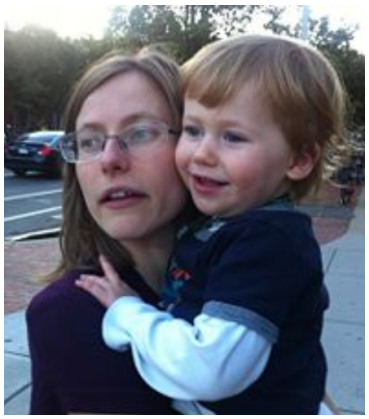
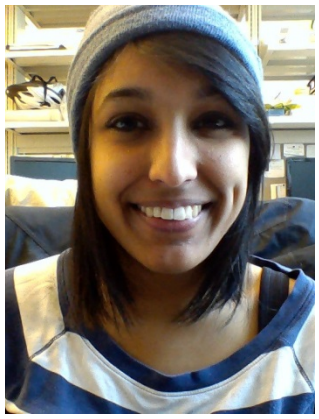
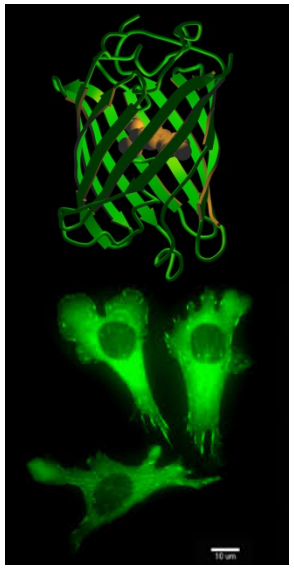
20.109 Spring 2014 Mod 2 – Lecture 4
System Engineering and Protein Foundations



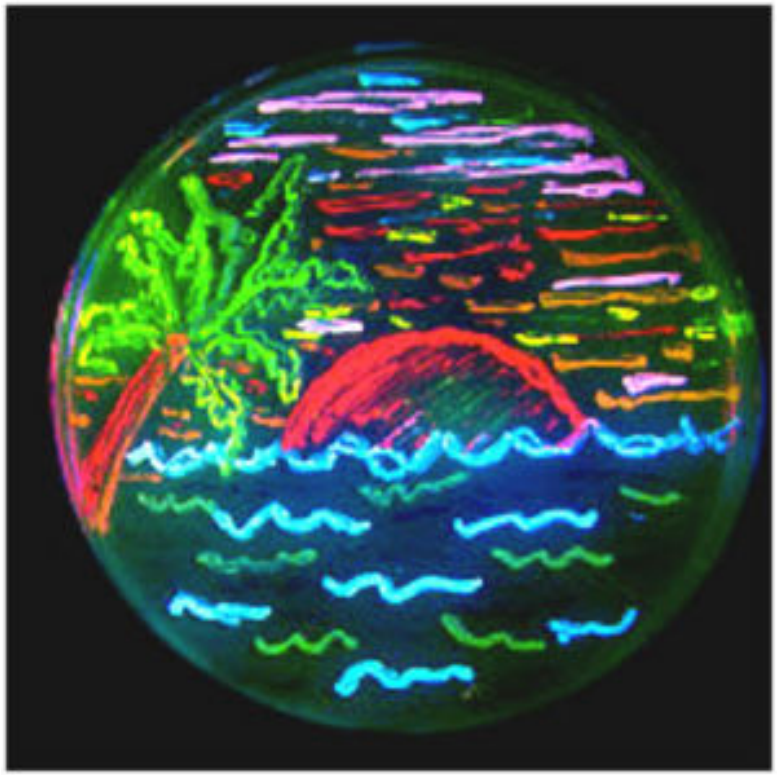



Agi Stachowiak
Shannon Hughes
Aneesh Ramaswamy
Suhani Vora (TA)
Leona Samson (Lectures)
Zachary Nagel (help with development)

Key Experimental Methods for Module 1

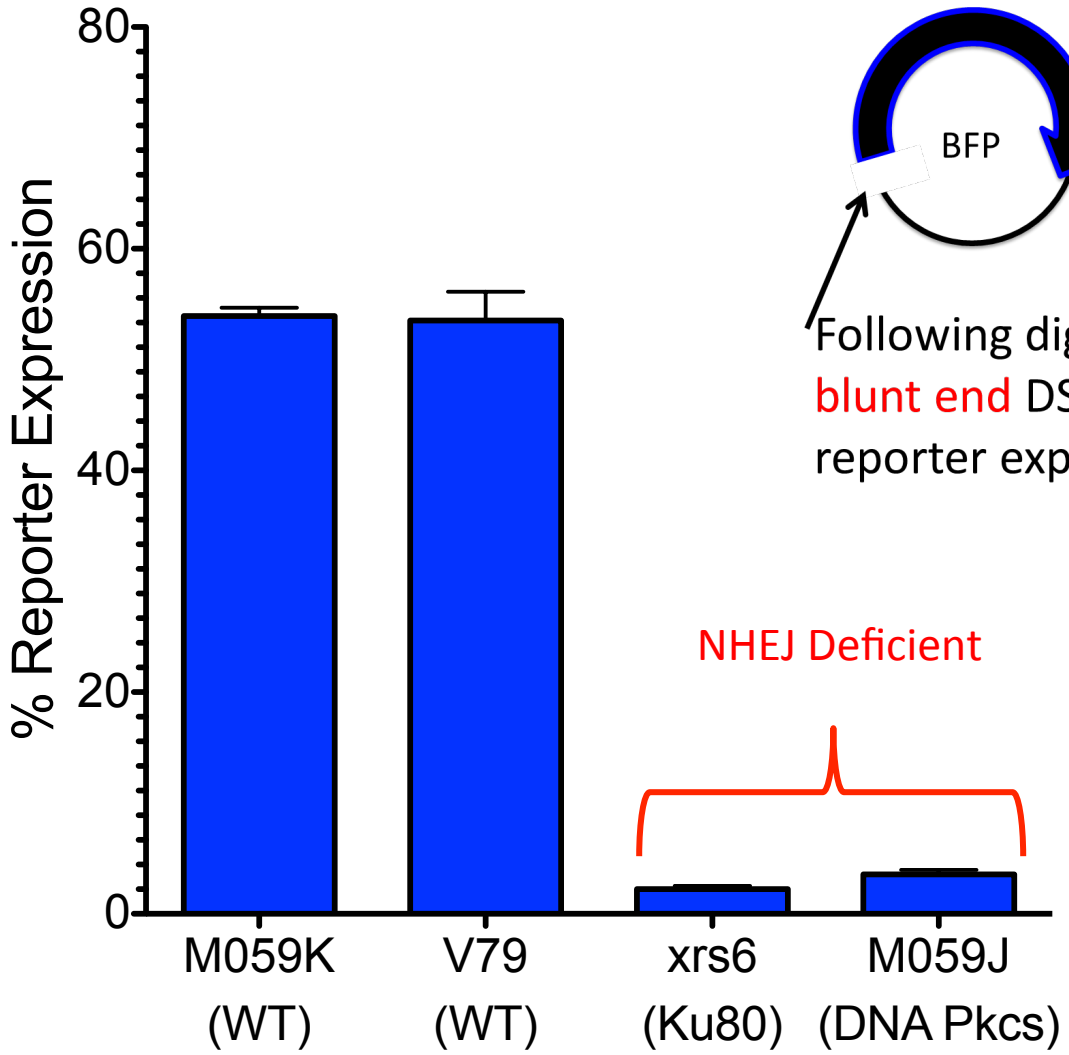


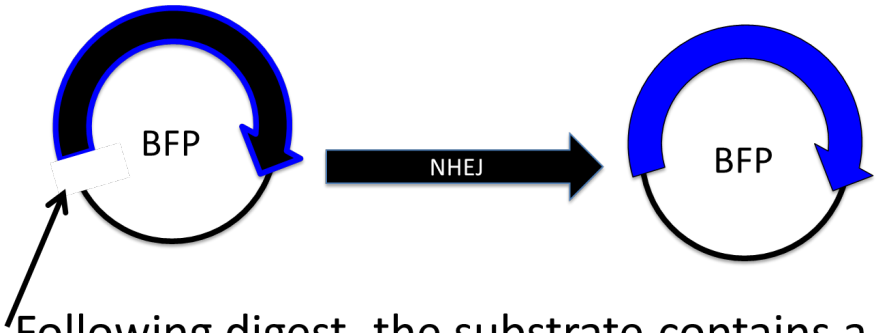
- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data



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.

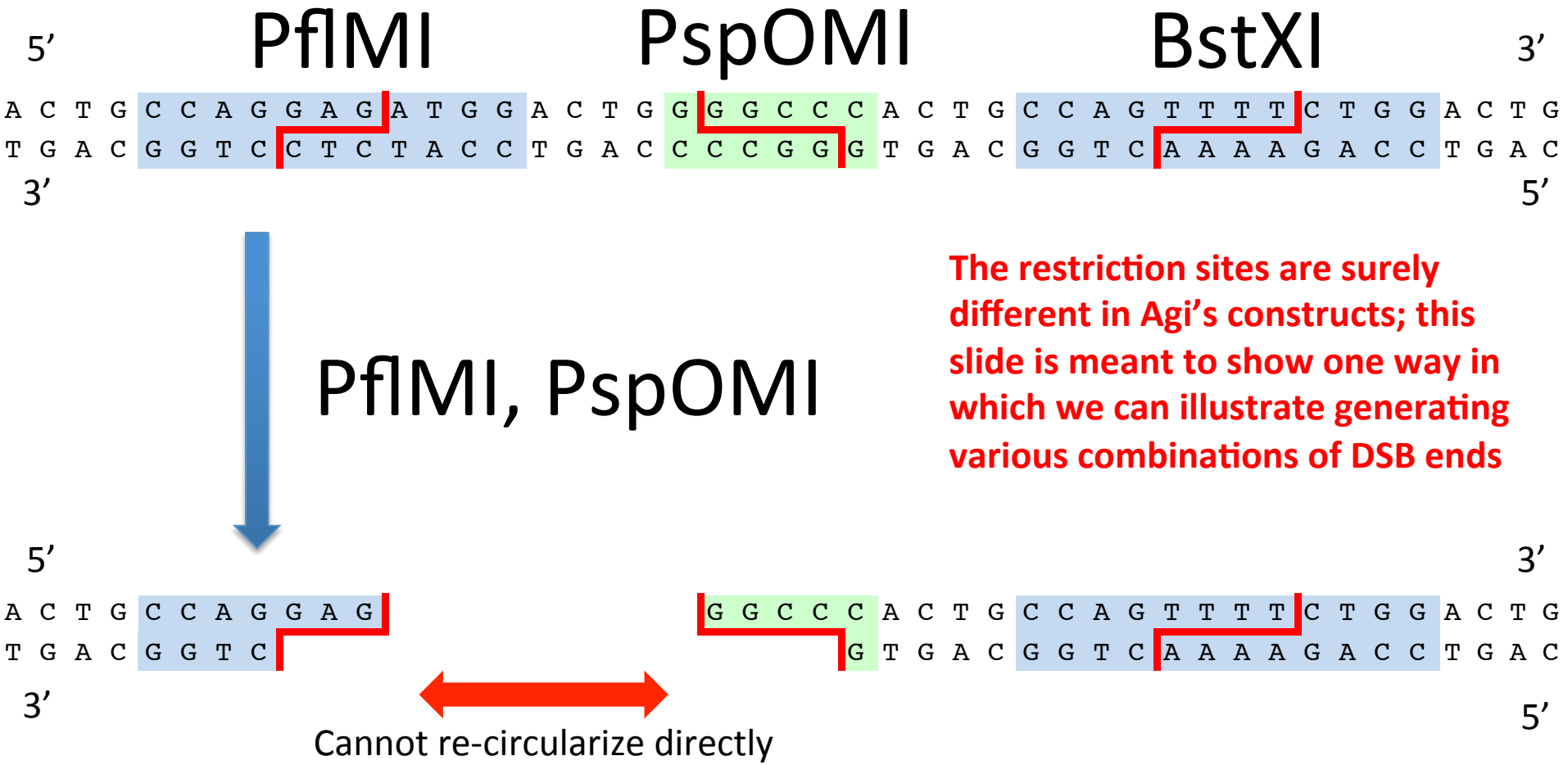
NHEJ HCR in WT and NHEJ defective cells at 18 hours post-transfection:



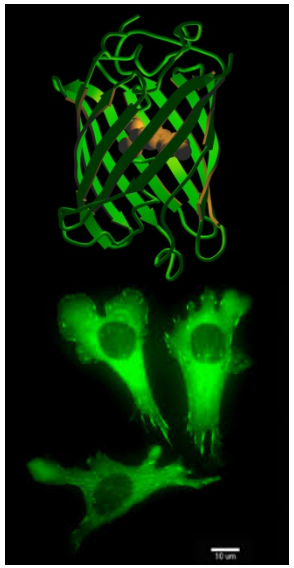


Following digest, the substrate contains a blunt end DSB that prevents fluorescent reporter expression

Double digest to produce DSBs with ends that are not compatible with ligation:



What experimental question will you ask in Module 2?

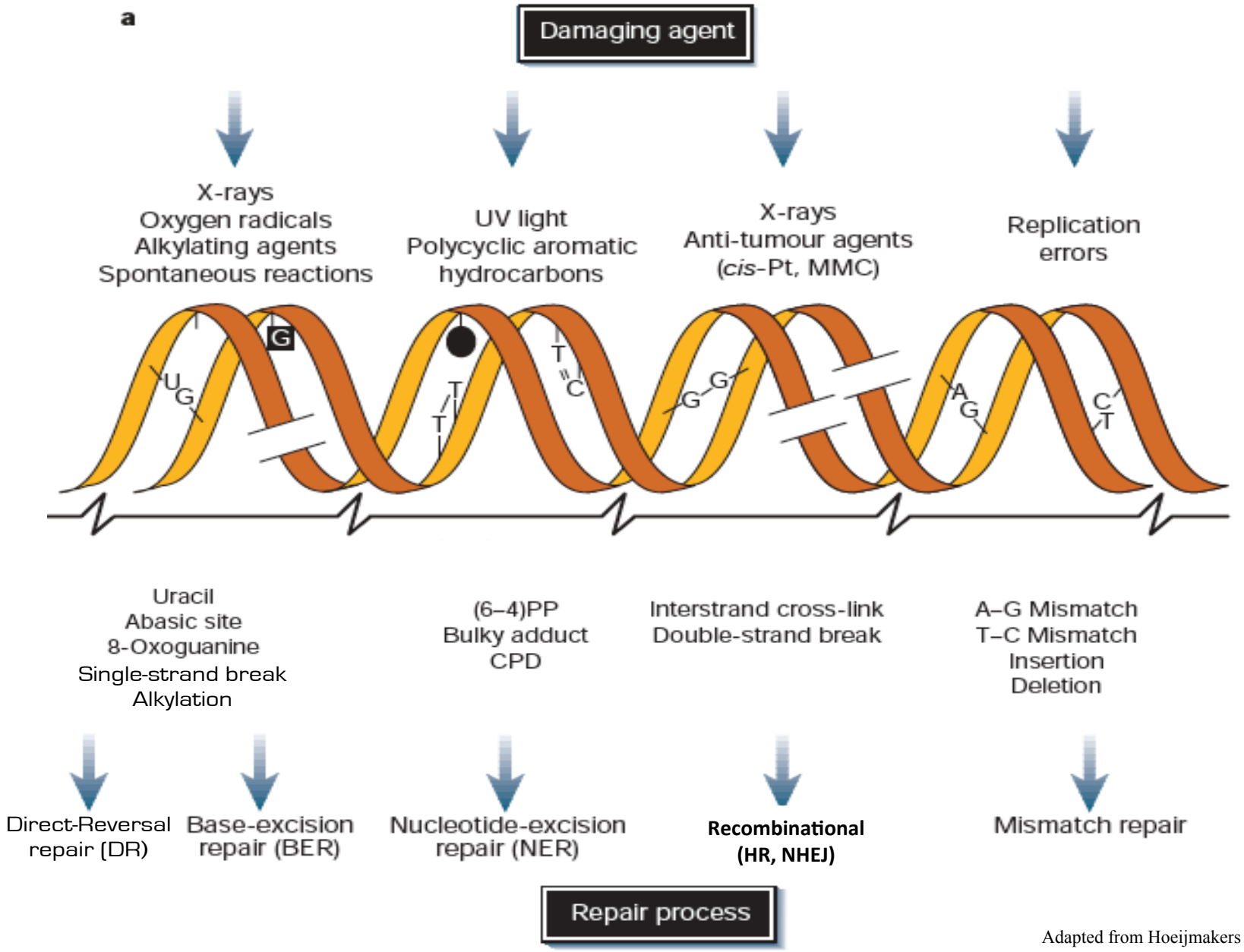


How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

This raises the following questions

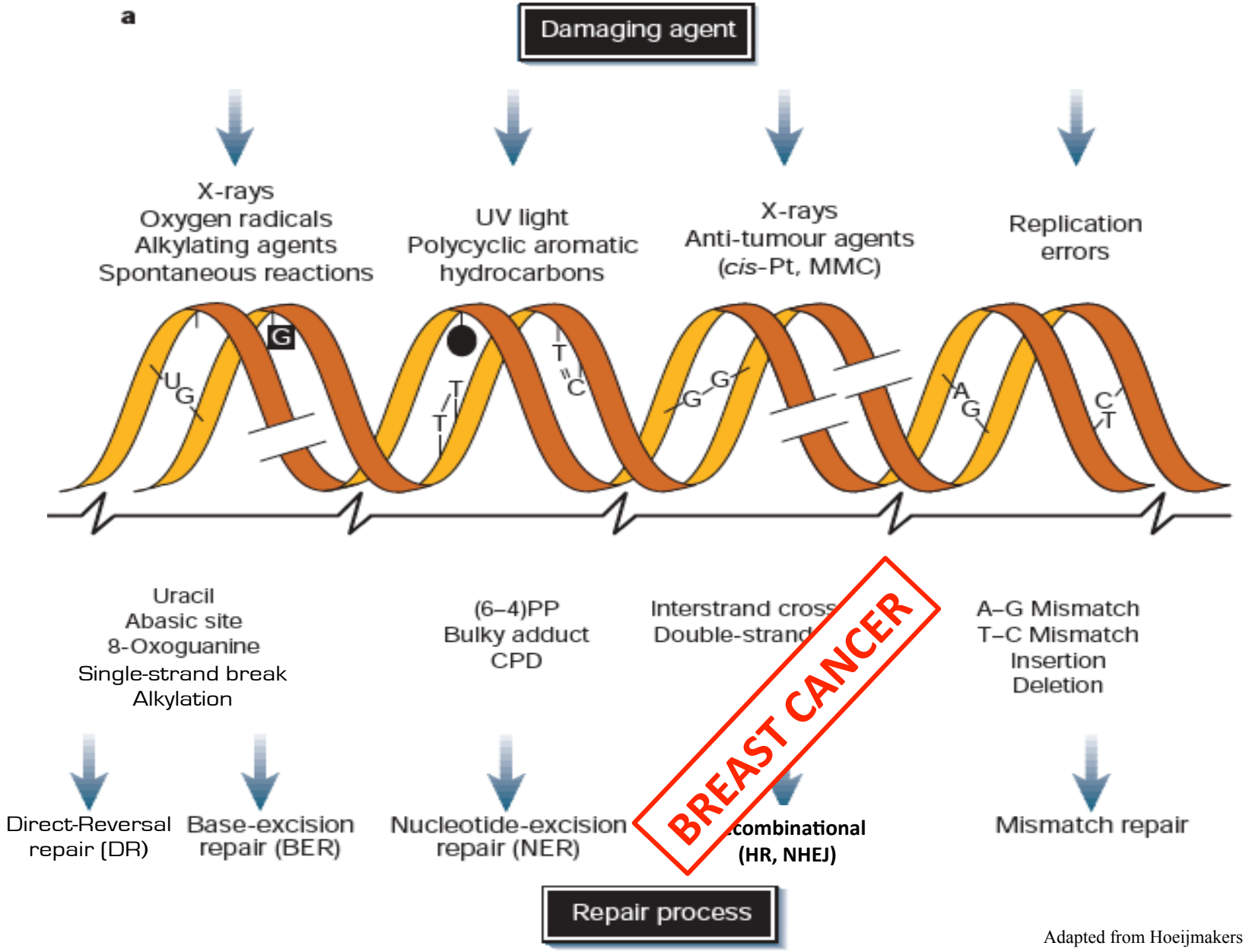
- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair efficiency?

DNA Damage and Repair



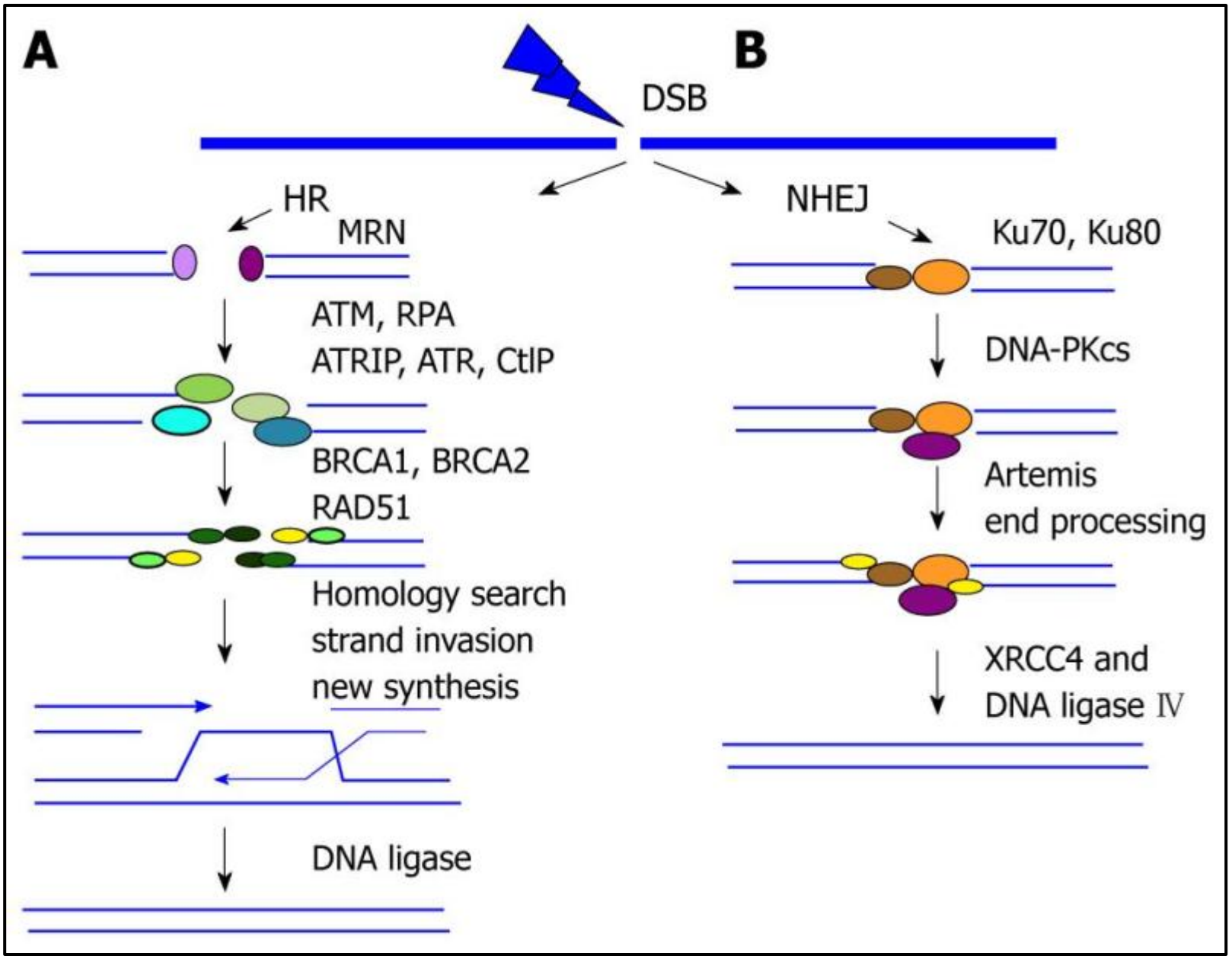
Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

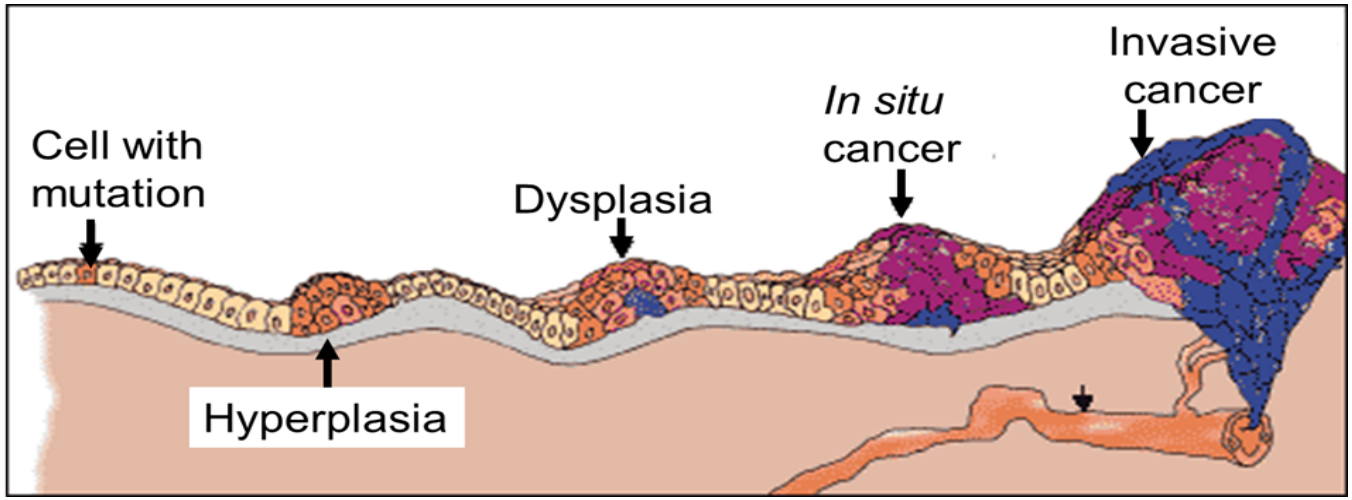
DNA Double Strand Break (DSB) Repair

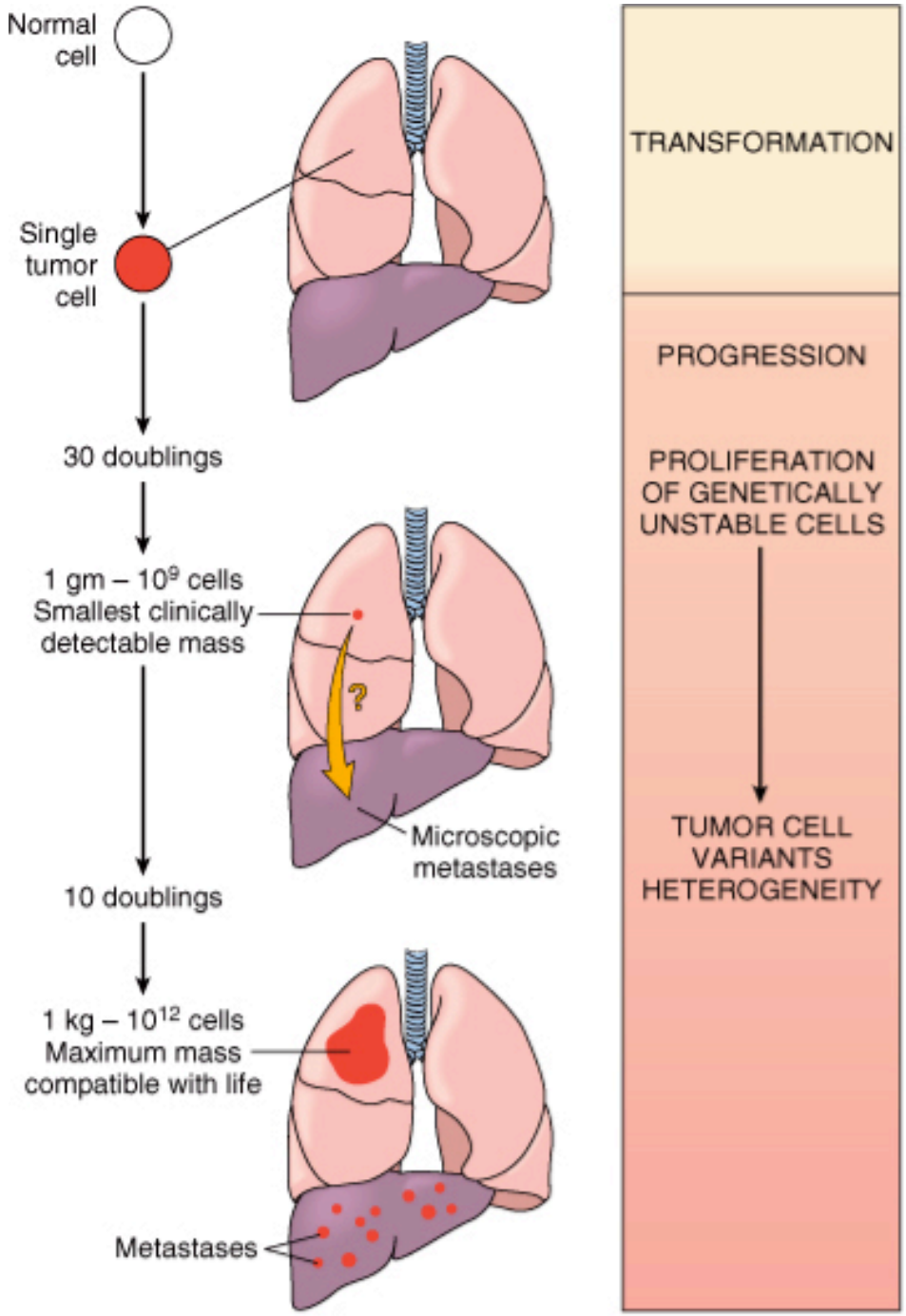


Cancers arise from the accumulation of heritable changes in gene function

Normal Epithelium → Neoplasia → Invasive Carcinoma → Metastasis

1st Mutation → 2nd Mutation → Additional Mutations

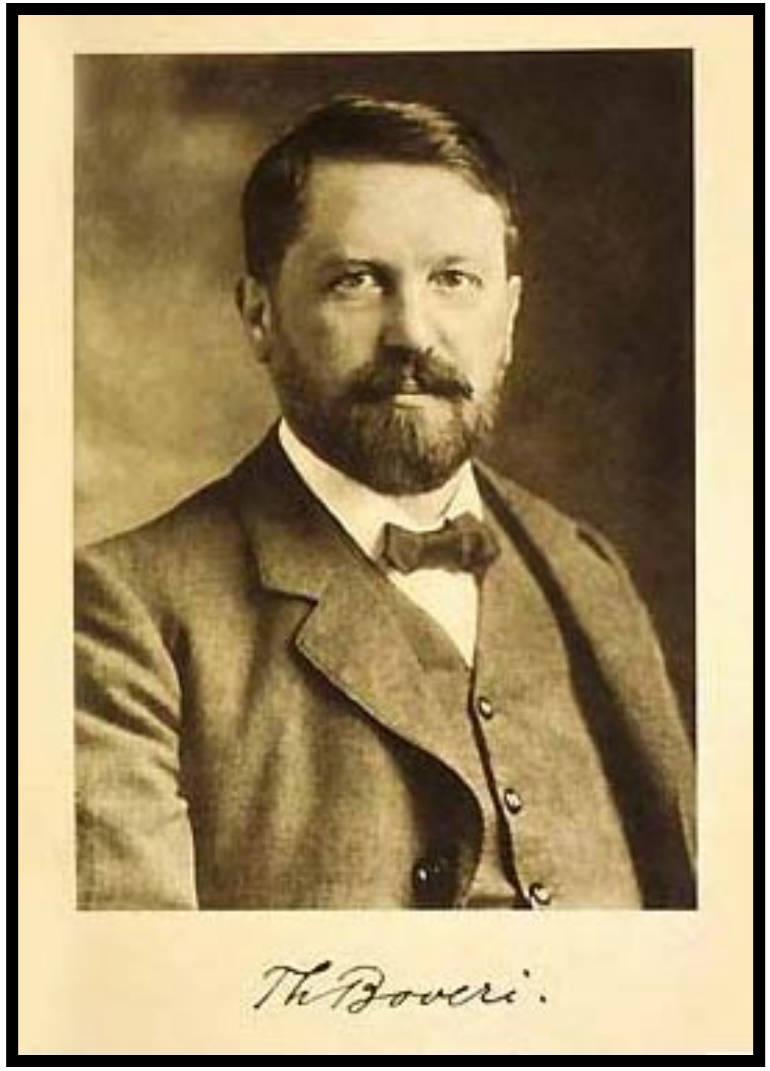




Multiple Mutations

More and more Mutations

The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915



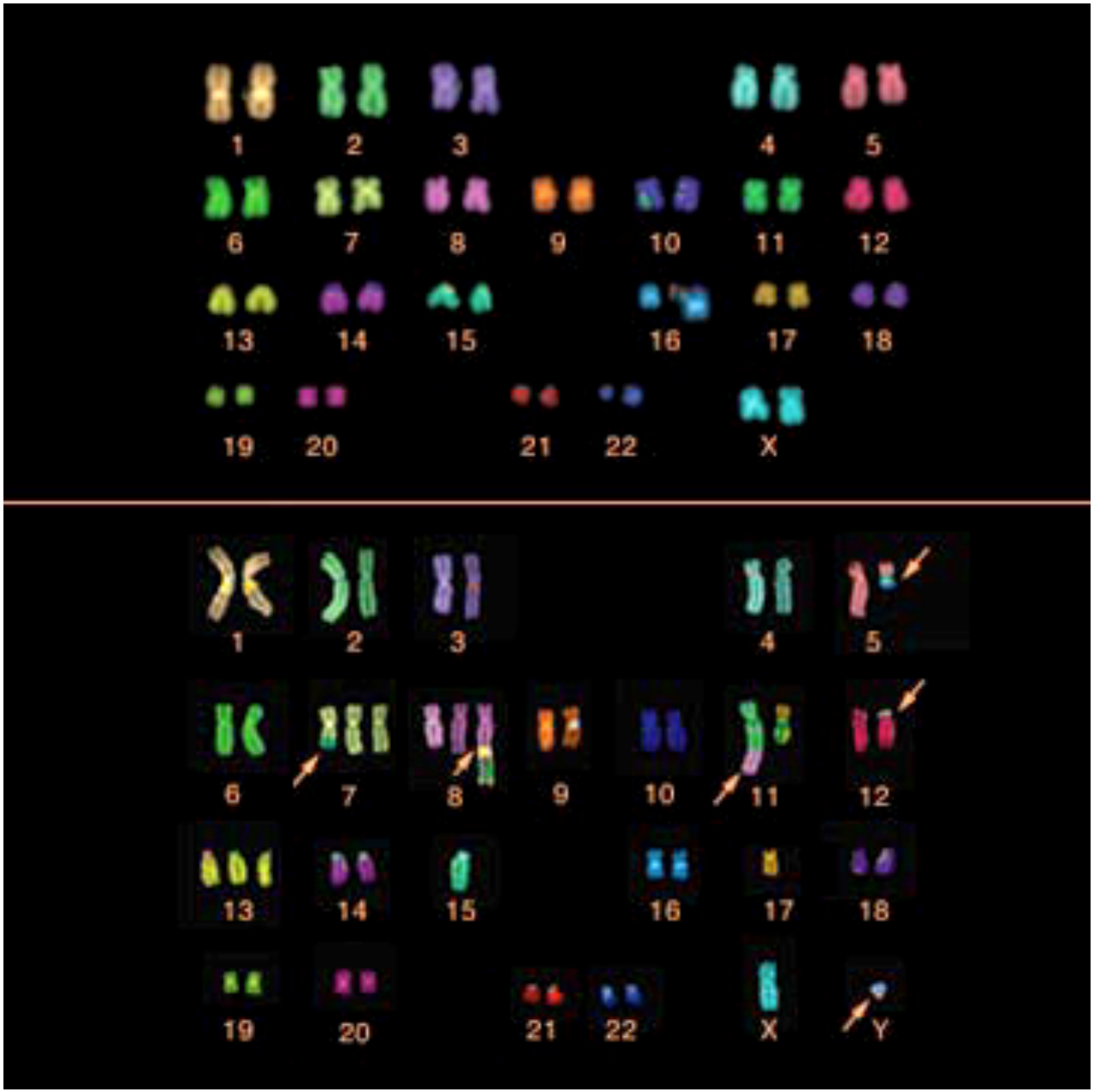
- Established that chromosomes carry the hereditary information by showing that aberrant segregation of chromosomes leads to certain phenotypes in sea urchin eggs.
- Suggested that aberrant segregation of human chromosomes could be responsible for a normal cell becoming a tumor cell
- Suggested that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



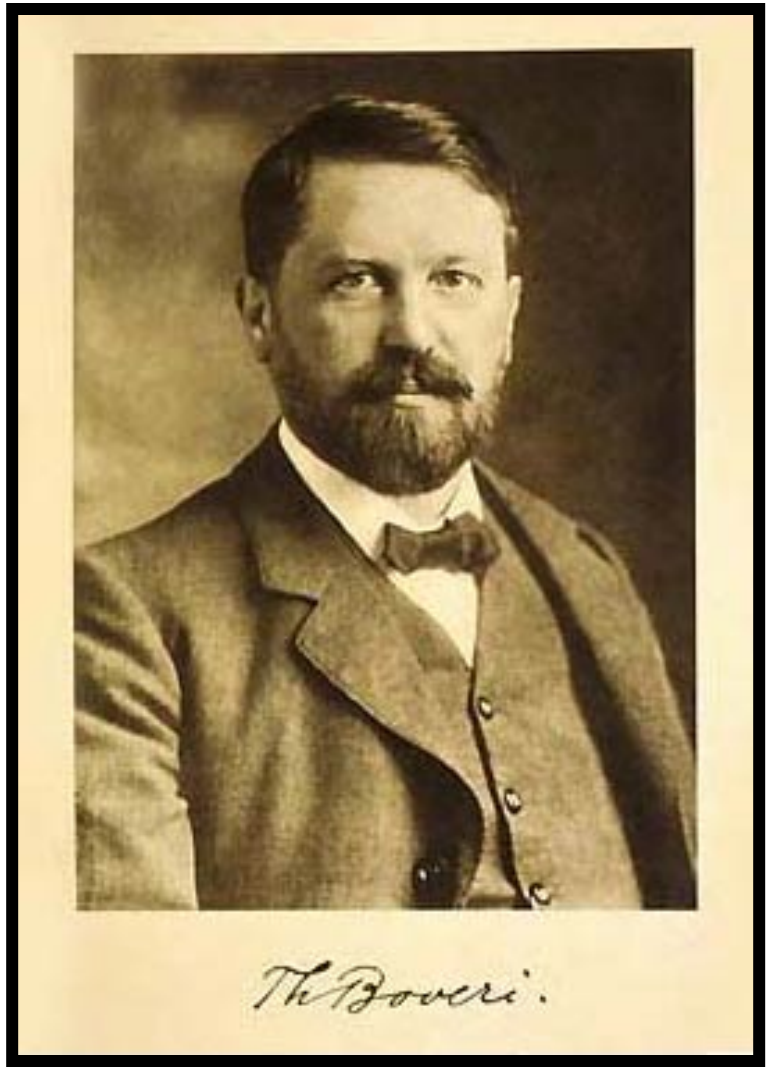
Chromosomes from a Normal cell

Chromosomes from a Tumor cell

Spectral Karyotyping (SKY)
"SKY Painted Chromosomes"

Chromosomes from a Pancreatic Tumor Cell

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

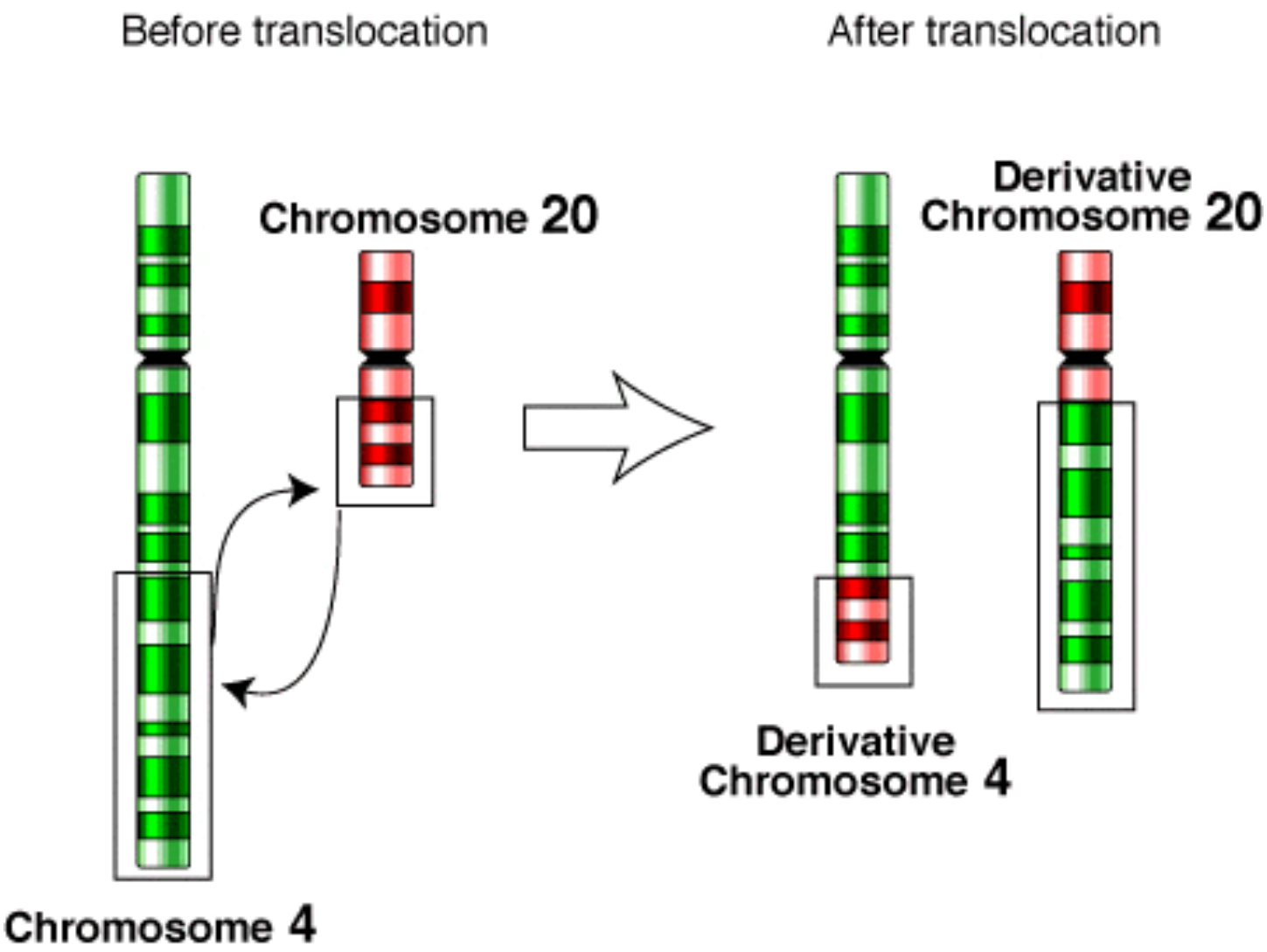
Tumor suppressor genes

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

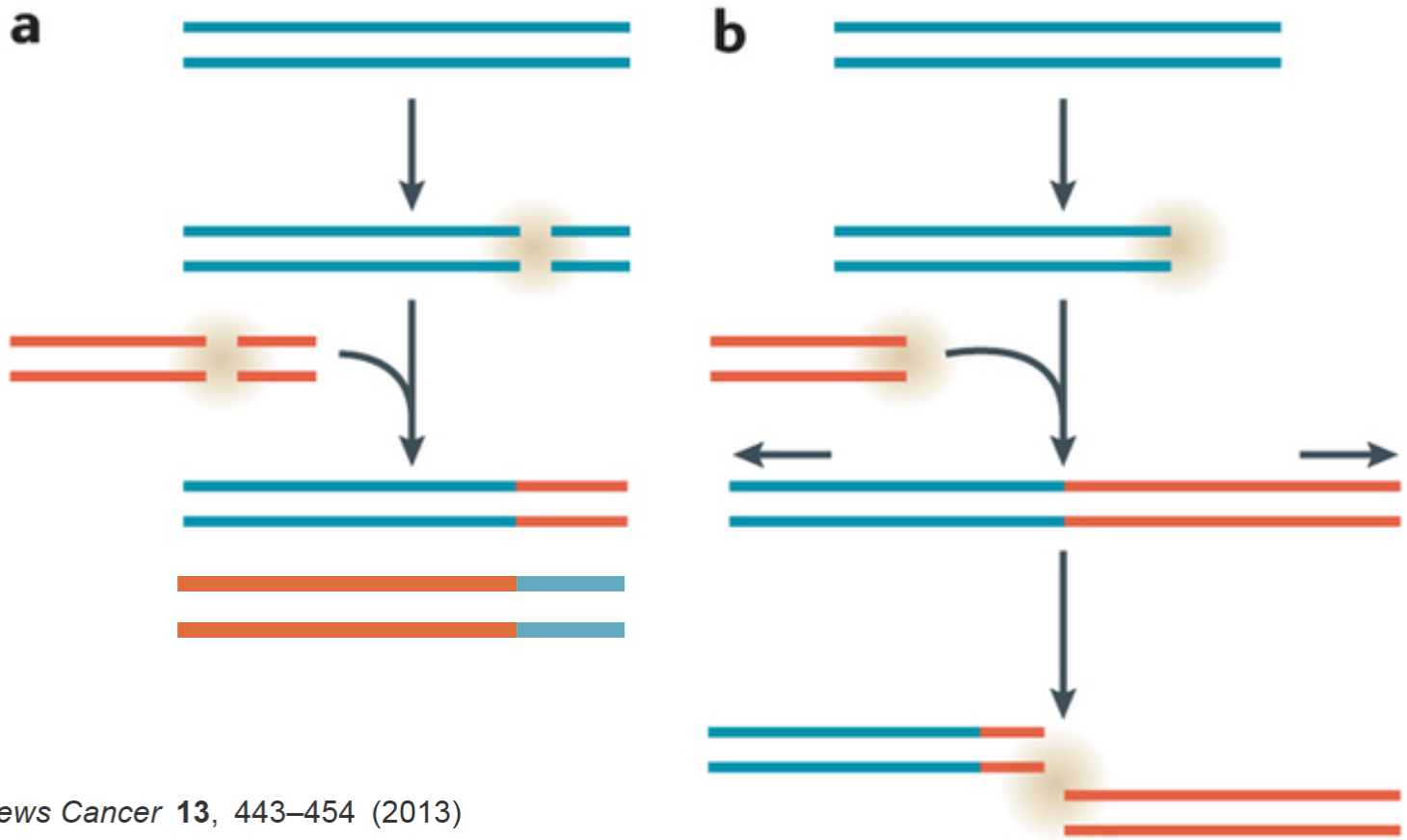
Mutator genes

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

Mechanisms of Chromosome Translocation



Mechanisms of Chromosome Translocation

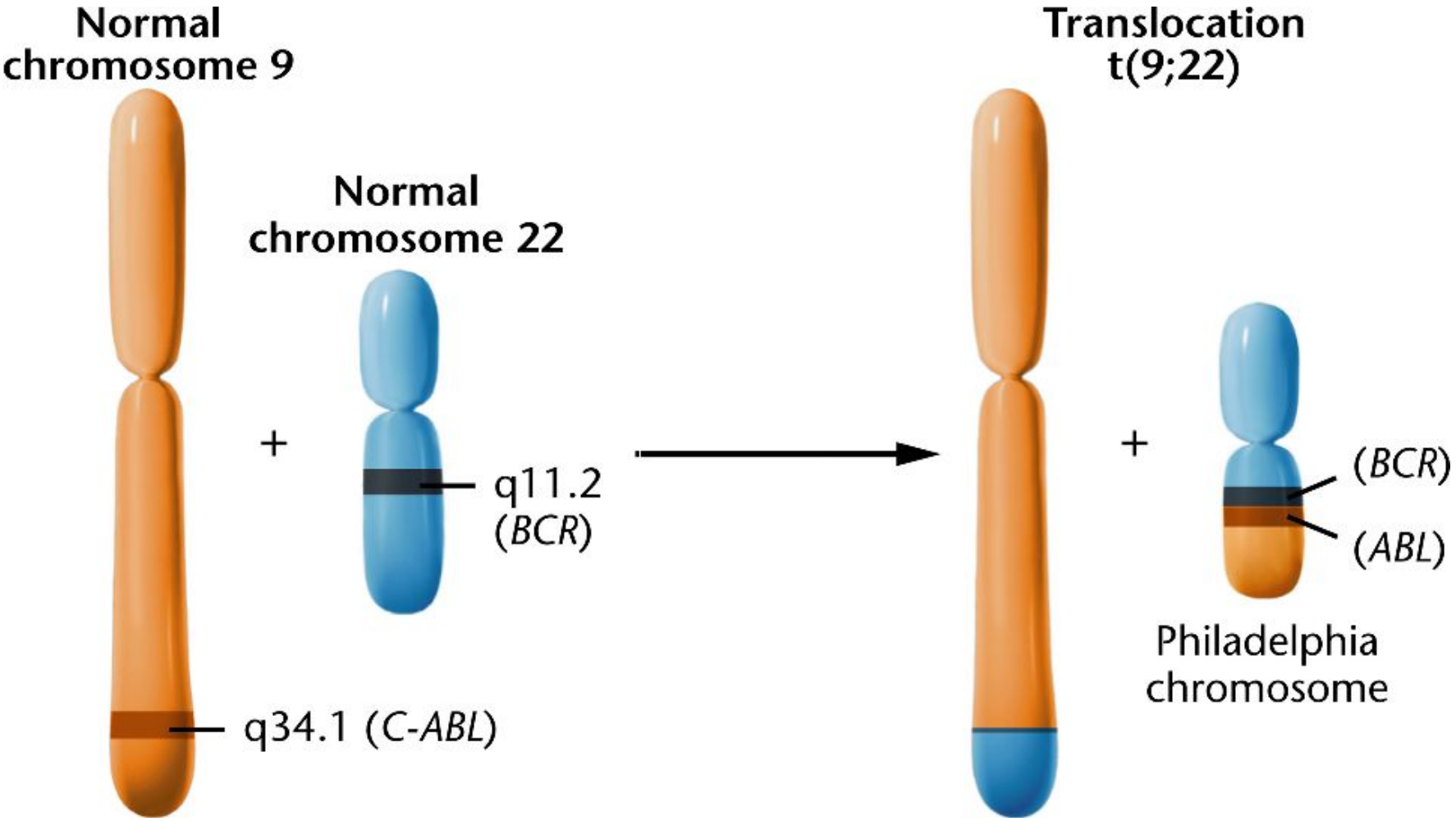


Nature Reviews Cancer **13**, 443–454 (2013)

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.

Chronic Myelogenous Leukemia

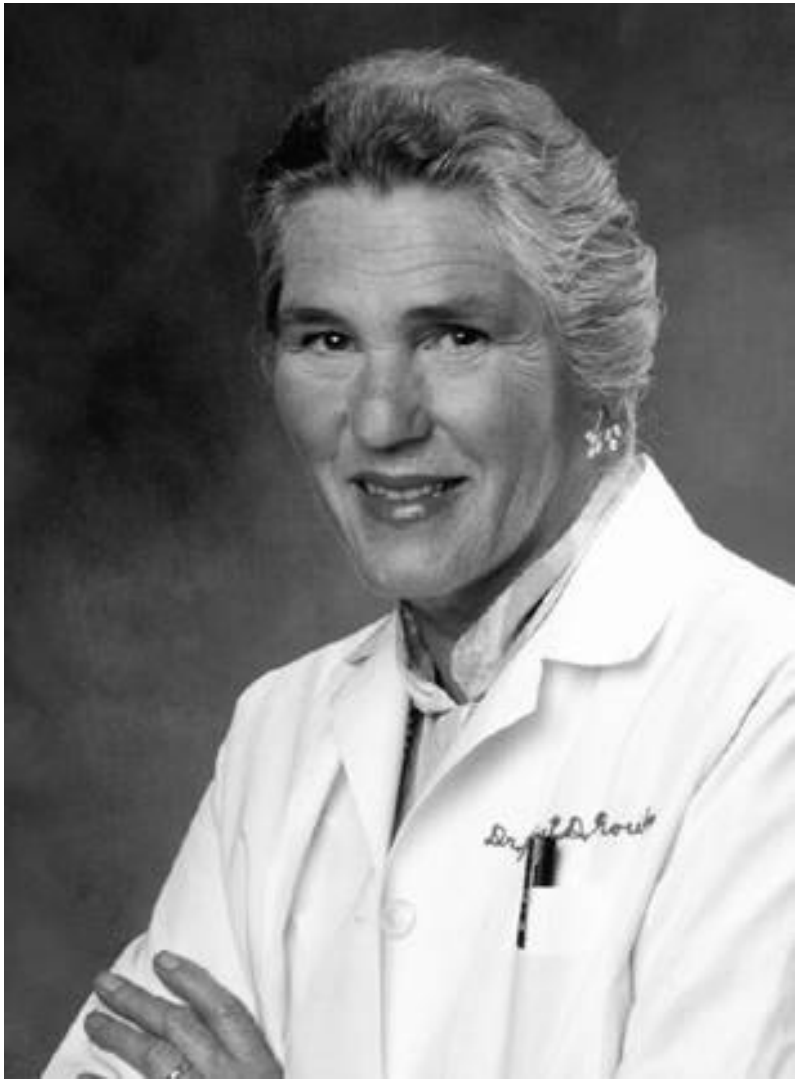


breakpoint cluster region protein (**BCR**) C-Abl receptor tyrosine kinase

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.



Large Deletions or Insertions

SKY chromosome painting: breast cancer

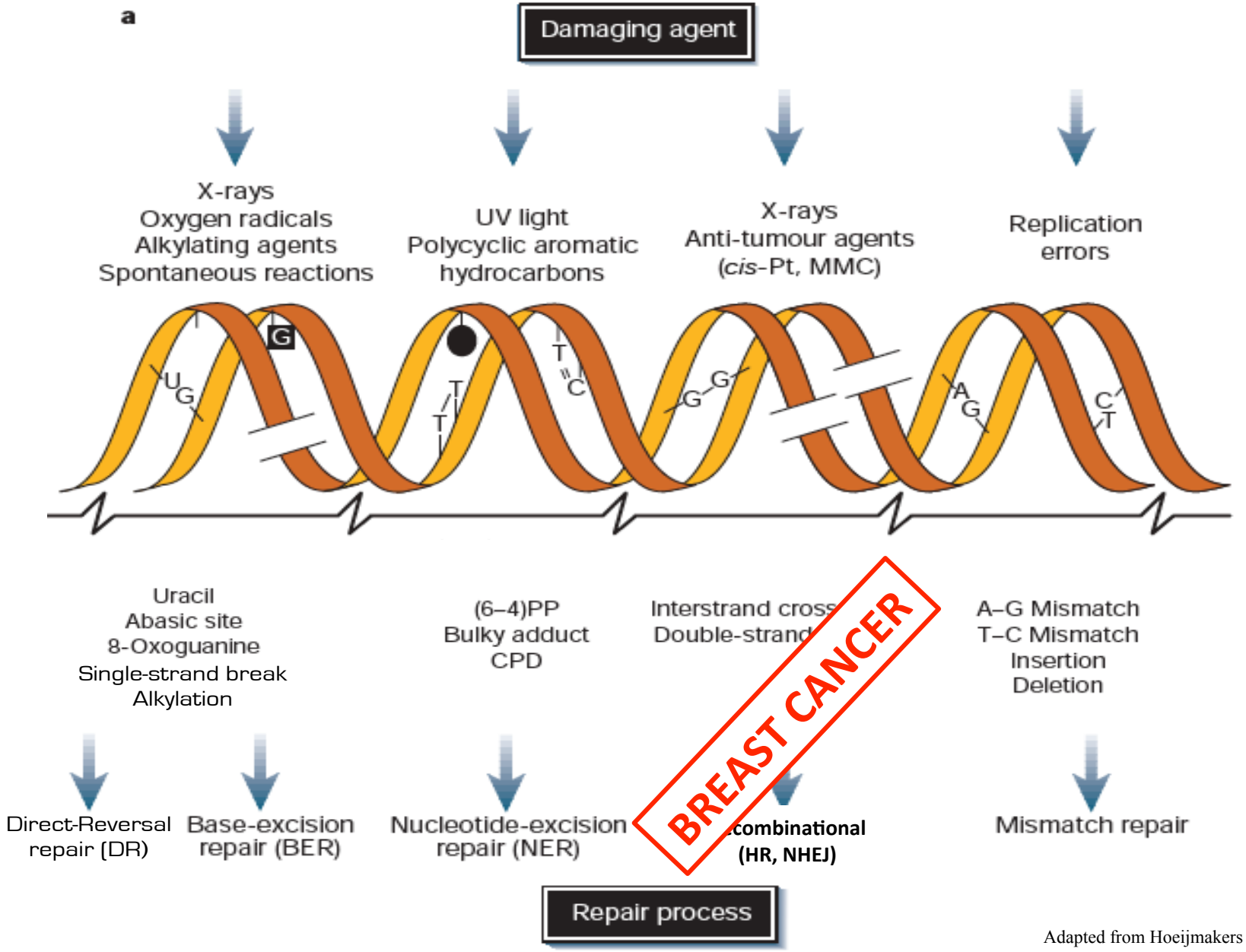
Artwork by Jeanne Kelly © 2004

Normal SKY chromosomes are not multicolored.

Chromosomes in breast cancer appear multicolored because they have exchanged genetic material.

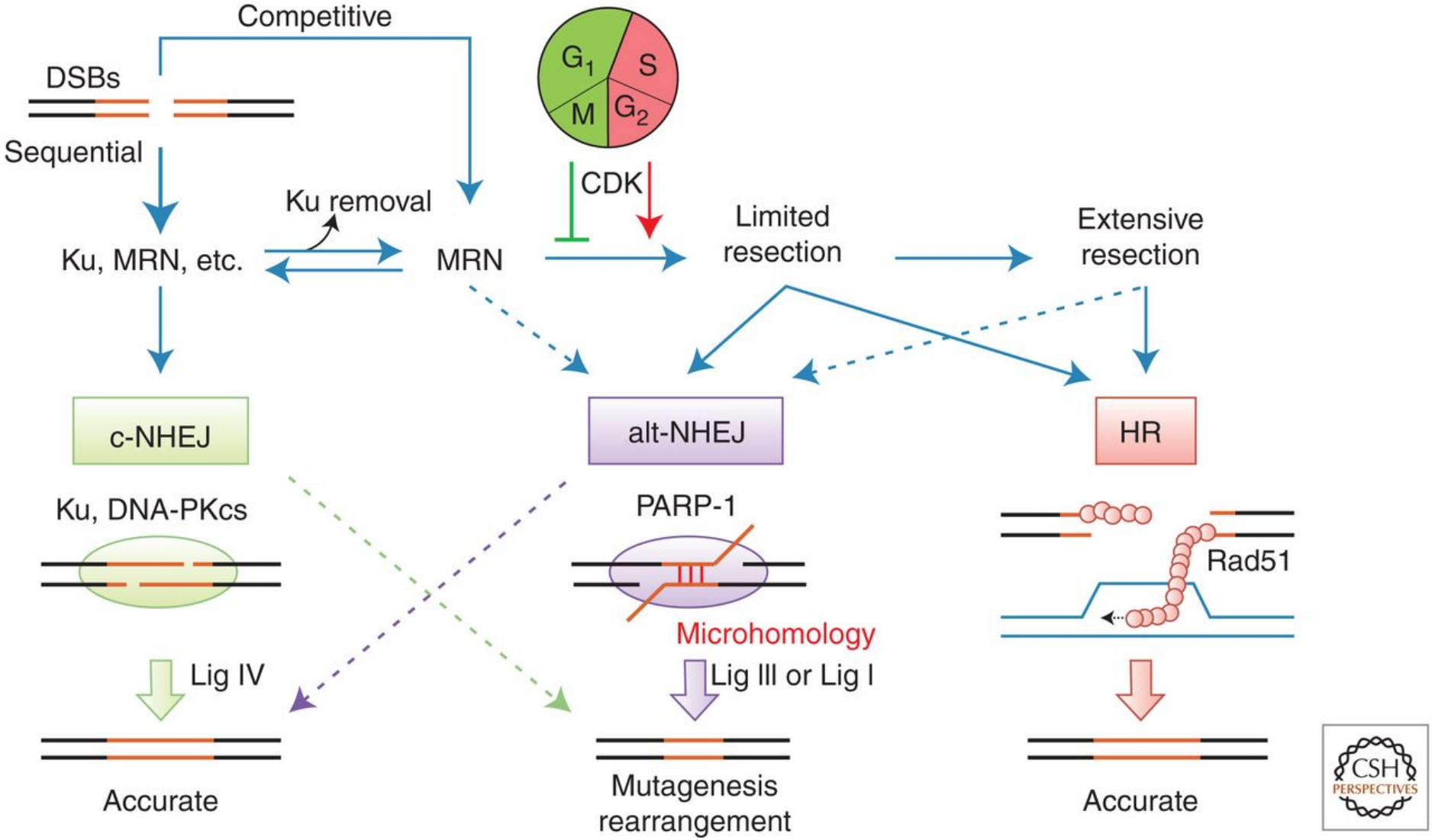
NATIONAL CANCER INSTITUTE

DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

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

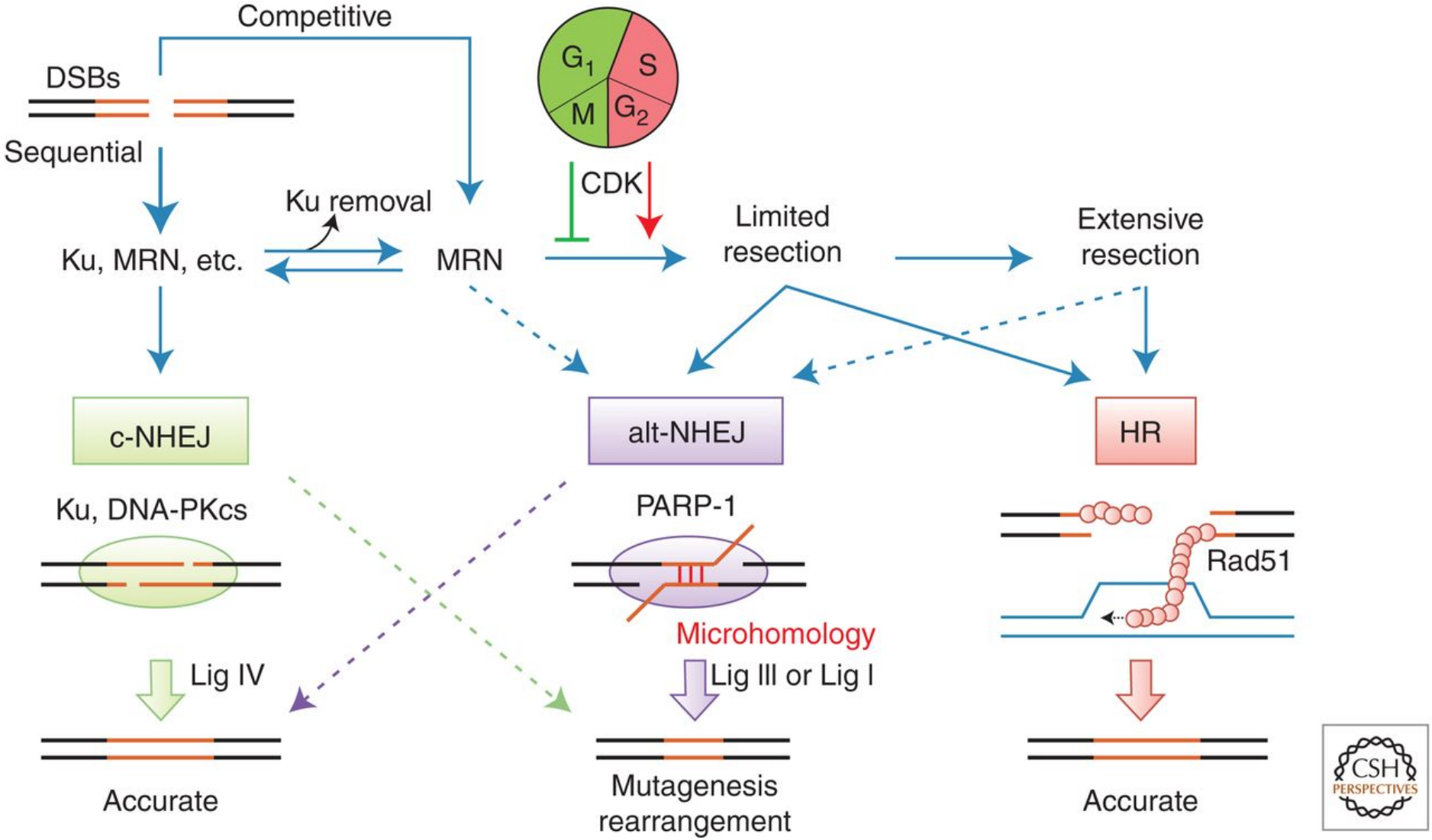
http://web.mit.edu/engelward-lab/animations/SSA.html

Synthesis-Dependent Strand Annealing (Homologous Recombination)

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

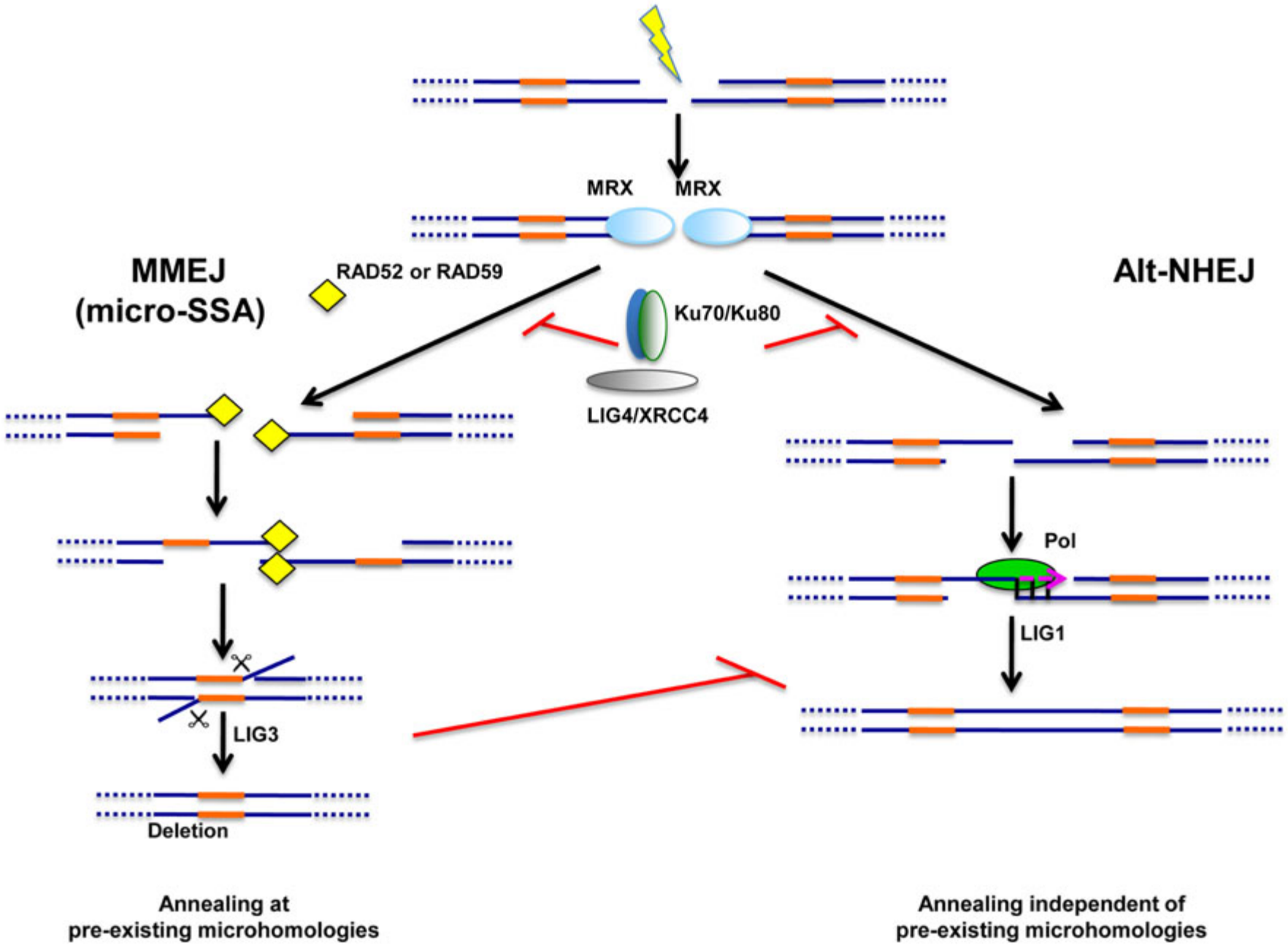
Engelward lab Animations

Disposition of DSBs between repair pathways.

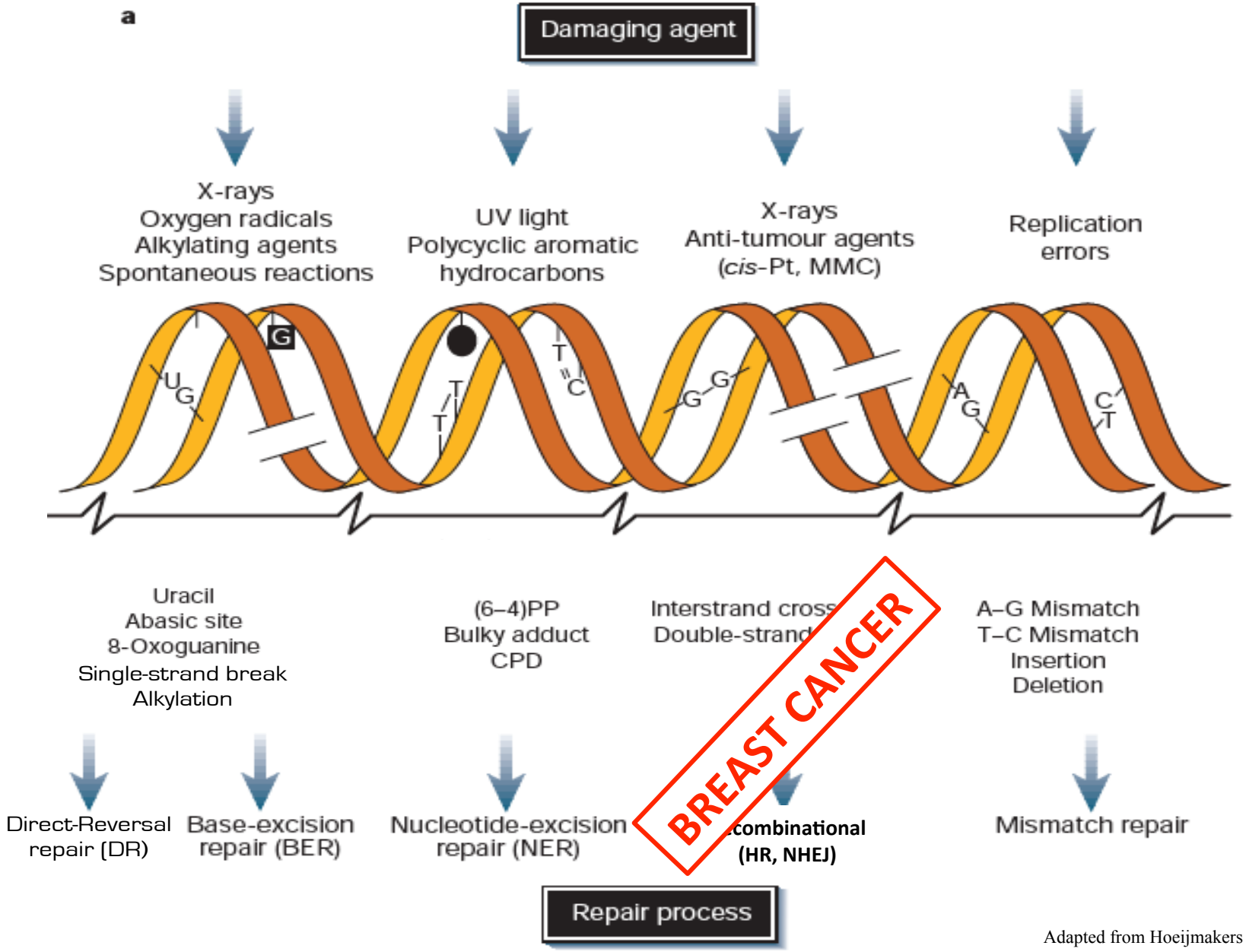


Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757

Ever more “Alternative Non Homologous End Joining Pathways



DNA Damage and Repair



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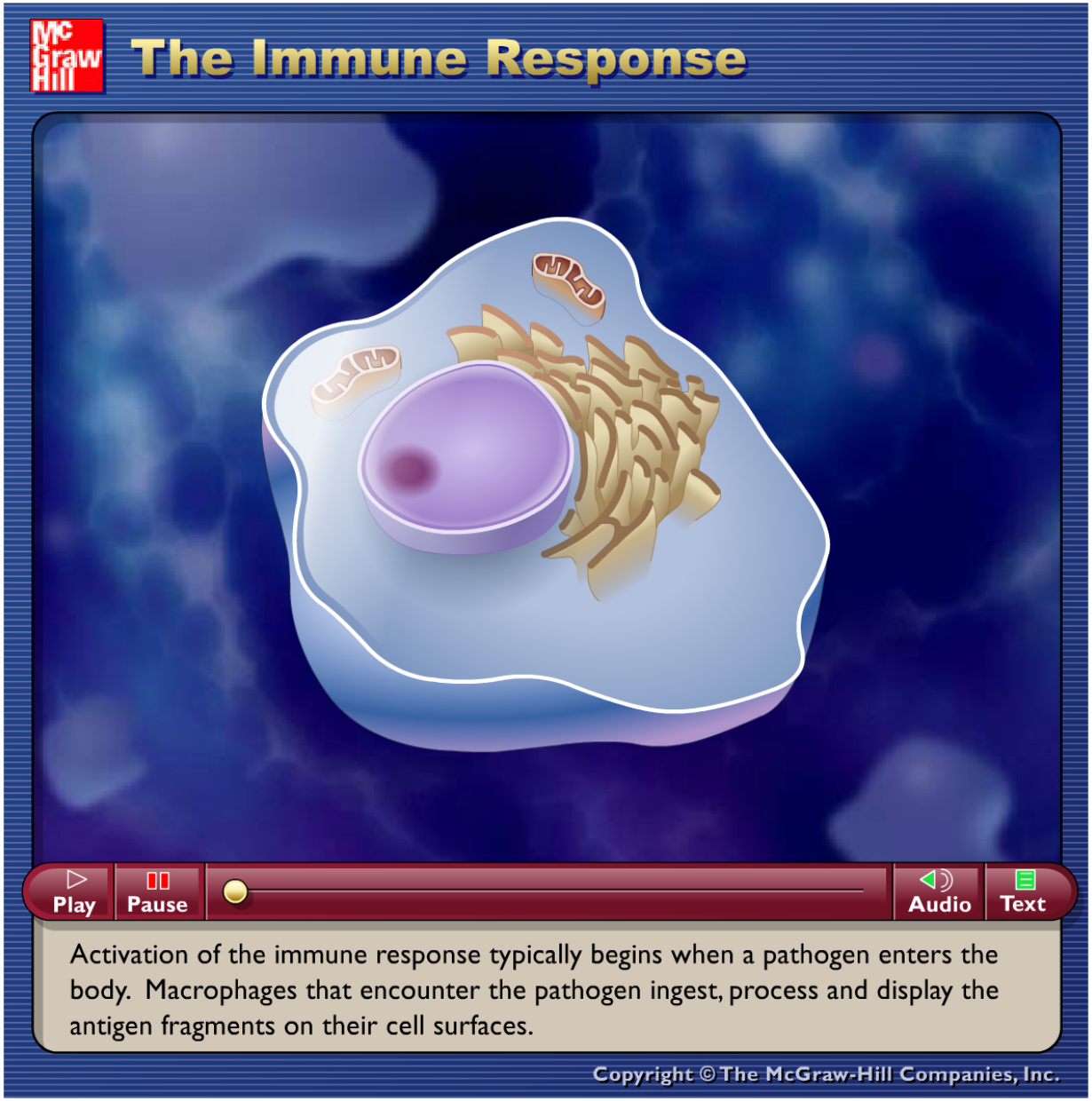
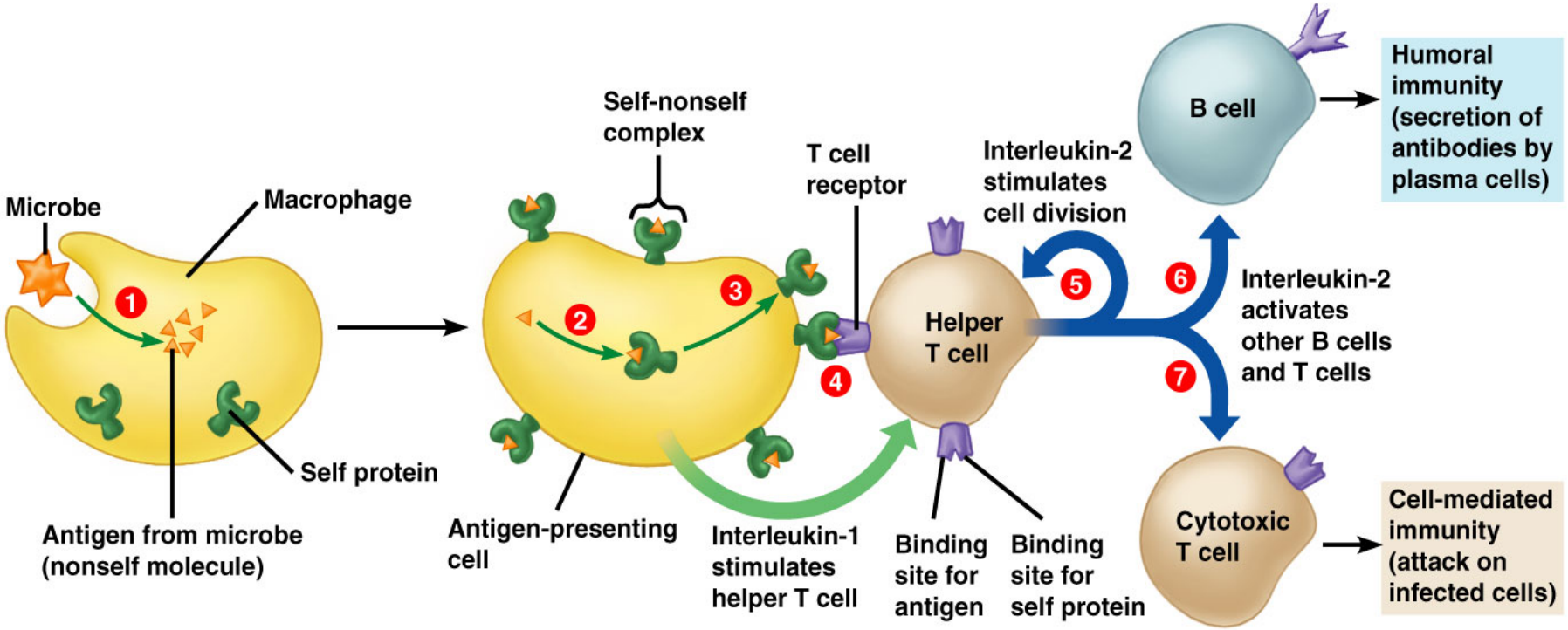
Non Homologous End Joining is REQUIRED for a functional immune system!



http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter24/animation__the_immune_response.html

The body contains millions of different T-cells and B-cells, each able to respond to one specific antigen.



http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

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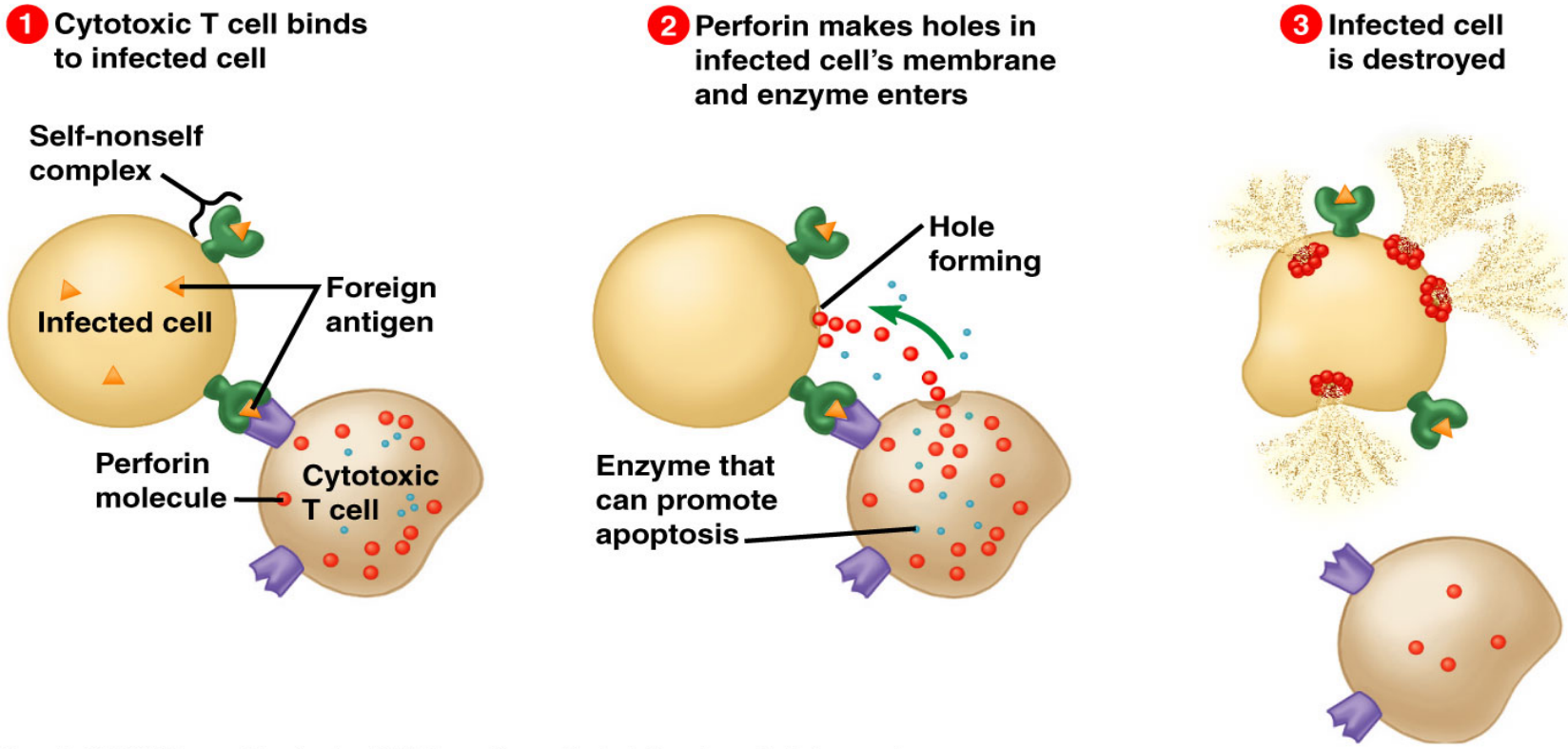

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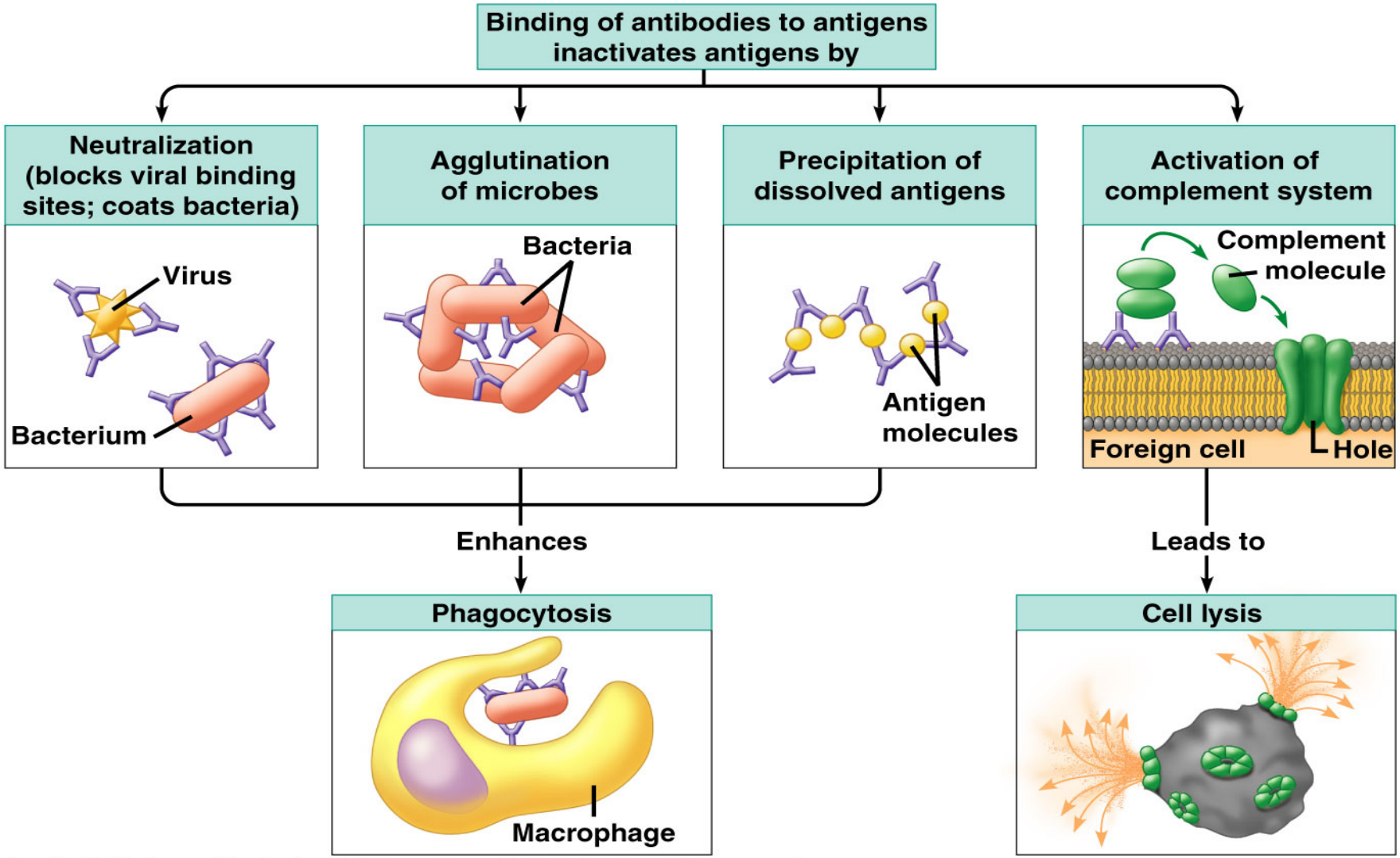
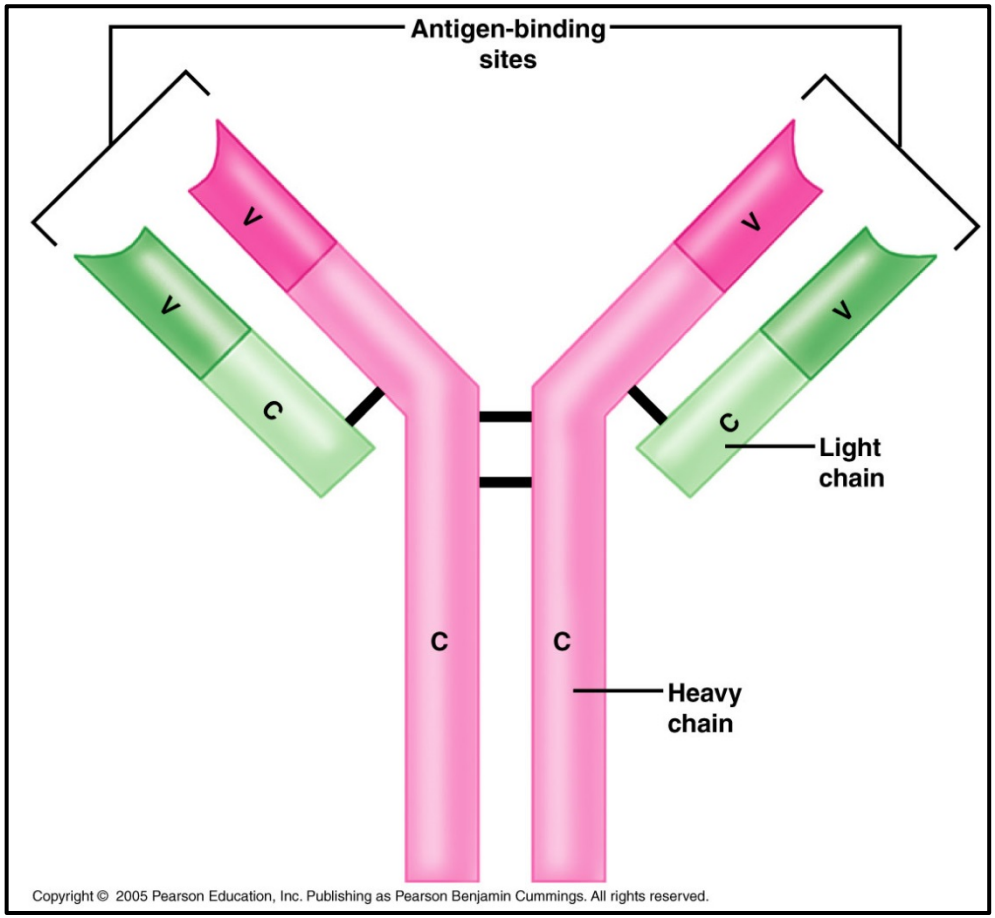
Antibodies work in different ways



http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

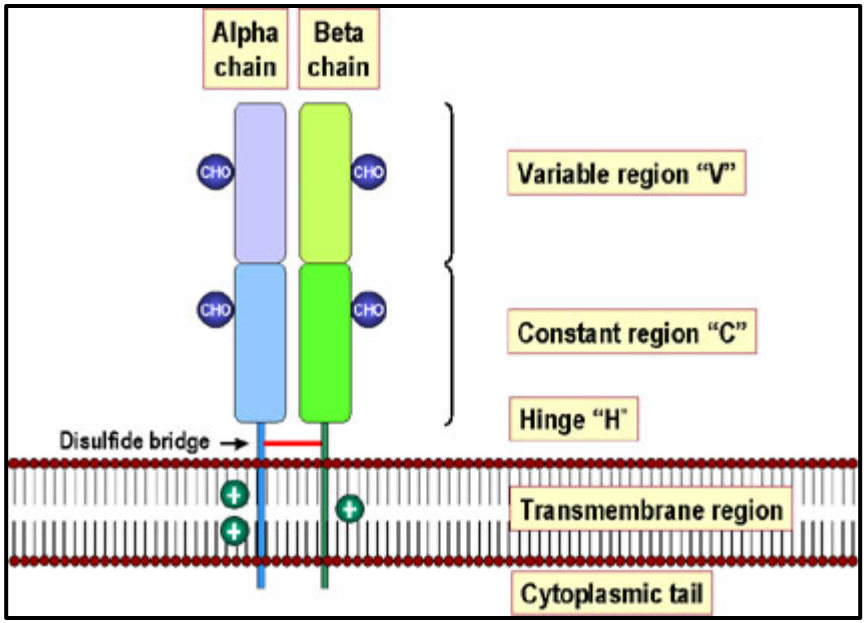
"antigen" comes from **ANTI**-body **GEN**erating substances



http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

B-cell Immunoglobulin

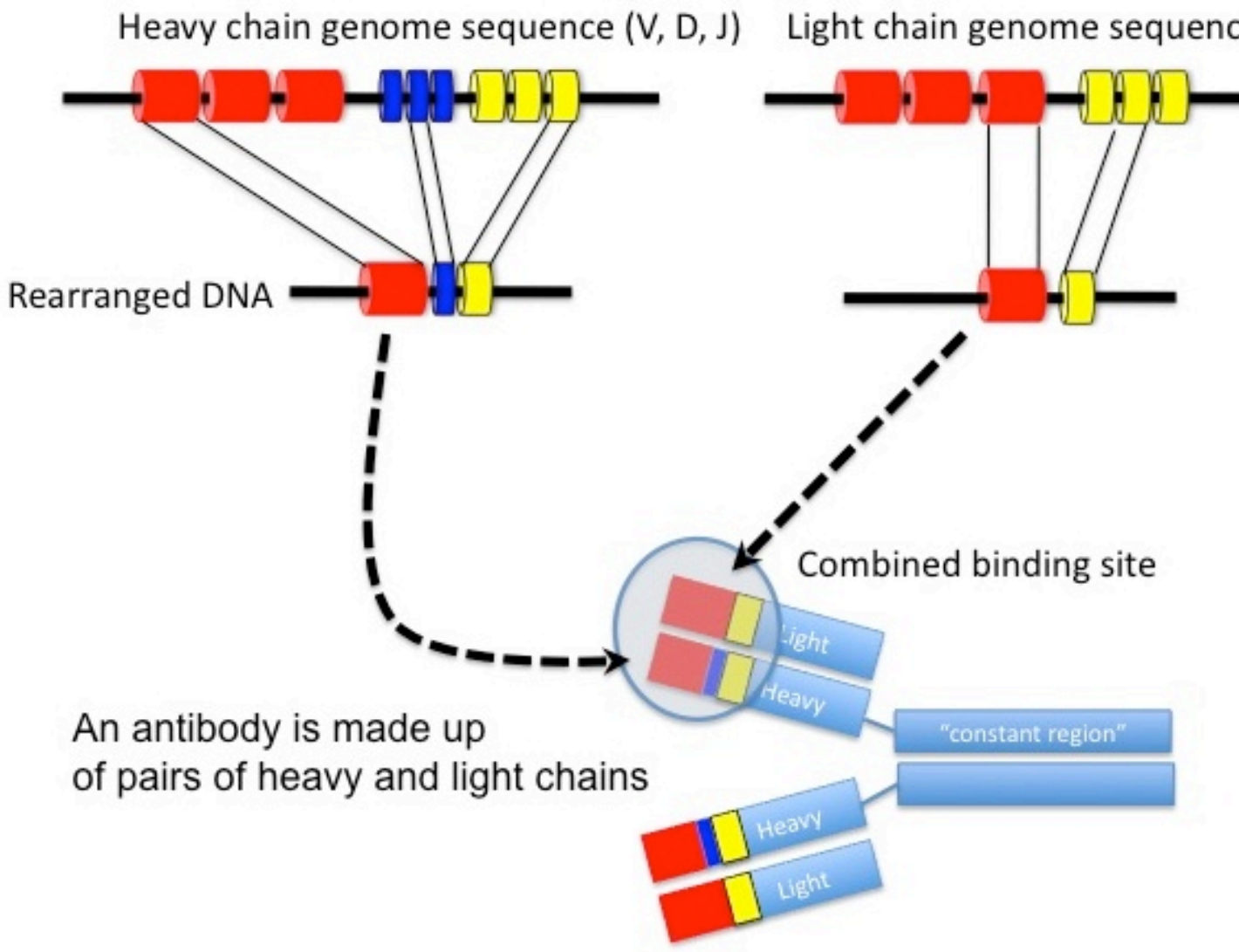
Antigen Binding Site



http://pathmicro.med.sc.edu/bowers/mhc.htm

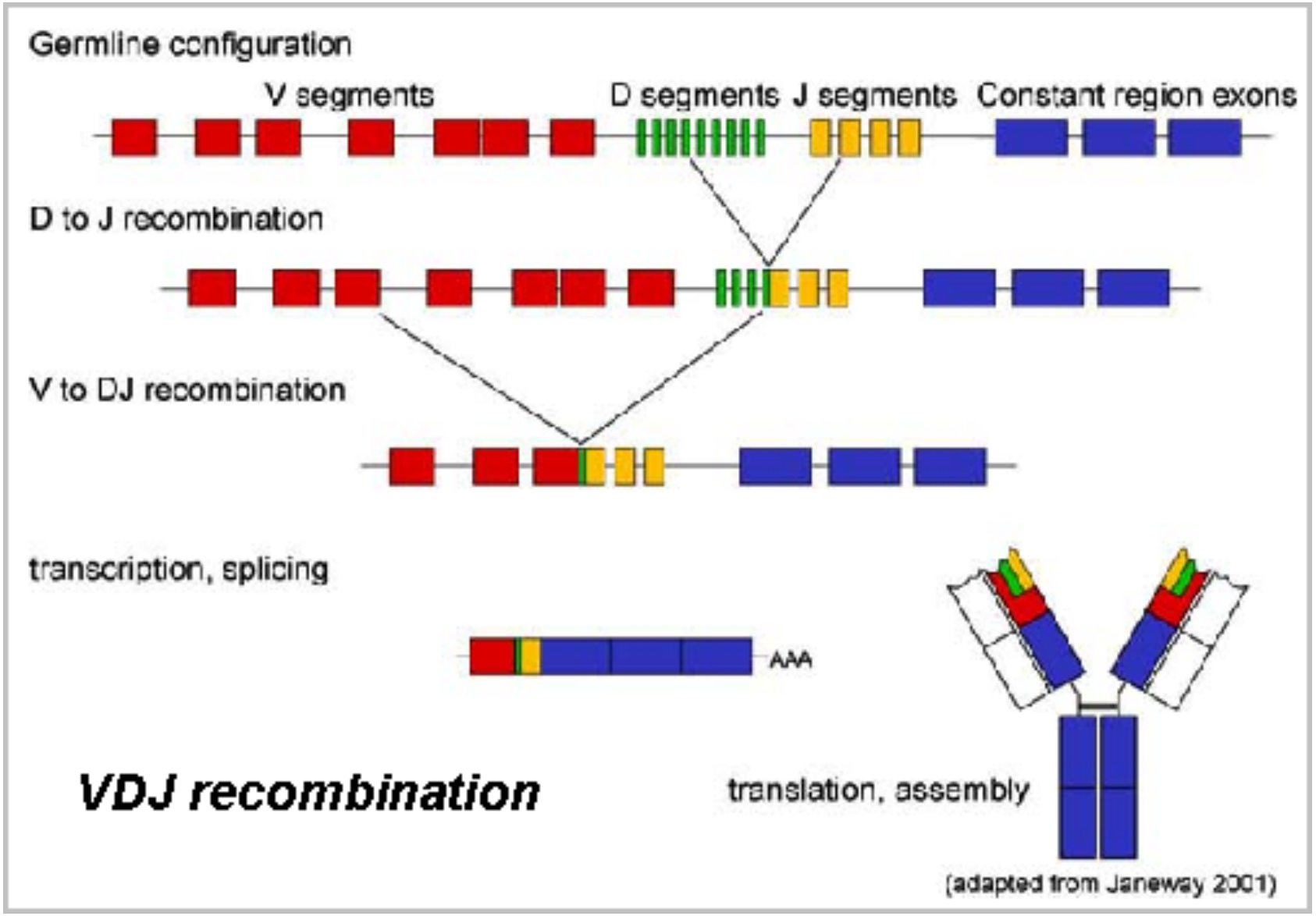
T-cell Receptor

How Do the Variable Regions become Variable? Through Programmed NHEJ!!



V -variable,
D - diversity
J- joining
gene
segments

How Do the Variable Regions become Variable? Through Programmed NHEJ!!



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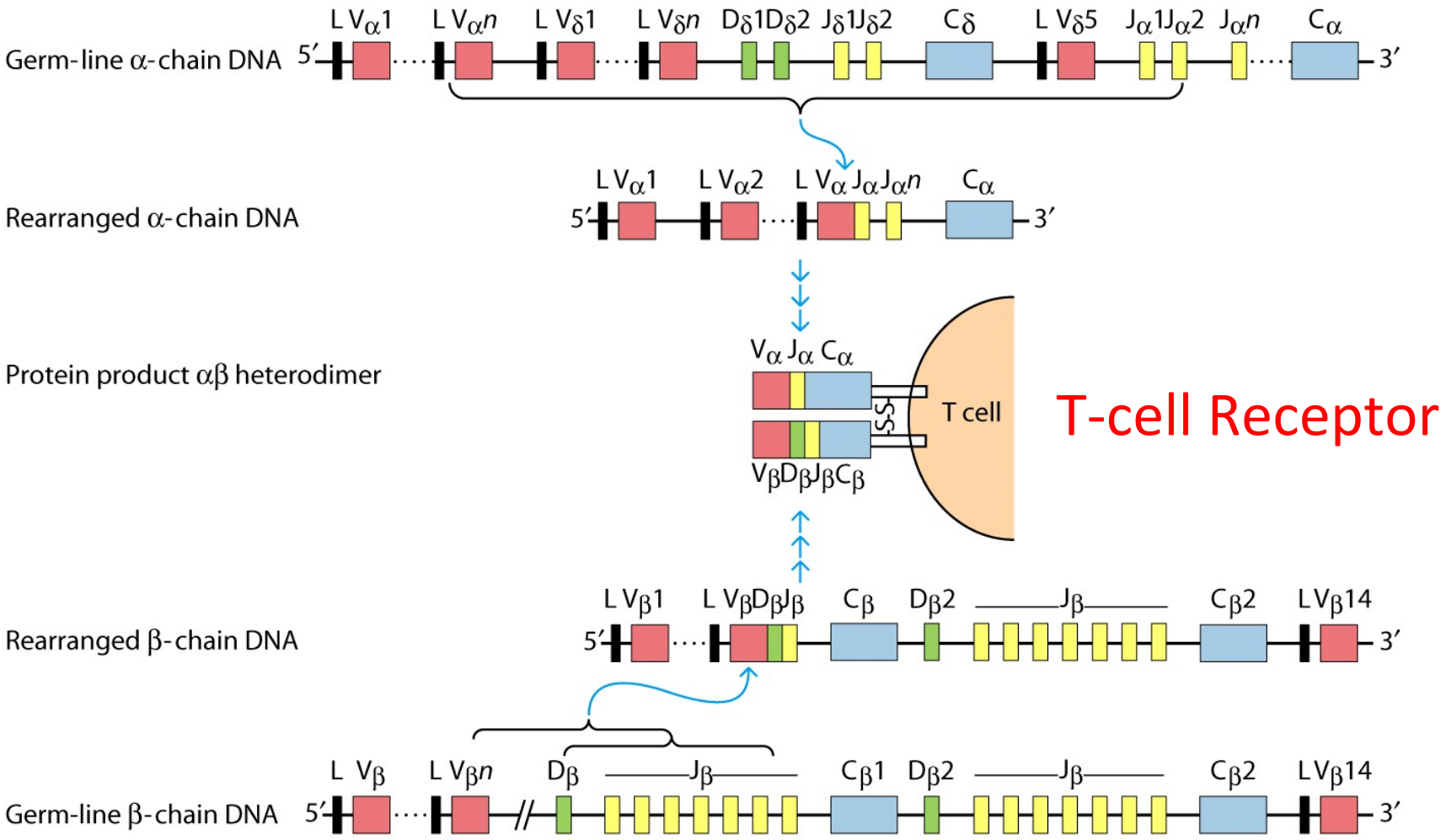
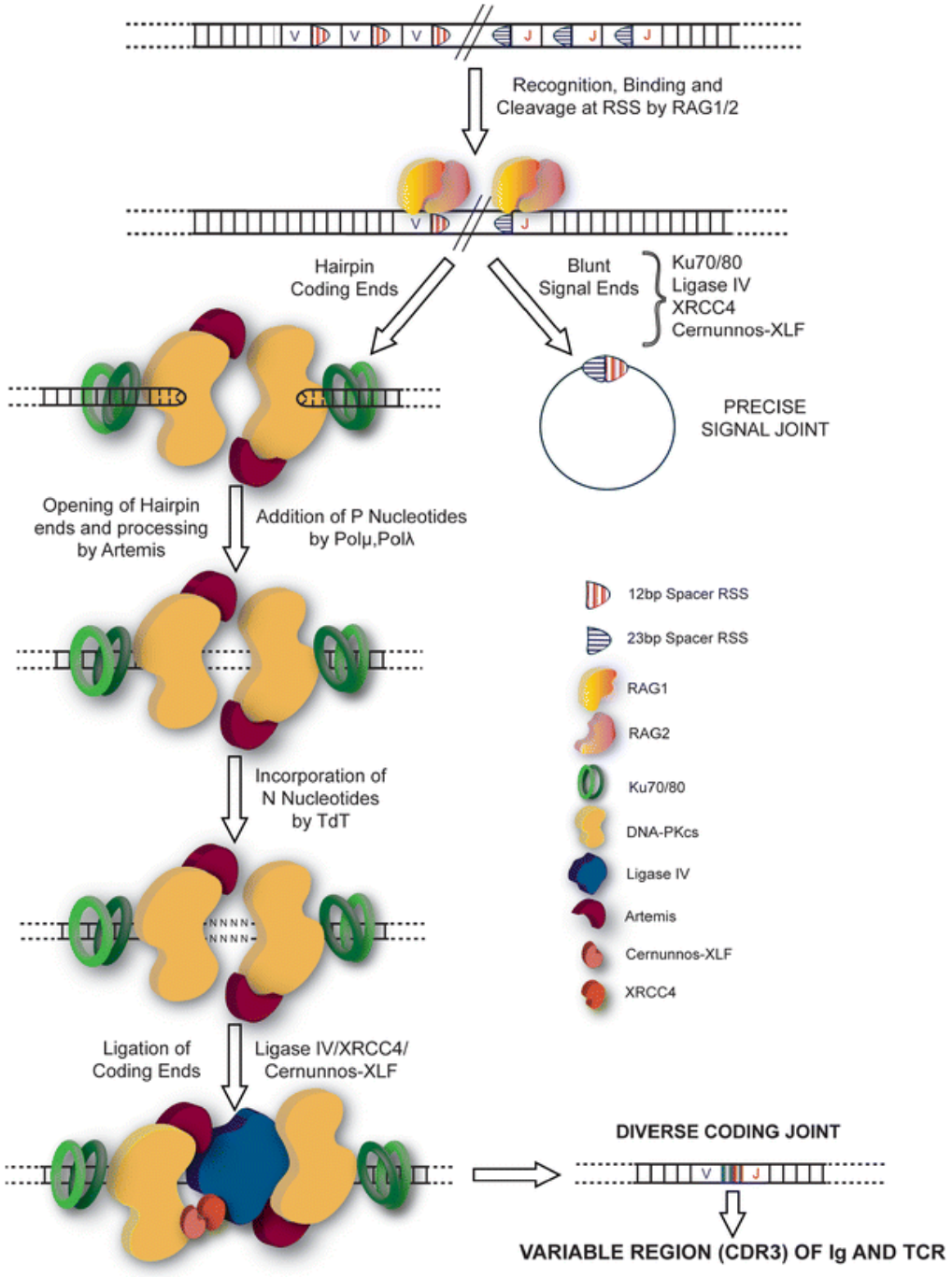


V(D)J Gene Recombination

http://www.youtube.com/watch?v=QTOBSFJWogE

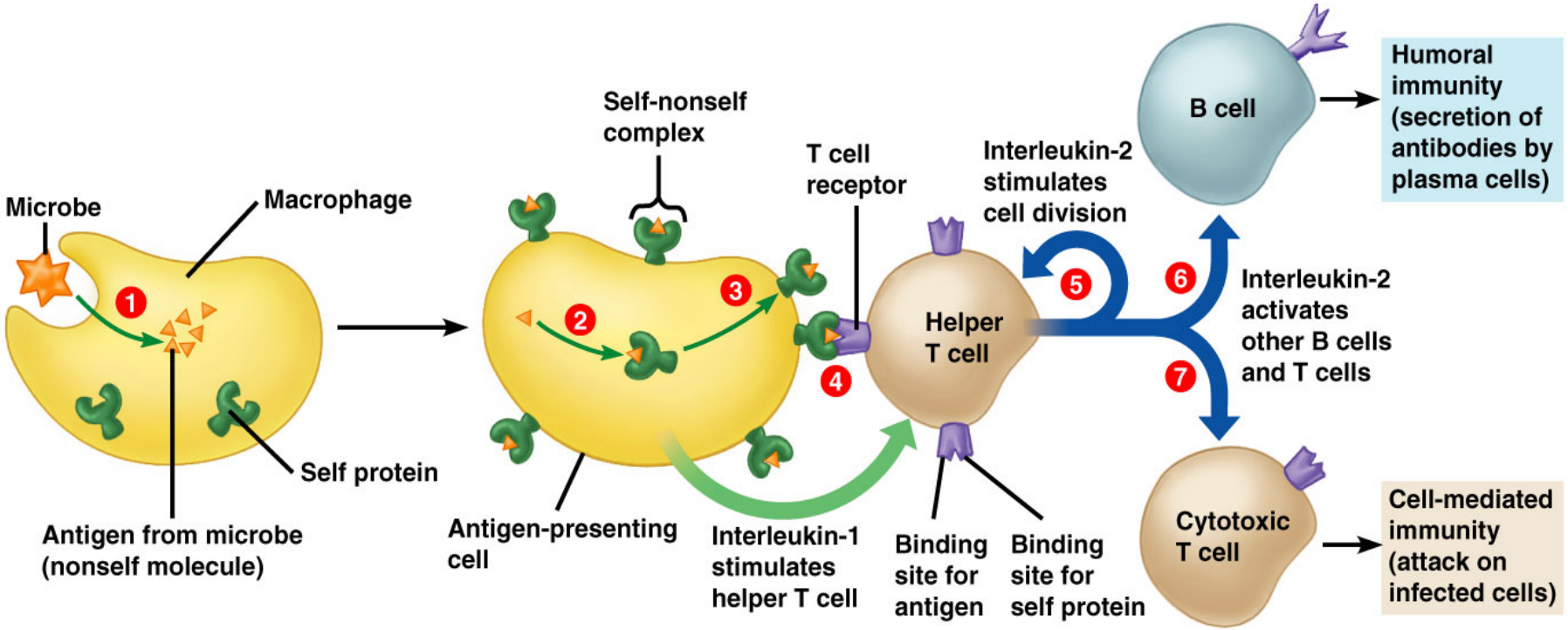


How Do the Variable Regions become Variable? Through NHEJ mediated DNA Recombination!

The rearrangement starts with the binding of products from recombination activating genes RAG1 and RAG2, whose expression is unique to lymphoid progenitor cells

Immunologic Research
December 2012, Volume 54, Issue 1-3, pp 233-246

The body contains millions of different T-cells and B-cells, each able to respond to one specific antigen.



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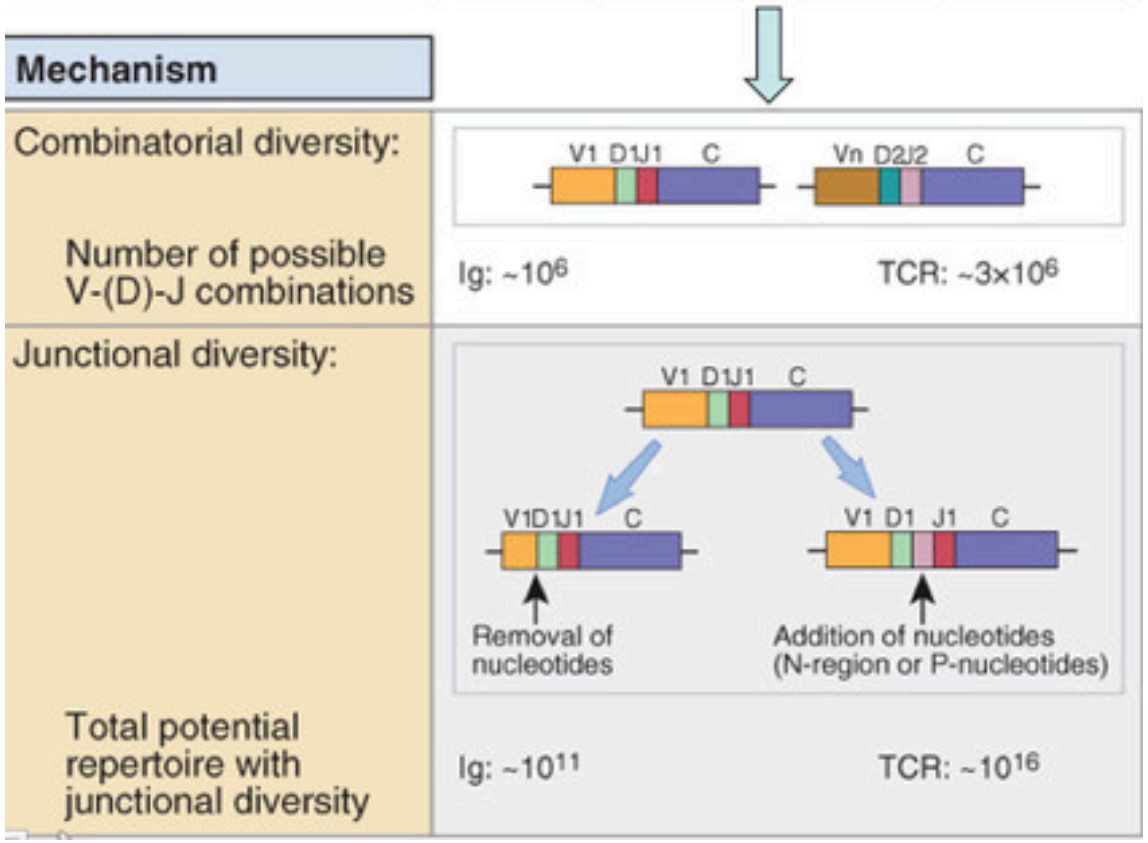
How Variable is Variable?

Number of functional gene segments in human immunoglobulin loci			
Segment	light chains		heavy chain
	κ	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Over 15,000,000 combinations of variable, diversity and joining gene segments are possible. Imprecise recombination and mutation increase the variability into billions of possible combinations.

How Variable is Variable?

	Immunoglobulin		T cell receptor	
	Heavy chain	κ	α	β
Number of V gene segments	~100	35	54	67
Number of diversity (D) gene segments	27	0	0	2
Number of joining (J) gene segments	6	5	61	4



1 - 3,000,000 combinations of variable, diversity and joining gene segments are possible. Imprecise recombination and mutation increase the variability into billions of possible combinations.

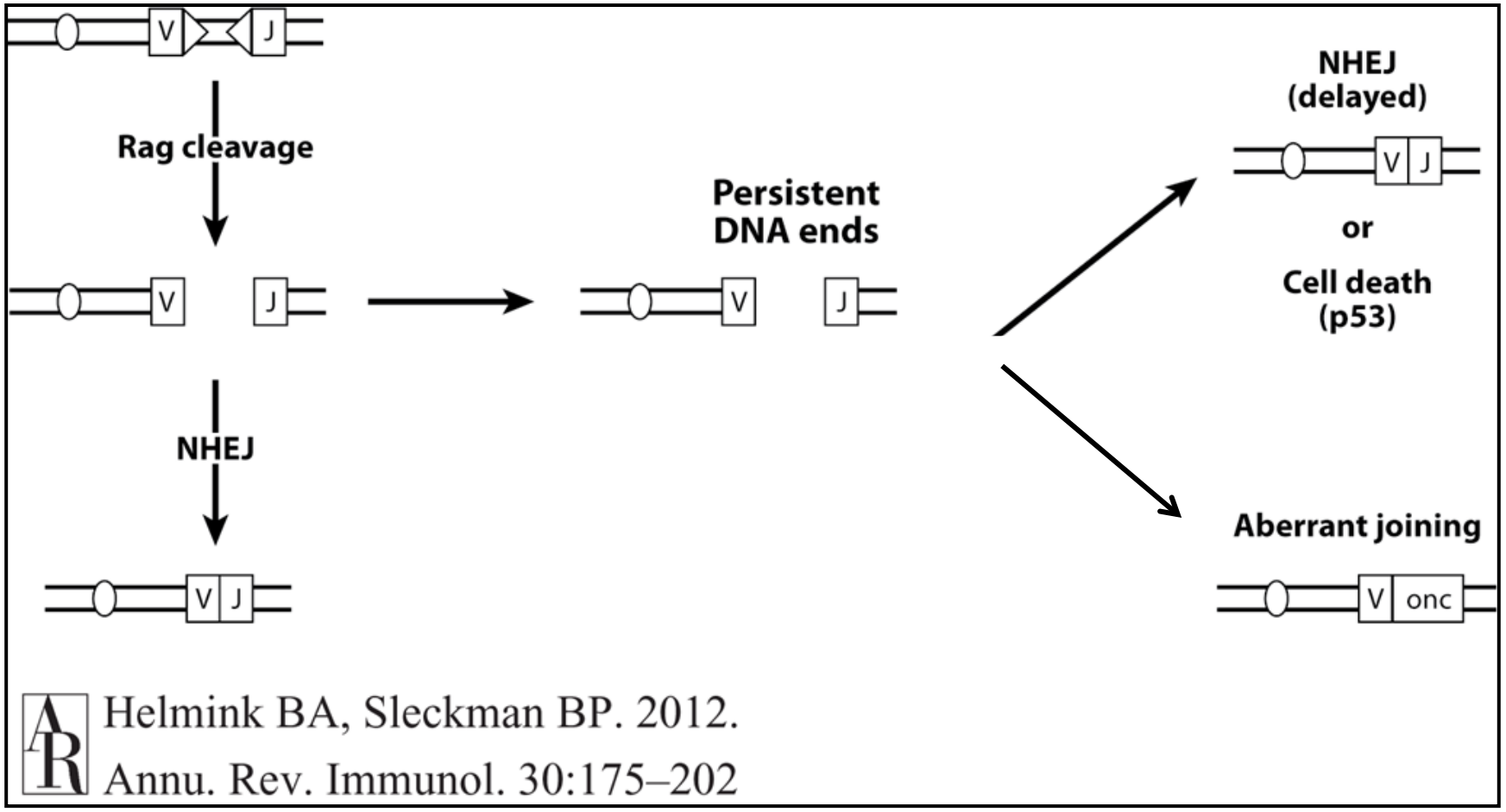
What happens if mice or people lose NHEJ capacity?

What happens if mice or people lose NHEJ capacity?

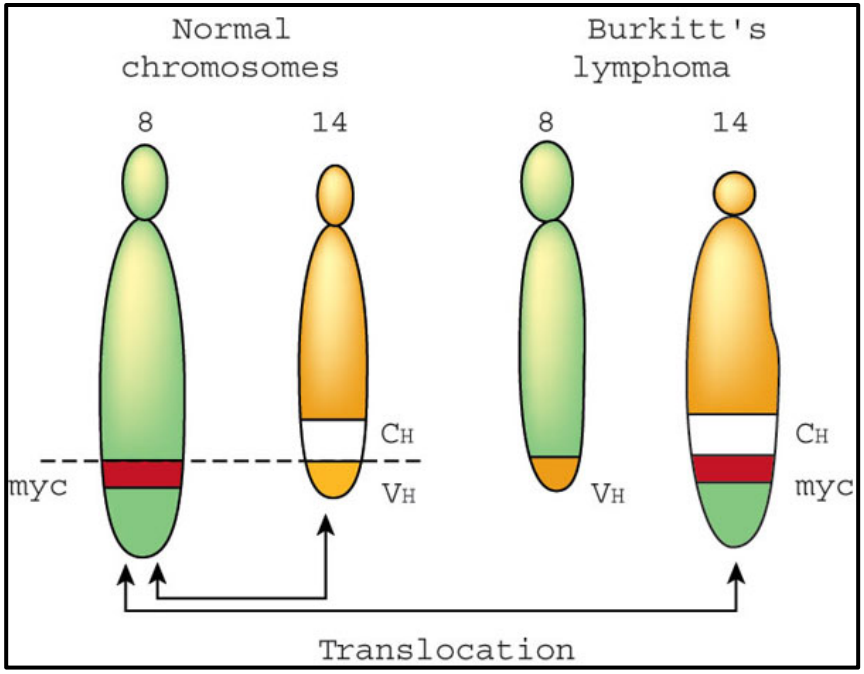
NHEJ gene	Mouse knockout phenotype	Patient phenotype
XRCC6 (encoding Ku70)	Viable, SCID, small size, radiosensitivity and thymoma[50,51]	None known
XRCC5 (encoding Ku80)	Viable, SCID, small size, radiosensitivity, genomic instability and tumours, especially with p53 deletion[47,52–54]	None known
PRKDC (encoding DNA-PKcs)	Viable, SCID, some genomic instability and tumours with p53 (REFS 55–57)	Human hypomorph has SCID and radiosensitivity[58]
DCLRE1C (encoding Artemis)	Viable, SCID, radiosensitivity and genomic instability[59]	Null results in SCID and radiosensitivity; hypomorph shows reduction in lymphocytes, genomic instability and lymphoma[60,61]
NHEJ1 (encoding XLF)	Mild lymphocytopaenia and radiosensitivity[62]	Cernunnos syndrome; immunodeficiency, developmental delay, microcephaly, reduced growth and genomic instability[63]
XRCC4	Null is lethal with neuronal apoptosis; rescue with p53 results in SCID, radiosensitivity, early B lymphoma and genomic instability[49,64]	None known
LIG4	Knockout is lethal with neuronal apoptosis; rescue with p53 results in pro-B lymphoma and radiosensitivity; hypomorph is small, lymphopaenic and has reduced haematopoietic stem cell function[65,66]	LIG4 syndrome; immunodeficiency, reduced growth, developmental issues, microcephaly and malignancy[67,68]

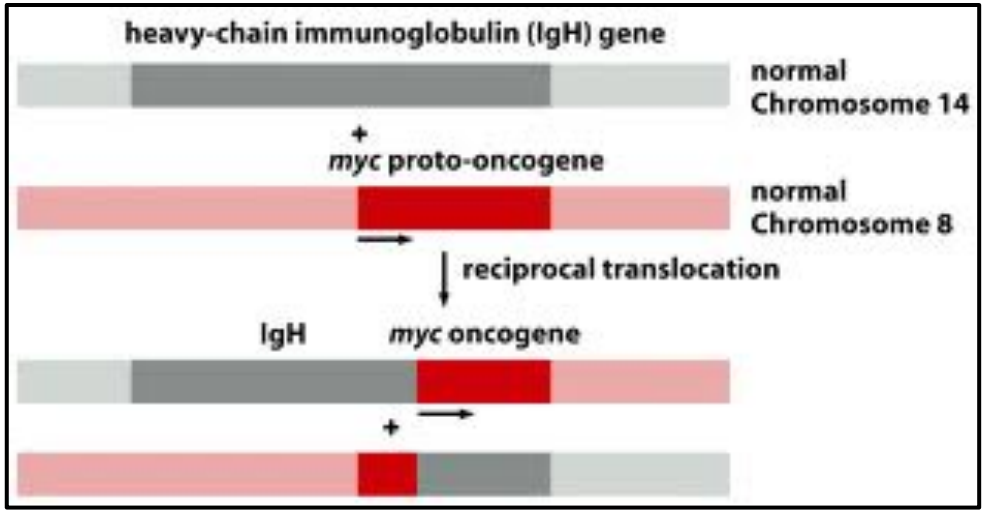
DCLRE1C, DNA cross-link repair 1C; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; LIG4, DNA ligase 4; NHEJ, non-homologous end-joining; NHEJ1, NHEJ factor 1; PRKDC, protein kinase, DNA-activated, catalytic polypeptide; SCID, severe combined immunodeficiency; XLF, XRCC4-like factor; XRCC, X-ray repair cross-complementing protein.

Can V(D)J Recombination Go Wrong?

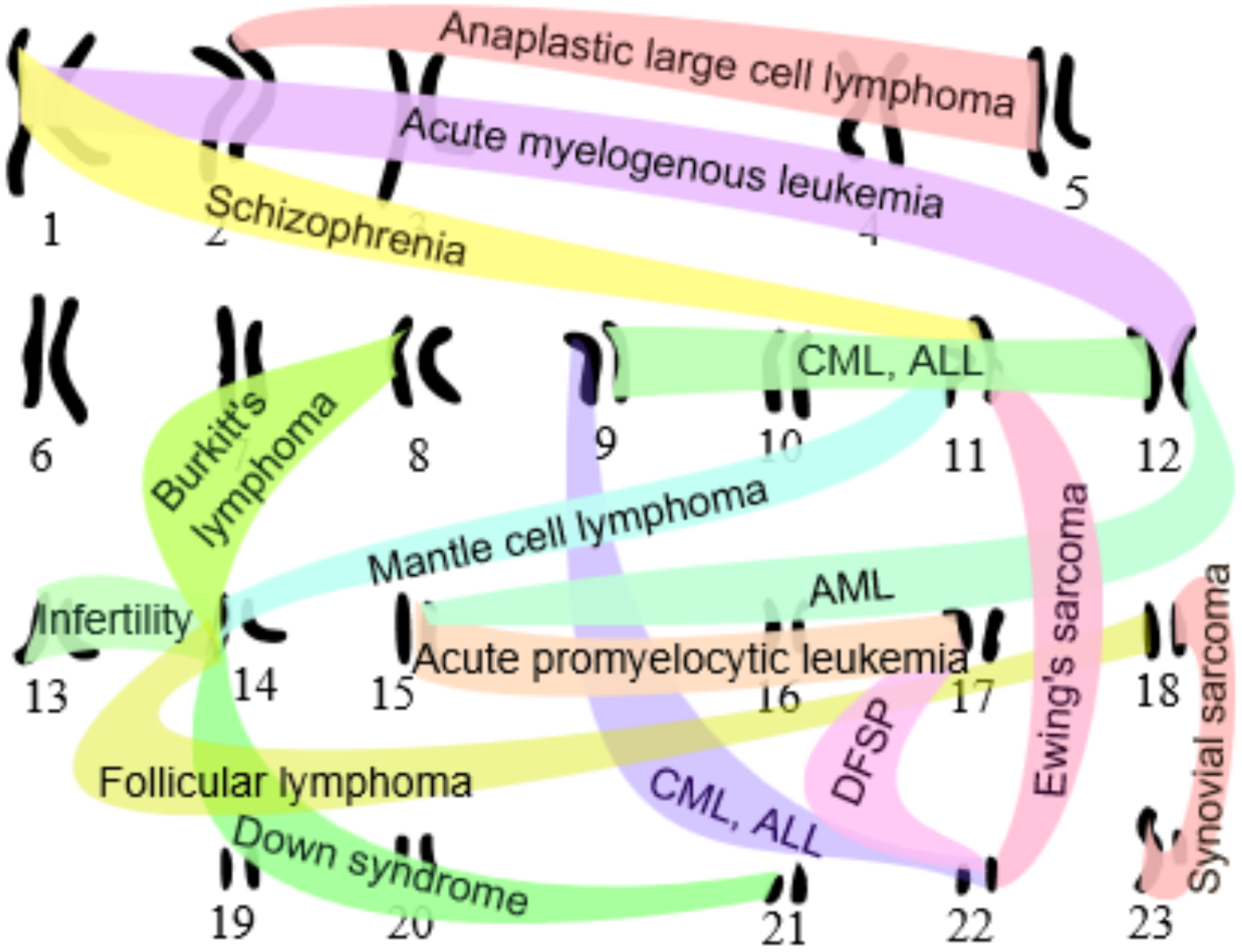


BURKITT's LYMPHOMA
B-cell Lymphoma

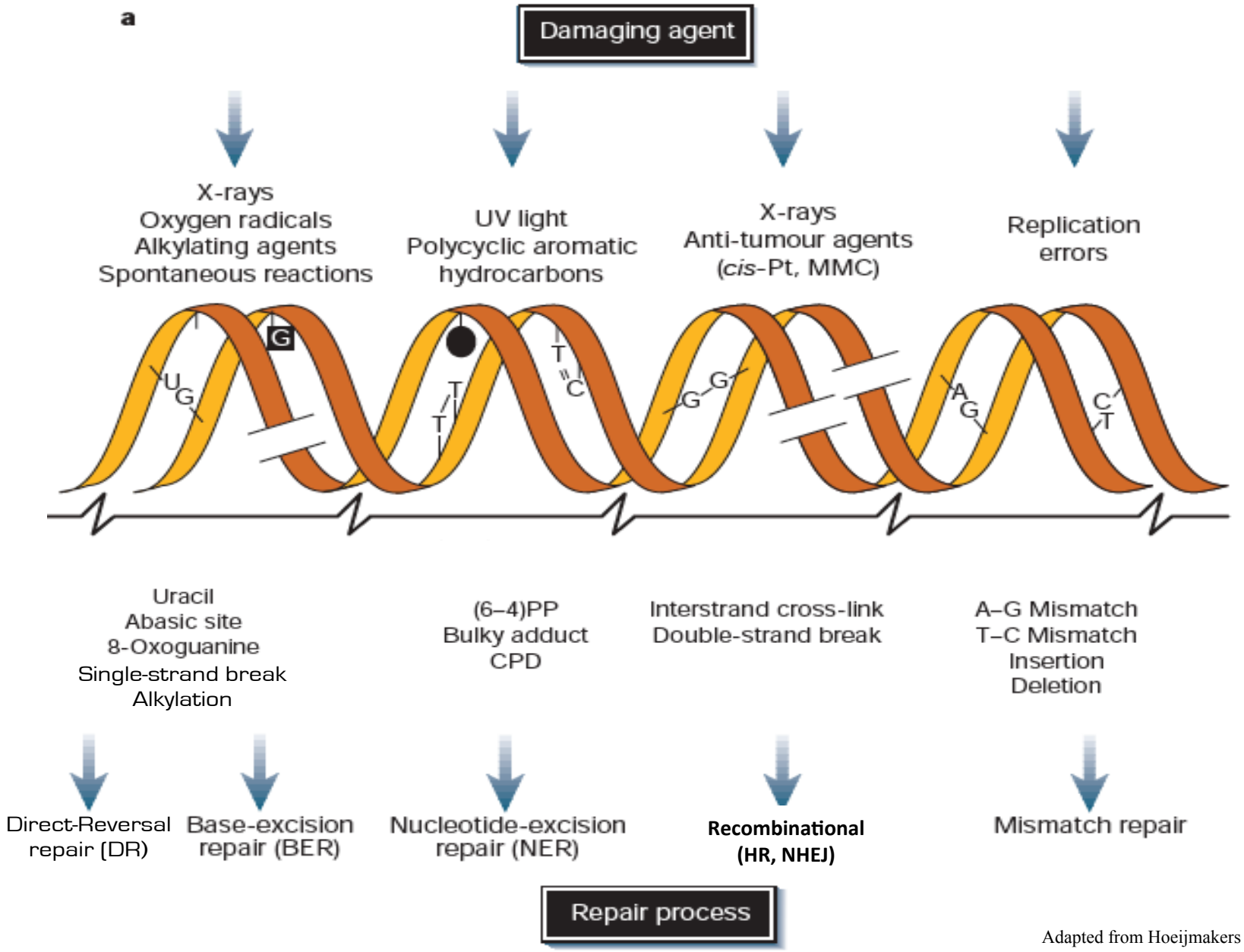




Diseases that involve Chromosome Translocations

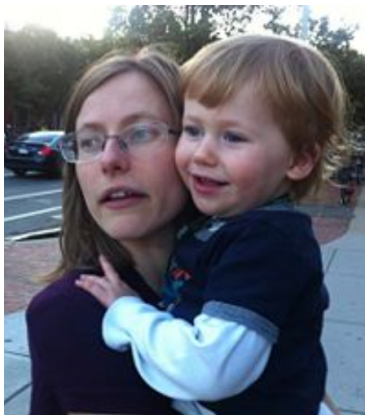


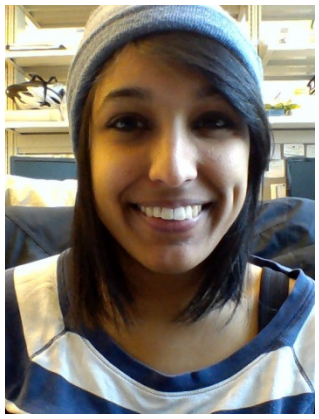
DNA Damage and Repair



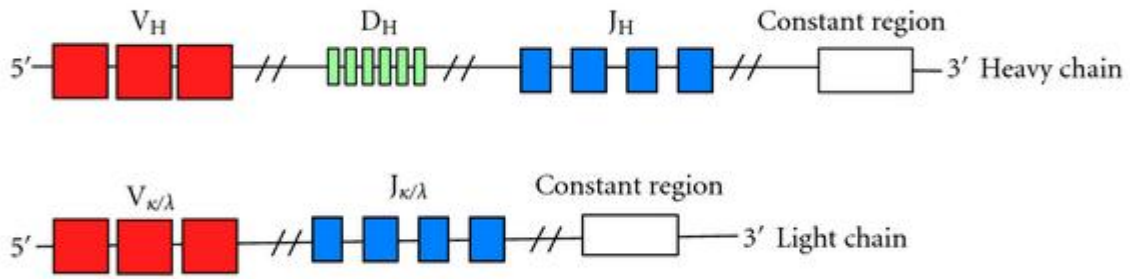
Adapted from Hoeijmakers **2001** *Nature*

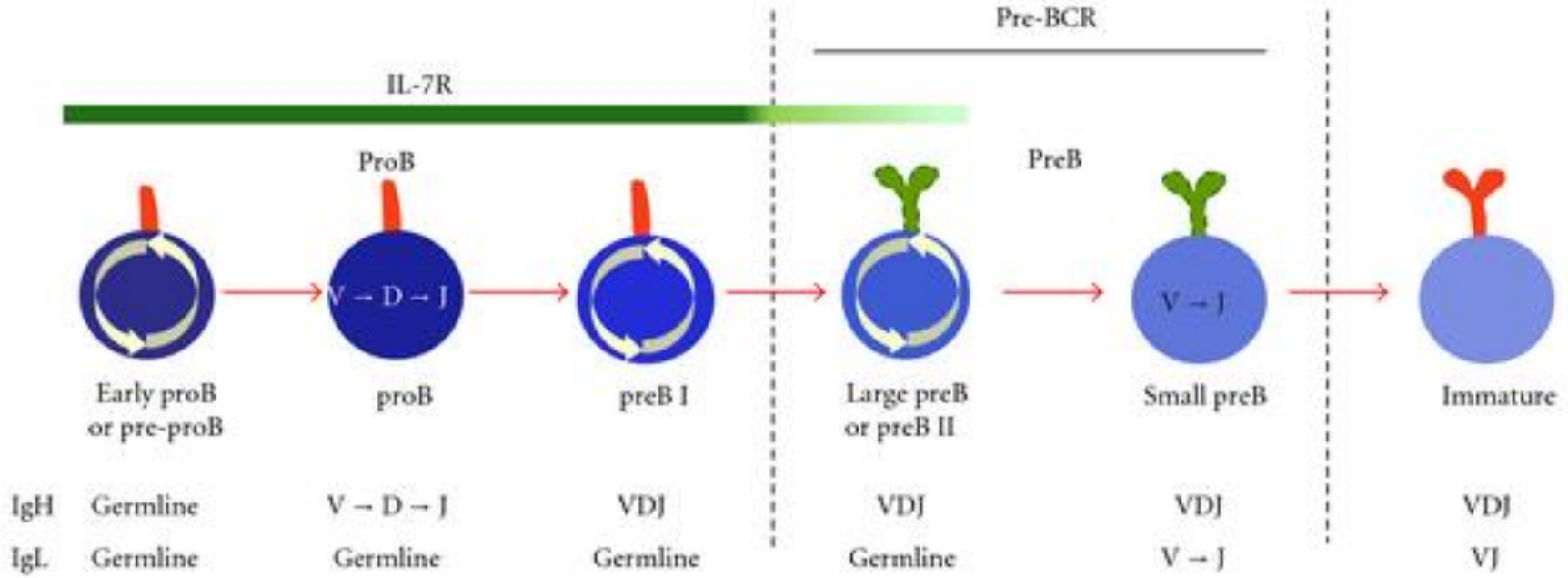
20.109 Spring 2014 Mod 2 – Lecture 4
System Engineering and Protein Foundations

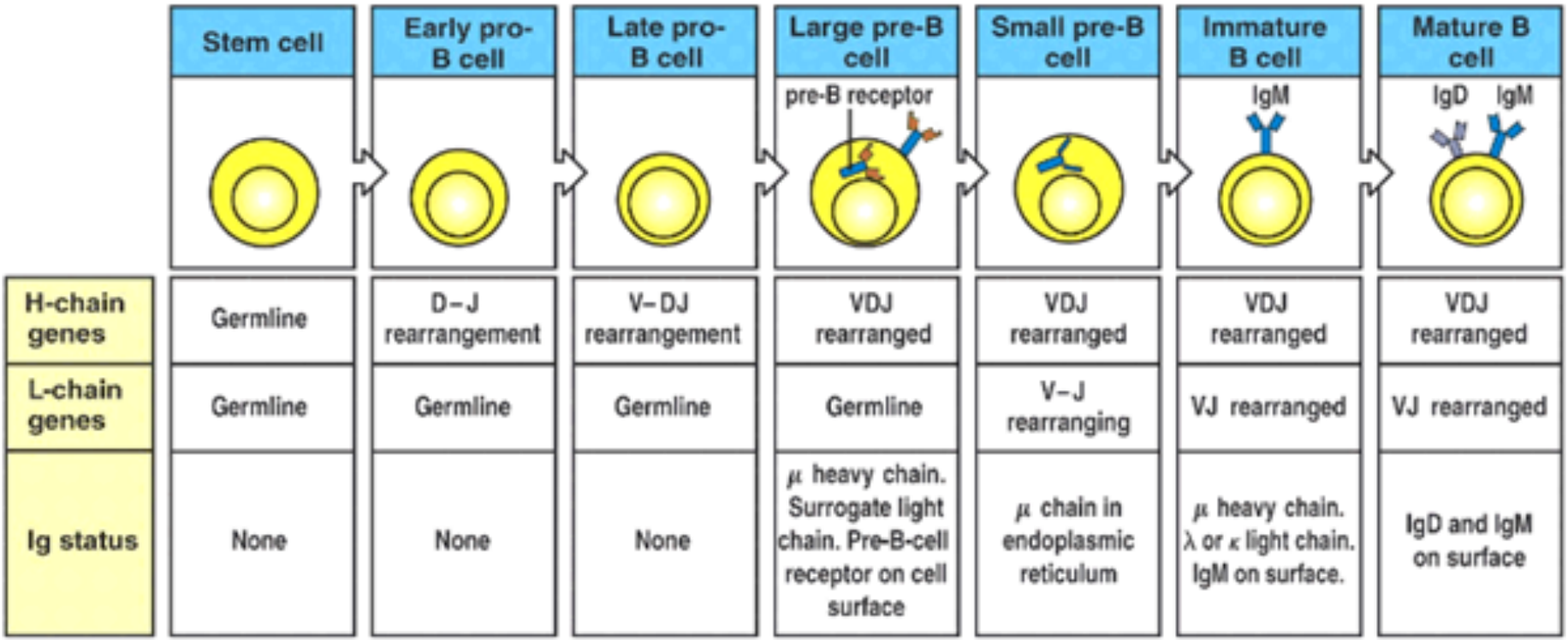


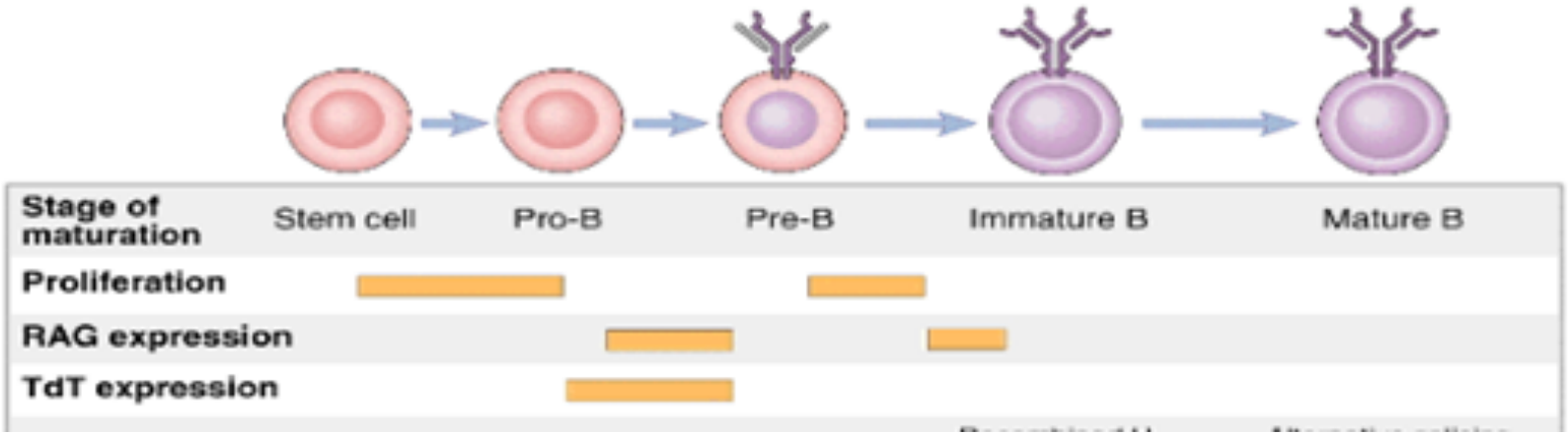



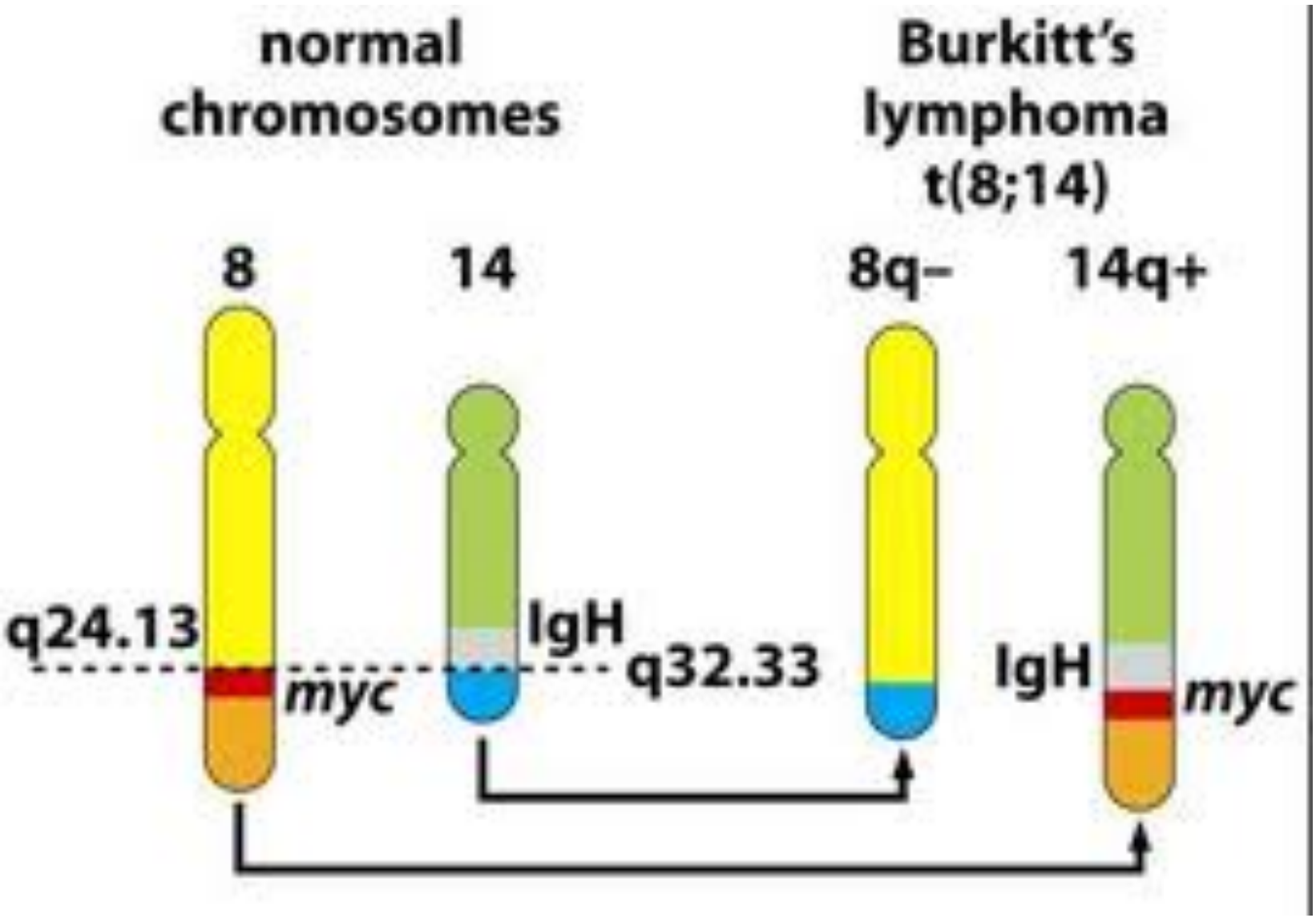
Agi Stachowiak
Shannon Hughes
Aneesh Ramaswamy
Suhani Vora (TA)
Leona Samson (Lectures)
Zachary Nagel (help with development)

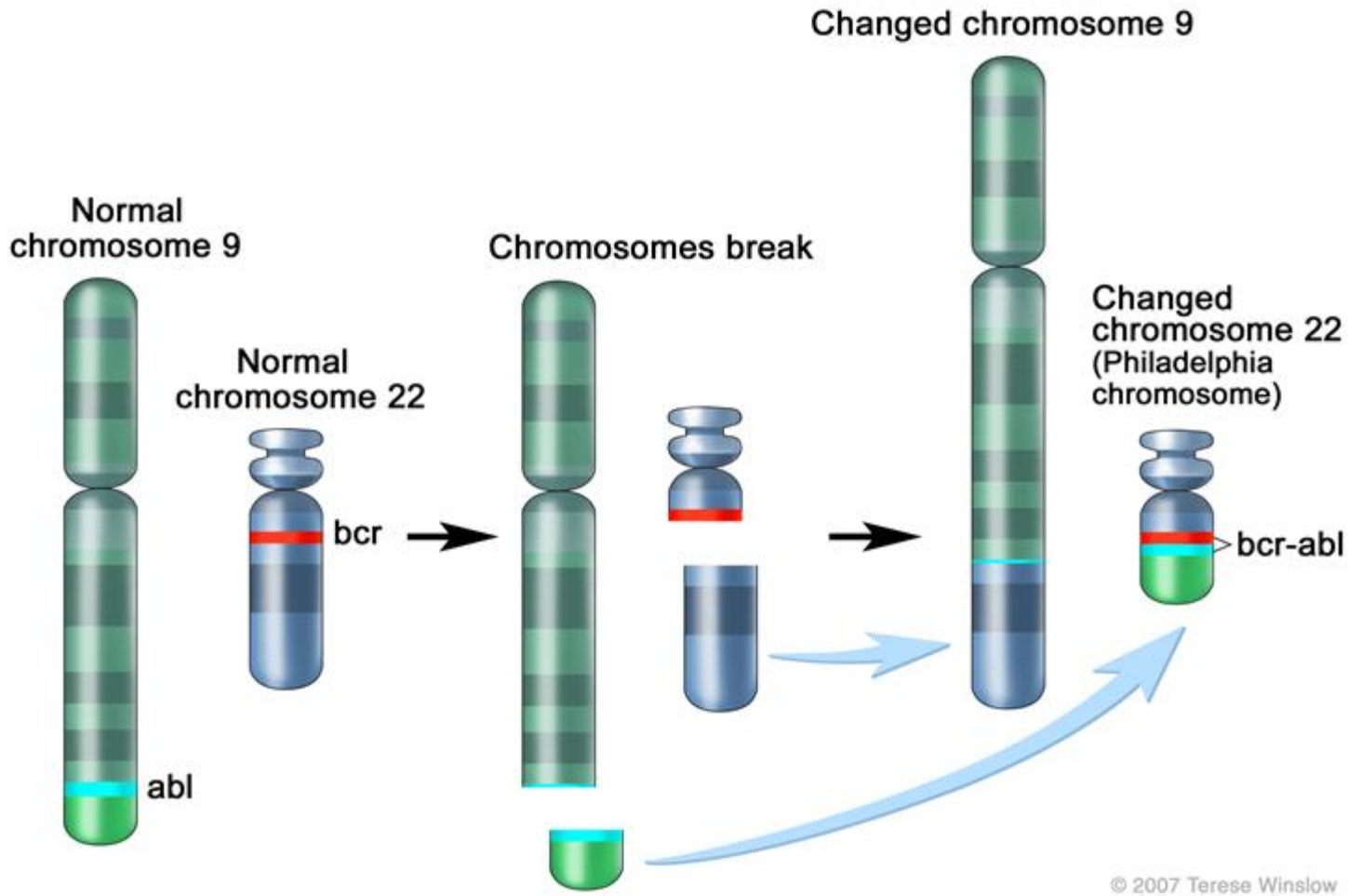

V_H
D_H
J_H
Constant region
5′
3′ Heavy chain
$V_{\kappa/\lambda}$
$J_{\kappa/\lambda}$
Constant region
5′
3′ Light chain


Pre-BCR
IL-7R
ProB
PreB
V → D → J
V → J
Early proB
or pre-proB
proB
preB I
Large preB
or preB II
Small preB
Immature
IgH Germline V → D → J VDJ VDJ VDJ VDJ
IgL Germline Germline Germline Germline V → J VJ


Stem cell
Early pro-B cell
Late pro-B cell
Large pre-B cell
pre-B receptor
Small pre-B cell
Immature B cell
IgM
Mature B cell
IgD IgM
H-chain genes
Germline
D–J rearrangement
V–DJ rearrangement
VDJ rearranged
VDJ rearranged
VDJ rearranged
VDJ rearranged
L-chain genes
Germline
Germline
Germline
Germline
V–J rearranging
VJ rearranged
VJ rearranged
Ig status
None
None
None
μ heavy chain. Surrogate light chain. Pre-B-cell receptor on cell surface
μ chain in endoplasmic reticulum
μ heavy chain. λ or κ light chain. IgM on surface.
IgD and IgM on surface


Stage of maturation
Stem cell
Pro-B
Pre-B
Immature B
Mature B
Proliferation
RAG expression
TdT expression


normal
chromosomes
Burkitt's
lymphoma
t(8;14)
8
14
8q–
14q+
q24.13
myc
IgH
q32.33
IgH
myc


Changed chromosome 9
Normal chromosome 9
Chromosomes break
Normal chromosome 22
Changed chromosome 22 (Philadelphia chromosome)
bcr
bcr-abl
abl
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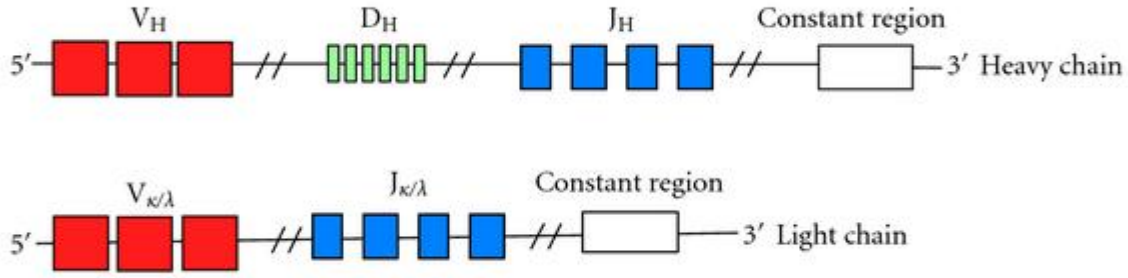
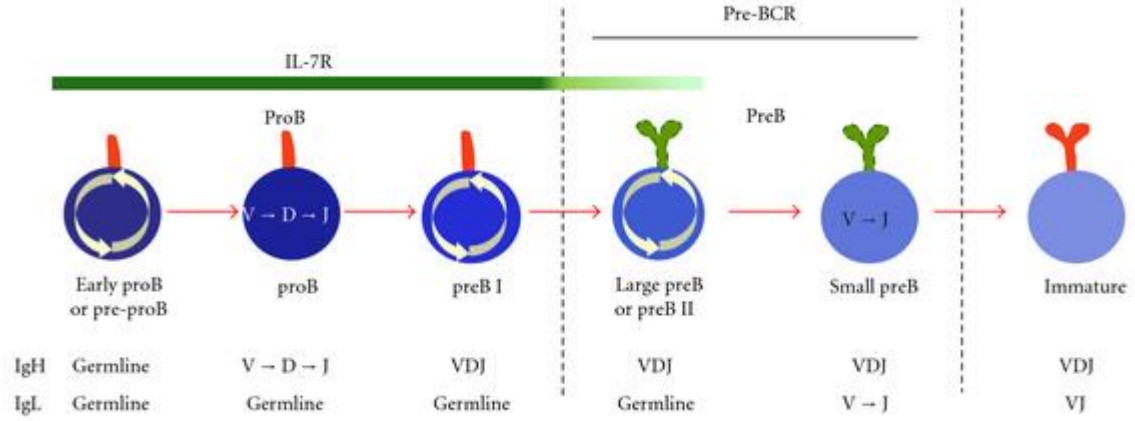
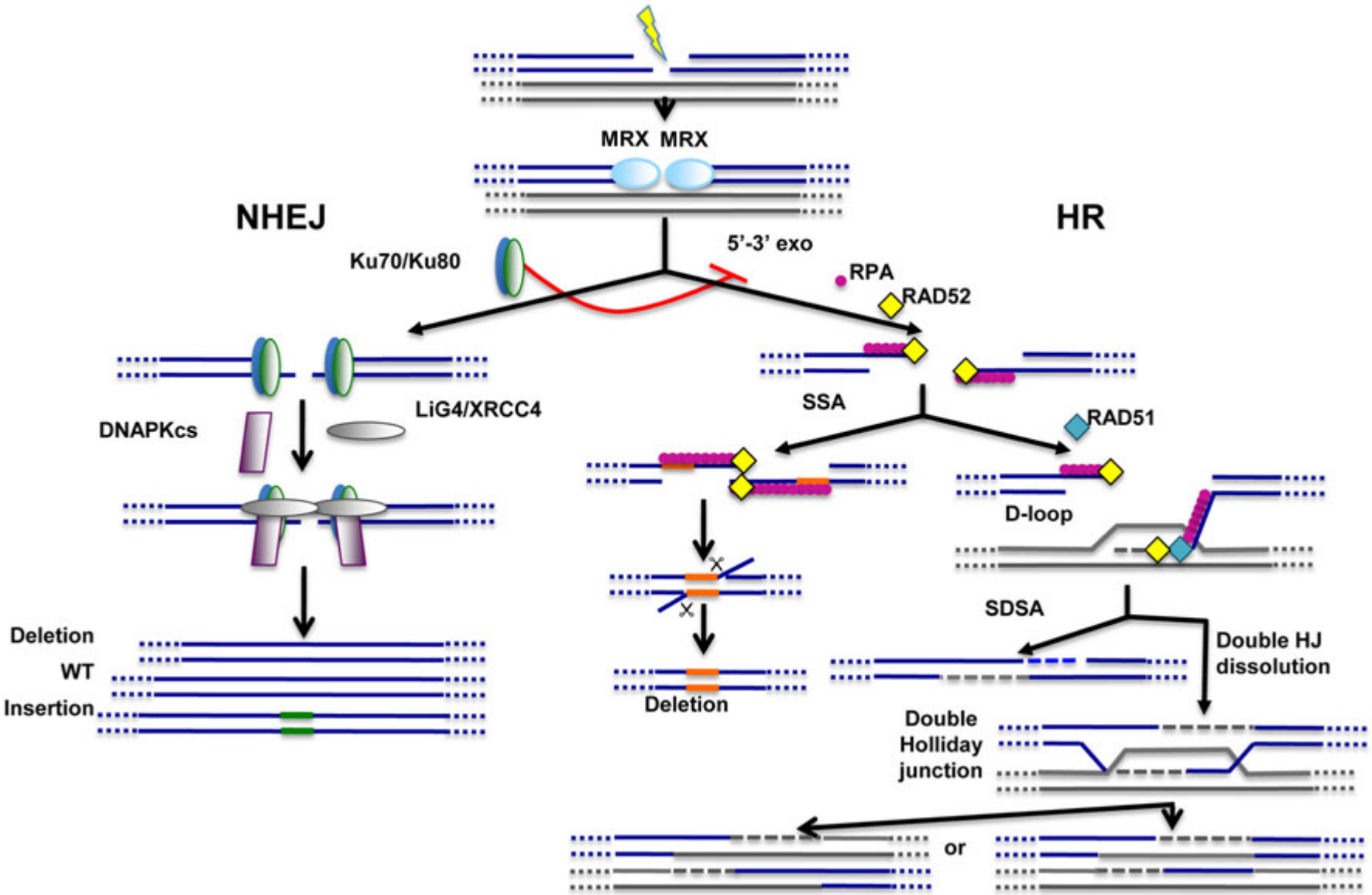
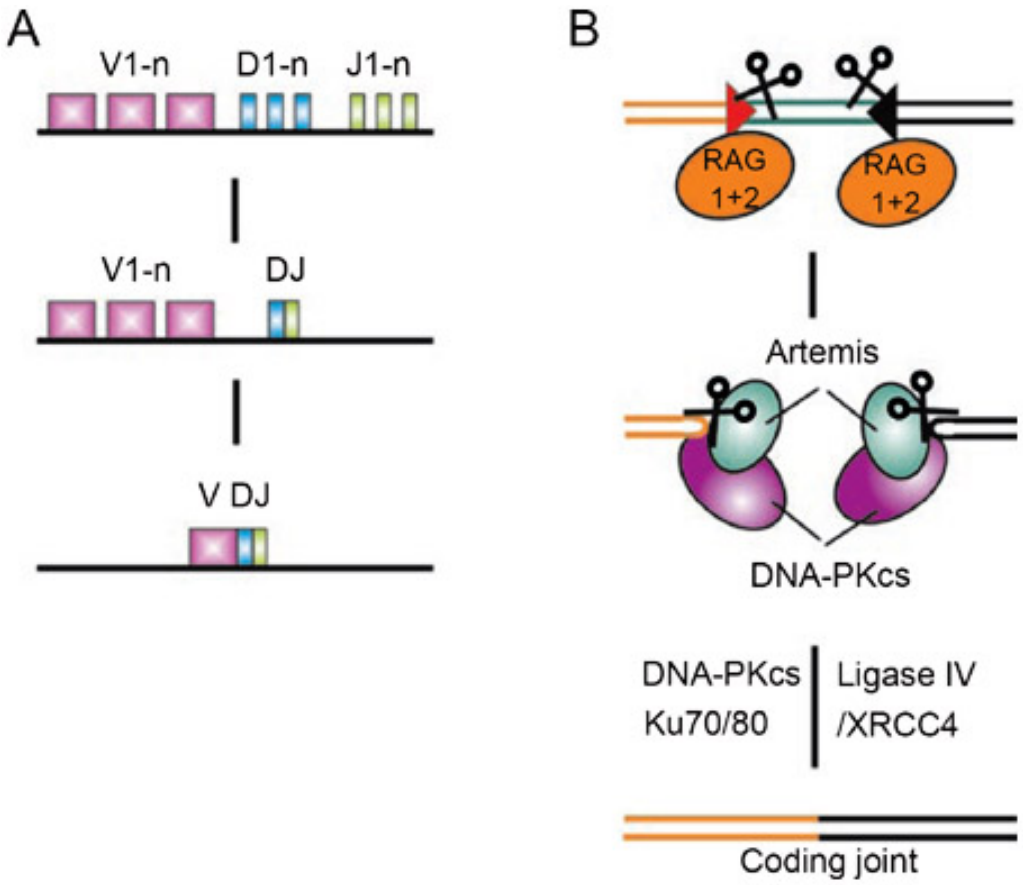

V_H
D_H
J_H
Constant region
5′
3′ Heavy chain
$V_{\kappa/\lambda}$
$J_{\kappa/\lambda}$
Constant region
5′
3′ Light chain


Pre-BCR
IL-7R
ProB
PreB
V → D → J
V → J
Early proB
or pre-proB
proB
preB I
Large preB
or preB II
Small preB
Immature
IgH Germline V → D → J VDJ VDJ VDJ VDJ
IgL Germline Germline Germline Germline V → J VJ


MRX MRX
NHEJ
HR
Ku70/Ku80
5'-3' exo
RPA
RAD52
DNAPKcs
LiG4/XRCC4
SSA
RAD51
D-loop
SDSA
Double HJ dissolution
Deletion
WT
Insertion
Deletion
Double Holliday junction
or



Simplified overview of V(D)J recombination. **(A)** Genes that encode immunoglobulins or T-cell receptors are not present in an active form in developing B- and T-lymphocytes, but need to be formed by the combination of gene segments. This process is called V(D)J recombination. Gene segments are classified into three groups: variable (V), diversity (D), and joining (J) segments. In the case of an IgH gene, D and J segments are first joined, followed by the combination of the DJ assembly with a V segment. **(B)** Gene segments are joined by the introduction of a DSB at the edges of selected segments by the RAG 1 and RAG 2 proteins, followed by removal of the intervening DNA and ligation of the segments. Before ligation can take place, the typical hairpin structure of the coding ends needs to be opened by the endonuclease Artemis. V(D)J recombination requires the NHEJ core enzymes (DNA-PK_{CS}, Ku70/80, ligase IV, and XRCC4), indicating that ligation of the gene segments is mediated by the NHEJ process.

Mechanisms of Chromosome Translocation

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