20.109 Spring 2016 Module 2 – Lecture 4
System Engineering (March 29th 2016)




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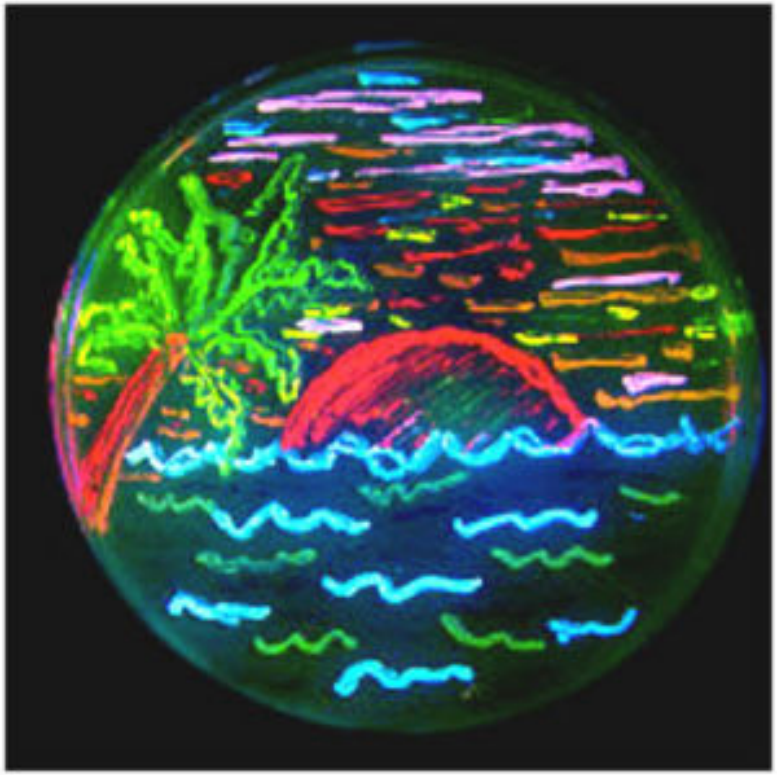
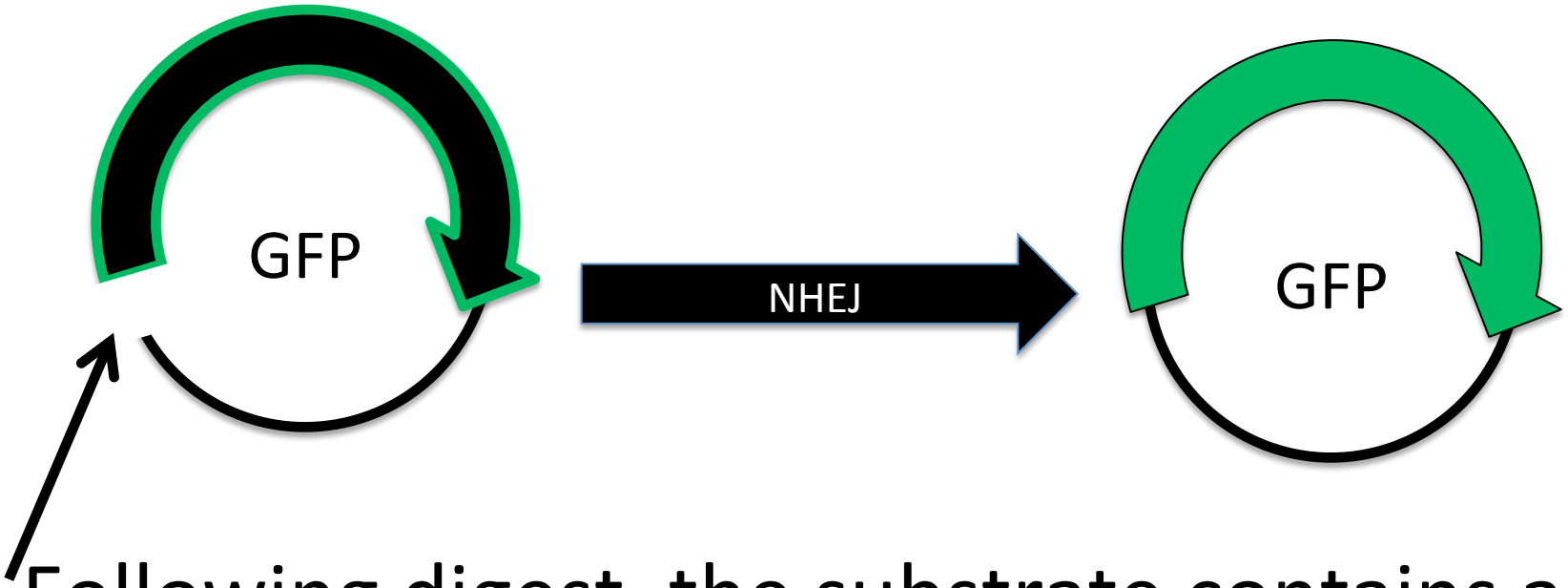
Key Experimental Methods for Module 2

- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data



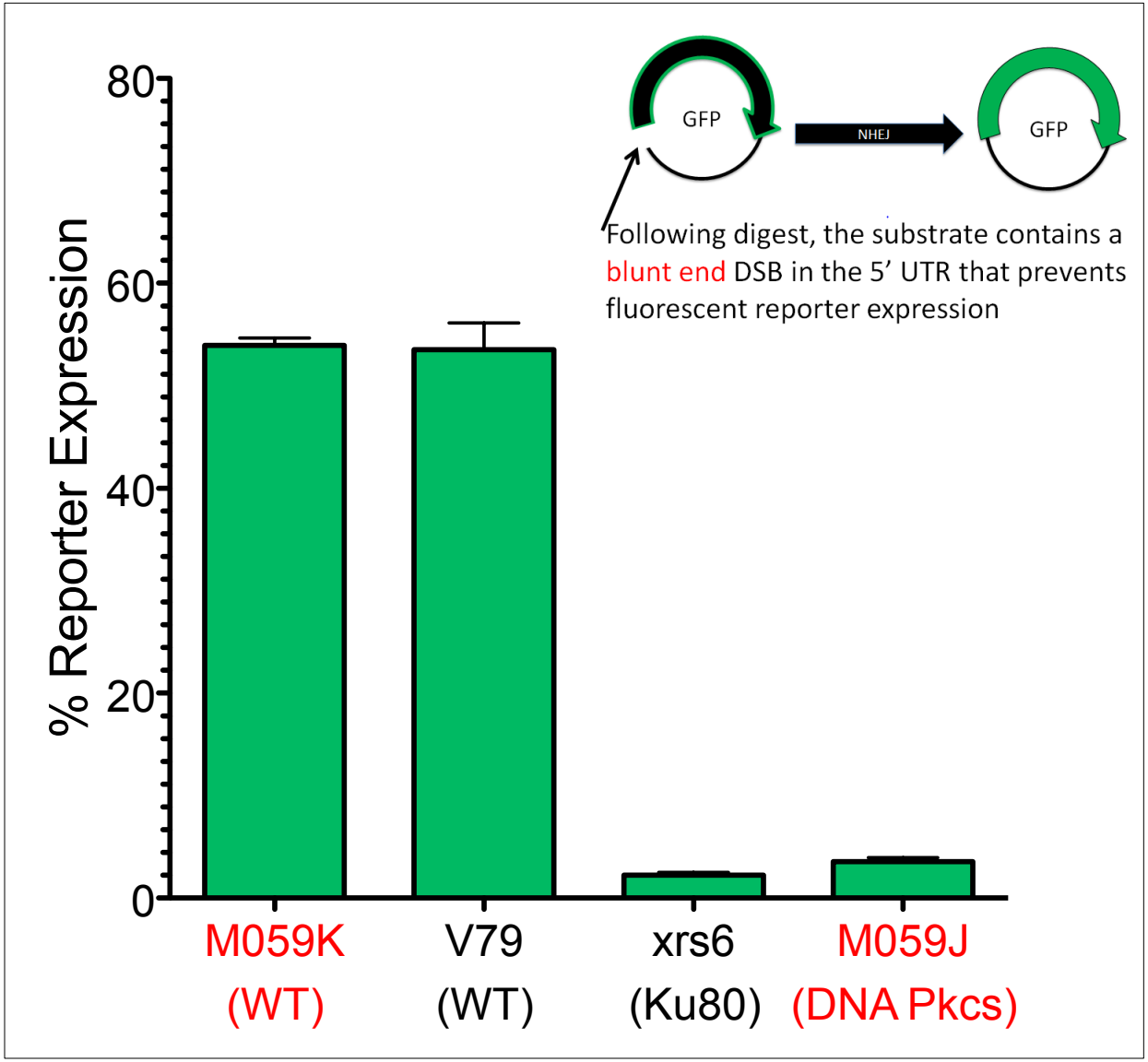
The diversity of fluorescent proteins and genetic mutations is illustrated by this San Diego beach scene drawn with living bacteria expressing 8 different colors of fluorescent proteins.

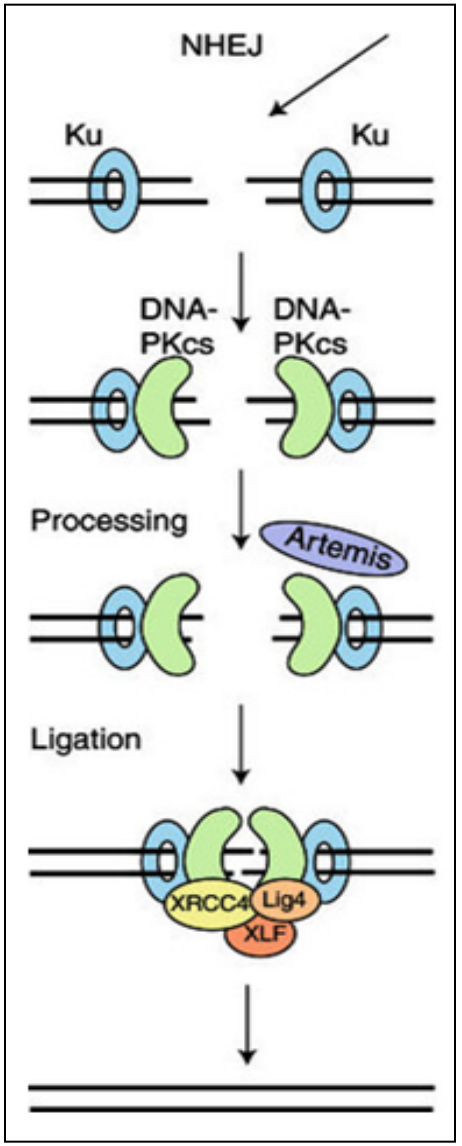
Basis for the fluorescent reporter assay:



Following digest, the substrate contains a DSB in the 5’ UTR that prevents fluorescent reporter expression

NHEJ HCR in WT and NHEJ defective cells at 18 hours post-transfection:





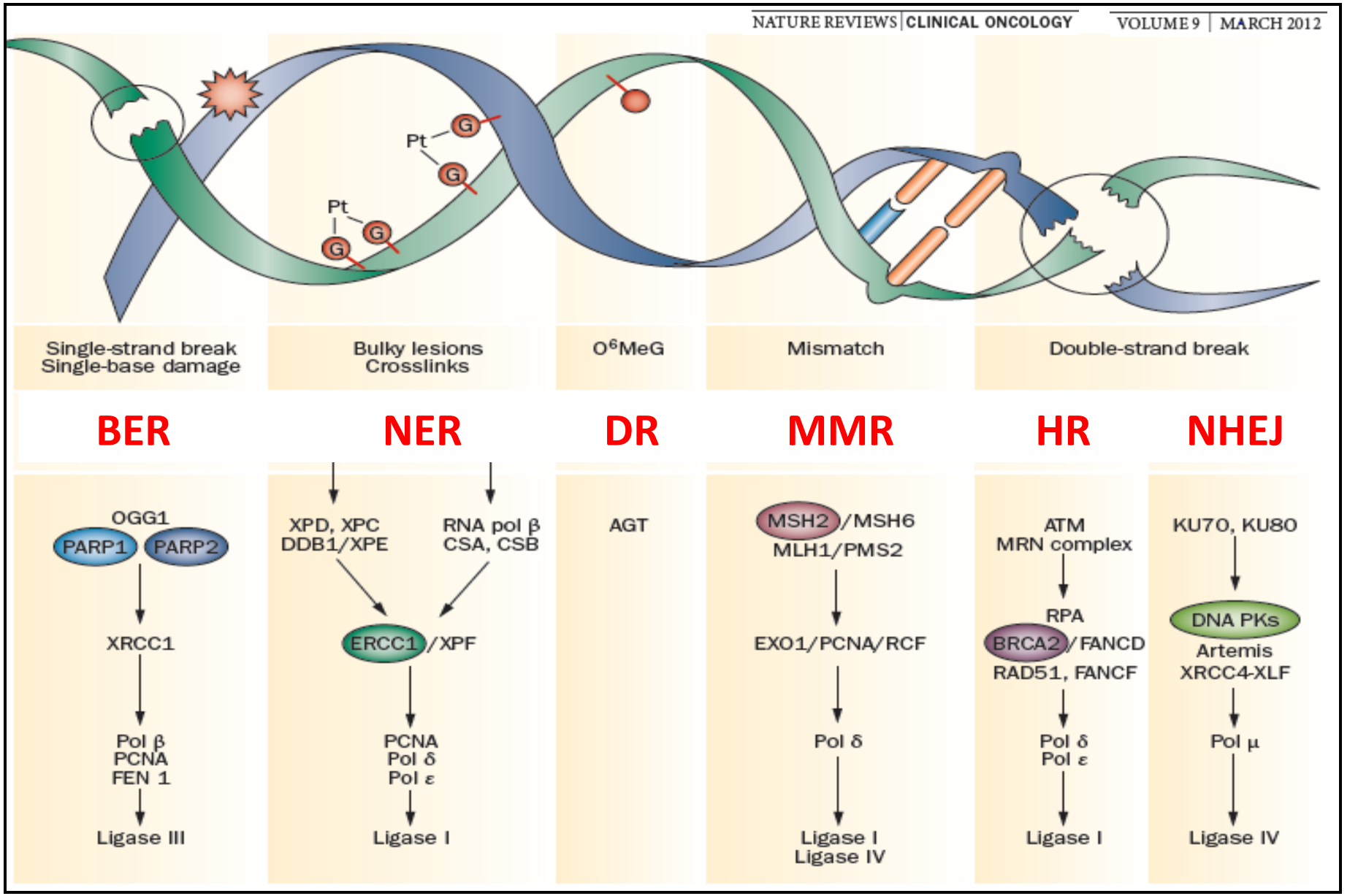
What experimental question will you ask in Module 2?

How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

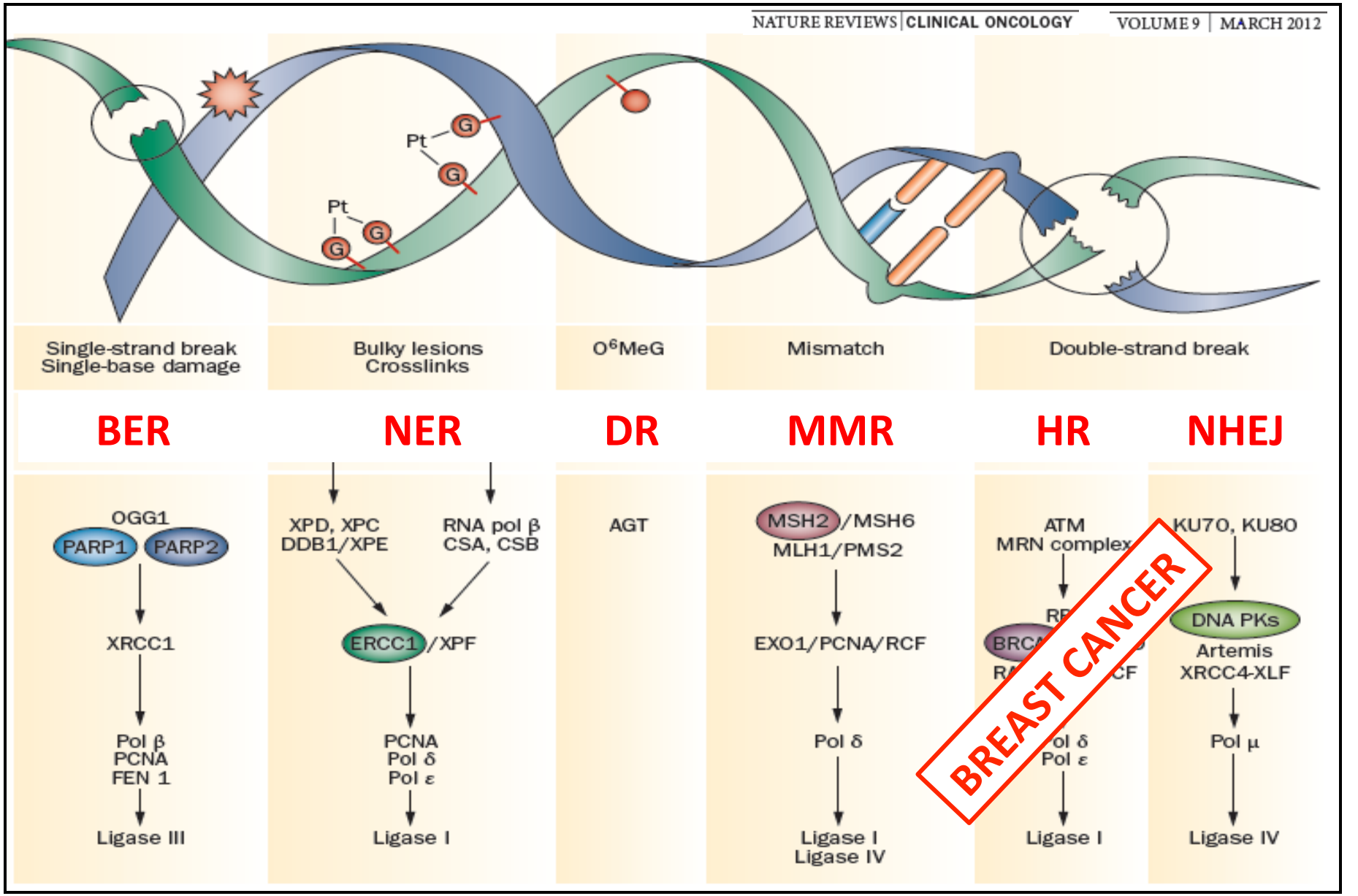
This raises the following questions

- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair efficiency?

Six Major DNA Repair Pathways



Six Major DNA Repair Pathways



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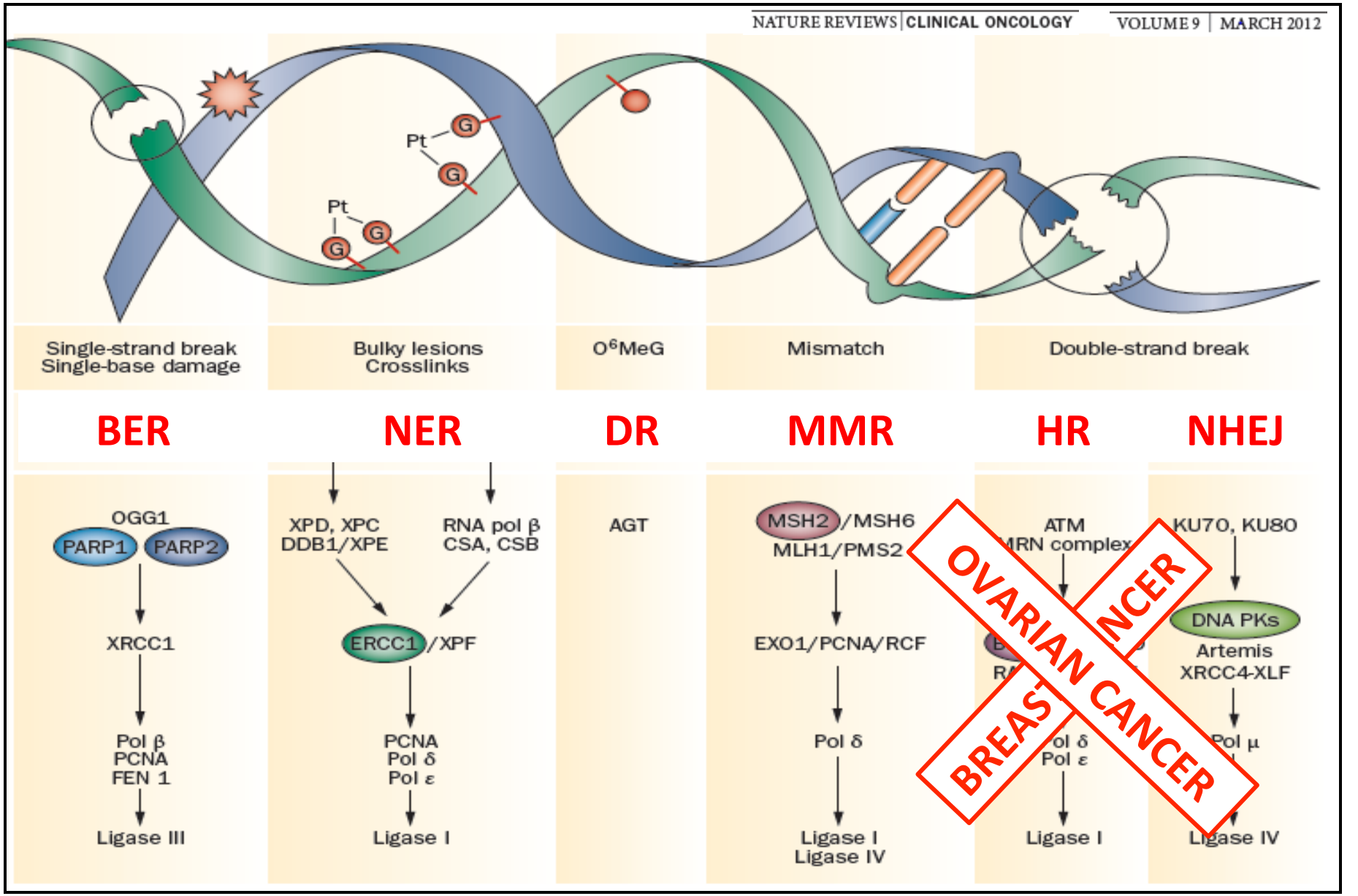

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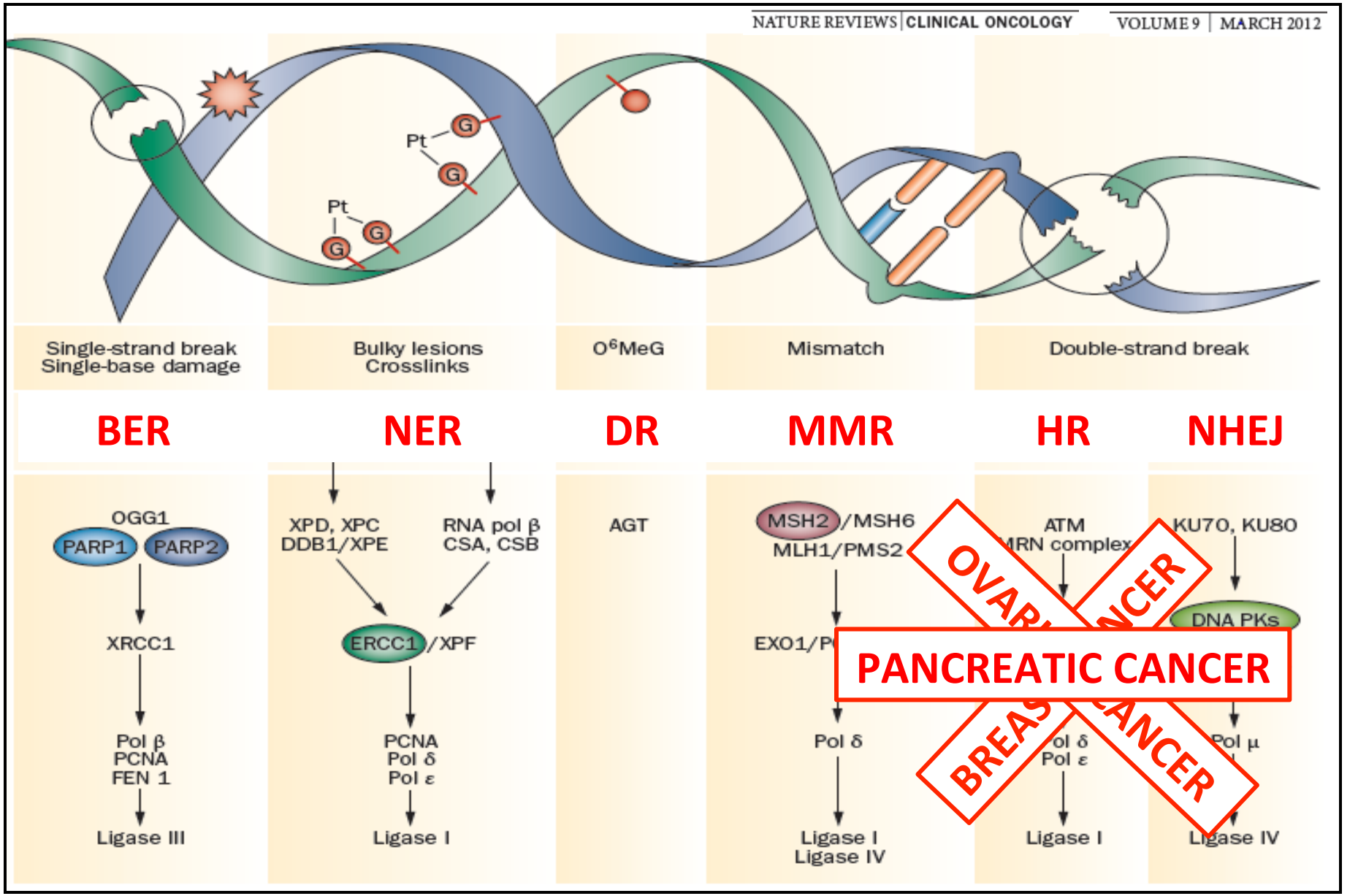

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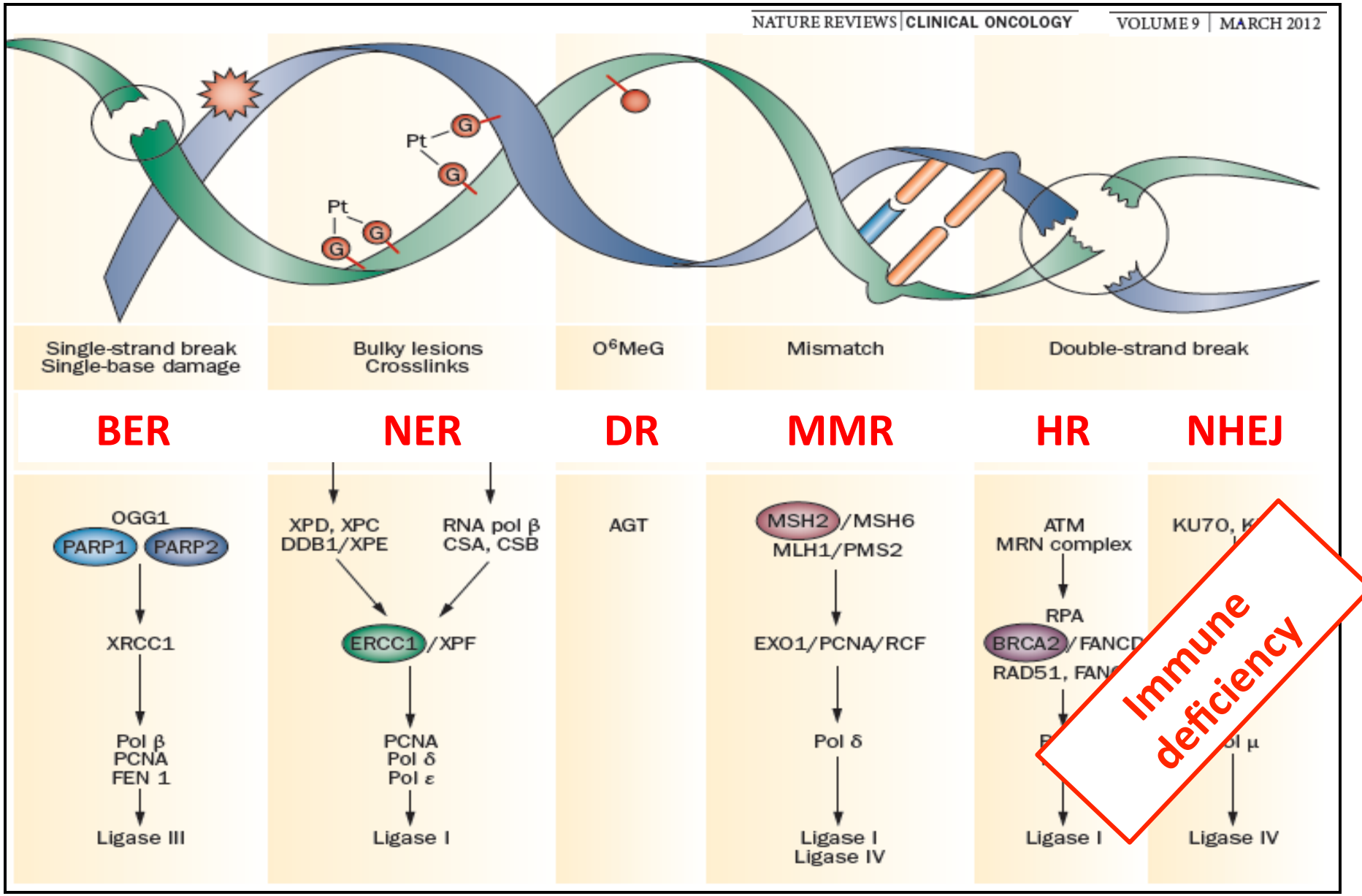
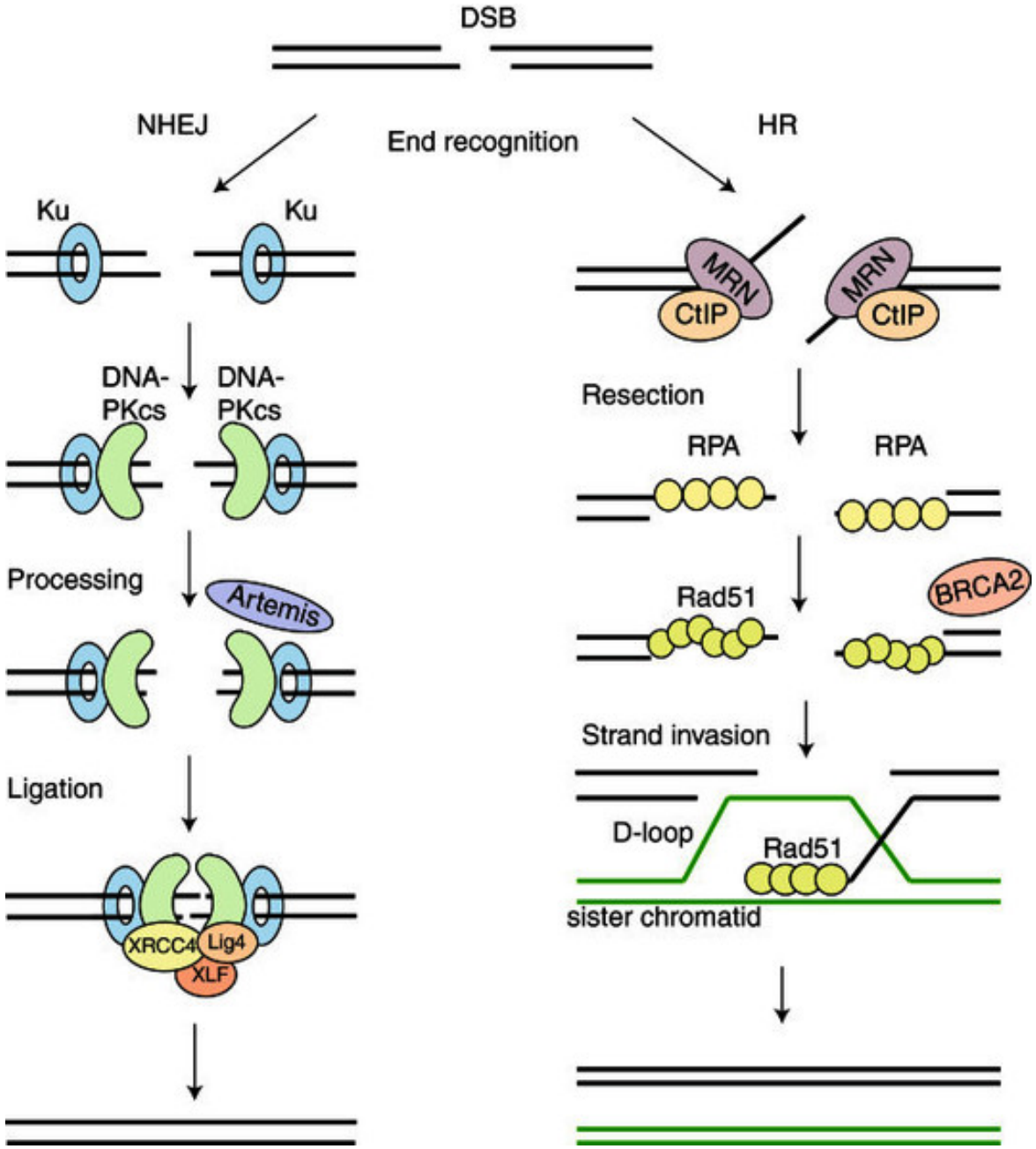




Error Prone

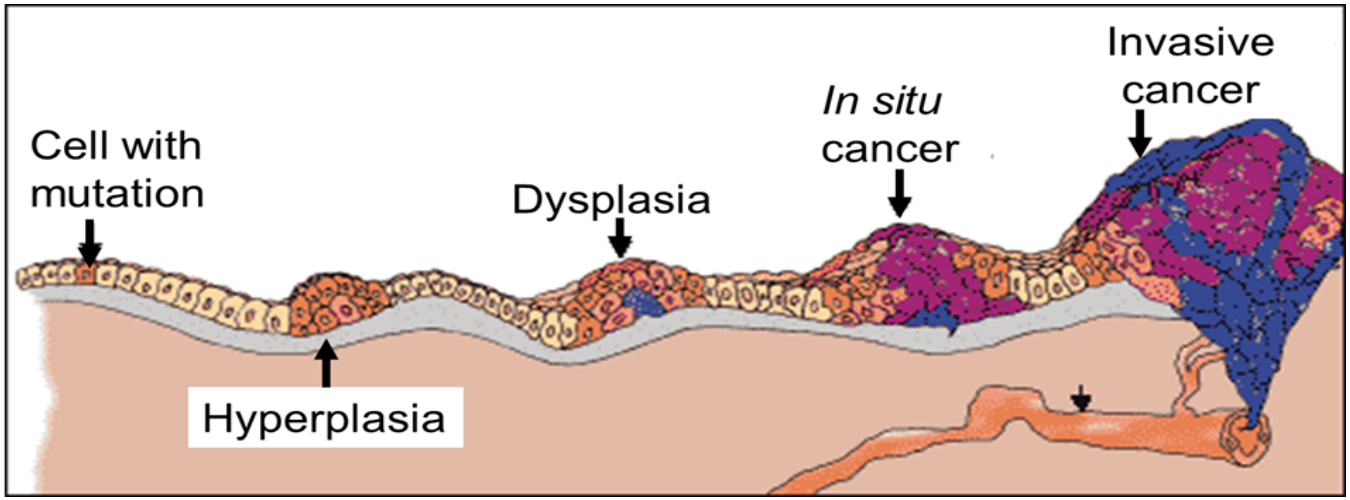
Error Free

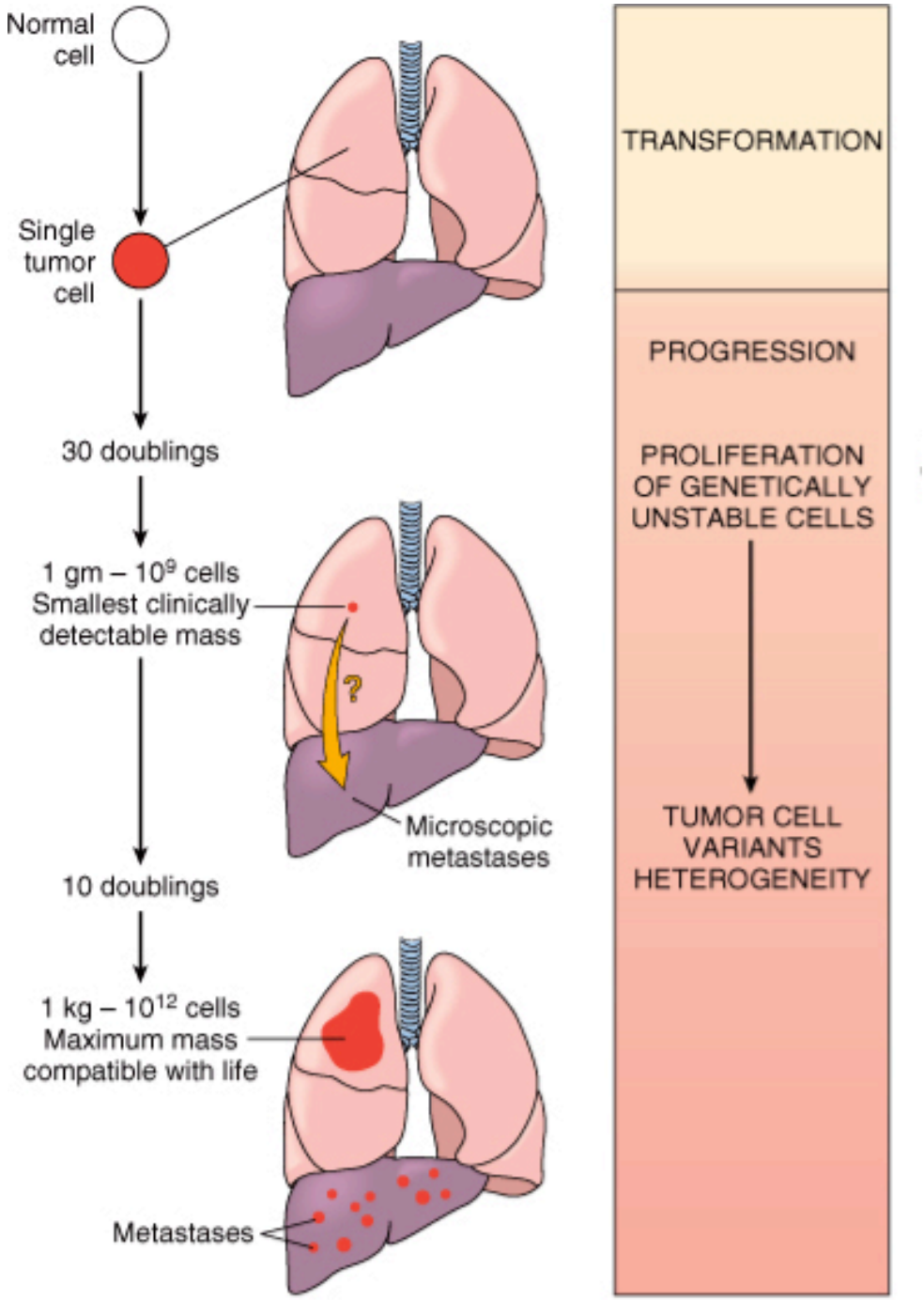
How does the cell decide which pathway to use?

Cancers arise from the accumulation of heritable changes in gene function

Normal Epithelium → Neoplasia → Invasive Carcinoma → Metastasis

1st Mutation → 2nd Mutation → Additional Mutations

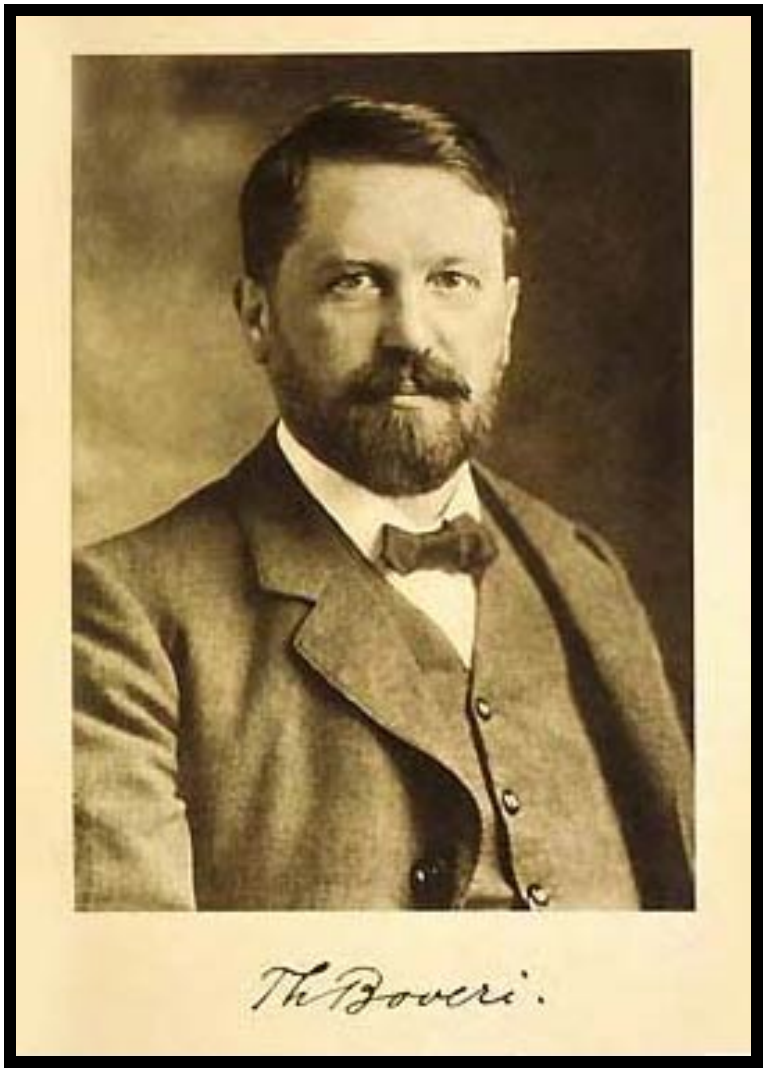




Multiple Mutations

More and more Mutations

The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915



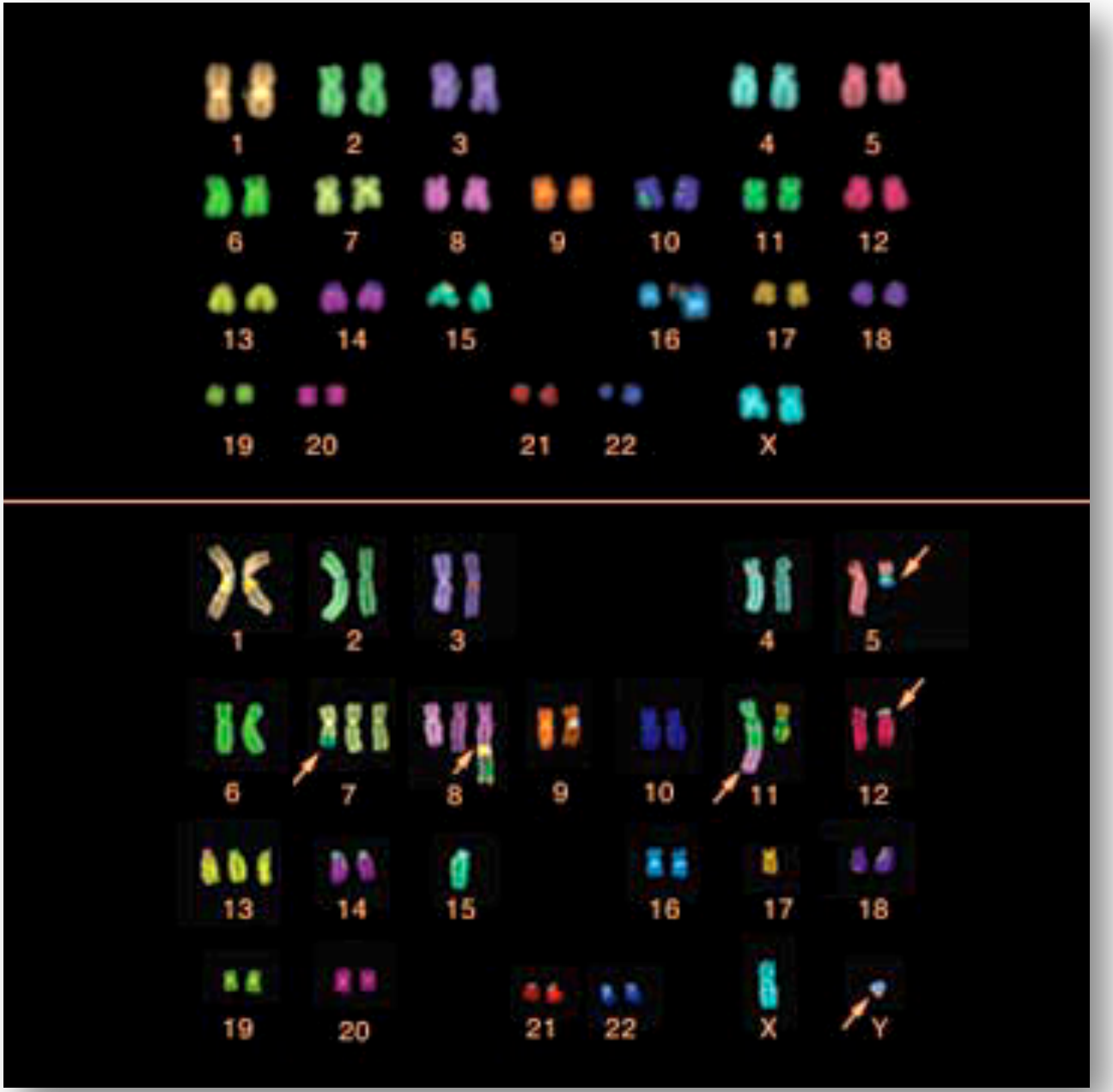
- Established that chromosomes carry the hereditary information by showing that aberrant segregation of chromosomes leads to certain phenotypes in sea urchin eggs.
- Suggested that aberrant segregation of human chromosomes could be responsible for a normal cell becoming a tumor cell
- Suggested that some chromosomes promoted cell growth and others inhibit cell growth

Marcella O'Grady Boveri (1865-1950) also contributed

Marcella O'Grady Boveri (1863-1950) also contributed to Boveri's theory

She was the first woman student to graduate from MIT with a Biology Major in 1885!

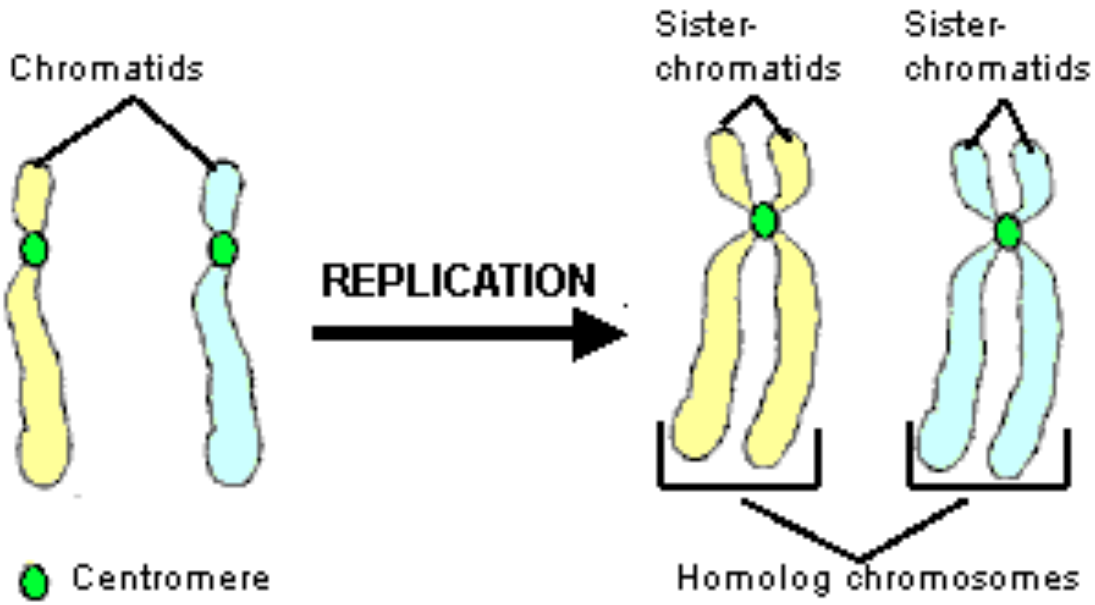
J Med Genet. 1985;22(6):431-40.
Marcella O'Grady Boveri (1865-1950) and the chromosome theory of cancer

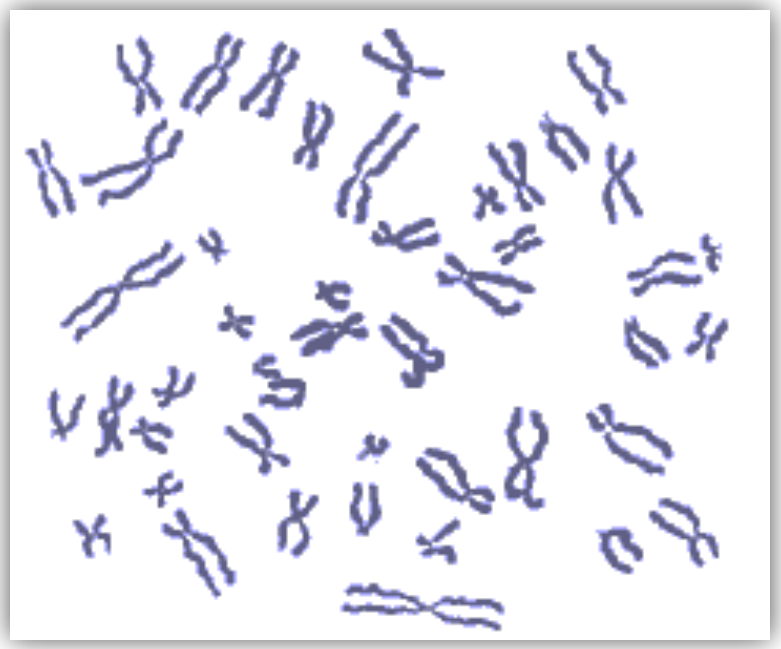


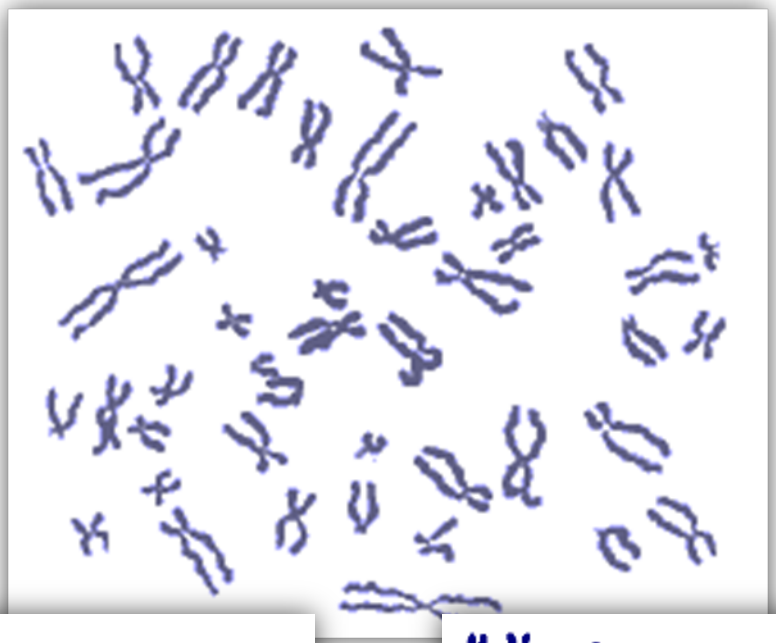
Chromosomes from a Normal cell

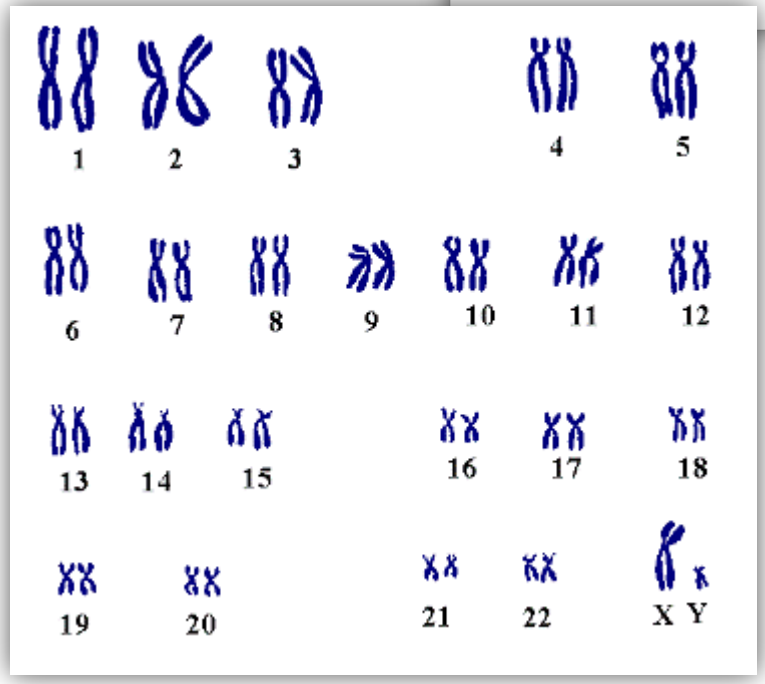
Chromosomes from a Tumor cell

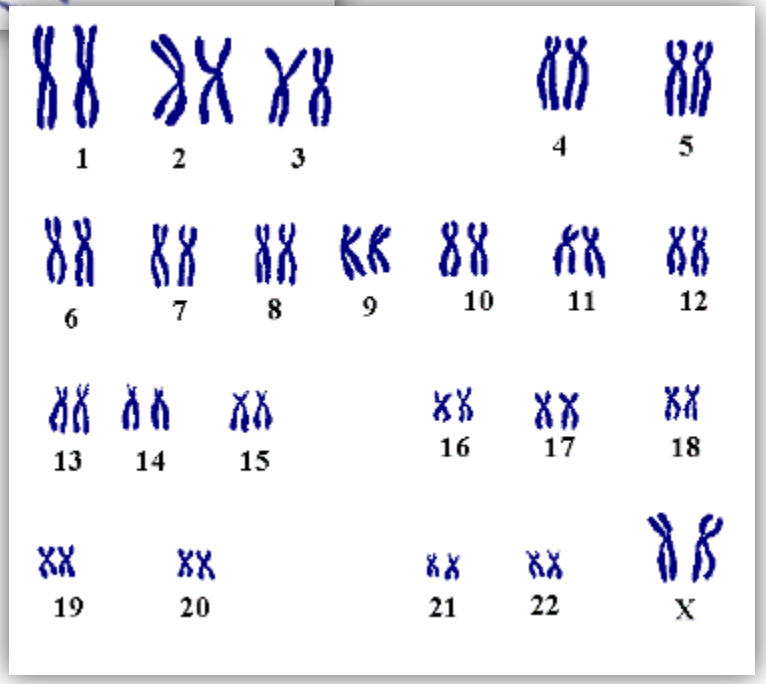
Spectral Karyotyping (SKY)
"SKY Painted Chromosomes"


Chromatids
Sister-
chromatids
Sister-
chromatids
REPLICATION
Centromere
Homolog chromosomes





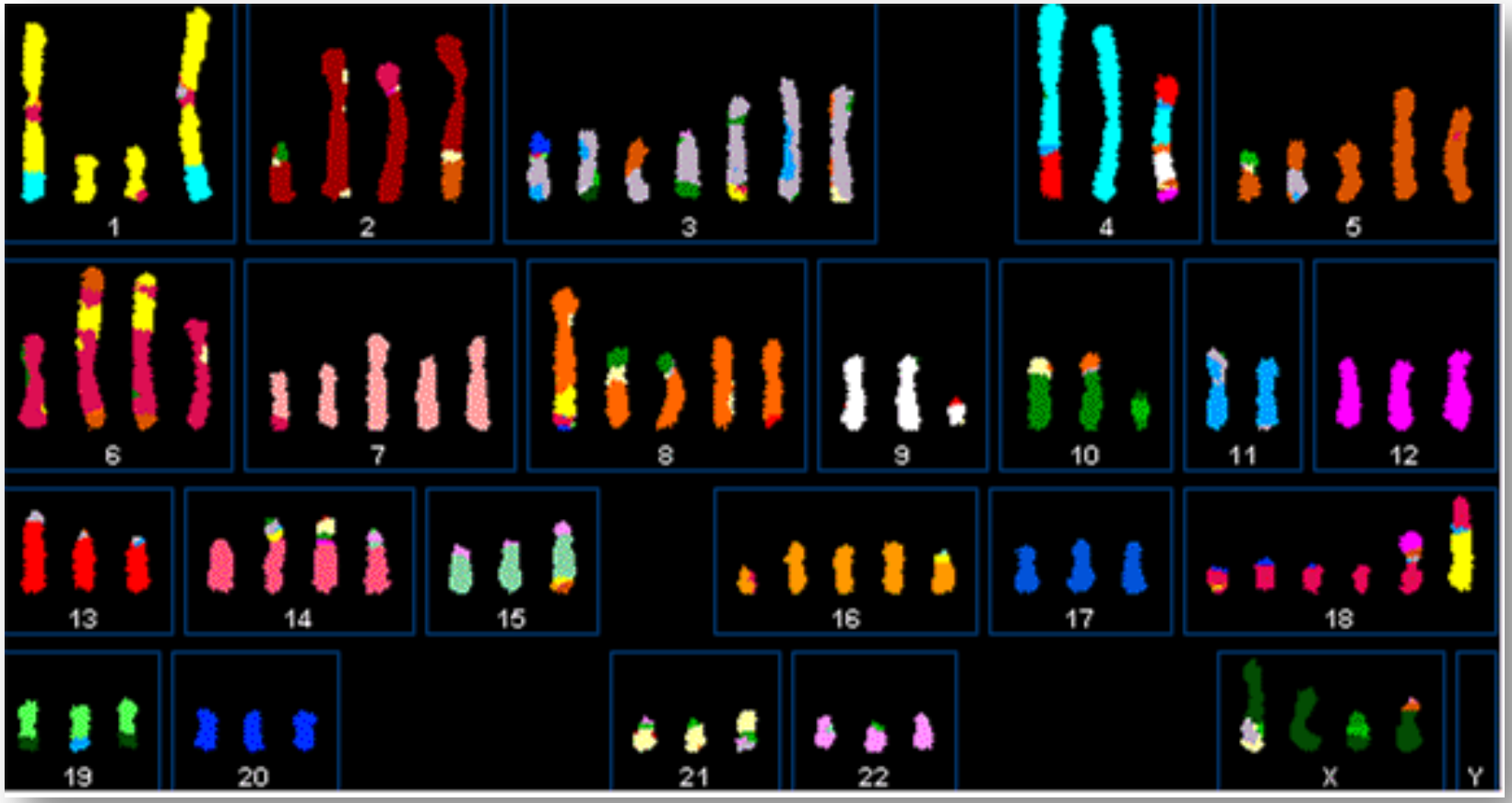




http://www.geocities.ws/genetrix4u/genetics/genetics2.html

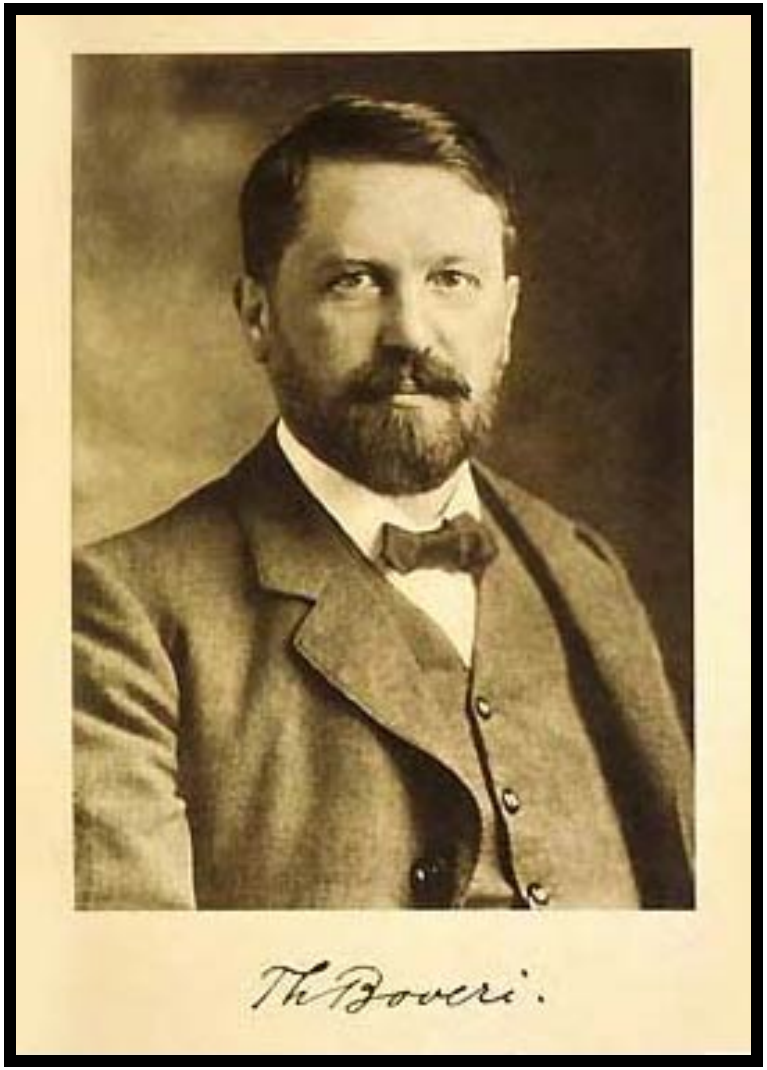
Chromosomes from a Pancreatic Tumor Cell
Probably BRCA1 or BRCA2 deficient

Chromosomes from a BRCA1 deficient Breast Tumor Cell



https://www.google.com/search?q=sky+chromosomes+brca1&rlz=1T4GGHP_enUS635US636&biw=1195&bih=758&source=lnms&tbm=isch&sa=X&ved=0ahUKEwigv9_j1cfLAhVK

The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915



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Alterations (mutations) in different kinds of Genes cause Cancer

Oncogenes

genes that ordinarily promote cell proliferation but when mutated or overexpressed promote uncontrolled growth

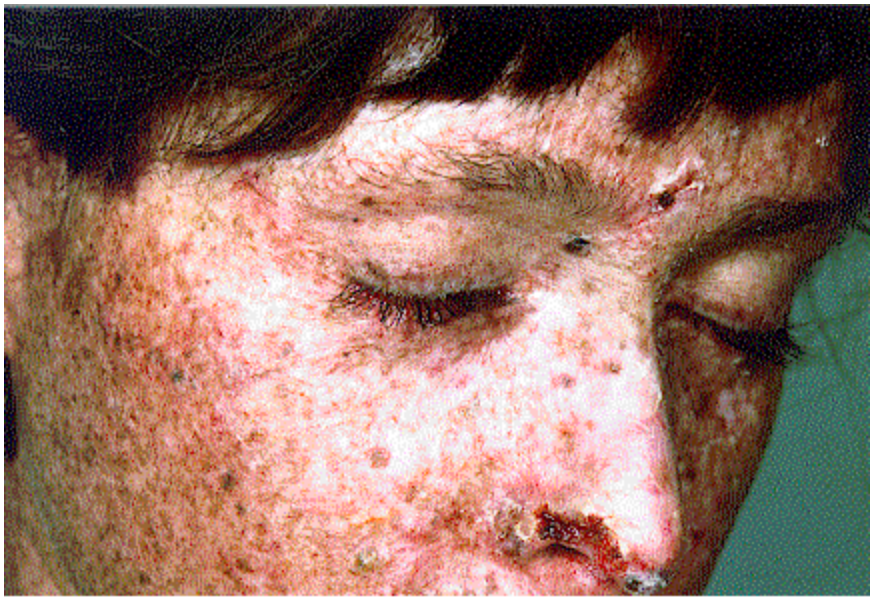
Tumor suppressor genes

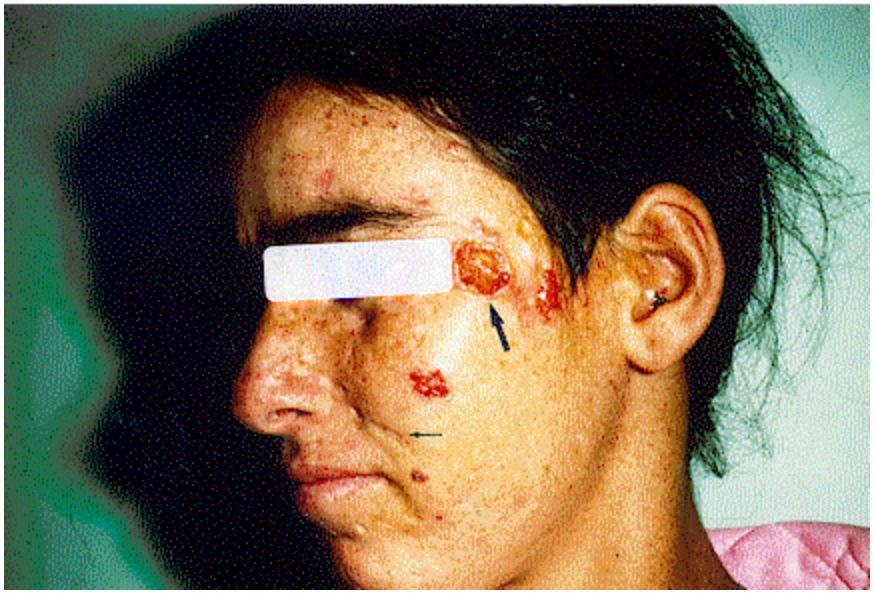
genes that ordinarily prevent inappropriate proliferation but when mutated allow uncontrolled growth

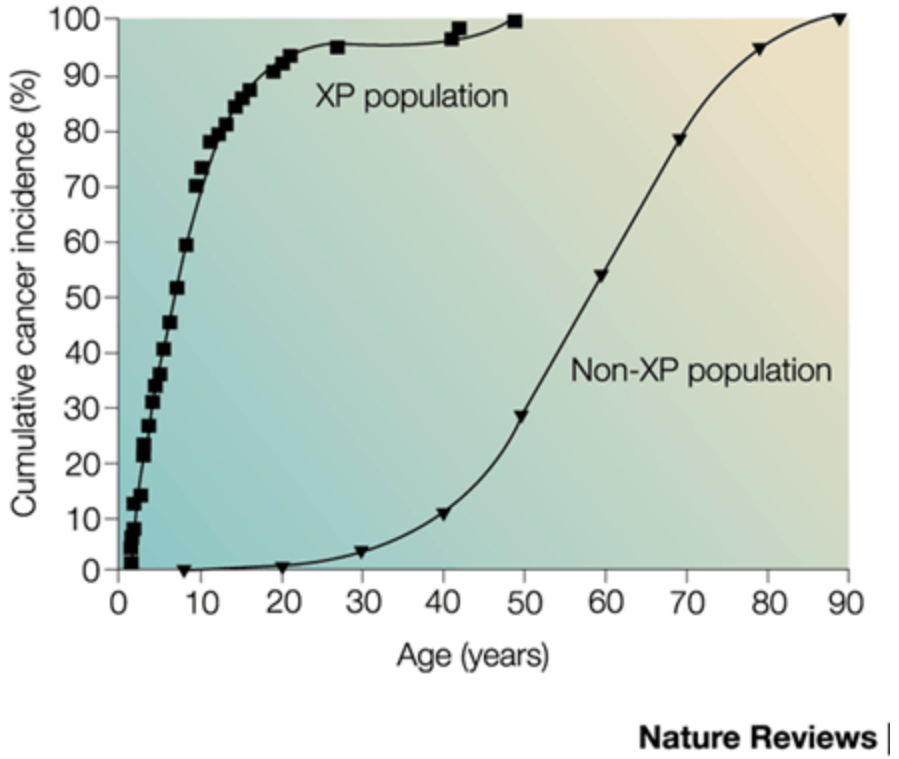
Mutator genes

genes that ordinarily prevent mutations; alterations in these genes allow increased mutation rates

Lack of DNA repair accelerates the onset of cancer

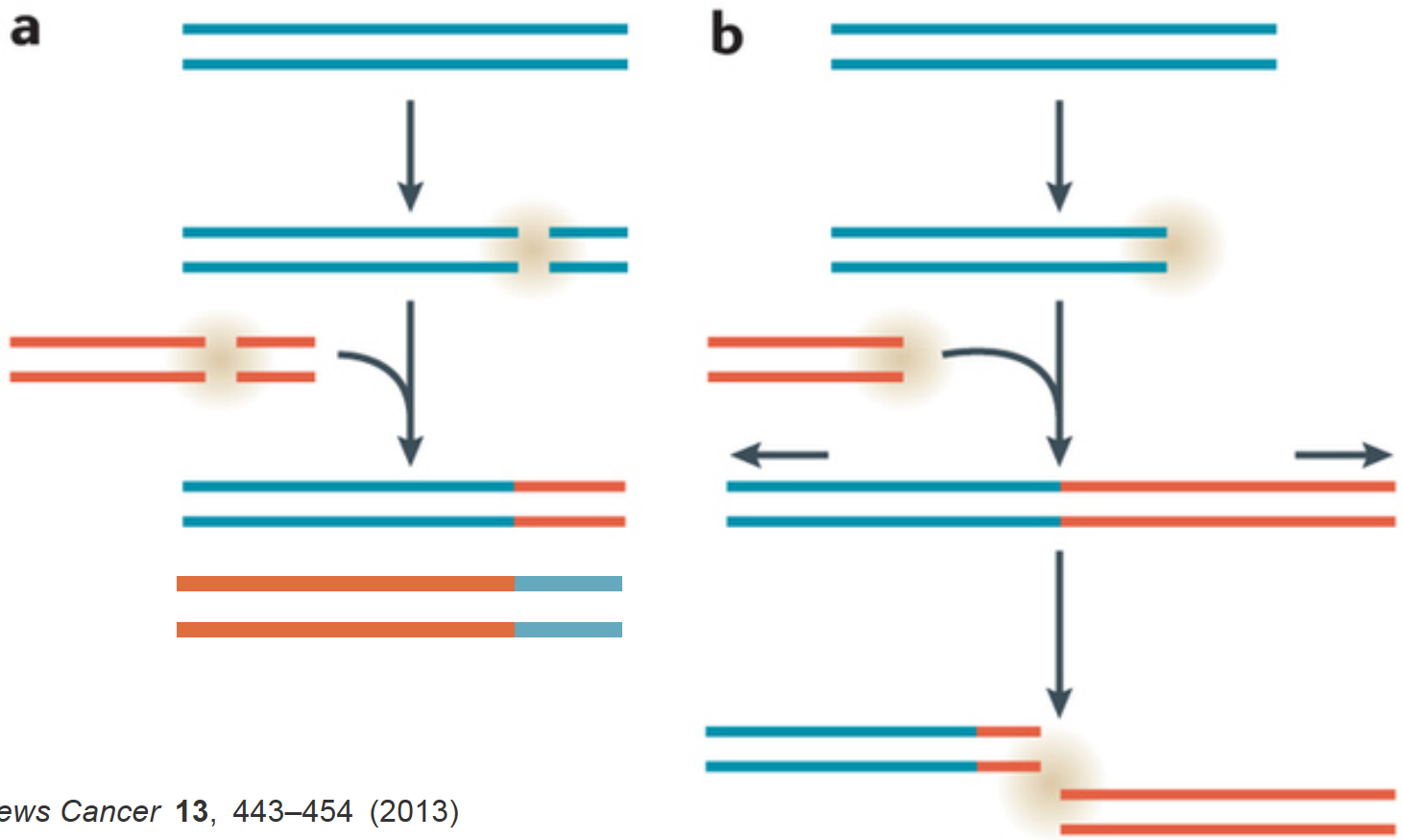






Nature Reviews

Mechanisms of Chromosome Translocation

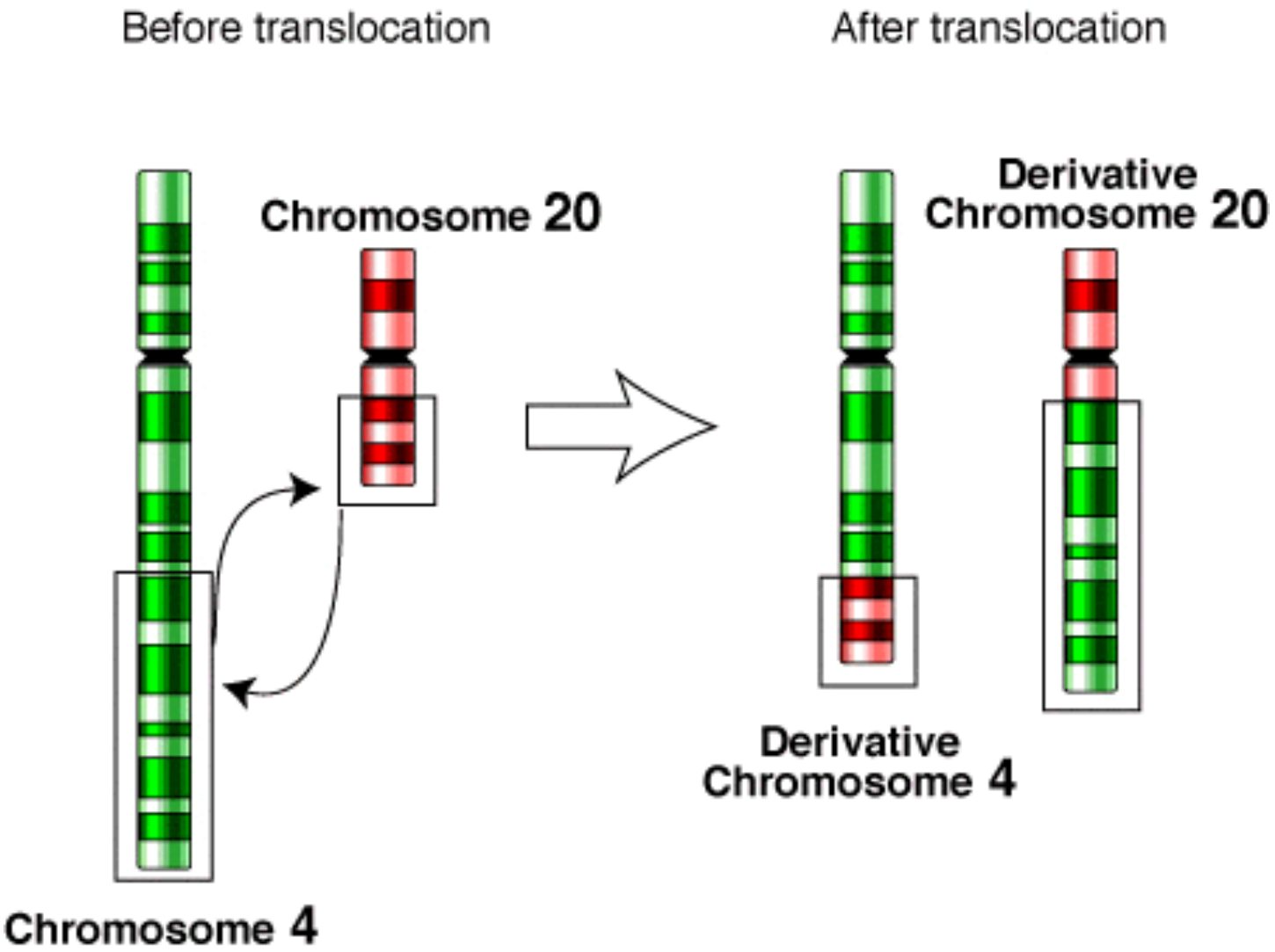


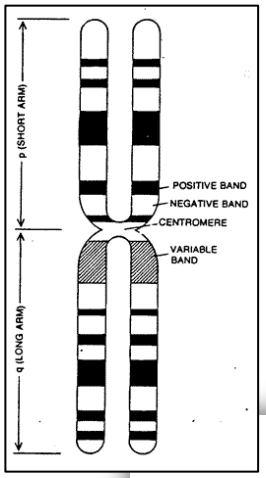
Nature Reviews Cancer **13**, 443–454 (2013)

a | Balanced reciprocal translocations from the fusion of two double-strand breaks that arise in the same cell; ligation of the free DNA ends is mediated by the **non-homologous end-joining pathway**. Red and blue strands represent different chromosomes.

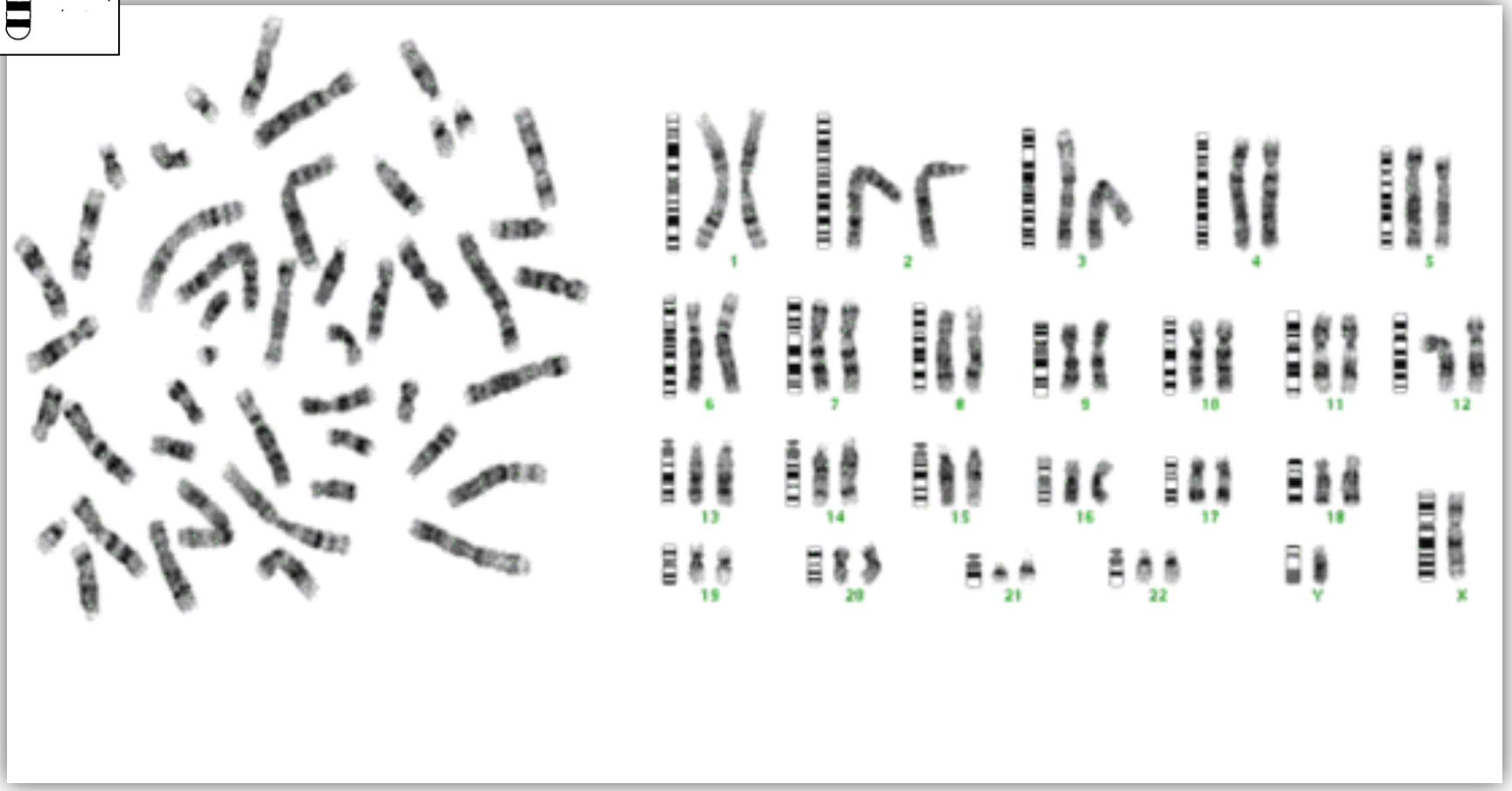
b Telomere uncapping or attrition generates a DNA double-strand break response, which potentially leads to the fusion of telomeres, generating end-to-end fusions. During anaphase, dicentric fusion chromosomes are pulled apart, leading to the formation of translocations and double-strand breaks. Broken chromosomes act as substrates for additional rounds of fusion and breakage, generating increasingly complex translocations.

Mechanisms of Chromosome Translocation



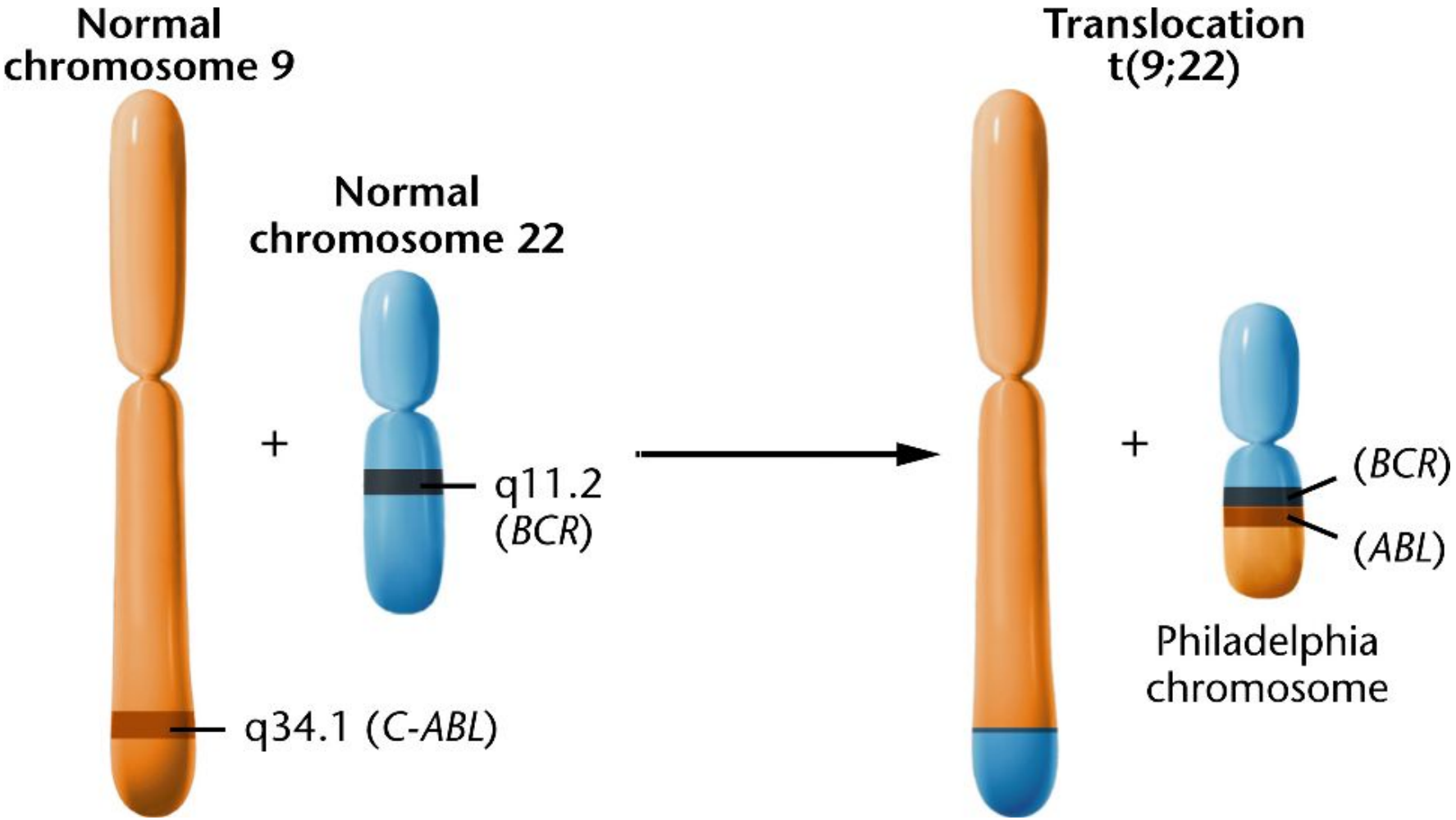


Chromosome Banding



http://geneticslab.wikispaces.com/Chromosomes

Chronic Myelogenous Leukemia

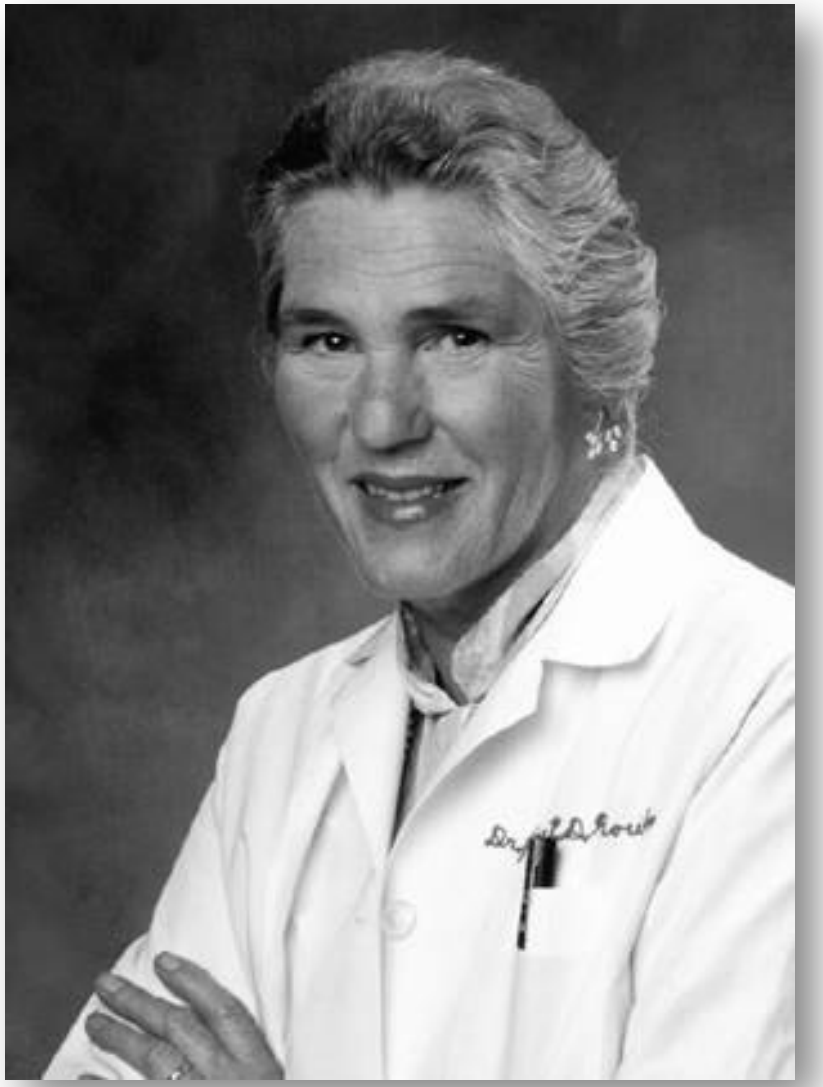


breakpoint cluster region protein (**BCR**) C-Abl non-receptor tyrosine kinase

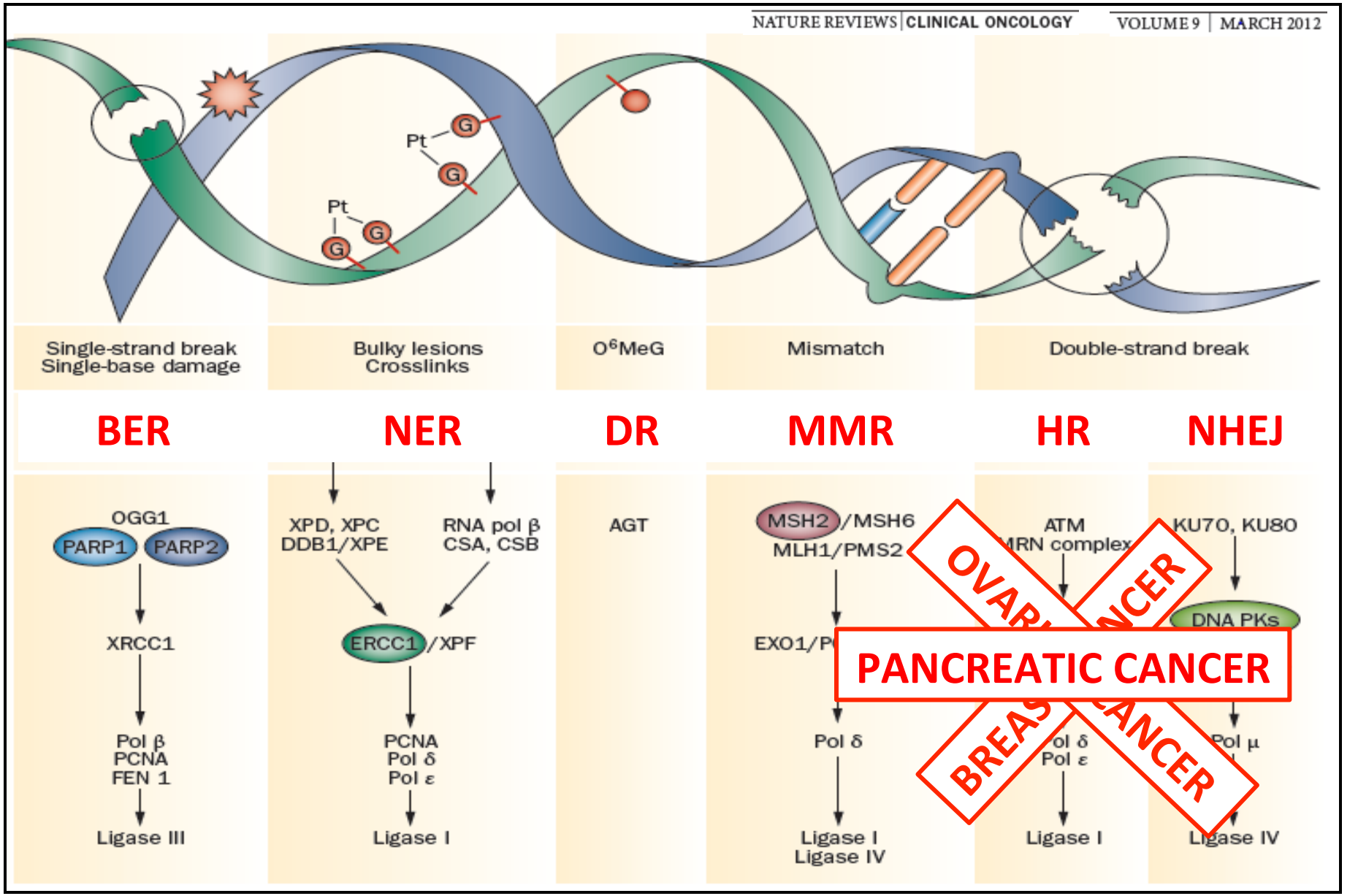
Janet Rowley

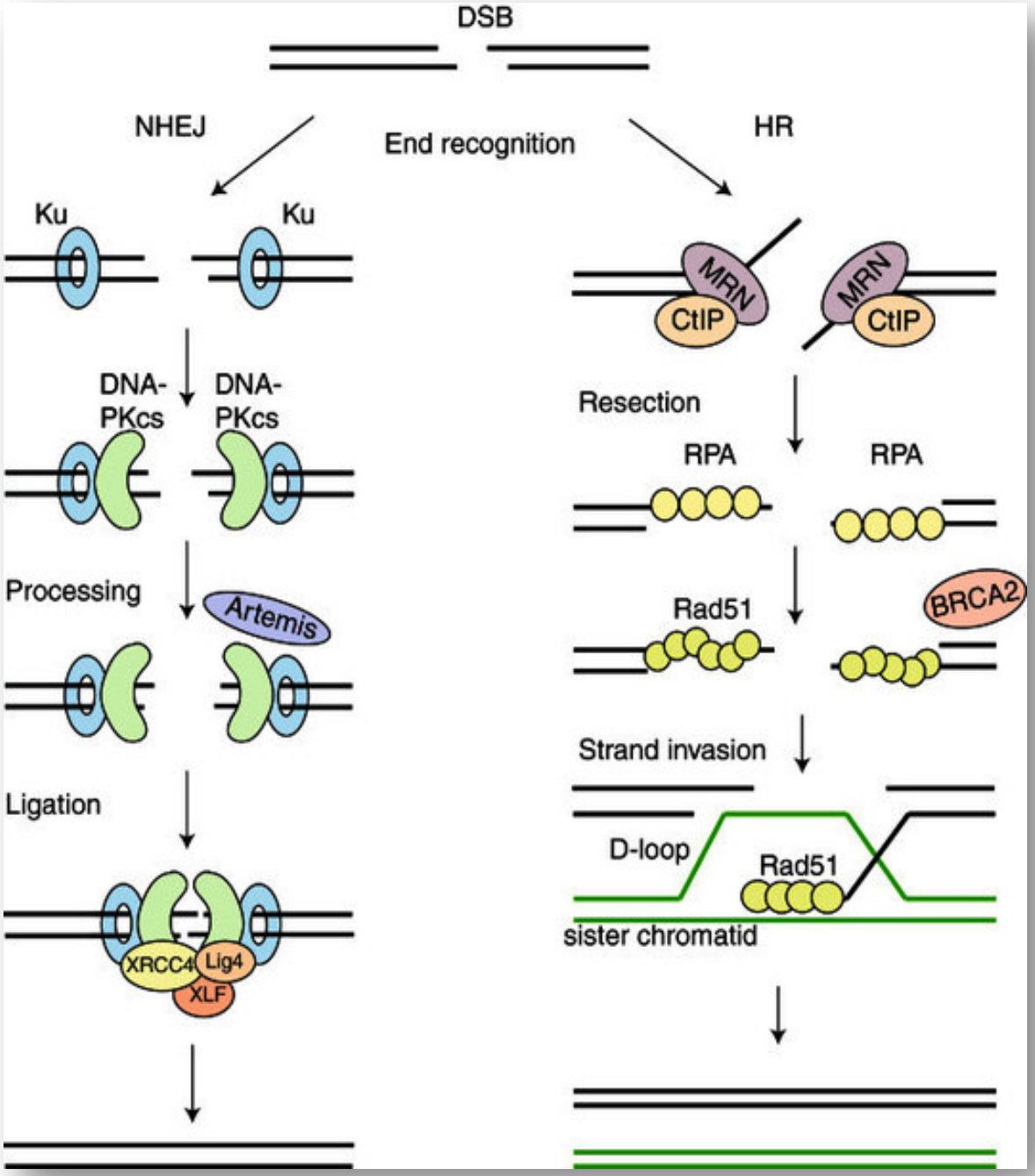
(April 5, 1925 – December 17, 2013)

American human geneticist and the first scientist to identify a chromosomal translocation as the cause of leukemia and other cancers.



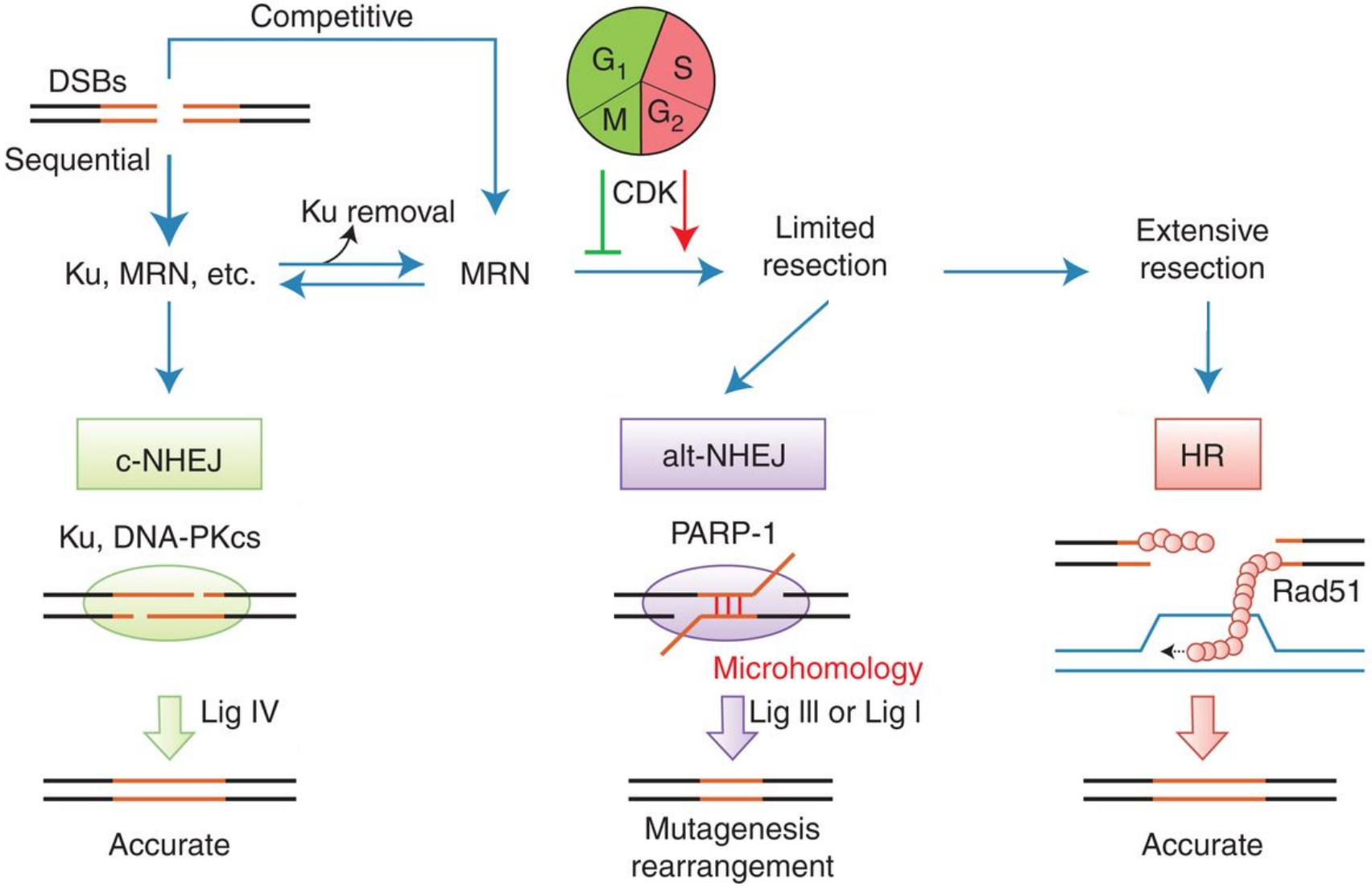
Six Major DNA Repair Pathways





How does the cell decide which pathway to use?

Disposition of DSBs between repair pathways.



Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757

Non-Homologous End Joining

http://web.mit.edu/engelward-lab/animations/NHEJ.html

Double-Strand Break Repair via Single Strand Annealing – Alternate NHEJ

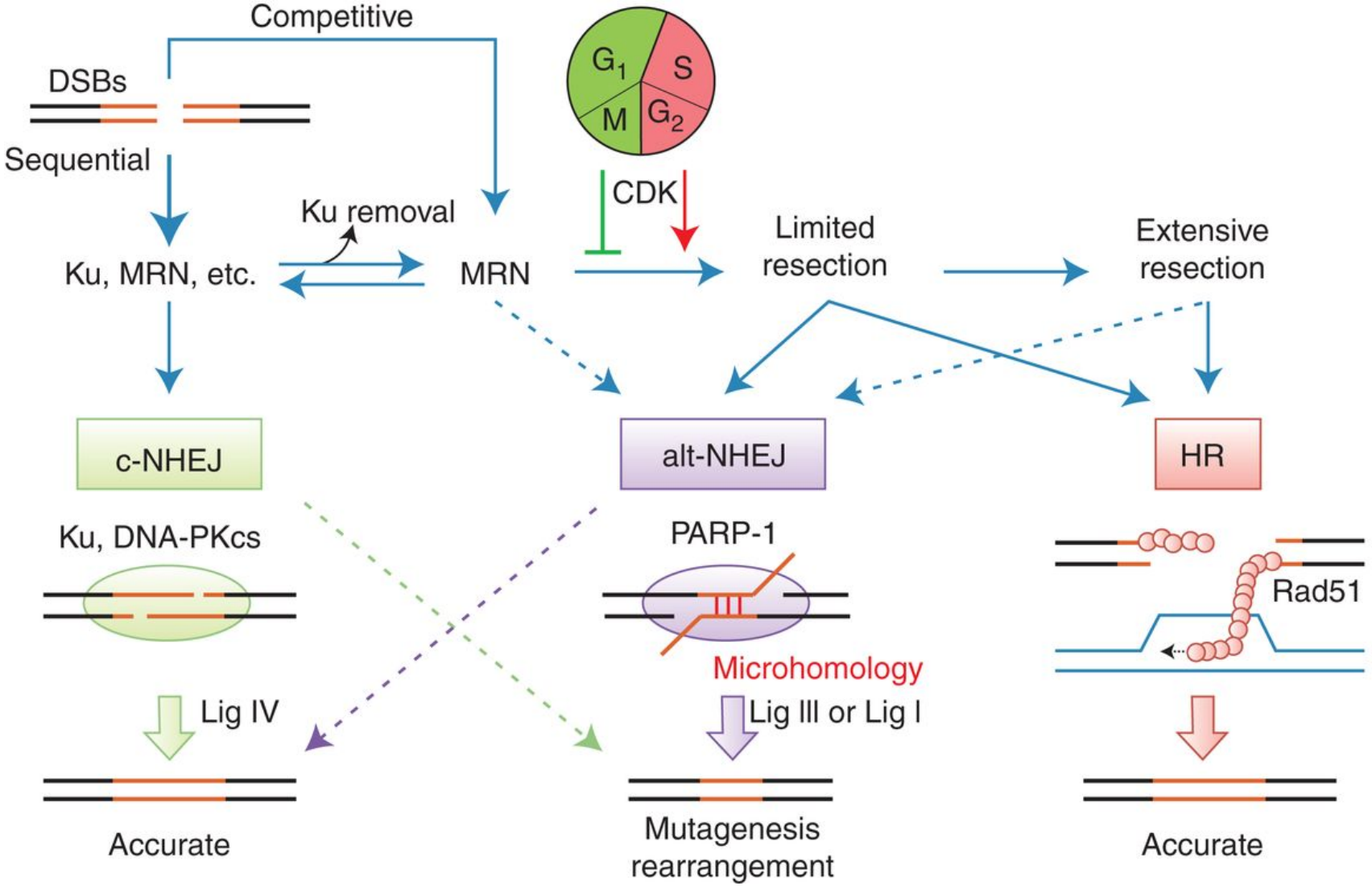
http://web.mit.edu/engelward-lab/animations/SSA.html

Synthesis-Dependent Strand Annealing (Homologous Recombination)

http://web.mit.edu/engelward-lab/animations/SDSA.html

Engelward lab Animations

Disposition of DSBs between repair pathways.



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