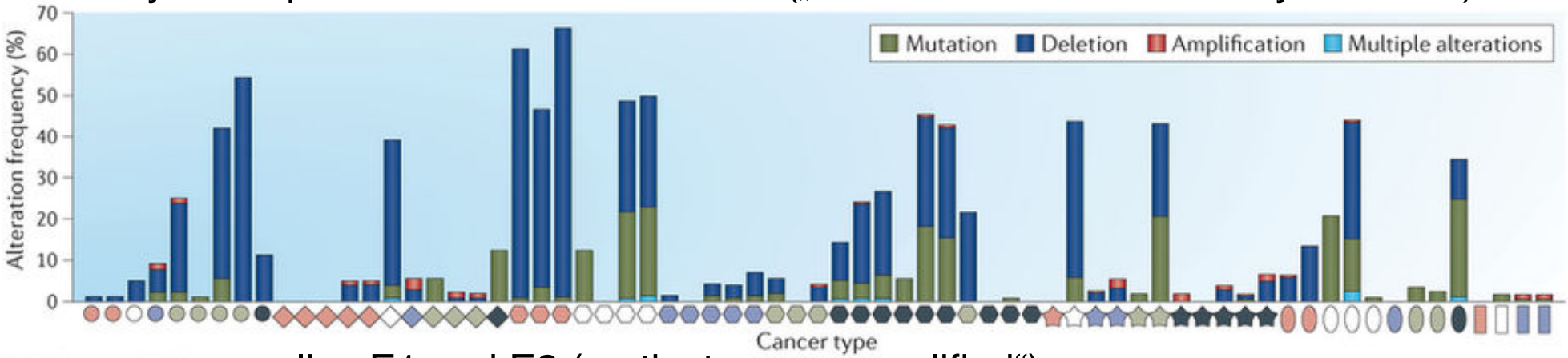
RB1 retinoblastoma 1 („tumor suppressor is mutated or deleted“)



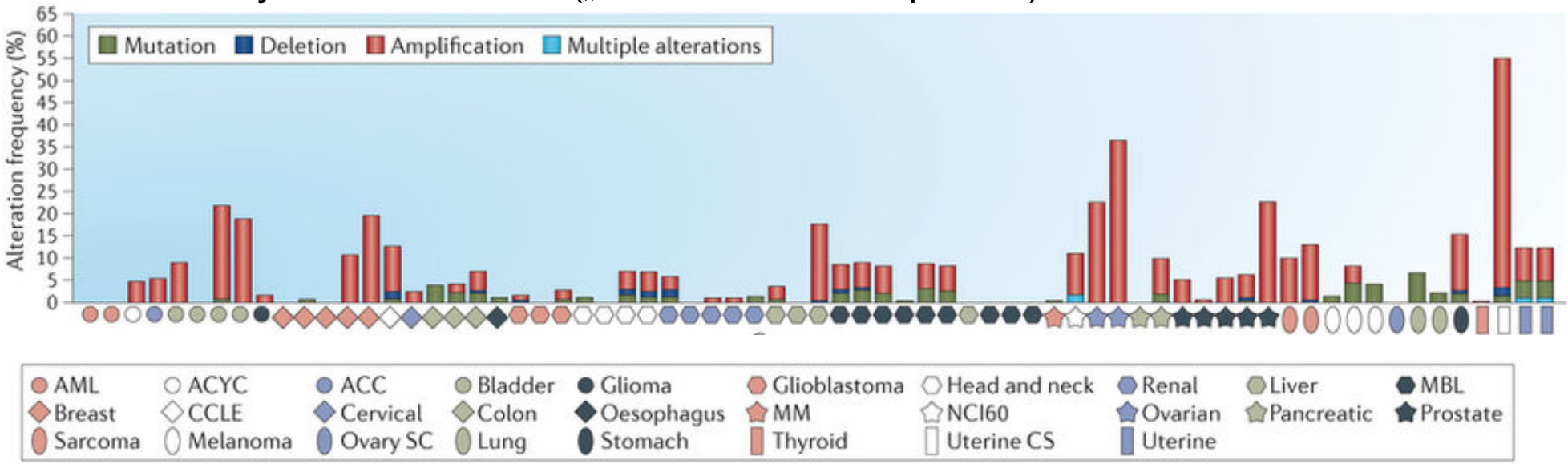
http://www.nature.com/articles/nrd4504

Deregulation of CDK regulatory genes in cancer.

CDKN2A cyclin-dependent kinase inhibitor 2A („inhibitors are shut down by deletion“)



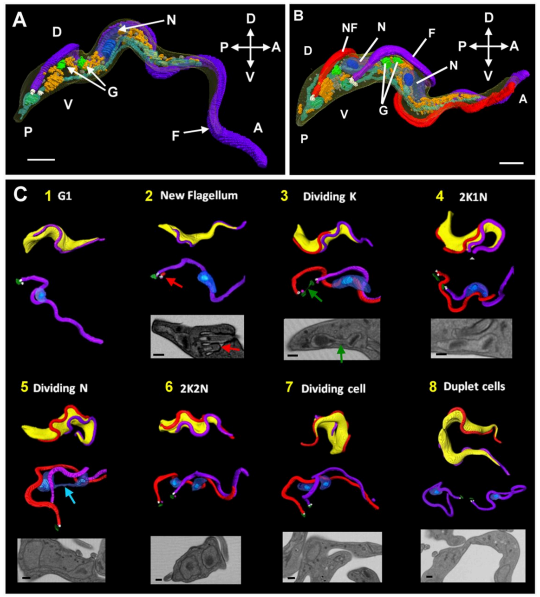
CCNE1 and *CCNE2* cyclins E1 and E2 („activators are amplified“)

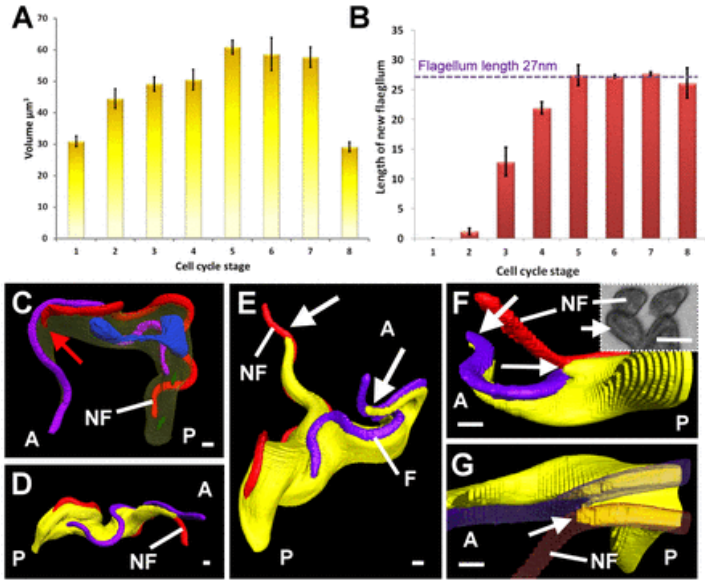


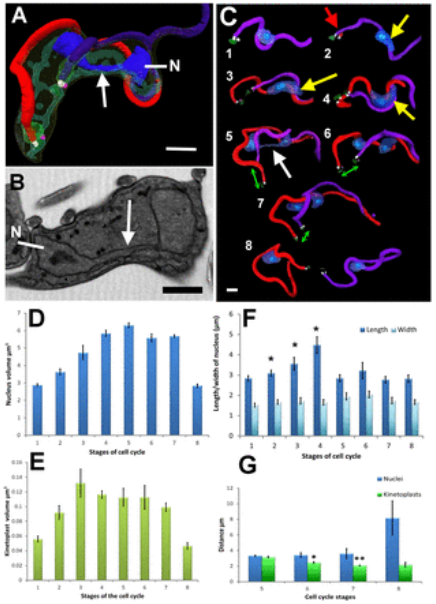
http://www.nature.com/articles/nrd4504

Content from paper 4 that is relevant for mini test #2

ONLY: methods and results related to Figs 1, 2, 3

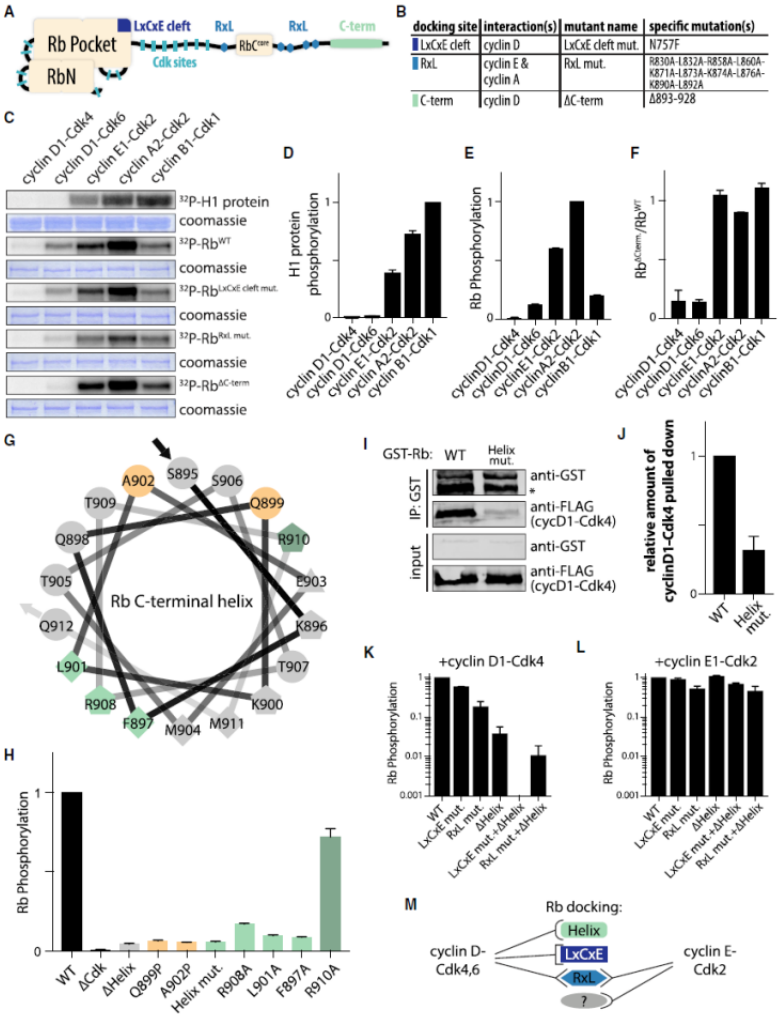


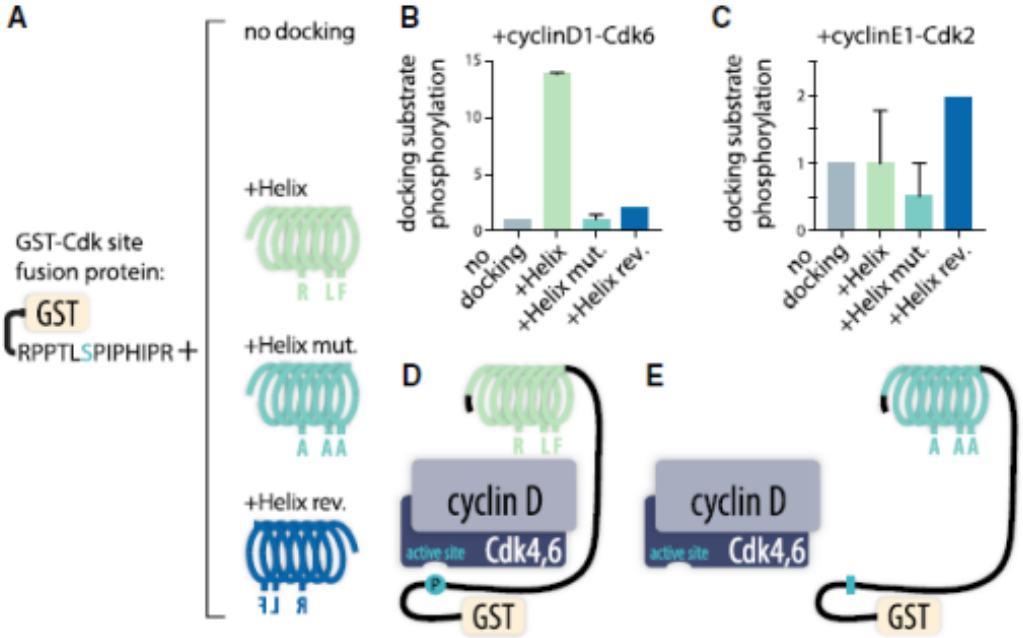




Content from paper 5 that is relevant for mini test #2

ONLY: methods and
results related
to Figs 1 and 2





Content from paper 6 that is relevant for mini test #2

ONLY: methods and
results related
to Figs 2 and 3