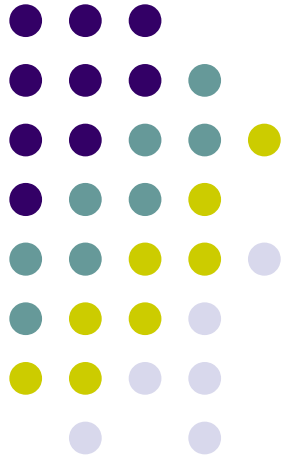
Curso de Radiobiología
UDELAR
Facultad de Ciencias
Unidad de Física Médica

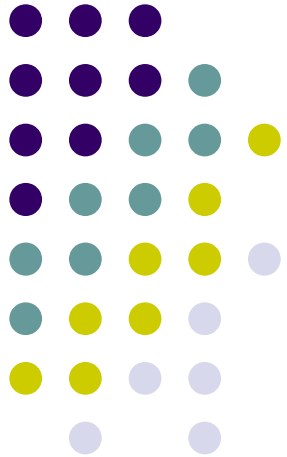
Dr. Eduardo Francisco Larrinaga Cortina



4R. Redistribución

Créditos:

Dr. Jerry Battista

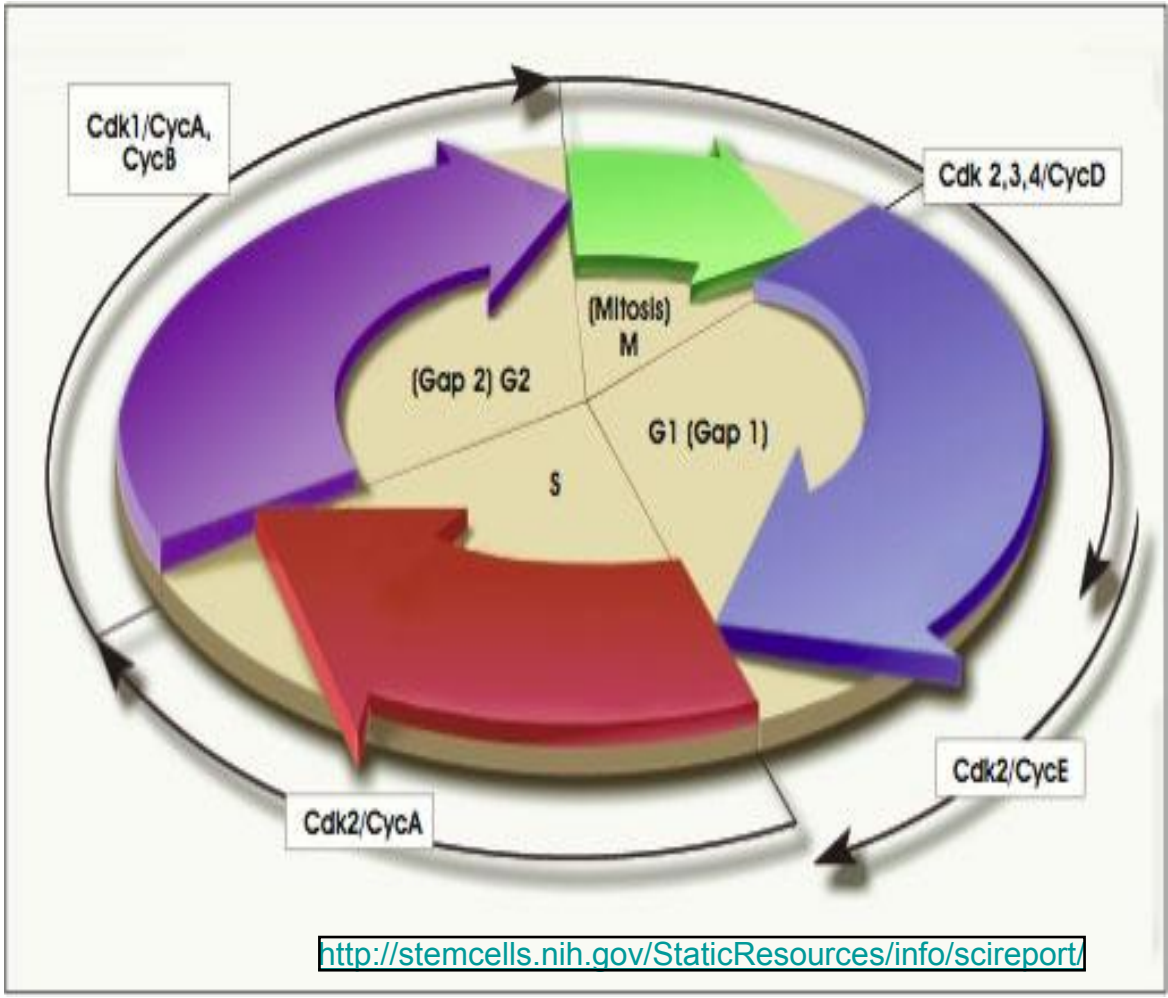


March
2011

R2

Cell Re-Assortment
or
Cell Redistribution

J. Battista, Ph.D.



http://stemcells.nih.gov/StaticResources/info/scireport/

Chapters 4,5,17,21

Radiobiology “4 R’s”

- R1 – Repair of Sublethal DNA Damage
  - DSB’s repaired by a set of enzymes
  - DSB Repair time on the order of <u>hours</u>

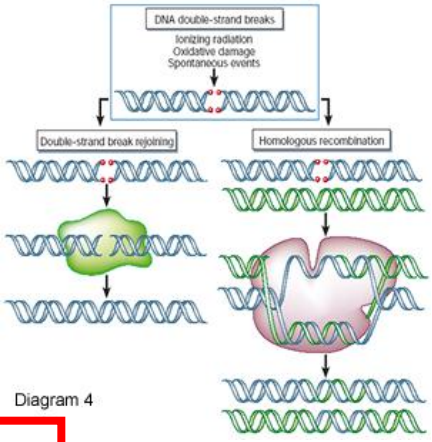
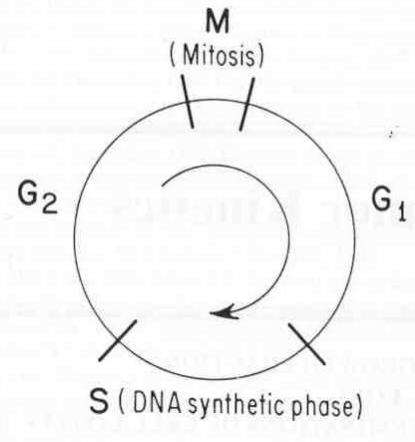


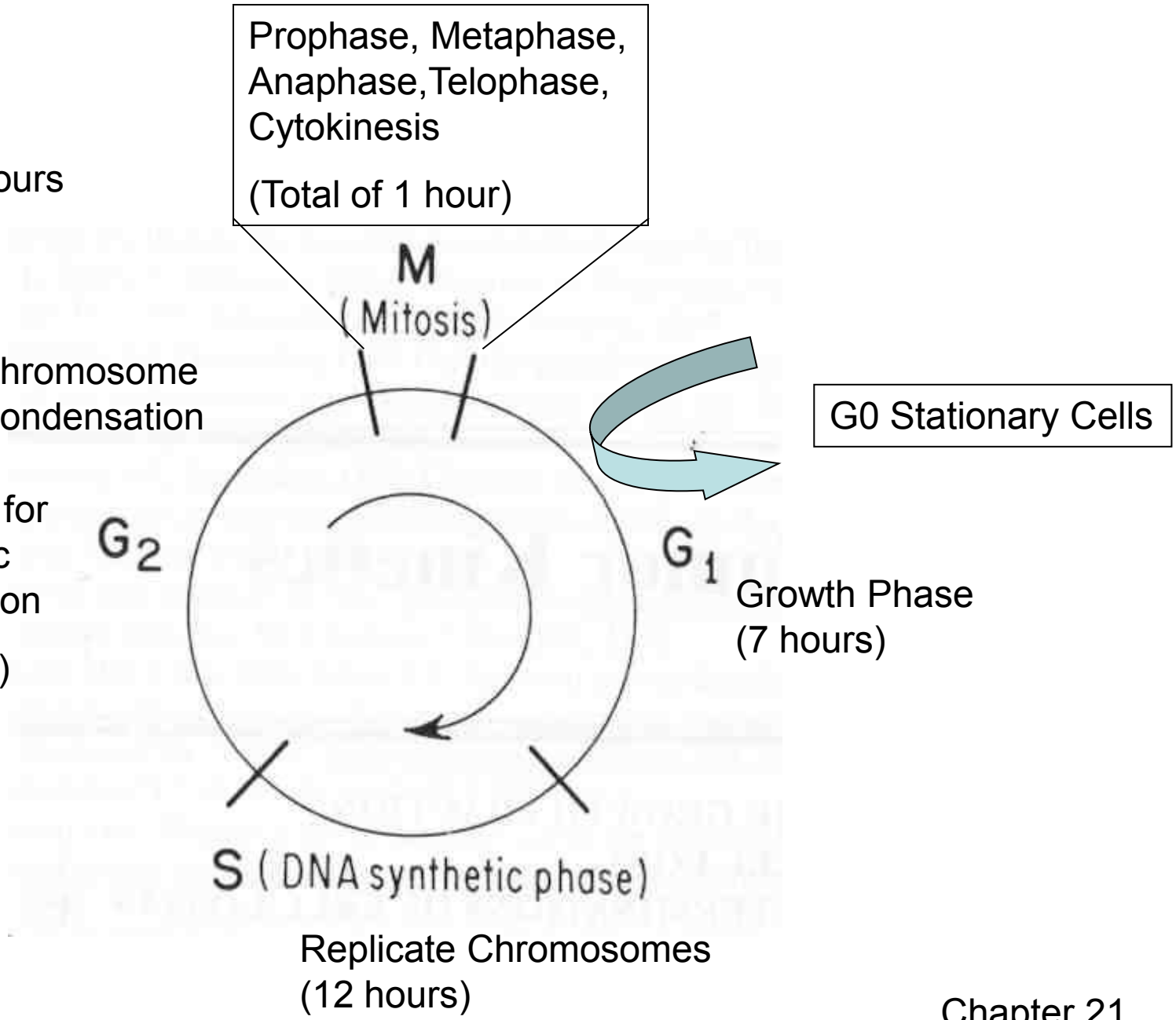
Diagram 4

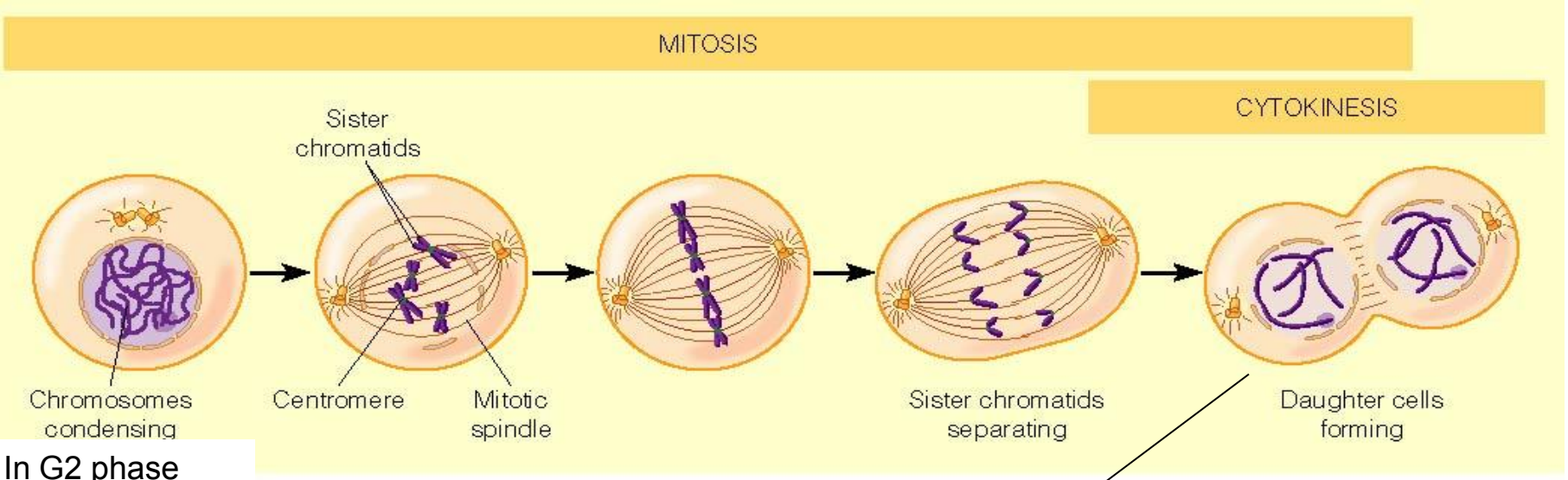
- R2 – Re-Assortment or Redistribution
  - Cell Cycle Effect or Cell Age effect
  - Explains Inverse Dose Rate Effect
  - Time scale on the order of < cell cycle period (< T_c)



CELL CLOCK

e.g. T_C = 24 hours

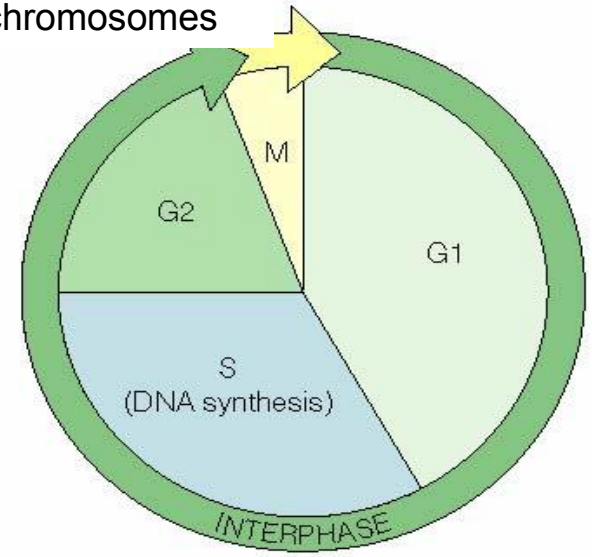




(a) The M (mitotic) phase

In G2 phase

2n = 46 'dual-legged' chromosomes



(b) The cell cycle

In G1 phase

2n = 46 single-legged chromatids

In human cells,

n = 23 chromosomes

per parent

http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-19/1901.jpg

Cell Phase Durations in Experimental Systems

TABLE 21.1. *The Constituent Parts of the Cell Cycle for Some Cells in Culture and Tumors in Experimental Animals*

Authors	Cell or Tissue	T_C, h	T_S, h	T_M, h	T_{G2}	T_{G1}
Bedford	Hamster cells *in vitro*	10	6	1	1	2
	HeLa cells *in vitro*	23	8	1	3	11
Steel	Mammary tumors in the rat					
	BICR/M1	19	8	~1	2	8
	BICR/A2	63	10	~1	2	50
Quastler and Sherman	Mouse intestinal crypt	18.75	7.5	0.5	0.5–1.0	9.5
Brown and Berry	Hamster cheek pouch epithelium	120–152	8.6	1.0	1.9	108–140
	Chemically induced carcinoma in pouch	10.7	5.9	0.4	1.6	2.8

More Variable

of the Cell Cycle (T_C) in 41 Human and Solid Tumors of Various Histologic Types

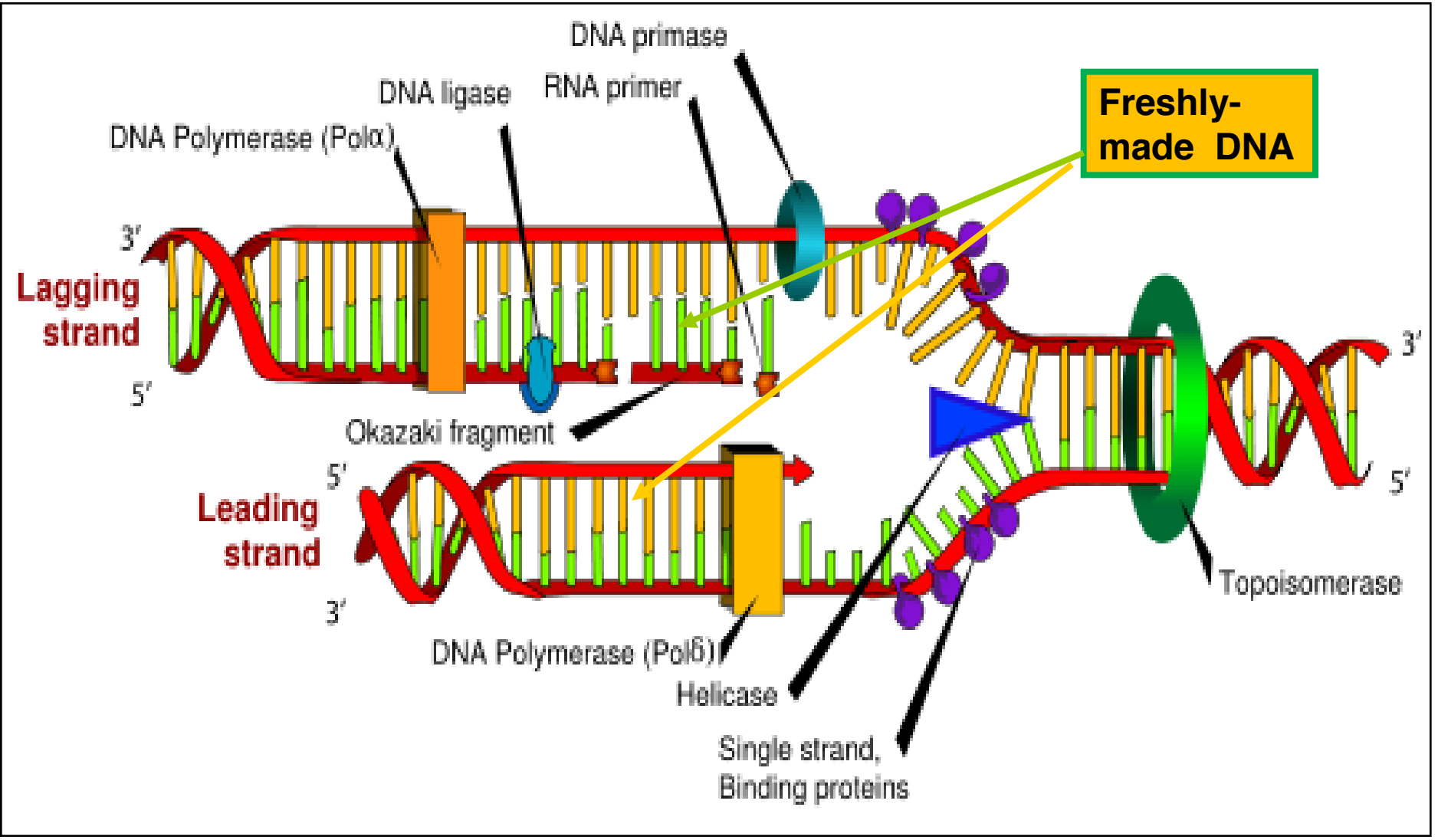
Authors	T_C, h
Frindel *et al.* (1968)	97, 51.5, 27.5, 48, 49.8
Bennington (1969)	15.5, 14.9
Young and de Vita (1970)	42, 82, 74
Shirakawa *et al.* (1970)	120, 144
Weinstein and Frost (1970)	217
Terz *et al.* (1971)	44.5, 31, 14, 25.5, 26,
Peckham and Steel (1973)	59
Estevez *et al.* (1972)	37, 30, 48, 30, 38, 96, 48
Terz and Curutchet (1974)[a]	18, 19, 19.2, 120
Malaise *et al.* (unpublished data)[a]	24, 33, 48, 42
Muggia *et al.* (1972)	64
Bresciani *et al.* (1974)	82, 50, 67, 53, 58

[a]Measured by the mean grain count halving time.

From Tubiana M, Malaise E: Growth rate and cell kinetics in human tumors: Some prognostic and therapeutic implications. In Symington T, Carter RL (eds): Scientific Foundations of Oncology, pp 126–136. Chicago, Year Book Medical Publishers, 1976, with permission.

Table 21.4

DNA Replication in S-Phase



http://en.wikipedia.org/wiki/Image:DNA_replication_editable.svg

Pulse/Flash S-Labeling

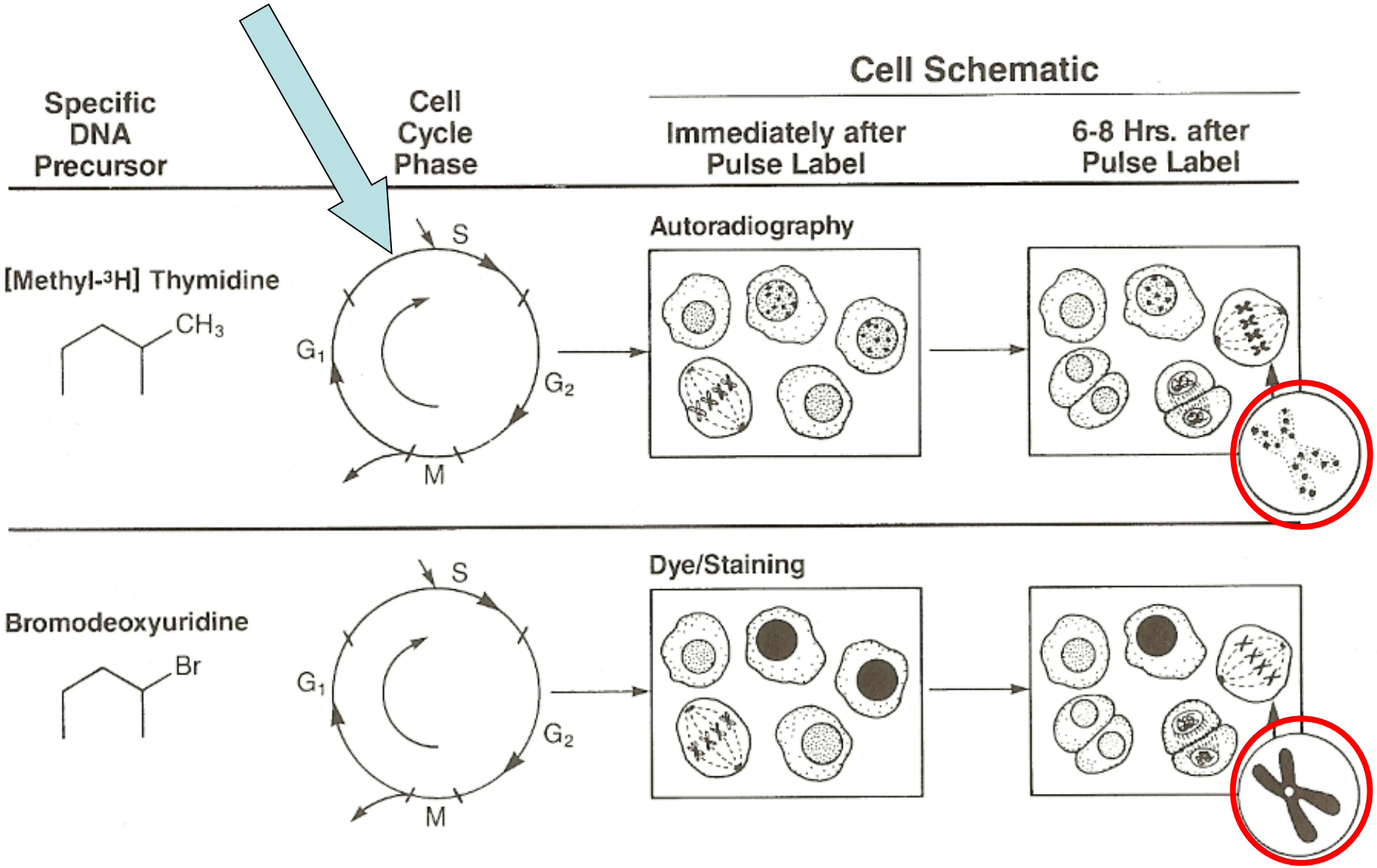


Fig 4.2

% Labeled Mitosis

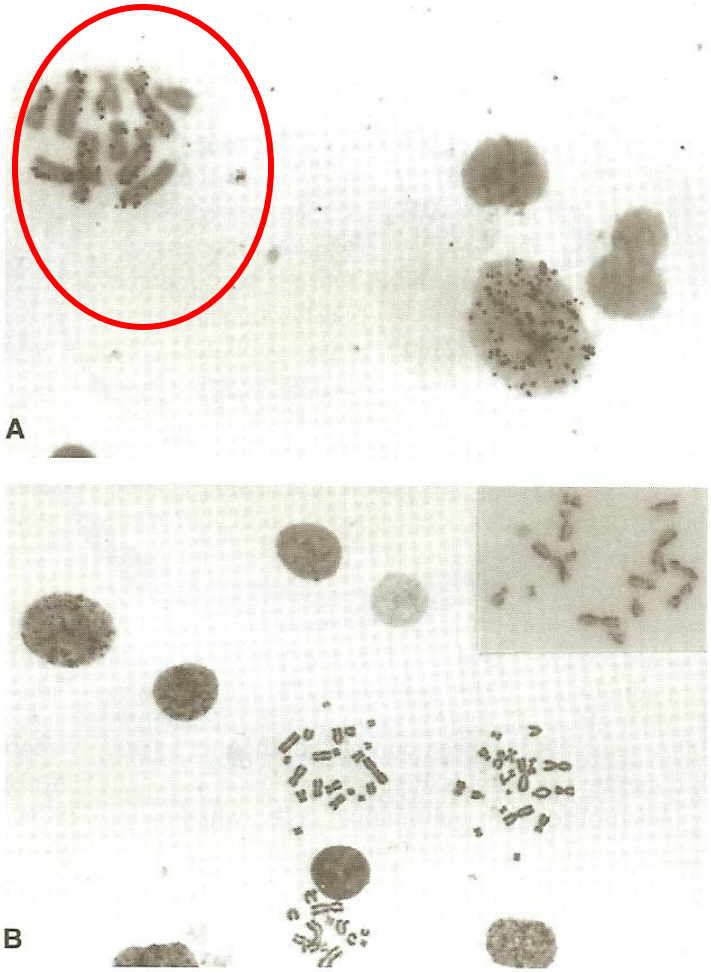
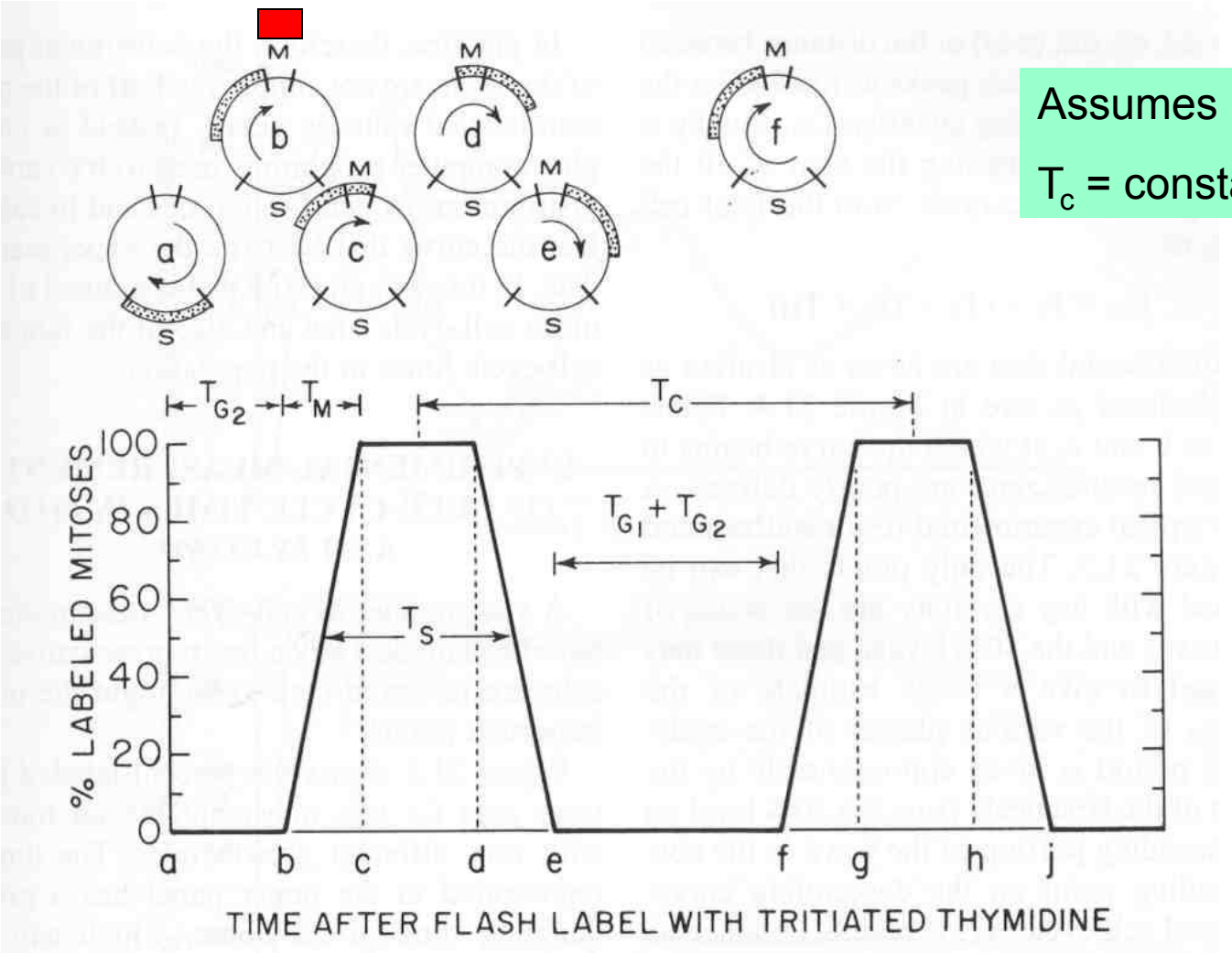


FIGURE 4.3 ● **A:** Autoradiograph of Chinese hamster cells in culture flash-labeled with tritiated thymidine. The black grains over some cells indicate that they were synthesizing DNA when they were labeled. Also shown is a labeled mitotic cell. This cell was in S phase when the culture was flash-labeled but moved to M phase before it was stained and autoradiographed. **B:** Photomicrograph showing cells labeled and unlabeled with bromodeoxyuridine. Cells were grown in the presence of bromodeoxyuridine and then fixed and stained 20 hours later. Incorporated bromodeoxyuridine stains purple, which shows up dark in this black-and-white print; the rest of the cell is light blue. The stained interphase cell indicates that it was in S phase during the time the bromodeoxyuridine was available. Also shown is a first-generation mitotic cell, which had been in S phase at the time the bromodeoxyuridine was available and had moved to M phase by the time it was fixed and stained. It can be identified as first-generation because both chromatids of each chromosome are stained uniformly. **Inset:** A second-generation mitotic cell, which passed through two S phases during bromodeoxyuridine availability. One chromatid of each chromosome is darker because both strands of the DNA double helix have incorporated bromodeoxyuridine. One chromatid is lighter because only one strand of the DNA has incorporated bromodeoxyuridine. (Courtesy of Dr. Charles Geard.)

Cell Cycle Analysis *via* % Labeled Mitosis

Observation window in mitosis



Assumes

T_c = constant

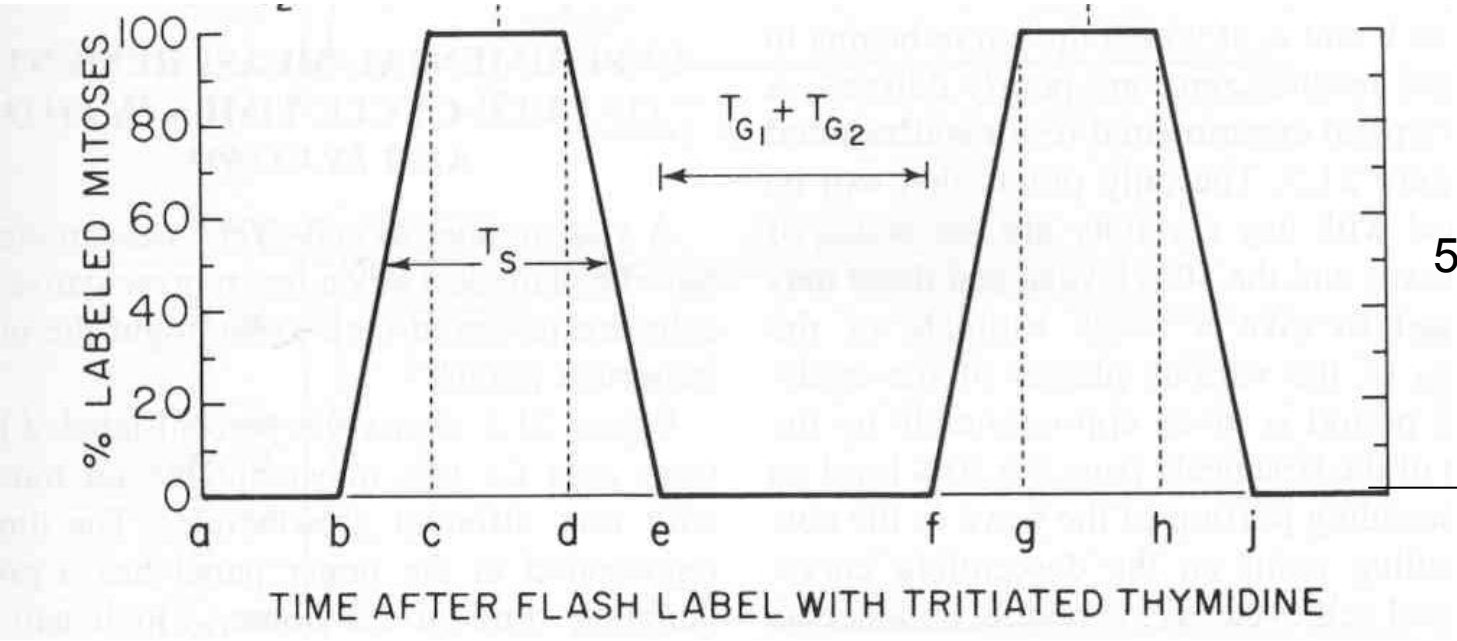
Fig 21.4

Multiple Cell Cycles

Dilution Effect

In 1st mitosis

In 2nd mitosis



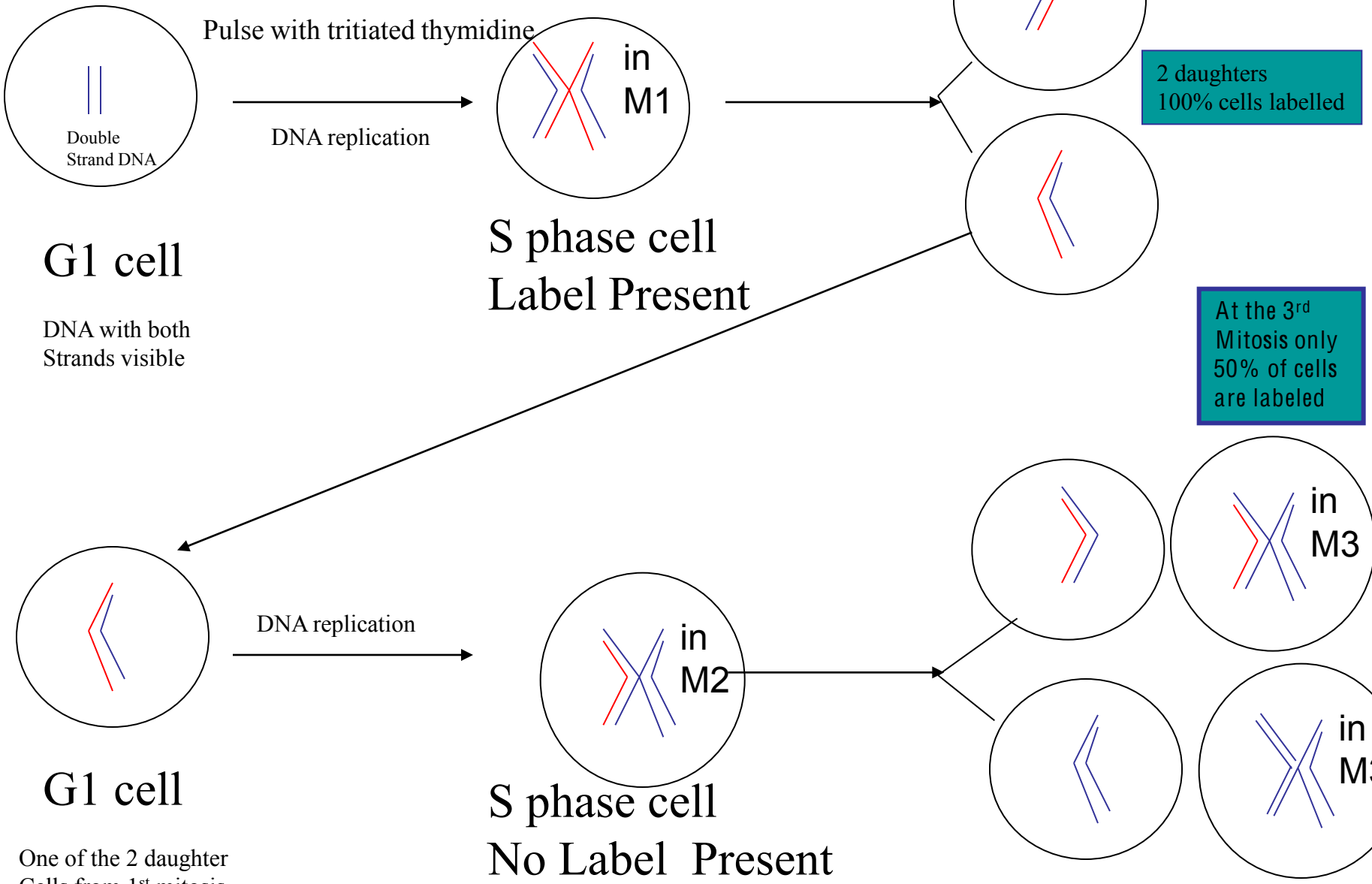
In 3rd mitosis

50 %

Assuming T_c = constant

DILUTION at M3

Labeled strand is red
Original strand is blue



Cell Cycle “Speed” Distribution

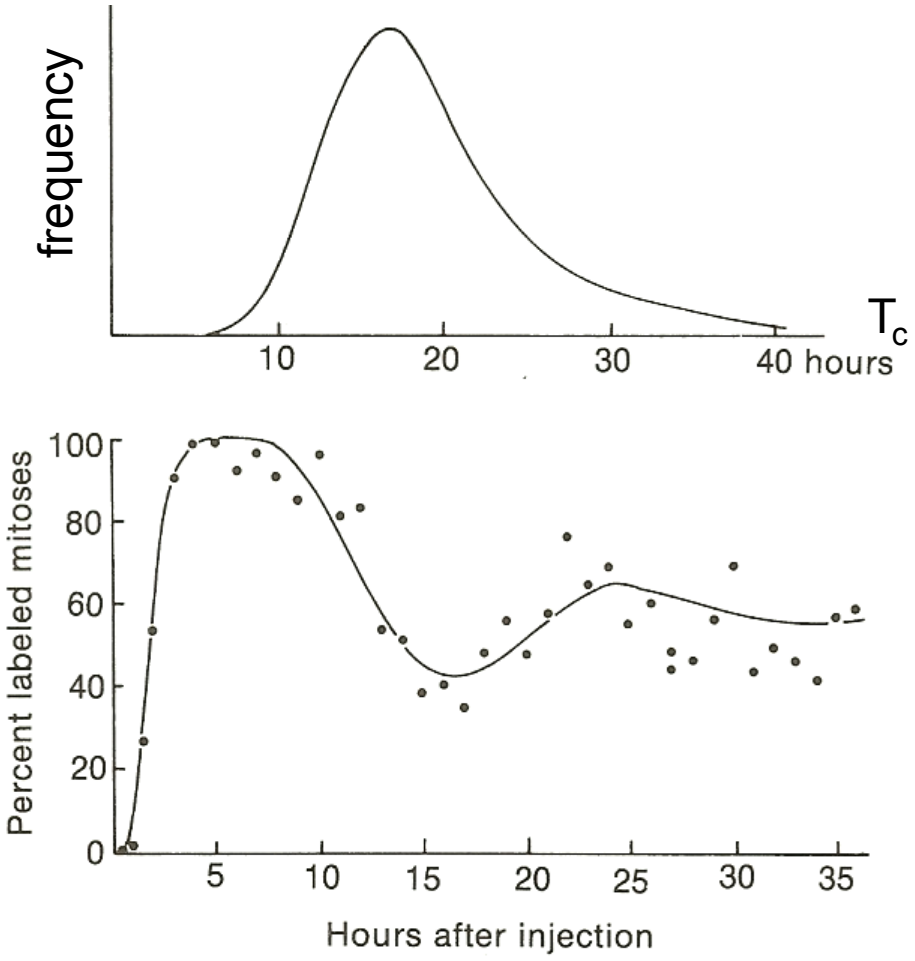
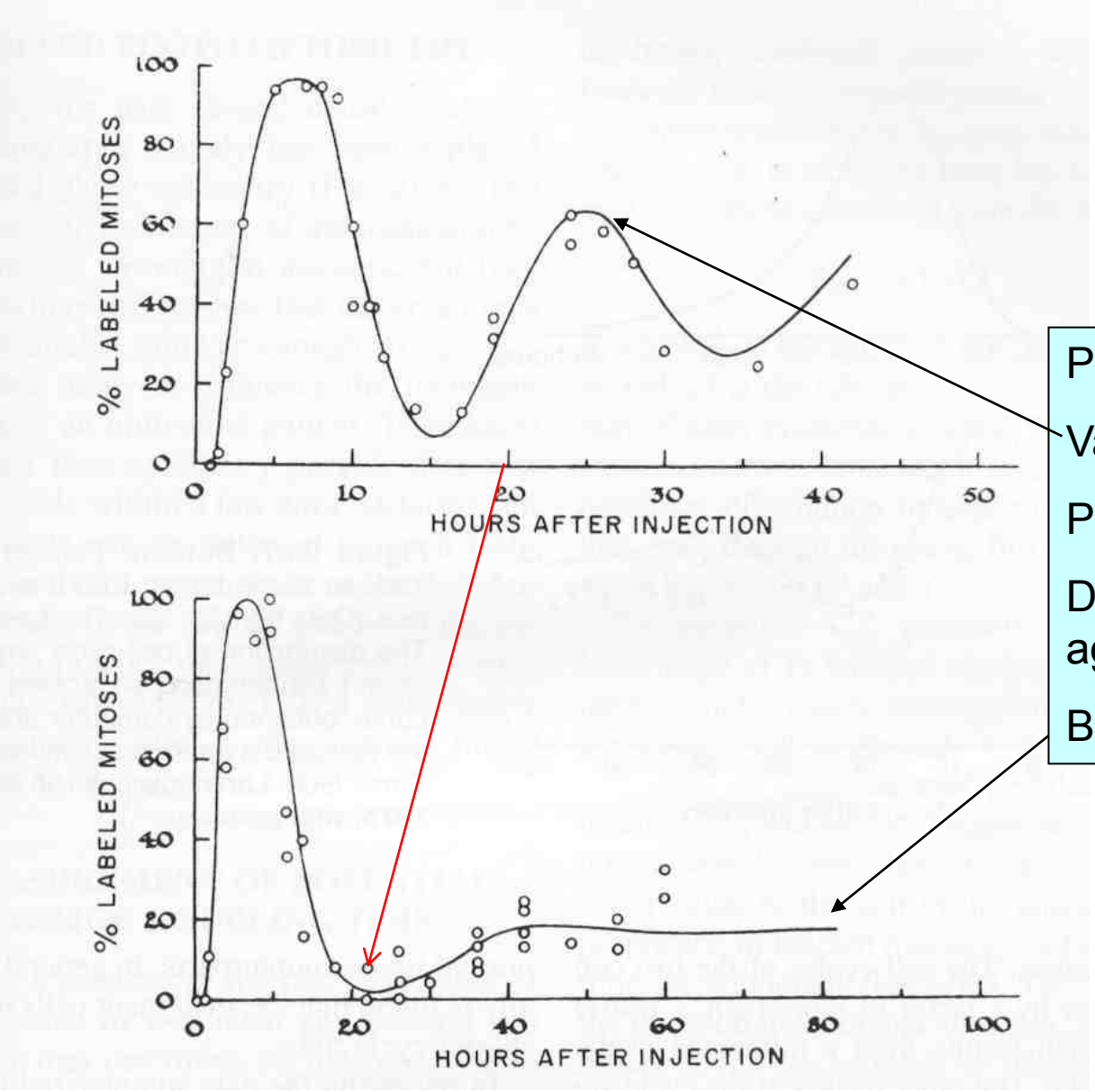


FIGURE 21.7 **Bottom:** Percent labeled mitoses curve for an EMT6 mouse tumor. (Data from Dr. Sara Rockwell.) **Top:** The distribution of cell-cycle times consistent with the damped labeled mitoses curve, obtained by computer analysis of the data and a mathematical model. (From Steel GG: The growth kinetics of tumors in relation to their therapeutic response. *Laryngoscope* 85:359–370, 1975, with permission.)

Real Flash Label Experiments



Peak fading is due to:

Variable Cell “speed”

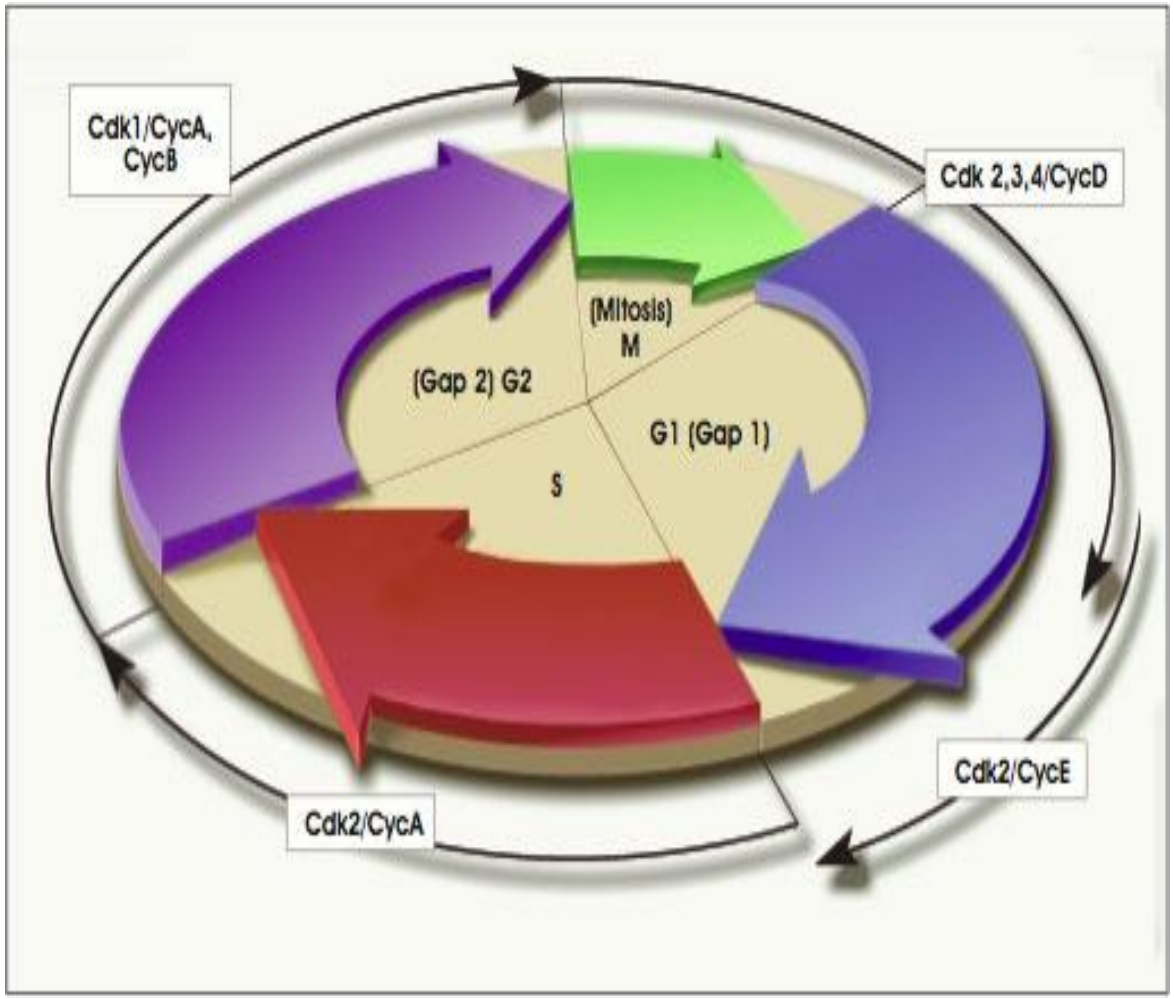
Plus

Dilution of labeling agent at ≥M3

Background Label

Fig 21.6

How are cells distributed around the cell cycle ?



Cell Subpopulation Size in Phases (Asynchronous Cells)

More cells here !
WHY ?

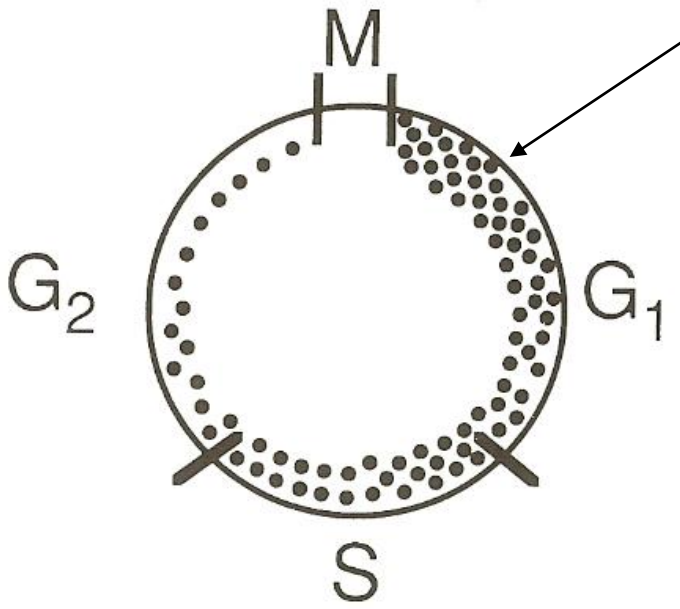
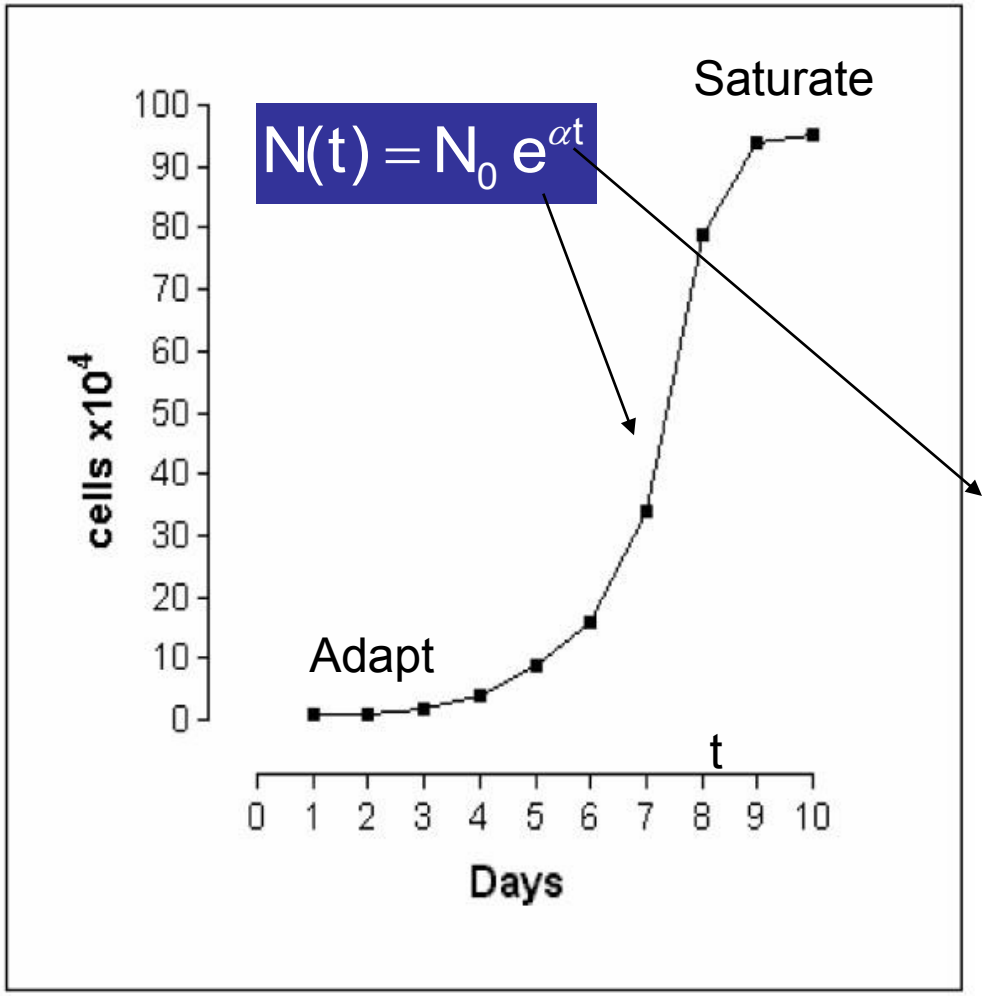


Figure 21.2. Diagram illustrating the fact that cells cannot be distributed uniformly in time around the cell cycle because they double in number during mitosis. The simplest assumption is that they are distributed as an exponential function of time.

Cell Exponential Growth

alpha is a growth constant

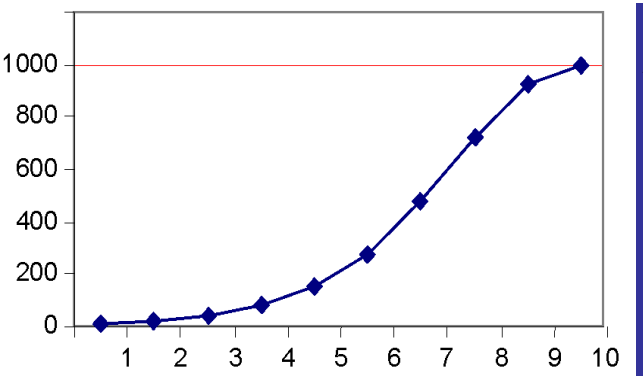


Cell Doubling Time

$T_D = 0.693/\alpha$

http://bmc.ub.uni-potsdam.de/1475-2867-5-13/1475-2867-5-13-4.jpg

Distribution of Cells in Cell Cycle



Population growth trajectory ($N_1 = N_0 = (Rm - sN_0)N_0$, Rm = maximum rate of increase, here = 1, s = interaction coefficient, here = 0.0001, and carrying capacity of environment = 1000.

$$N(t) = N_0 e^{\alpha t}$$

$$\alpha = \frac{\ln 2}{T}$$

$$n(t,\tau) = \alpha N(t) e^{\alpha \tau}$$

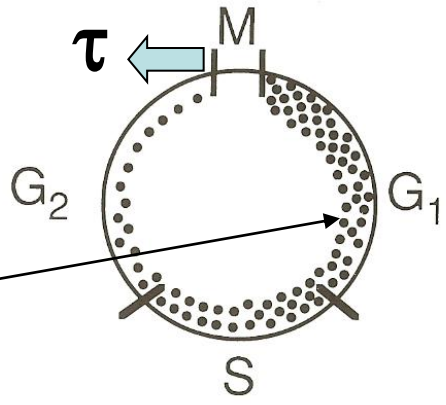


Figure 21.2. Diagram illustrating the fact that cells cannot be distributed uniformly in time around the cell cycle because they double in number during mitosis. The simplest assumption is that they are distributed as an exponential function of time.

where **t** ≡ observation real time

a lot of young cells in G1 compare to the later phases

τ ≡ time before cell division

T ≡ total cell cycle duration

T- τ **= cell age**

N_0 ≡ initial number of cells

N(t) ≡ number of cells at time t

n(t,τ) ≡ number of cells at real time (t) "flowing" past a point on the cell cycle preceding division by τ

Fraction of Cells in Mitosis (Mitotic Index)

$$M(t) = \frac{\int_0^{\tau_m} n(t,\tau)\,d\tau}{\int_0^{T} n(t,\tau)\,d\tau}$$

$$M(t) = e^{\alpha\tau_m} - 1$$

$$M(t) = \alpha\,\tau_m \quad \text{for } \tau_m \ll T$$

where $\tau_m \equiv$ period of mitotic phase

M(t) $\equiv$ fraction of cells in mitotic phase

Hall's estimate:

$M = \lambda\, T_M/T_c$

Where

$\lambda = 0.693$

Fraction of Cells with Label Uptake (Labeling Index)

$$L(t) = \begin{cases} \dfrac{\int_{G_2}^{G_2+S+t} n(t,0)\,dt}{N(t)} & \text{for} \quad t < G_2 \\ \dfrac{\int_{G_2}^{G_2+S+t} n(t,0)\,dt + \int_{T+G_2}^{T+t} n(t,0)\,dt}{N(t)} & \text{for} \quad t > G_2 \end{cases}$$

where **t** ≡ time after adding labelling agent

S ≡ period of S phase

G_2 ≡ period of G2 phase

L(t) ≡ fraction of labelled cells

Hall's estimate:

$L = \lambda\, T_S/T_c$

Where

λ = 0.67 to 1.00

A Great Reference

DNA SYNTHESIS IN INDIVIDUAL L-STRAIN MOUSE CELLS

C. P. STANNERS* AND J. E. TILL

Department of Medical Biophysics, University of Toronto, and Physics Division, The Ontario Cancer Institute, Toronto, Ontario (Canada)

(Received April 21st, 1959)

SUMMARY

The time relationship between DNA synthesis and mitosis has been determined for L-strain mouse cells cultivated *in vitro*. DNA synthesis was detected autoradiographically by following the uptake of [^{3}H]thymidine into the cell nucleus. DNA synthesis in this cell system was found to take place in an approximately linear fashion over a single period of six to seven hours, ending three to four hours before mitosis, for a total generation time of twenty hours.

A mathematical treatment for exponentially multiplying cultures is presented.

INTRODUCTION

It is obvious that a cell must double its content of deoxyribose nucleic acid (DNA) before it divides. For many years it was believed that the cell synthesized this DNA during prophase and metaphase, so that when the two sets of chromosomes separated at anaphase, each had a full complement of DNA. Recently, many workers have shown, by the use of photometric and autoradiographic techniques, that DNA synthesis actually occurs during interphase over a period which ends at a certain time before mitosis, and which has a definite length[1–3]. This was found to be true for many different types of cells, both animal and plant, but the exact timing of the DNA synthesis period appeared to vary with the cell type. We have investigated, by the method of autoradiography, DNA synthesis in exponentially multiplying mouse cells, cultivated *in vitro*. This investigation is preliminary to studies of the perturbing influence of various chemical and physical agents upon DNA metabolism in the cell.

MATERIALS AND METHODS

Detection of DNA synthesis

Since thymidine has been shown to be a specific precursor of DNA[4], the uptake of [^{3}H]thymidine (^{3}HTDN) into the cell nucleus was used as a measure of DNA synthesis. The β-decay of tritium was detected autoradiographically. The low energy of tritium β-particles affords excellent resolution on the autoradiographs[5] (see Fig. 1).

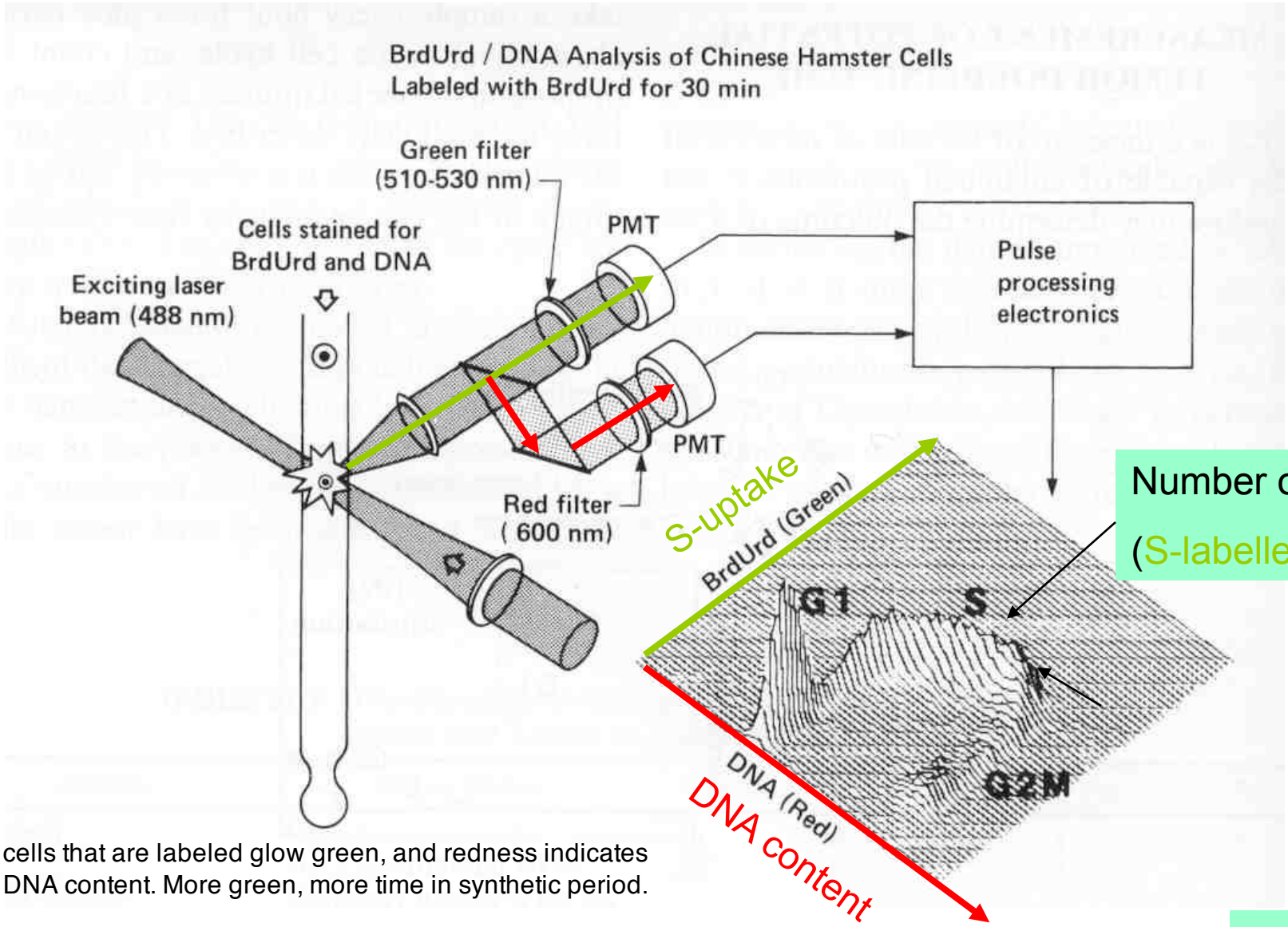
* Graduate student, Department of Medical Biophysics, University of Toronto, and Fellow in Radiation Physics of the National Cancer Institute of Canada.

Paper is posted

Cell Cycle Analysis by Flow Cytometry

cells exponentially distributed

Fluorescence-activated cell sorting (FACS)



cells that are labeled glow green, and redness indicates DNA content. More green, more time in synthetic period.

Fig 21.9

S – DNA Synthesis Phase

use DNA amt as clock to tell duration of S-phase

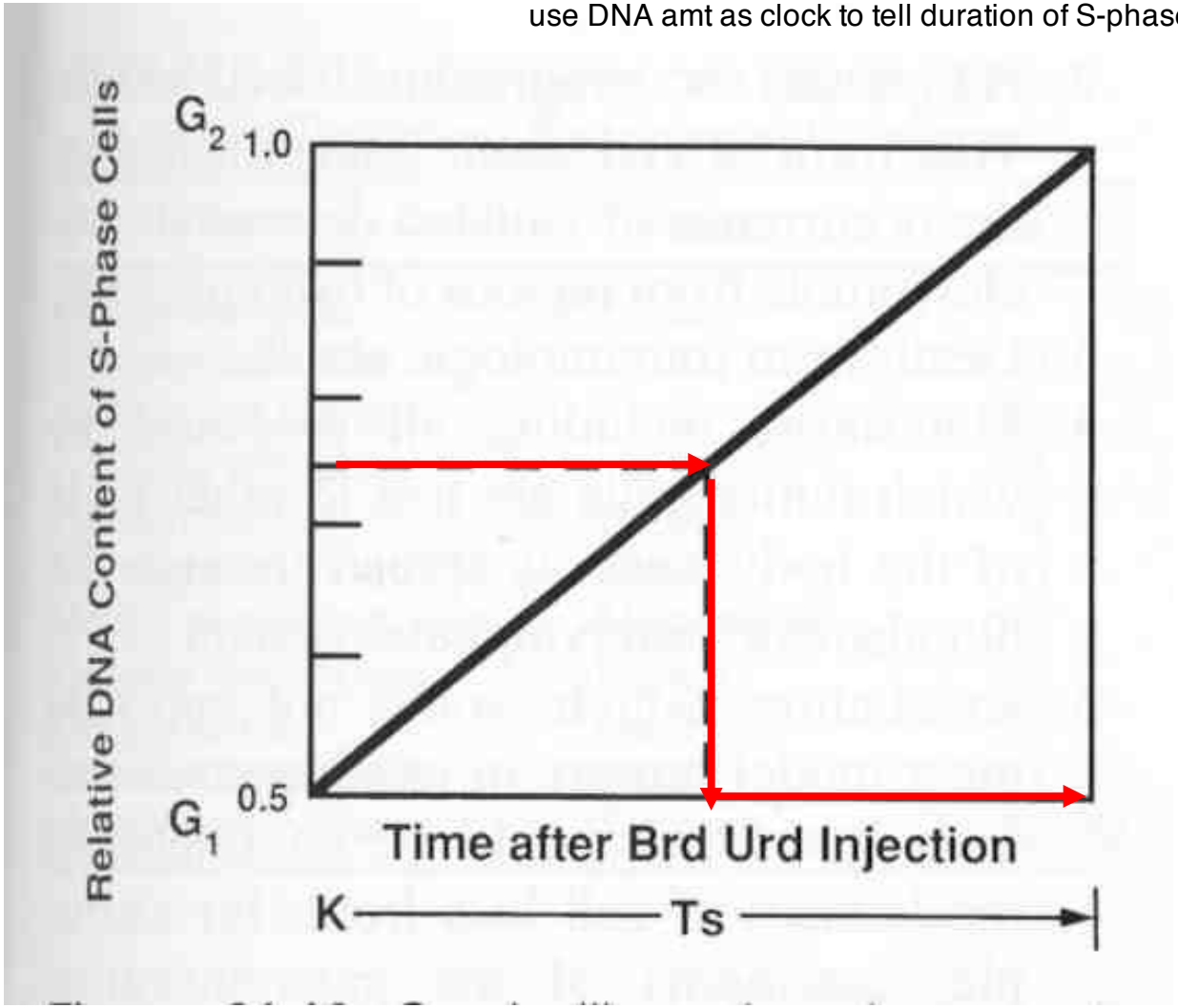
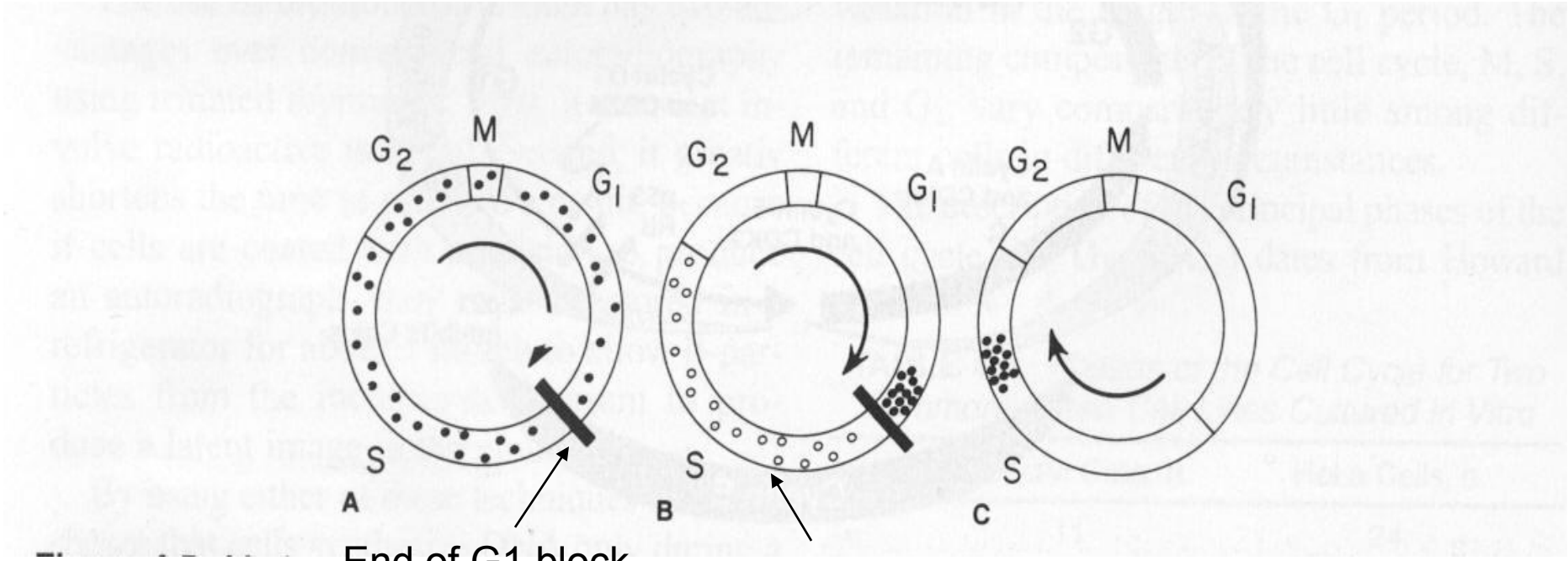


Fig 21.10

Cell Age Effect

Chapter 4

Cells can be Synchronized using drugs (e.g hydroxyurea)



hydroxyurea piles up cells in G1 phase. When hydroxyurea is remove, cycle starts. S phase highly radioresistant. G2 and M more radiosensitive.

Fig 4.5

Single Dose Experiment of Synchronized Cells

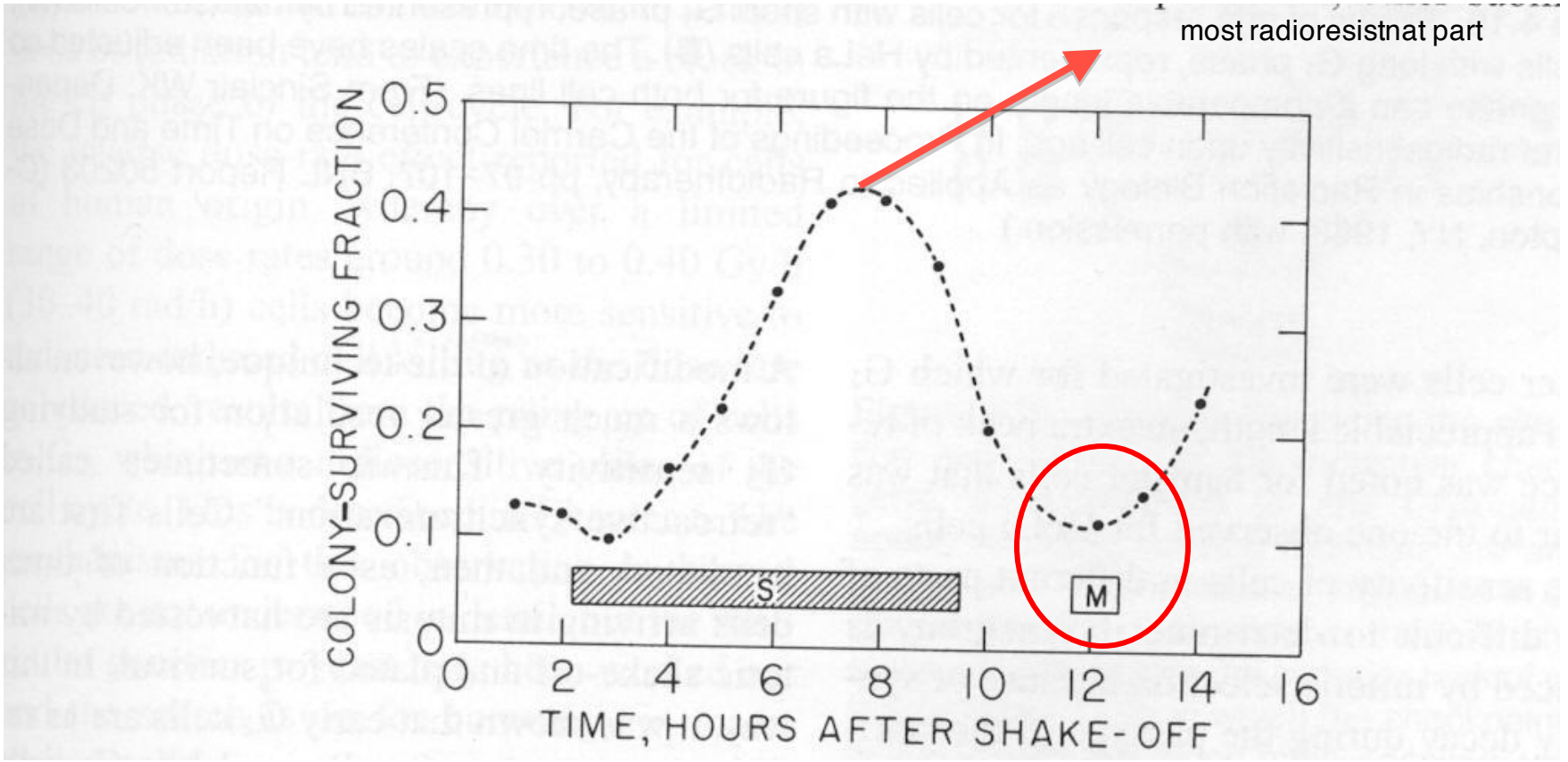


Fig 4.7

Cell Survival of Synchronized Cells

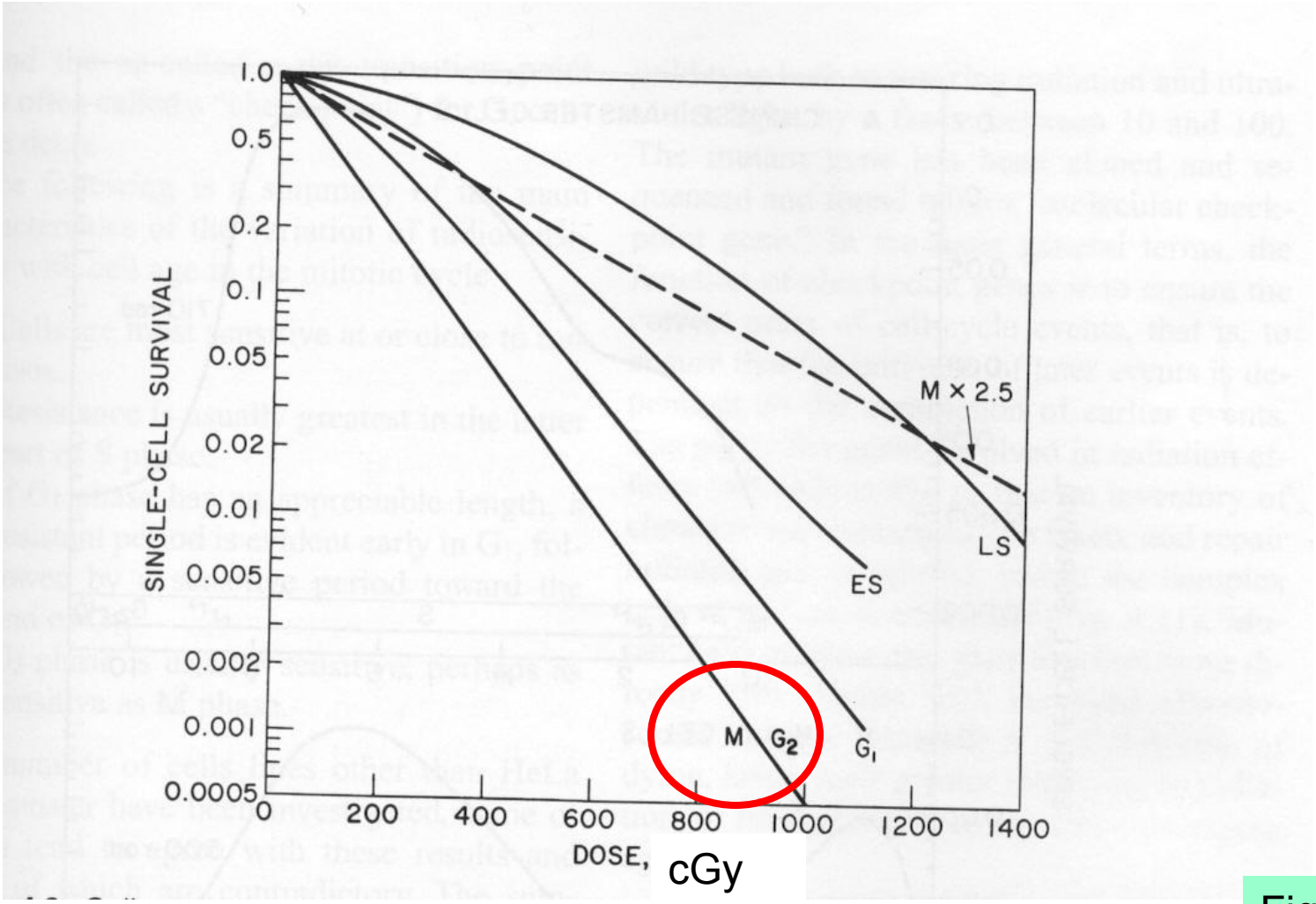


Fig 4.8

R2 - Cell Cycle Phase Radiosensitivity

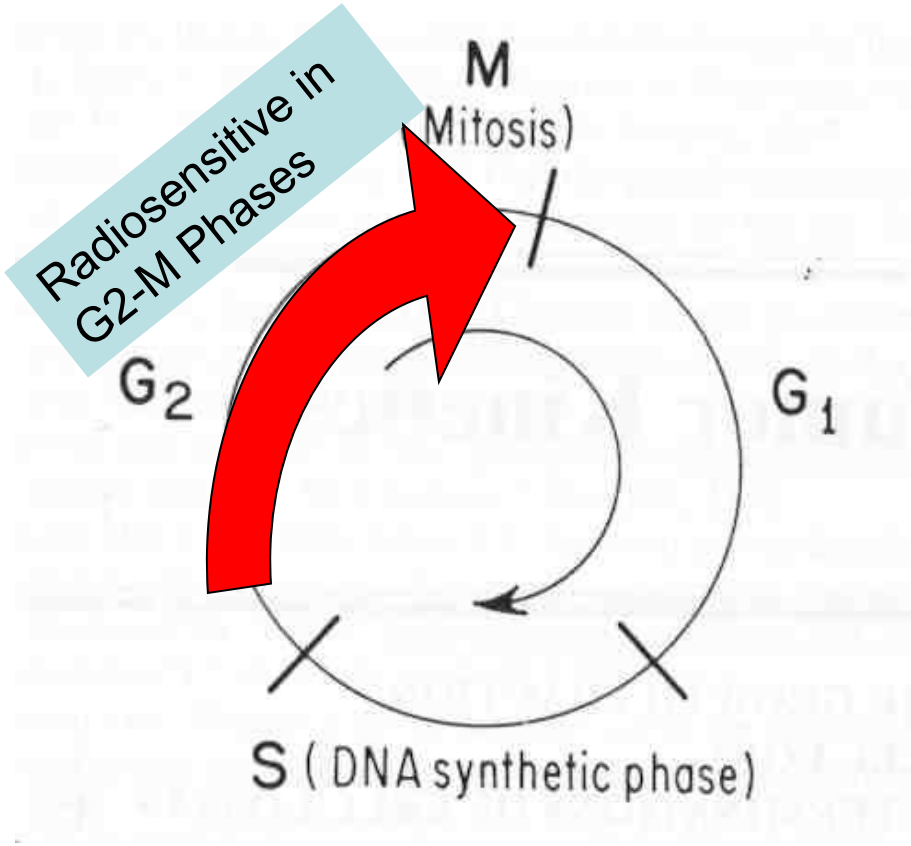


Fig 4.1

COMPACTED CHROMATIN AND RADIOTHERAPY

When cancer doctors treat with radiation
Cell killing can display wide variation
And this in turn as consequence assures
That their therapy will fail to yield some cures
This saddles our profession with bad stigma
And the basis for resistance is still enigma

Now X-rays kill most cells by single-hits
At least when dose is given in 2 Gy bits
We think that's due to damage very focal
In DNA, that's multiple and local
When chromatin in cells is found condensed
Effects of radiation are enhanced

Mitotic cells are always at great risk
Their genes are well compacted to resist
The trauma of genetic segregation
A critical event in cell division
Cells whose DNA dispersion is defective
Will be radiation sensitive, selective

How to put all cancer cells into this state
Before we close the linac door to 'radiate
Is a challenge for the scientist and doc
Hope this effort was promoted by this talk
And to thwart a cancer's threat of strong persistence
We must undo the major causes of resistance

J. D. Chapman, 1998

Cell Cycle Regulation

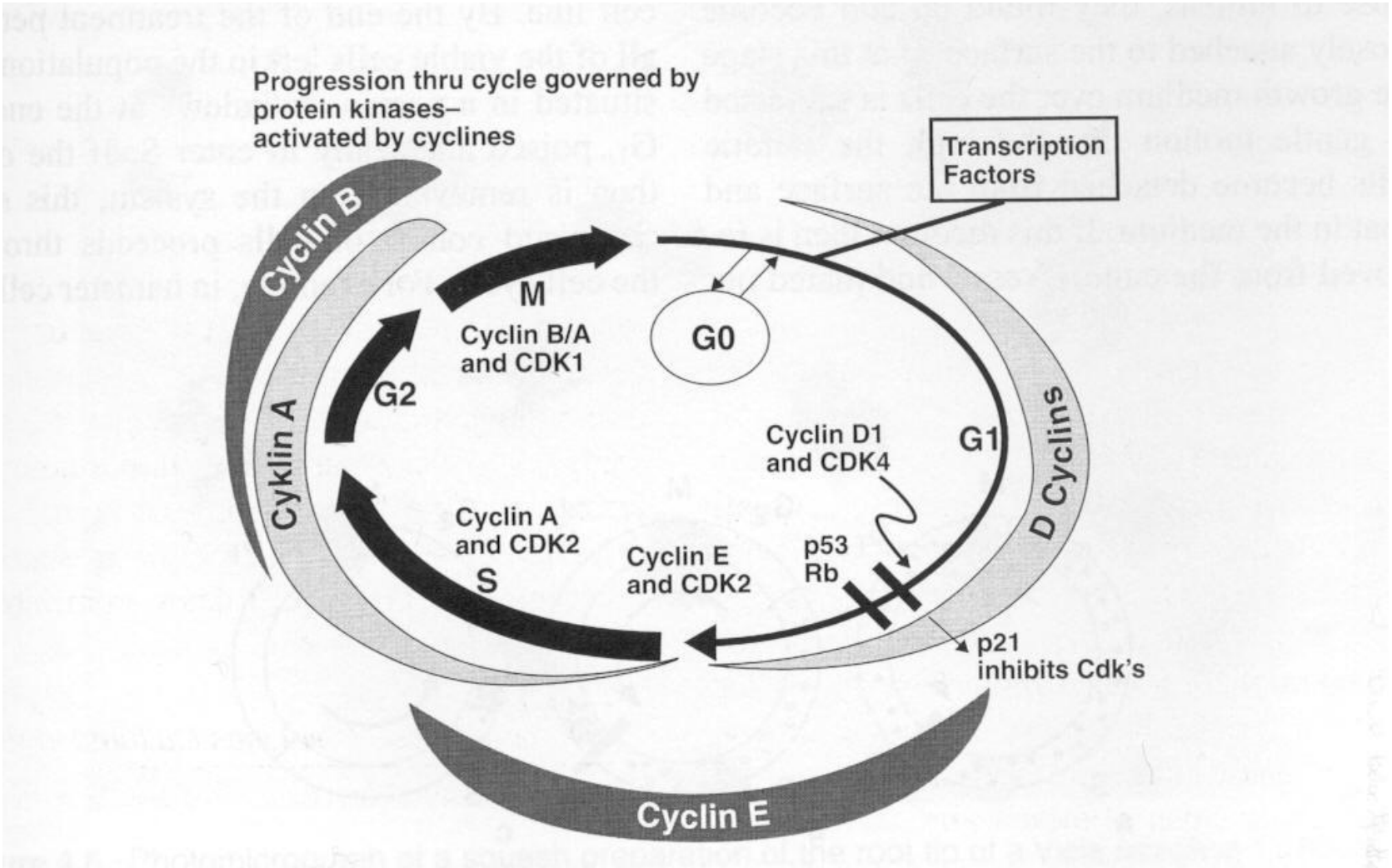
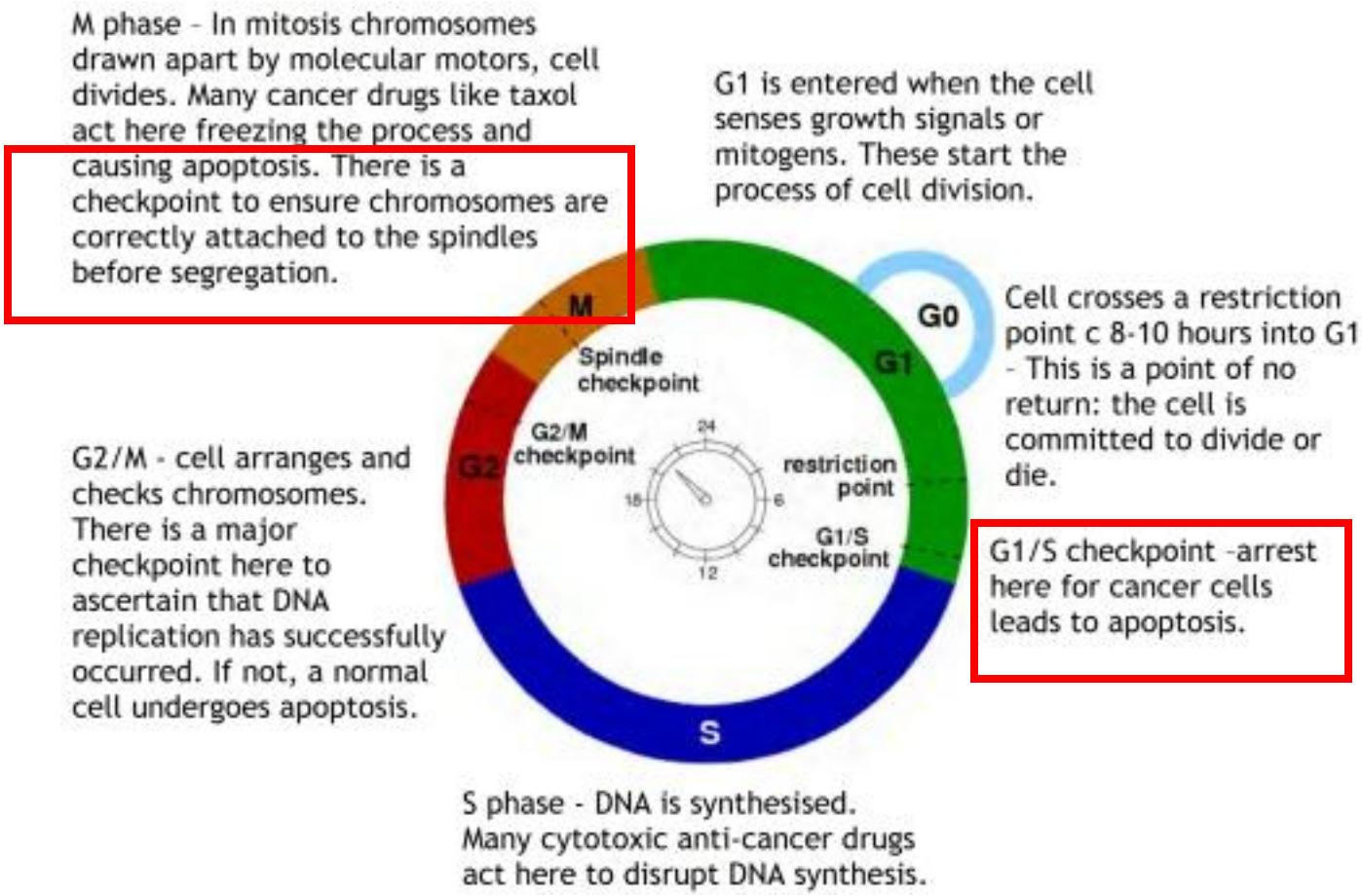


Fig 17.15

Cell Cycle Check Points



http://www.physiomics-plc.com/images/Cell%20Cycle11-12-05.jpg

Damaged Chromosomes can stop Cell from Cycling

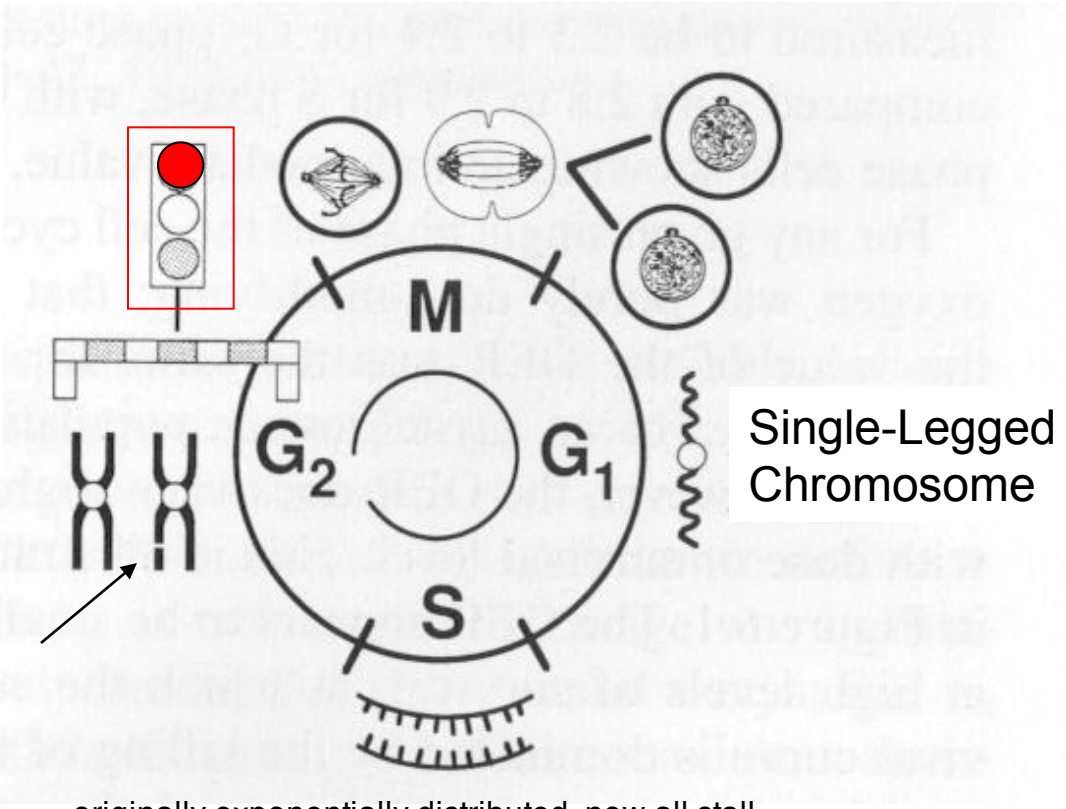
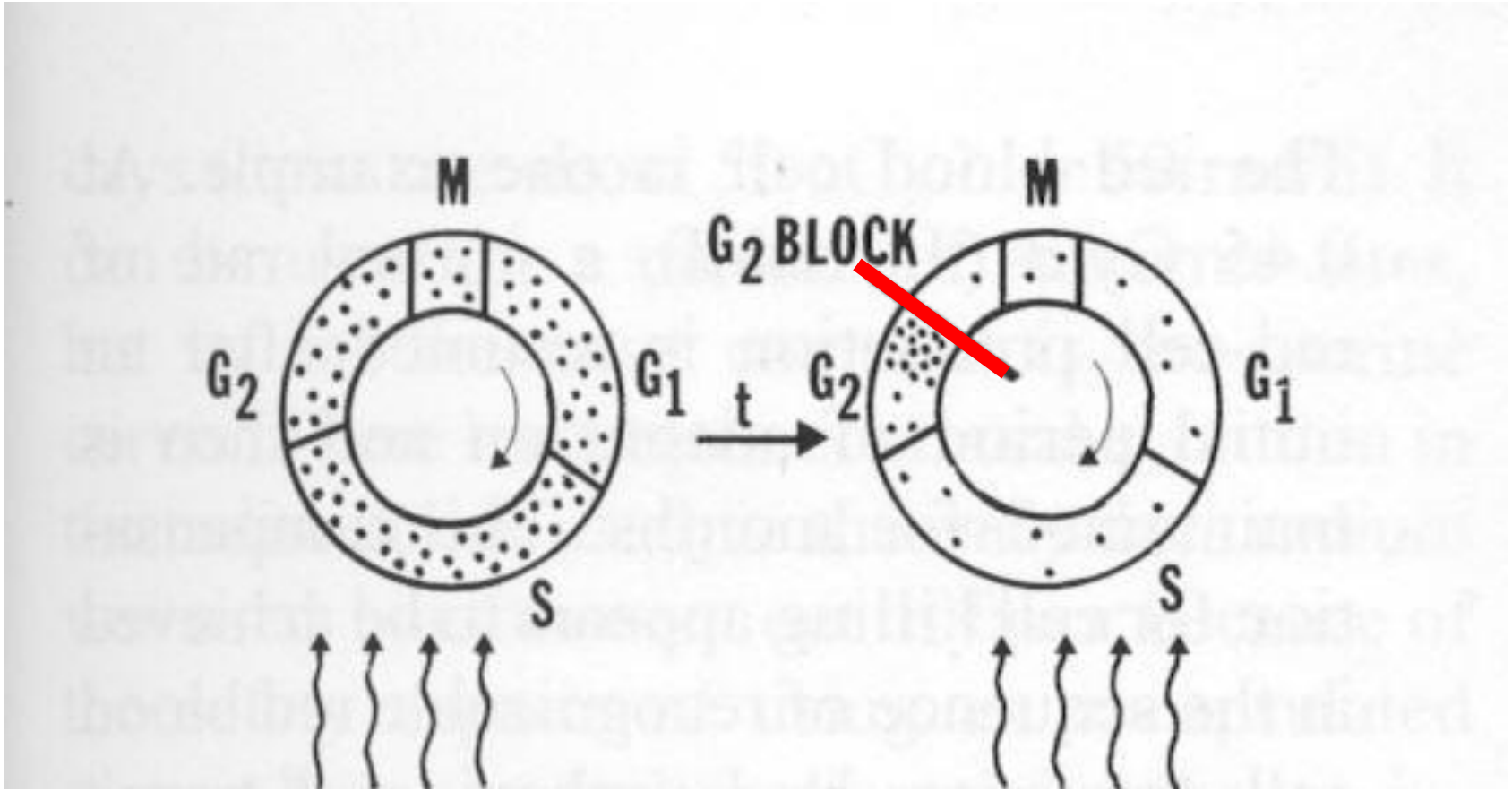


Fig 4.11

Radiation-induced G2 Pileup



taken them out of S phase, pile up in G2, the more sensitive part

Fig 5.17

Dose Rate Experiments

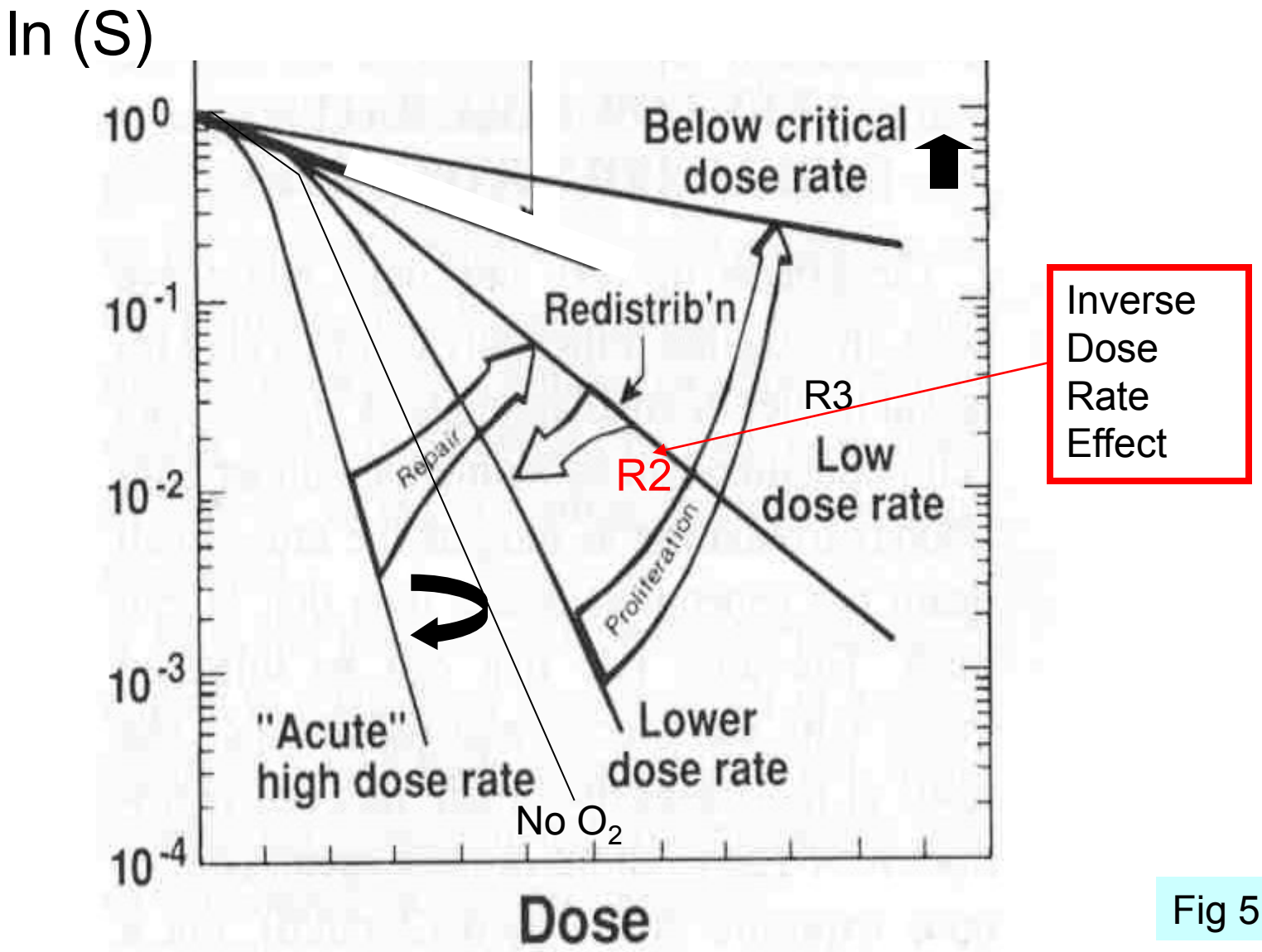
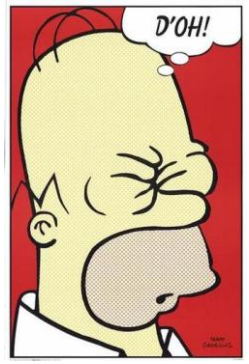


Fig 5.18

Does a lower dose rate <u>always</u> improve cell survival ? Nope



- Repair time for DNA injury (R1)

BUT…

- Re- assortment/bunching in G2 phase (R2)

Repair improves cell survival, whereas Re-assortment causes more radiation death