20.109 Spring 2016 Module 2 – Lecture 5
System Engineering (March 31st 2016)




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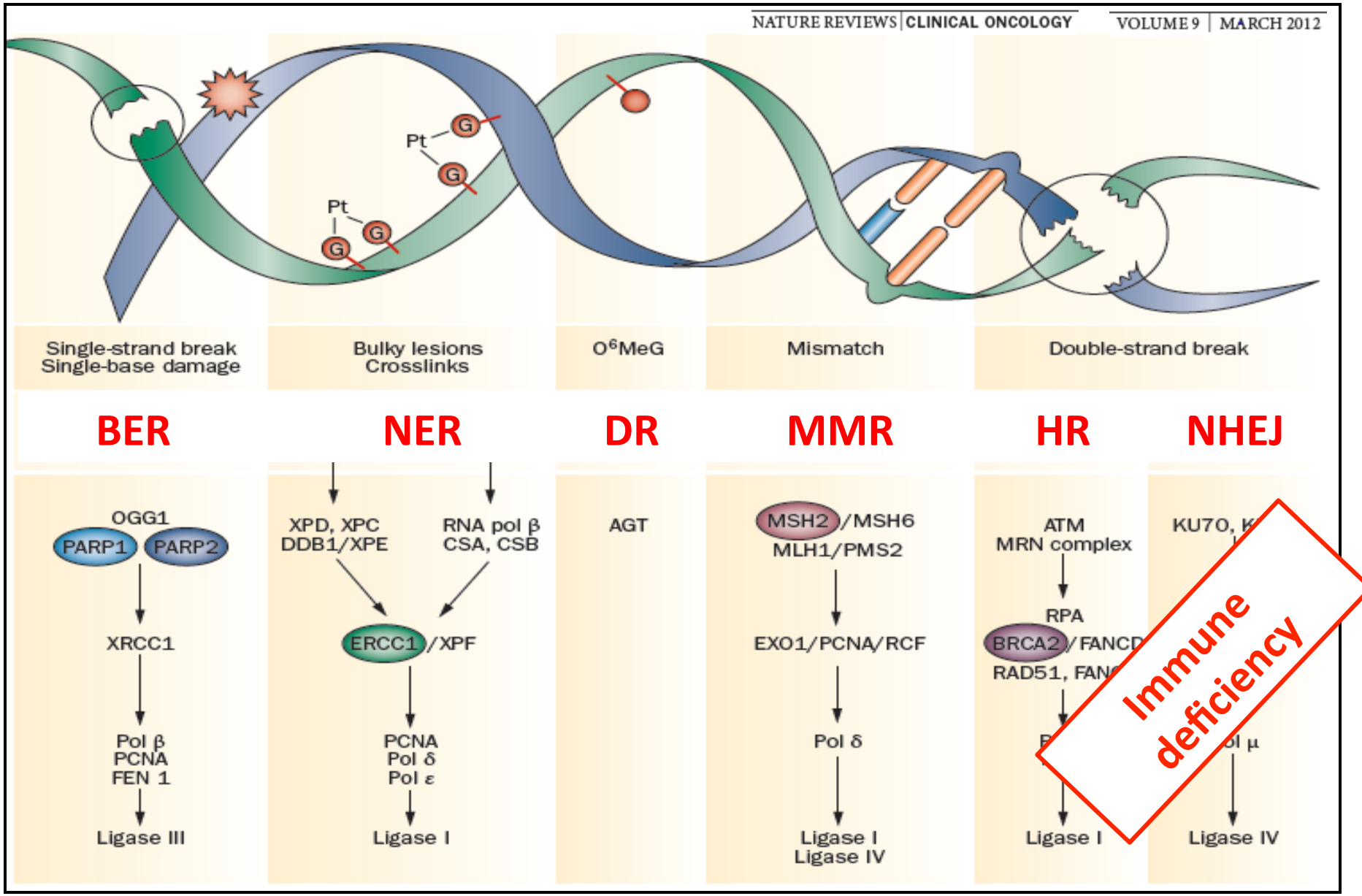
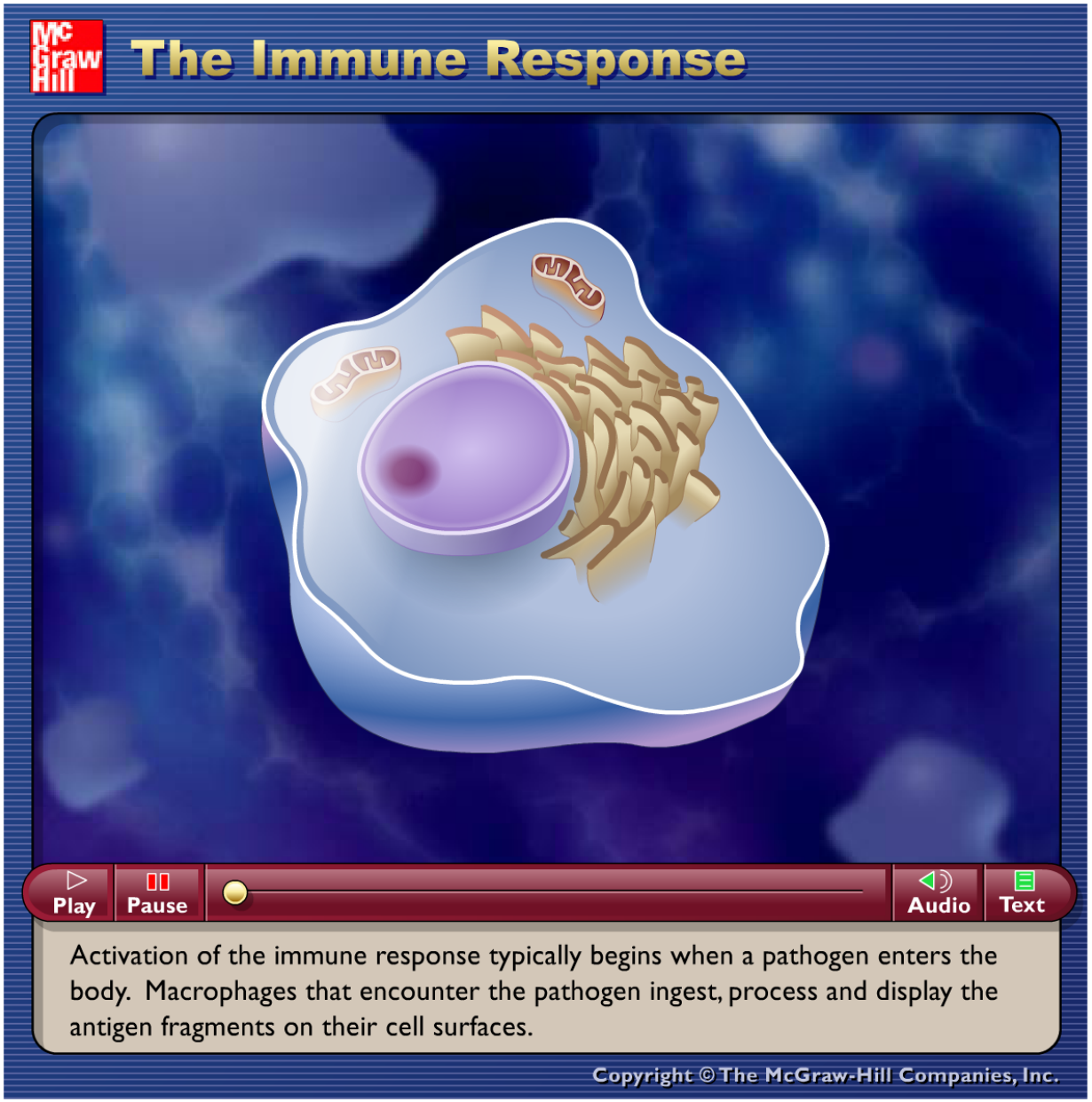
Six Major DNA Repair Pathways

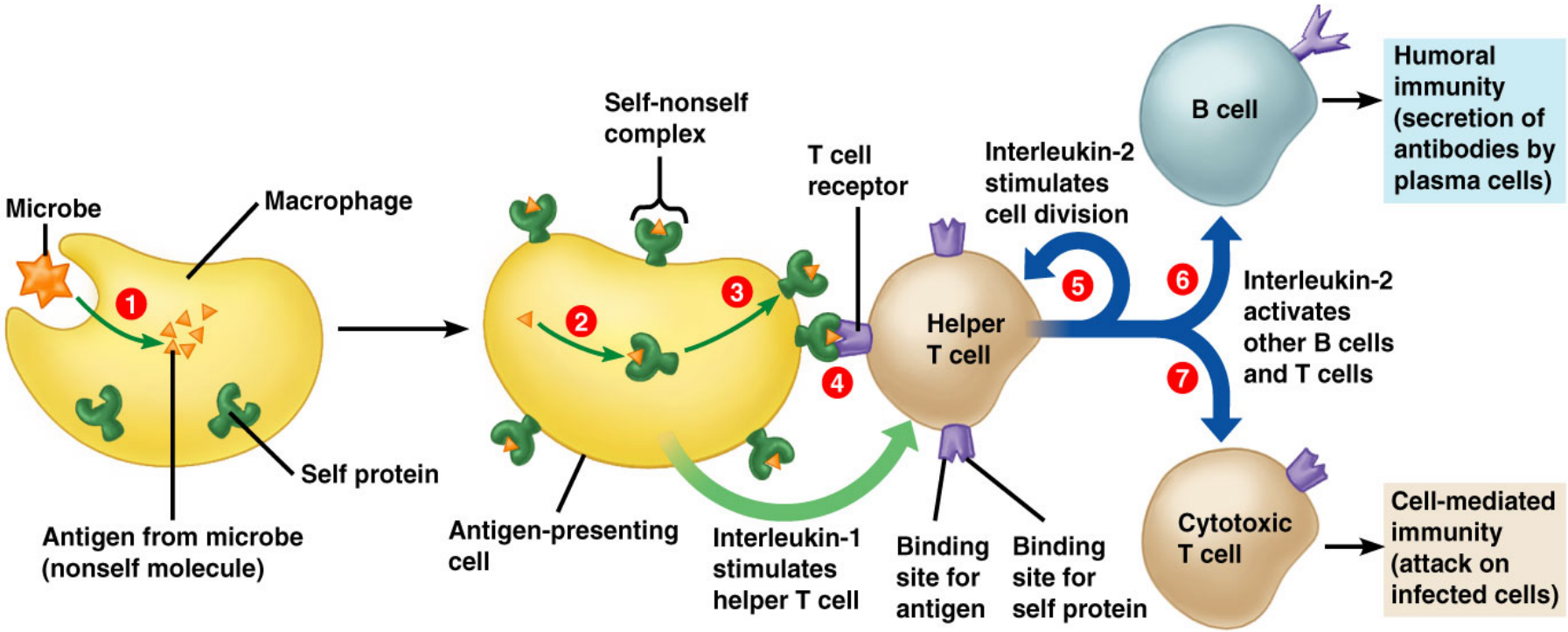


Non Homologous End Joining is REQUIRED for a functional immune system!



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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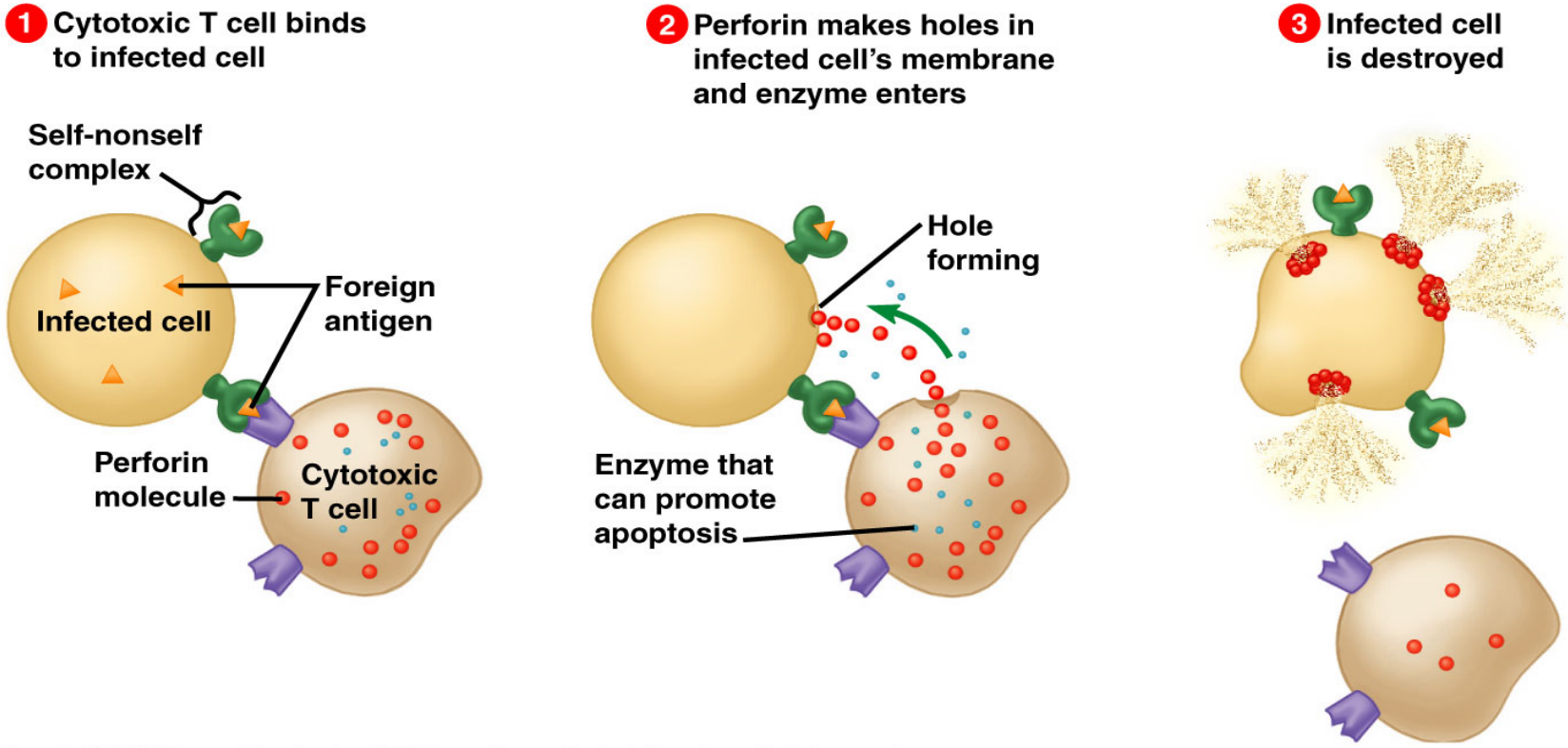

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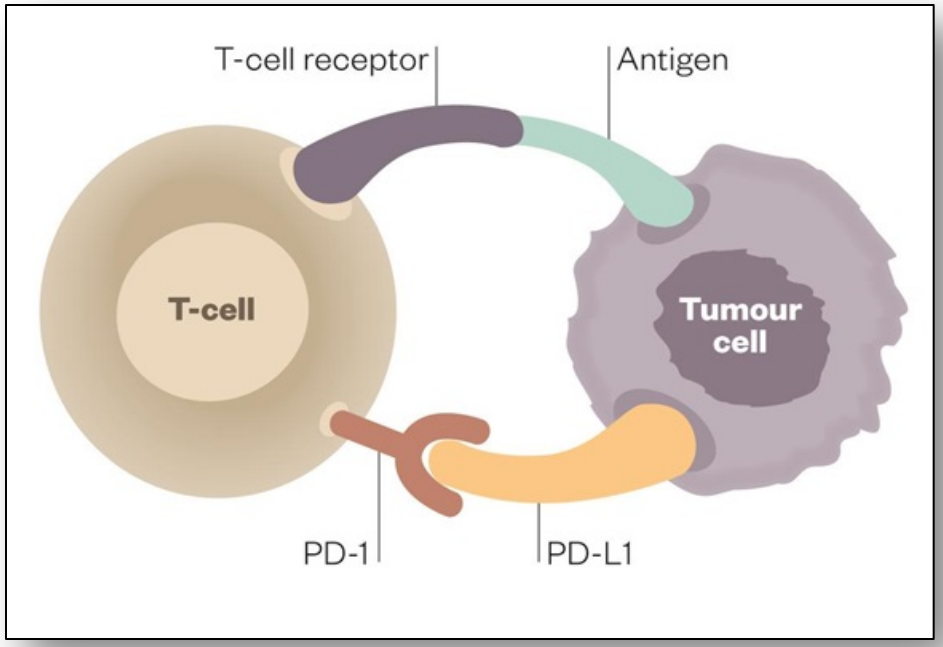
Immune Checkpoint Cancer Therapy

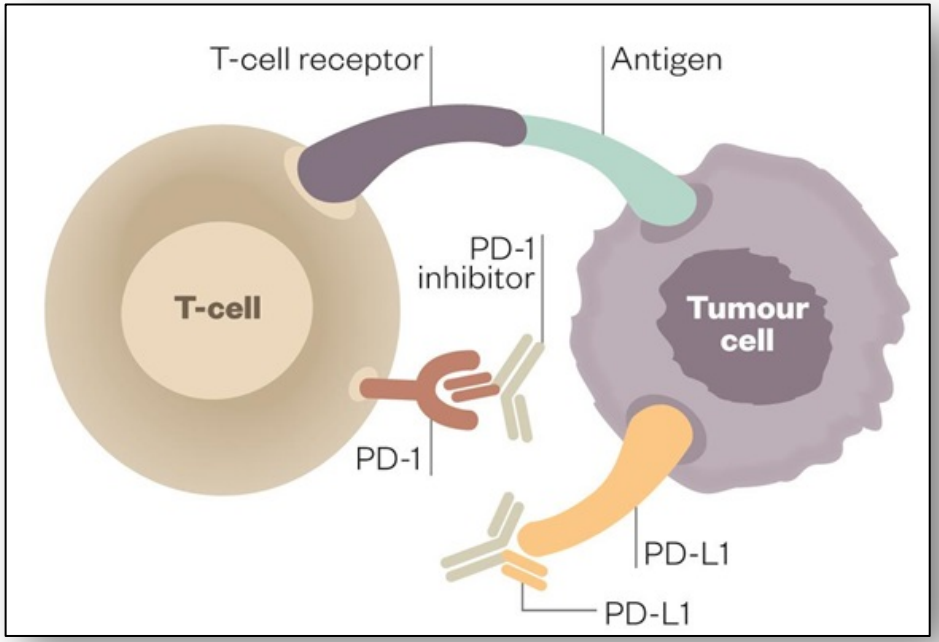
Deactivated T-cell



Tumor cells launch Immune Checkpoint (PD-L1 ligand binds PD-1 receptor to deactivate killer T cells).

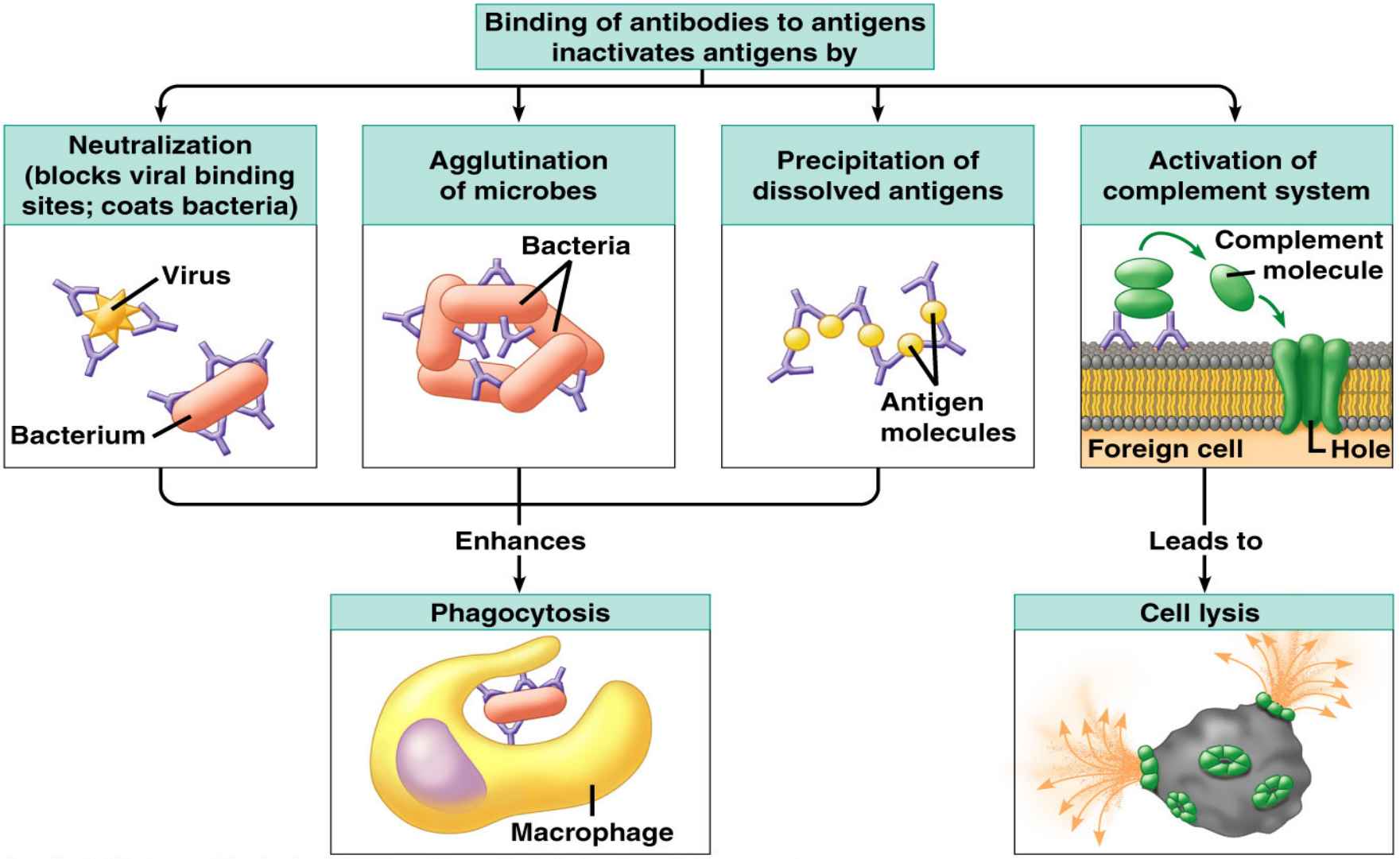
Evading the Immune System

Activated T-cell



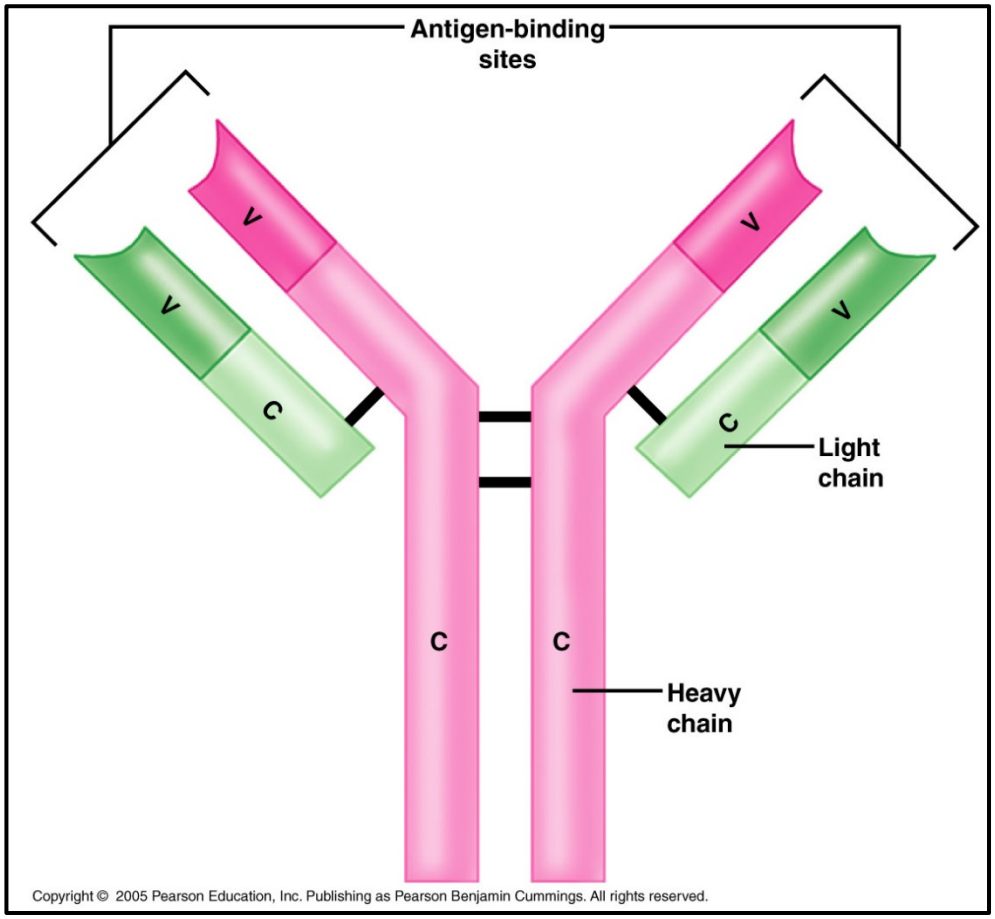
Treatment with Antibodies to block PD-Ligand1 and PD-1 receptor allowing Activation of Killer T cells

Antibodies work in different ways



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

"ANTIGEN" comes from ANTI-body GENerating substances



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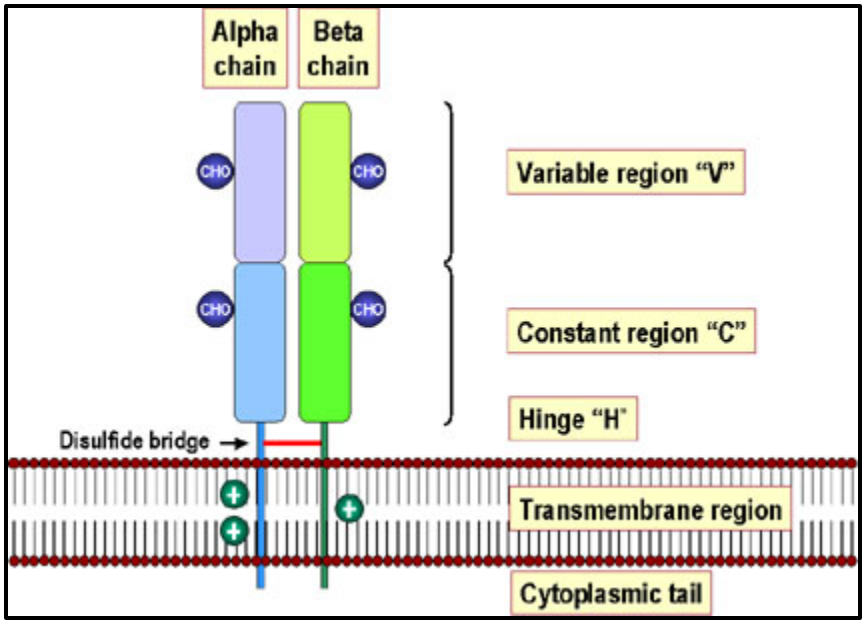
B-cell Immunoglobulin

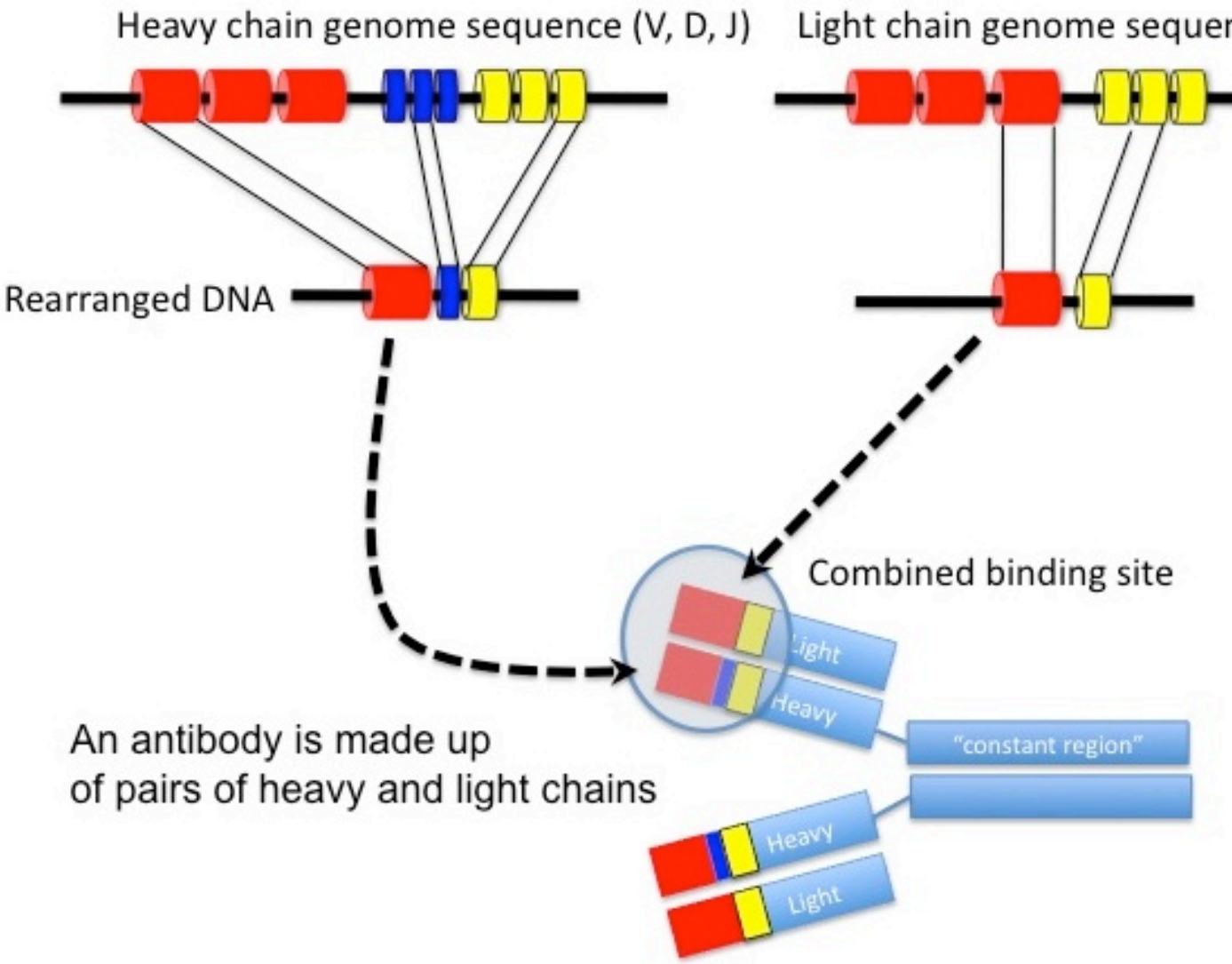
Antigen Binding Site



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

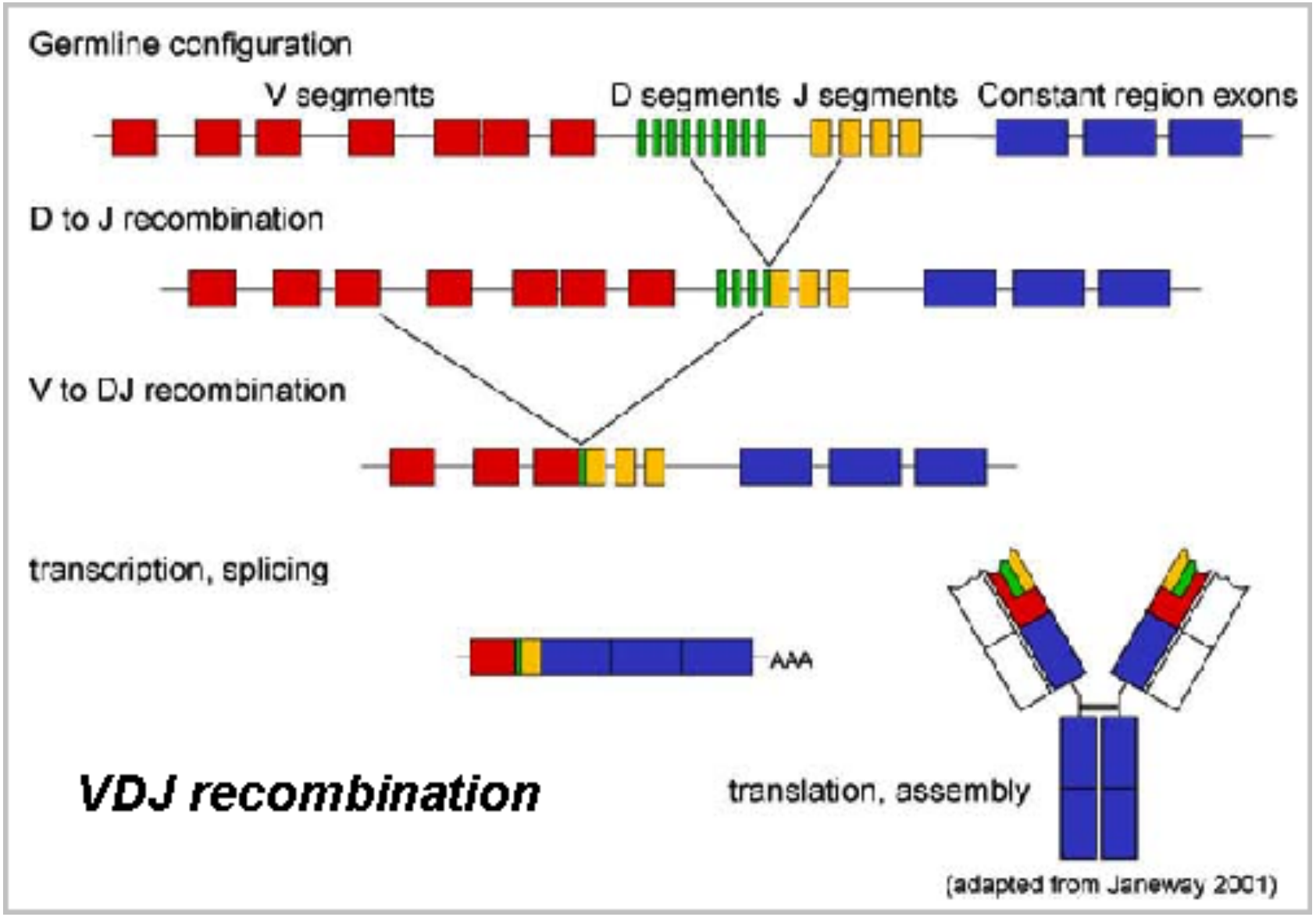
T-cell Receptor

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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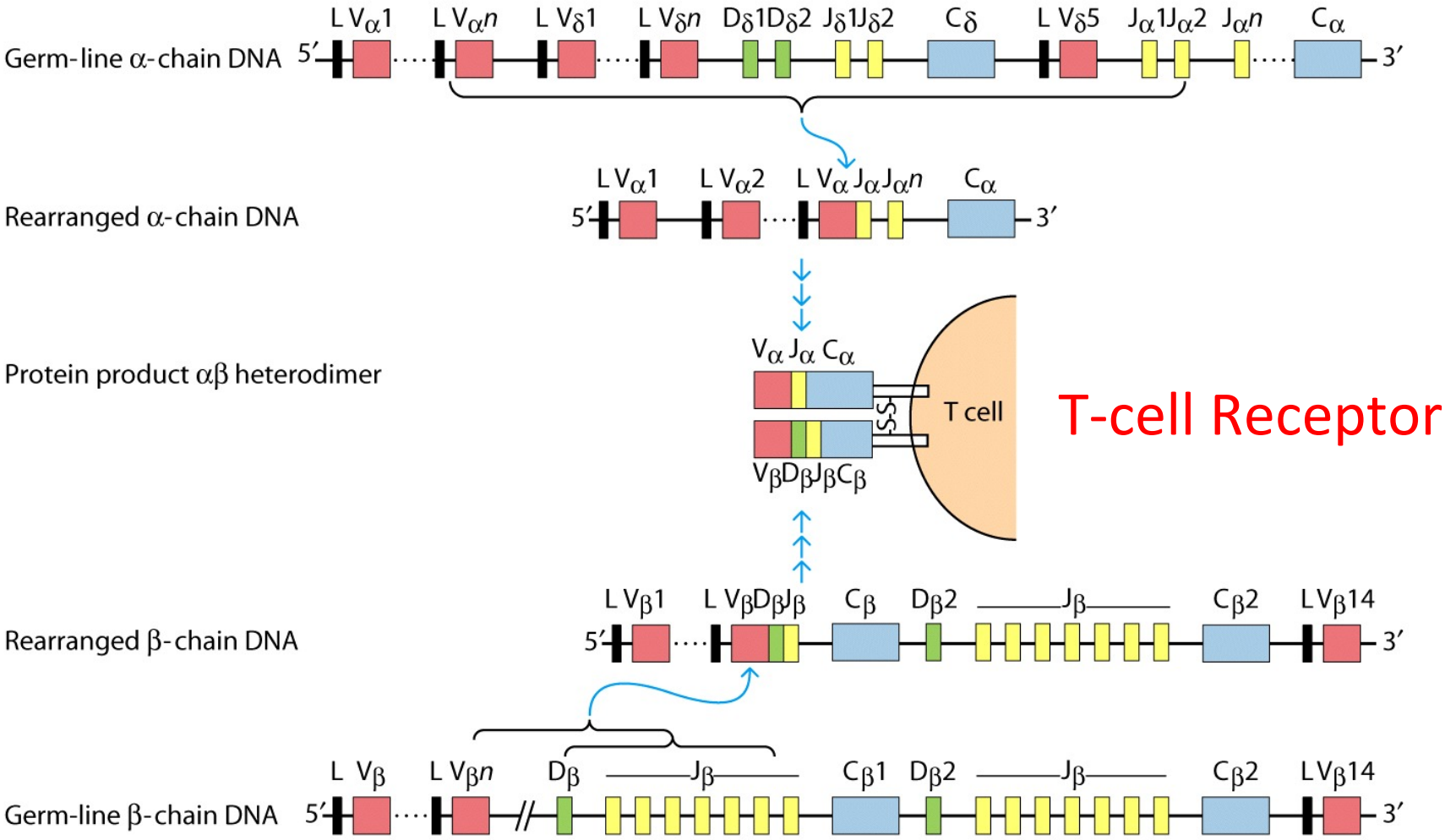
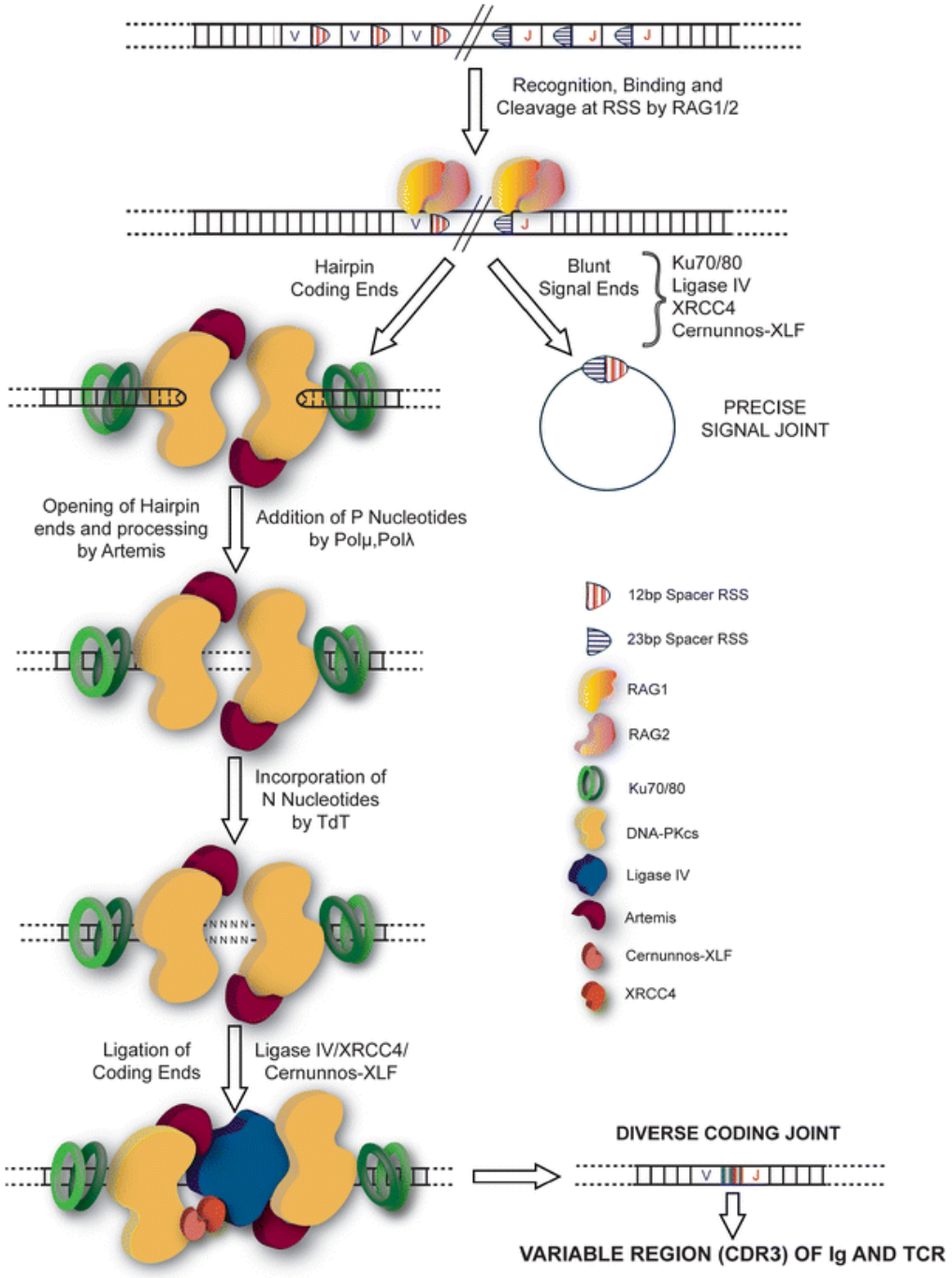


V(D)J Gene Recombination

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

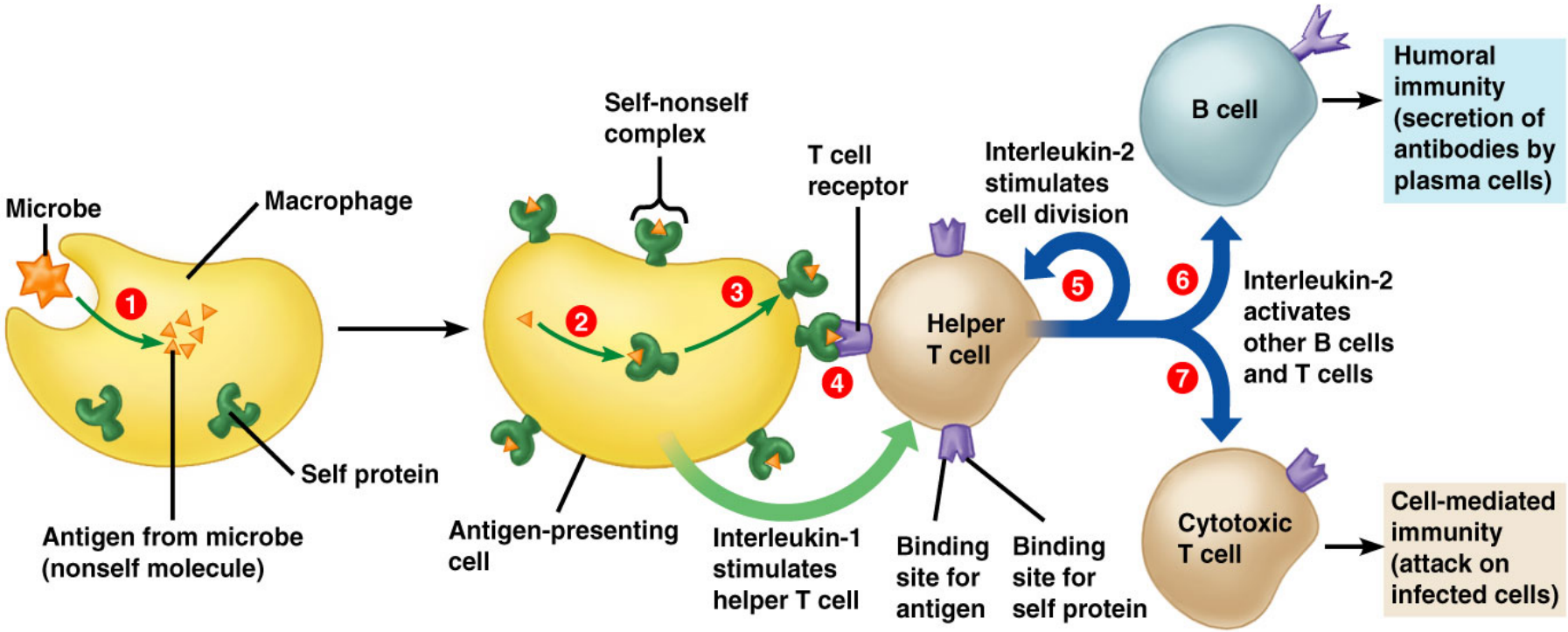


How Do the Variable Regions become Variable? Through NHEJ mediated DNA Recombination!

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.



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

How Variable is Variable?

Number of functional gene segments in human immuniglobulin 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, V(D)J, 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.

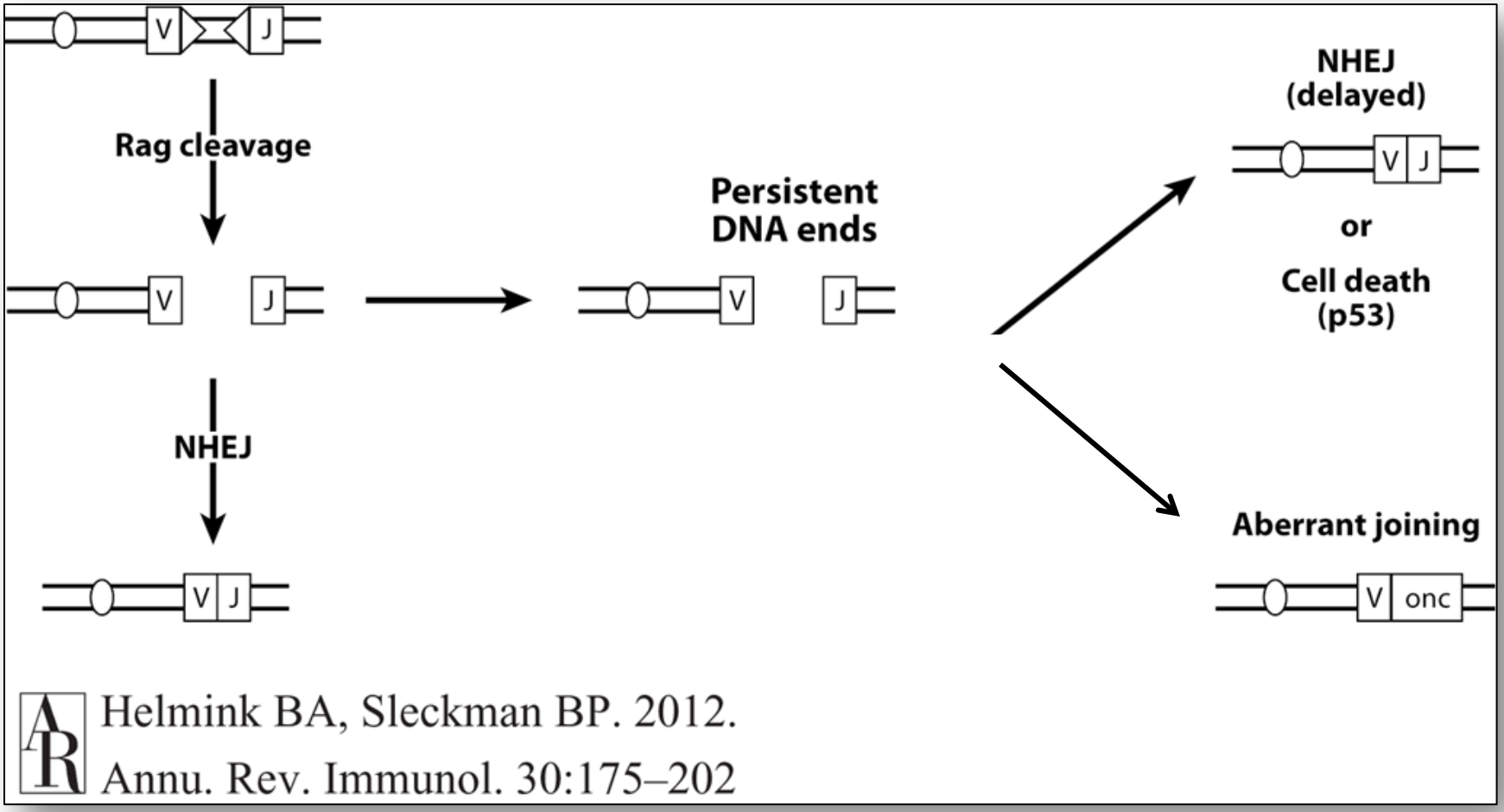
What happens if mice or people lose NHEJ capacity?

What happens if mice or people lose NHEJ capacity? SCID – Severe Combined ImmunoDeficiency

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]

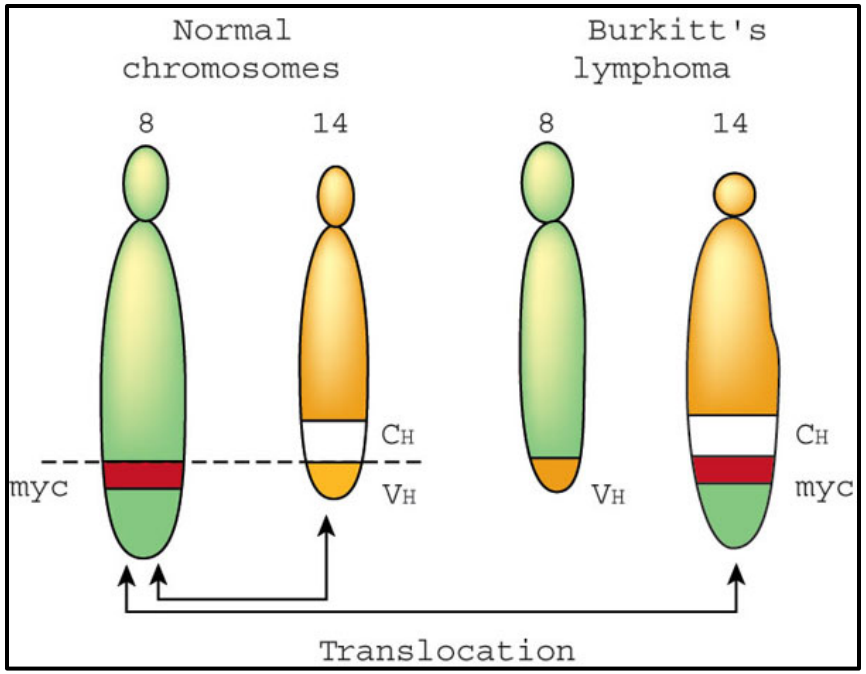
DCLRE1C, DNA cross-link repair 1C; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; LIG4, DNA ligase 4; NHEJ, non-homologous end-joining; NHEJ1, NHEJ factor 1; PRKDC, protein kinase, DNA-activated, catalytic polypeptide; SCID, severe combined immunodeficiency; XLF, XRCC4-like factor; XRCC, X-ray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

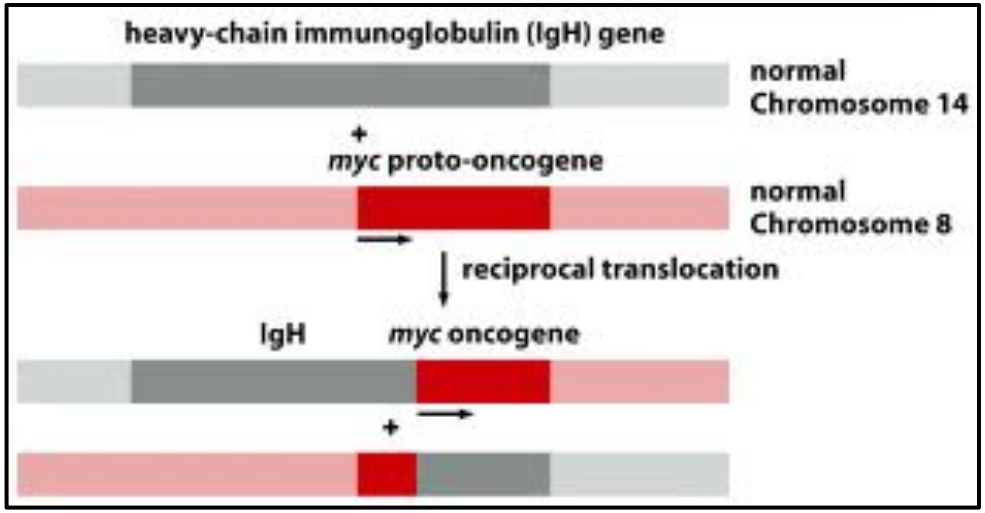
Can V(D)J Recombination Go Wrong?



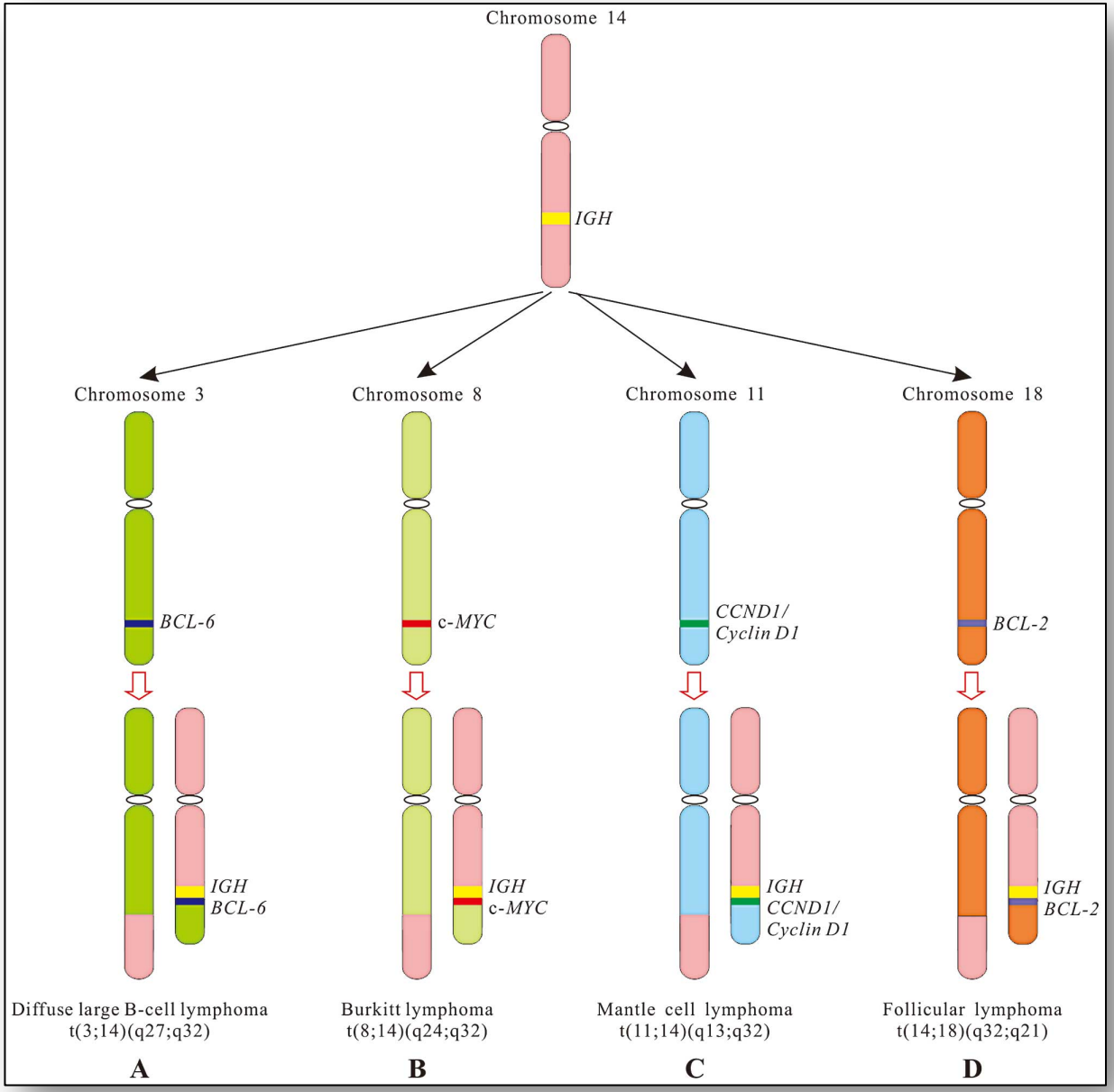
Helmink BA, Sleckman BP. 2012.
Annu. Rev. Immunol. 30:175–202

BURKITT's LYMPHOMA
B-cell Lymphoma

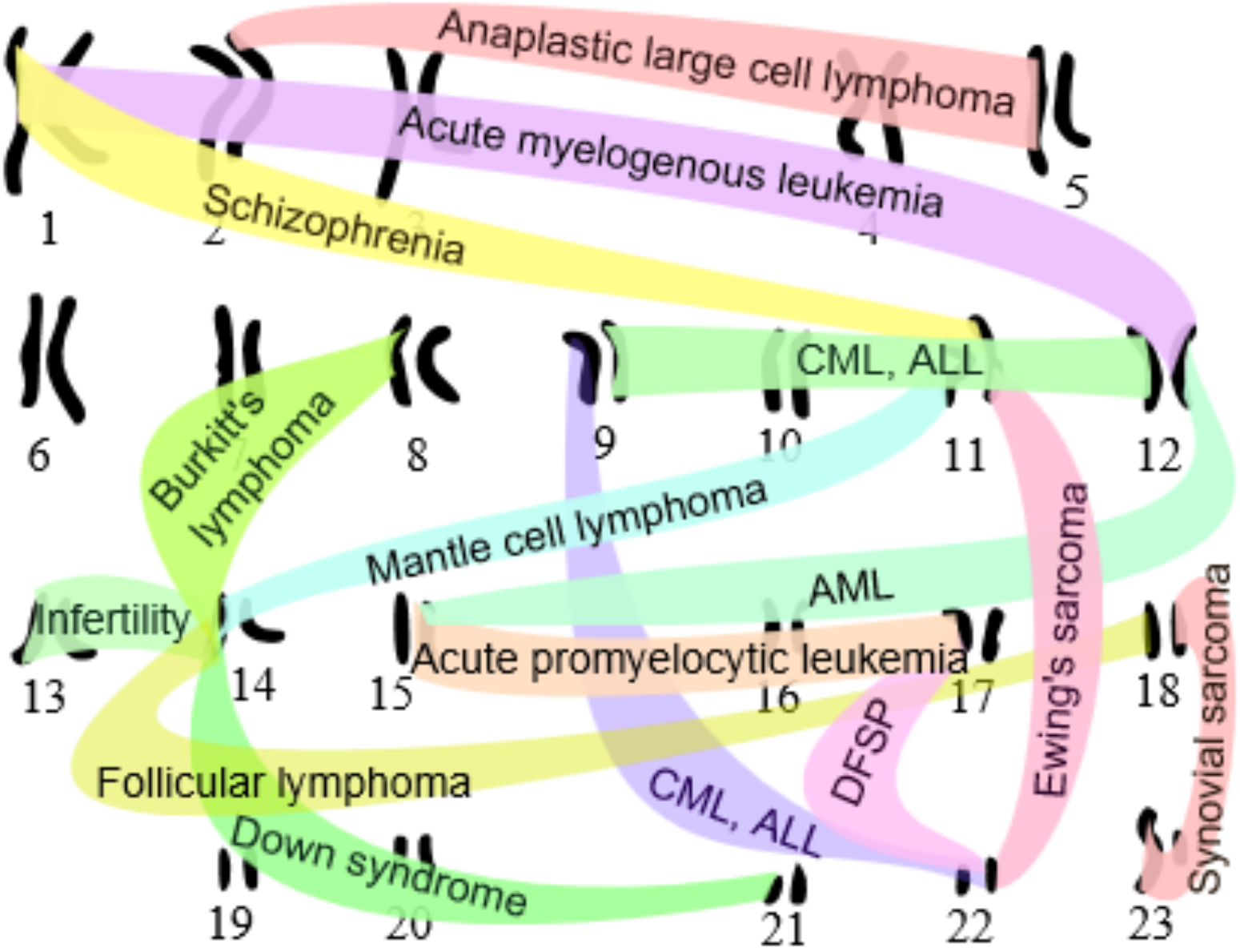




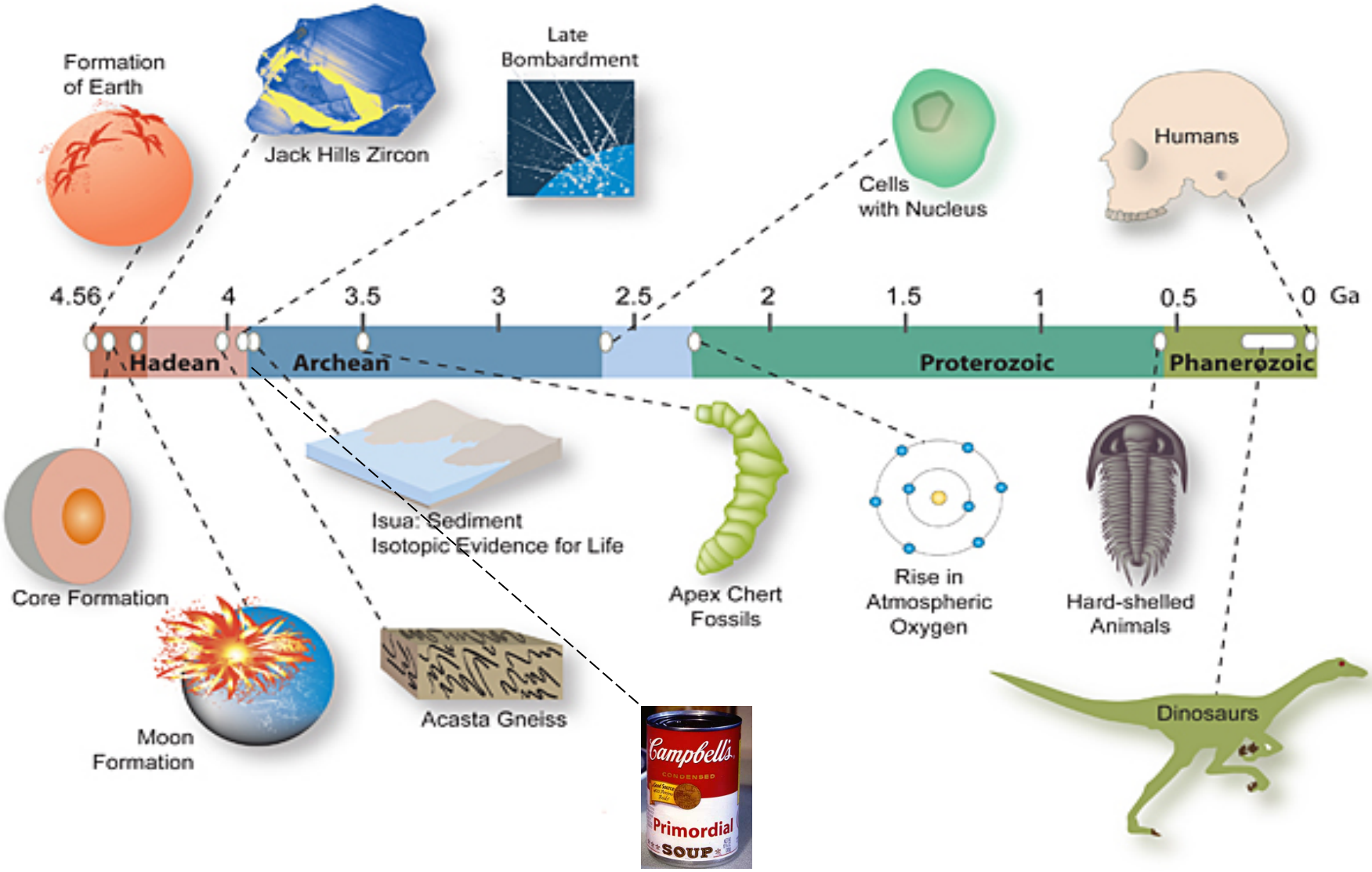
Other B-cell Lymphomas



Diseases that involve Chromosome Translocations



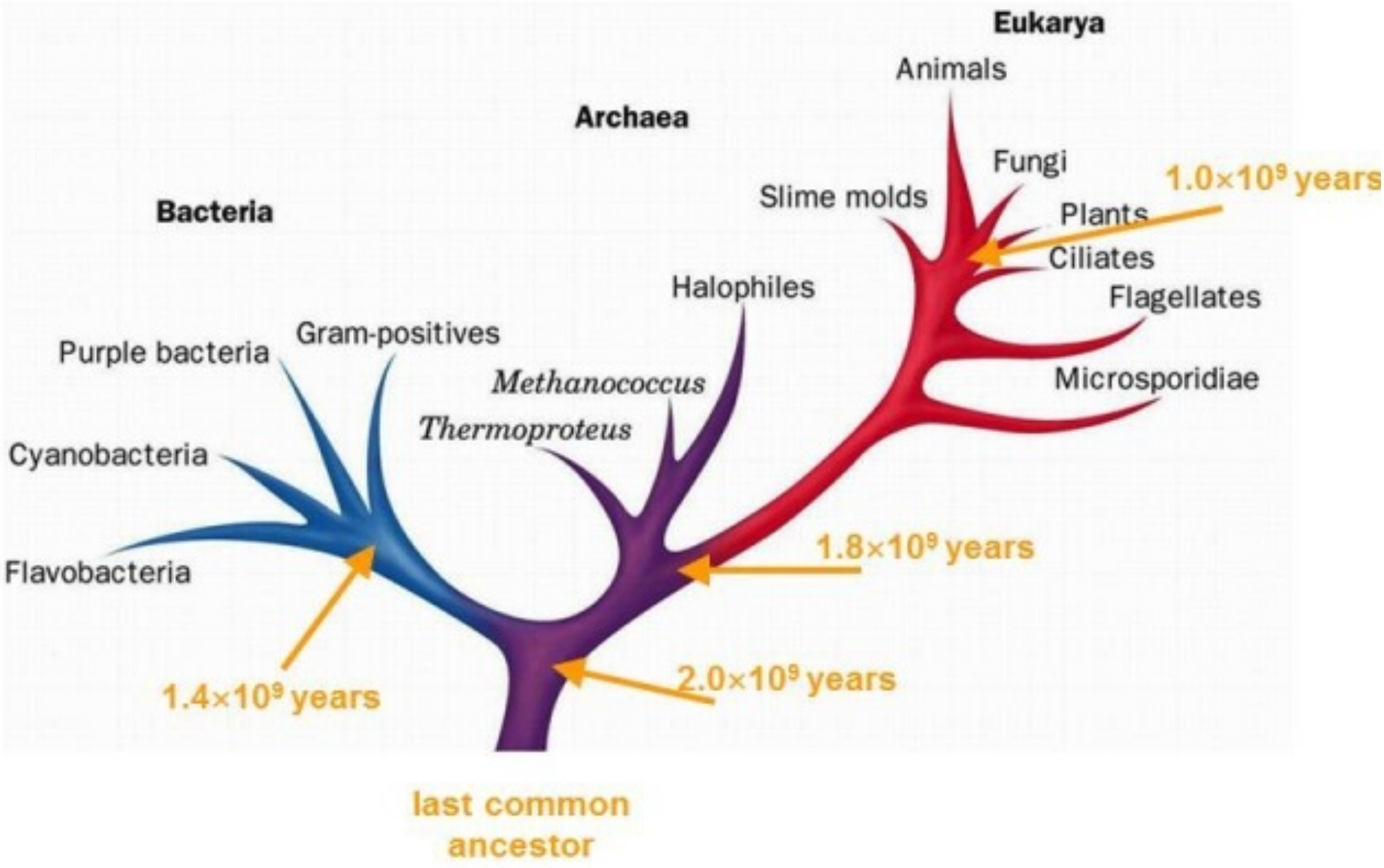
Evolution of life on Earth



http://astrobiology.com/2014/02/oldest-piece-of-earths-crust-suggests-a-cool-early-history.html

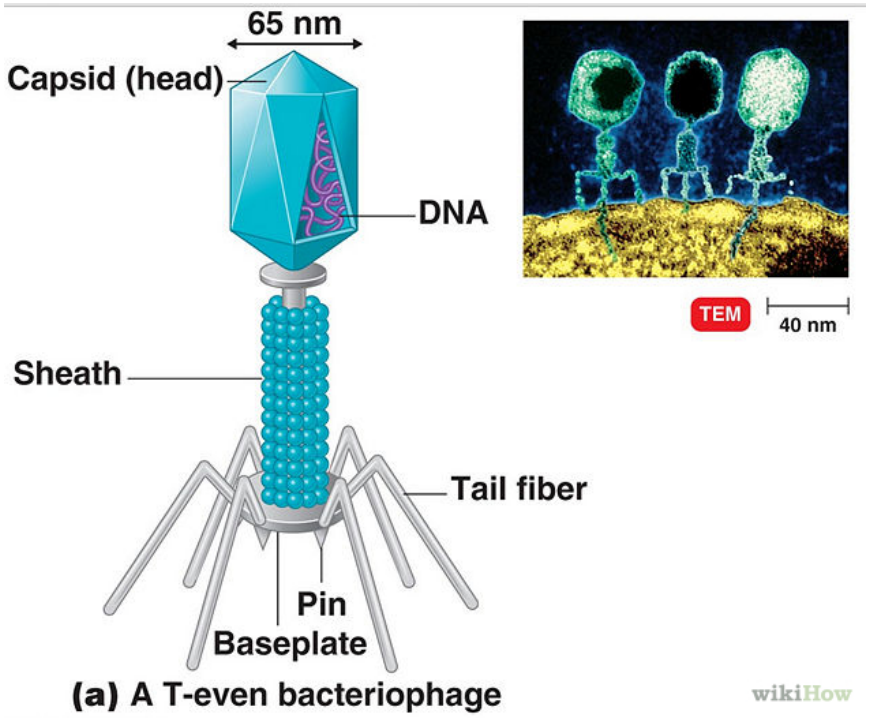
Andree Valley

All known life forms are based on DNA

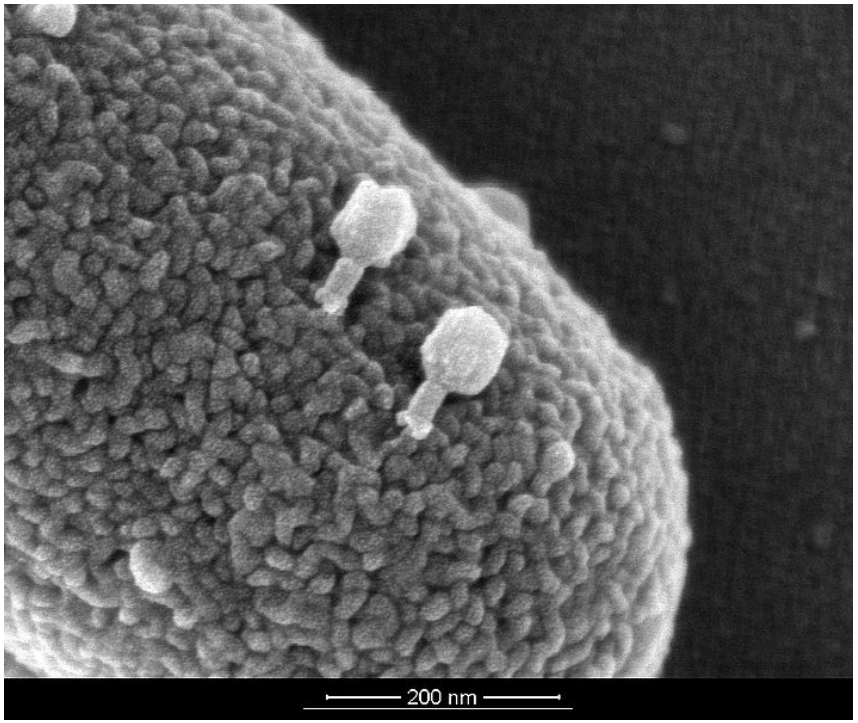


http://biologicalphysics.iop.org/cws/article/lectures/47042

Bacteriophage (bacterial virus) infecting *E. coli*



(a) A T-even bacteriophage



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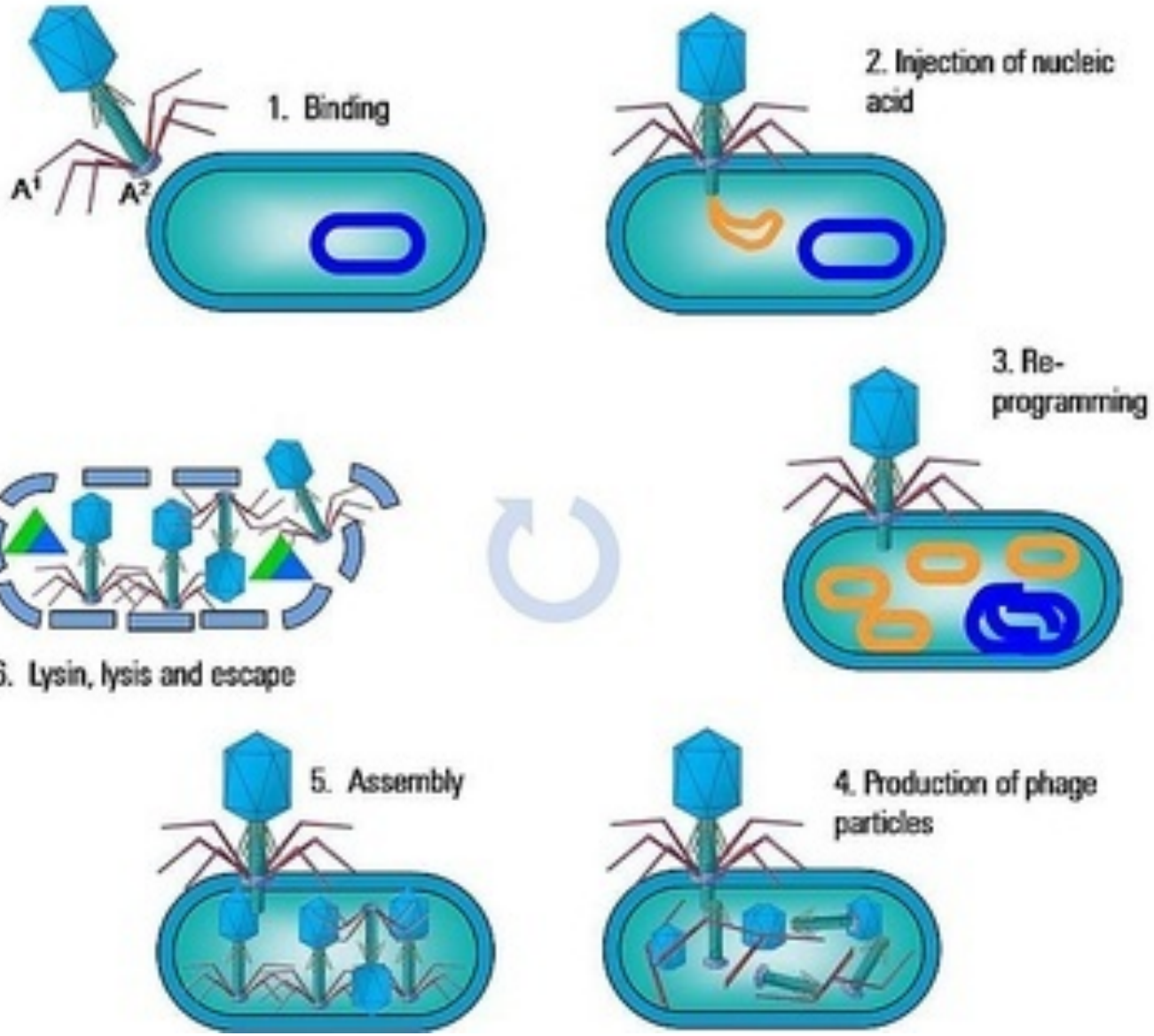


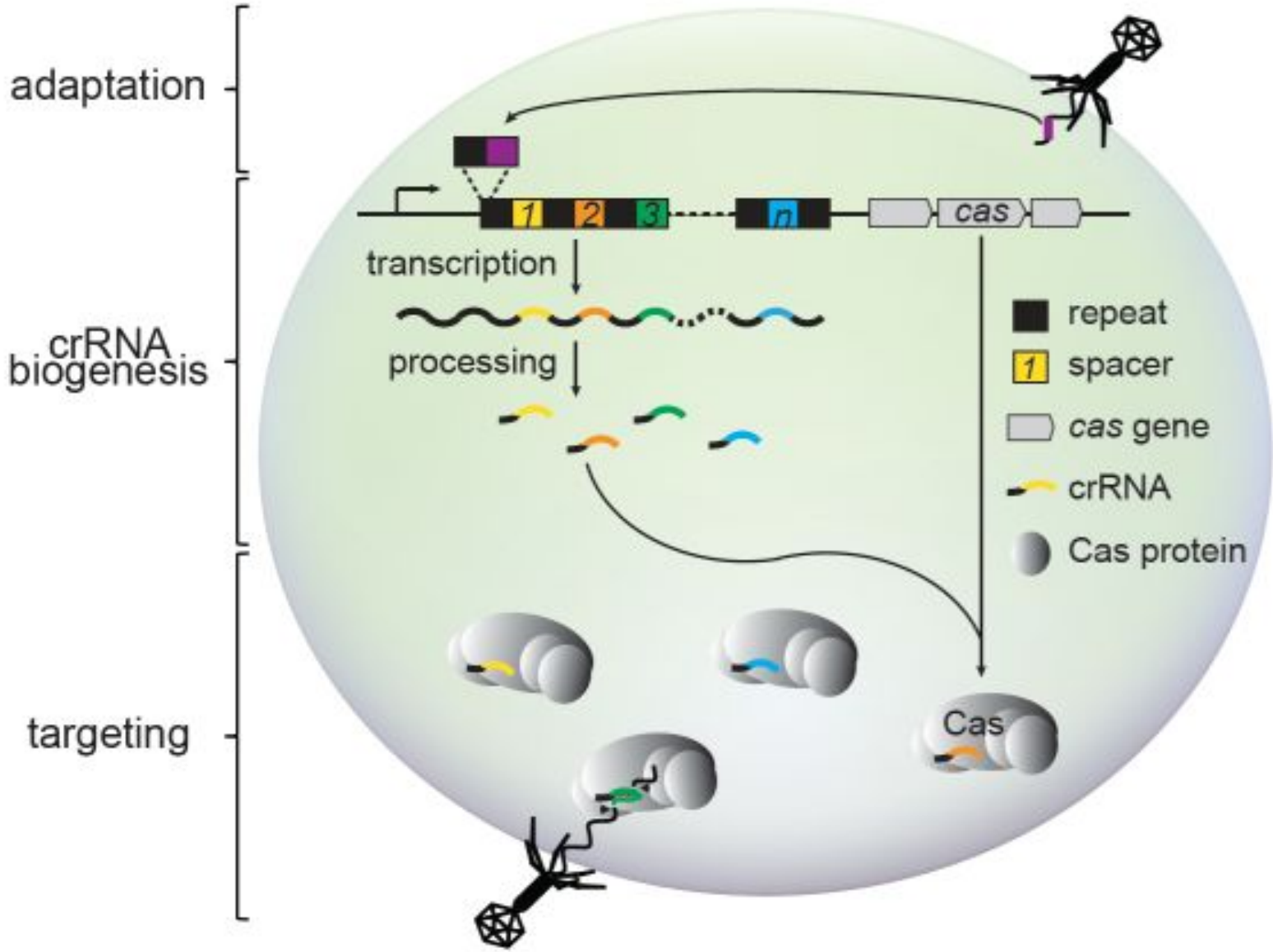
Hygiea technology – the 3 key components:

A: Two types of tail protein ligands

B: Lytic enzyme (disruption of the bacteria from the inside)

C: Binding ligand from the lytic enzyme – called the 'Cell wall Binding Domain' (or CBD)

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



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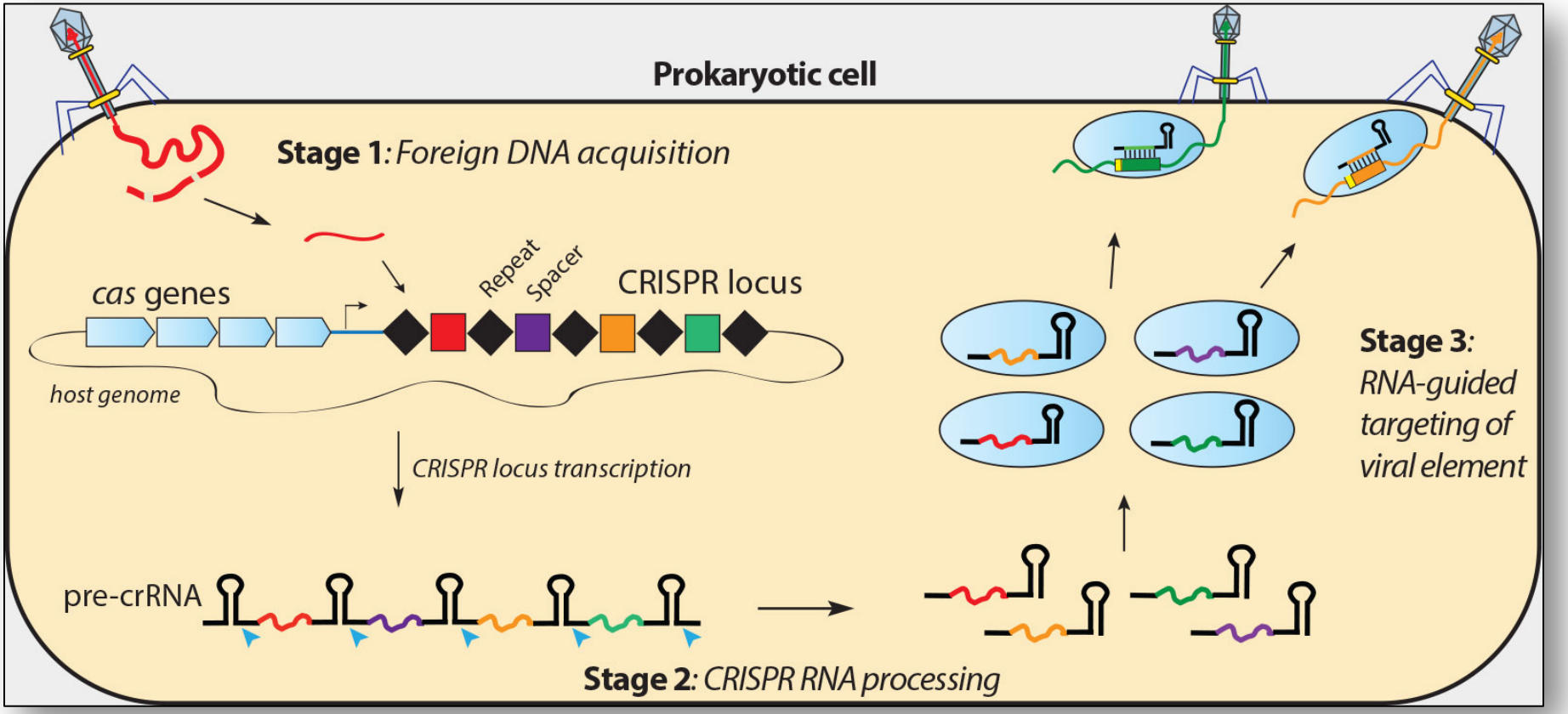
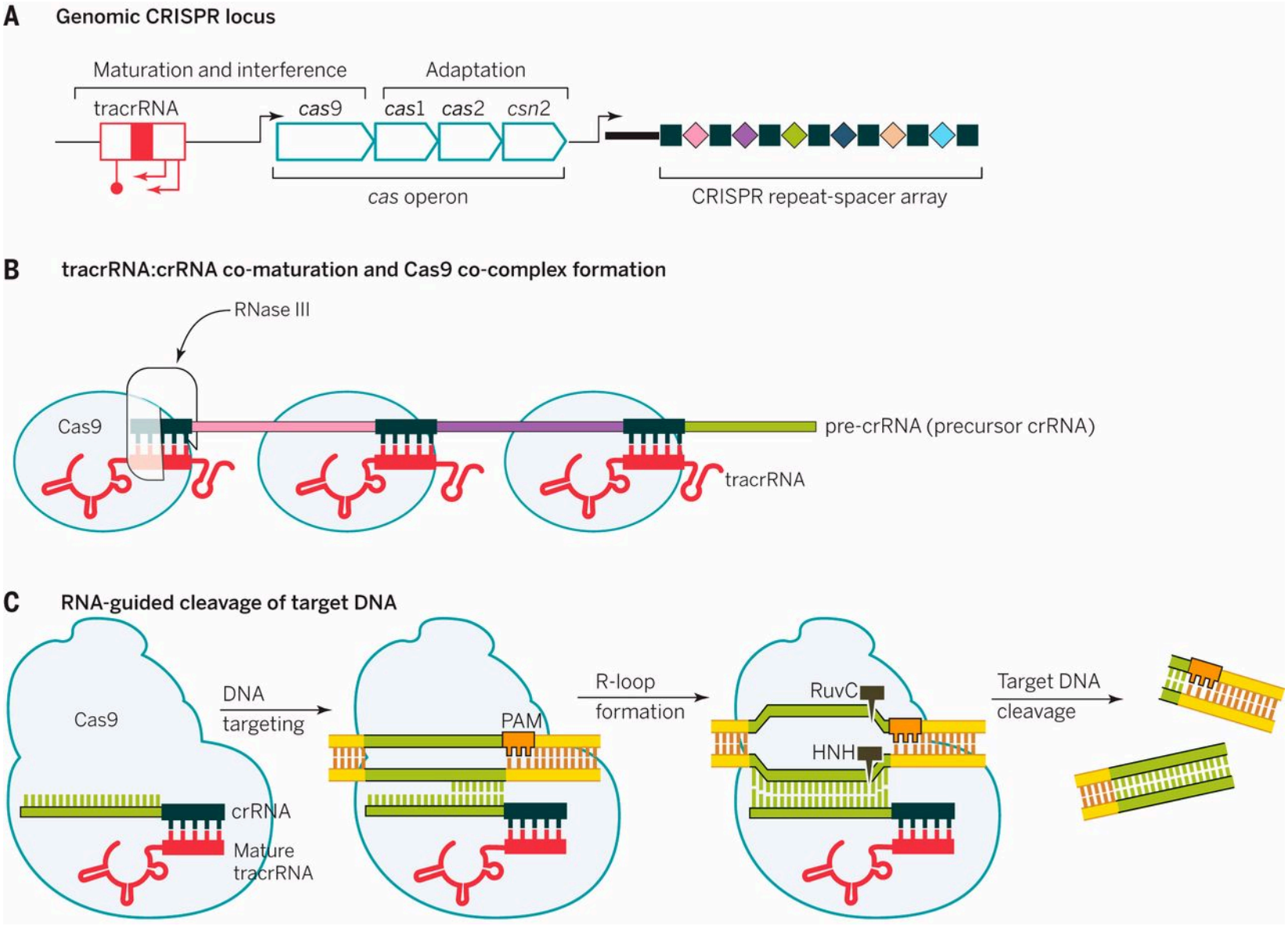
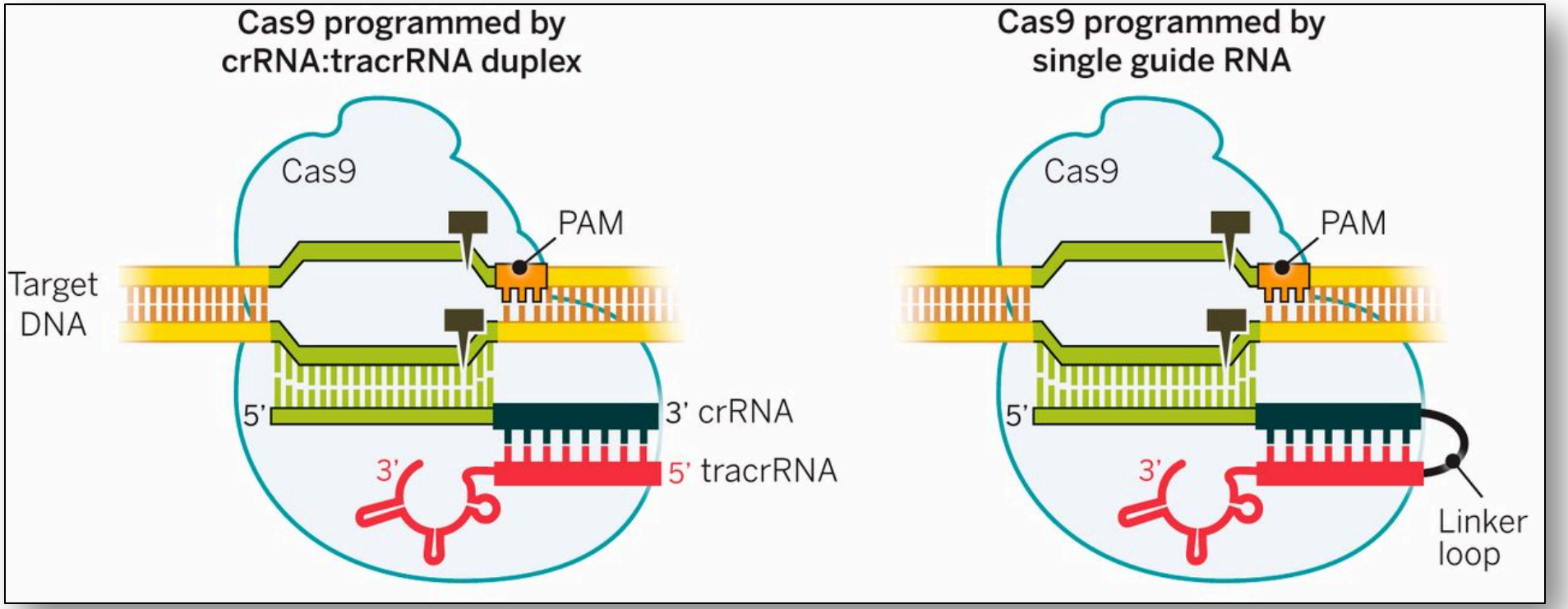


http://rna.berkeley.edu/crispr.html



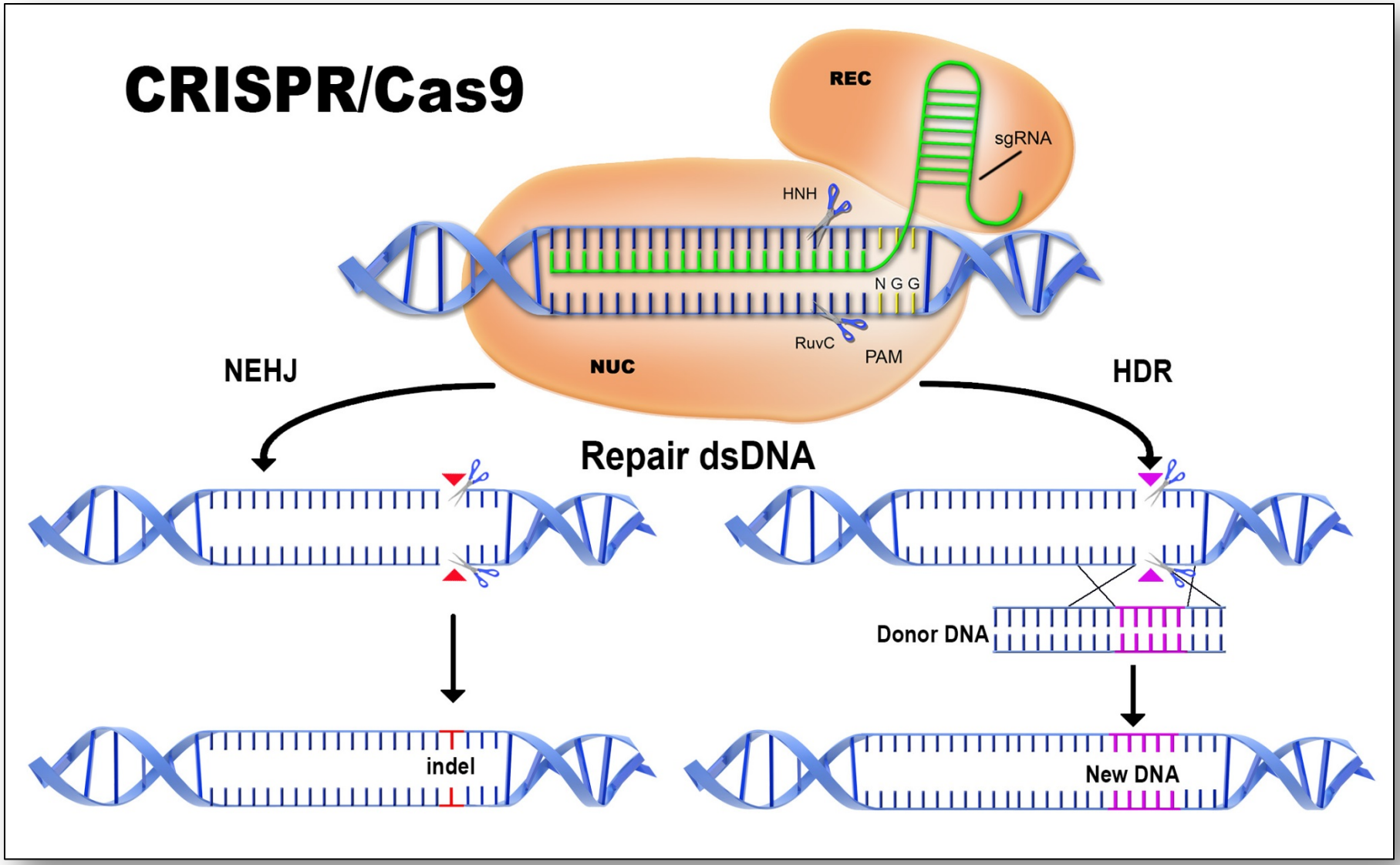
http://science.sciencemag.org/content/346/6213/1258096.figures-only

BREAKTHROUGH – fuse the crRNA and tracrRNA to make a single "Guide RNA"



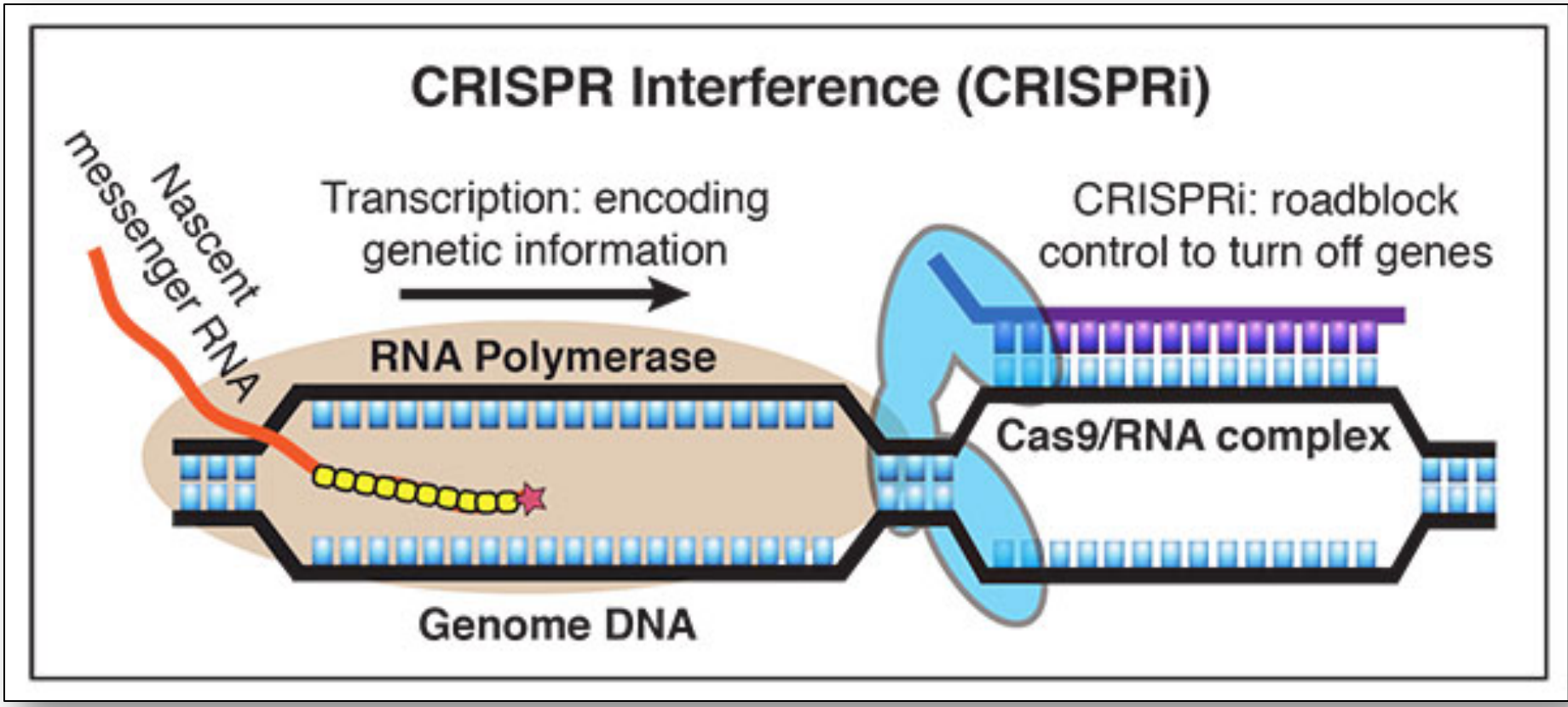
http://science.sciencemag.org/content/346/6213/1258096.figures-only

CRISPR/Cas9 can help **make** a mutation or **fix** a mutation
NHEJ vs Homologous Recombination



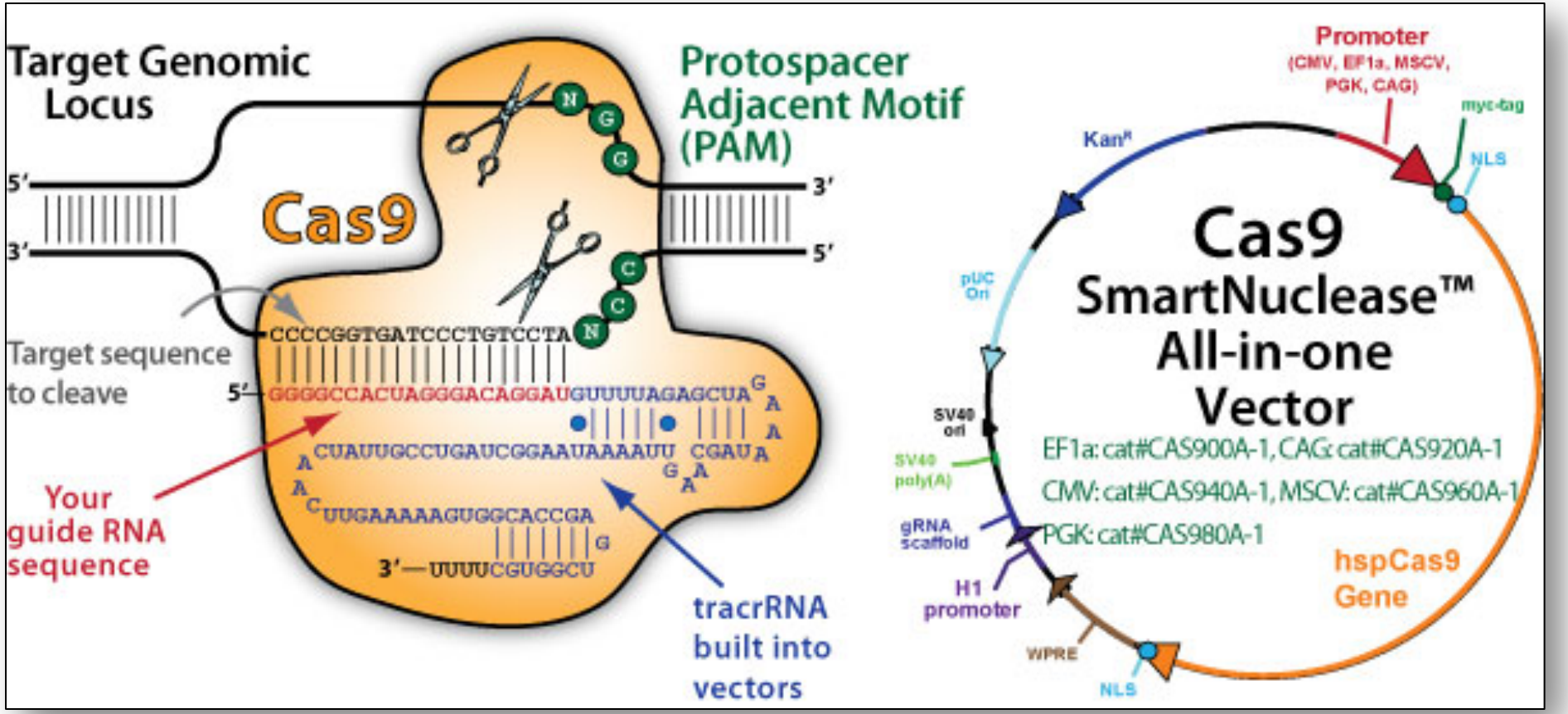
http://www.timeone.ca/designer-genes-getting-crispr-and-crispr/#sthash.3wiIRuzX.dpbs

Non cleaving Cas9 can block gene expression



http://www.cancer.gov/about-cancer/causes-prevention/research/crispr

Genome Editing Kits Galore



https://www.systembio.com/crispr-cas9-plasmids

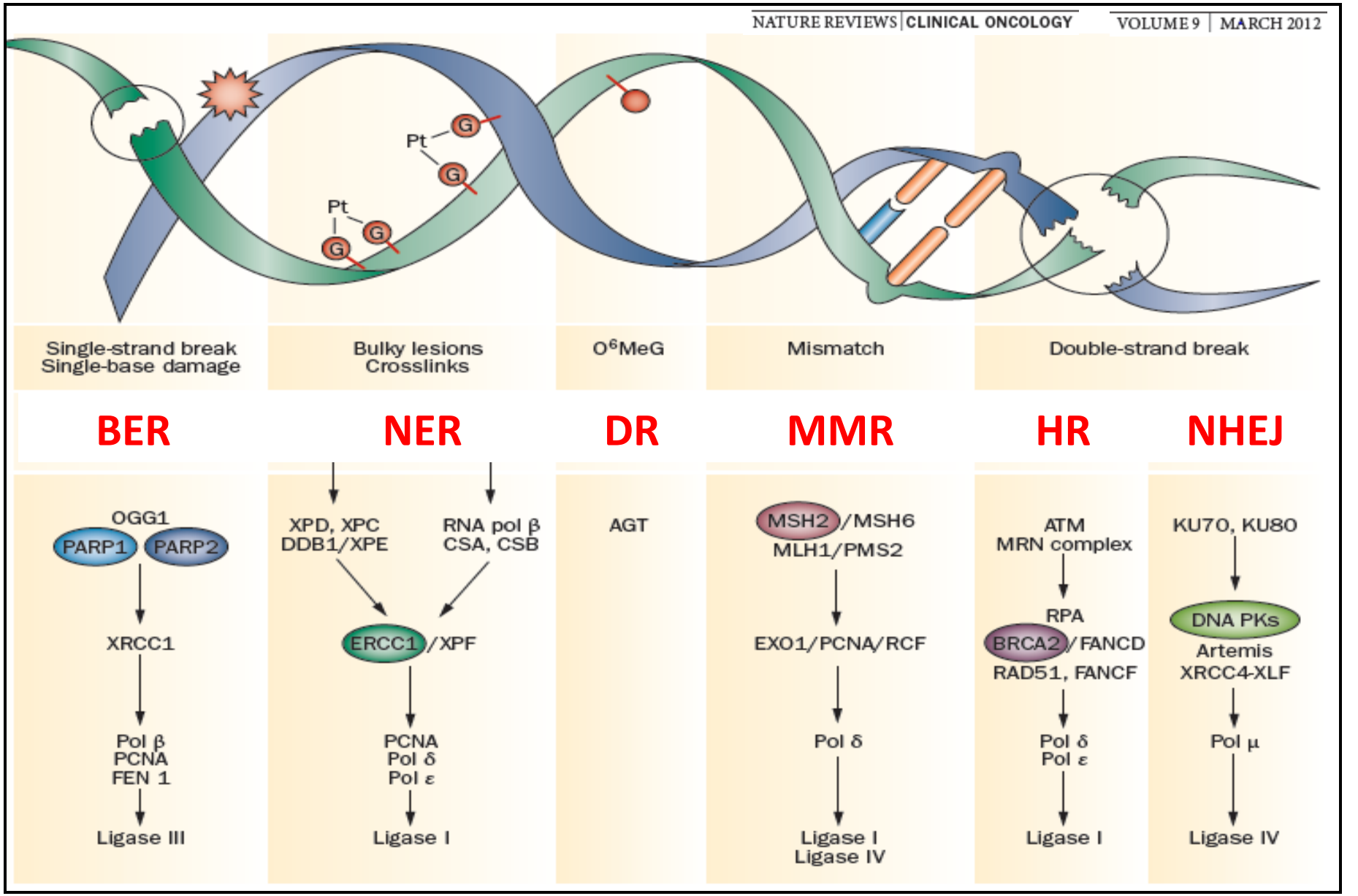
Awards Galore

Emmanuelle Charpentier and Jennifer Doudna

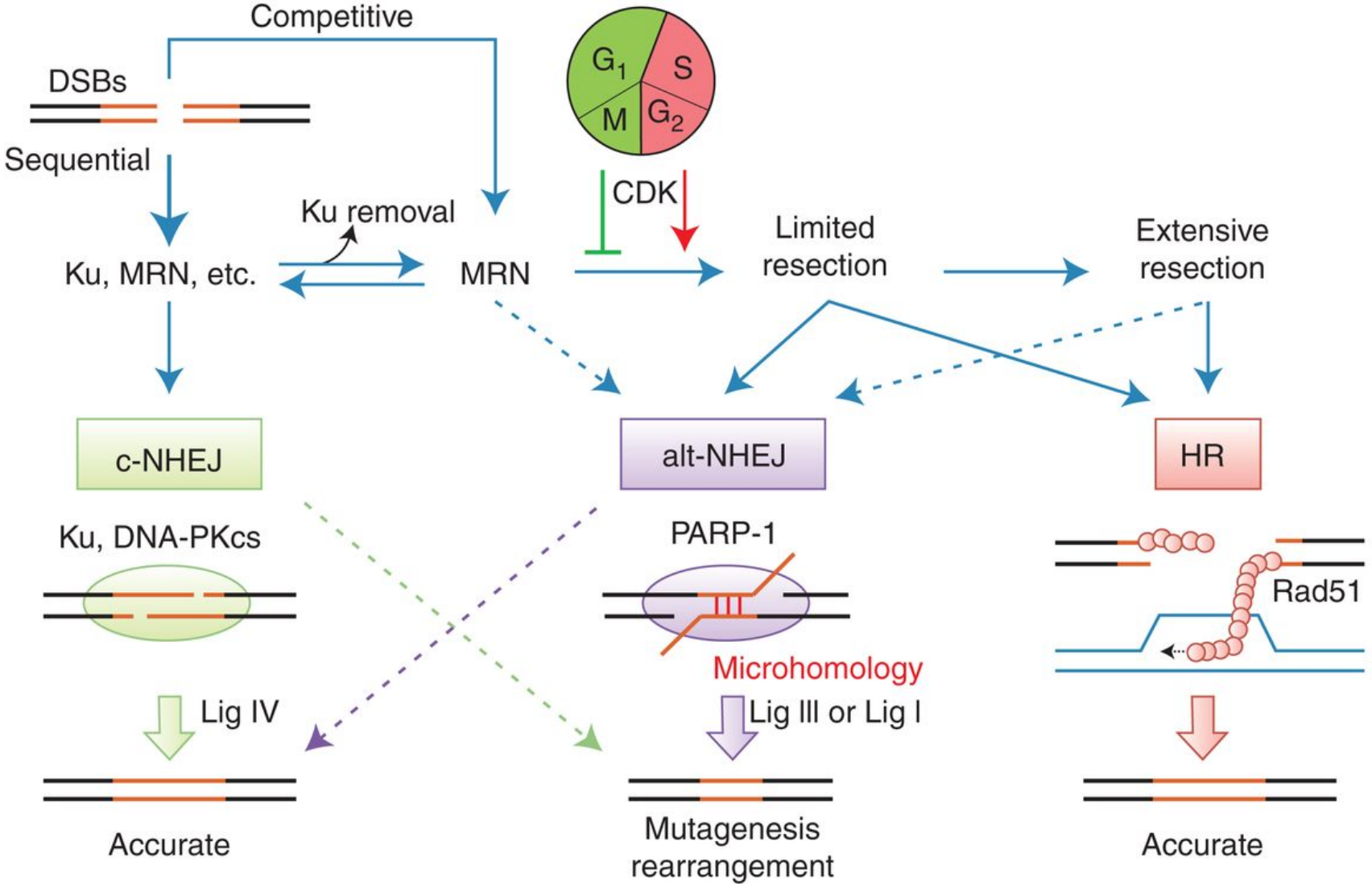
Awards Galore

Feng Zhang - MIT

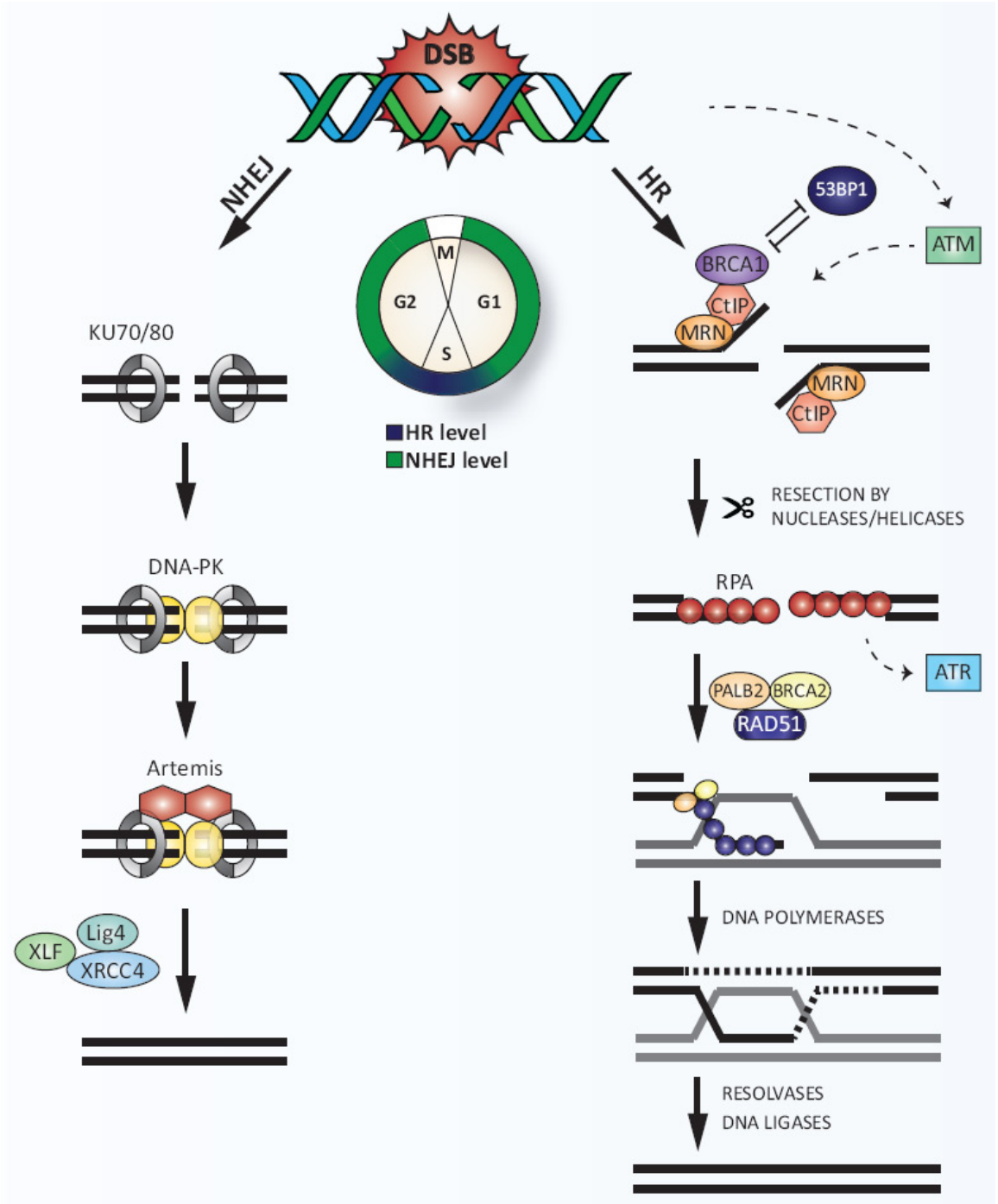
Six Major DNA Repair Pathways



Disposition of DSBs between repair pathways.

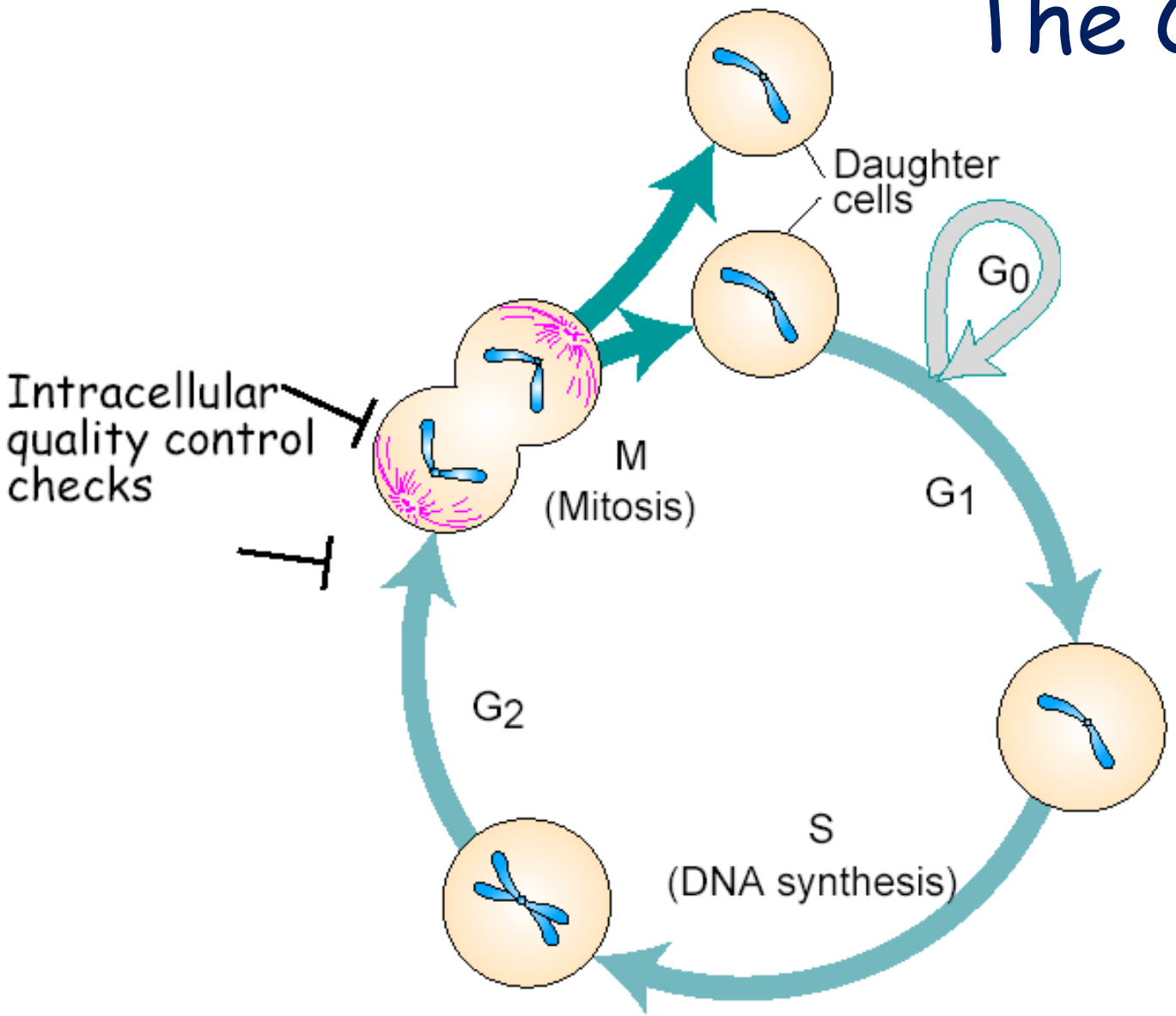


Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757



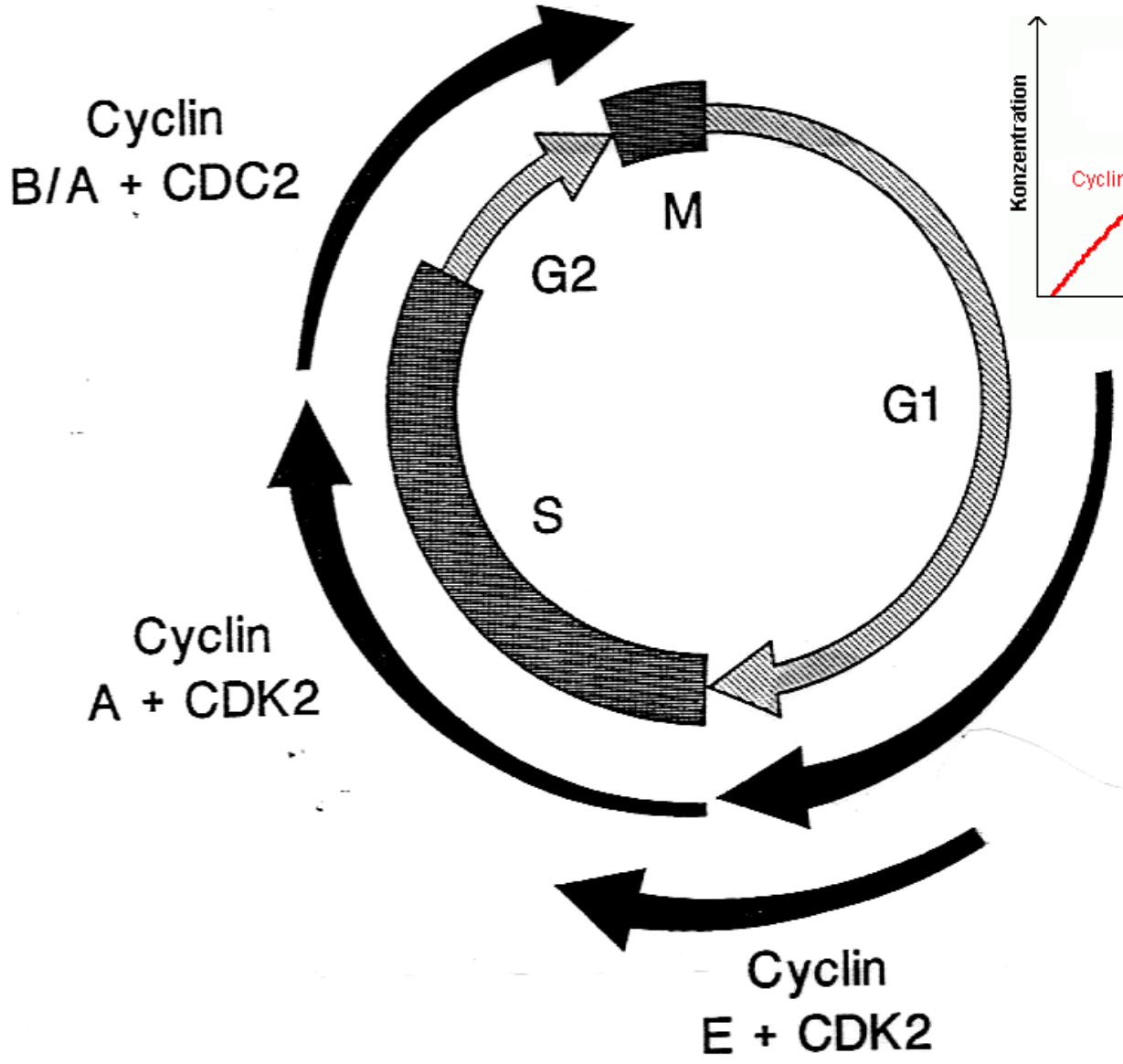
How does the cell decide which pathway to use?

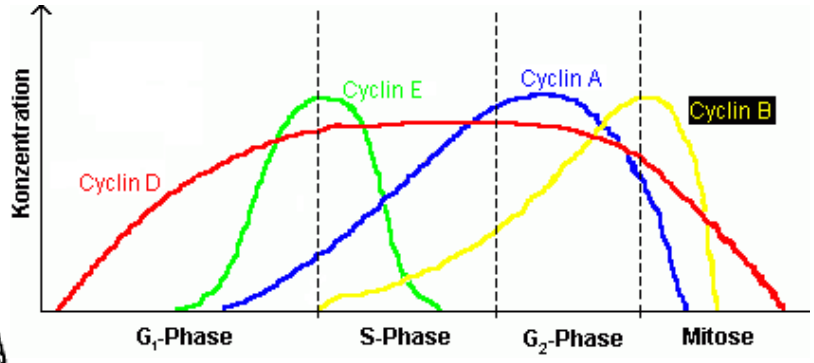
The Cell Cycle

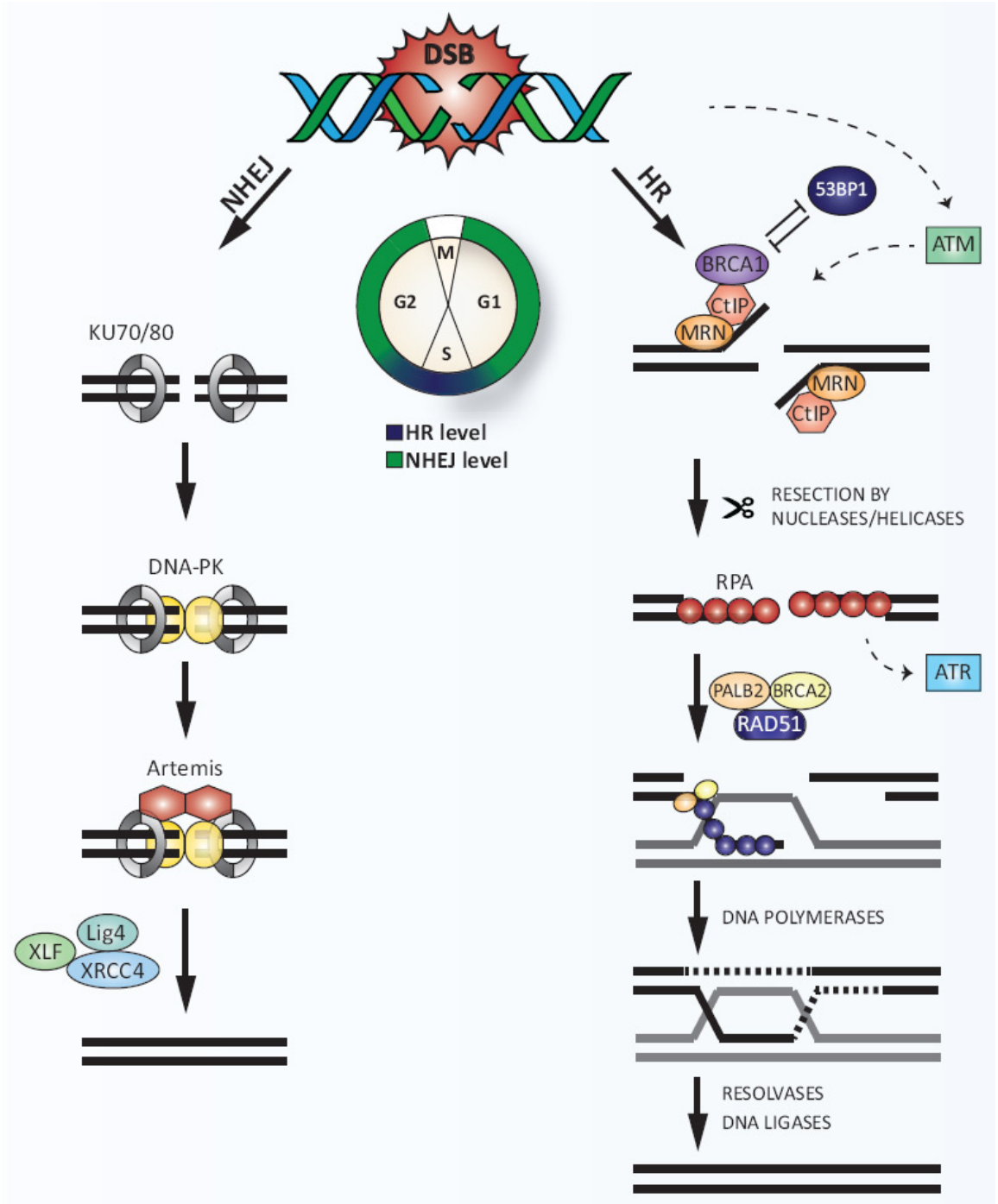


Duplication of chromosomes
DNA Replication

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







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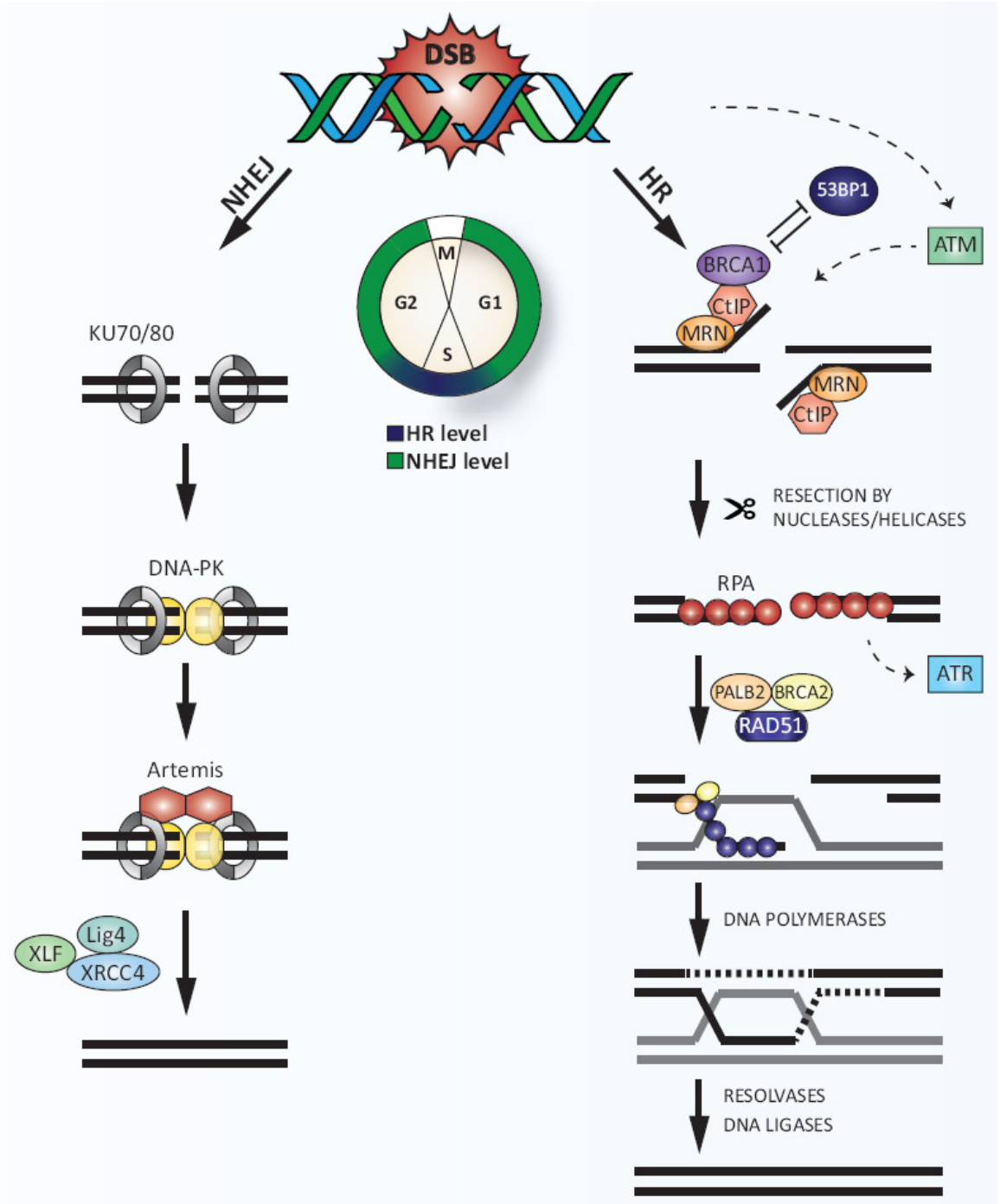
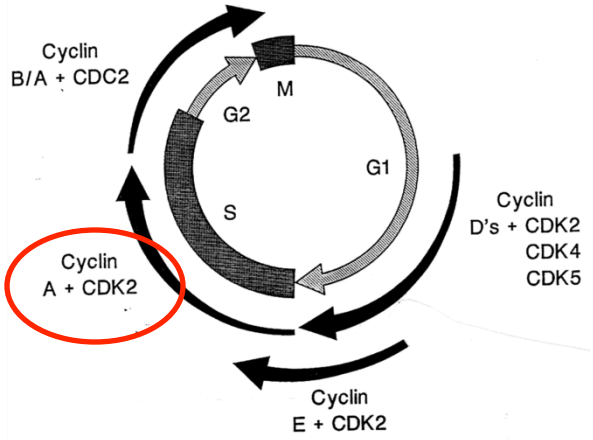
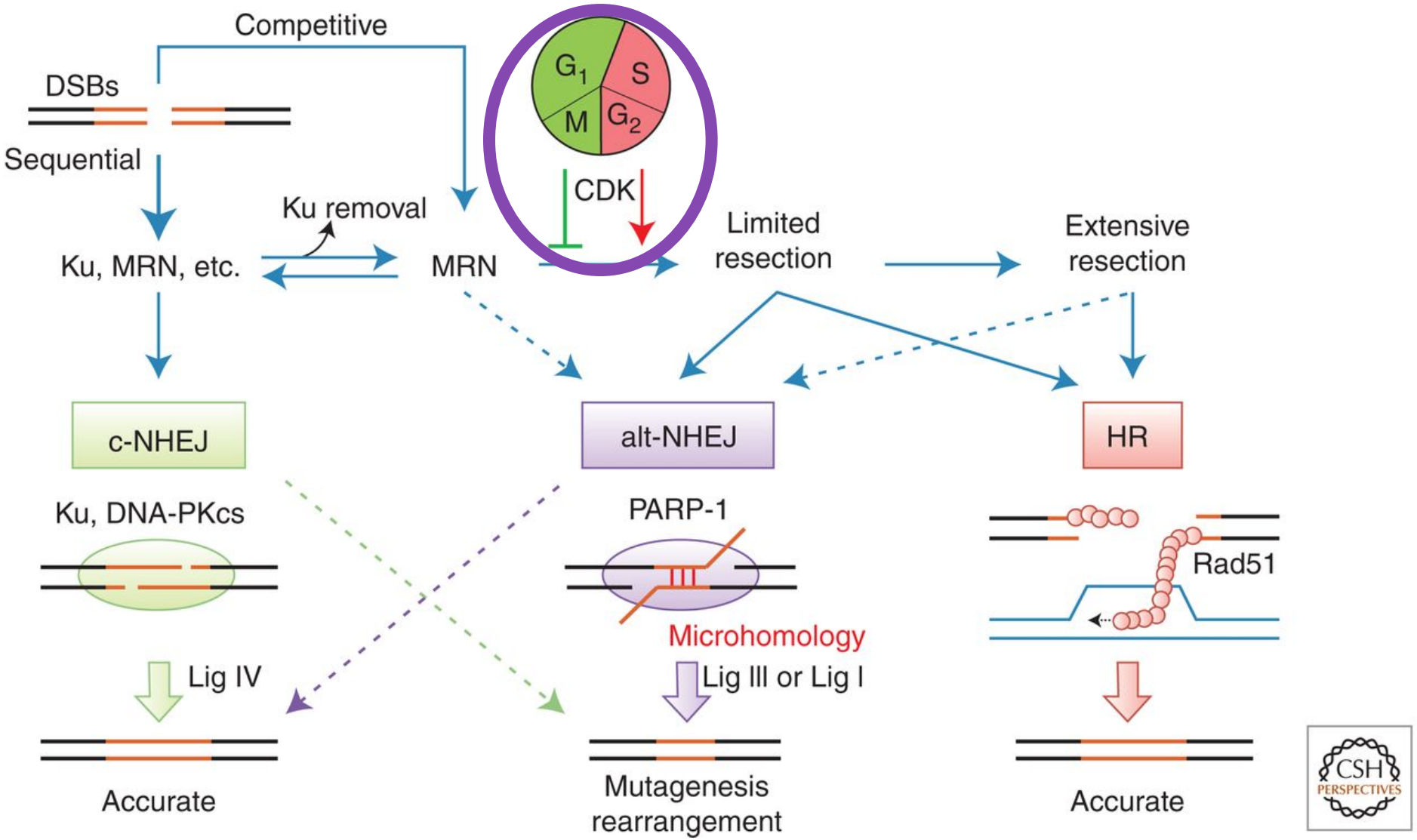


CyclinA-CDK2 targets the CtIP/BRCA1 complex



Disposition of DSBs between repair pathways.



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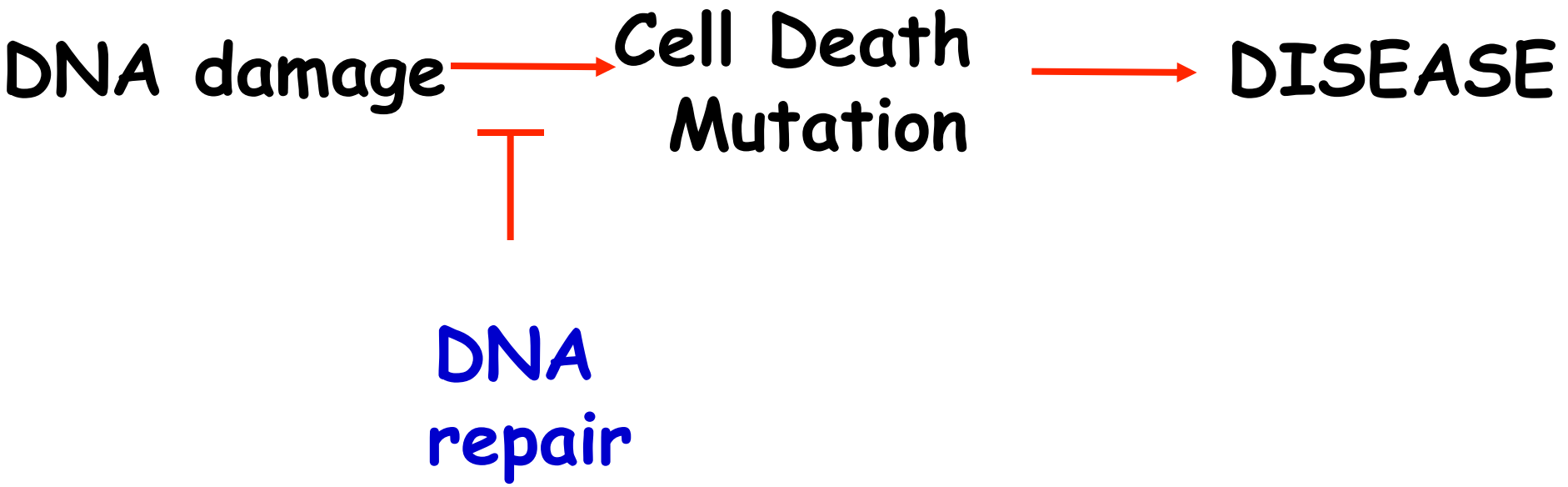
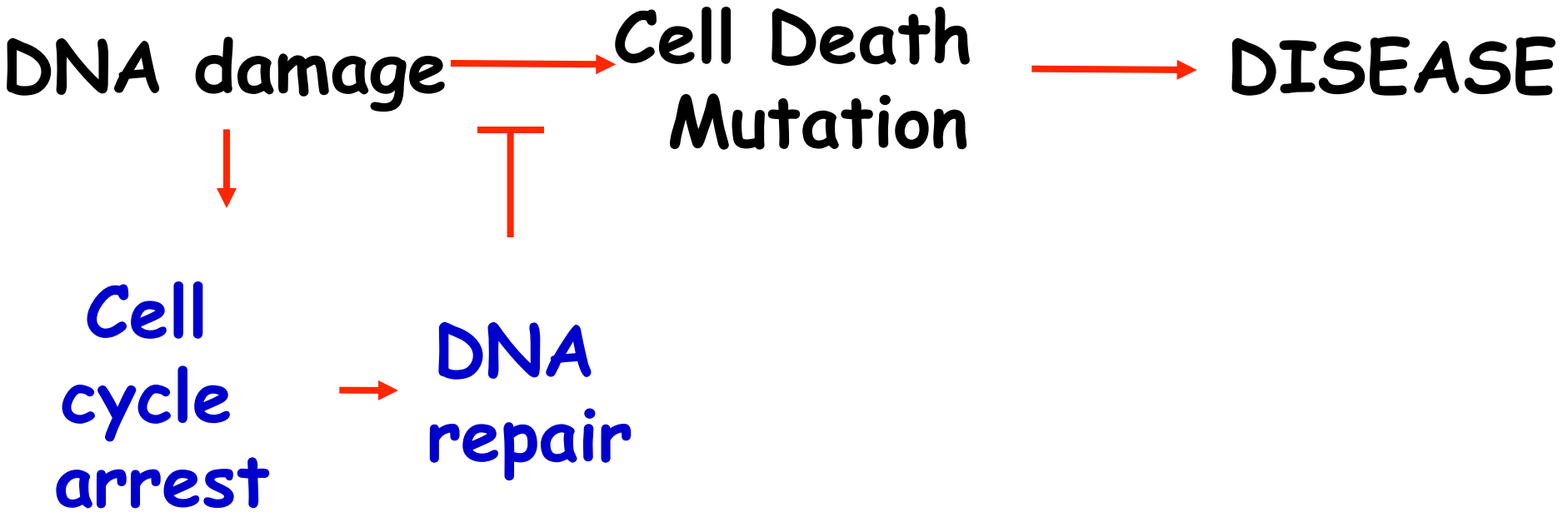
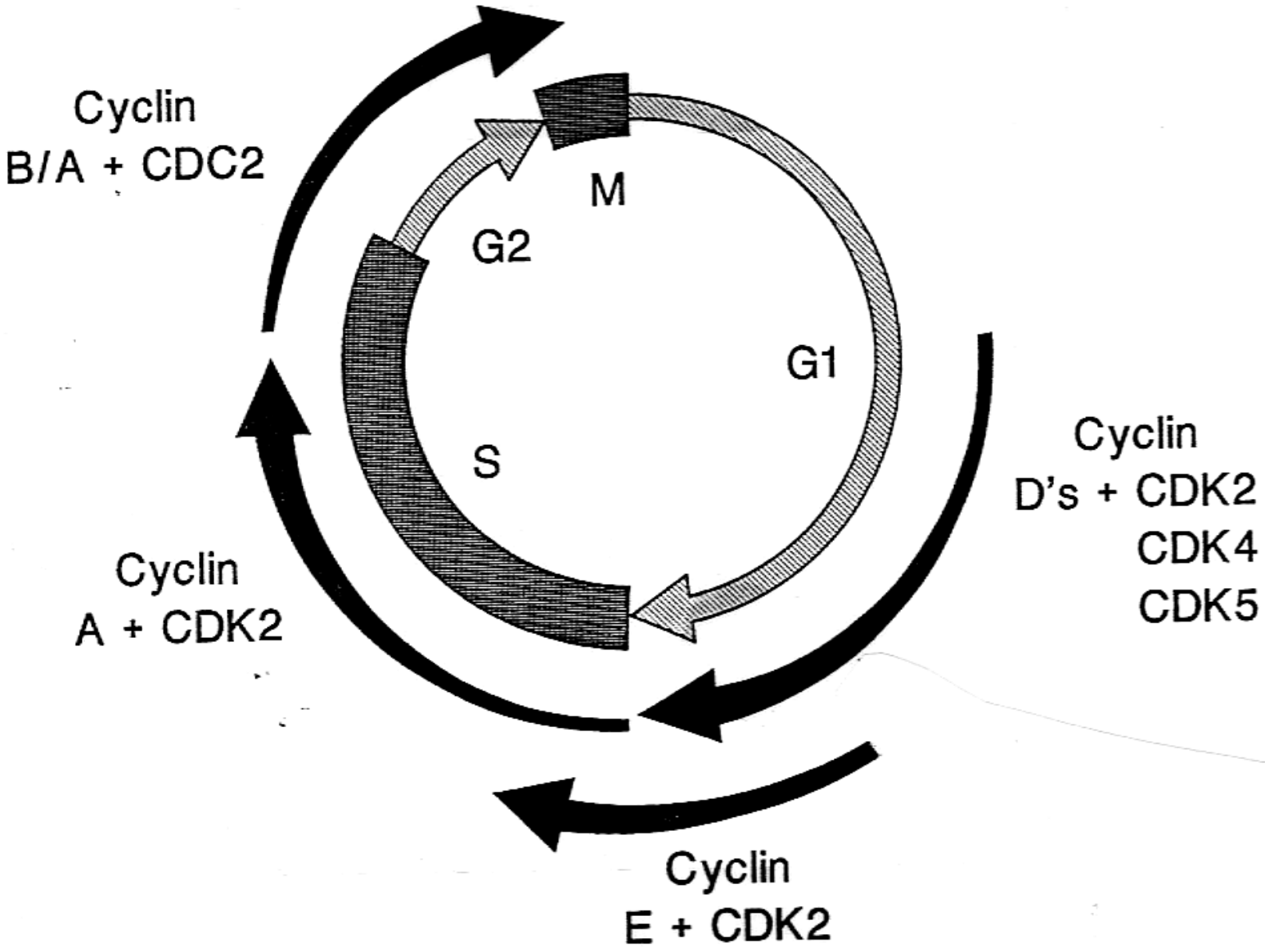
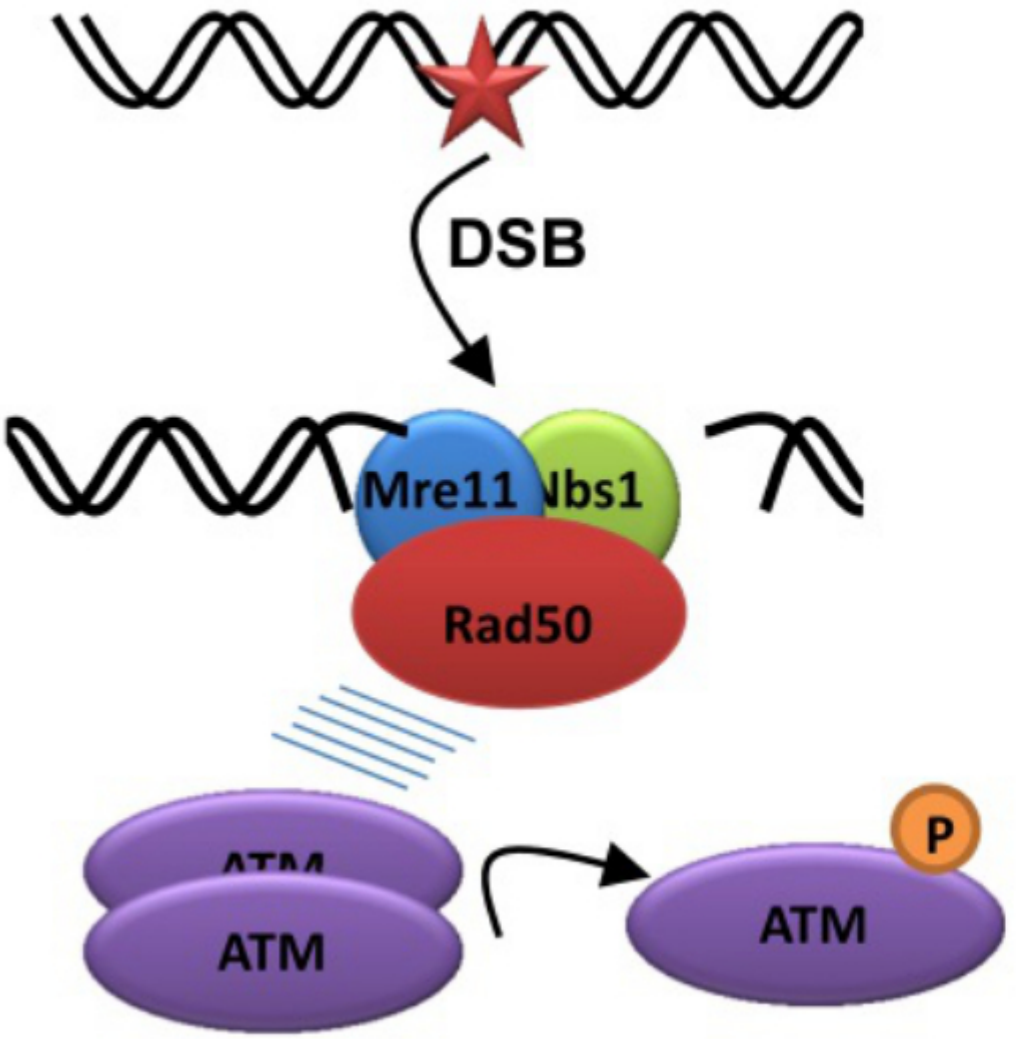
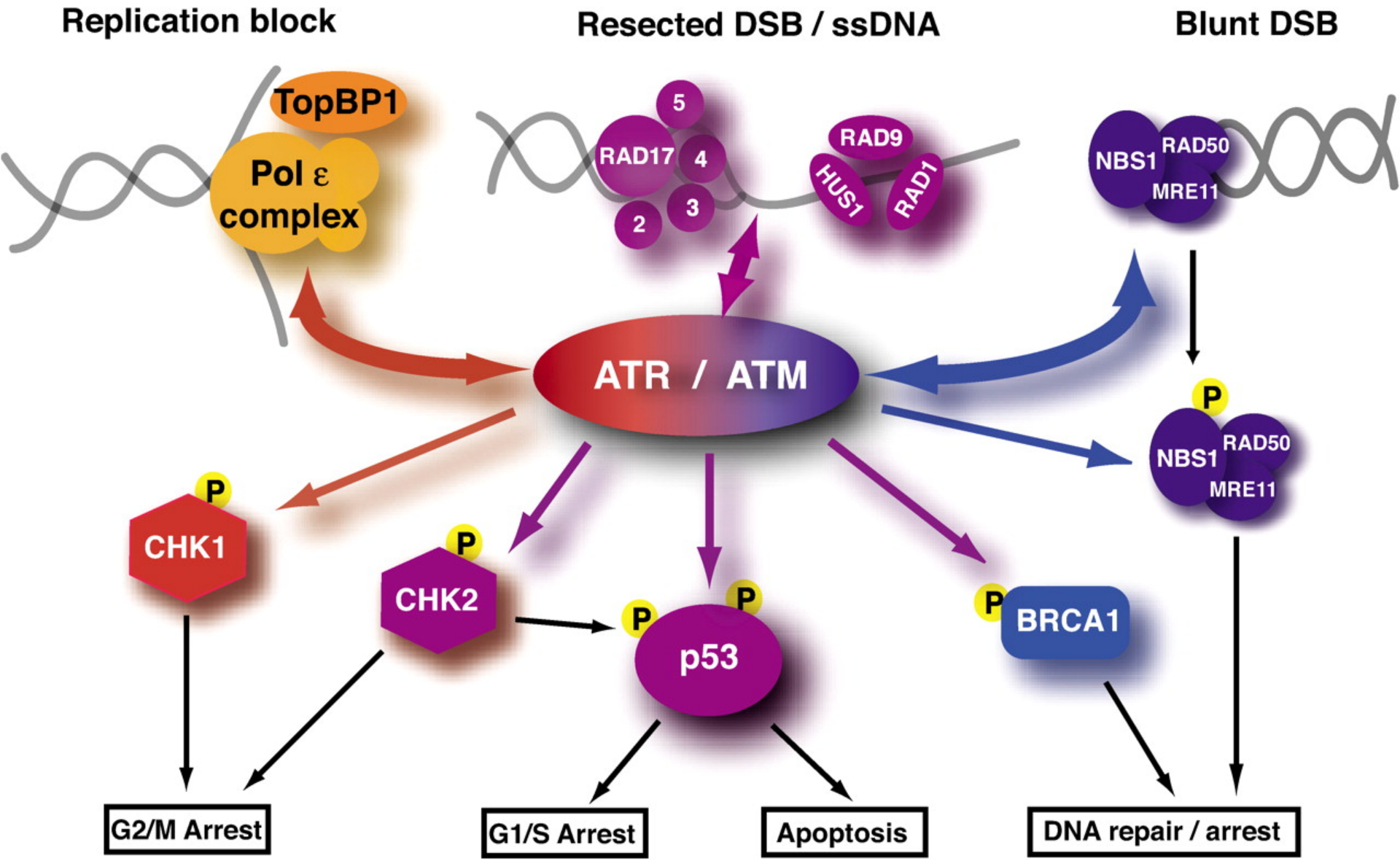

DNA damage
Cell Death
Mutation
DISEASE
DNA
repair

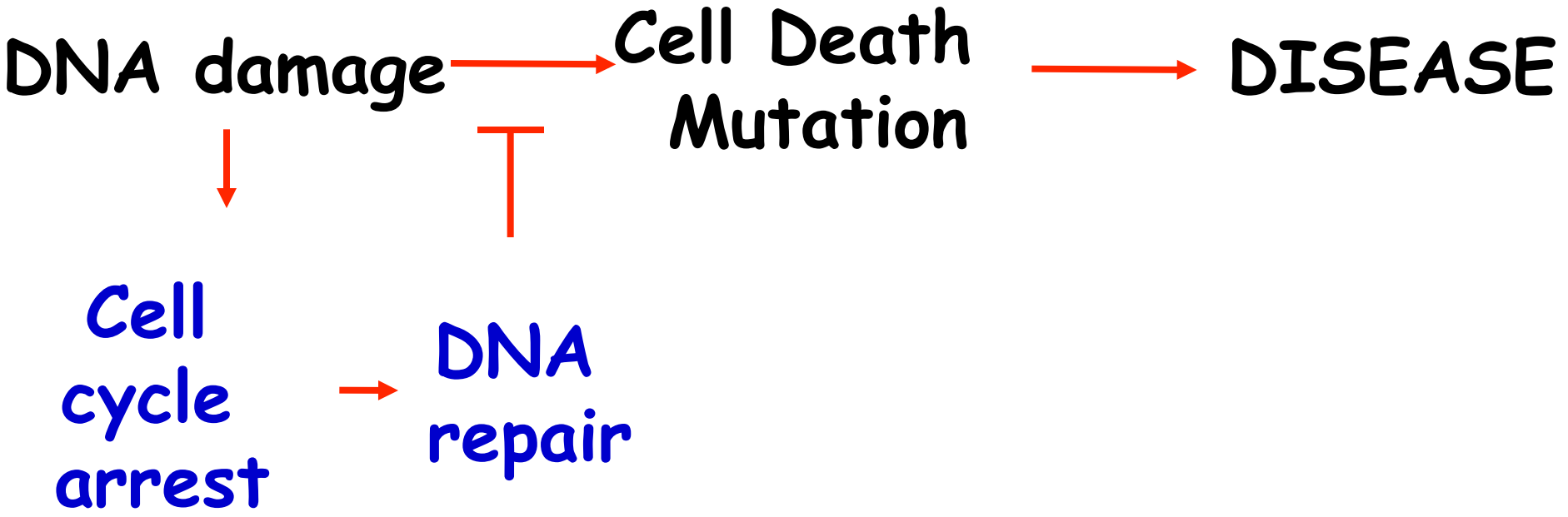

DNA damage
Cell Death
Mutation
DISEASE
Cell
cycle
arrest
DNA
repair


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

Signaling at DSBs – ATM kinase activated




Replication block
Resected DSB / ssDNA
Blunt DSB
TopBP1
Pol ε complex
5
RAD17
4
3
2
RAD9
HUS1
RAD1
NBS1
RAD50
MRE11
ATR / ATM
P
NBS1
RAD50
MRE11
P
CHK1
P
CHK2
P
P
p53
P
P
BRCA1
G2/M Arrest
G1/S Arrest
Apoptosis
DNA repair / arrest


DNA damage
Cell Death
Mutation
DISEASE
Cell
cycle
arrest
DNA
repair

Ataxia Telangiectasia – Cancer Prone

Defective DNA Damage Responses can affect both neurodegeneration and cancer susceptibility

Ataxia Telangiectasia

- 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

20.109 Spring 2016 Module 2 – Lecture 5
System Engineering (March 31st 2016)

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