Curso de Radiobiología
UDELAR

Facultad de Ciencias

Unidad de Física Médica

Dr. Eduardo Francisco Larrinaga Cortina

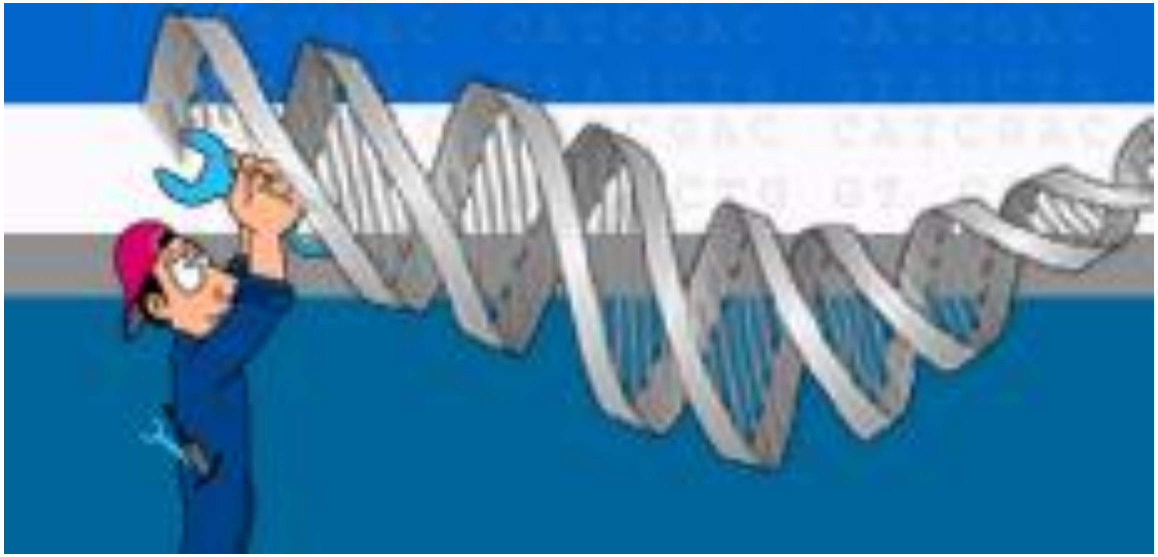
4R. Reparación del ADN

Créditos:

Dr. Jerry Battista

MBP 4467

Version: 2011

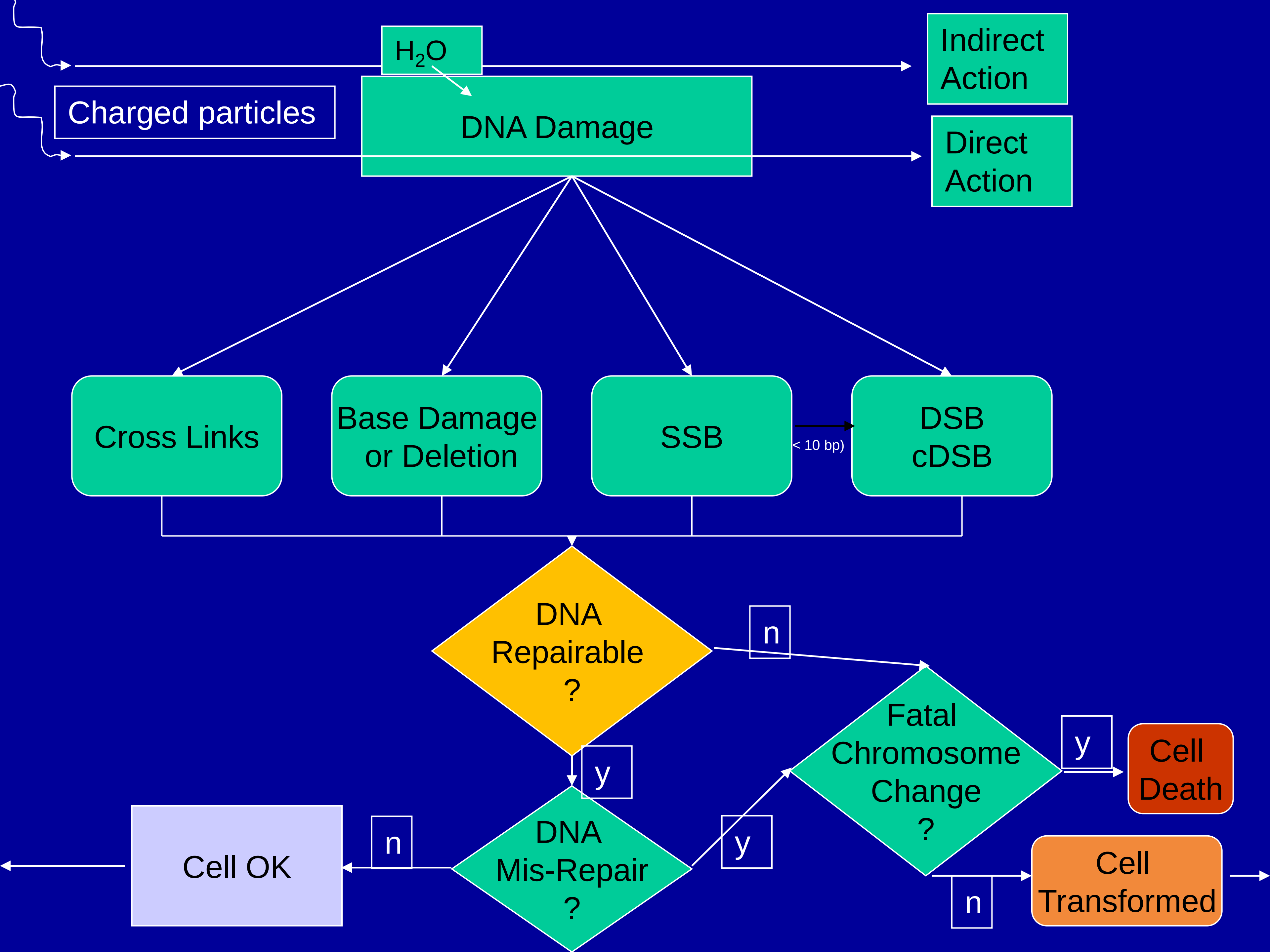


DNA Repair

J. Battista

Chapters 2, 5, and 17

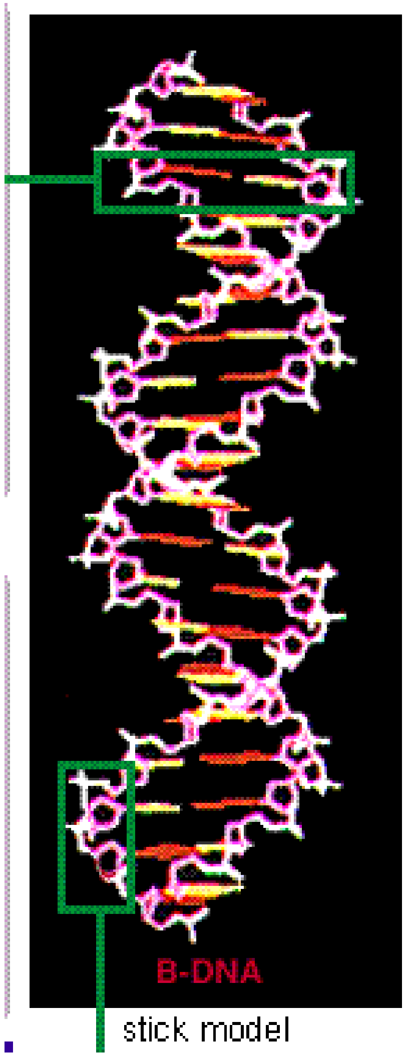
www.weizmann.ac.il/



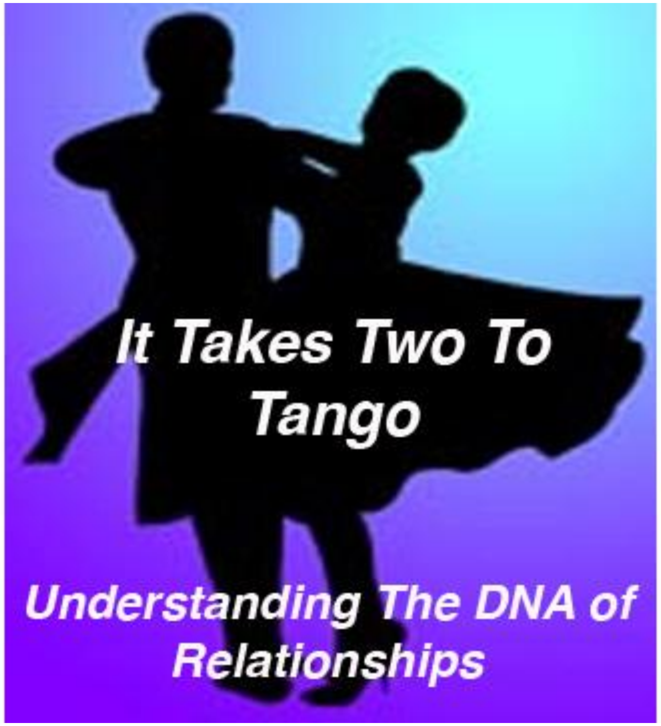
H_2O
Indirect Action
Charged particles
DNA Damage
Direct Action
Cross Links
Base Damage or Deletion
SSB
< 10 bp)
DSB cDSB
DNA Repairable ?
n
Fatal Chromosome Change ?
y
Cell Death
y
DNA Mis-Repair ?
n
Cell OK
y
n
Cell Transformed

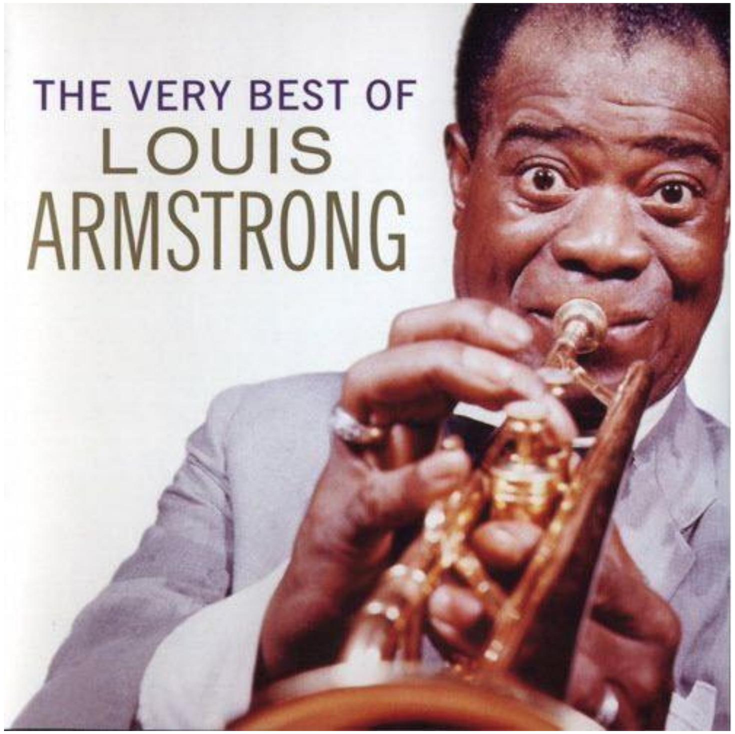
Types of DNA DAMAGE

- Single-strand breaks (SSB)
  - Direct energy depositions in sugar/phosphate backbone
  - OH-deoxyribose "attack"
  - Combine to form
- Double Strand Breaks
  - Opposite strand SSBs
  - Distance < 10 bp
  - Time << Repair Time
- Base damage
  - Direct energy depositions
  - OH-base reactions
  - e^-_{aq}-base reactions



Another Famous J^2B Saying # 7





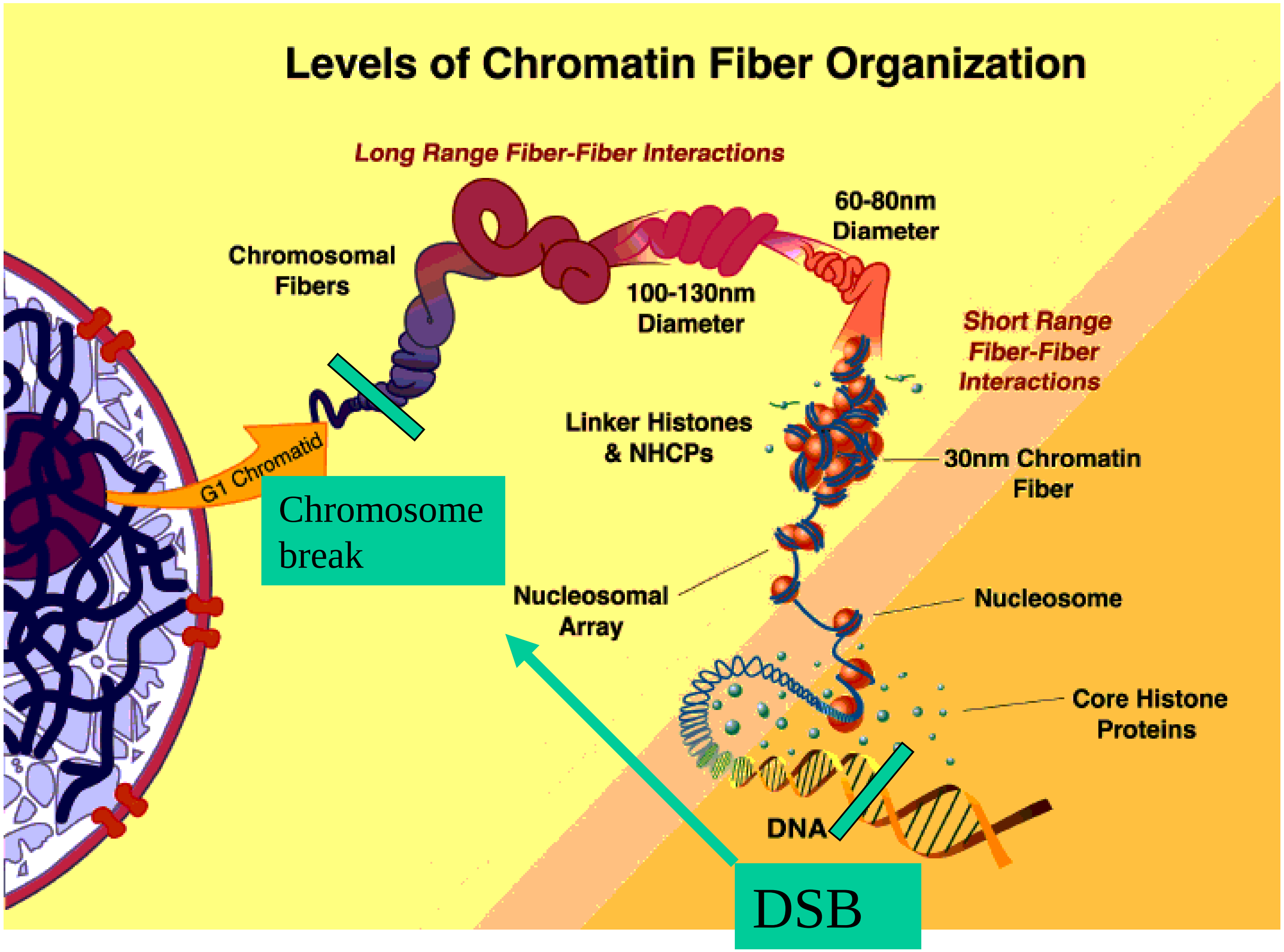
http://www.ebcminden.com/Two%20To%20Tango.jpg

Saying # 7

“It takes two to tango !”

it takes a pair of SSB’s to form a DSB!

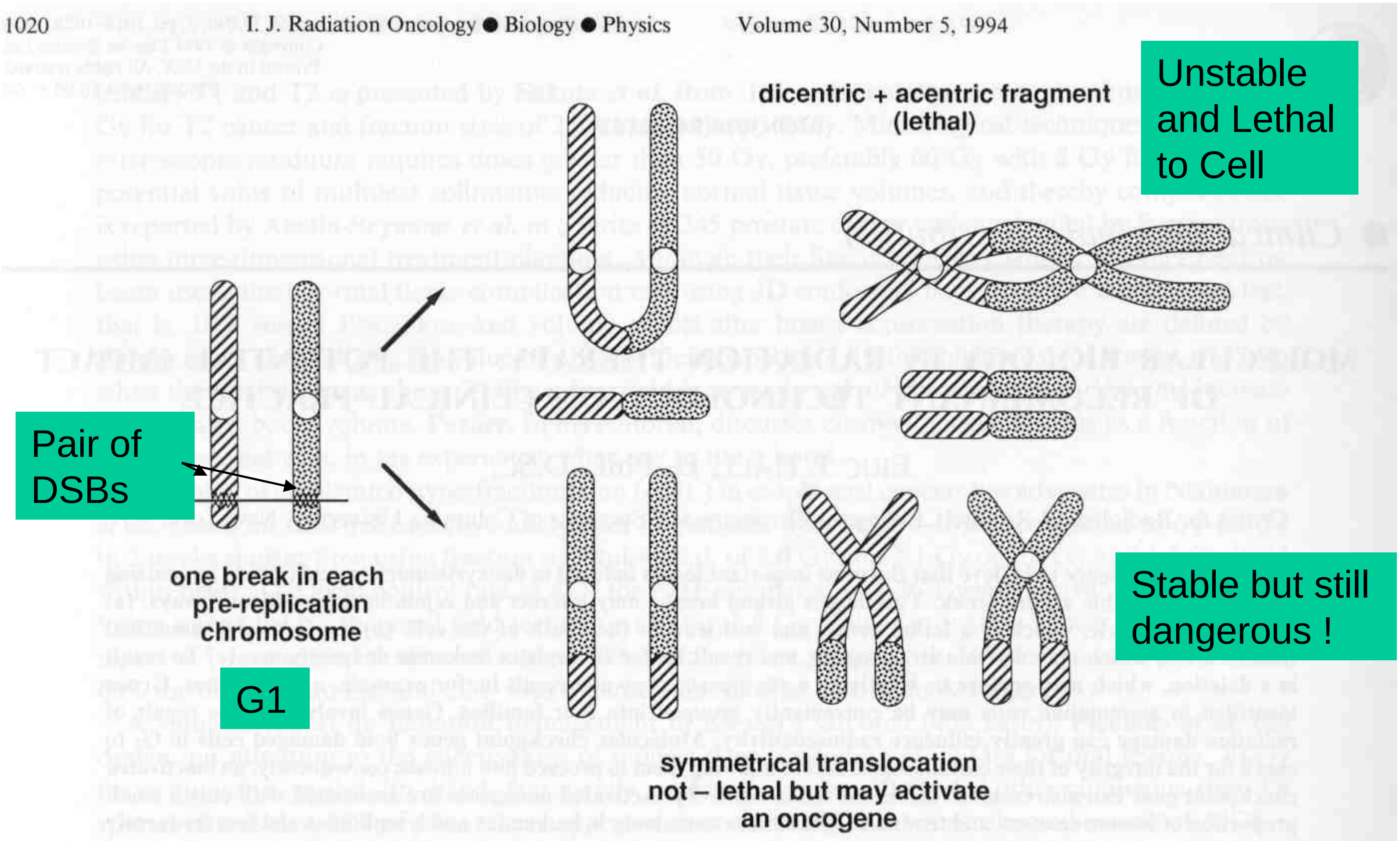
A DSB break leads to a break in a chromosome



Saying # 7

“It takes two to tango !”

e.g. it takes a pair of DSB’s to form a faulty chromosome


1020 I. J. Radiation Oncology ● Biology ● Physics Volume 30, Number 5, 1994
dicentric + acentric fragment
(lethal)
Unstable and Lethal to Cell
Pair of DSBs
one break in each
pre-replication
chromosome
G1
Stable but still dangerous !
symmetrical translocation
not – lethal but may activate
an oncogene

It takes two to tango !

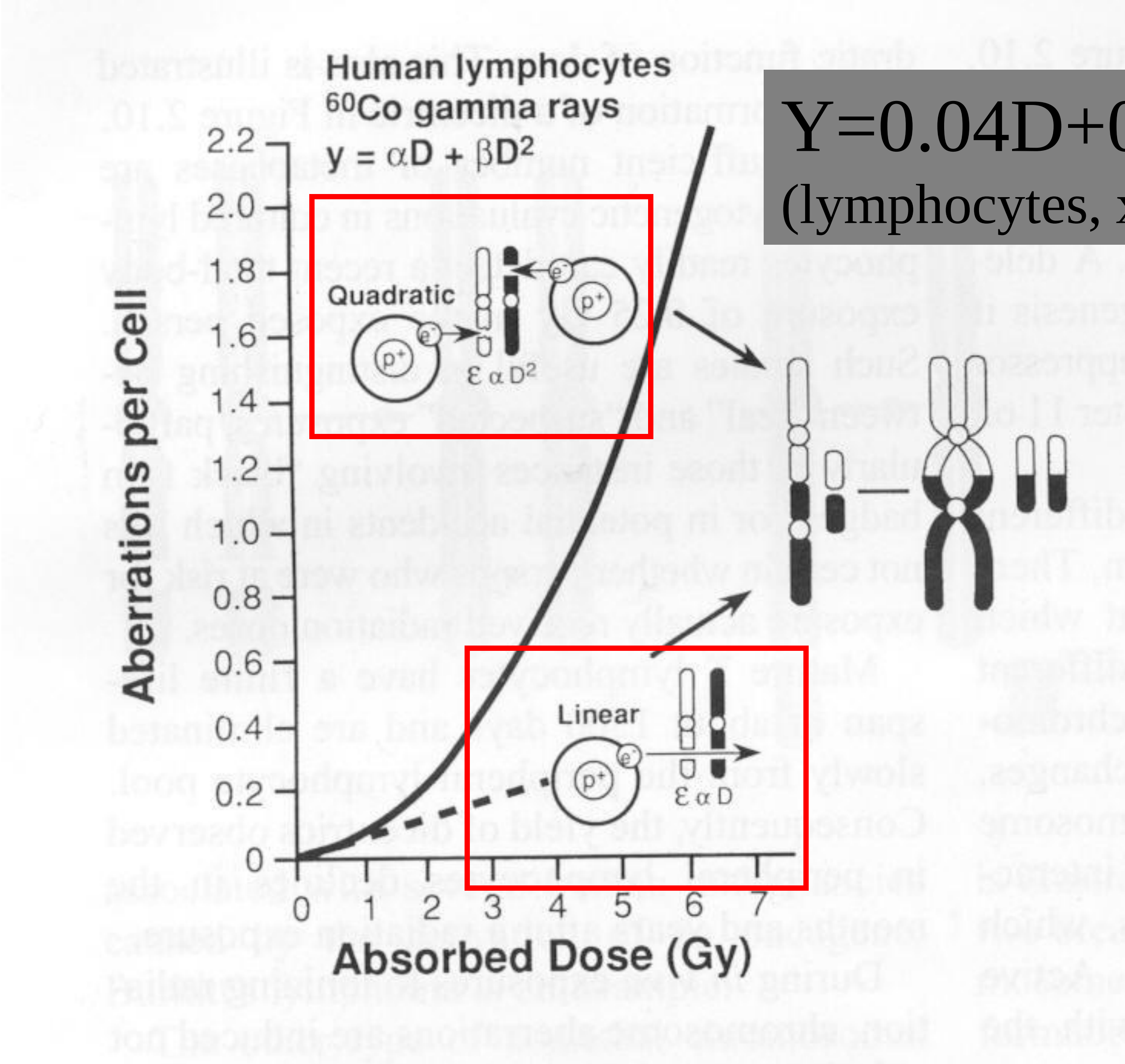
α and β effects

The DSB pair can be formed by either one (linear term) or two cooperating radiation tracks (quadratic term).

If two tracks are involved, dose rate is important.

It affects the observed β value.

Fatal Chromosome Yield

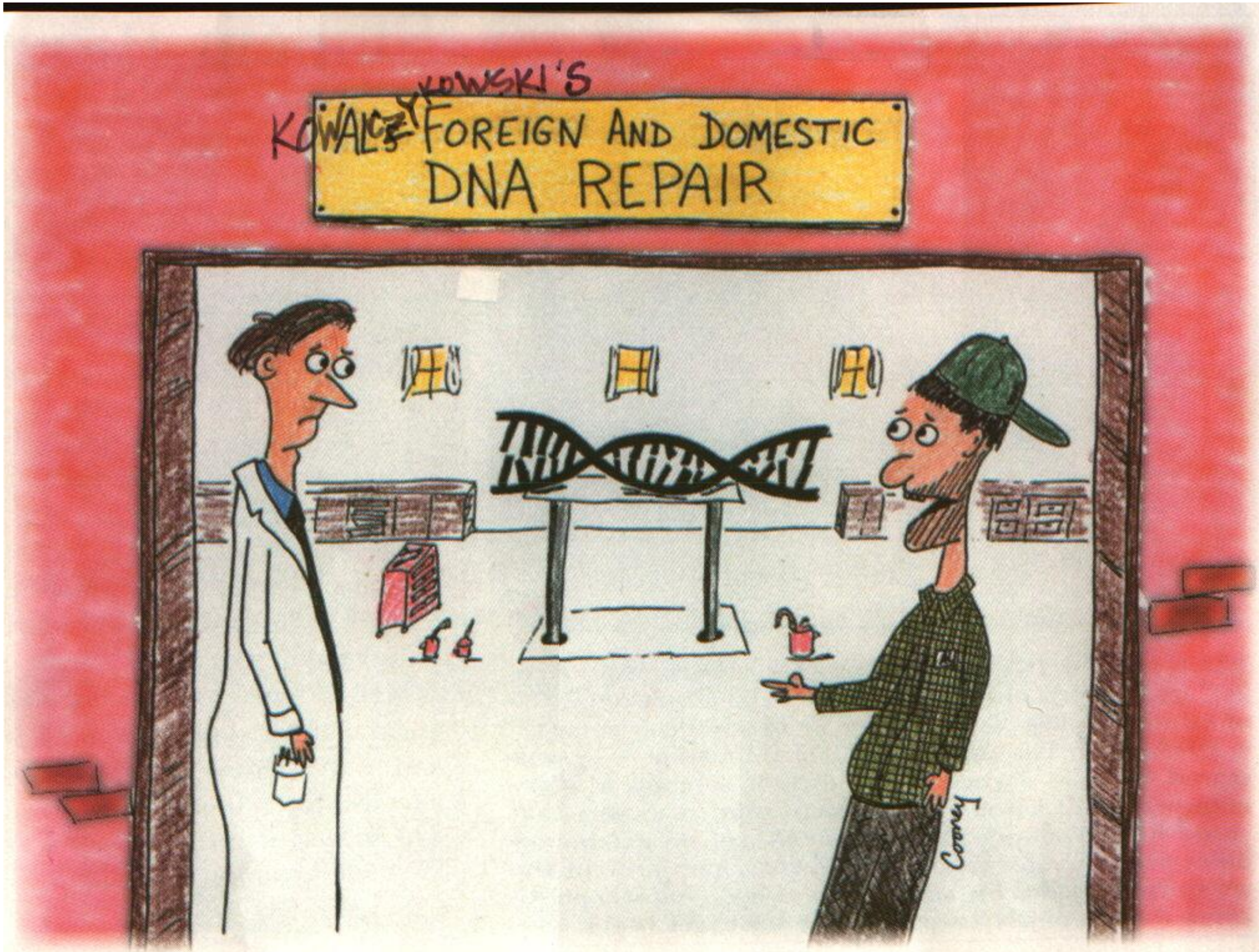


$Y=0.04D+0.06D^2$

(lymphocytes, x-rays)

Summary of DNA Damage

- Yield per cell per Gy of radiation
  - Damaged Bases 1,000
  - SSB's 1,000
  - DSB's 40 (typical value)
- Yield of fatal Chromosome Aberrations (CA) is far less
  - 0.1 CA's per cell per Gy (1 in 10 cells affected)
  - Much less than above yields !
- Not all DSB-pair "hits" materialize into fatality
- <u>Repair of DNA DSB's plays a key role in survival</u>



"You're lucky nobody was injured. Your base pairs are out of alignment and that has your reading frames all messed up."

www-mic.ucdavis.edu/ sklab/genetic%20recomb.htm

DNA Repair and Dose Rate Effects

Intrinsic Radiosensitivity

+ Repair (R1) Time Effects

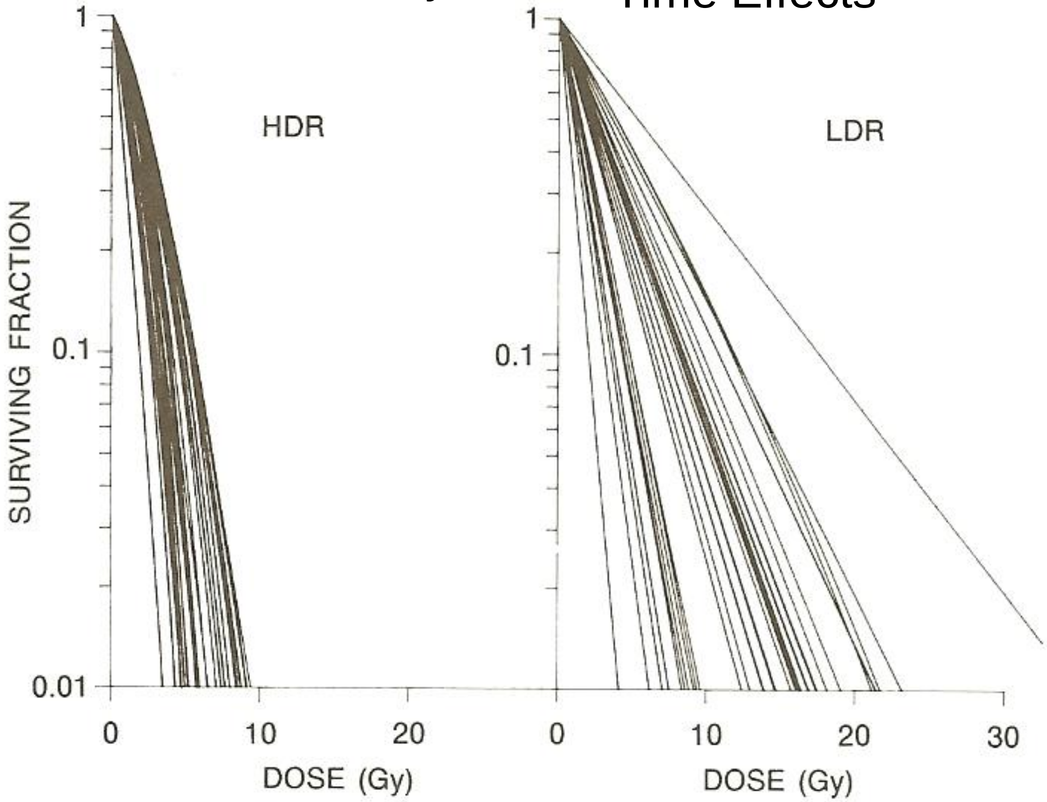


FIGURE 5.14 Dose–survival curves at high dose rates (HDR) and low dose rates (LDR) for a large number of cell lines of human origin cultured *in vitro*. Note that the survival curves fan out at low dose rates because in addition to a range of inherent radiosensitivities (evident at HDR), there is also a range of repair times of sublethal damage.

Ataxia Telangiectasia (AT)

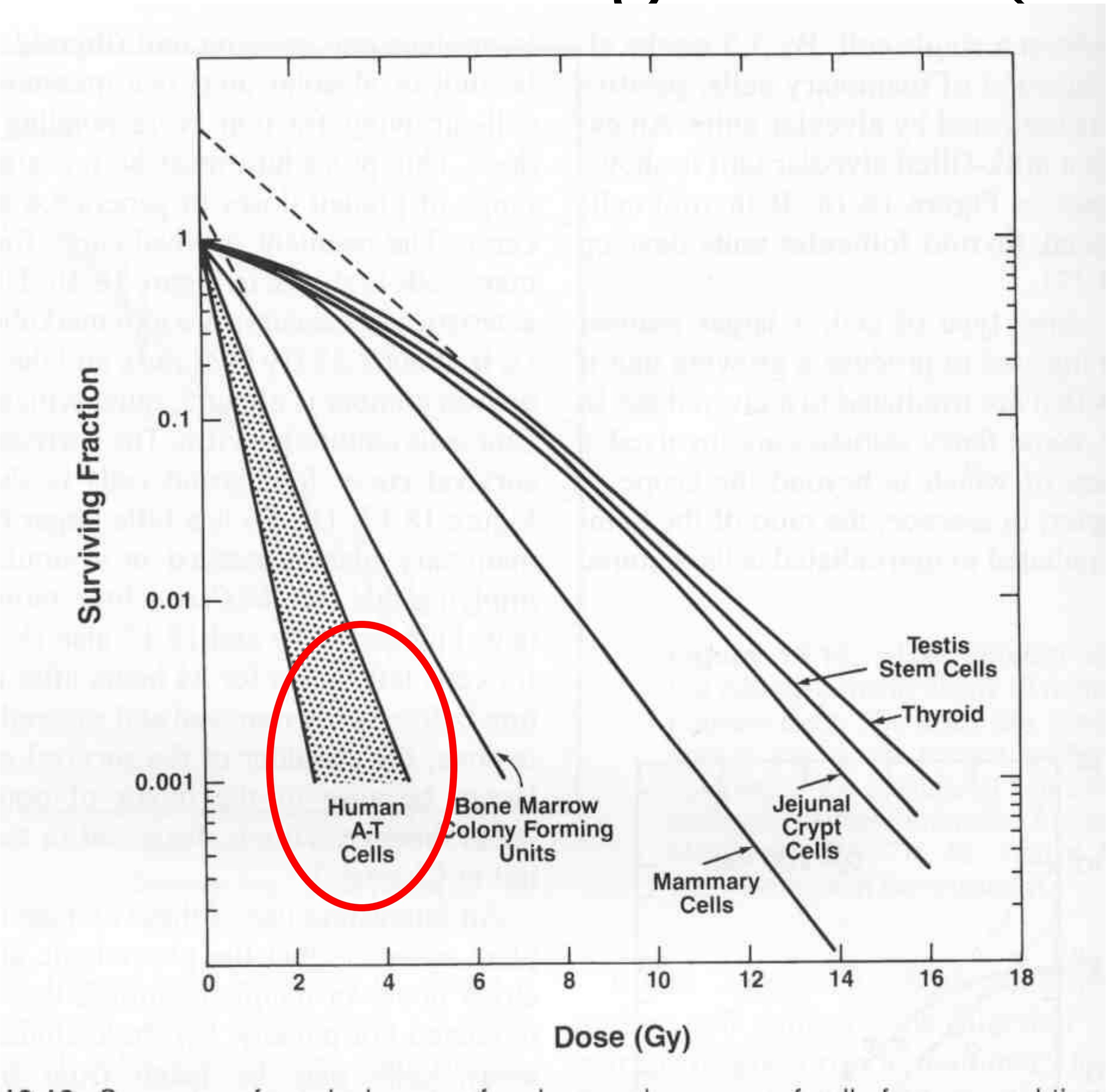
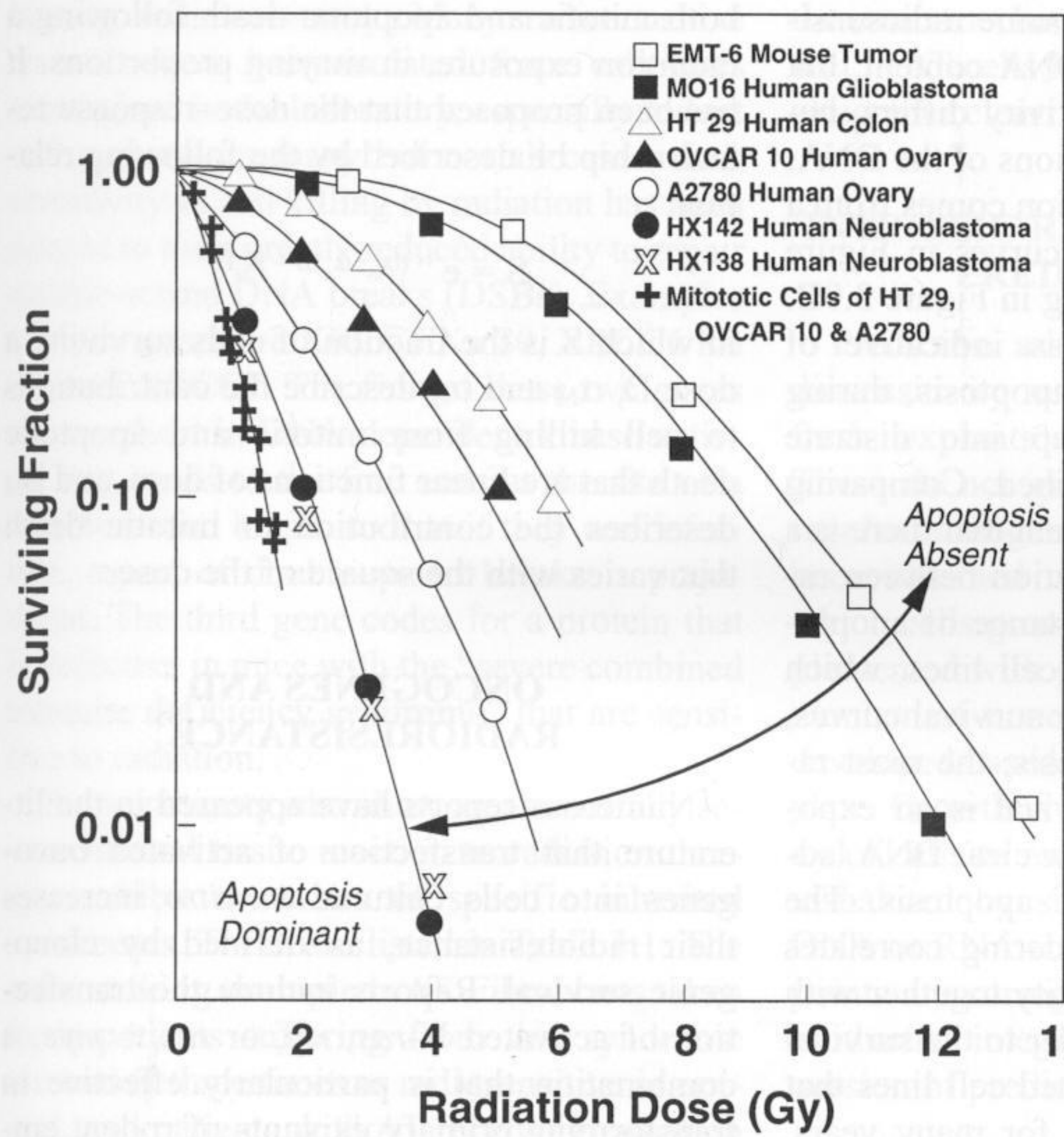


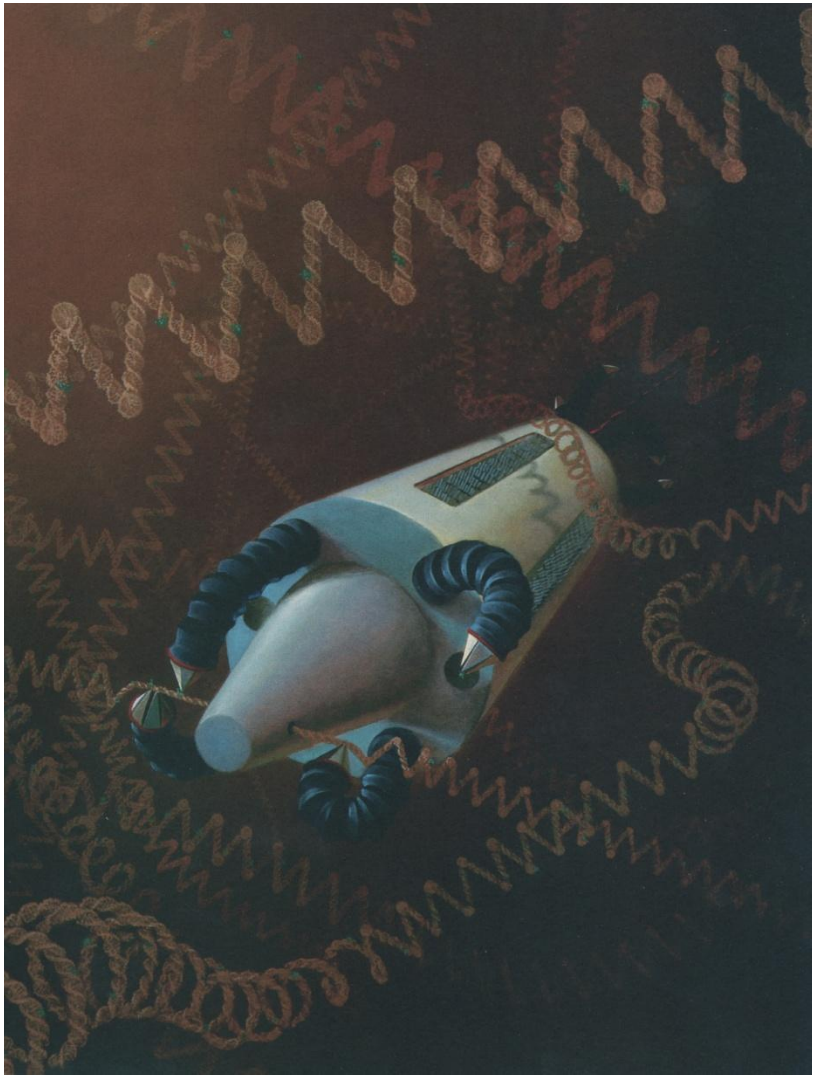
Fig 18.8



EMT-6 Mouse Tumor
MO16 Human Glioblastoma
HT 29 Human Colon
OVCAR 10 Human Ovary
A2780 Human Ovary
HX142 Human Neuroblastoma
HX138 Human Neuroblastoma
Mitotic Cells of HT 29, OVCAR 10 & A2780
Surviving Fraction
1.00
0.10
0.01
Apoptosis Absent
Apoptosis Dominant
0
2
4
6
8
10
12
14
Radiation Dose (Gy)

A

DNA Repair – how is it done ?



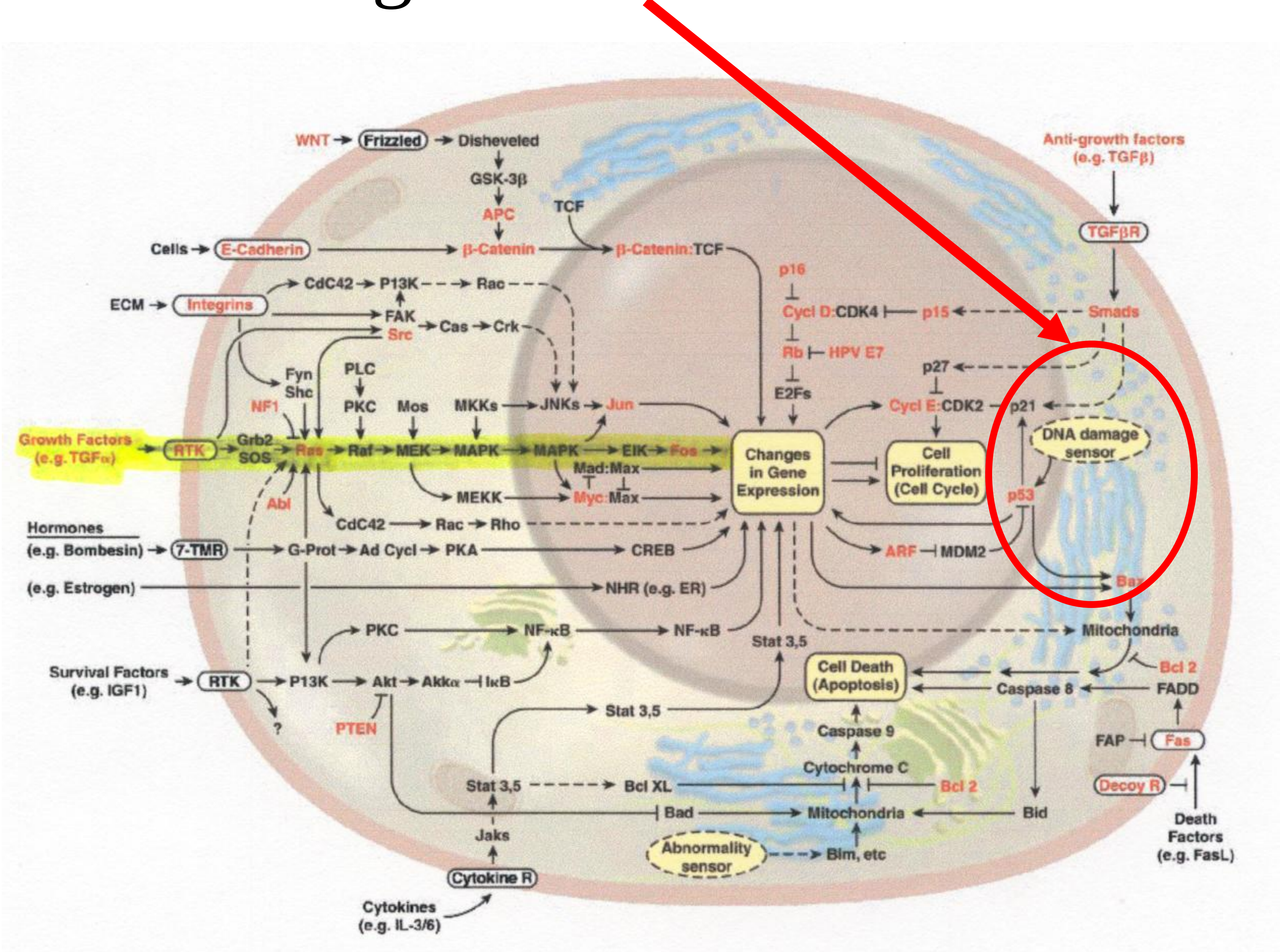
http://www.foresight.org/Nanomedicine/Gallery/Images/TimeLifeNMB.jpeg

How does the Cell cope ?

- Cells experience 100,000’s DNA lesions per day
  - Replication errors, base corruption, chemical reactions, radiation damage
- However, observed mutation rate is low
- Therefore, cells must have efficient pathways to

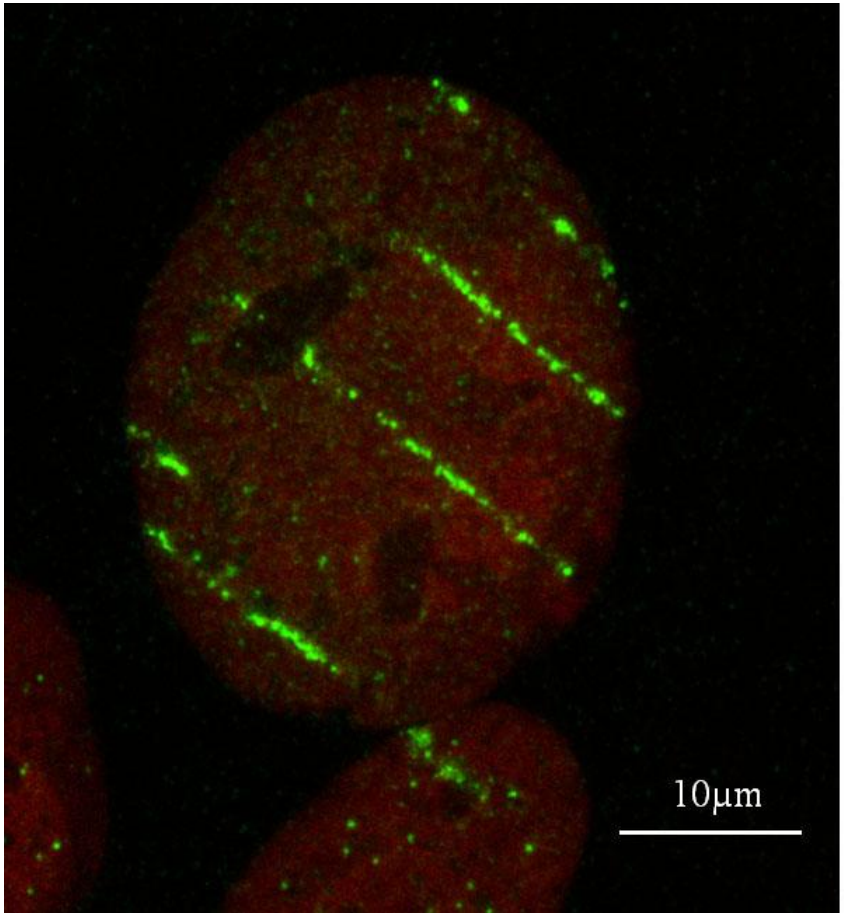
Sense DNA damage
Initiate Repair
Seal it up

DNA Damage Sensing is Regular Cell Business

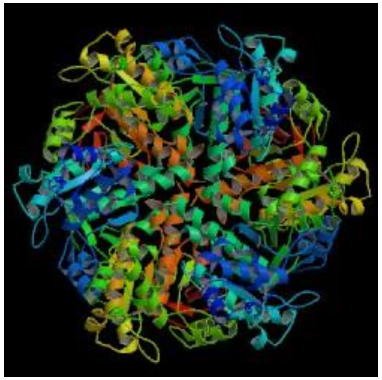


30. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57–70.

Repair Enzyme (Mre11) at Work



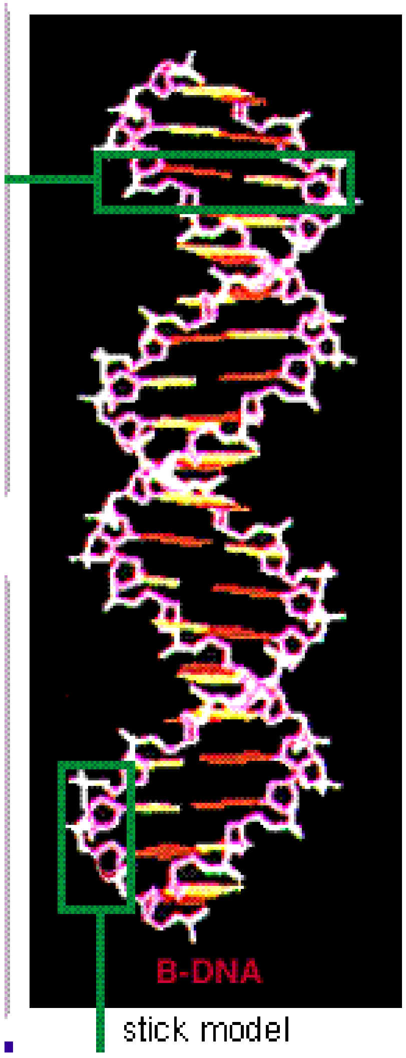
Microscopic visualization of the extremely localized DNA damage induced in nuclei of mammalian cells following irradiation with accelerated ions. Immuno-fluorescence stained repair proteins accumulate at the lesions along the individual ion tracks traversing the nucleus of a human cell, appearing as parallel streaks. Red: DNA counterstain (Propidium Iodide). Green: Repair protein (Mre11).
(B.Jakob, M. Scholz and G. Taucher-Scholz, Radiat. Res. 2003)



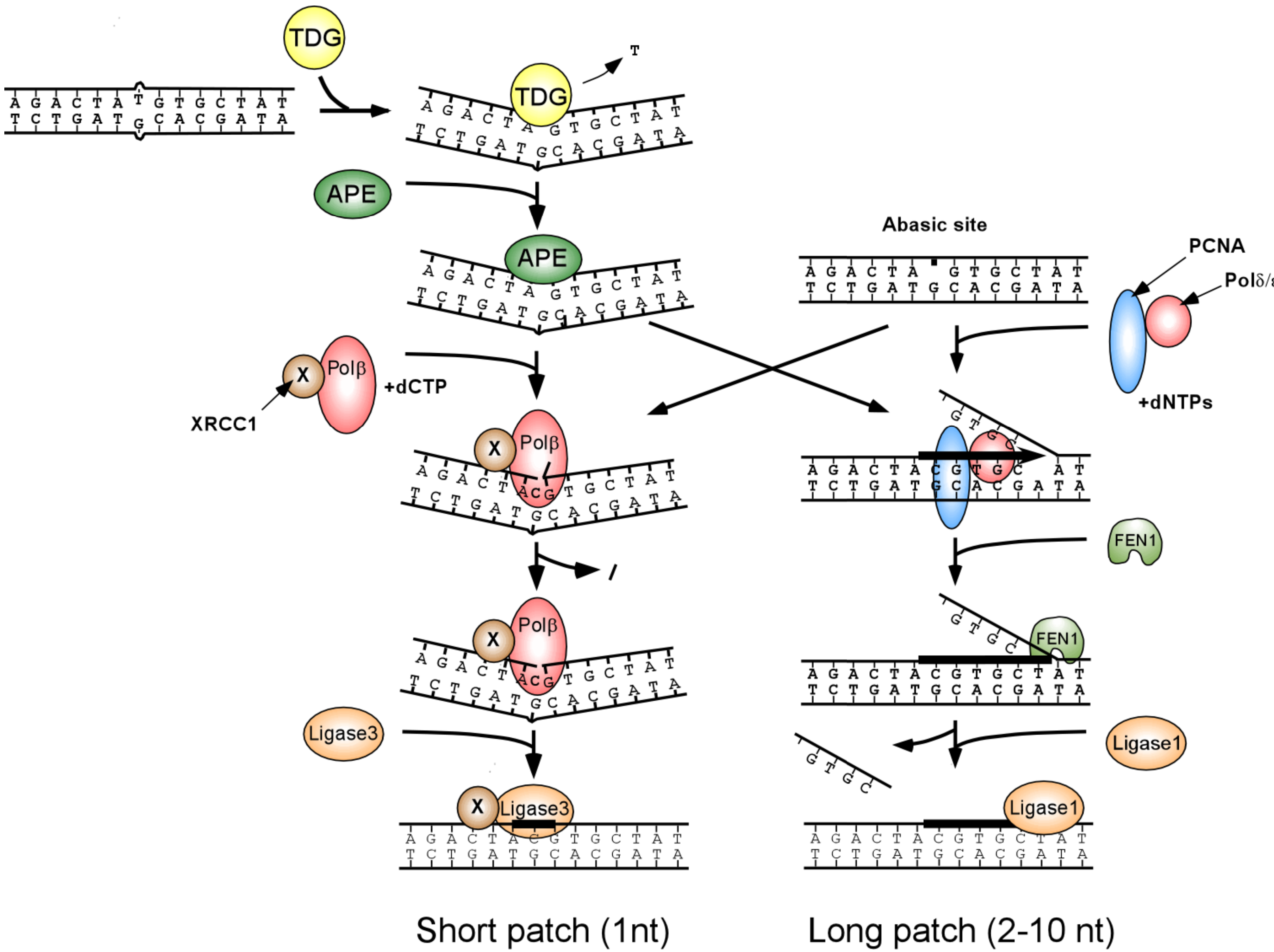
Rad 51 Protein

Types of DNA DAMAGE

- Single-strand breaks (SSB)
  - Direct energy depositions in sugar/phosphate backbone
  - OH-deoxyribose "attack"
  - Combine to form
- Double Strand Breaks
  - Opposite strand SSBs
  - Distance < 10 bp
  - Time << Repair Time
- Base damage
  - Direct energy depositions
  - OH-base reactions
  - e^-_{aq}-base reactions

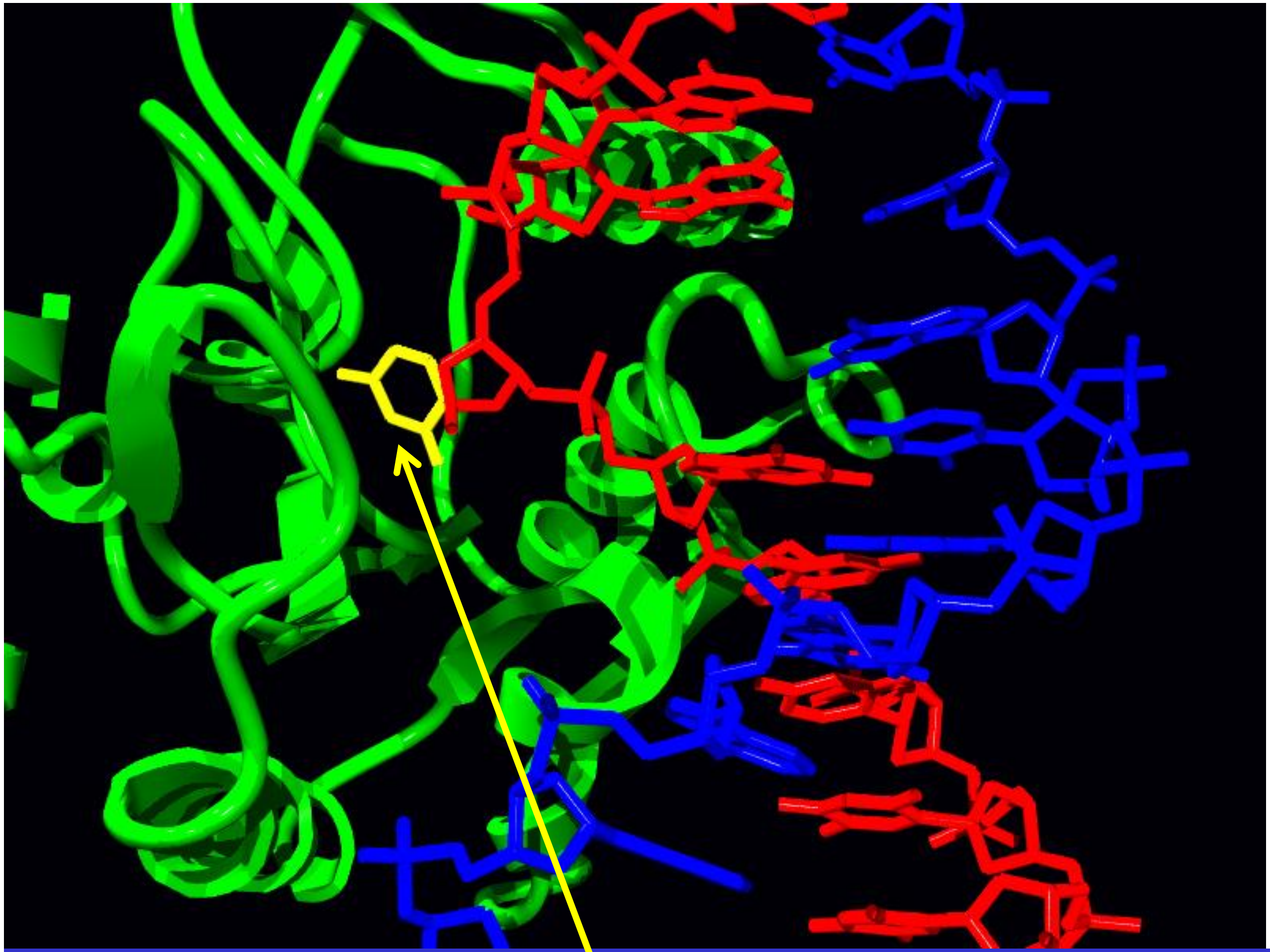


Base Excision Repair



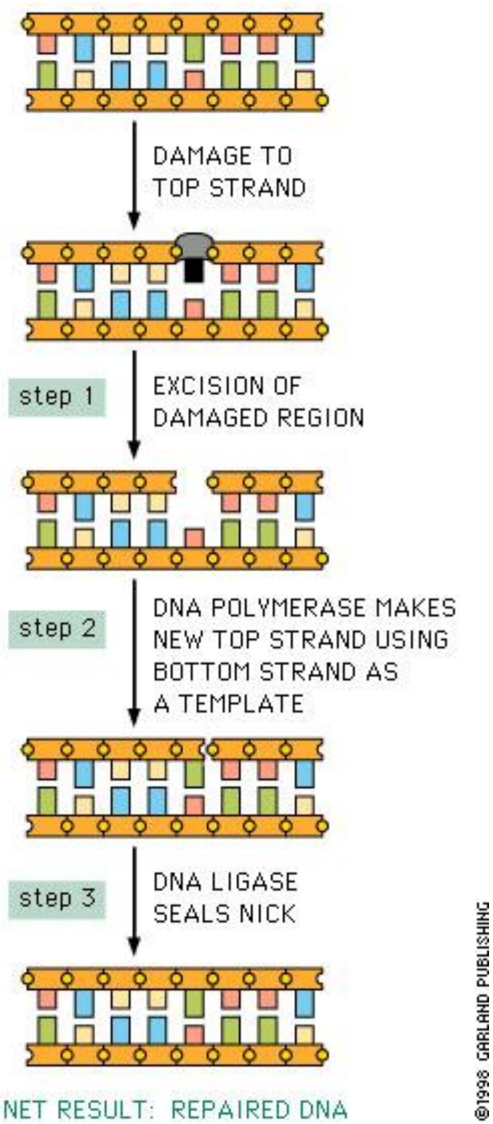
Copyright 2001/MarcTini

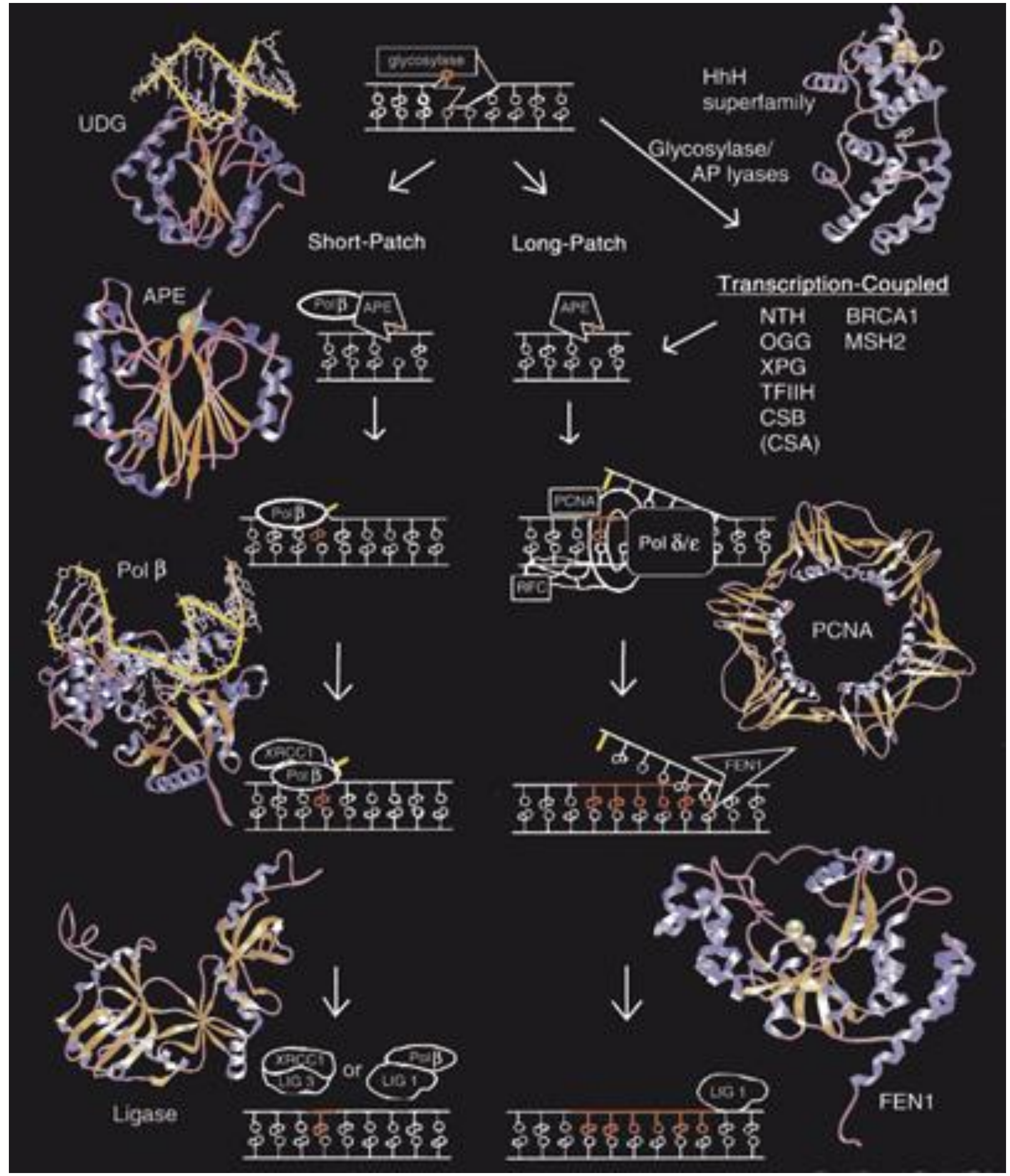
Base Excision Repair



Structure of the base-excision repair enzyme uracil-DNA glycosylase. The targeted uracil residue is shown in yellow

SSB Repair

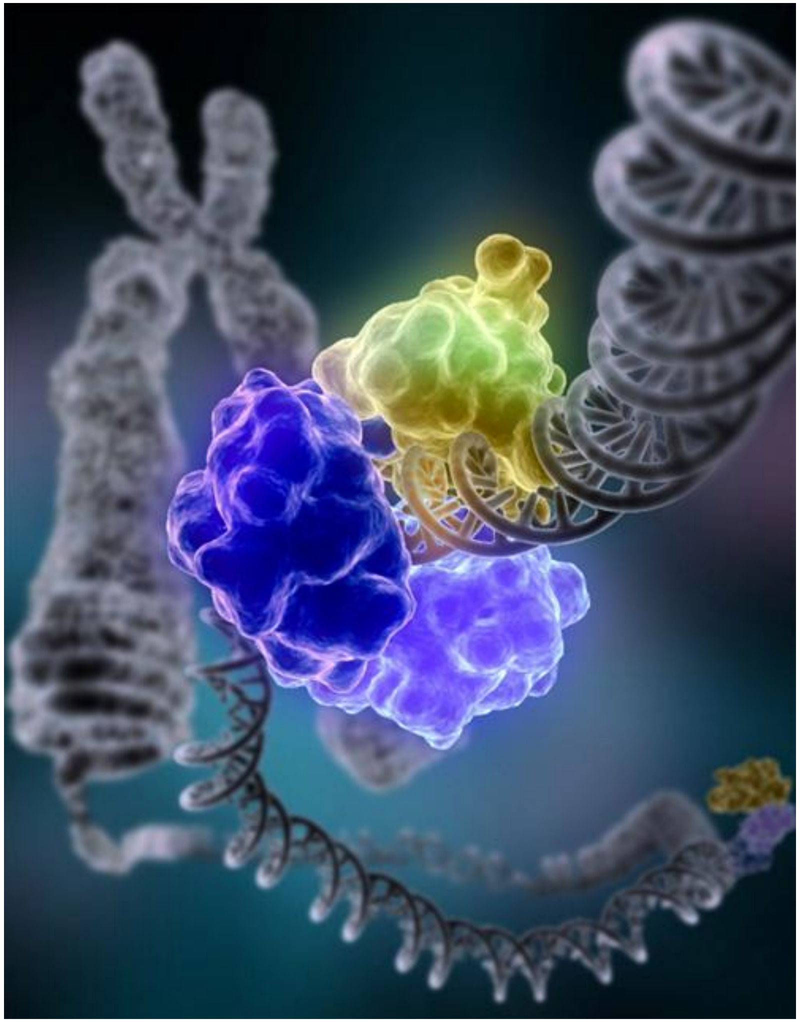




http://www.csu.edu.au/faculty/health/biomed/subjects/molbol/images/6_26.jpg

http://www.ic-rm.mlib.cnr.it/pasc1.jpg

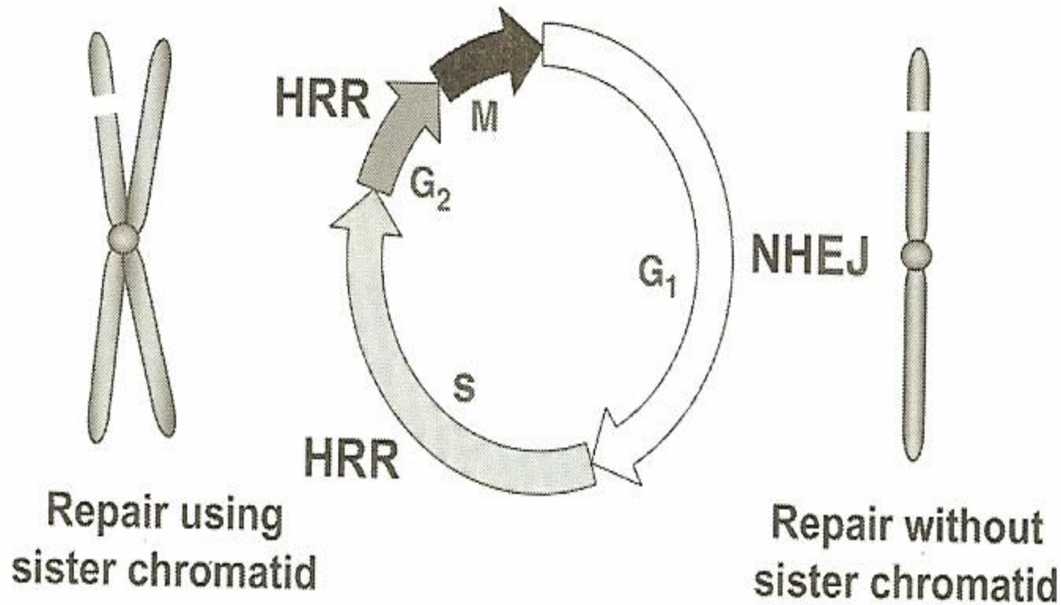
DNA Ligase fixes DSB's



DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 100,000's lesions per cell per day. A special enzyme, DNA ligase (shown here in color), encircles the double helix to repair a broken strand of DNA. DNA ligase is responsible for repairing the millions of DNA breaks generated during the normal course of a cell's life. Without molecules that can mend such breaks, cells can malfunction, die, or become cancerous.

DSB Repair is more complex and can occur in G1 or S-G2 Phases

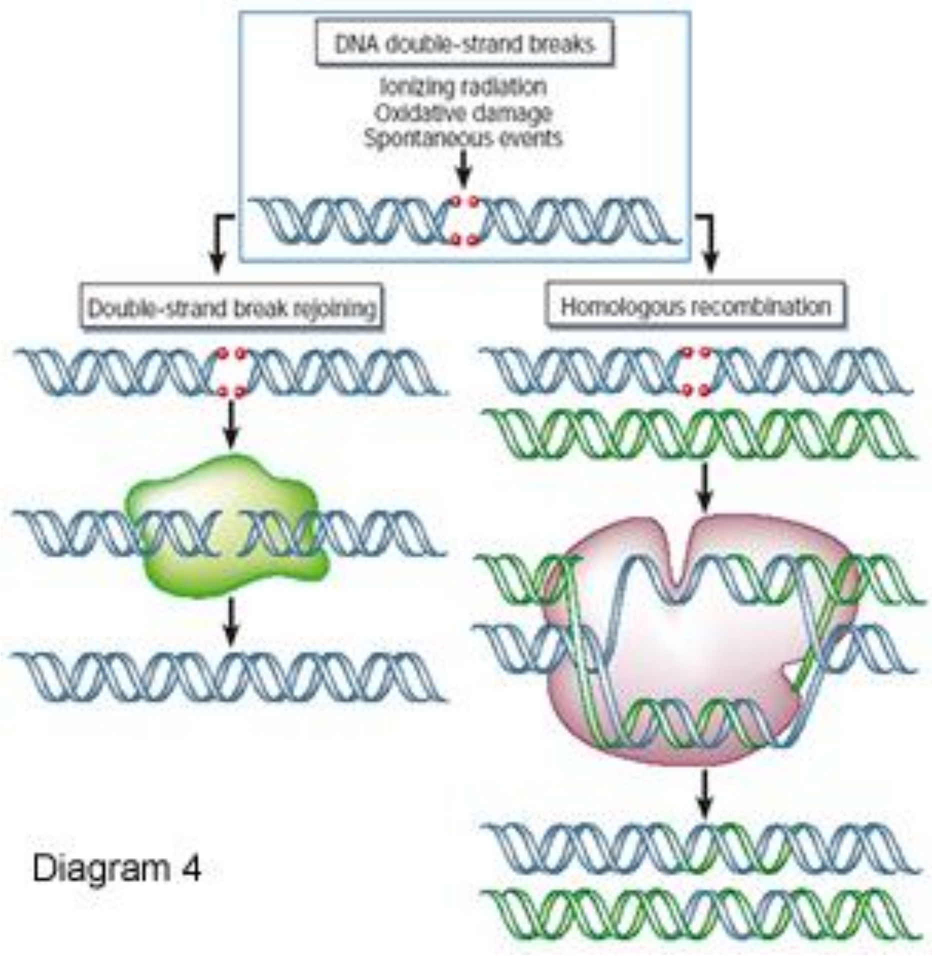
FIGURE 5.2 • Illustration showing that nonhomologous recombination occurs in the G_1 phase of the cell cycle, at which stage there is no sister chromatid to use as a template for repair. In contrast, homologous recombination occurs in the S and G_2 phases of the cell cycle, when there is a sister chromatid to use as a template in repair.



B. Homologous Repair

A. Non-Homologous Repair

DSB Repair Modes



A B

http://people.bath.ac.uk/pr1cemb/HR.png

DSB Repair in More Detail

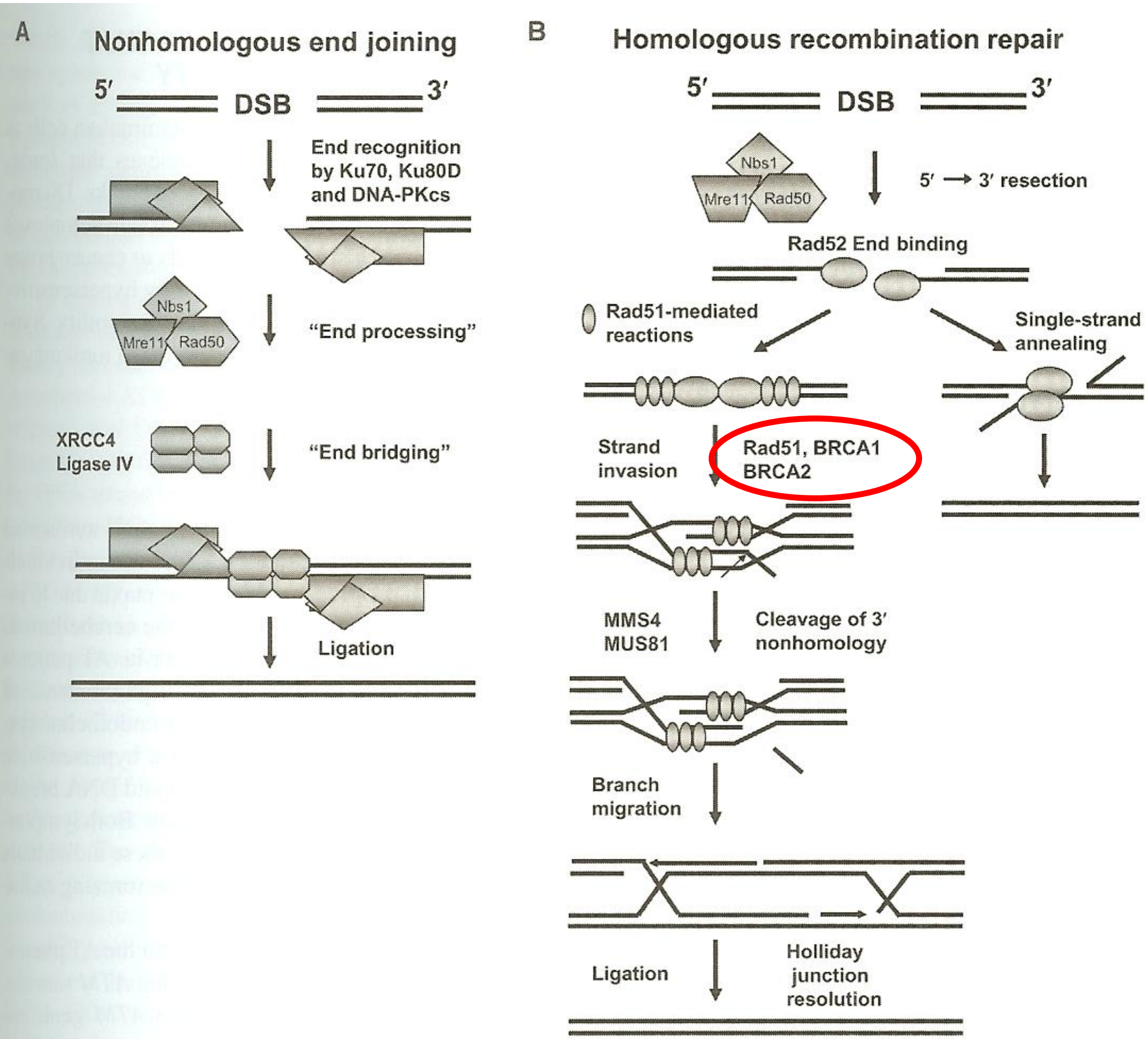
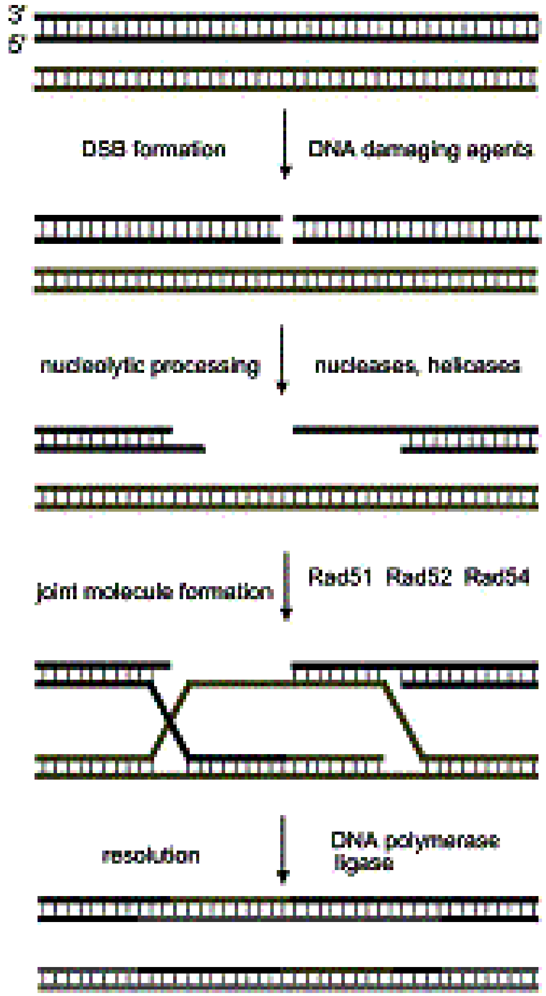
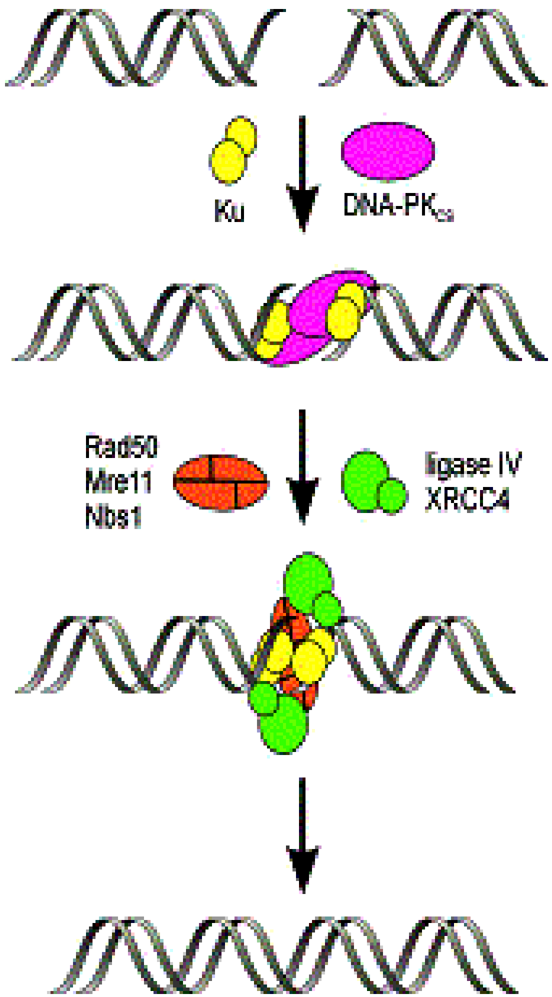


FIGURE 5.3 DNA double-strand break repair via homologous and nonhomologous recombination. **A:** A double-strand break with no template to guide gap filling. Consequently, errors are more likely to occur in this process, which is called nonhomologous end joining. See text for details. **B:** A double-strand break that has occurred after replication (in S or G_2 phase of the cell cycle), so that identical sister chromatids are available. In homologous recombination (also termed single-strand annealing), the exposed 3′ end invades the homologous duplex, so that the complementary strand acts as a template for gap filling.

B. Homologous Repair

It takes Two to Tango !





Happens in Hours

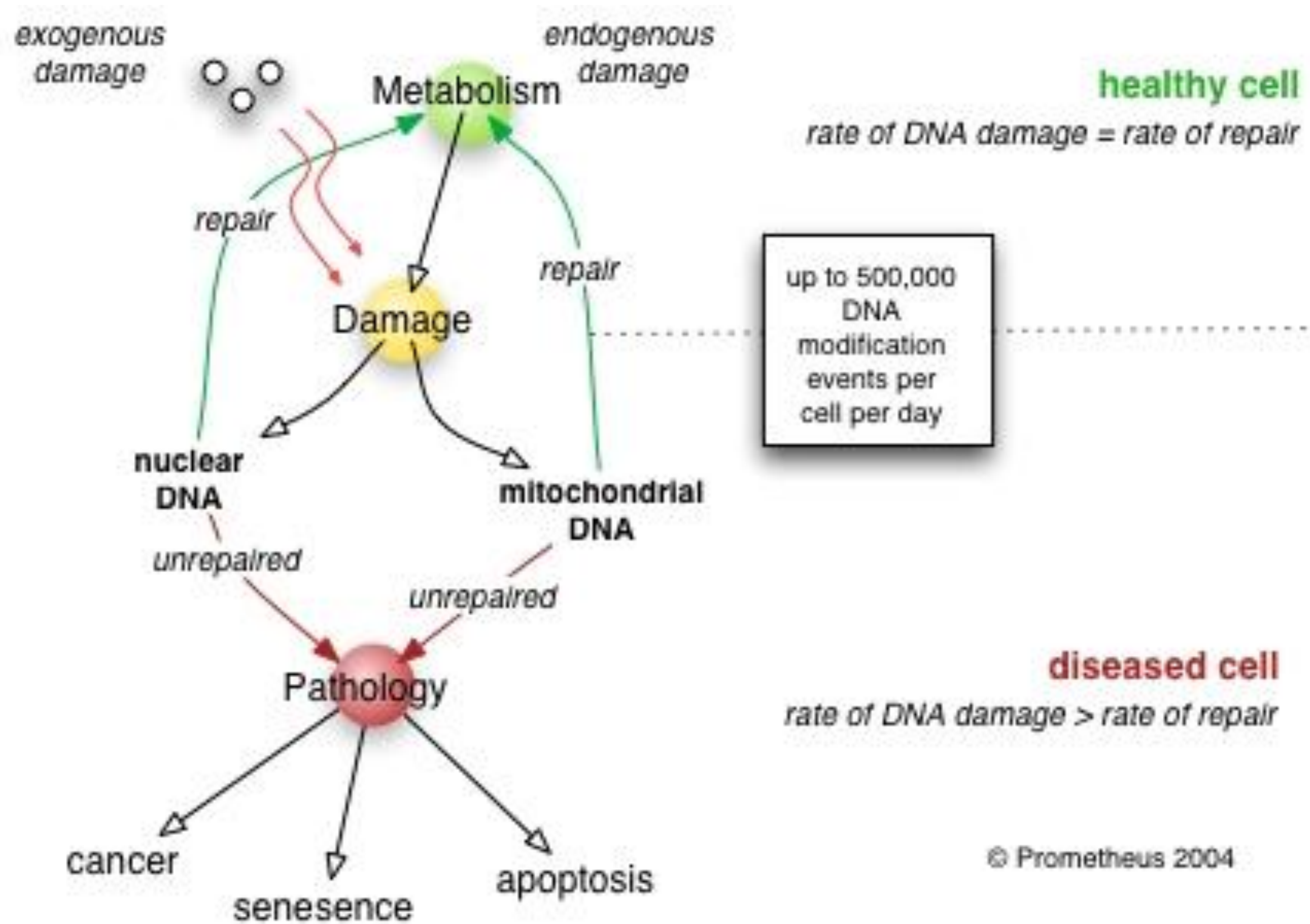
http://www.mb.tn.tudelft.nl/projects/dna_repair/pathway.gif

Human Error Rates

U.S. Postal Service on-time delivery of local first-class mail	13 late deliveries per 100 parcels
Airline luggage system	1 lost bag per 200
A professional typist typing at 120 words per minute	1 mistake per 250 characters
Driving a car in the United States	1 death per 10^4 people per year
DNA replication (without mismatch repair)	1 mistake per 10^7 nucleotides copied
DNA replication (with mismatch repair)	1 mistake per 10^9 nucleotides copied

http://www.csu.edu.au/faculty/health/biomed/subjects/molbol/images/6_26.jpg

DNA Equilibrium



The Bigger Picture
Genomic Instability

FIGURE 17.1 ● The process of malignant transformation results from mutations in three groups of genes: gain-of-function mutations that activate oncogenes, loss-of-function mutations that inactivate tumor-suppressor genes, and loss of activity of DNA stability (e.g., repair) genes that increase the probability for genomic instability. This figure depicts how the stimulatory effects of oncogenes on the cell cycle are opposed by the inhibitory effects of tumor-suppressor genes on the cell cycle that can lead to apoptosis. R indicates the restriction point that is regulated by the *p53* and *pRb* tumor-suppressor genes. The consequences of oncogene activation and tumor-suppressor gene and DNA integrity gene inactivation are immortalization, transformation, and metastasis.

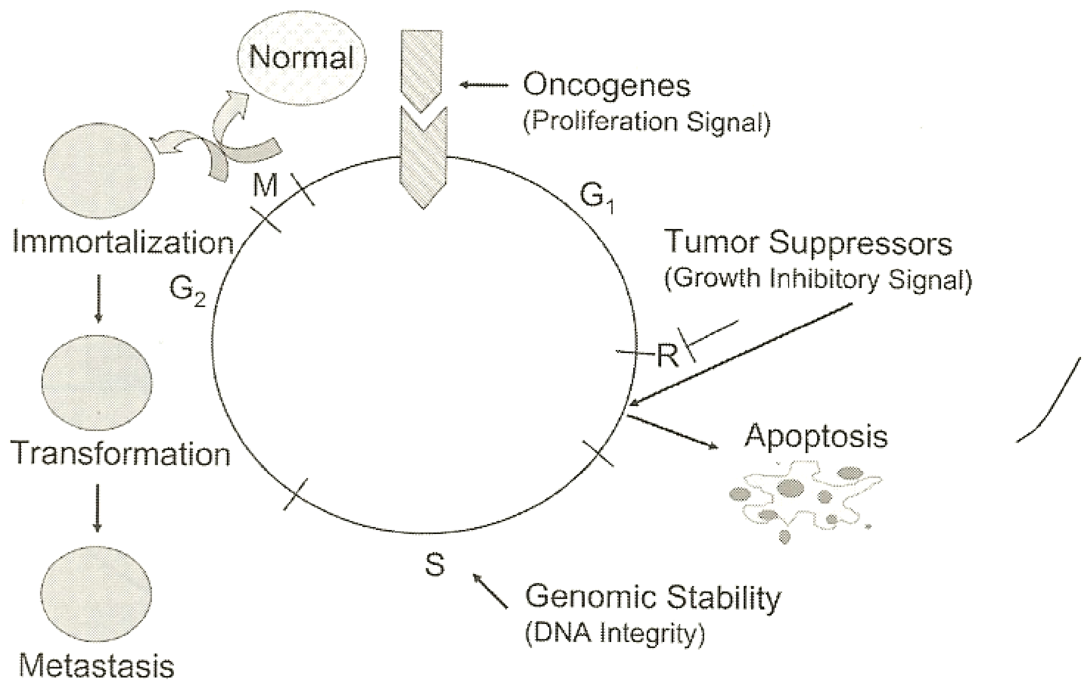
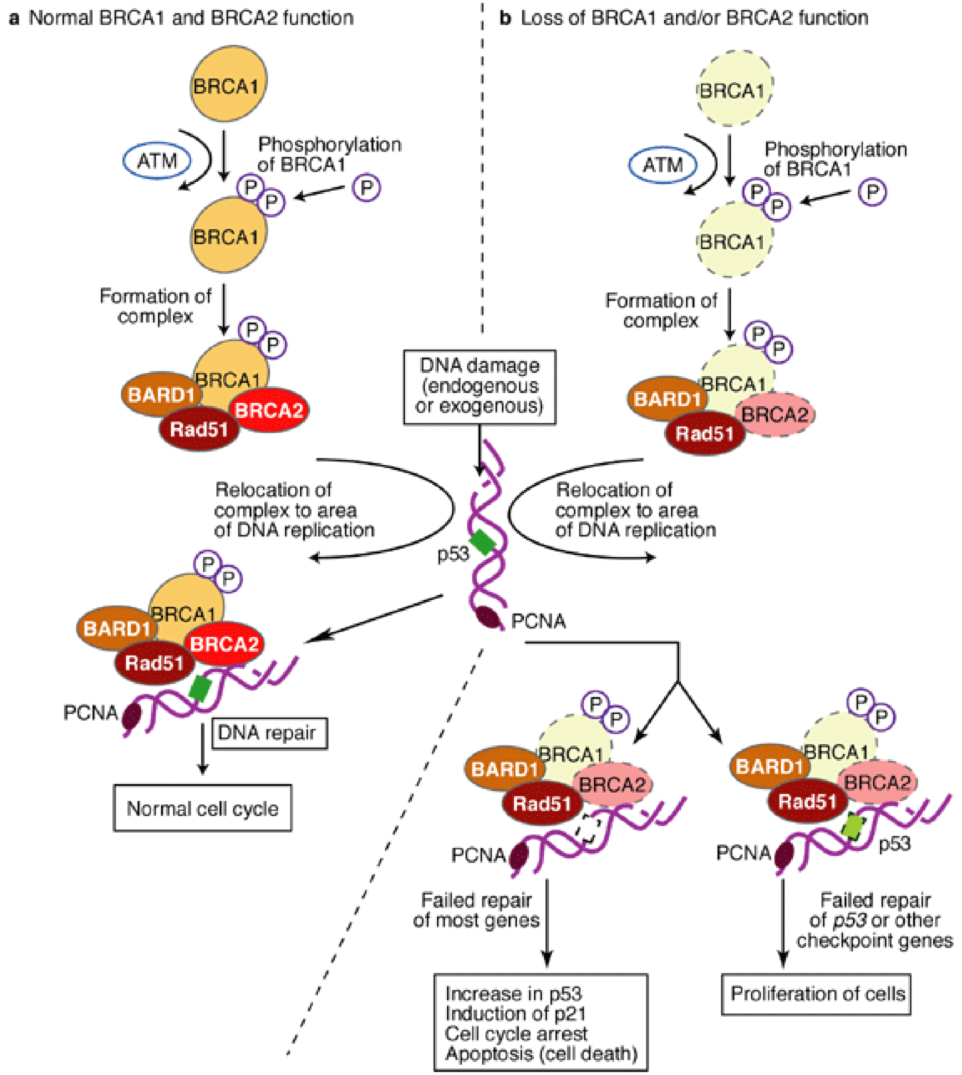


TABLE 17.3

DNA Repair and Stability Genes and Their Associated Syndromes

Suppressor	Syndrome	Tumor
ATM	Ataxia-telangiectasia	Leukemia, lymphoma
XP	Xeroderma pigmentosum	Skin
BRCA1	Hereditary breast cancer 1	Breast
BRCA2	Hereditary breast cancer 2	Breast, ovary
FANC	Fanconi's anemia	Leukemia
NBS	Nijmegen breakage syndrome	Lymphoma
hMSH2	Hereditary nonpolyposis colorectal cancer	Colon
hMLH1	Hereditary nonpolyposis colorectal cancer	Colon
hMSH6	Hereditary nonpolyposis colorectal cancer	Colon
hPMS1	Hereditary nonpolyposis colorectal cancer	Colon
hPMS2	Hereditary nonpolyposis colorectal cancer	Colon

DNA Repair Failure Linked to Cancer



The roles of BRCA1 and BRCA2 in DNA repair

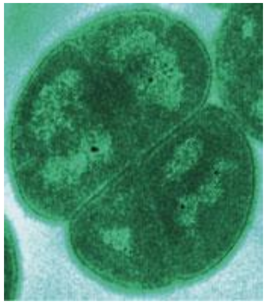
Published in Expert Reviews in Molecular Medicine by Cambridge University Press (2001)

http://www-ermm.cbcu.cam.ac.uk/fig002mgb.gif

Summary

- DNA integrity is monitored and damage is sensed.
- DNA damage triggers cell-wide rescue activity.
- DNA Repair takes place through "enzyme teams"
  - e.g. endonuclease, exonuclease, polymerase, ligase
- Single-strand breaks (SSB) are very efficiently repaired
- Double-strand break (DSB) repair is more complex
  - Mis-repairs happen (rarely)
- Altered DNA can lead to cell death or abnormalities, including DNA repair defects !
- DNA damage can "accumulate" leading to <u>genome instability</u>
- *"Cancer is the "perfect storm" of cumulative DNA alterations"*

Conan the Bacterium



- ***Deinococcus radiodurans*** ("strange berry that withstands radiation", formerly called *Micrococcus radiodurans*) is an extremophilic bacterium, and is the most radioresistant organism known.
- While a dose of 10 Gy is sufficient to kill a human, and a dose of 60 Gy is sufficient to kill all cells in a culture of *E. coli*, *D. radiodurans* is capable of withstanding an instantaneous dose of up to 5,000 Gy with no loss of viability
- An instantaneous dose of up to 15,000 Gy leads to 37% viability.
- It can survive heat, cold, dehydration, vacuum, and acid.