

Of complexes and maintenance of genome stability

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CSB, Ceitec, MU

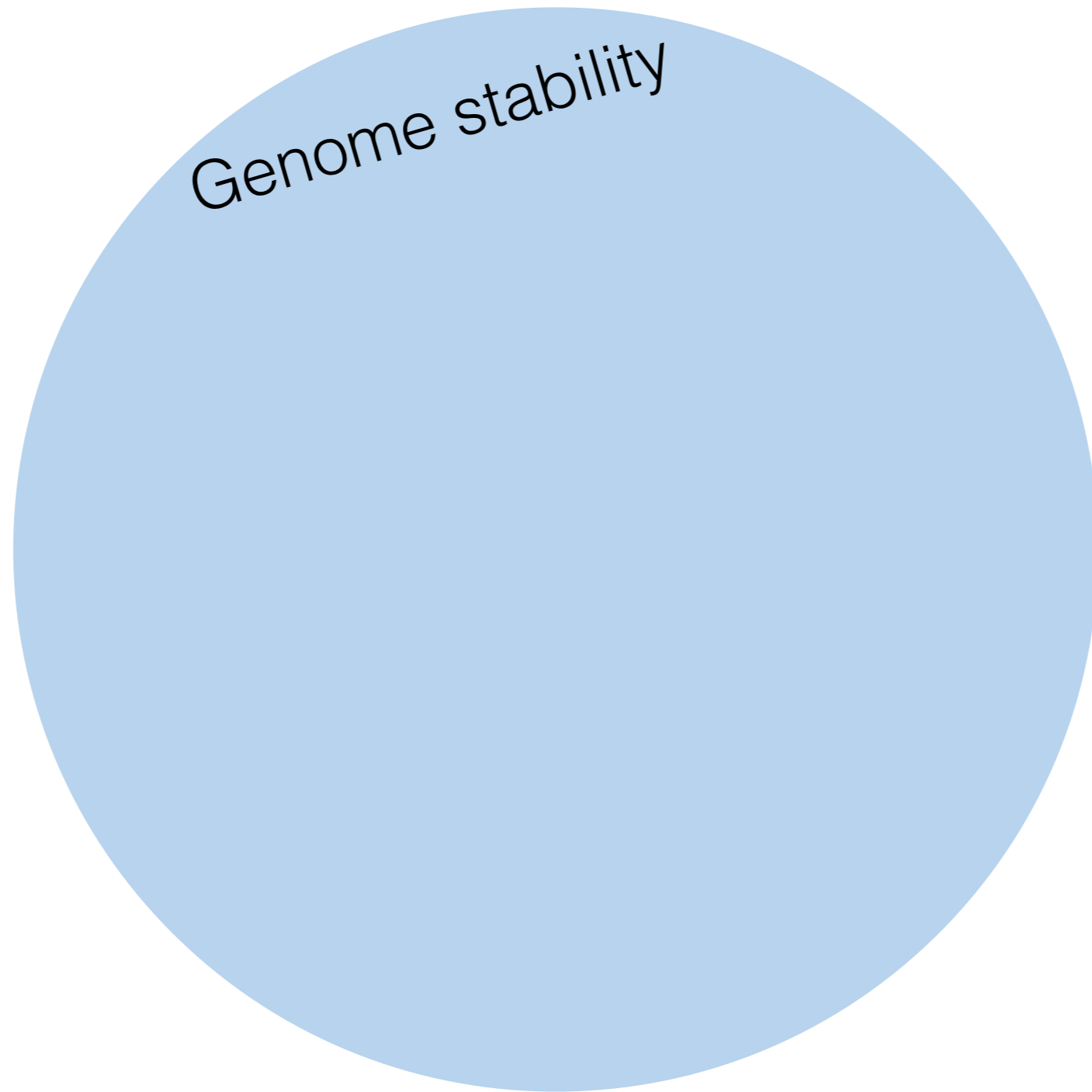
24-Mar-22

Content

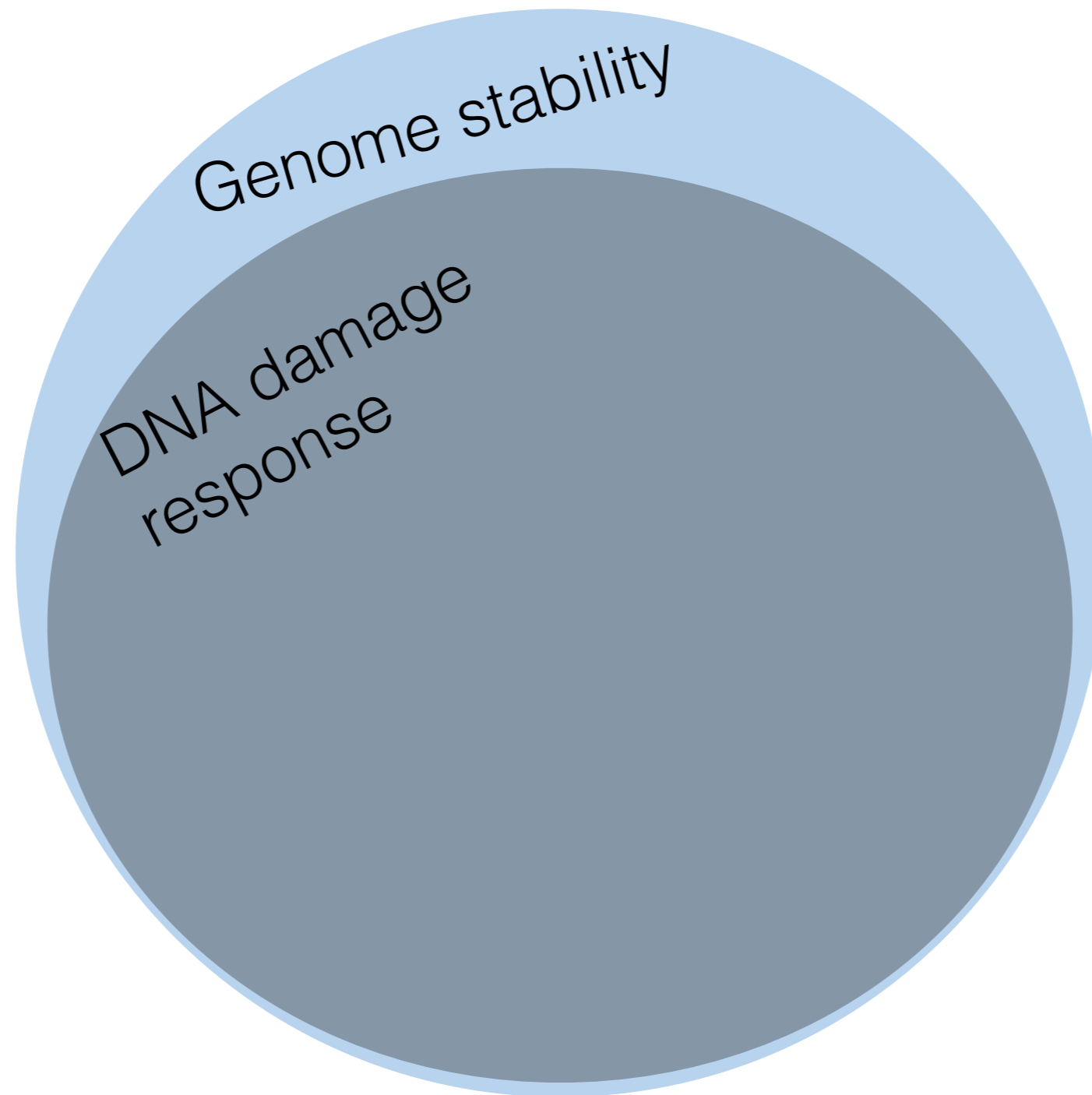
1. What is maintenance of genome stability?
2. What are the challenges to the genome stability?
3. How do cells know the genome stability has been compromised?
4. How do cells maintain the genome stability?
5. How to study the genome stability maintenance?
(Case study on Homologous recombination)

What is the maintenance of genome stability?

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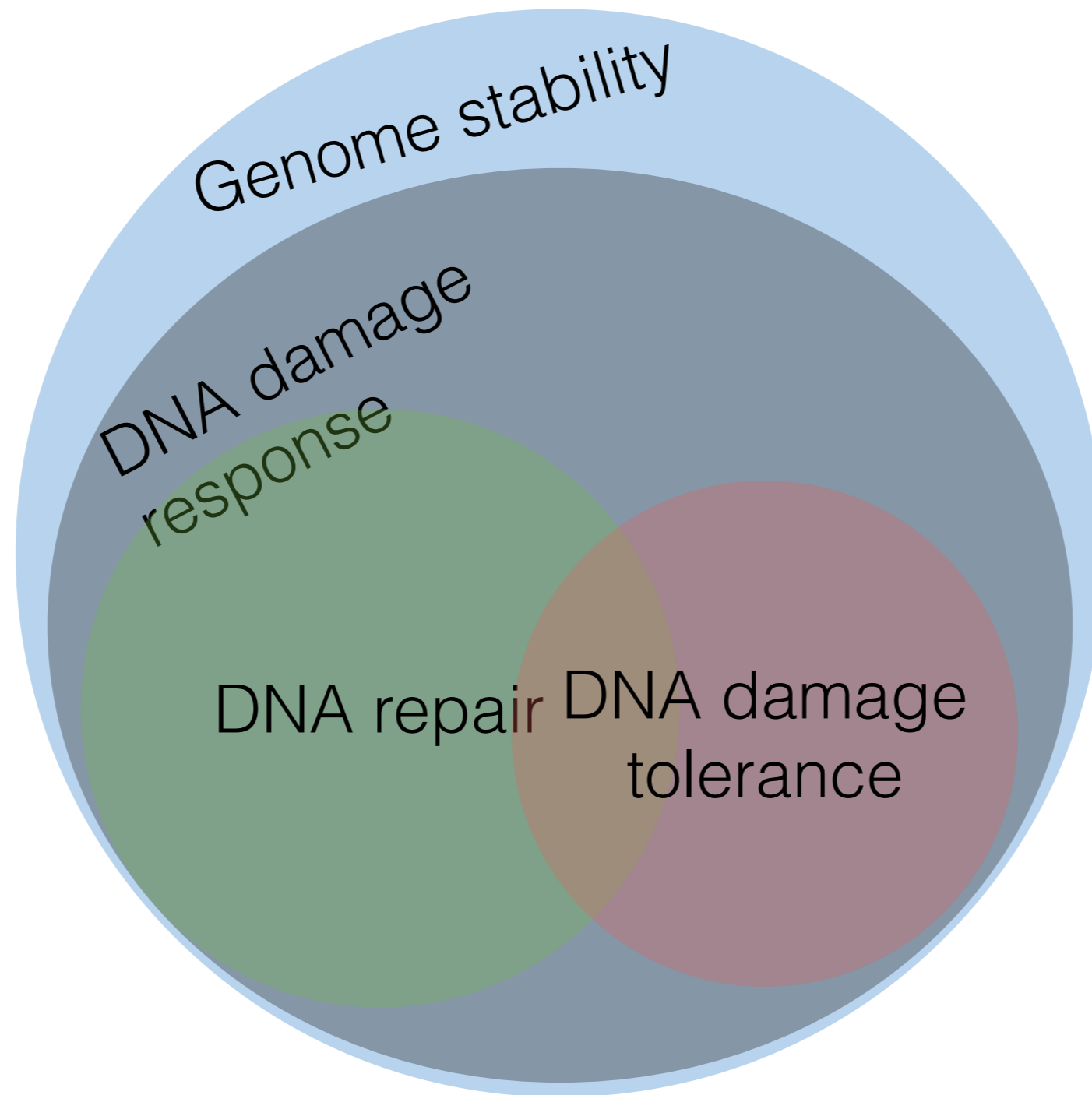
What is the maintenance of genome stability?



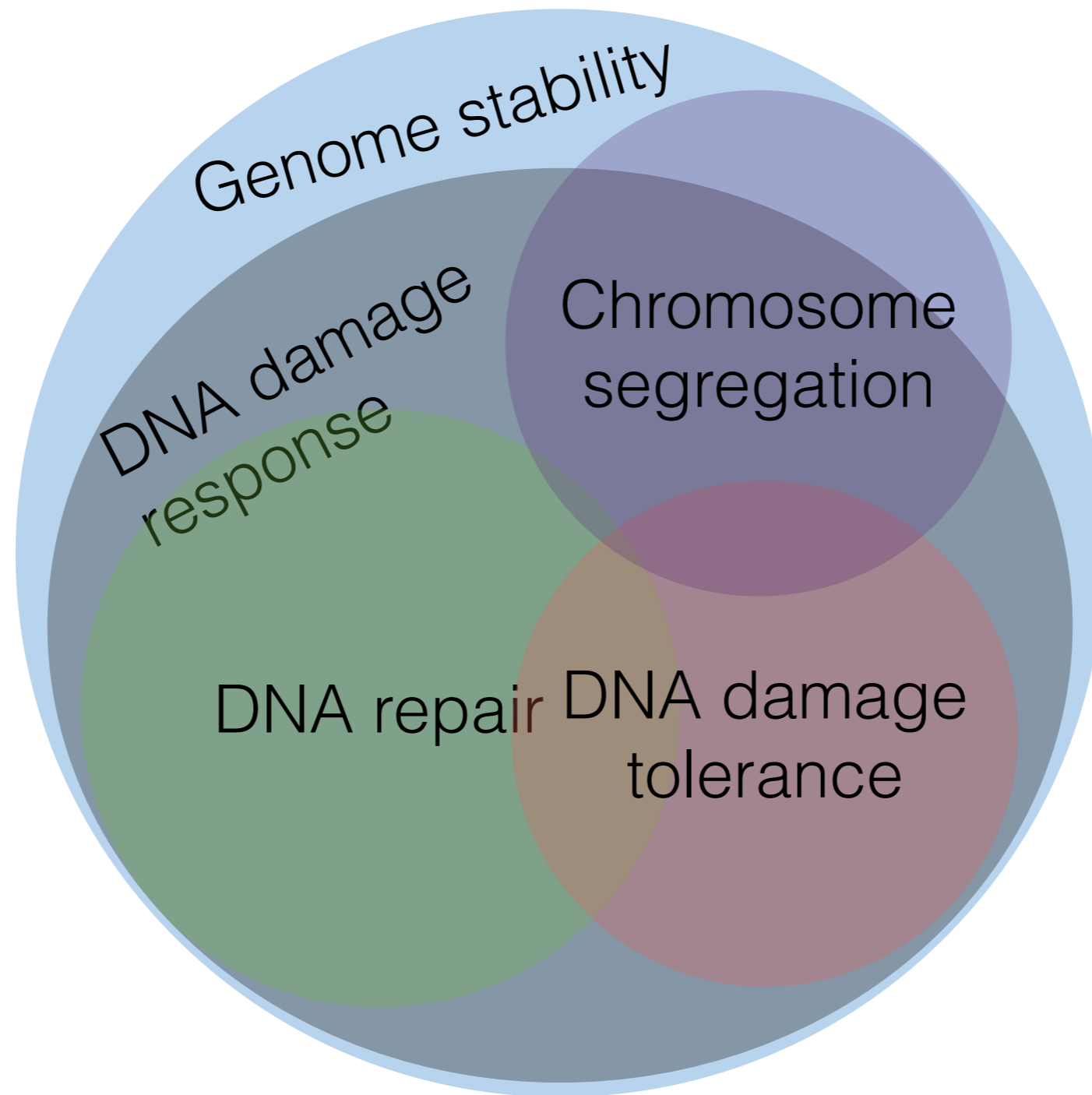
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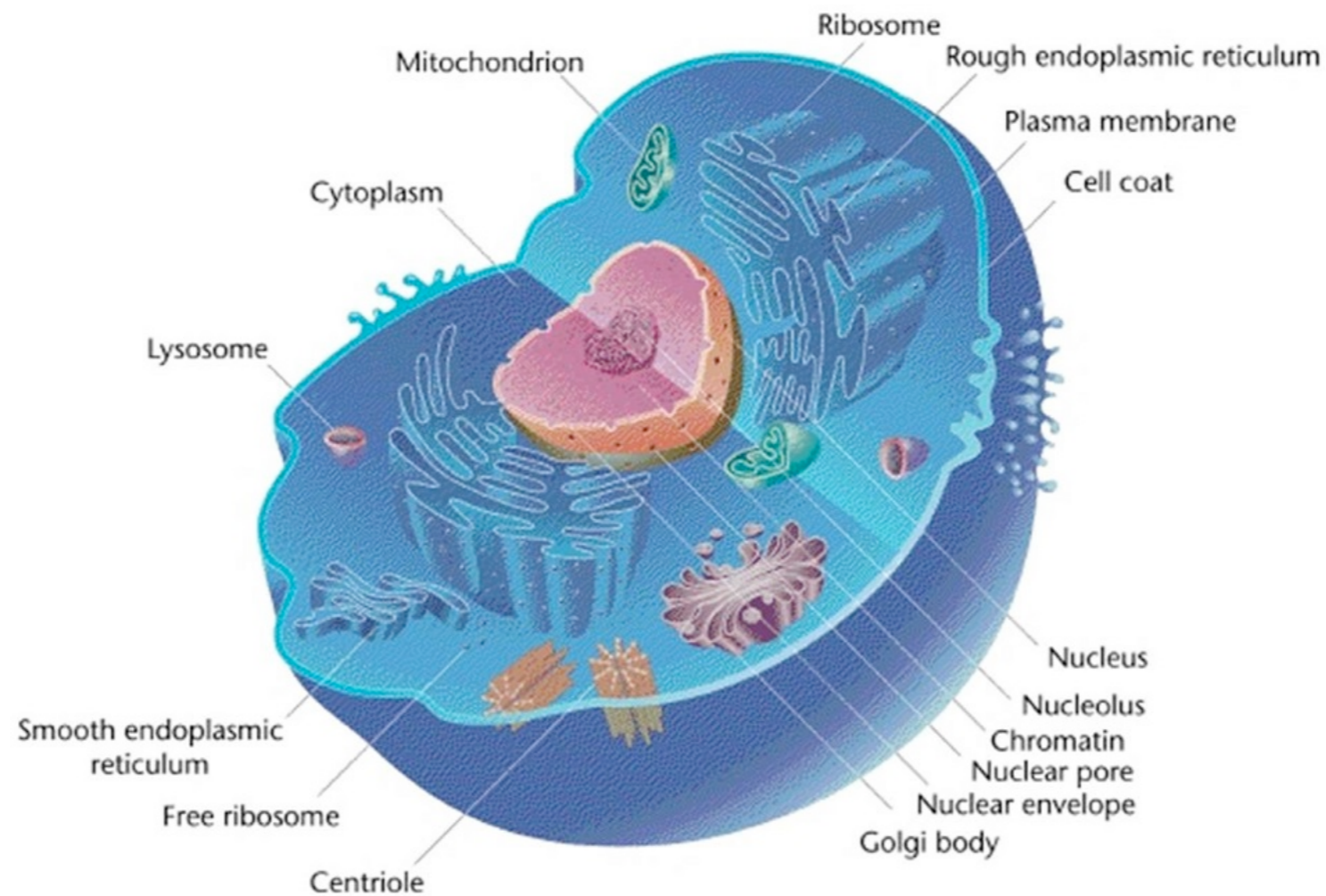
What is the maintenance of genome stability?

What is the maintenance of genome stability?

It is the ability of living organisms to preserve its genetic material in time and across generations.

What are the challenges to genome stability?

All living matter is constantly exposed to environment that challenges genome stability



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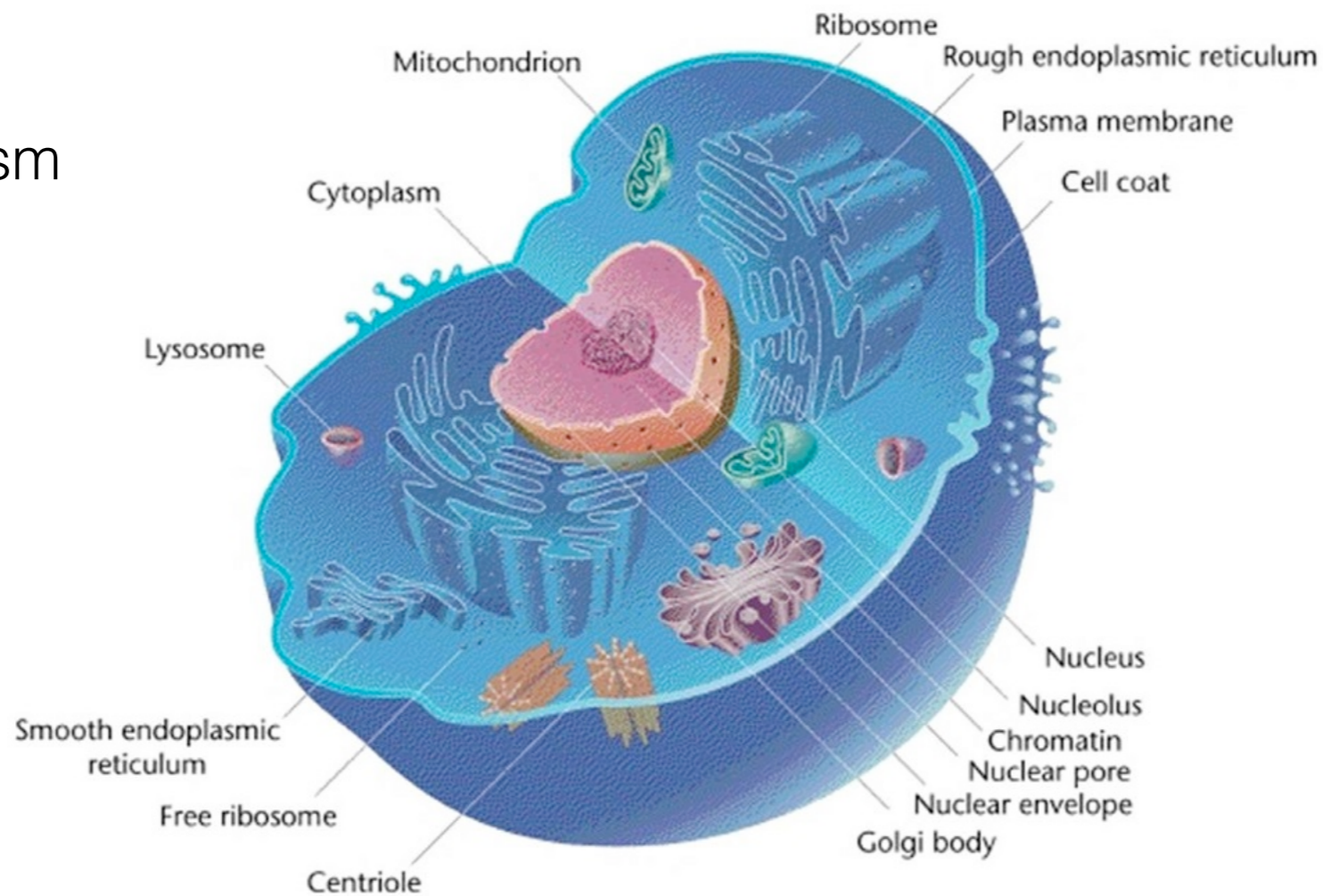
Endogenous

Cellular metabolism

DNA replication

Transcription

Spontaneous
modification
of the DNA



What are the challenges to genome stability?

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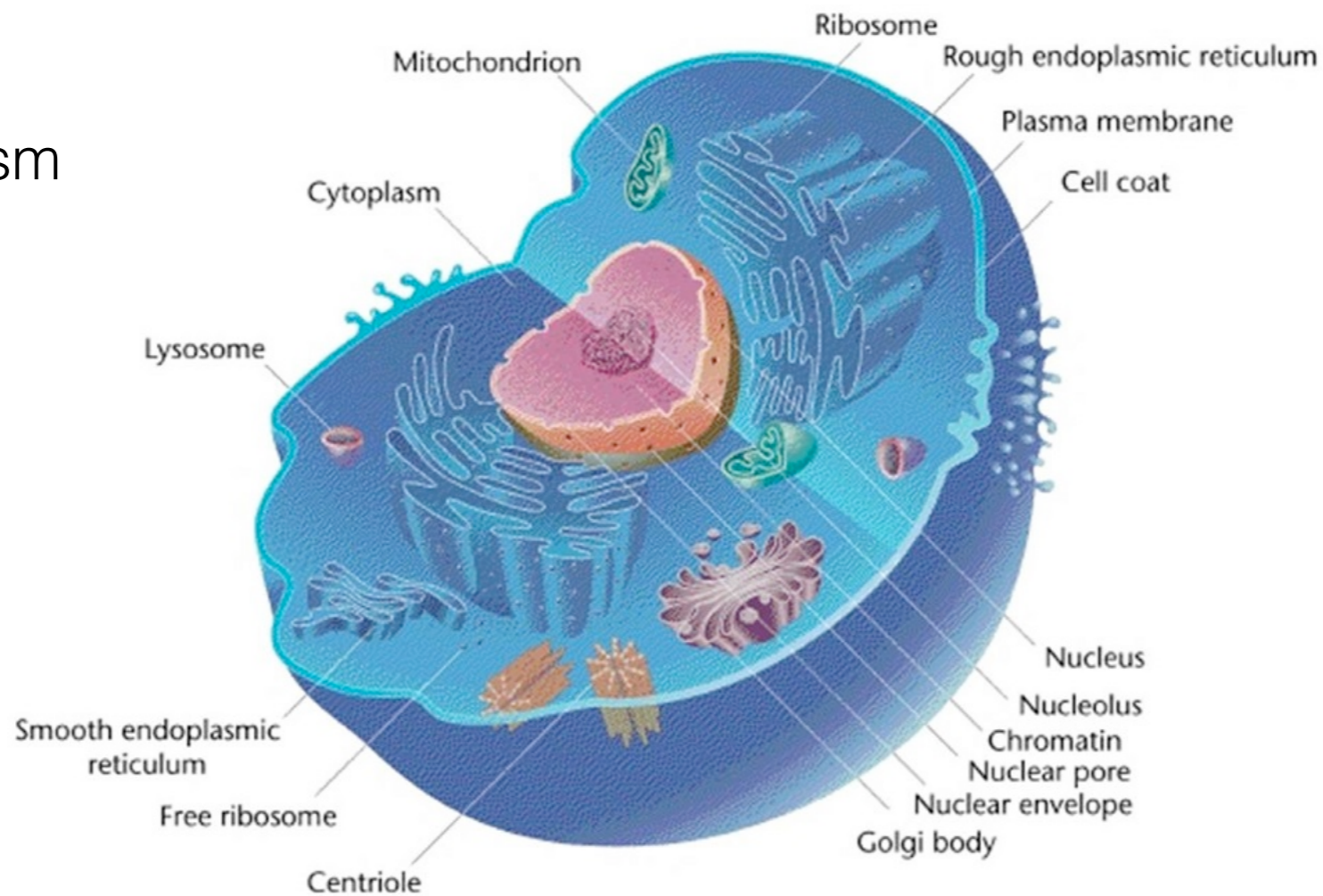
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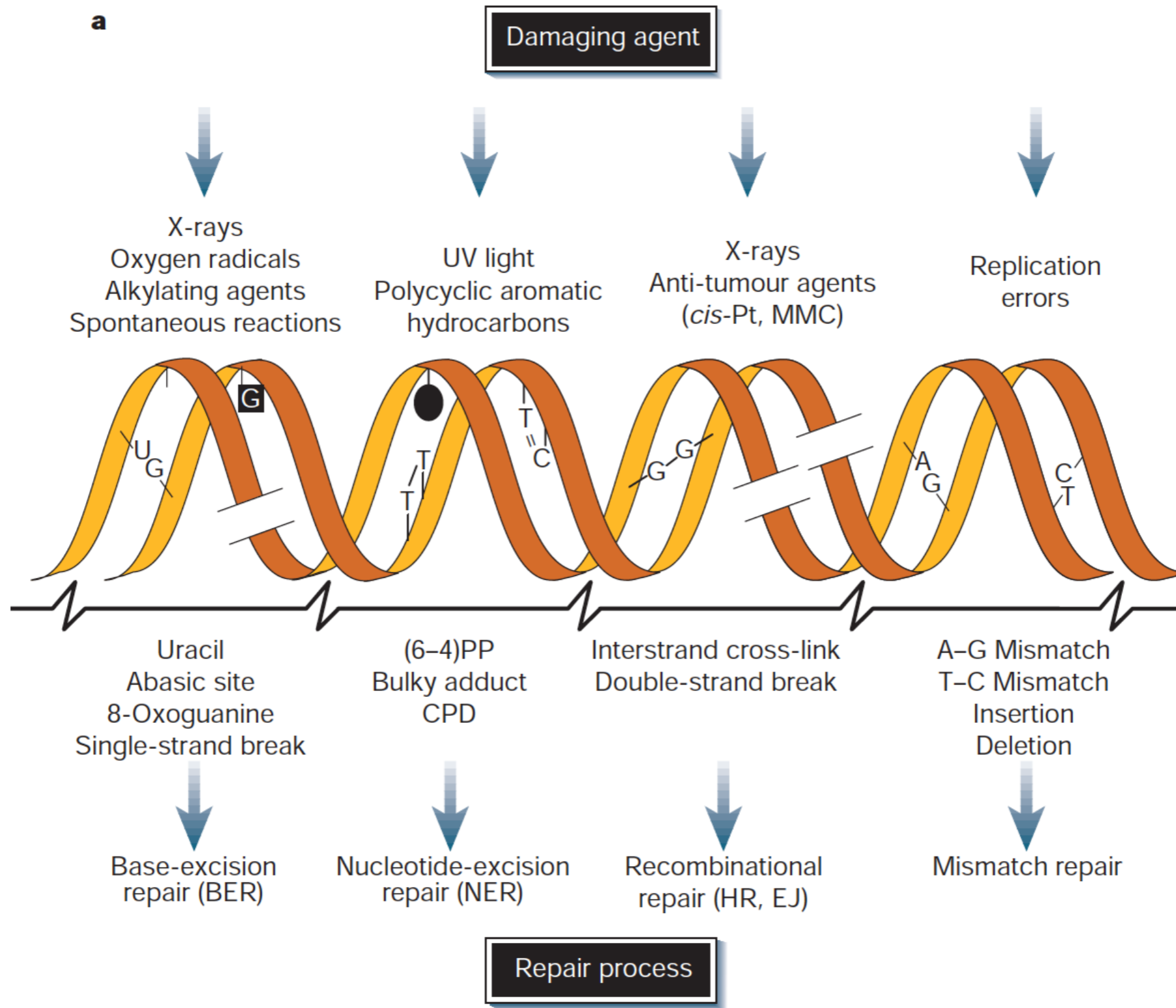
Exogenous

Radiation

Diet

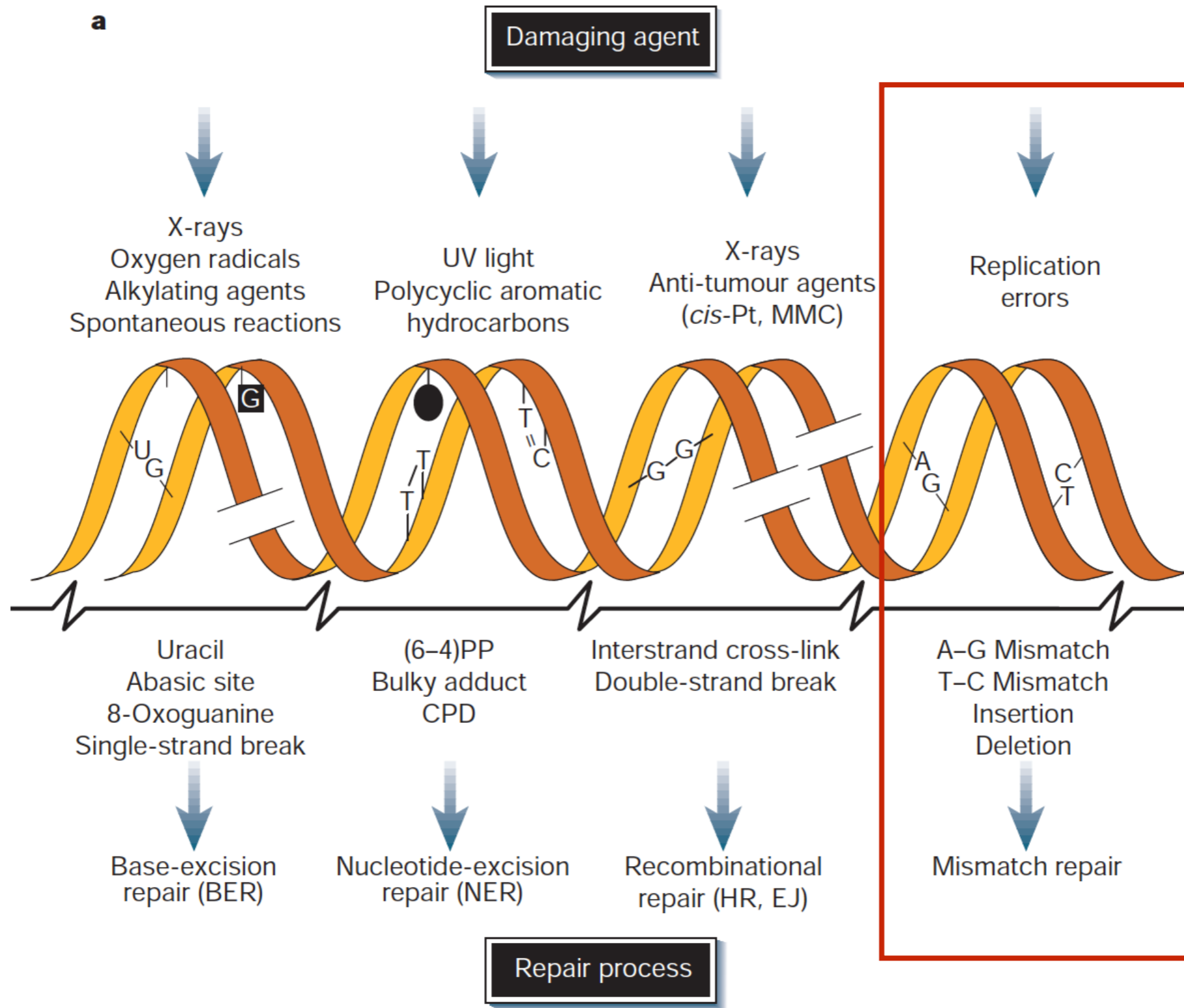
Stress

What are the challenges to genome stability?



Hoeijmakers, 2001

What are the challenges to genome stability?



Hoeijmakers, 2001

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What are the challenges to genome stability?

What is more prevalent? Exogenous or endogenous damage?

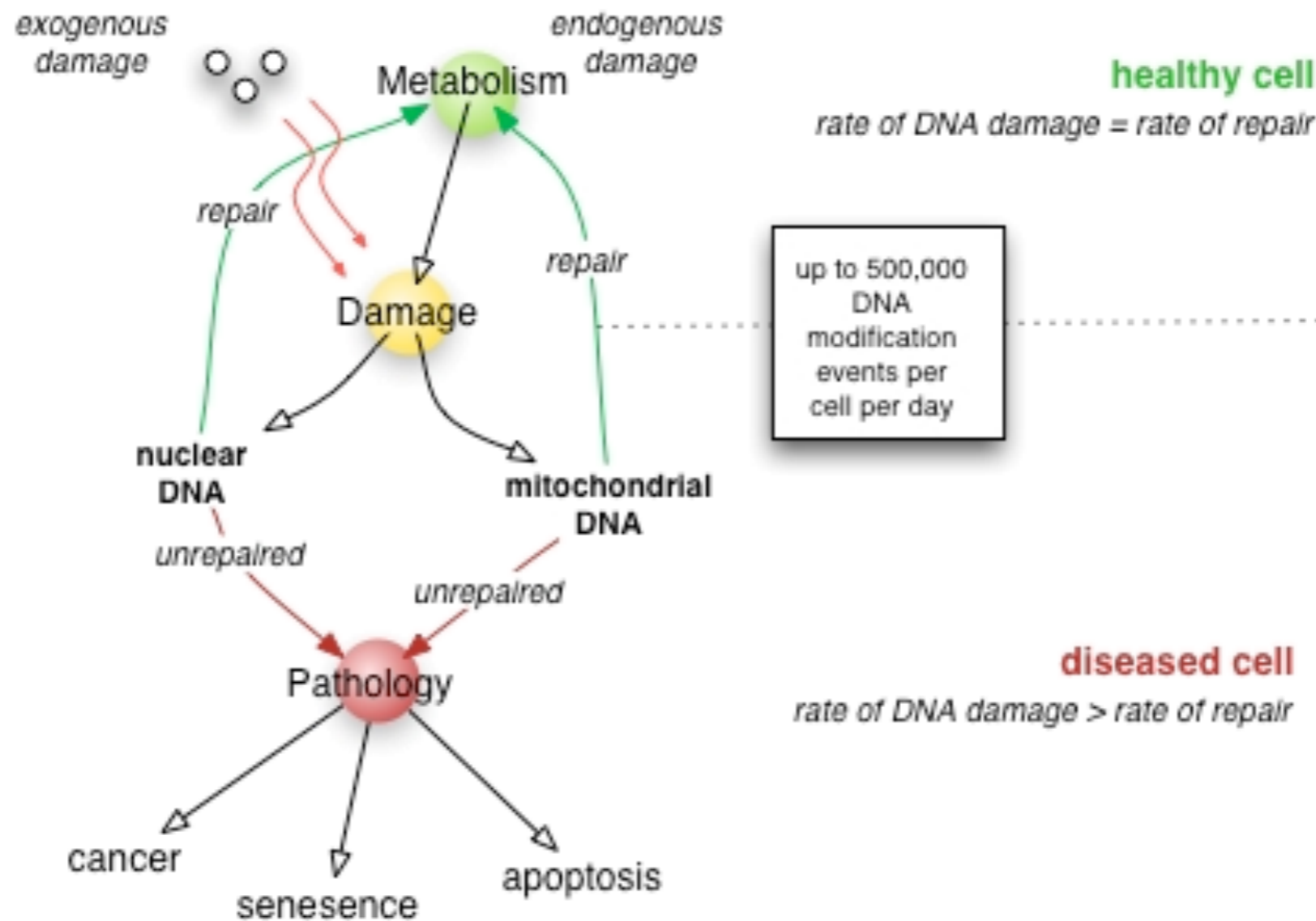
What are the challenges to genome stability?

What is more prevalent? Exogenous or endogenous damage?

Even-though, historically, exogenous DNA damage was considered to be the prime cause of mutagenesis, recently, as the methodology has progressed, the cellular DNA metabolism pathways (replication and transcription) are being recognised as the more prevalent cause of mutations.

What are the challenges to genome stability?

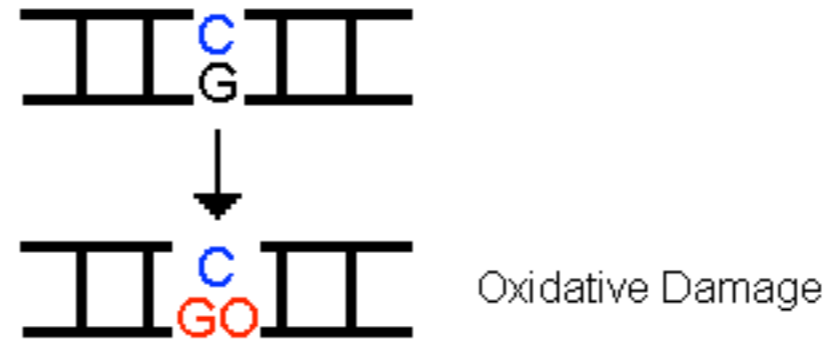
Inability to repair properly the damage may lead to cancer, senescence, or apoptosis.



What is the difference between a primary lesion and a mutation?

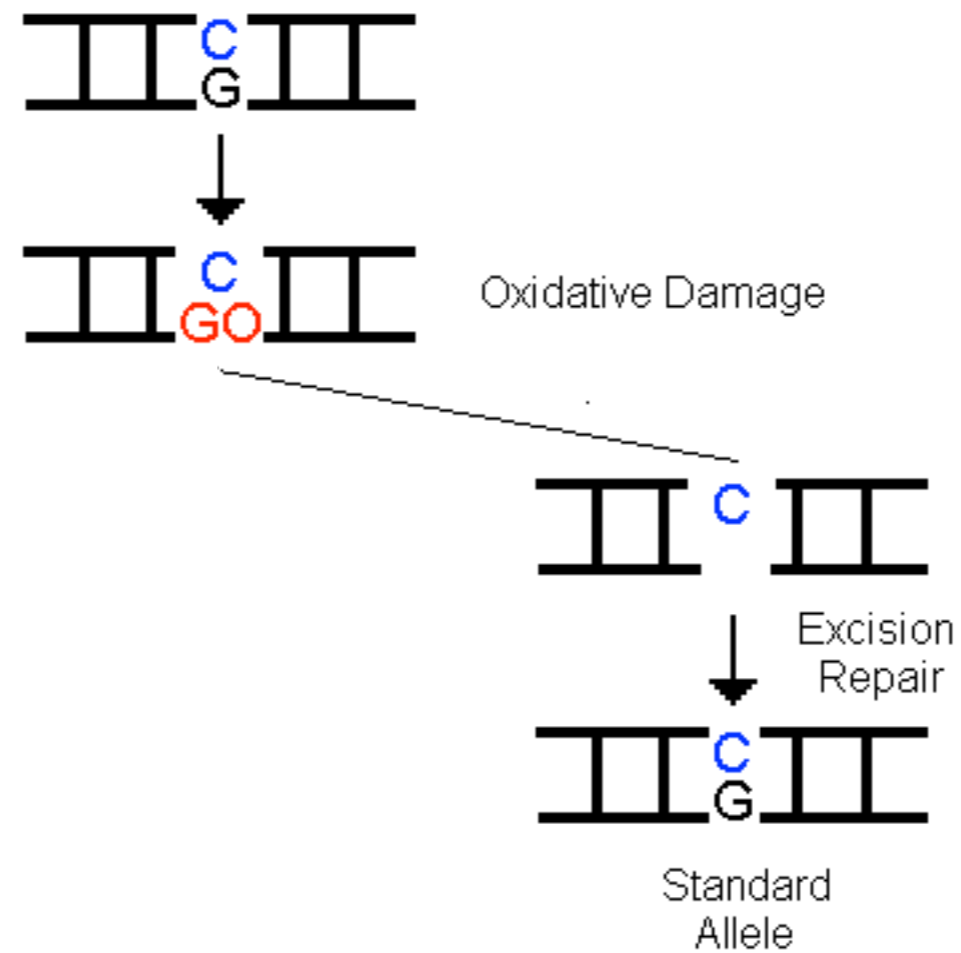
What is the difference between a primary lesion and a mutation?

(Carr 1999)



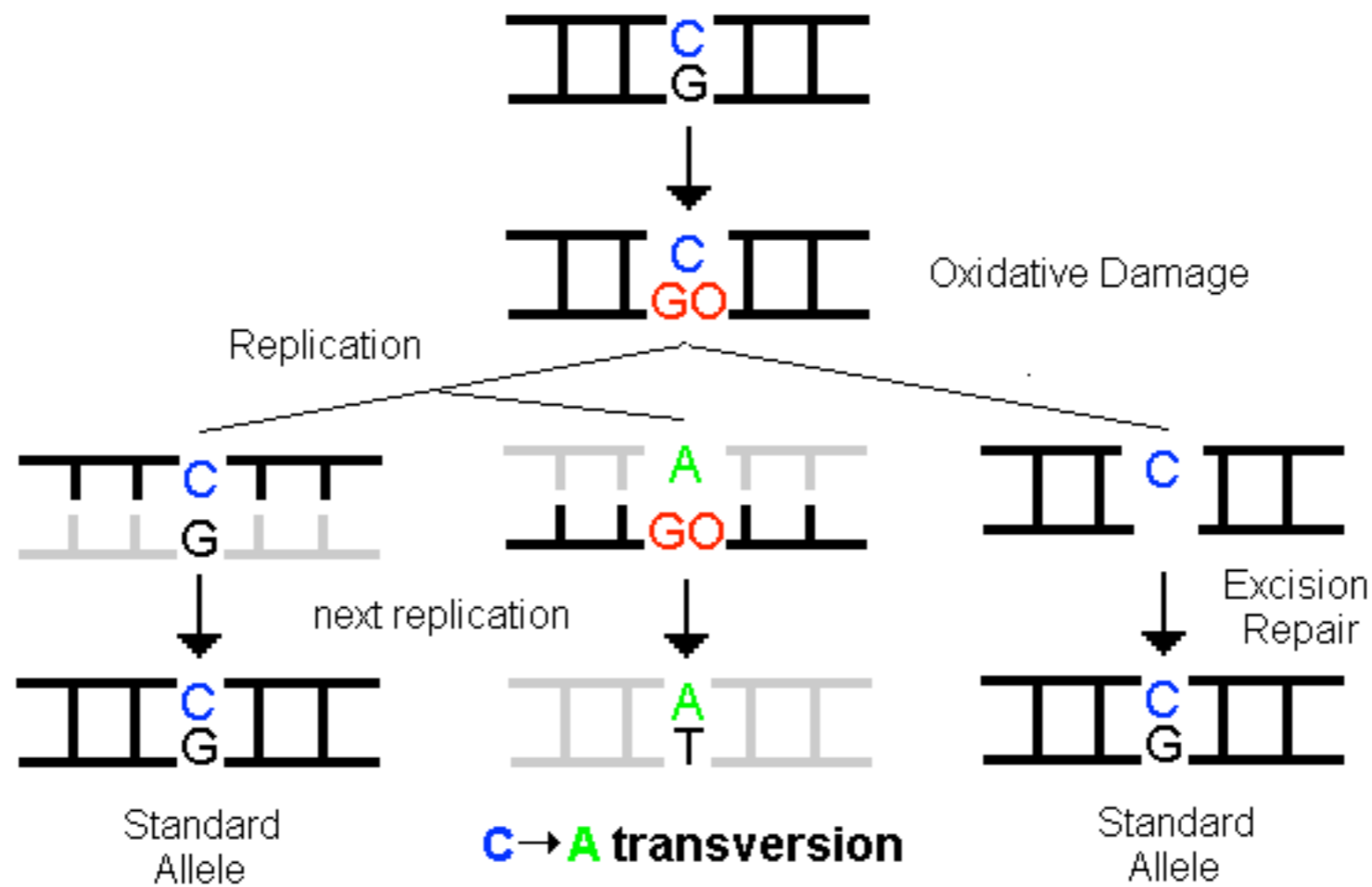
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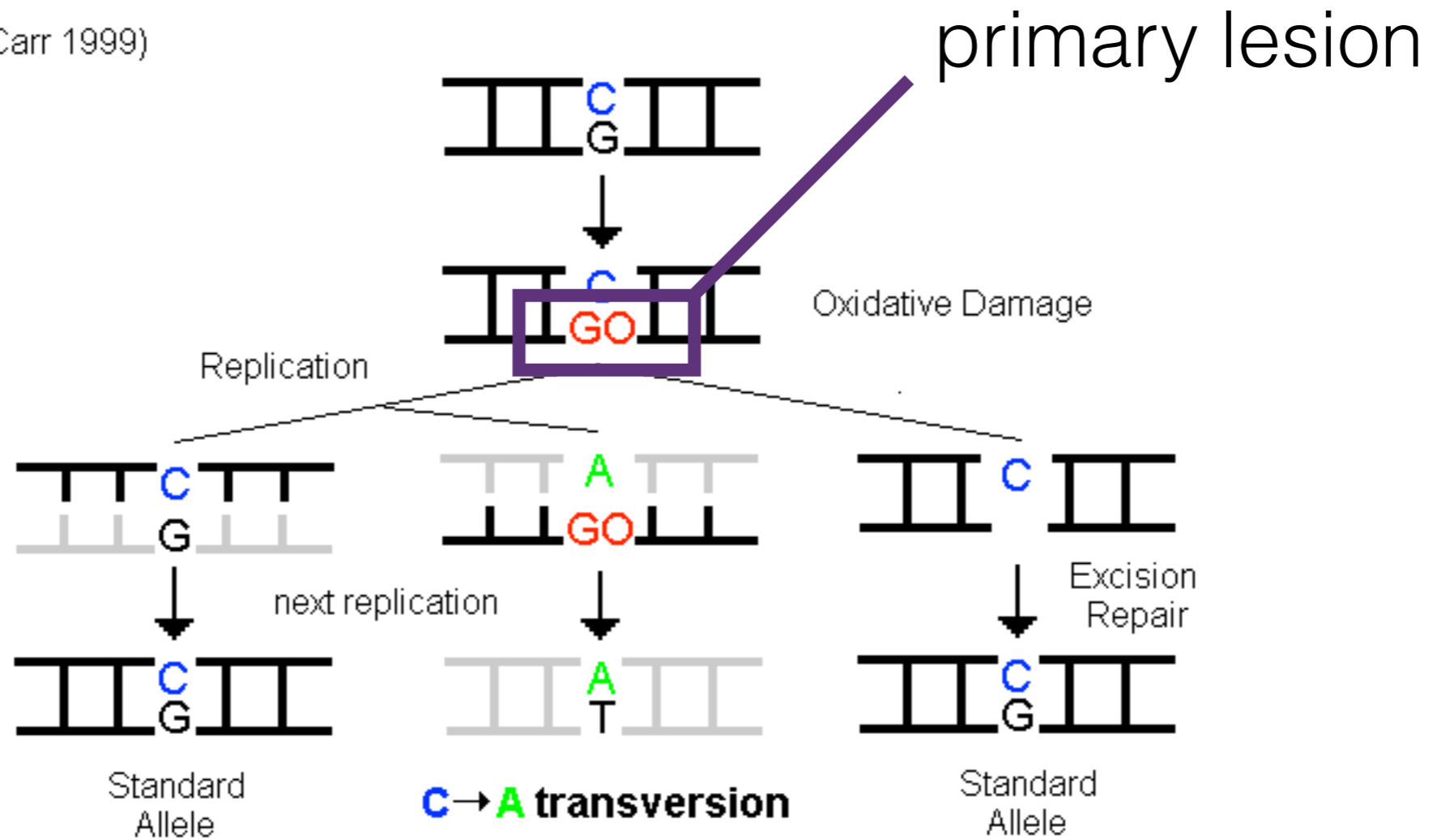
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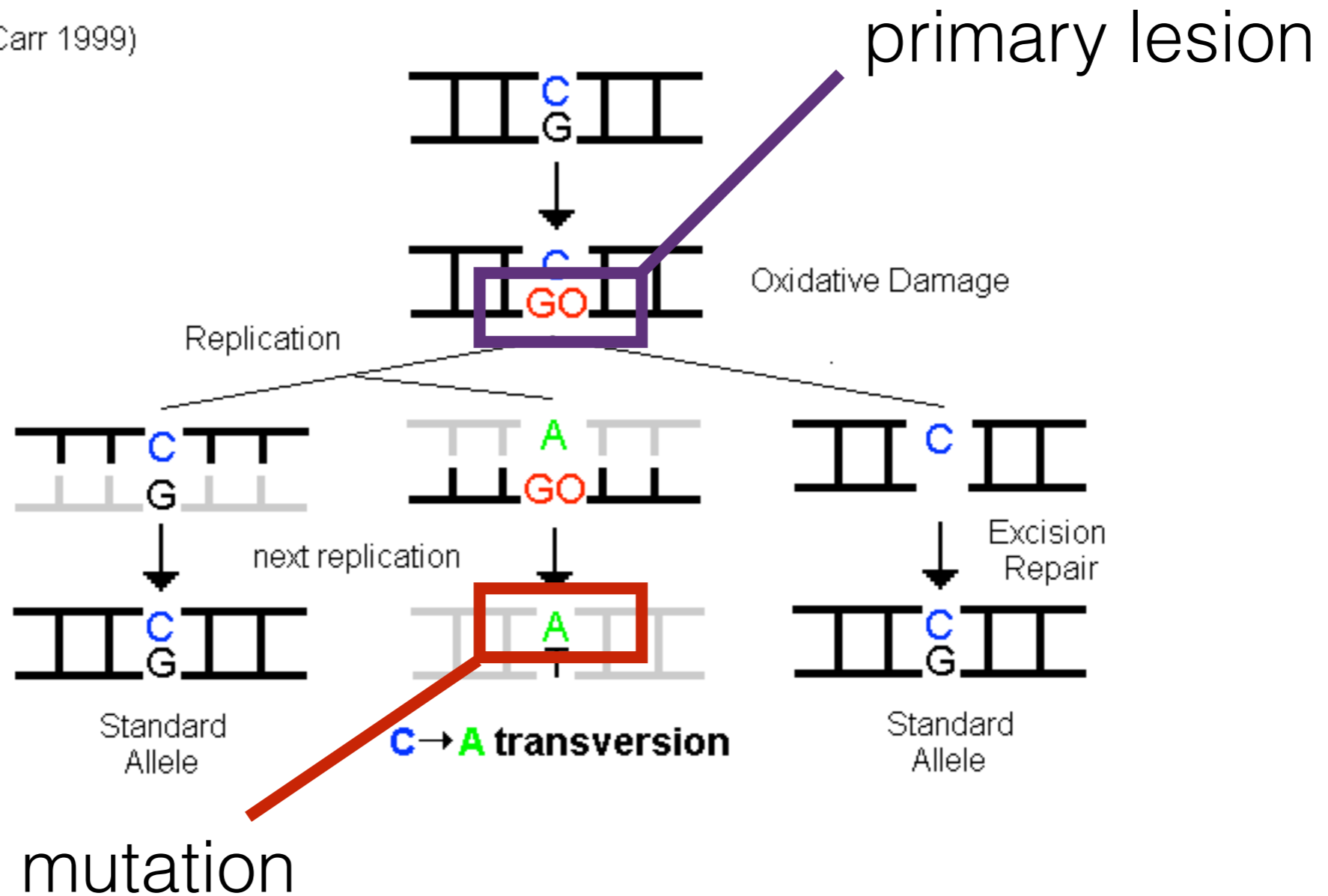
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Transient summary I

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Cells are continuously exposed to wide variety of DNA damage

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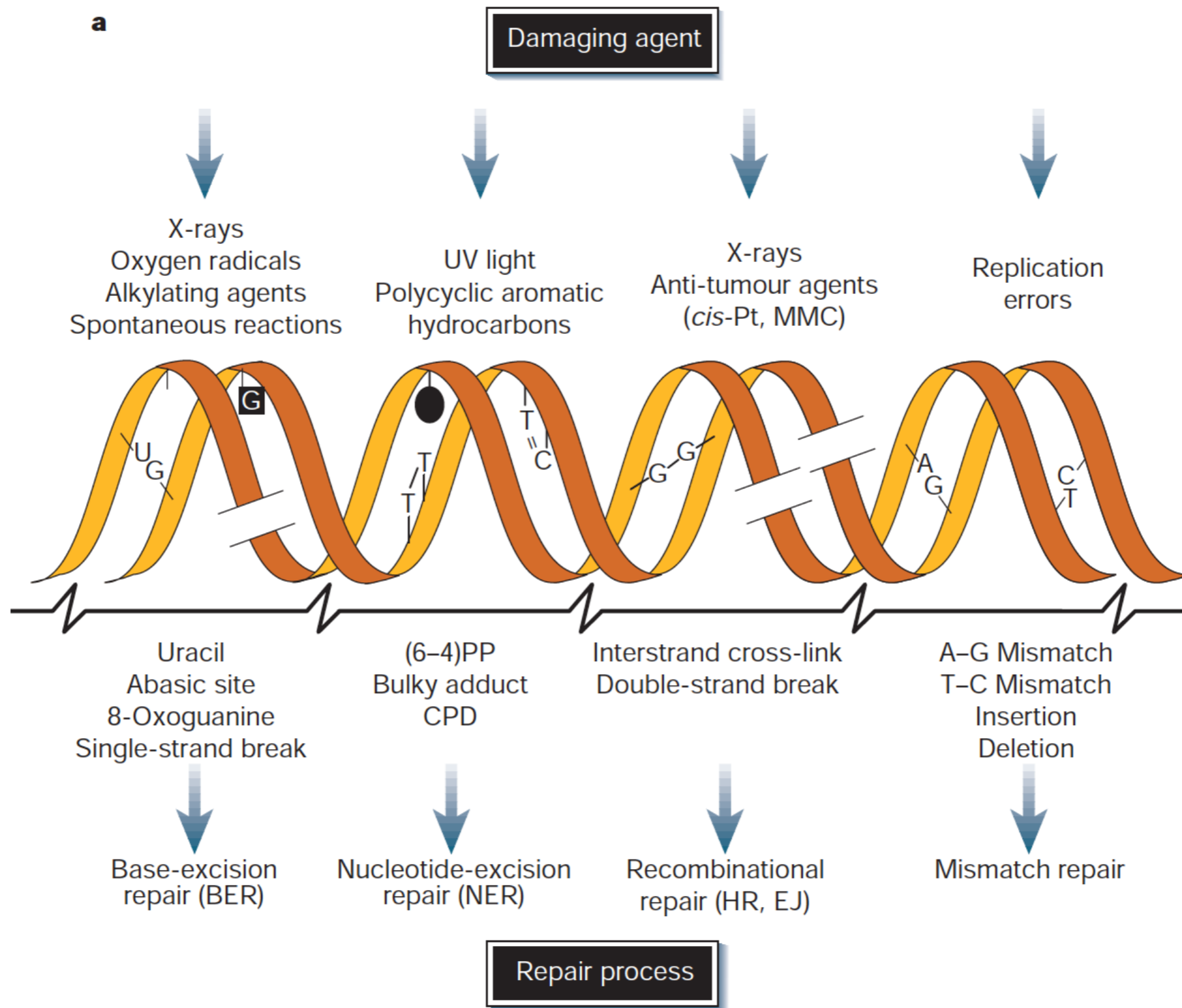
Terms Genome stability, DNA damage response, DNA repair, DNA damage tolerance denote closely related, yet not interchangeable terms

Cells are continuously exposed to wide variety of DNA damage

Failure to properly deal with the damage may have fatal consequences to cells

How do cells know genome stability has been compromised?

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Hoeijmakers, 2001

How do cells know genome stability has been compromised?

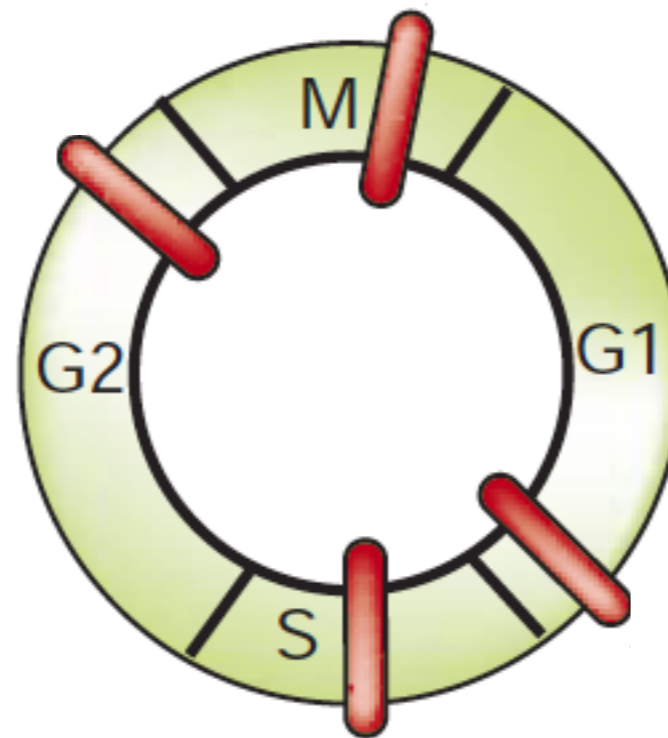
The challenges

- different types of DNA damage

How do cells know genome stability has been compromised?

The challenges

- different types of DNA damage
- cell-cycle stage

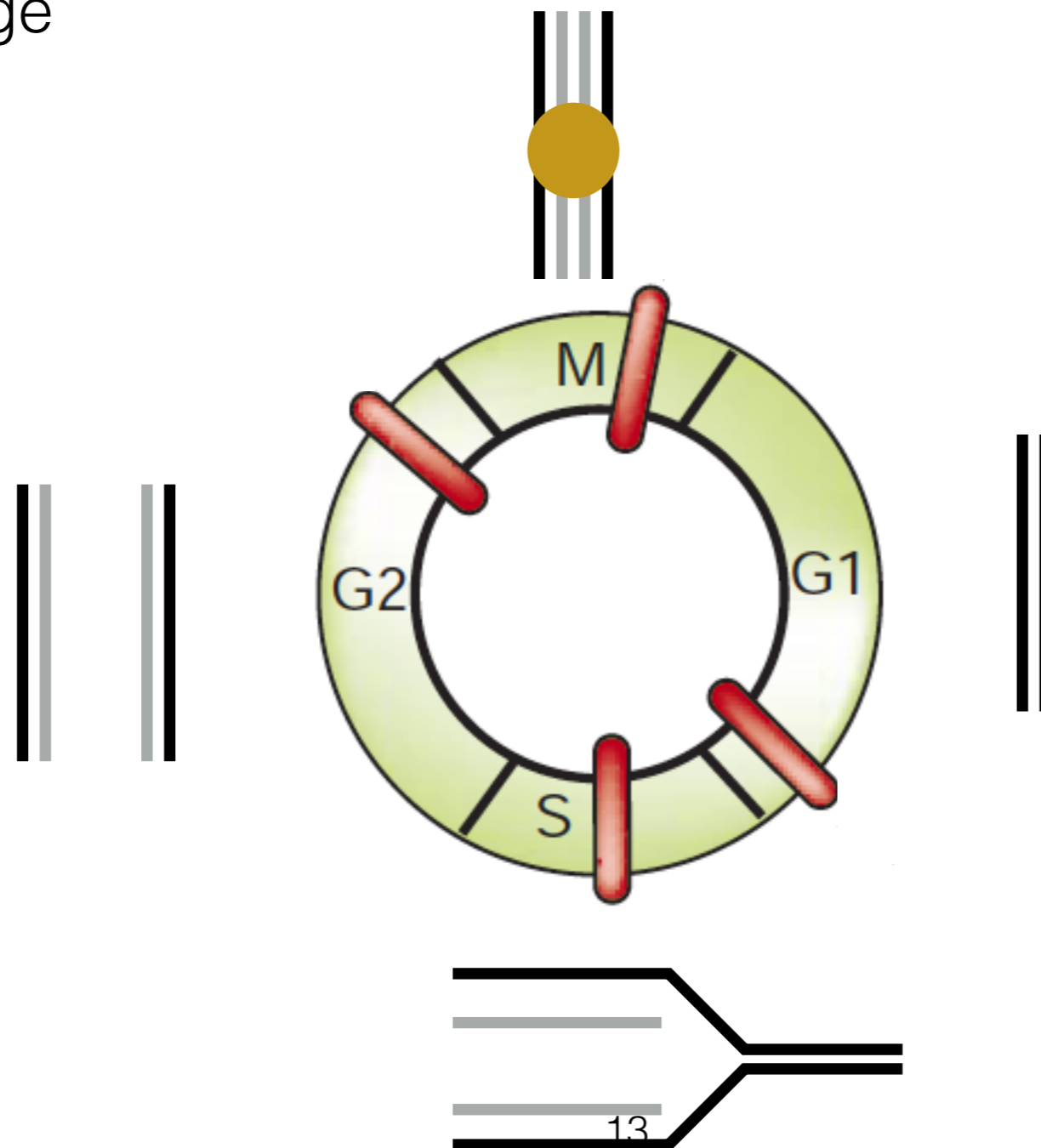


Hoeijmakers, 2001

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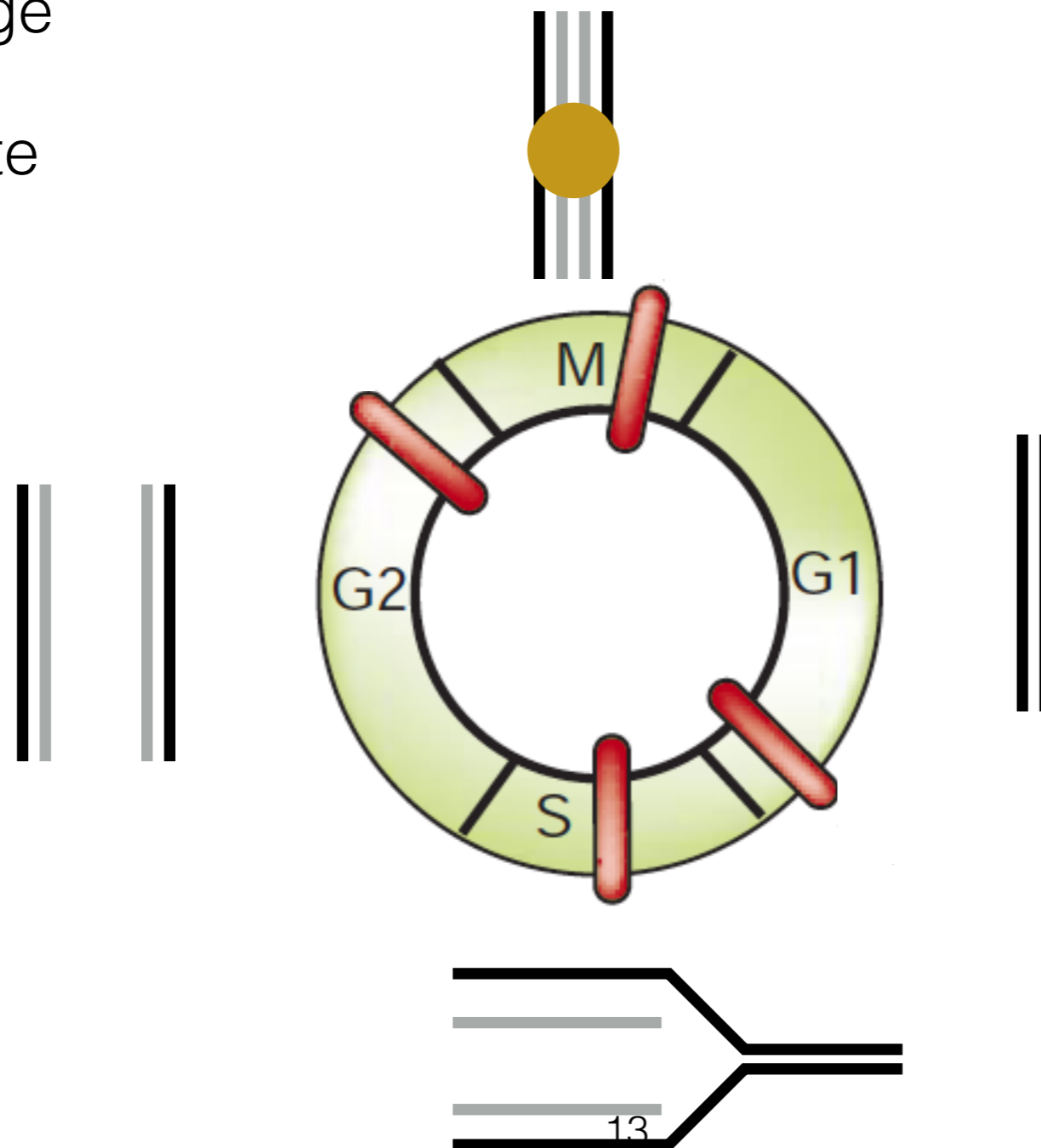


Hoeijmakers, 2001

How do cells know genome stability has been compromised?

The challenges

- different types of DNA damage
- cell-cycle stage
- metabolic state



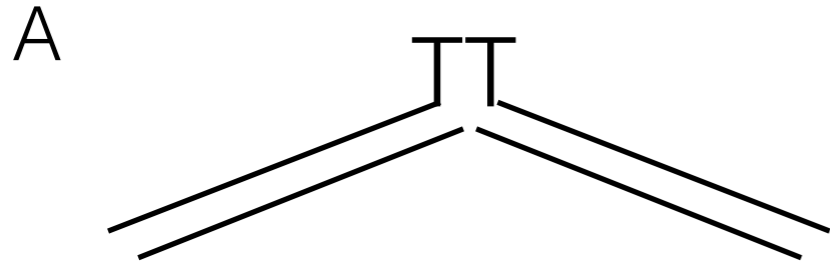
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How do cells know genome stability has been compromised?

Cells possess context-specific sensors that recognise signals from the damaged DNA

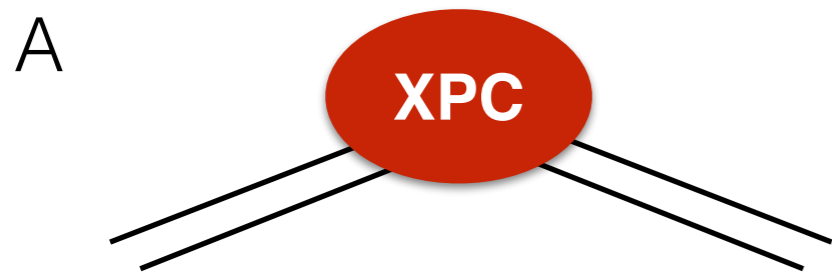
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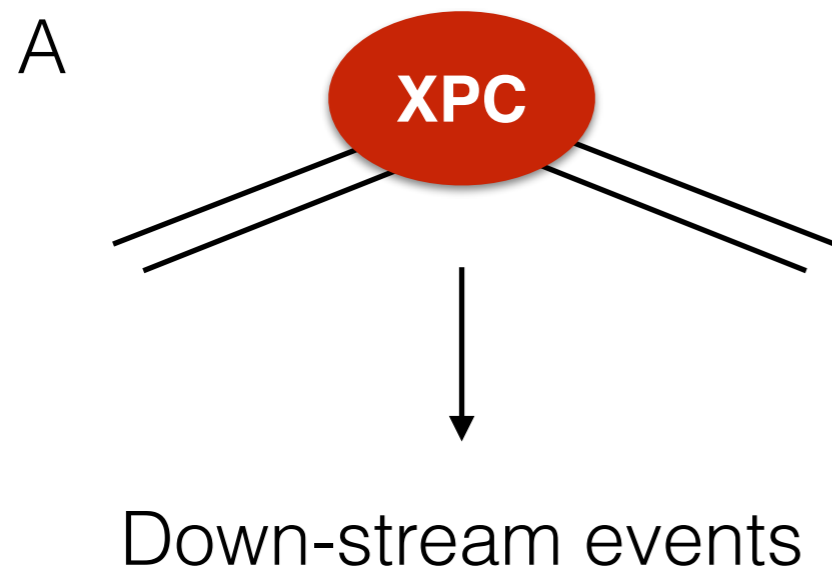
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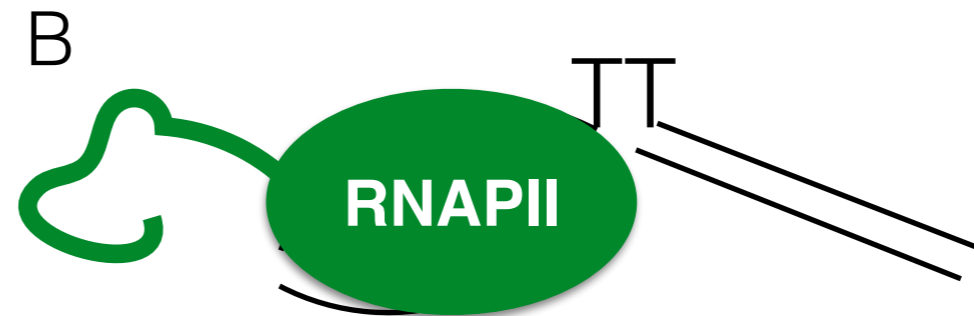
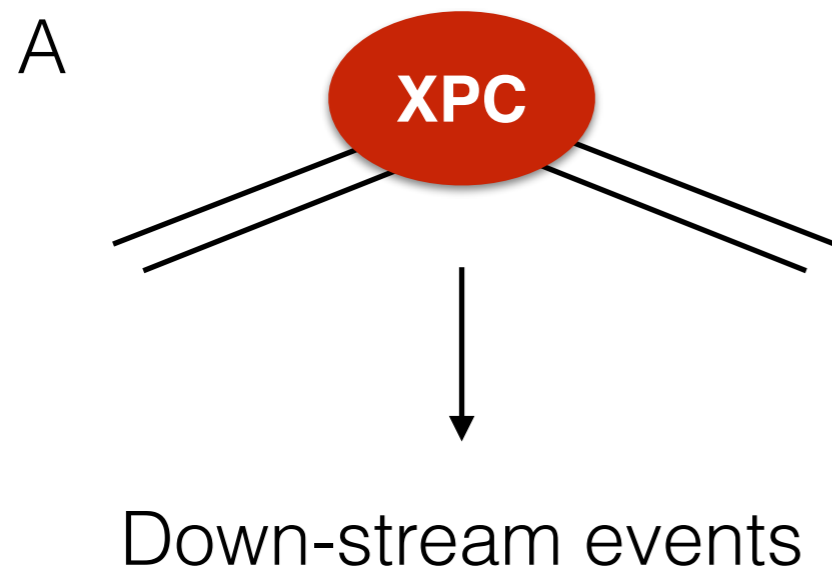
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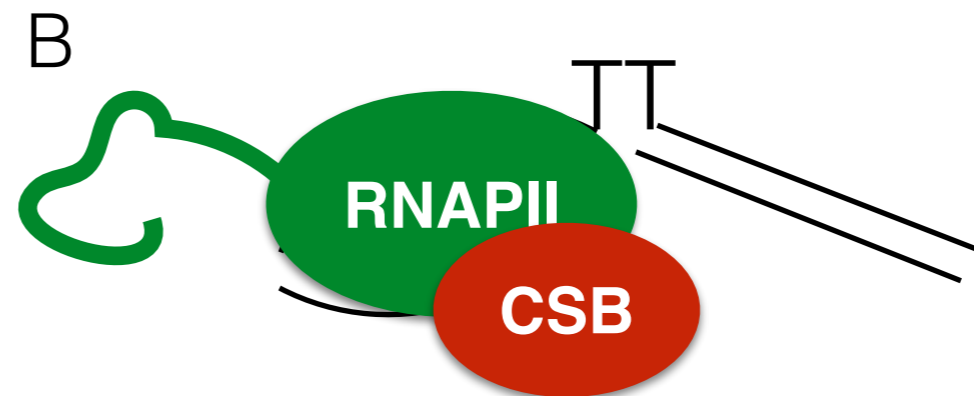
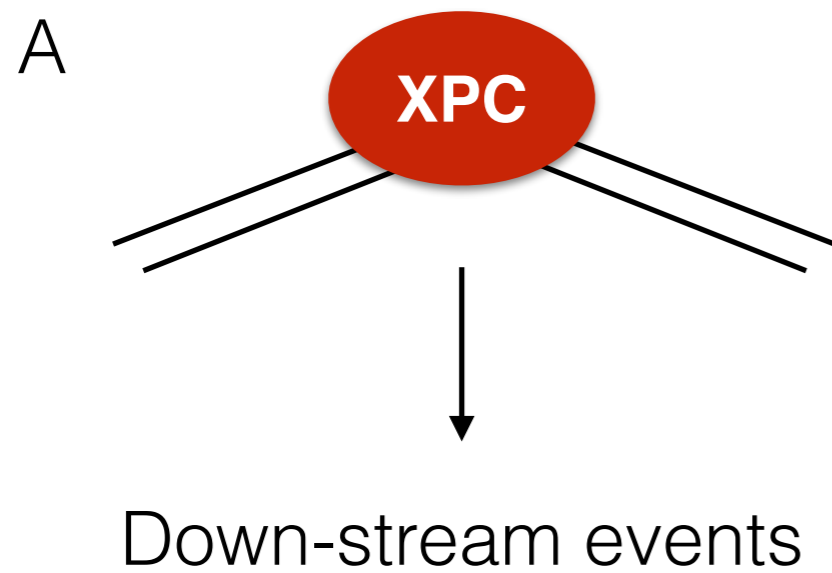
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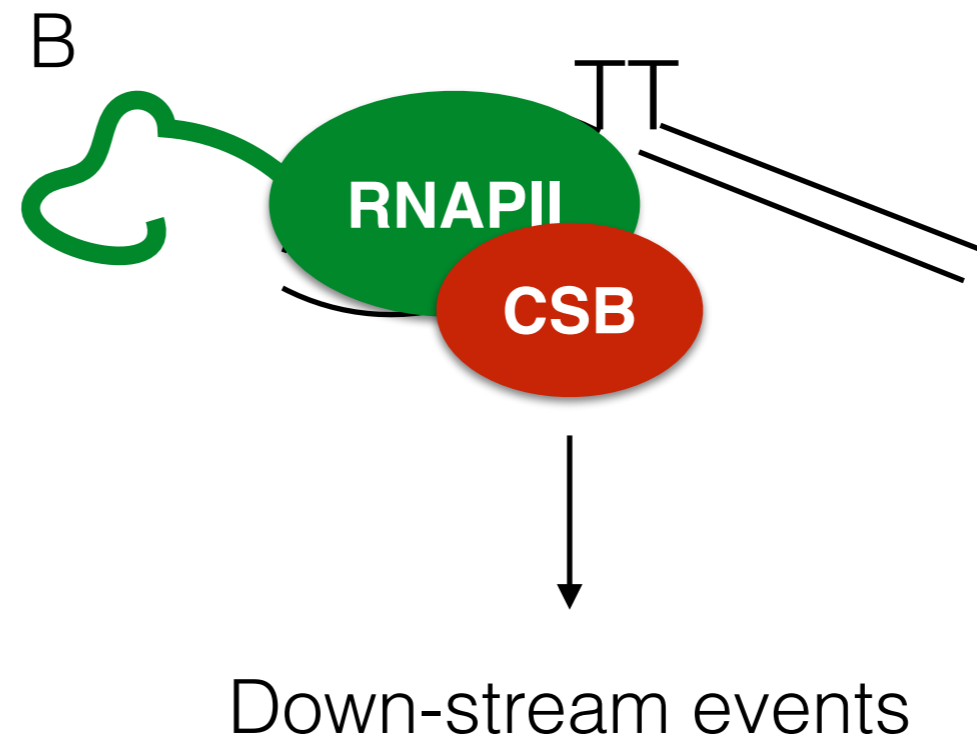
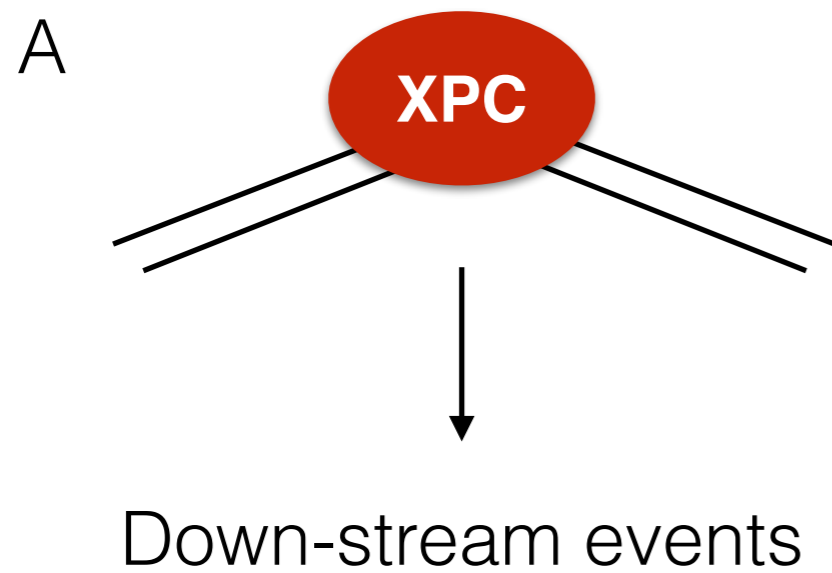
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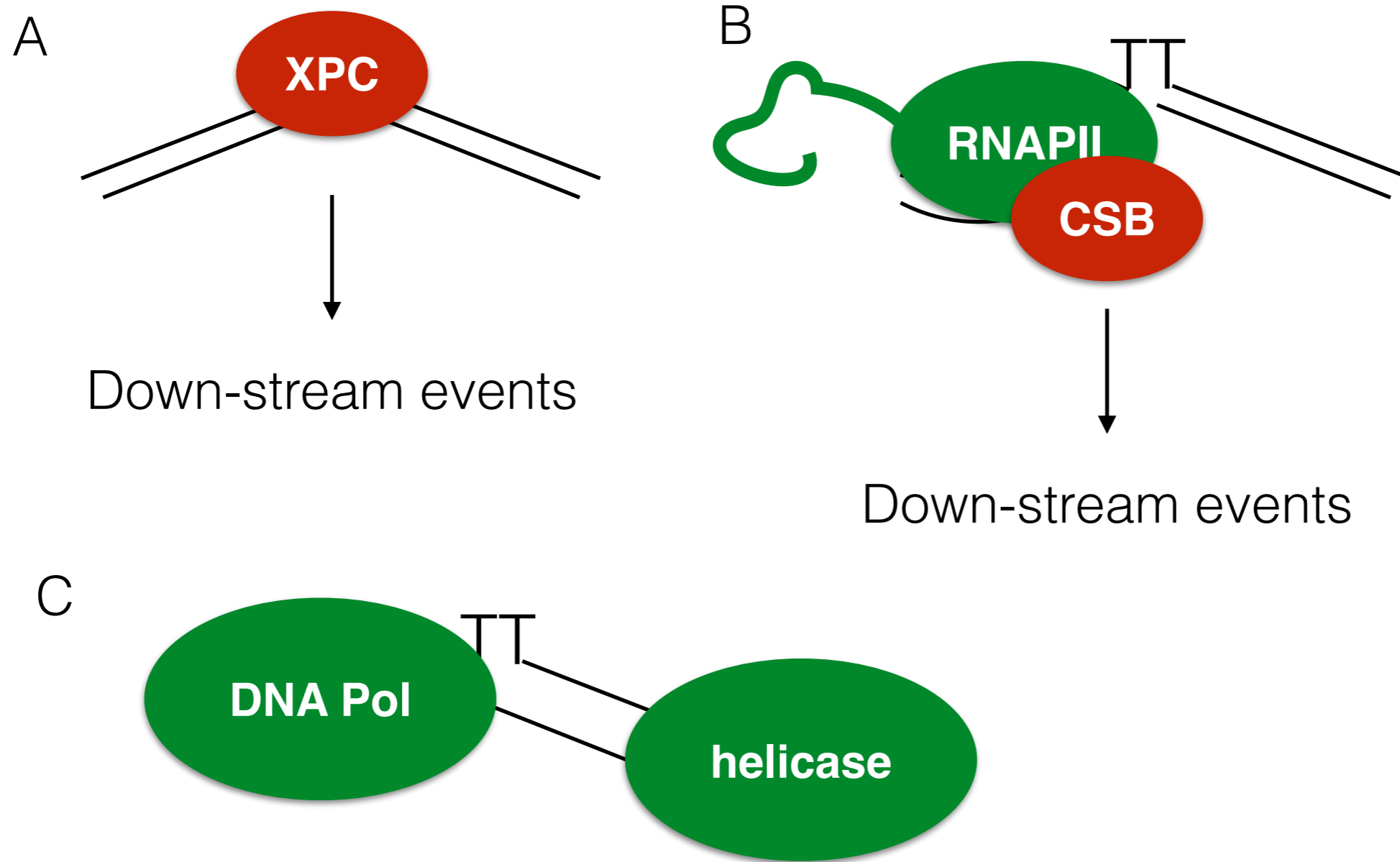
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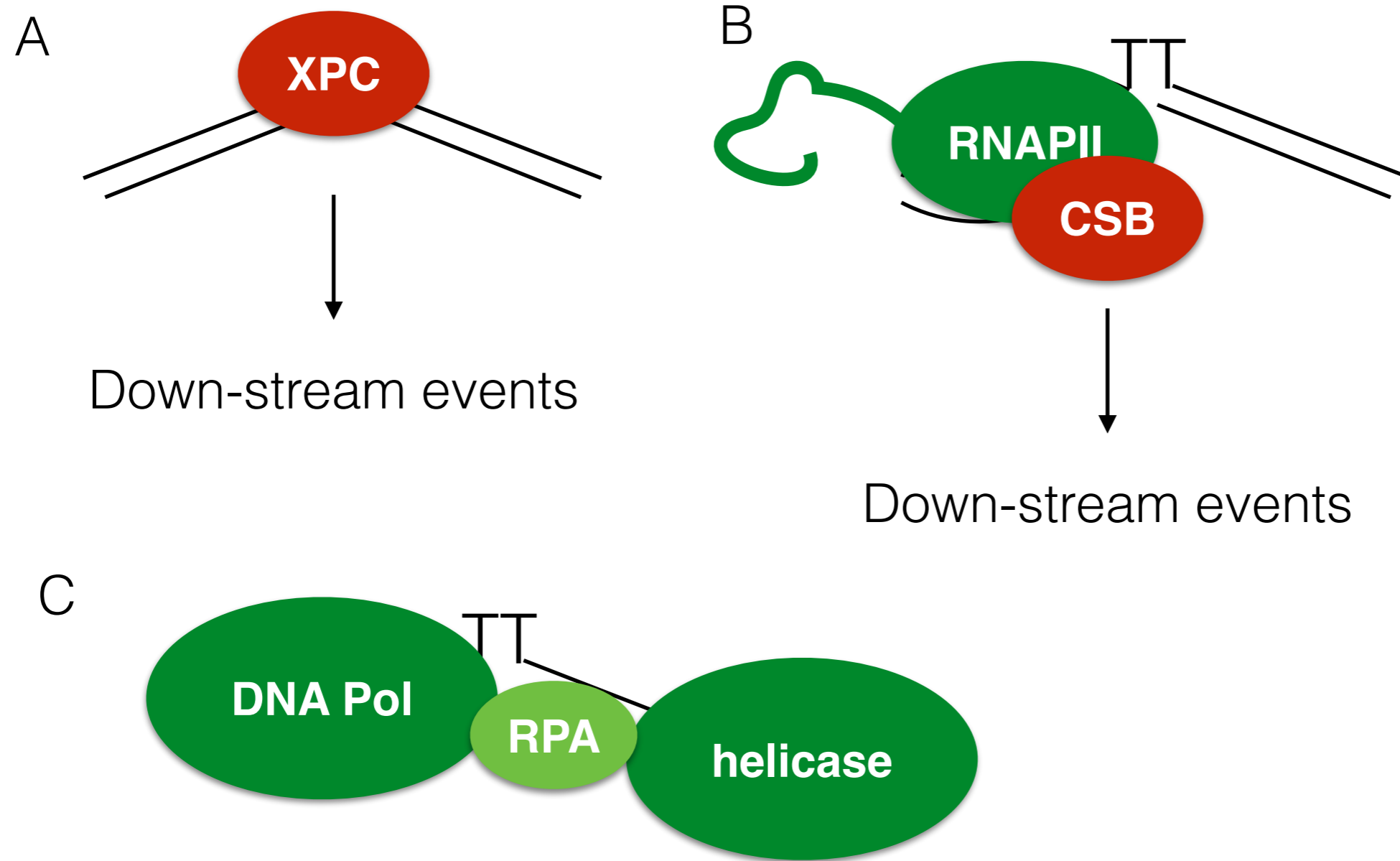
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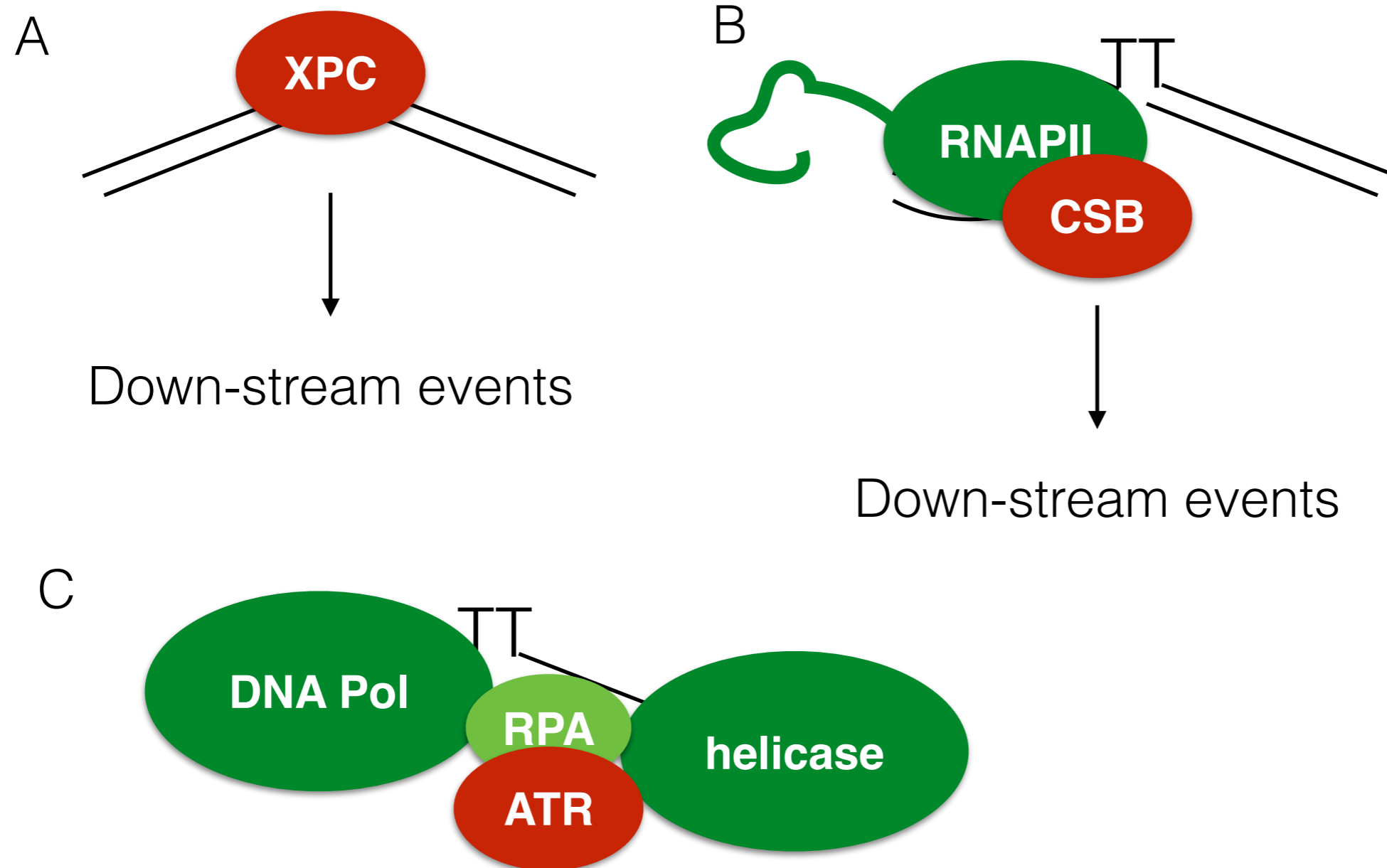
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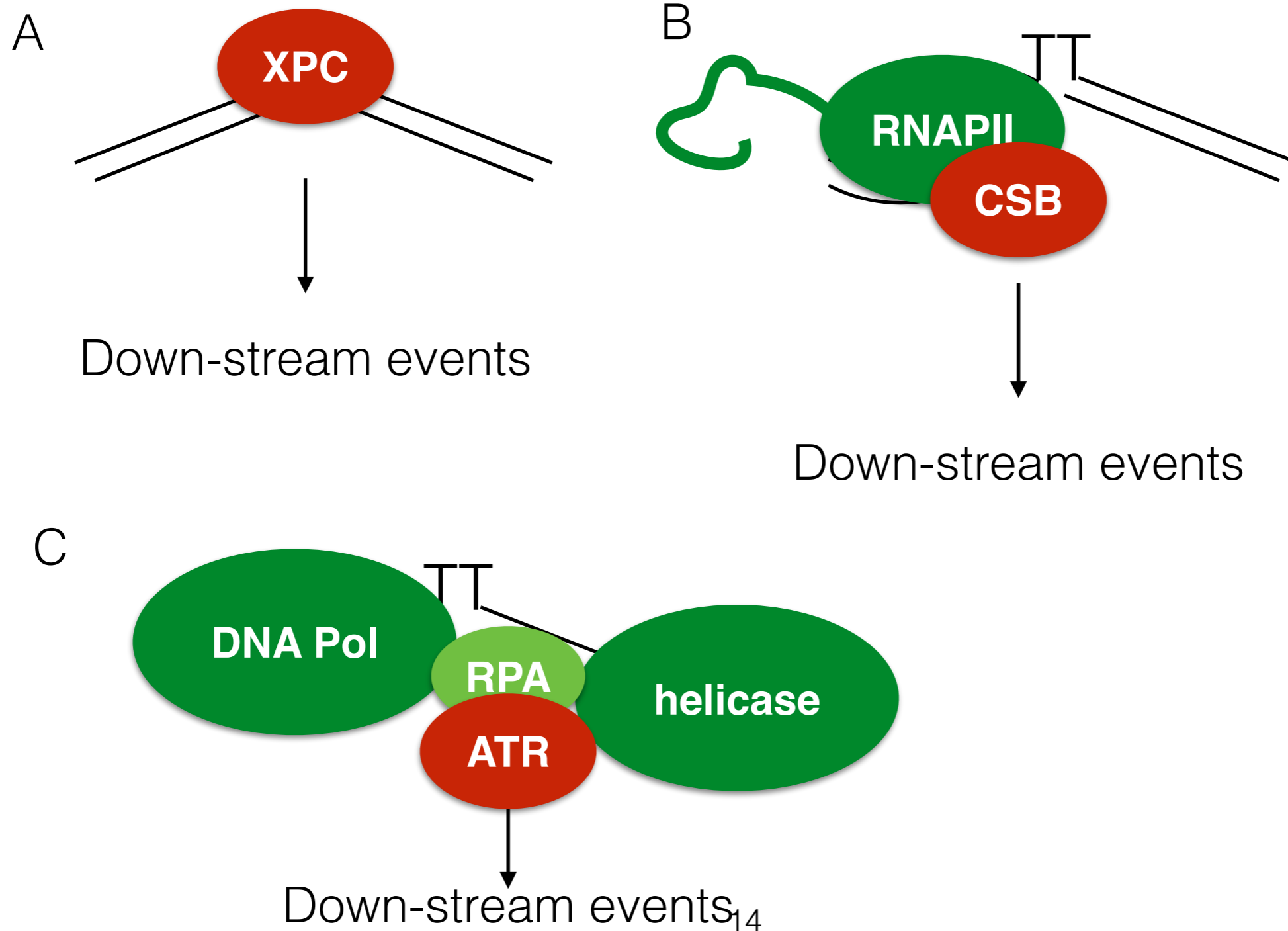
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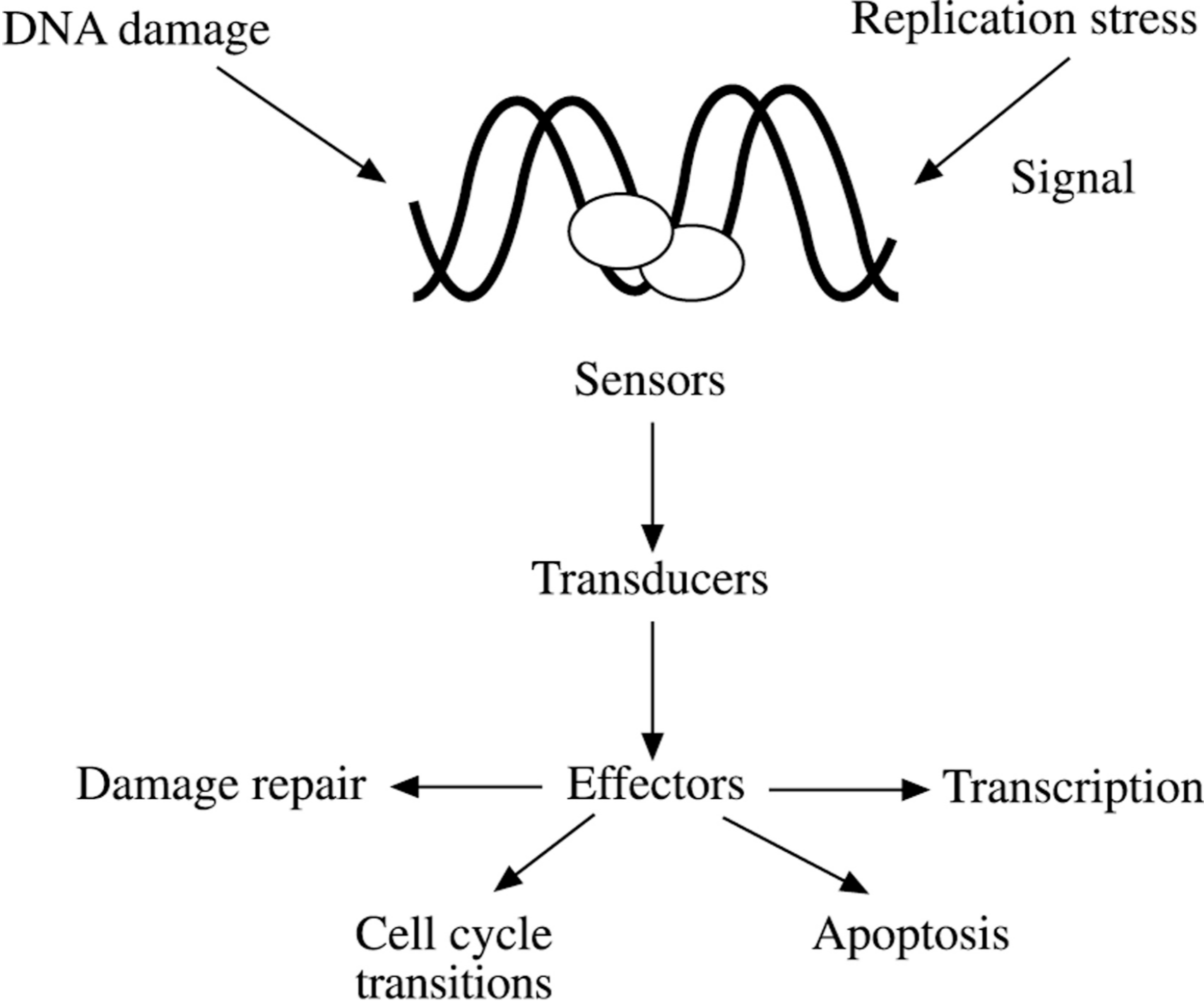


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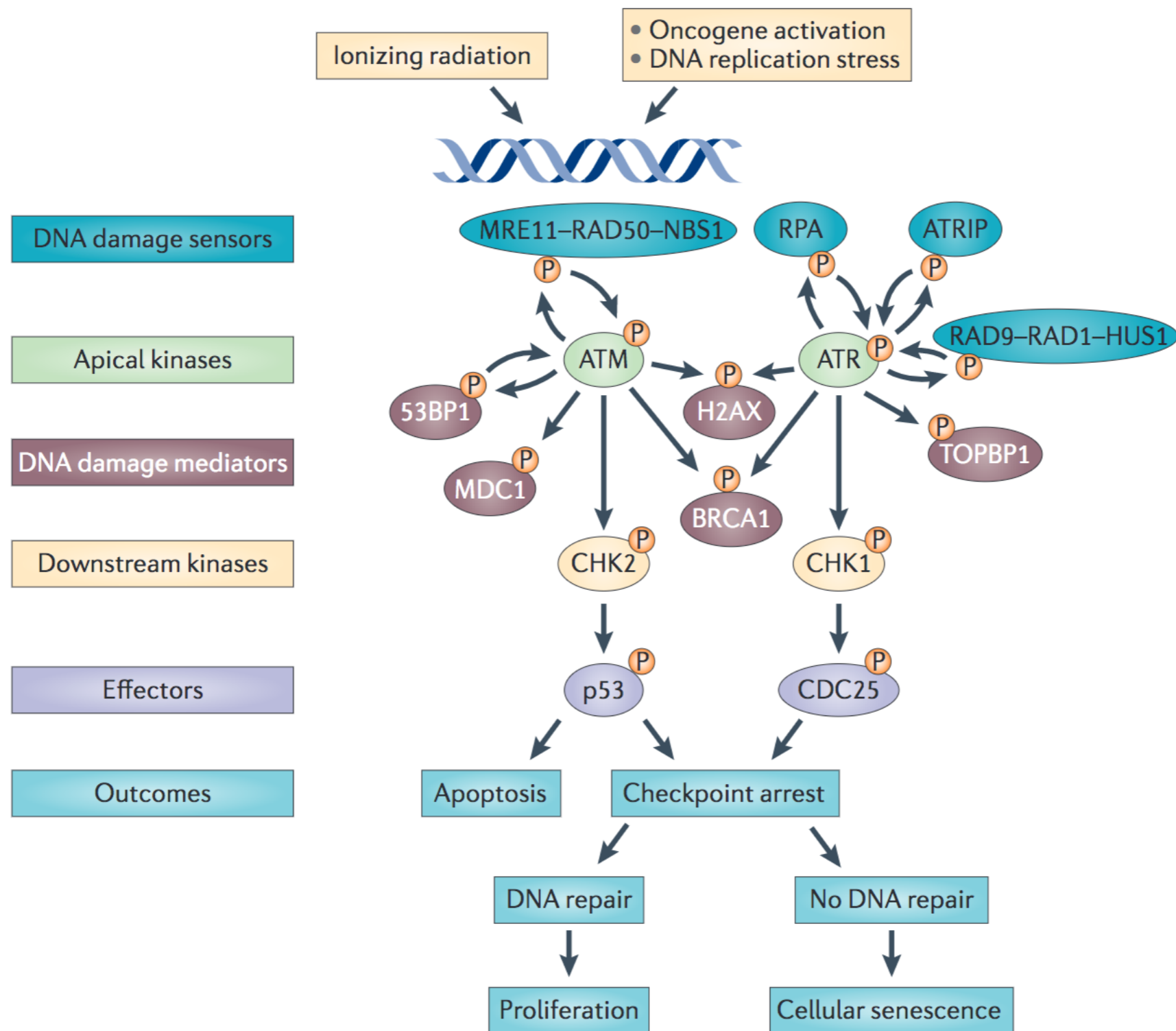


How do cells react to DNA damage?



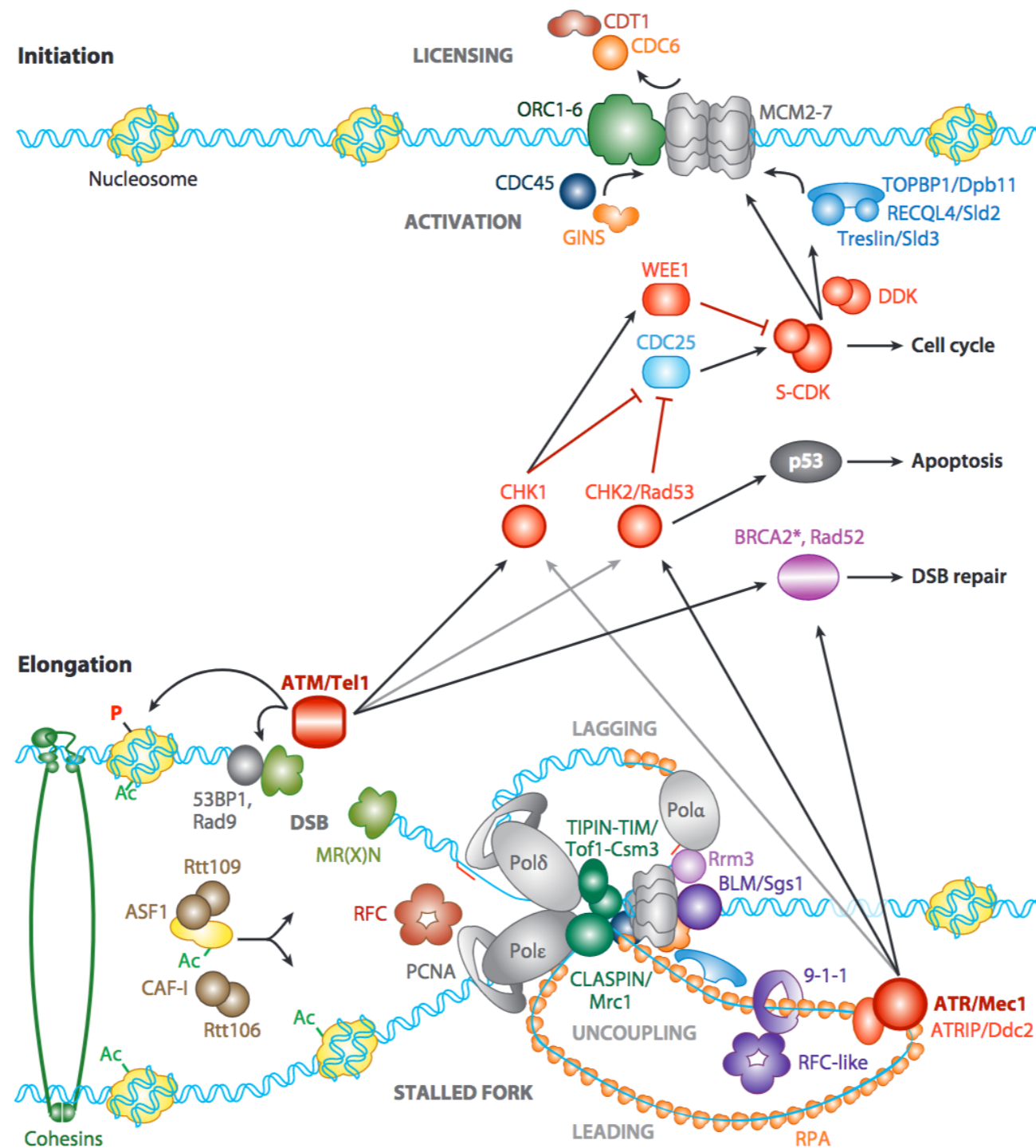
How do cells react to DNA damage?

A simplified picture



How do cells react to DNA damage?

A more comprehensive picture



Transient summary II

Transient summary II

Cells possess specific factors - sensors - that recognise insults to DNA structure, DNA breaks, or stalled machineries like transcription and replication.

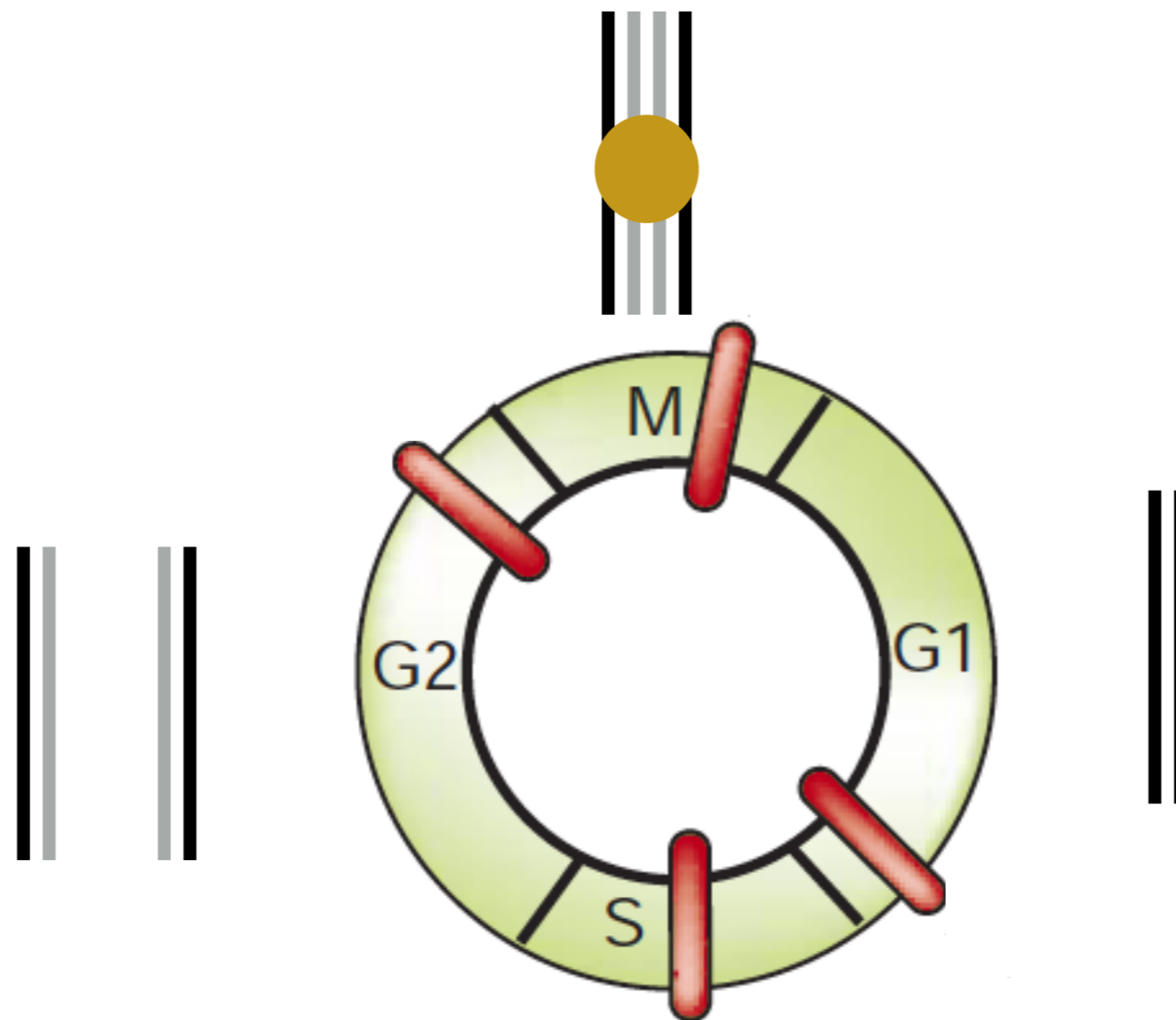
Transient summary II

Cells possess specific factors - sensors - that recognise insults to DNA structure, DNA breaks, or stalled machineries like transcription and replication.

The sensors subsequently activate complex signalling pathways that lead to halt of cell-cycle, as well as to decision as of which pathway is to be used; balancing the cell-cycle stage and other needs of the cell.

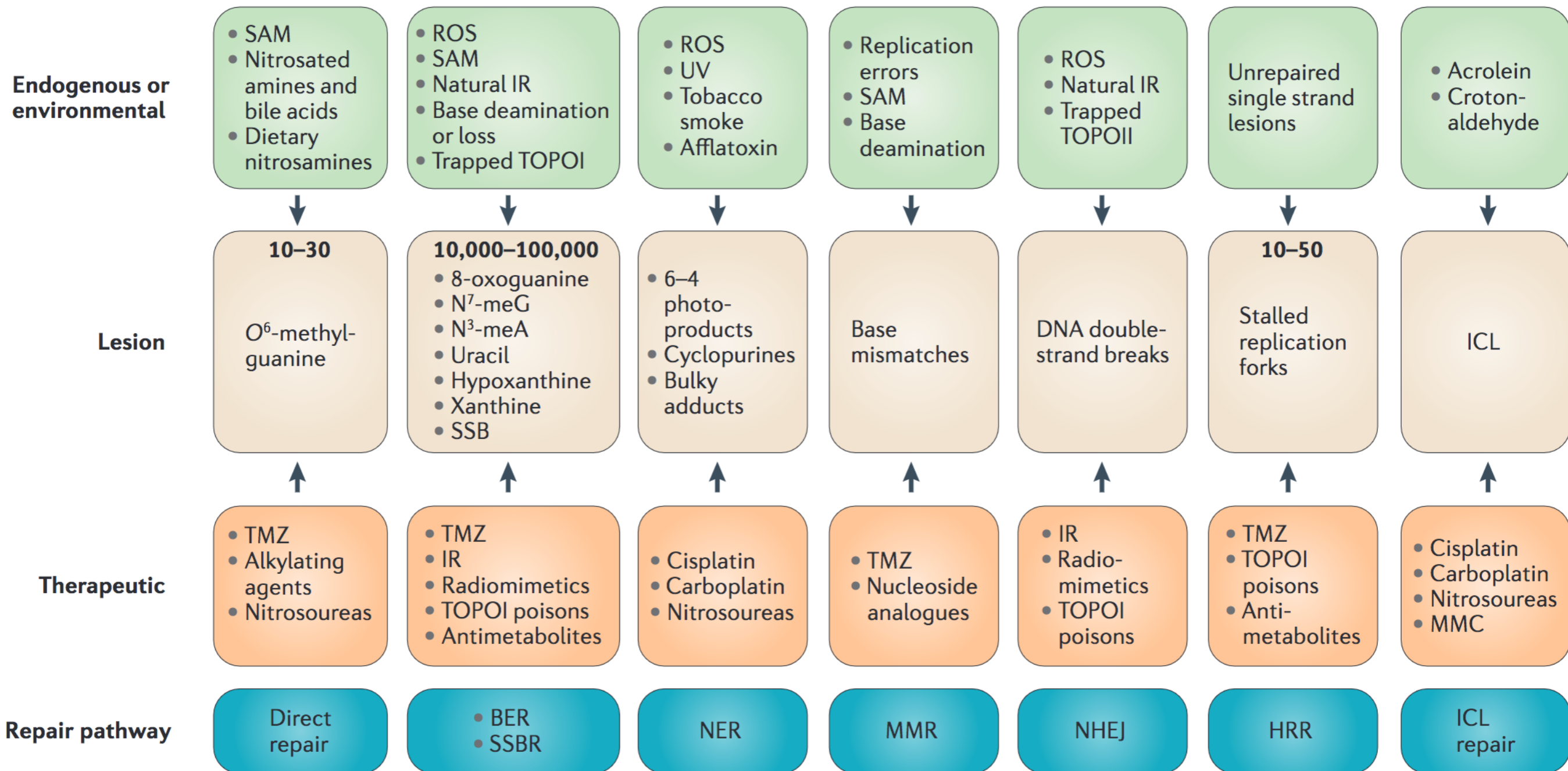
How do cell maintain genome stability?

DNA repair is prevalent outside the S-phase, in which DNA damage tolerance is preferred.



Hoeijmakers, 2001

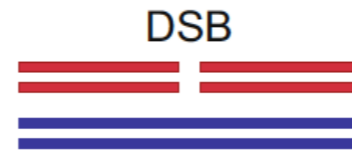
How do cells maintain genome stability?



Curtin et al., 2012

How do cells maintain genome stability?

Double-stranded DNA breaks (DSB) repair



NHEJ: non-homologous end joining

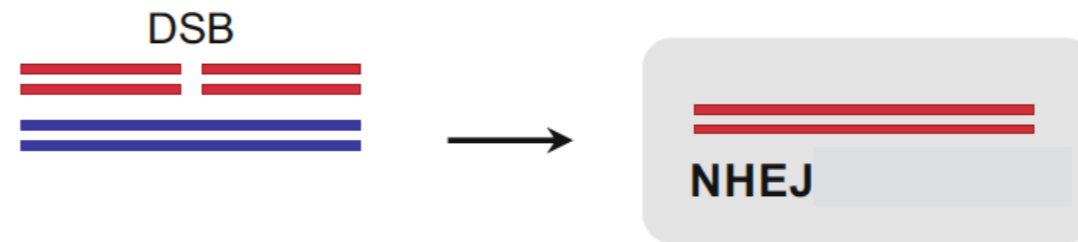
SSA: single strand annealing

SDSA: synthesis-dependent strand-annealing

DSBR: DSB repair

How do cell maintain genome stability?

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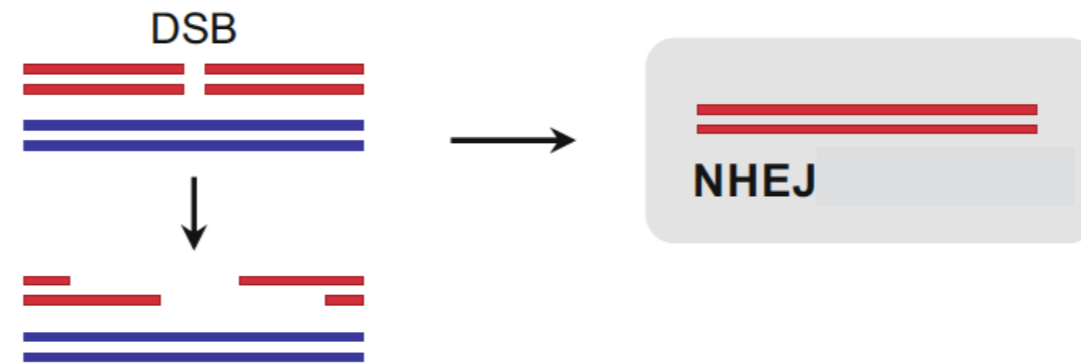
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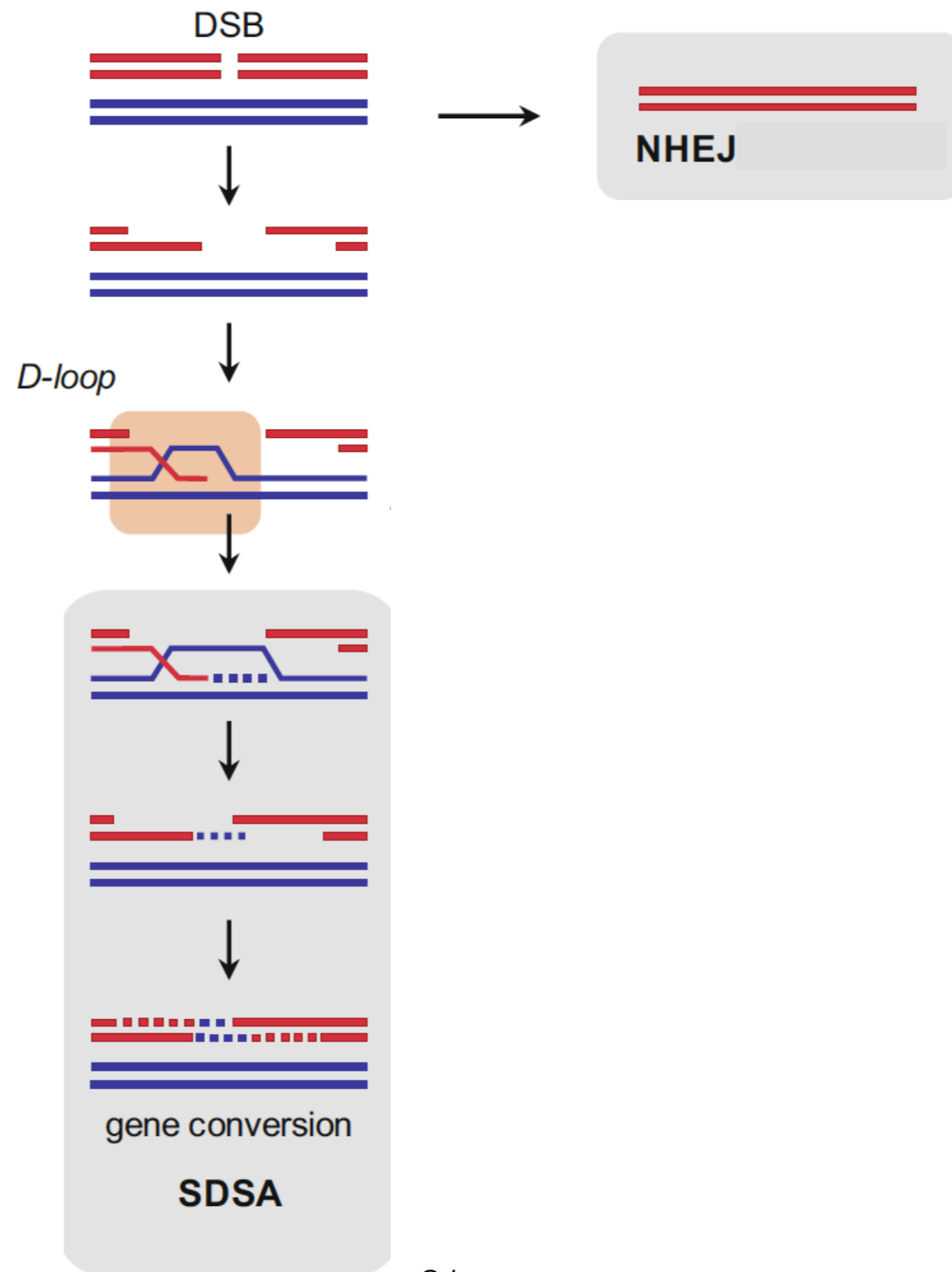
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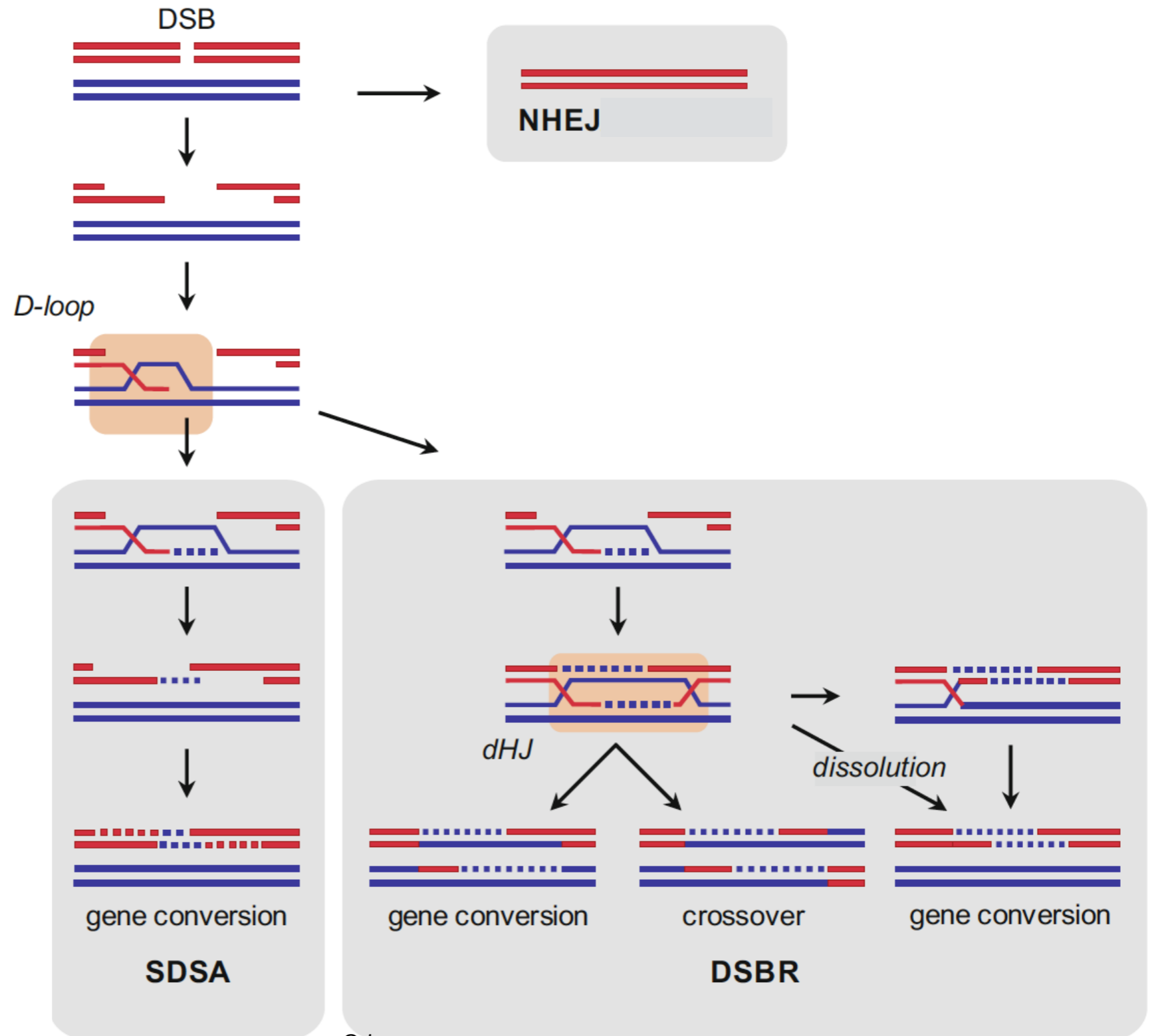
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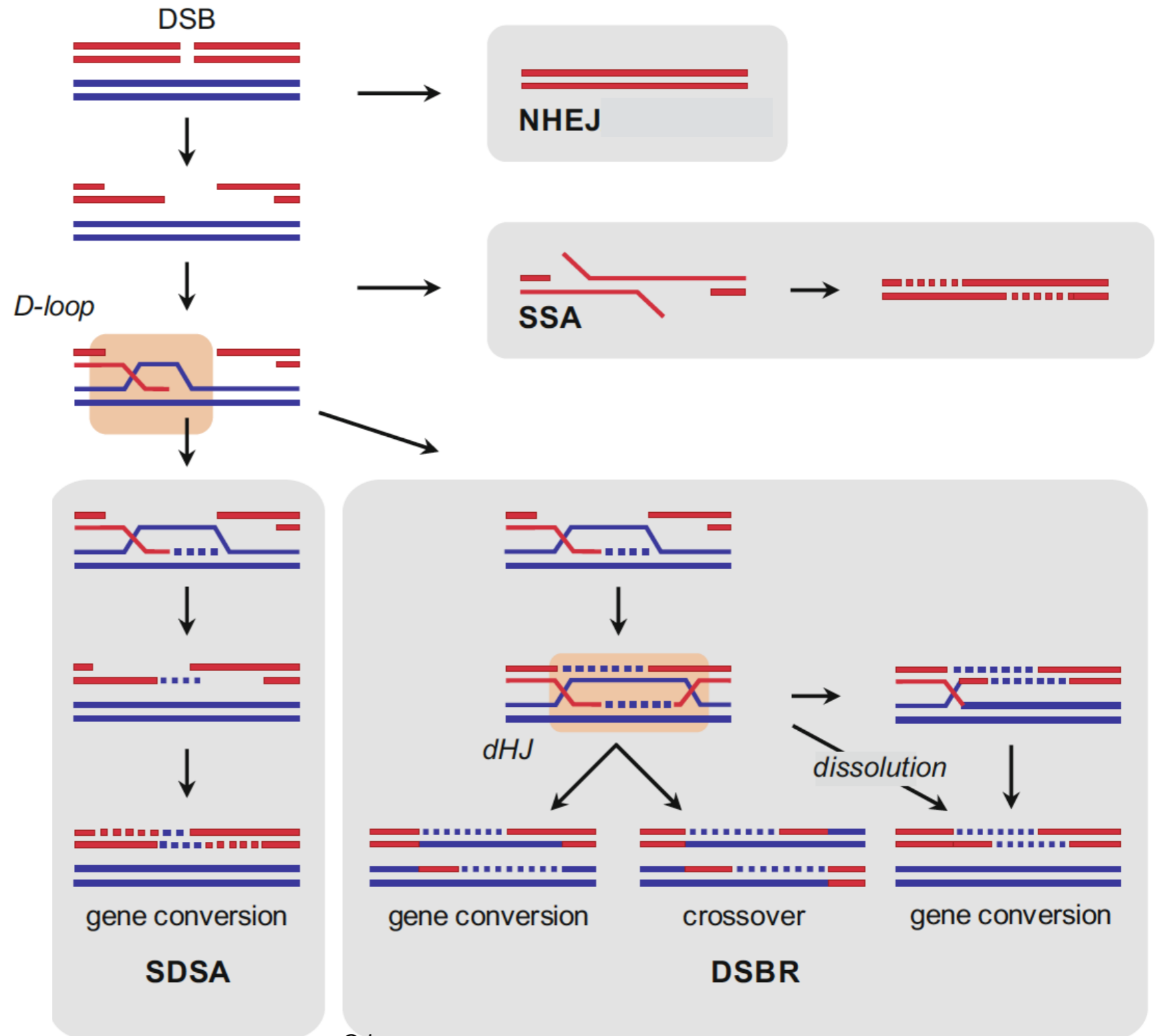
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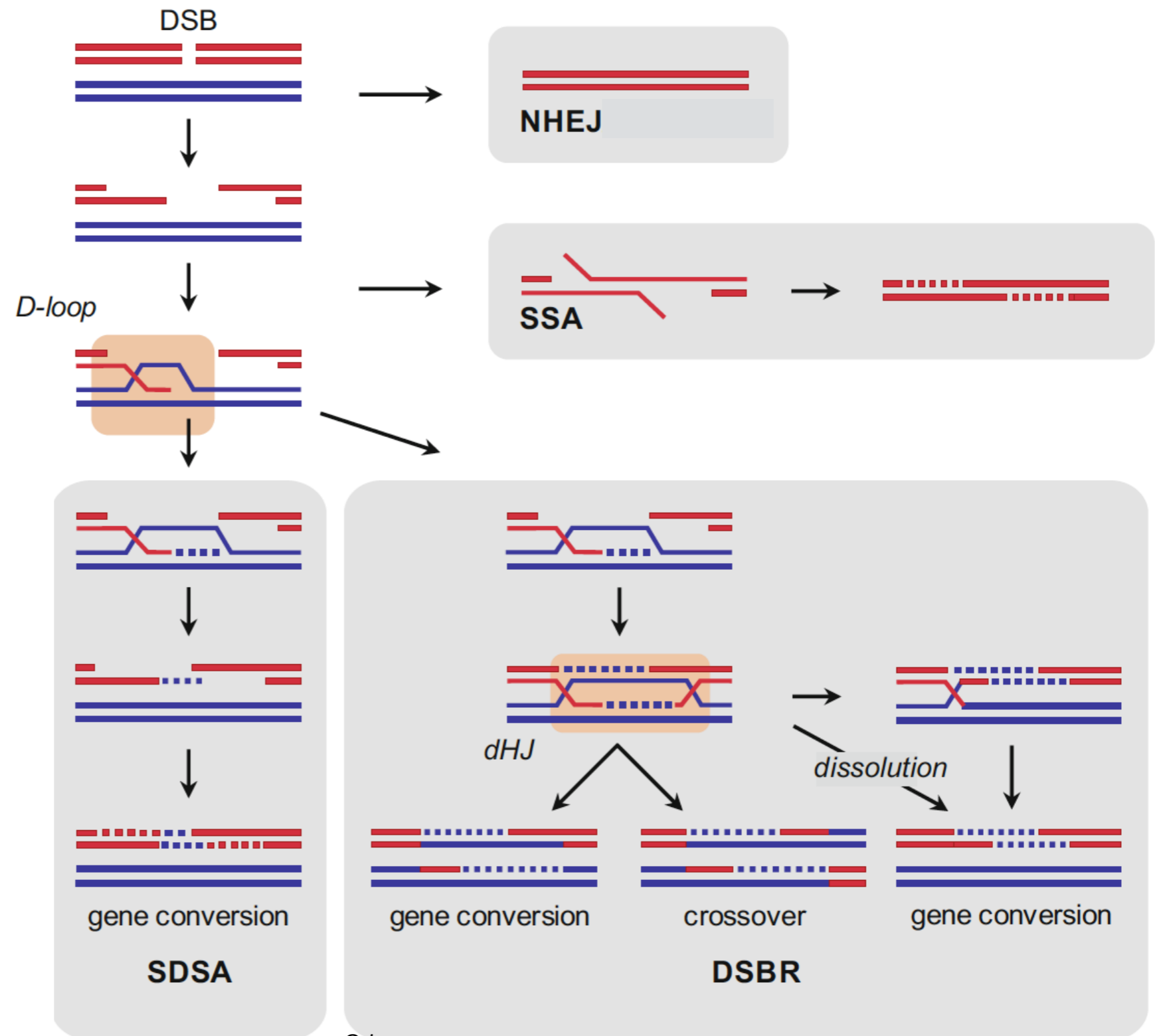
Error-prone

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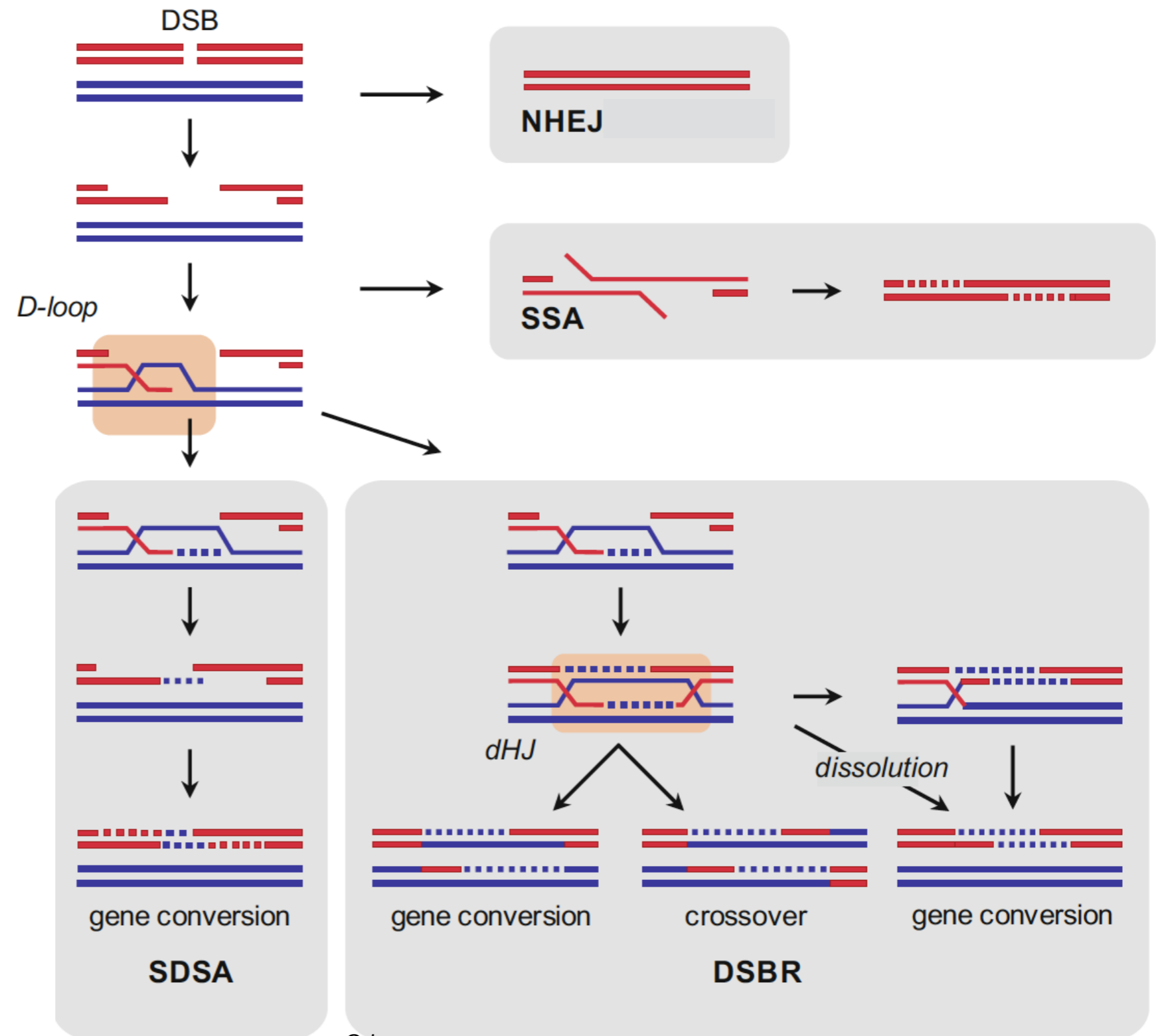
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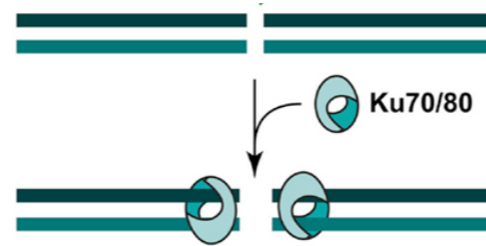
Error-free



How do cell maintain genome stability?

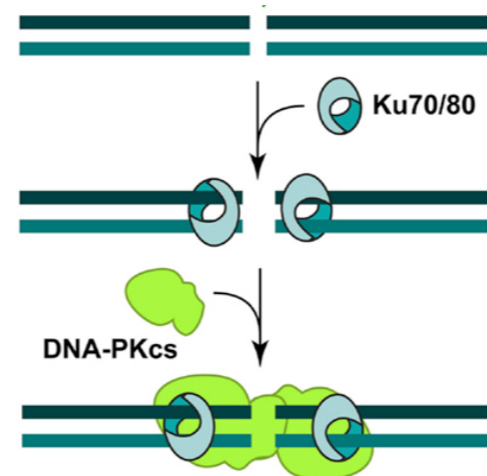
Double-stranded DNA breaks (DSB) repair

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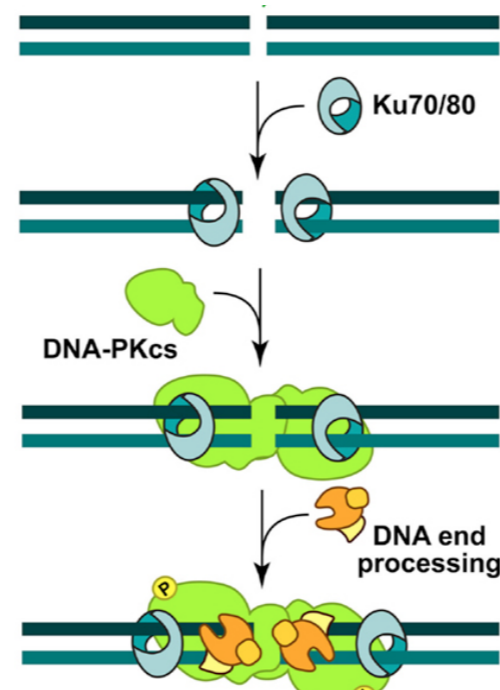
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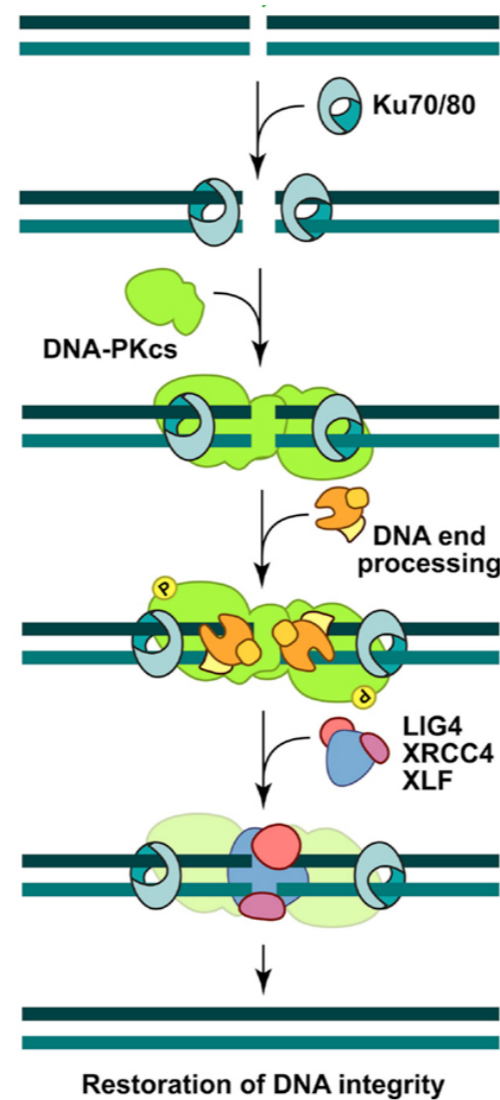
Double-stranded DNA breaks (DSB) repair
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How do cell maintain genome stability?

Double-stranded DNA breaks (DSB) repair
Non-homologous end joining

NHEJ is an error-prone pathway



How do cell maintain genome stability?

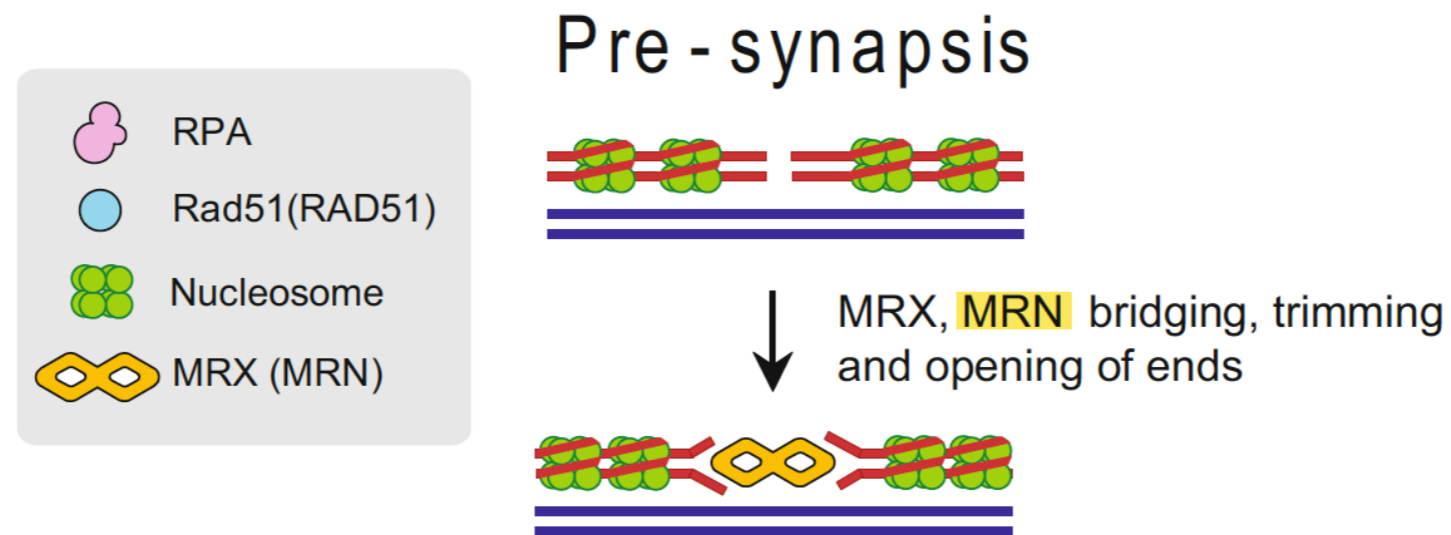
Double-stranded DNA breaks (DSB) repair

Homologous recombination

Pre - synapsis

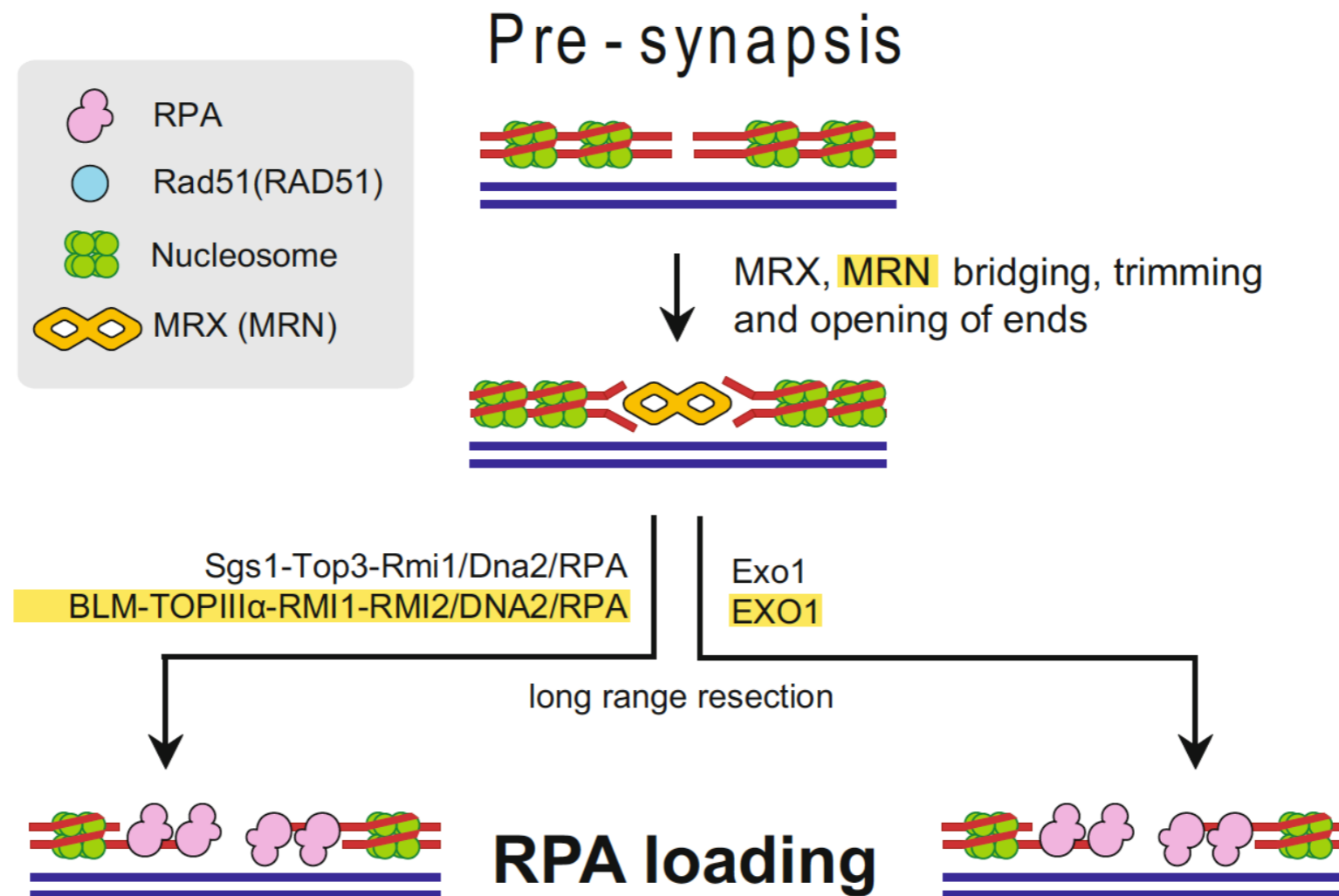
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Double-stranded DNA breaks (DSB) repair
Homologous recombination



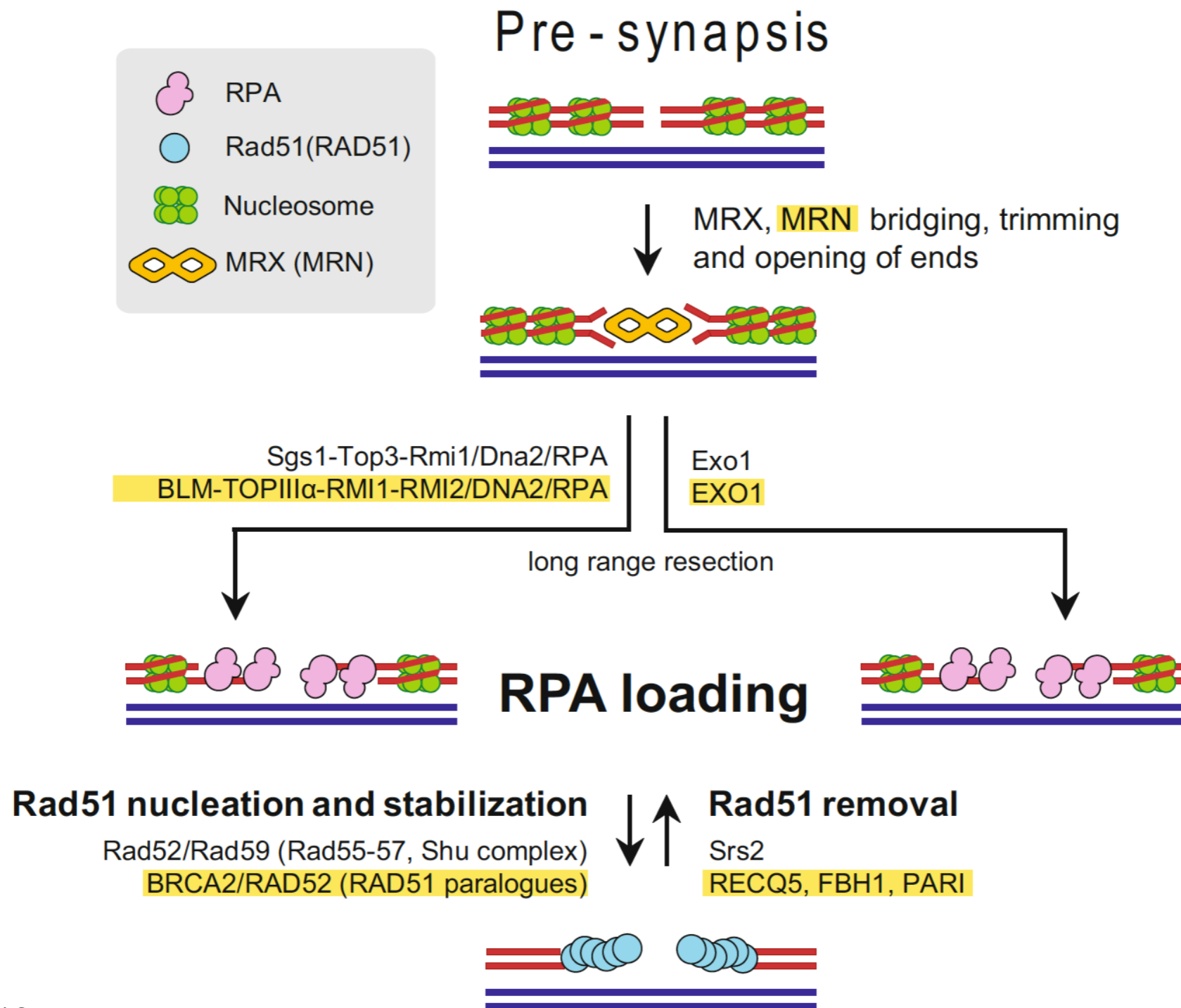
How do cell maintain genome stability?

Double-stranded DNA breaks (DSB) repair Homologous recombination



How do cell maintain genome stability?

Double-stranded DNA breaks (DSB) repair Homologous recombination

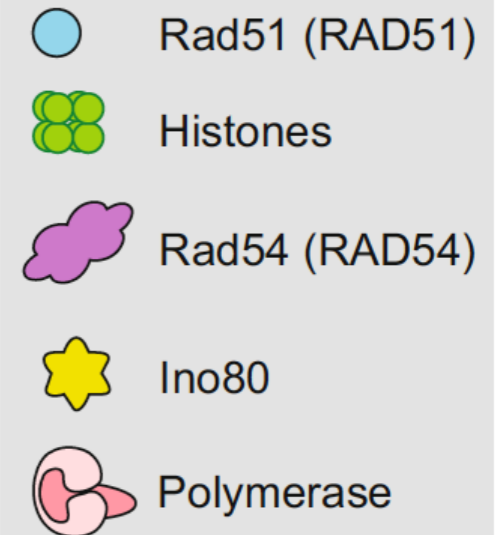


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Double-stranded DNA breaks (DSB) repair

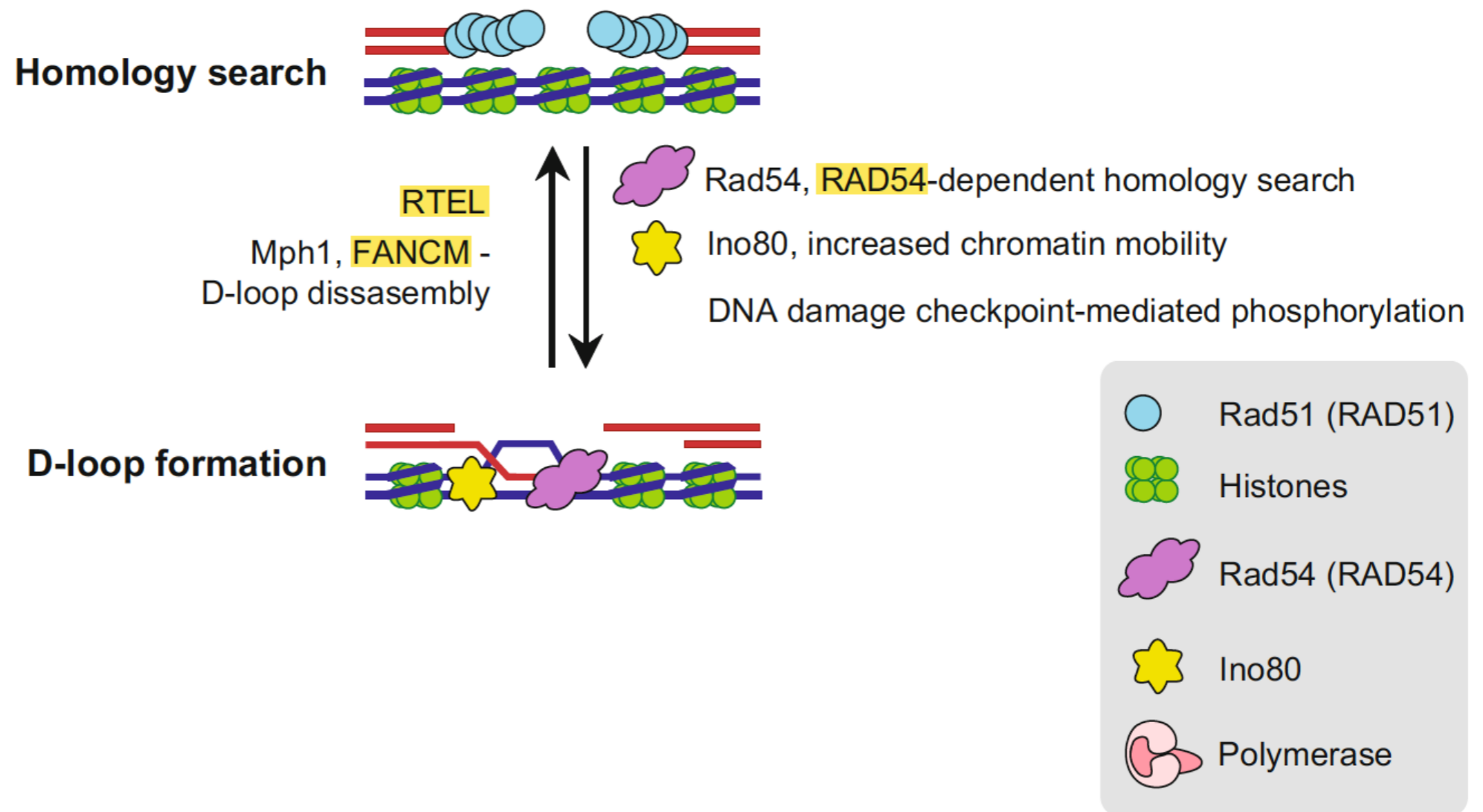
Homologous recombination

Synapsis



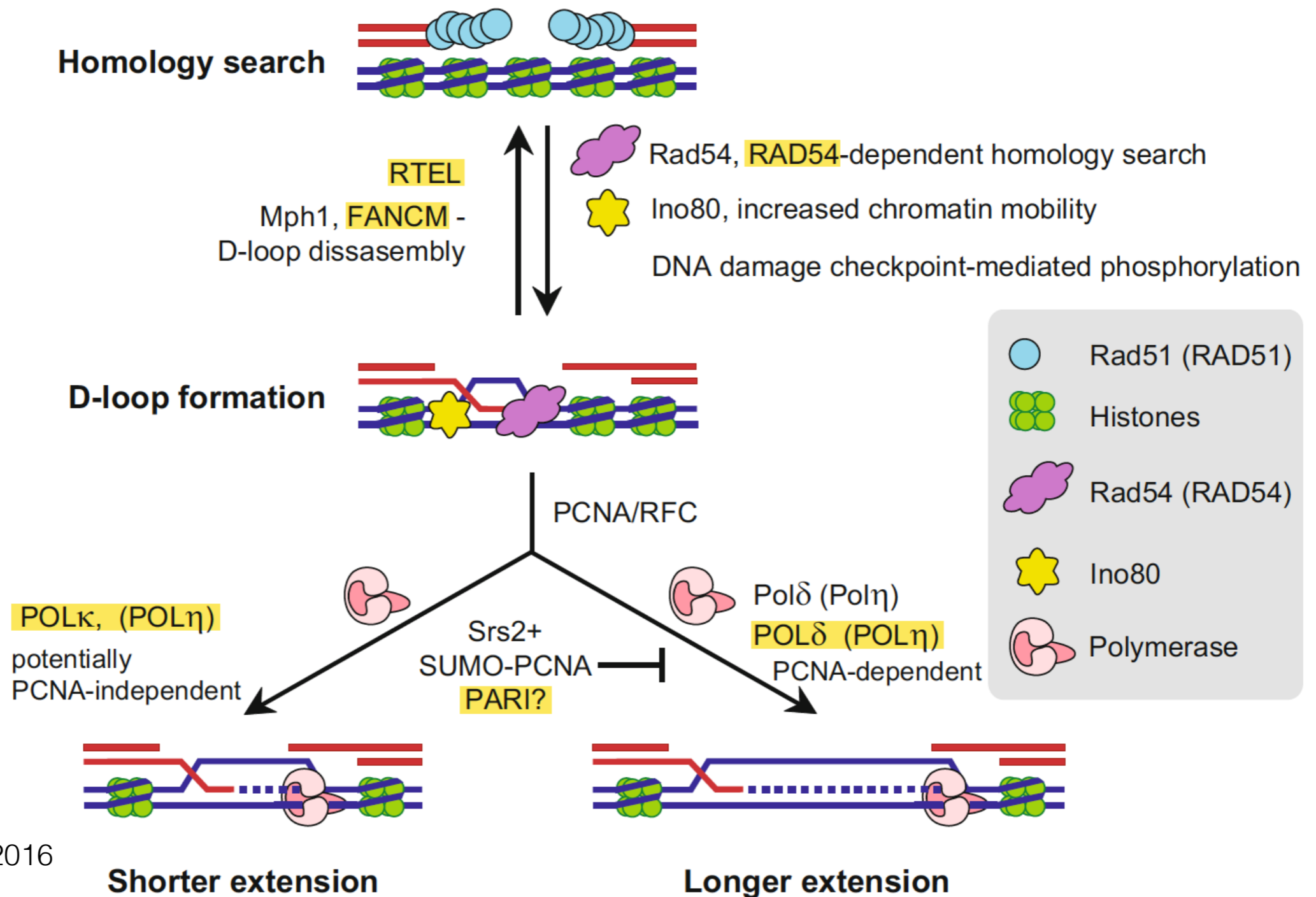
How do cells maintain genome stability?

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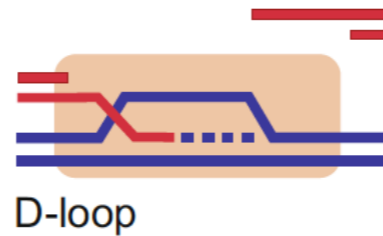


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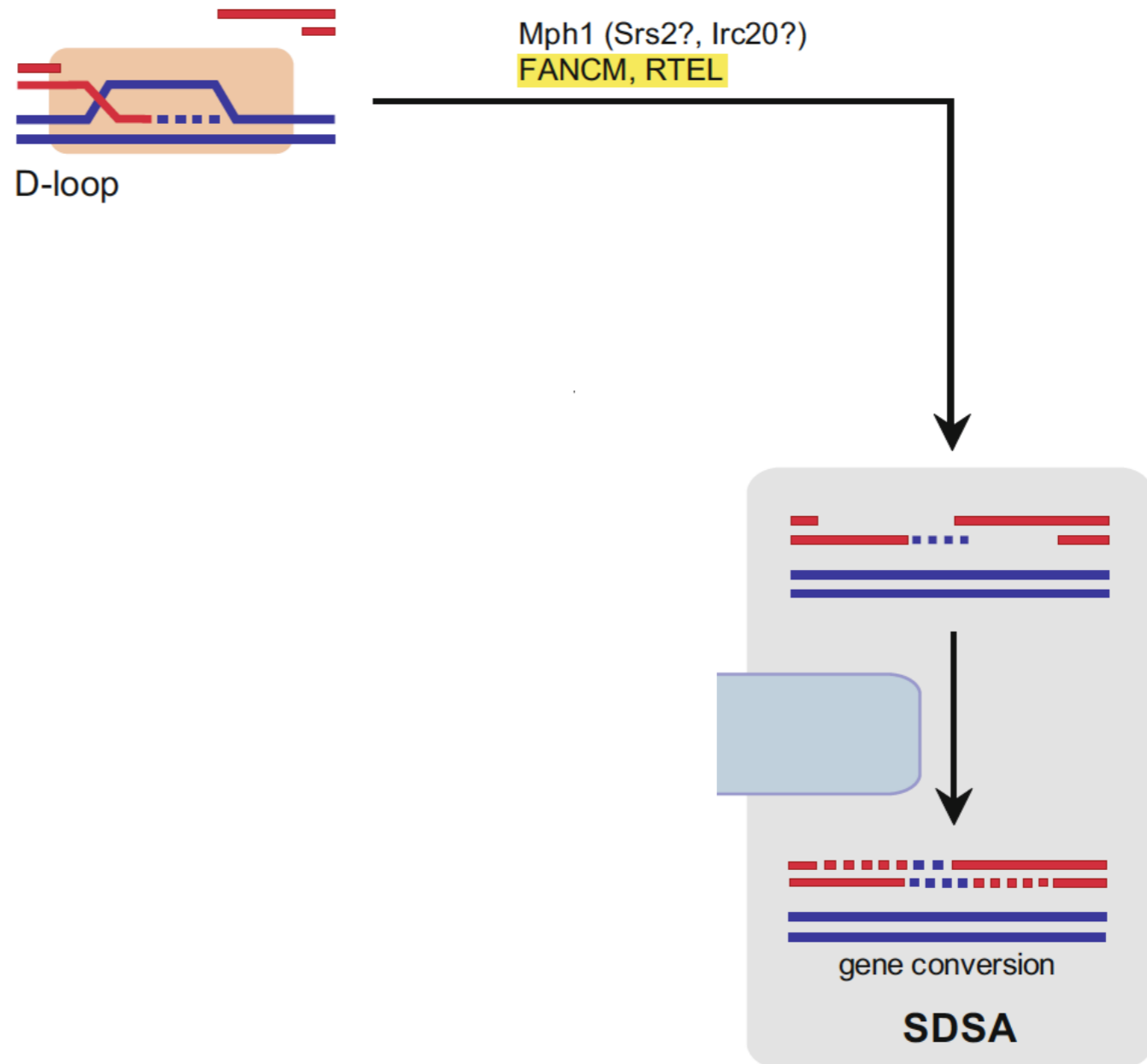
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How do cell maintain genome stability?

Double-stranded DNA breaks (DSB) repair
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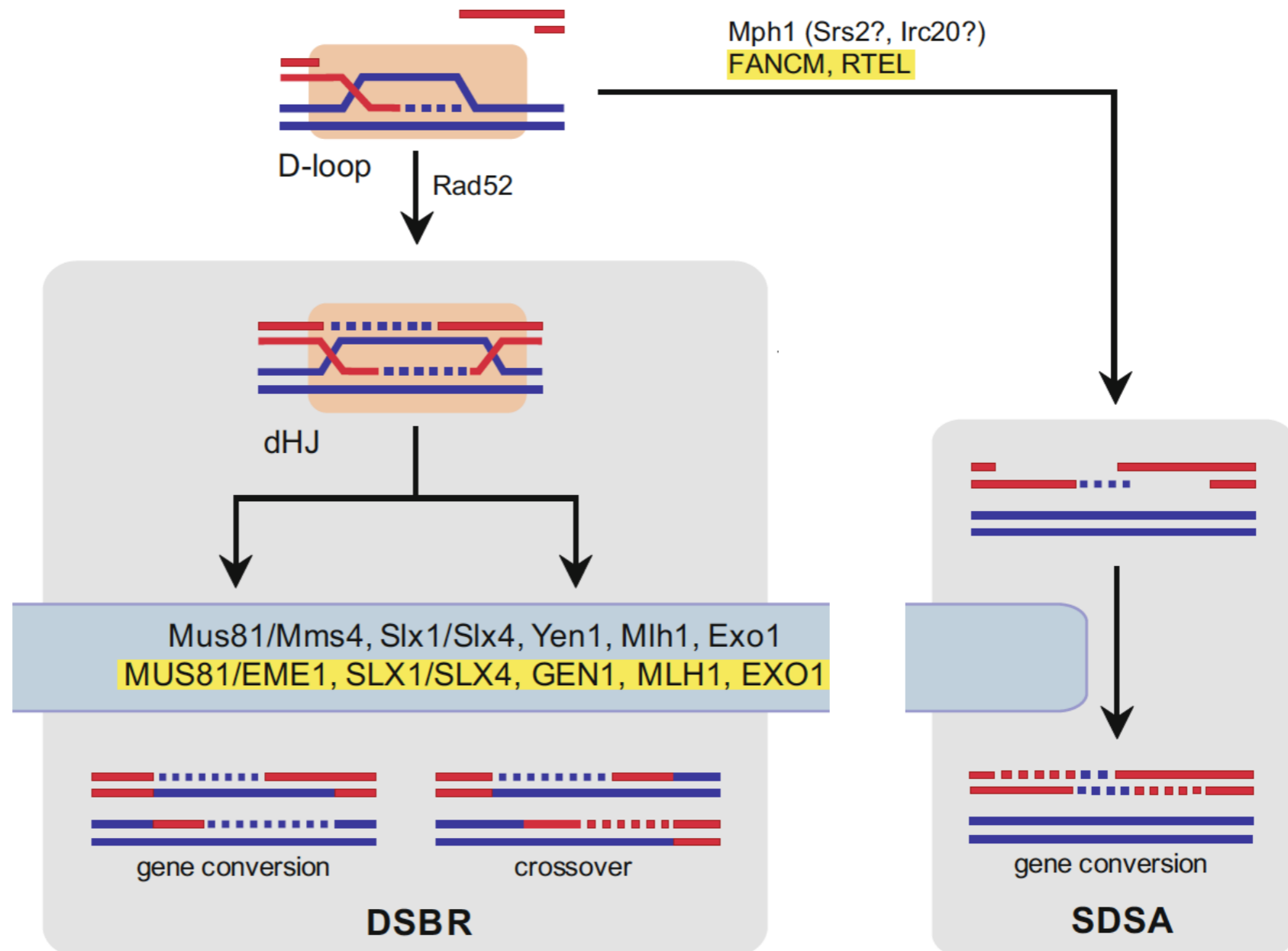
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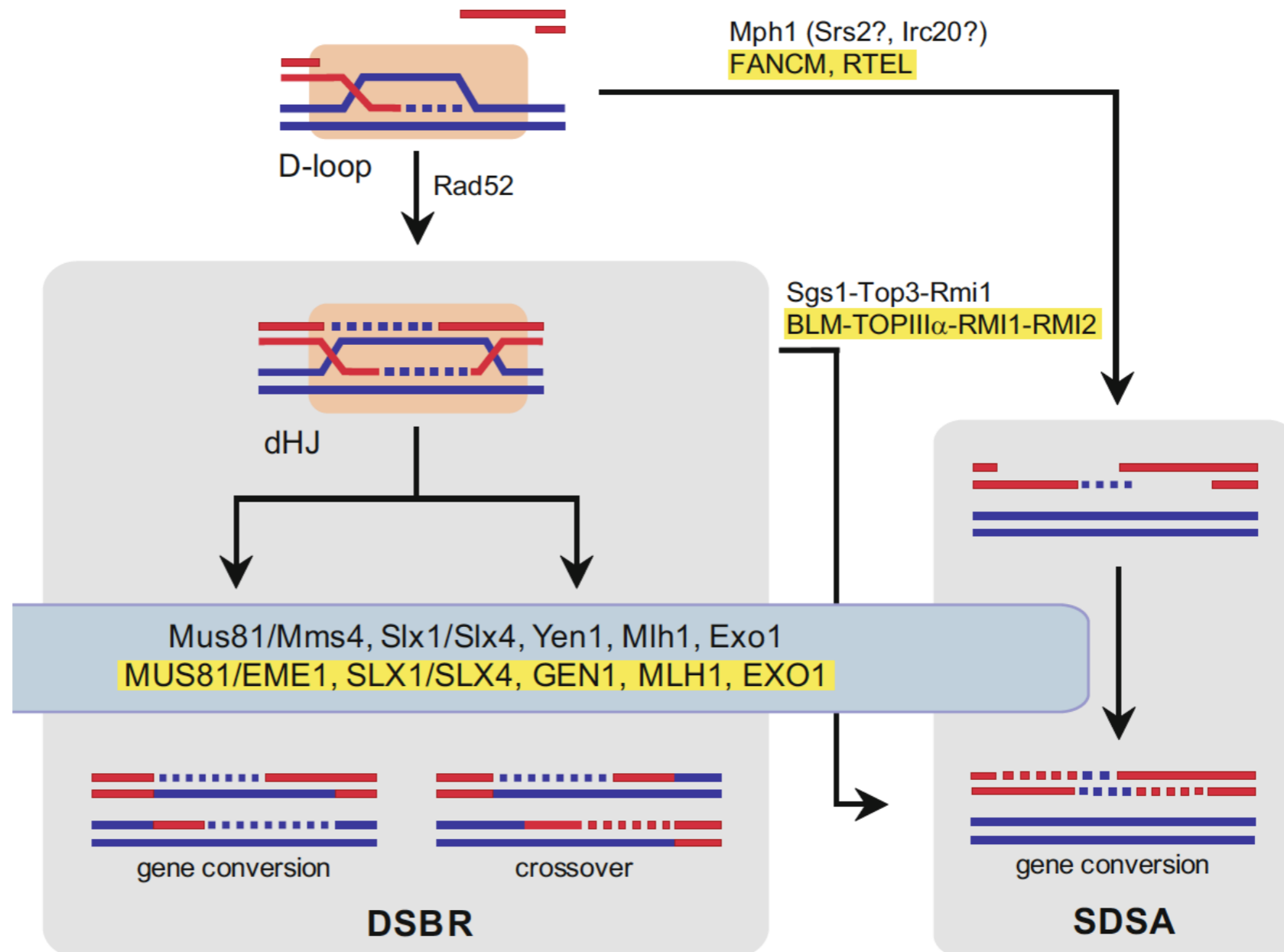
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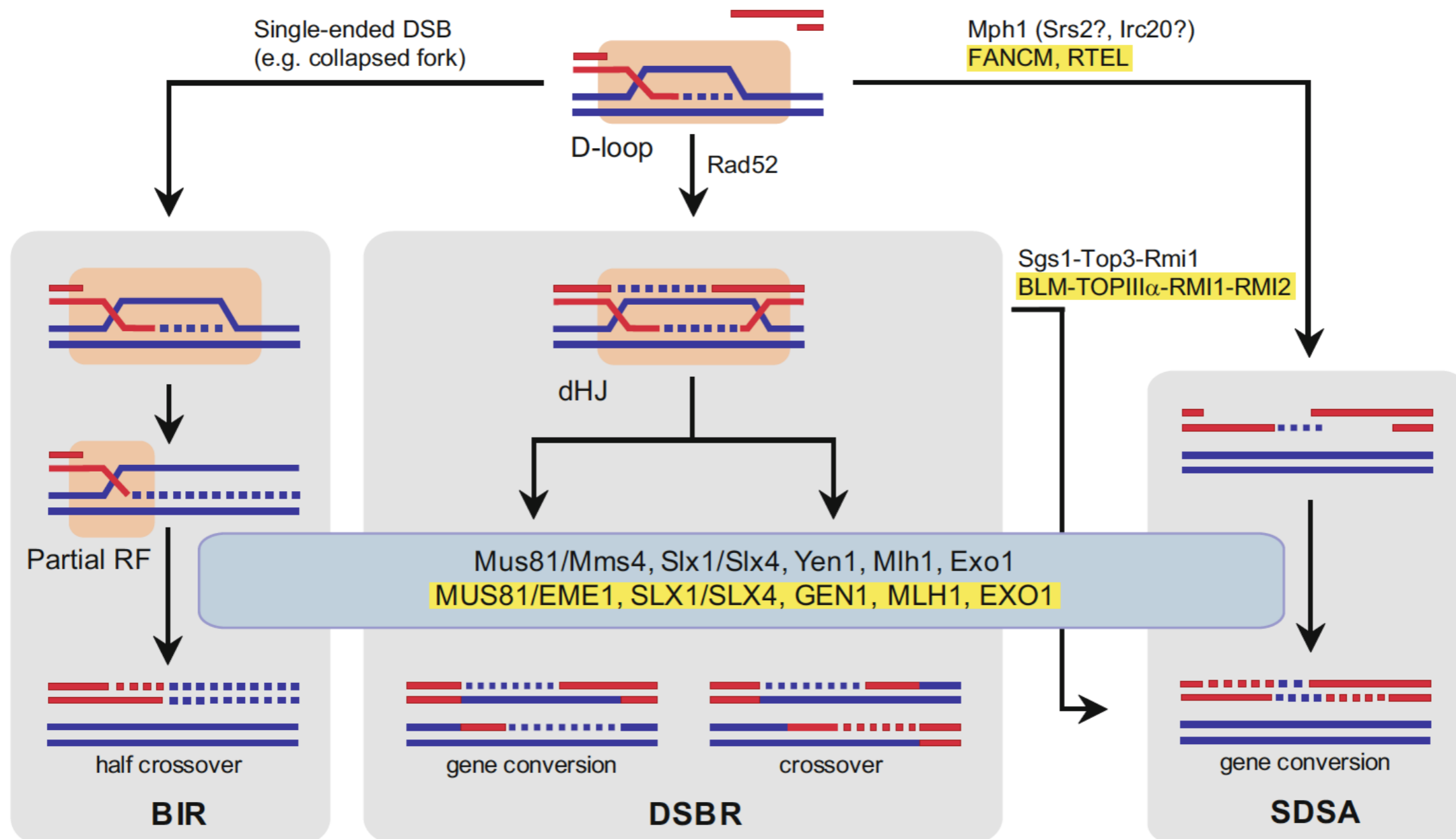
Post-synapsis



How do cells maintain genome stability?

Double-stranded DNA breaks (DSB) repair Homologous recombination

Post-synapsis



Transient summary III

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Different types of DNA damage are repaired by specific repair pathway

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The repair is generally error-free, except for NHEJ and SSA

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Different types of DNA damage are repaired by specific repair pathway

The repair is generally error-free, except for NHEJ and SSA

In S-phase, cells activate tolerance mechanisms that allow timely completion of DNA replication

How to study genome stability maintenance? (Case study on Homologous recombination)

Guidelines for DNA recombination and repair studies: Mechanistic assays of DNA repair processes

Hannah L Klein^{1,*}, Kenny K.H. Ang², Michelle R. Arkin², Emily C. Beckwitt^{3,4}, Yi-Hsuan Chang⁵, Jun Fan⁶, Youngho Kwon^{7,8}, Michael J. Morten¹, Sucheta Mukherjee⁹, Oliver J. Pambos⁶, Hafez el Sayyed⁶, Elizabeth S. Thrall¹⁰, João P. Vieira-da-Rocha⁹, Quan Wang¹¹, Shuang Wang^{12,13}, Hsin-Yi Yeh⁵, Julie S. Biteen¹⁴, Peter Chi^{5,15}, Wolf-Dietrich Heyer^{9,16}, Achillefs N. Kapanidis⁶, Joseph J. Loparo¹⁰, Terence R. Strick^{12,13,17}, Patrick Sung^{7,8}, Bennett Van Houten^{3,18,19}, Hengyao Niu^{11,*} and Eli Rothenberg^{1,*}

How to study genome stability maintenance? (Case study on Homologous recombination)

Different strategies exist

Genetic tools

Enable us to identify genes and the relationships among, thereby building a pathway

Microscopic tools

Give us a glimpse at spacial and temporal relationships of genes of interests

Biochemical tools

Enable us to understand mechanisms and complex formations within a studied pathway

Structural tools

Enable us to understand molecular mechanisms at atomic resolution

Single molecule techniques

Enable us to understand behaviour at of single molecules as compared to bulk biochemical reactions

How to study genome stability maintenance? Step 1: identify the genes

Molec. gen. Genet. 125, 197—216 (1973)
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Interactions among Genes Controlling Sensitivity to Radiation and Alkylation in Yeast

Martin Brendel and Robert H. Haynes

Department of Biology, York University, Toronto, Canada

Received March 27, 1973

How to study genome stability maintenance?

Step 1: identify the genes

Molec. gen. Genet. 125, 197—216 (1973)
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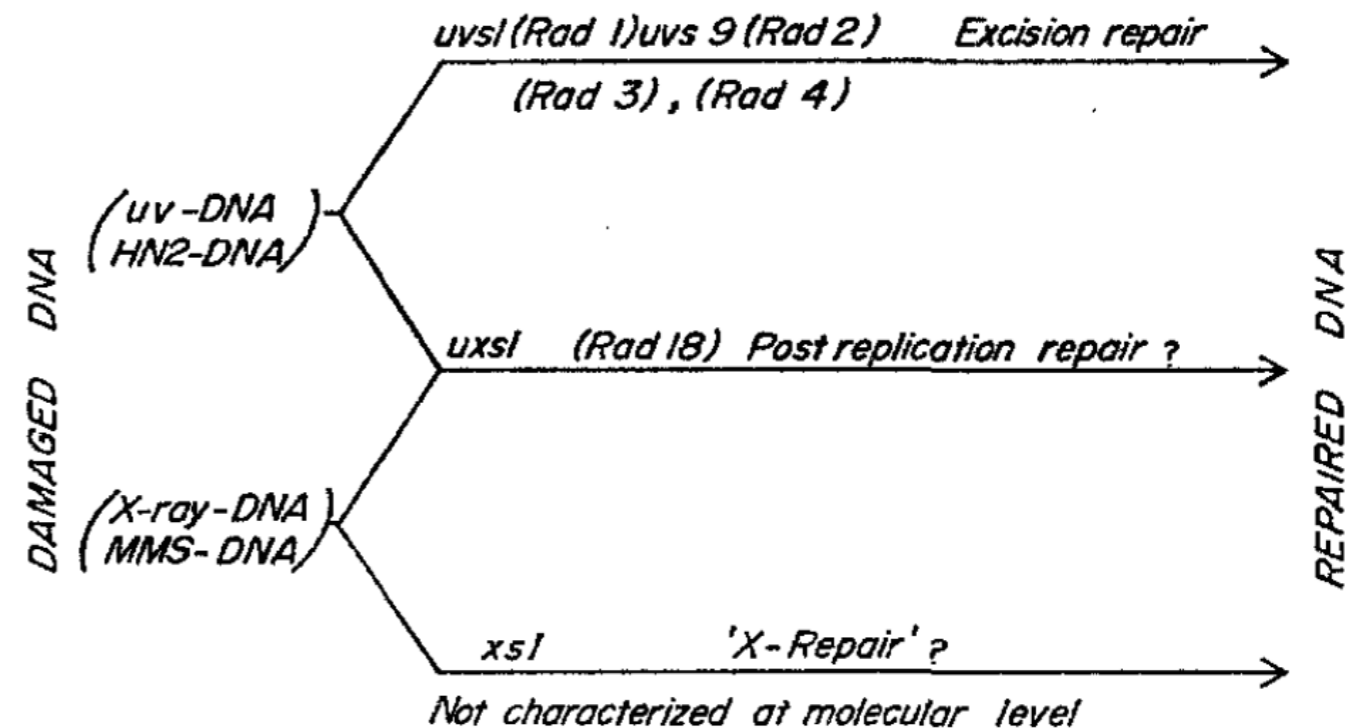
Interactions among Genes Controlling Sensitivity to Radiation and Alkylation in Yeast

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Using a thorough genetic analysis of the isolated mutants, they were able to build a first model of multiple pathways dealing with DNA damage.



How to study genome stability maintenance? Step 1: identify the genes

nature

Vol 455 | 9 October 2008 | doi:10.1038/nature07312

ARTICLES

Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing

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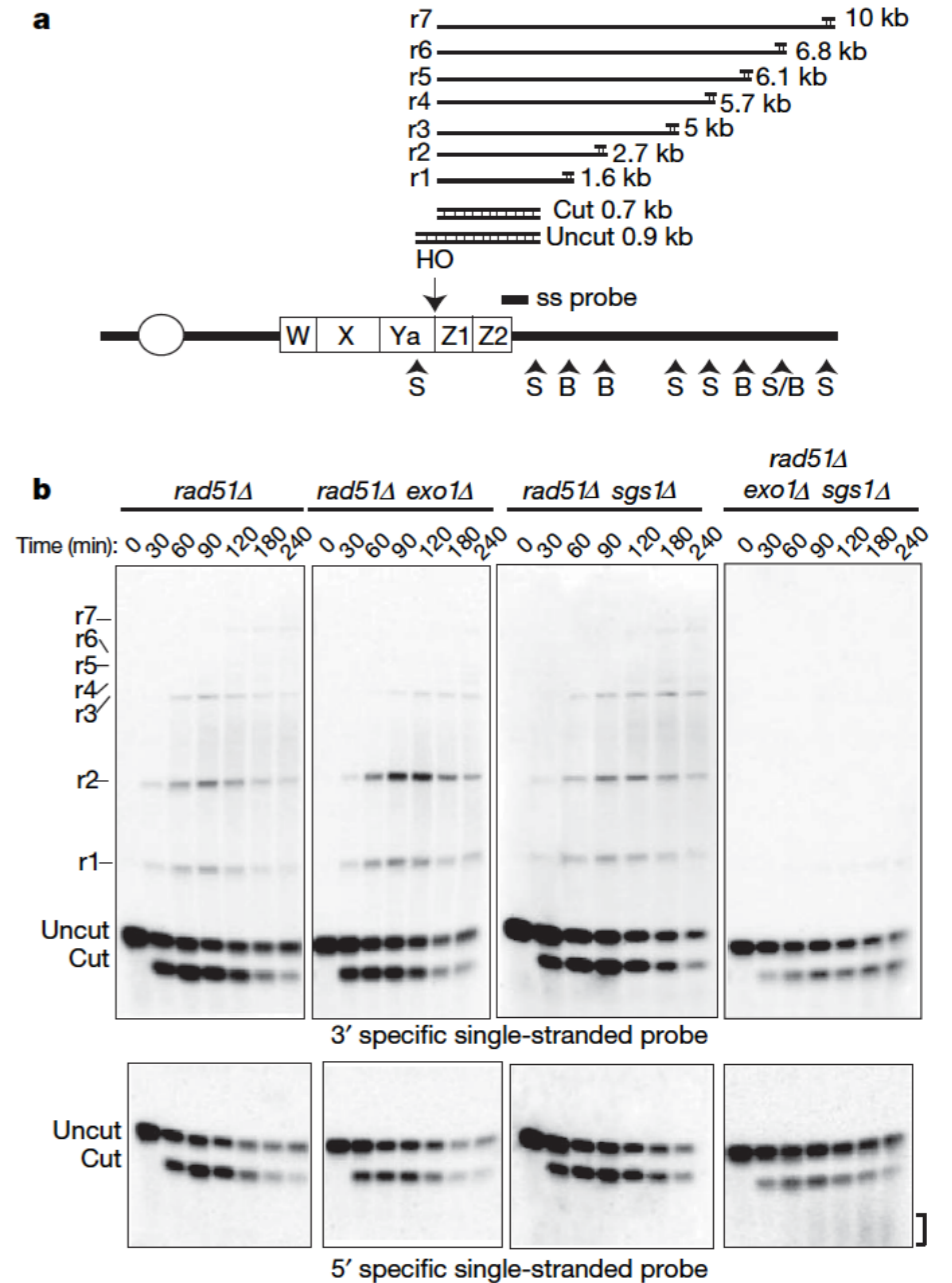


Figure 3 | Single-stranded intermediates fail to form in the absence of Exo1 and Sgs1. **a**, Representation of the method used to detect single-stranded

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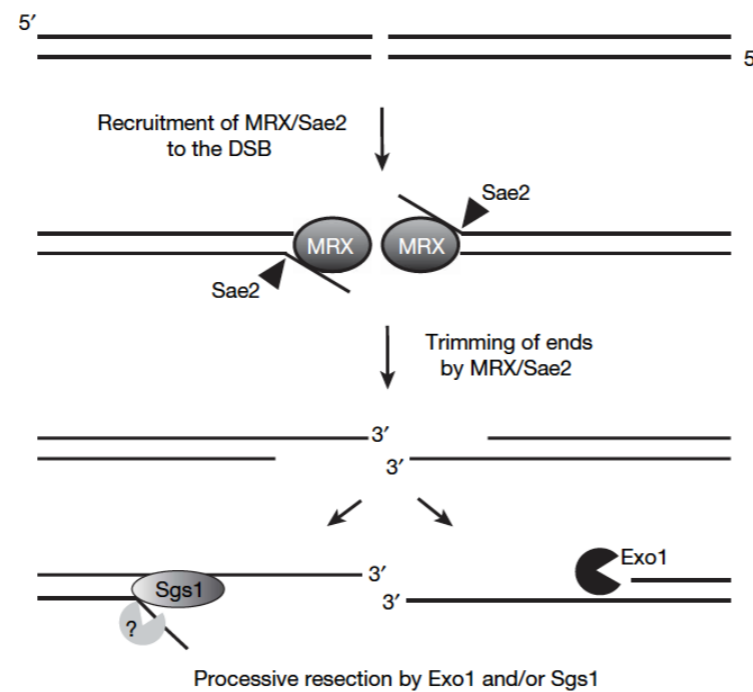


Figure 5 | Two-step mechanism for DSB resection. After a DSB is formed

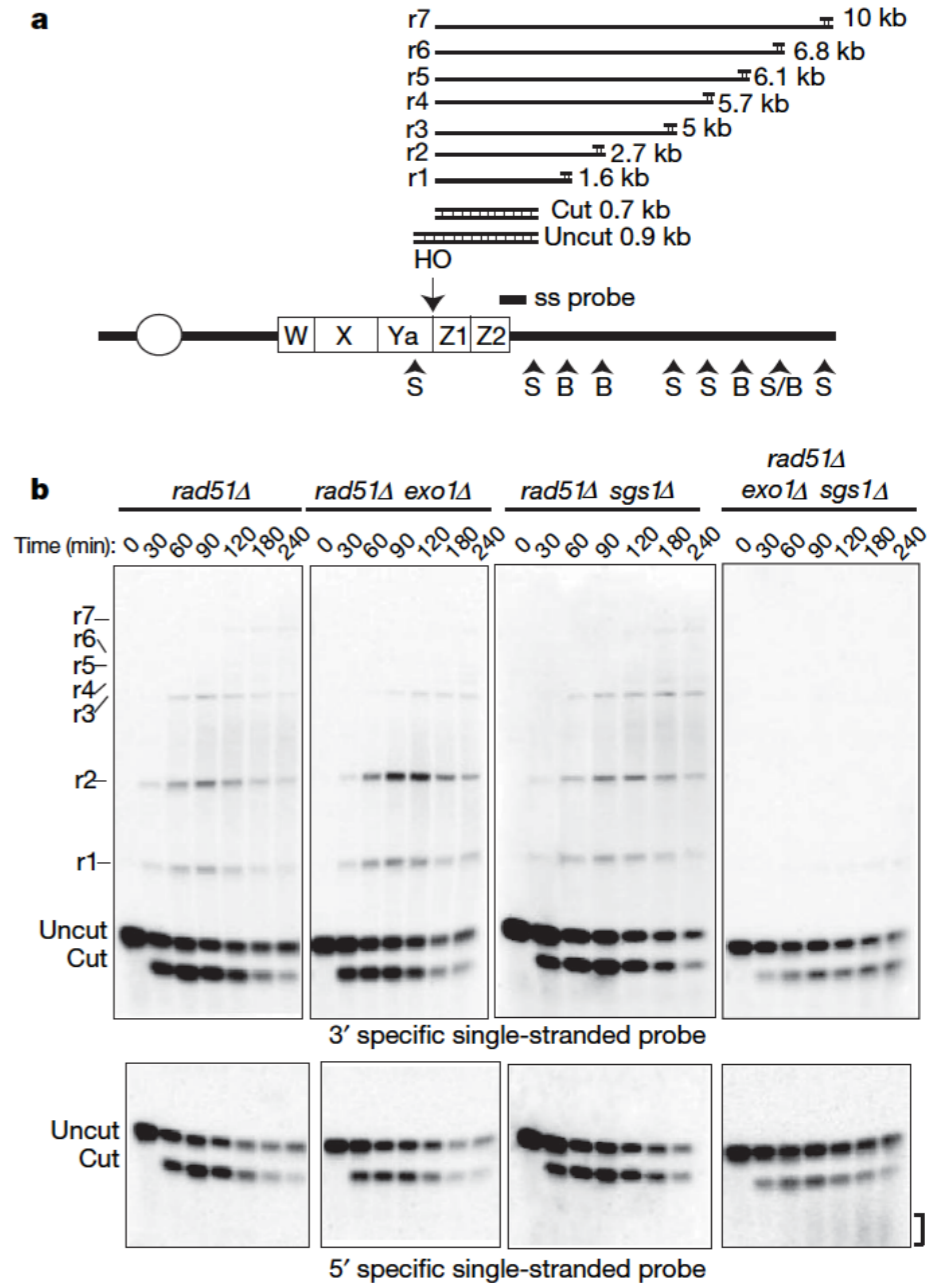


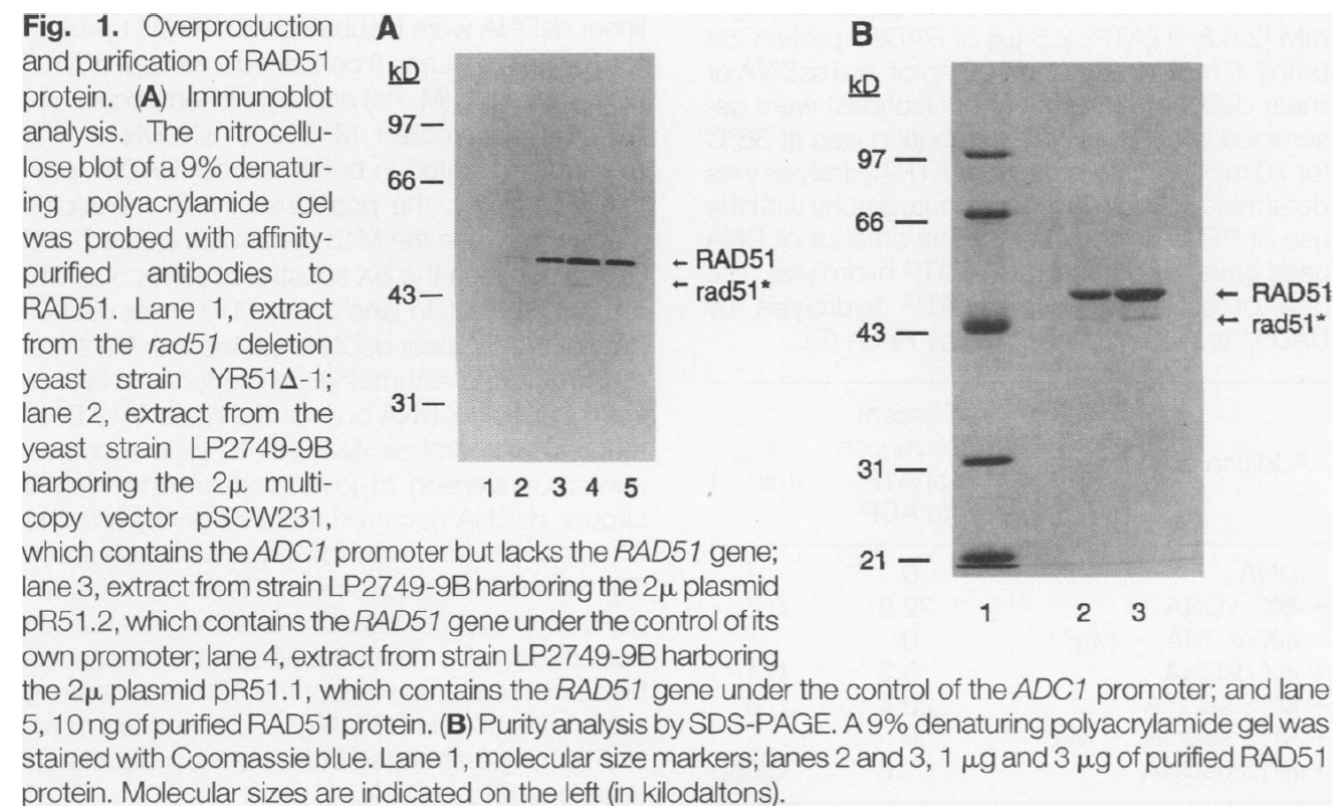
Figure 3 | Single-stranded intermediates fail to form in the absence of Exo1 and Sgs1. a, Representation of the method used to detect single-stranded

Using a genetic approach Mimitou and Symington, were able to show for the first time the mechanism by which cells resect the ends of broken DNA.

How to study genome stability maintenance?
Step2: purify and study the proteins alone

Catalysis of ATP-Dependent Homologous DNA Pairing and Strand Exchange by Yeast RAD51 Protein

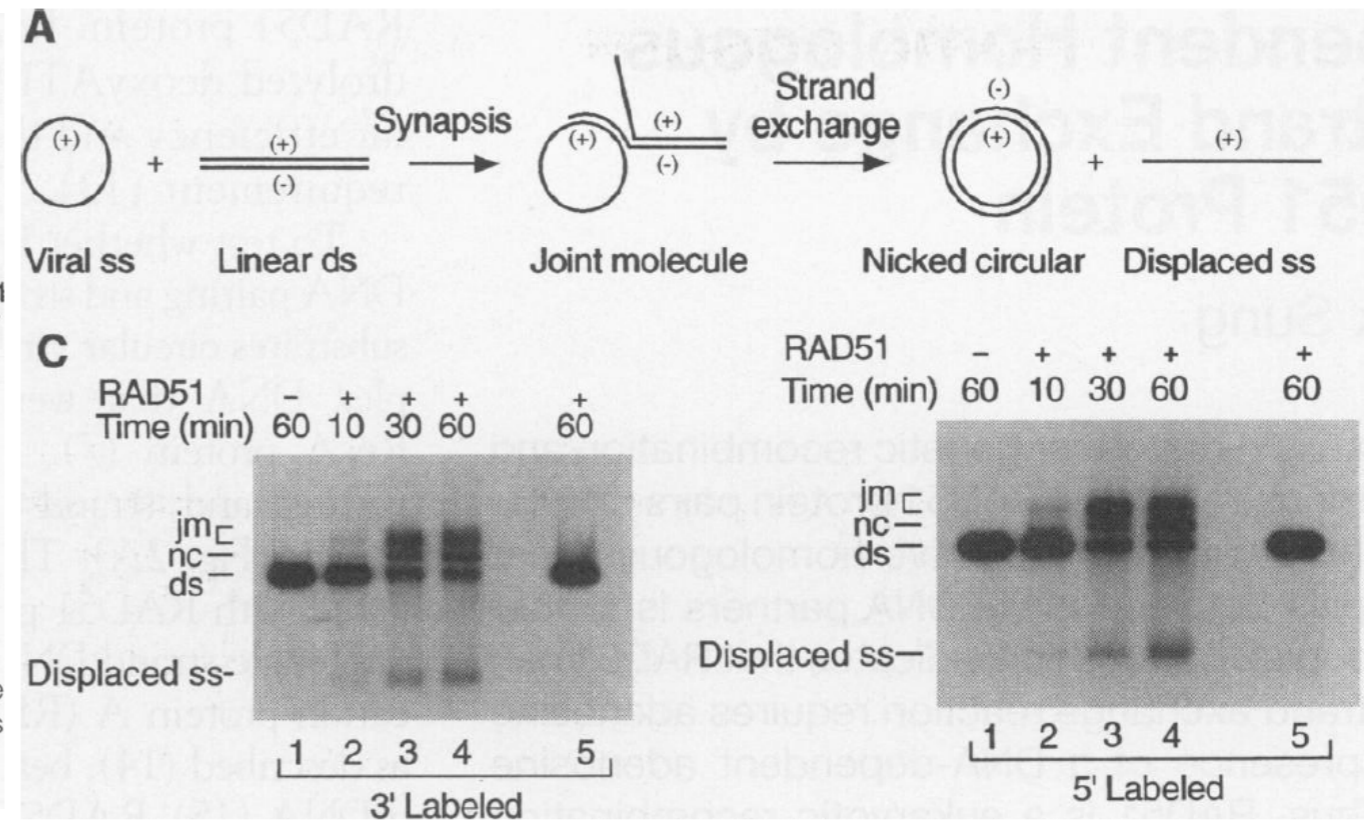
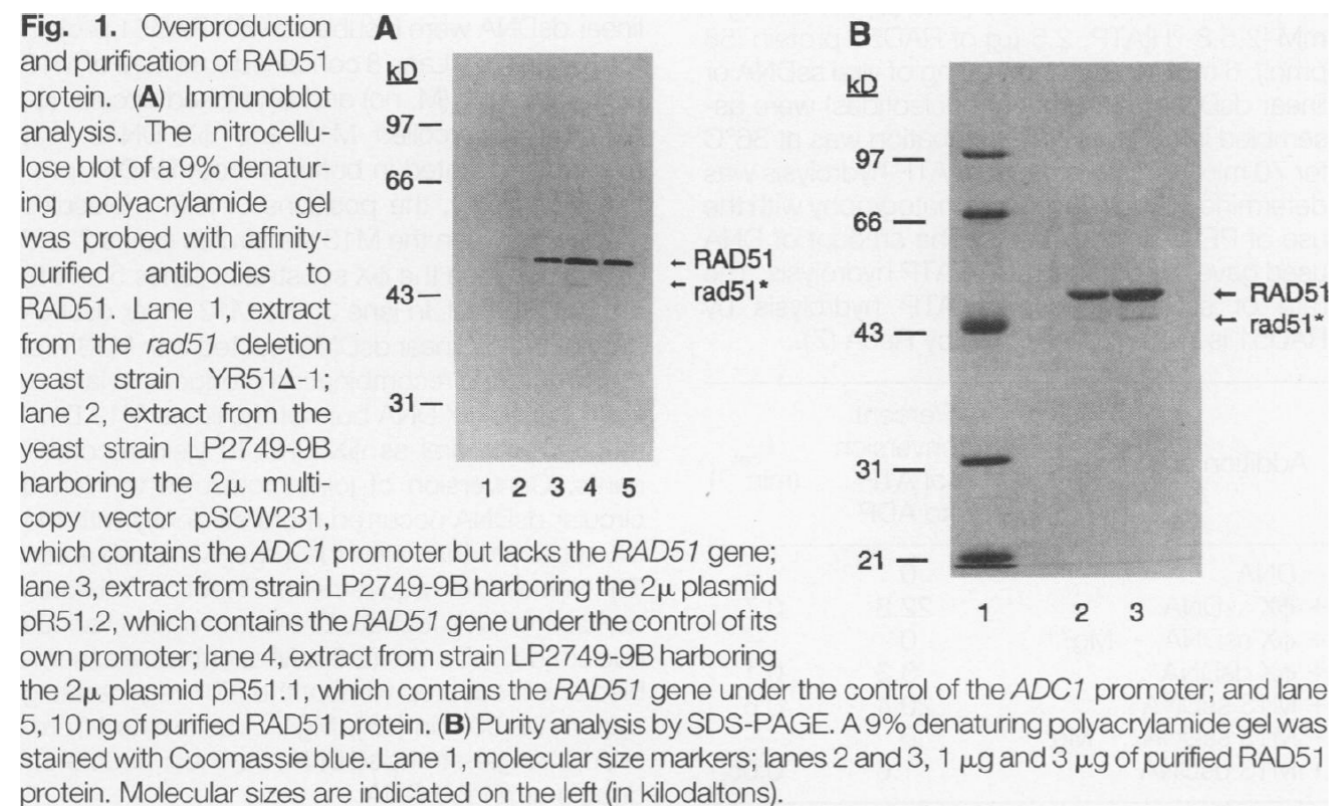
Patrick Sung



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Catalysis of ATP-Dependent Homologous DNA Pairing and Strand Exchange by Yeast RAD51 Protein

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Using a purified protein, Patrick Sung was able to show that Rad51 is a bona fide recombinase.

How to study genome stability maintenance? Step2: purify and study the proteins in assemblies

nature

Vol 467|2 September 2010|doi:10.1038/nature09355

LETTERS

DNA end resection by Dna2–Sgs1–RPA and its stimulation by Top3–Rmi1 and Mre11–Rad50–Xrs2

Petr Cejka^{1,2}, Elda Cannavo^{1,2}, Piotr Polaczek³, Taro Masuda-Sasa³, Subhash Pokharel³, Judith L. Campbell³
& Stephen C. Kowalczykowski^{1,2}

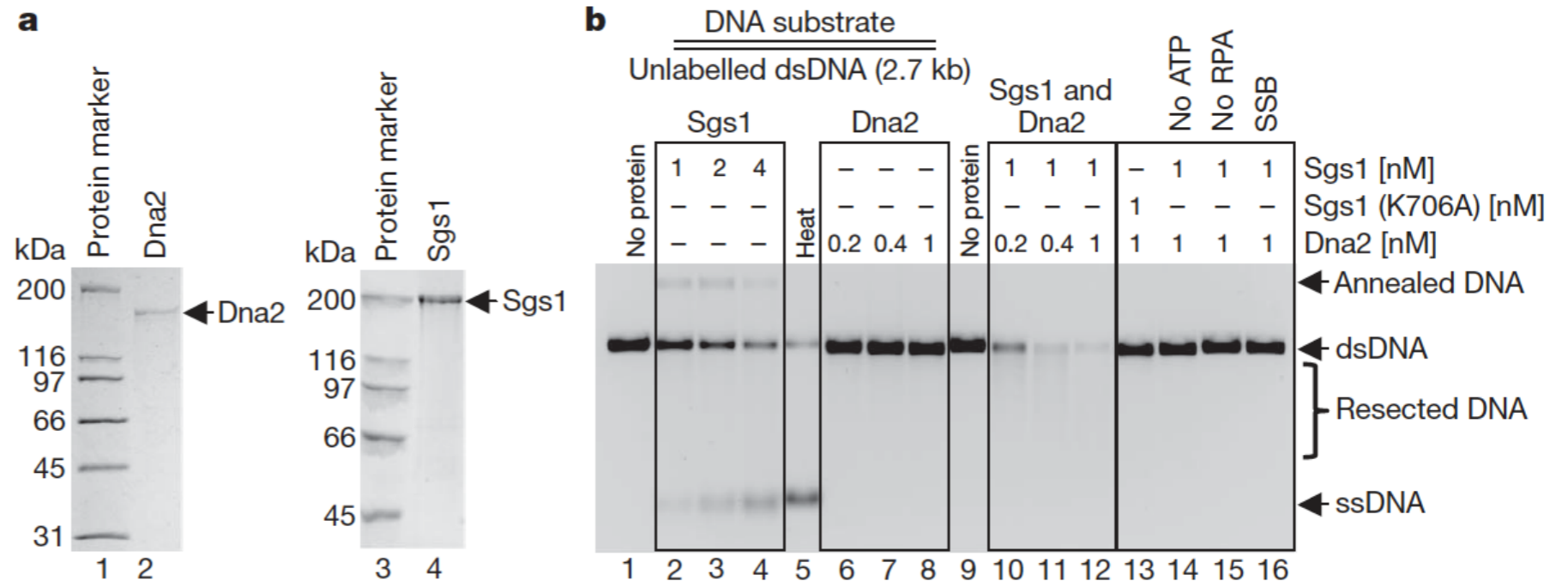
How to study genome stability maintenance?

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Using purified proteins, Cejka et al., were able to reconstitute end resection *in vitro*.

How to study genome stability maintenance? Step3: study the proteins in time and space

Cell, Vol. 118, 699–713, September 17, 2004, Copyright ©2004 by Cell Press

Choreography of the DNA Damage Response: Spatiotemporal Relationships among Checkpoint and Repair Proteins

Michael Lisby,^{1,3} Jacqueline H. Barlow,
Rebecca C. Burgess,² and Rodney Rothstein*

How to study genome stability maintenance? Step3: study the proteins in time and space

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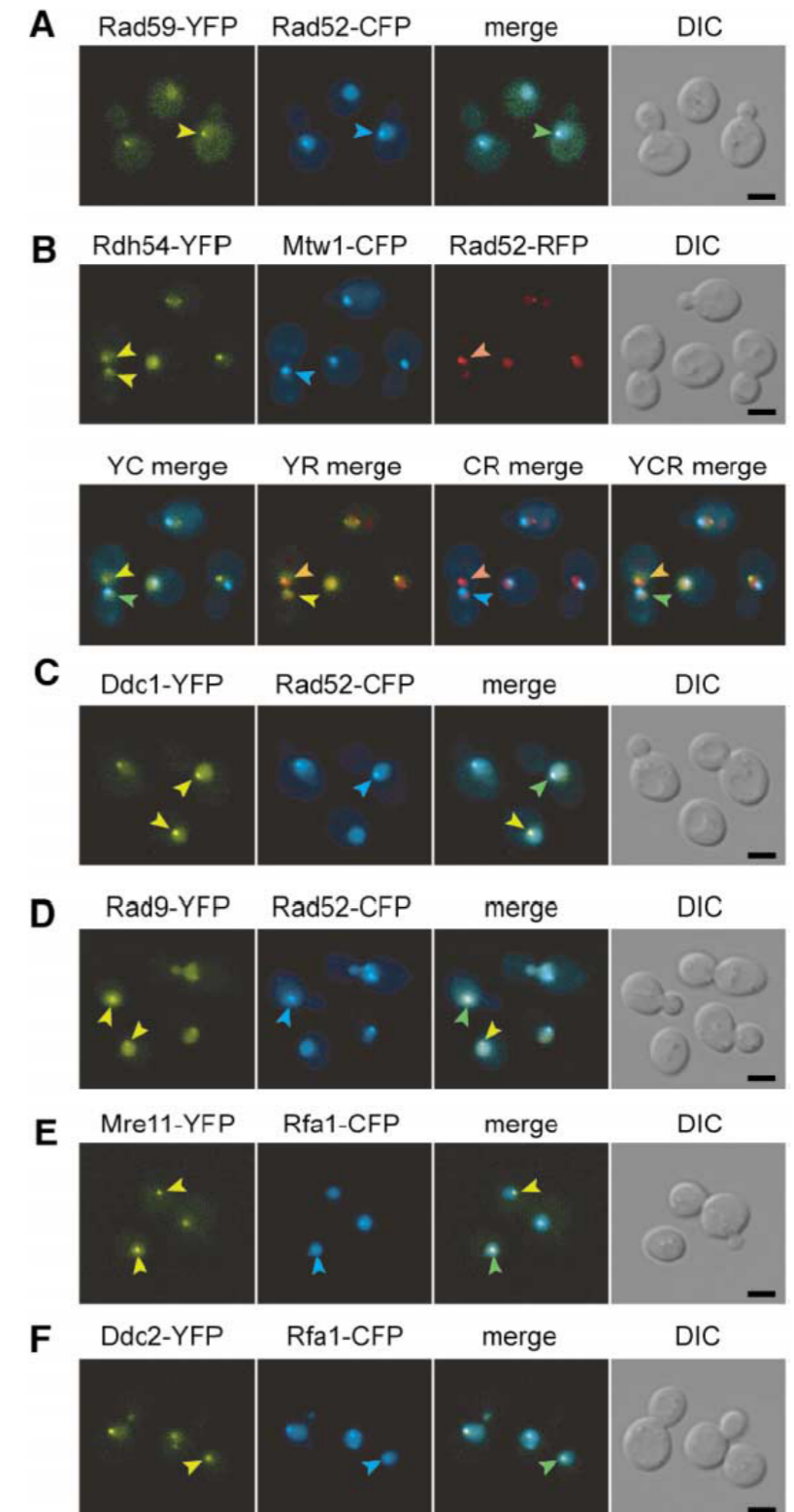


Figure 1. Colocalization of Checkpoint and Repair Foci

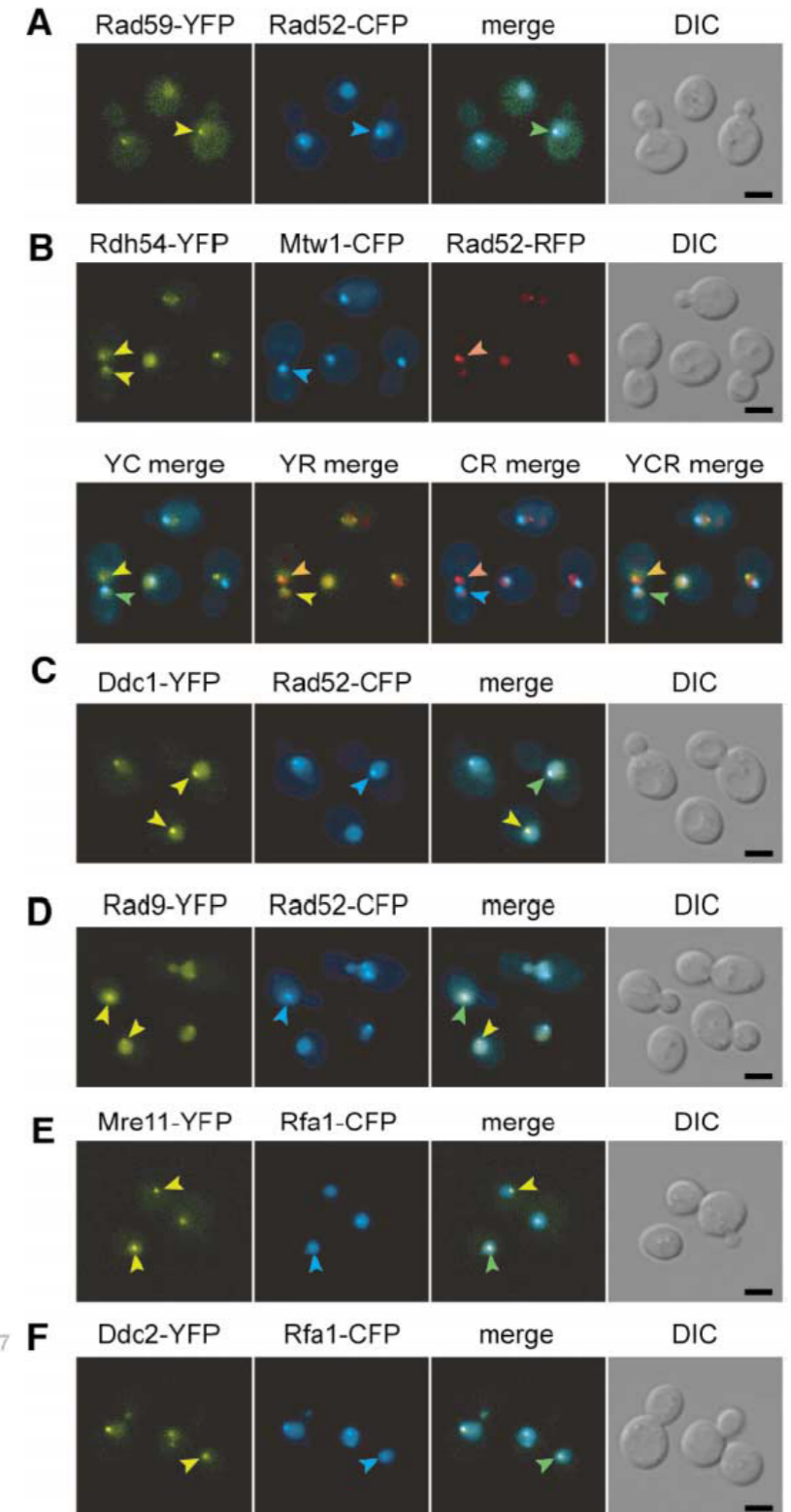
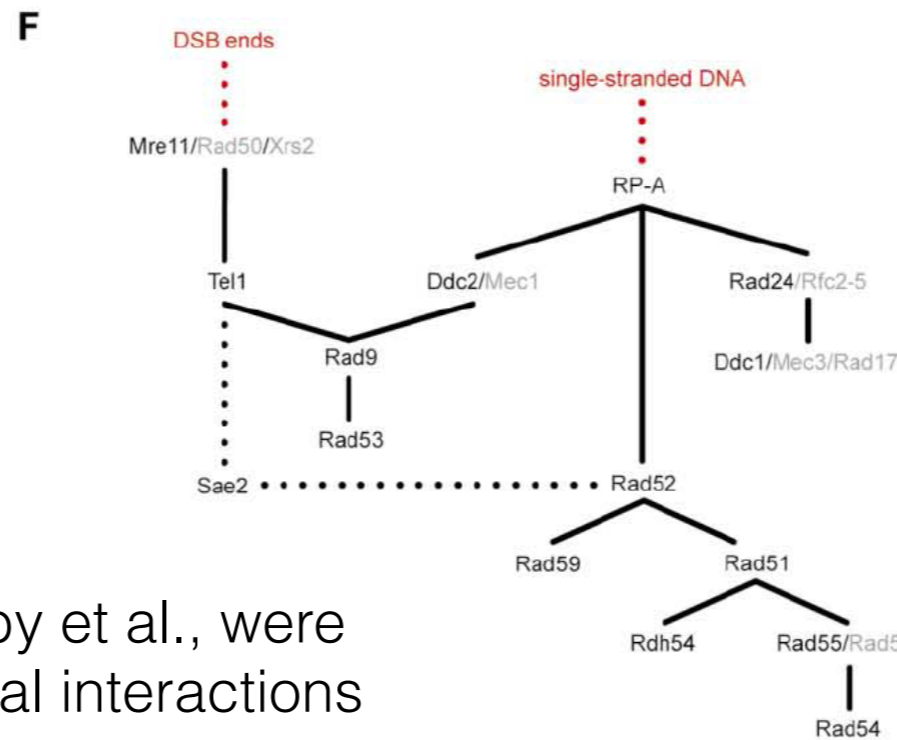
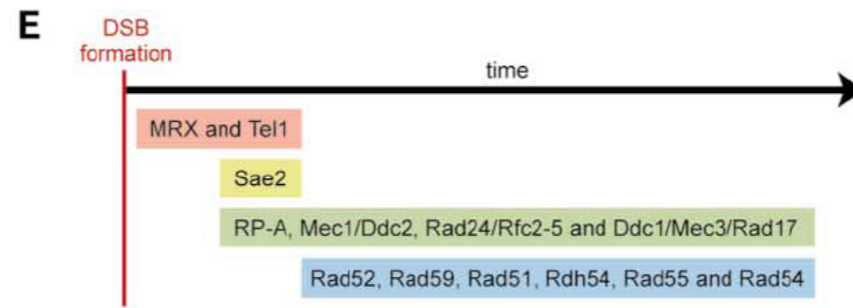
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Using life-cell microscopy, Lisby et al., were able to study the spatiotemporal interactions among recombination factors.

How to study genome stability maintenance?
Step4: study the role of protein complex formation?

Protein Group Modification and Synergy in the SUMO Pathway as Exemplified in DNA Repair

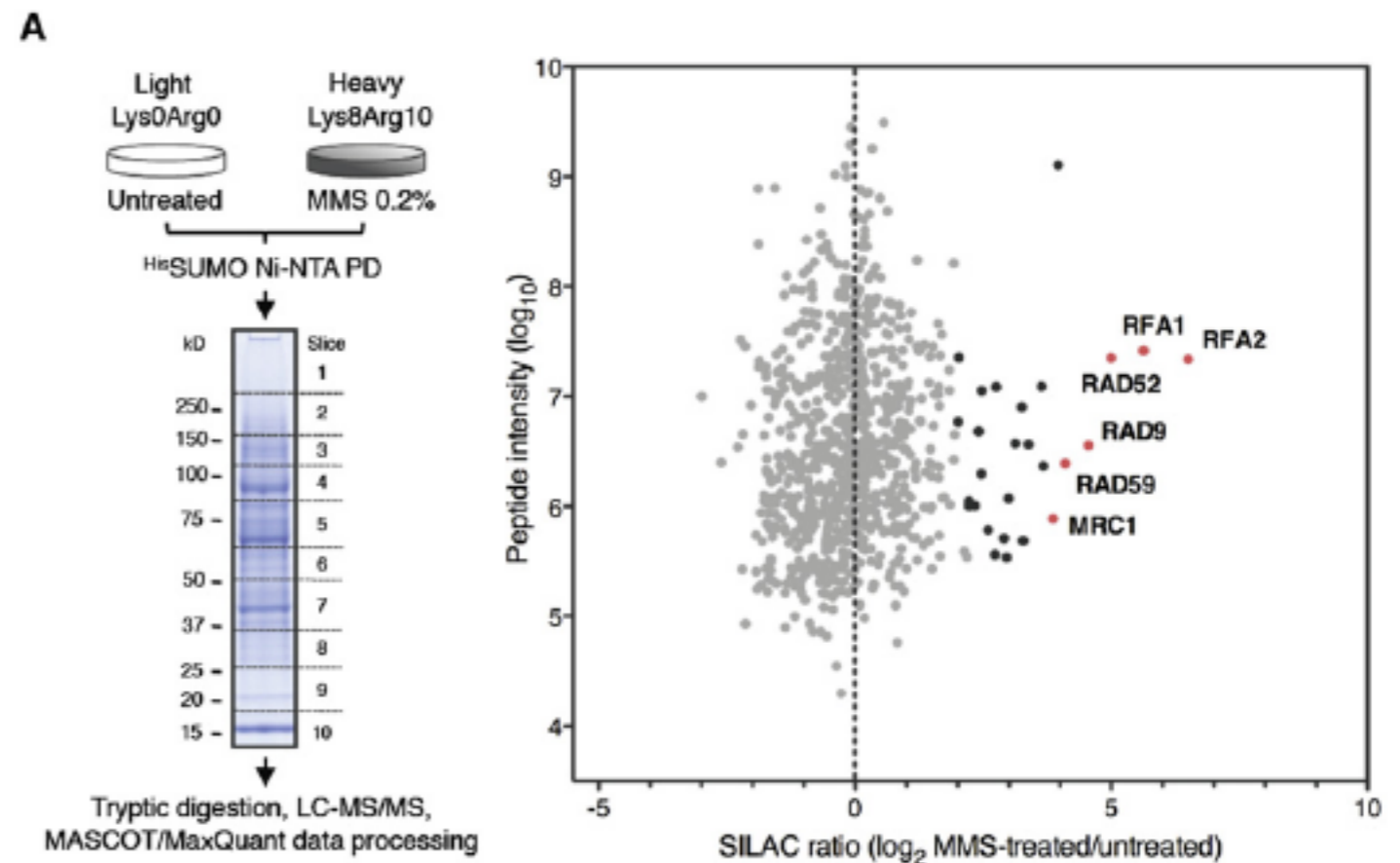
Ivan Psakhye¹ and Stefan Jentsch^{1,*}

¹Department of Molecular Cell Biology, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

*Correspondence: jentsch@biochem.mpg.de

<http://dx.doi.org/10.1016/j.cell.2012.10.021>

Using SILAC approaches, Psakhye and Jentsch showed that majority of HR proteins are Sumoylated upon DSBs induction.



How to study genome stability maintenance?
Step4: study the role of protein complex formation?

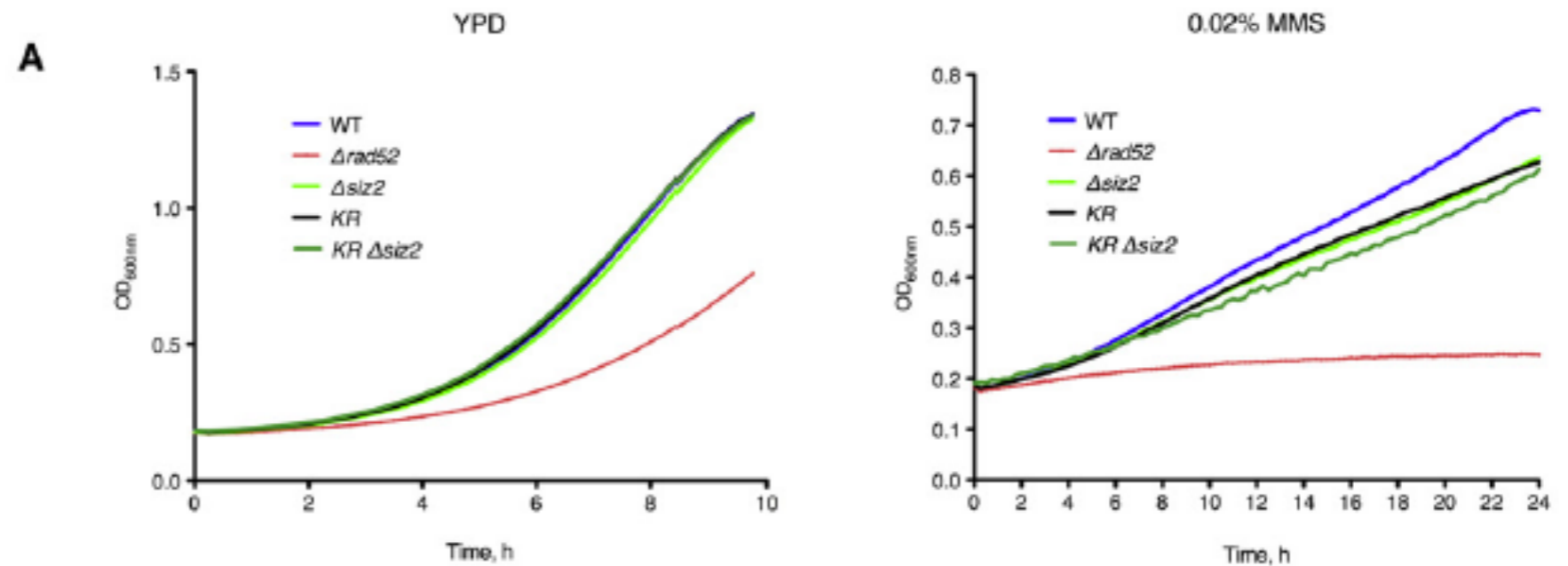
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This Sumo-SIM mediated interactions are trigger timely completion of HR.

How to study genome stability maintenance? Step4: study the role of protein complex formation?

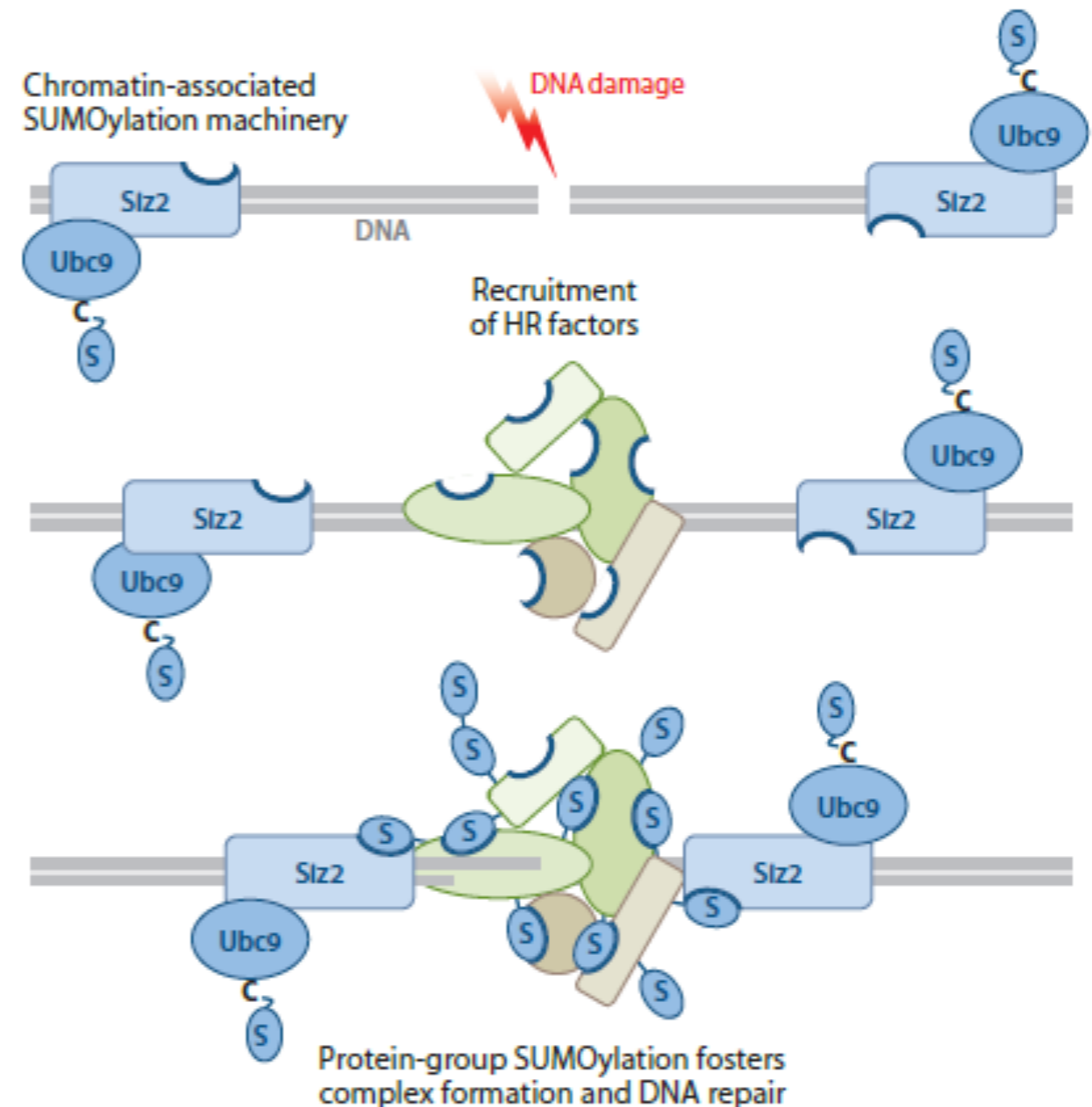
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How to study genome stability maintenance? Step5: study the molecular mechanisms by the means of structural biology

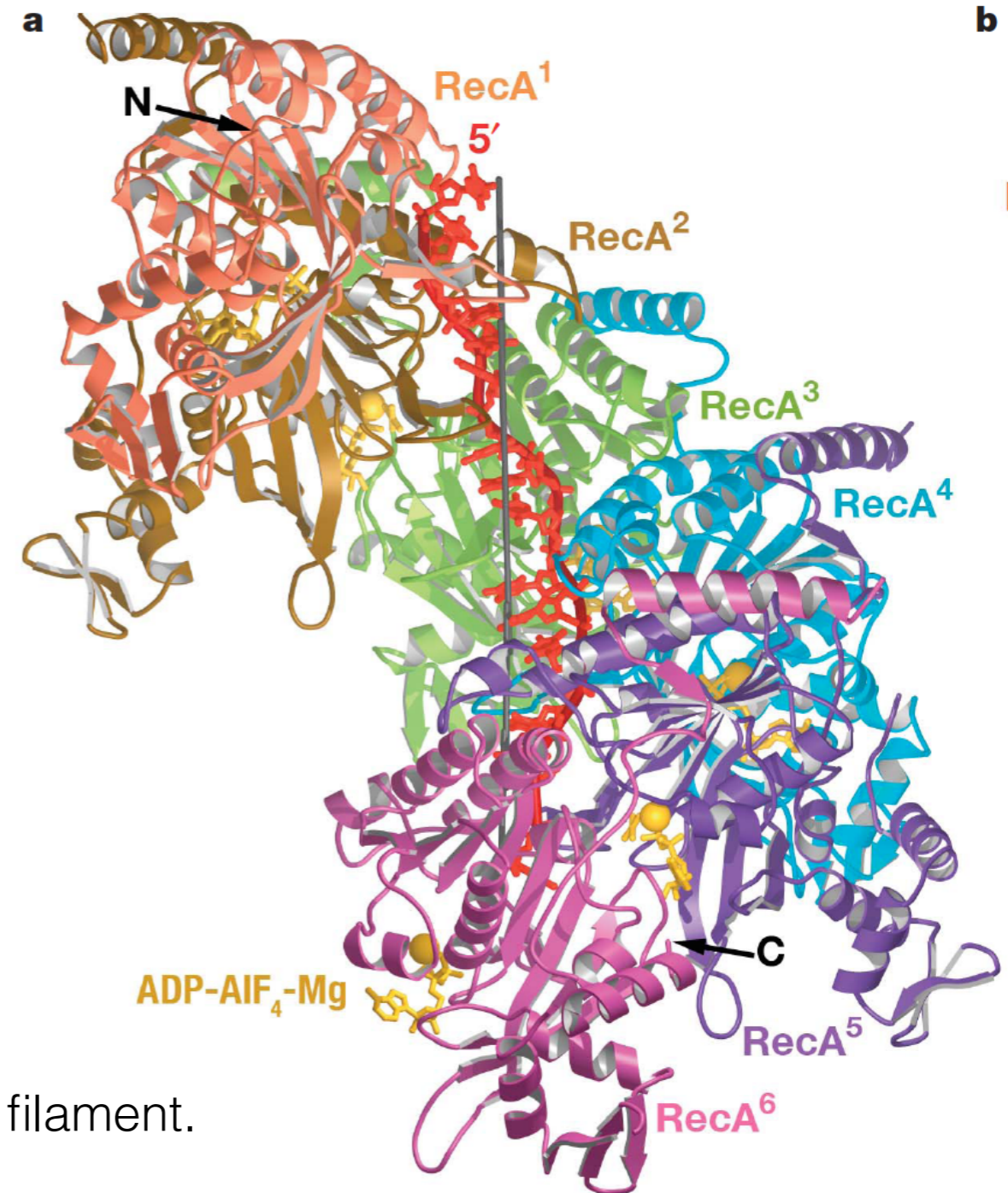
Vol 453 | 22 May 2008 | doi:10.1038/nature06971

nature

ARTICLES

Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures

Zhucheng Chen^{1,3}, Haijuan Yang¹ & Nikola P. Pavletich^{1,2}



Crystal structure of presynaptic filament.

How to study genome stability maintenance? Step5: study the molecular mechanisms by the means of structural biology

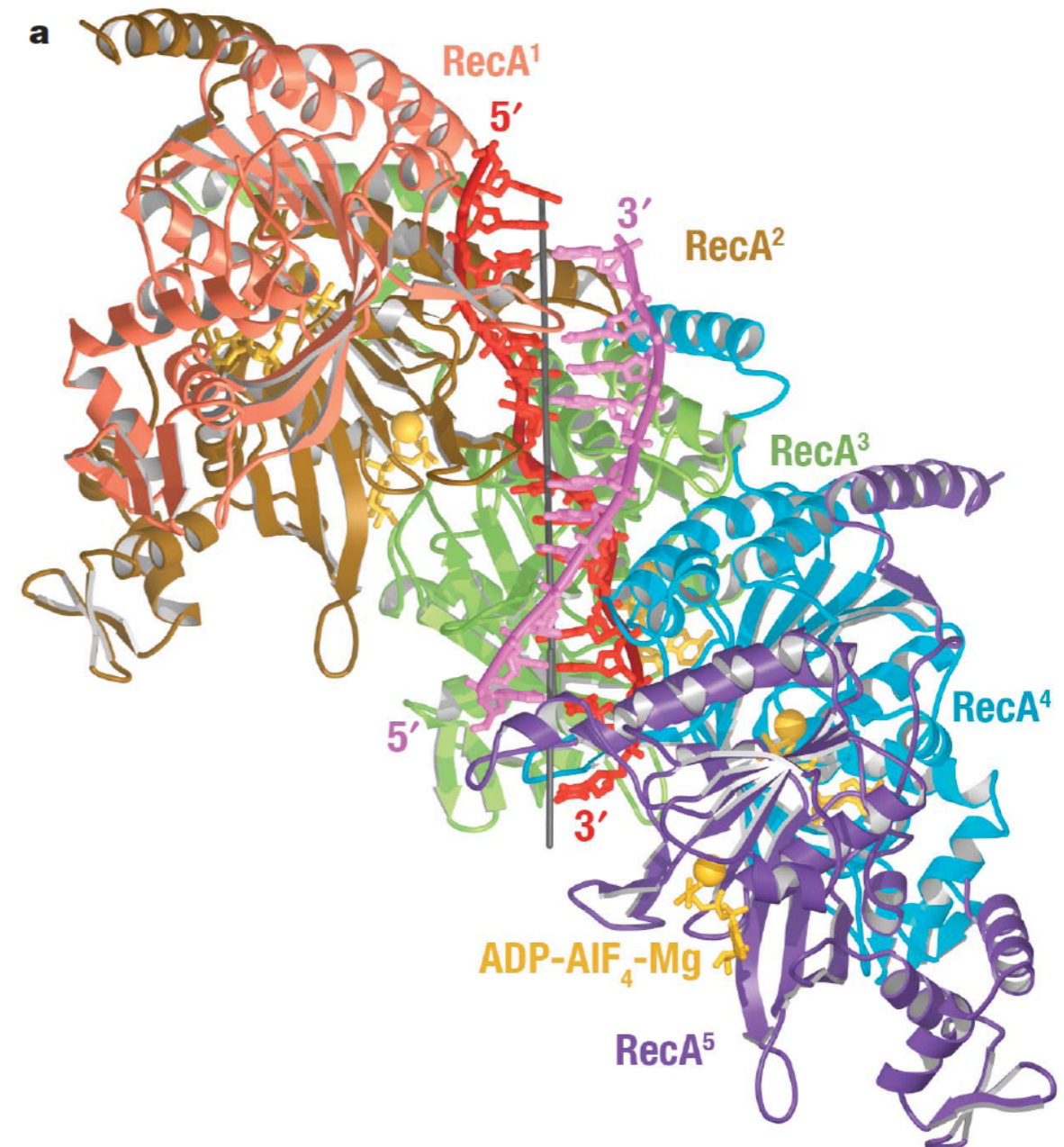
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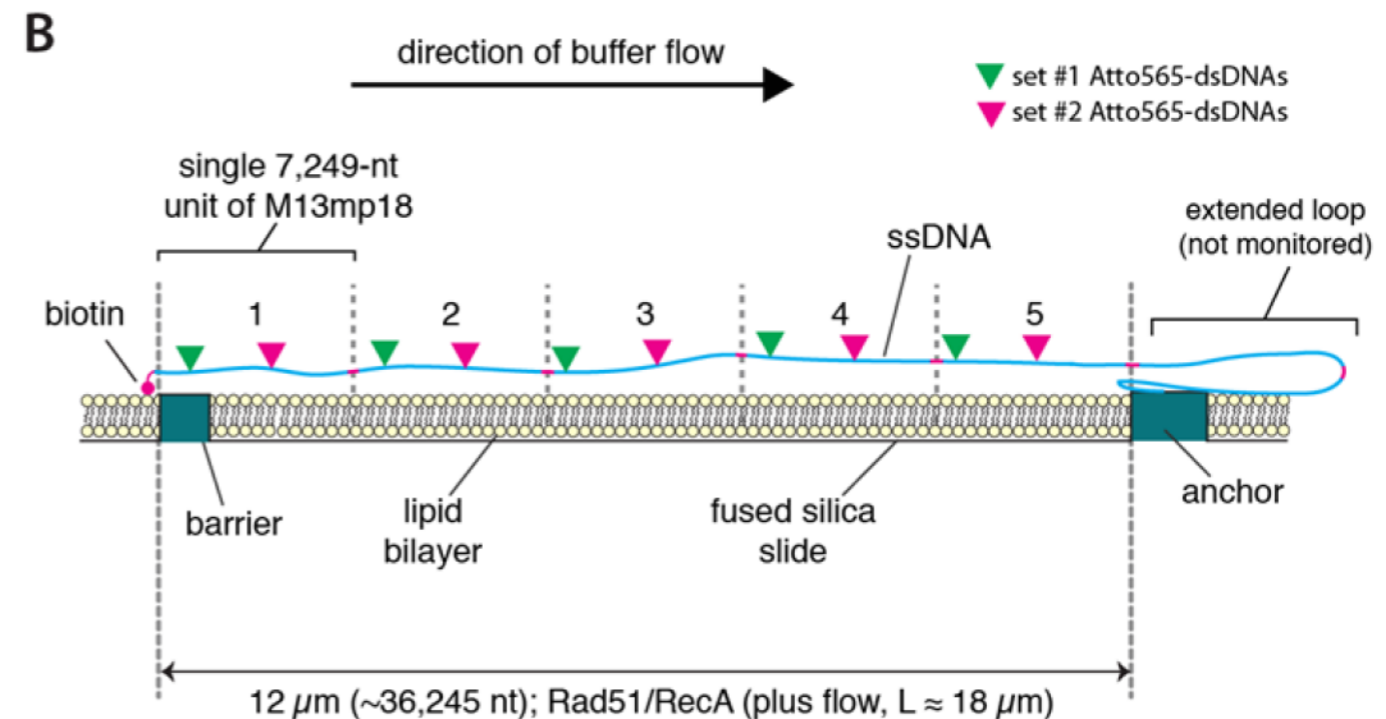
By comparing the two structure a detailed, molecular mechanism of the strand exchange reaction can be inferred.

How to study genome stability maintenance?
Step6: study the molecular mechanisms by the means of single-molecule techniques.

DNA RECOMBINATION

Base triplet stepping by the Rad51/RecA family of recombinases

Ja Yil Lee,¹ Tsuyoshi Terakawa,^{1,2*} Zhi Qi,^{1*} Justin B. Steinfeld,¹ Sy Redding,^{3†}
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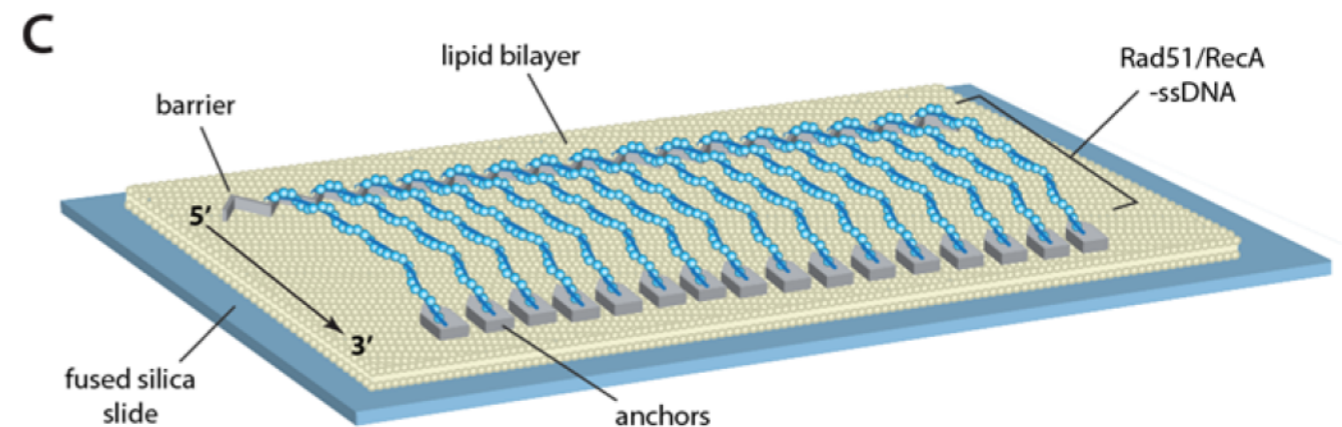
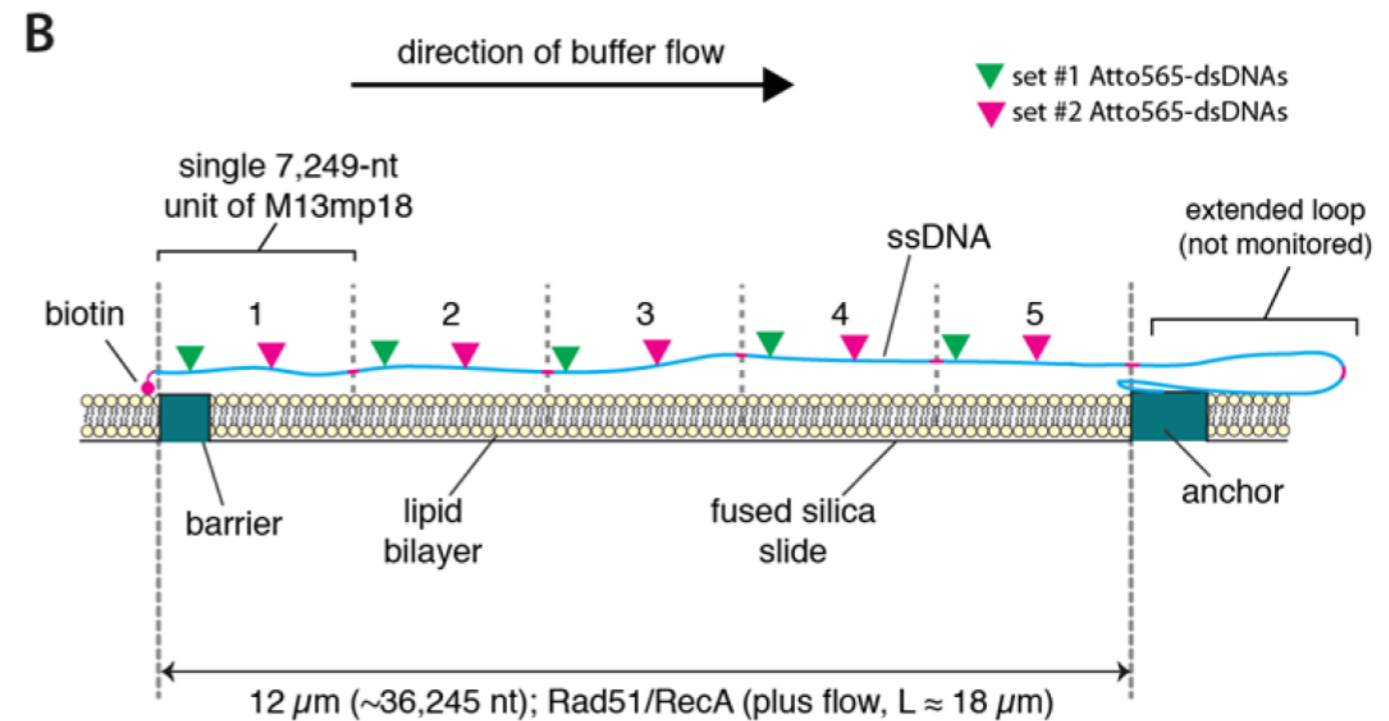


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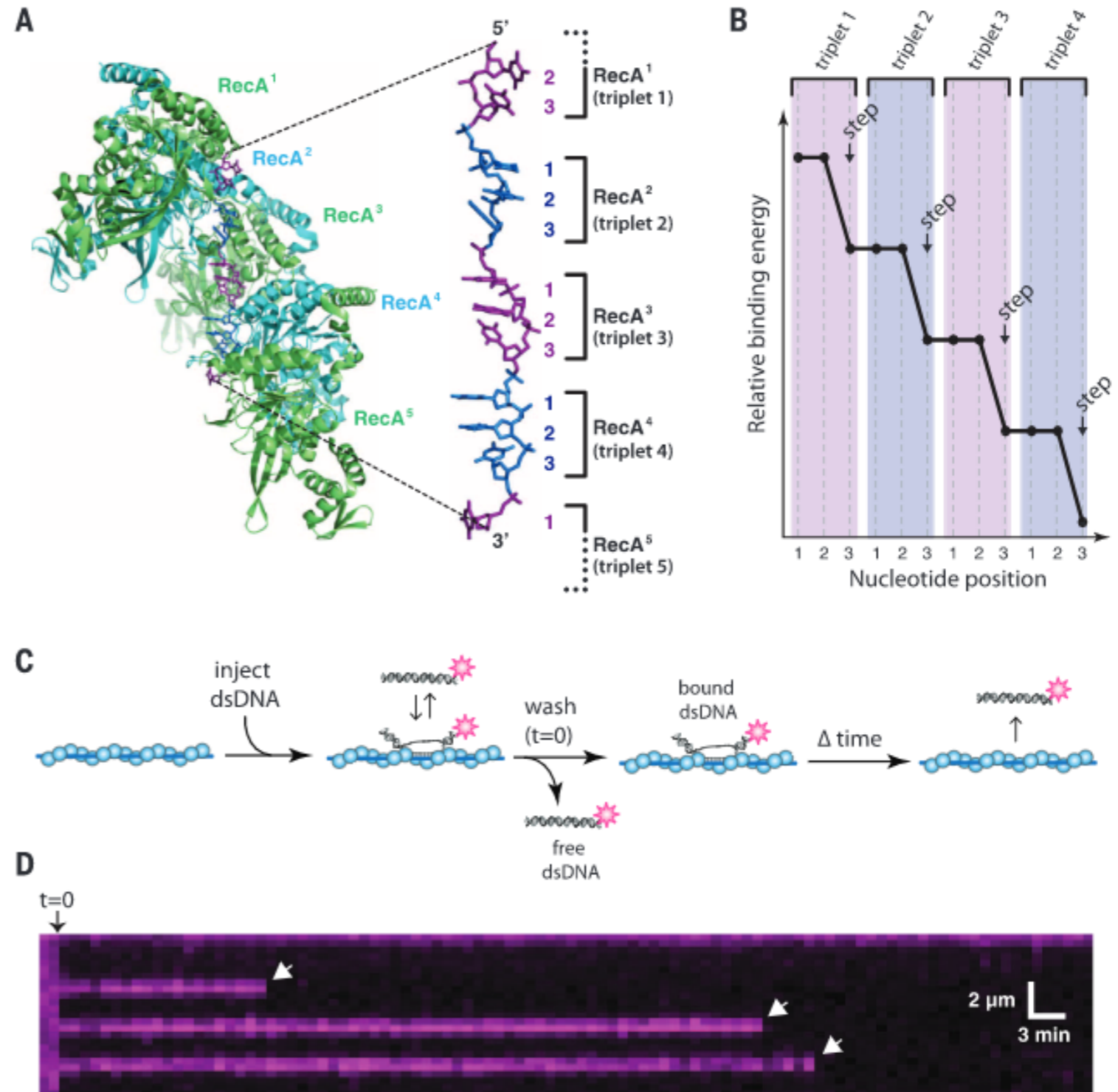


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The techniques must be combined, in order to get a full picture of the pathway

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Use whatever technique at hand that will help you answer your scientific question

Summary

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Majority of factors responsible for maintaining genome stability acts in complexes, let those be dynamic or not

