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Cell Cycle Lectures

L1: Introduction to the Cell Cycle

L2-4: Cytoskeleton Structure and Dynamics

L5-7: Chromosomes in the Cell Cycle

L8: Cell Cycle Control

L9: Cell Cycle Checkpoints

L10: The Cell Cycle and Cancer

Lecture 1

Introduction to the Cell Cycle

Outline:

Cell cycle basics

Interphase and mitosis

Cell cycle regulation

Variant cell cycles

Consequences of defective cell division

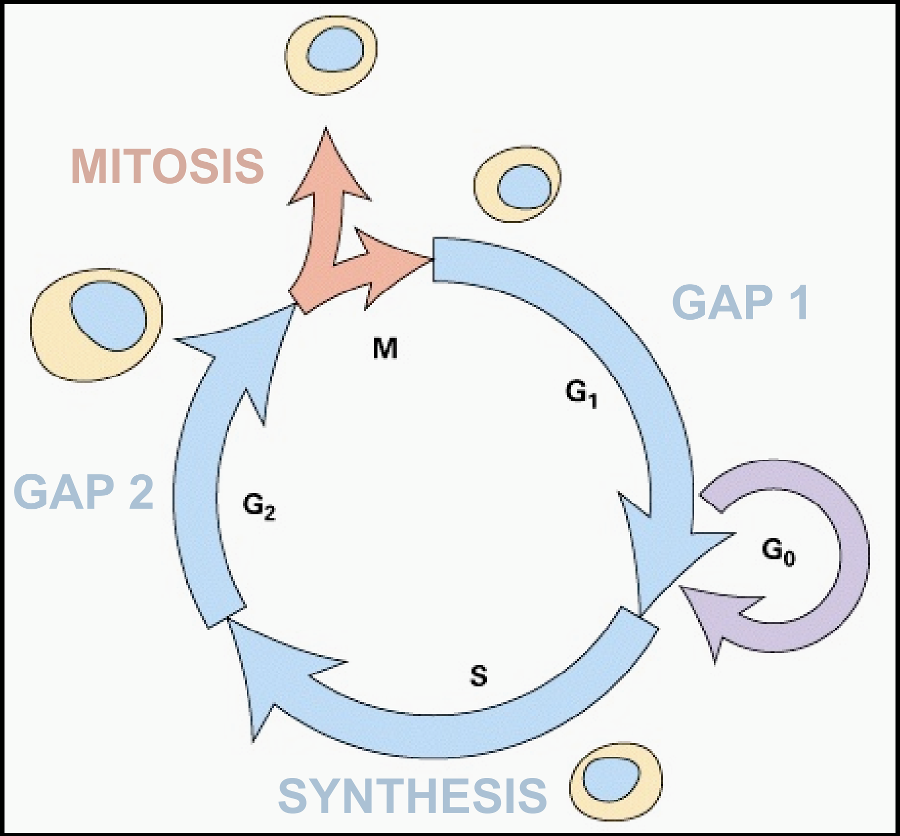
Methods for studying the cell cycle

Paper: check the website tonight

Paul Nurse “Controlling the Cell Cycle” !!!
Thu 4 PM, 100 GPBB

Cell Cycle Basics

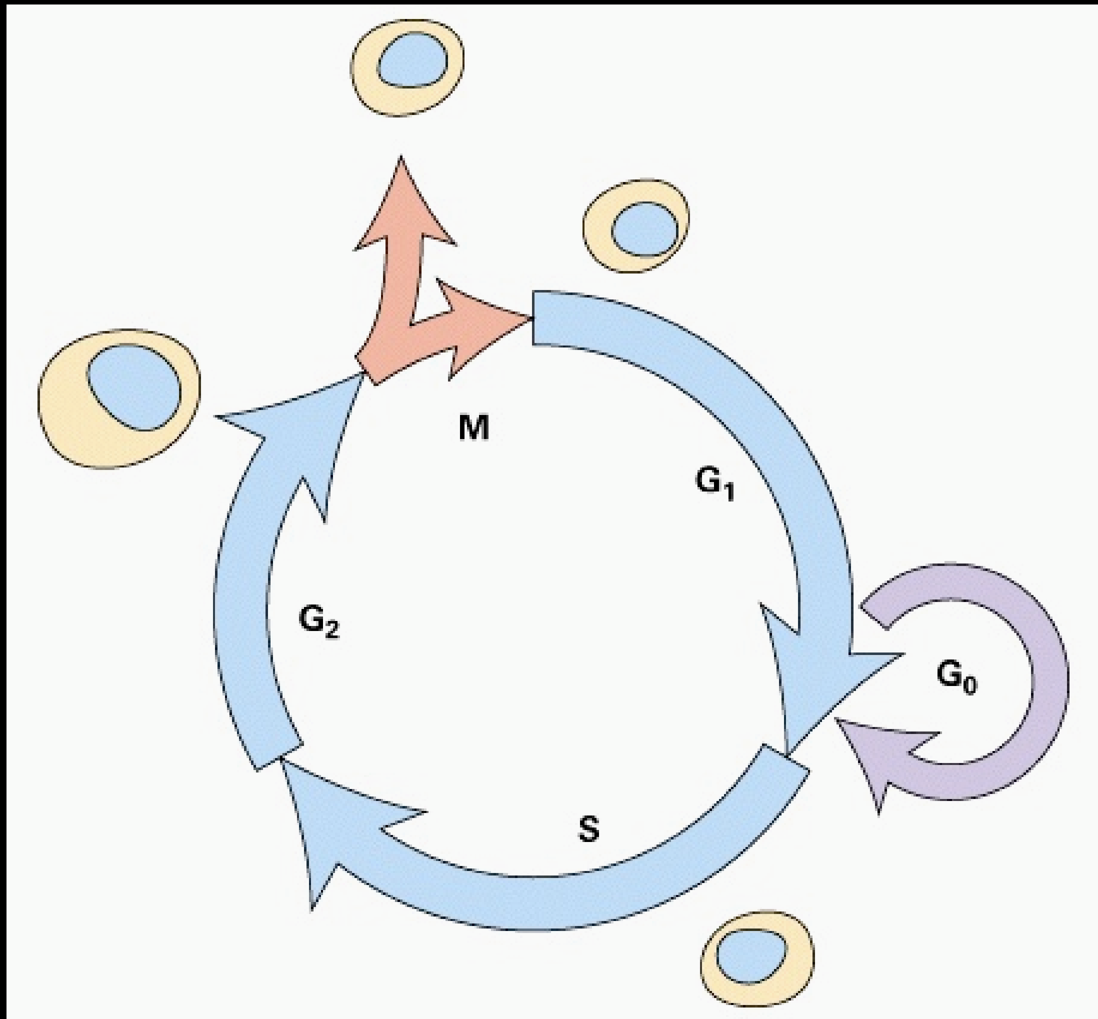
sequence of events for one round of cell duplication



great example of how cells integrate internal and external signals and structures to accomplish a dynamic, essential function

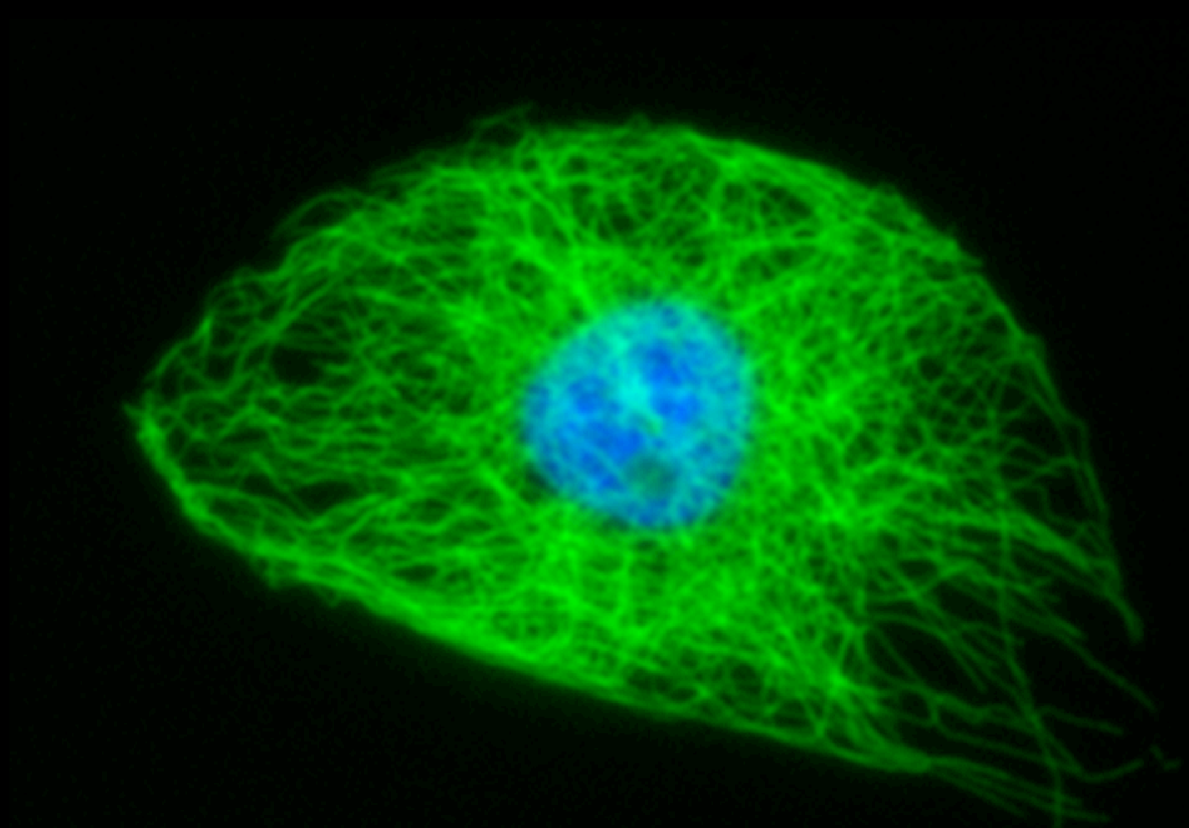
very complex, but significant knowledge generated over past 20 years

Cells spend most of the time in interphase



sit around, looking bored and boring...but...

Interphase



Microtubules

DNA

G1	S	G2
cell growth gene expression	DNA replication gene expression	cell growth prepare for mitosis

Reason for mitosis - accurate segregation of replicated DNA

.....requires exquisite, dynamic coordination with cytoskeleton & other cellular components

Mitosis: Dynamic Cytoskeletal and Nuclear Events

nuclear envelope:
breaks down and reforms

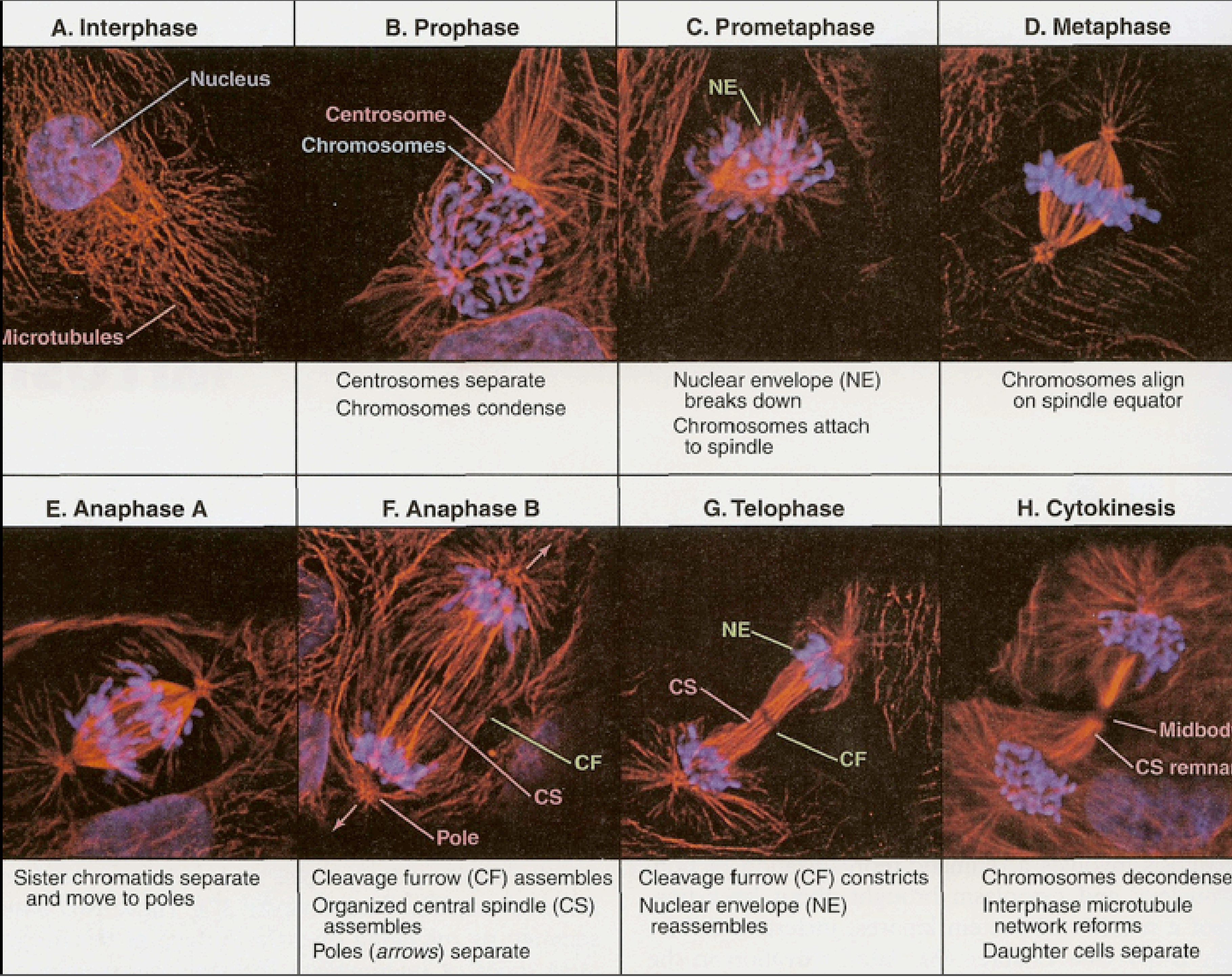
nucleolus:
breaks down and reforms

chromosomes:
replicate, condense, segregate

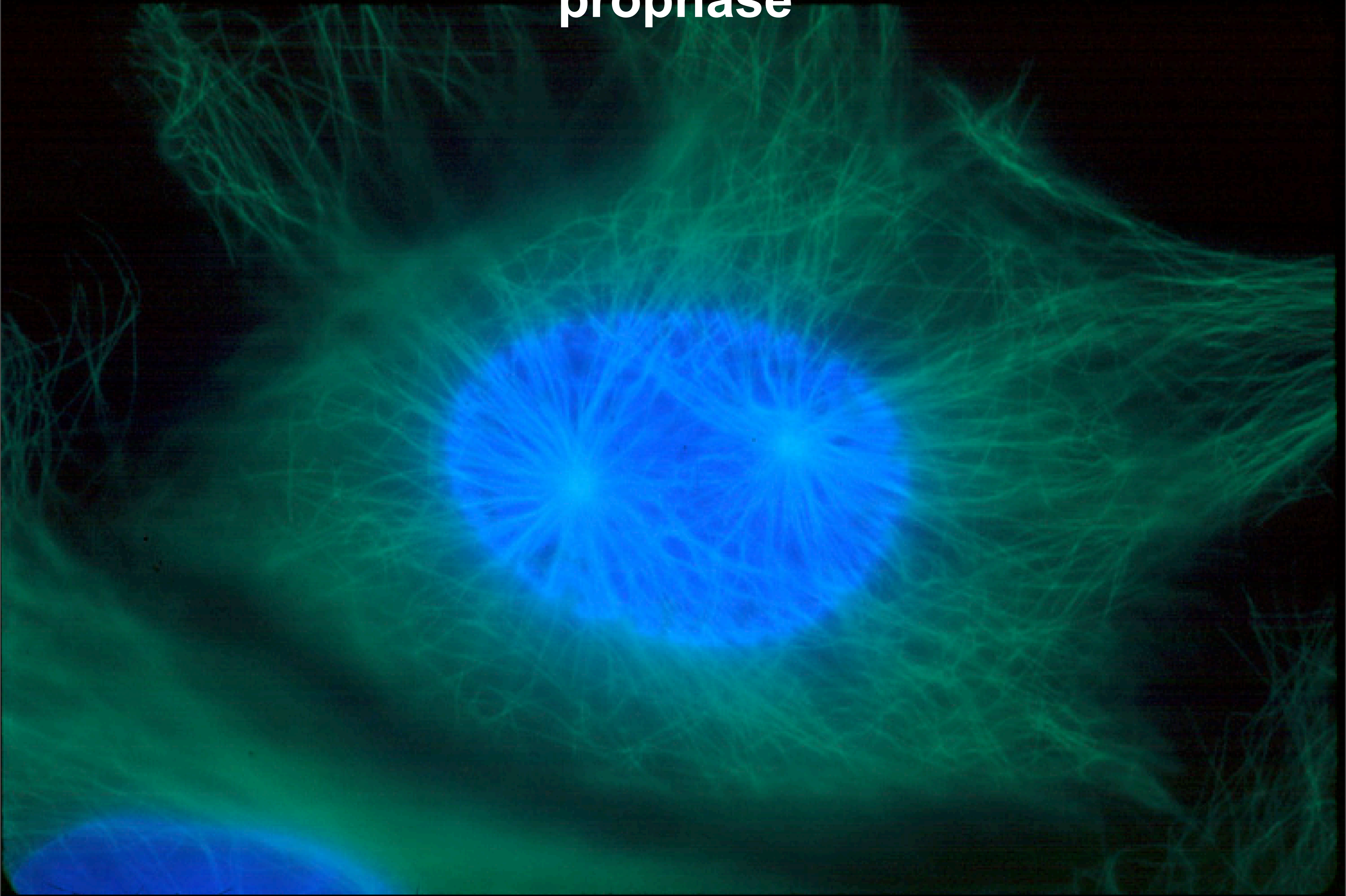
centrioles:
duplicate and separate

microtubules:
radial array becomes bipolar spindle

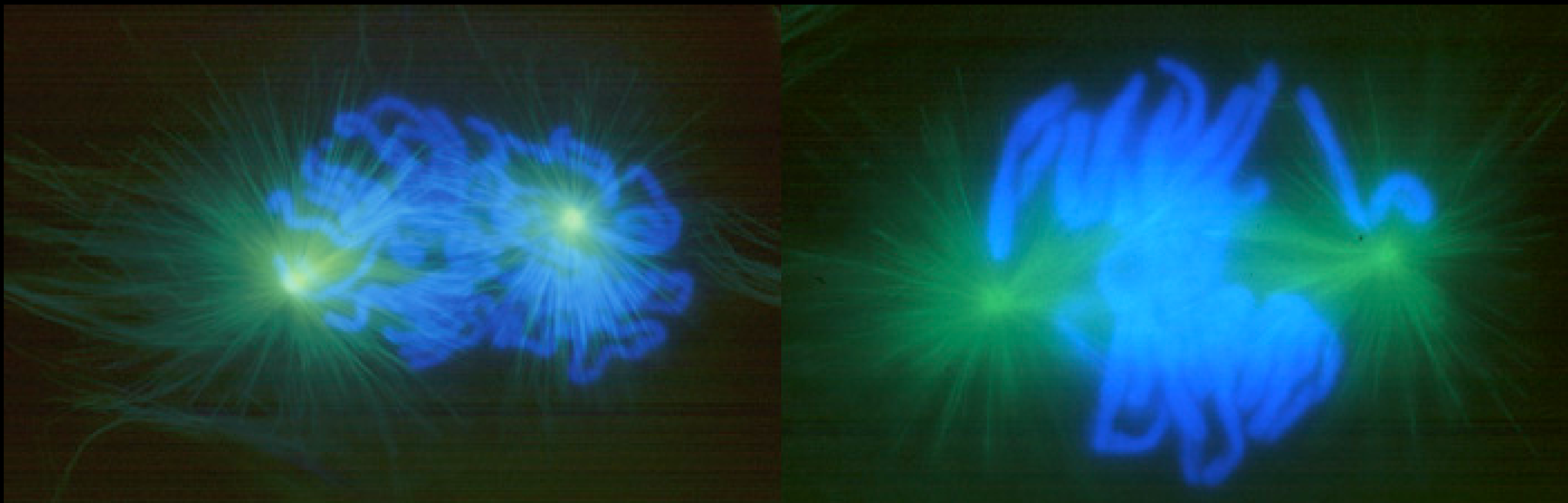
actin:
cytokinetic furrow formation


A. Interphase
Nucleus
icrotubules
B. Prophase
Centrosome
Chromosomes
Centrosomes separate
Chromosomes condense
C. Prometaphase
NE
Nuclear envelope (NE) breaks down
Chromosomes attach to spindle
D. Metaphase
Chromosomes align on spindle equator
E. Anaphase A
Sister chromatids separate and move to poles
F. Anaphase B
CF
CS
Pole
Cleavage furrow (CF) assembles
Organized central spindle (CS) assembles
Poles (*arrows*) separate
G. Telophase
NE
CS
CF
Cleavage furrow (CF) constricts
Nuclear envelope (NE) reassembles
H. Cytokinesis
Midbod
CS remna
Chromosomes decondense
Interphase microtubule network reforms
Daughter cells separate

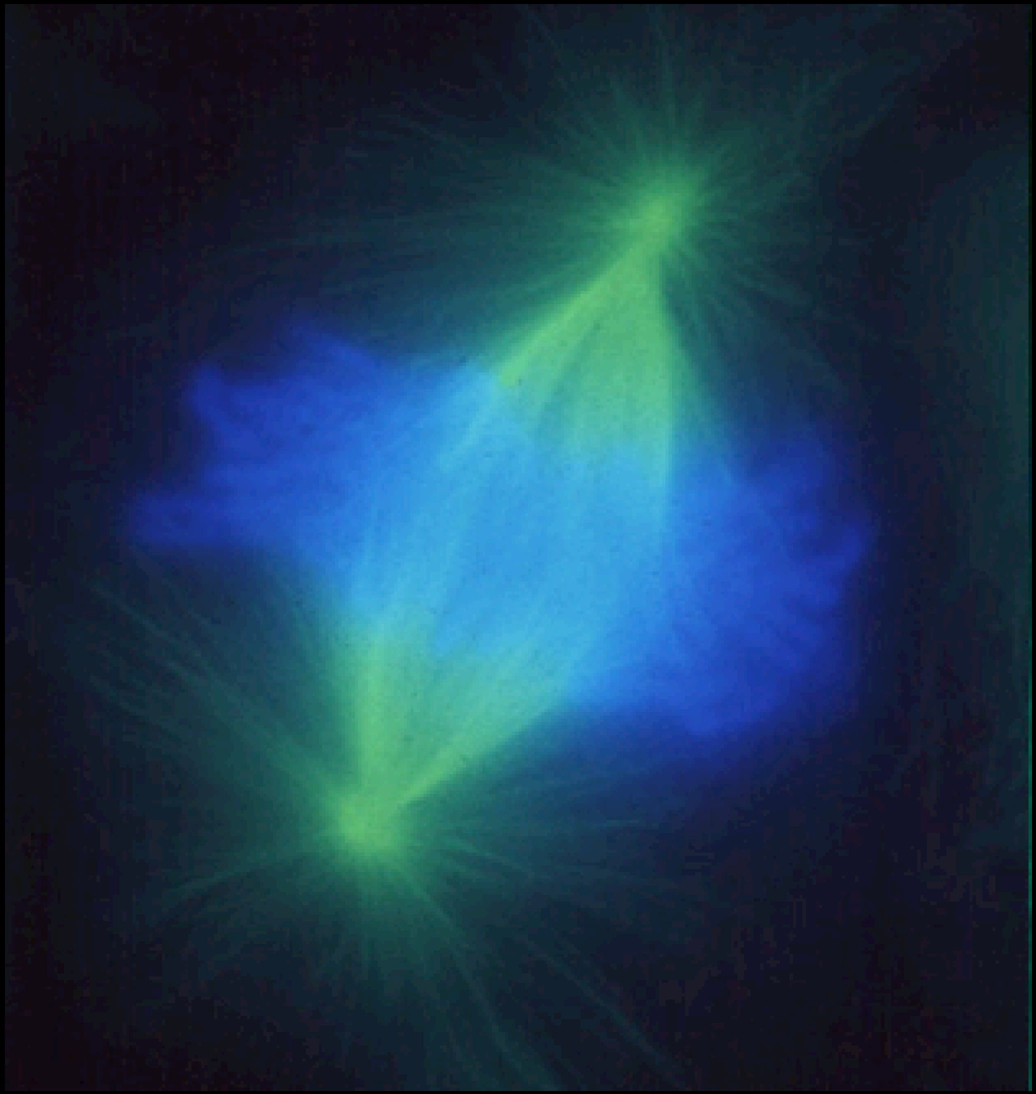
prophase



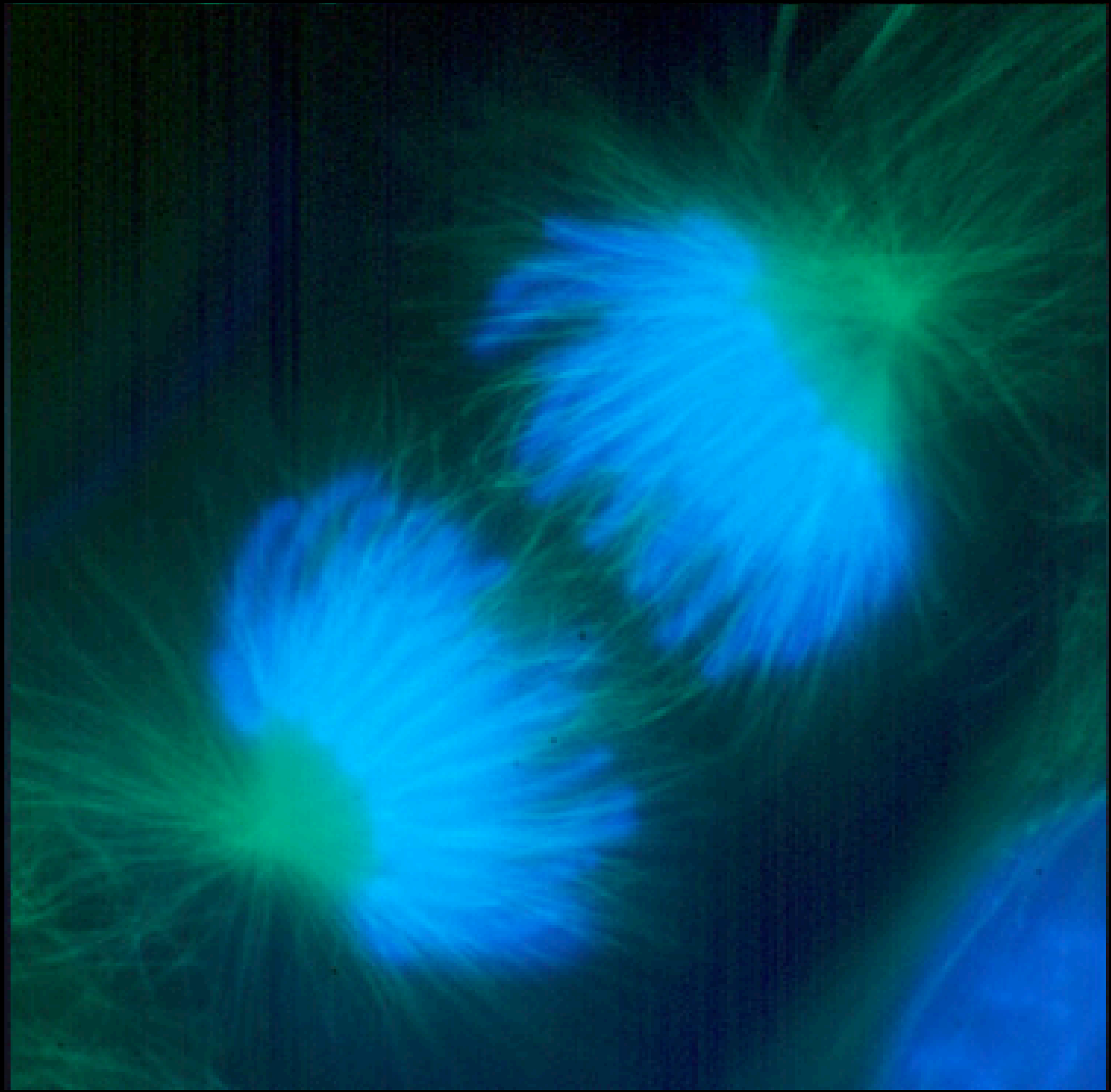
prometaphase



metaphase

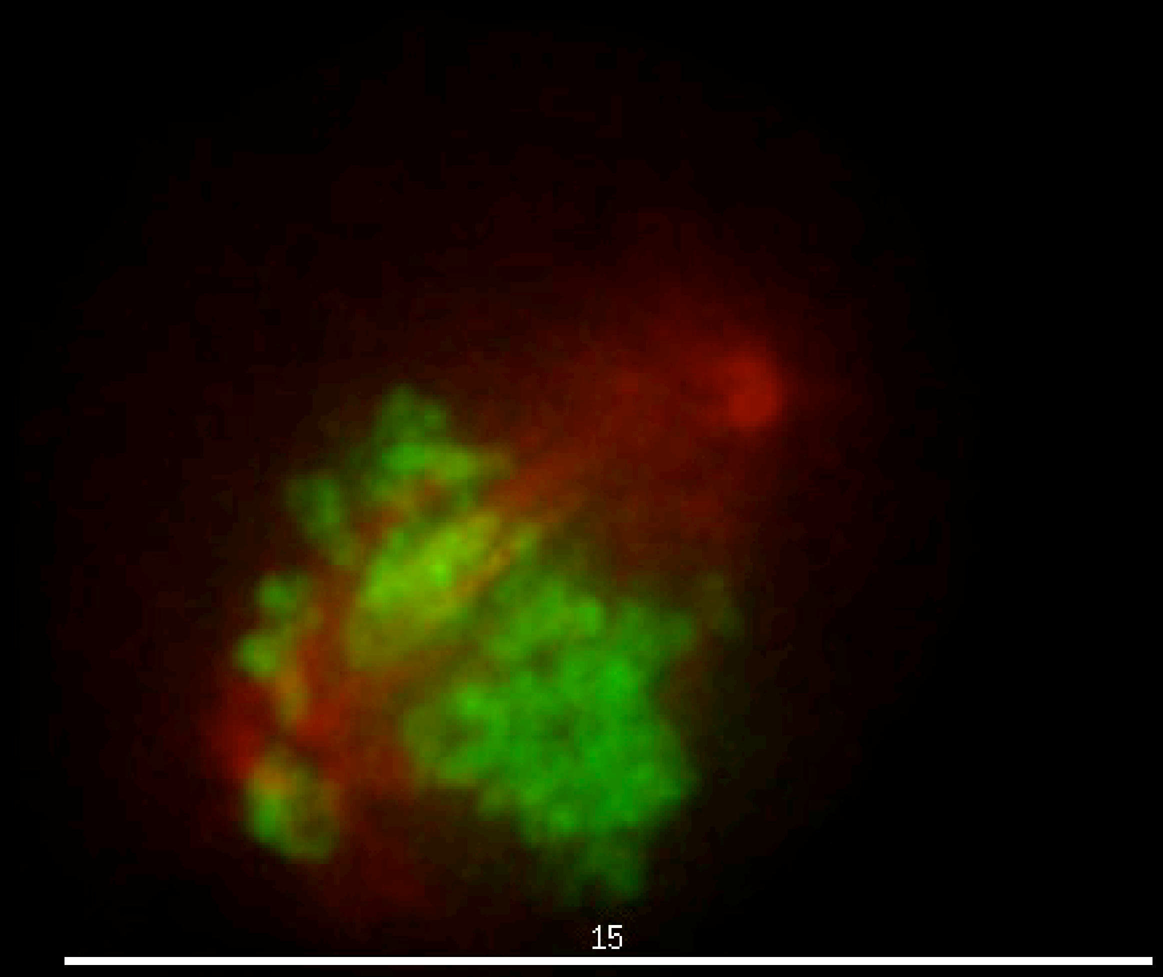


anaphase



Drosophila tissue culture cell in mitosis

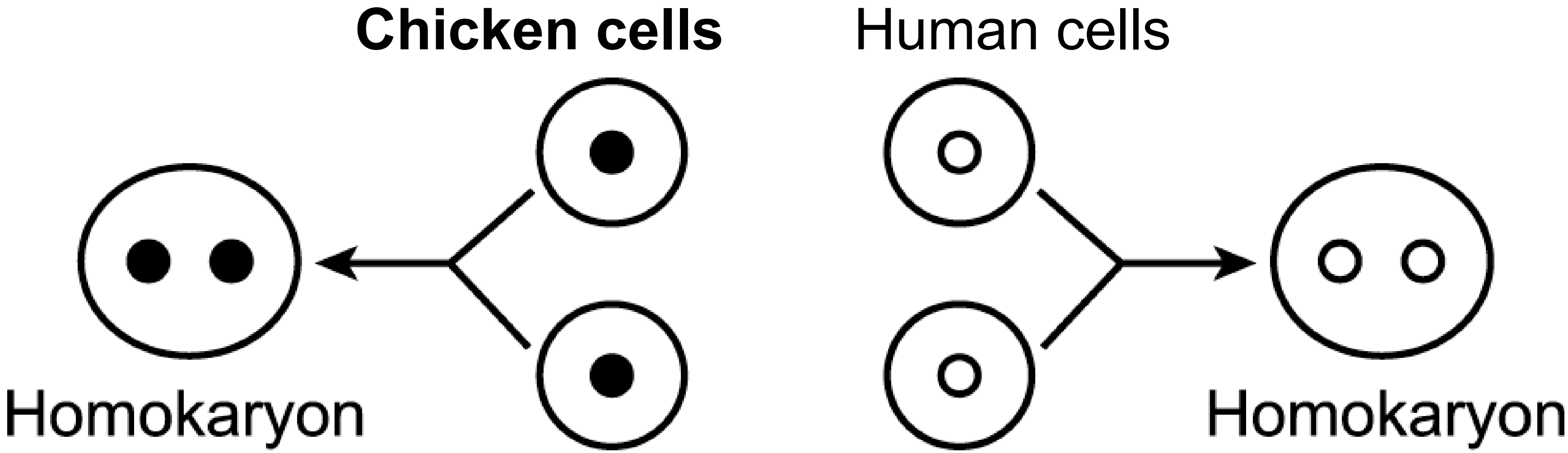
Histone-GFP
Tubulin-RFP

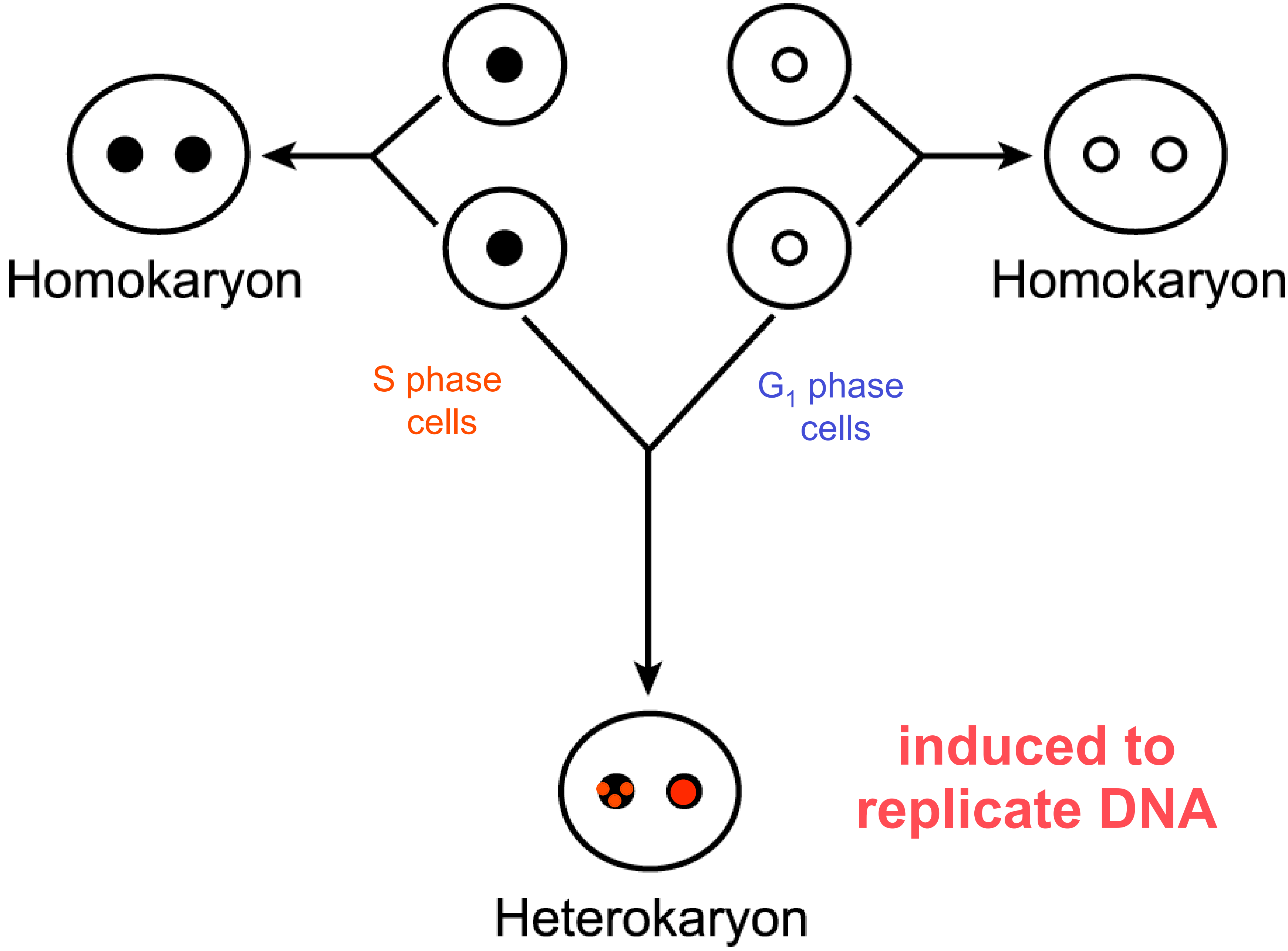


Cell Cycle Regulation

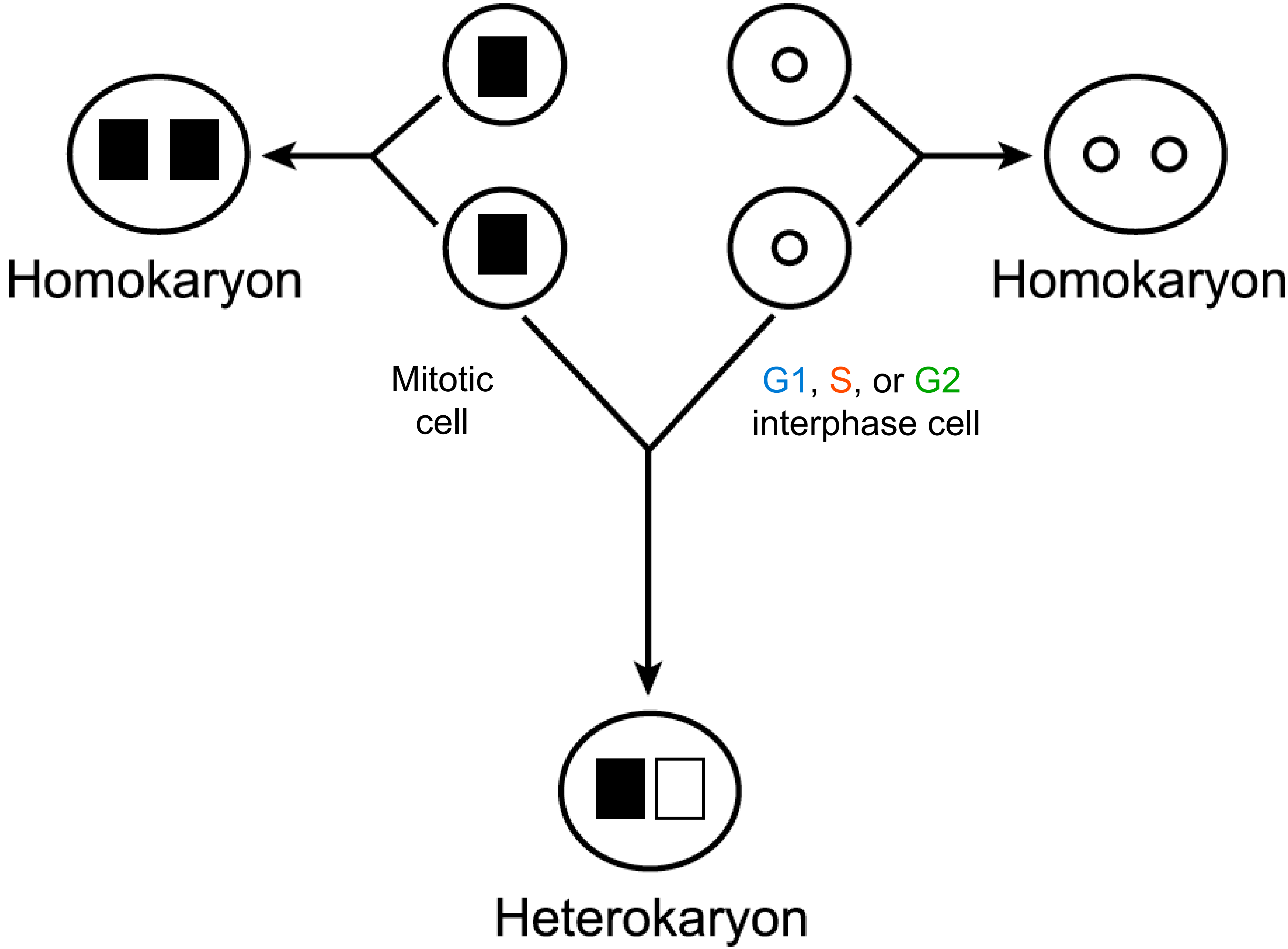
How do we know cell division is regulated?

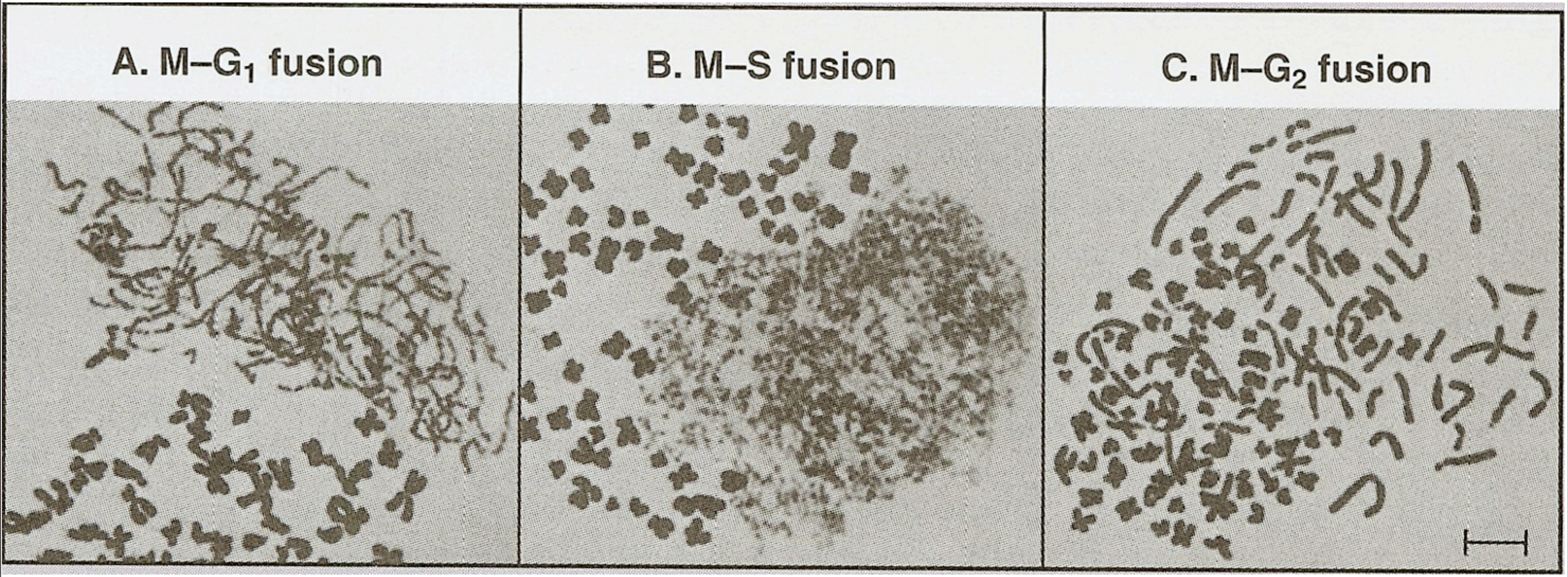
in our bodies daily!


Chicken cells
Human cells
Homokaryon
Homokaryon

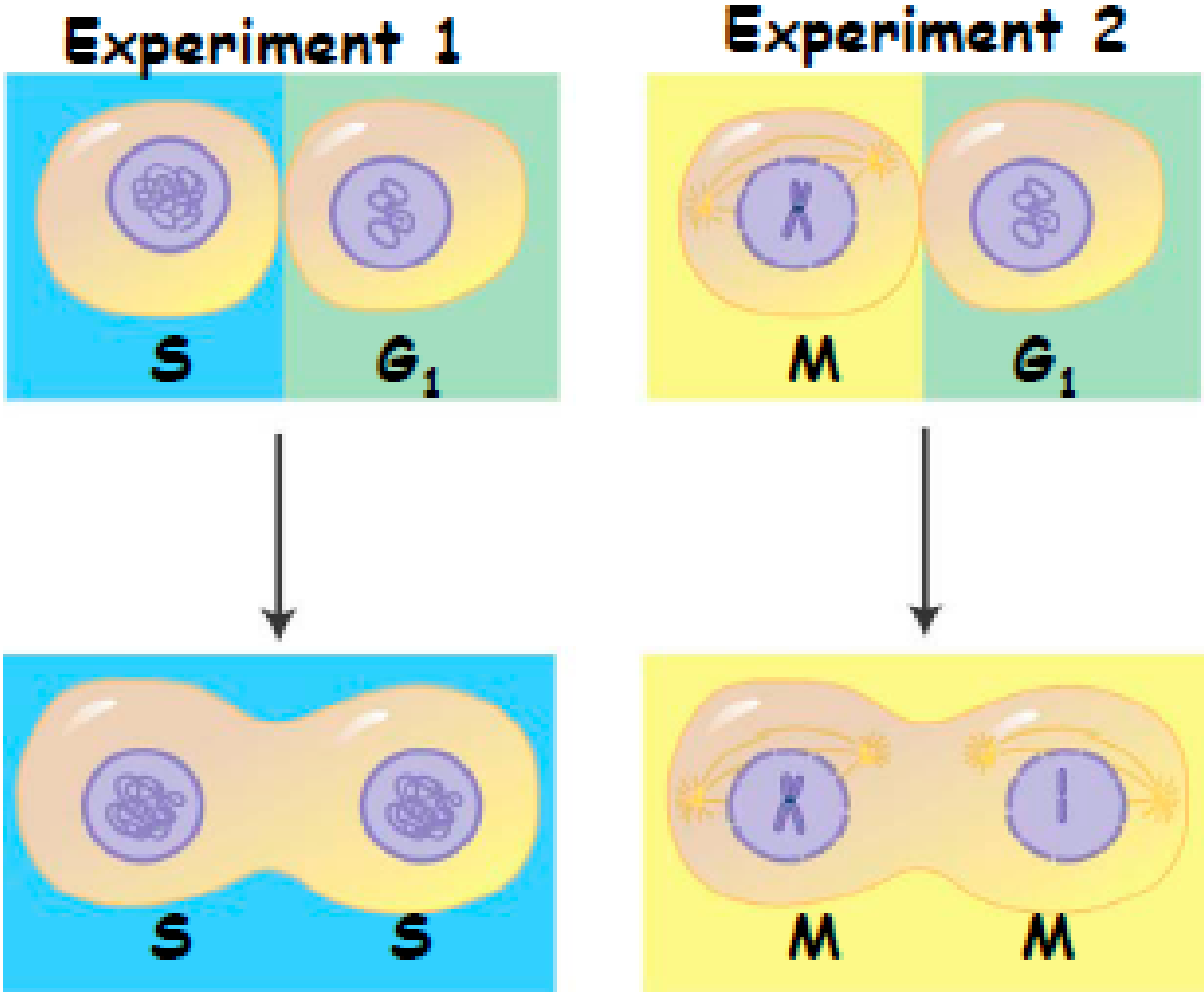


induced to replicate DNA


Homokaryon
Homokaryon
Mitotic cell
G1, S, or G2 interphase cell
Heterokaryon

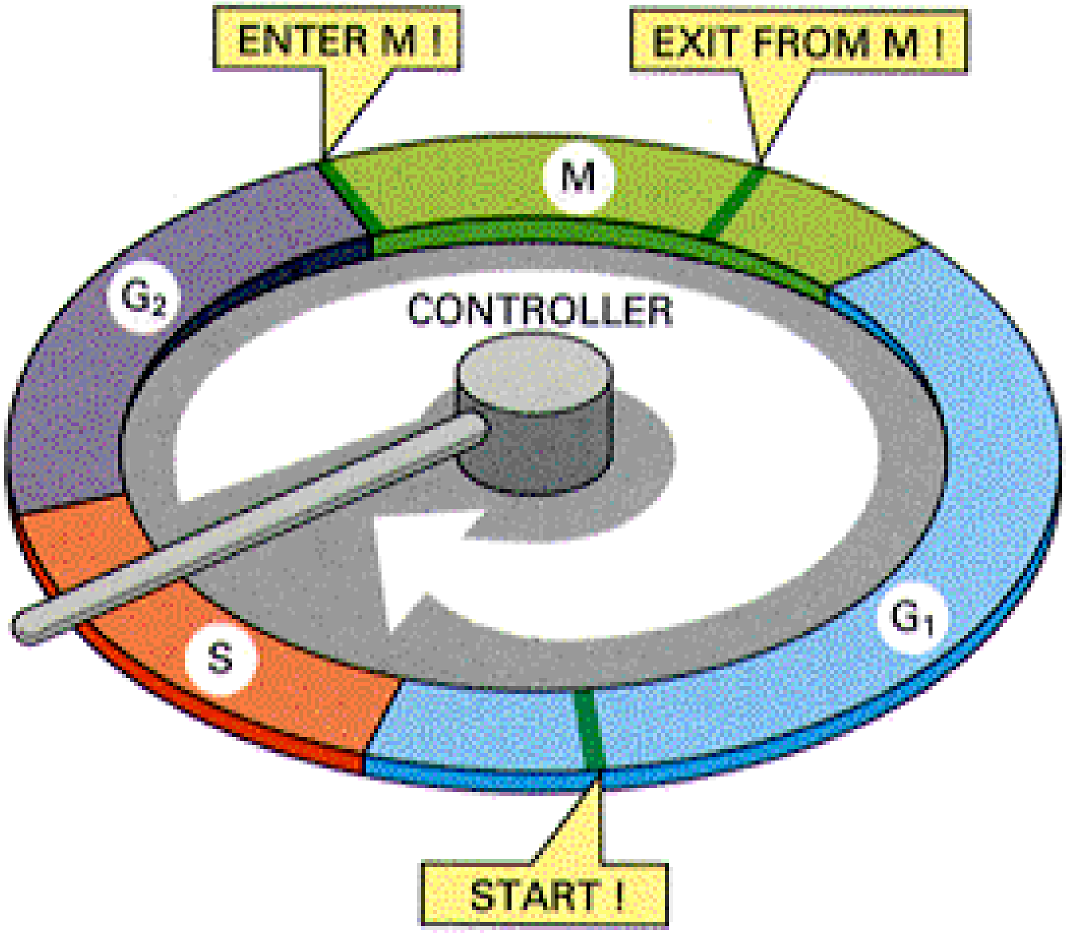


why do these chromosomes look so crappy?



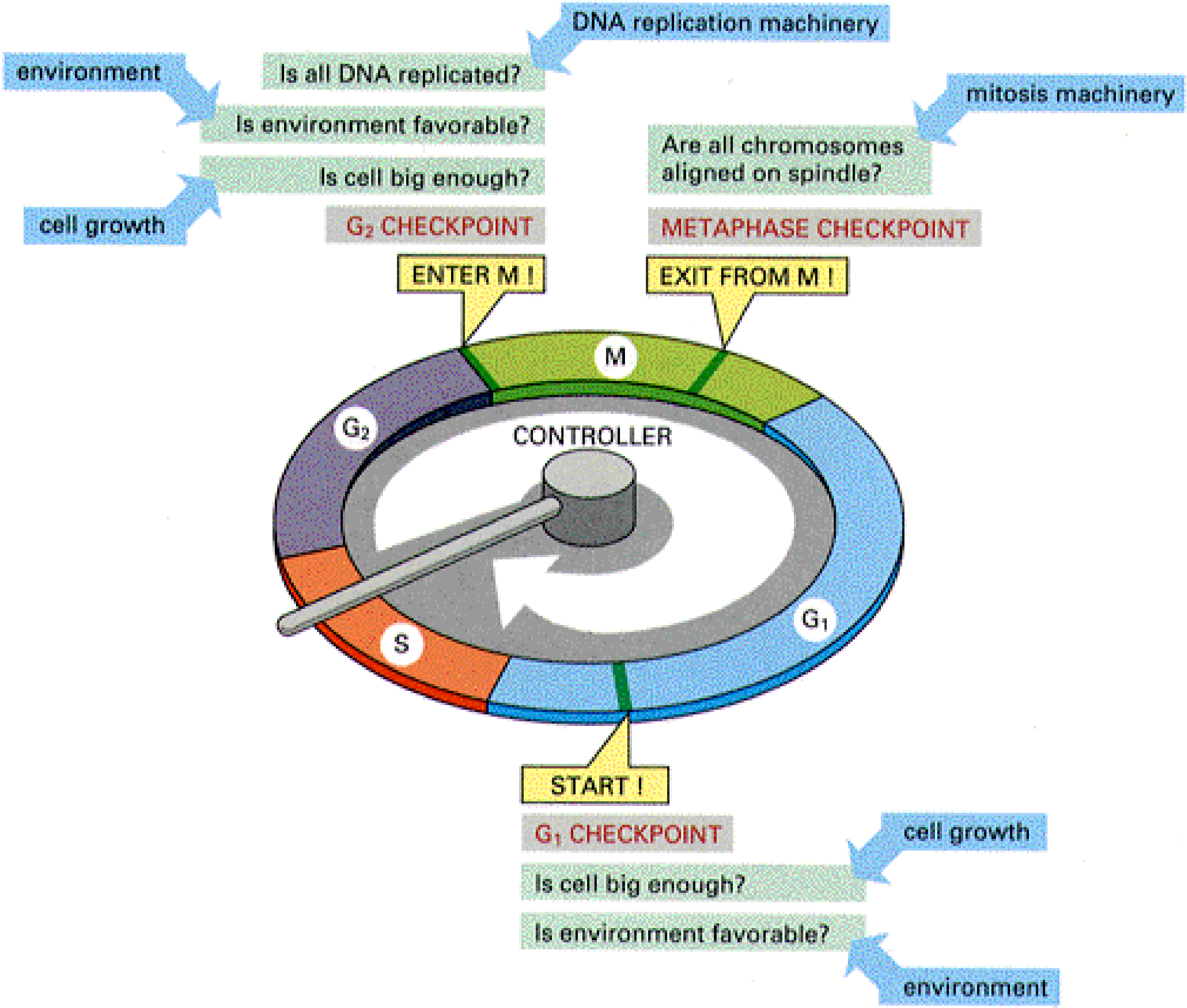
S phase 'cytoplasm' dominant over G1 M 'cytoplasm' dominant over interphase

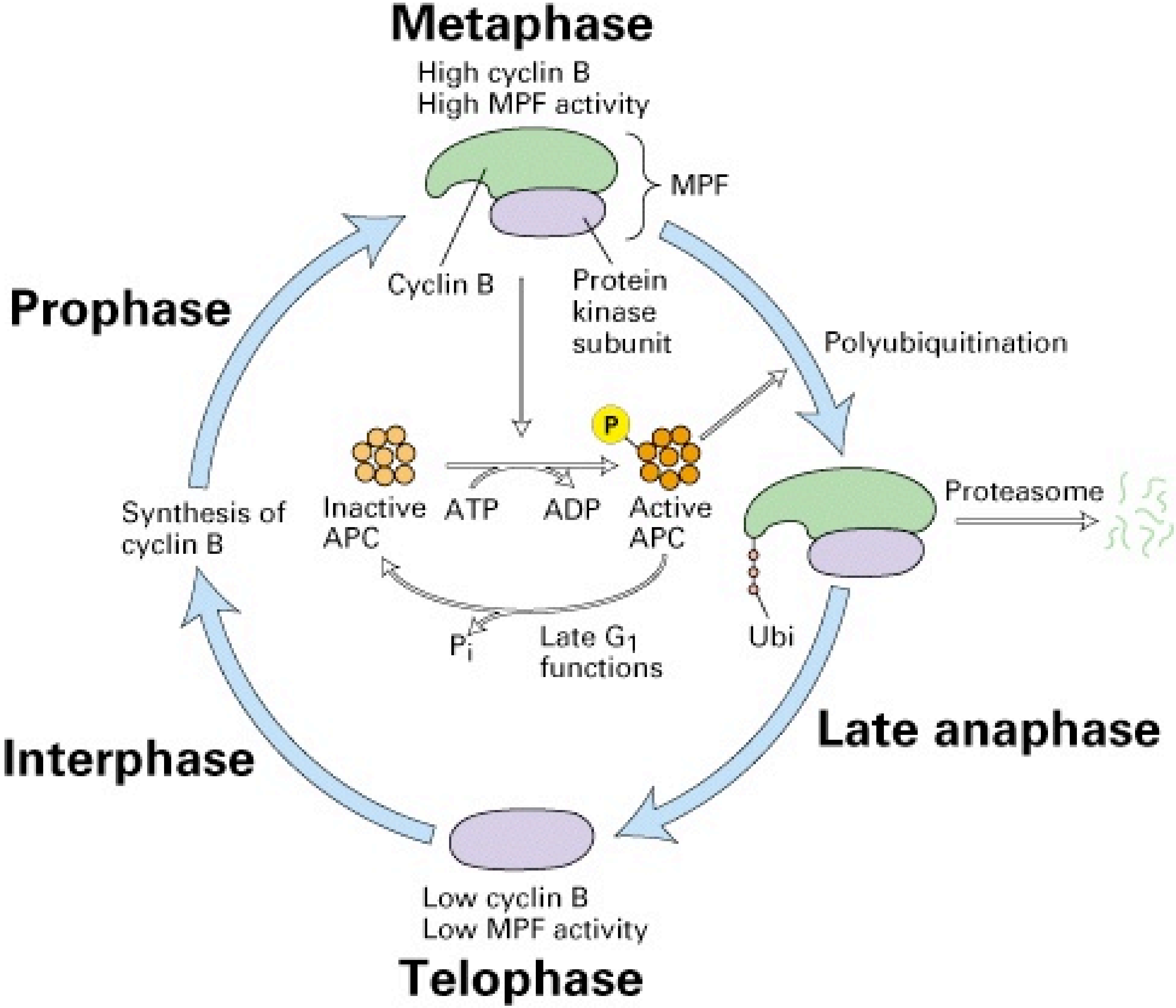
G1/S is the major point of regulation



environment, cell size

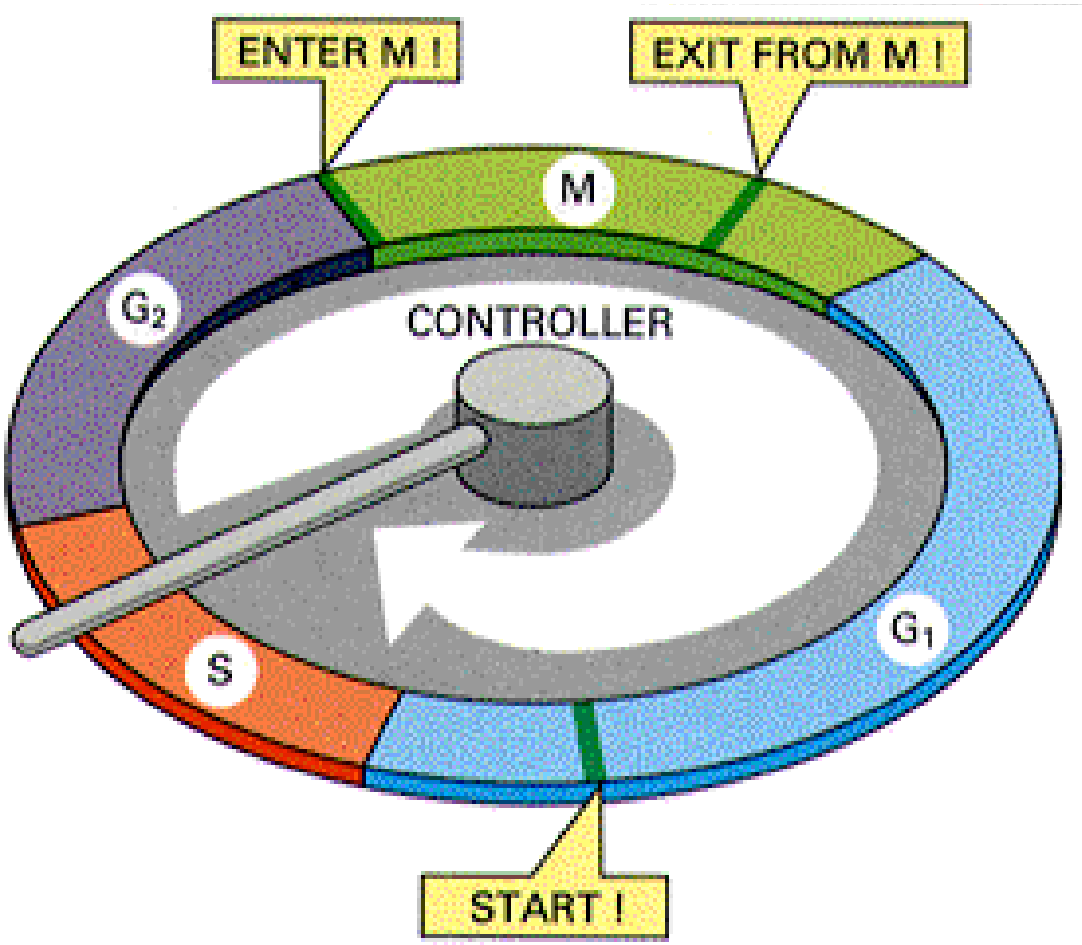
(nutrients, contact with other cells, extracellular signals)


DNA replication machinery
environment
Is all DNA replicated?
Is environment favorable?
mitosis machinery
Are all chromosomes aligned on spindle?
Is cell big enough?
cell growth
G_2 CHECKPOINT
METAPHASE CHECKPOINT
ENTER M !
EXIT FROM M !
M
G_2
CONTROLLER
G_1
S
START !
G_1 CHECKPOINT
cell growth
Is cell big enough?
Is environment favorable?
environment



Metaphase
High cyclin B
High MPF activity
MPF
Cyclin B
Protein kinase subunit
Prophase
Polyubiquitination
P
Synthesis of cyclin B
Inactive APC
ATP
ADP
Active APC
Proteasome
P_i
Late G_1 functions
Ubi
Late anaphase
Interphase
Low cyclin B
Low MPF activity
Telophase

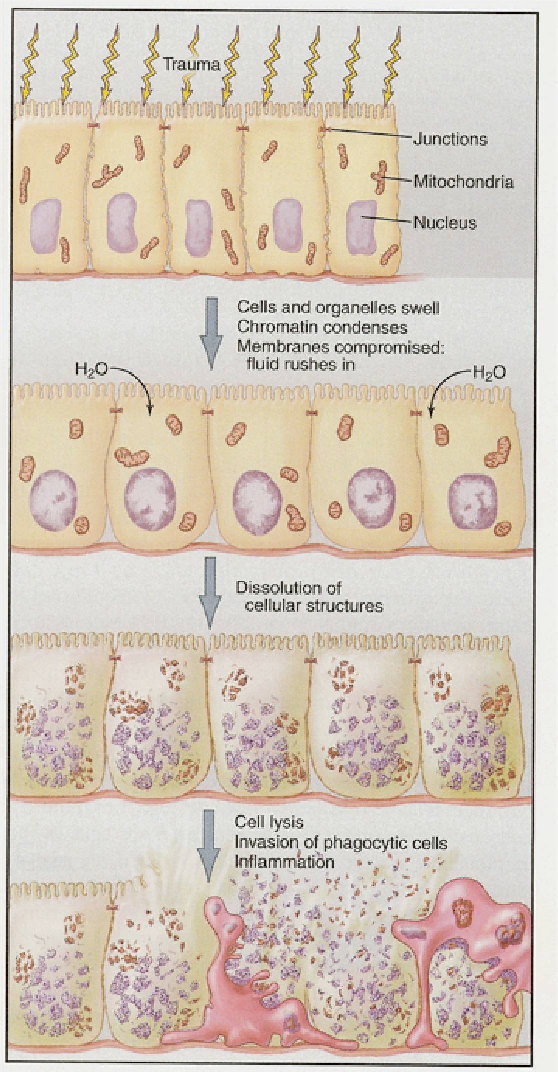
Cell Death is an Integral Part of Cell Cycle Regulation



Apoptosis - programmed cell death, 'suicide'

Necrosis - not programmed, eg due to injury

Necrosis


Trauma
Junctions
Mitochondria
Nucleus
Cells and organelles swell
Chromatin condenses
Membranes compromised:
fluid rushes in
H_2O
H_2O
Dissolution of
cellular structures
Cell lysis
Invasion of phagocytic cells
Inflammation

‘The Departed’

DiCaprio

HMGB1-YFP in HeLa Cells (UV-treated)

Time-lapse interval = 2 mins
Frame rate = 10 fps

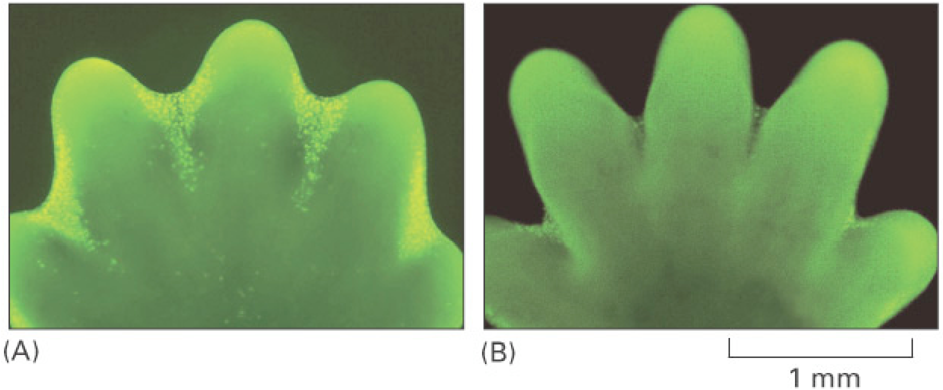
Damon

HeLa Cells, UV damage

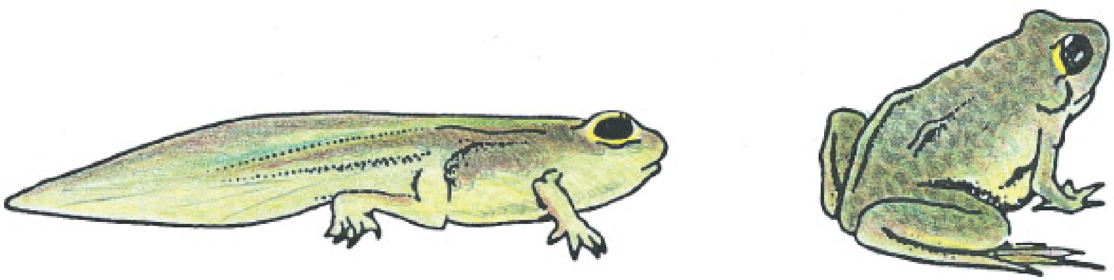
CHROMATIN

Apoptosis and Development

sculpting of embryonic forms
development of nervous system
elimination of self-reactive parts of the immune system

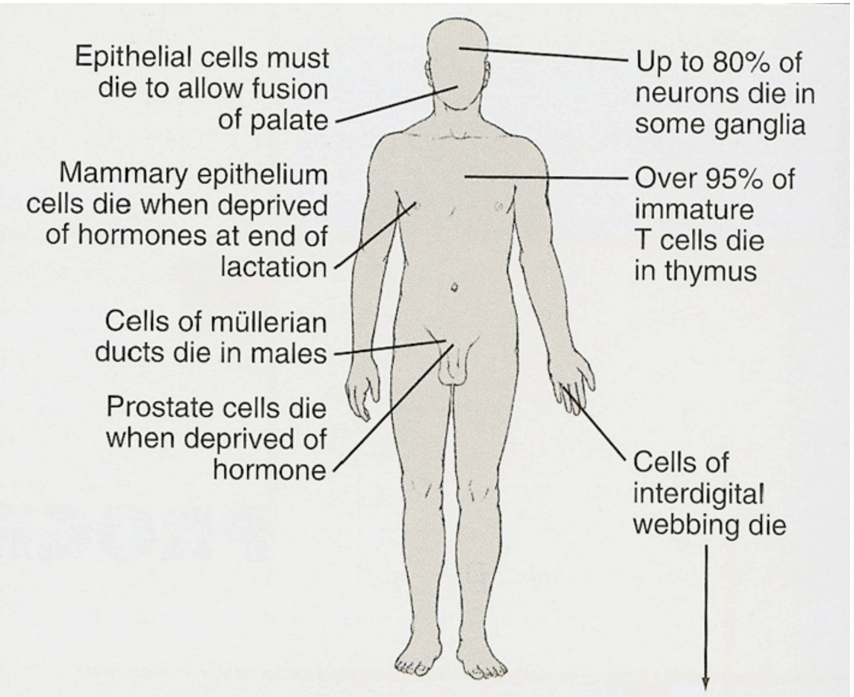


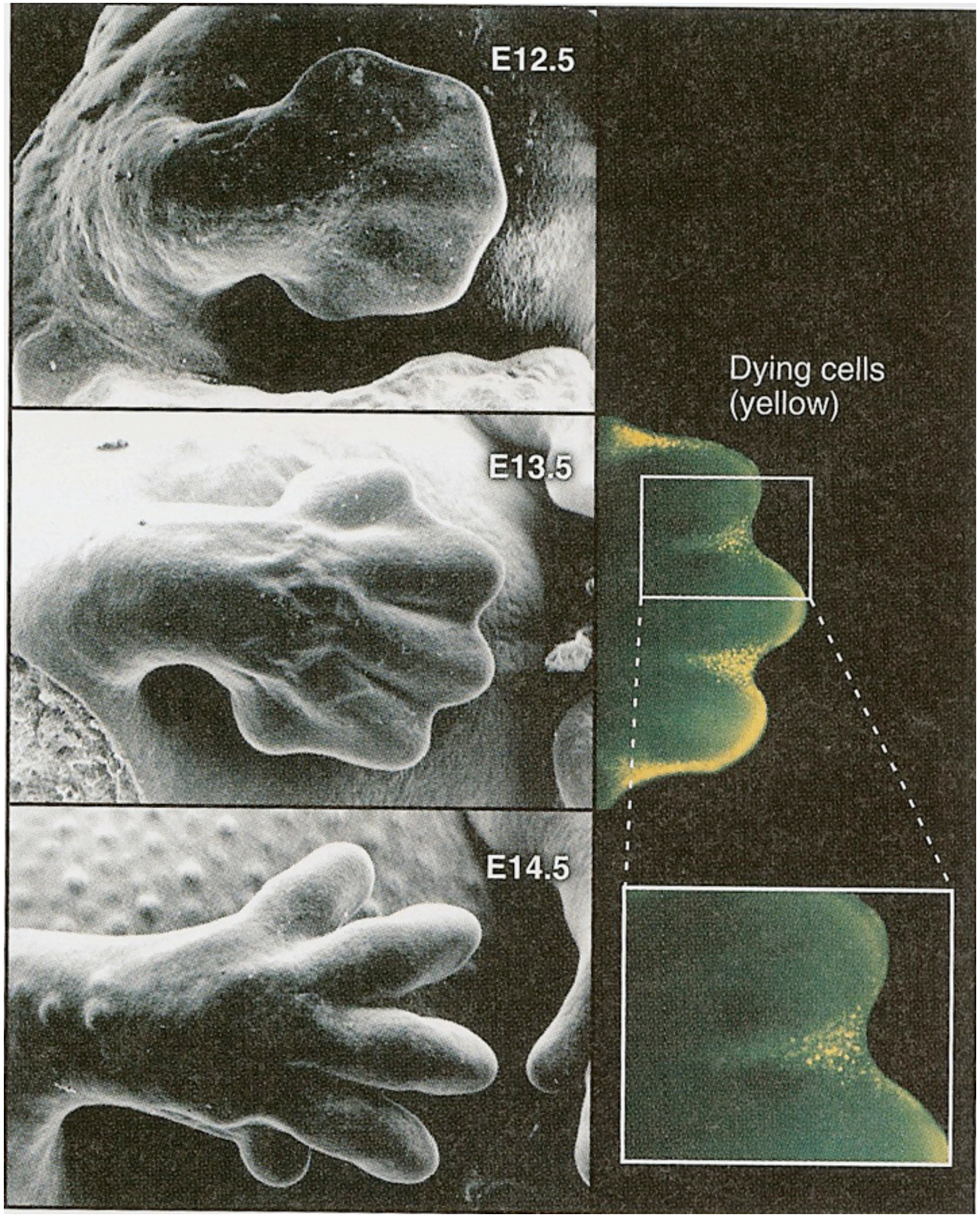
Interdigital cell death elaborates digits



Apoptosis eliminates a tadpole’s tail

Programmed Cell Death in Human Development

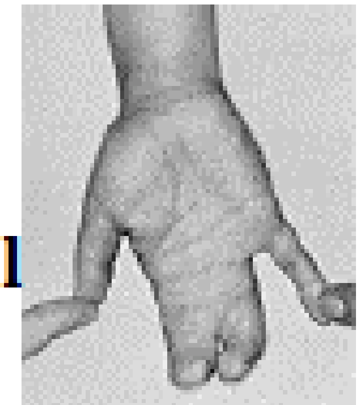




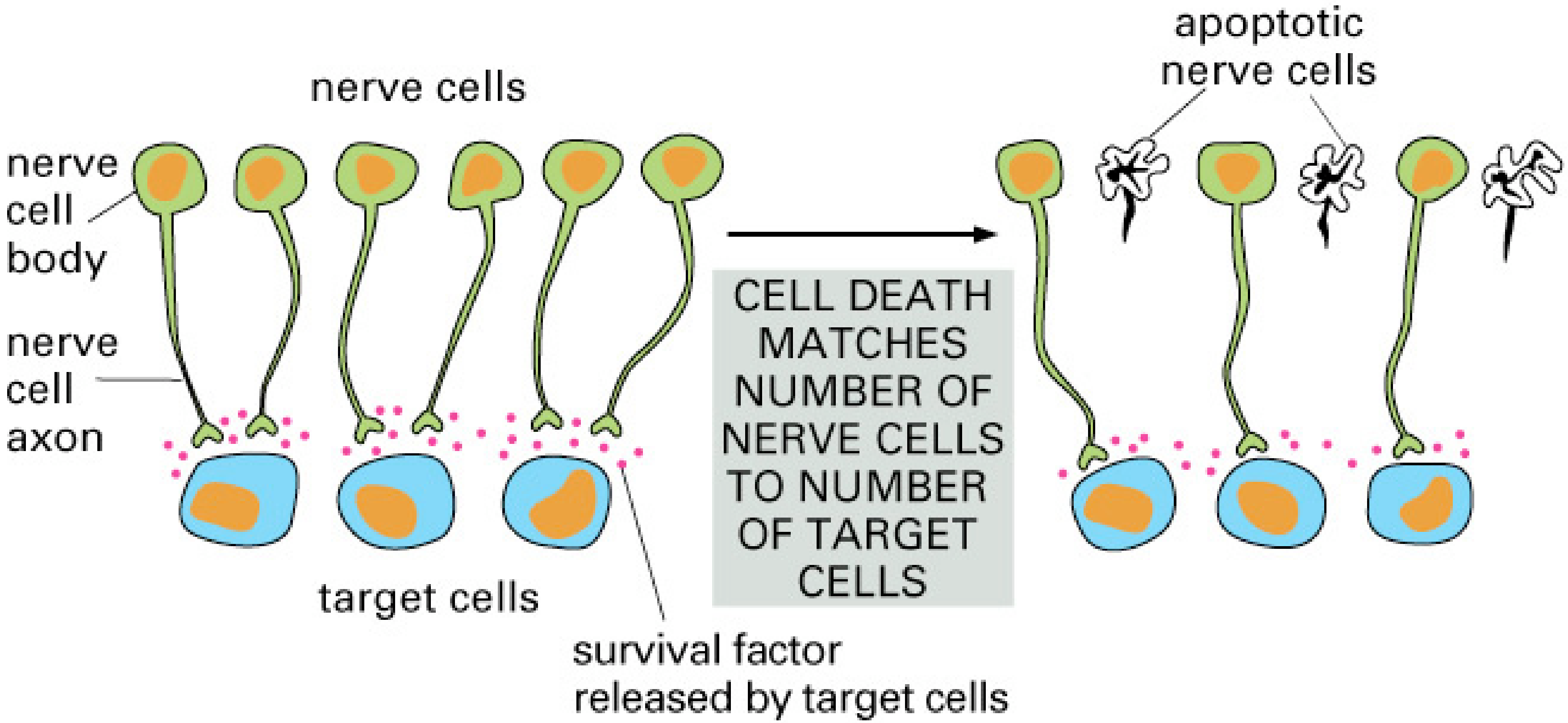
Apoptosis Required for Normal Development

Normal
Apoptosis

Dysfunctional
Apoptosis



Cell death is essential during formation of the nervous system



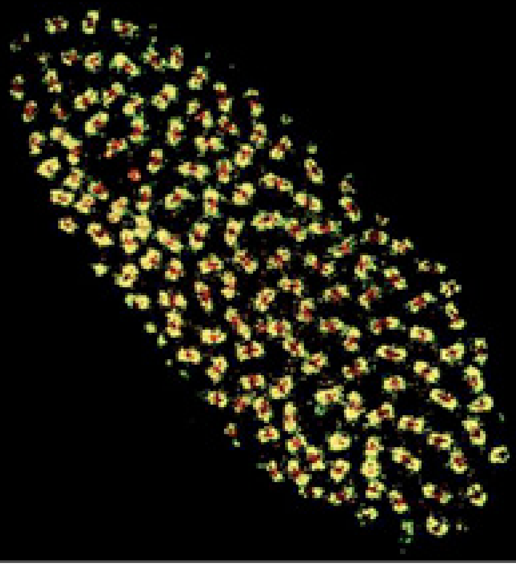
Variations on a Theme.. Many Types of Cell Divisions

Somatic cell cycles - slow (~24 hrs), have G1 and G2

Embryonic cell cycles - fast, only S & M

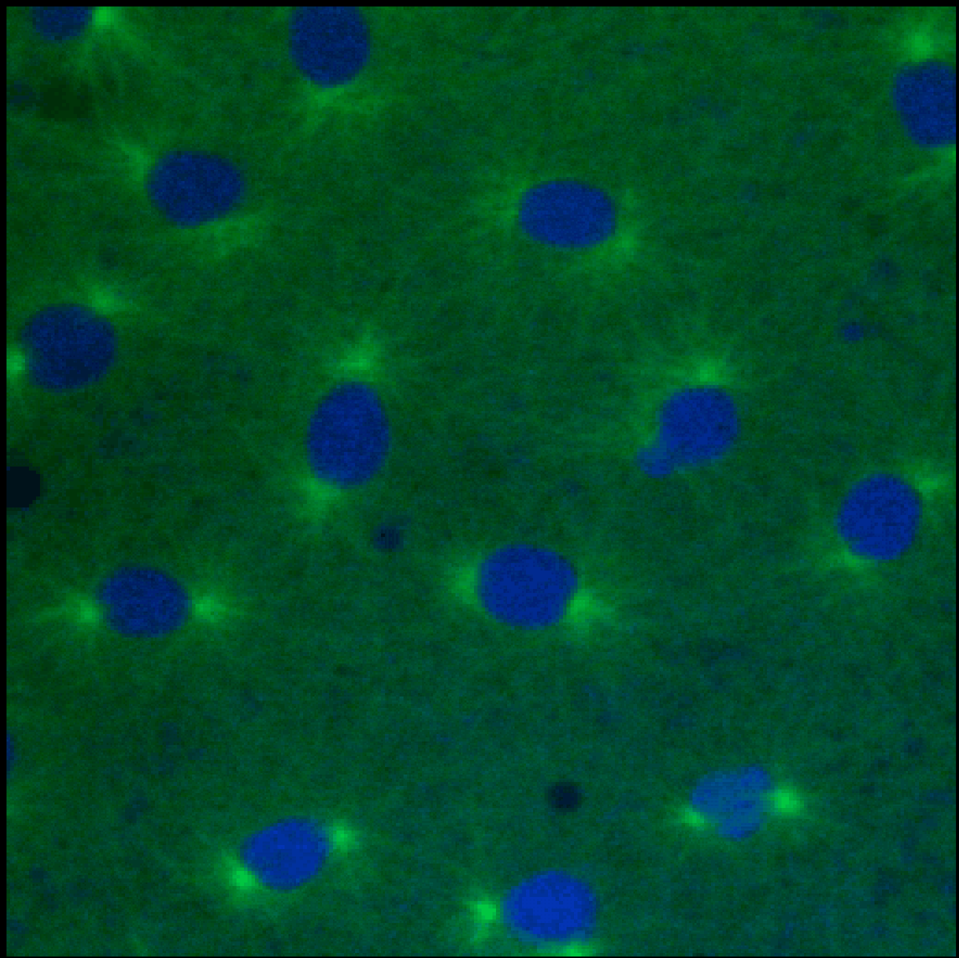
Drosophila

6-10 minute cycles,
synchronous nuclear division
in one cell (syncytium)



DNA

Tubulin



Variations on a Theme.. Many Types of Cell Divisions

Embryonic cell cycles - fast, only S & M

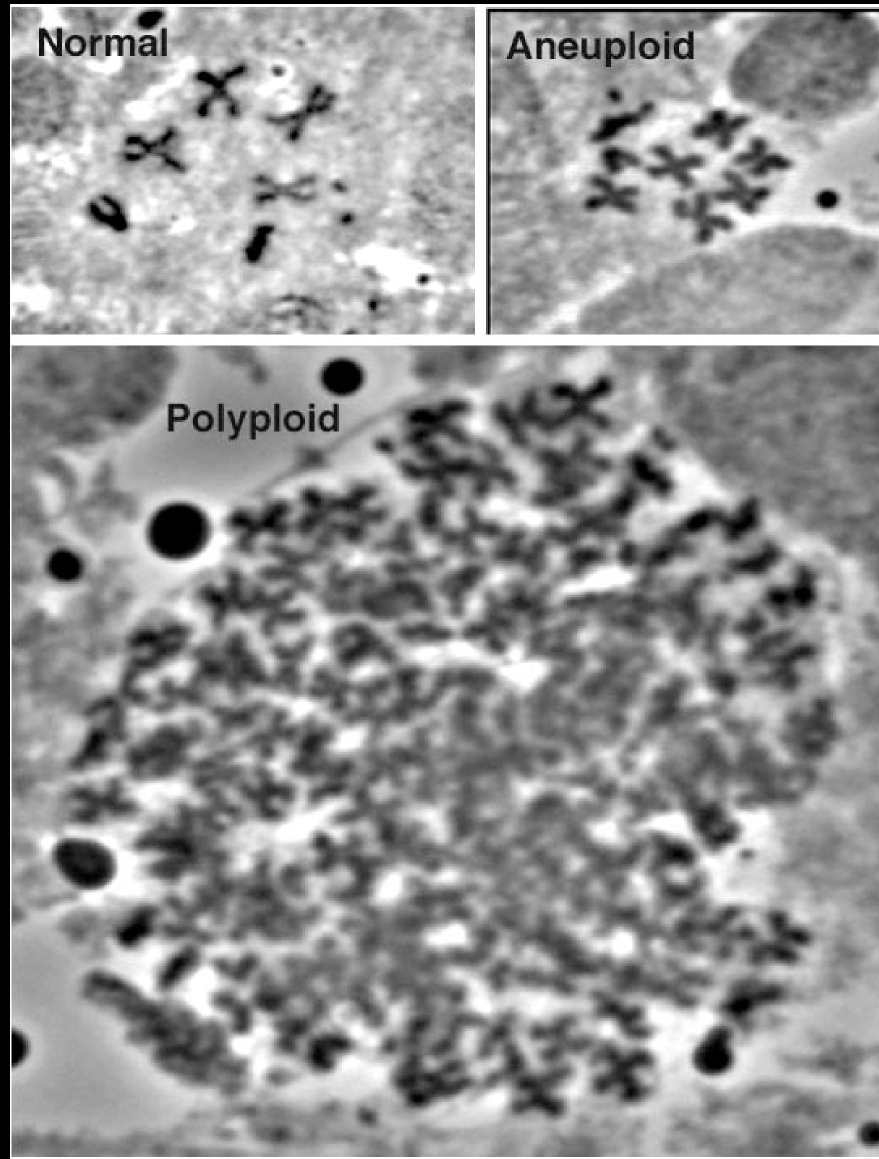
Vertebrates, Sea Urchins

**30-60 minute cycles,
cell divisions**

Variations on a Theme.. Many Types of Cell Cycles

Polyploidy-
multiple copies of
normal genome (eg 4 instead of 2)

occurs normally, e.g. liver cells hexaploid

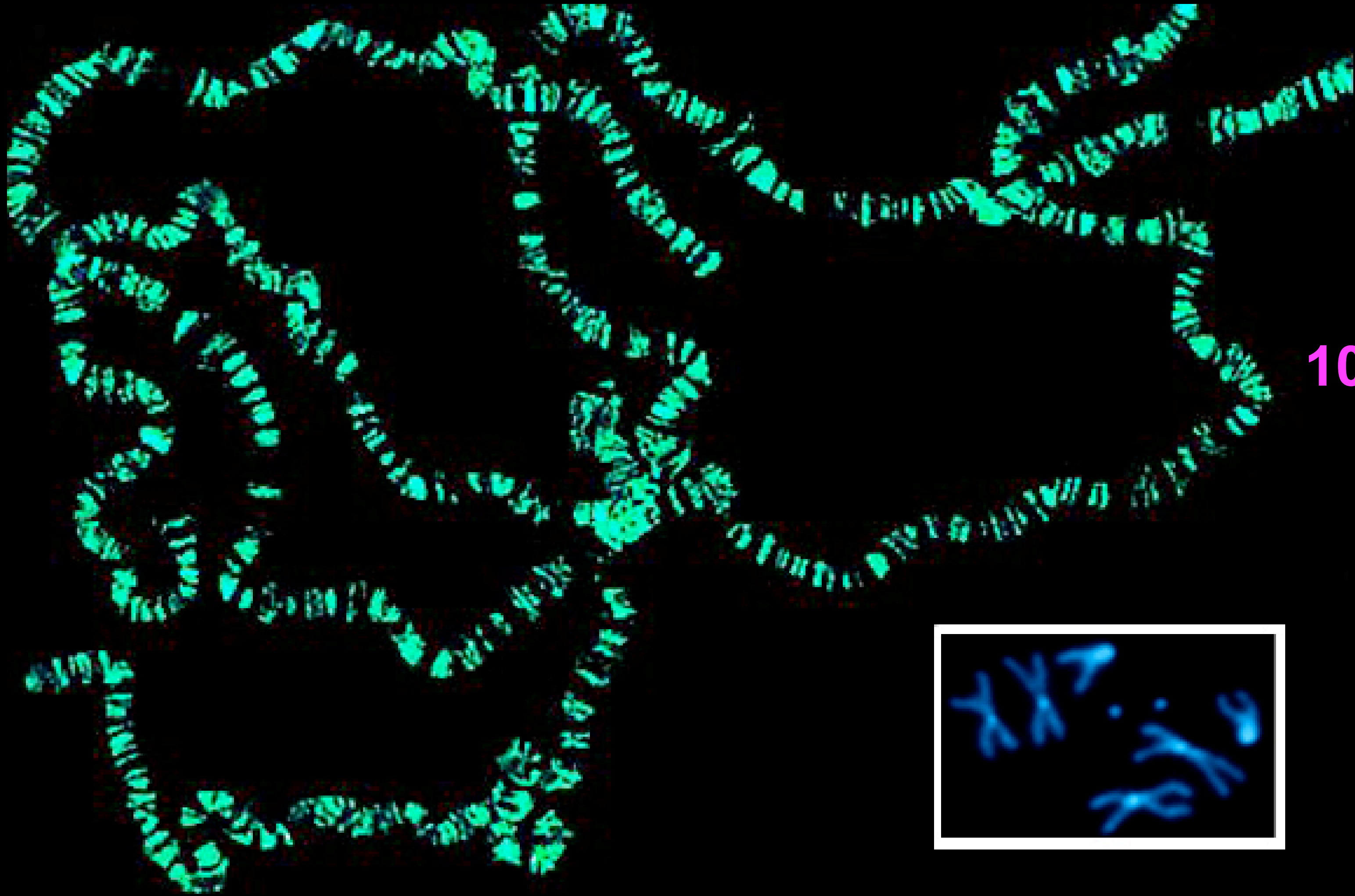


Endoreplication - continued replication and growth without division

Variations on a Theme.. Many Types of Cell Cycles

Endoreplication - continued replication and growth without division

Polyteny- homologous chromosomes retain alignment

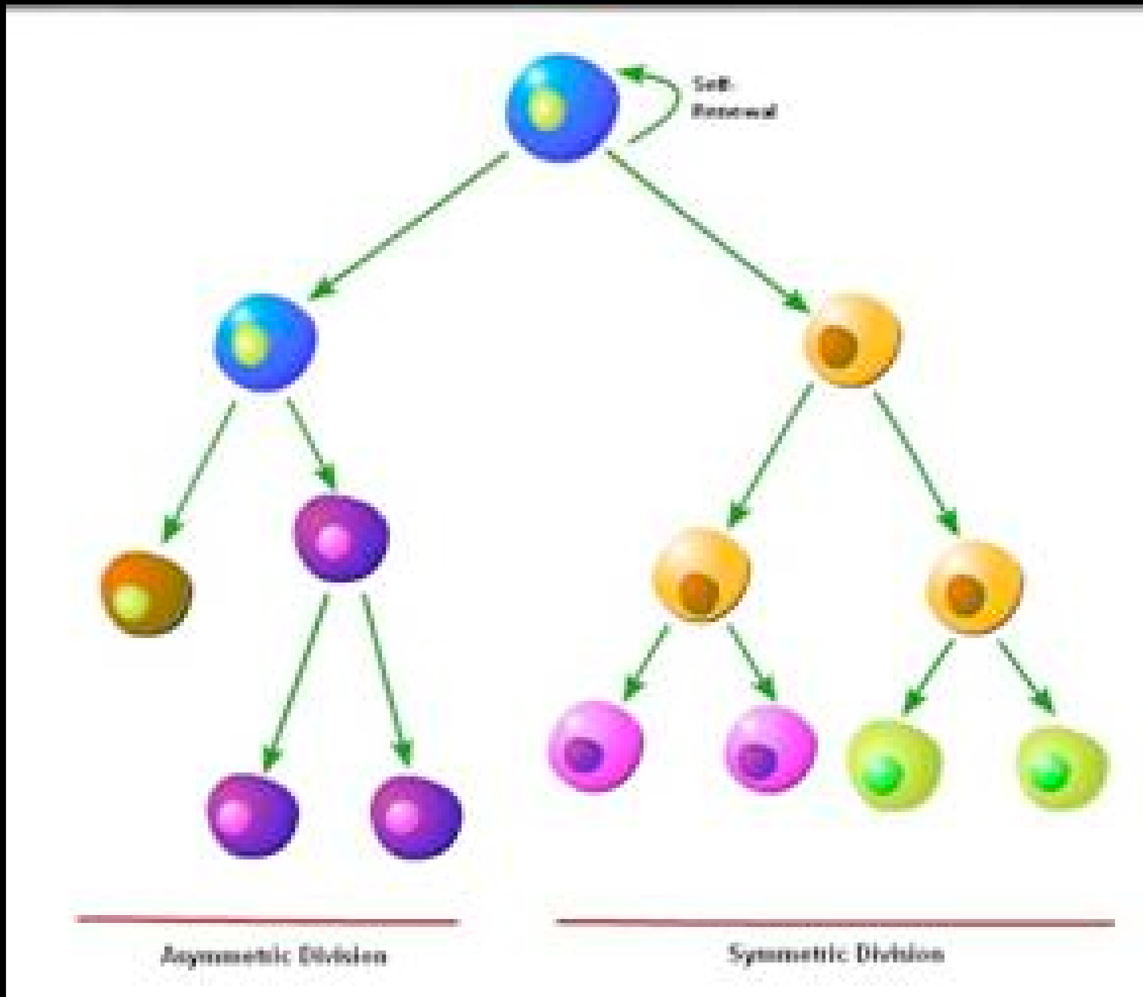


1000-2000C

Variations on a Theme.. Many Types of Cell Cycles

Asymmetric divisions - unequal segregation of cell fate determinants

Stem cells

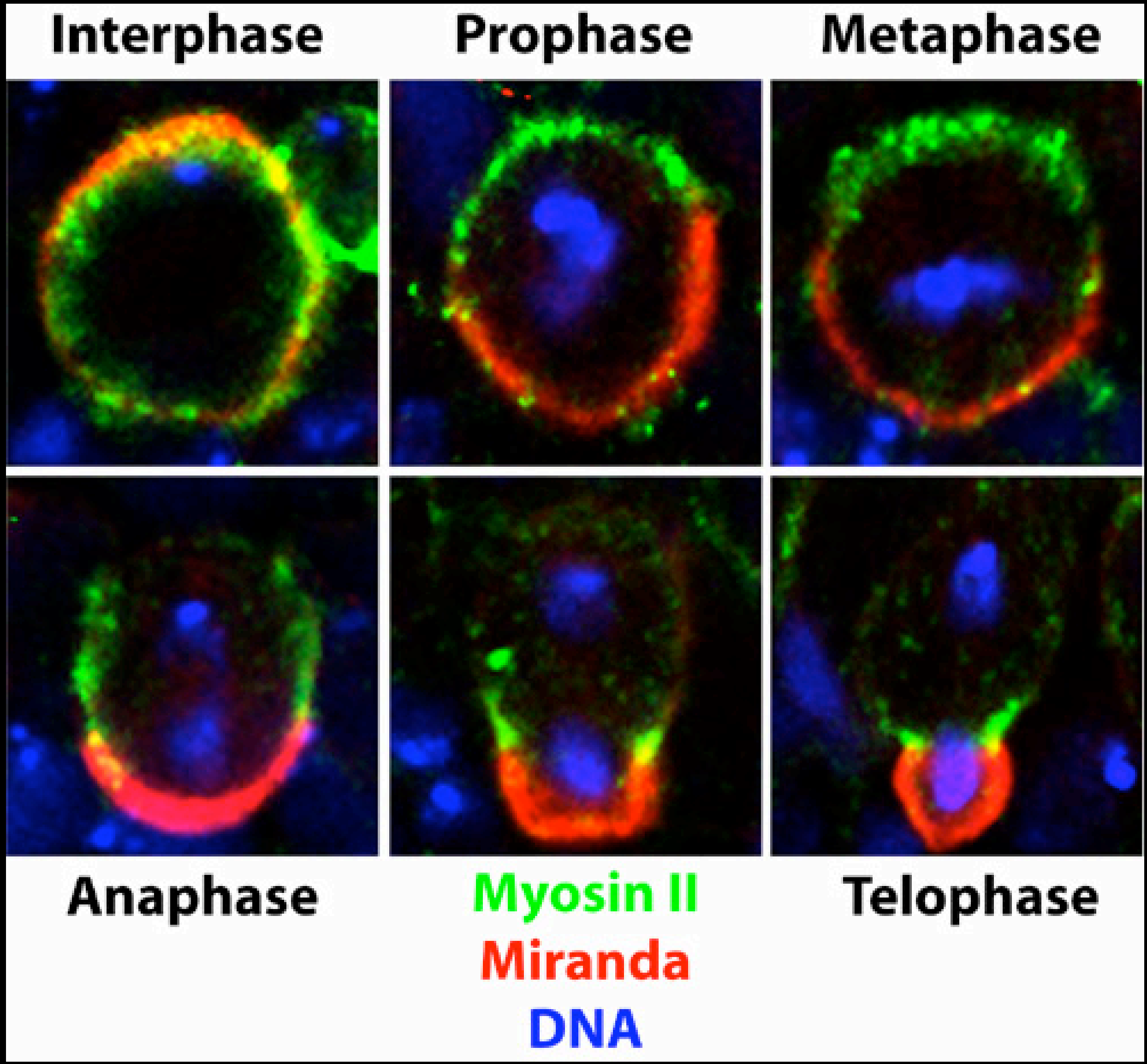


Variations on a Theme.. Many Types of Cell Cycles

Asymmetric divisions - unequal segregation of cell fate determinants

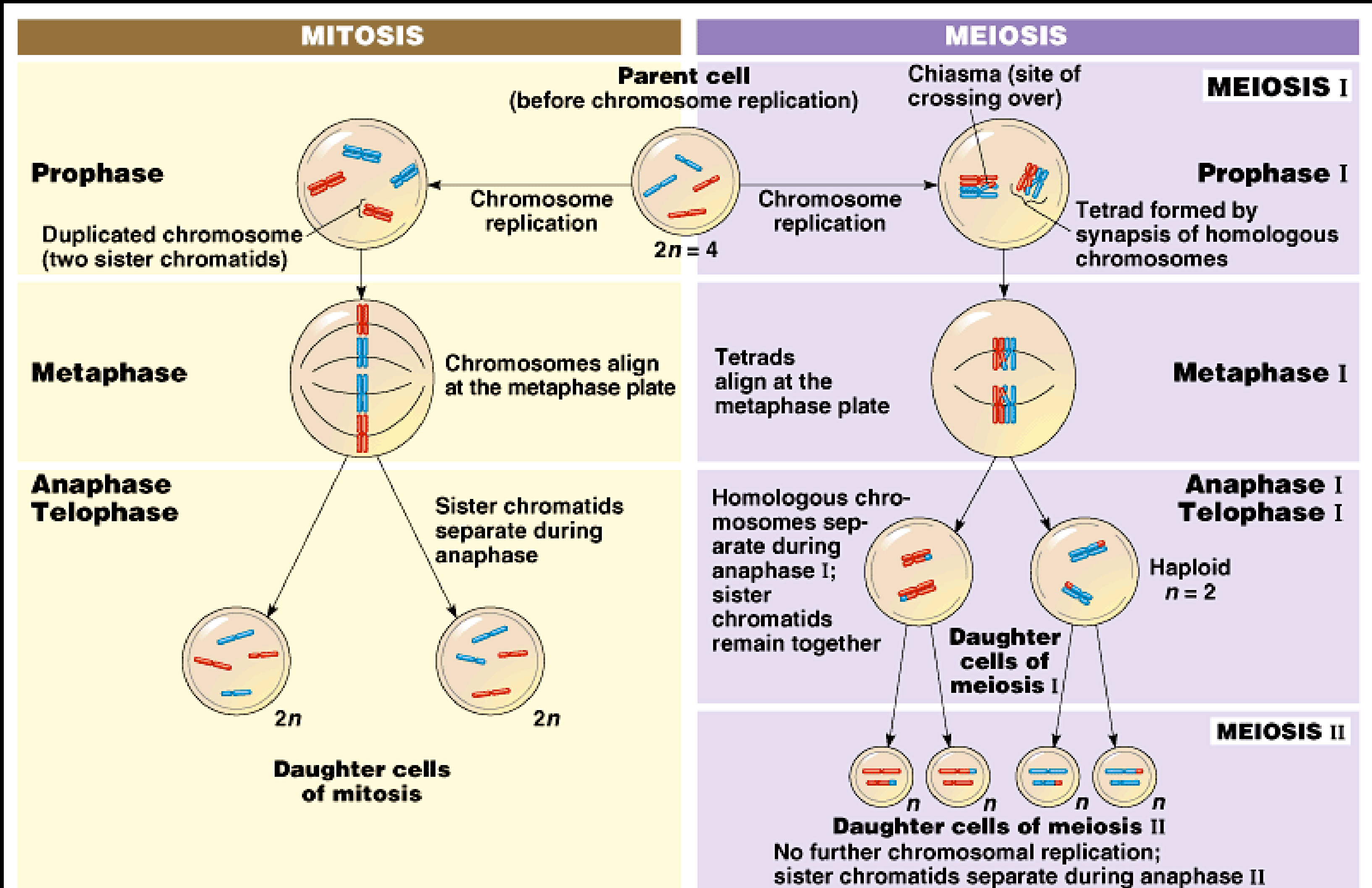
Neuroblasts

Miranda maintains undetermined state

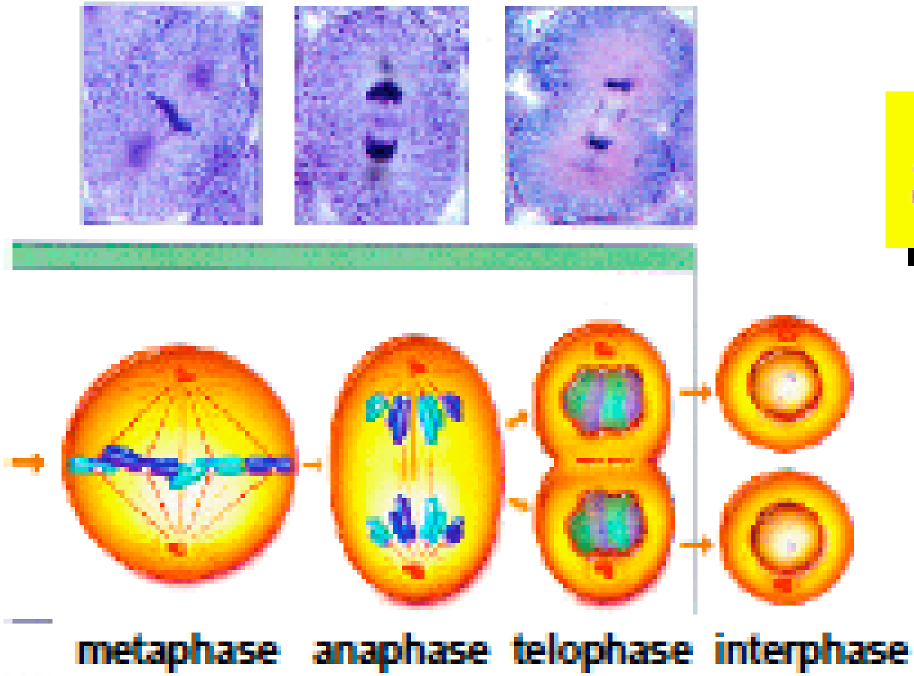


Variations on a Theme.. Many Types of Cell Cycles

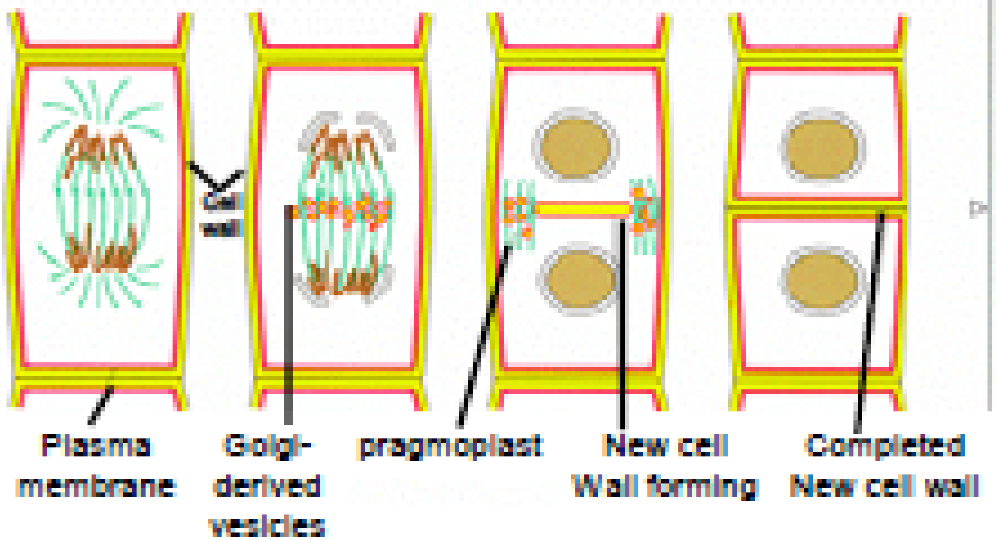
Meiosis- production of haploid germ cells

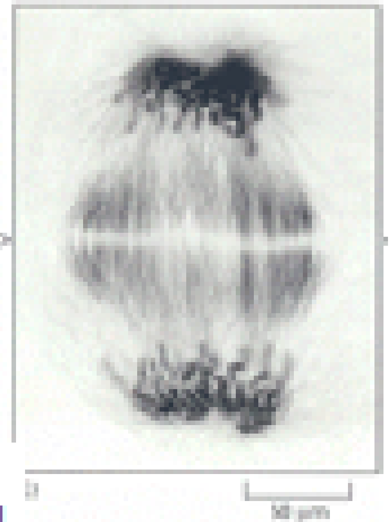


animal



plants



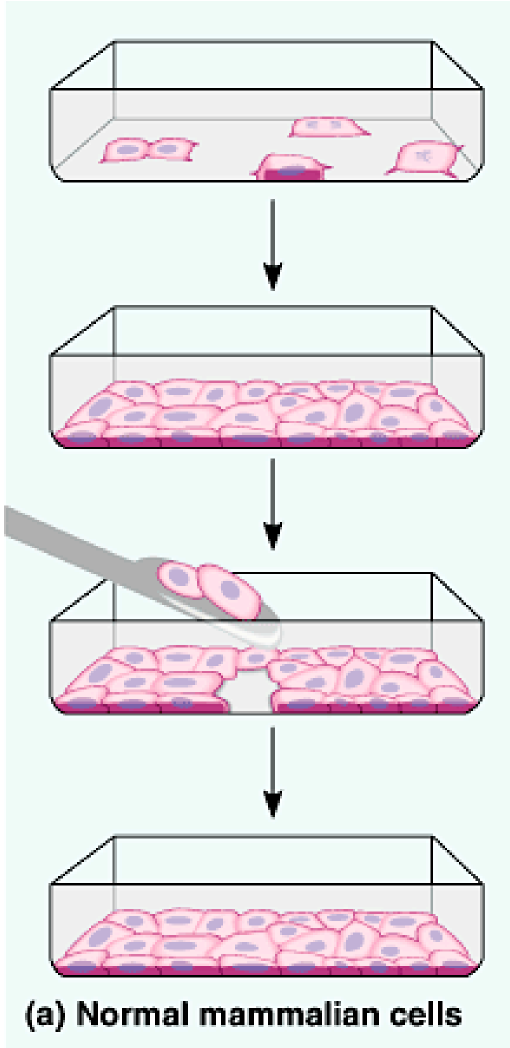


Consequences of Defective Cell Divisions

unrestricted cell growth & cancer

chromosome missegregation & aneuploidy

loss of DNA integrity



seed new culture, growth and division
‘anchorage dependent’

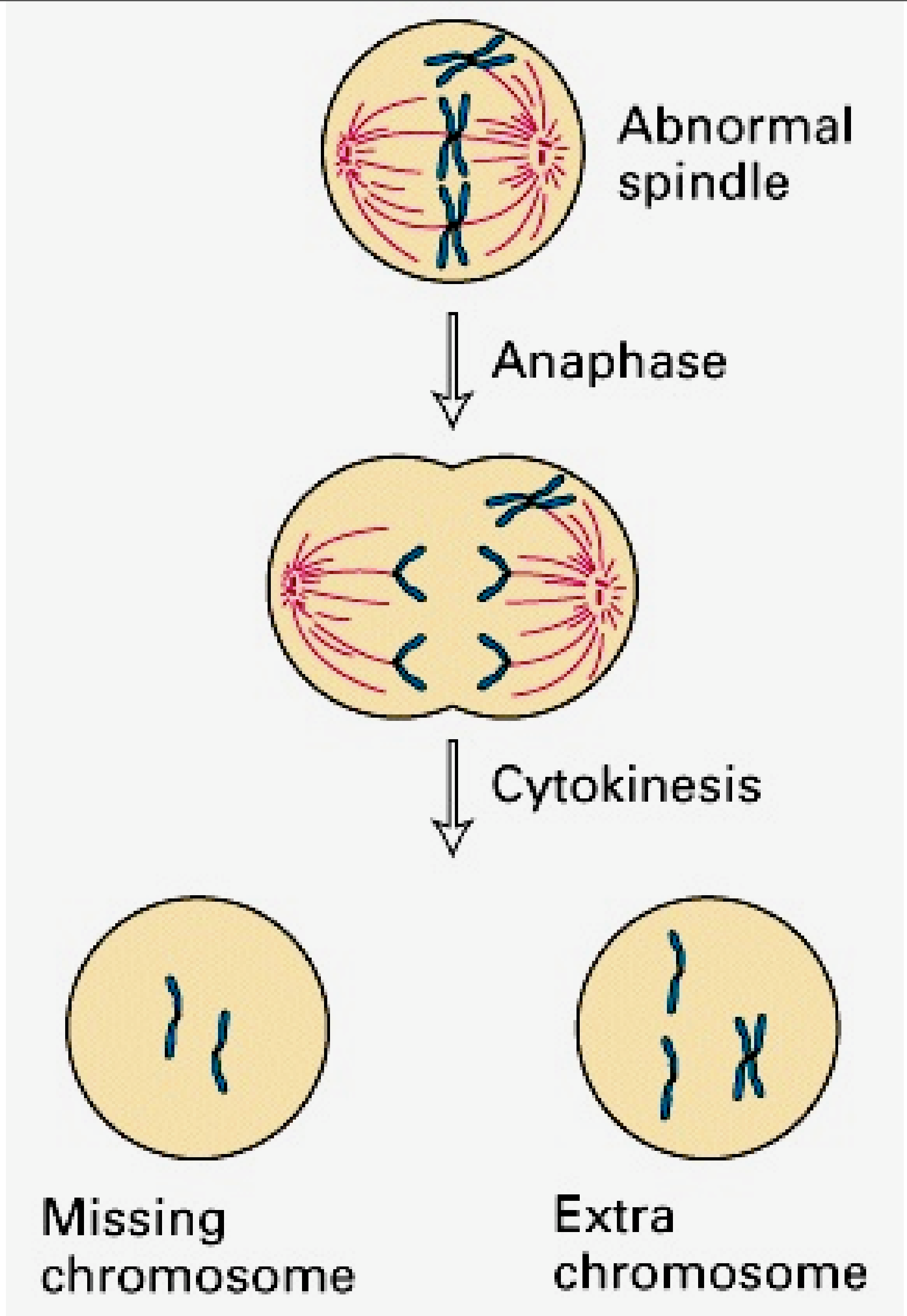
cells fill plate, stop growth and division
‘contact’ or ‘density-dependent’ inhibition

remove cells
new round of growth

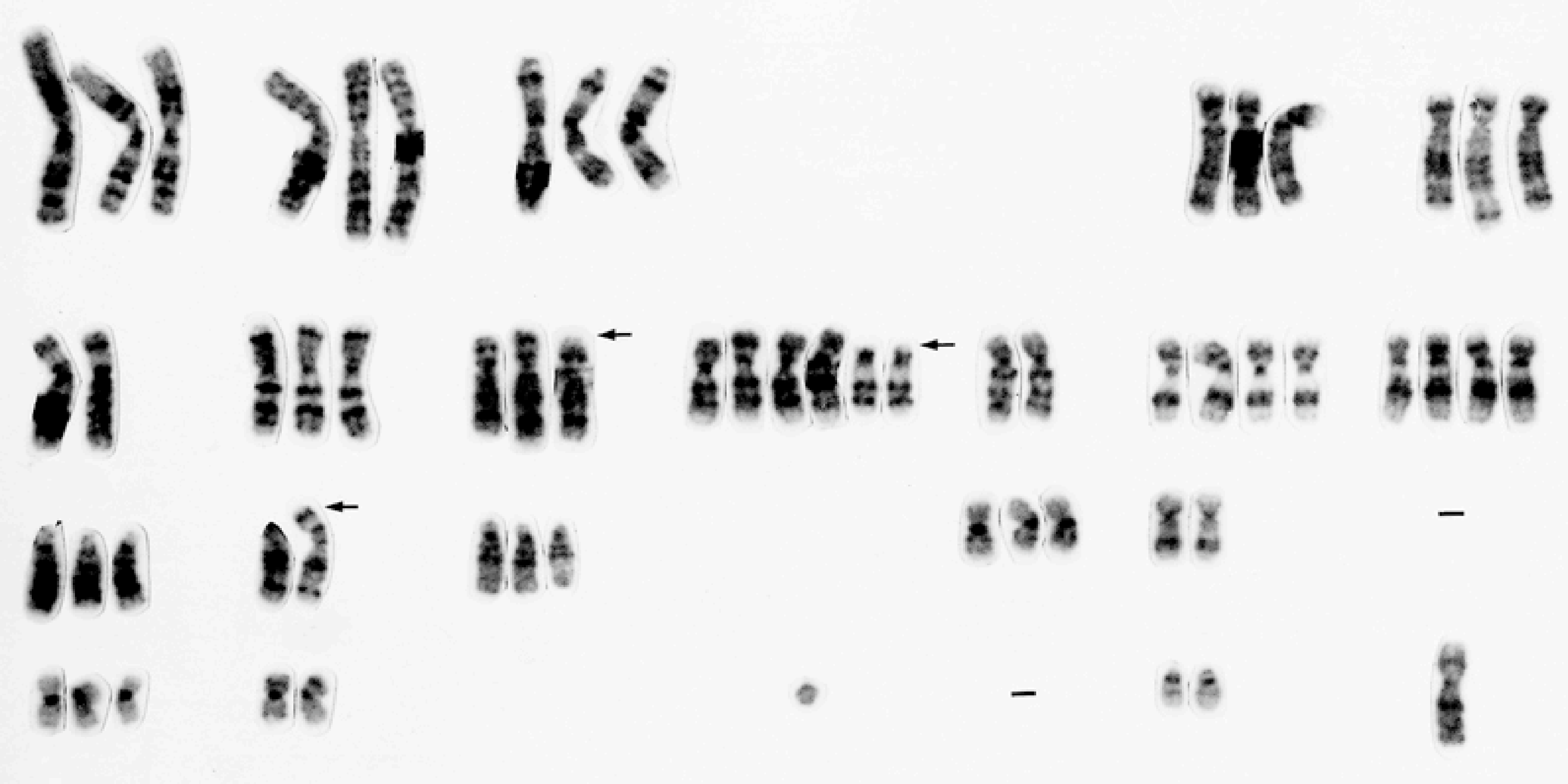
stop growth and division

Chromosome non-disjunction: aneuploidy

➔ TOAST



Karyotype of malignant pancreatic tumor

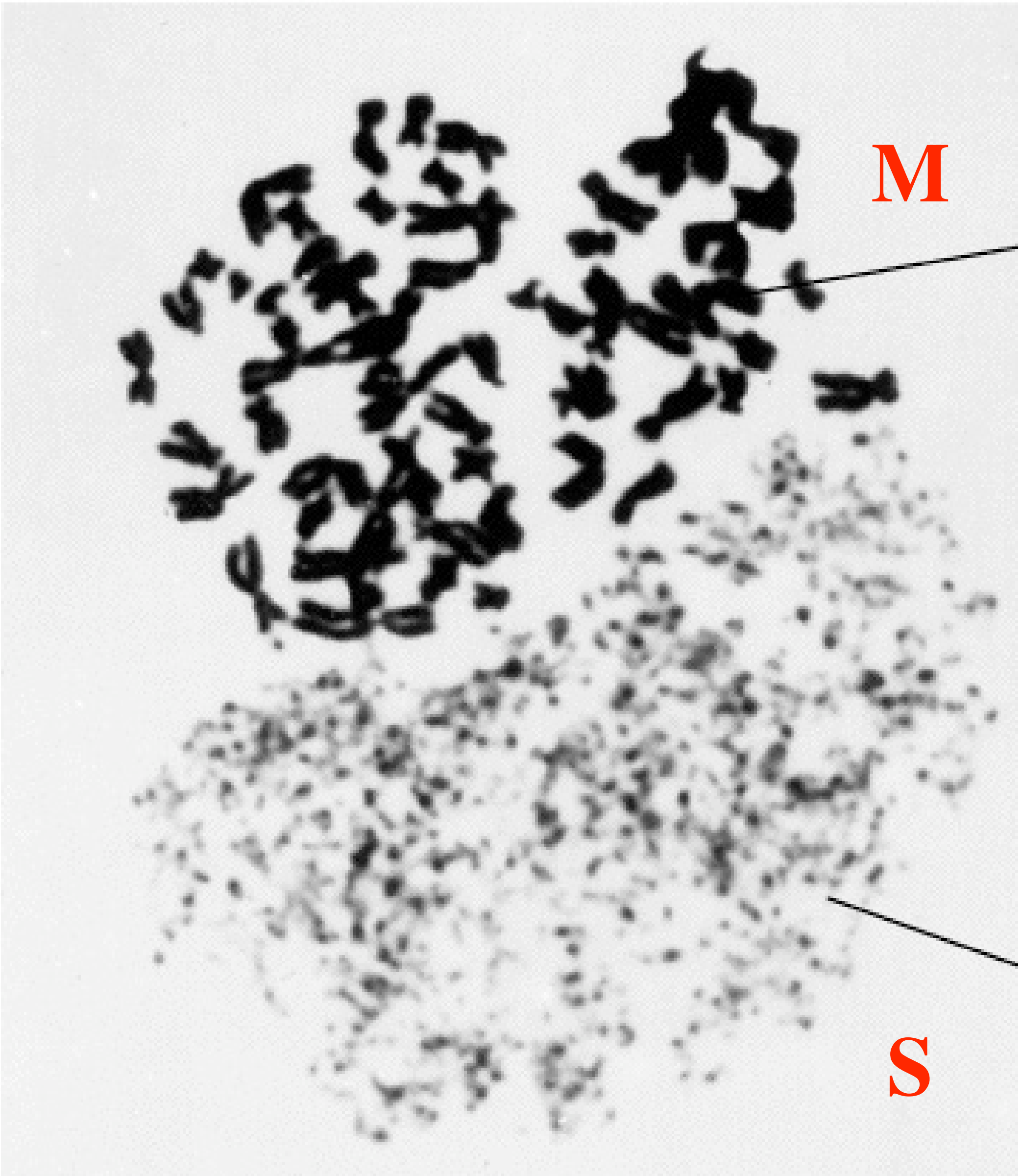


From Suresh C. Jhanwar, Memorial Sloan-Kettering Cancer Center.

defects in chromosome segregation:

→ birth defects

→ associated with tumor progression



chromosome condensation during S-phase

➔ TOAST

DNA damage:

chemicals
radiation
normal DNA metabolism

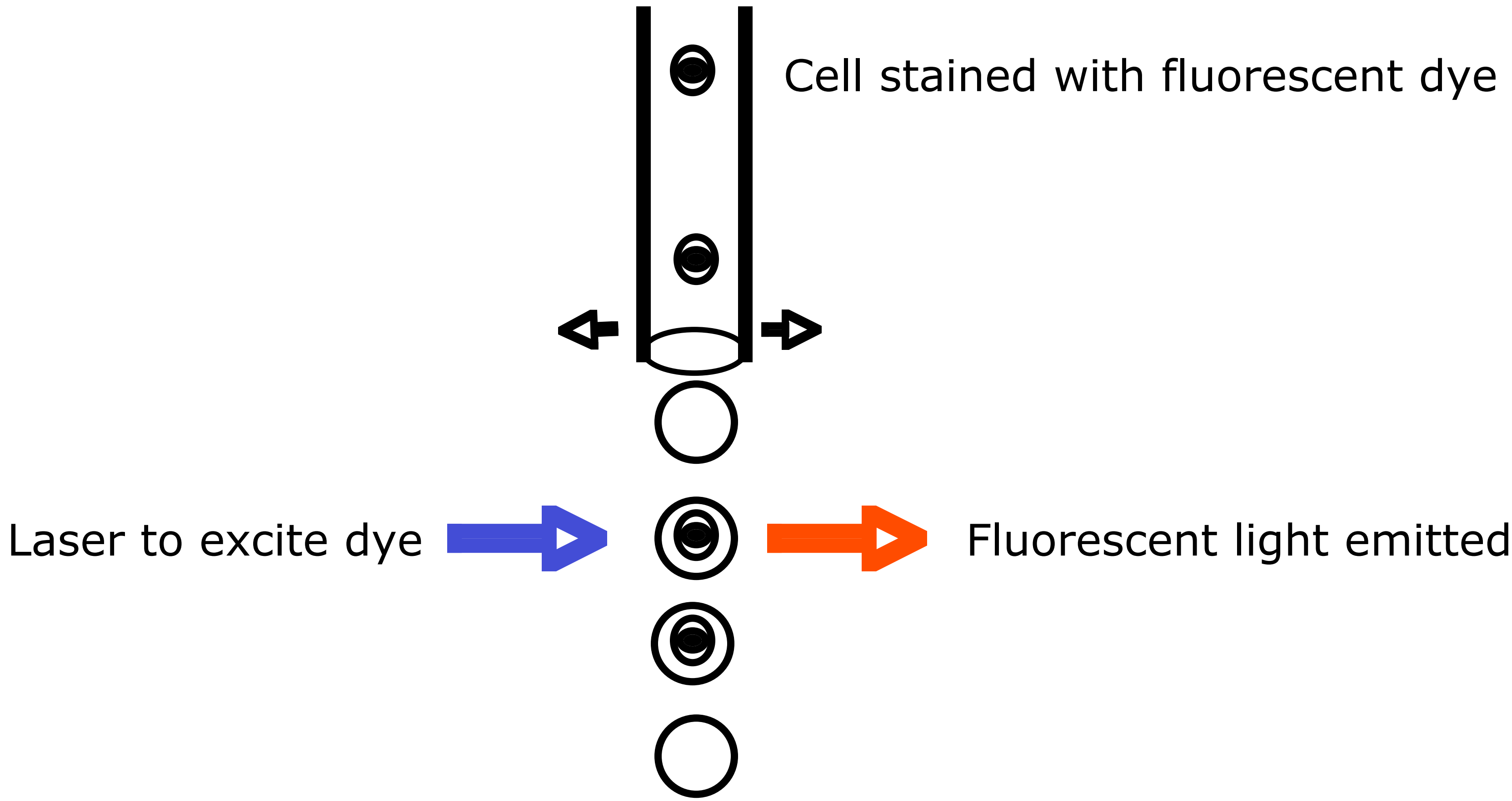
Methods for Studying the Cell Cycle

cell fusion
live and fixed imaging

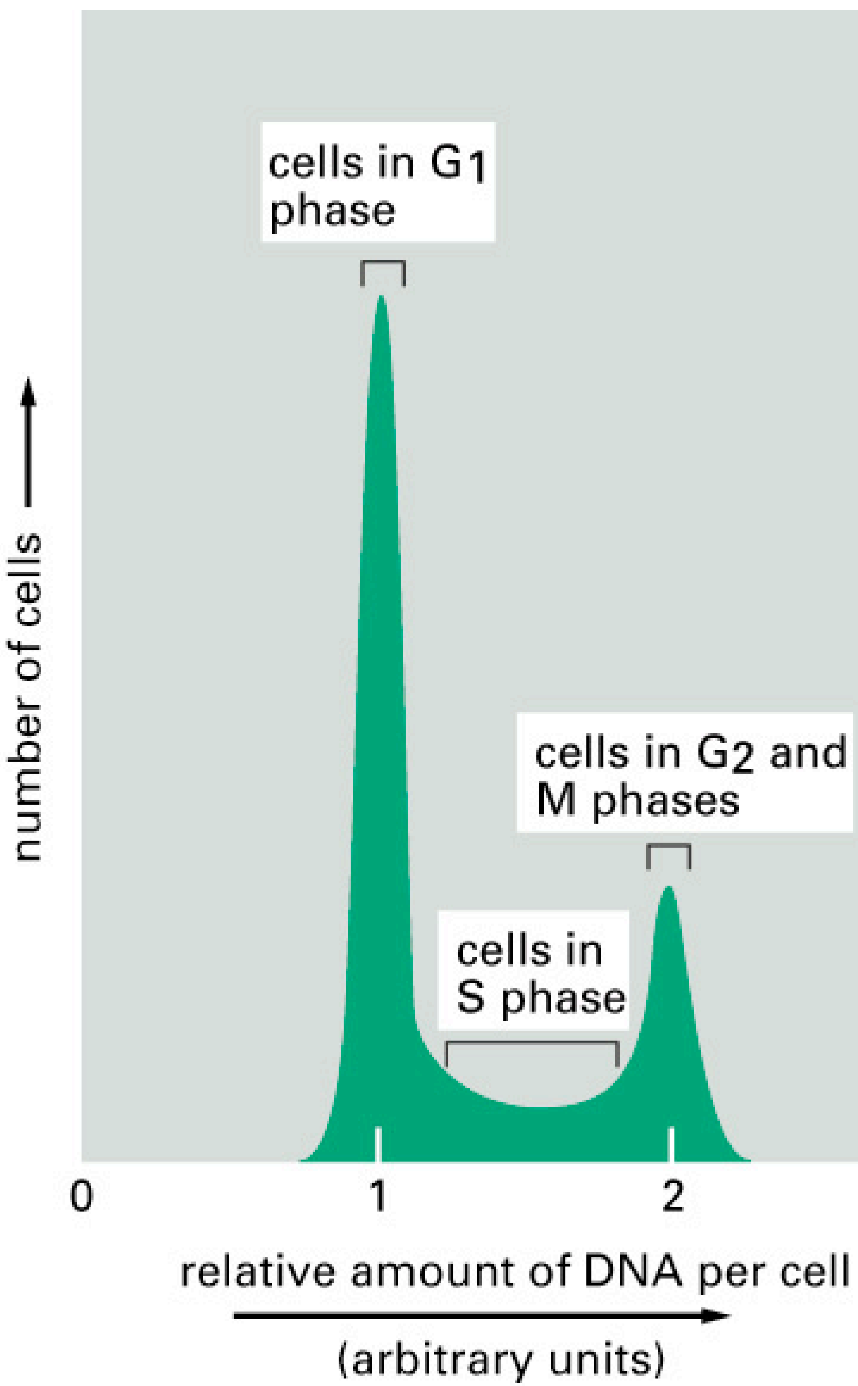
genetics
biochemistry
in vitro systems

inhibitors of cellular processes
(transcription, replication, microtubules)

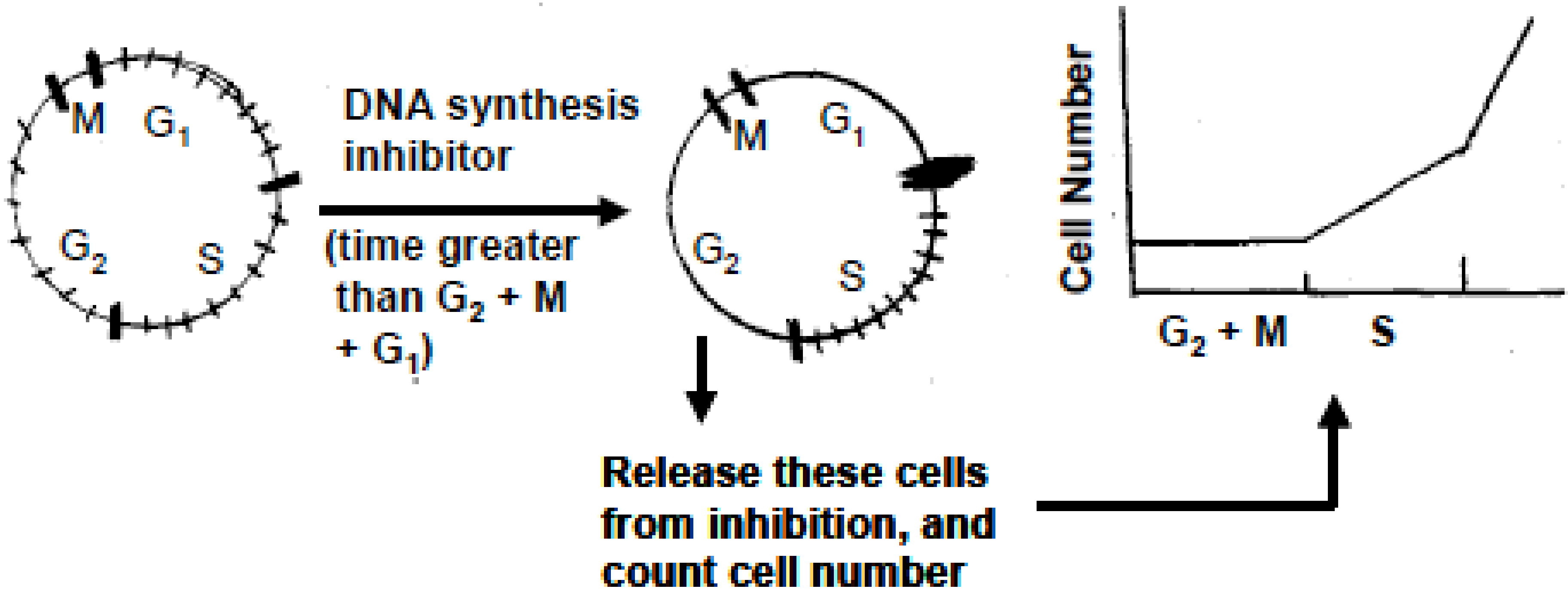
Cell Sorting



sort cell cycle stages based on total amount of DNA

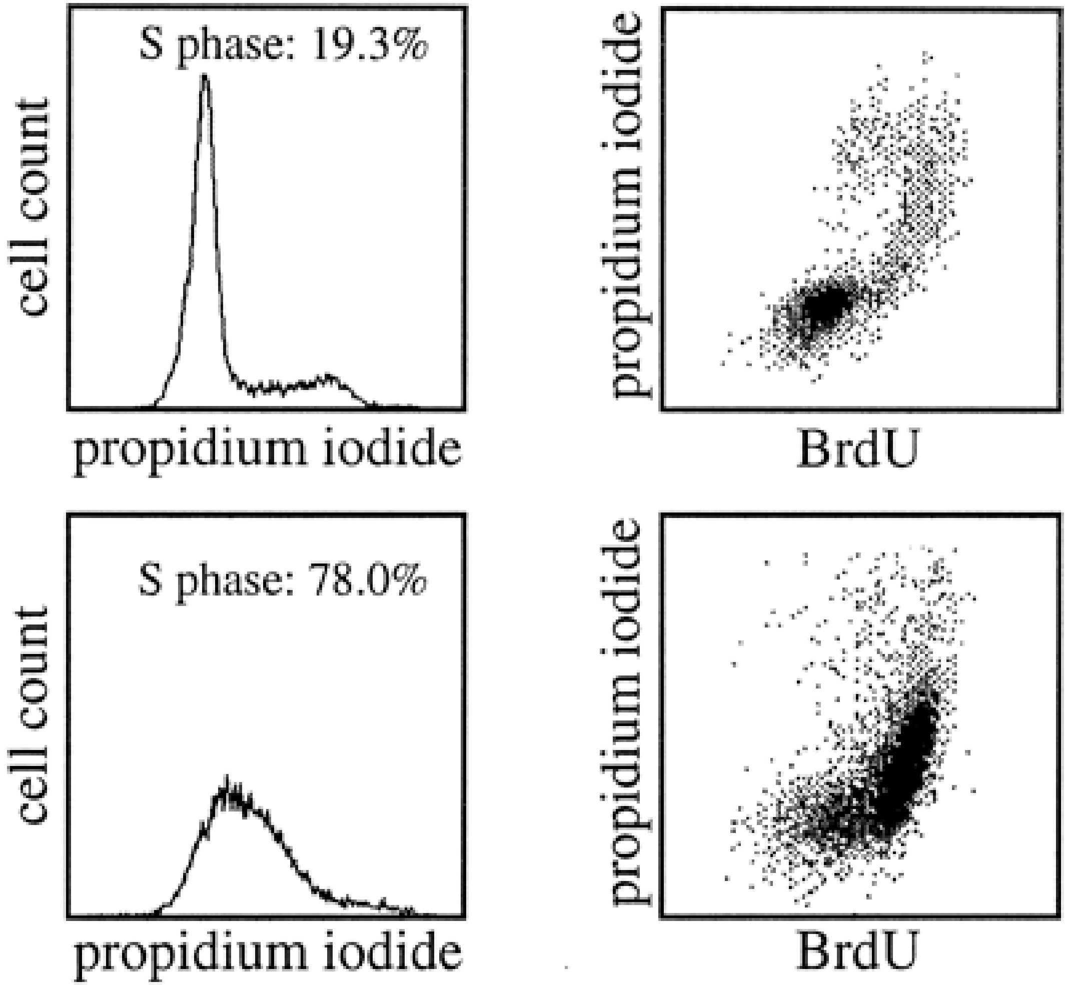

cells in G1 phase
cells in G2 and M phases
cells in S phase
number of cells
0
1
2
relative amount of DNA per cell
(arbitrary units)

Synchronizing Cells by Replication Inhibition



Synchronizing Cells by Replication Inhibition

add BrdU to determine which cells are in S phase, and how much DNA is replicated



no synchronization

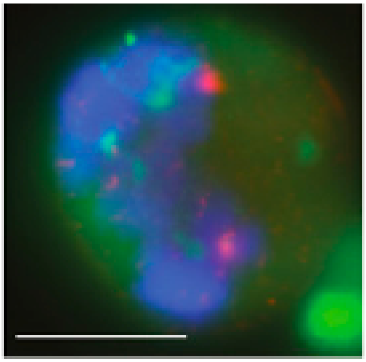
after synchronization

Synchronizing Cells by Mitotic Inhibition

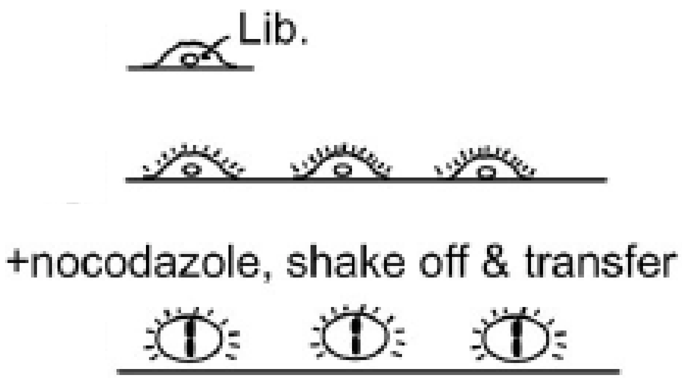
colchicine / colcemid / nocodazole - microtubule assembly inhibitors

taxol - microtubule disassembly inhibitor

+ nocodazole

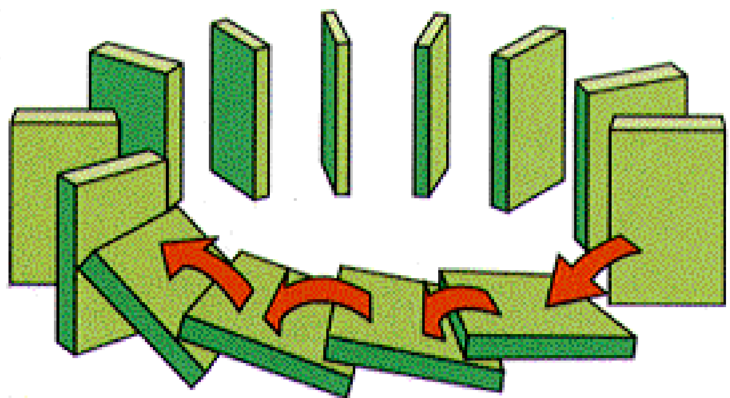


prometaphase arrest



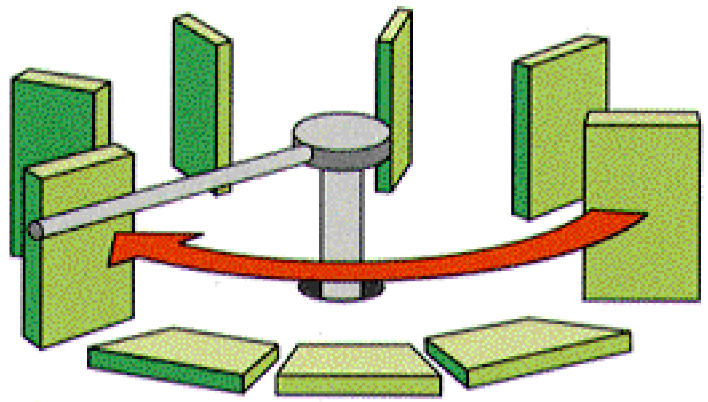
Genetic Screens: Yeast ‘Cell Division Cycle’ (CDC) Mutants

‘Dominos’
sequential, dependent events



(A)

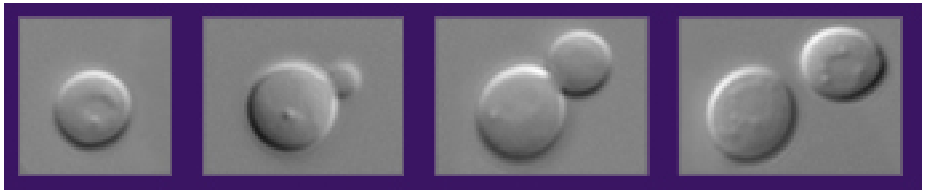
‘Oscillator’
central controller

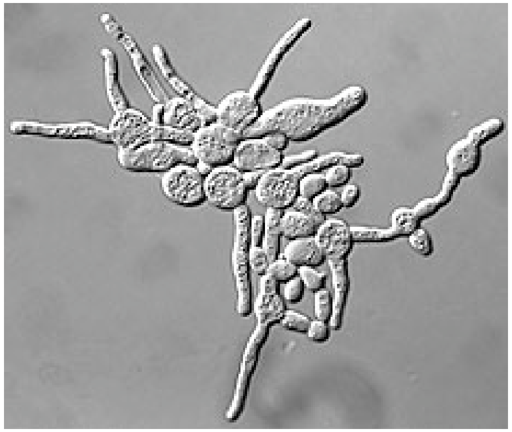


(B)

can individual protein mutations block steps or whole process ?

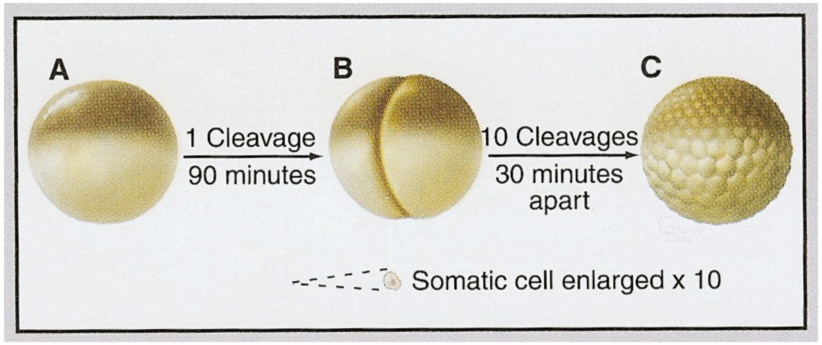
Genetic Screens: Yeast ‘Cell Division Cycle’ (CDC) Mutants

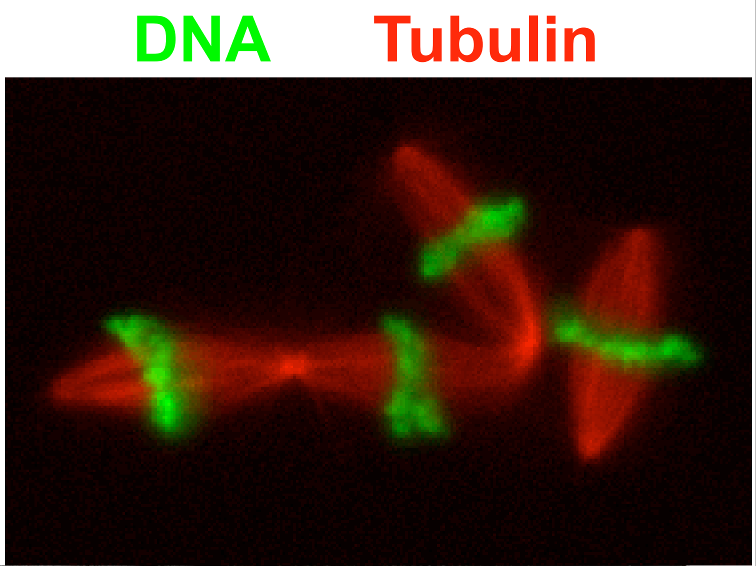


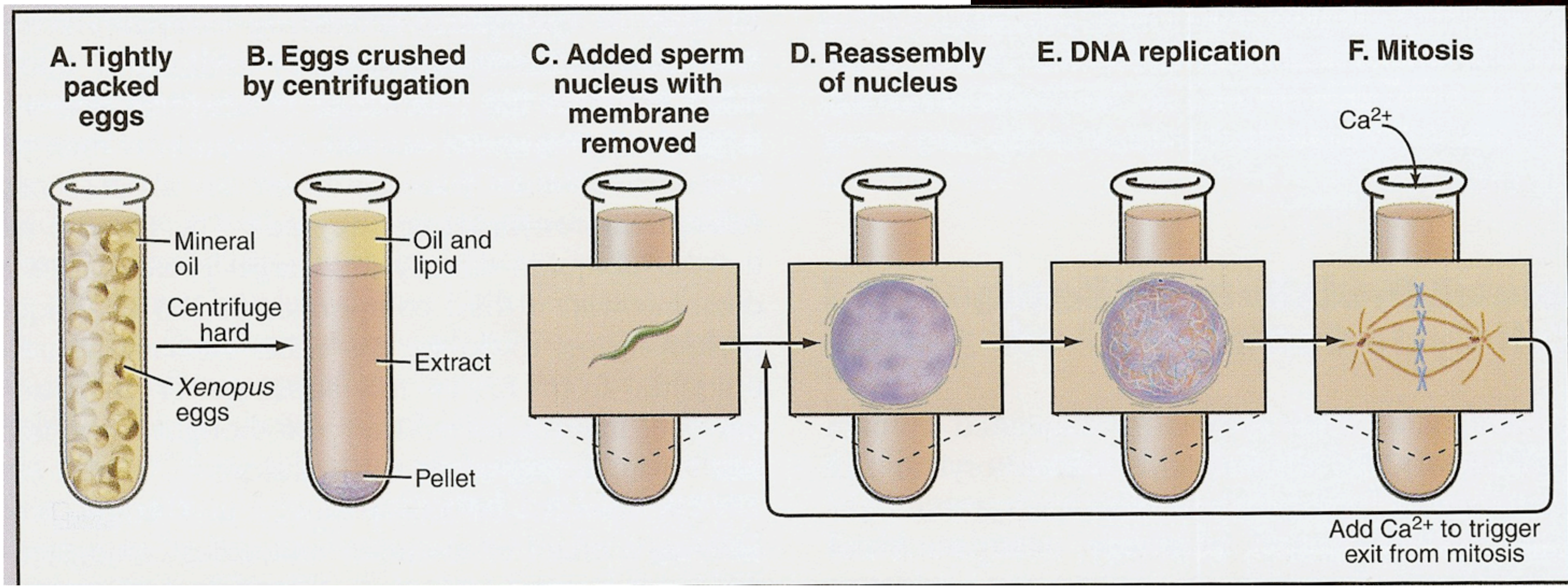


- Lee Hartwell (cerevisiae); Paul Nurse (pombe)
- **Goal:** find mutants unable to transit the cell cycle
- **Why yeast?**
  - Cell shape --> cell cycle stage
  - Grow as haploids (easier to find mutants), or diploids (can do genetics)
- **Problem:**
  - the screen is for cells that can't grow
- **Solution:**
  - temperature sensitive mutants
  - Replica plating

In vitro Dissection of the Cell Cycle - Xenopus Egg Extracts







use to isolate proteins present at particular stages
manipulate proteins-deplete and observe changes to cell cycle

Thursday

L2-4: Cytoskeleton Structure and Dynamics