20.109 Spring 2017 Module 2 – Lecture 4

Gene Expression Engineering (March 21st 2017)




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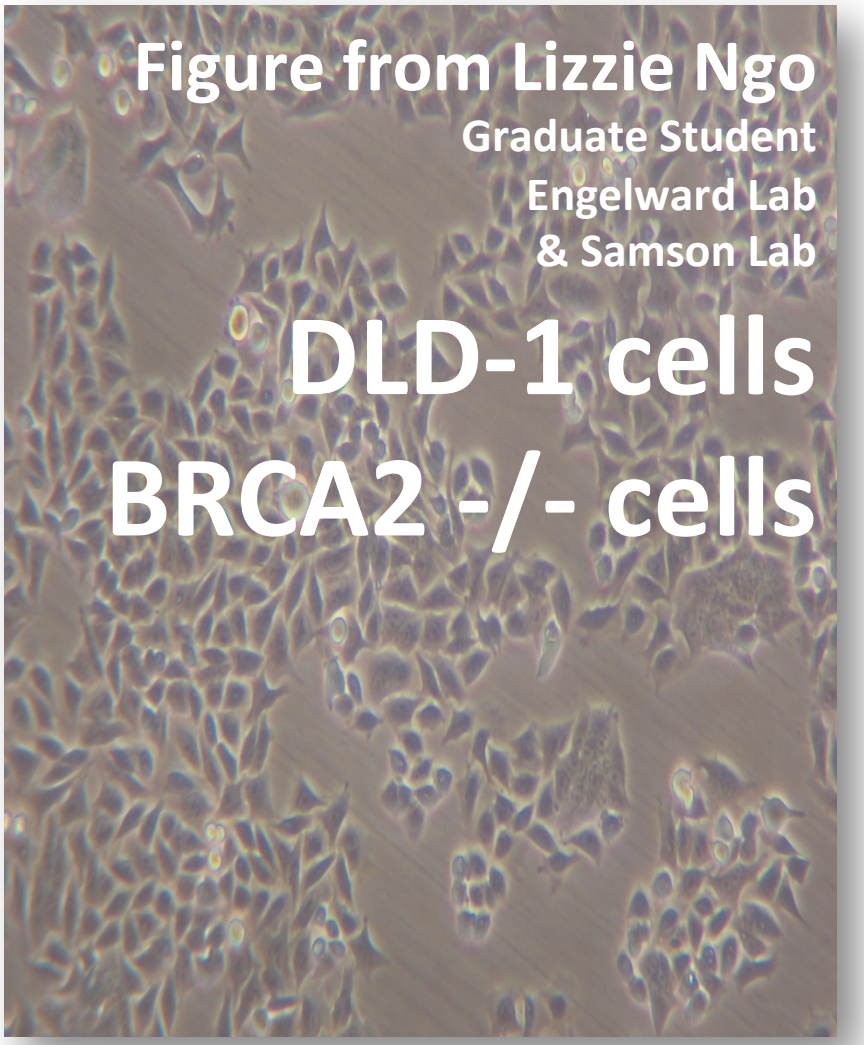
Maxine Jonas

Rob Wilson

Leona Samson (Lectures)

Here's how you will treat your cells today



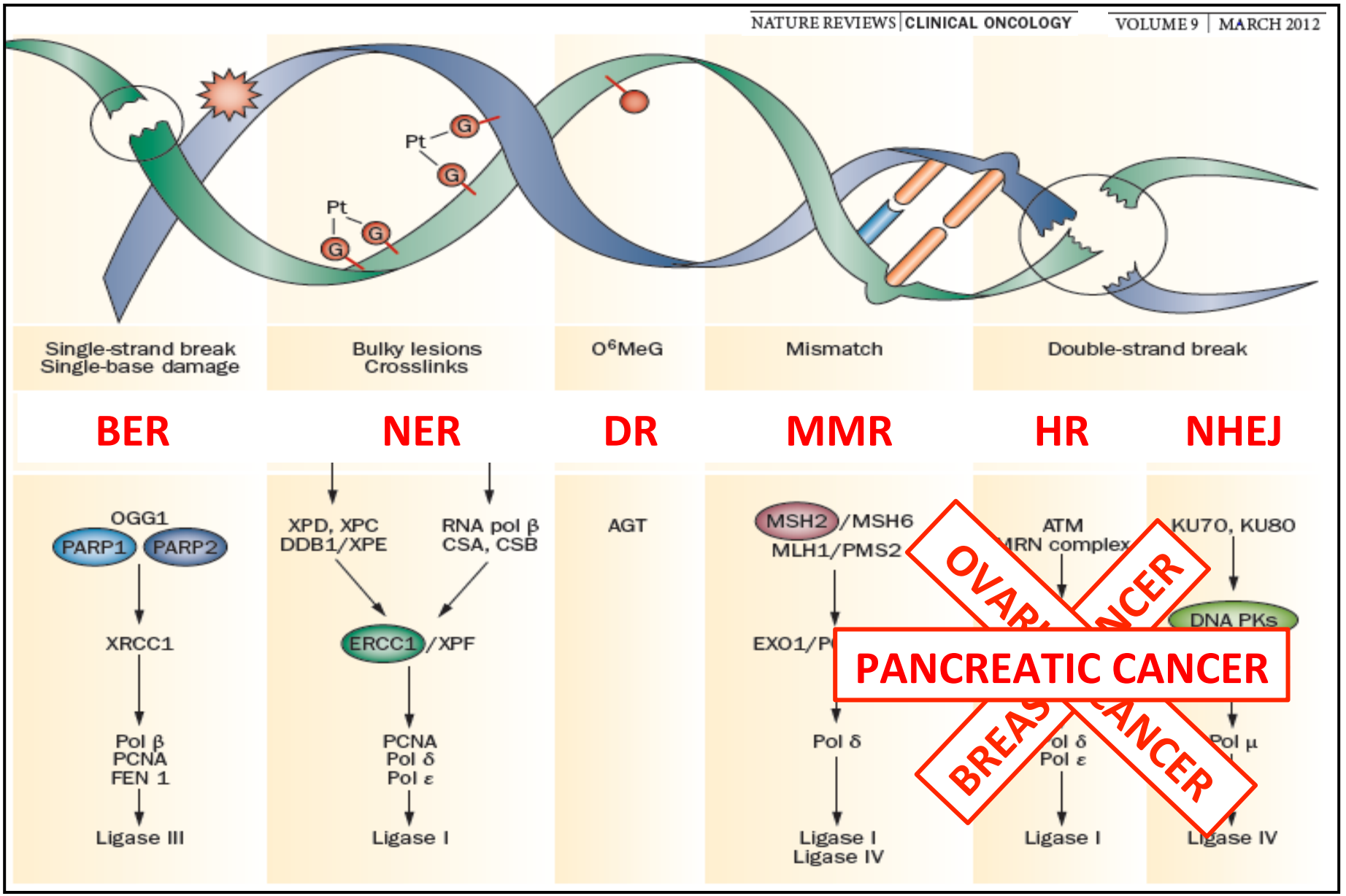
[CANCER RESEARCH 39, 1020–1025, March 1979]
0008-5472/79/0039-0000$02.00

- Etoposide to inhibit TopoII leading to DNA DSBs
- Compound 401 to inhibit Non Homologous End Joining (NHEJ)
- Etoposide + Compound 401
- Olaparib to inhibit PARP1 to stabilize DNA SSBs leading to DNA DSBs at collapsed replication forks

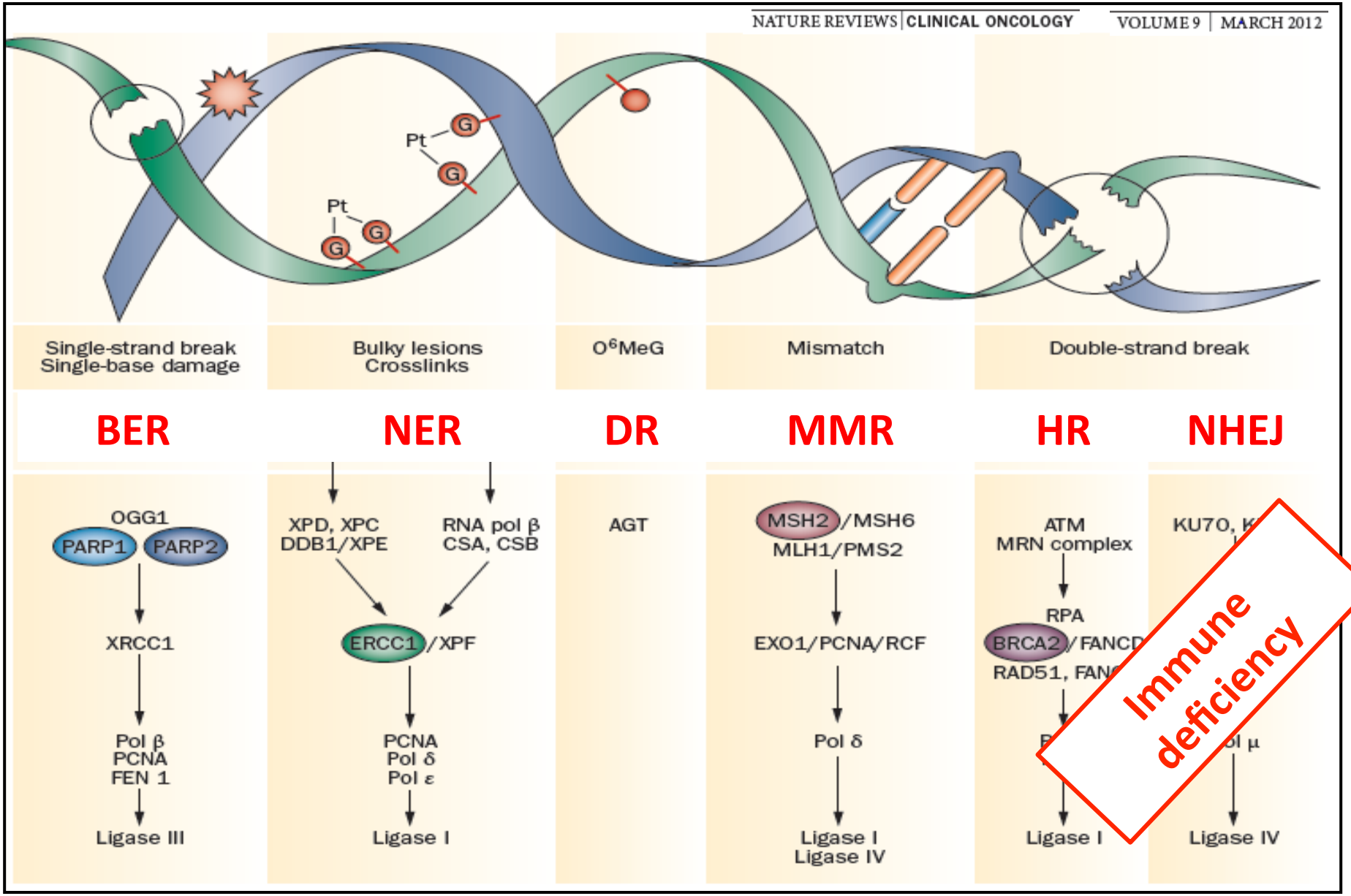
Key Experimental Methods for Module 2

- Grow human cancer cells in tissue cell culture
- Monitor specific protein levels by Western blot
- Kill cancer cells with chemotherapy drugs
- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

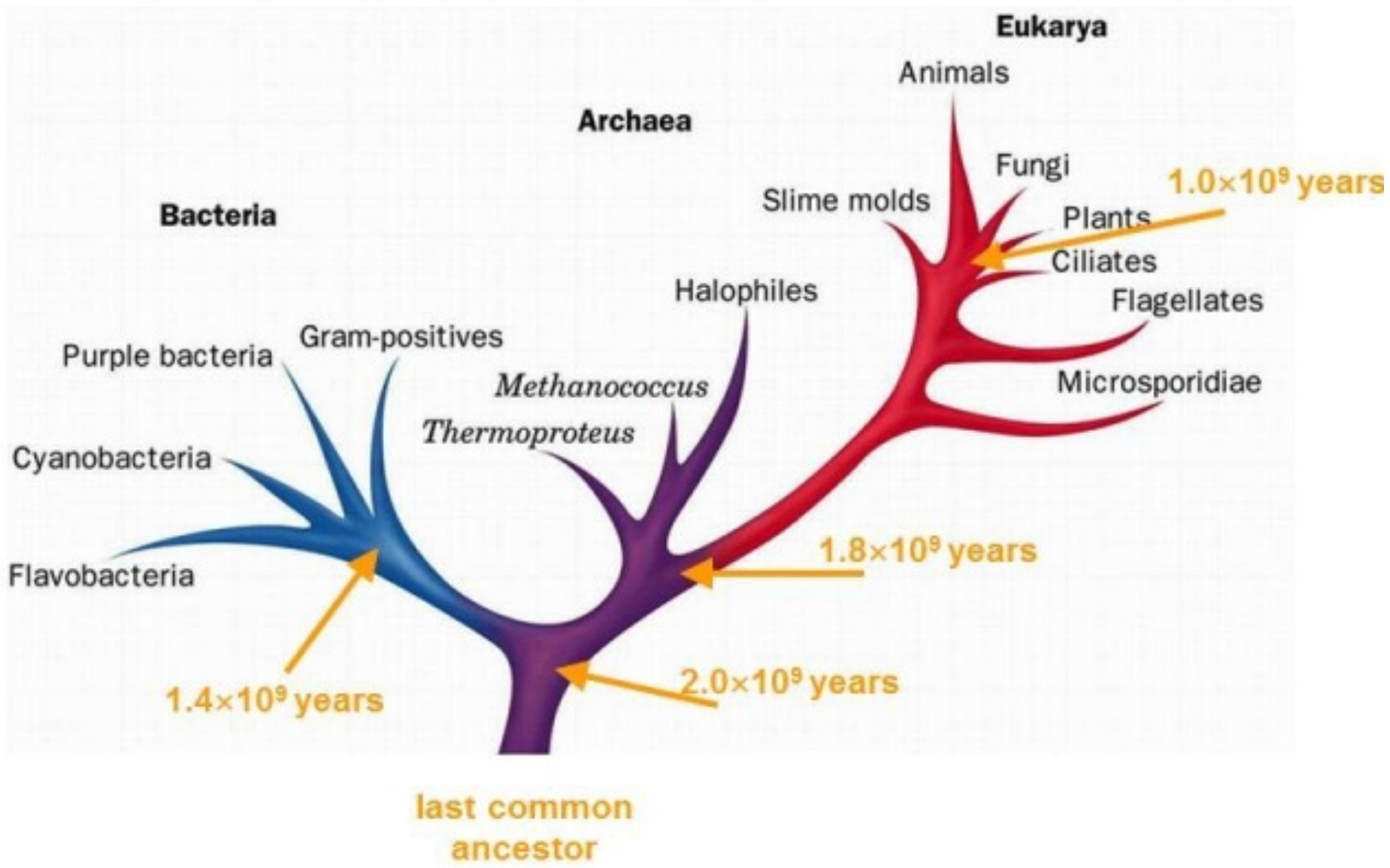
Six Major DNA Repair Pathways



Six Major DNA Repair Pathways

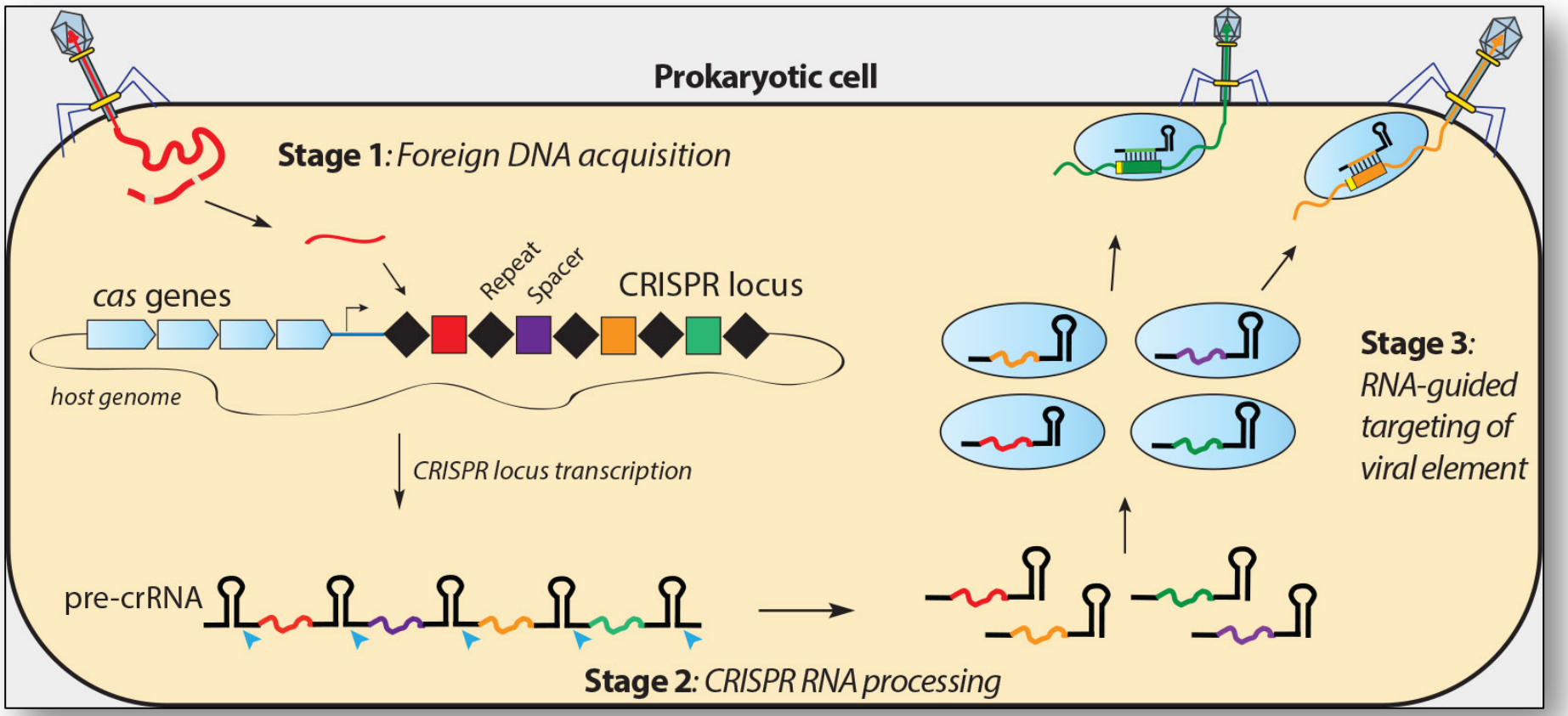


All known life forms are based on DNA



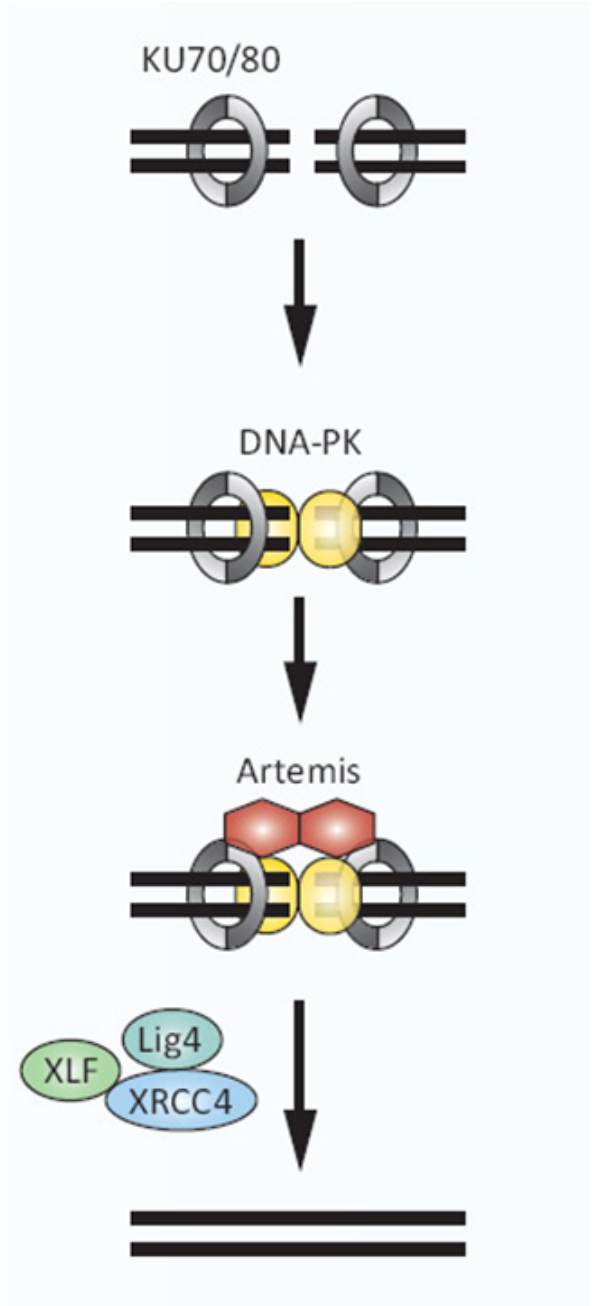
http://biologicalphysics.iop.org/cws/article/lectures/47042

CRISPR - **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
CAS genes – **C**RISPR **AS**sociated genes



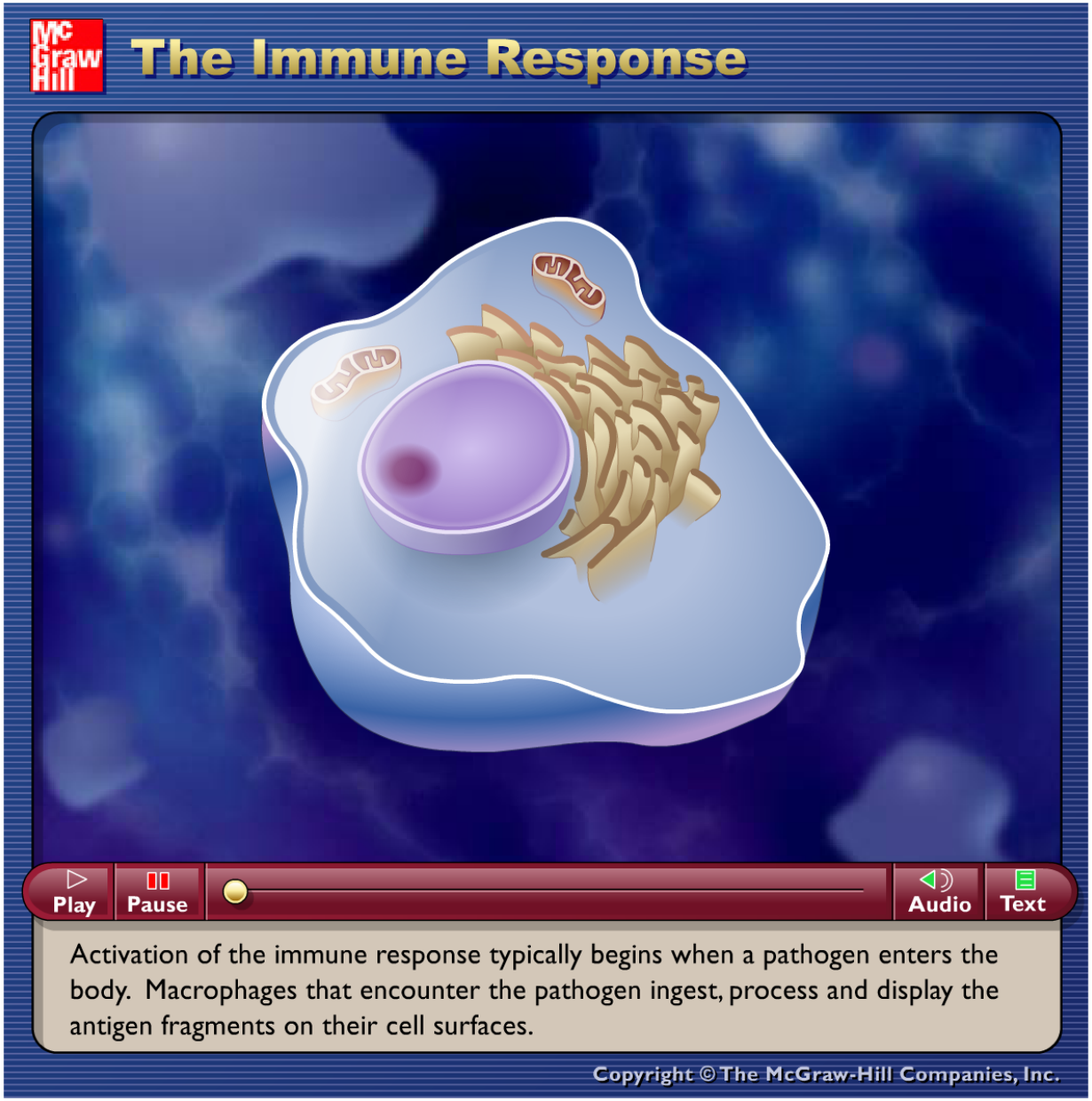
http://rna.berkeley.edu/crispr.html

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



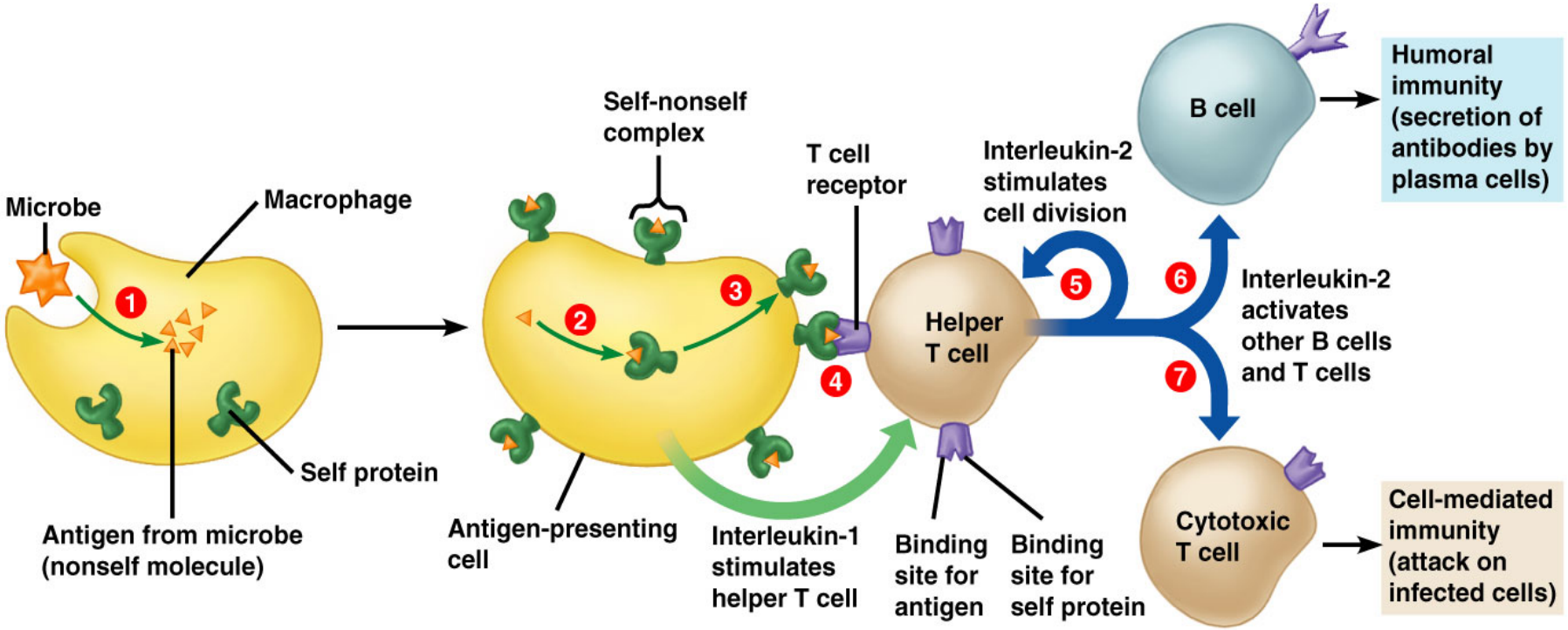
Non Homologous End Joining

NHEJ



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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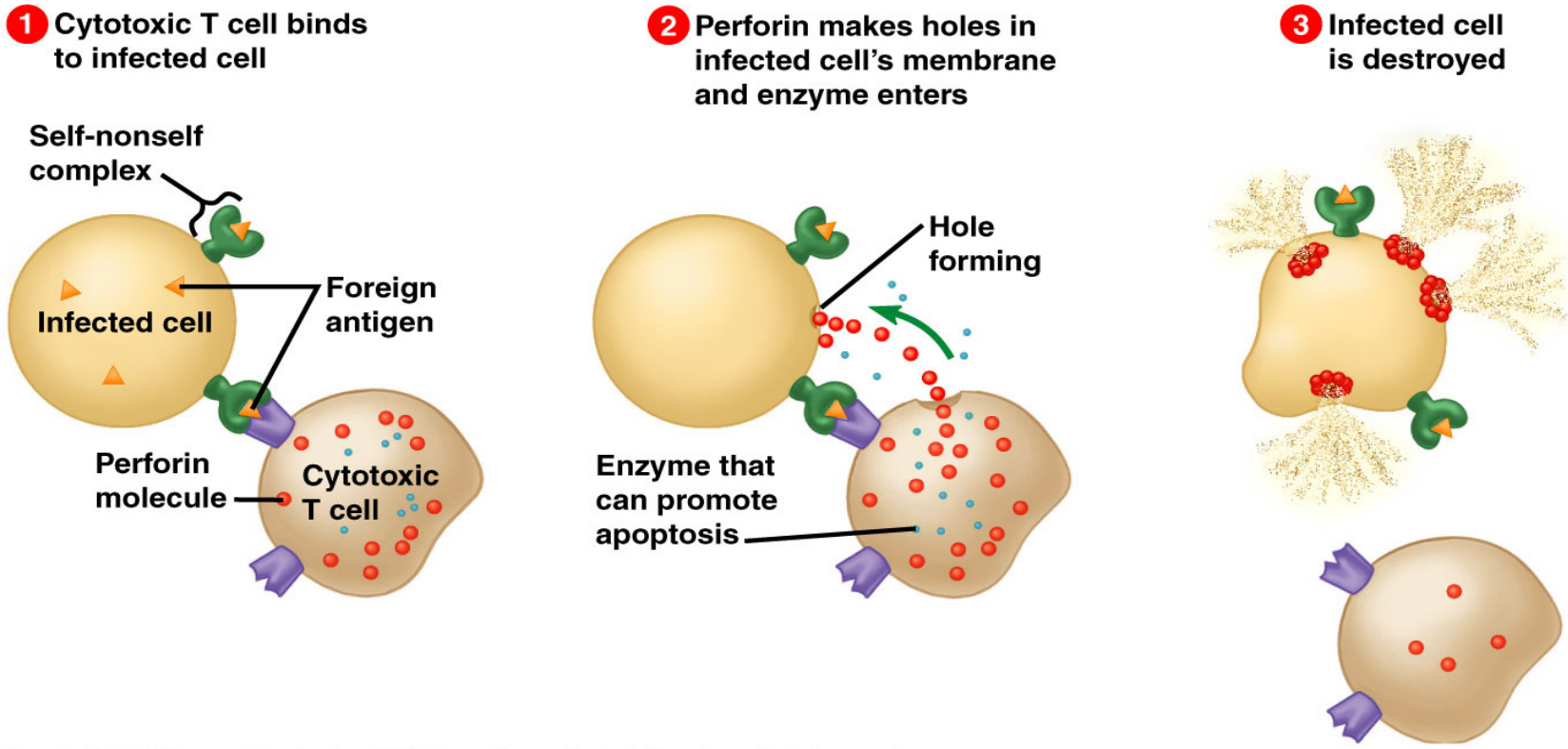
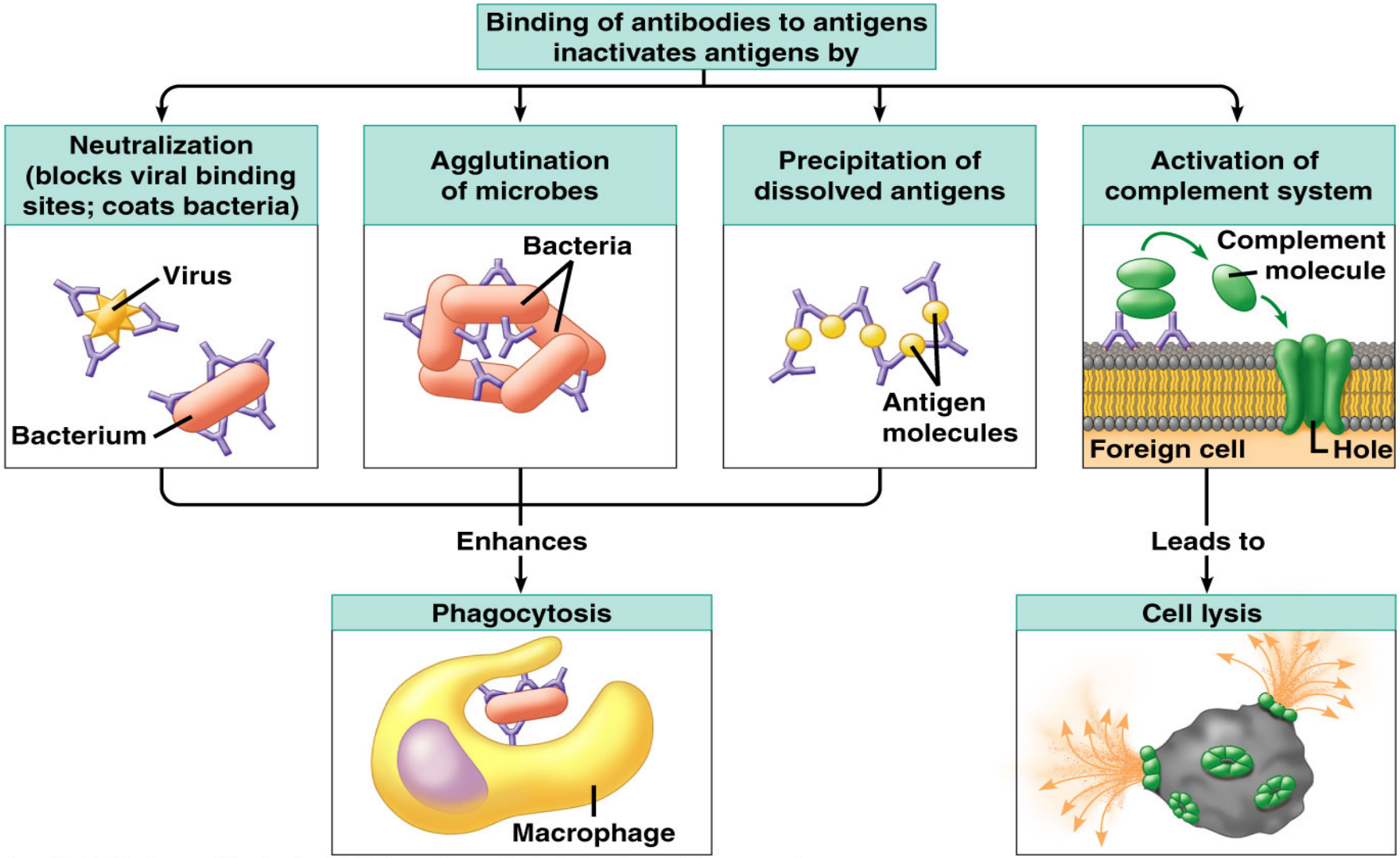


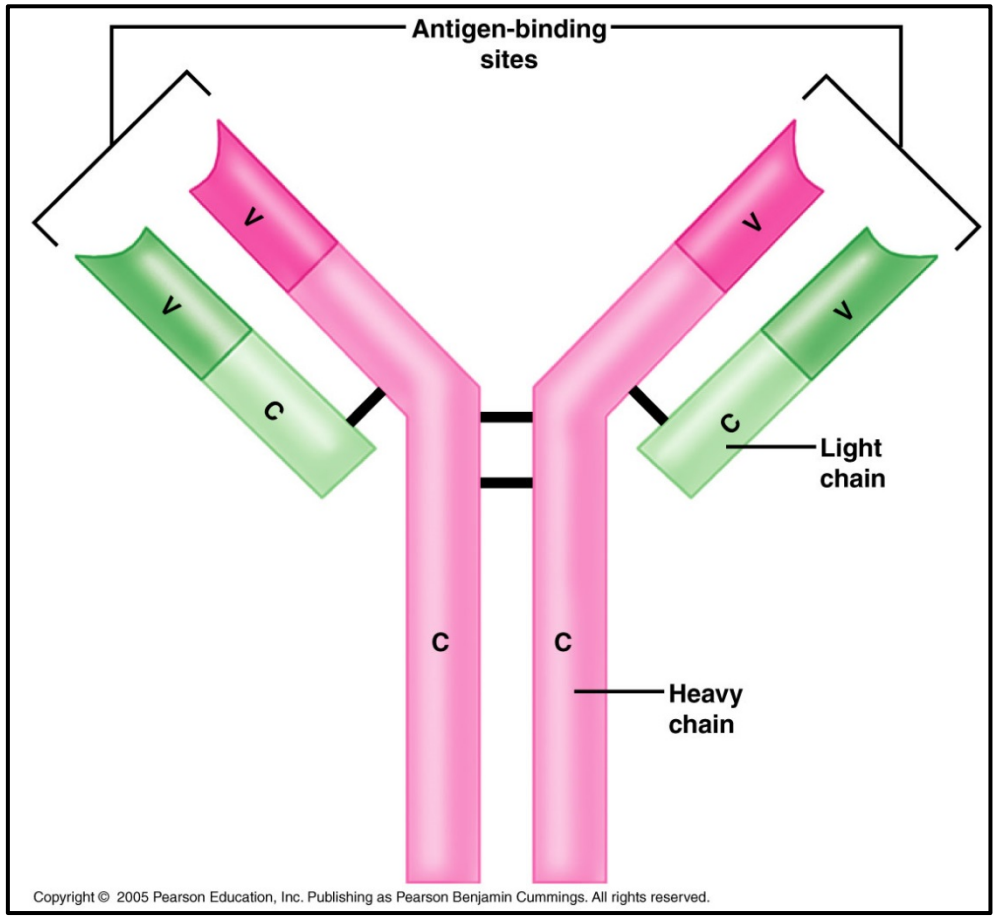
http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

Antibodies work in different ways



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

"ANTIGEN" comes from ANTI-body GENerating substances



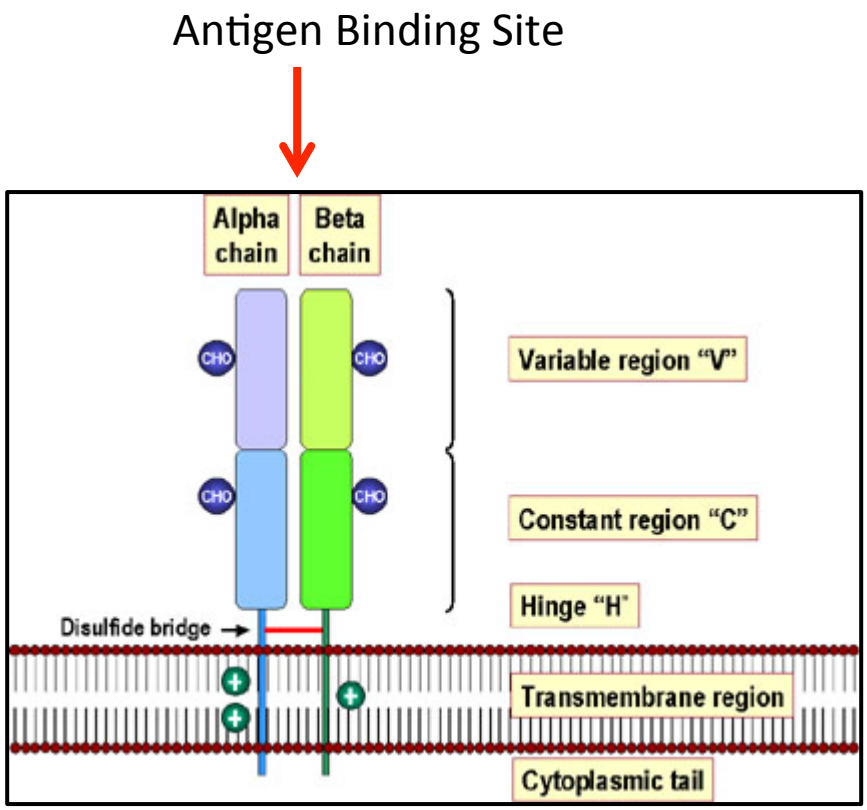
V = Variable

C = Constant

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

B-cell Immunoglobulin

“**ANTIGEN**" comes from **ANTI**-body **GEN**erating substances

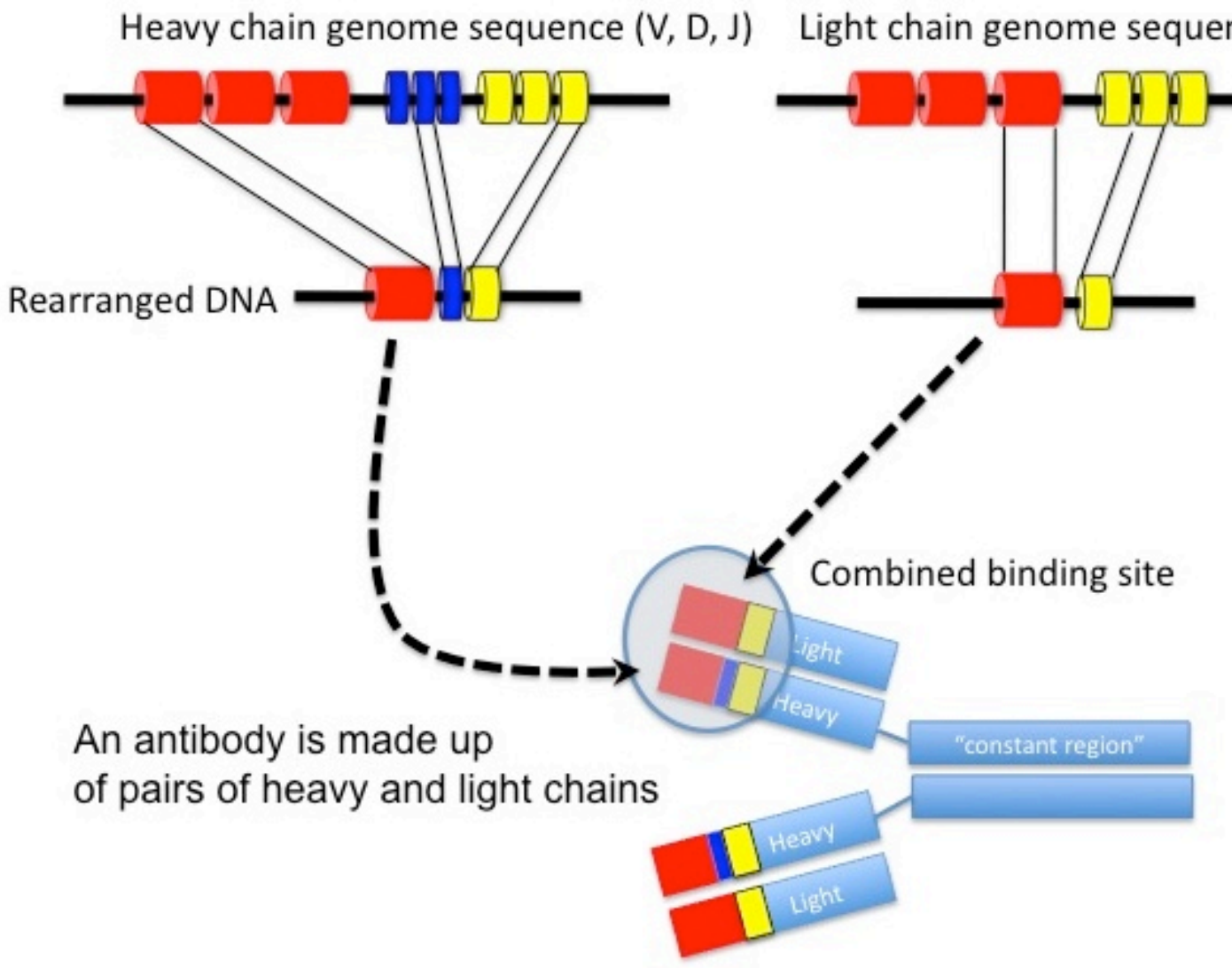


http://pathmicro.med.sc.edu/bowers/mhc.htm

T-cell Receptor

How Do the Variable Regions become Variable?

Through Programmed NHEJ!!



V -variable,

D - diversity

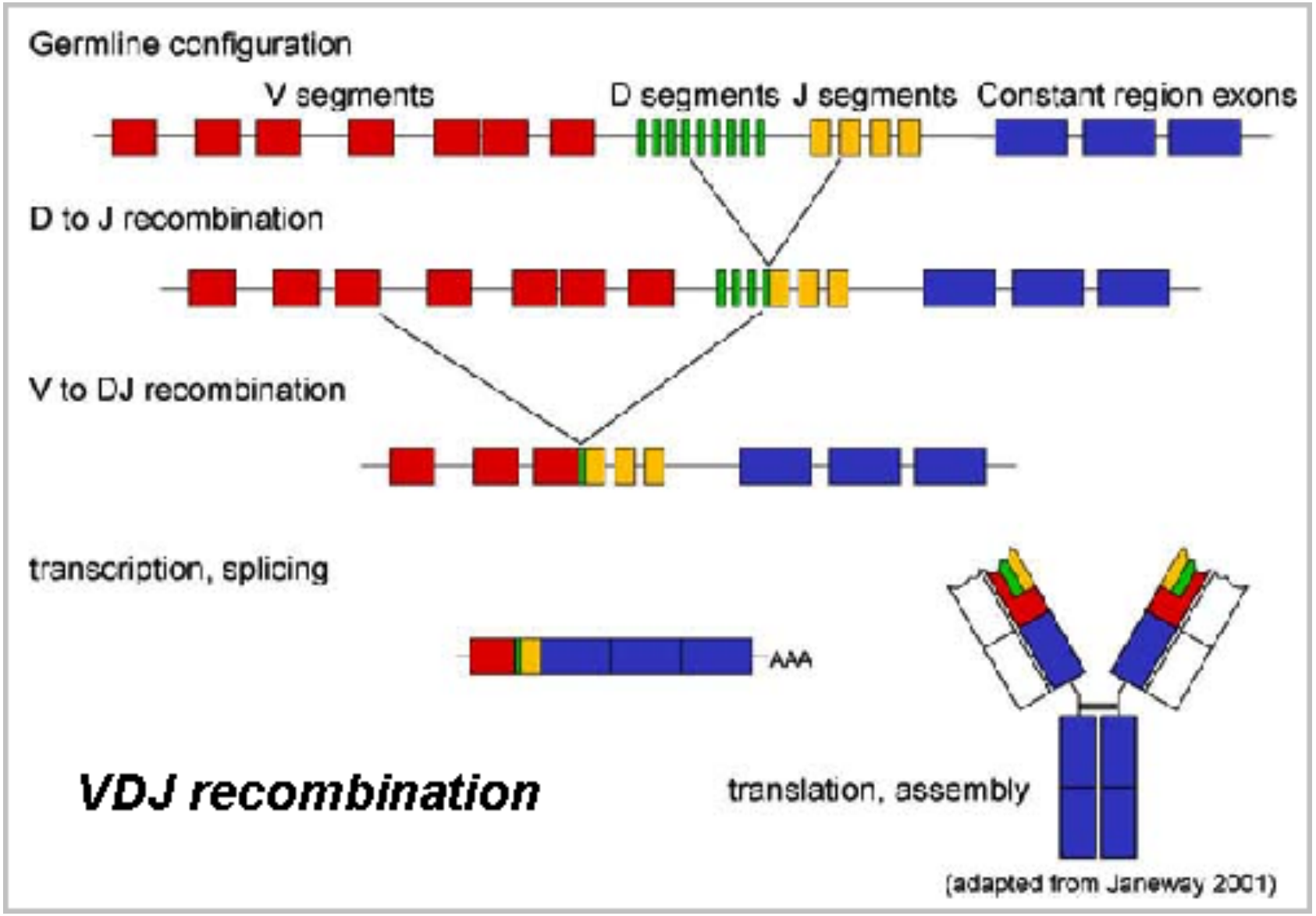
J- joining

gene

segments

How Do the Variable Regions become Variable?

Through Programmed NHEJ!!



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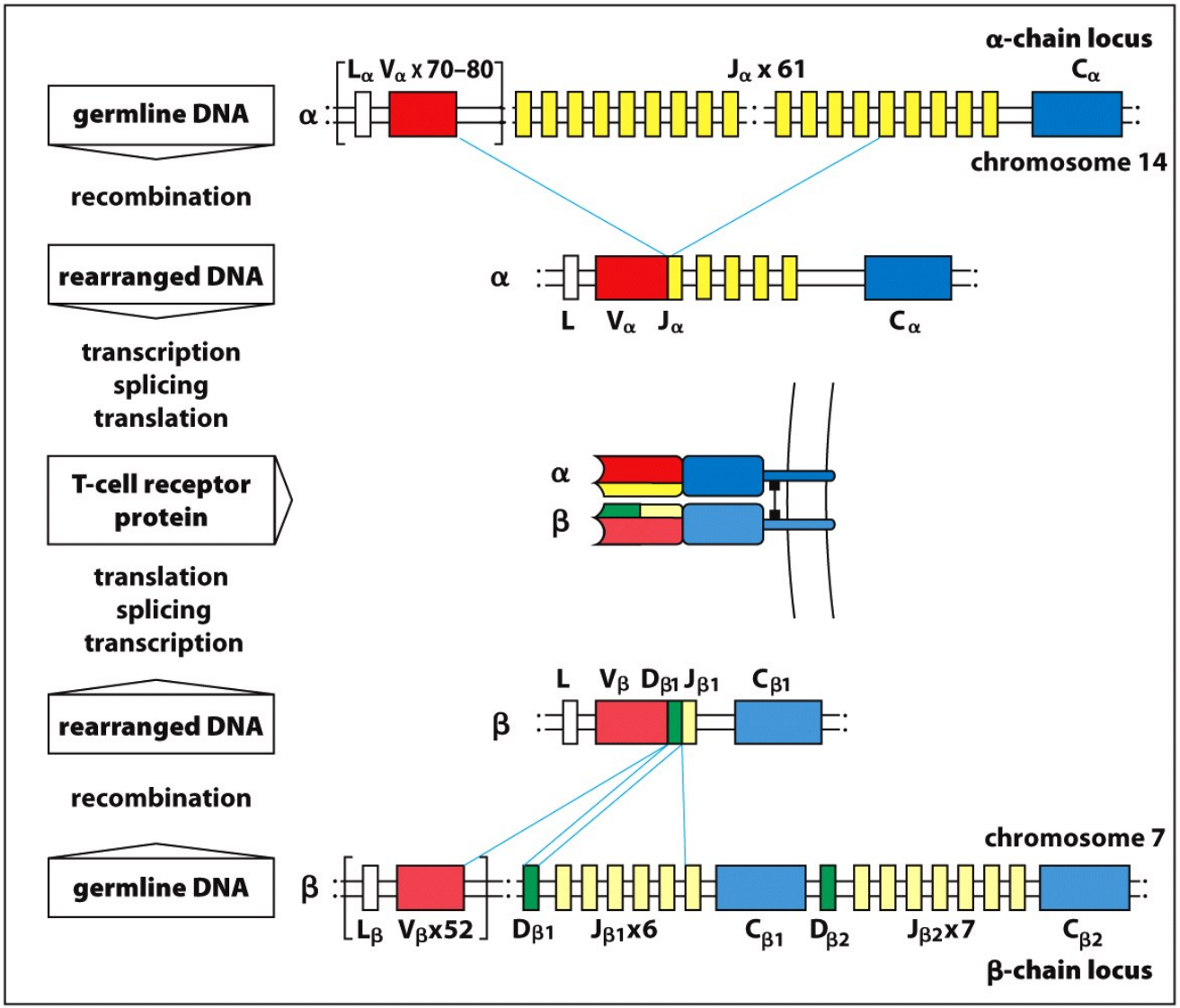
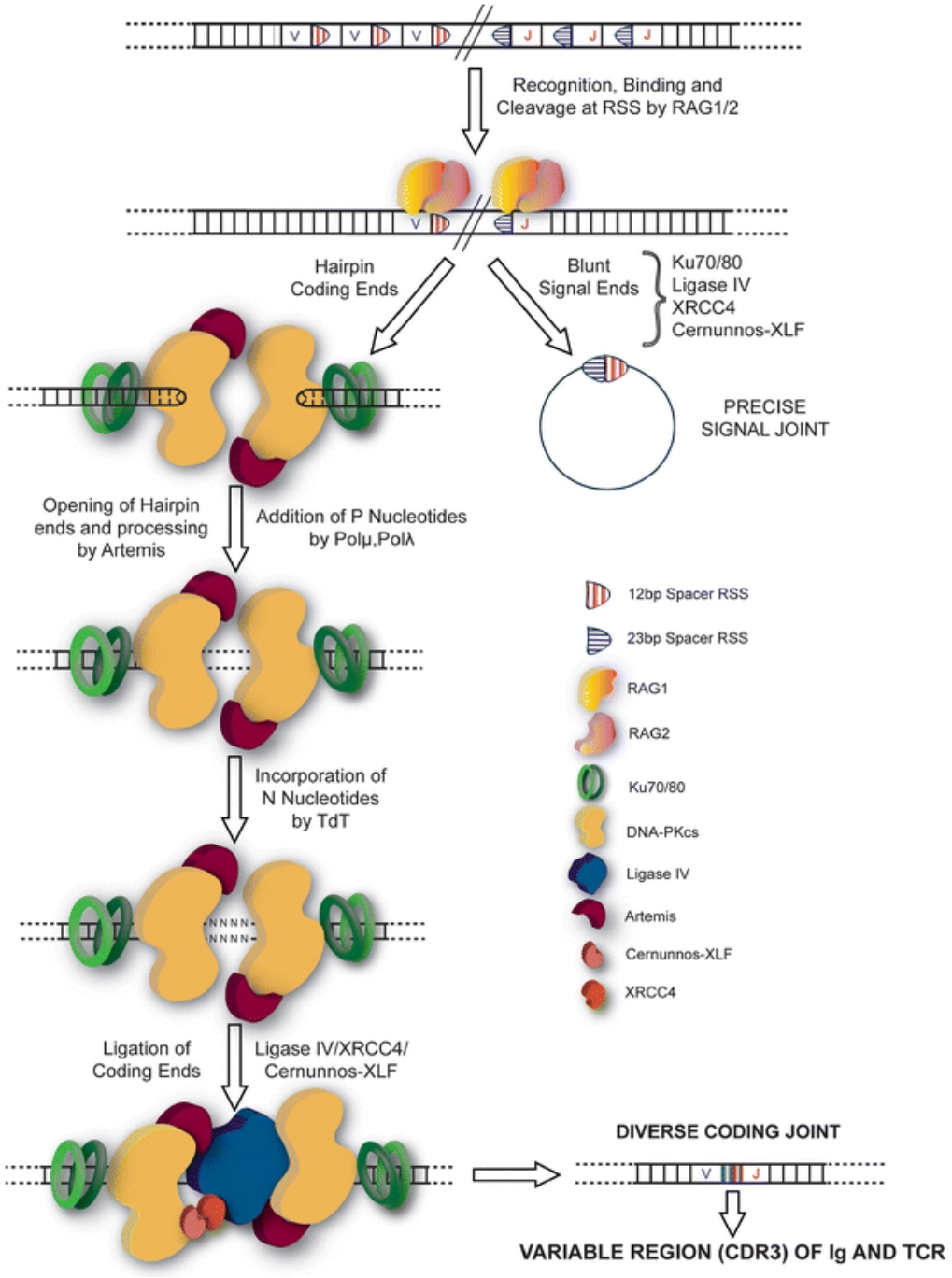


Figure 5.3 The Immune System, 3ed. (© Garland Science 2009)

V(D)J Gene Recombination

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

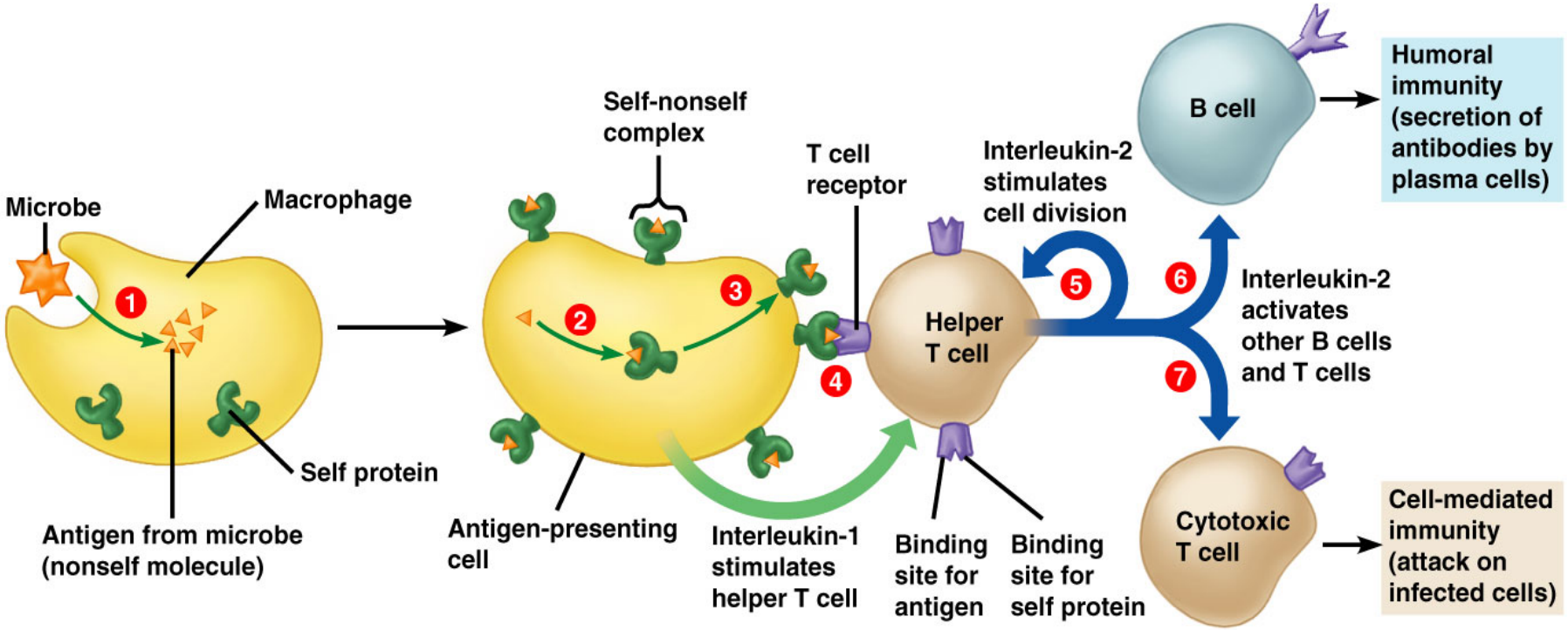


How Do the Variable Regions become Variable? Through NHEJ mediated DNA Recombination!

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.



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

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?

	T cell receptor	
	α	β
Number of V gene segments	54	67
Number of diversity (D) gene segments	0	2
Number of joining (J) gene segments	61	4

Over 3,000,000 combinations of variable, diversity and joining, V(D)J, gene segments are possible. Imprecise recombination and mutation increase the variability into billions of possible combinations.

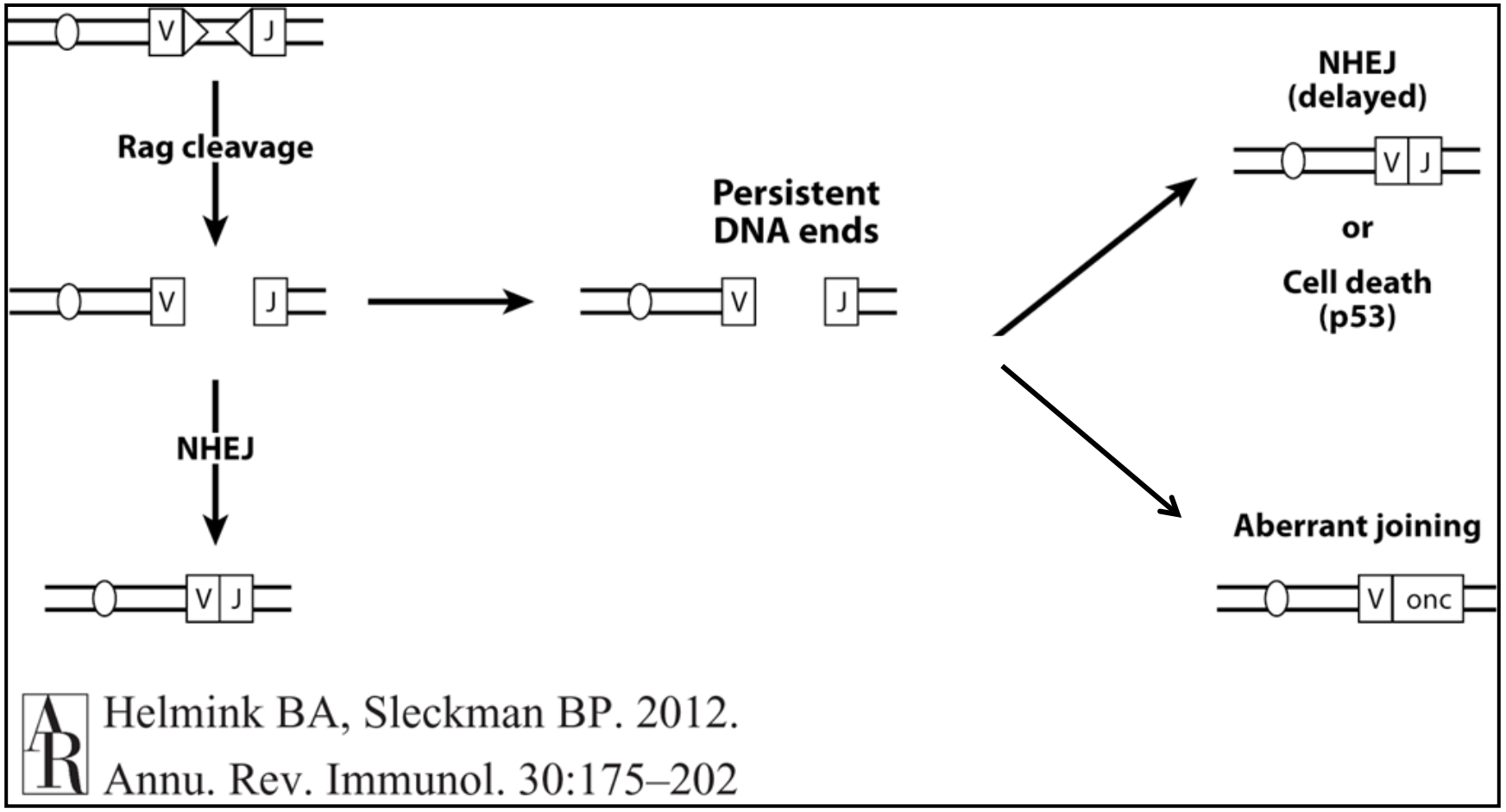
What happens if mice or people lose NHEJ capacity?

What happens if mice or people lose NHEJ capacity?

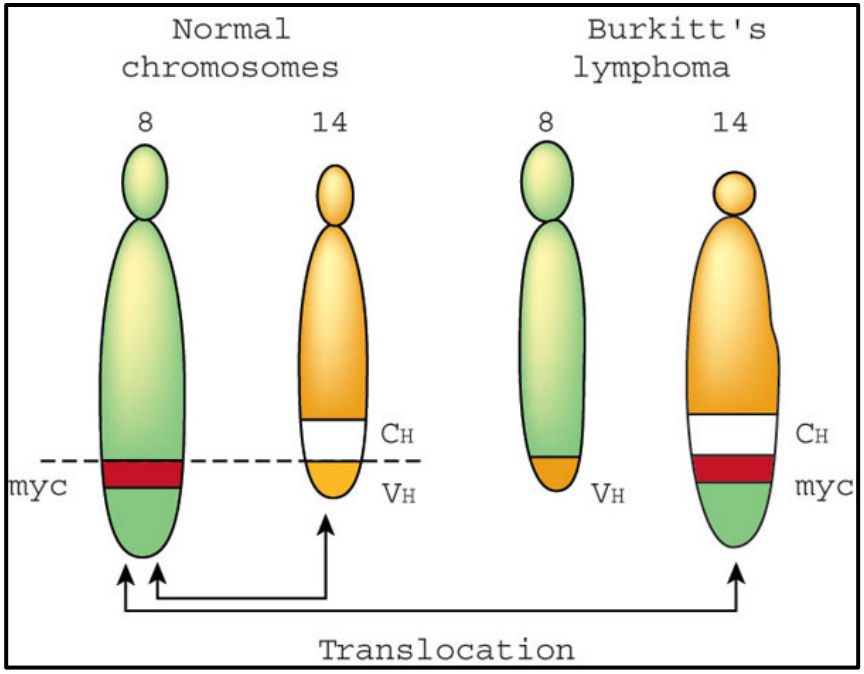
NHEJ gene	Mouse knockout phenotype	Patient phenotype
XRCC6 (encoding Ku70)	Viable, SCID, small size, radiosensitivity and thymoma[50,51]	None known
XRCC5 (encoding Ku80)	Viable, SCID, small size, radiosensitivity, genomic instability and tumours, especially with p53 deletion[47,52–54]	None known
PRKDC (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]

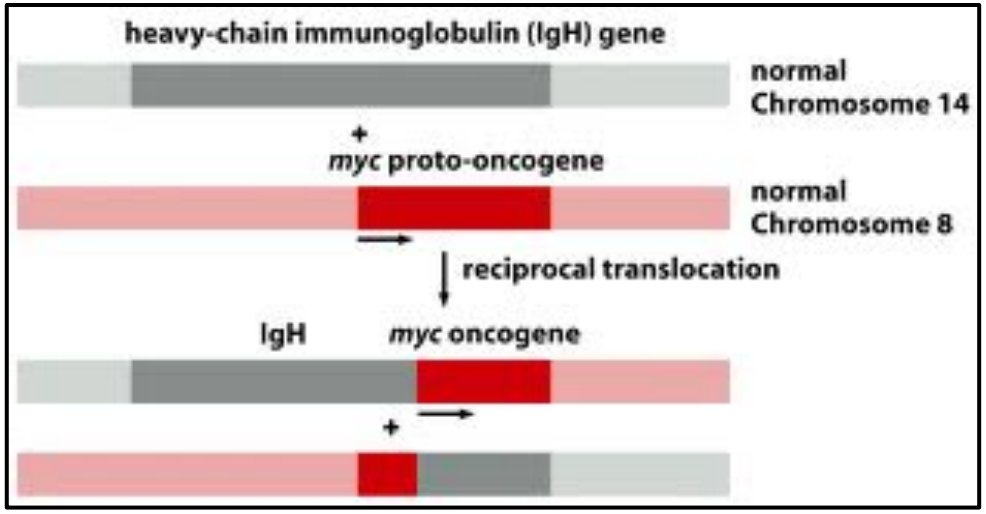
DCLRE1C, DNA cross-link repair 1C; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; LIG4, DNA ligase 4; NHEJ, non-homologous end-joining; NHEJ1, NHEJ factor 1; PRKDC, protein kinase, DNA-activated, catalytic polypeptide; SCID, severe combined immunodeficiency; XLF, XRCC4-like factor; XRCC, X-ray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

Can V(D)J Recombination Go Wrong?



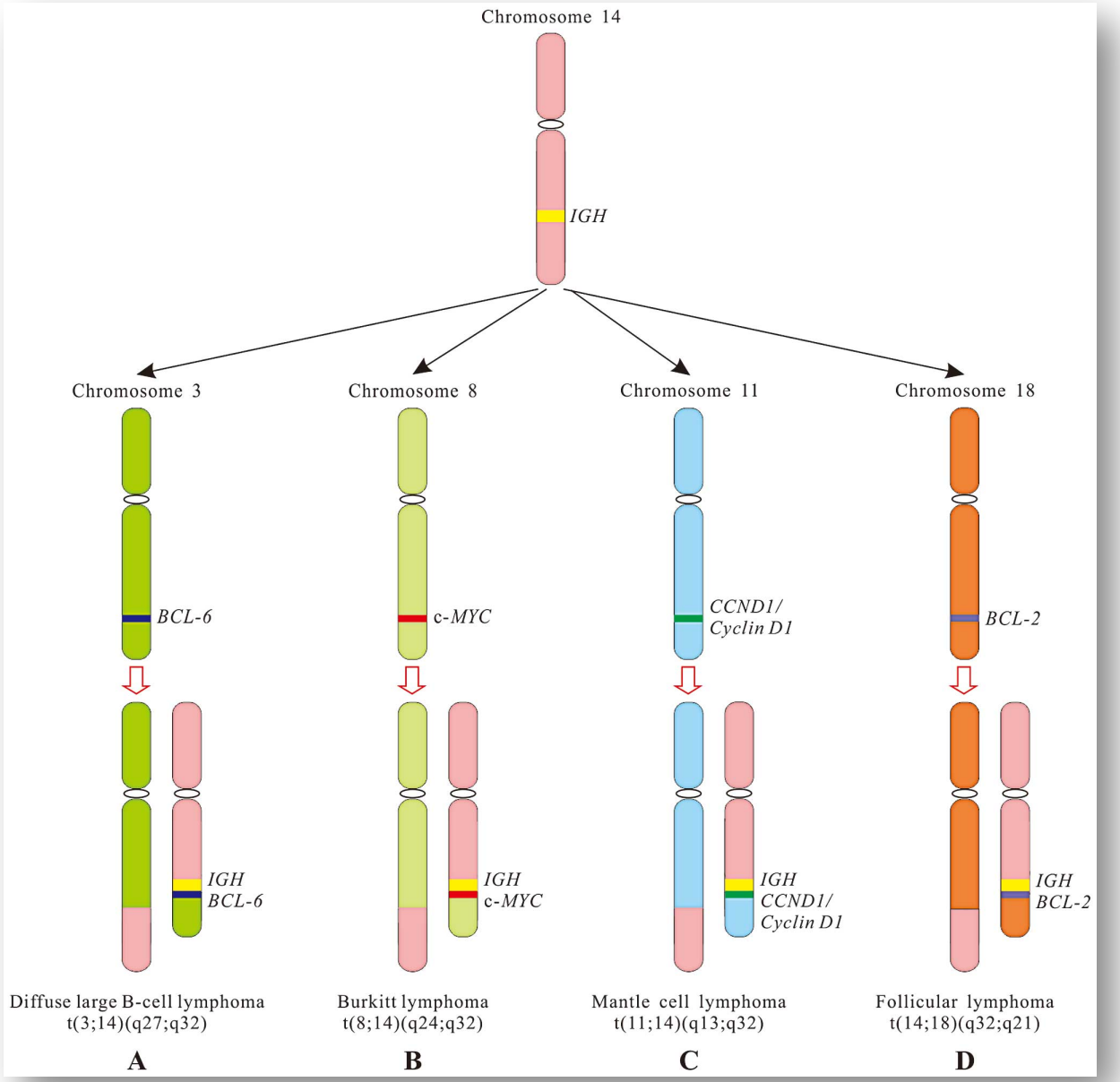
BURKITT's LYMPHOMA
B-cell Lymphoma





MYC-overexpression stimulates cell proliferation

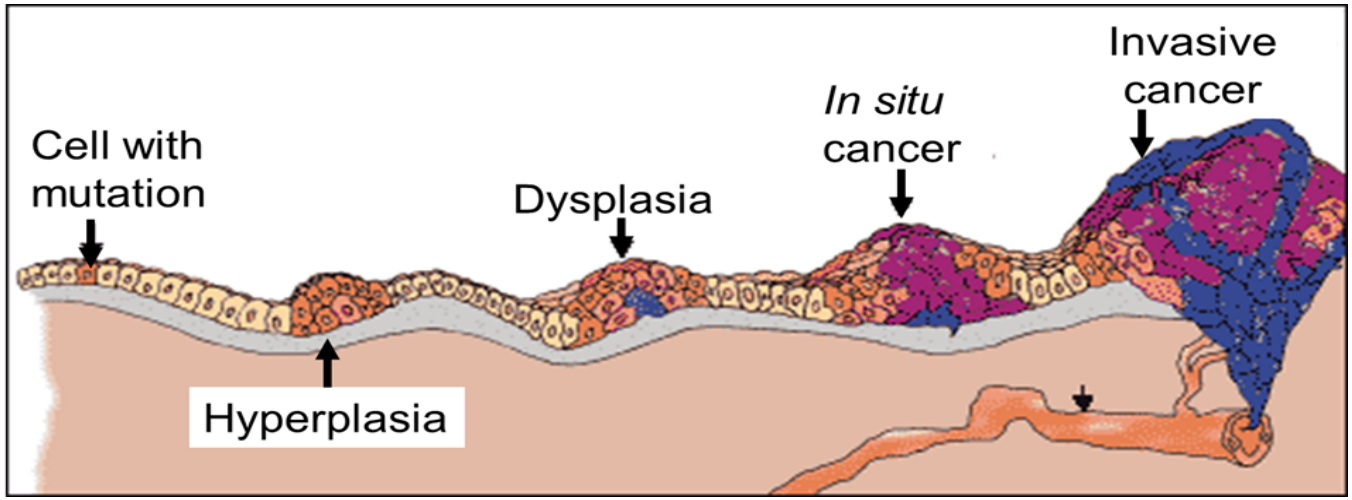
Other B-cell Lymphomas



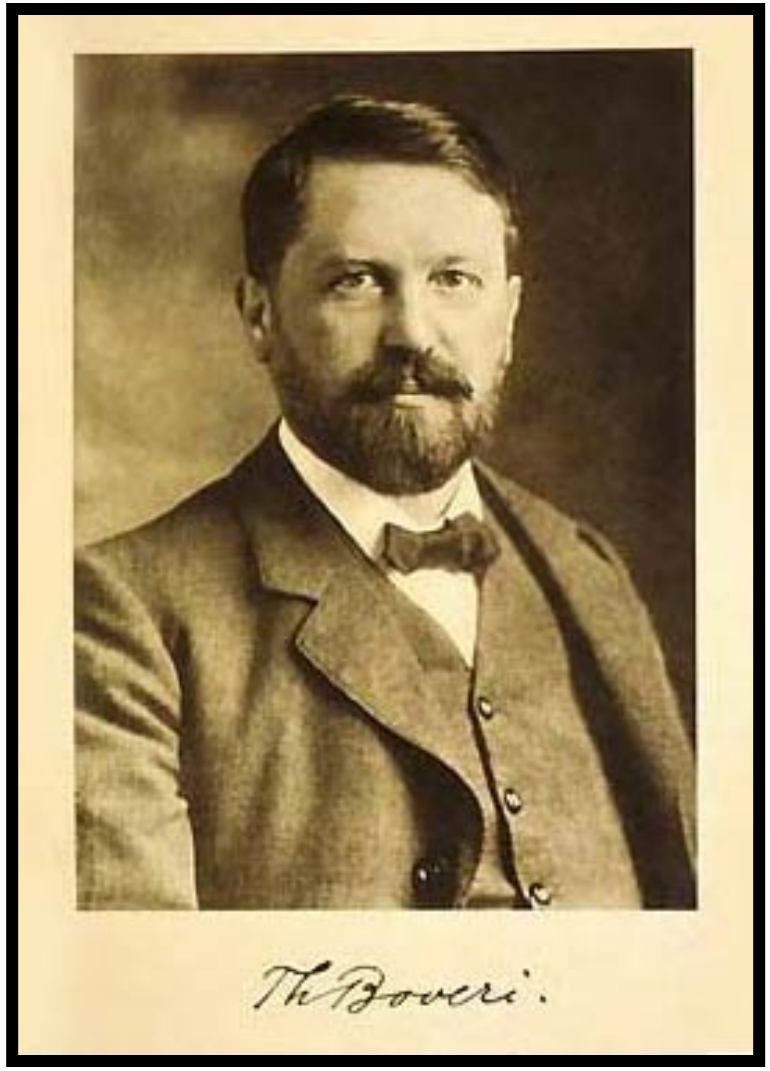
Cancers arise from the accumulation of heritable changes in gene function

Normal Epithelium → Neoplasia → Invasive Carcinoma → Metastasis

1st Mutation → 2nd Mutation → Additional Mutations



The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915



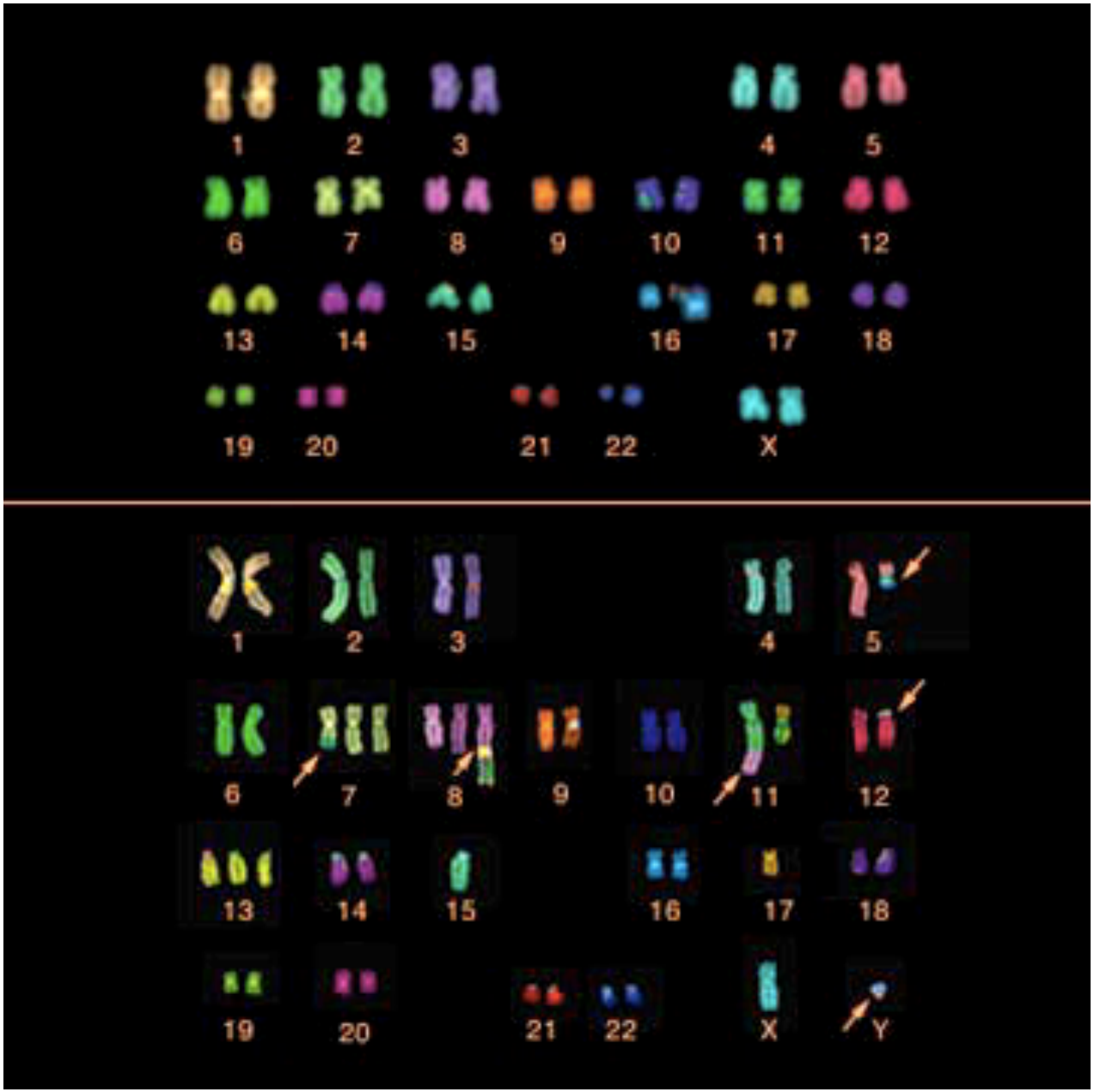
- Established that chromosomes carry the hereditary information by showing that aberrant segregation of chromosomes leads to certain phenotypes in sea urchin eggs.
- Suggested that aberrant segregation of human chromosomes could be responsible for a normal cell becoming a tumor cell
- Suggested that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

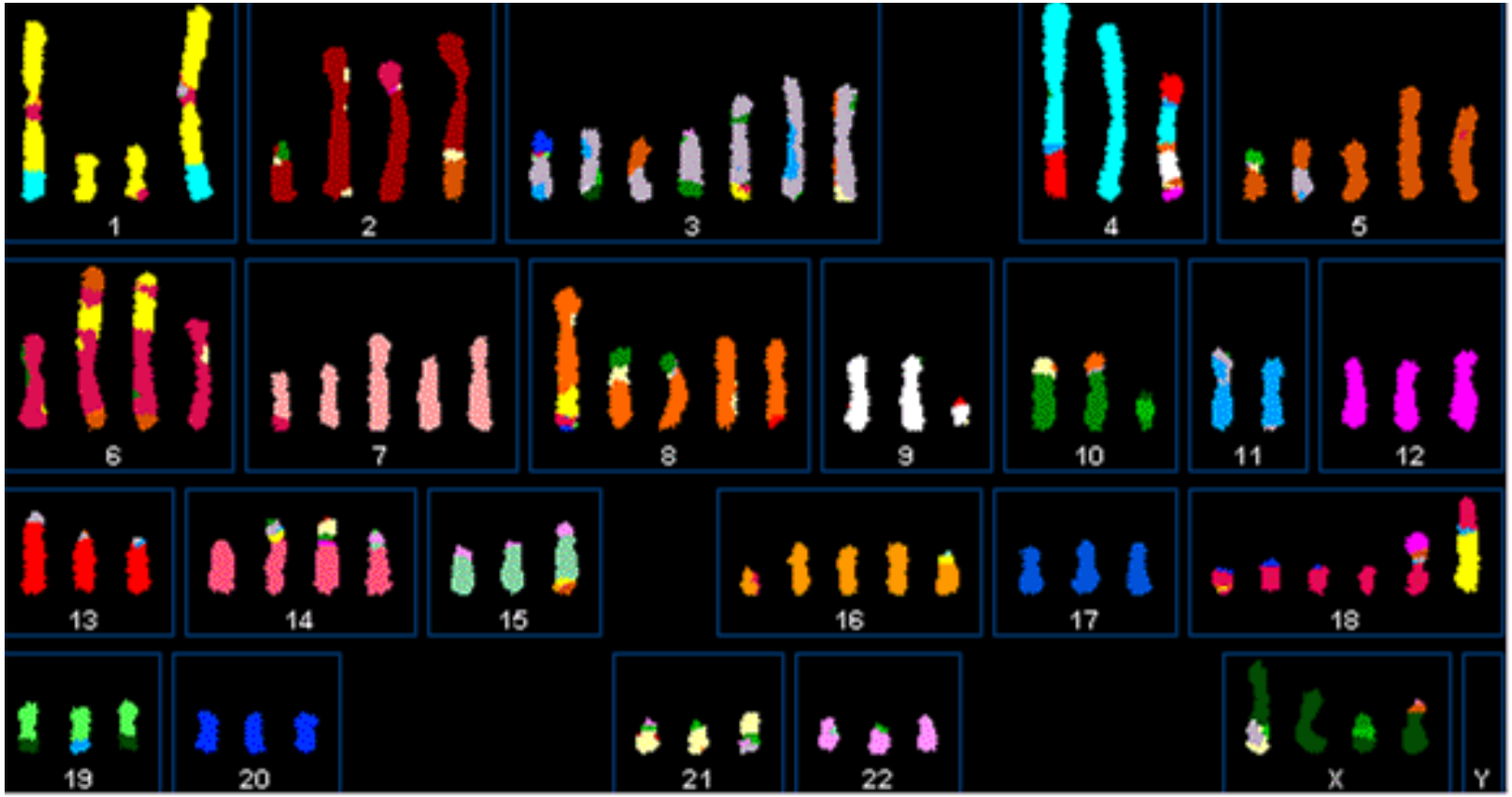


Chromosomes from a Normal cell

Chromosomes from a Tumor cell

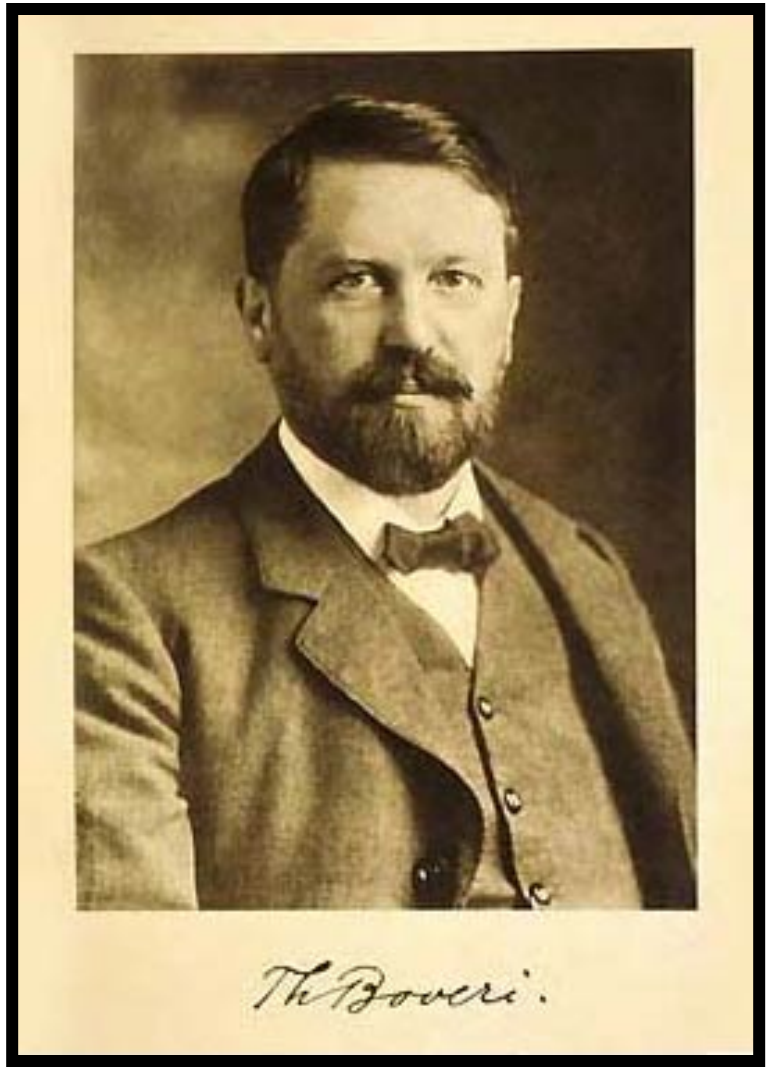
Spectral Karyotyping (SKY)
"SKY Painted Chromosomes"

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

Alterations (mutations) in different kinds of Genes cause Cancer

Oncogenes

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

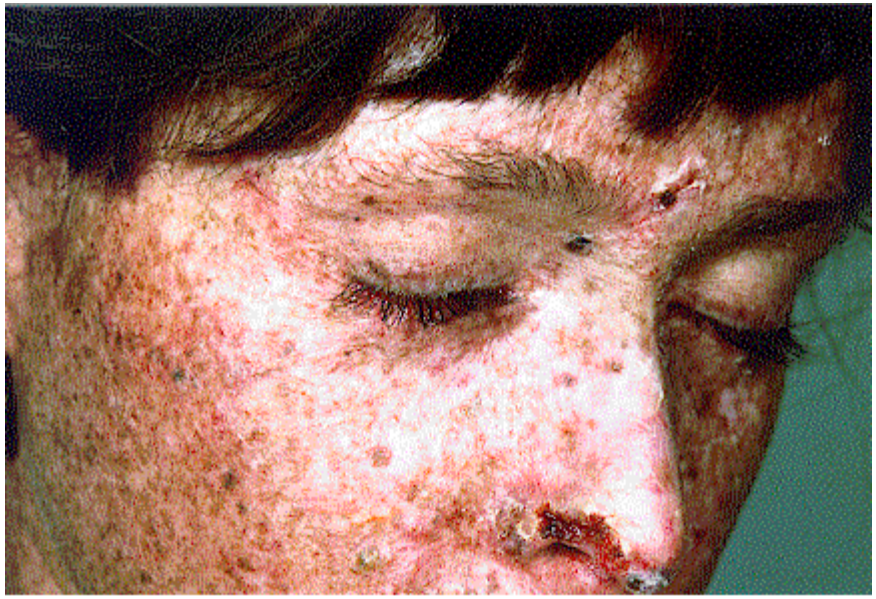
Tumor suppressor genes

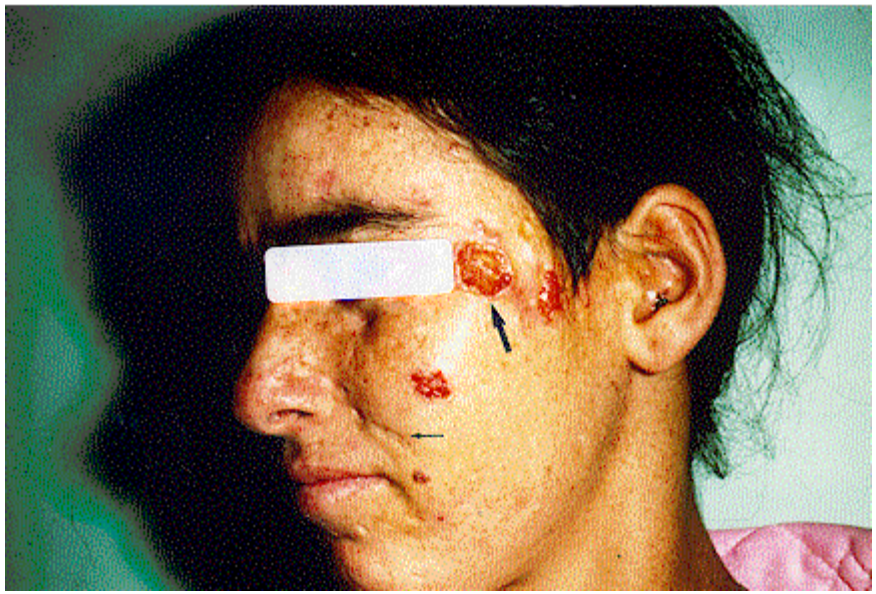
genes that ordinarily prevent inappropriate proliferation but when mutated allow uncontrolled growth

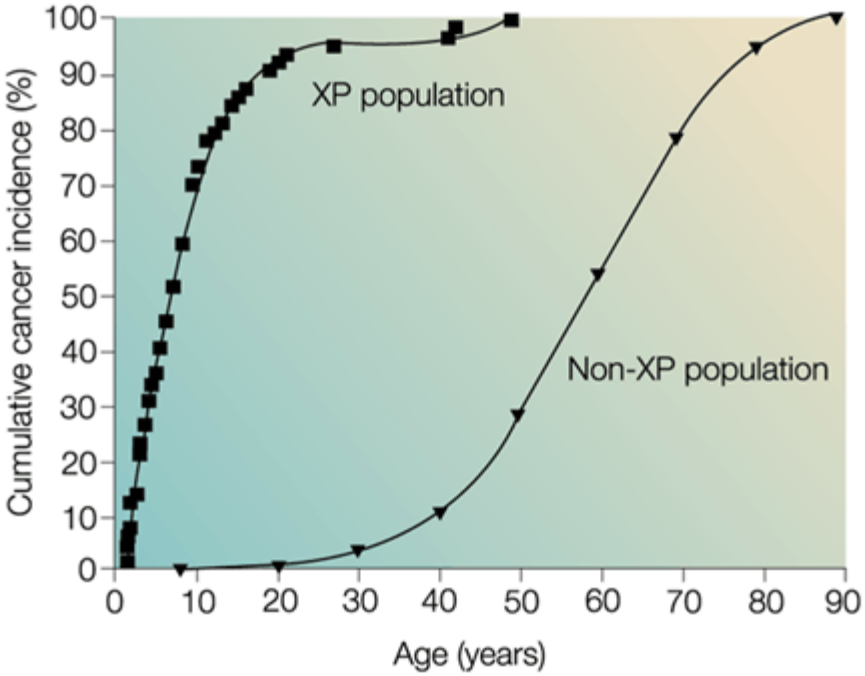
Mutator genes

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

Lack of DNA repair accelerates the onset of cancer

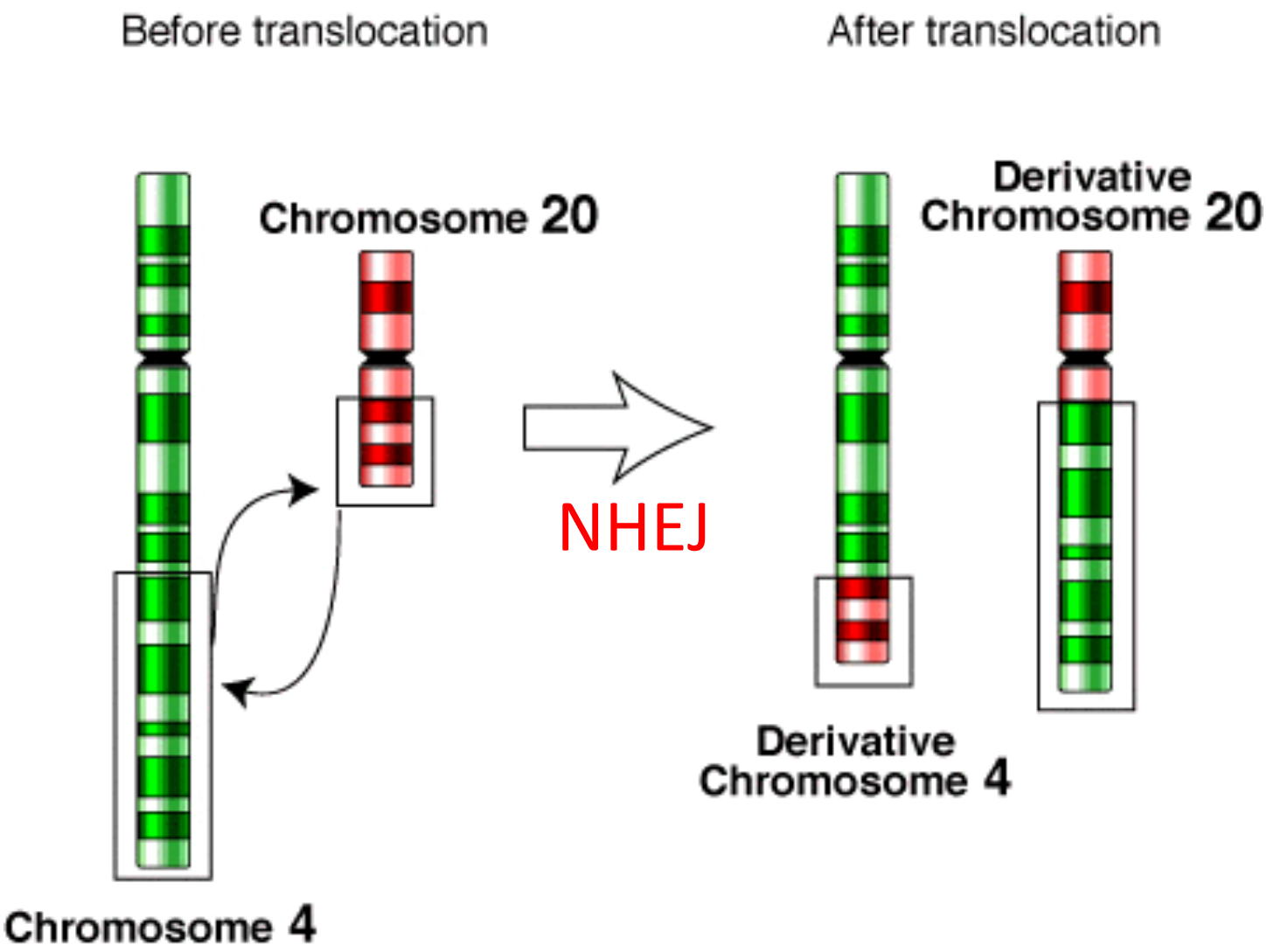




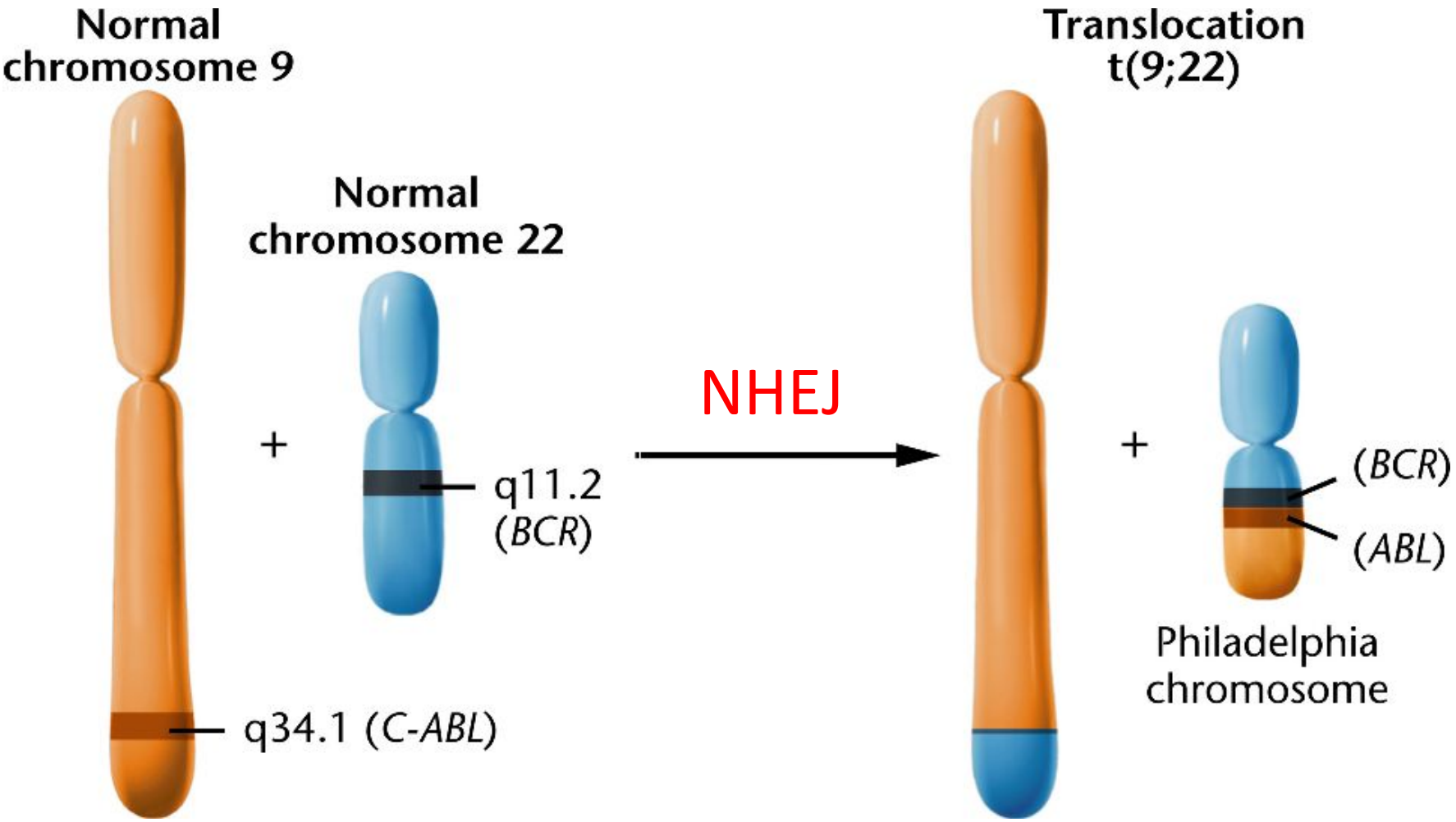


Nature Reviews |

Mechanisms of Chromosome Translocation



Chronic Myelogenous Leukemia

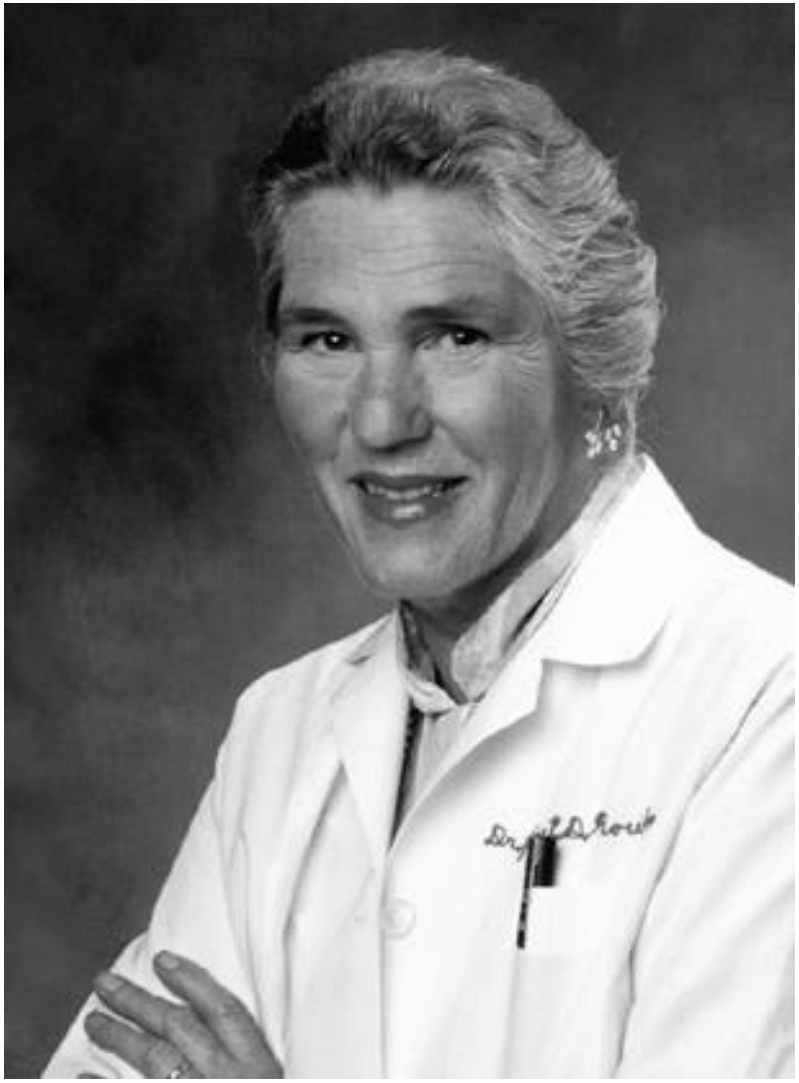


Breakpoint Cluster Region protein (BCR)
C-Abl non-receptor tyrosine kinase – stimulates cell growth

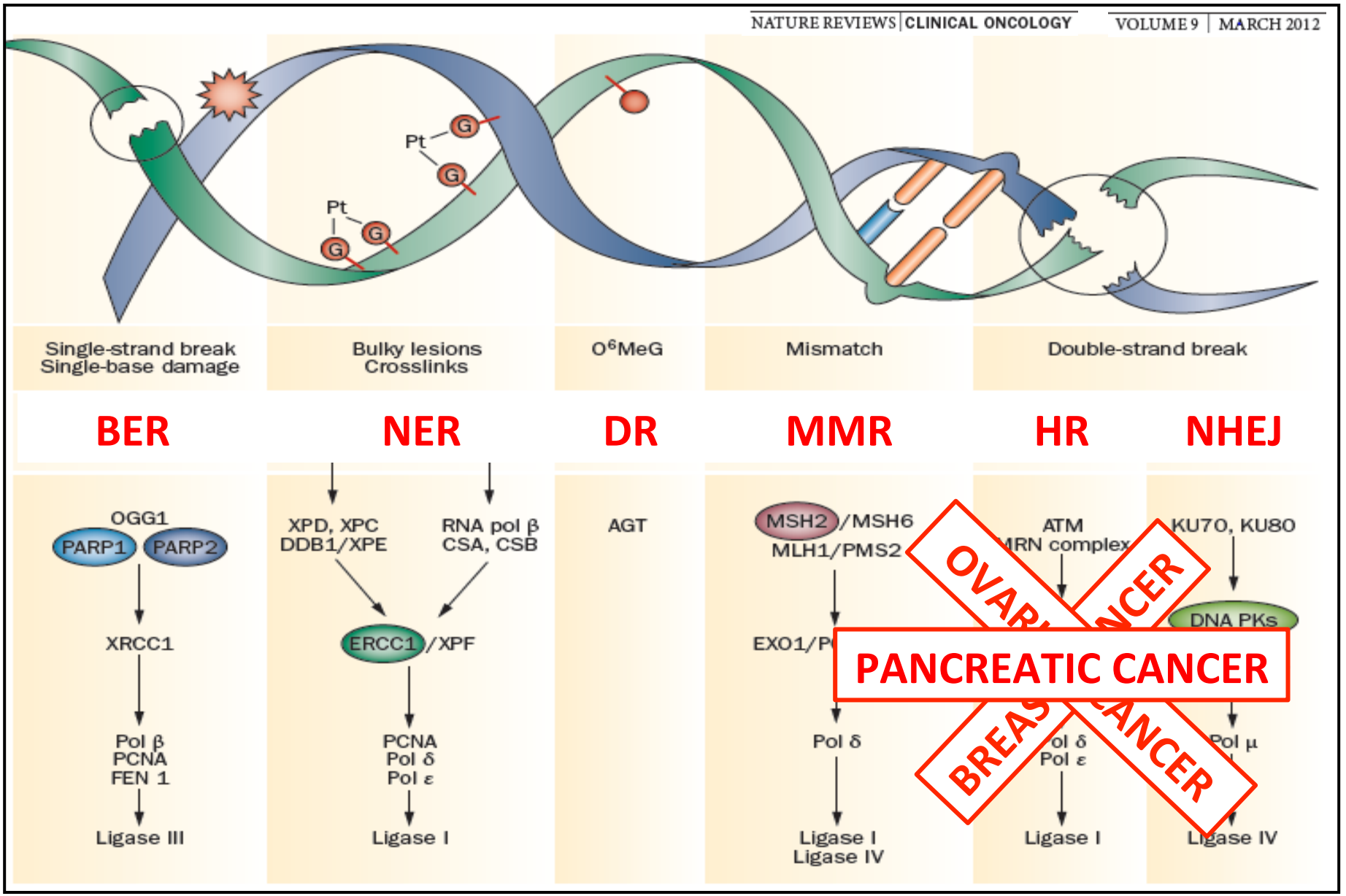
Janet Rowley

(April 5, 1925 – December 17, 2013)

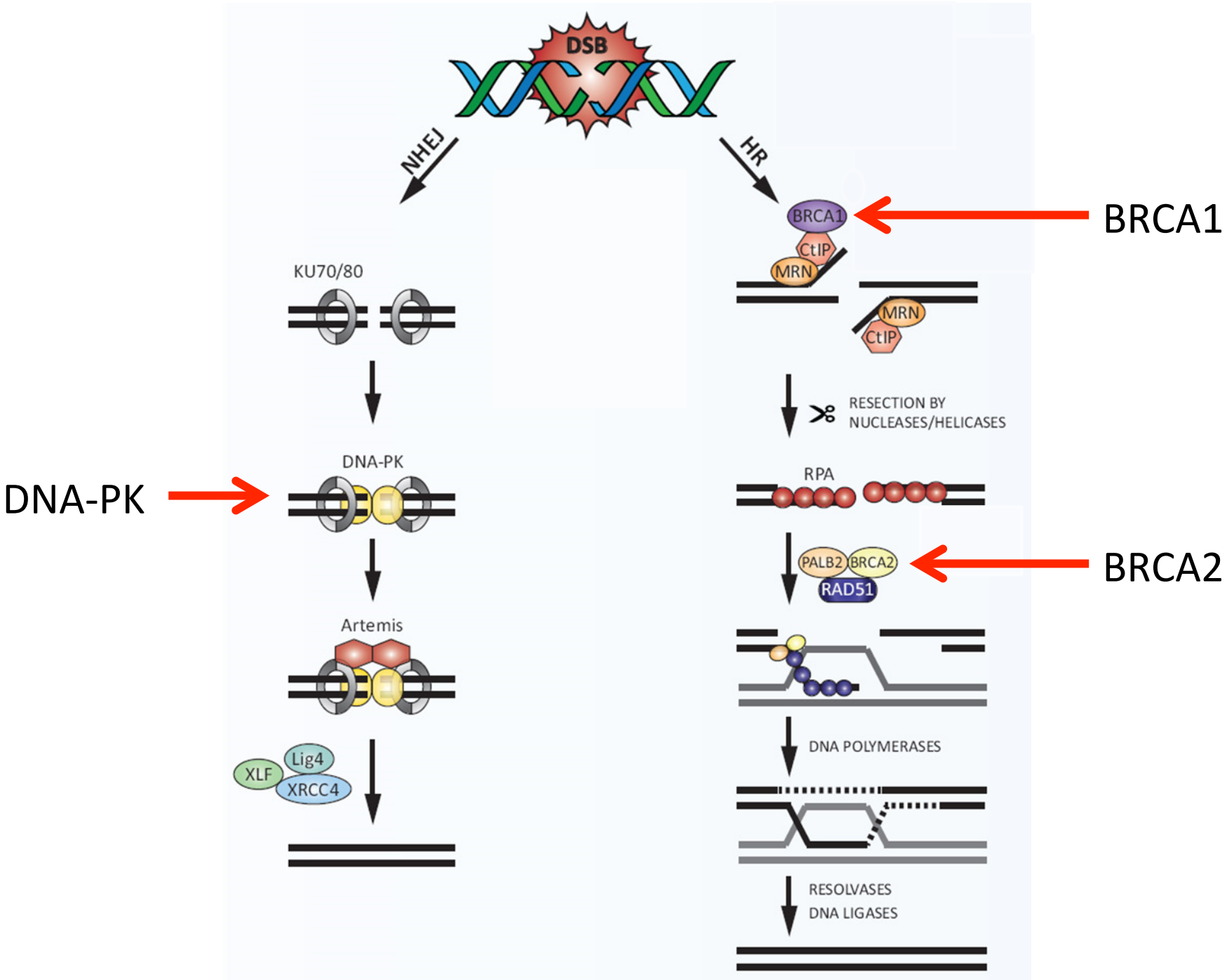
American human geneticist and the first scientist to identify the mechanism by which a chromosomal translocation causes Leukemia and other cancers.

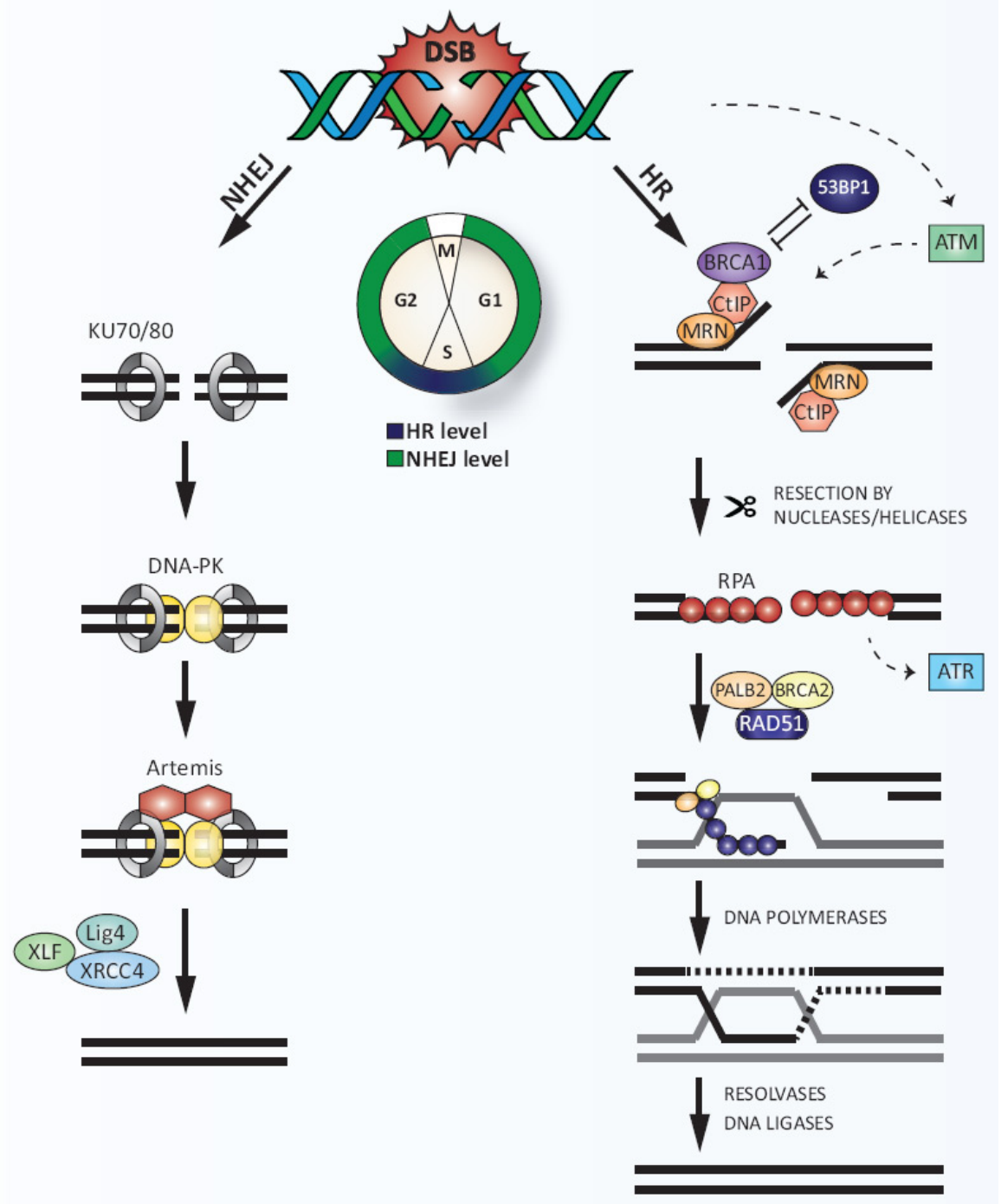


Six Major DNA Repair Pathways



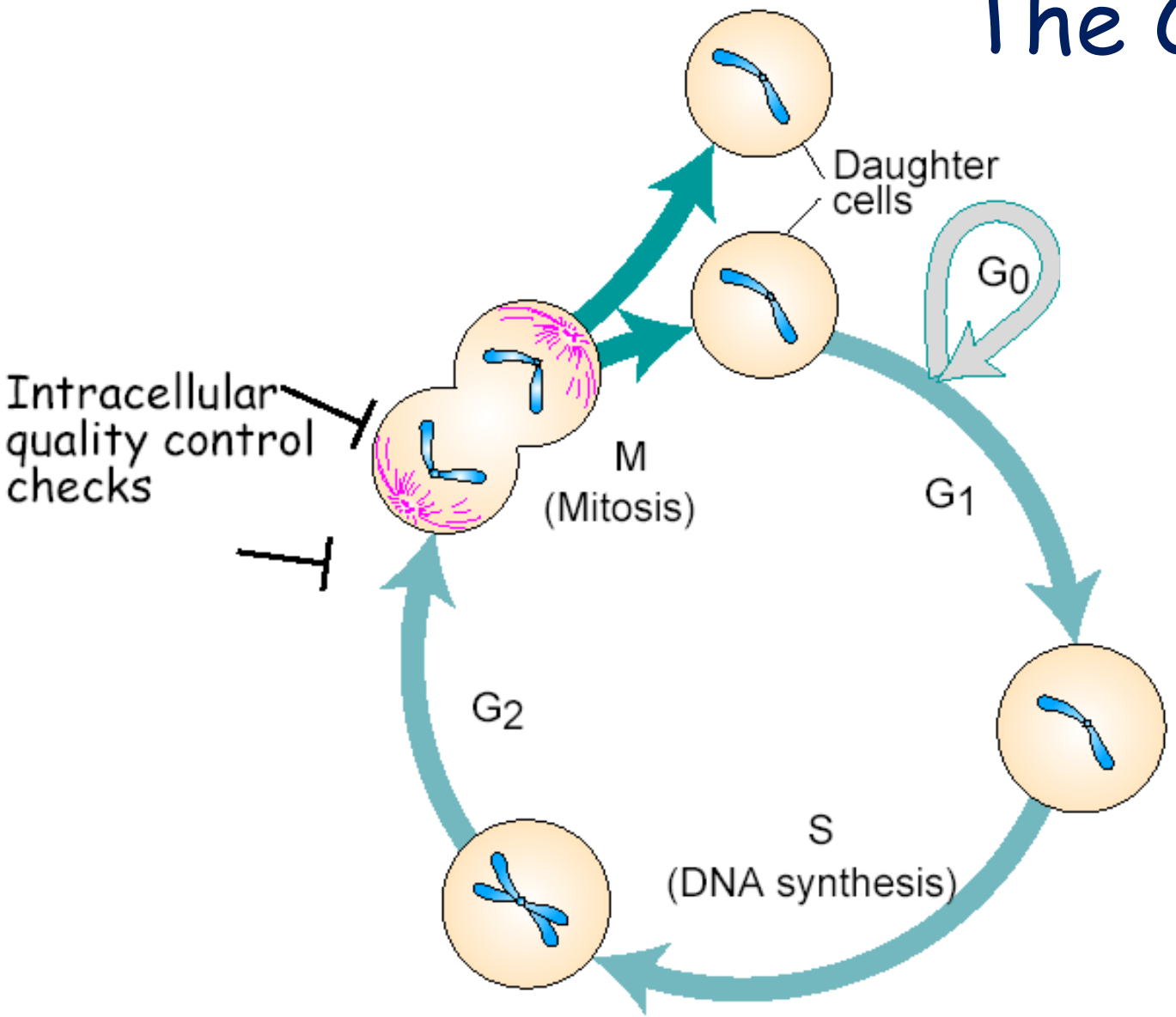
Chromosomes from a Pancreatic Tumor Cell


DSB
NHEJ
HR
KU70/80
DNA-PK
Artemis
XLF
Lig4
XRCC4
BRCA1
CtIP
MRN
MRN
CtIP
RESECTION BY
NUCLEASES/HELICASES
RPA
PALB2
BRCA2
RAD51
DNA POLYMERASES
RESOLVASES
DNA LIGASES
DNA-PK
BRCA1
BRCA2



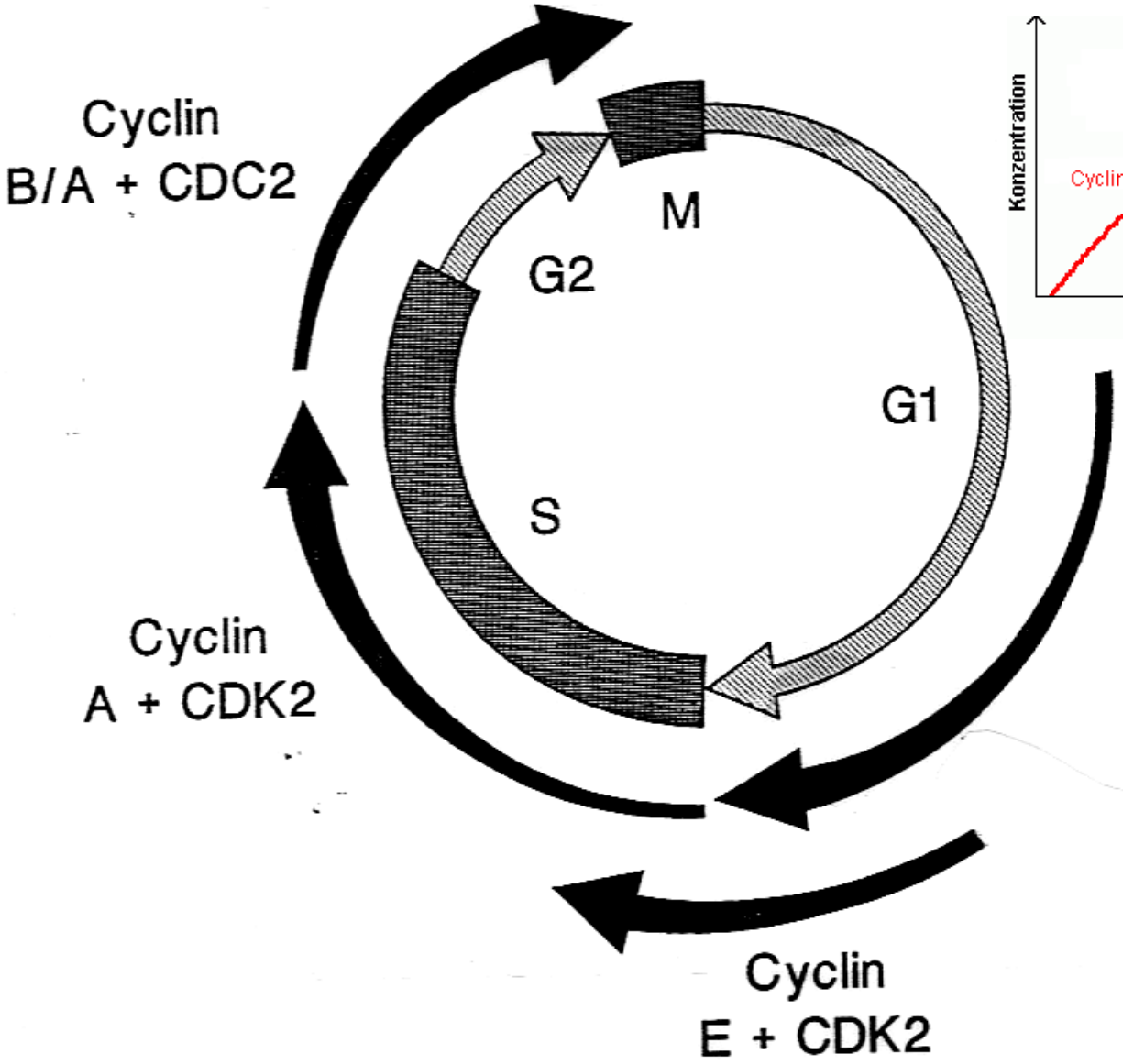
How does the cell decide which pathway to use?

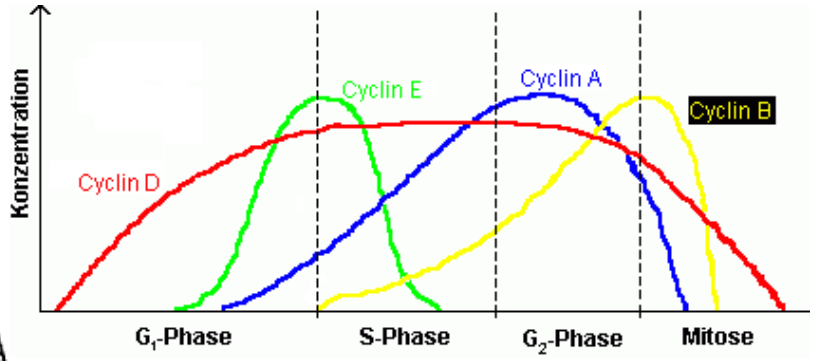
The Cell Cycle

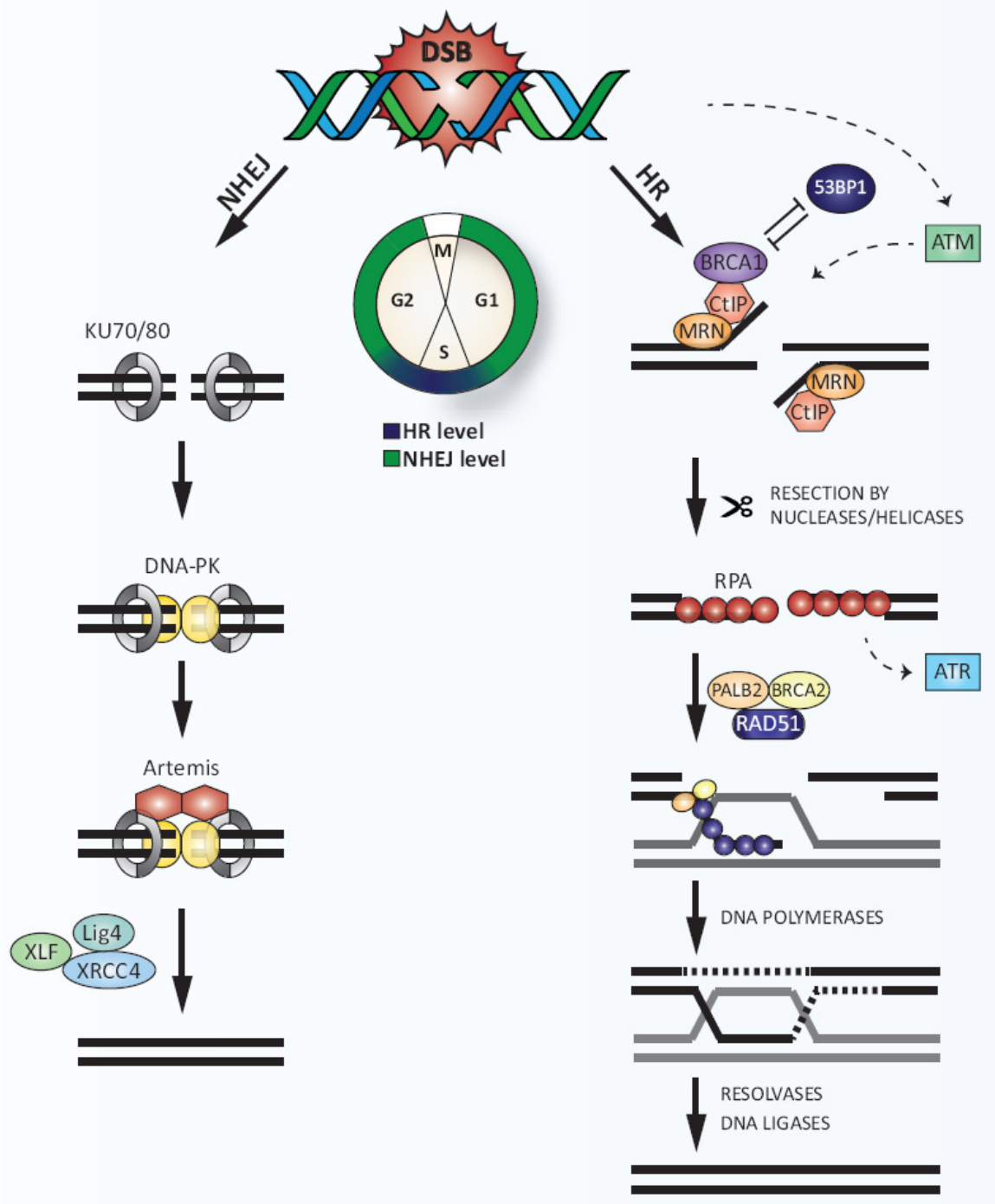


Duplication of chromosomes
DNA Replication

Progression through the Cell Cycle REQUIRES a series of cyclins and cyclin-dependent-kinases (CDKs)

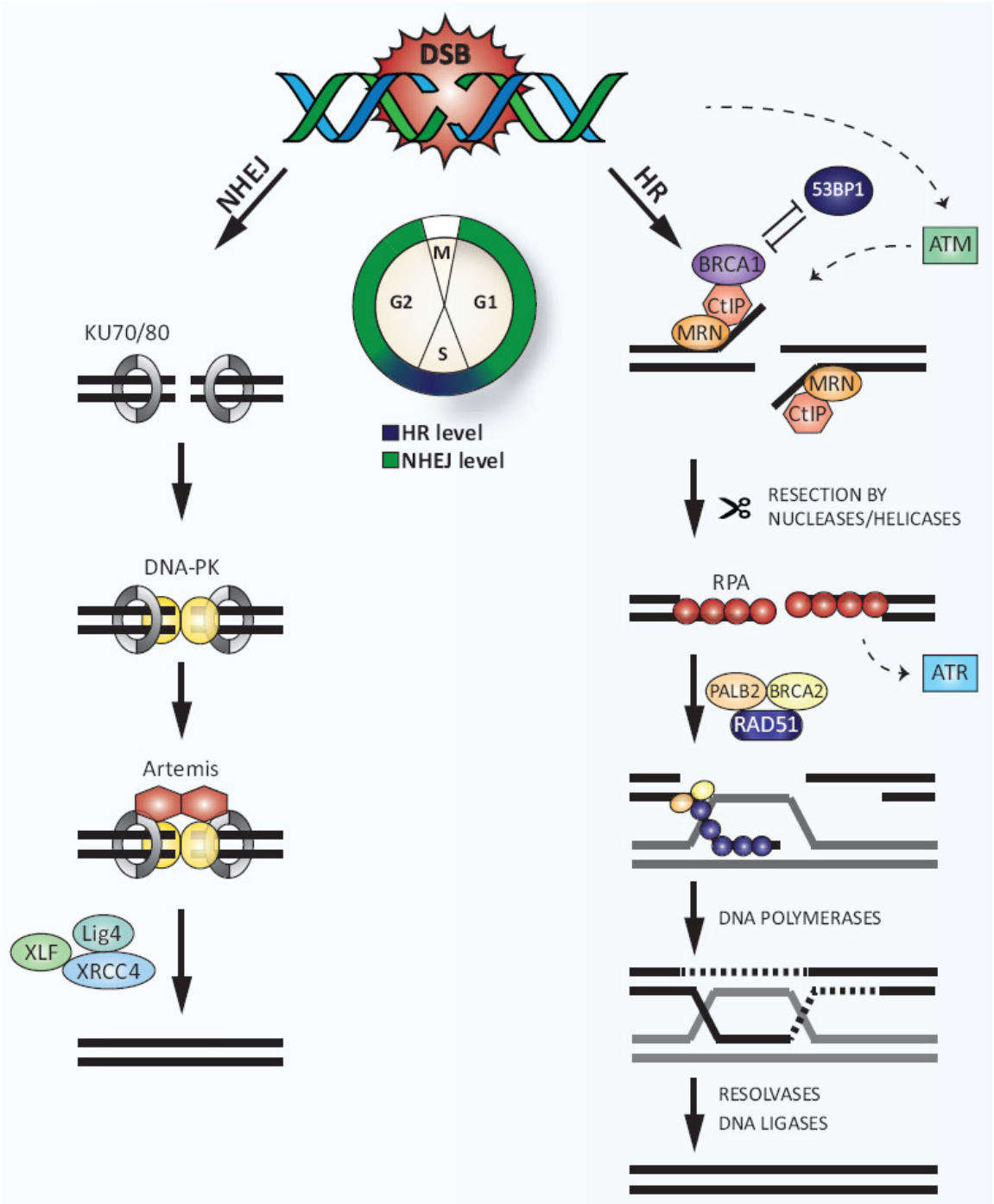


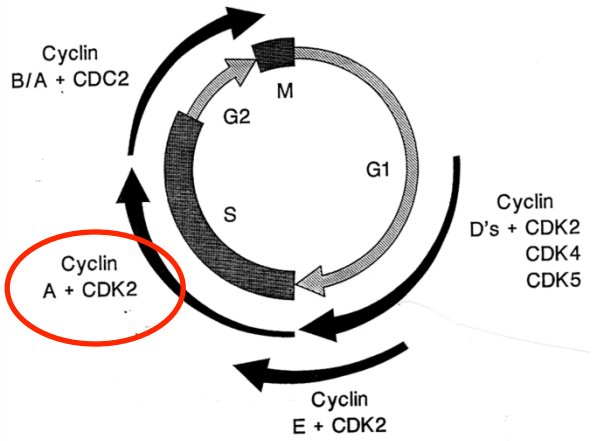




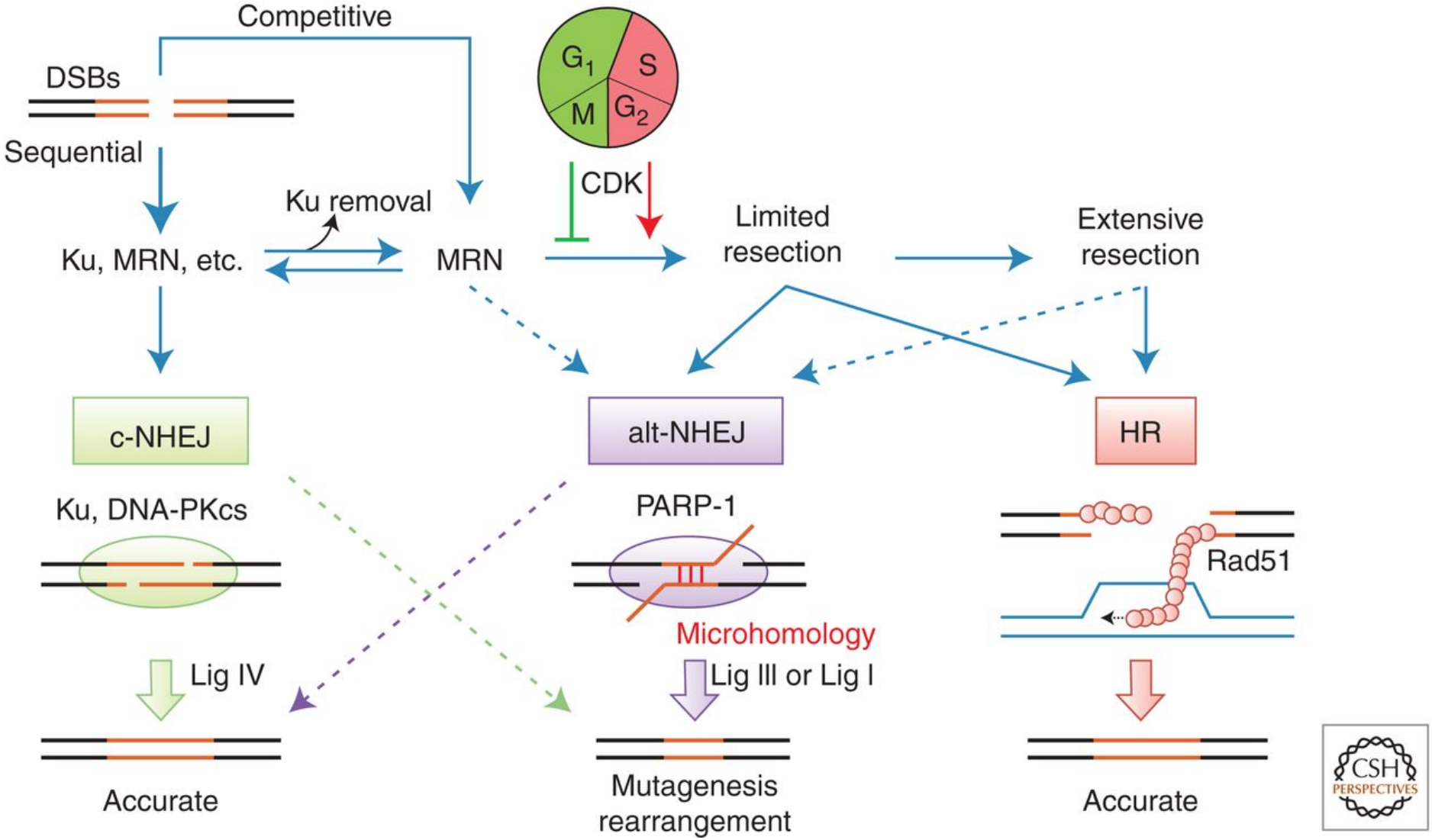
How does the cell decide which pathway to use?

CyclinA-CDK2 targets the CtIP/ BRCA1/MRN complex





Disposition of DSBs between repair pathways.



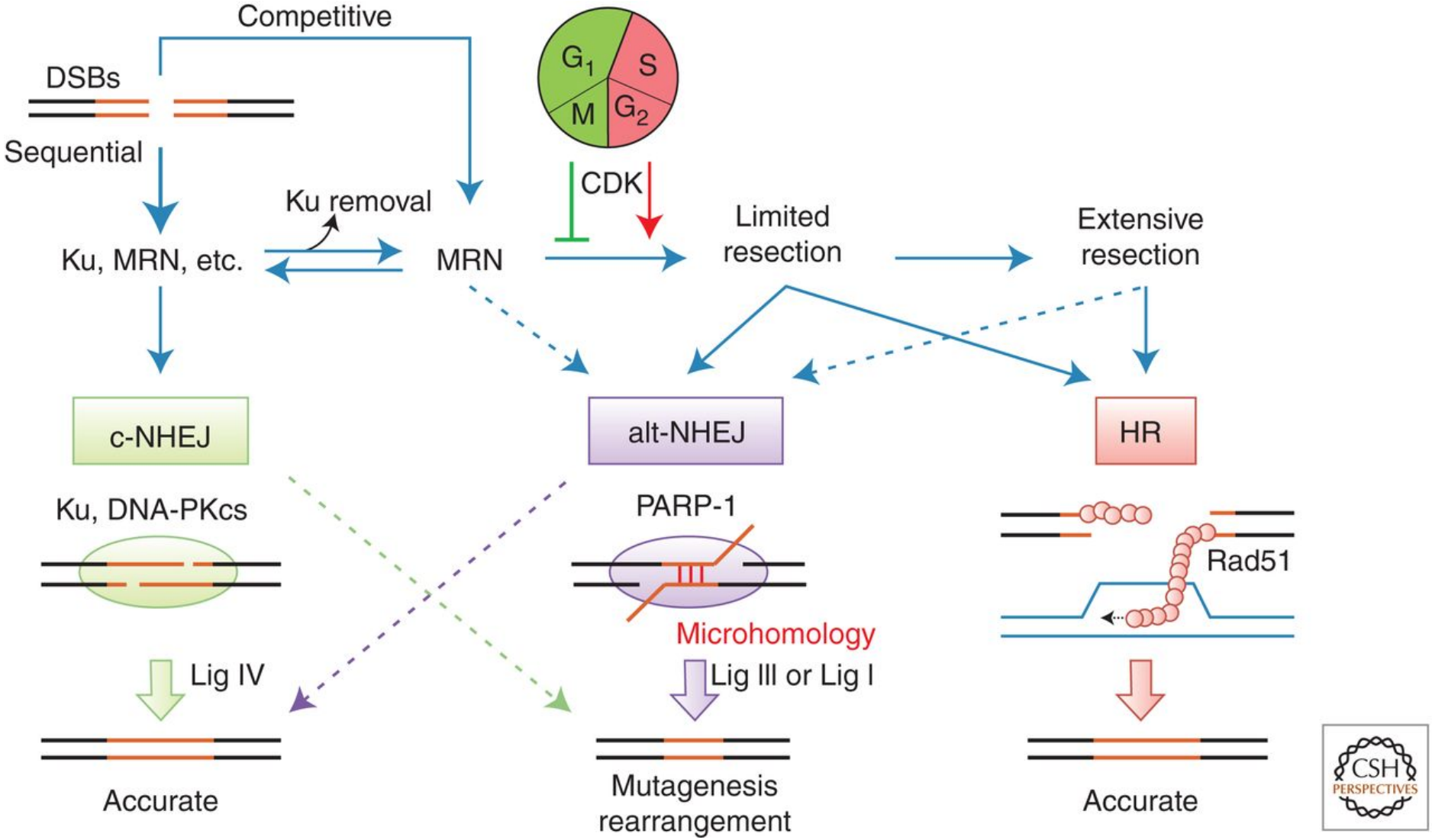
Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757

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

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

Engelward lab Animations

Disposition of DSBs between repair pathways.



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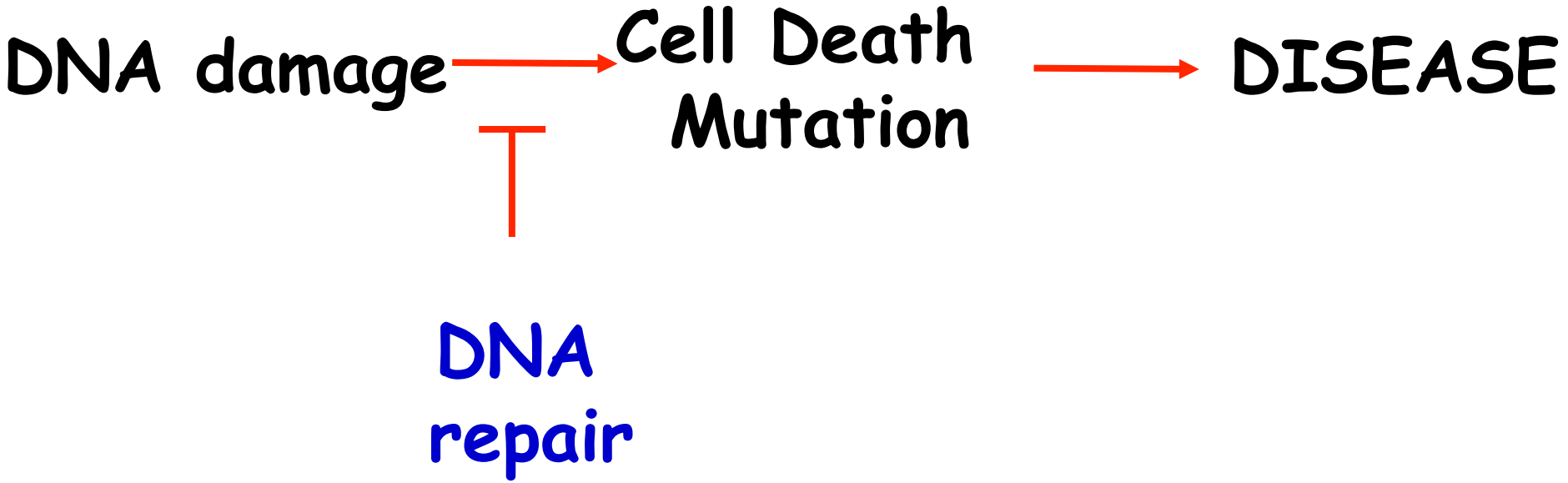
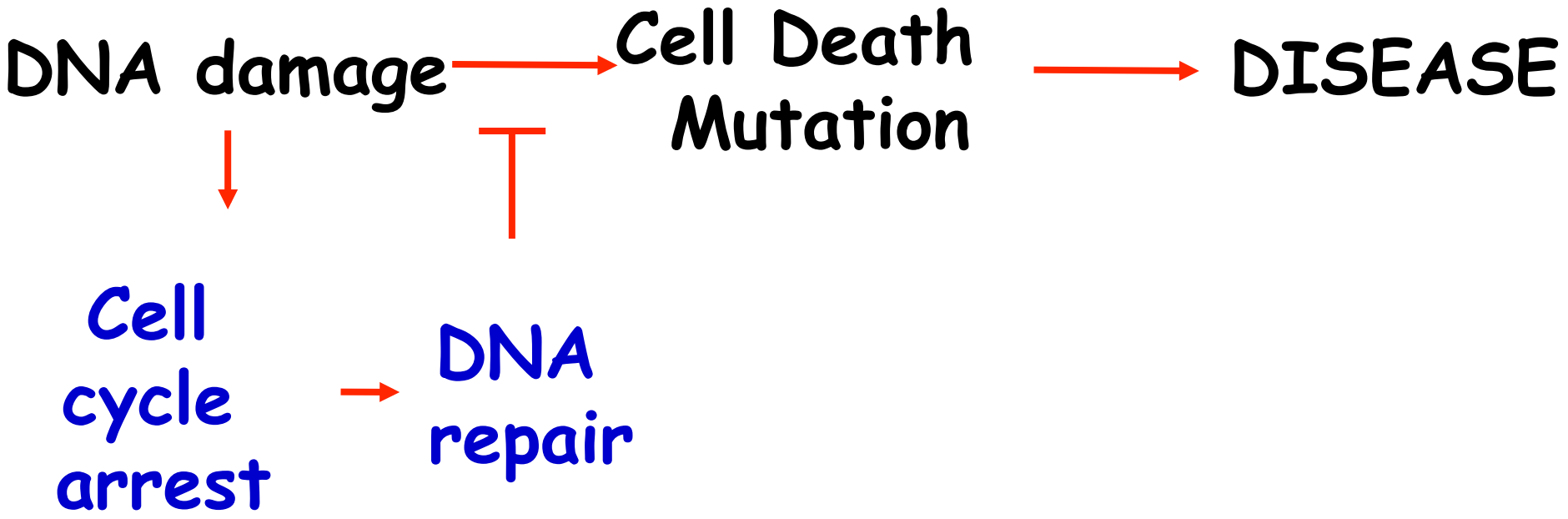

DNA damage
Cell Death
Mutation
DISEASE
DNA
repair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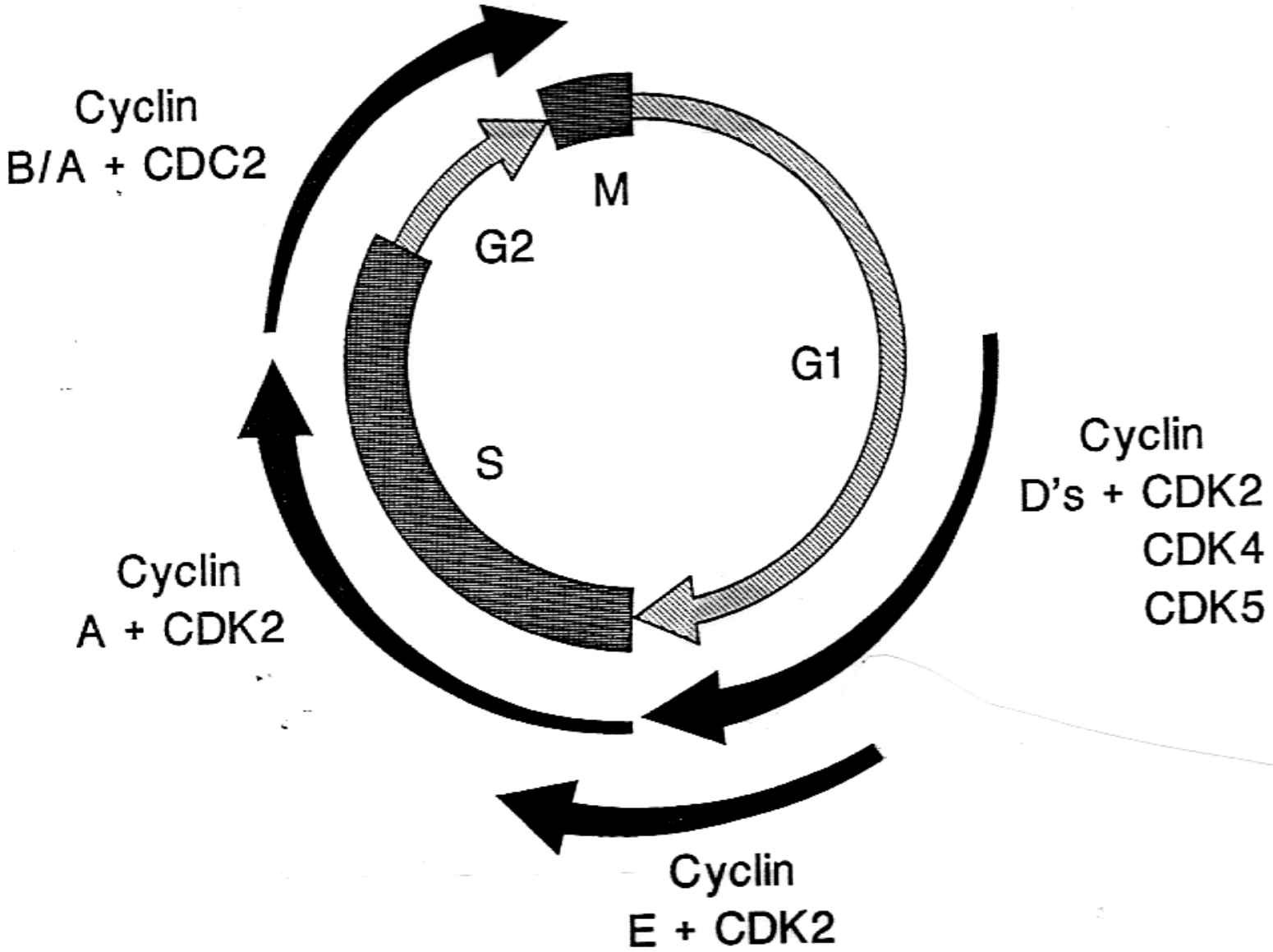
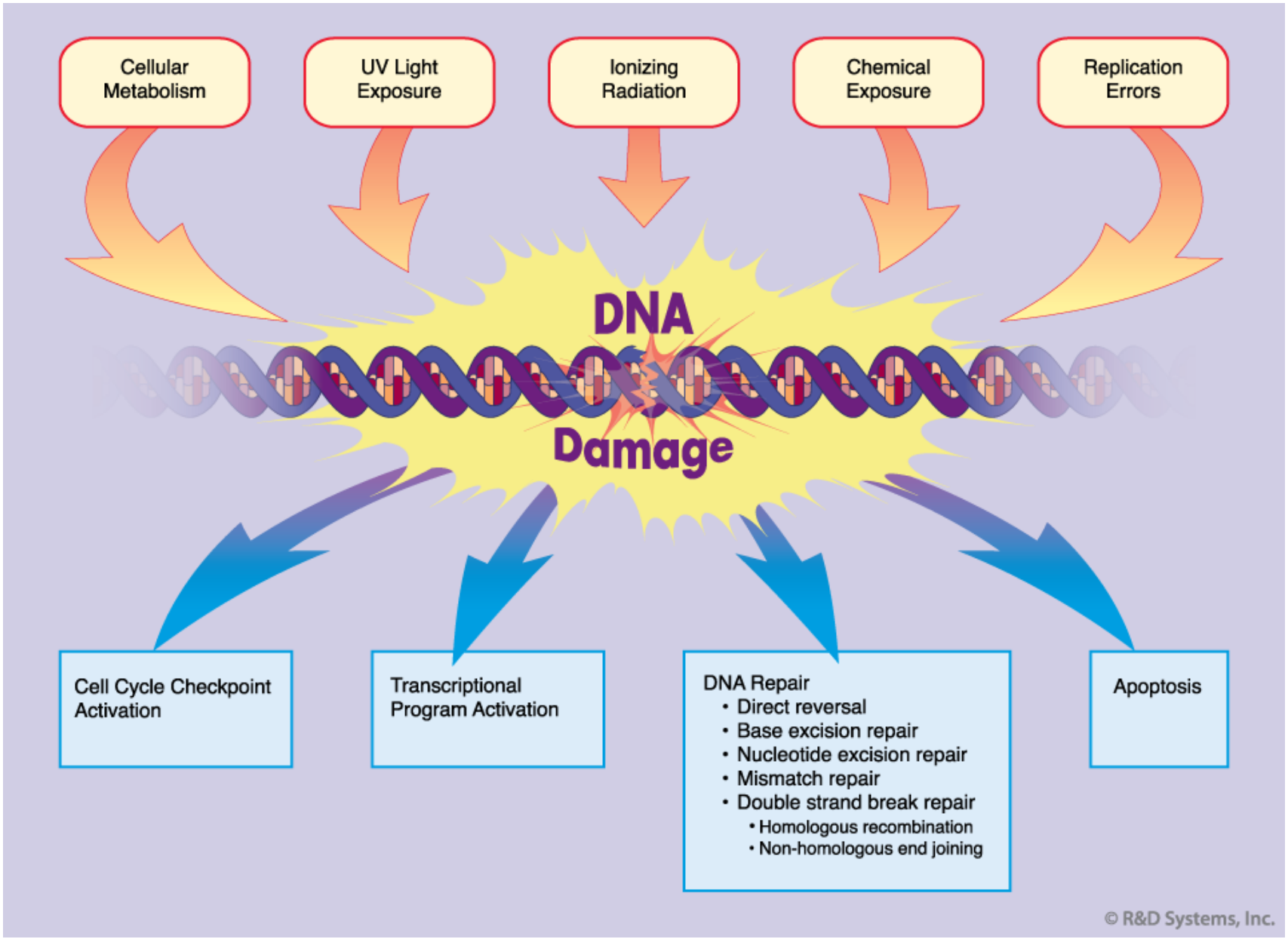
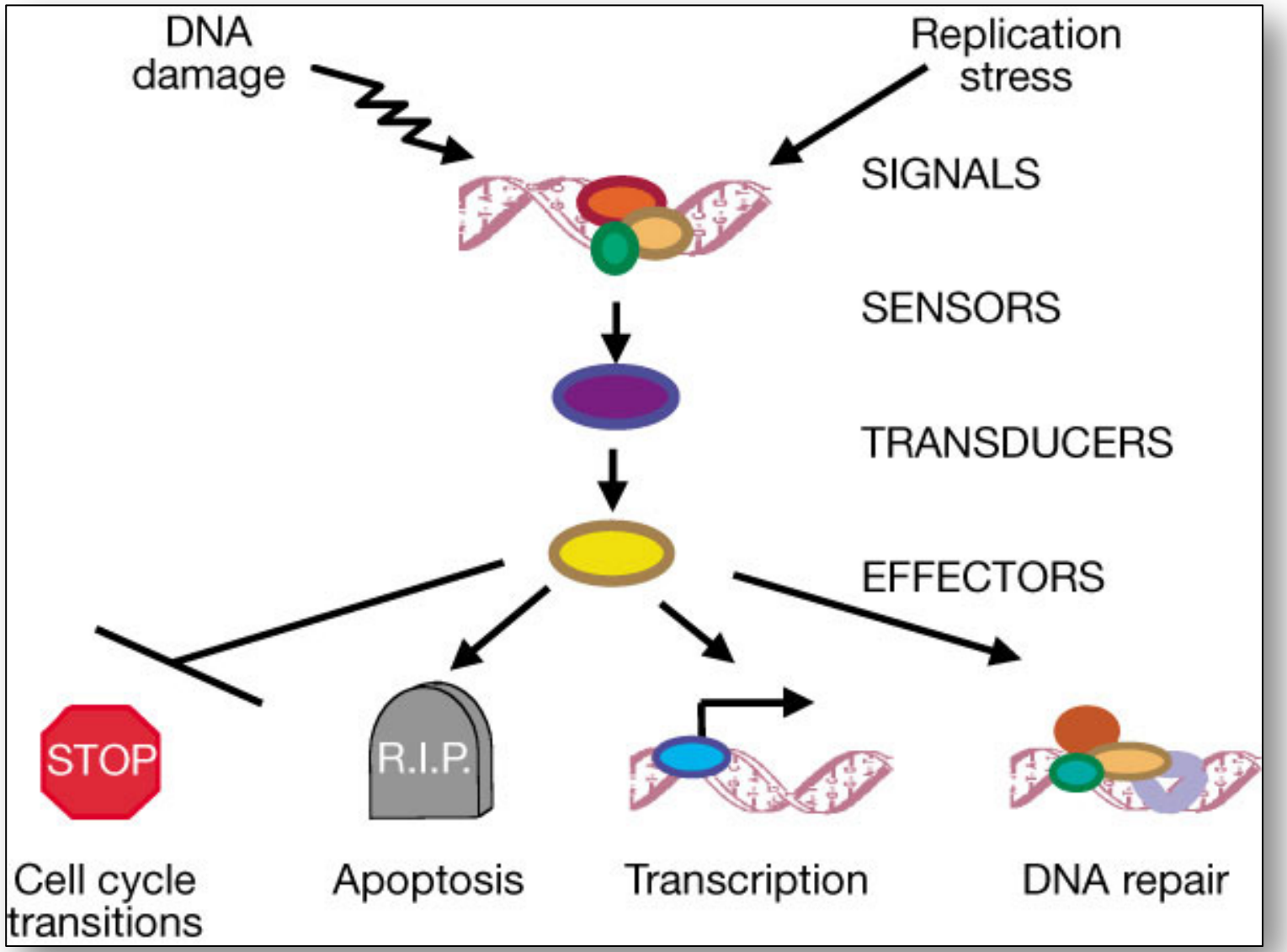

Cyclin
B/A + CDC2
M
G2
G1
S
Cyclin
D's + CDK2
CDK4
CDK5
Cyclin
A + CDK2
Cyclin
E + CDK2

The DNA Damage Response - DDR



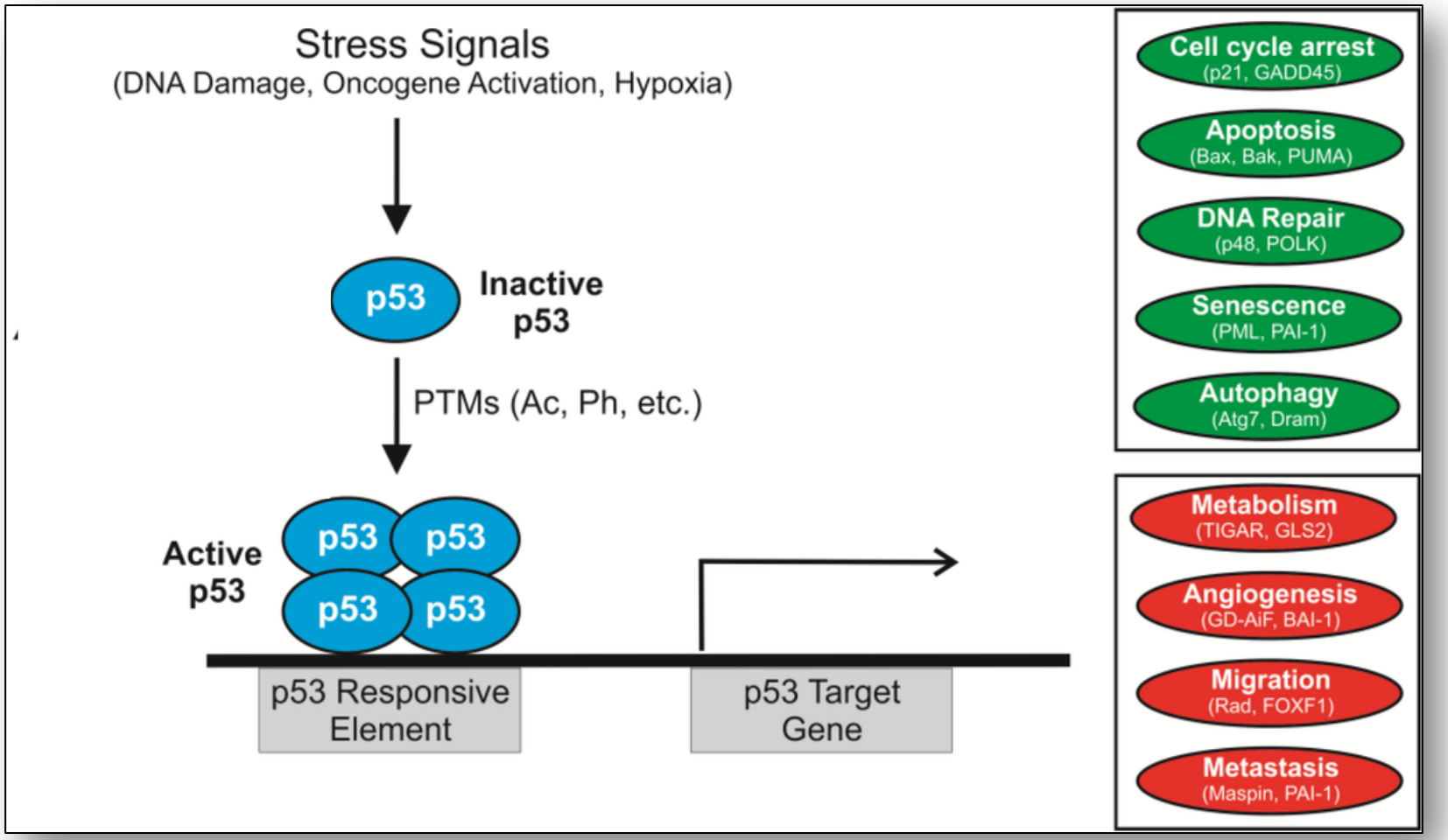
https://www.rndsystems.com/resources/articles/dna-damage-response

The DNA Damage Response - DDR



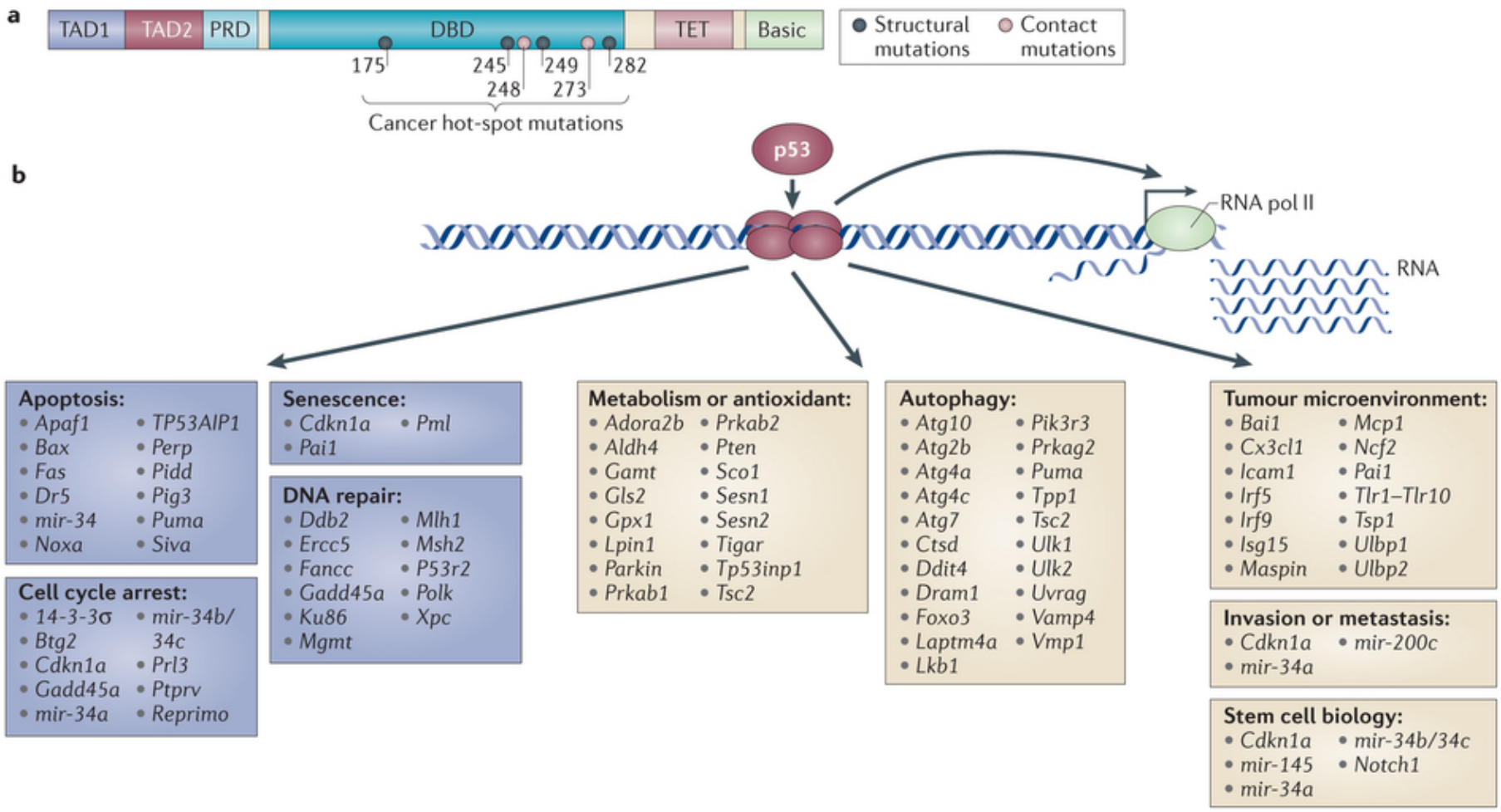
http://www.nature.com/nature/journal/v408/n6811/full/408433a0.html

P53 Regulates the transcription of MANY genes in MANY pathways



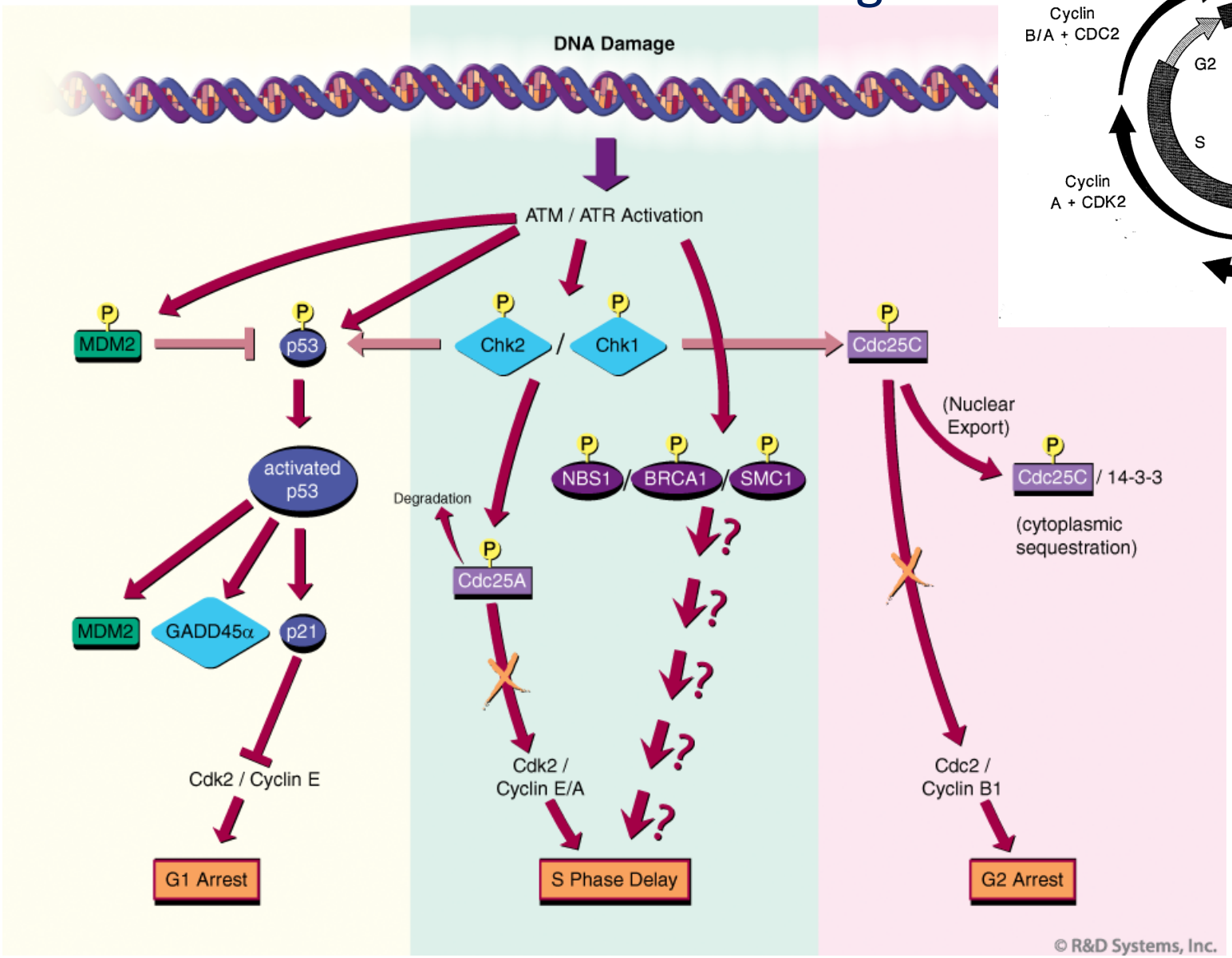
http://www.mdpi.com/2072-6694/7/1/30/htm

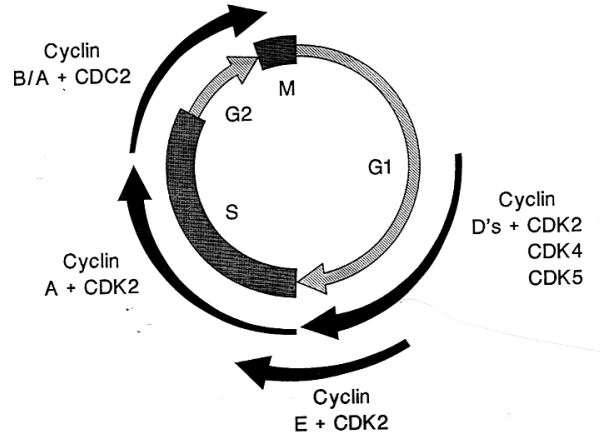
Activated p53 Target Genes



http://www.nature.com/nrc/journal/v14/n5/fig_tab/nrc3711_F3.html

ATM and ATR protein kinases are activated by DNA damage

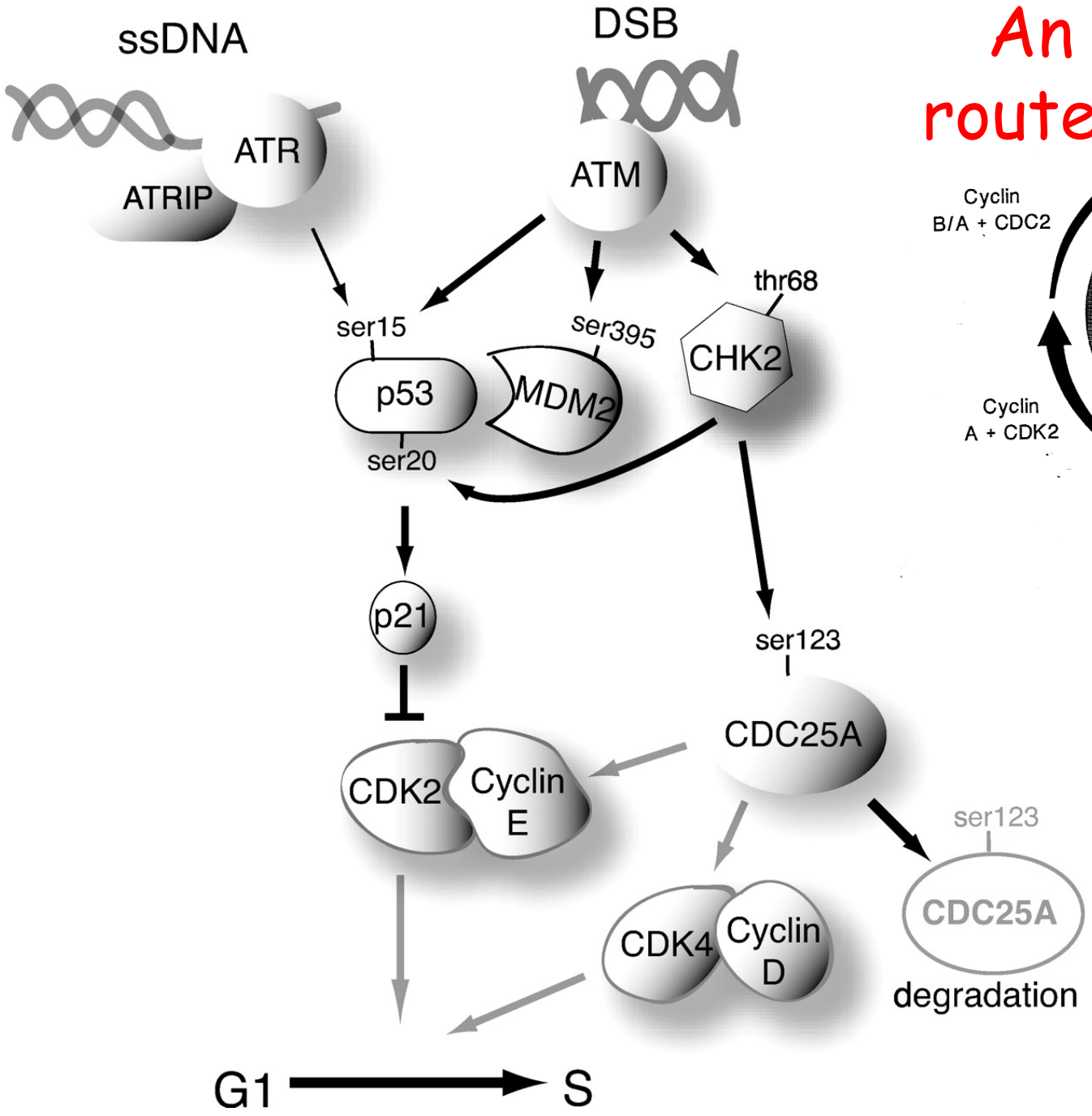


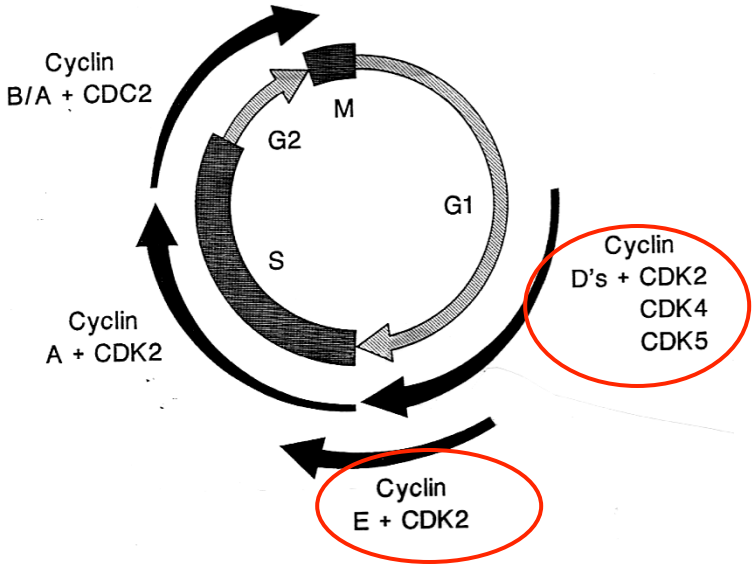


Cell Cycle Arrest

https://www.rndsystems.com/resources/articles/dna-damage-response

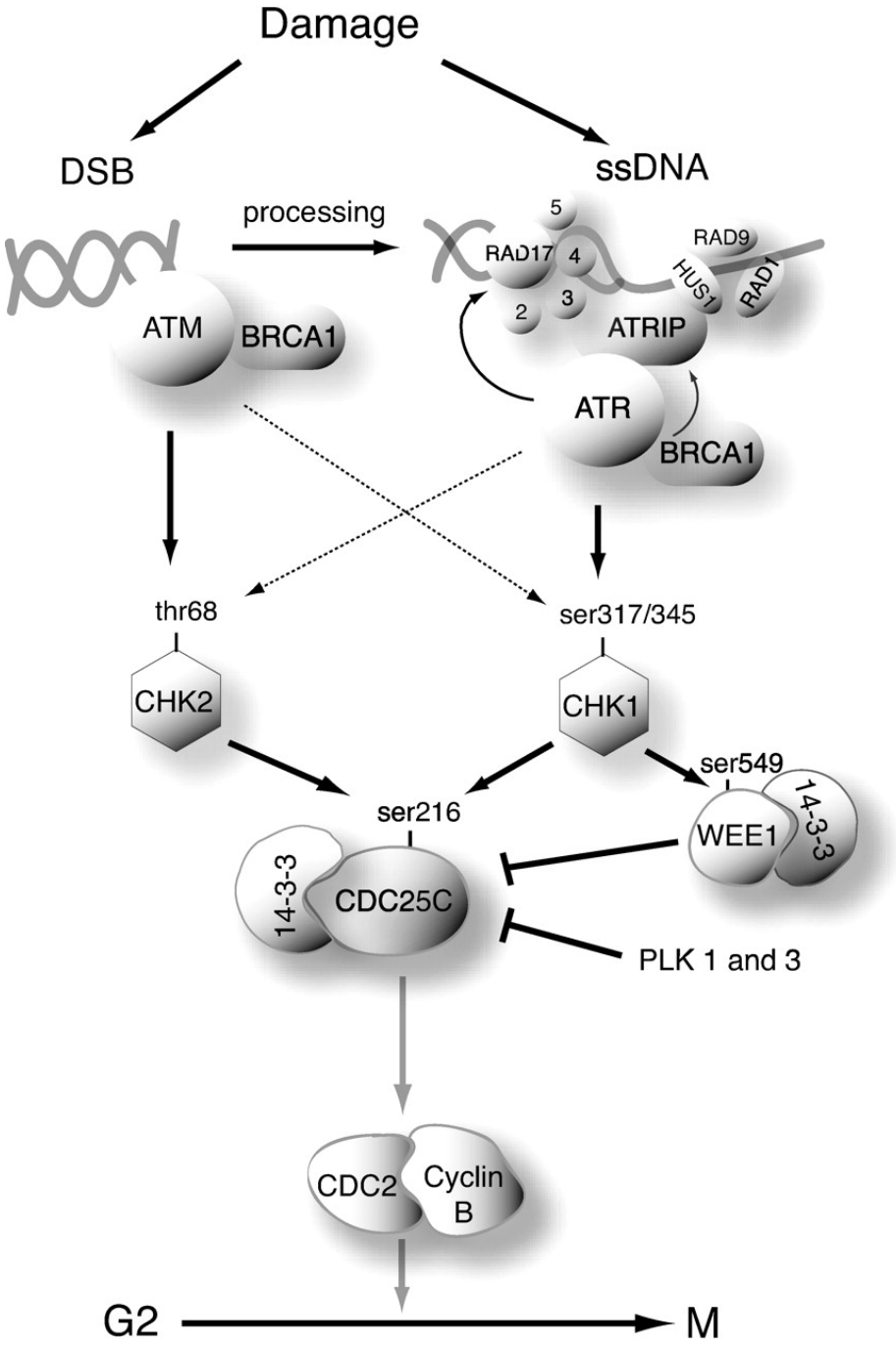
An alternative route to G1 arrest

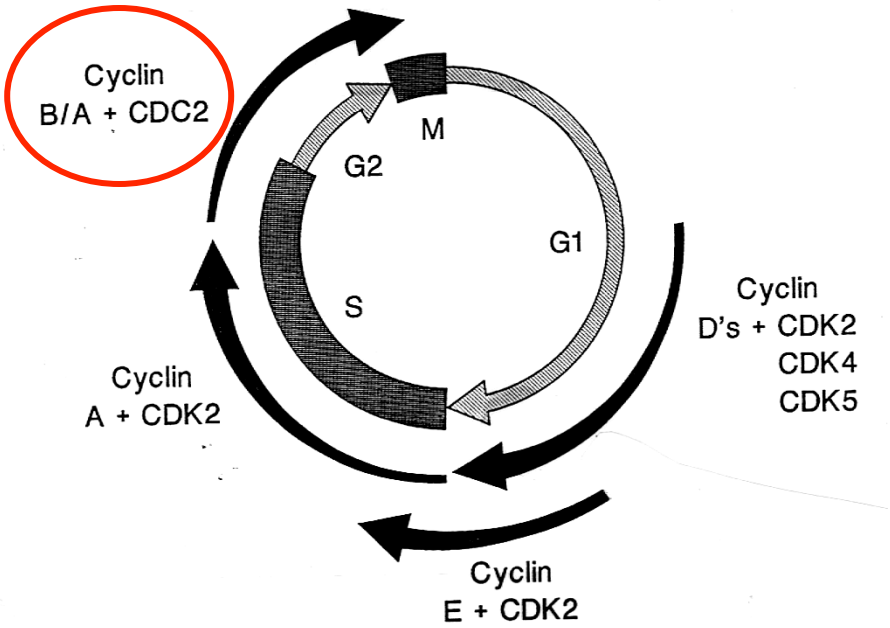




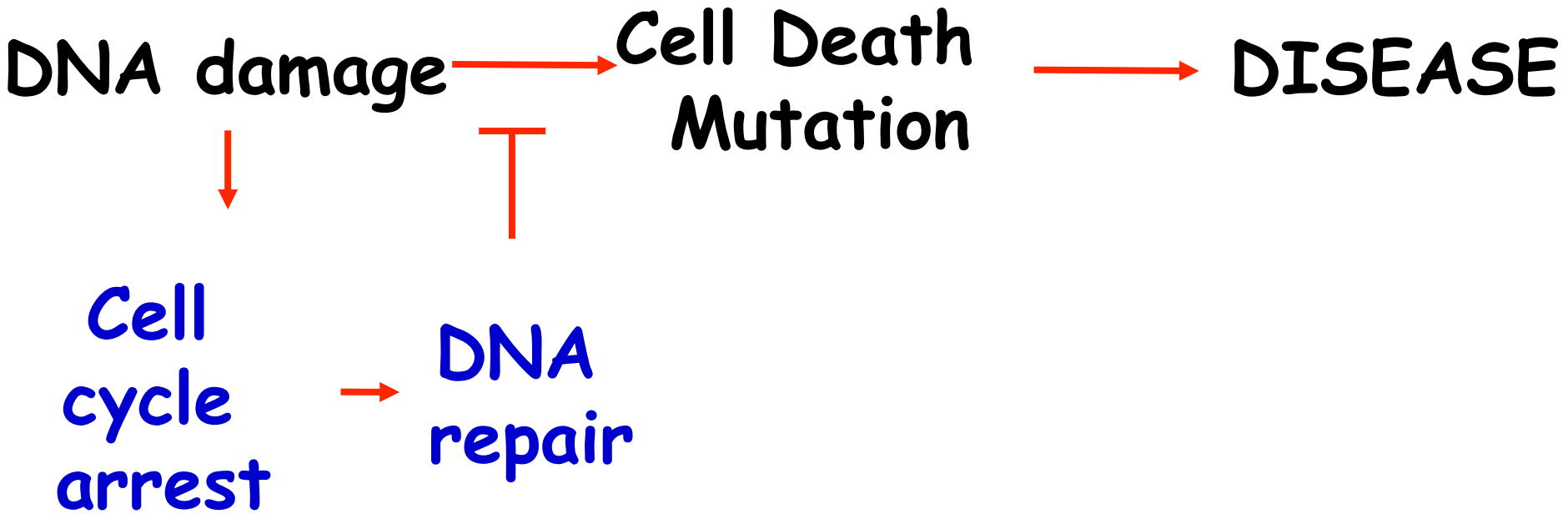
CDC25A is a phosphatase that must act on CDK2 and CDK4 to activate the complexes

G2/M Arrest





CDC25C has different substrate..Cdc2 to target G2/M transition


DNA damage
Cell Death
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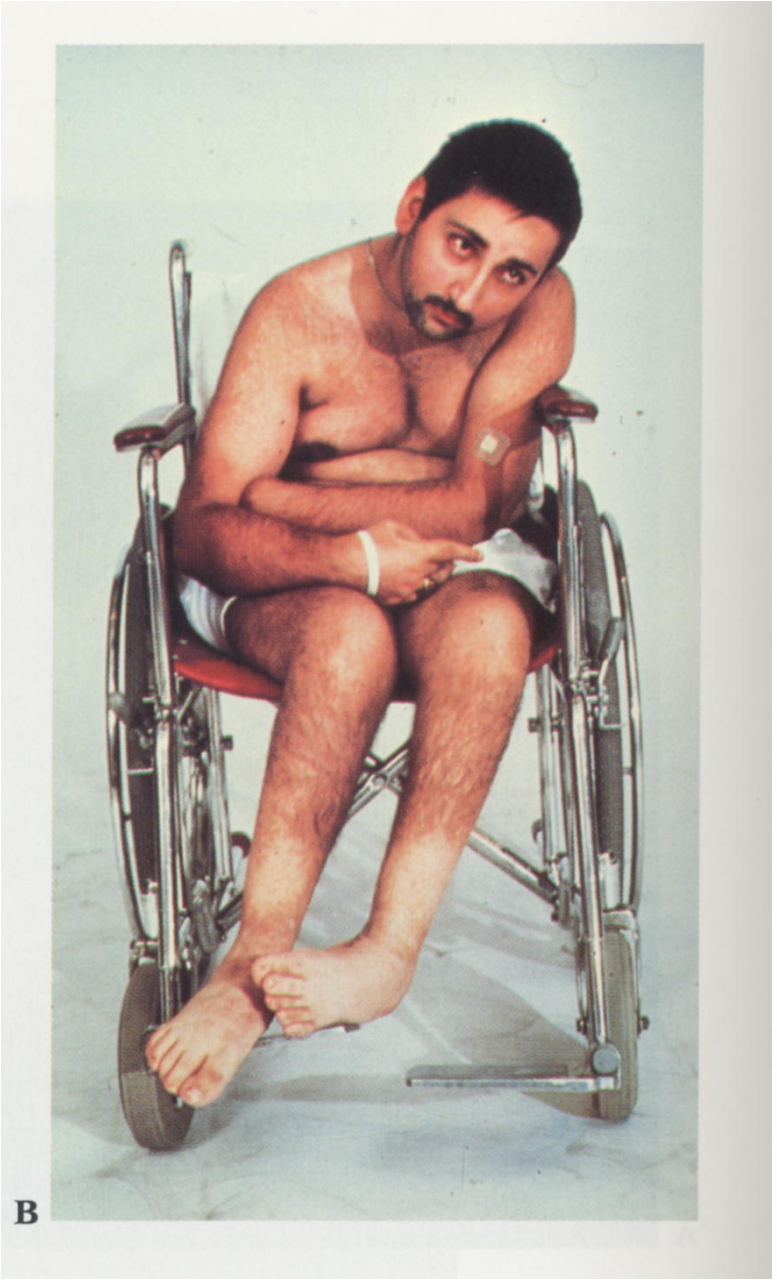
Ataxia Telangiectasia patients – Cancer Prone

ATM kinase gene mutated

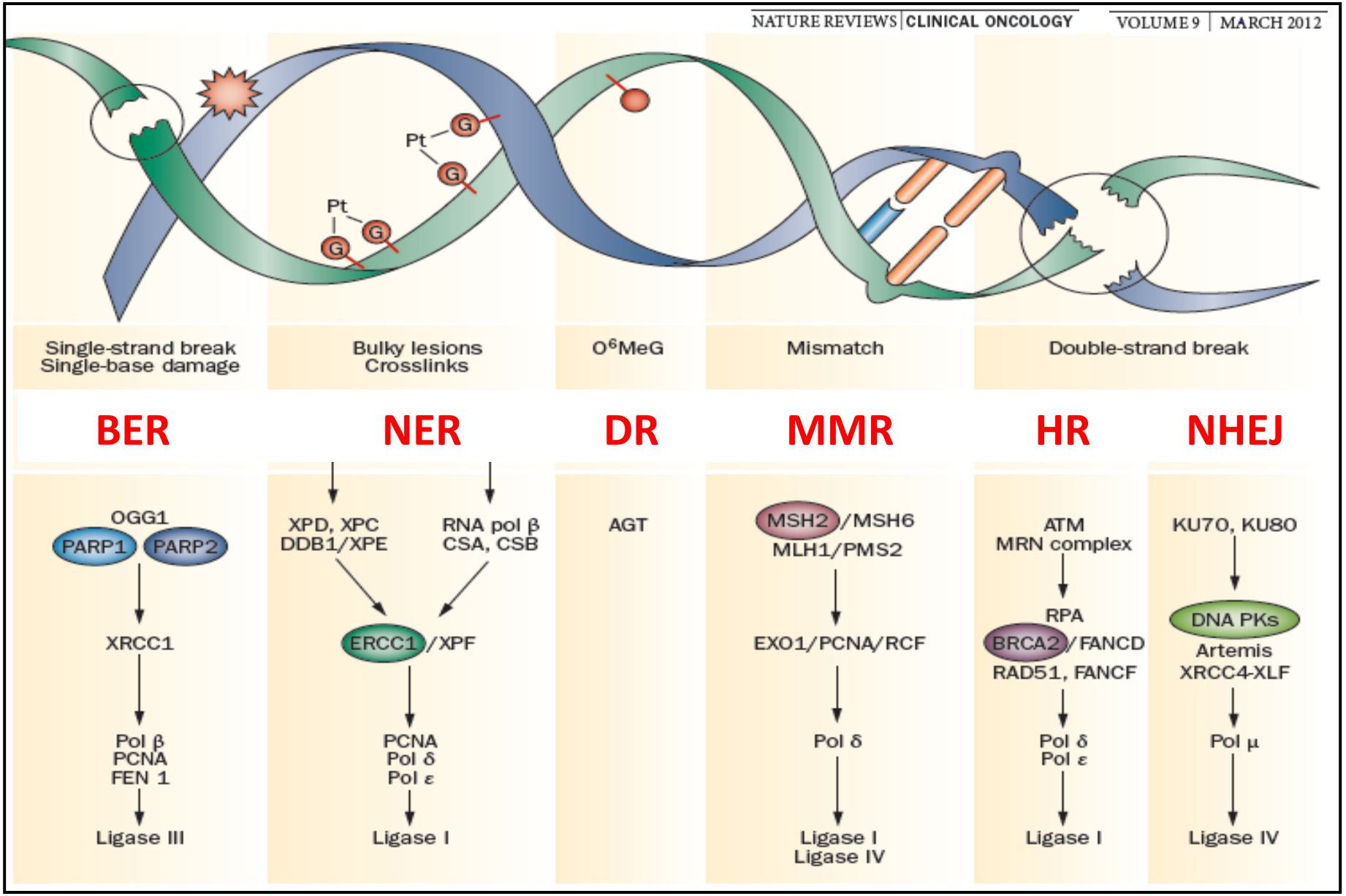
Defective DNA Damage Responses can affect both neurodegeneration and cancer susceptibility



Ataxia Telangiectasia patients
ATM gene mutated

- Staggering gait
- Muscular un-coordination
- Mental retardation
- Dilation of small blood vessels
- Immune dysfunction
- Cancer prone…lymphomas
- Cells from AT patients have lost cell cycle checkpoints and have abnormal DDR

Six Major DNA Repair Pathways



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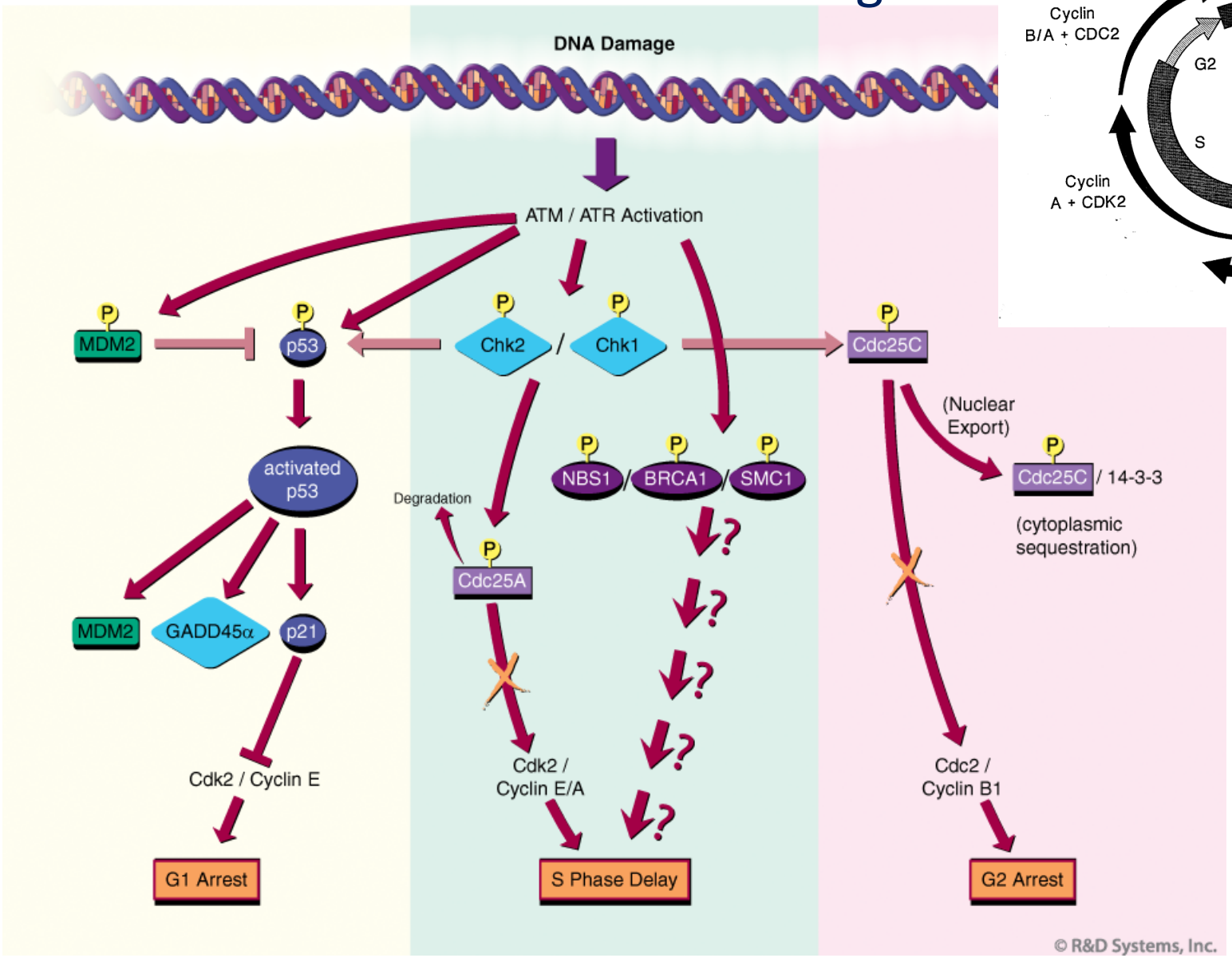

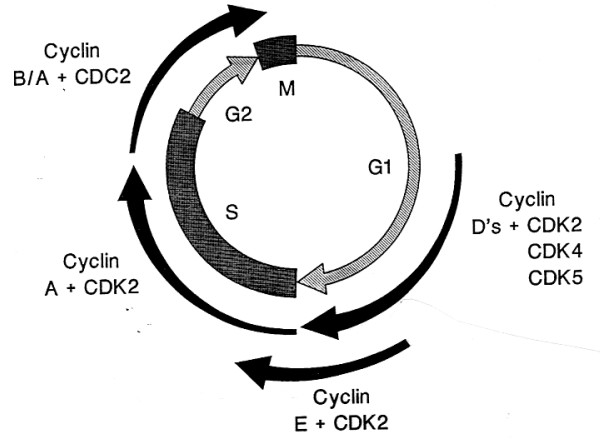

Cell Cycle Arrest

https://www.rndsystems.com/resources/articles/dna-damage-response

Key Experimental Methods for Module 2

- Grow human cancer cells in tissue cell culture
- Monitor specific protein levels by Western blot
- Kill cancer cells with chemotherapy drugs
- Engineer the inhibition of DNA Repair pathways
- Monitor changes in a gene's expression (qPCR)
- Analyze RNAseq dataset measuring expression of ~ 20,000 genes (BIG DATA!)
- Statistical analysis of all biological data

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20.109 Spring 2017 Module 2 – Lecture 2
Gene Expression Engineering (March 14th 2017)




Noreen Lyell

Leslie McLain

Maxine Jonas

Rob Wilson

Leona Samson (Lectures)