DNA damage, DNA Repair and Signal Transduction pathways

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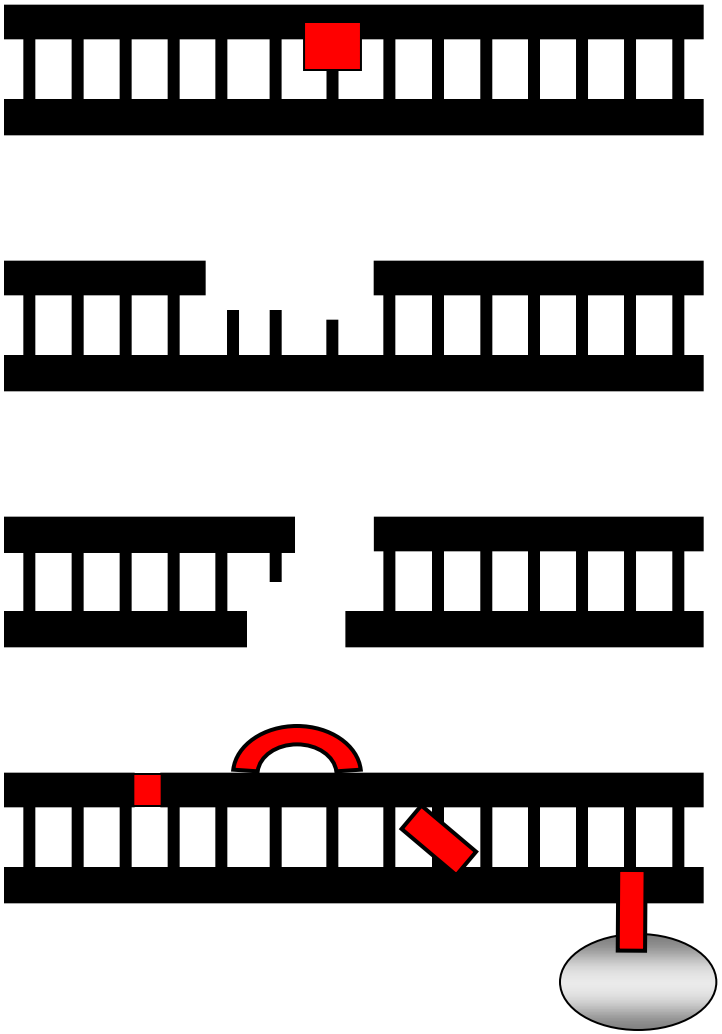
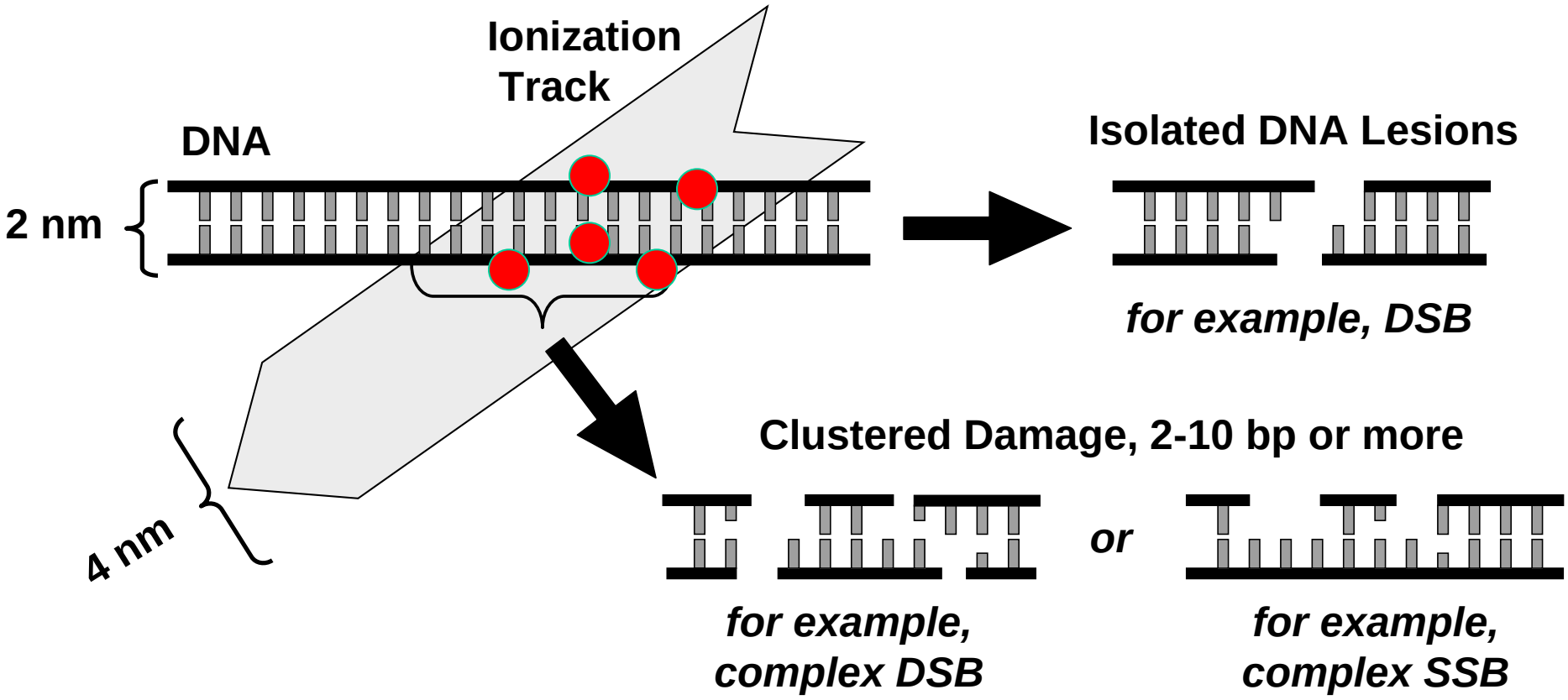
Radiation Oncology
University of Maryland

IR-induced DNA Damage is heterogeneous



Damage type	No./Gy/cell
base damage	> 1000
single-strand break (SSB)	500-1000
double-strand break (DSB)	~ 40
sugar damage, DNA-DNA and DNA-protein cross links	*various*

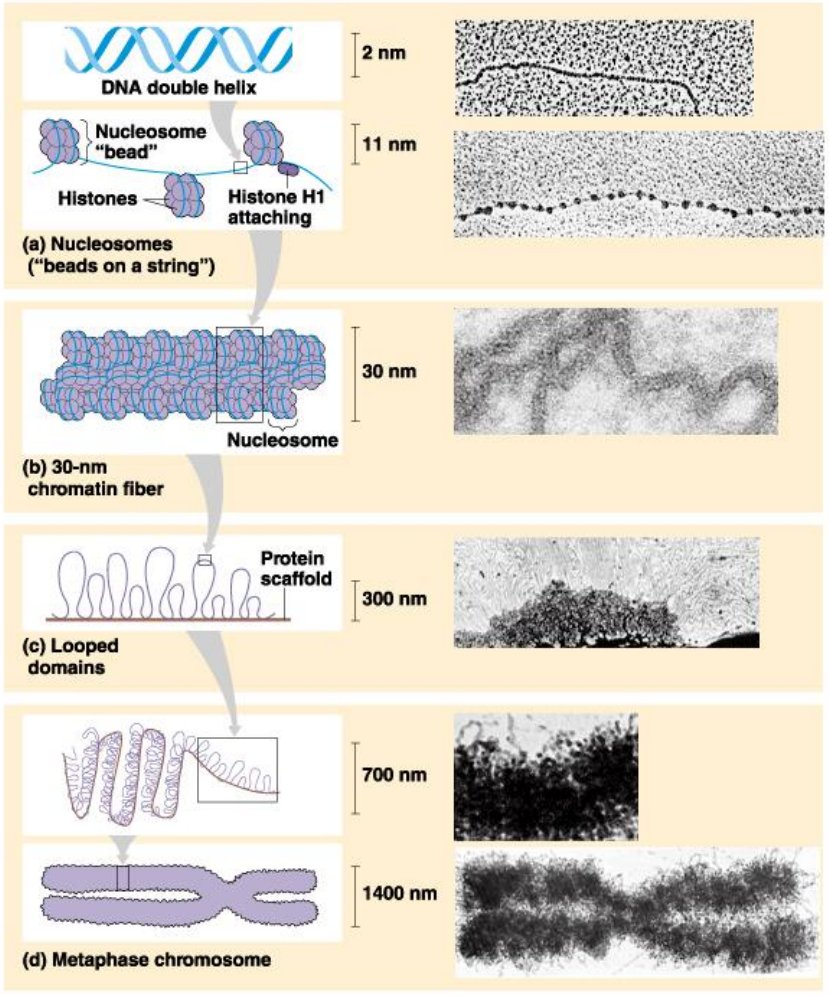
IR Causes Clustered DNA Damages



IR typically induces oxidized purines and pyrimidines, abasic (AP) sites and SSB.

DSB may be clustered or non-clustered or arise during BER repair.

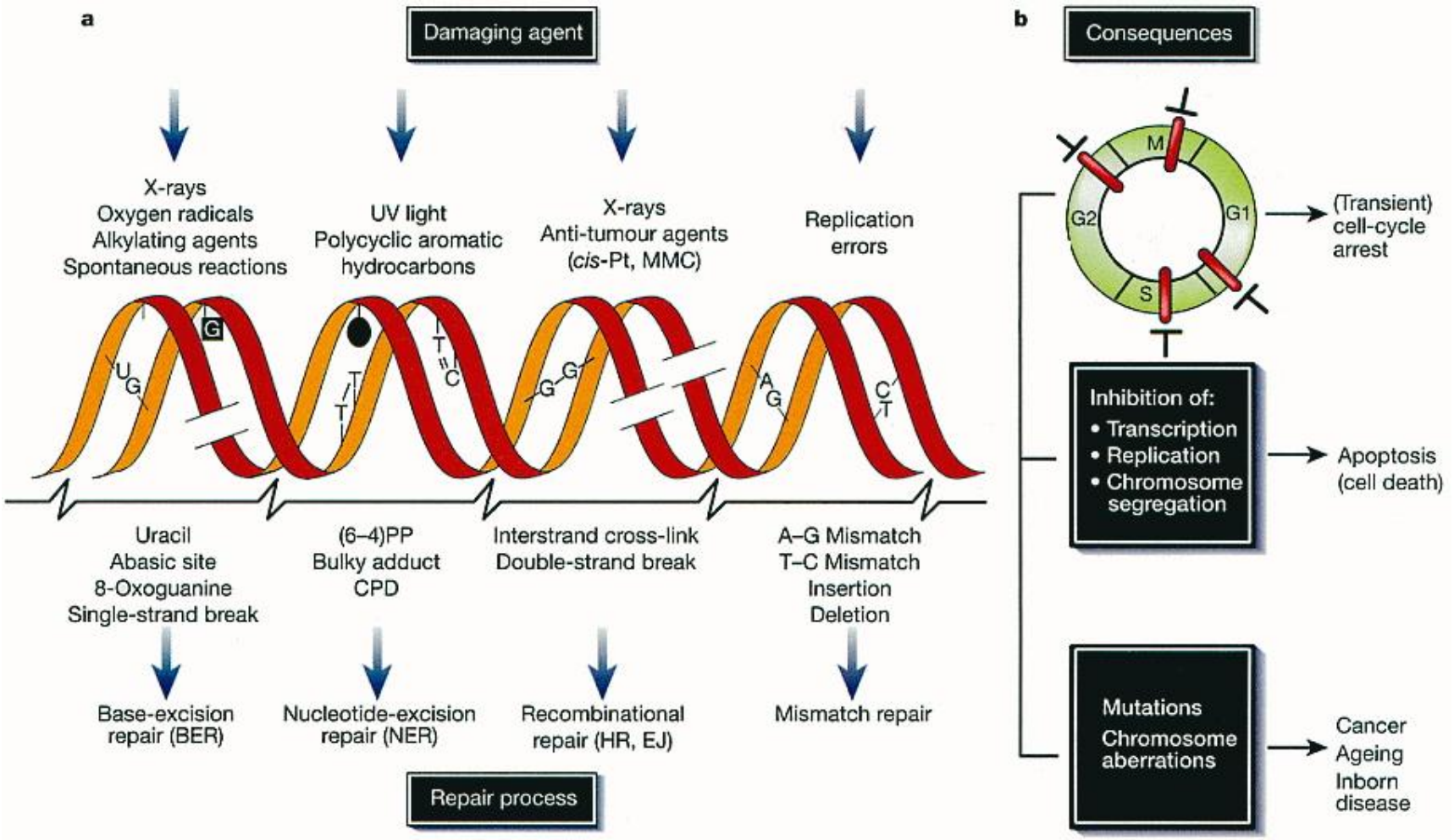
Chromatin Structure



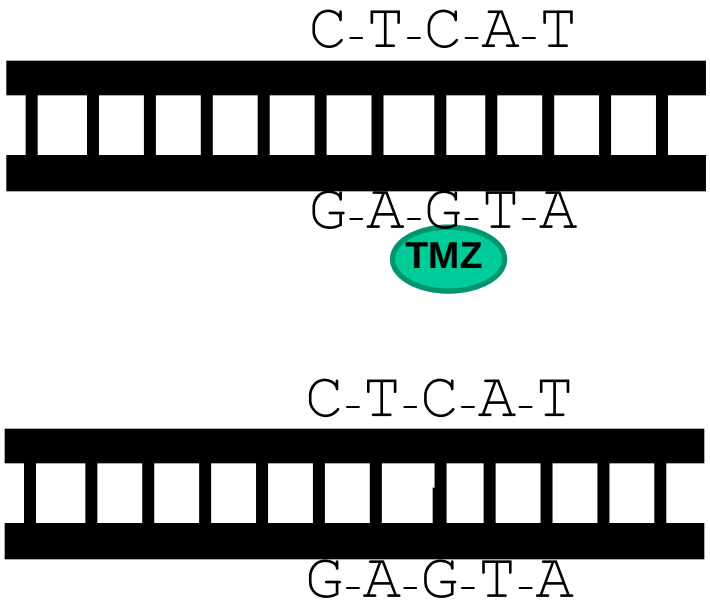
- IR induced DNA lesions are clustered.

- Locally multiply damaged sites (LMDS) within 20 bp.

DNA Repair Mechanisms

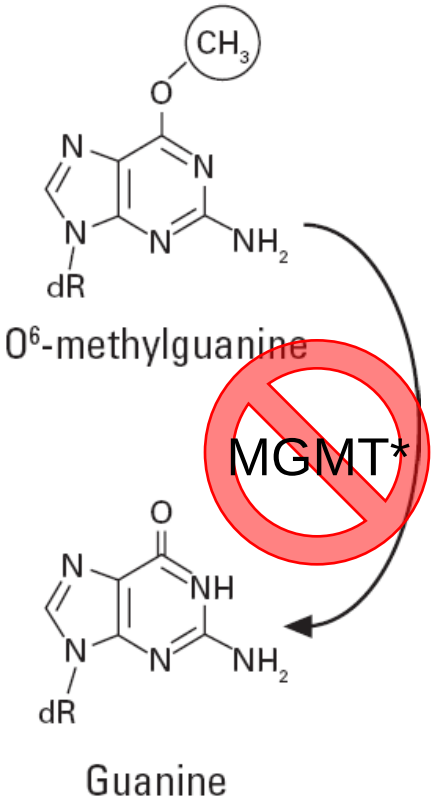


Direct Base Damage Reversal



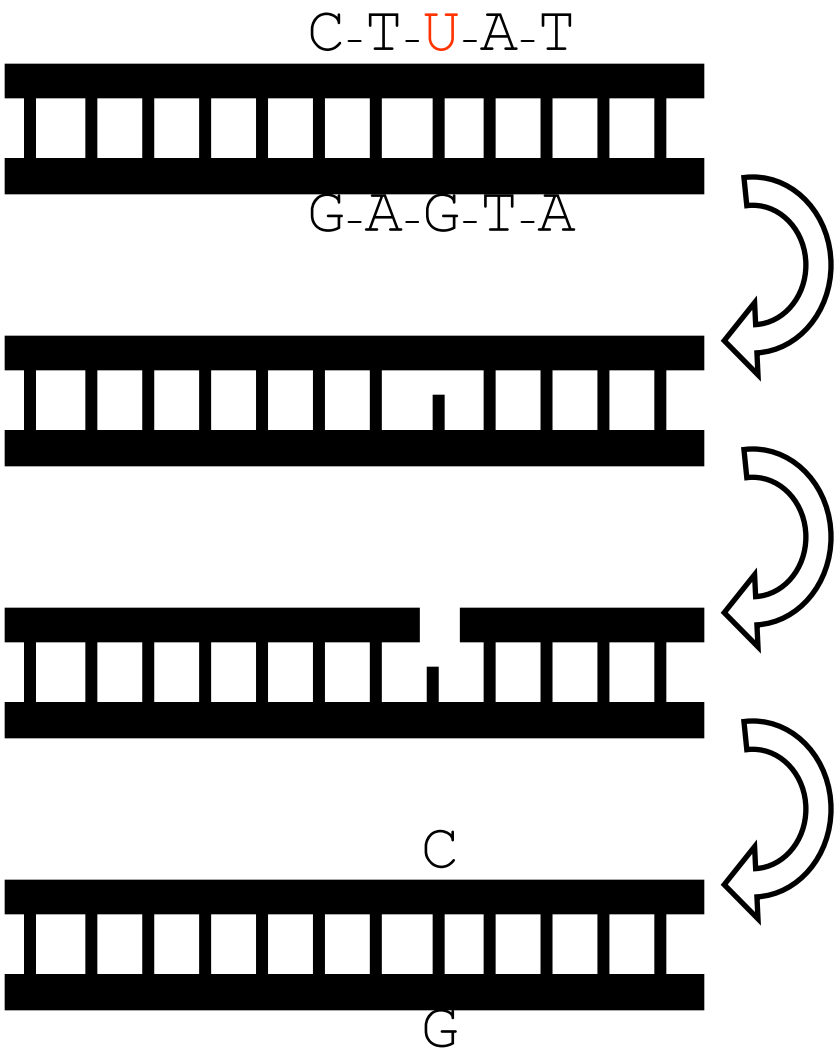
MGMT

Temozolomide



*O6-methylguanine-DNA methyltransferase

Base Excision Repair (BER)



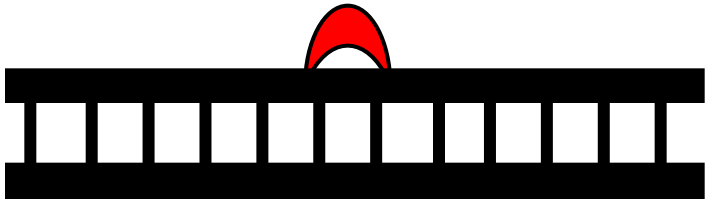
DNA glycosylase

AP endonuclease (APE) and phosphodiesterase

DNA polymerase β adds in "C" and DNA ligase III seals the nick

NER: Global Genome Repair (GGR) and Transcription-Coupled Repair (TCR)

Bulky lesions
such as UV damage



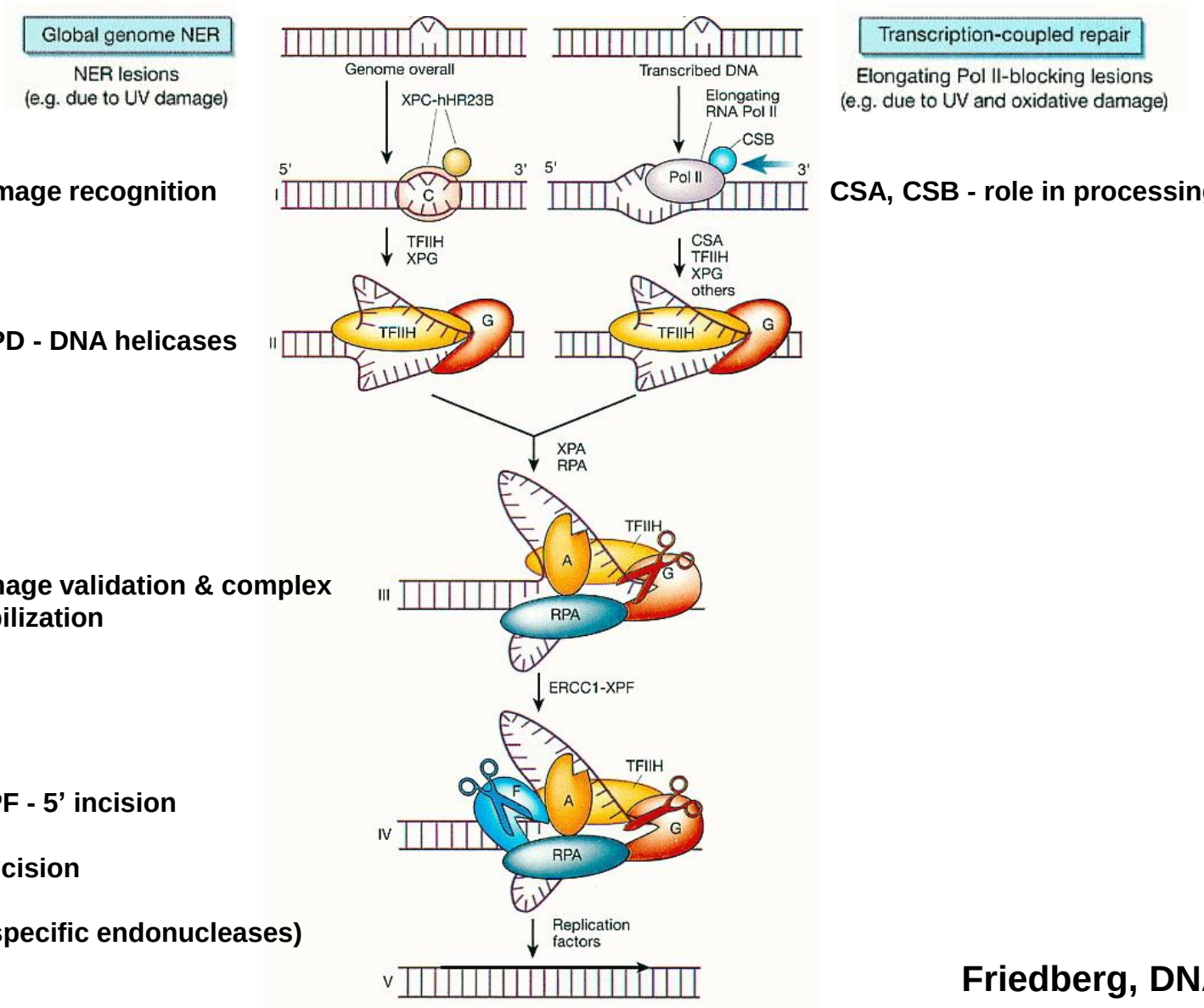
GGR

TCR

defective in *Xeroderma Pigmentosum* (XP)

= repair of transcribed strand in active genes, defective in *Cockayne's Syndrome* (CS) and in *XP*

Nucleotide Excision Repair



XPC - damage recognition

CSA, CSB - role in processing RNAP II?

XPB & XPD - DNA helicases

XPA & RPA - damage validation & complex stabilization

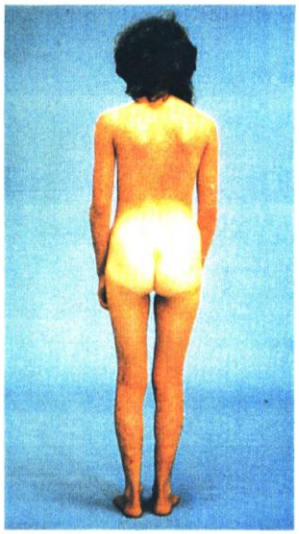
ERCC1-XPF - 5' incision

XPG - 3' incision

(junction specific endonucleases)

Friedberg, DNA repair

Xeroderma pigmentosum



-Extreme sensitivity to UV light (ie sunlight)

-1000X increase in cancer incidence

-7 Complementation groups

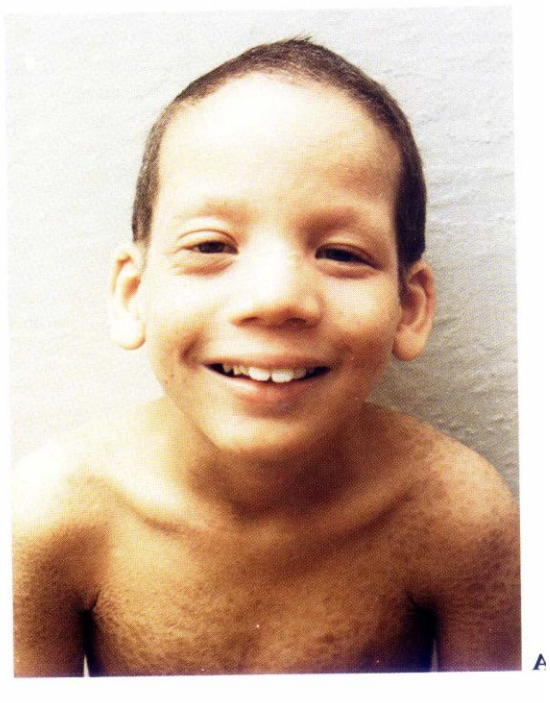
From DNA Repair and Mutagenesis
Friedberg, Walker and Siede, 1995

CS: Cockayne's Syndrome

Symptoms:
- Dwarfism
- Light Sensitivity (sometimes)
- Facial and limb abnormalities
- Neurological abnormalities
- Early death due to neurodegeneration (12.5yrs)
- Physical & mental retardation
- No predisposition to cancer

Complementation tests Reveal Mutations in:
- CS-A (encodes protein with WD repeats)
- CS-B (encodes DNA-dep ATPase of SNF2 family)
- XPB/CS
- XPD/CS
- XPG/CS
- Defects in CSA and/or CSB lead to loss of TCR
- CS mutant forms of XPB, D, and G also TCR defective…but different symptoms of XP!??

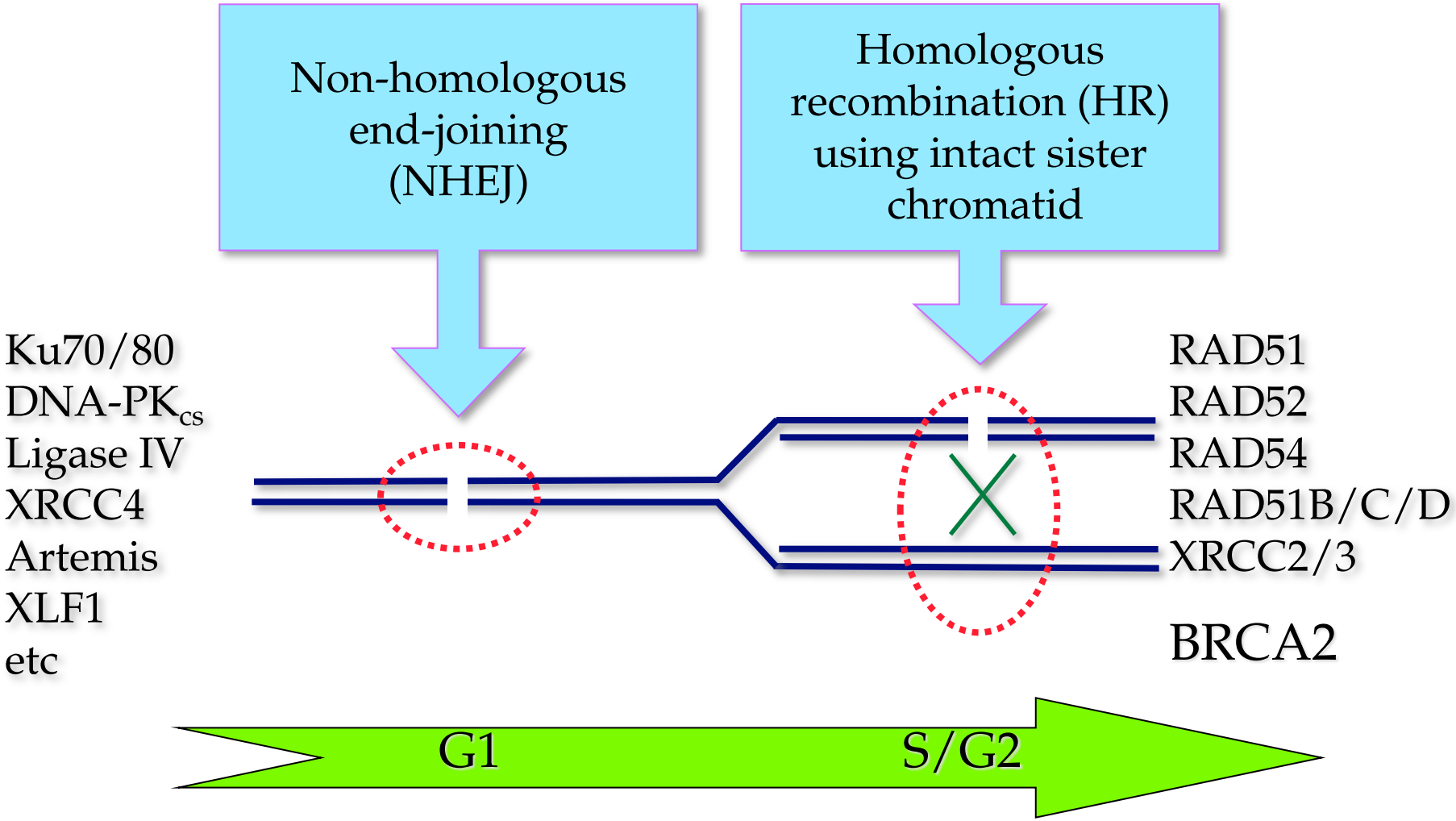
TTD: Trichothiodystrophy



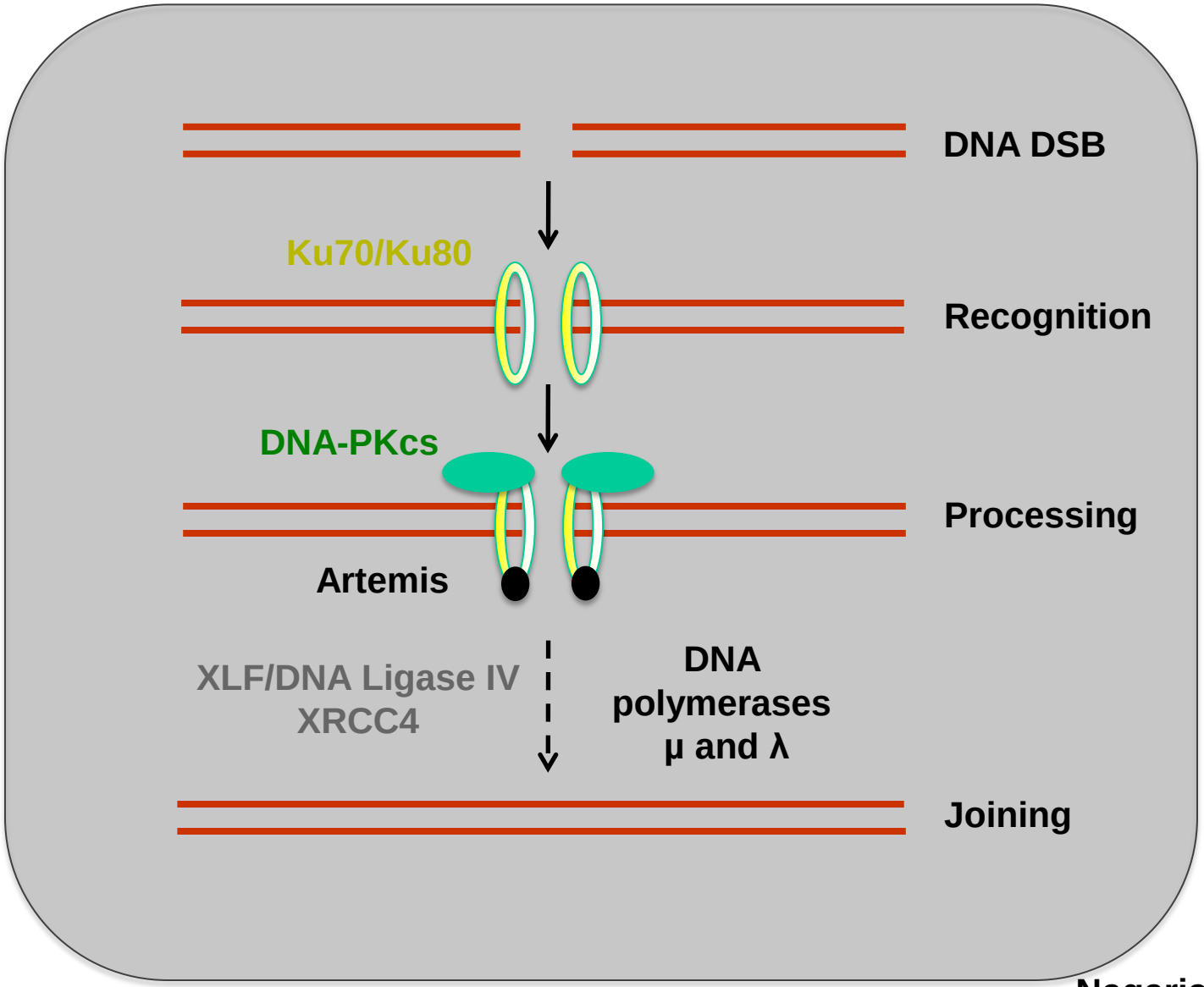
Symptoms:
- Sulfur deficient brittle hair
- Facial abnormalities
- Growth retardation
- Fish-like scales on skin
- Mental & physical retardation
- Light sensitivity (50%)
- No predisposition to cancer

Complementation tests Reveal Mutations in:
- TTD-A (possibly a part of TFIIH)
- XPB/TTD (a part of TFIIH)
- XPD/TTD (a part of TFIIH)

Primary Mechanisms of DSB Repair in Human Cells



Pathway of mammalian non-homologous end joining (NHEJ) repair

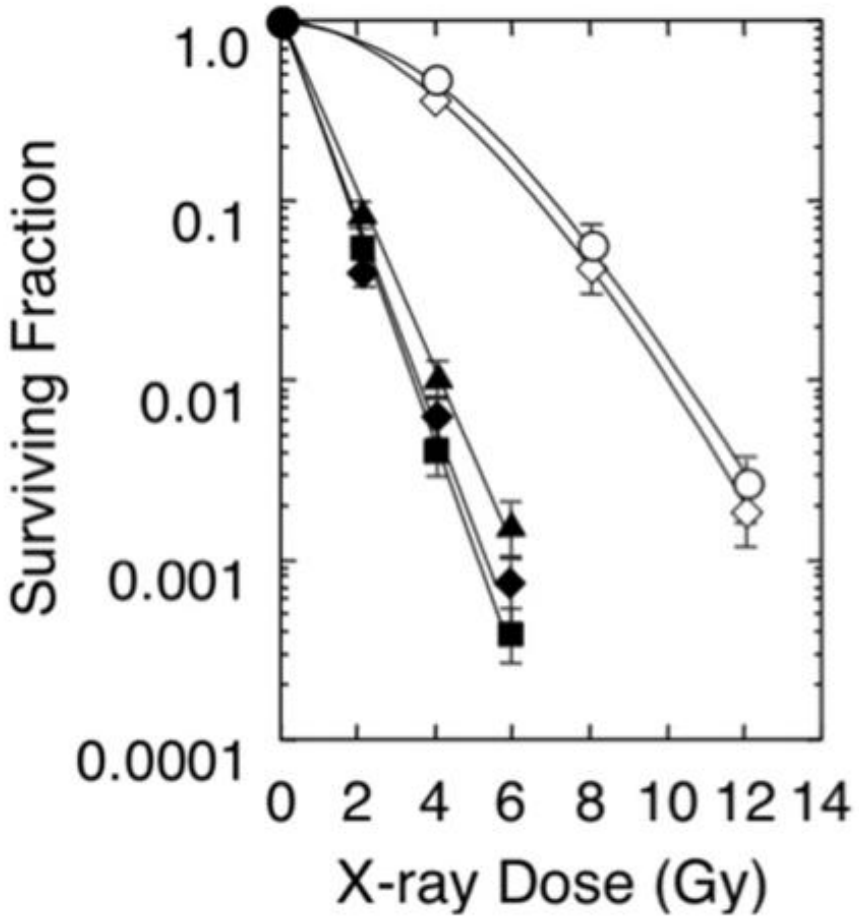


Nagaria et al, 2012

Effects of defective NHEJ

1. Impaired ability to rejoin DNA ends
2. Cellular hypersensitivity to IR
3. Impaired V(D)J recombination $\rightarrow$ immune defect
   e.g. SCID (severe combined immune deficiency syndrome)
4. Cancer predisposition in mice; however,
   NOT (yet) linked to human cancer predisposition
5. Developmental defects: growth and neurological

Survival curves of CHO cells and Ku-DNA-PK-deficient cells



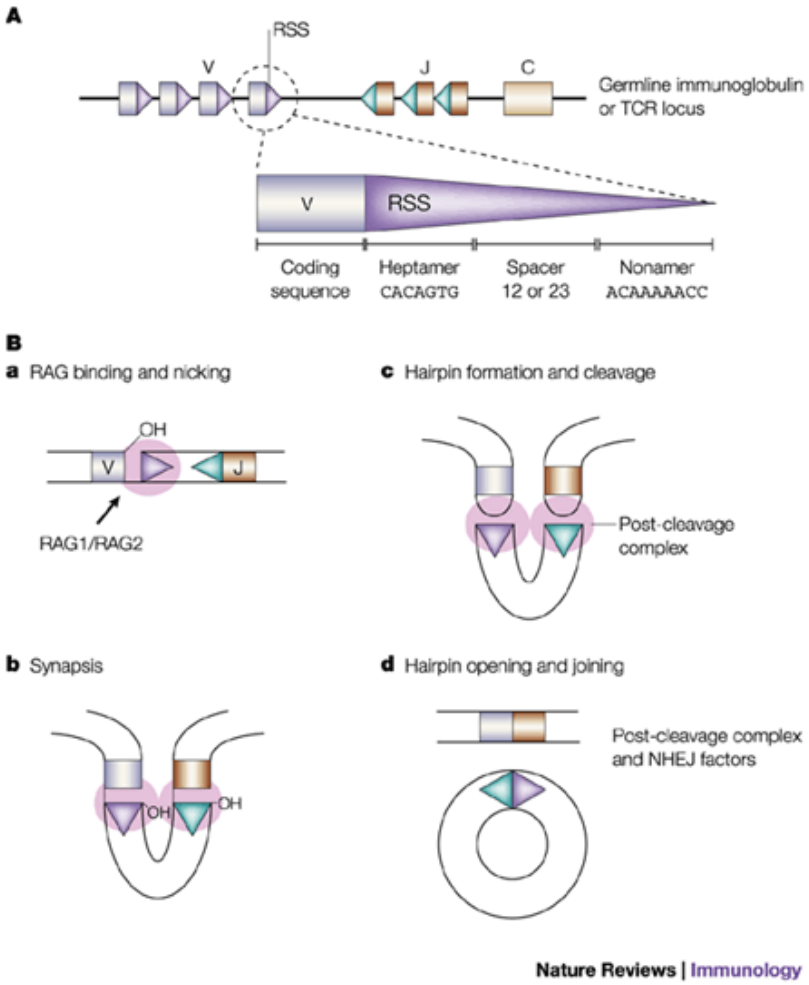
Suzuki et al, 2012

The RAG recombinase induces transient double strand breaks (DSBs) that are joined by NHEJ during VDJ recombination

Developing T and B lymphocytes undergo a series of somatic gene rearrangements leading to the generation of the lymphocyte antigen receptor.

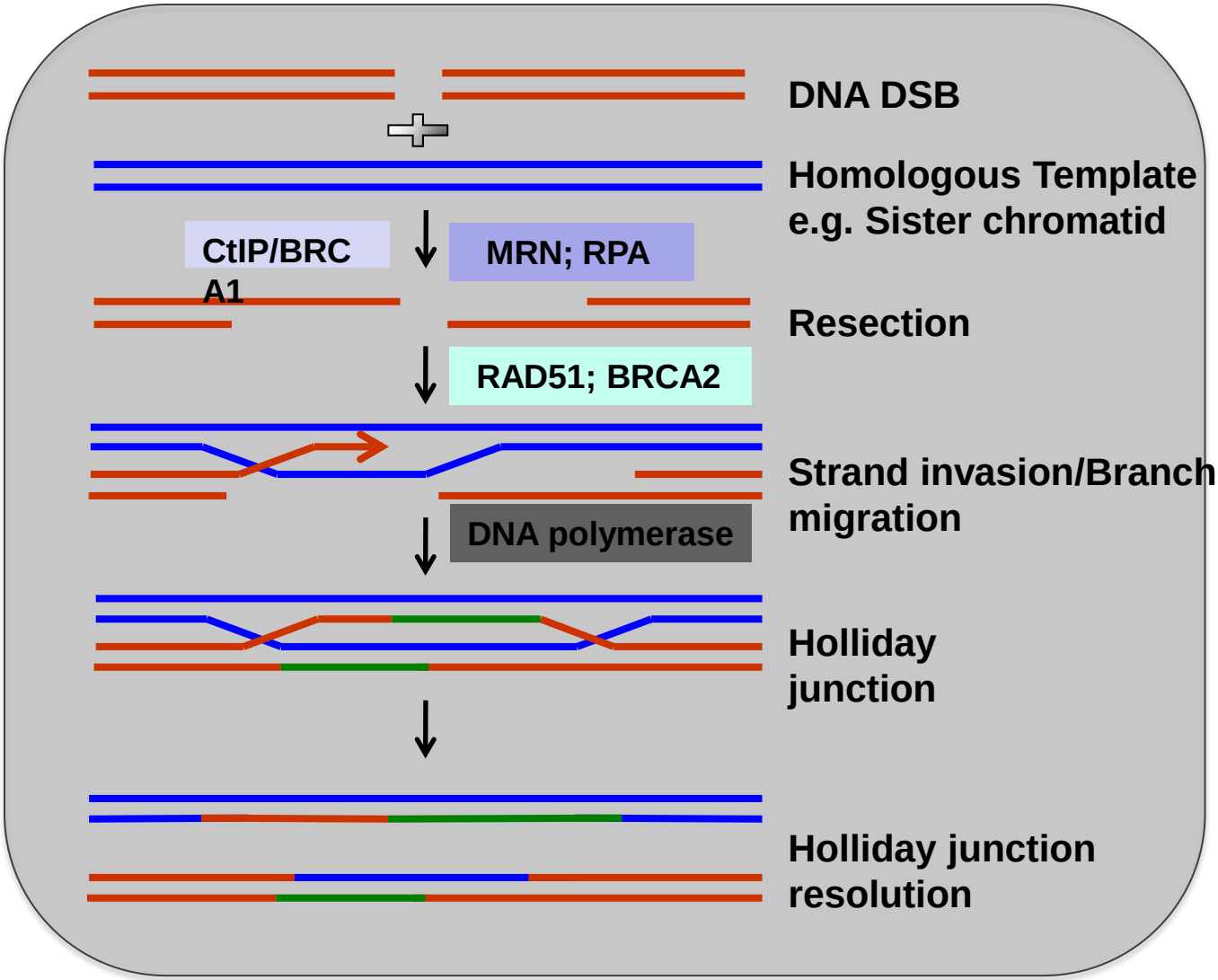
The RAG1/2 complex binds recombination signal sequences flanking V, D, and J gene segments at antigen receptor loci and induces a DSB that allows joining of the coding end to a non-adjacent gene segment.

The break is resolved by components of the non-homologous end joining (NHEJ) pathway.



Nature Reviews Immunology 3, 656-666 (August 2003)

Pathway of mammalian homologous recombination (HR) repair

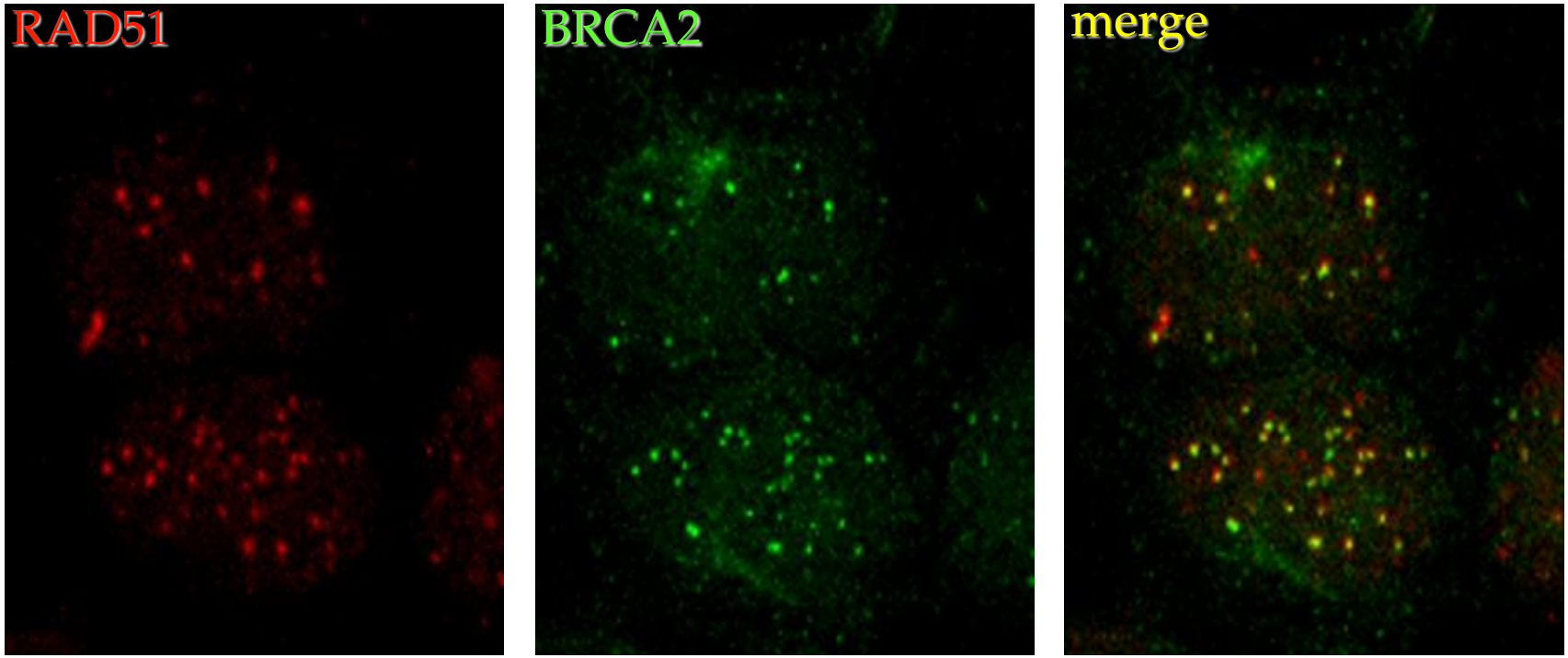


Nagaria et al, 2012

Effects of defective HR

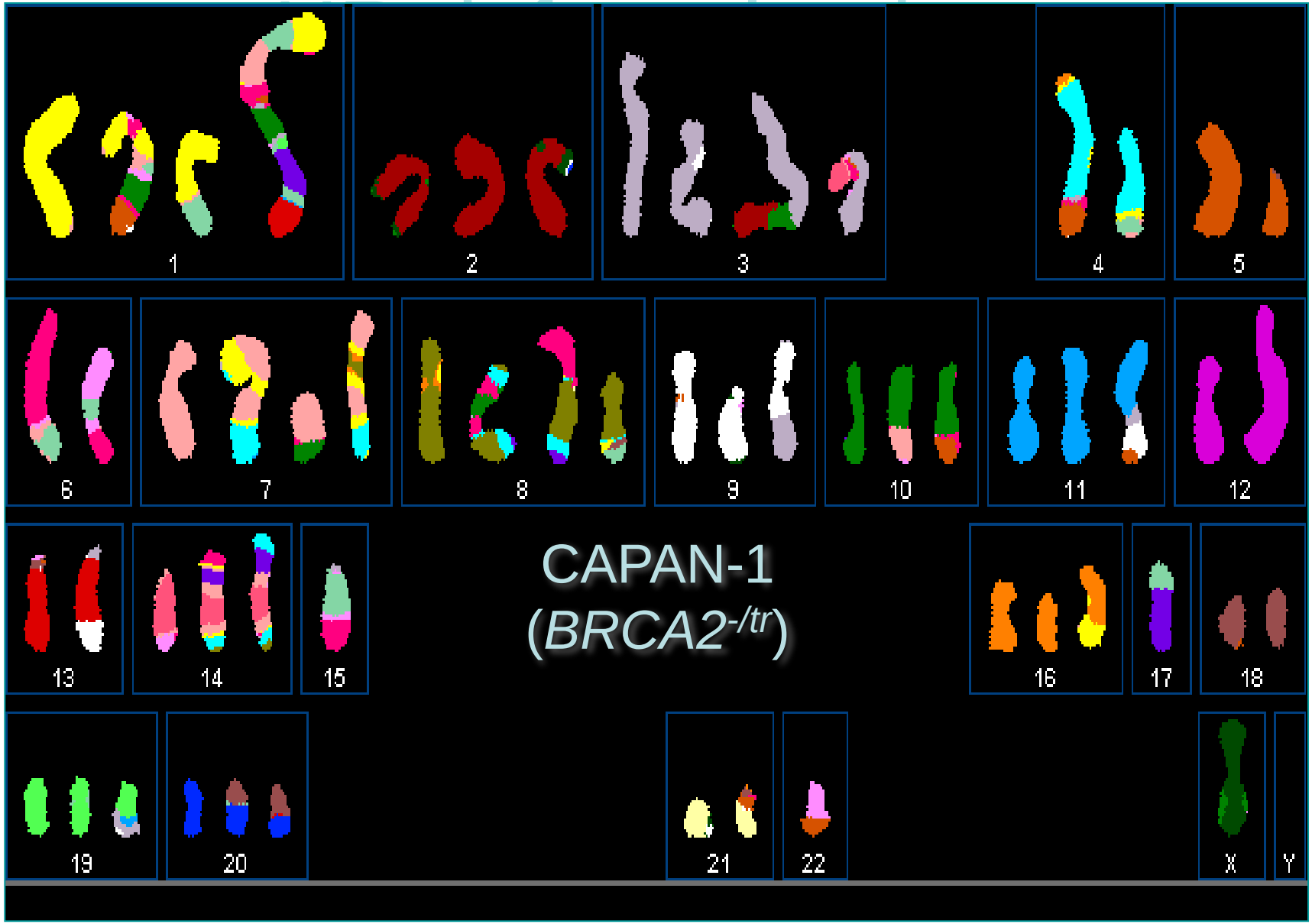
1. Cellular hypersensitivity to DNA Crosslinks
2. Chromosomal aberrations (chromatid-type)
3. Proliferating cells (and Stem Cells)
4. Cancer predisposition
5. Developmental defects: ko mice mostly lethal

BRCA2 associates with RAD51 Recombinase



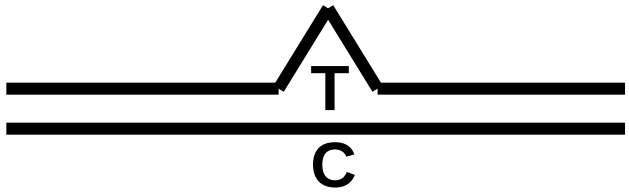
BRCA2 deletions lead to:

- Spontaneous gross chromosomal rearrangements
- X-ray sensitivity
- Recombination deficiency



Photograph courtesy of Joanne Davidson & Paul Edwards (Cambridge)

Mismatch Repair (MMR) functions in DNA Replication and HR



Functions of the MMR system:

(1) removes base-base and small insertion-deletion mismatches that arise during DNA replication

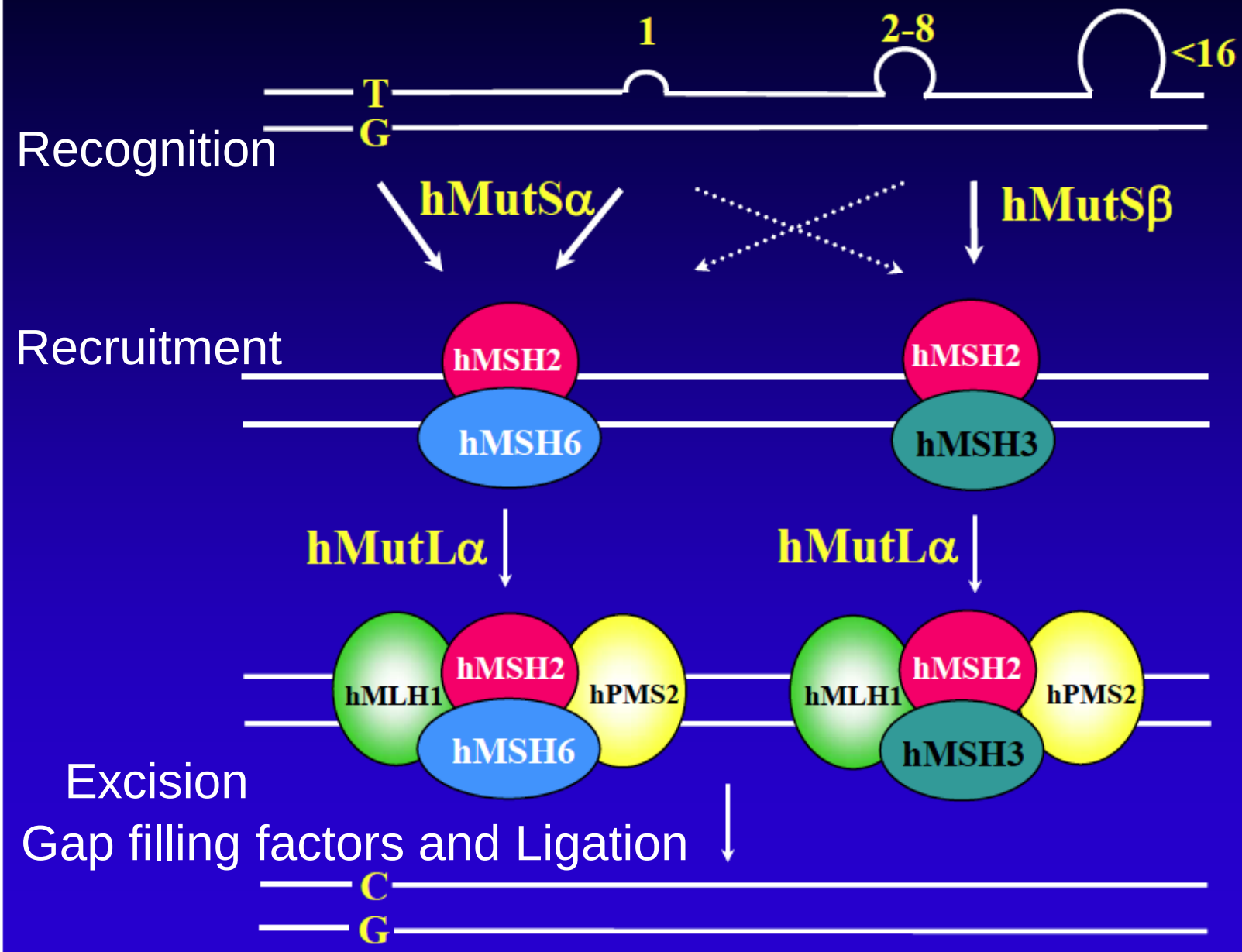
(2) removes base-base mismatches in HR intermediates

(3) may block HR in meiosis if there is too much sequence divergence

(4) Mutations correlate with microsatellite instability, a mutator phenotype in inherited cancer syndrome hereditary non-polyposis colorectal cancer (HNPCC).

Defective MMR does not lead to radiation sensitivity.

Recognition of mismatched DNA



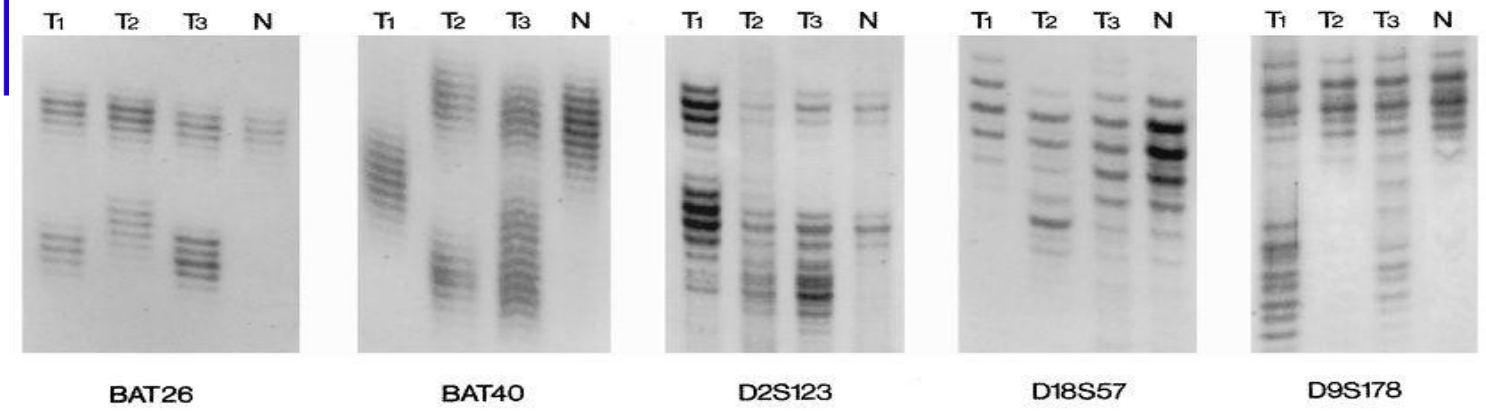
Allele Frequency of MMR Genes in HNPCC

Genes	Frequency
hMSH2	50 - 60%
hMLH1	15 - 20%
hPMS2	5 %
hPMS1	5%
hMSH6 (GTBP)	5%
hMSH3	< 5%
Other	10%

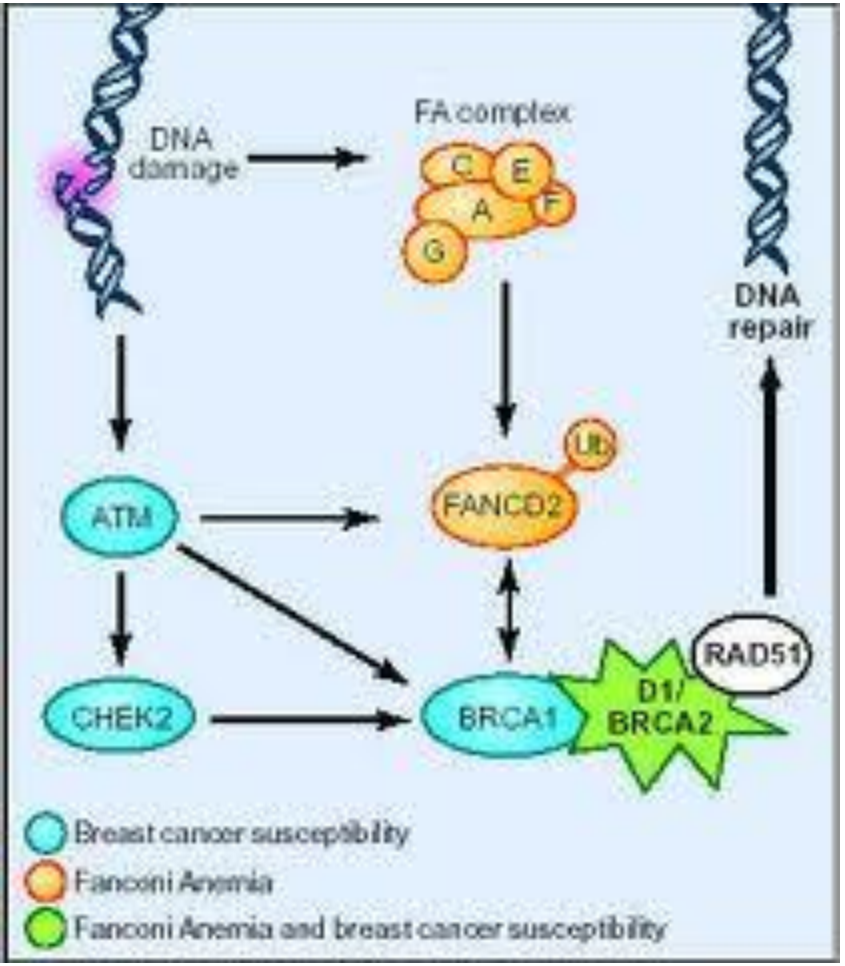
NCI-designed panel for Microsatellite instability (MSI) analysis.

Name	Locus	Primers	Size
BAT 25	1p13.1	5′ TCGCCTCCAAGAATGTAAGT 5′ TCTGCATTTTAACTATGGCTC	~125 bp
BAT26	2p	5′ TGACTACTTTTGACTTCAGCC 5′ AACCATTCAACATTTTTAACCC	~120 bp
BAT40	1p13.1	5′ ATTAACTTCCTACACCACAAC 5′ GTAGAGCAAGACCACCTTG	~125 bp
D2S123	2p16	5′ AAACAGGATGCCTGCCTTTA 5′ AACCATTCAACATTTTTAACCC	~220 bp
APC(D5S346)	5q21/22	5′ ACTCACTCTAGTGATAAATCG 5′ AGCAGATAAGACAGTATTACTAGTT	~120 bp
Mfd15(D17S250)	17q11.2-q12	5′ GGAAGAATCAAATAGACAAT 5′ GCTGGCCATATATATATTTAAACC	~155 bp

MSI-H: ≥ 30% instability on 5 sites (≥ 2/5).
MSI-L: < 30% instability on 5 sites (< 2/5).



FA pathway deals with inter-strand crosslinks occurring during replication



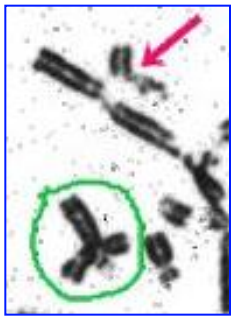
- Fanconi Anemia (FA) pathway is important for repairing inter-strand crosslinks during recombinational repair.
- A central event in the activation of the Fanconi anemia pathway is the mono-ubiquitylation of the FANCI-FANCD2 complex,
- Lack of obvious enzymatic activities among most FA members has made it challenging to unravel precise mechanistic details.
- FA is an inherited genomic instability disorder, caused by mutations in genes regulating replication-dependent removal of interstrand DNA crosslinks.
  - It is caused by mutations in 13 *Fanc* genes.

Fanconi anemia

- Autosomal recessive disorder (<1 in 100,000)

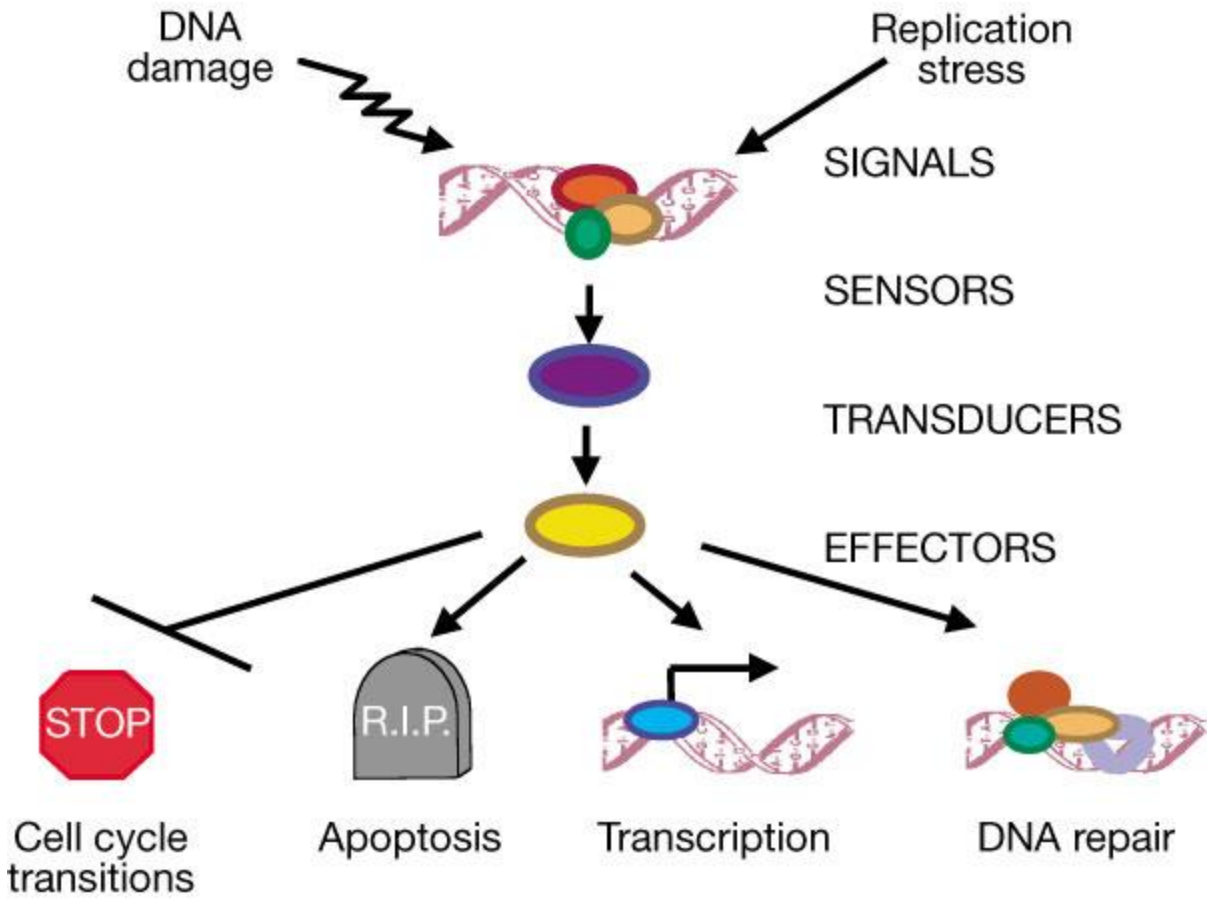
- Phenotype of FA patients
- childhood skeletal abnormalities (thumb, arm)
- bone marrow failure (aplastic anemia)
- increased risk of acute myeloid leukemia & solid tumors (head & neck squamous cell carcinomas)

- Cellular phenotype
- sensitivity to crosslinking agents
- chromosome breakage and multiple radial structures



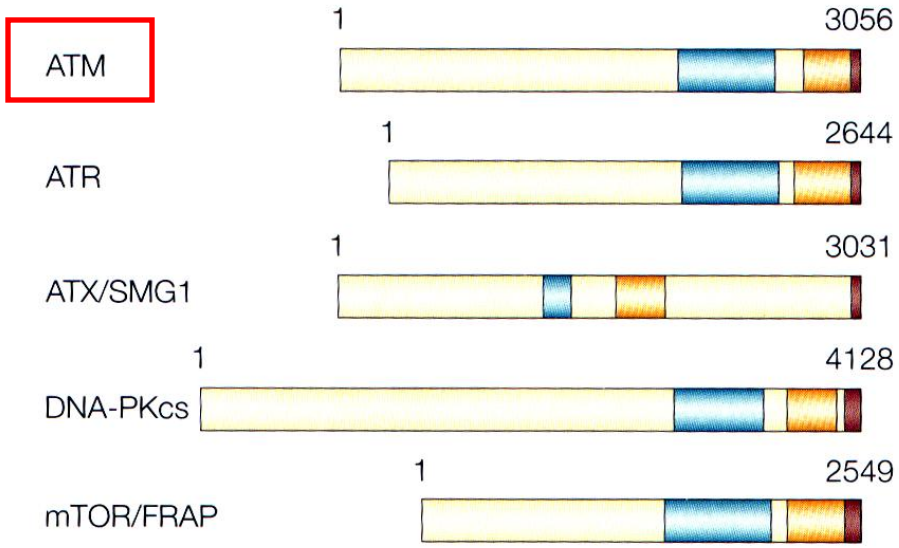
- 13 groups: FA-A, B, C, D1 (BRCA2), D2, E, F, G, I, J, L, M and N

The DNA Damage Response



Zhou and Elledge *Nature* **408**, 433 - 439 (2000)

ATM Protein

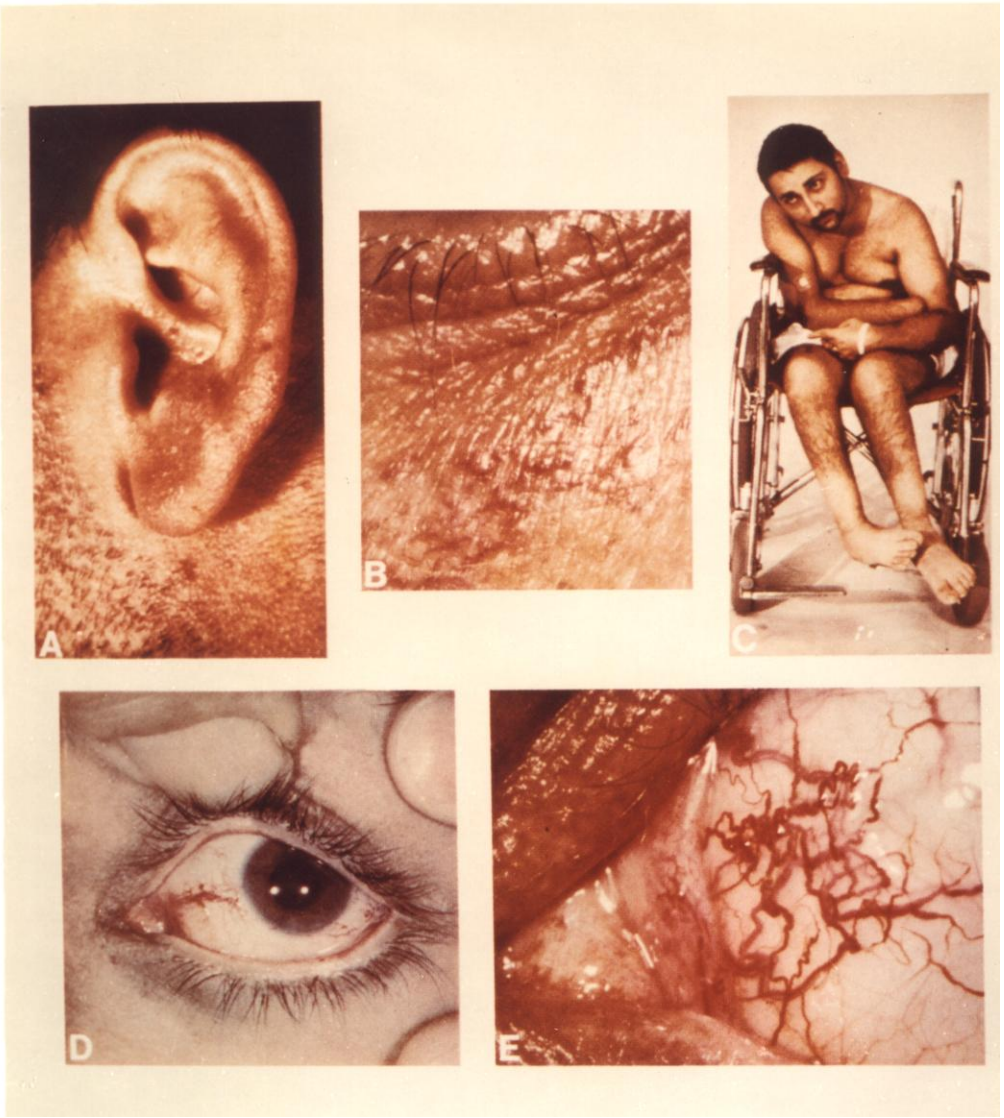


- Central mediator of many checkpoint responses (particularly to DNA DSBs)
- Large (370 kDa) protein kinase (PIK-related)
- Binds DNA directly
- Mutated in ataxia telangiectasia
  - Patients/cells radiation sensitive with checkpoint defects

ATM Pathway
ATAXIA TELANGIECTASIA

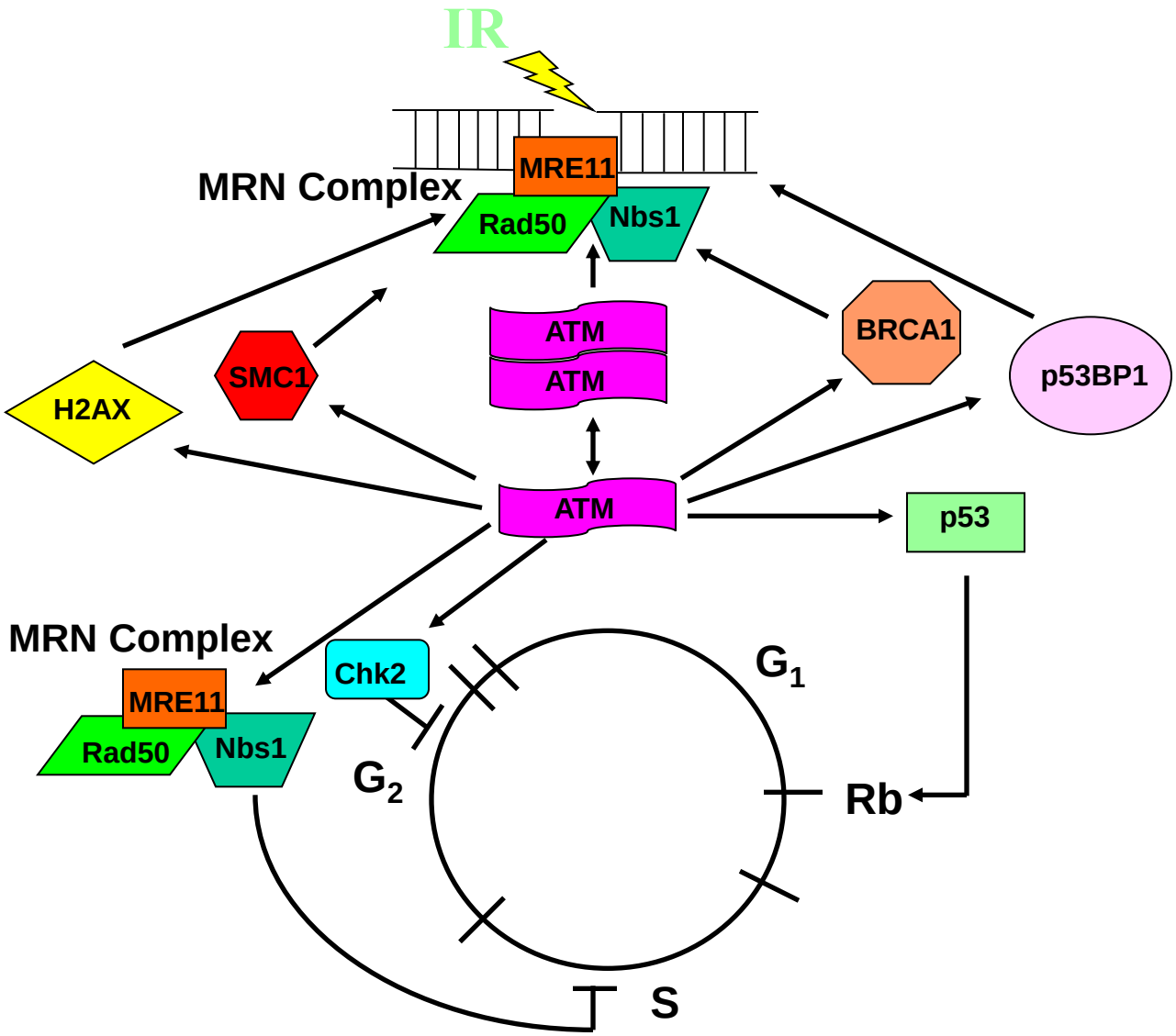
Autosomal recessive

- clinical radiosensitivity
- cerebellar degeneration
- cancer predisposition
- Immunodeficiency
- genome instability
- premature aging



Kraemer, K.H. Cellular Senescence and Somatic Cell Genetics: DNA Repair Process, Nichols and Murphy eds.1977

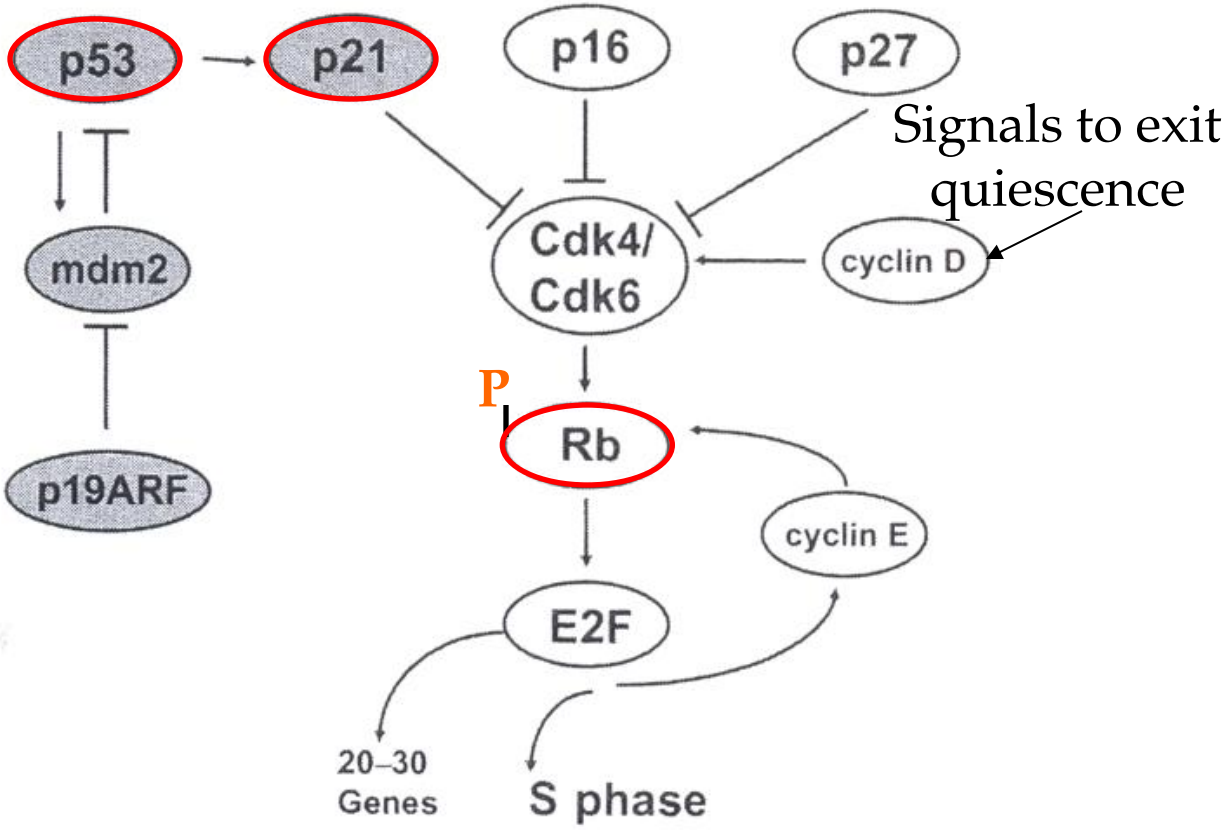
ATM Pathway


IR
MRN Complex
MRE11
Rad50
Nbs1
ATM
ATM
SMC1
H2AX
BRCA1
p53BP1
ATM
p53
MRN Complex
MRE11
Rad50
Nbs1
Chk2
G1
G2
Rb
S

Diseases with Aberrant Response to DNA DSBs

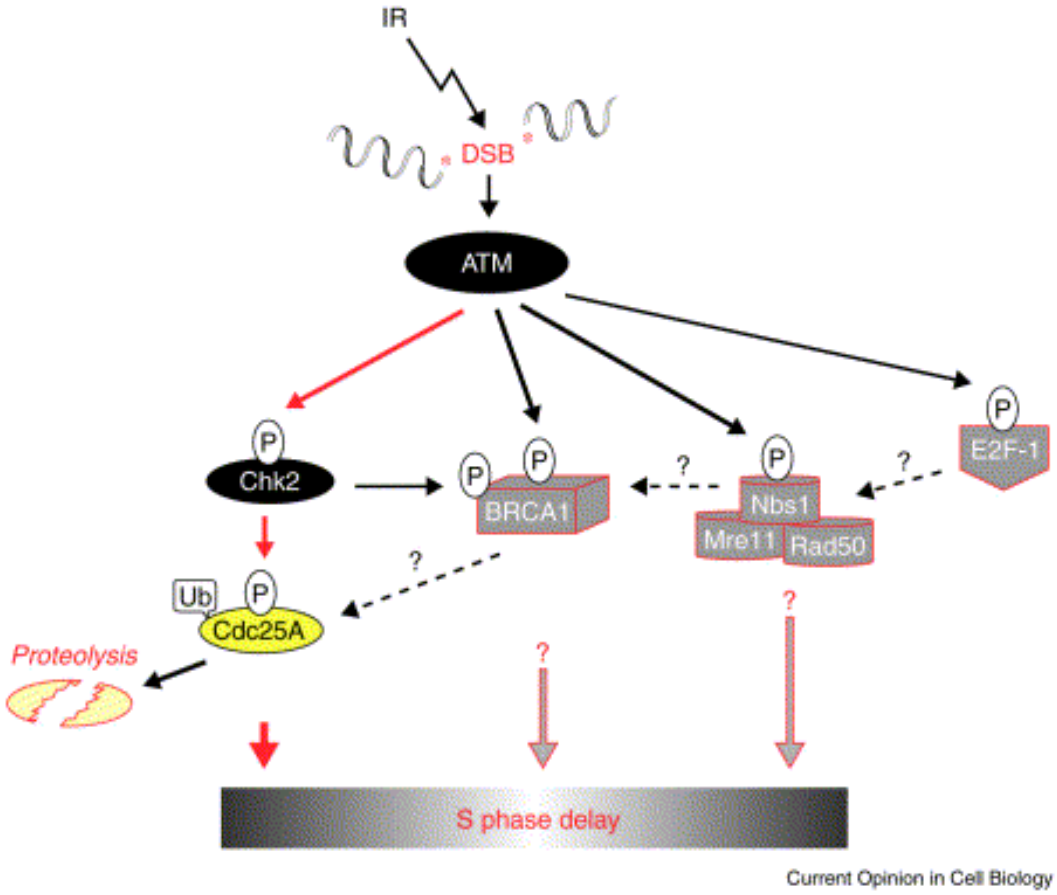
- Ataxia telangiectasia
  - -Radiation sensitive
  - -Increased cancer incidence
  - -Mutation of the ATM gene
  - -Codes for Atm- protein kinase invovled in damage-induced cell cycle checkpoint
- Nijmegen breakage syndrome
  - –Radiation sensitive
  - –Increased cancer incidence
  - –Mutation of the NBS1 gene
  - –Codes for Nbs1 protein
- AT-Like Disorder (ATLD)
  - –Radiation sensitive
  - –Increased cancer incidence
  - –Hypomorphic mutation of the hMRE11 gene
  - –Leads to low levels of aberrant hMre11 proteins

Key Players in G1checkpoint: Role of ATM



- Activation of G1 checkpoint is in large part mediated by ATM signaling to P53

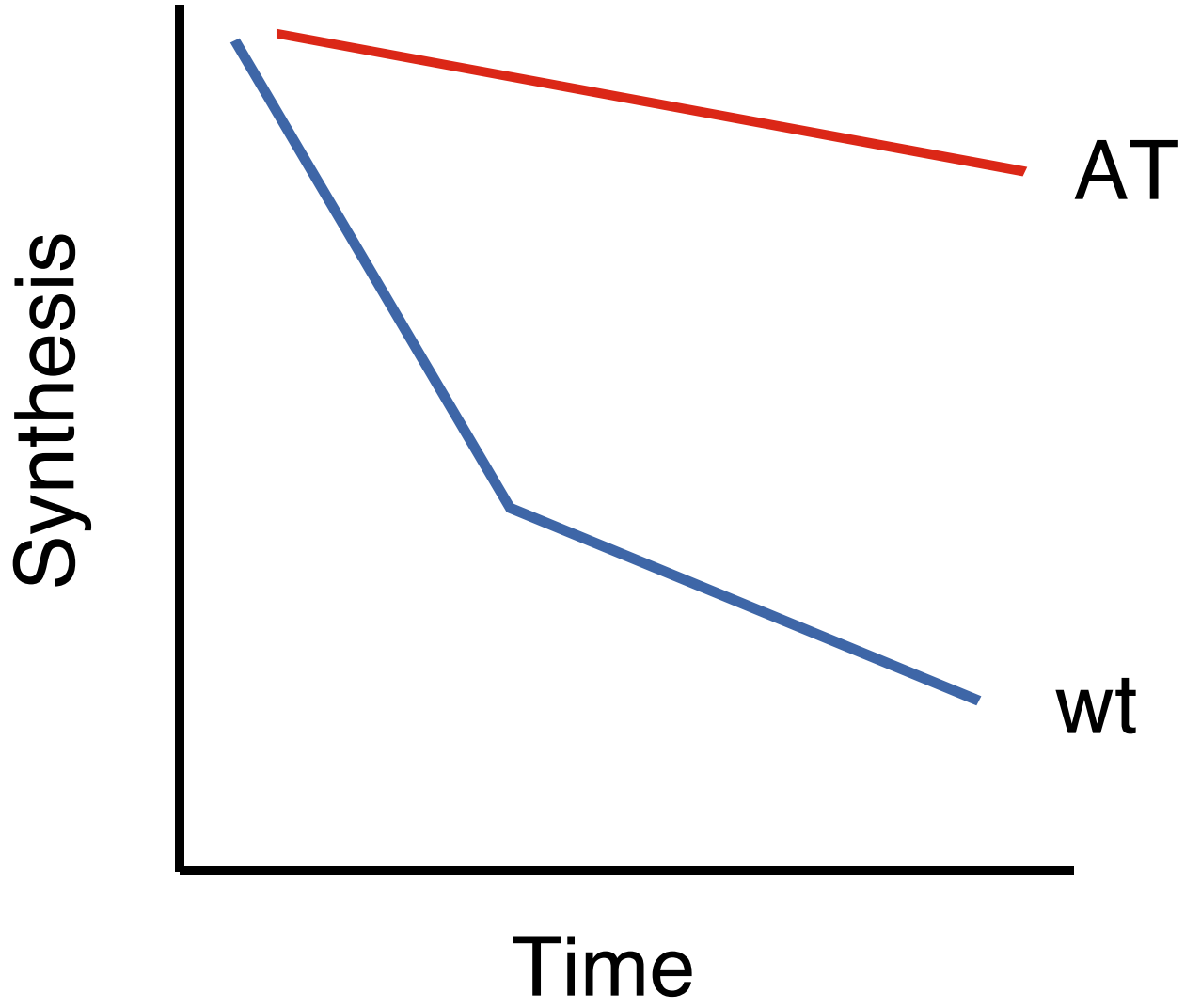
S-Phase Checkpoint: Role of ATM



- In part mediated by Cdc25A phosphatase
- Phosphorylation of NBS by ATM
- Protects replication forks

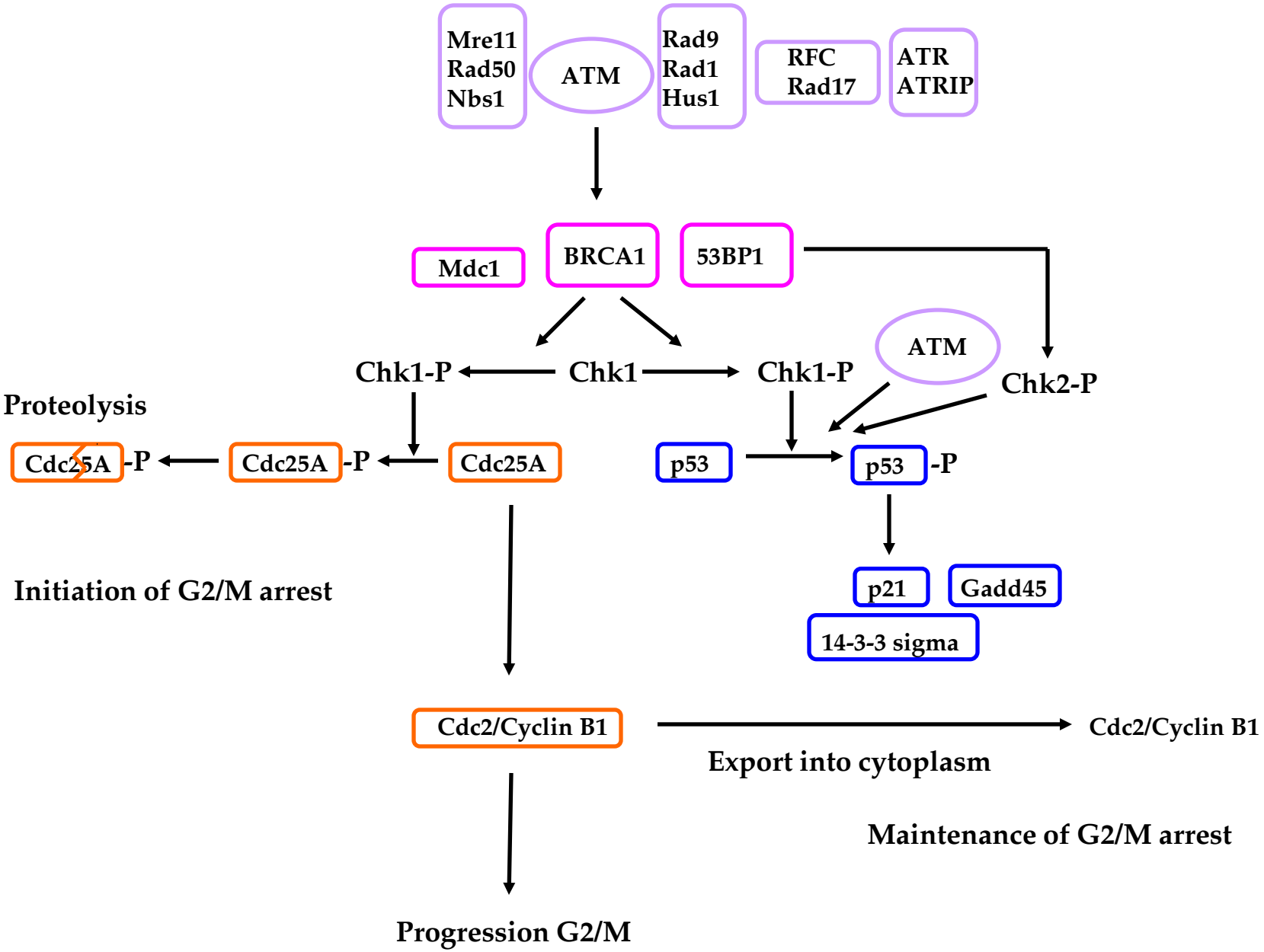
Bartek and Lukas (2001) Curr. Op. Cell Bio.13:738-47

AT Radioresistant DNA Synthesis



G2/M Most Important Checkpoint Following IR

- Prevents cells with damaged DNA to enter mitosis
- Cells lacking the G2 checkpoint are radiosensitive
  - Targeting components of G2 checkpoint to increase radiosensitivity
- Most regulated checkpoint
  - Initiation: p53 independent
  - Maintenance:p53 dependent



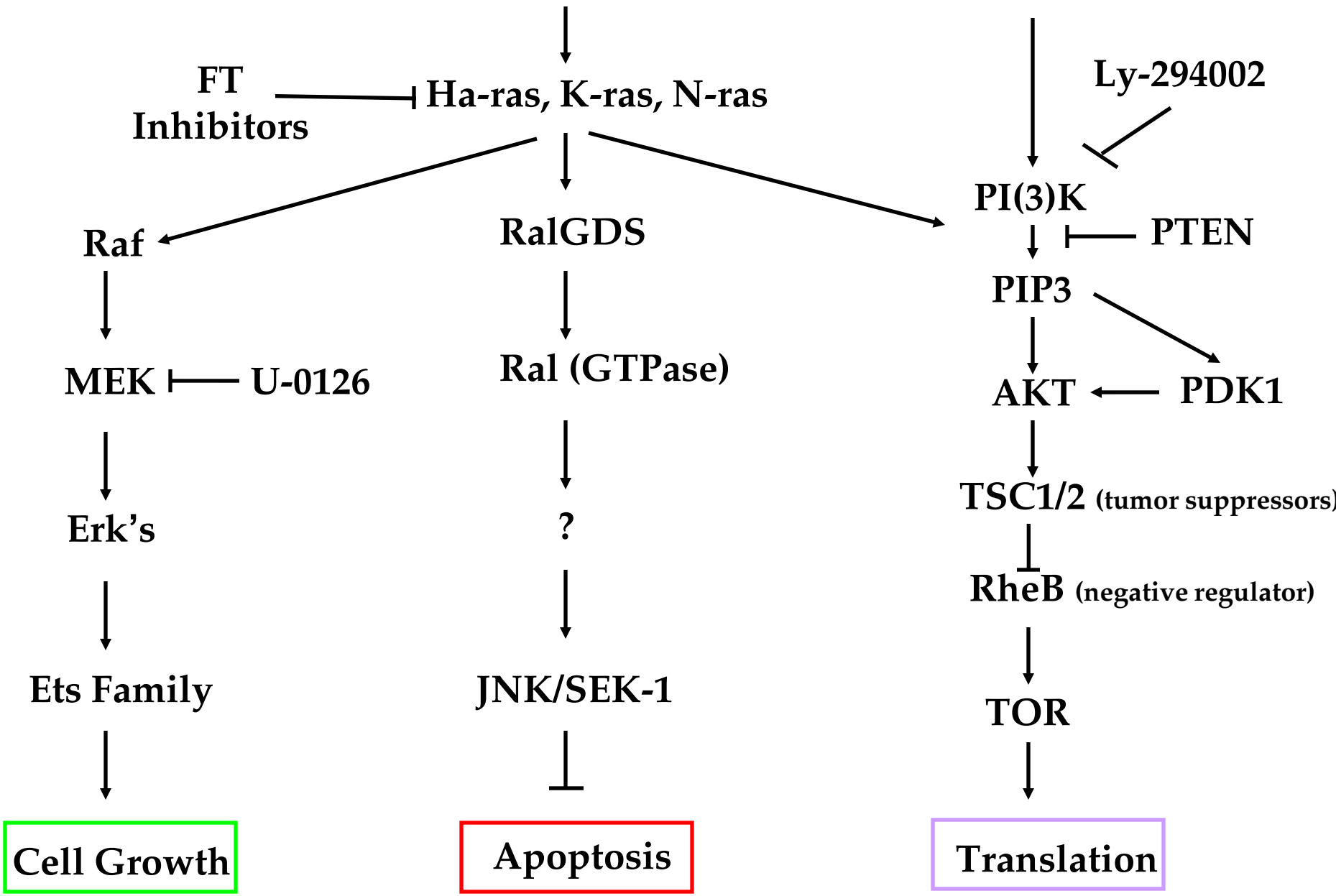
Shimada, M. et al. J.Mol.Hist, 2006, 37:253-260

Radiation-induced Signal Transduction

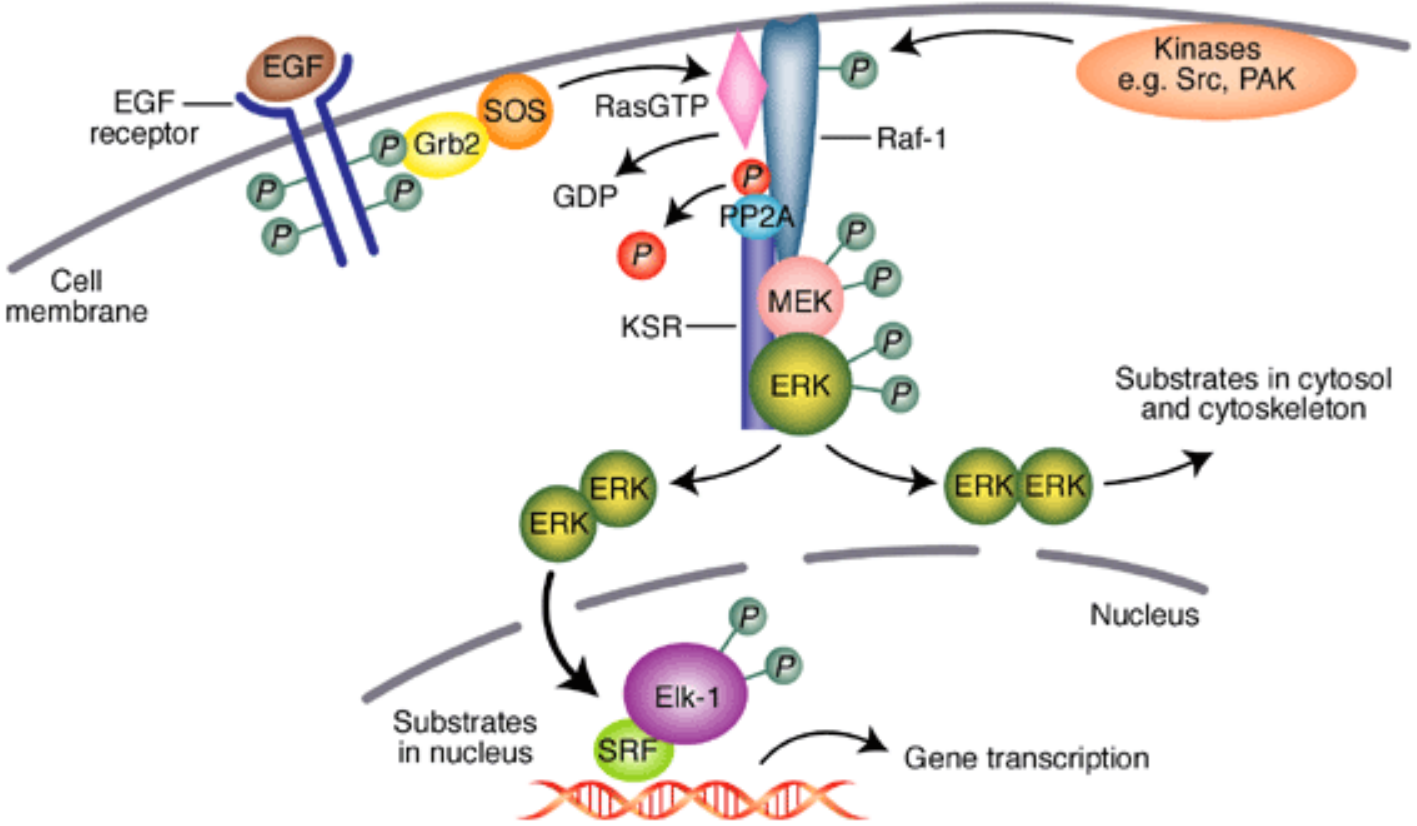
Cellular response to IR

- Membrane-cytoplasmic
  - Activation of multiple signaling pathways
    - Early response genes: c-fos, c-jun and c-myc
  - Receptor tyrosine kinases
  - Ras
  - Ceramide
  - NF-kB

- Nuclear (DNA damage)
  - Chromatin structure
  - Cell cycle checkpoints


Receptor Tyrosine Kinases
Insulin
FT Inhibitors
Ha-ras, K-ras, N-ras
Ly-294002
Raf
RalGDS
PI(3)K
PTEN
PIP3
MEK
U-0126
Ral (GTPase)
AKT
PDK1
Erk's
?
TSC1/2 (tumor suppressors)
RheB (negative regulator)
Ets Family
JNK/SEK-1
TOR
Cell Growth
Apoptosis
Translation

Ras Pathway



The organisation and function of the Ras-Raf-MEK-ERK pathway

Ras Pathway

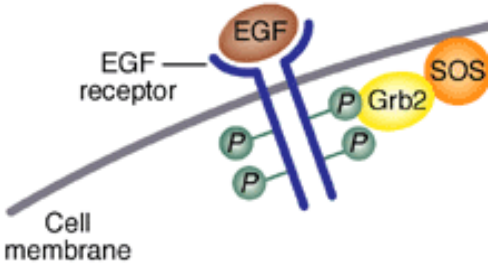
- Ras and its isoforms (H-ras, K-ras, and N-ras) are a family of GTP-binding proteins located on the cytoplasmic side of the cell membrane and are the most frequently mutated oncogene (~1/3) in human cancers.

- Proto-oncogenes: components of signaling networks, positive growth regulators. A gain of function in only one copy of a proto-oncogene results in a dominant acting oncogene.

Ras activation in cancer

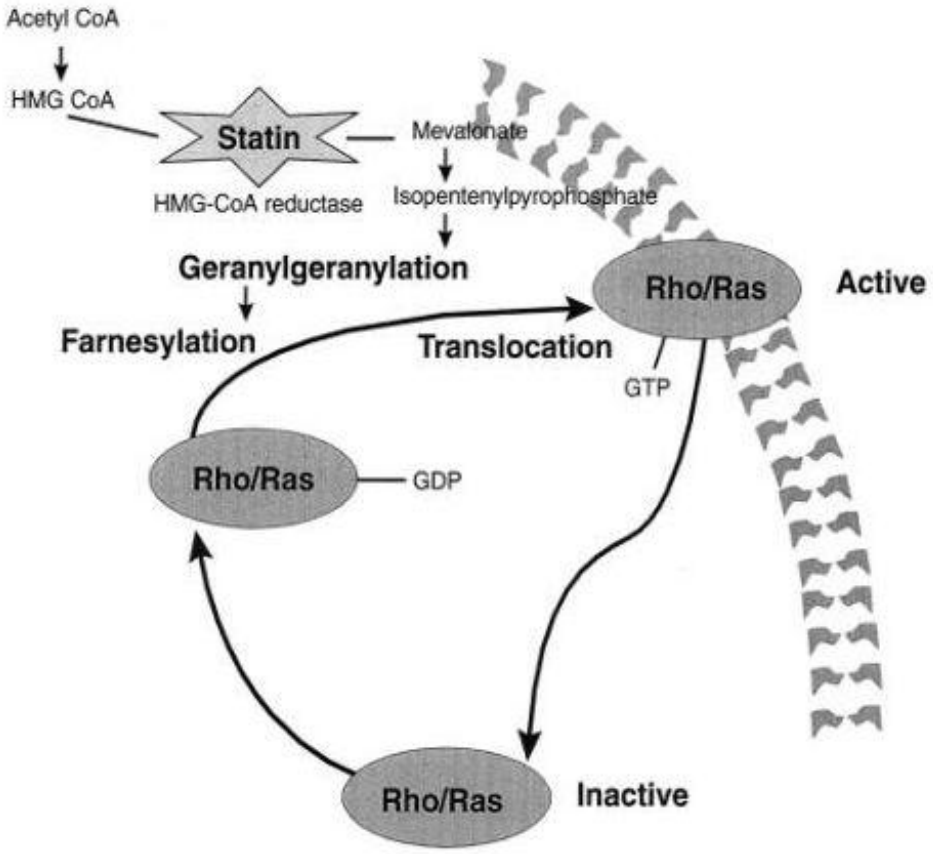
- Point mutations in the Ras genes are found in over 90% of human pancreatic carcinomas and 50% of human colon cancers. As a consequence, Ras loses its GTPase activity and permanently stays in the GTP-bound active state sending constantly signals into the nucleus.

- Over-expression of growth factors receptors can lead to uncontrolled proliferation. Although mutations in all three domains have been found, mutations in the ligand-binding domains are a common alteration that results in constitutive kinase activation.

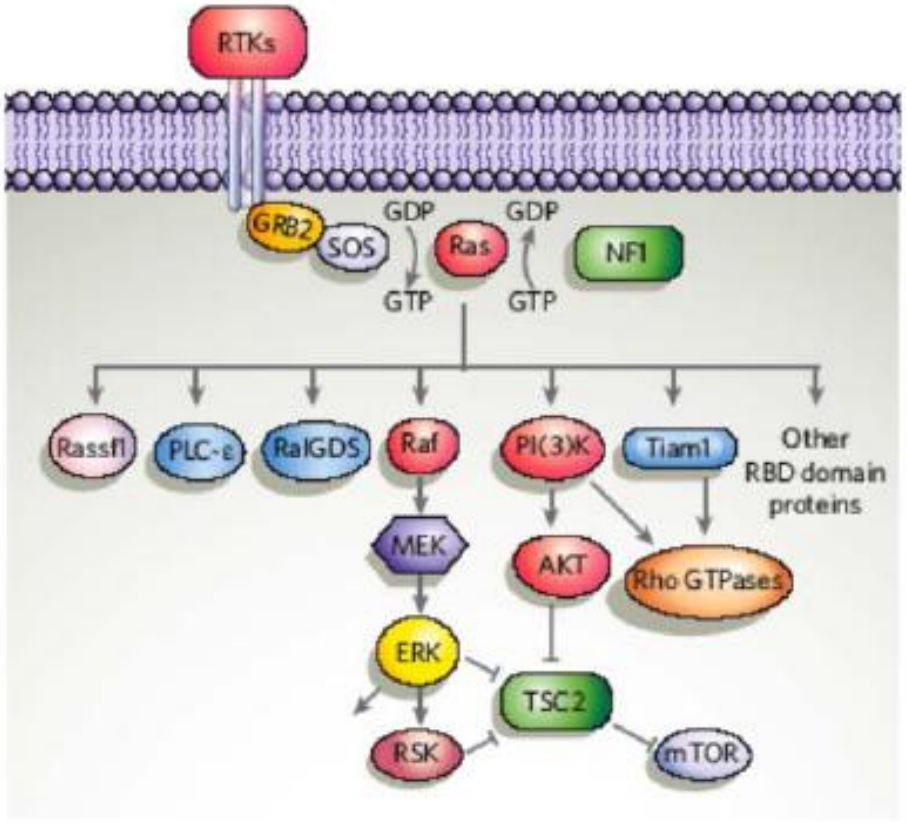


Targeting Ras to increase Radiosensitivity

- Activation of Ras requires the addition of isoprenoid lipid (prenylation) to attach to cell membrane. Prenylation is performed by farensyltransferase.
  - Inhibition of farnensyltransferase to inhibit Ras.



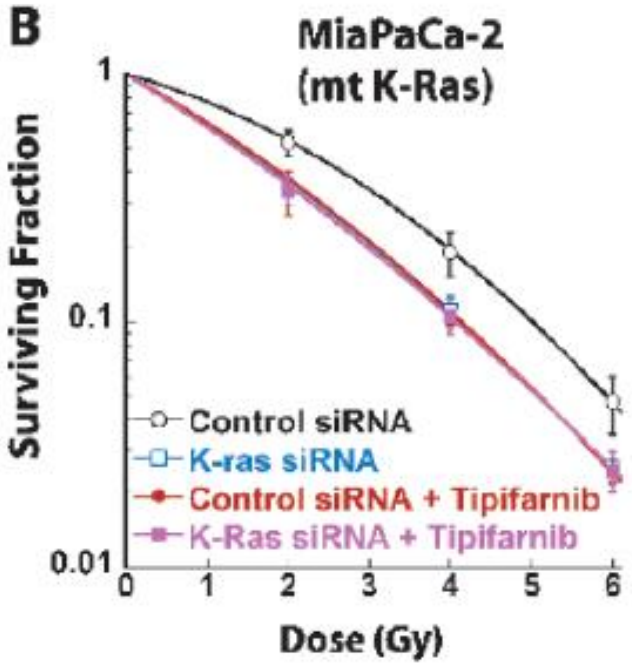
Ras inhibition



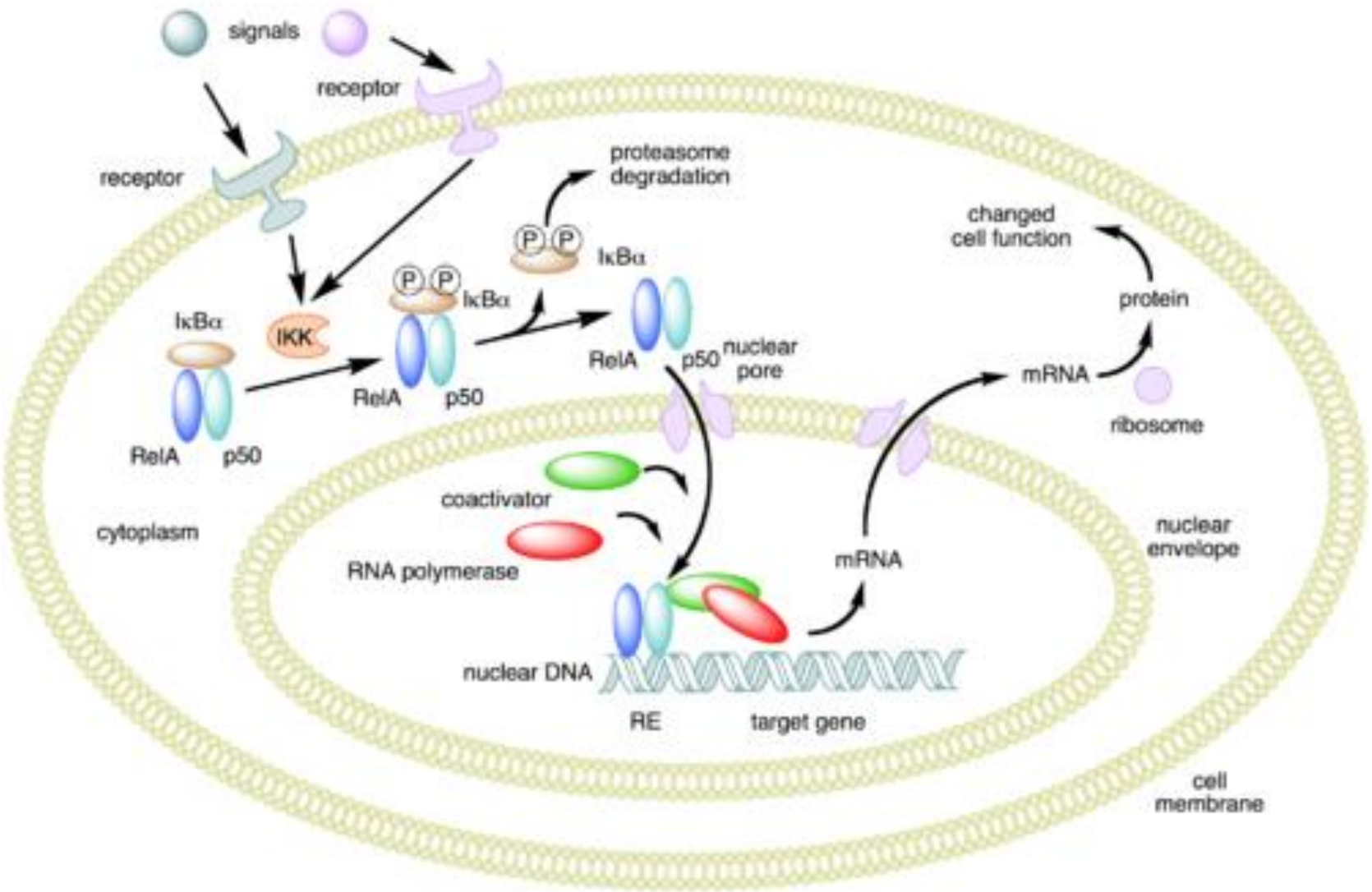
Oncogene RAS mutated in 20% of cancers

Ras controls proliferation, survival, differentiation, migration and angiogenesis

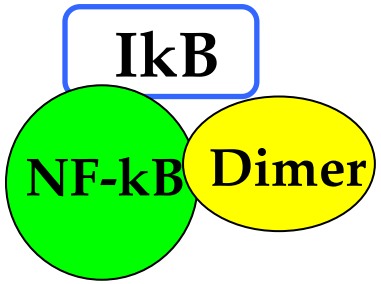
- Activation of Ras leads to radioresistance
- Inhibition of Ras activity by siRNA or farnesyl transferase inhibitor increases radiosensitivity



Nuclear Factor kB



Mechanisms and consequences of NFKB activation in tumor cells



Mutation of
IkB or NF-kB

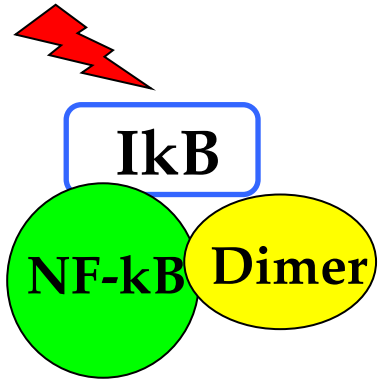
↓

Constitutive
NF-kB Activity

↓

- Proliferation
- Antiapoptosis
- Angiogenesis
- Migration

Stress



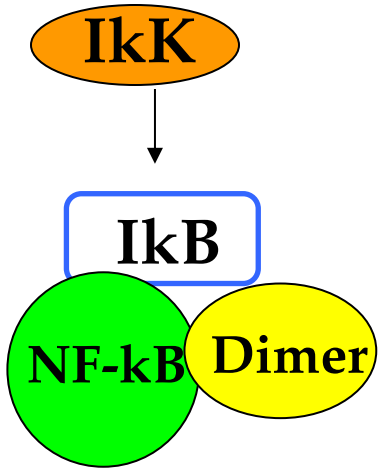
Stress-Induced
IkB Inactivation

↓

Inducible
NF-kB Activity

↓

- Proliferation
- Antiapoptosis
- Angiogenesis
- Migration



Constitutive Activation
of IkK

↓

Constitutive
NF-kB Activity

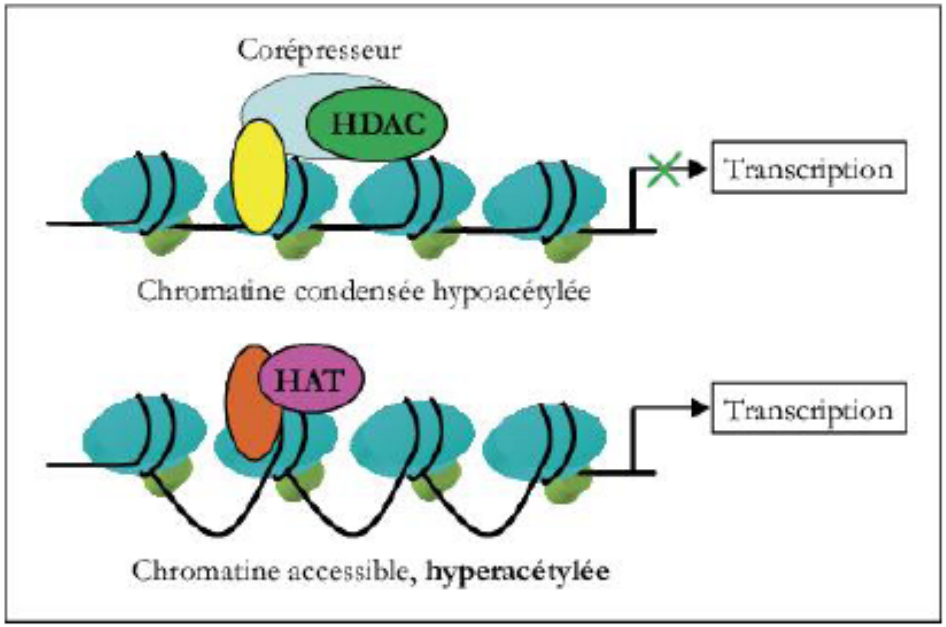
↓

- Proliferation
- Antiapoptosis
- Angiogenesis
- Migration

Inhibition of NFKB activity increases radiosensitivity

- Fail to proliferate in soft agar
- Become more sensitive to apoptotic cell death induced by IR or chemotherapy
- Exhibit decreased migration
- Drugs available to inhibit IKB degradation and prevent NFKB from entering the nucleus

HDAC inhibitors



- 16 HDAC inhibitors
- radiosensitization *in vitro*
- not well known mechanisms
- differential effect tumor/normal tissues
- > 100 clinical trials on going
- one published trial with pelvic radiation therapy (PRAVO)

Histone deacetylase (HDAC)

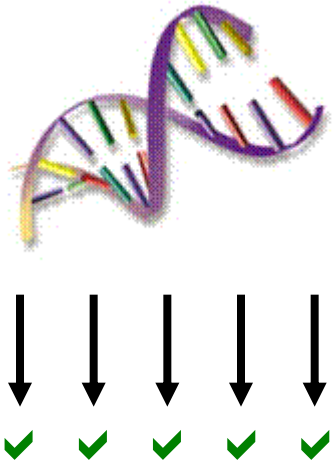
regulation of chromatine structure, gene transcription, and radiosensitivity?

HDAC overexpression is oncogenic

→ 4 phase I trials on going (pancreas, lung, prostate, esophagus, HN) and 1 phase II/III trial (glioblastoma)

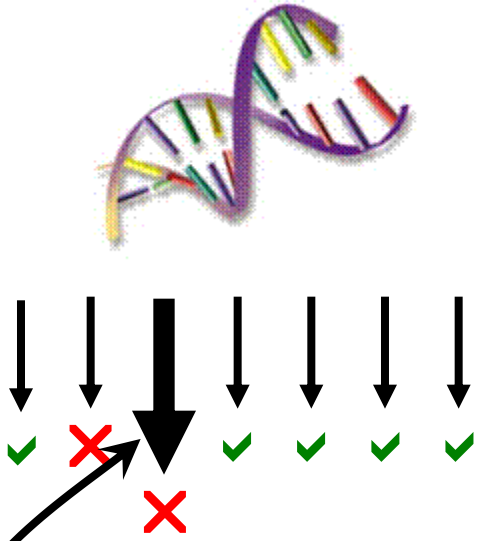
Principle of synthetic Lethality

Normal cells



Seven normal DNA repair pathways

Cancer cells



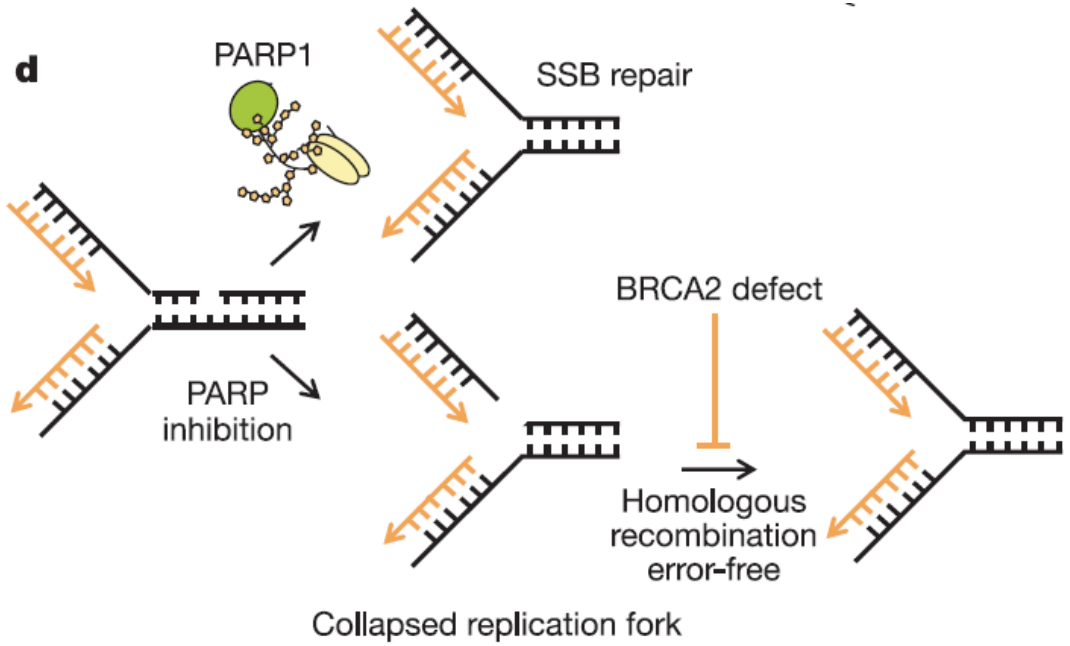
One defective pathway leads to hyper-dependence on a second pathway

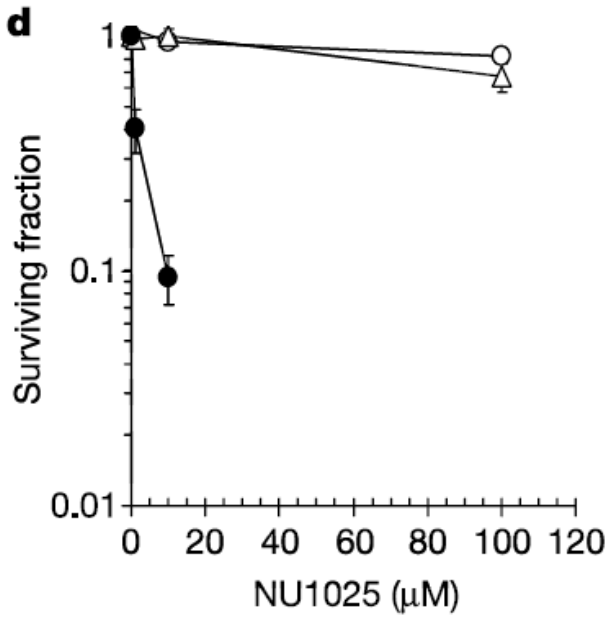
Principle of "synthetic lethality"

An inhibitor for the second pathway will kill the cancer cell

Synthetic Lethality

PARP inhibition toxic to BRCA2-deficient cells





Bryant et al. 2005, Nature 434: 913-17

Synthetic Lethality and Modifiers

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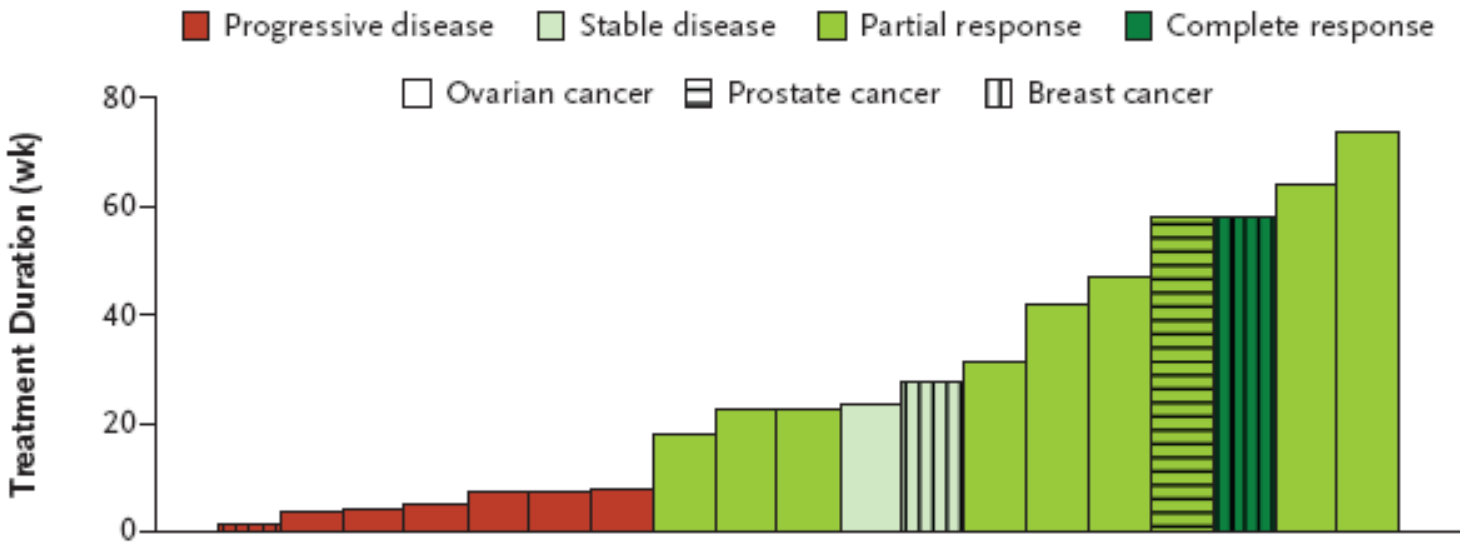
Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from *BRCA* Mutation Carriers

Peter C. Fong, M.D., David S. Boss, M.Sc., Timothy A. Yap, M.D., Andrew Tutt, M.D., Ph.D., Peijun Wu, Ph.D., Marja Mergui-Roelvink, M.D., Peter Mortimer, Ph.D., Helen Swaisland, B.Sc., Alan Lau, Ph.D., Mark J. O'Connor, Ph.D., Alan Ashworth, Ph.D., James Carmichael, M.D., Stan B. Kaye, M.D., Jan H.M. Schellens, M.D., Ph.D., and Johann S. de Bono, M.D., Ph.D.



Rational Combinations of DNA Repair Targeting Cytotoxic and Biological Therapy

Ionizing Radiation + SSB repair inhibitor (e.g. PARPi)	SSB → Fork Collapse HR not NHEJ
Cisplatin/MMC (interstrand crosslinks) + HR inhibitor (?possible without toxicity)	HR
Topoisomerase I poisons + TDP1 inhibitors	HR in S phase TDP1/PNK
Topoisomerase II poisons + cdk inhibitor?	HR in G2 phase
Alkylating agents (small) + SSB repair inhibitor + MGMTi	BER saturation?
Taxanes + checkpoint override (chk inhibitors)	Mitotic checkpoint

Intrinsic radiosensitivity