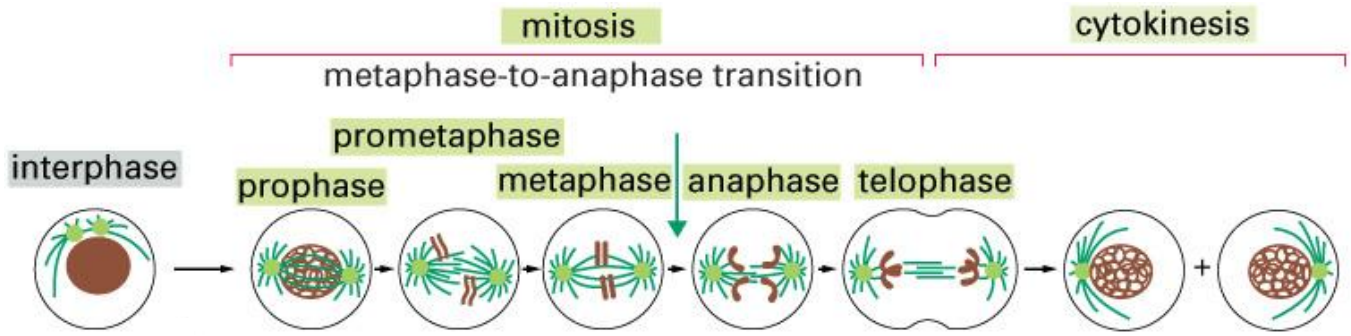
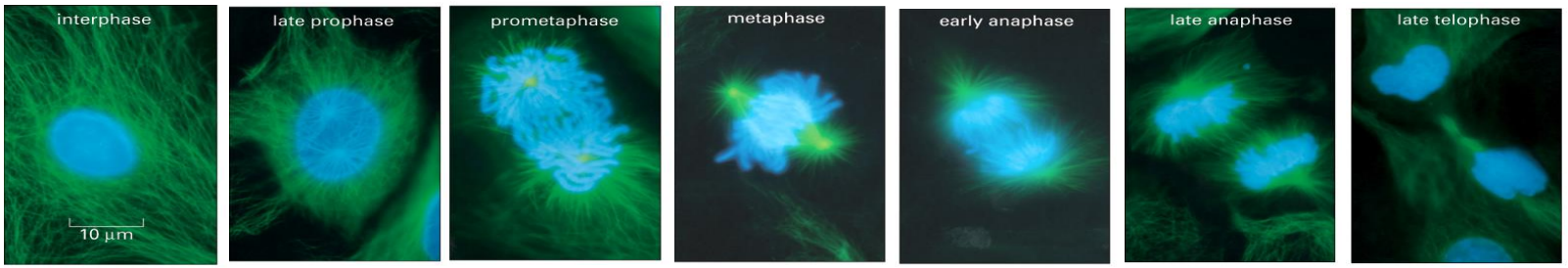
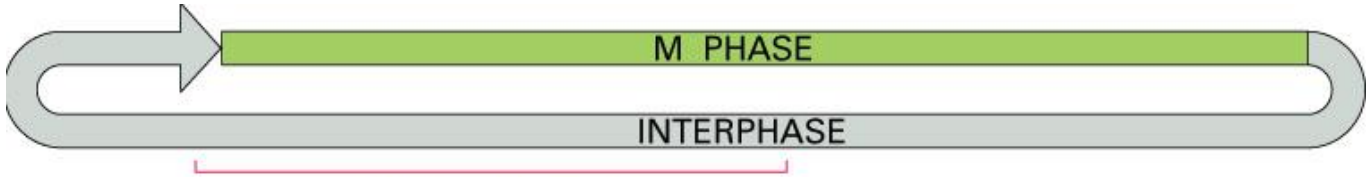
Mitosis, cell cycle, cell proliferation

Manfred Kubbies, Dept. Human Genetics, Univ. Würzburg, 2014

Mitosis / cell division





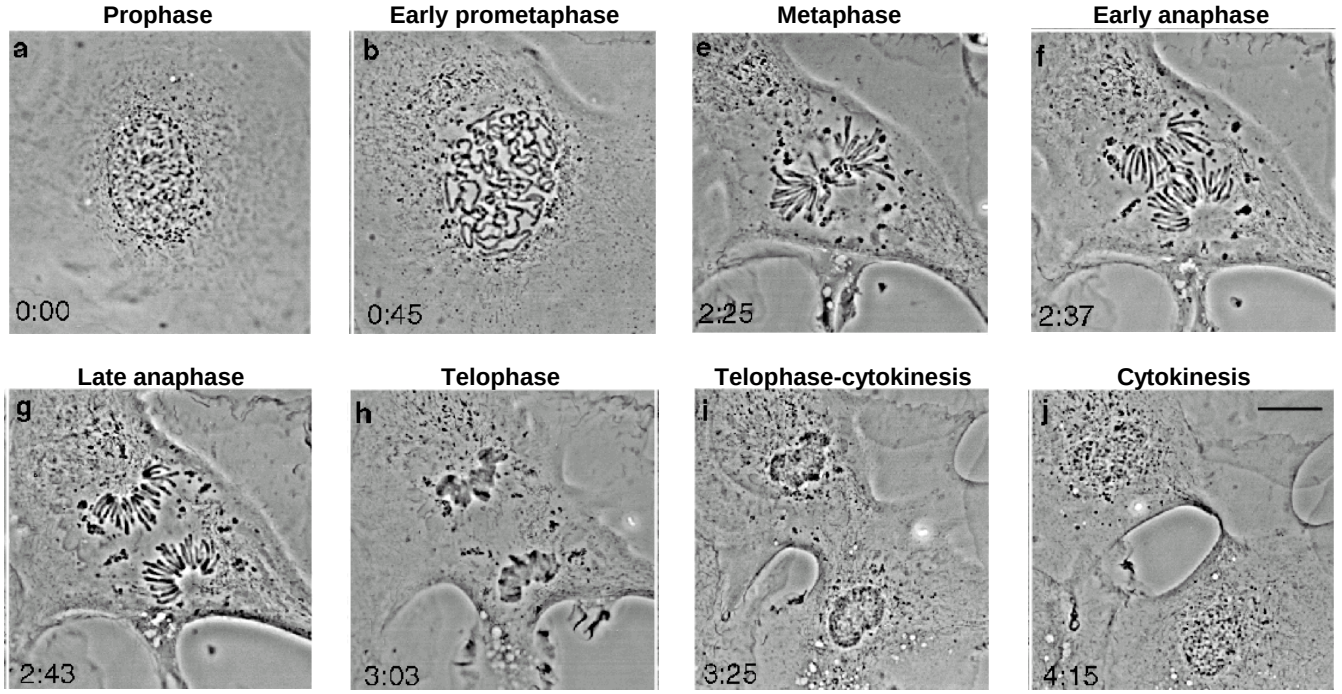


G2 phase **DNA replication** **G1 phase**

Note: the cell size increases during interphase !

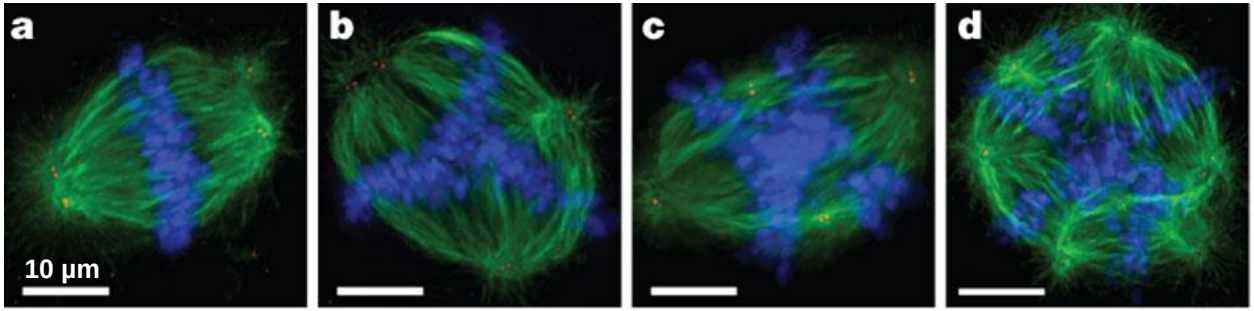
Alberts, Molecular Biology of the Cell, 2002

Mitosis and cytokinesis



Multipolar mitoses

Multipolar mitotic structures were detected in cultured hepatocytes by visualizing DNA (blue), microtubules (green) and centrioles (red). 4c hepatocytes contained bipolar (a) or multipolar spindles (b, c). Multipolar spindles were also seen in 8c hepatocytes (d).

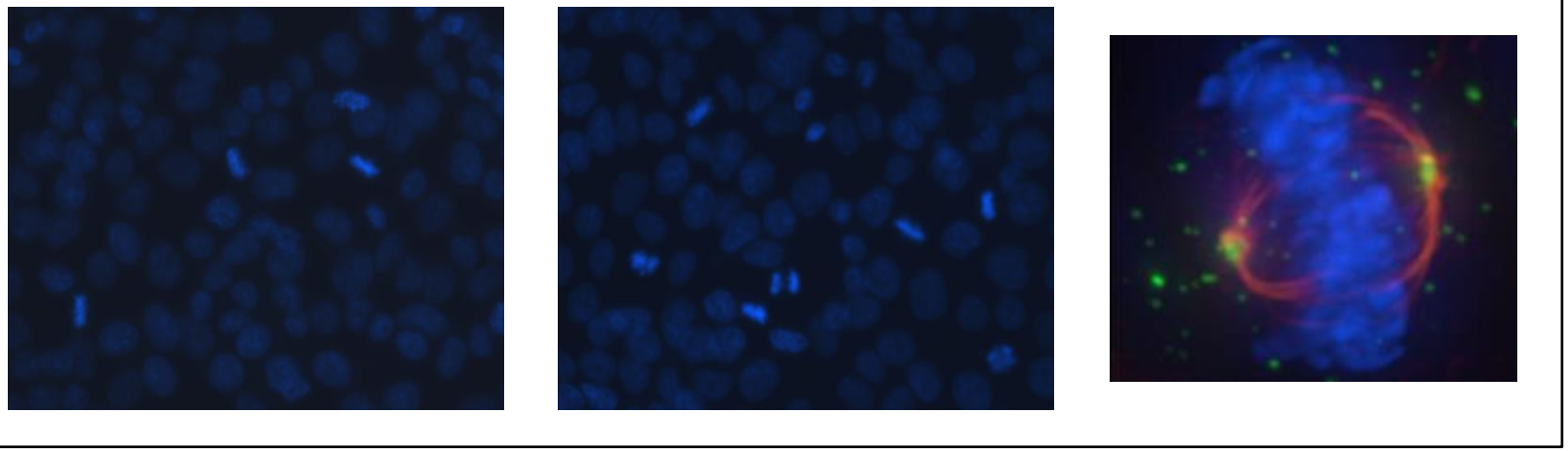


Duncan AW, Nature 467:707, 2010

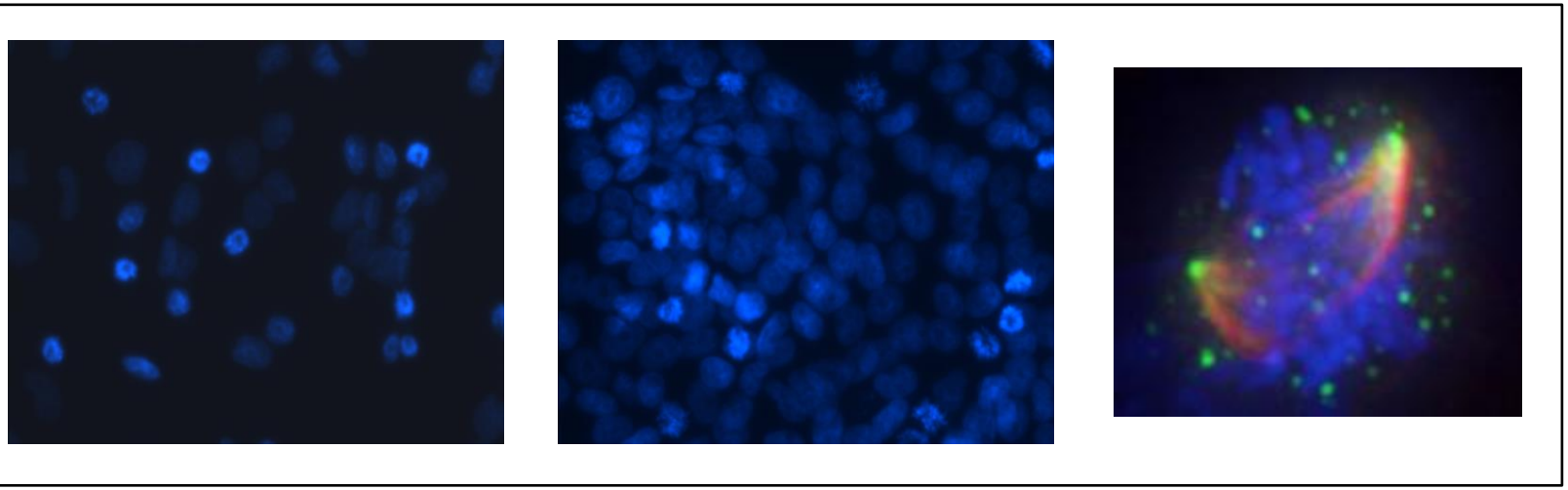
Mitosis in vitro: orientation of metaphase plates

In untreated cell cultures the orientation of most metaphase plates is perpendicular to gravity.
An abnormal orientation of metaphase plates in cell cultures is indicative of the disturbance of microtubules and/or centrosomes.

Normal orientation (90^0 relative to gravity)



Abnormal orientation (parallel to gravity)



HCT116 cells, 24 h cell culture, cell fixed with PFA. anti-tubulin-Cy3, anti-aurora A-FITC, DNA: Dapi.

Mitotic index

$$\textbf{MI} = \frac{\textbf{Number of metaphases}}{\textbf{Total number of cells}}$$

e. g. $$\text{MI} = \frac{\text{Number of metaphases}}{\text{1000 cells}} \times 100 \quad (\%)$$

Usual ~ 0.2 - 0.5 %

The MI value can be increased by inhibition of cells in metaphase:

e. g. colcemid for 1 h ~ 0.5 - 2.0 %

Terms of genome size and ploidy

c = content = DNA-content

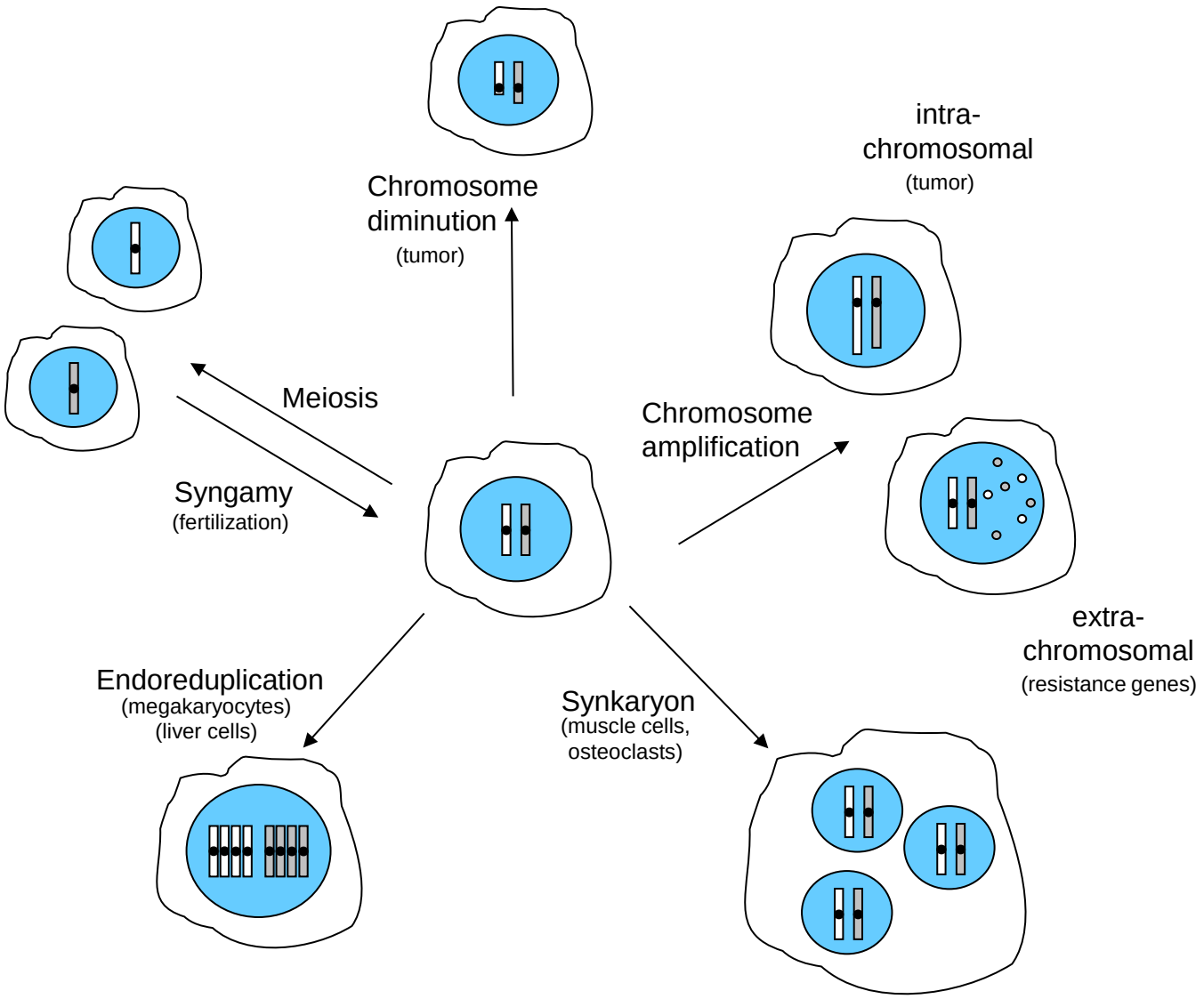
n = number of paternal/maternal chromosome complements

Normal (diploid): 2 c = 2 n

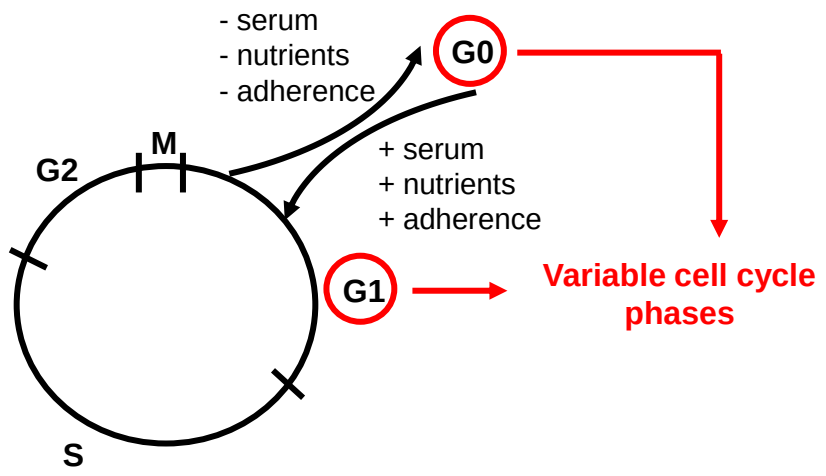
Tumor cells (e. g.): 2.7 c ≠ 2.7 n

Haploid	1 c	Normal cells
Diploid	2 c	
Tetraploid	4 c	
Aneuploid	≠ 2 c	
Hypodiploid	< 2 c	Tumor cells
Hyperdiploid	> 2 c	
Triploid	3 c	
Hypotetraploid	< 4 c	
Tetraploid	4 c	
Hypertetraploid	> 4 c	

Chromosomes: alterations of number and structure



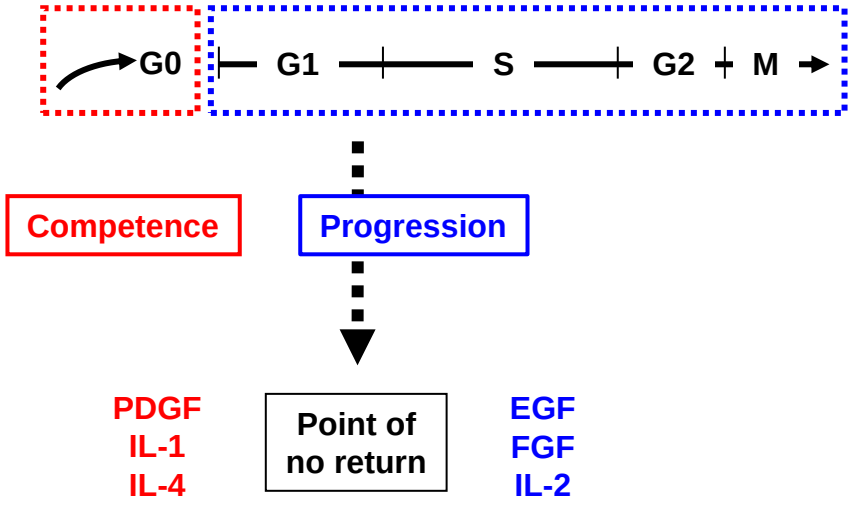
The cell cycle



Proliferation: G1-S-G2 = interphase, M = cell division

Differentiation / dormancy: G0

Cell cycle activtion via growth factors:



Duration of cell cycle compartments in vitro

(typical example skin fibroblasts)

G1 ~ 4 - 10 h
S ~ 7 - 12 h
G2 ~ 3 - 5 h
M ~ 1 - 2 h

G0 ~ days / weeks / years

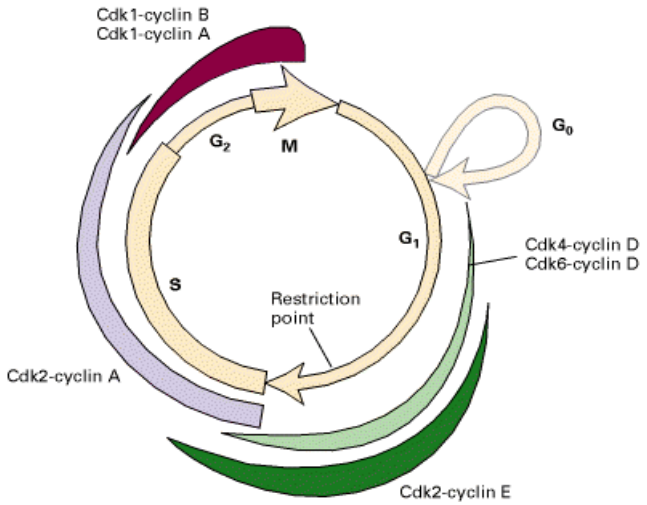
G0/G1-lag phase of activated human lymphocytes ~ 30 - 40 h

Cell proliferation in vitro:

Generation time $\neq$ cell cycle duration

Generation time = duration of population doubling (represents mean value of rapid-, slow- and non-proliferating cells)

Cell cycle: cyclin regulation



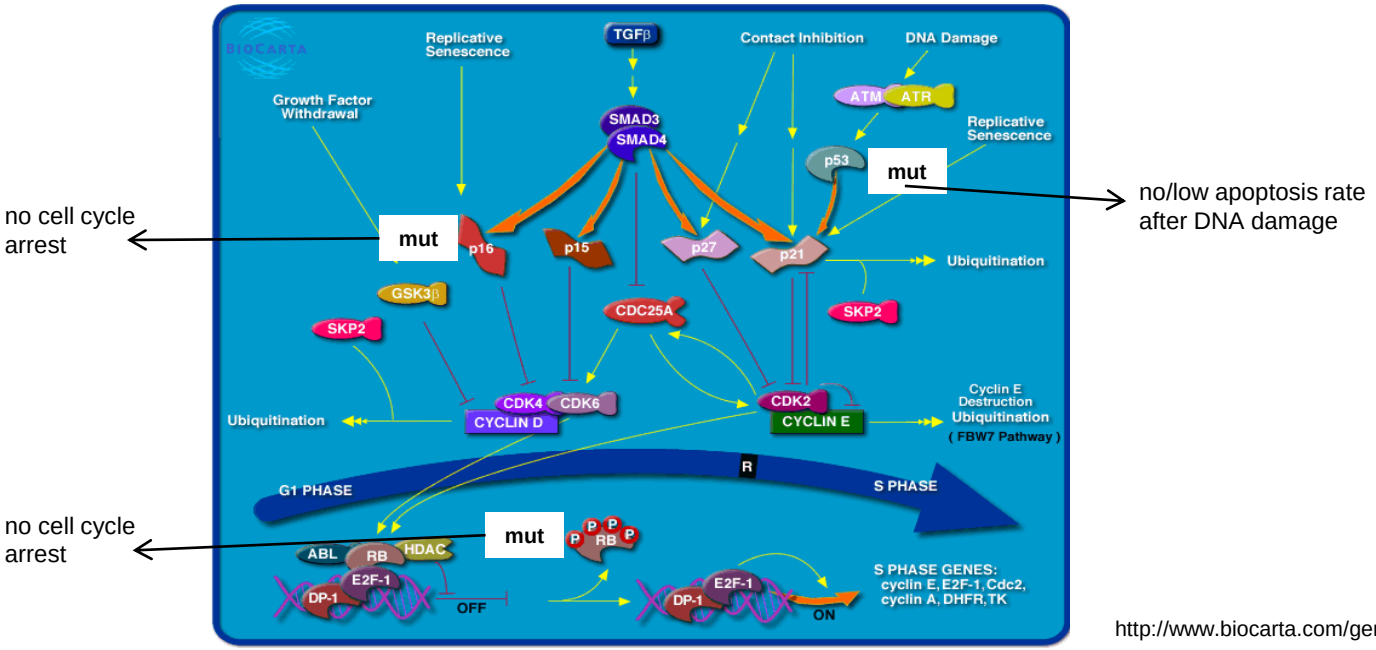
Kinase	Regulators	Putative substrates
Cdk1	Cyclin A, B	Rb, NF, histone H1
Cdk2	Cyclin A, E, D	Rb, p27
Cdk3	Cyclin E	E2F-1/DP-1
Cdk4	Cyclin D1, D2, D3	Rb
Cdk5	Cyclin D, p35	NF, Tau
Cdk6	Cyclin D1, D2, D3	Rb
Cdk7	Cyclin H	Cdc2, Cdk4/6
Cdk8	Cyclin C	RNA pol II
Cdk9	Cyclin T	Rb, mBp

INK4 family inhibitors (e.g. p16)

Cip/Kip family inhibitors (e.g. p21)

Lodish, Molecular Cell Biology, 2000

Tumor suppressor mutation dysregulation in cancer: G1-S cell cycle transit.

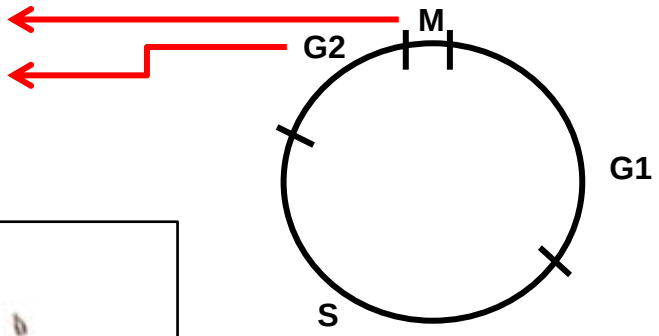


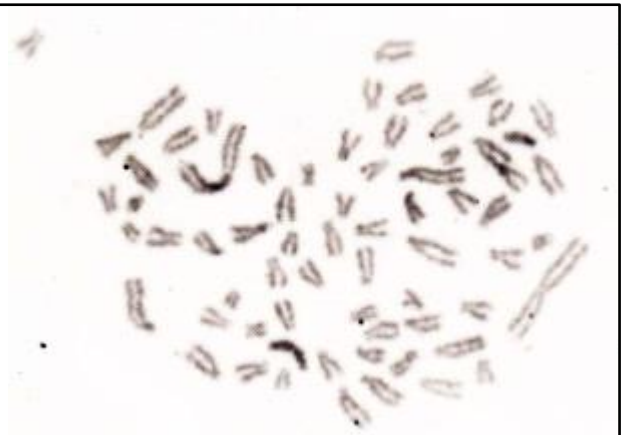
http://www.biocarta.com/genes/allpathways.asp

Cell cycle: spontaneous in vitro transformation - endoreduplication

Murine Micromys minutus fibroblasts cultivated for several weeks in culture. Spontaneous transformation of diploid cells shown by increasing numbers of metaphase chromosomes.

endoreduplication
(tetraploidy)
(2c ⟶ 4c)

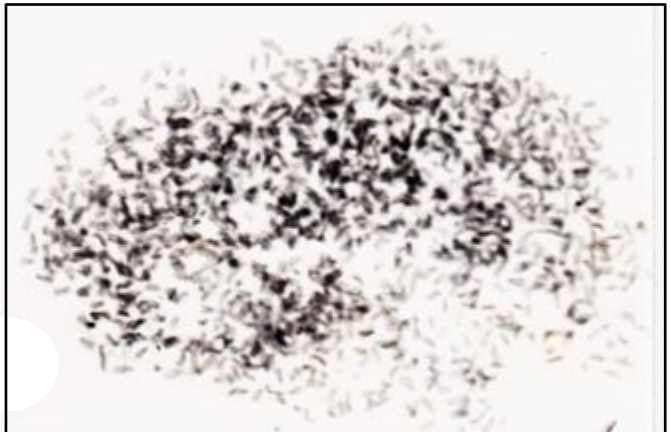




diploid metaphase (n=68 chr)

tetraploid metaphase (n=136 chr)

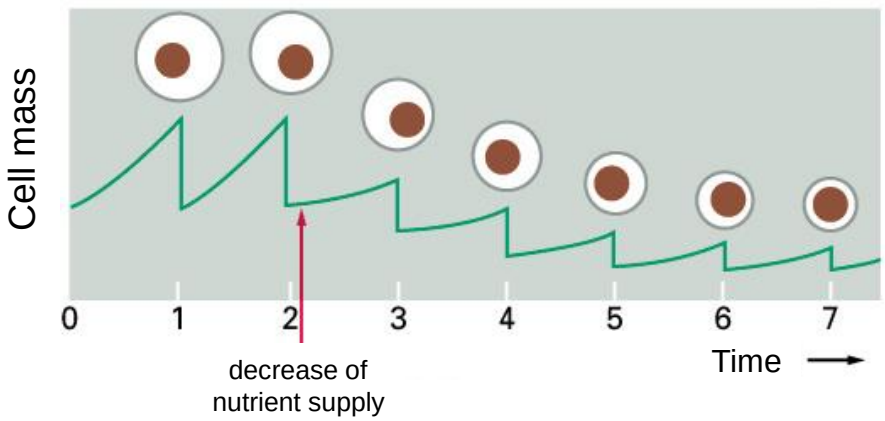
hypooctaploid metaphase (n>250 chr)



hyperoctaploid metaphase (n~500 chr)

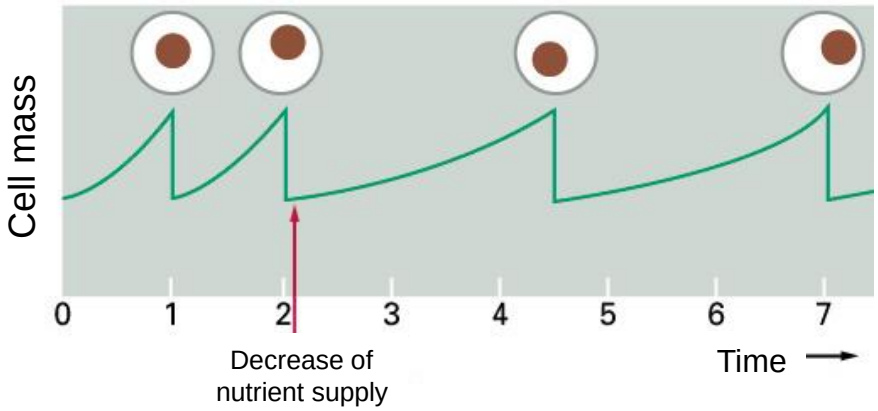
Cell division and cell growth

No nutrient dependent cell cycle control



Nutrient dependent cell cycle control

Cell cycle control of higher eukaryotes/mammalian cells: prolongation of G1-Phase until sufficient cell size.



Alberts, Molecular Biology of the Cell, 2002

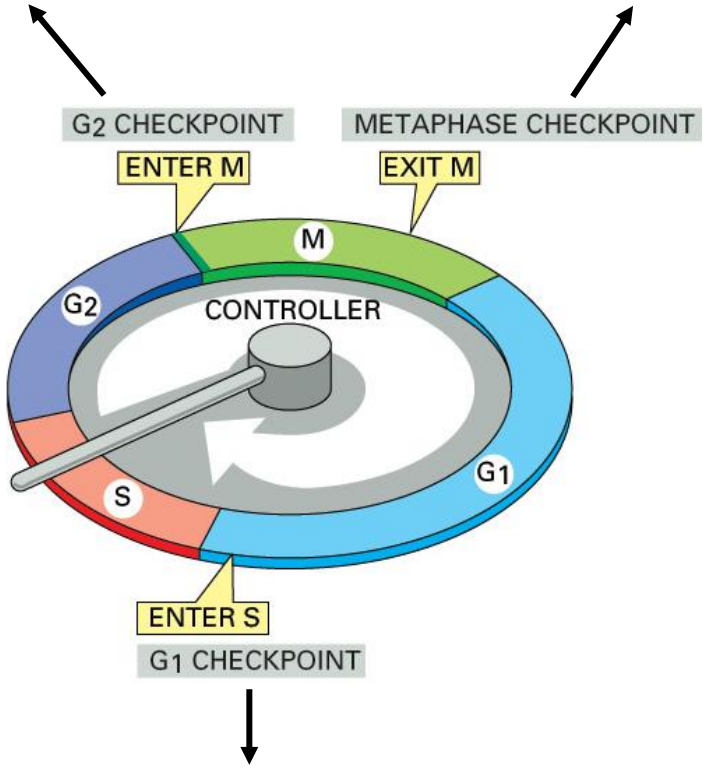
Cell cycle checkpoints

Genetic information: DNA duplicated
(DNA proof-reading)

Cell growth: sufficient size of cells
(energy, biochemical building blocks)

Environment: favorable
(adhesion, pH, temperature)

Mitosis: spindle attachment of
all chromosomes

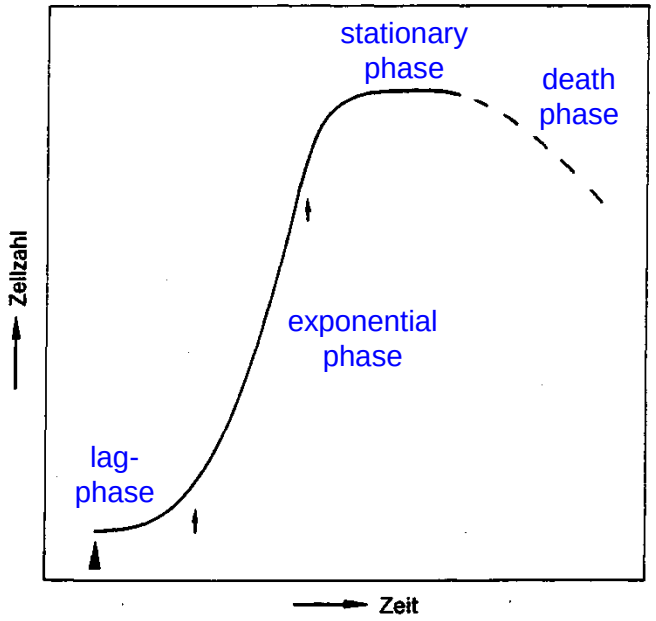


Cell growth: sufficient size of cells
(energy, biochemical building blocks)

Environment: favorable
(adhesion, pH, temperature)

Alberts, Molecular Biology of the Cell, 2002 (mod)

Growth curve, number of cell divisions, generation time



Half-log display of cell number (y-axis, log) and time (x-axis, lin) results in a straight line (cells in the exponential growth phase). This curve section is used for the calculation of the number of population doublings:

$$2^n = N / N_0$$

$$n \times \log 2 = \log N - \log N_0$$

$$\boxed{n = (\log N - \log N_0) / \log 2}$$

N = cell number at the end point of experiment
N_0 = cell number at begin of experiment
n = number of population doublings

Cells in stationary phase please note:

Suspension cell density increases (per volume)
(cell aggregation tendency)

Adherent cell culture becomes confluent (per area)
(contact inhibition, multilayer)

$$\boxed{T_G = T / n}$$

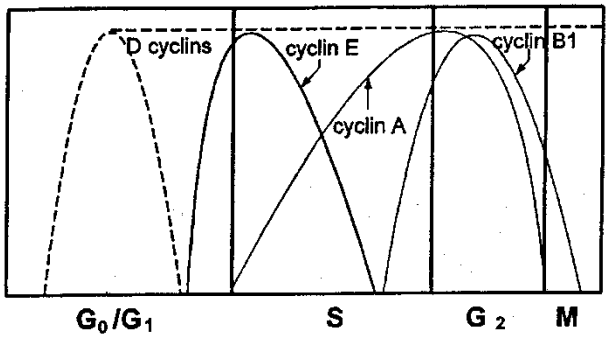
T_G = Generation time
T = Observation period from N_0 to N
n = number of population doublings

Cell cycle synchronization

The expression of many genes varies as cells progress through the cell cycle (e. g. cyclin expression).

Cyclin proteins, FACS analysis

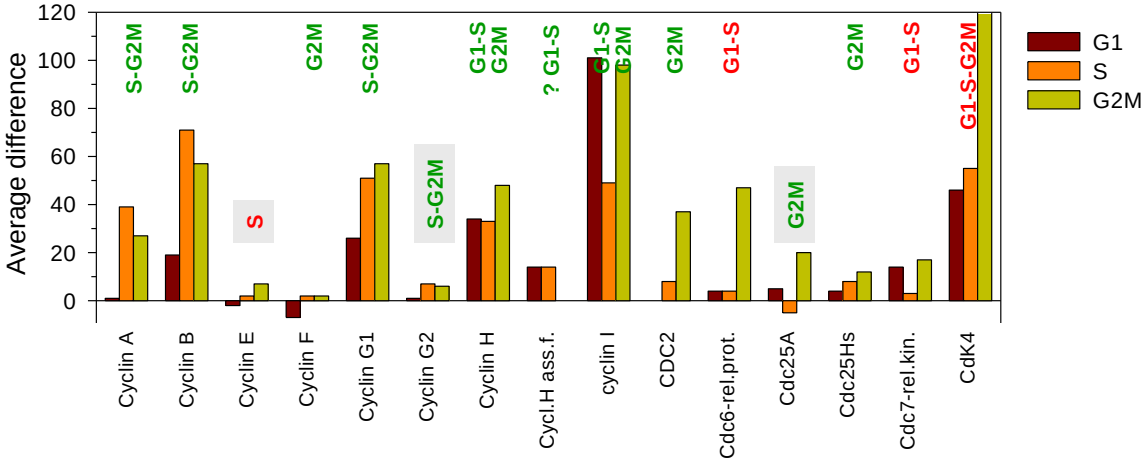
Darzynkiewcz Z et al, Cytometry 1996



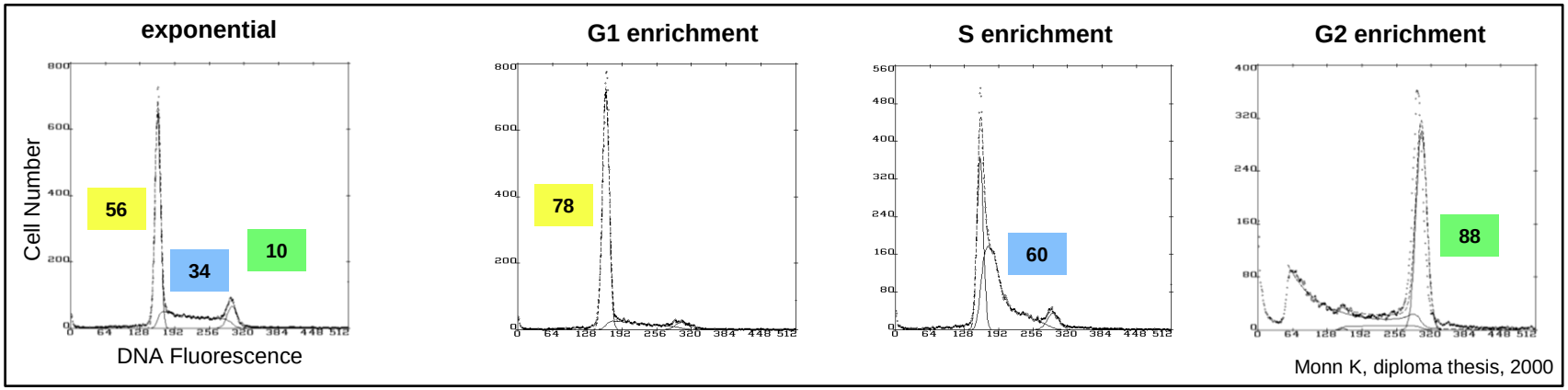
This expression pattern is typical for normal, diploid cells. However, in tumor cells the expression pattern may be abnormal (e.g. constitutive expression of D-cyclins).

Cyclin mRNA, Affymetrix mRNA analysis

Monn K, diploma thesis, 2000



Cell can be enriched in distinct cell cycle phases by different physical, chemical or biochemical means.



Monn K, diploma thesis, 2000

Cell cycle synchronization

Removal of growth factors

Mitoses knock-off (mechanical shearing)

Thymidine block

Chemical inhibitors (metabolism, signal cascades)

FACS Sorting

Elutriation

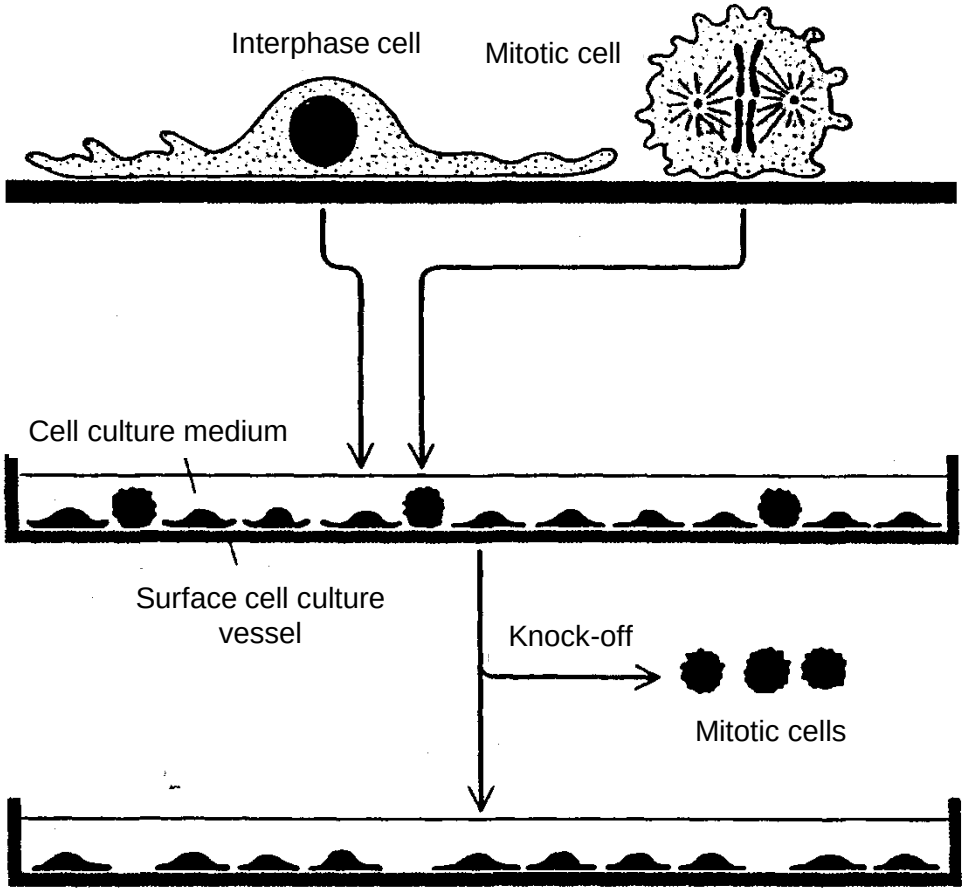
! Note !

Transformed cells are more difficult to synchronize in comparison to normal, diploid cells. Tumor cells often exhibit higher death rates during synchronization.

Cell cycle synchronization

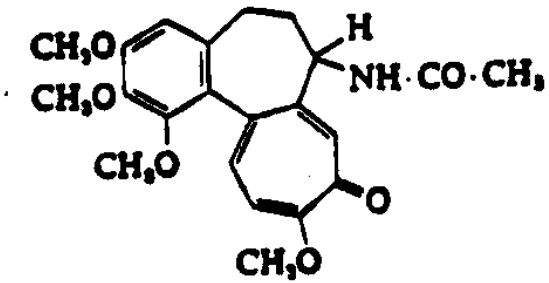
Mitoses knock-off

Due to ist lower adhesion mitotic cells are more weakly bound to the cell culture vessel surface. Knocking the cell culture flask onto a tight surface sheares the mitoses off the flask surface.

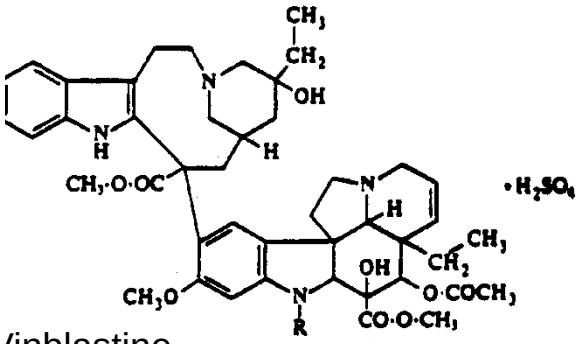


Cell cycle synchronization: chemical inhibitors

M-phase arrest
Microtubule destabilization

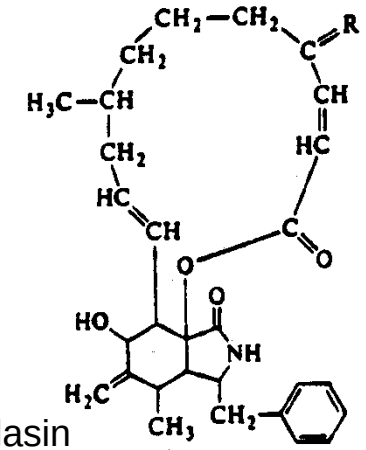


Colchicine



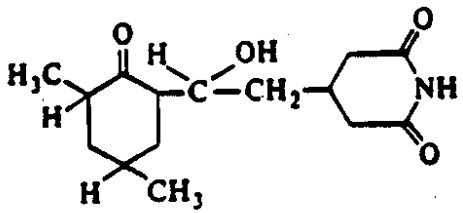
Vinblastine

G1-G2M-phase arrest
Actin filament destabilization



Cytochalasin

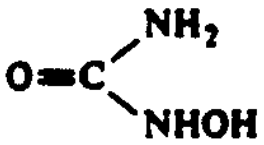
G1-phase arrest
Protein synthesis inhibition



Cycloheximid

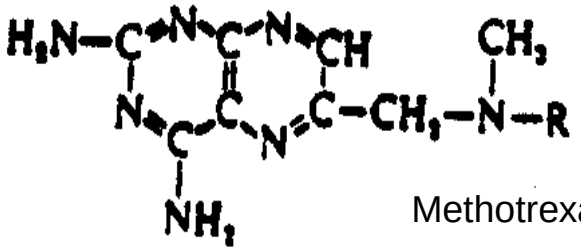
Cell cycle synchronization: chemical inhibitors

G1-phase arrest
DNA synthesis inhibition
Ribonucleotide reductase



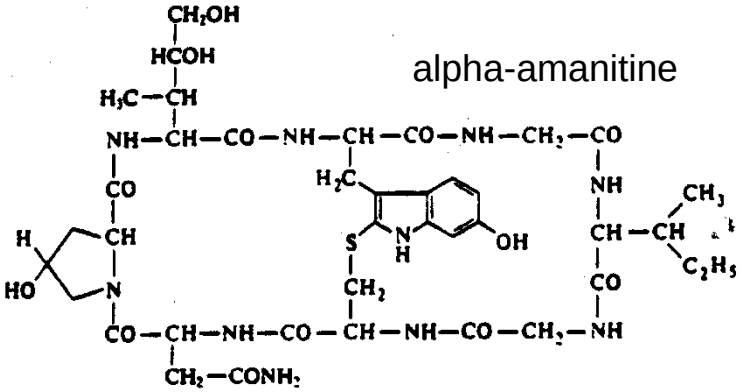
Hydroxyurea

G1-phase arrest
DNA synthesis inhibition
Dihydrofolate reductase



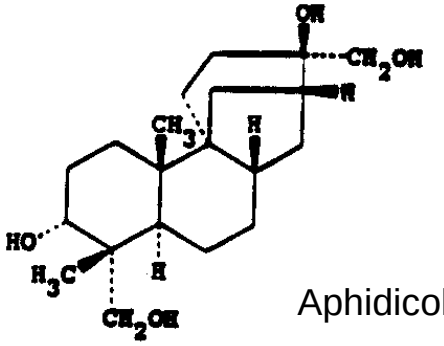
Methotrexate

G1-S-phase arrest
RNA synthesis inhibition
RNA polymerase II



alpha-amanitine

S-phase arrest
DNA synthesis inhibition
DNA polymerase alpha

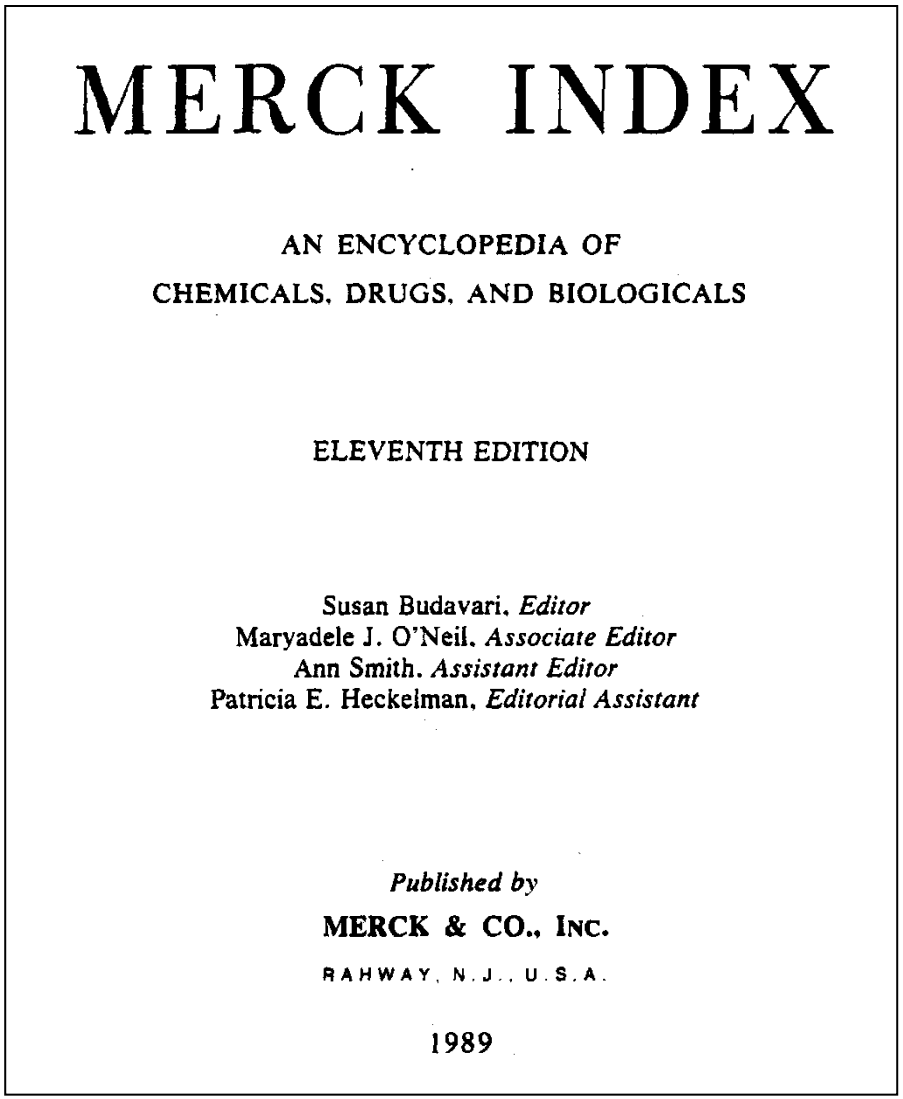


Aphidicoline

Many cell cycle inhibitors exhibit a concentration dependent cell cycle compartment specifity !

Informations on toxic compounds

Besides informations from the red list (Rote Liste), toxicity informations from public institutions and from the internet (e. g. Wikipedia), the MERCK INDEX gives background data of the physical, chemical and toxicological properties of many compounds.



MERCK INDEX

AN ENCYCLOPEDIA OF
CHEMICALS, DRUGS, AND BIOLOGICALS

ELEVENTH EDITION

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RAHWAY, N.J., U.S.A.

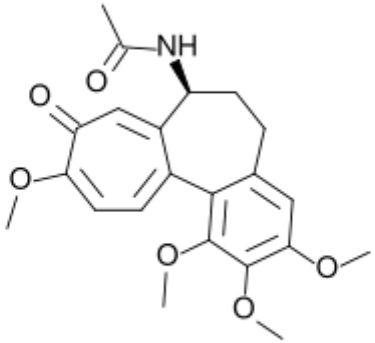
1989

Informations on toxic compounds

e. g. colchicine from Wikipedia

(http://de.wikipedia.org/wiki/Colchicin)

Formula



General remarks

Name	Colchicin
Andere Namen	(−)-(a*R*,7*S*)-Colchicin
Summenformel	$C_{22}H_{25}NO_6$
CAS-Nummer	64-86-8
Kurzbeschreibung	blassgelbes kristallines Pulver welches sich bei Lagerung an der Luft dunkel färbt

Properties

Molmasse	399,43 $g \cdot mol^{-1}$
Aggregatzustand	fest
Dichte	?
Aggregatzustand	fest
Dichte	?
Schmelzpunkt	277 °C
Siedepunkt	?
Dampfdruck	unbekannter Wert oder fehlende Angabe!
Löslichkeit	löslich in Wasser, Ethanol und Chloroform; in Petrolether praktisch unlöslich

Safety aspects

Gefahrstoffkennzeichnung

Gefahrensymbole

T+

Sehr giftig

R- und S-Sätze	R: ?
	S: ?

More safety aspects

MAK	?
LD_{50} (oral, Ratte)	Mensch: 1,6 $mg \cdot kg^{-1}$

Analytical technologies for cell proliferation analysis

Cell counting (Coulter Counter, counting chamber)

Enzymatic analysis (WST, MTT, CellTiterGlo ATP)

DNA / protein quantification (spectroscopic analysis)

^{3}H thymidine /BrdU labeling (radioisotope/ELISA analysis)

DNA histogram (FACS analysis)

Fluorochrome dilution labeling techniques (FACS analysis)

Proliferation marker (FACS, IHC analysis: Ki67, PCNA)

Impedance analysis (electrical contact coated microtiter plates)