Of complexes and the maintenance of genome stability

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2. What are the challenges to the genome stability?

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4. How do cells maintain the genome stability?

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

What is the maintenance of genome stability?

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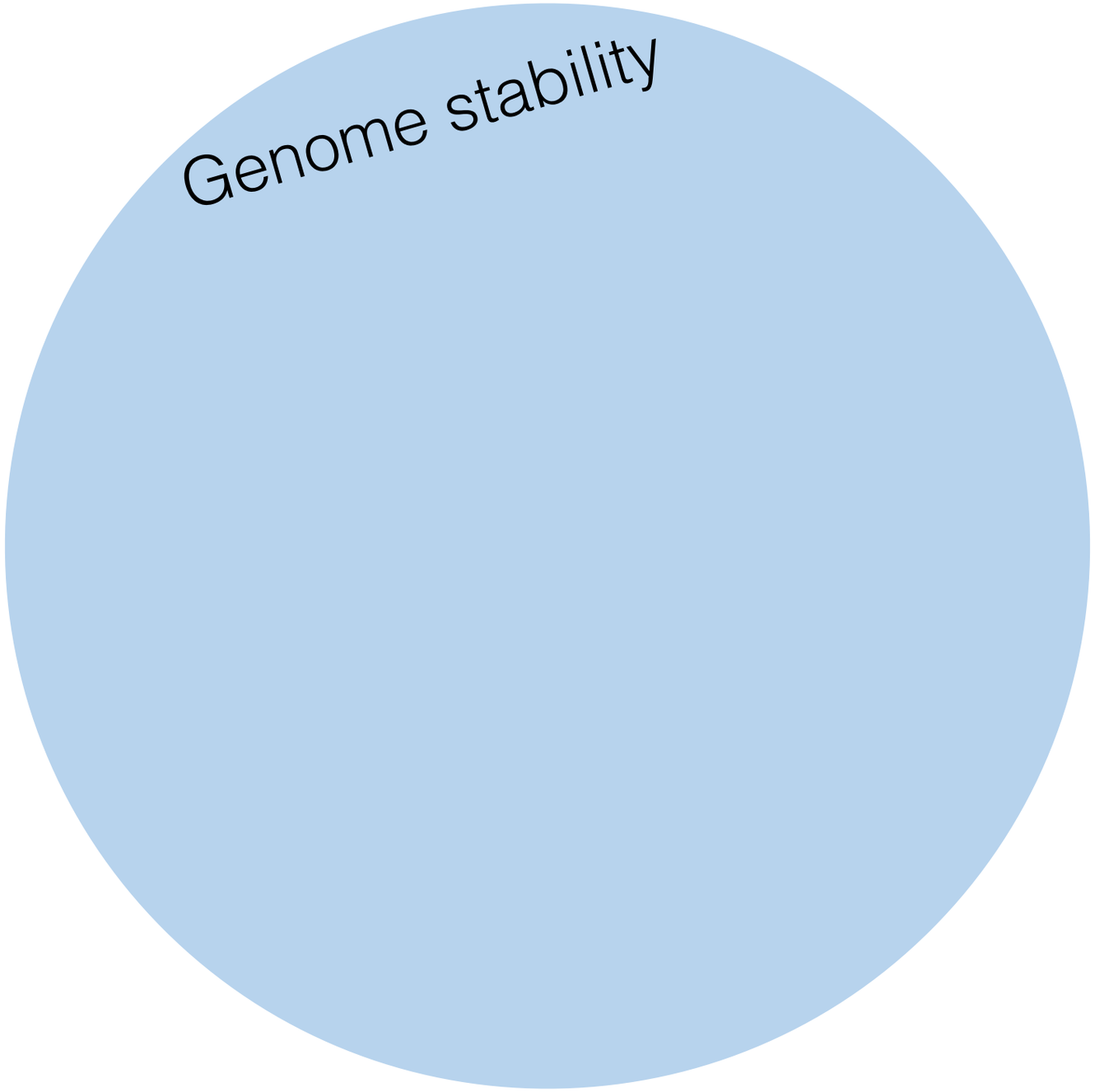

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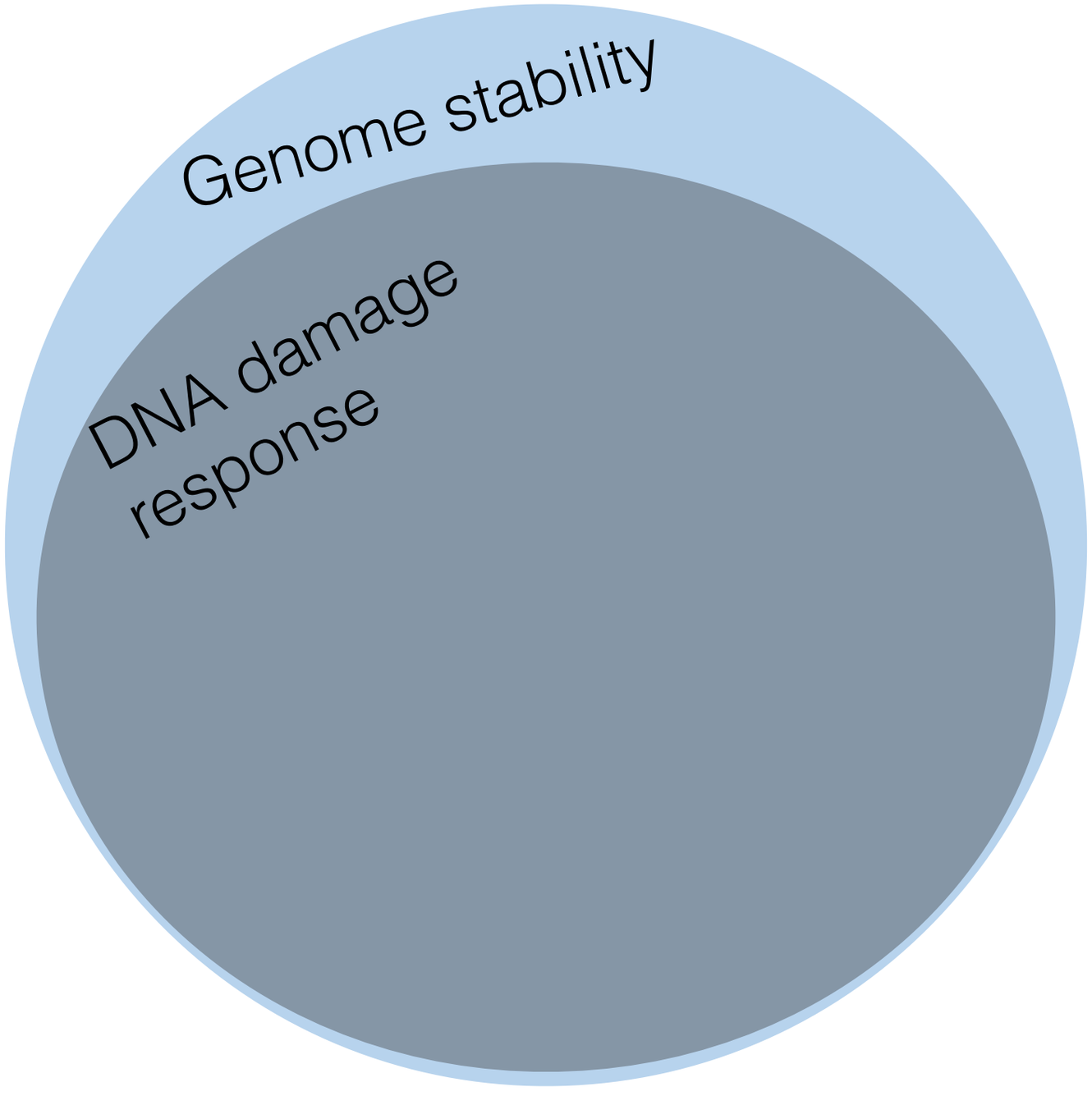

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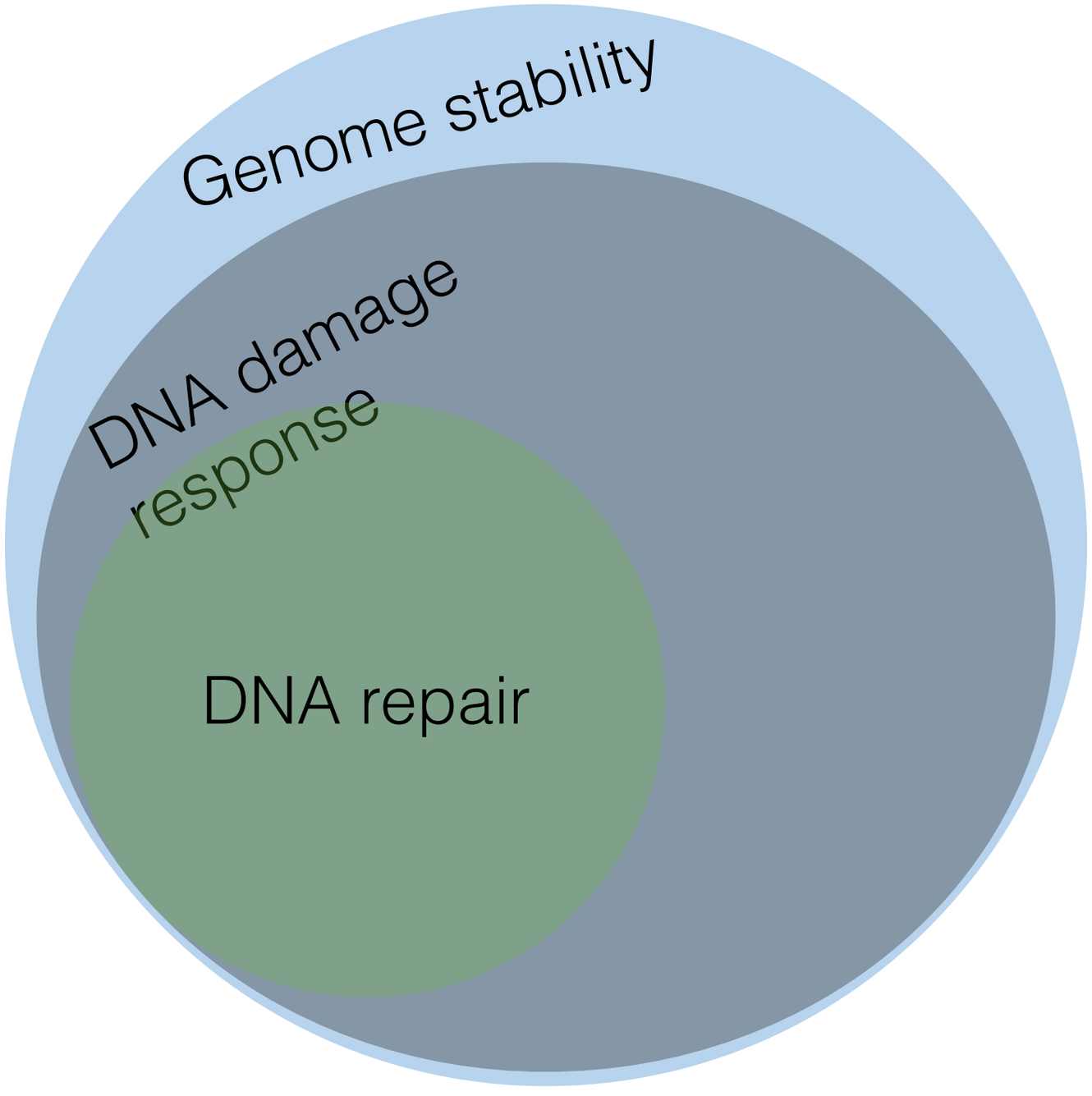

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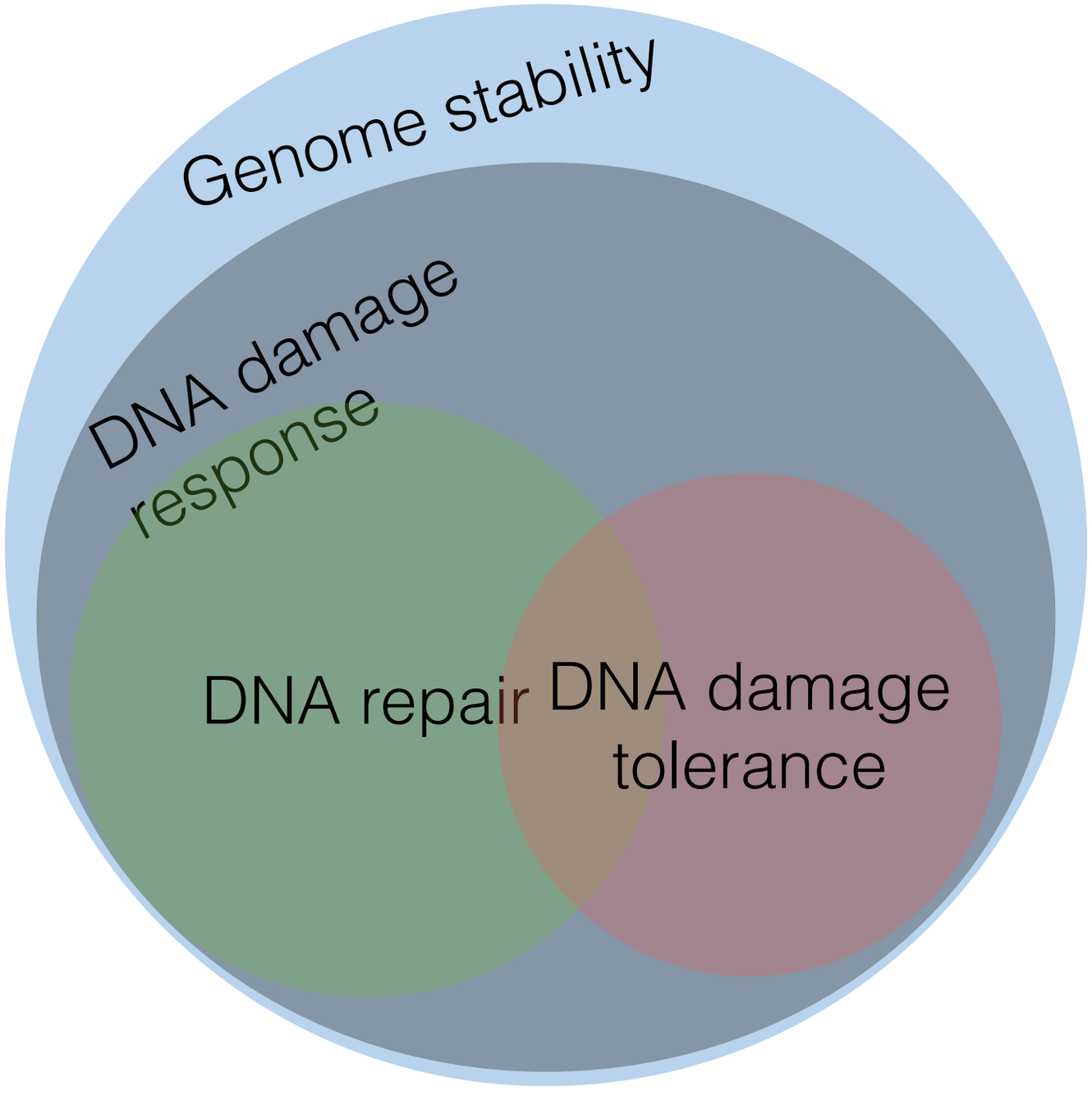

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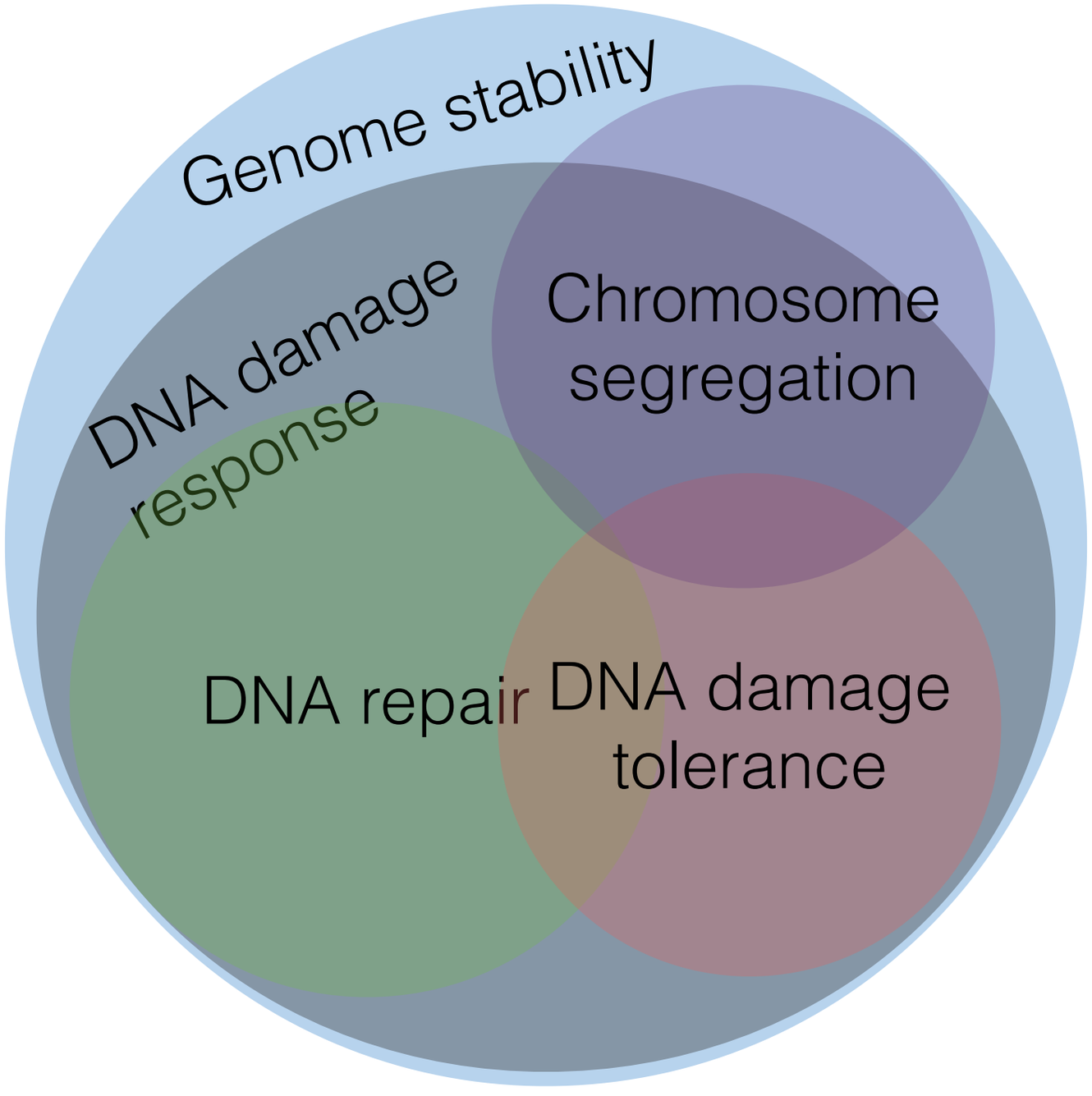

What is the maintenance of genome stability?

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

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

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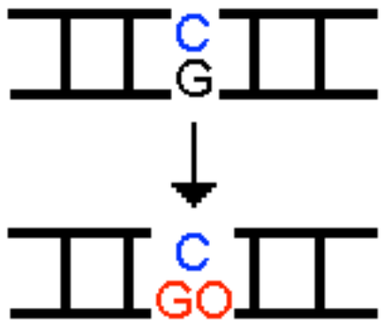
(Carr 1999)



Oxidative Damage

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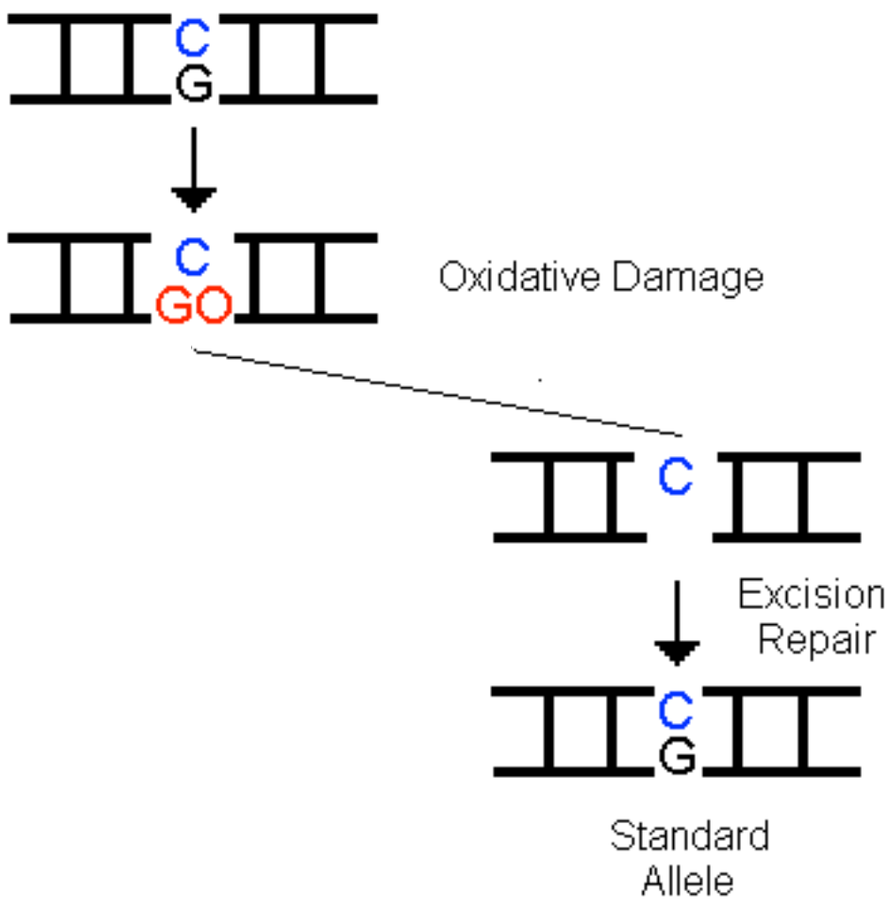

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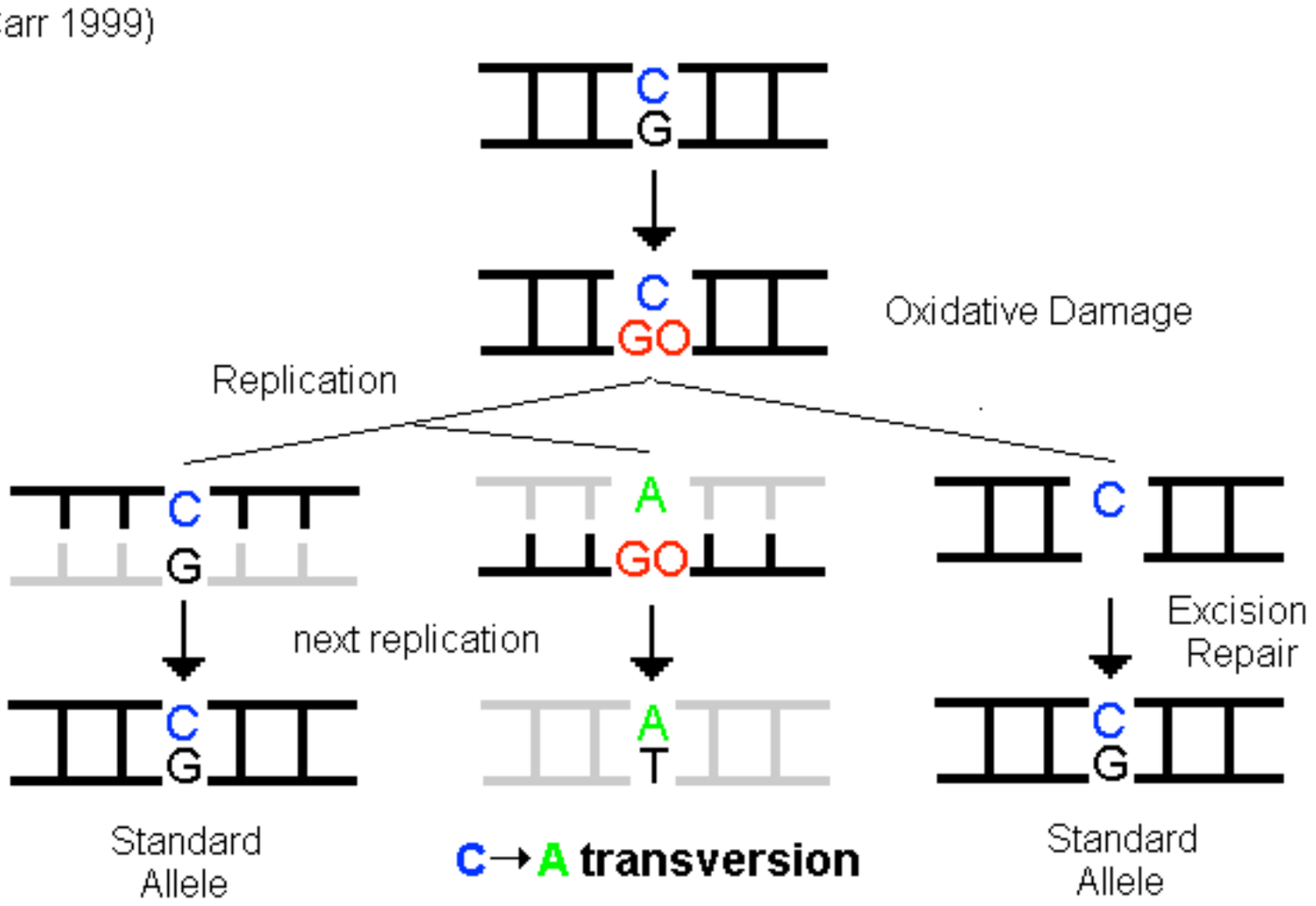

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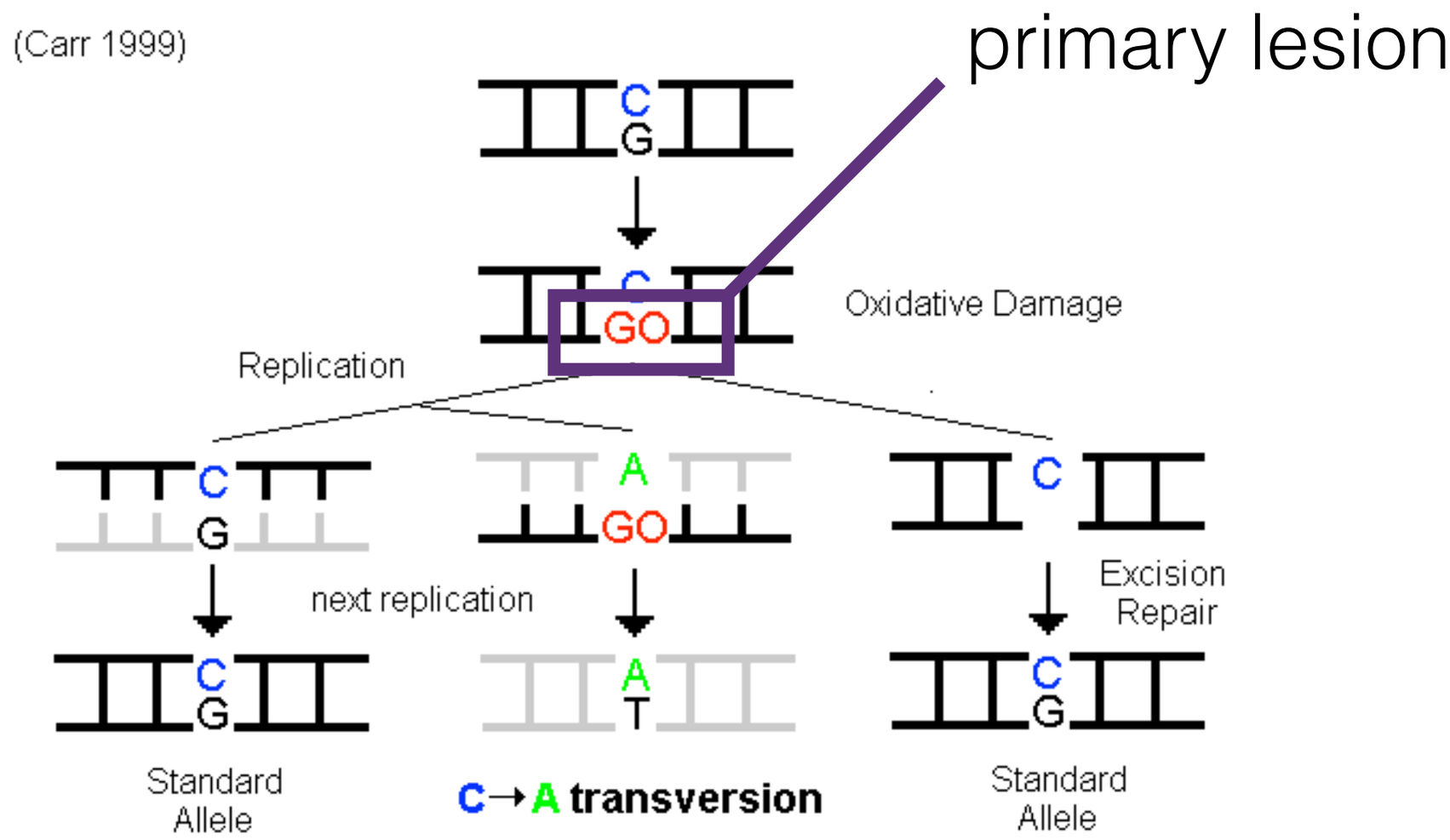

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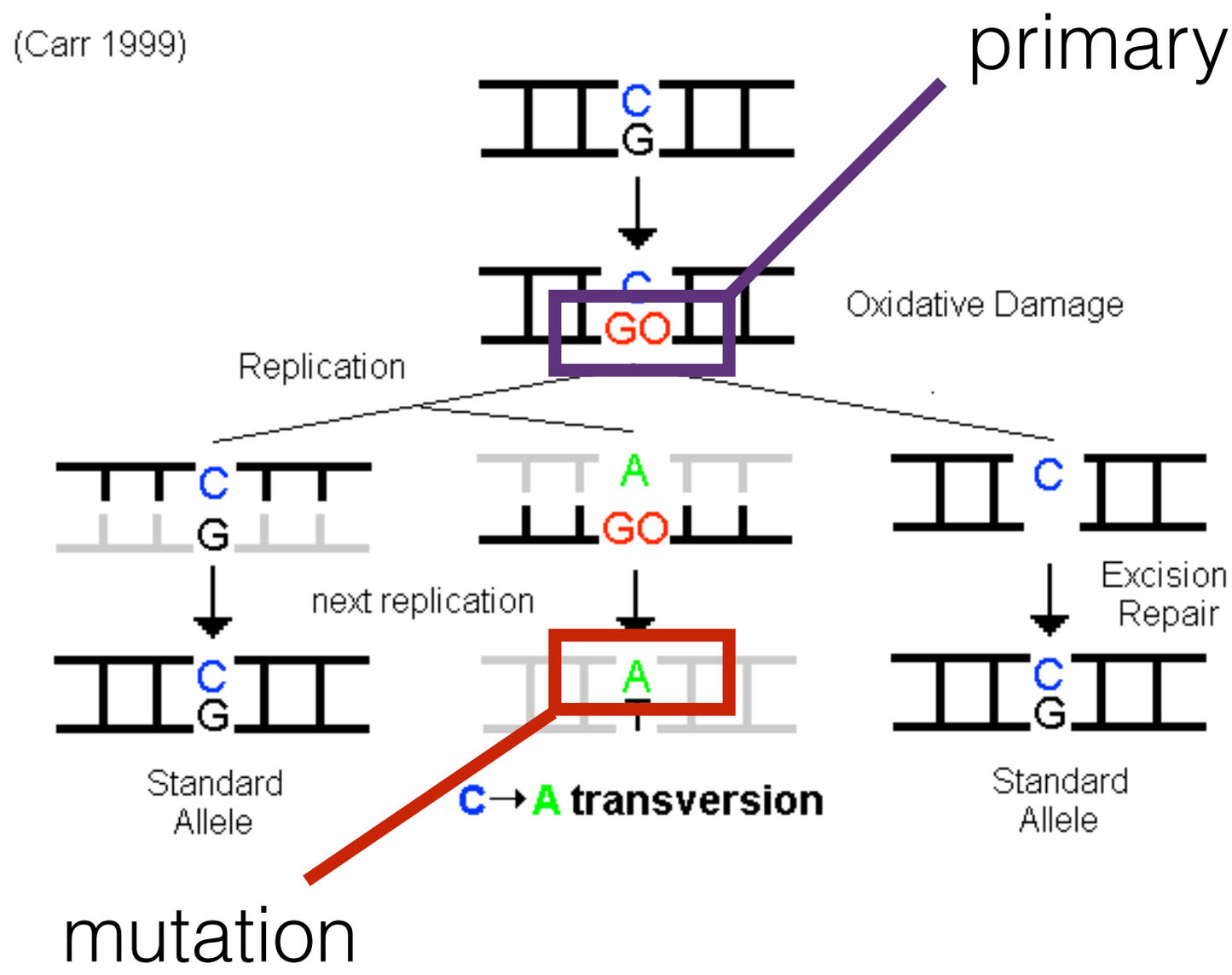

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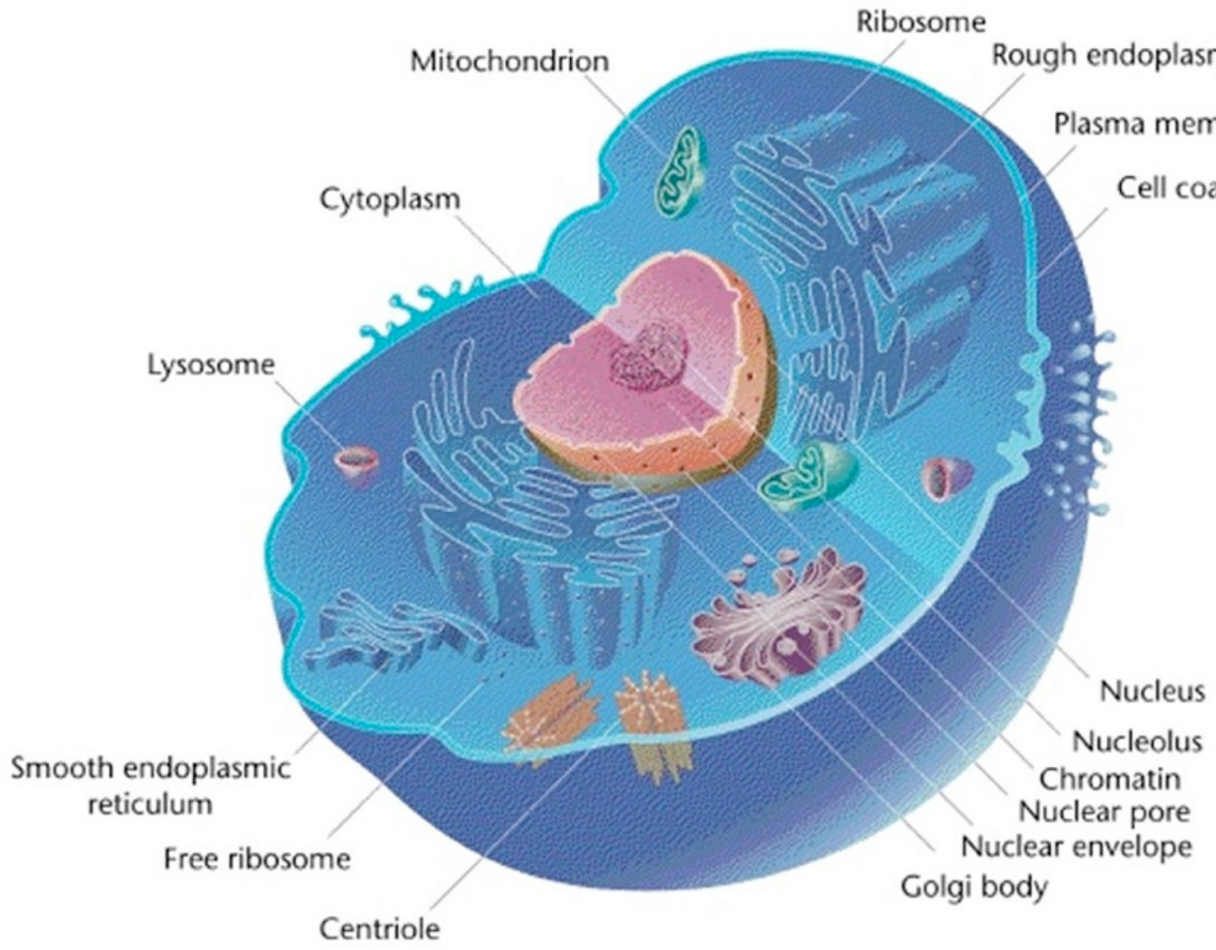


What are the challenges to genome stability?

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

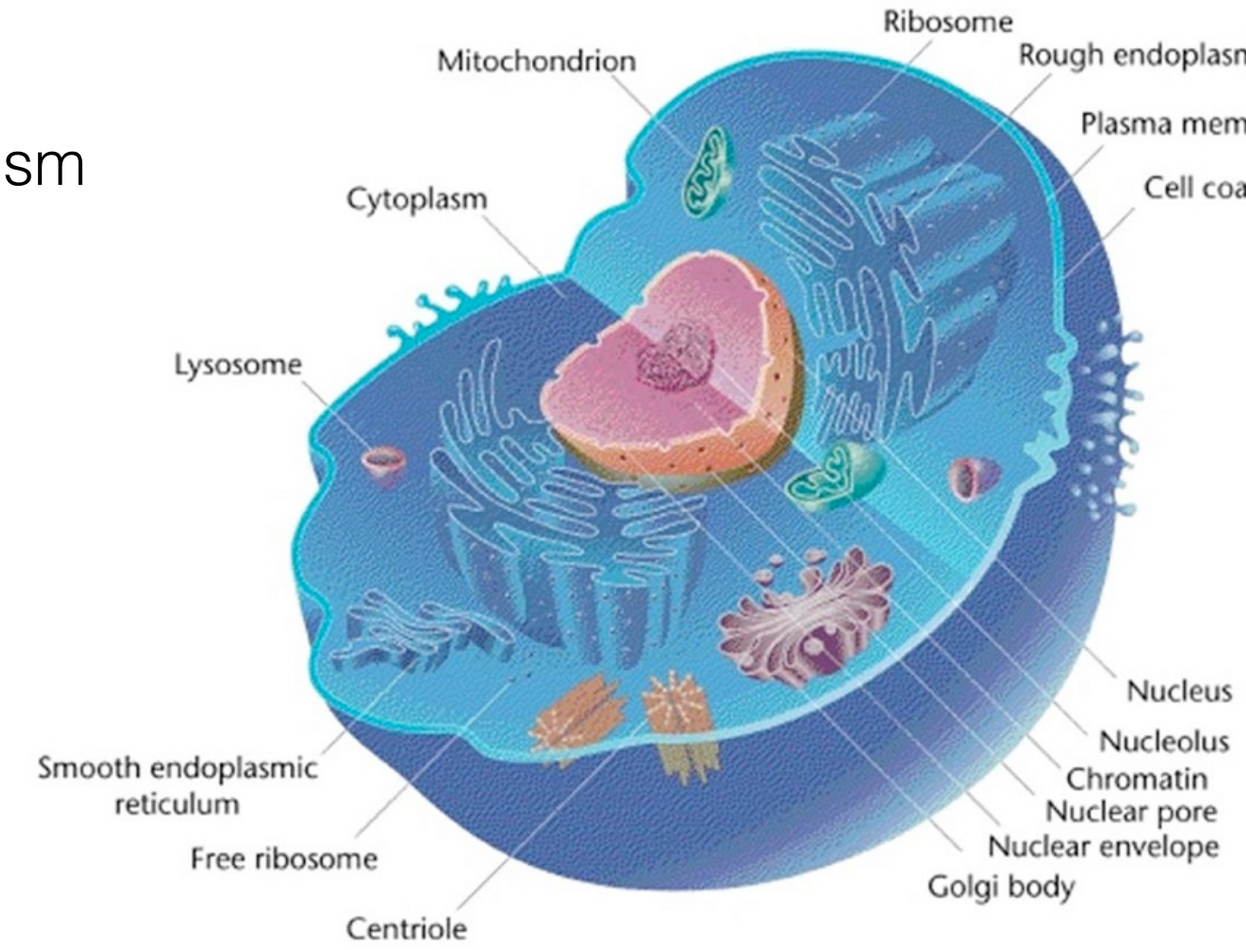
Endogenous

Cellular metabolism

DNA replication

Transcription

Spontaneous modification of the DNA



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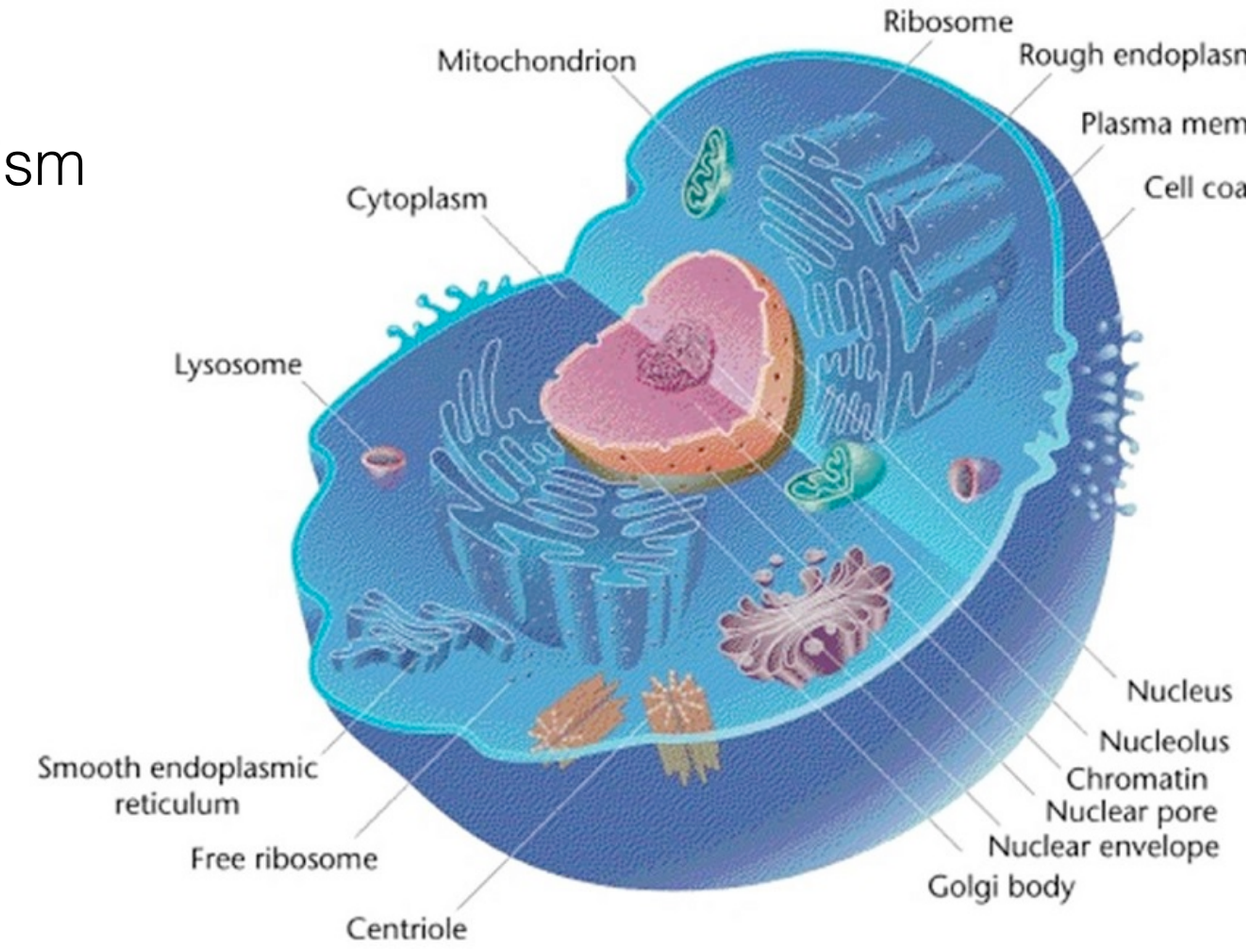


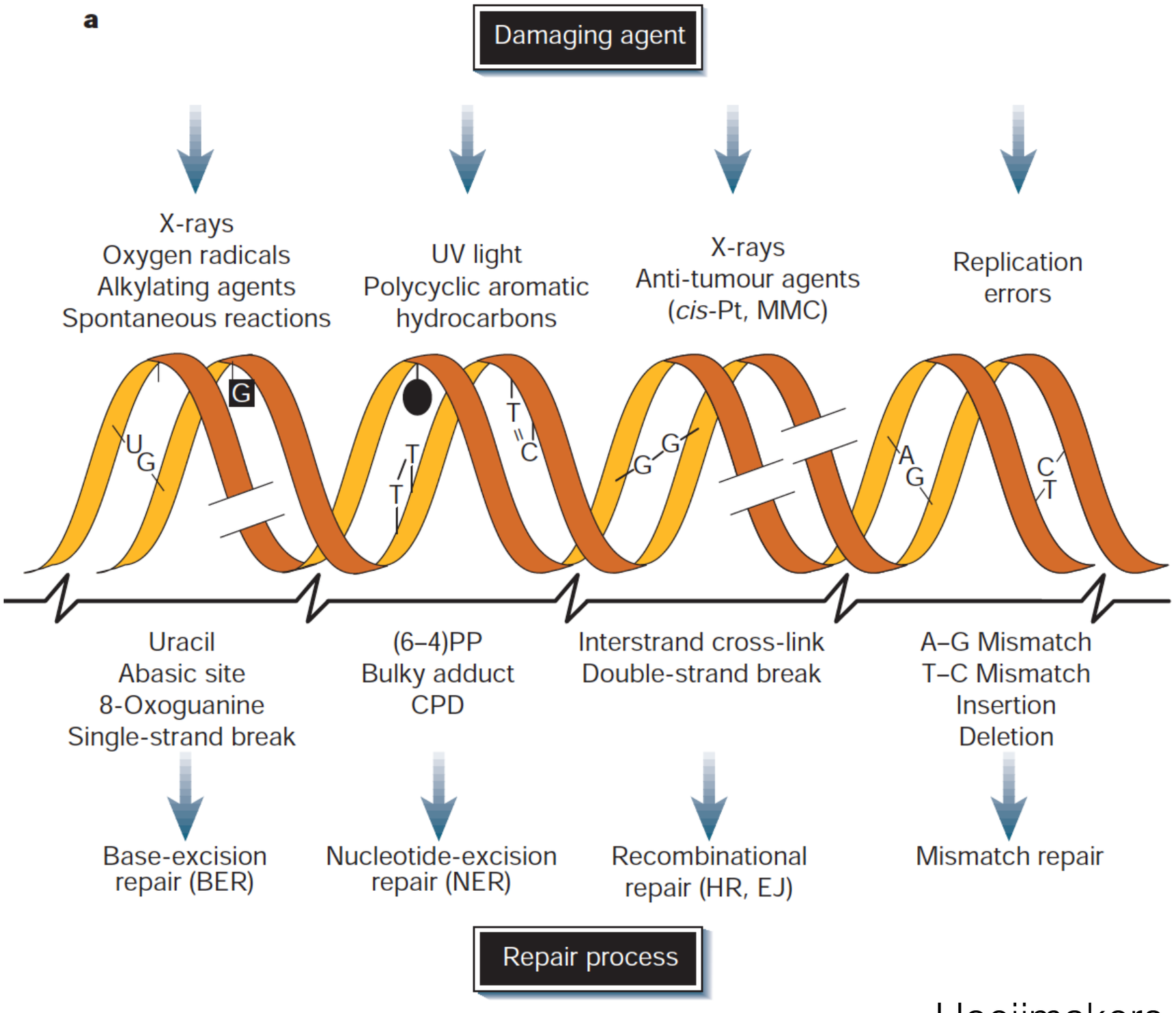
Exogenous

Radiation

Diet

Stress

What are the challenges to genome stability?



Hoeijmakers, 2001

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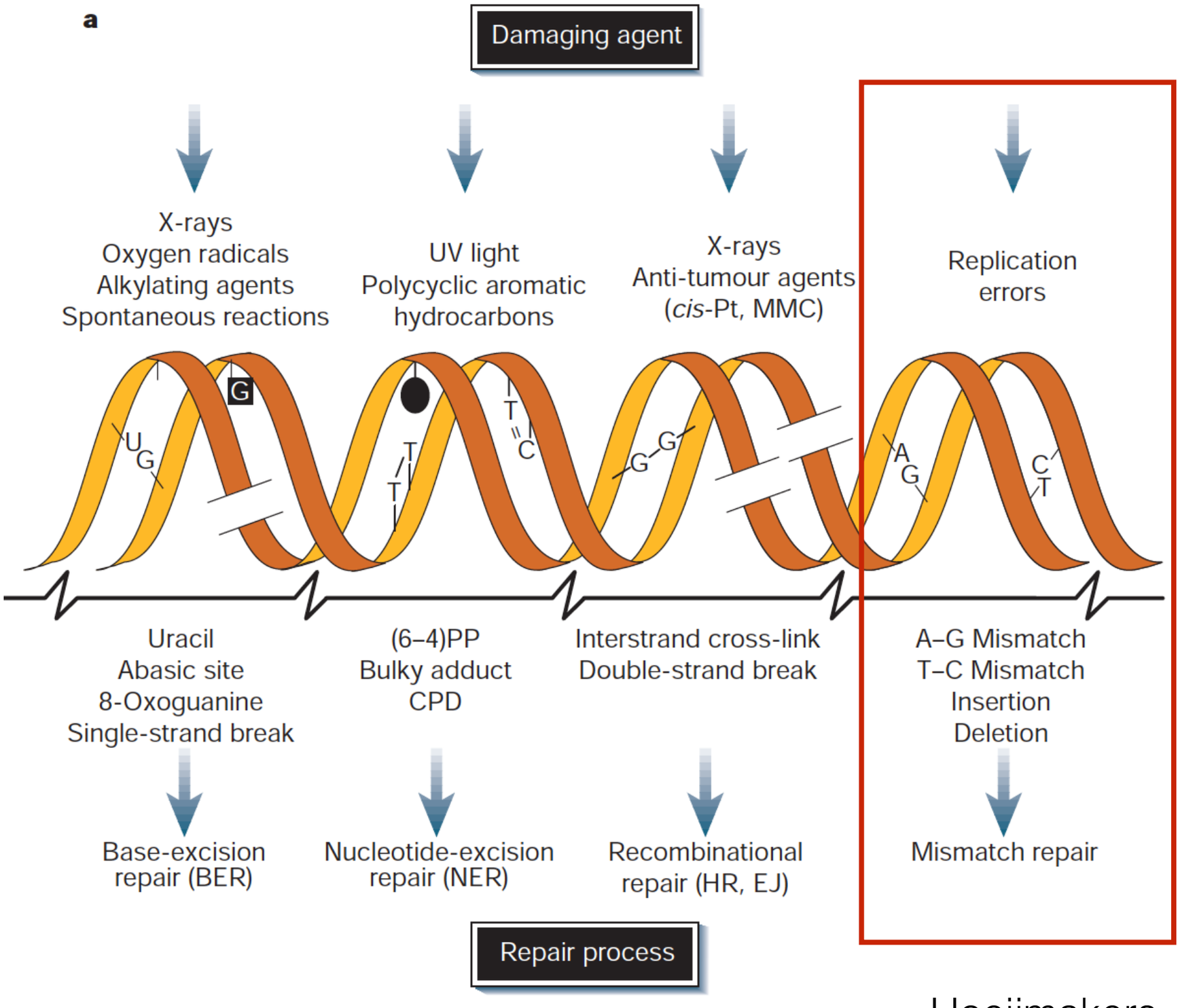

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What are the challenges to genome stability?

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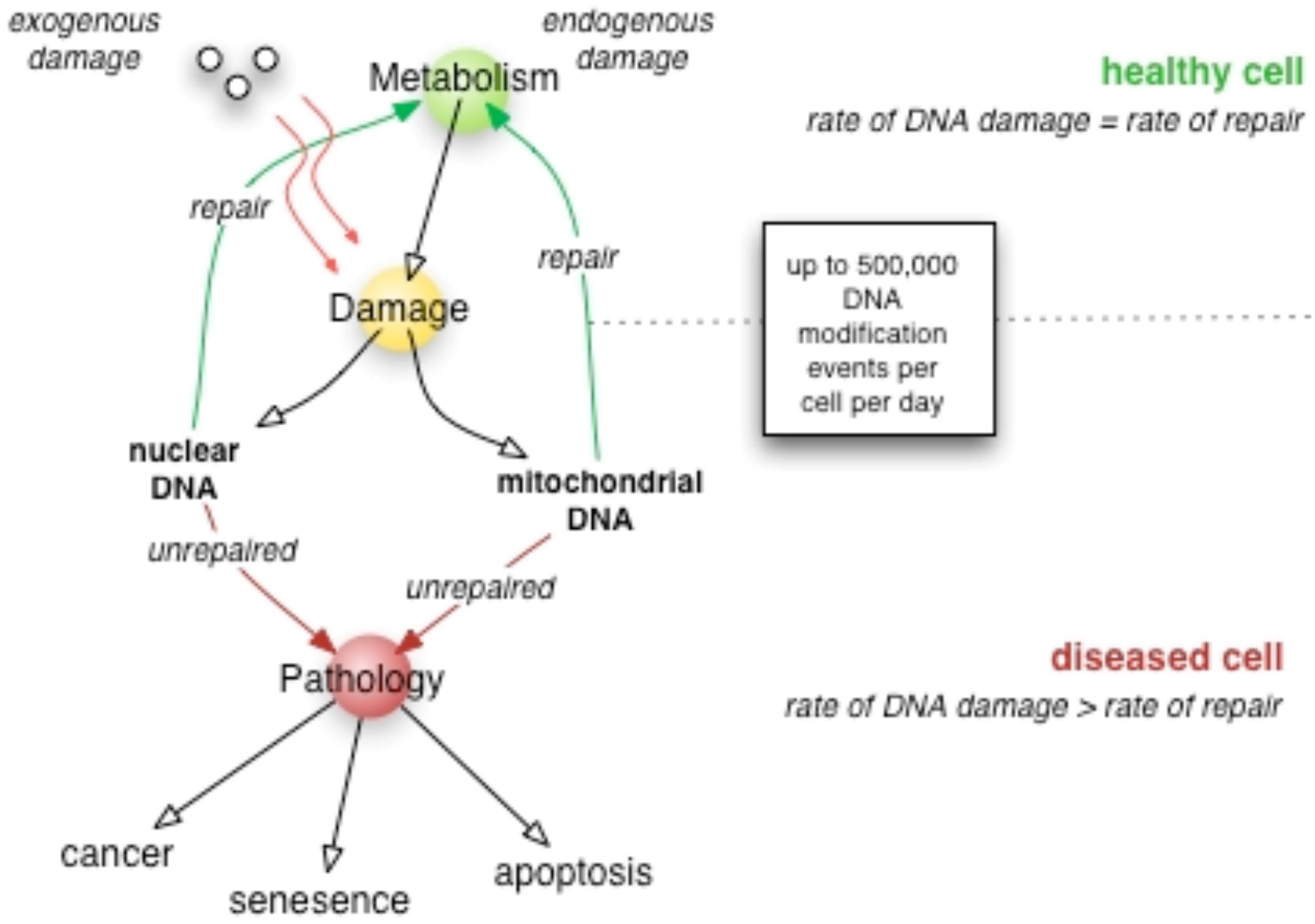
Even-though, historically, exogenous DNA damage was considered to be the prime cause of mutagenesis, recently, as the methodology has progressed, the cellular DNA metabolism pathways (replication and transcription) are being recognised as the more prevalent cause of mutations.

What are the challenges to genome stability?

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



Transient summary I

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

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Cells are continuously exposed to wide variety of DNA damage

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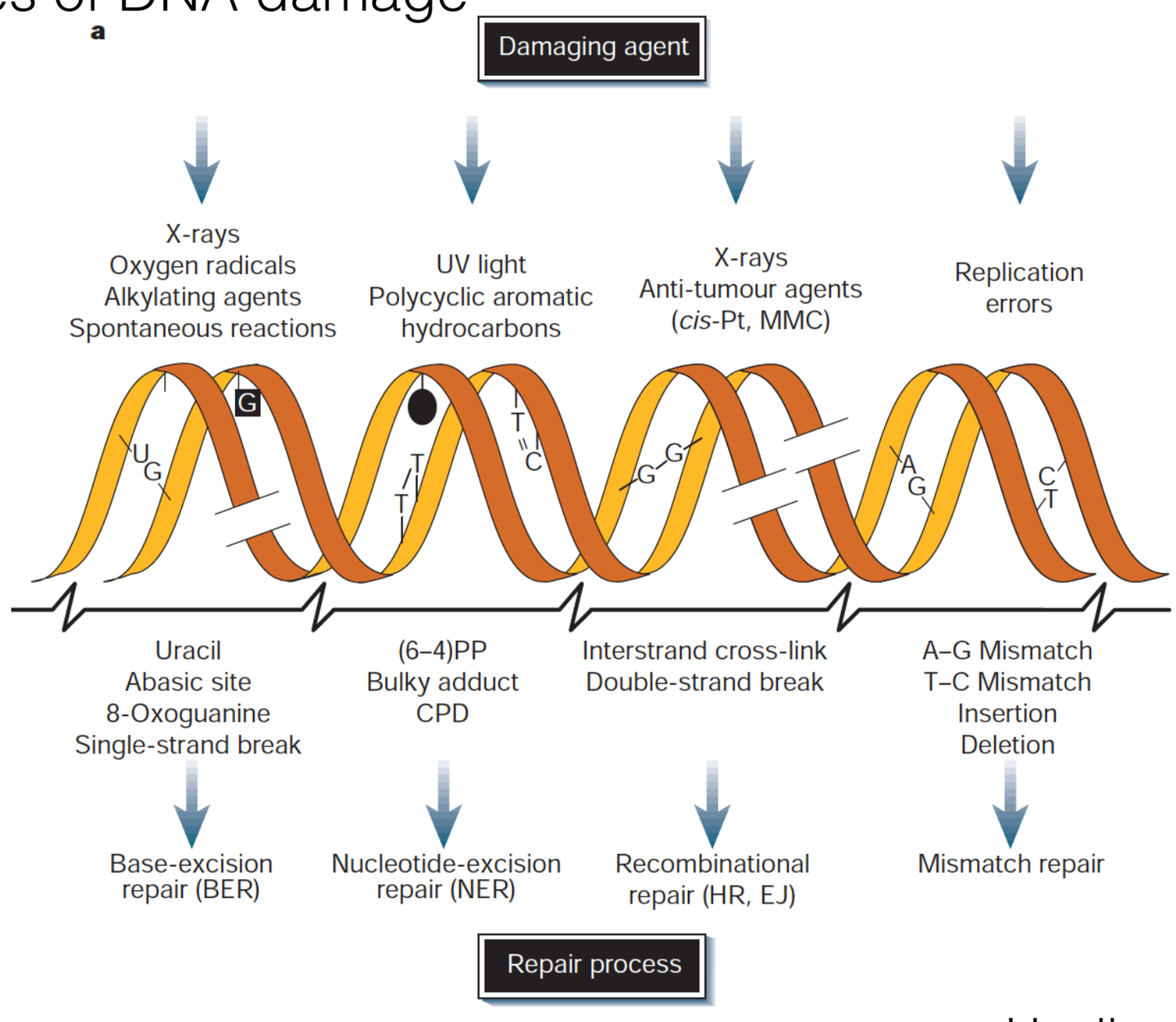
Cells are continuously exposed to wide variety of DNA damage

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

How do cells know genome stability has been compromised?

The challenges

- different types of DNA damage



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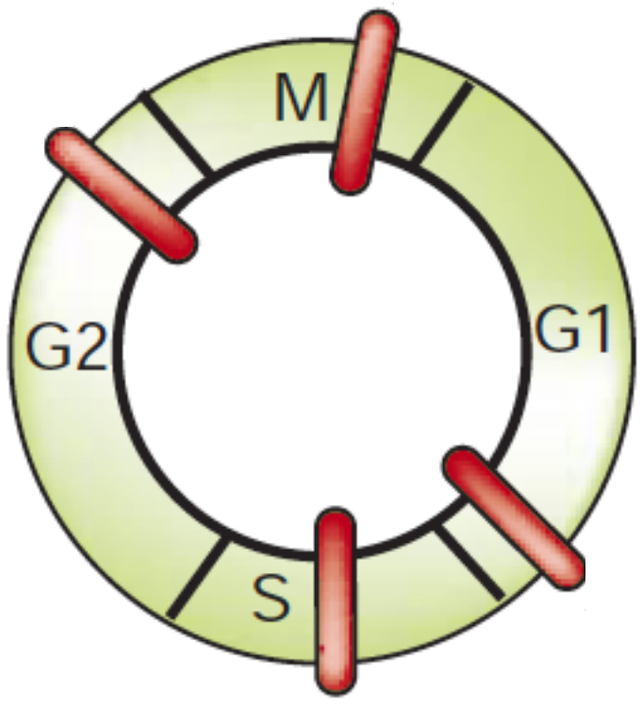
- cell-cycle stage



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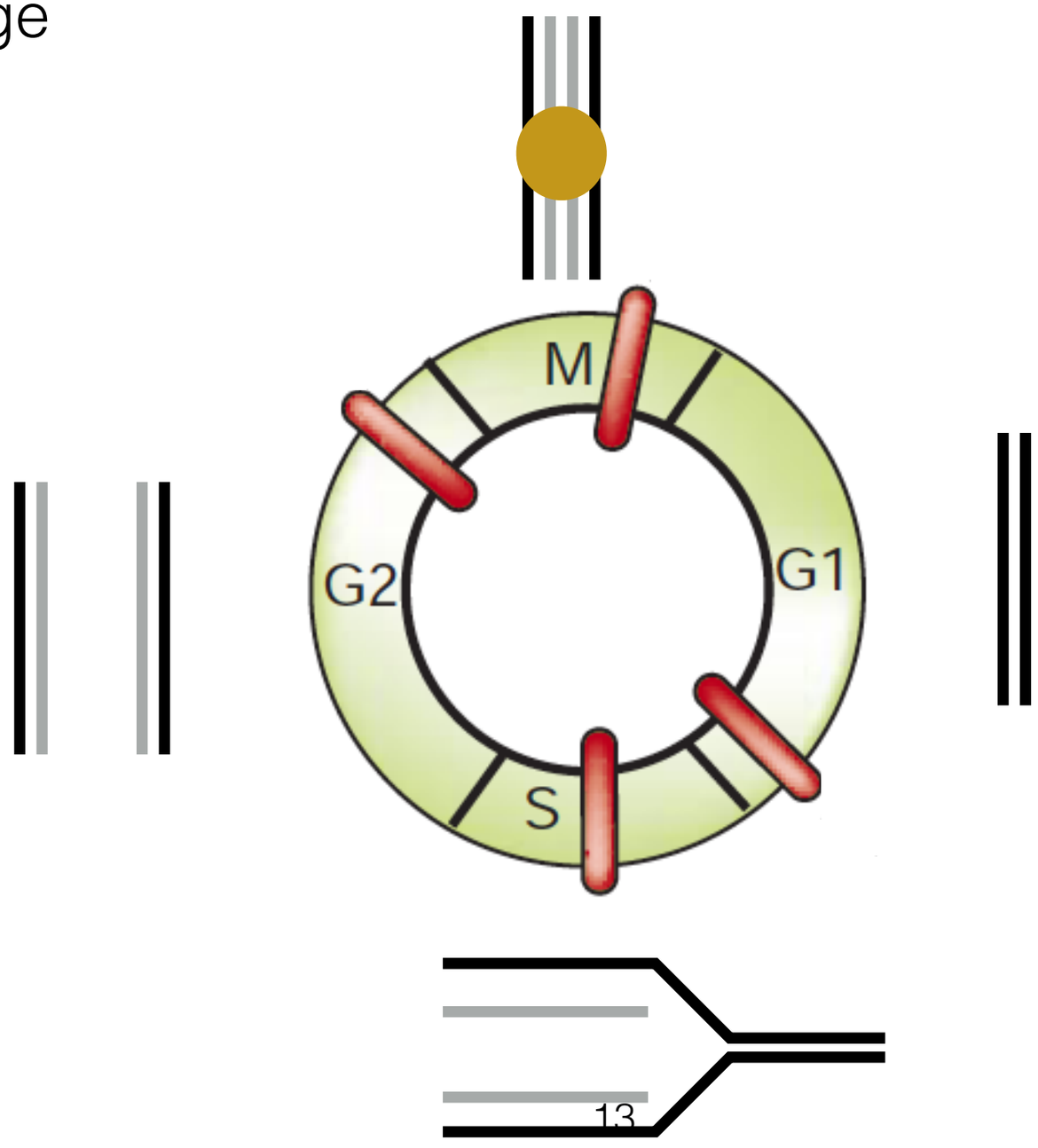


Hoeijmakers, 2001

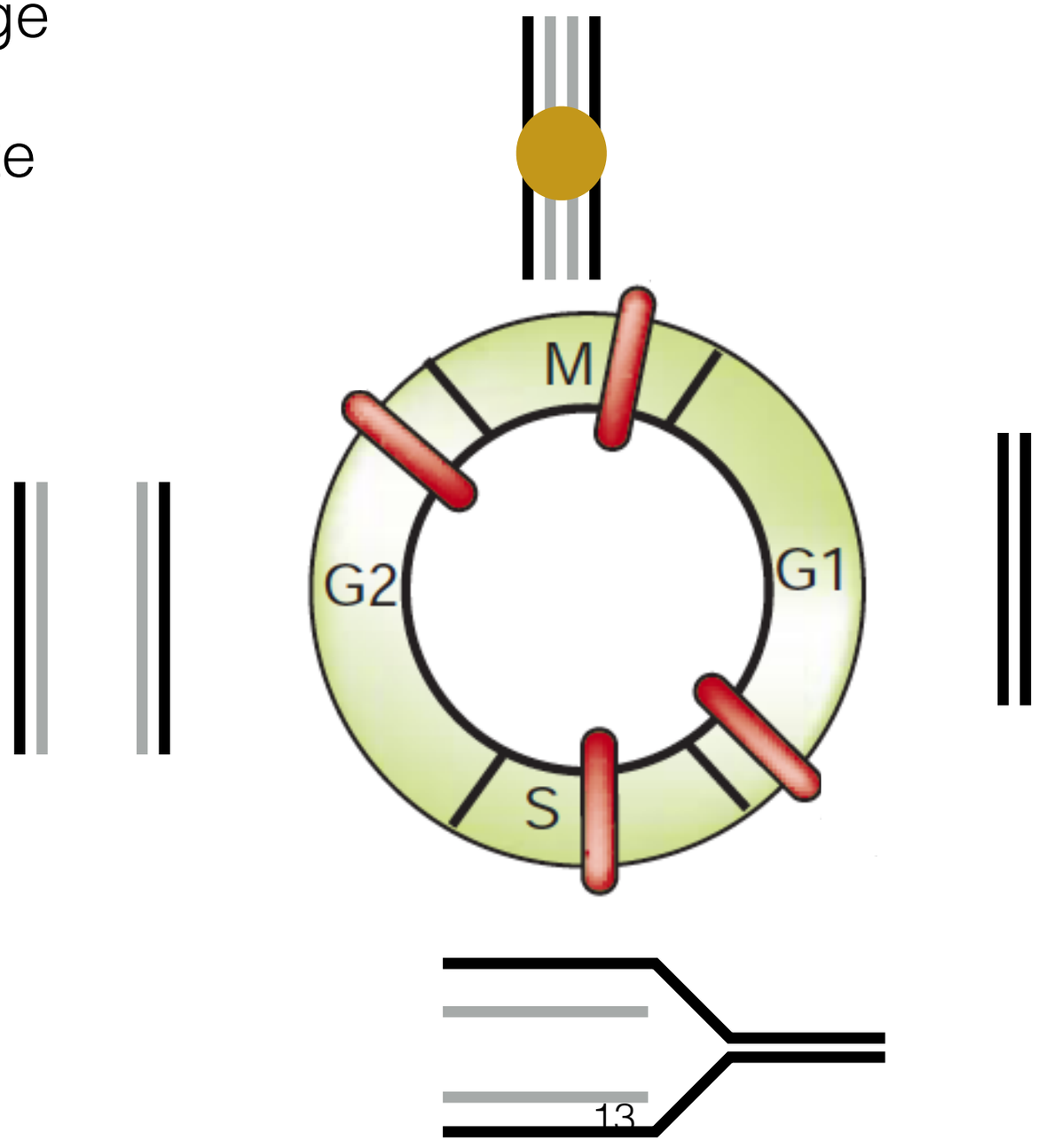
How do cells know genome stability has been compromised?

The challenges

- different types of DNA damage

- cell-cycle stage

- metabolic state



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

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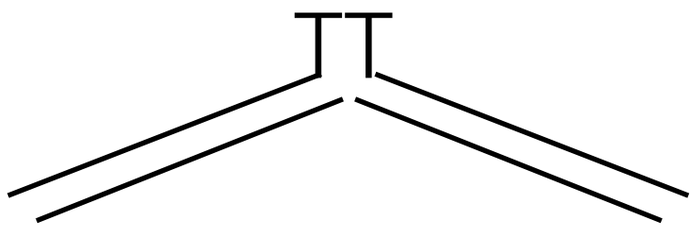

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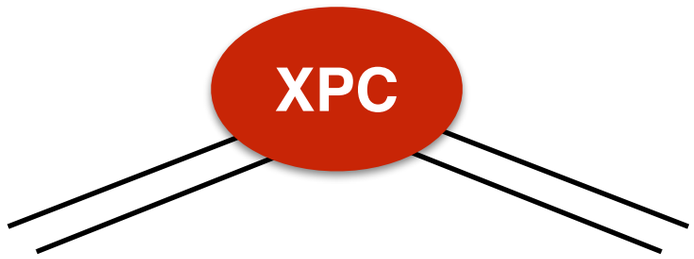

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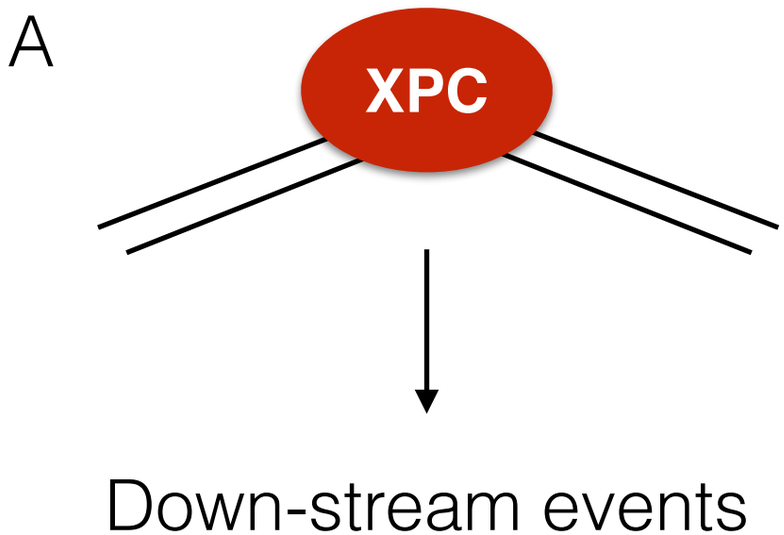

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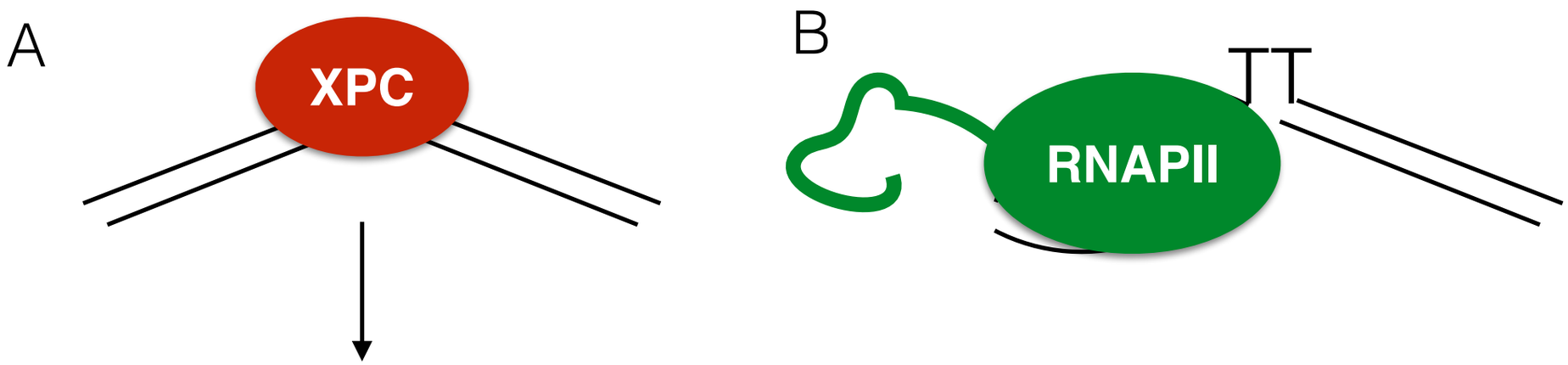

Down-stream events

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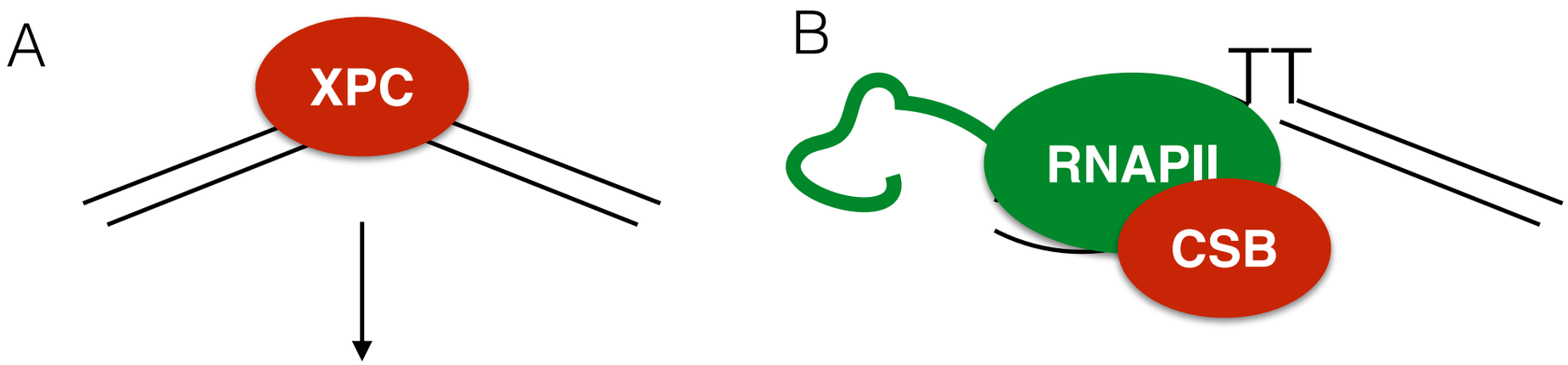

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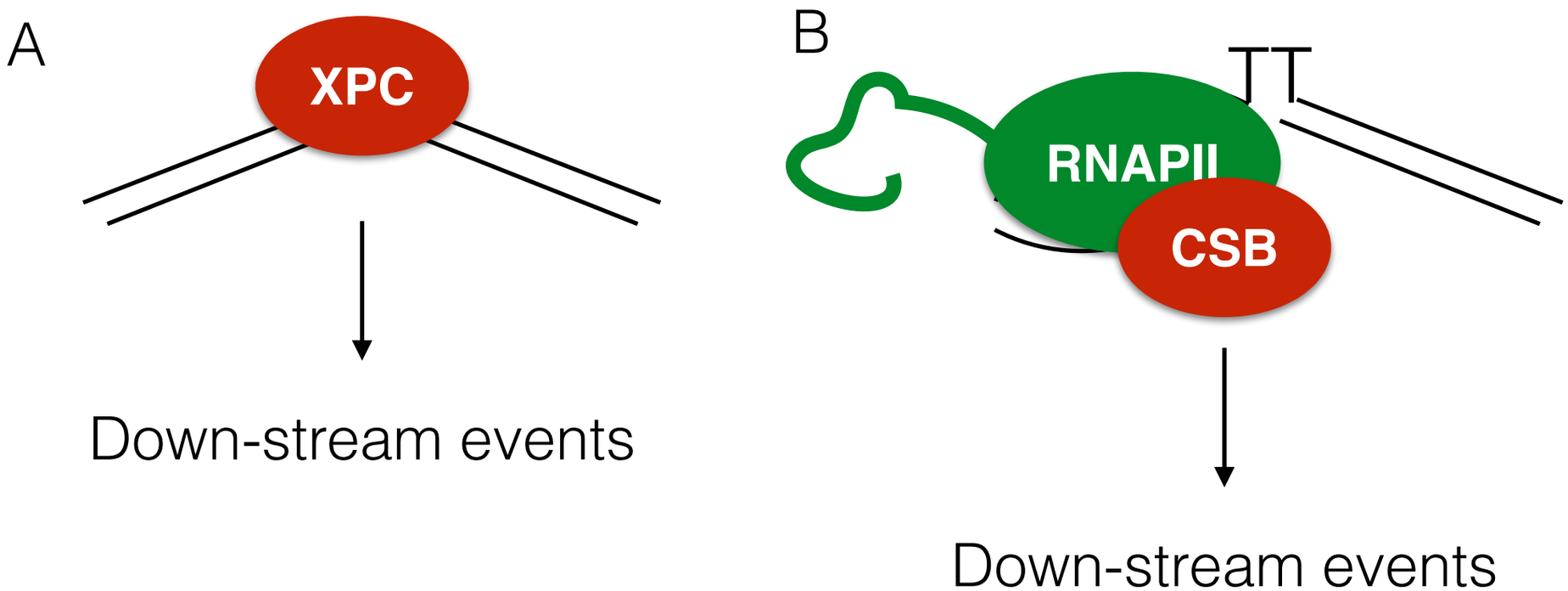

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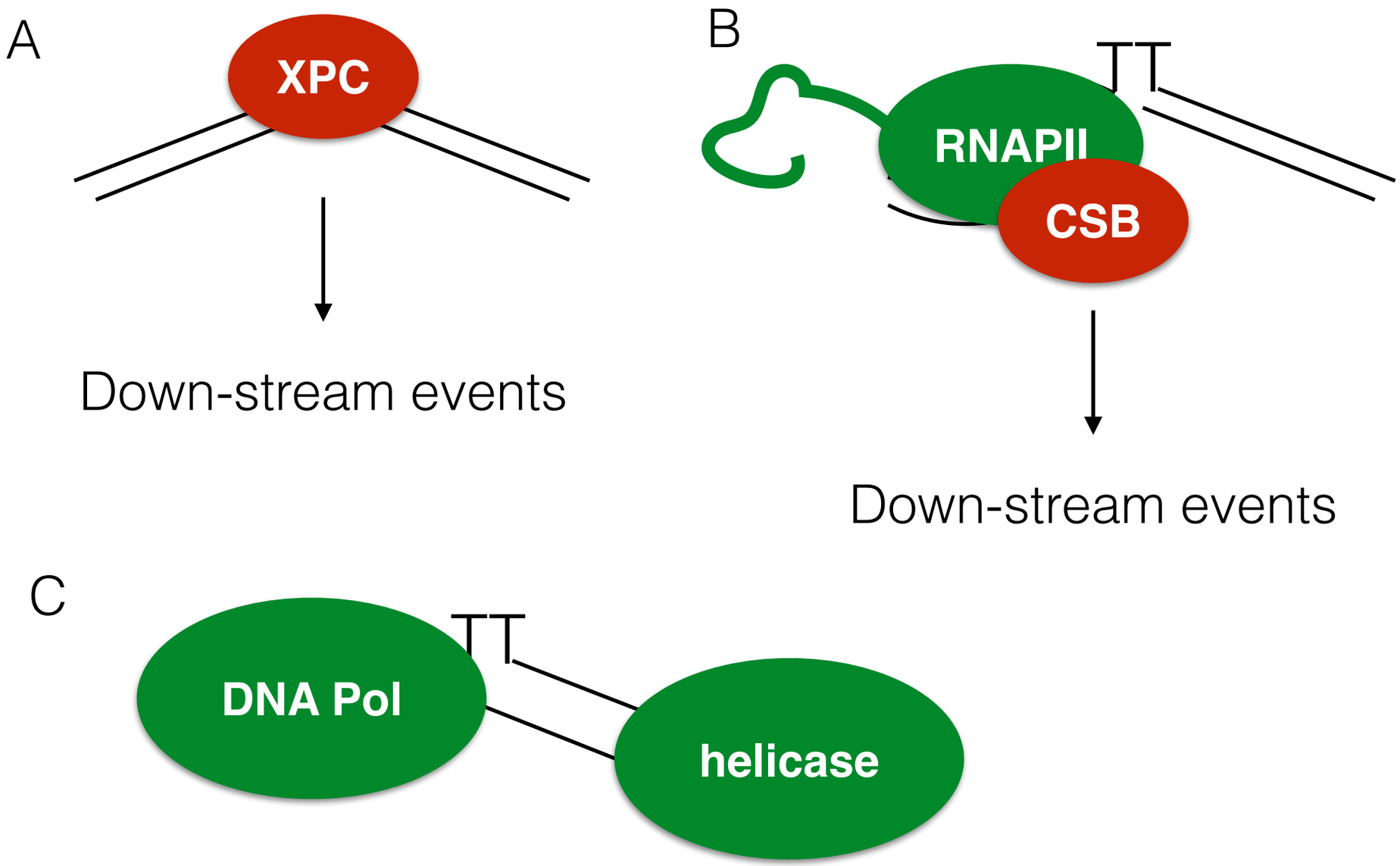

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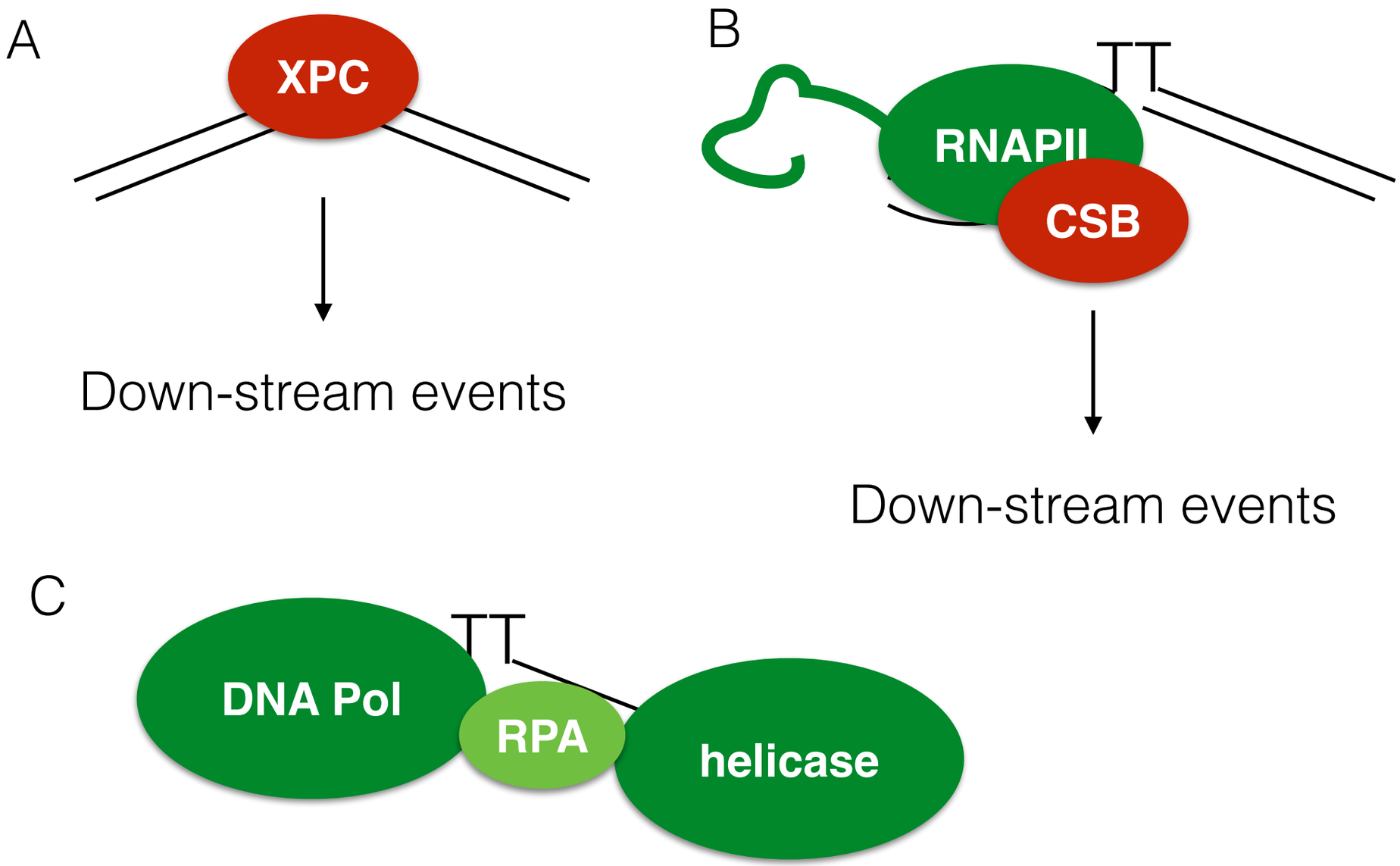

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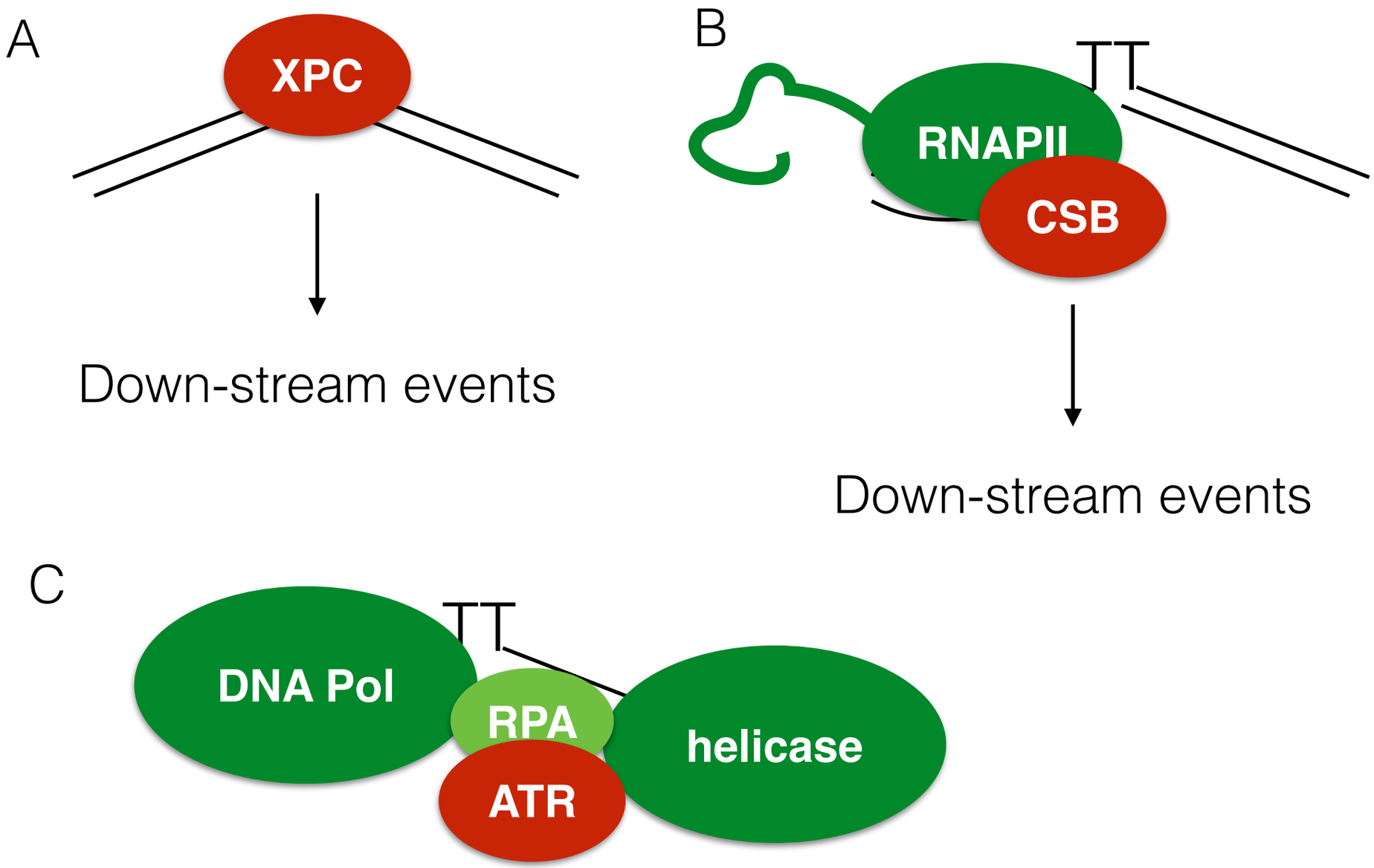

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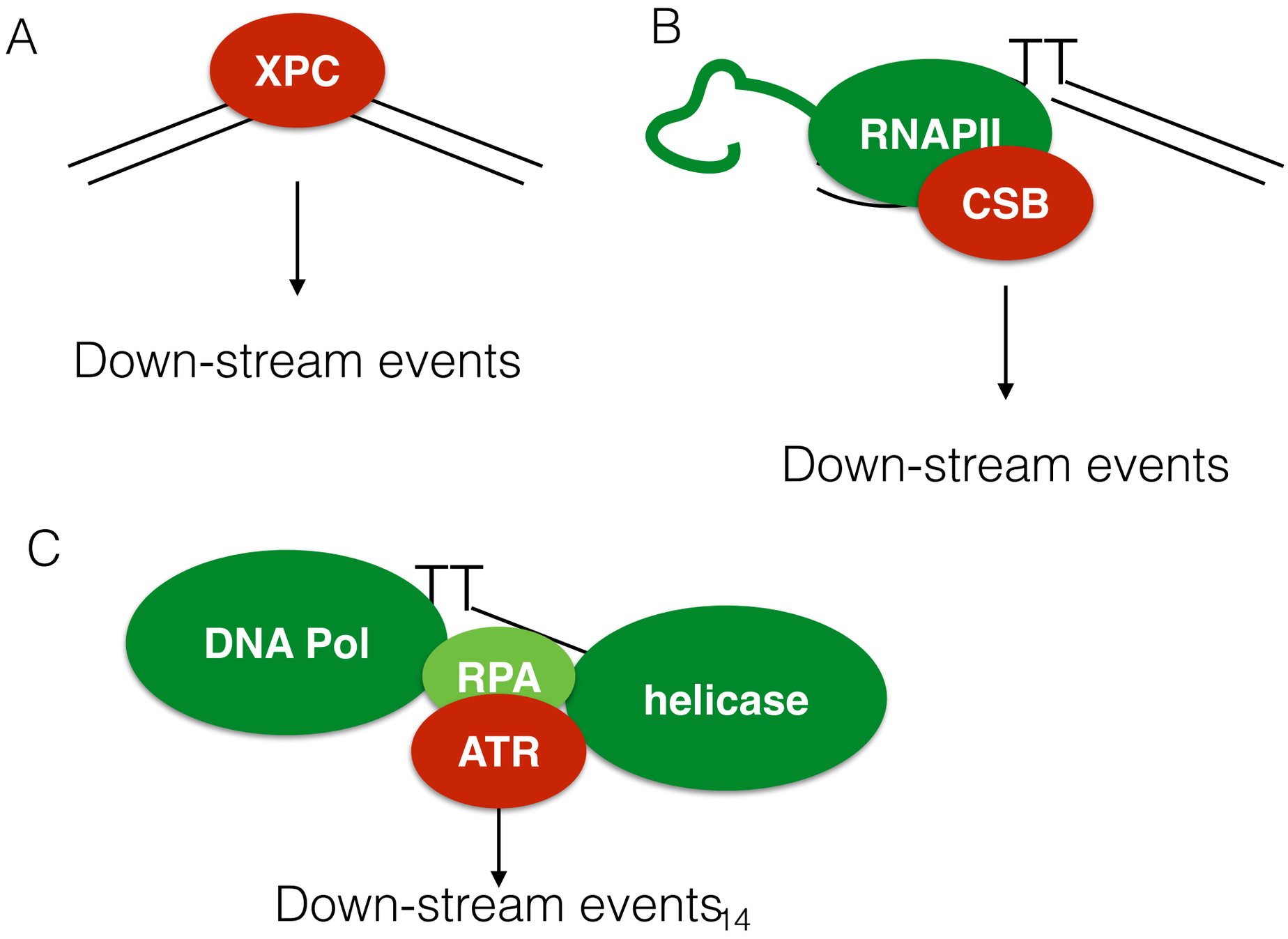
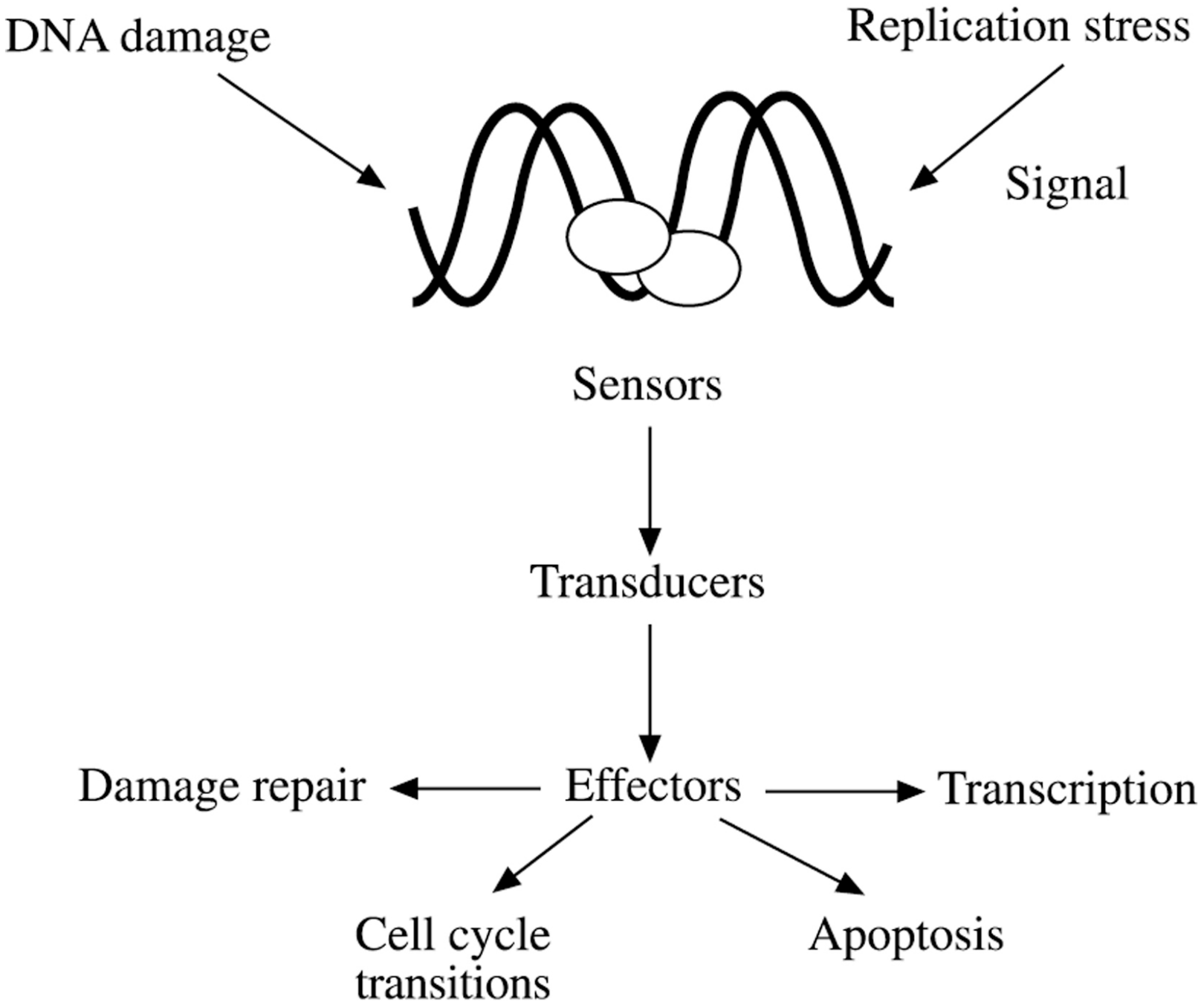
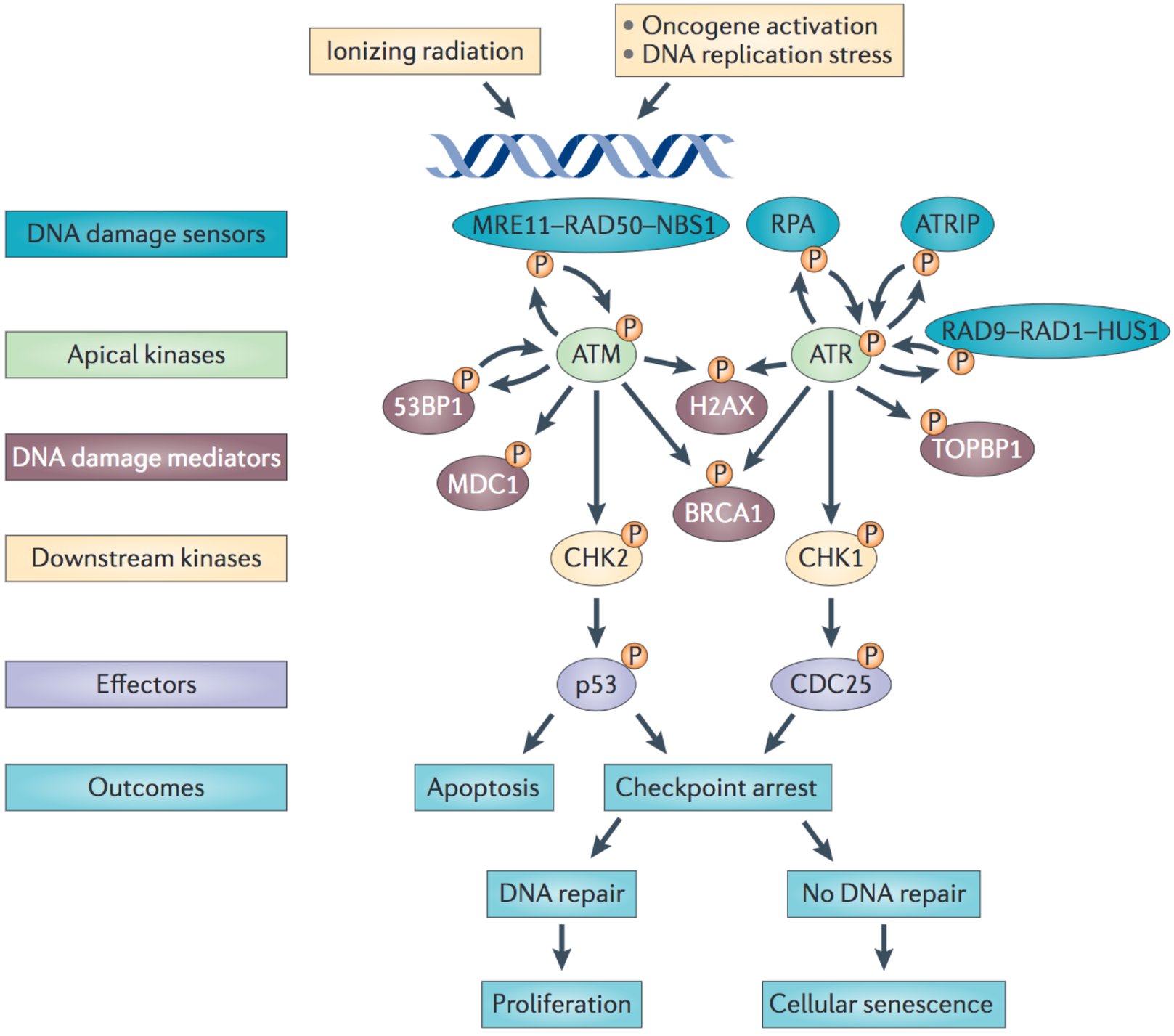


How do cells react to DNA damage?



How do cells react to DNA damage?

A simplified picture



d'Adda d'Fagagna, 2012

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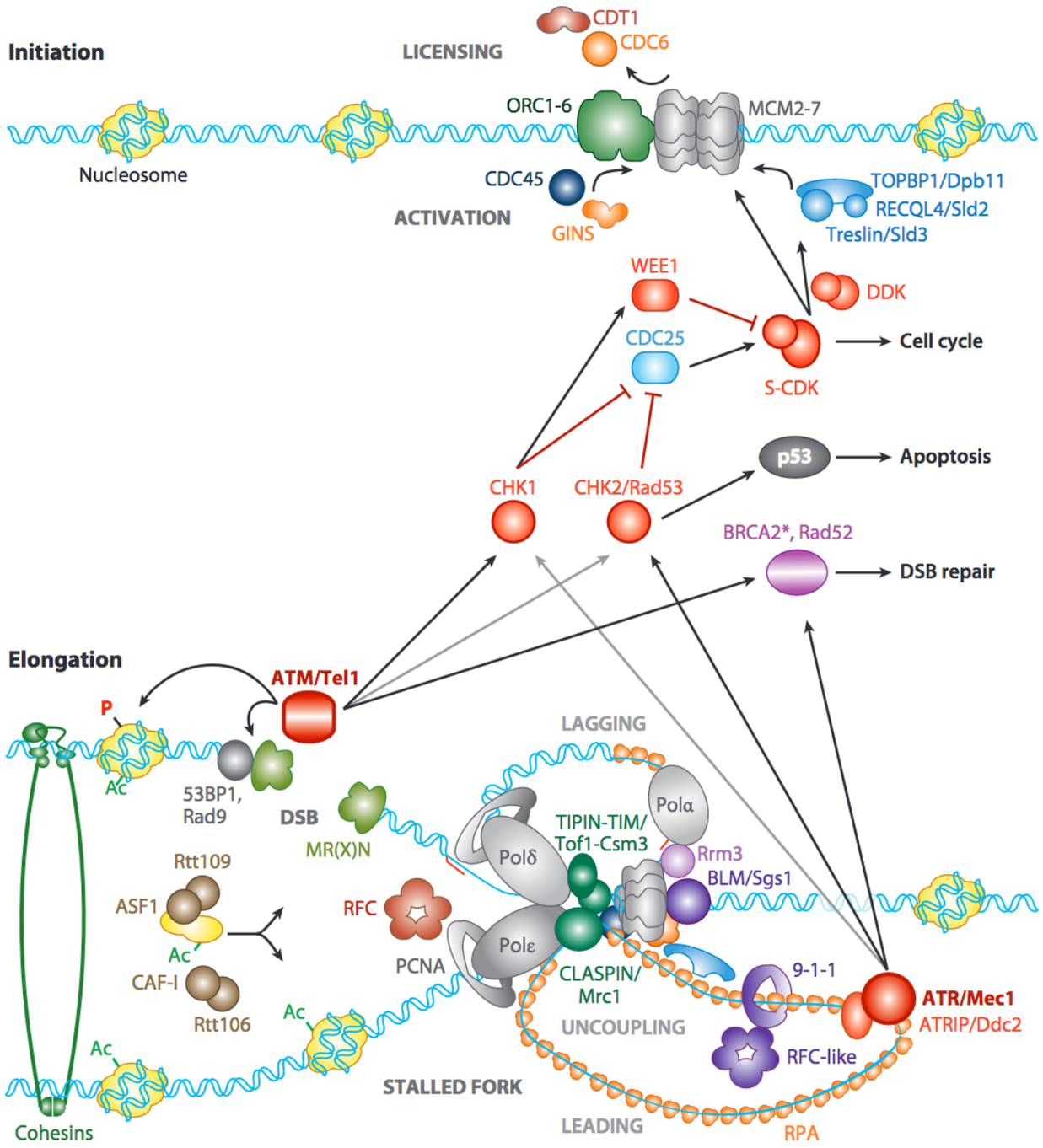
A more comprehensive picture



Aguilera and García-Muse, 2013

Transient summary II

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

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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?

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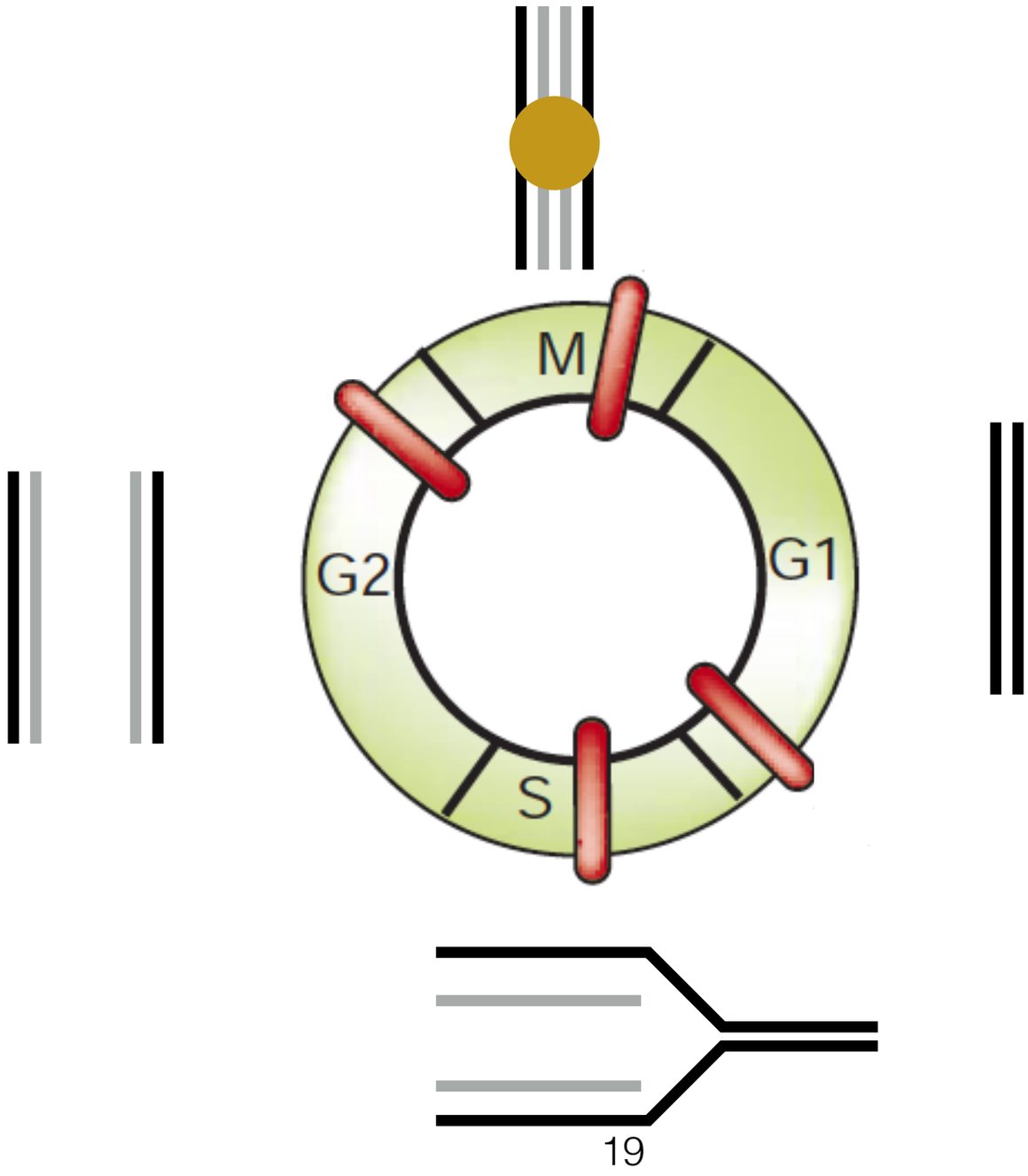
DNA repair is prevalent outside the S-phase, in which DNA damage tolerance is preferred.



Hoeijmakers, 2001

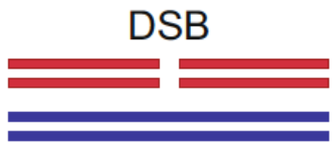
How do cell maintain genome stability?

Endogenous or environmental	• SAM • Nitrosated amines and bile acids • Dietary nitrosamines	• ROS • SAM • Natural IR • Base deamination or loss • Trapped TOPOI	• ROS • UV • Tobacco smoke • Afflatoxin	• Replication errors • SAM • Base deamination	• ROS • Natural IR • Trapped TOPOII	Unrepaired single strand lesions	• Acrolein • Croton-aldehyde
Lesion	**10–30** O^6-methyl-guanine	**10,000–100,000** • 8-oxoguanine • N^7-meG • N^3-meA • Uracil • Hypoxanthine • Xanthine • SSB	• 6–4 photo-products • Cyclopurines • Bulky adducts	Base mismatches	DNA double-strand breaks	**10–50** Stalled replication forks	ICL
Therapeutic	• TMZ • Alkylating agents • Nitrosoureas	• TMZ • IR • Radiomimetics • TOPOI poisons • Antimetabolites	• Cisplatin • Carboplatin • Nitrosoureas	• TMZ • Nucleoside analogues	• IR • Radio-mimetics • TOPOI poisons	• TMZ • TOPOI poisons • Anti-metabolites	• Cisplatin • Carboplatin • Nitrosoureas • MMC
Repair pathway	Direct repair	• BER • SSBR	NER	MMR	NHEJ	HRR	ICL repair

Curtin et al., 2012

How do cell 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

Sebesta and Krejci, 2016

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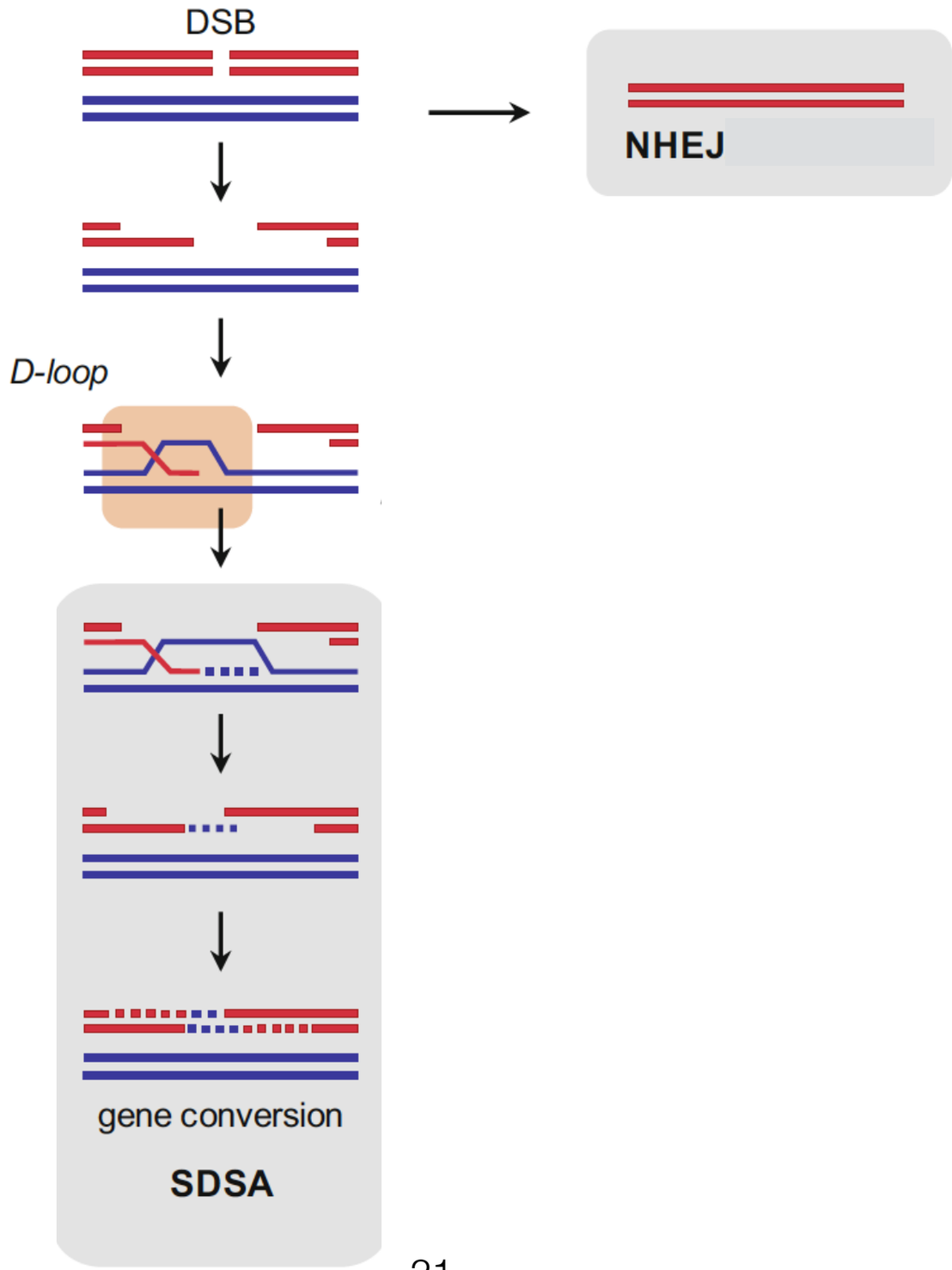

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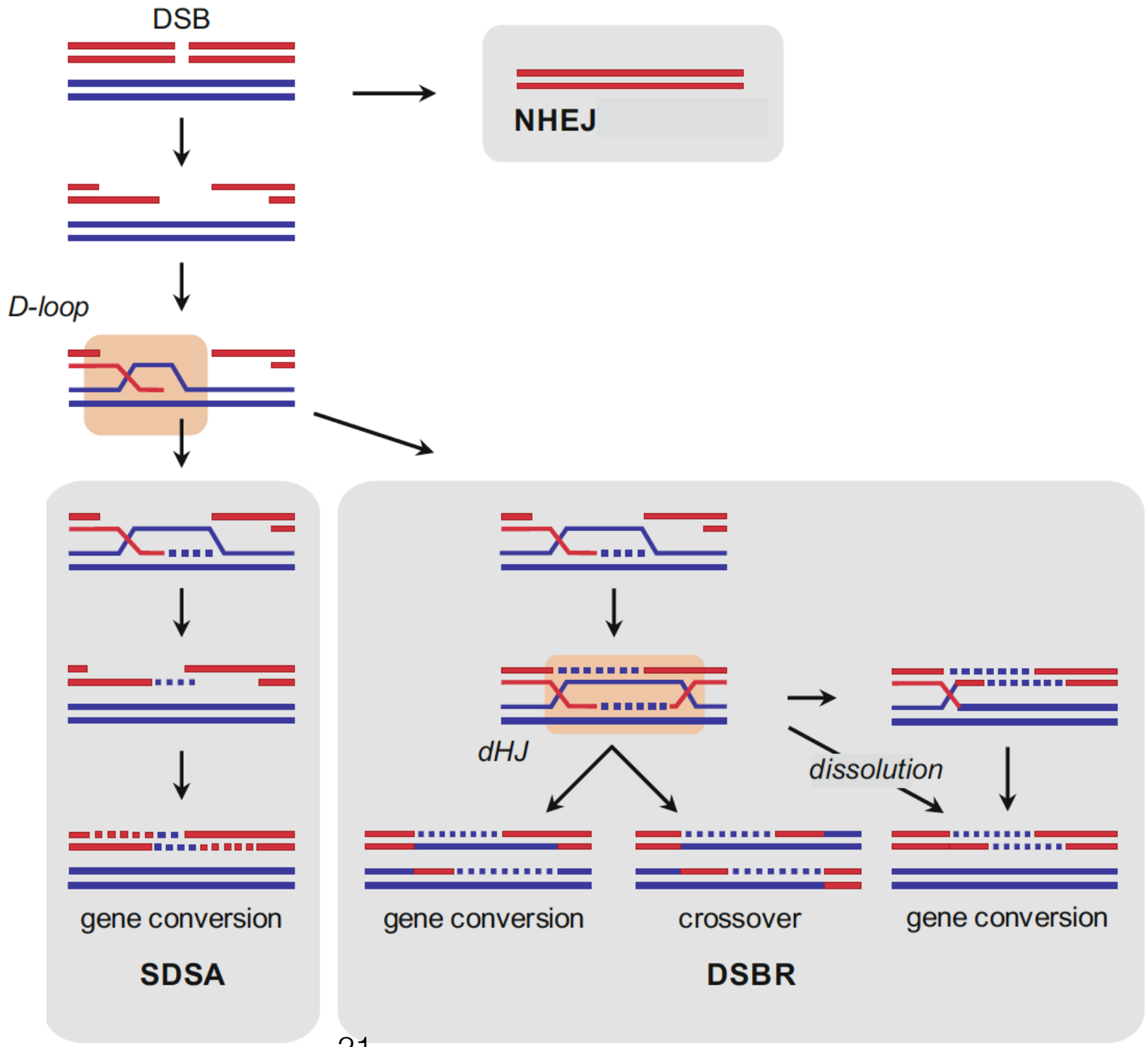

Sebesta and Krejci, 2016

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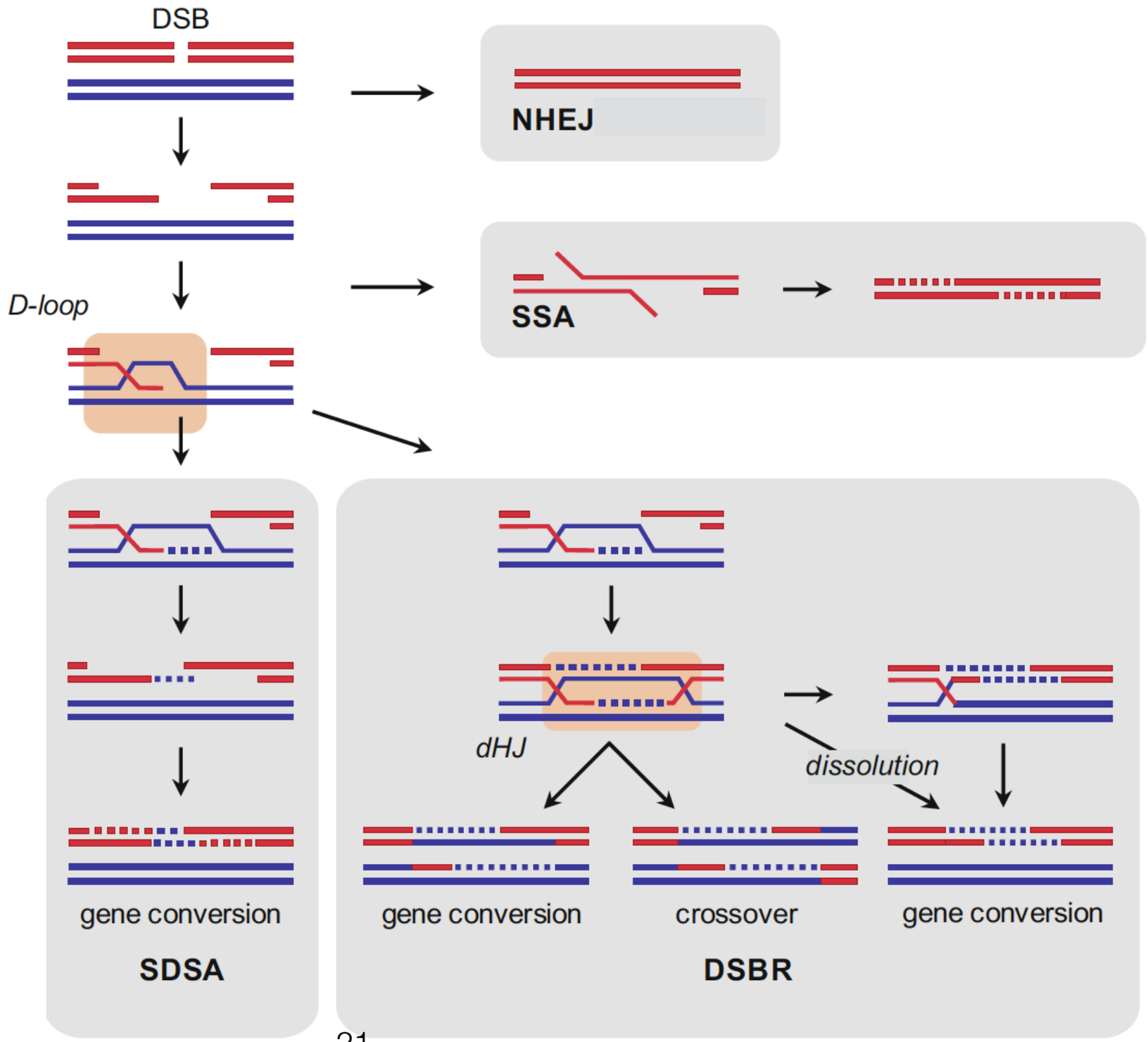

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Error-prone

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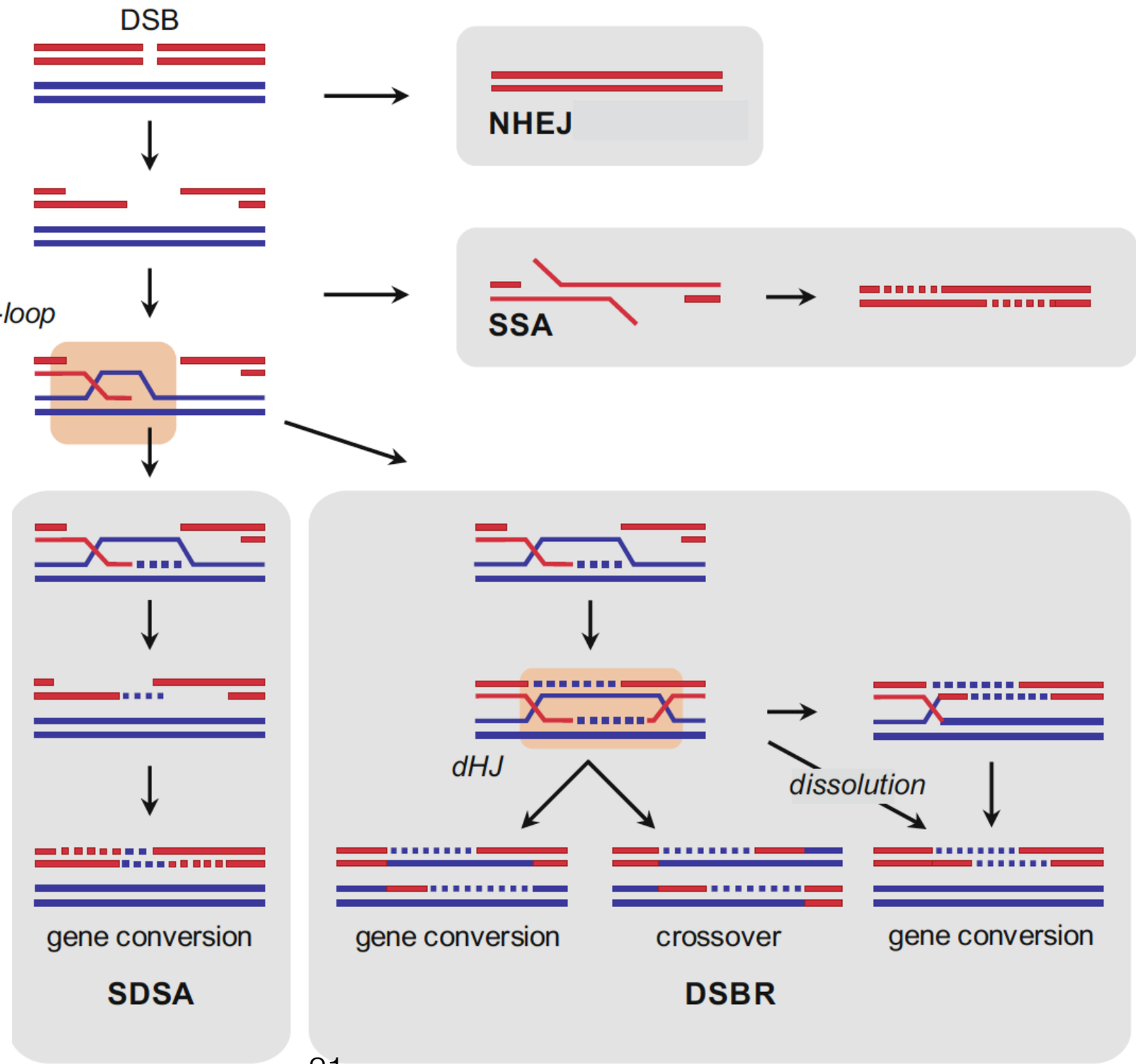

Sebesta and Krejci, 2016

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Double-stranded DNA breaks (DSB) repair

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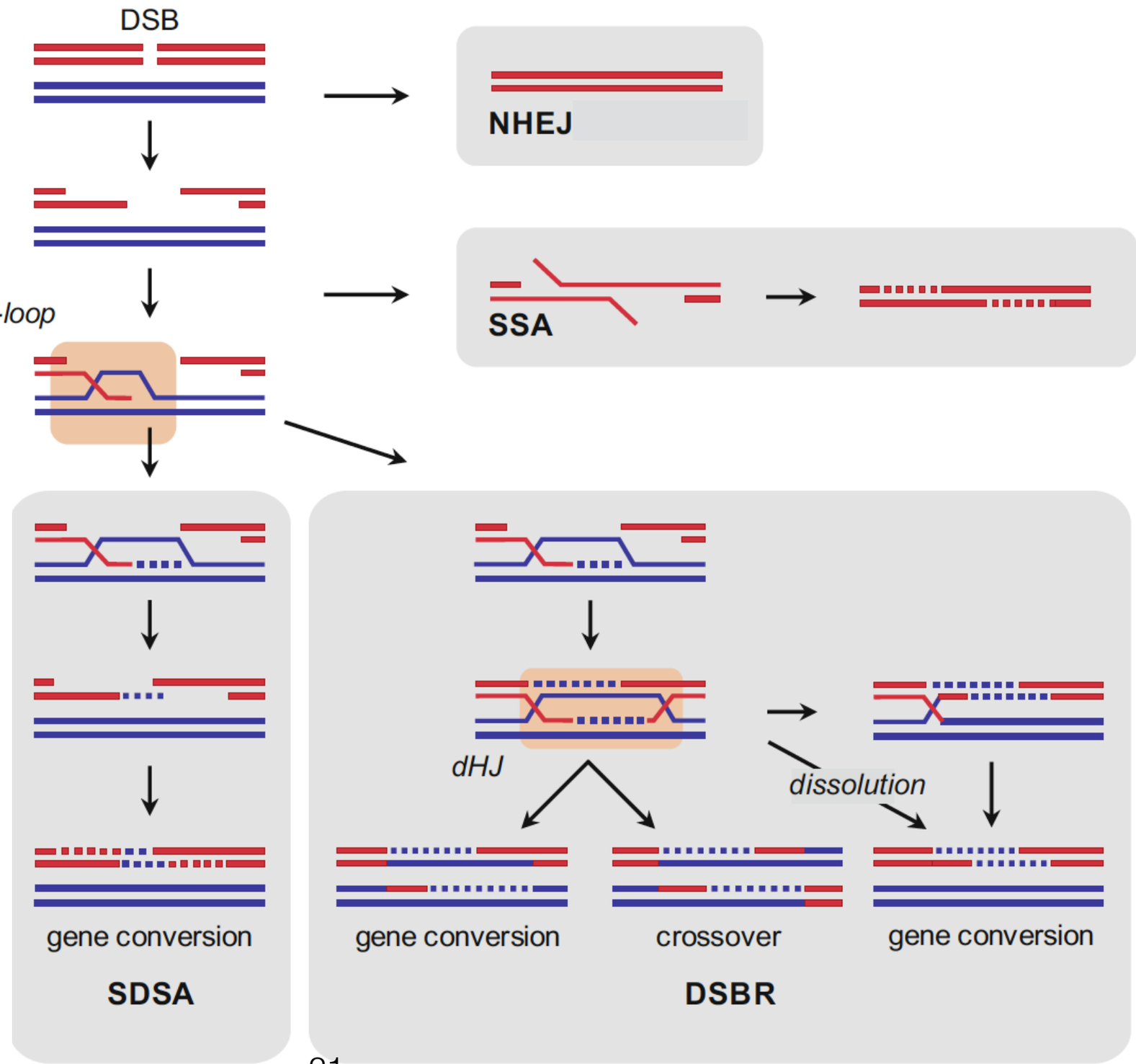
Error-free



Sebesta and Krejci, 2016

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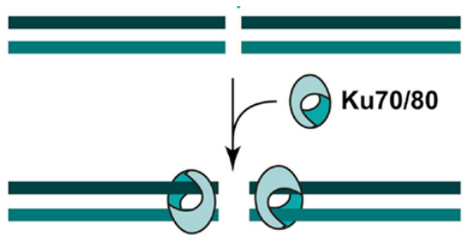

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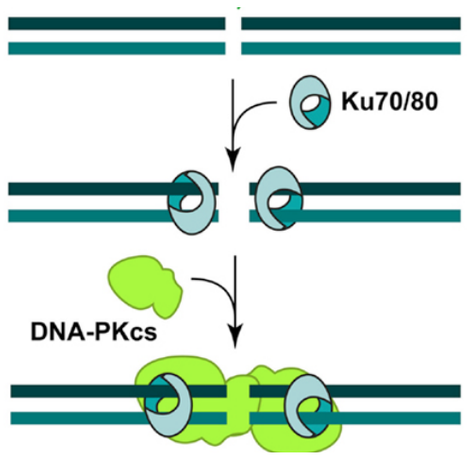

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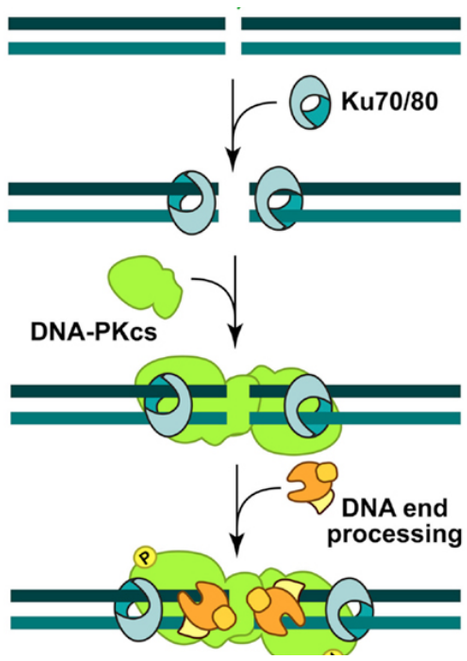
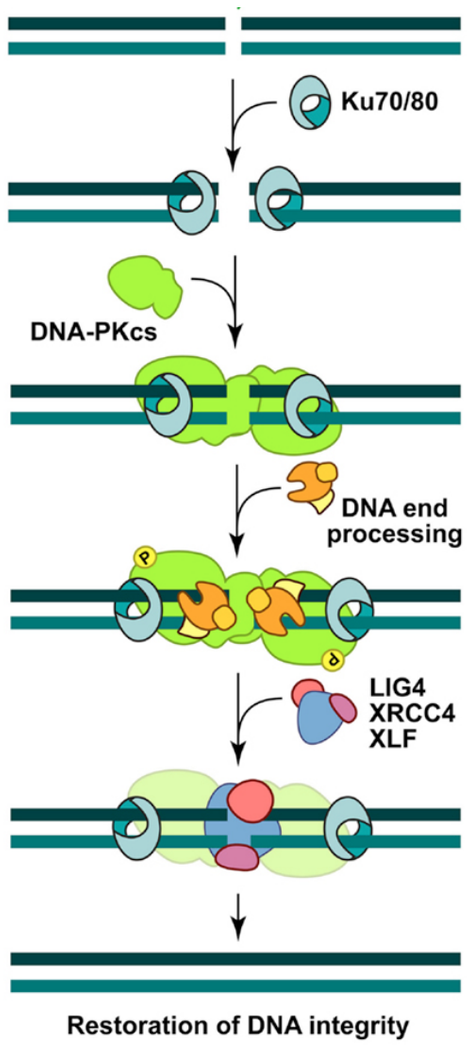


How do cell maintain genome stability?

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

Sebesta and Krejci, 2016

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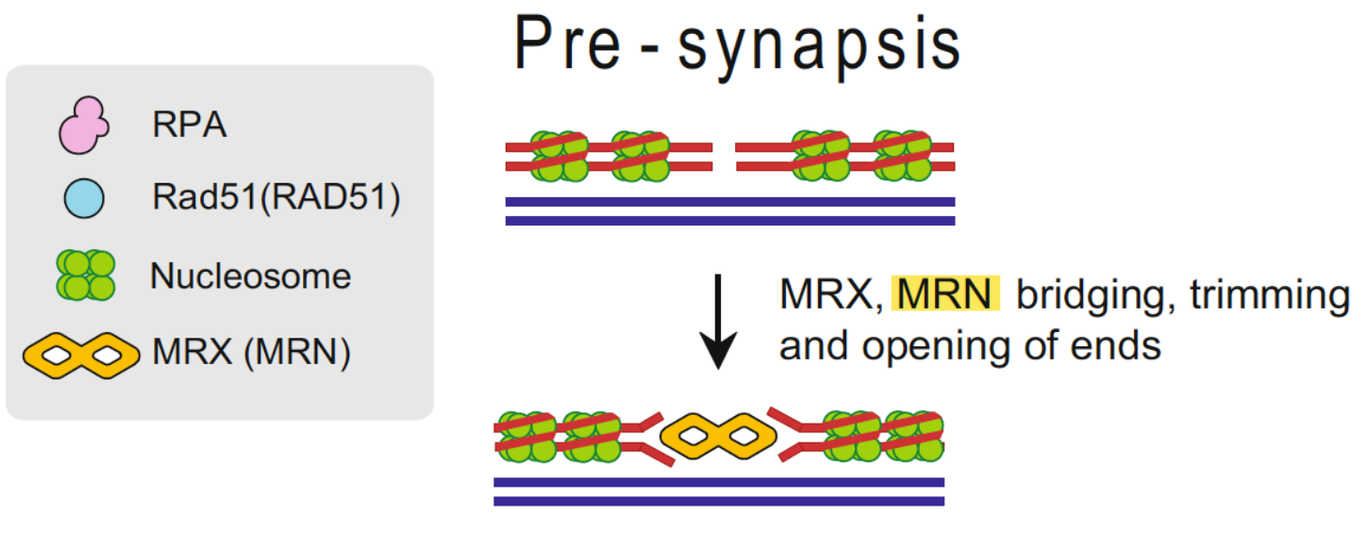

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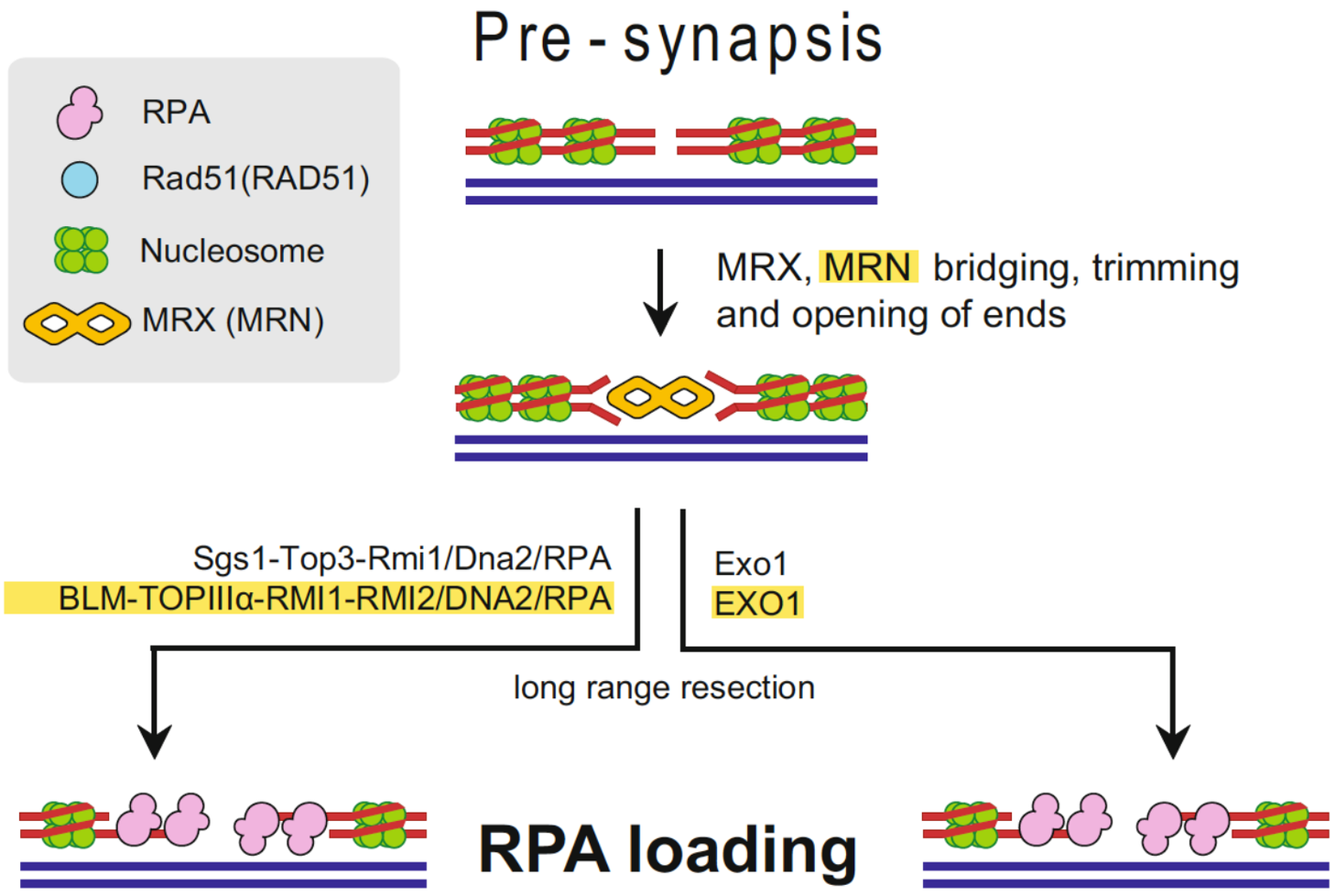

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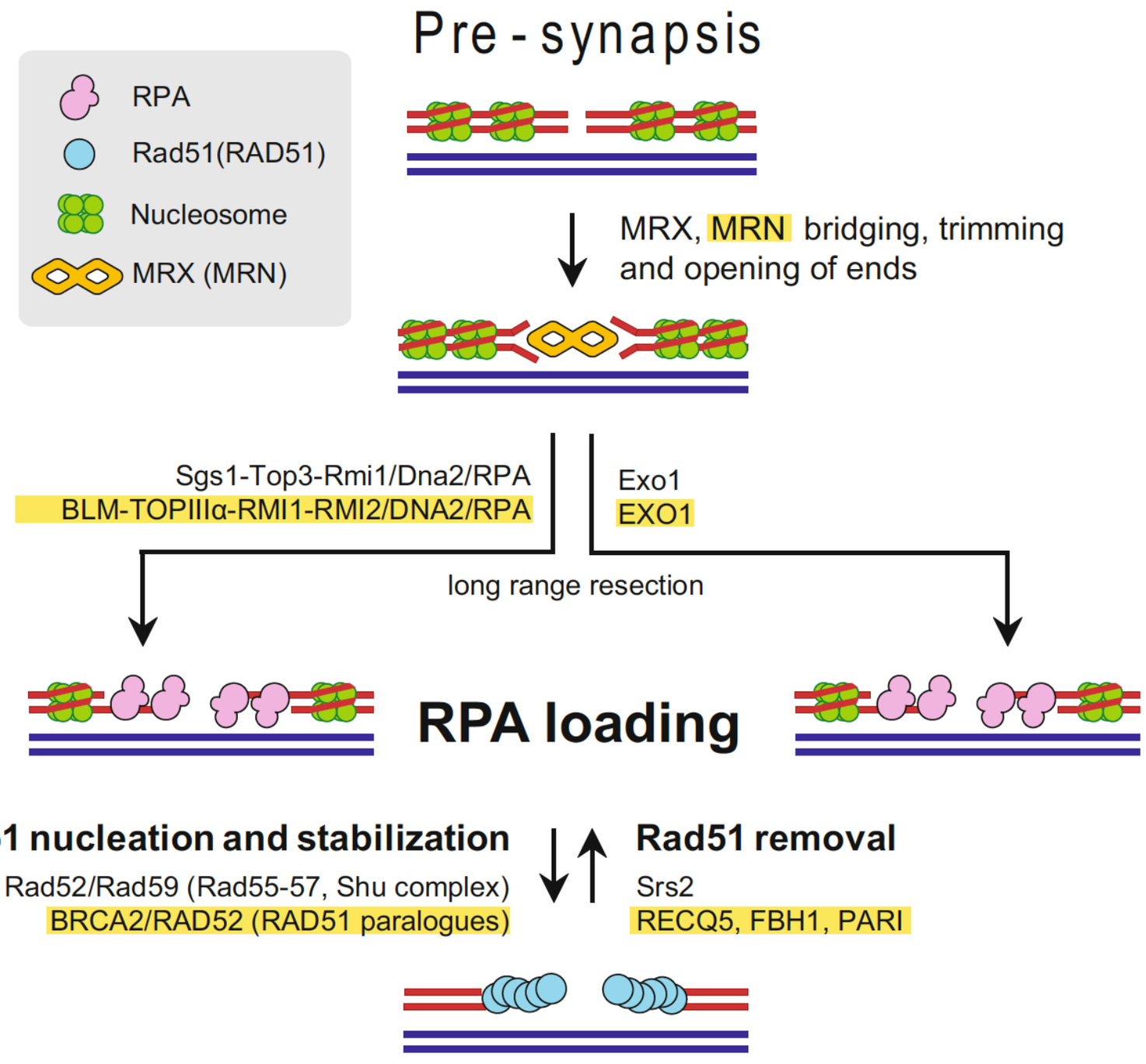

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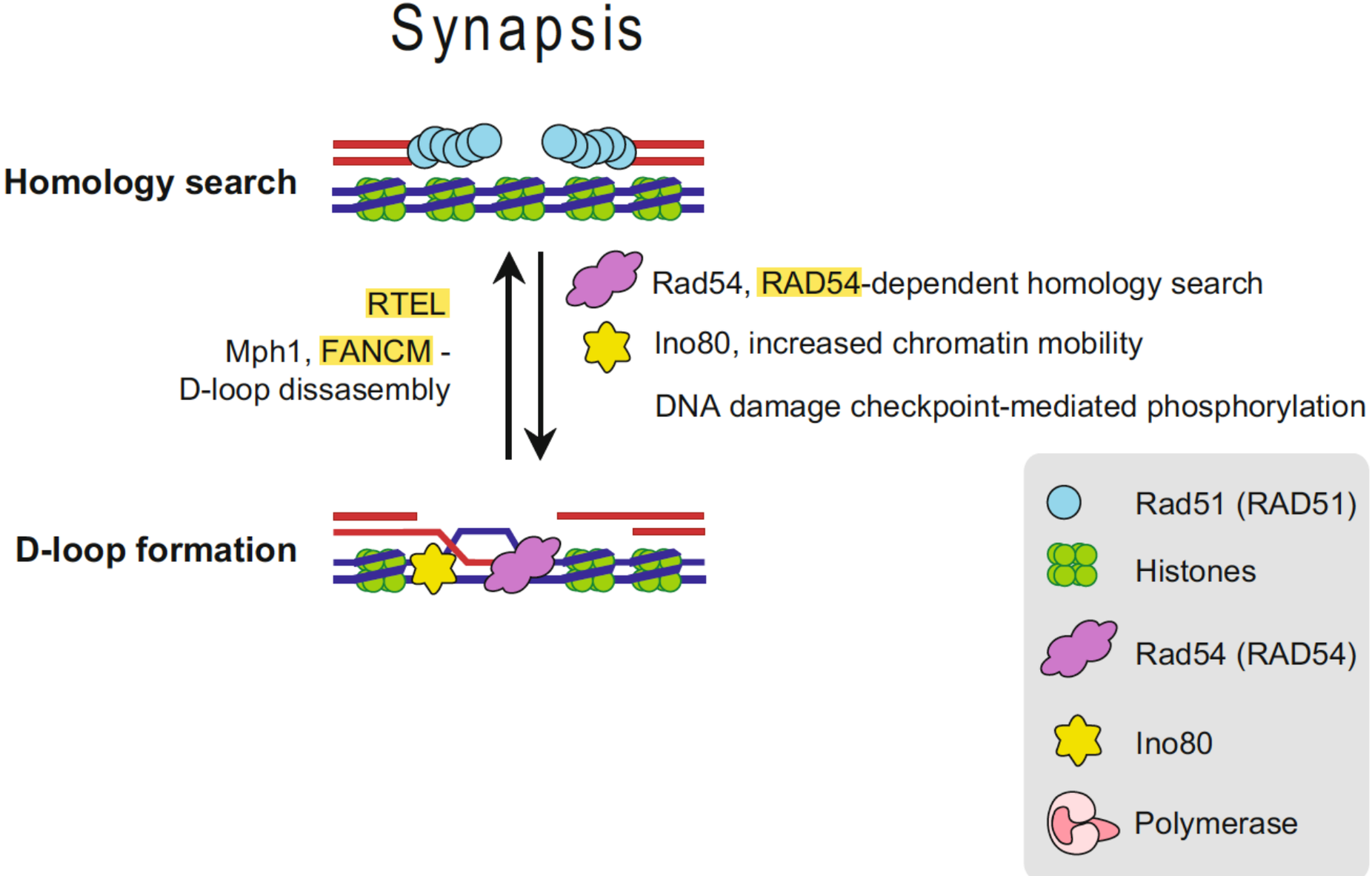

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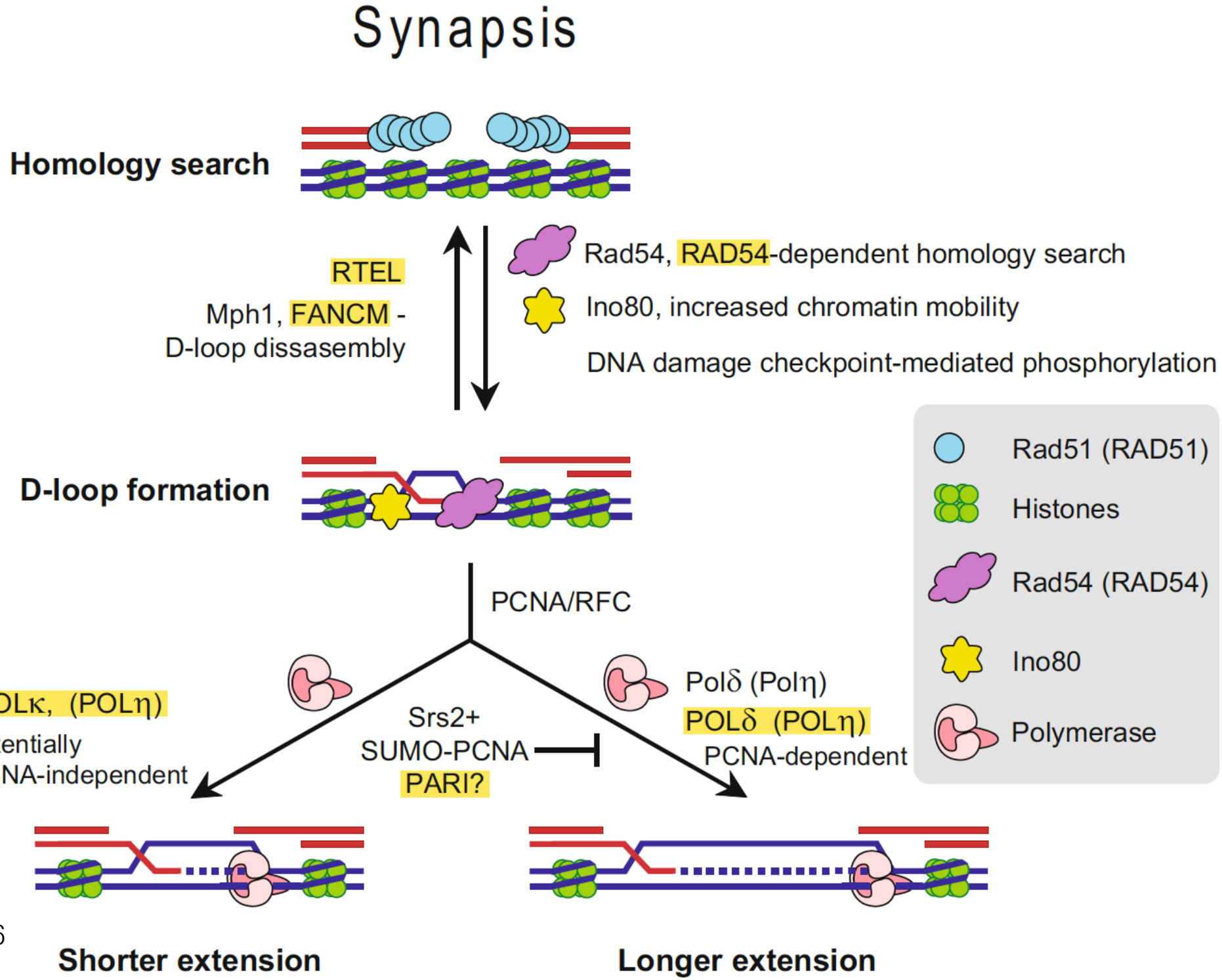

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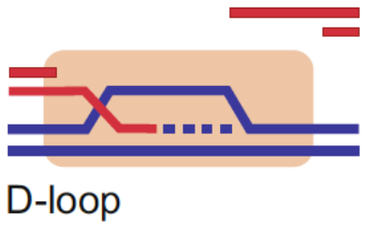
Post-synapsis



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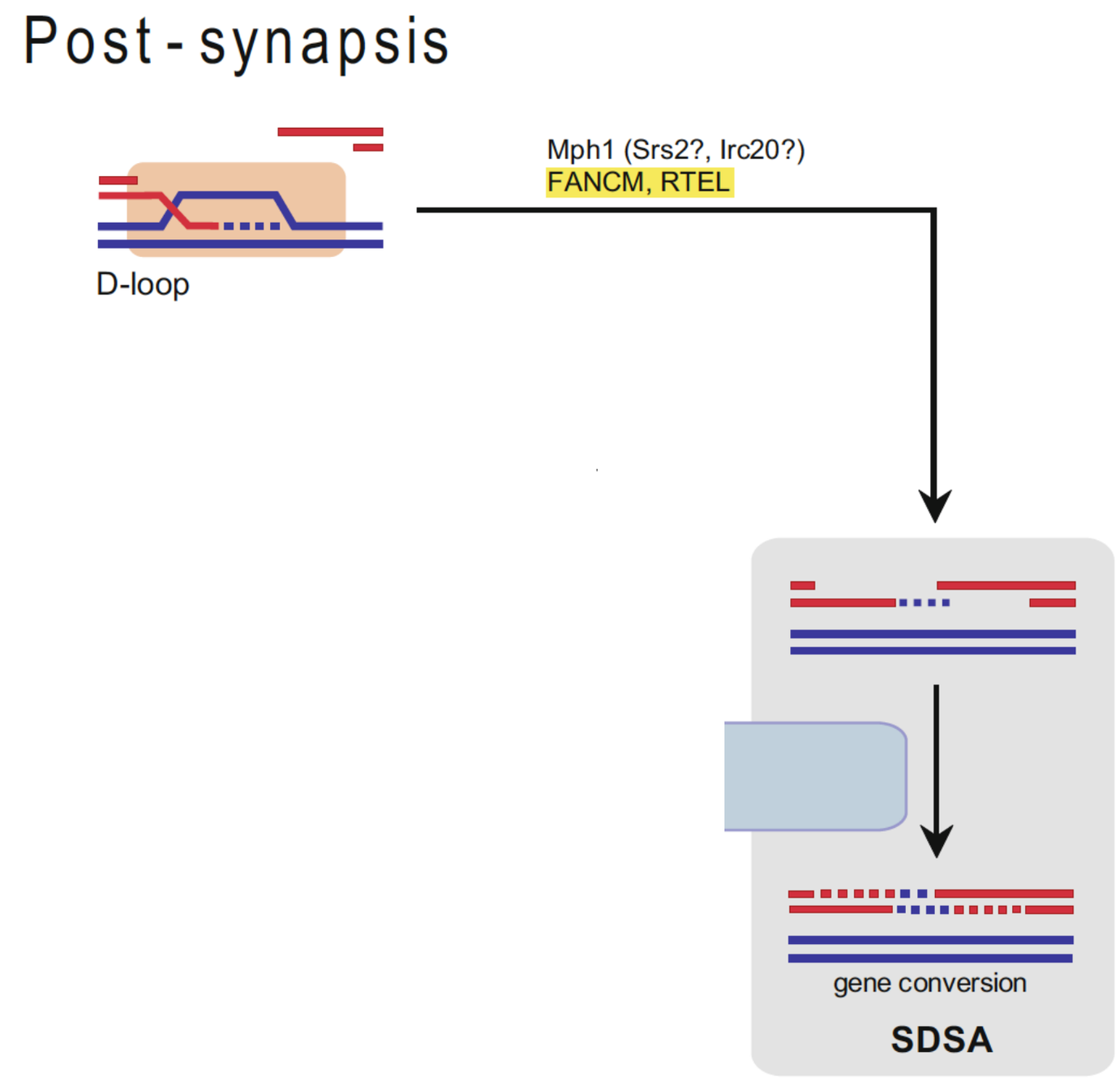

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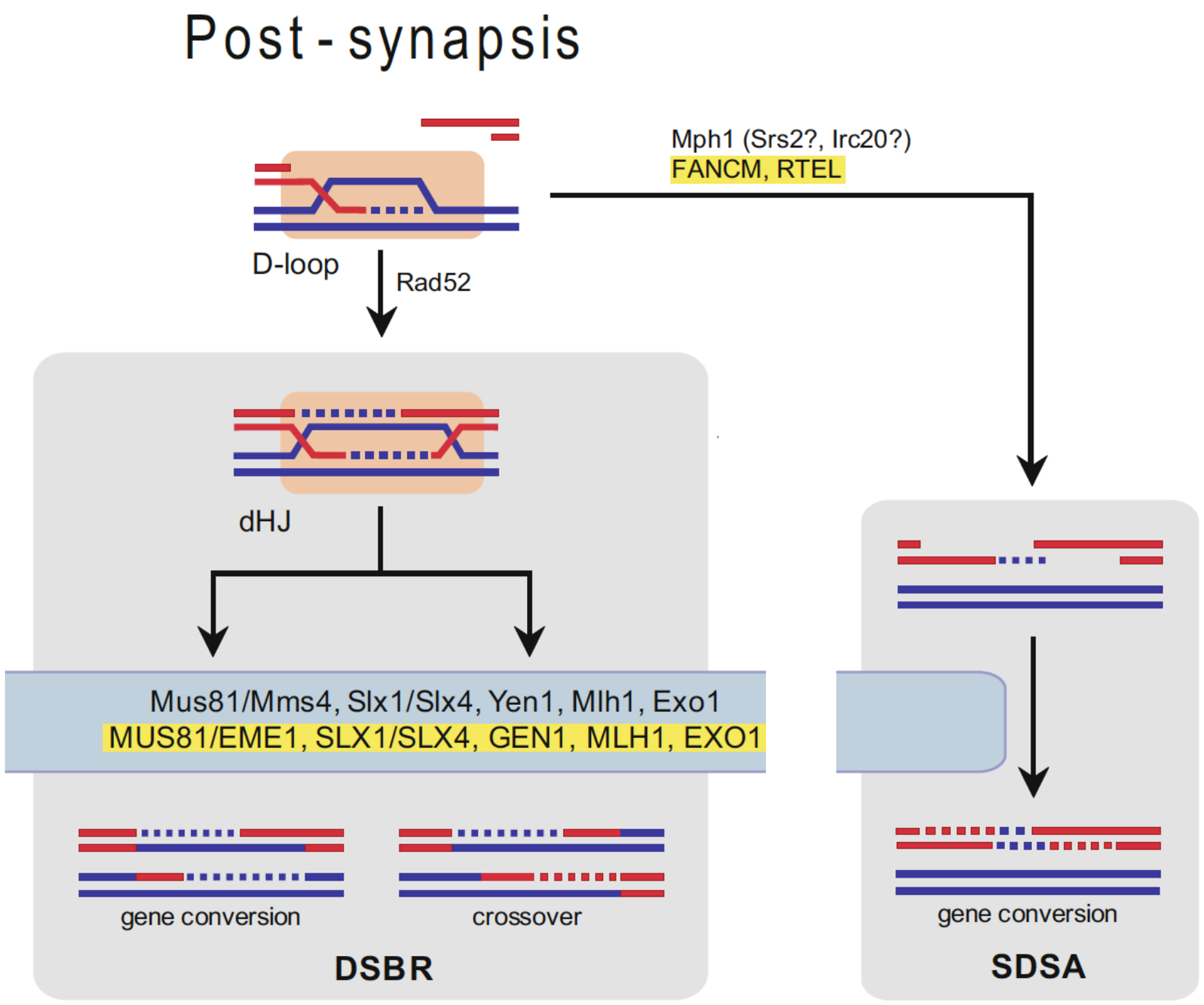

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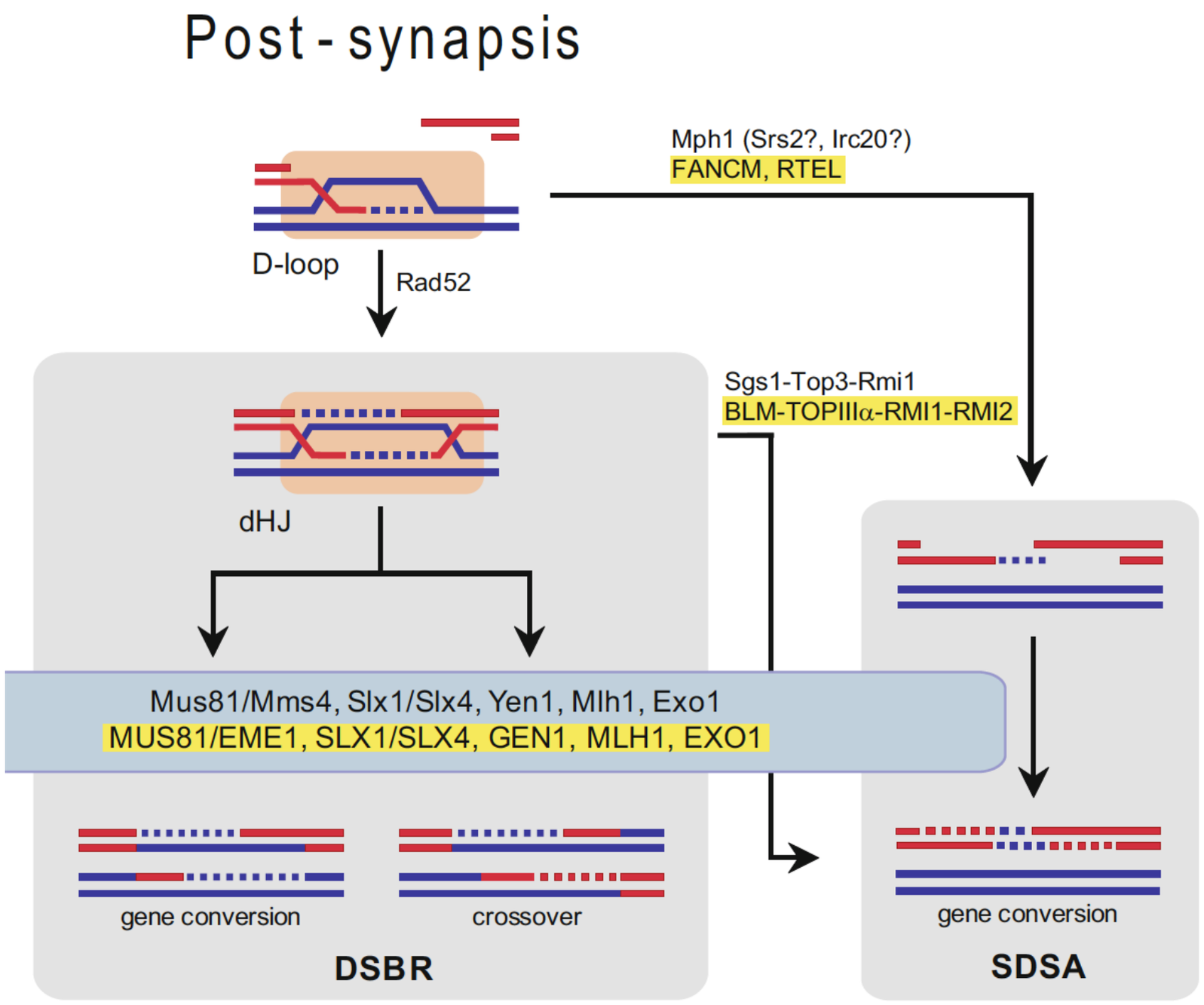

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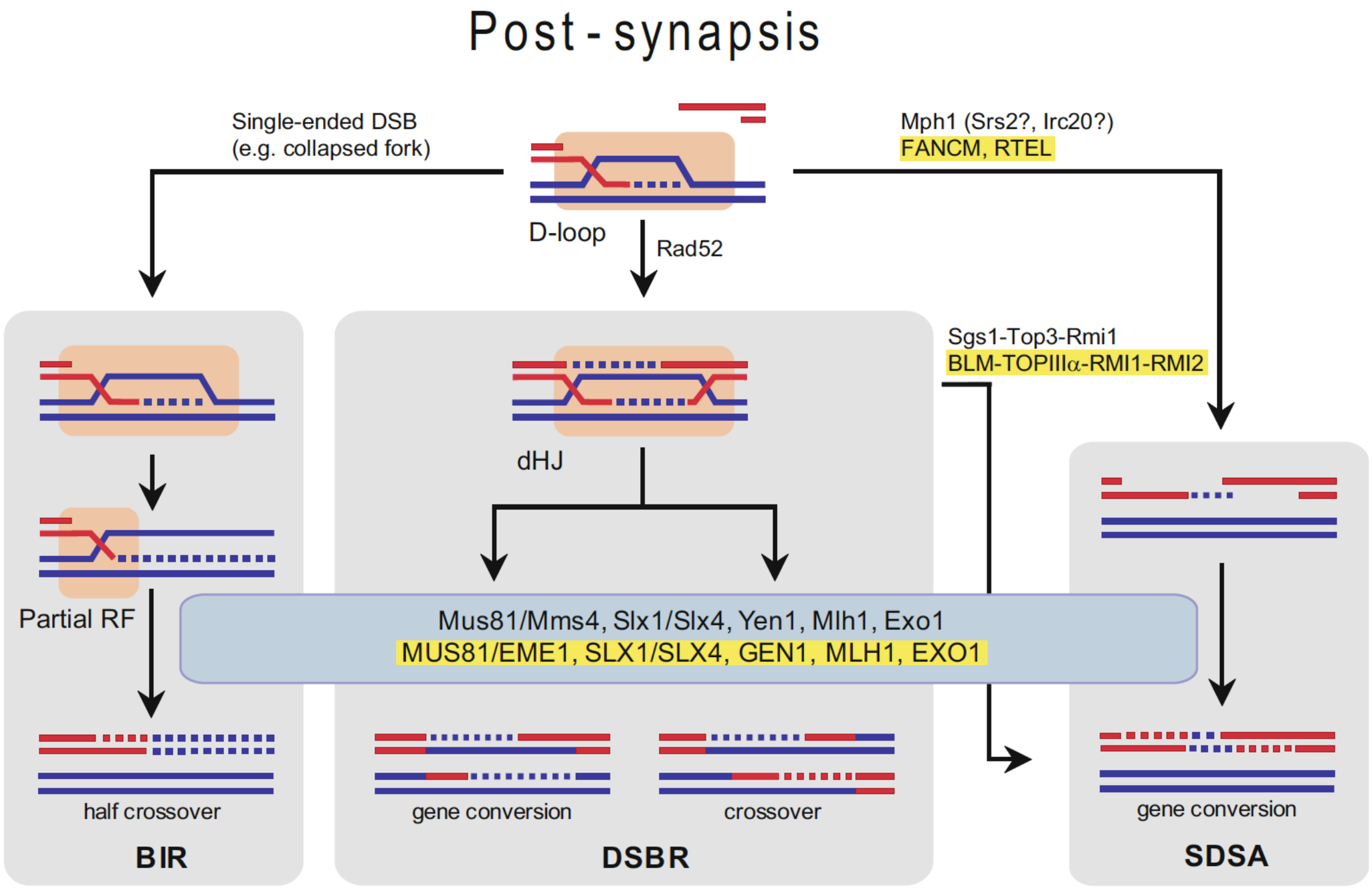

Sebesta and Krejci, 2016

Transient summary III

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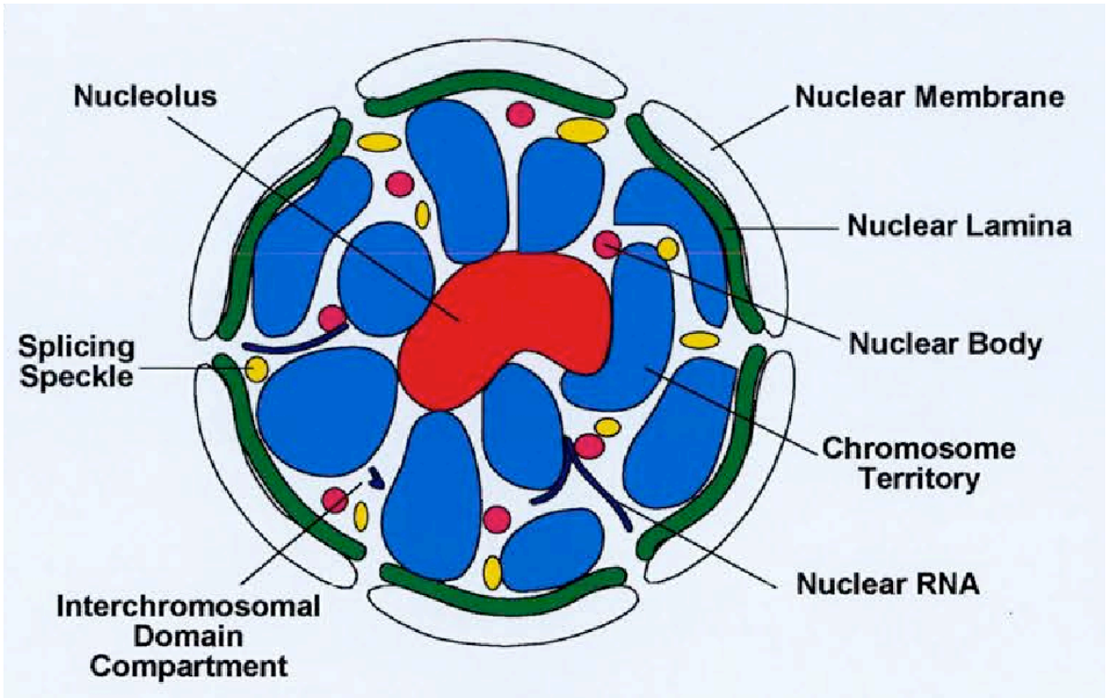
In S-phase, cells activate tolerance mechanisms that allow timely completion of DNA replication

How do cells organise their repair machineries to effectively repair DNA damage?

The challenges

- timely recruitment of repair factors to the sites of DNA damage



Yang *et al.*, 2018

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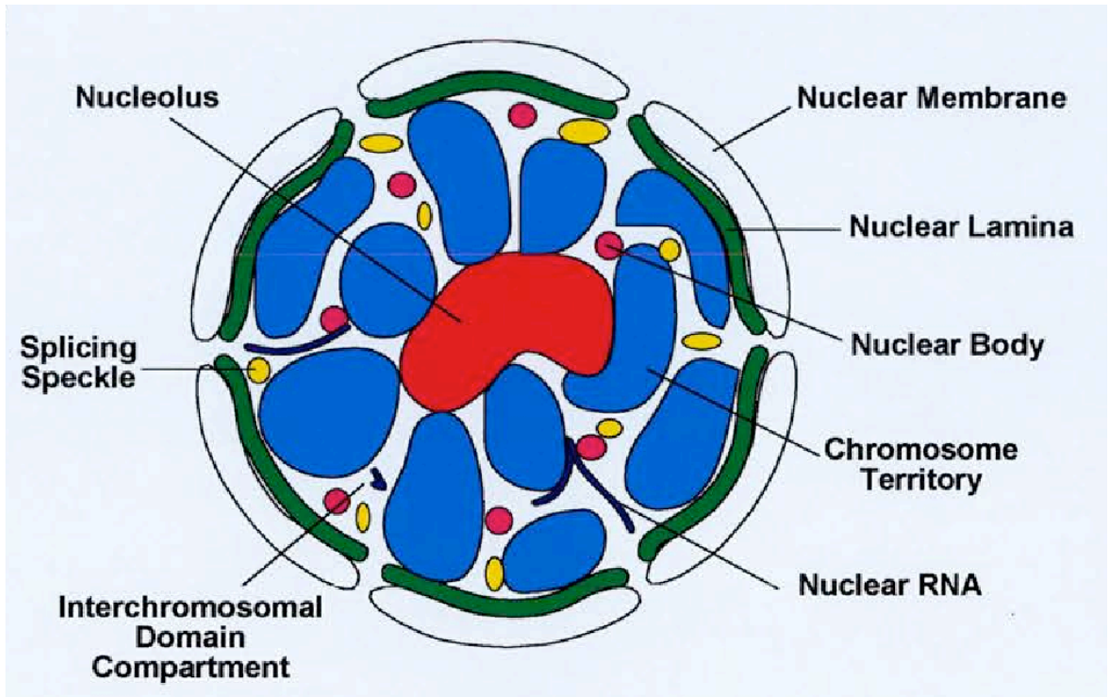

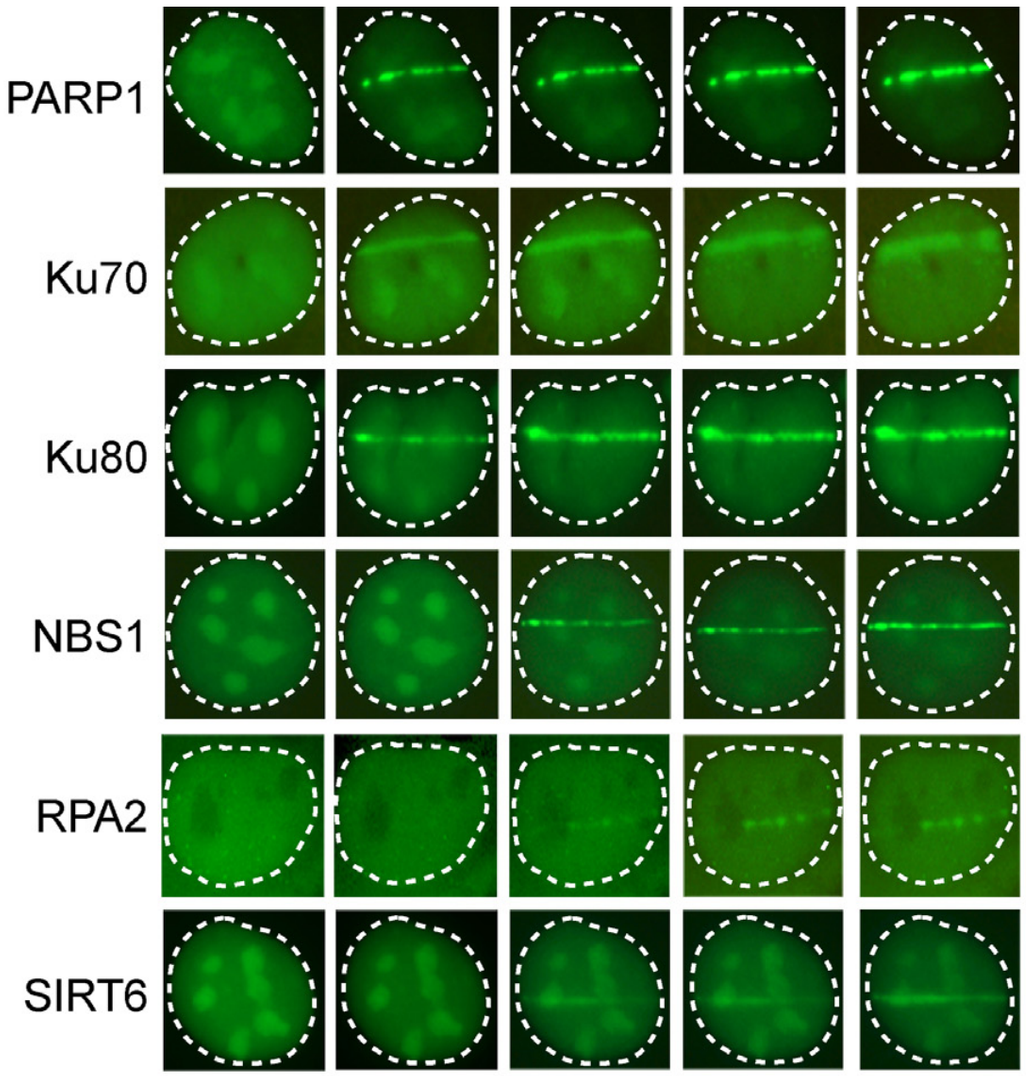

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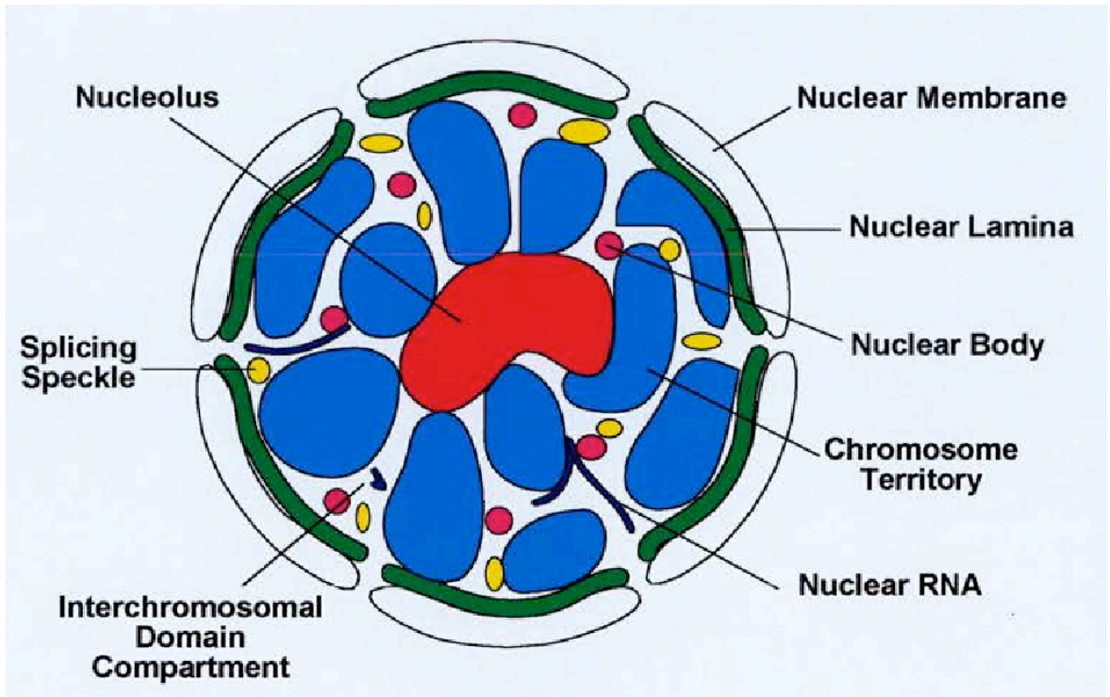

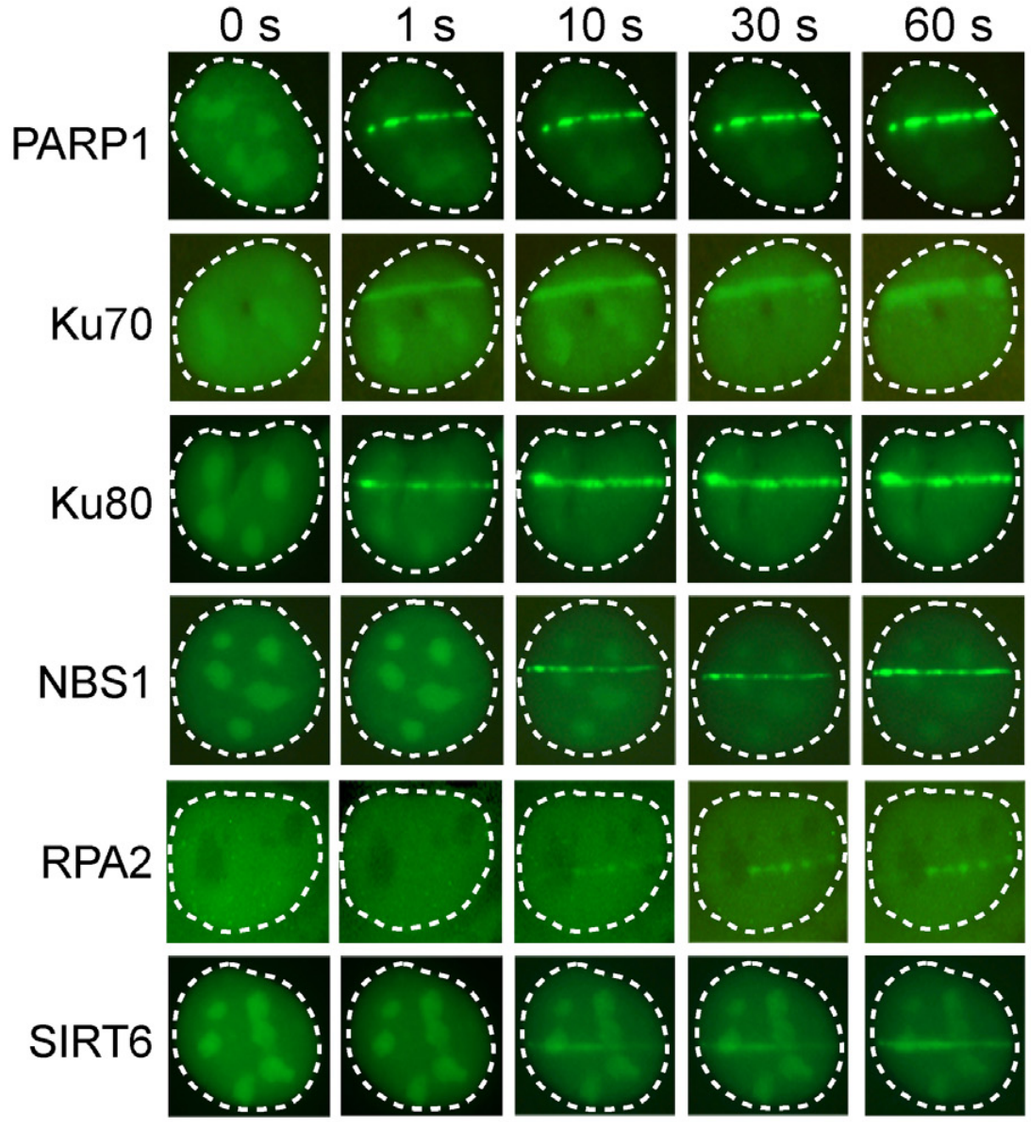

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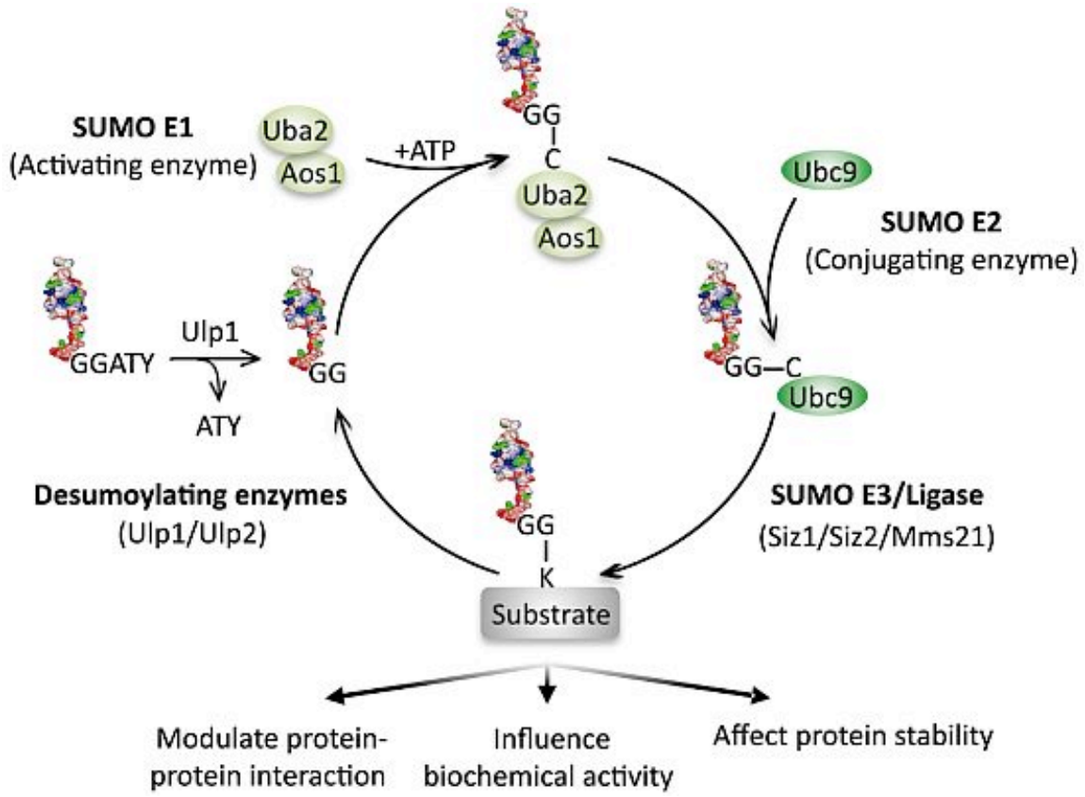
How do cells organise their repair machineries to effectively repair DNA damage?

Post-translational modifications promote complex-formation

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

Ivan Psakhye[1] and Stefan Jentsch[1,*]
[1]Department 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



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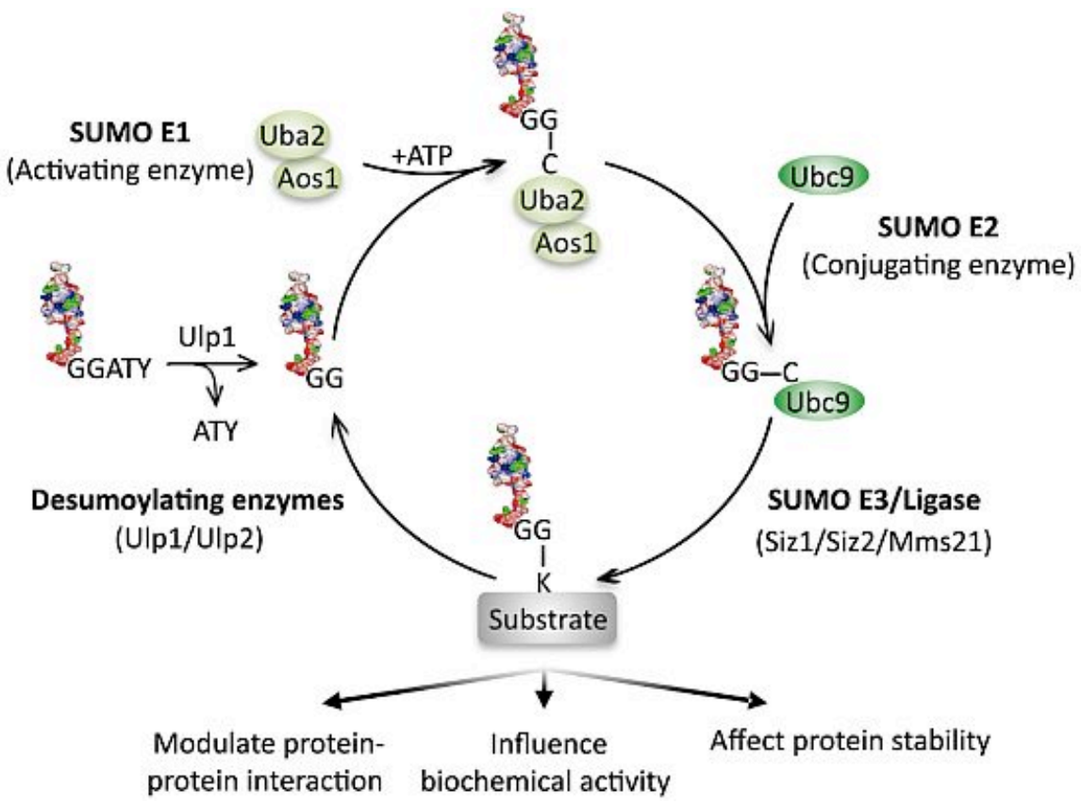

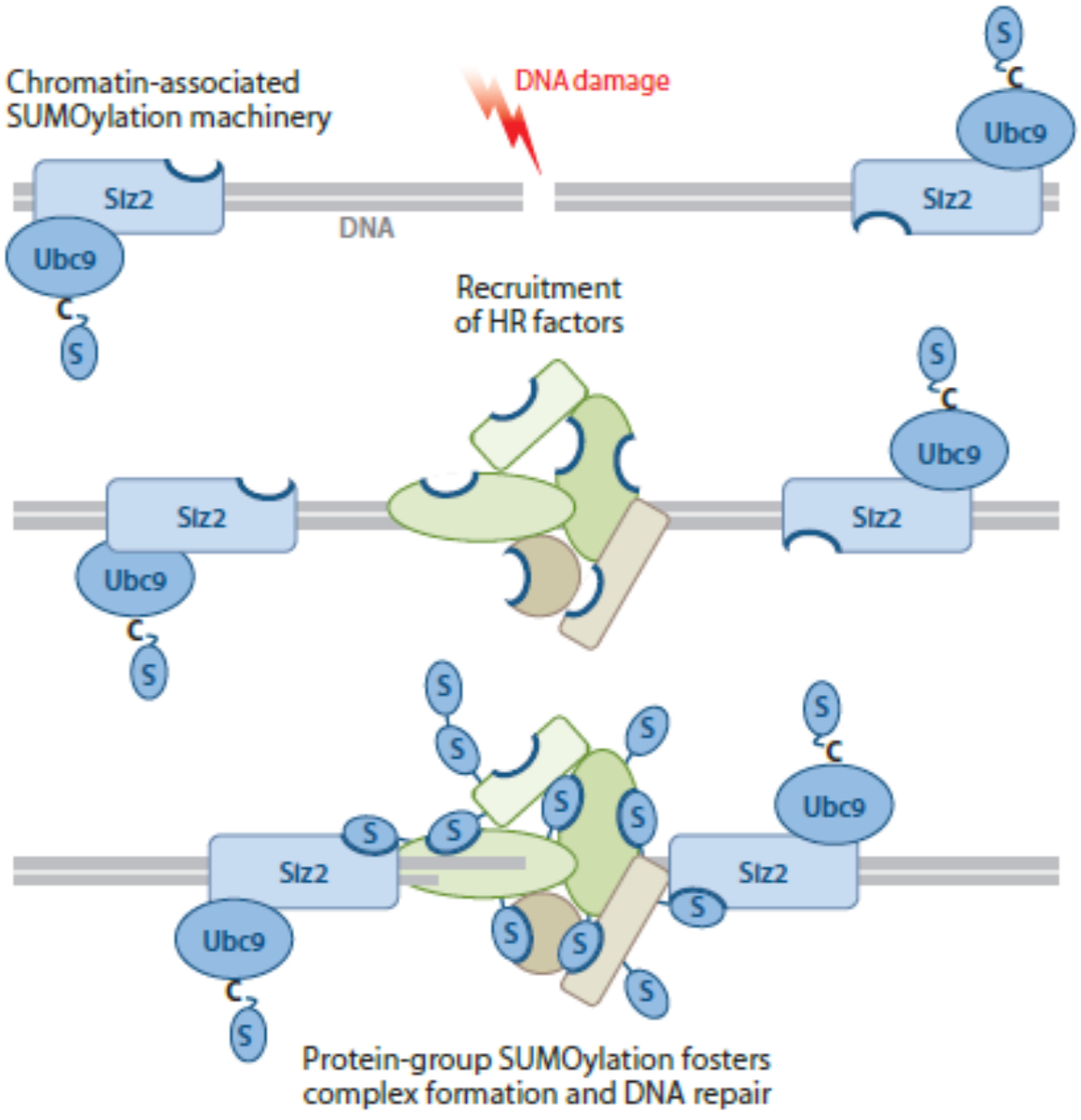

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Liquid-liquid phase-separation (a.k.a condensation with liquid-like properties)

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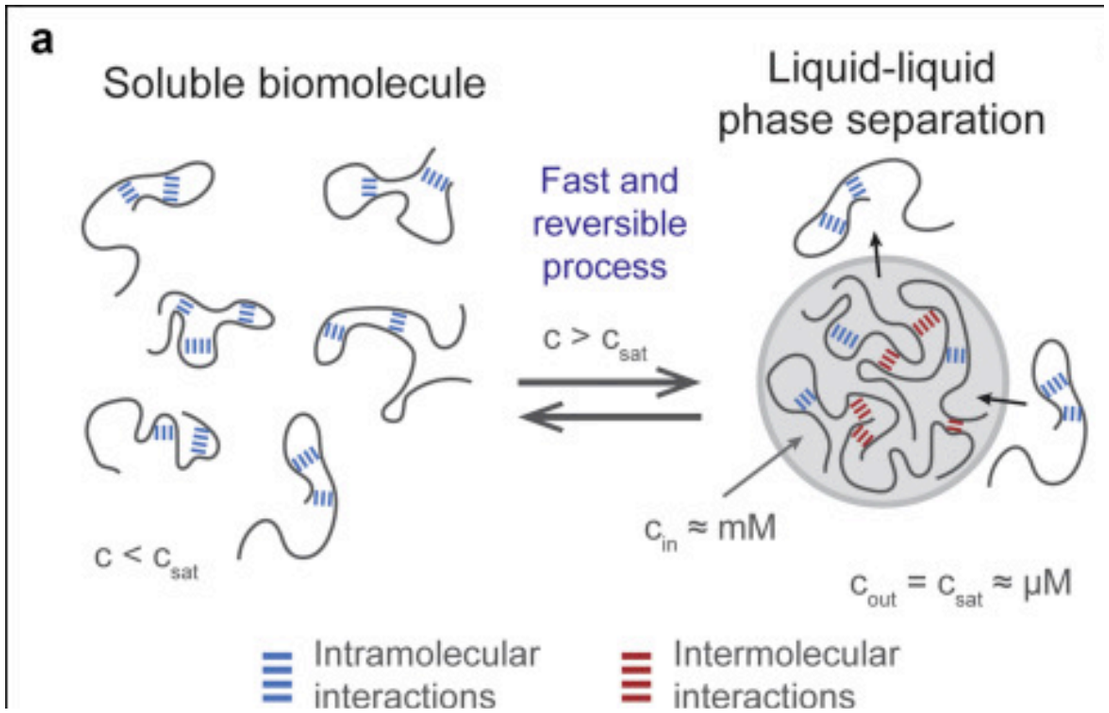

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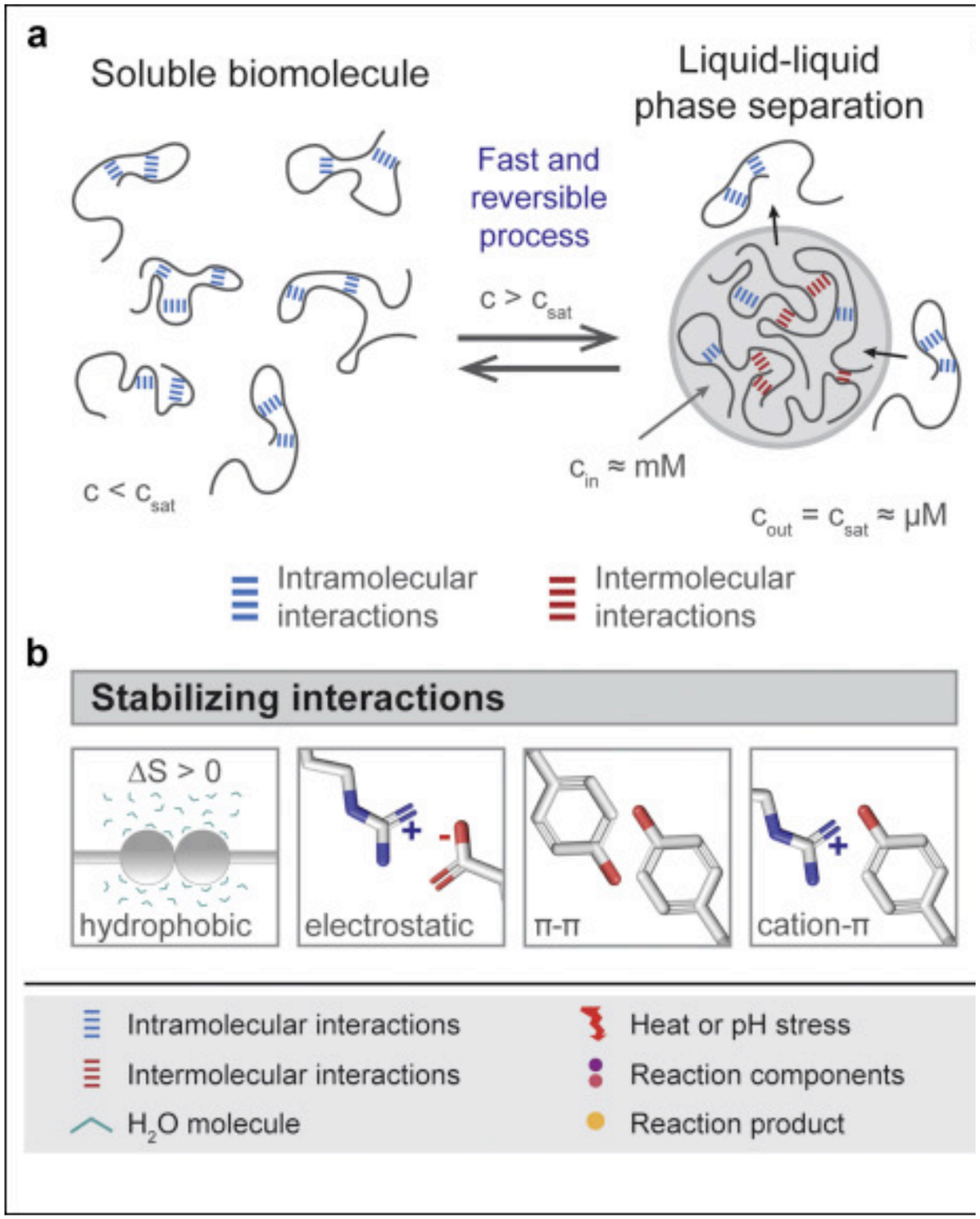

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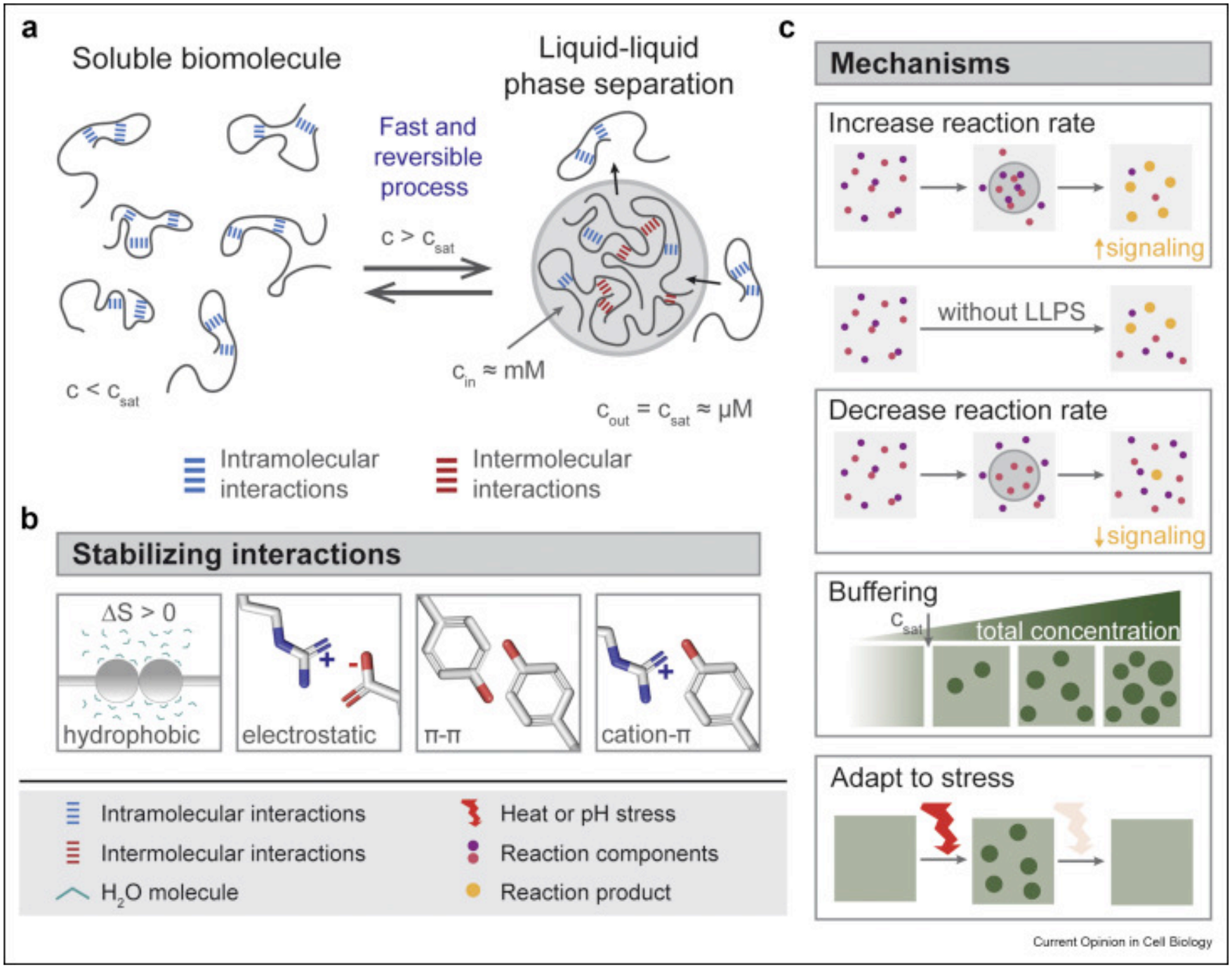

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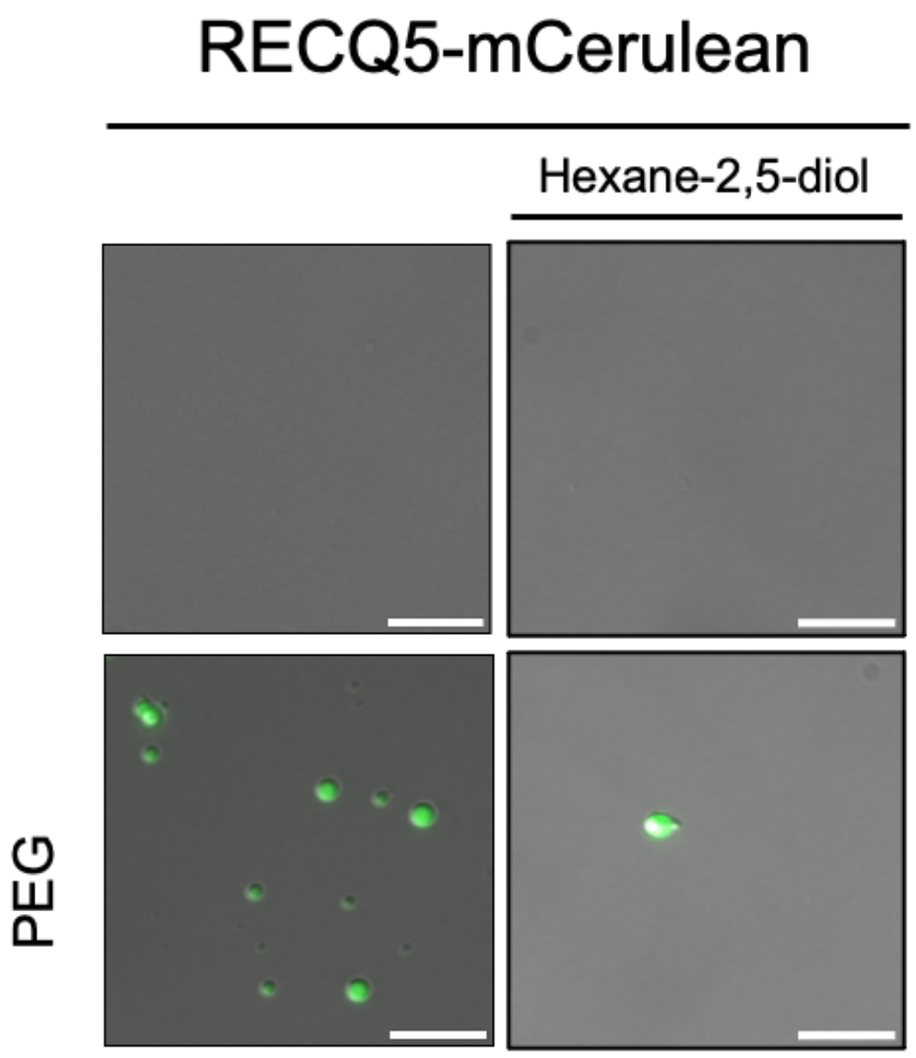

In vitro

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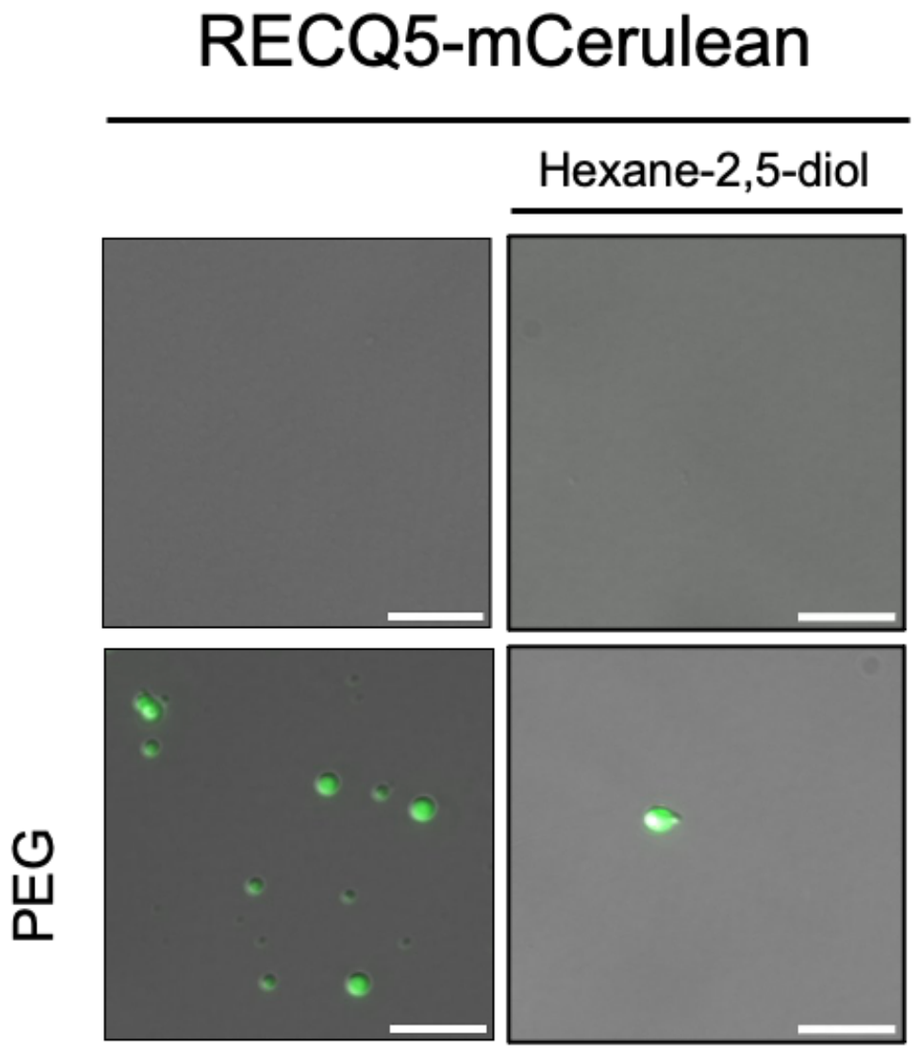

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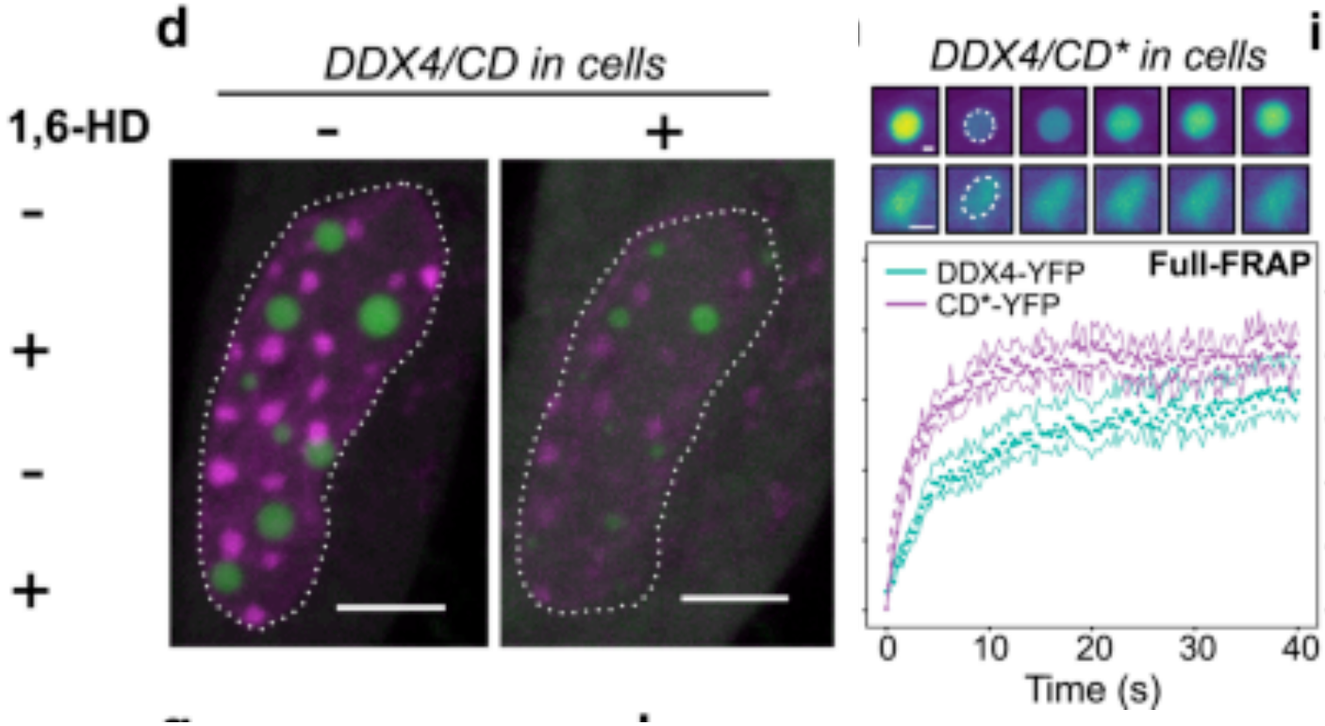

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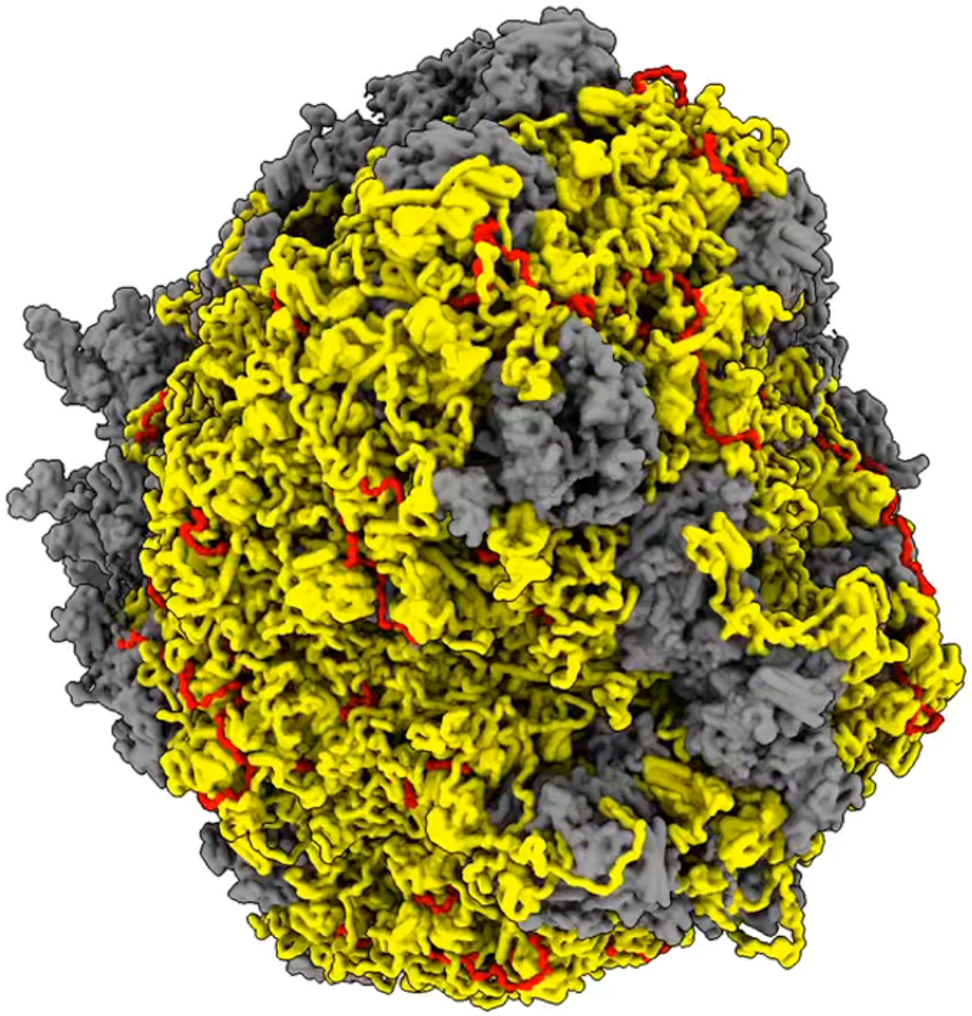

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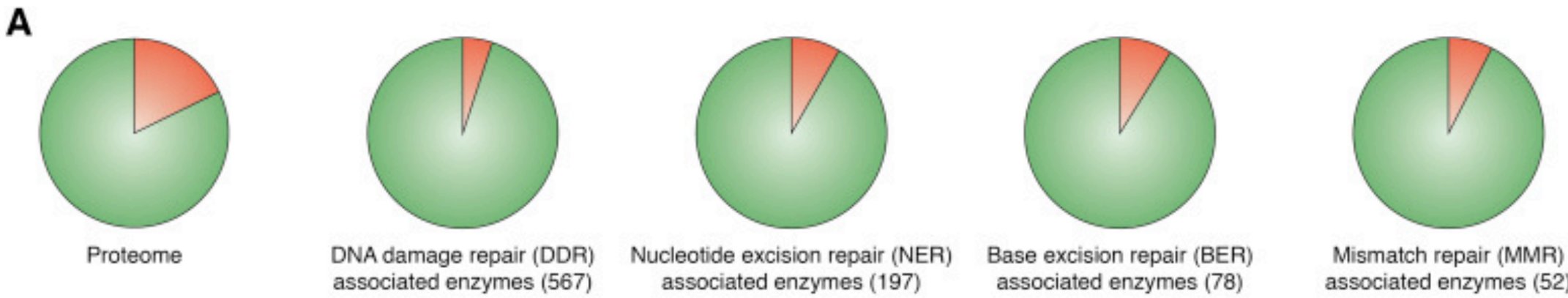

A substantial portion of DNA repair factors contain intrinsically-disordered regions (IDR)

Liquid-liquid phase separation may promote the efficiency of DNA repair pathways by organising the proteins into dedicated “repair factories”

Transient summary IV

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Despite highly dense environment in the nuclei, DNA repair factors are recruited to the sites of DNA damage within seconds

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Complexes may be formed by group modifications (*e.g.*, by SUMO) to form transient repair complexes at the sites of DNA damage

A novel concept – liquid-liquid phase separation may provide a clue, whereby proteins may be self-organising into repair factories, promoting efficient DNA repair

microbial cell

Review

www.microbialcell.com

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

Hannah L Klein[1,*], Kenny K.H. Ang[2], Michelle R. Arkin[2], Emily C. Beckwitt[3,4], Yi-Hsuan Chang[5], Jun Fan[6], Youngho Kwon[7,8], Michael J. Morten[1], Sucheta Mukherjee[9], Oliver J. Pambos[6], Hafez el Sayyed[6], Elizabeth S. Thrall[10], João P. Vieira-da-Rocha[9], Quan Wang[11], Shuang Wang[12,13], Hsin-Yi Yeh[5], Julie S. Biteen[14], Peter Chi[5,15], Wolf-Dietrich Heyer[9,16], Achillefs N. Kapanidis[6], Joseph J. Loparo[10], 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?
Step1: identify the genes

Molec. gen. Genet. 125, 197—216 (1973)

Interactions among Genes Controlling Sensitivity to Radiation and Alkylation in Yeast

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Department of Biology, York University, Toronto, Canada

Received March 27, 1973

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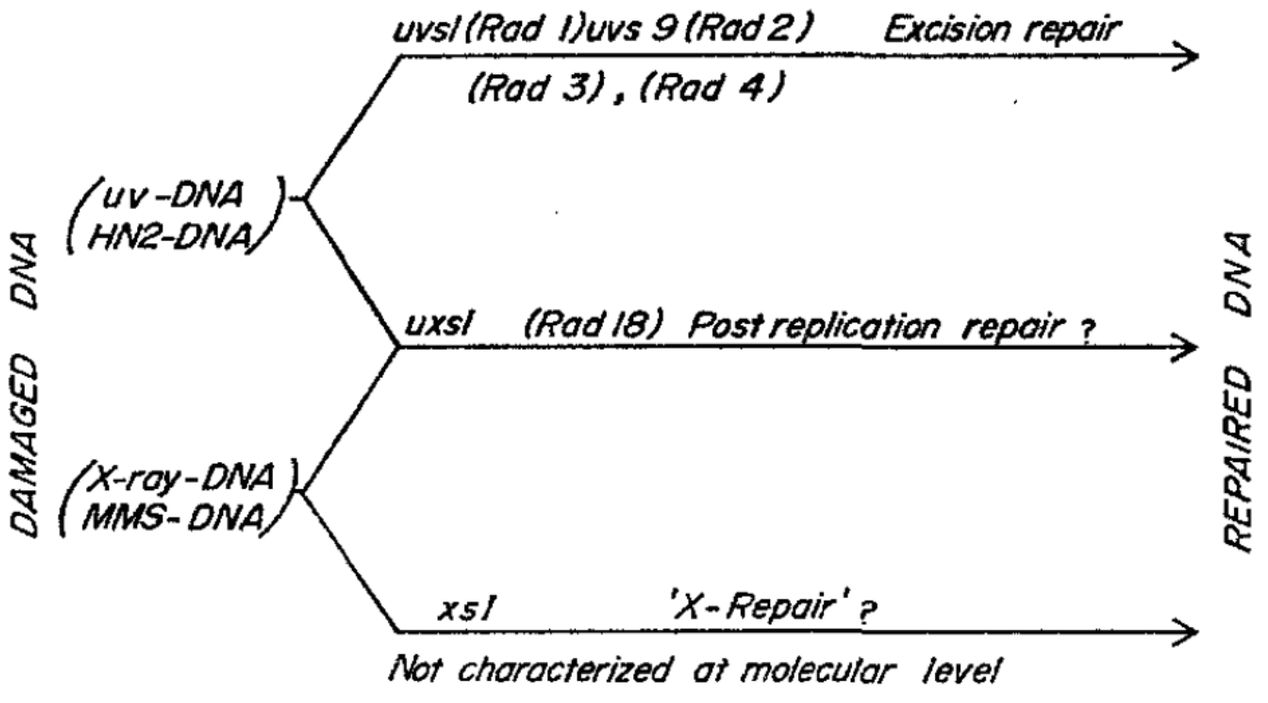
Using a thorough genetic analysis of the isolated mutants, they were able to build a first model of multiple pathways dealing with DNA damage.



How to study genome stability maintenance?
Step1: 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

Eleni P. Mimitou[1] & Lorraine S. Symington[1]

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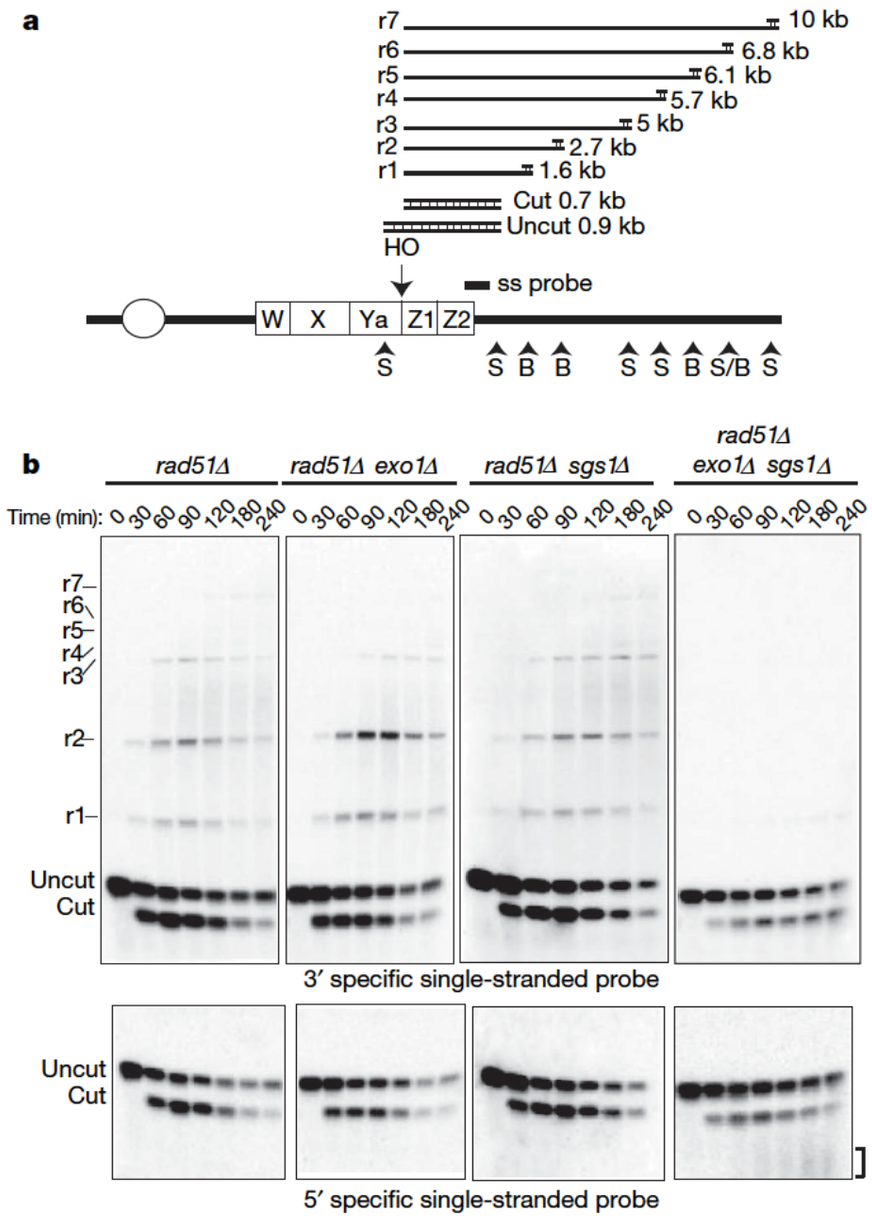

Figure 3 | Single-stranded intermediates fail to form in the absence of Exo1 and Sgs1. **a**, Representation of the method used to detect single-stranded

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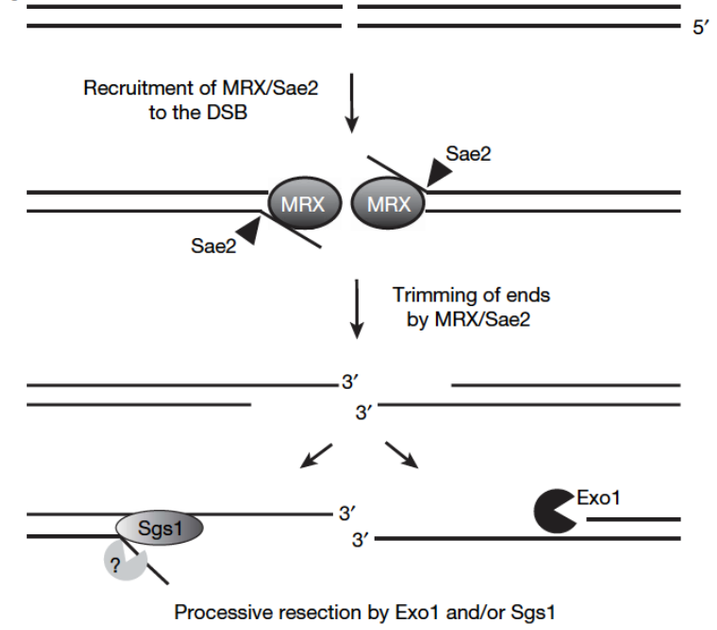

Figure 5 | Two-step mechanism for DSB resection. After a DSB is formed

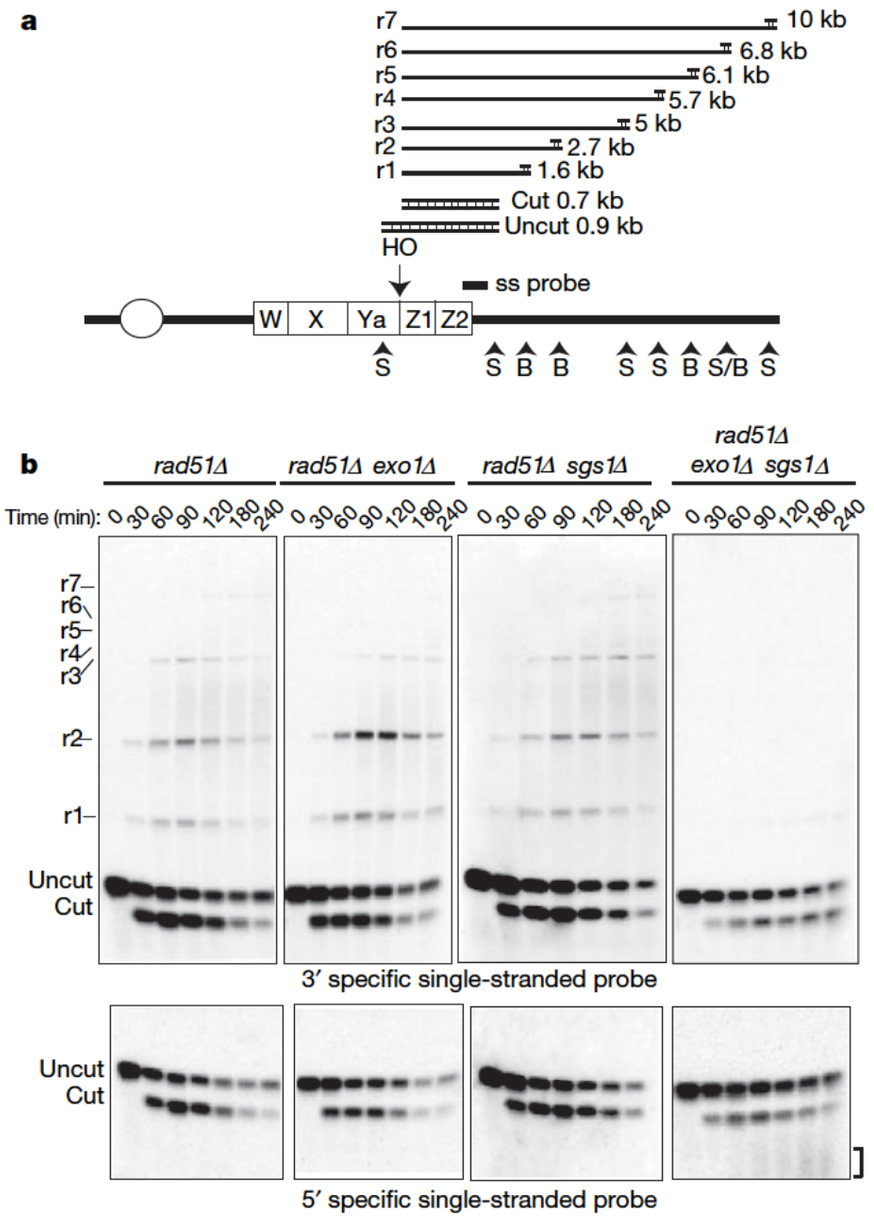

Figure 3 | Single-stranded intermediates fail to form in the absence of Exo1 and Sgs1. a, Representation of the method used to detect single-stranded

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

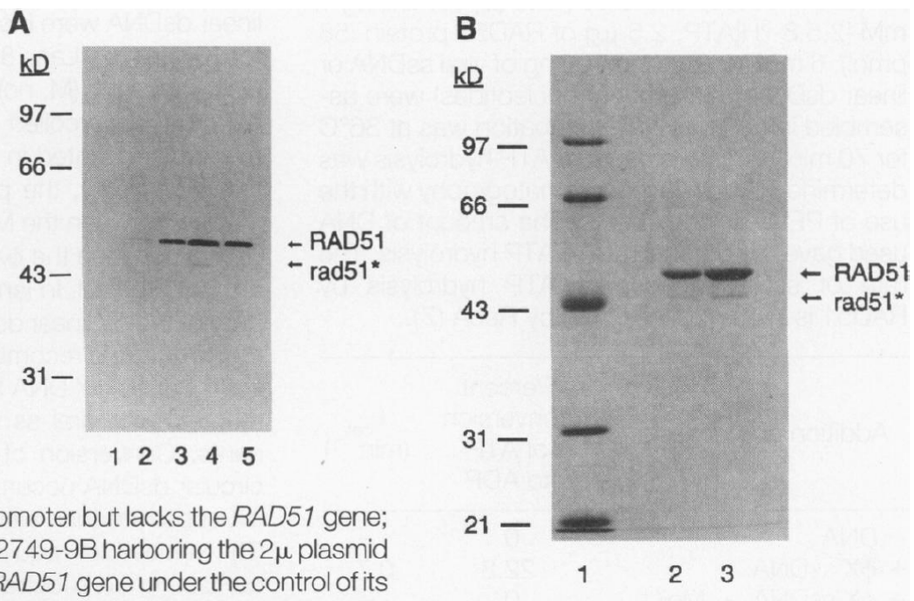

Fig. 1. Overproduction and purification of RAD51 protein. (**A**) Immunoblot analysis. The nitrocellulose blot of a 9% denaturing polyacrylamide gel was probed with affinity-purified antibodies to RAD51. Lane 1, extract from the *rad51* deletion yeast strain YR51Δ-1; lane 2, extract from the yeast strain LP2749-9B harboring the 2μ multicopy vector pSCW231, which contains the *ADC1* promoter but lacks the *RAD51* gene; lane 3, extract from strain LP2749-9B harboring the 2μ plasmid pR51.2, which contains the *RAD51* gene under the control of its own promoter; lane 4, extract from strain LP2749-9B harboring the 2μ plasmid pR51.1, which contains the *RAD51* gene under the control of the *ADC1* promoter; and lane 5, 10 ng of purified RAD51 protein. (**B**) Purity analysis by SDS-PAGE. A 9% denaturing polyacrylamide gel was stained with Coomassie blue. Lane 1, molecular size markers; lanes 2 and 3, 1 μg and 3 μg of purified RAD51 protein. Molecular sizes are indicated on the left (in kilodaltons).

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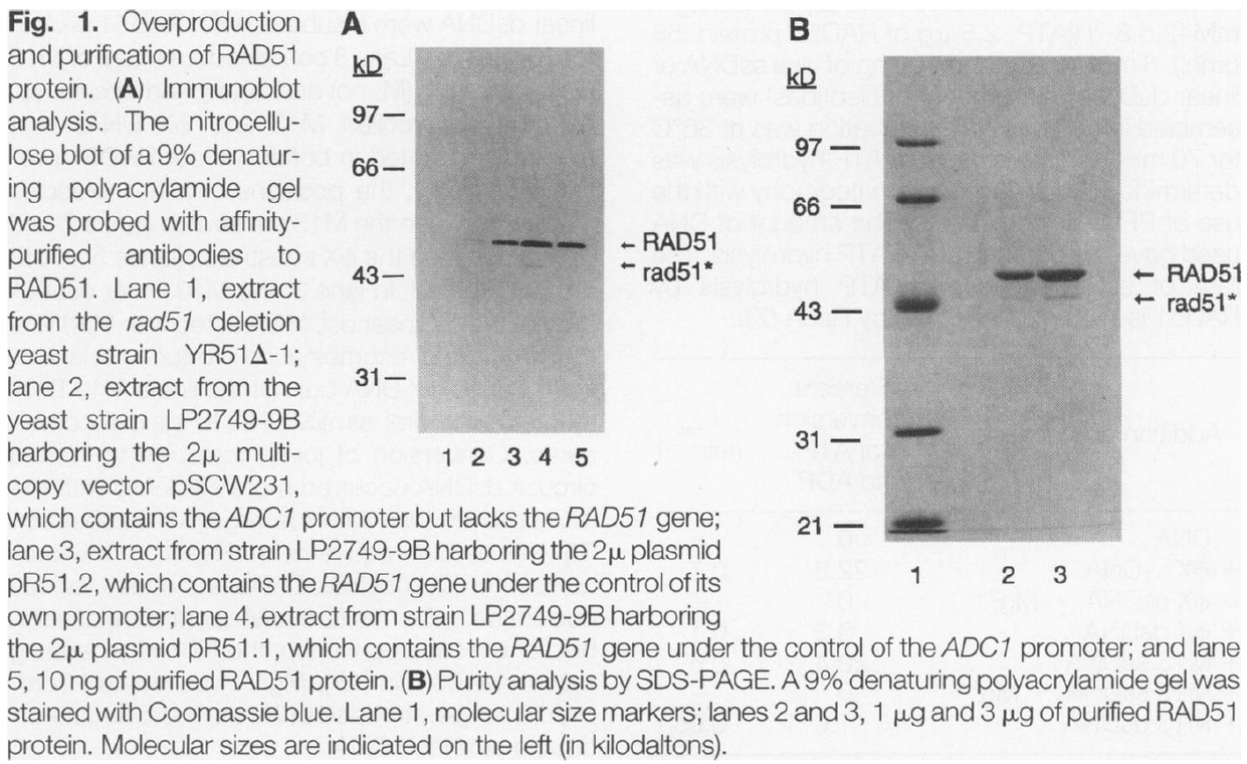

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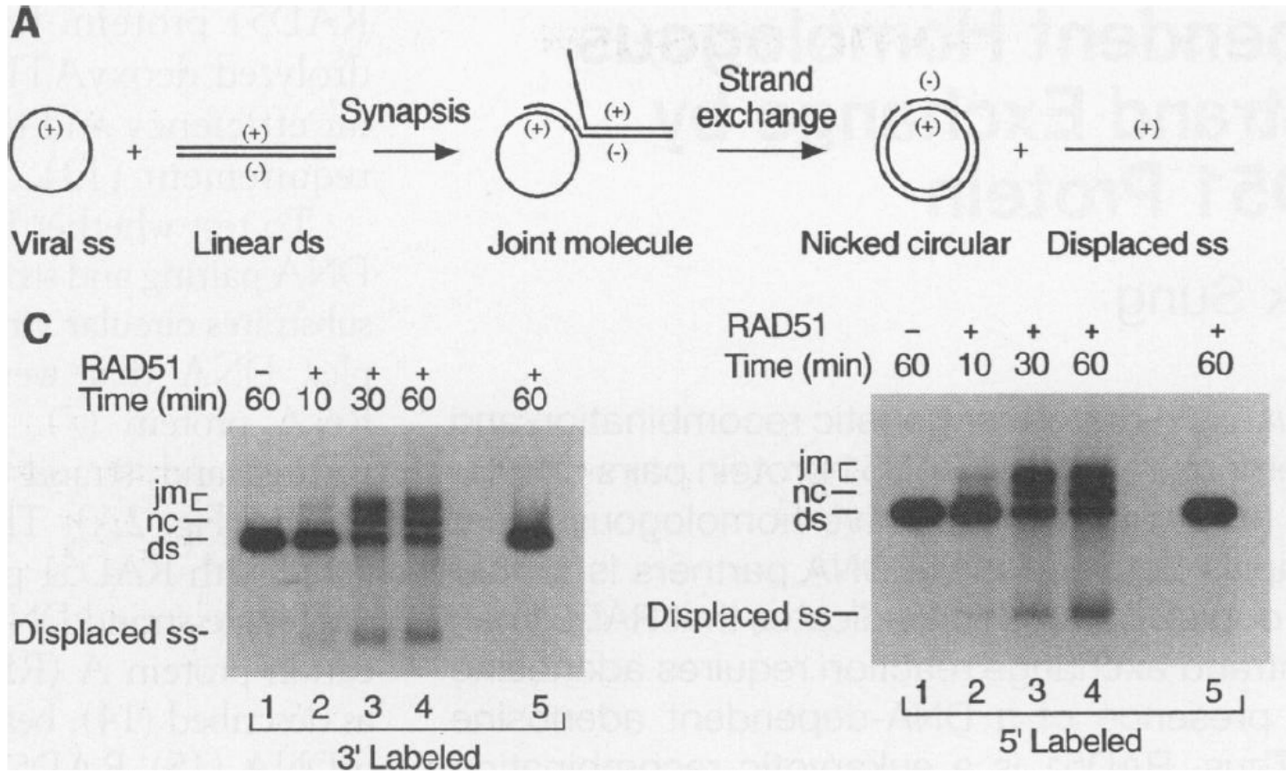

Using a purified protein, Patrick Sung was able to show that Rad51 is a bona fide recombinase.

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 Polaczek[3], Taro Masuda-Sasa[3], Subhash Pokharel[3], Judith L. Campbell[3] & Stephen C. Kowalczykowski[1,2]

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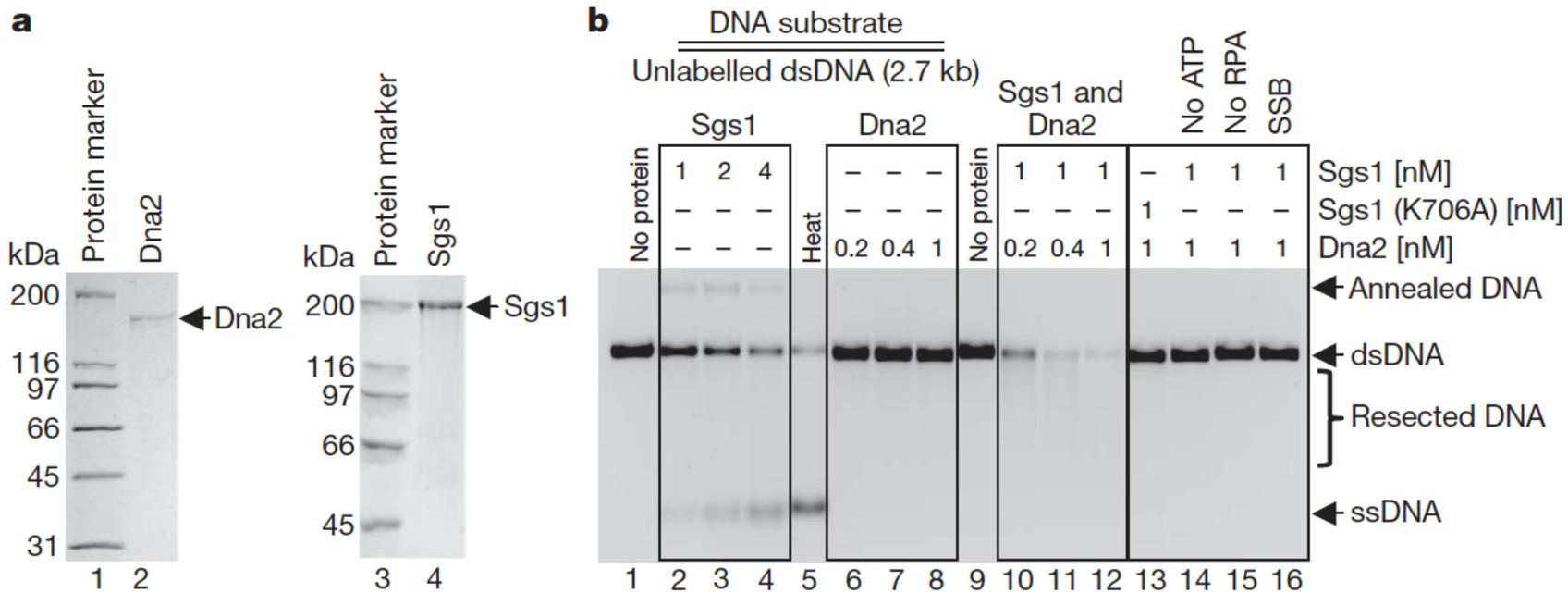

Using purified proteins, Cejka et al., were able to reconstitute end resection *in vitro*.

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

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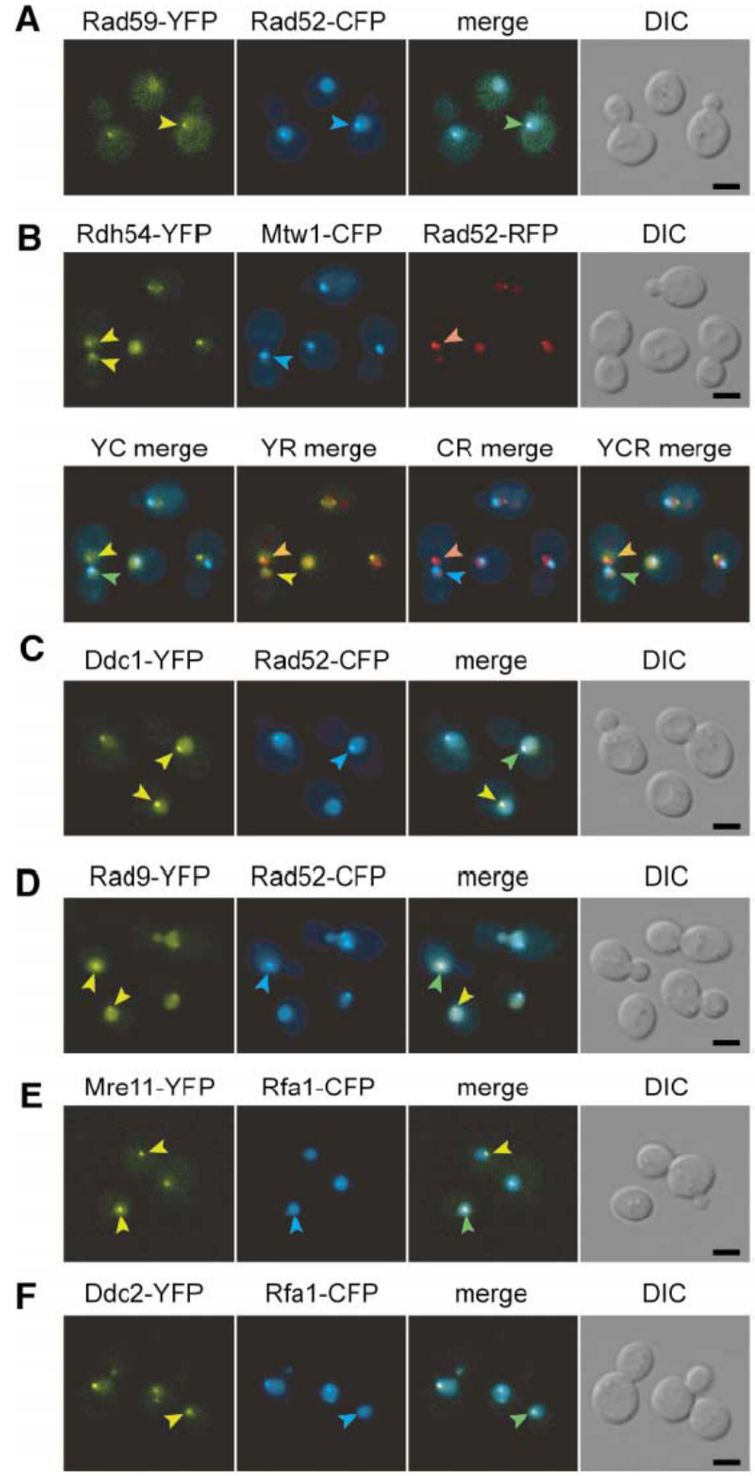

Figure 1. Colocalization of Checkpoint and Repair Foci

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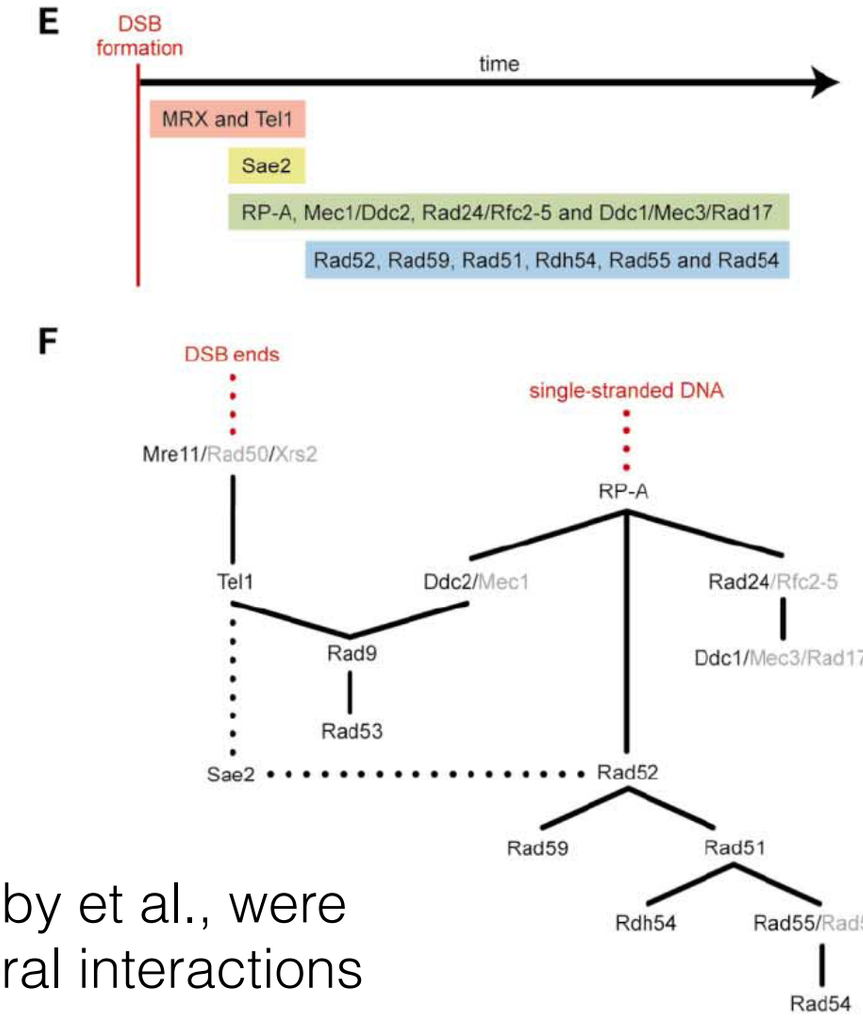


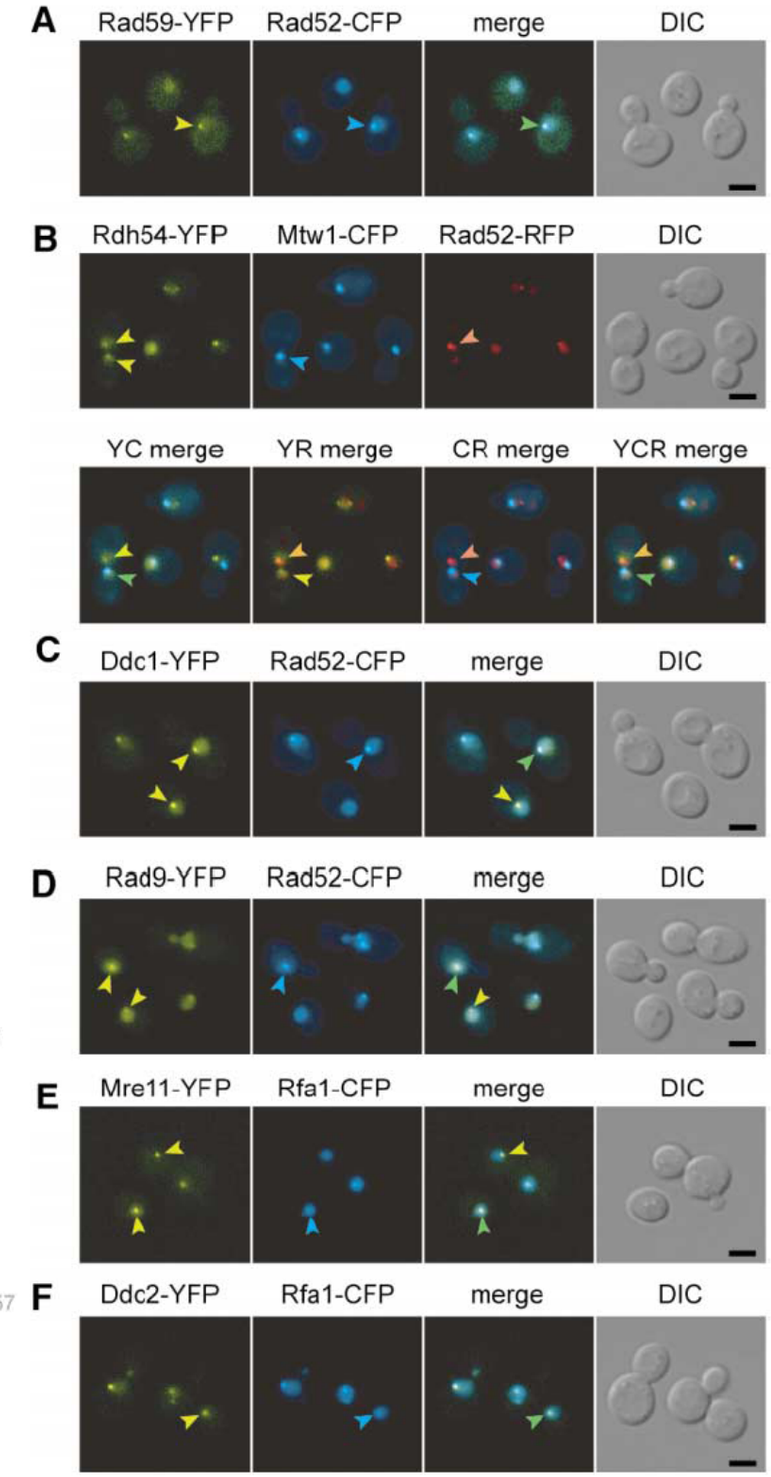


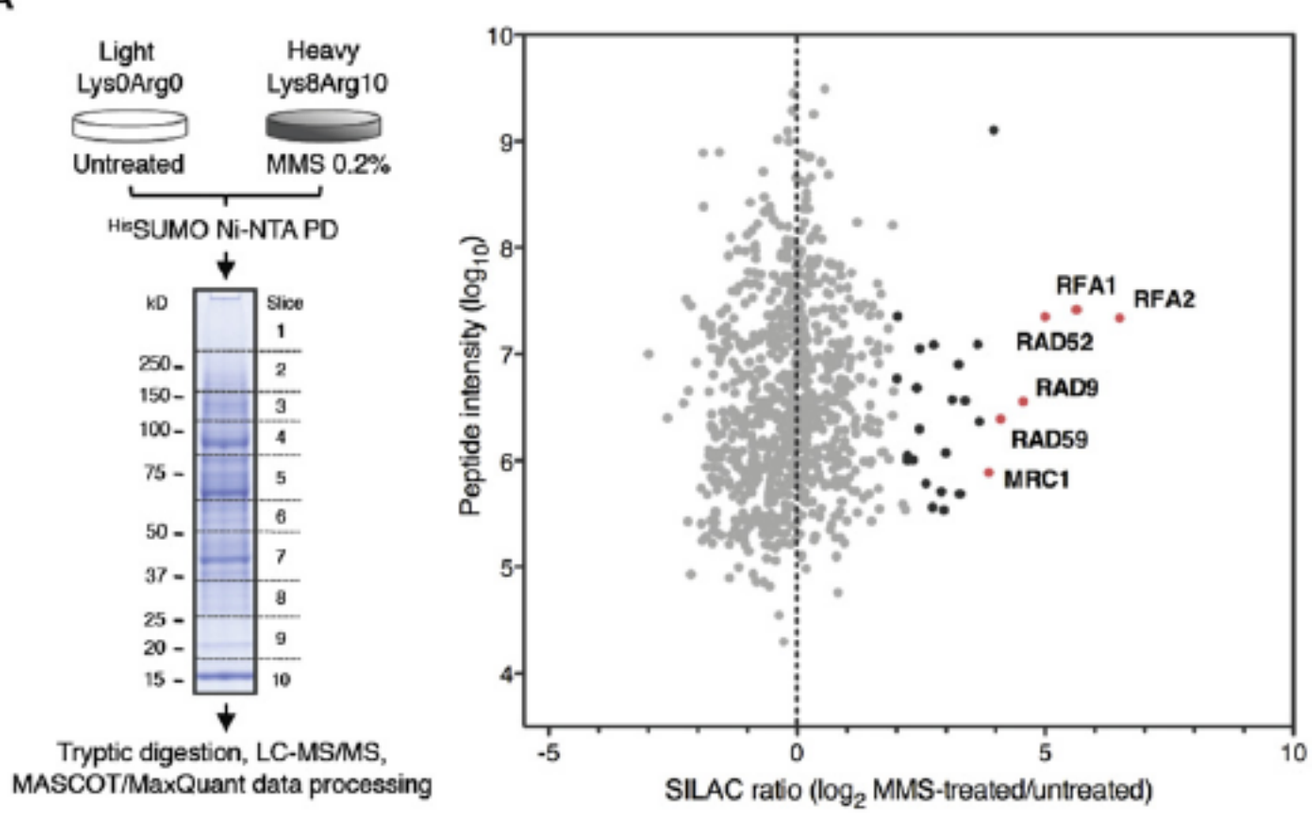
Figure 1. Colocalization of Checkpoint and Repair Foci

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 Psakhye[1] and Stefan Jentsch[1,*]
[1]Department 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.

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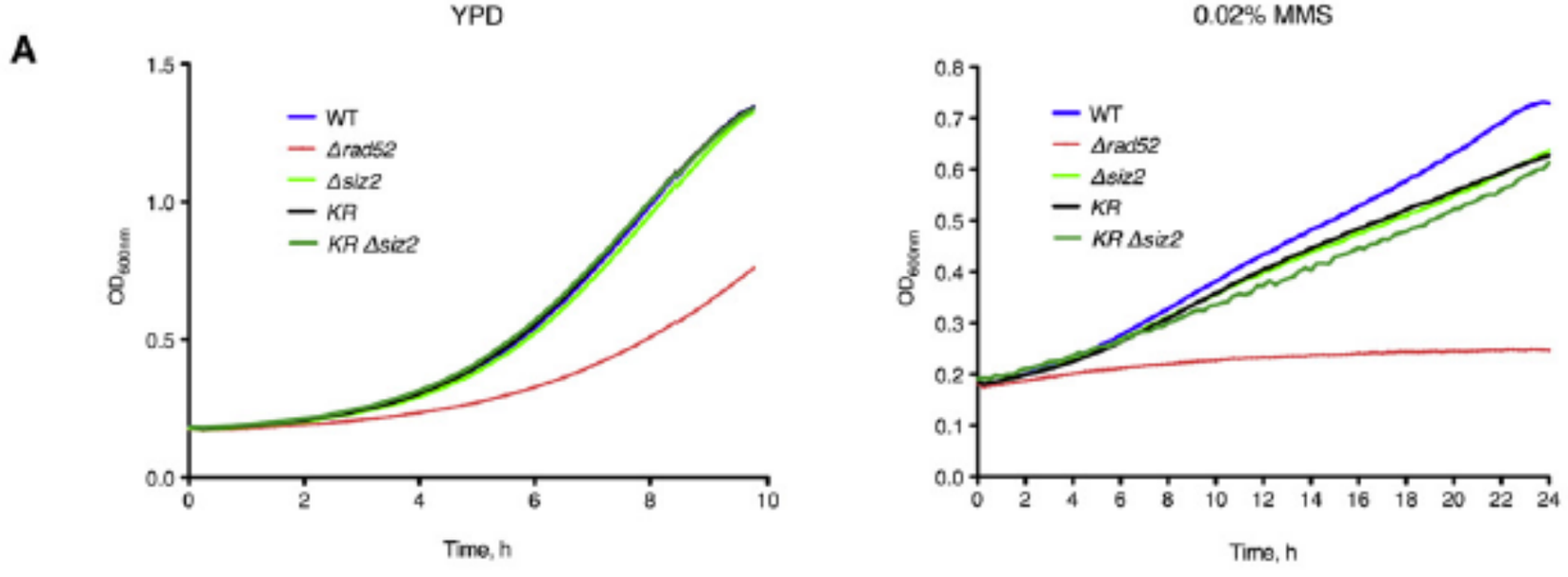

This Sumo-SIM mediated interactions are trigger timely completion of HR.

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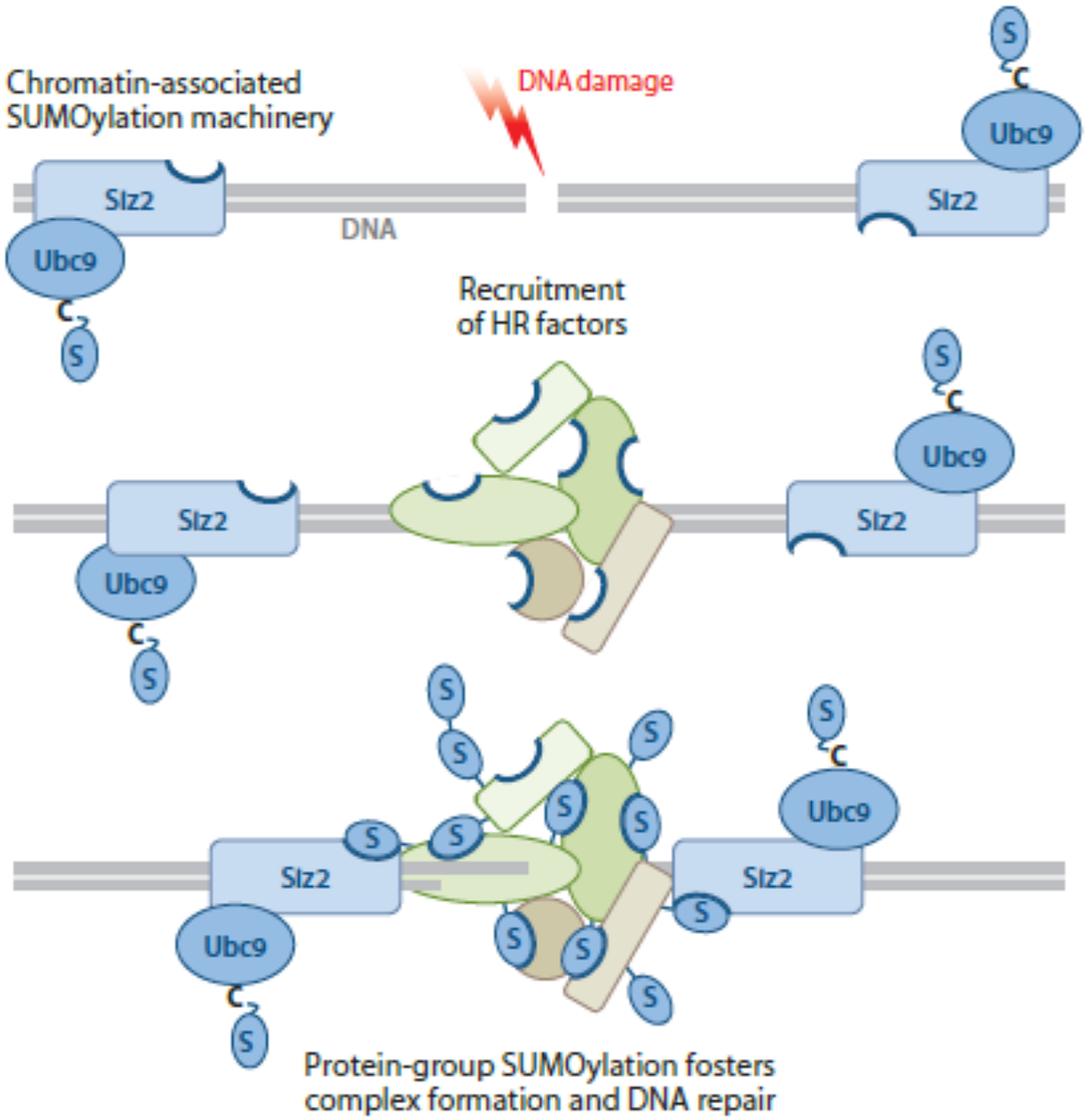

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How to study genome stability maintenance? Step5: study the molecular mechanisms by the means of structural biology

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

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

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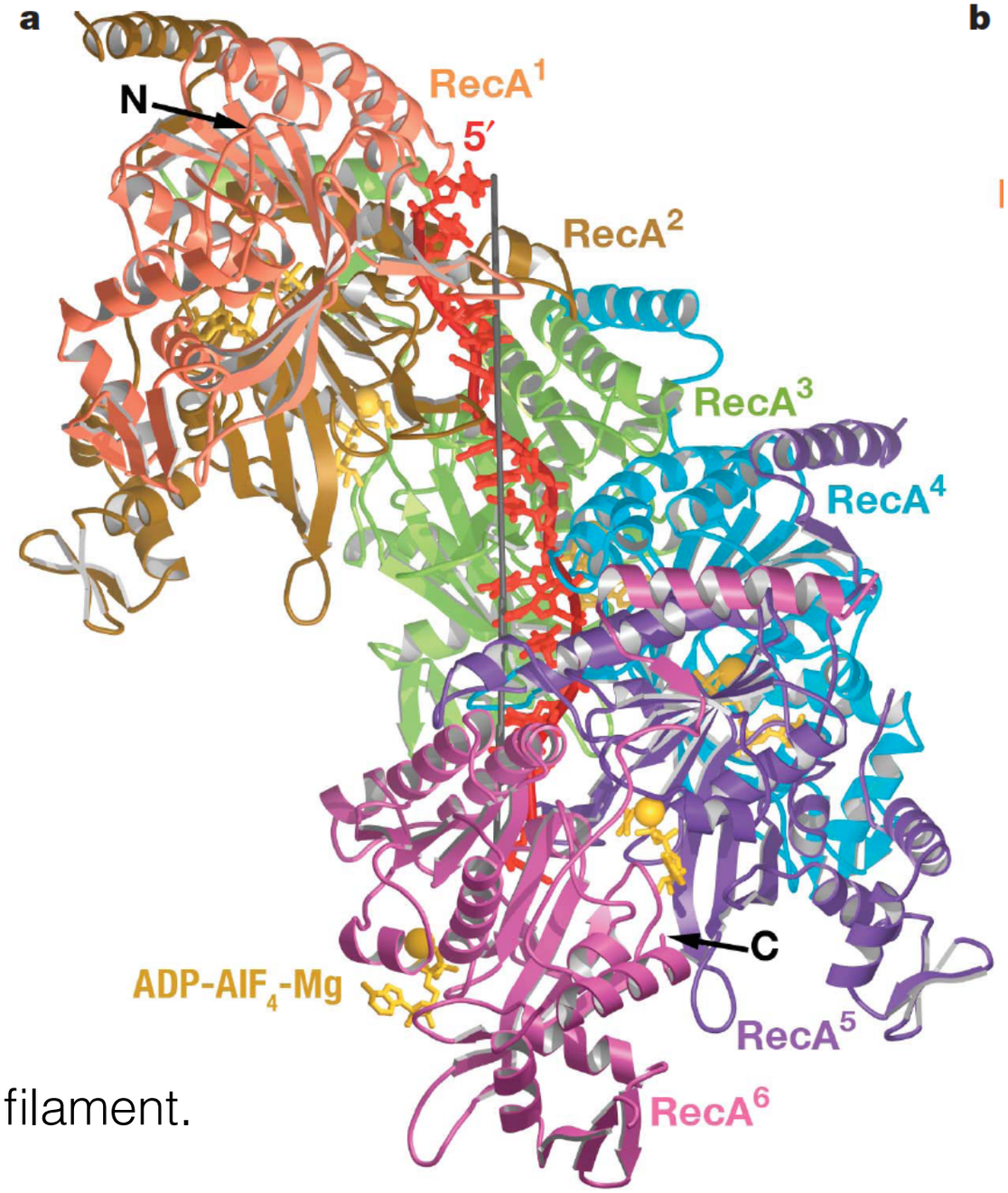

Crystal structure of presynaptic filament.

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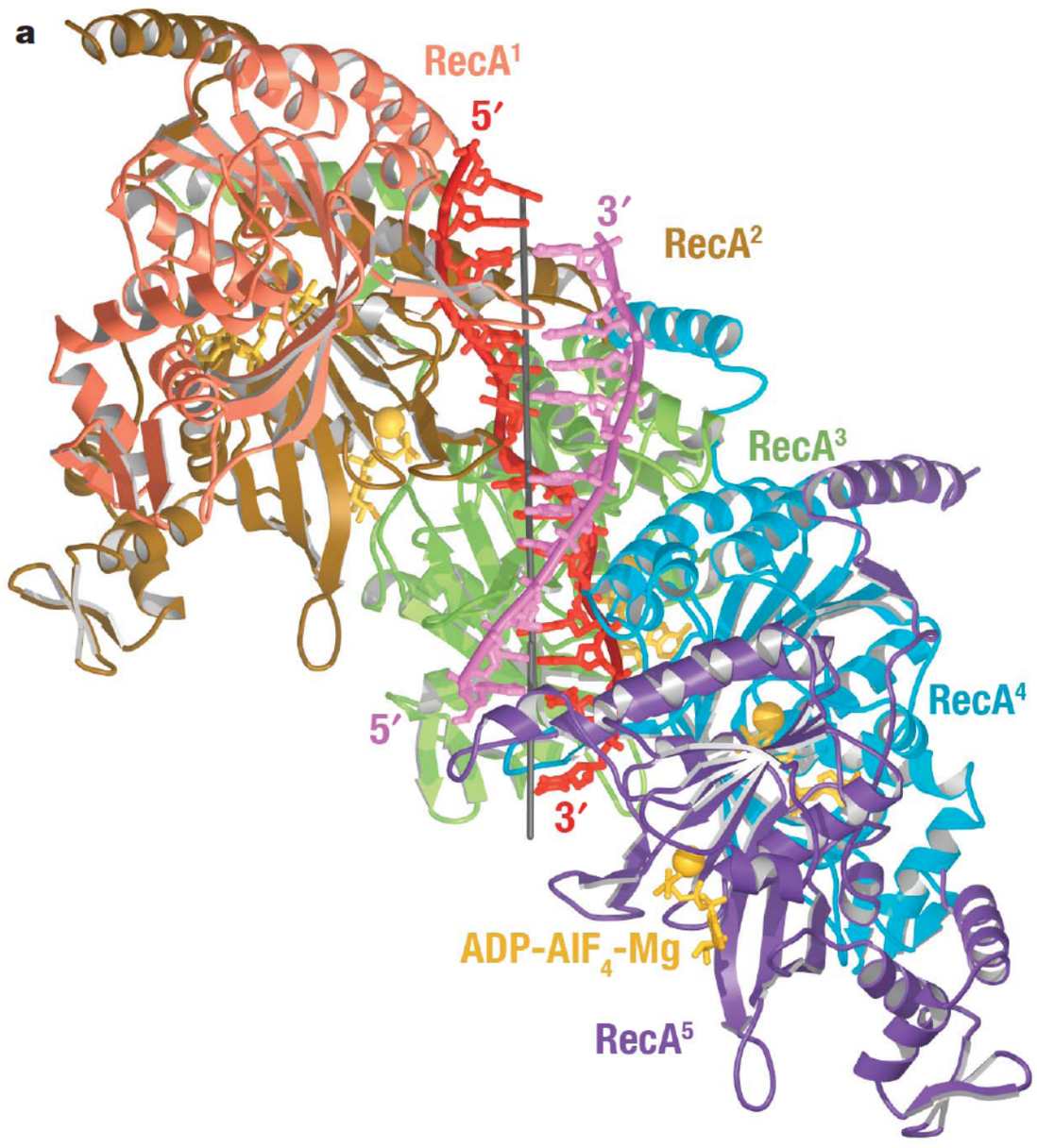

Crystal structure of postsynaptic filament.

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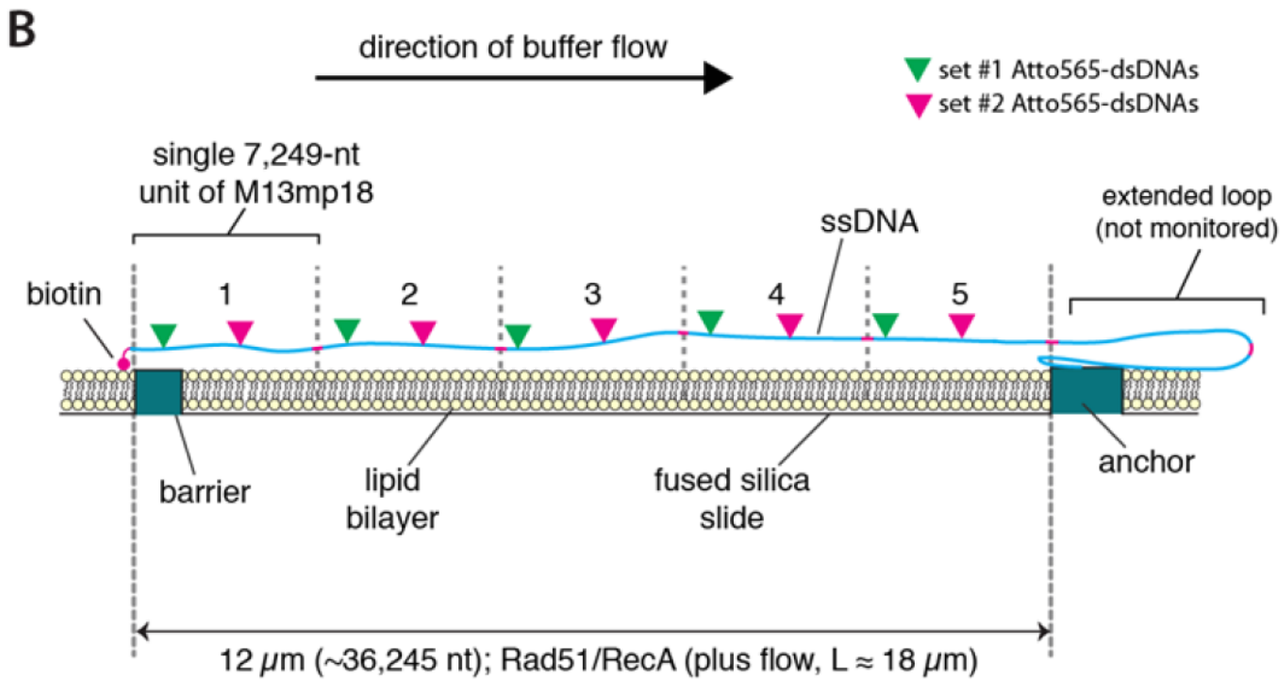
By comparing the two structure a detailed, molecular mechanism of the strand exchange reaction can be inferred.

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]†
YoungHo Kwon,[4] William A. Gaines,[4] Weixing Zhao,[4] Patrick Sung,[4] Eric C. Greene[1,5]‡



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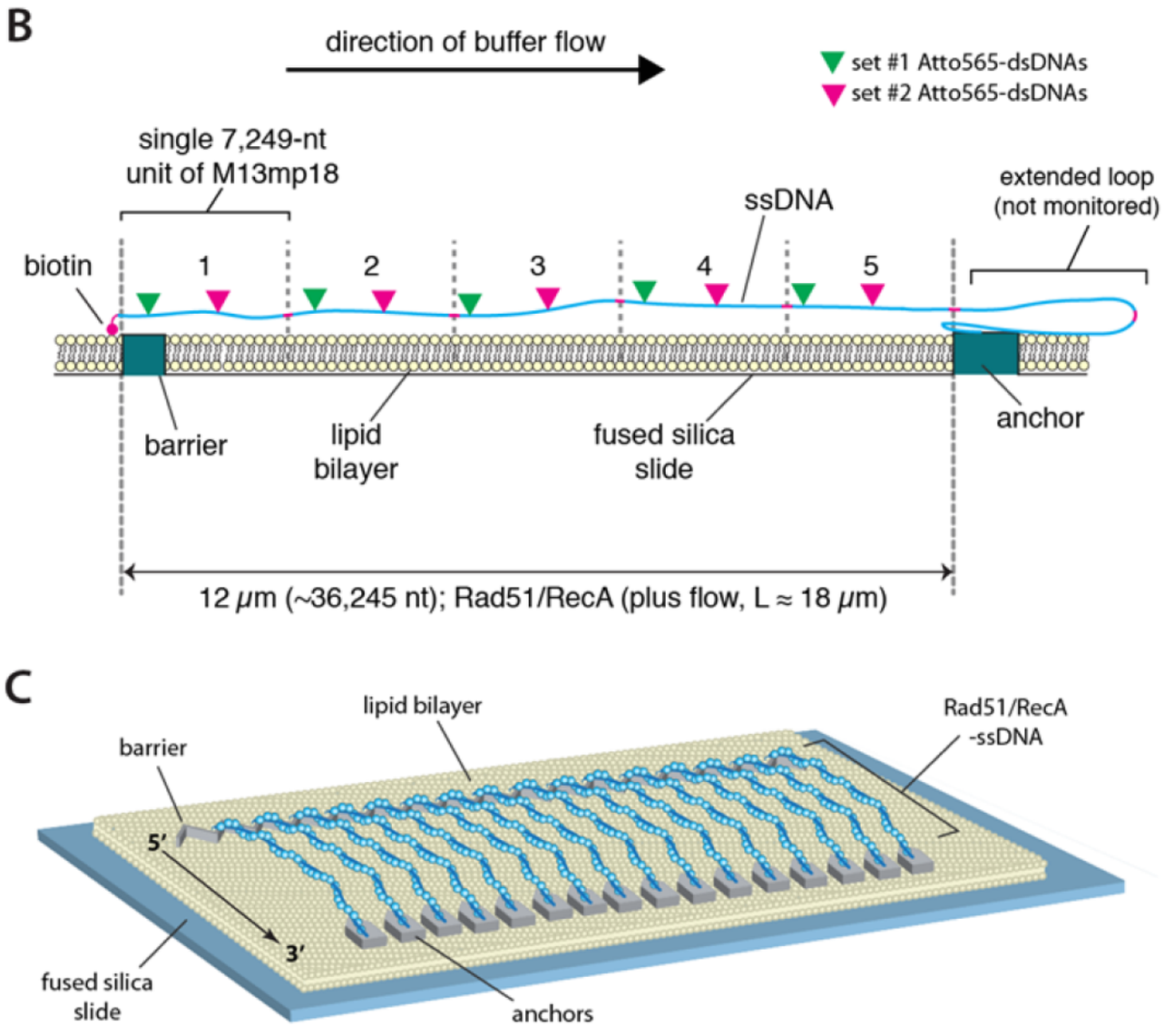

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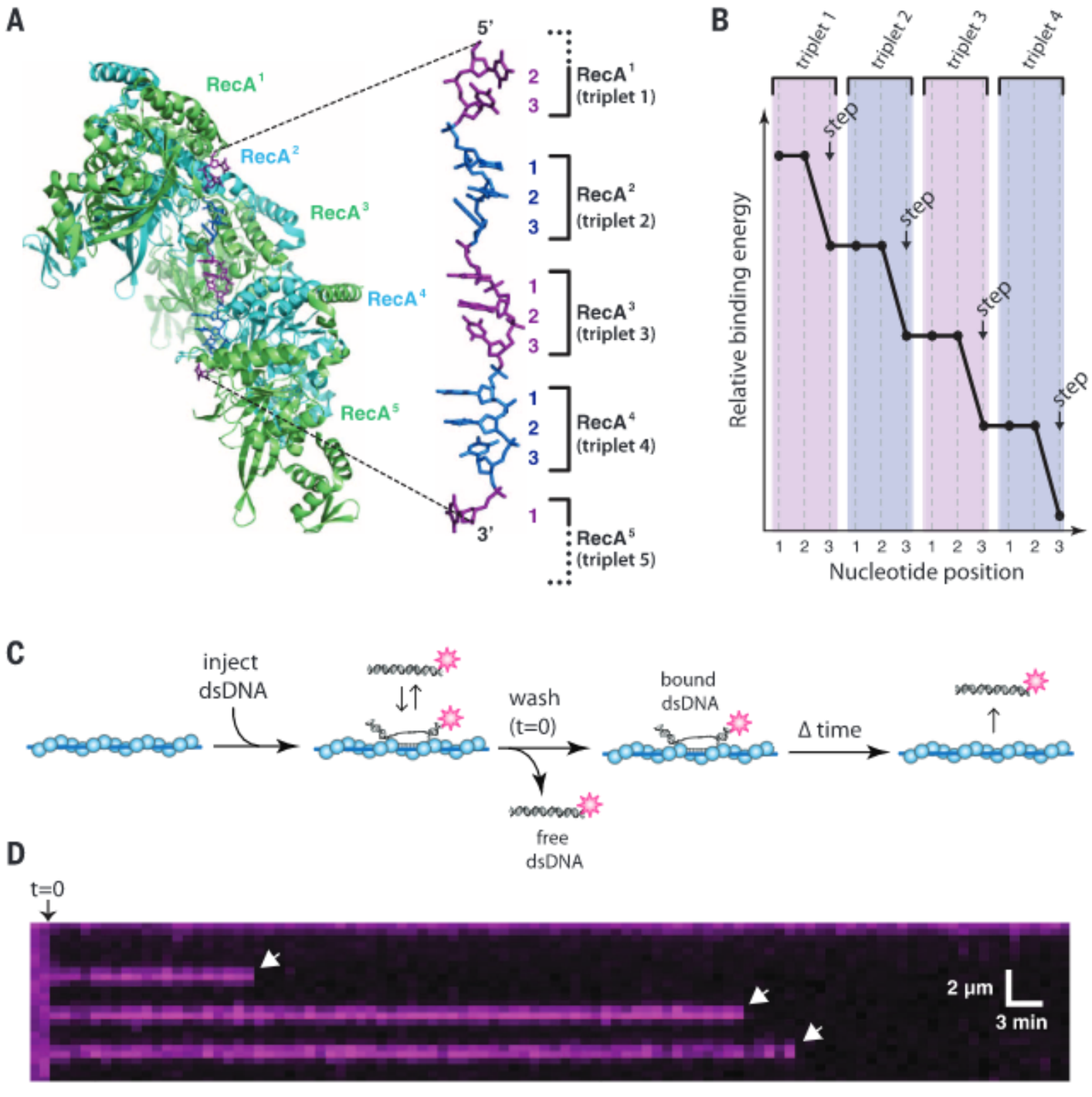

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The techniques must be combined, in order to get a full picture of the pathway

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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