Cellular Stress Response

Literature Talk

Chun (Alice) Li

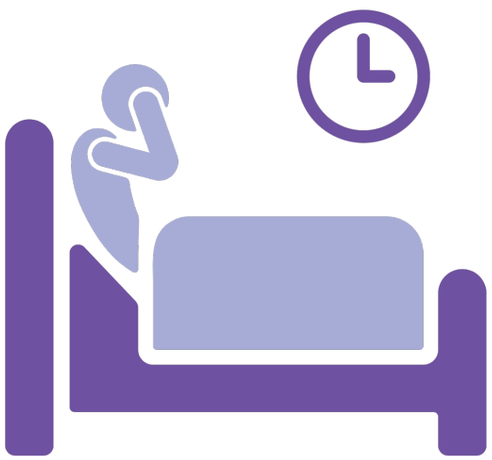
April 2nd, 2024

When we are stressed…

Physical injuries

Working too hard

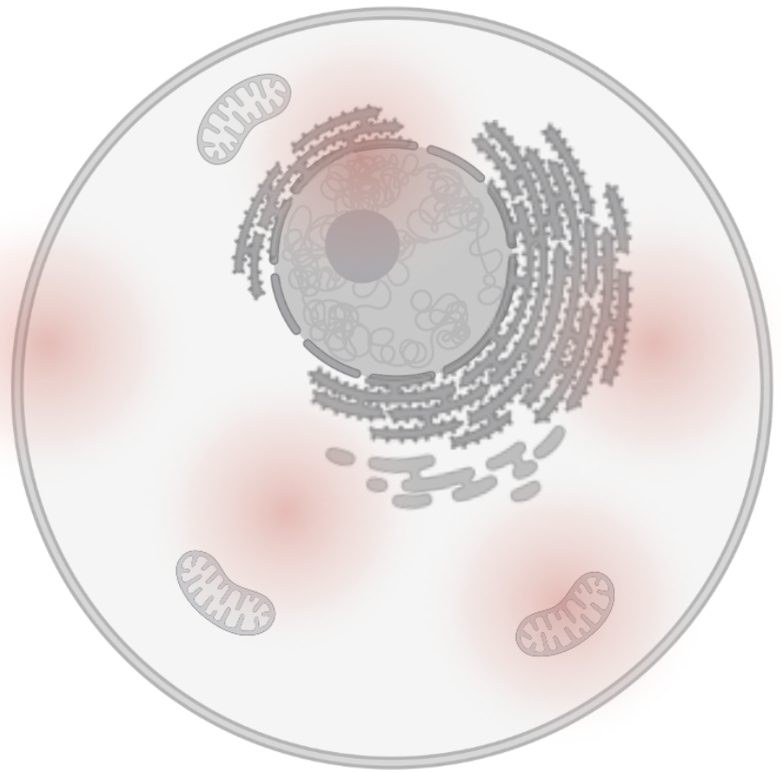
Excessive eating



Bad night of sleep

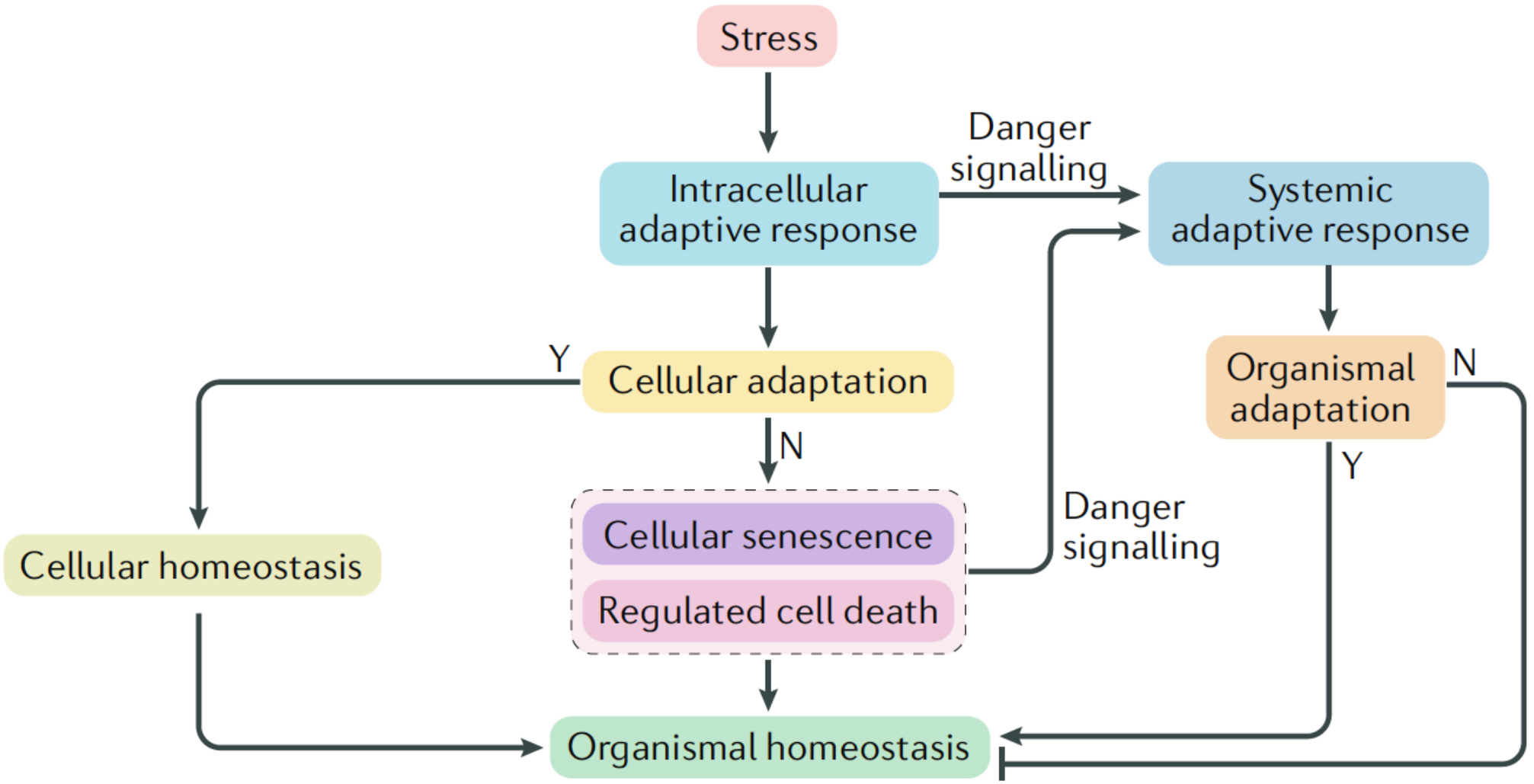
To destress

What would the cell do if it is stressed?



Cells will initiate relevant stress response pathways to destress as well!

Why do we need cellular stress response?

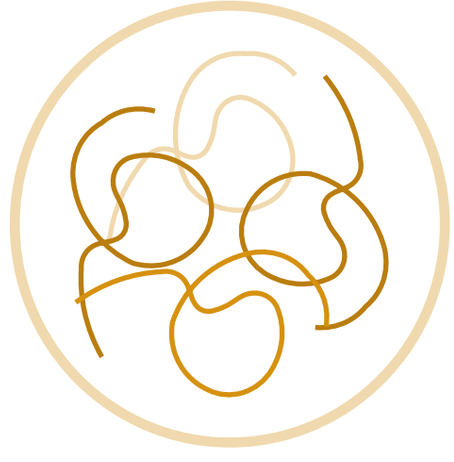


Stress response is key to the return to cellular and/or organismal homeostasis

Galluzzi, L.; Yamazaki, T.; Kroemer, G. *Nat. Rev. Mol. Cel. Biol.* **2018**, *19,* 731.

Key types of stress response

Heat shock response



Unfolded protein response

DNA damage response

Response to oxidative stress

Key types of stress response

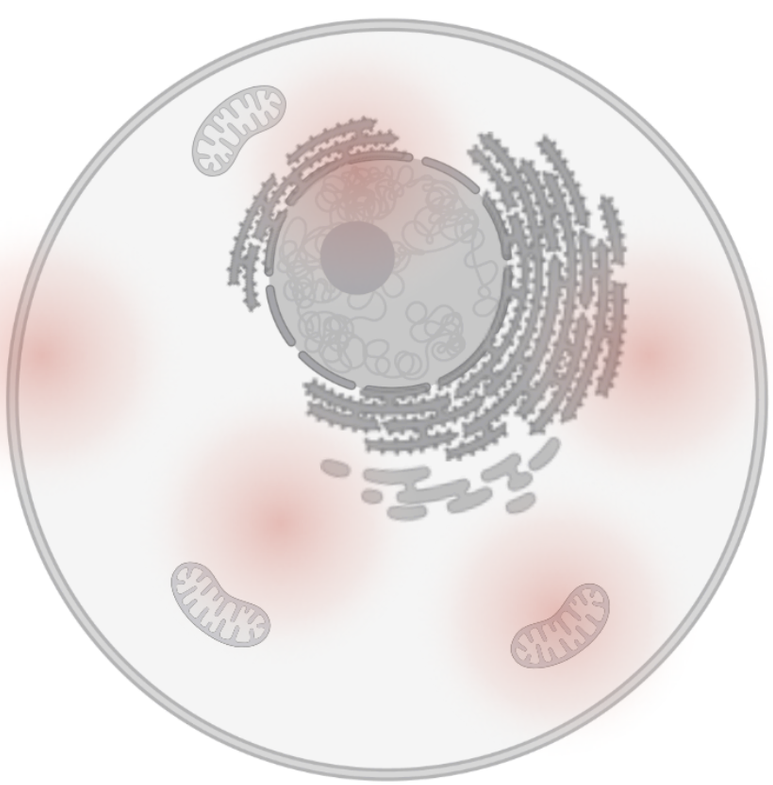
Heat shock response

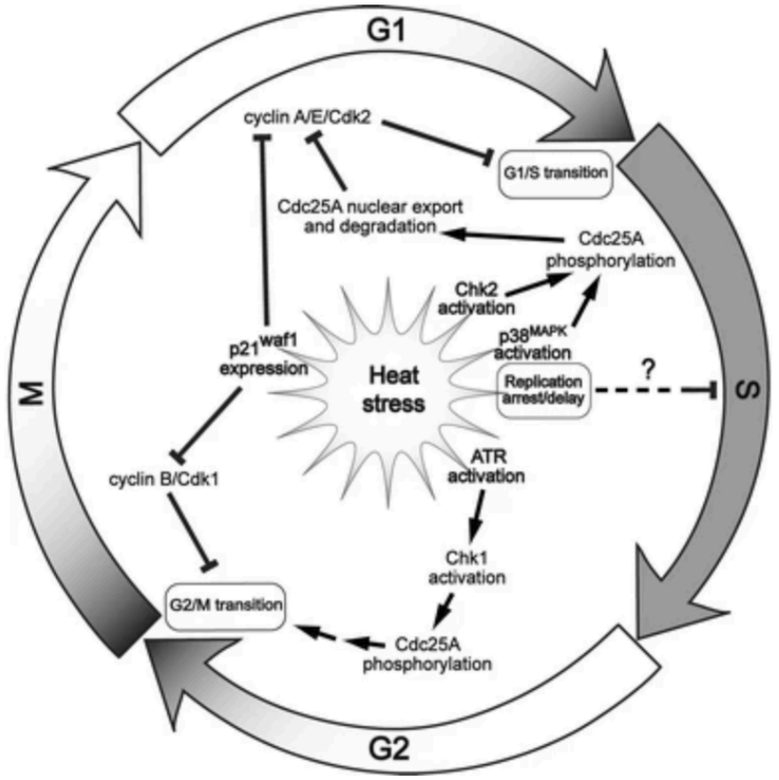
Unfolded protein response

DNA damage response

Response to oxidative stress

Cellular Heat Shock Effect





Protein/lipid/DNA destabilization due to elevated temperature;
Increased cell cycle arrest and cell death

Velichko, A. K. et al. *Cell. Nol. Life. Sci.* **2013**, *70,* 4229.

Heat Shock Response (HSR)

Discovery

First described by

Ferruccio Ritossa in 1962 in Italy

De Maio, A. et al. *Cell Stress Chaperones.* **2012**, *17,* 139.

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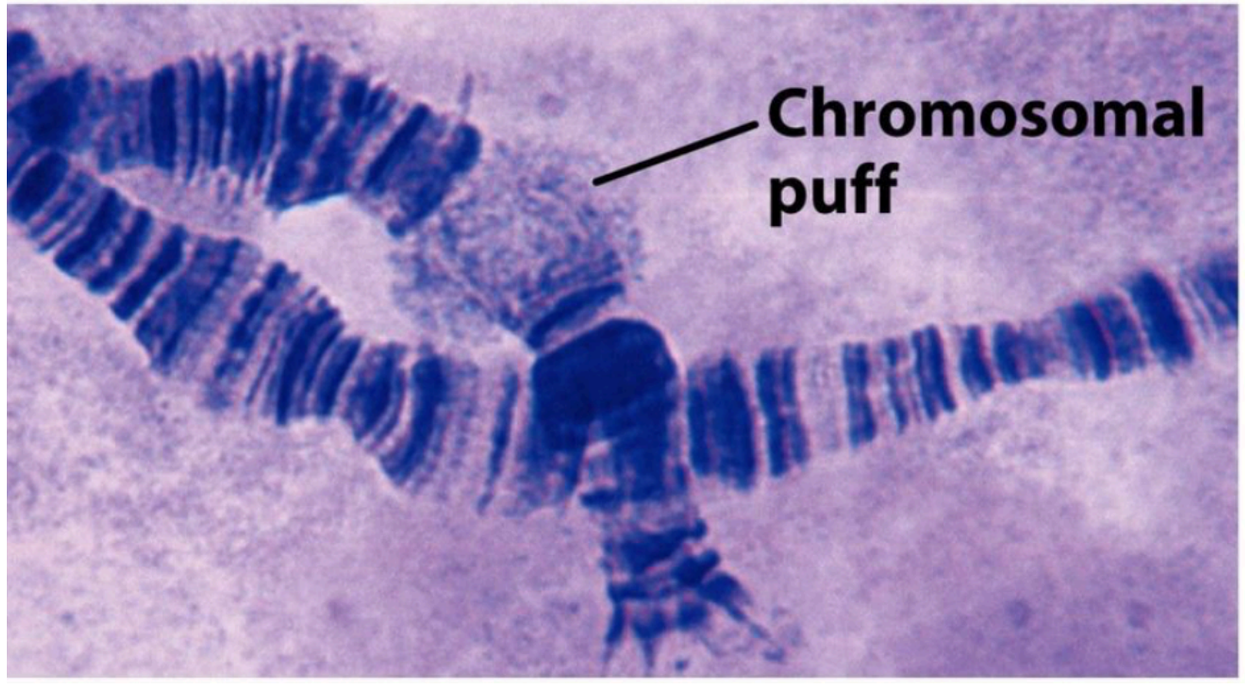

Observed ***different chromosomal puffs in Drosophila genome***
during elevated temperatures

= increased expression of an unknown protein

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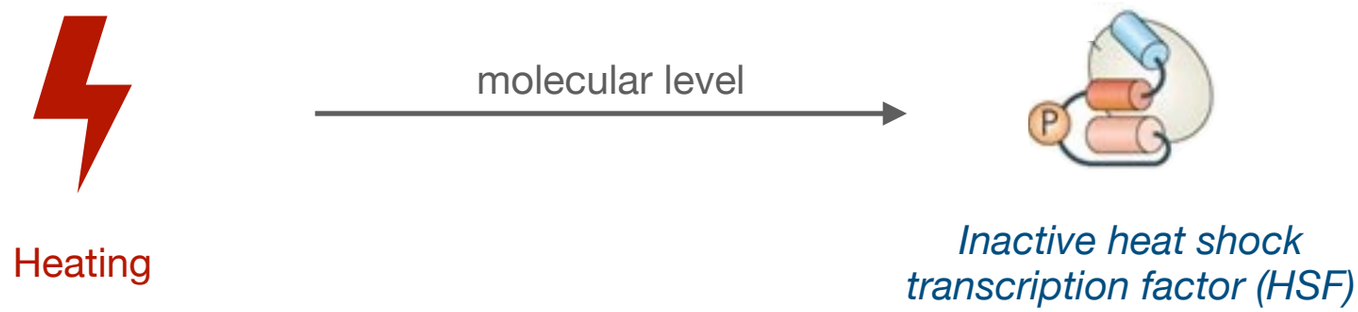
Molecular Heat Shock Response

Initiation



Akerfelt, M.; Morimoto, R. I.; Sistonen, L. *Nat. Rev. Mol. Cel. Biol.* **2010**, *11,* 545.

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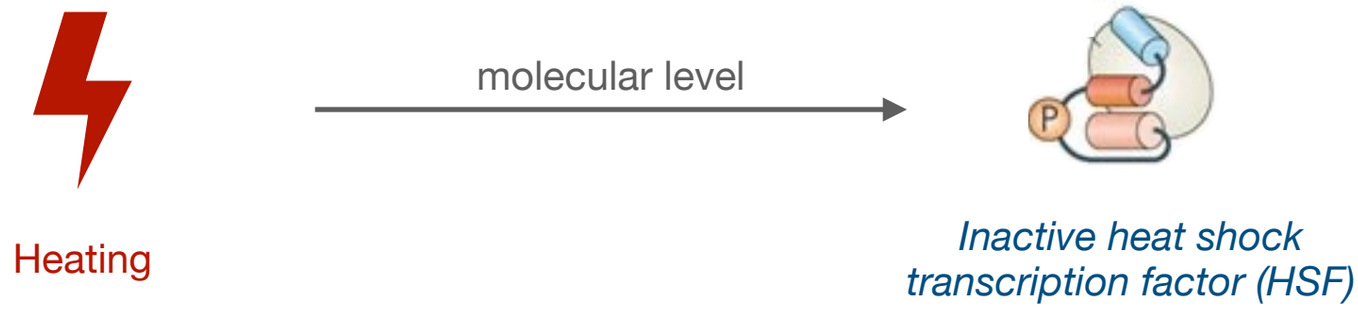

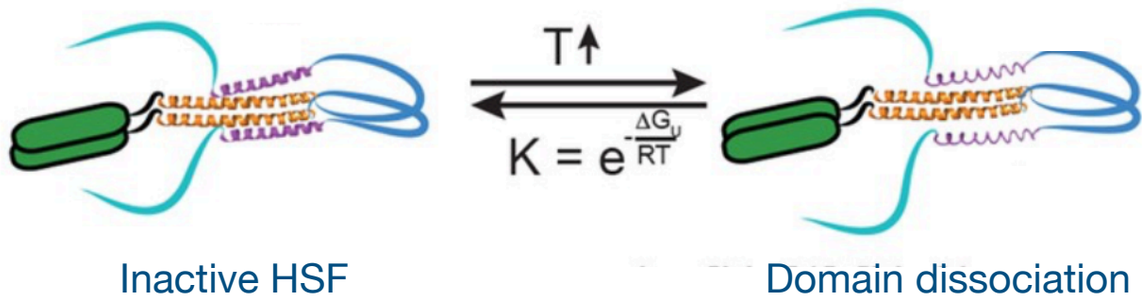

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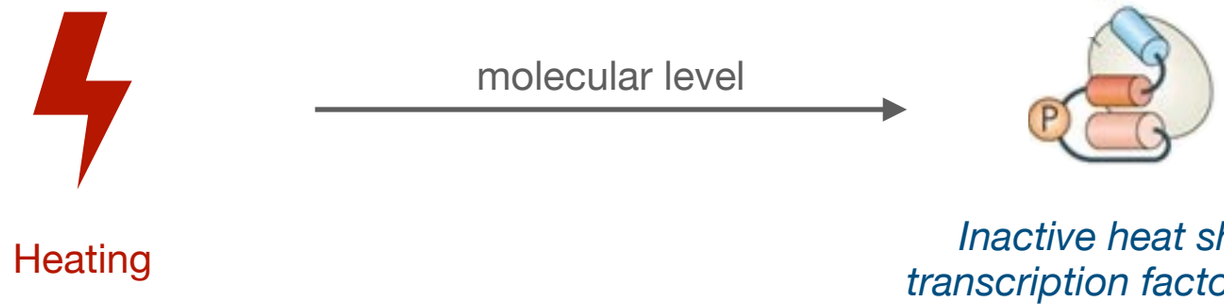

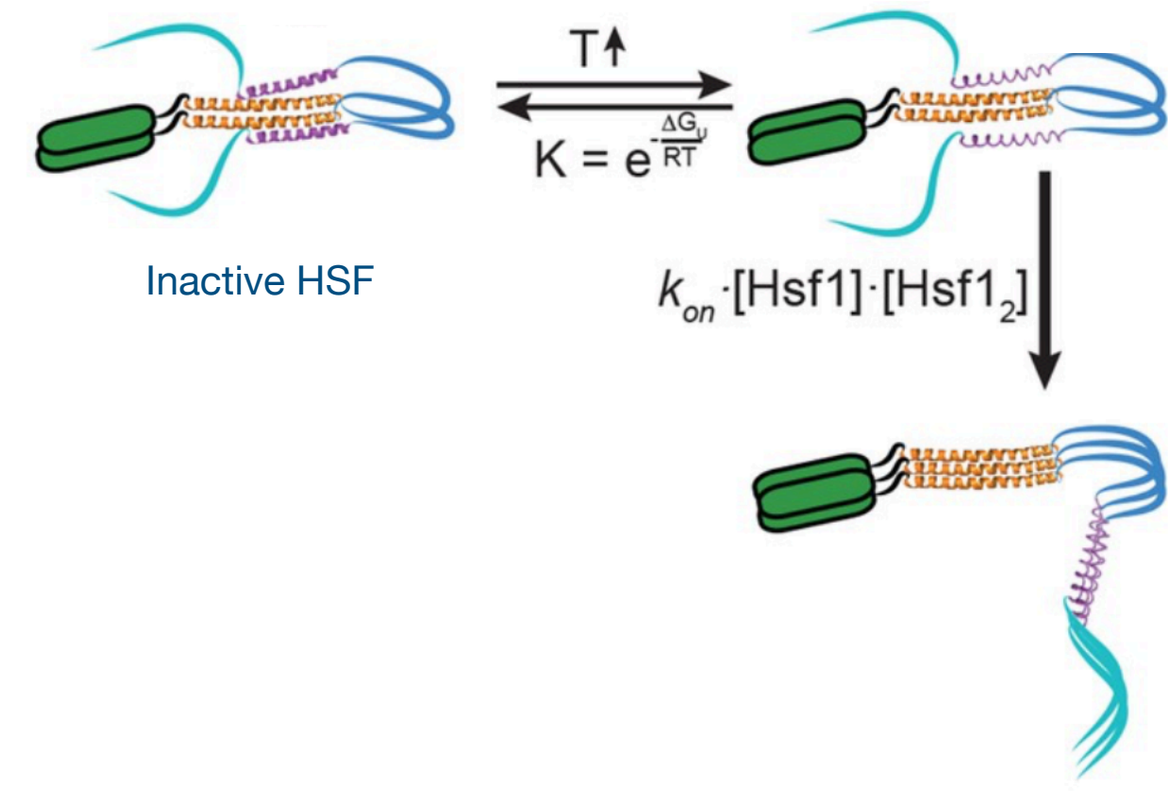

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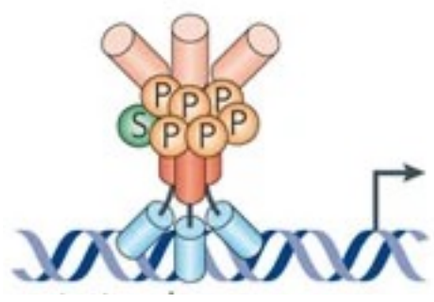
Molecular Heat Shock Response

Initiation

Heating

molecular level

Inactive heat shock transcription factor (HSF)



HSF trimerization, Transcriptional activation

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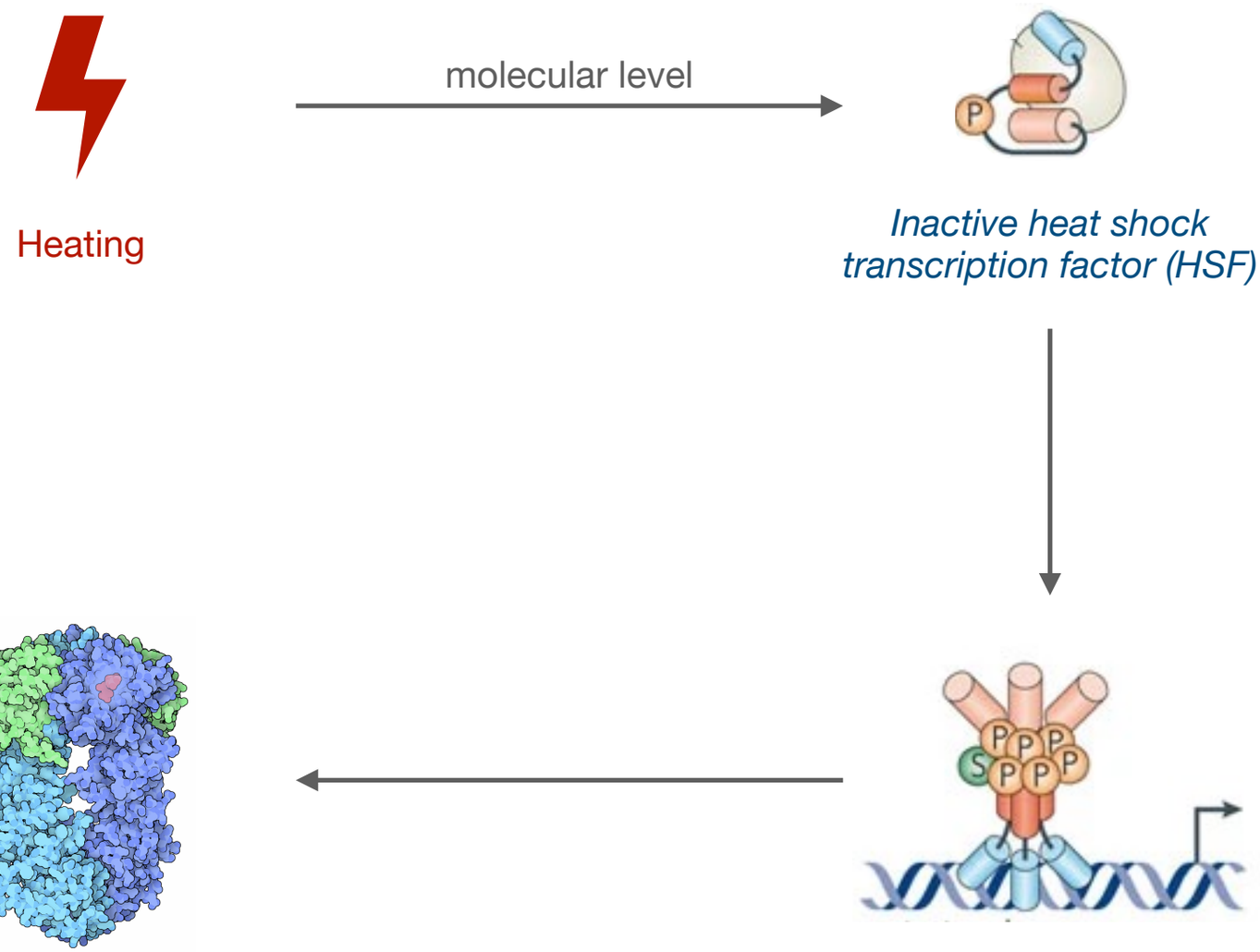

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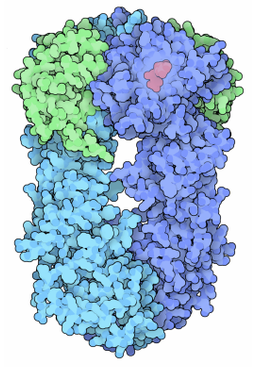
Heat Shock Protein (Hsp)



Conserved amount prokaryotes and eukaryotes

5-10% of total cellular protein content

Hu, C. et al. *MedComm (2020).* **2022**, *3,* e161.

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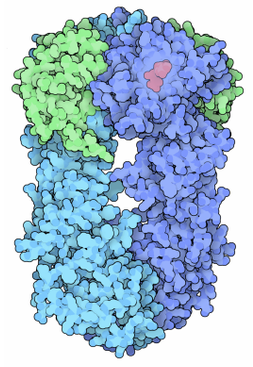

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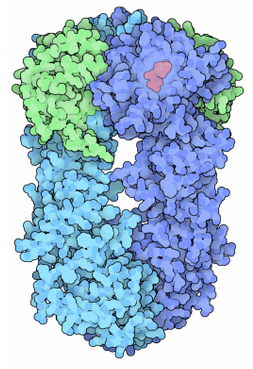

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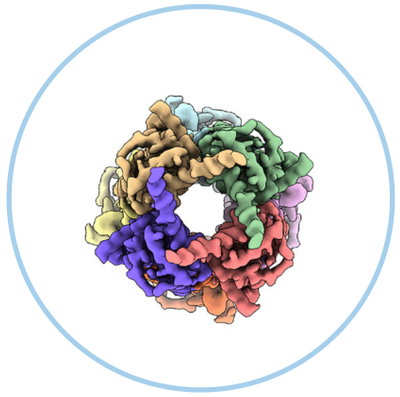
Assist folding of newly synthesized polypeptide



Protein complex assembly

Ensure correct folding

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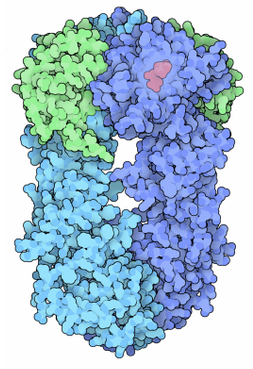

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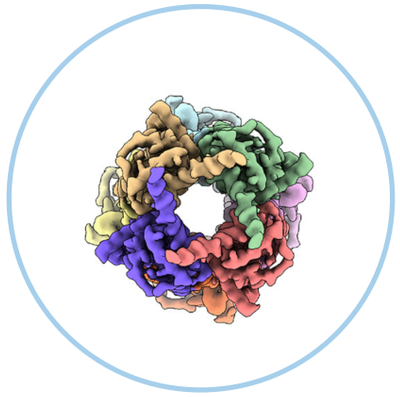
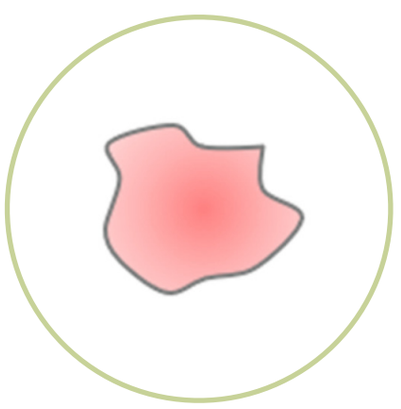


Protein complex assembly

Degradation of misfolded proteins



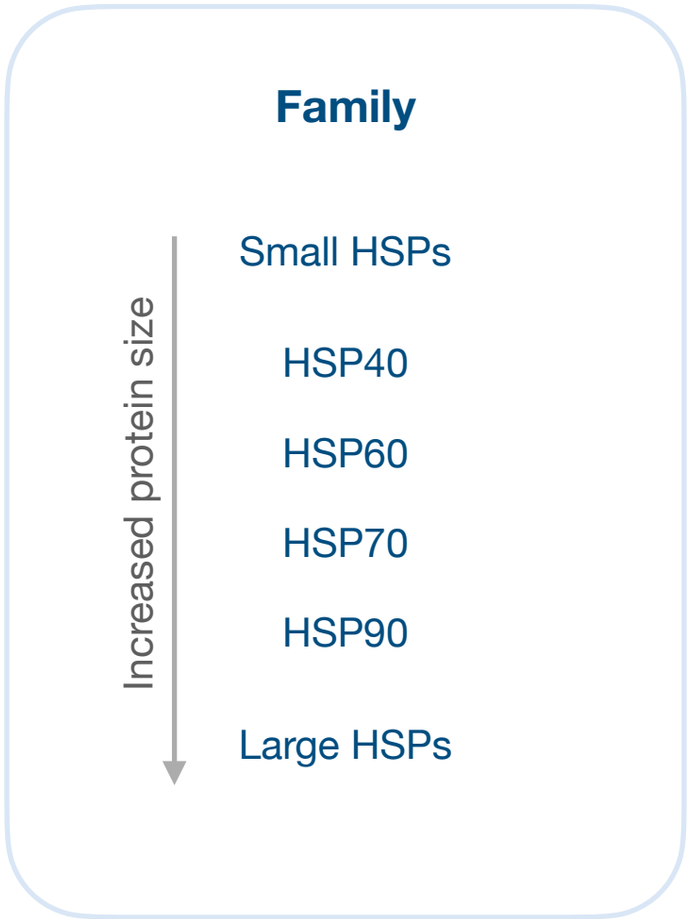
Dissociating protein aggregates

Ensure correct folding

Eliminate unwanted proteins

Hu, C. et al. *MedComm (2020).* **2022**, *3,* e161.

Heat shock protein (Hsp) classification



Classified by size/molecular weight

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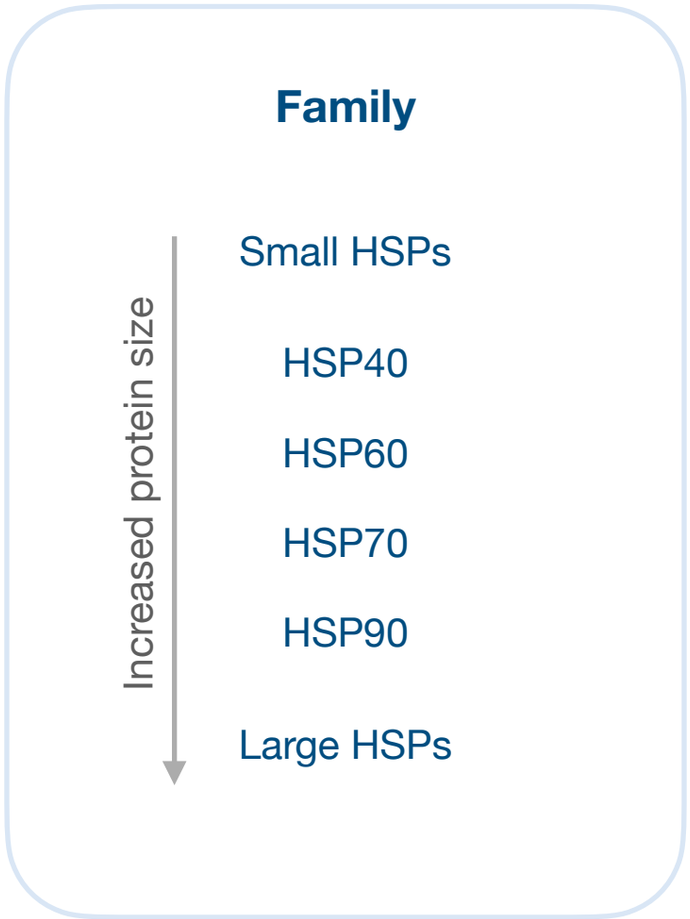

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Each class contains multiple members

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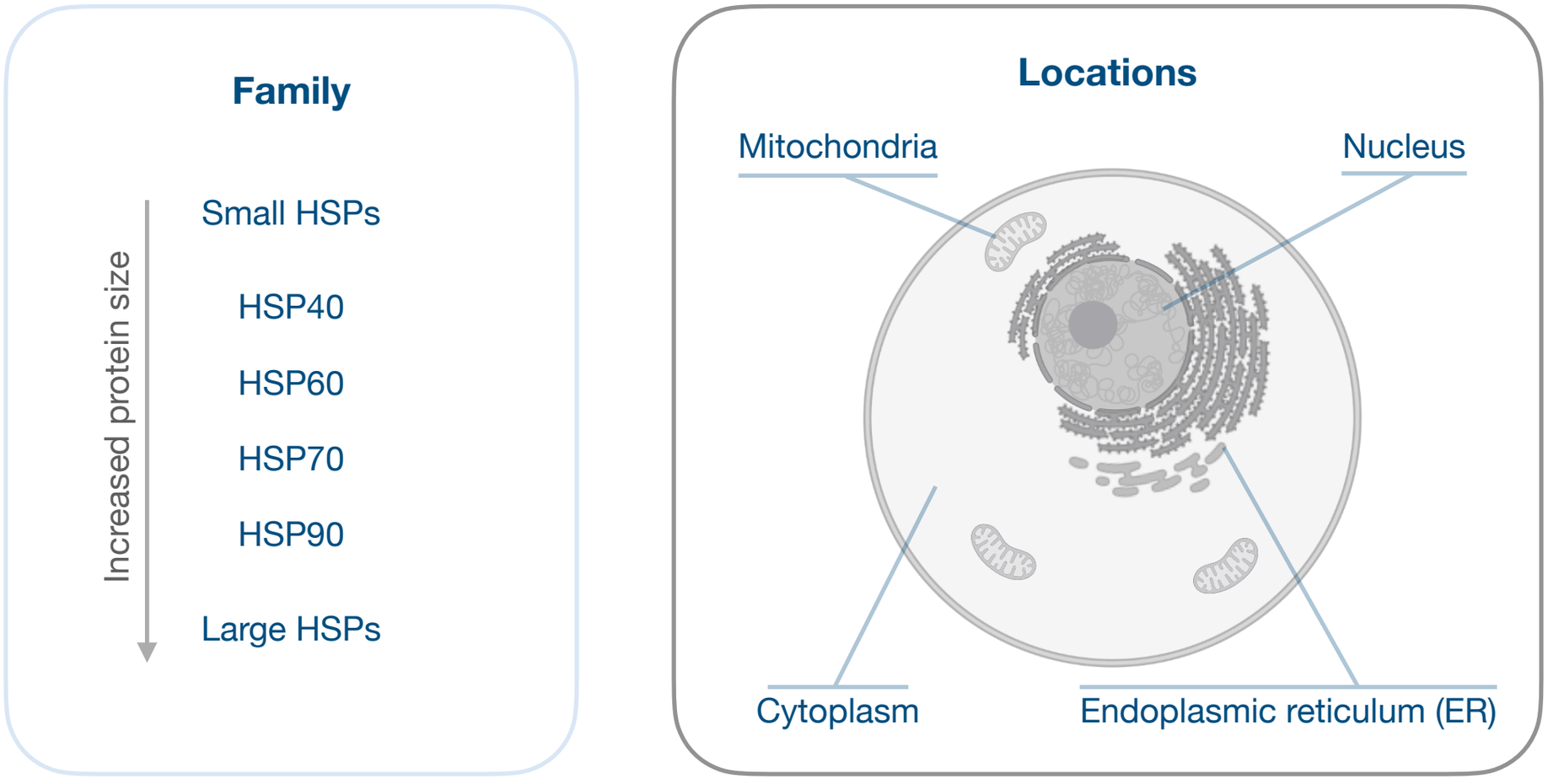

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Each class contains multiple members

Different Hsp families/members have different functions and locations

Hu, C. et al. *MedComm (2020).* **2022**, *3,* e161.

Heat shock protein (Hsp) classification

HSP70

HSP90

Heat shock protein (Hsp) classification

HSP70

HSP90

1) Ubiquitous substrate

2) assist de novo folding of proteins, prevent unfolded protein aggregation, and refold aggregated proteins

Richter, K.; Haslbeck, M.; Buchner, J. *Mol. Cell.* **2010**, *40,* 253.

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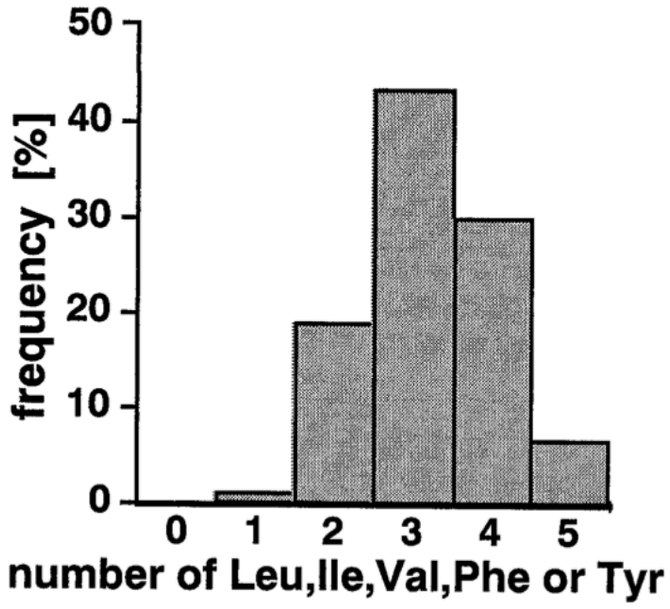
Hsp70 substrate recognition

Recognize 5-residue hydrophobic core (preferably aliphatic residues)



Amino acid	Amino acid content (%)
	Core
Leu	29.1
Ile	10.7
Val	12.4
Phe	4.9
Trp	0.9
Tyr	6.7
Ala	7.6
Gly	3.6
Met	0.4
Pro	2.9
Cys	0.0
His	1.1
Asn	1.6
Gln	2.2
Ser	2.9
Thr	5.6
Lys	3.6
Arg	2.7
Asp	0.9
Glu	0.4

Hydrophobic residues usually buried in globular proteins — exposure in solution indicates unfolded/misfolded

Rüdiger, S.; Germeroth, L.; Schneider-Mergener, J.; Bukau, B. *EMBO J.* **1997**, *16,* 1501.

Heat shock protein (Hsp) classification

HSP70

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Hsp70 mechanism

De novo synthesized/ unfolded/misfolded proteins

Ambrose, A. J.; Chapman, E. *J. Med. Chem.* **2021**, *64,* 7060.

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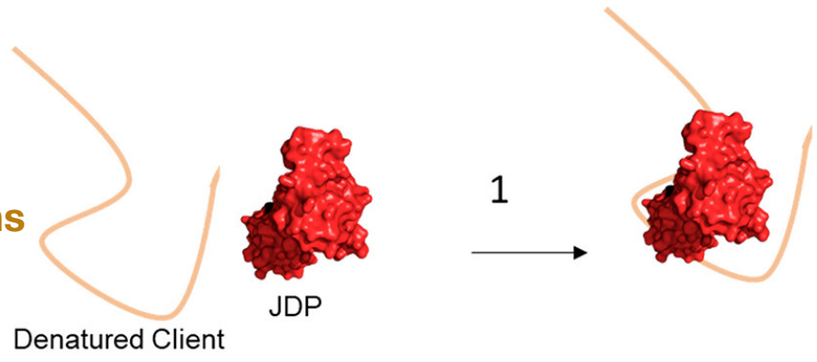

JDP (Hsp40) = J-domain proteins;
act as co-chaperone to Hsp70

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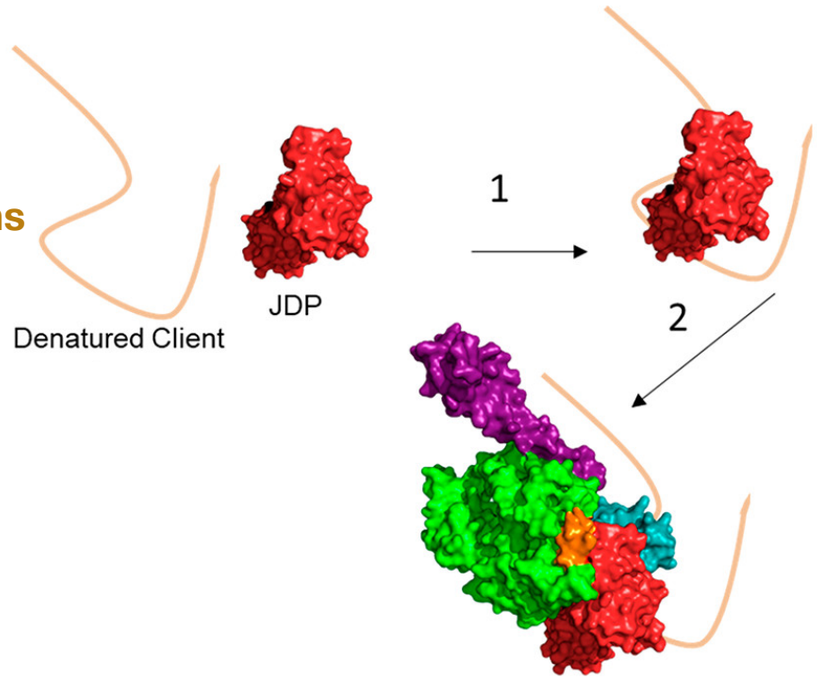

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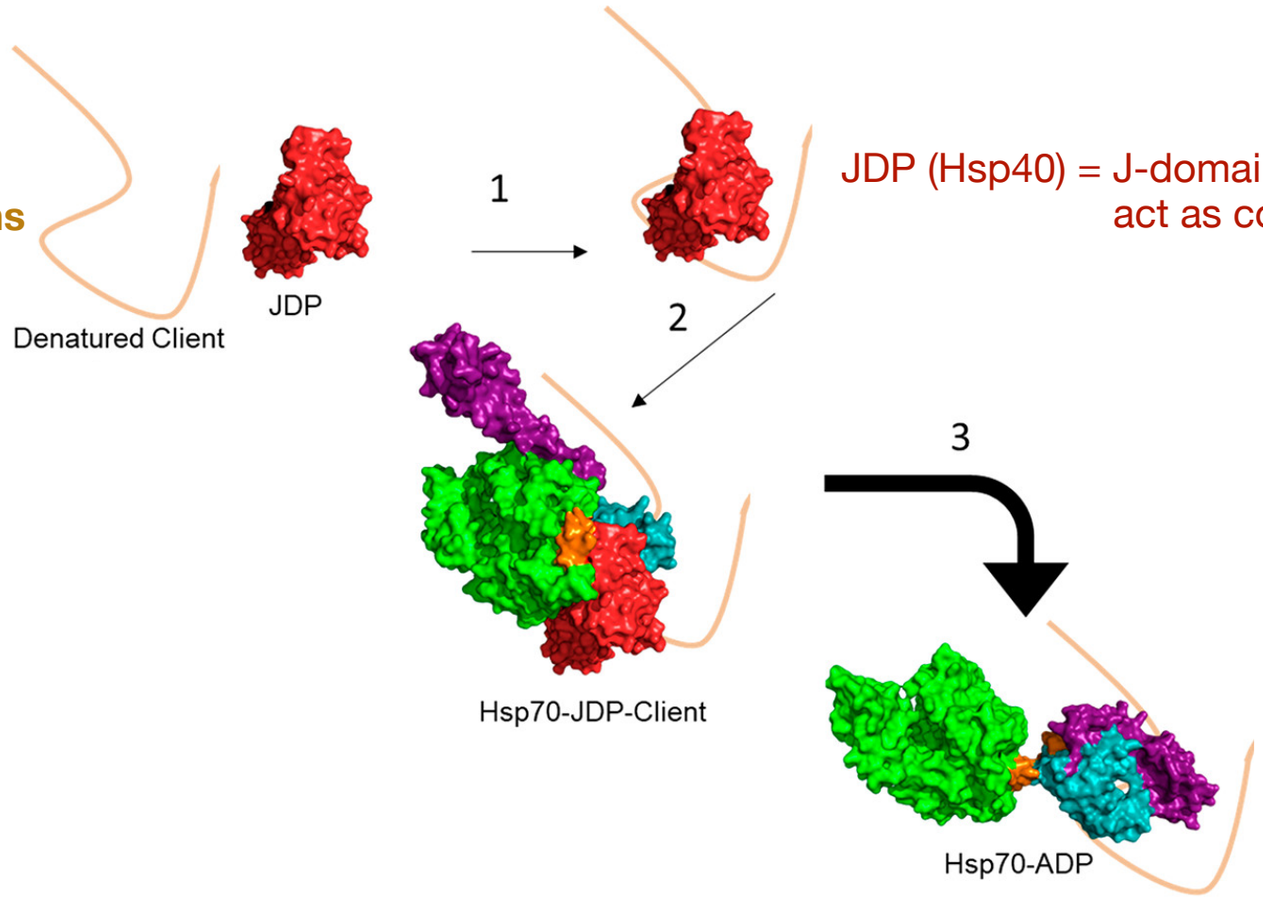

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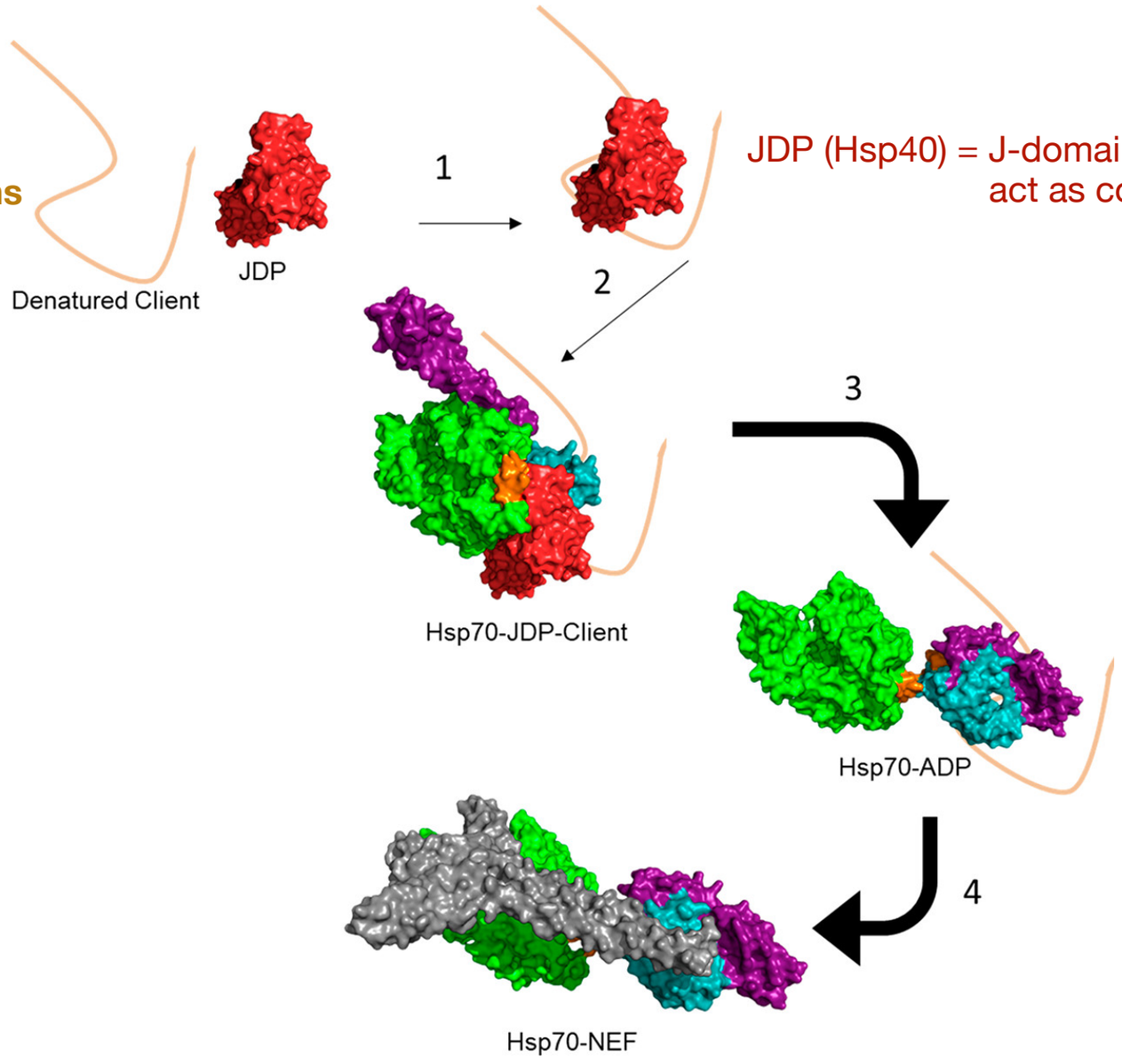

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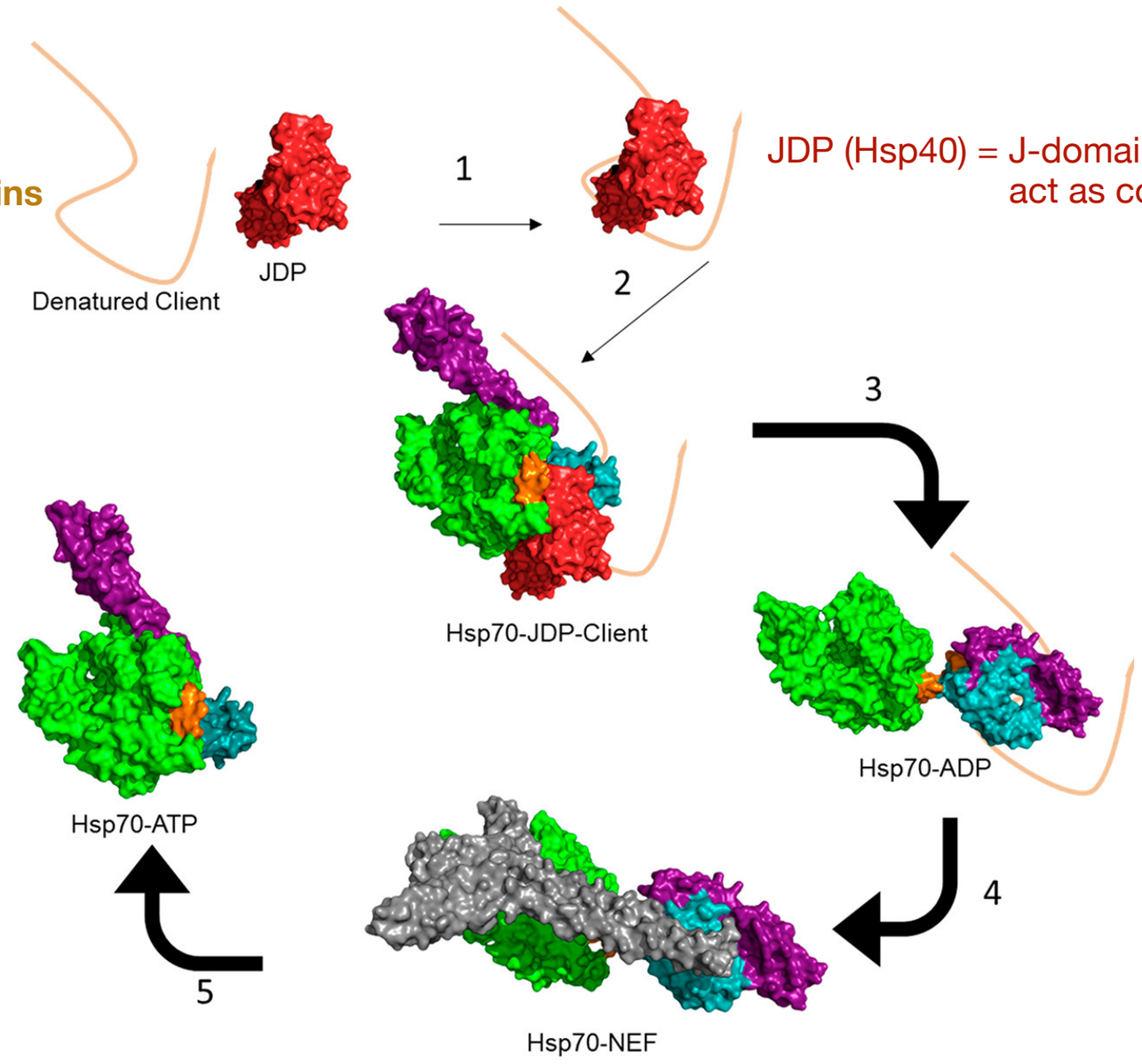

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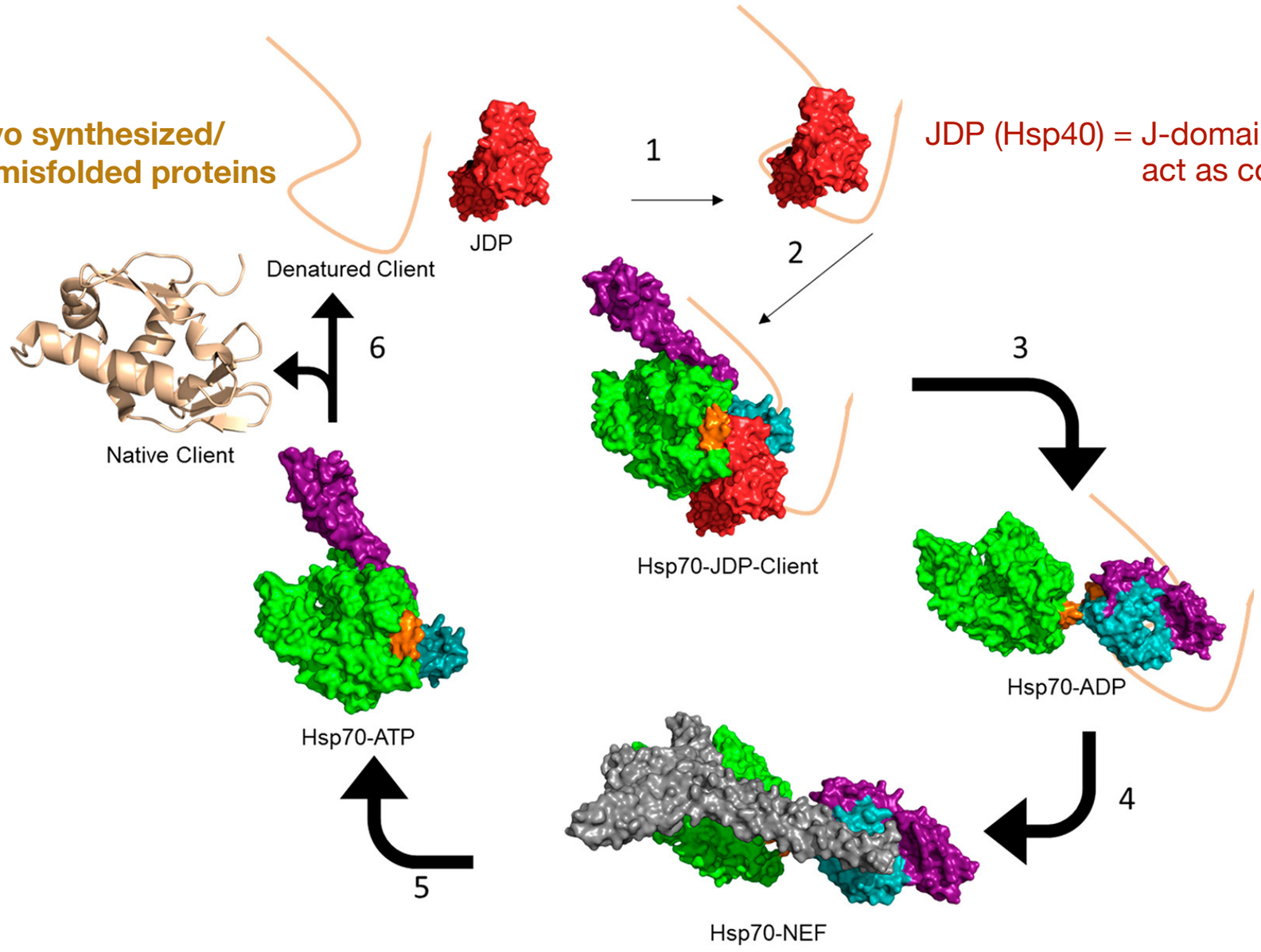

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Heat shock protein (Hsp) classification

HSP70

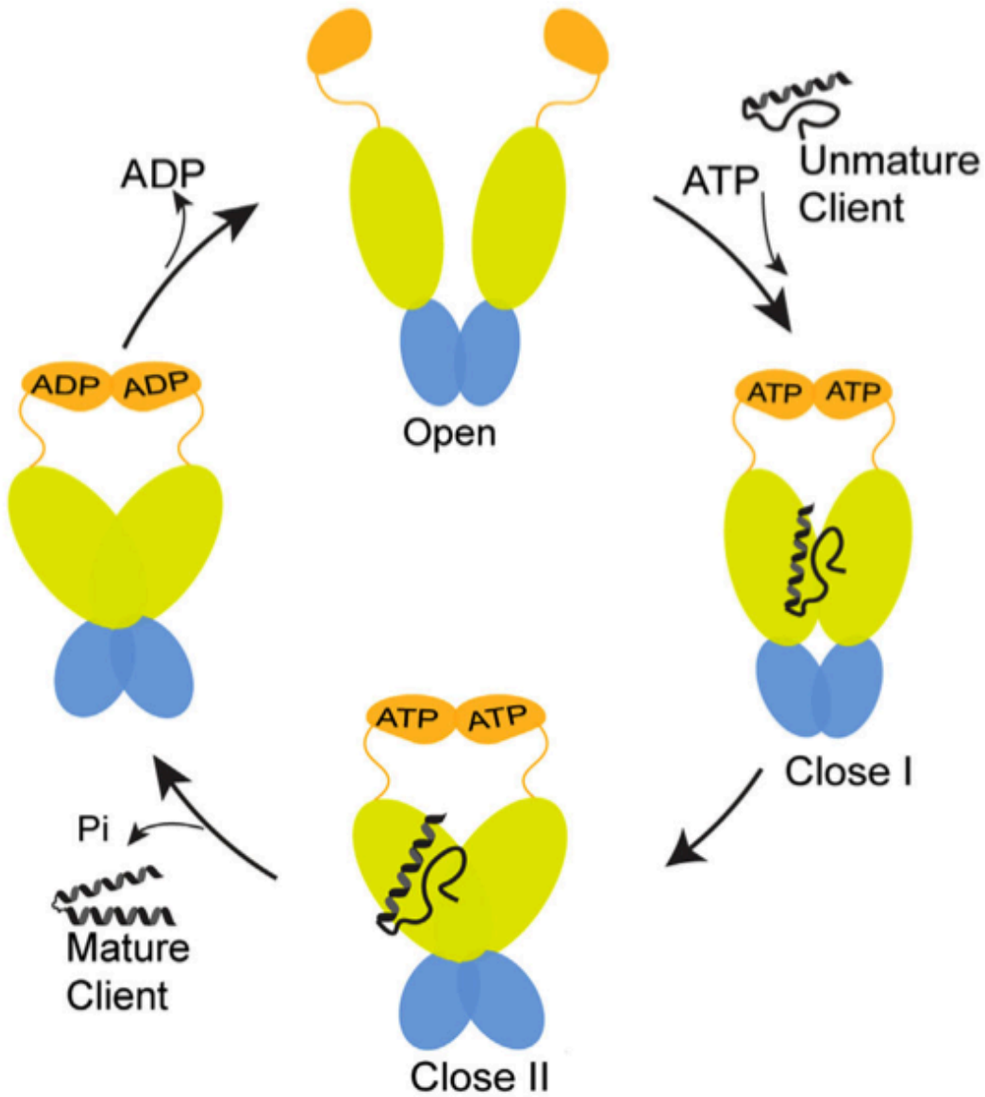
HSP90

*1) only binds to **native-like proteins***

*2) not just as a foldase, but also assist **protein conformational maturation***

Hu, C. et al. *MedComm (2020).* **2022**, *3,* e161.

Hsp90 mechanism



Similar to Hsp70, both requires ATP; can accept partially folded structures from Hsp70

Hu, C. et al. *MedComm (2020).* **2022**, *3,* e161.

Heat Shock Proteins — not just for hyperthermia

Heat shock proteins important in oncological/neurological disease settings as molecular chaperones

Yang, S.; Xiao, H.; Cao, L. *Biomed. Pharmacother.* **2021**, *142,* 112074.

Heat Shock Proteins — not just for hyperthermia

Heat shock proteins important in oncological/neurological disease settings as molecular chaperones

Cancer Cell

Tumor environment

eg. Hypoxia, acidosis, nutrient-deprivation

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Heat shock protein overexpression

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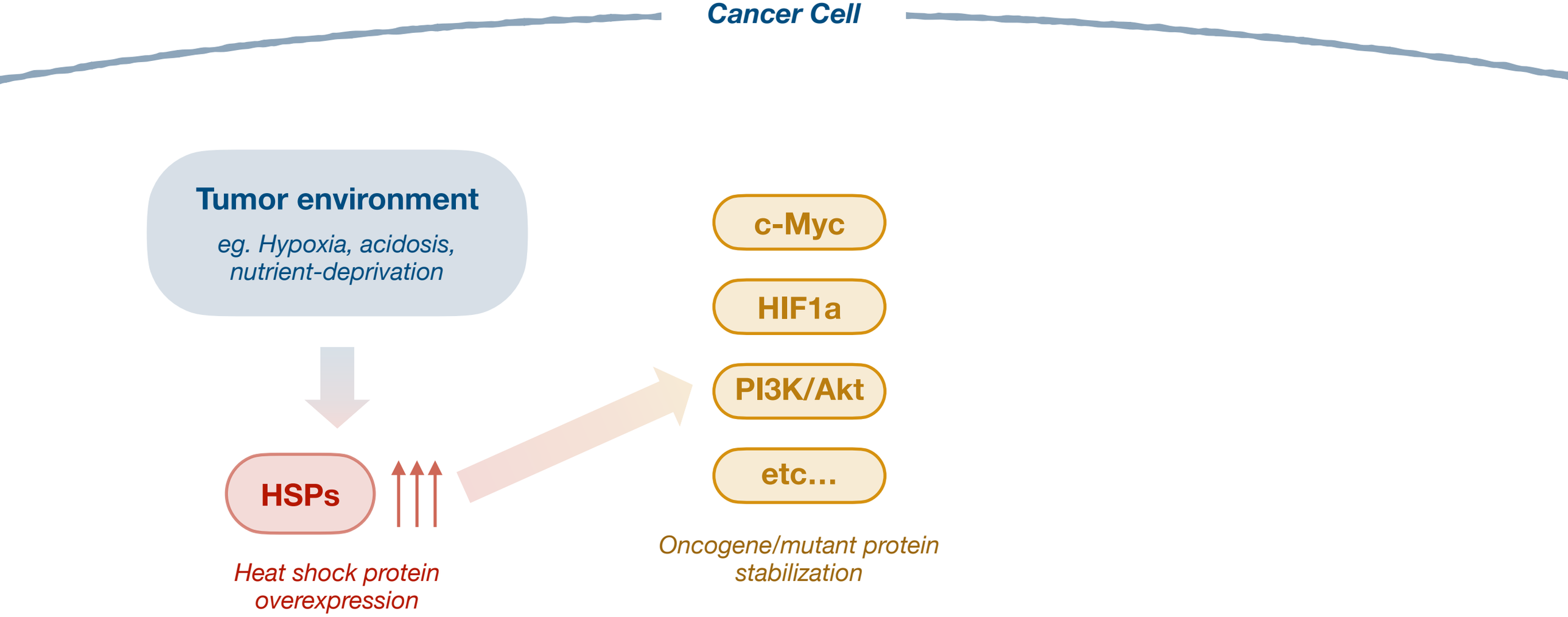

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Tumor environment

eg. Hypoxia, acidosis, nutrient-deprivation

HSPs

Heat shock protein overexpression

c-Myc

HIF1a

PI3K/Akt

etc…

Oncogene/mutant protein stabilization

Cancer cell survival, growth, proliferation

Yang, S.; Xiao, H.; Cao, L. *Biomed. Pharmacother.* **2021**, *142,* 112074.

What if Hsp or other chaperones not working properly? Or overloaded with unfolded proteins?

Key types of stress response

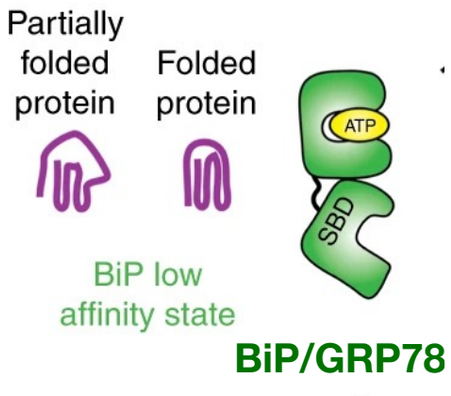
Heat shock response

Unfolded protein response

DNA damage response

Response to oxidative stress

Unfolded Protein Response (UPR)

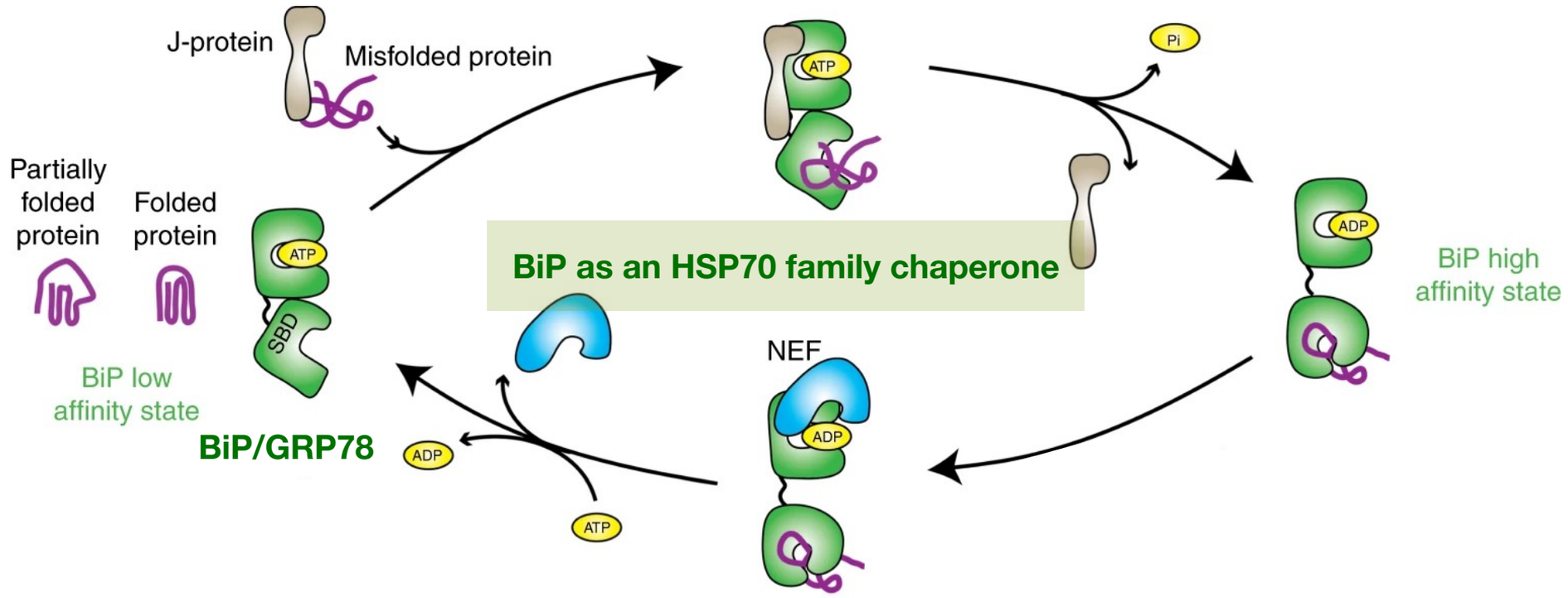


BiP/GRP78

BiP as an HSP70 family chaperone

Kopp, M. C. et al. *Nat. Struct. Mol Biol.* **2020**, *26,* 1053.

Unfolded Protein Response (UPR)



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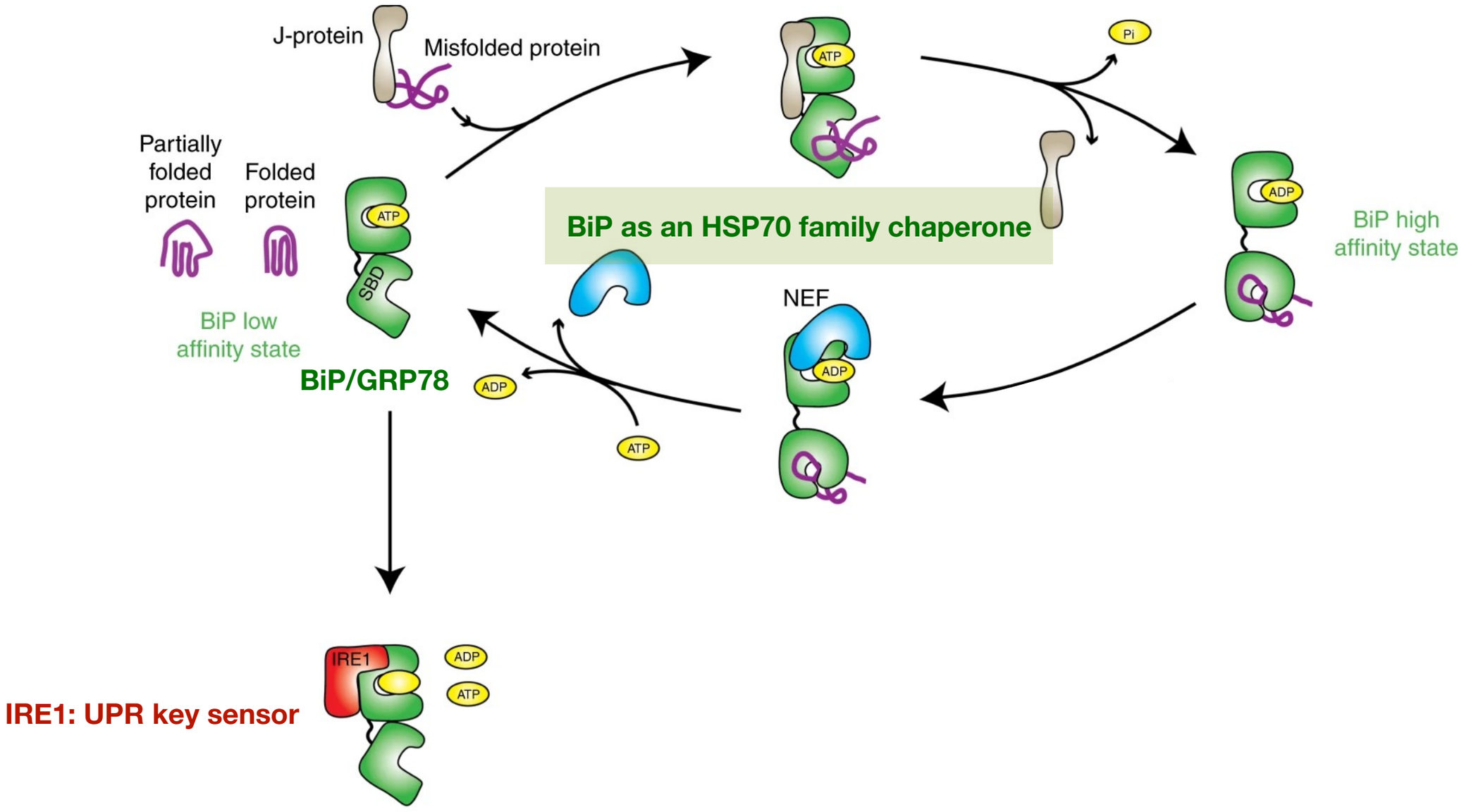

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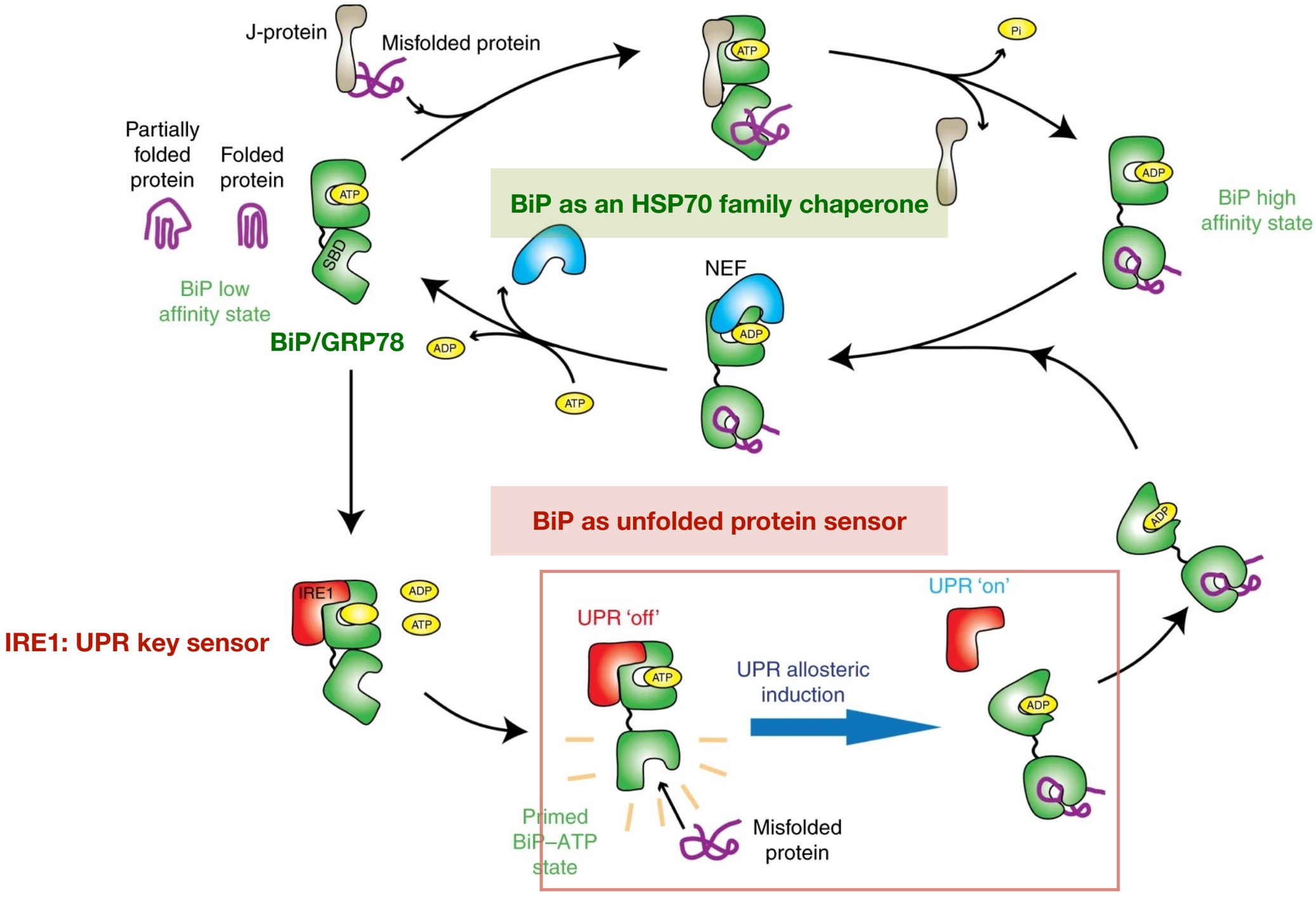

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Endoplasmic Reticulum (ER)

Unfolded protein response happening in the ER

Hetz, C. *Nat. Rev. Mol. Cell. Biol.* **2012**, *13,* 89.

Endoplasmic Reticulum (ER)

Unfolded protein response happening in the ER

Endoplasmic Reticulum (ER)

Initial protein folding and maturation

Ca^{2+} reservoir

Gluconeogenesis

Lipid synthesis

Biogenesis of autophagasomes and peroxisomes

Hetz, C. *Nat. Rev. Mol. Cell. Biol.* **2012**, *13,* 89.

Endoplasmic Reticulum (ER) environment important for protein folding

Endoplasmic Reticulum (ER) environment important for protein folding

Difference in folding conditions

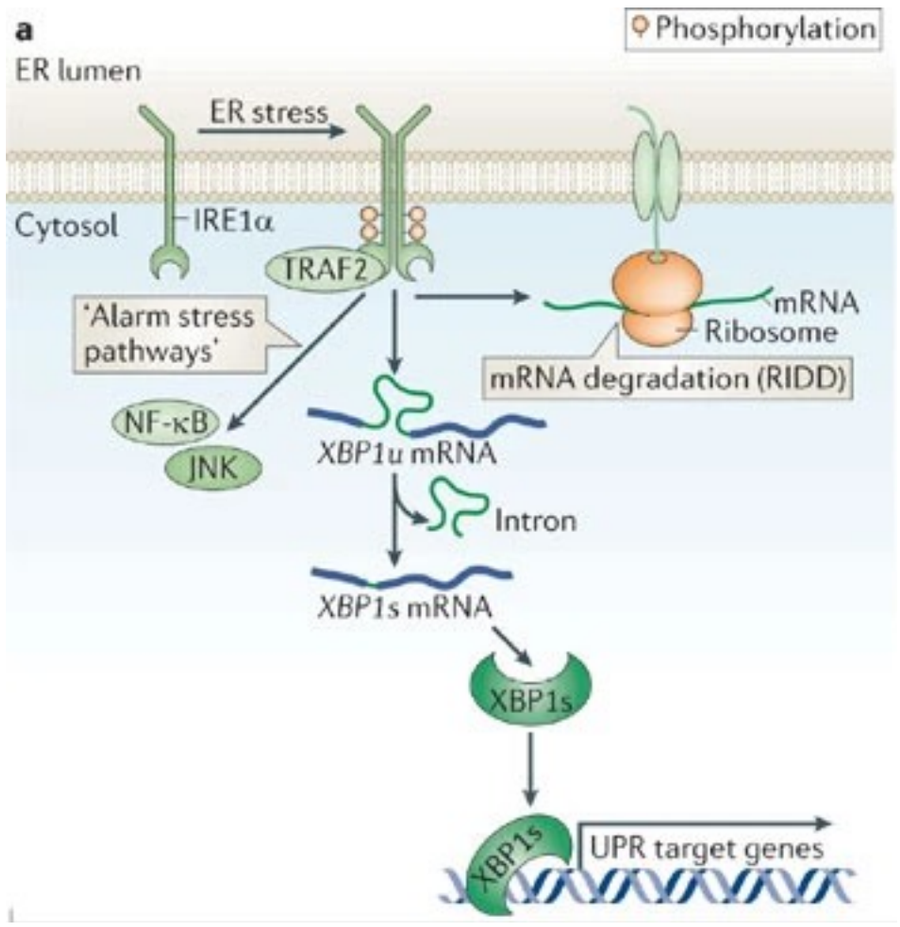
Parameter	ER	Cytosol
Redox state	Oxidizing	Reducing
Calcium	From 0 to 1 mM free Ca^{++} major protein-bound storage	<1 μM
Energy generating system	No	Yes
Glycosylation machinery	Yes	No
Proteolytic machinery	No	Many
HSP70 chaperones	BiP/GRP78, GRP170	HSP70 ,HSC70
HSP90 chaperones	GRP94	HSP90
Stress response	ER stress response = unfolded protein response	Heat shock response and metabolic stress

Comparison of conditions that affect protein folding and disposal of misfolded proteins (parameter) and the response to changes in these conditions between the ER and the cytosol.

Many chaperones/folding assisting proteins are Ca^{2+} dependent

3 major pathways of unfolded protein response (UPR)

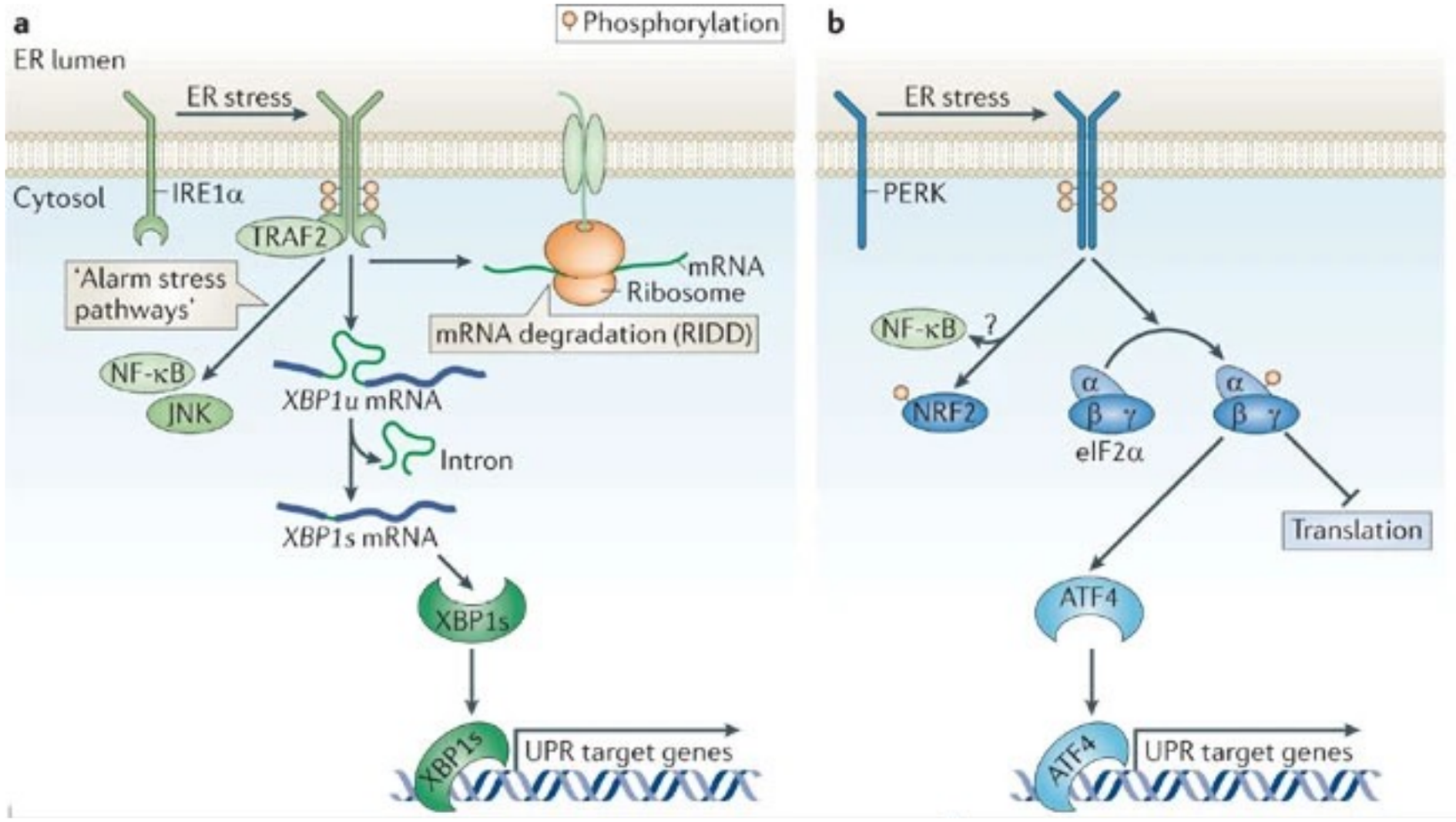
IRE1a



Hetz, C. *Nat. Rev. Mol. Cell. Biol.* **2012**, *13,* 89.

3 major pathways of unfolded protein response (UPR)

IRE1a | **PERK**



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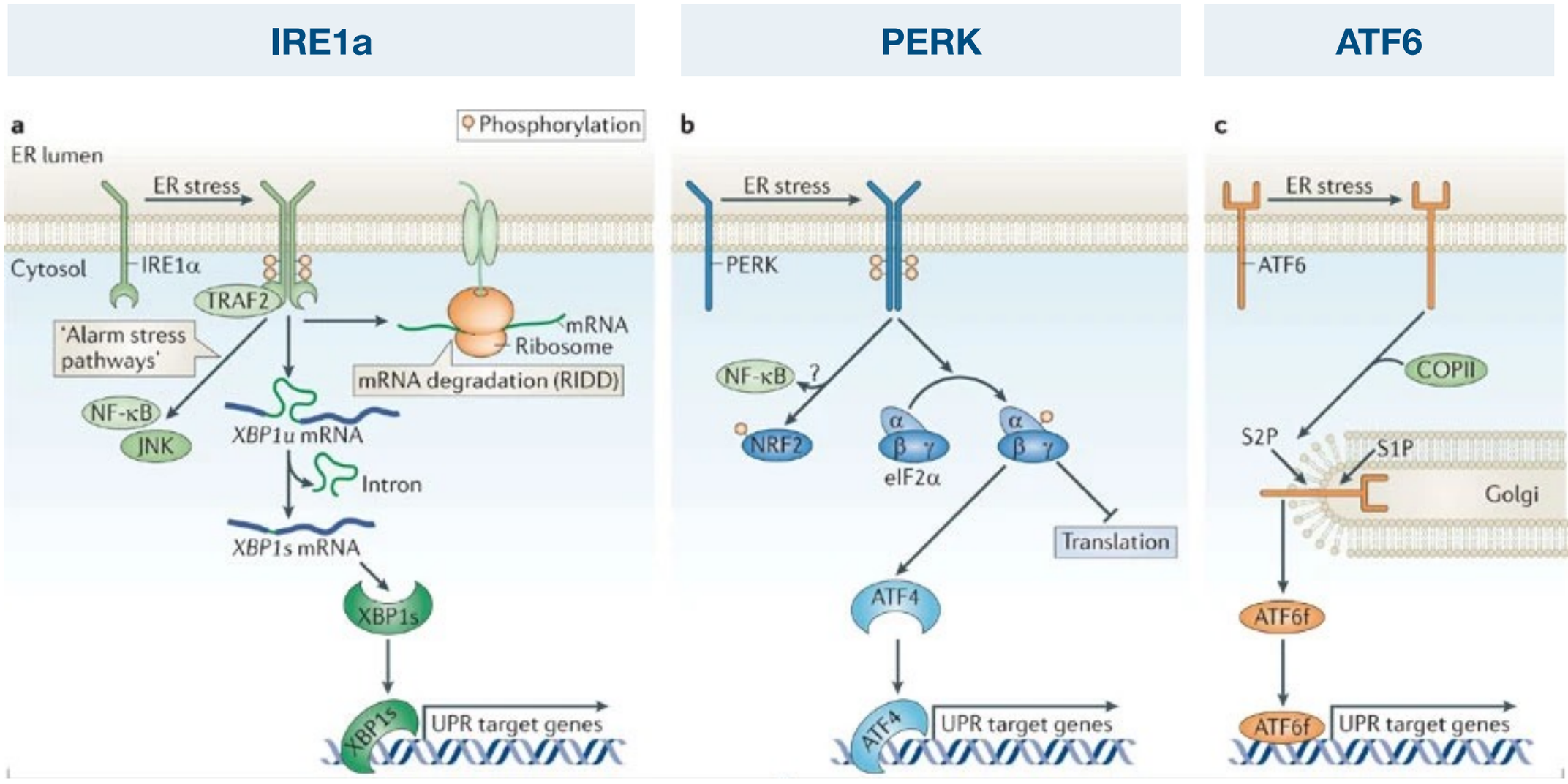
3 major pathways of unfolded protein response (UPR)



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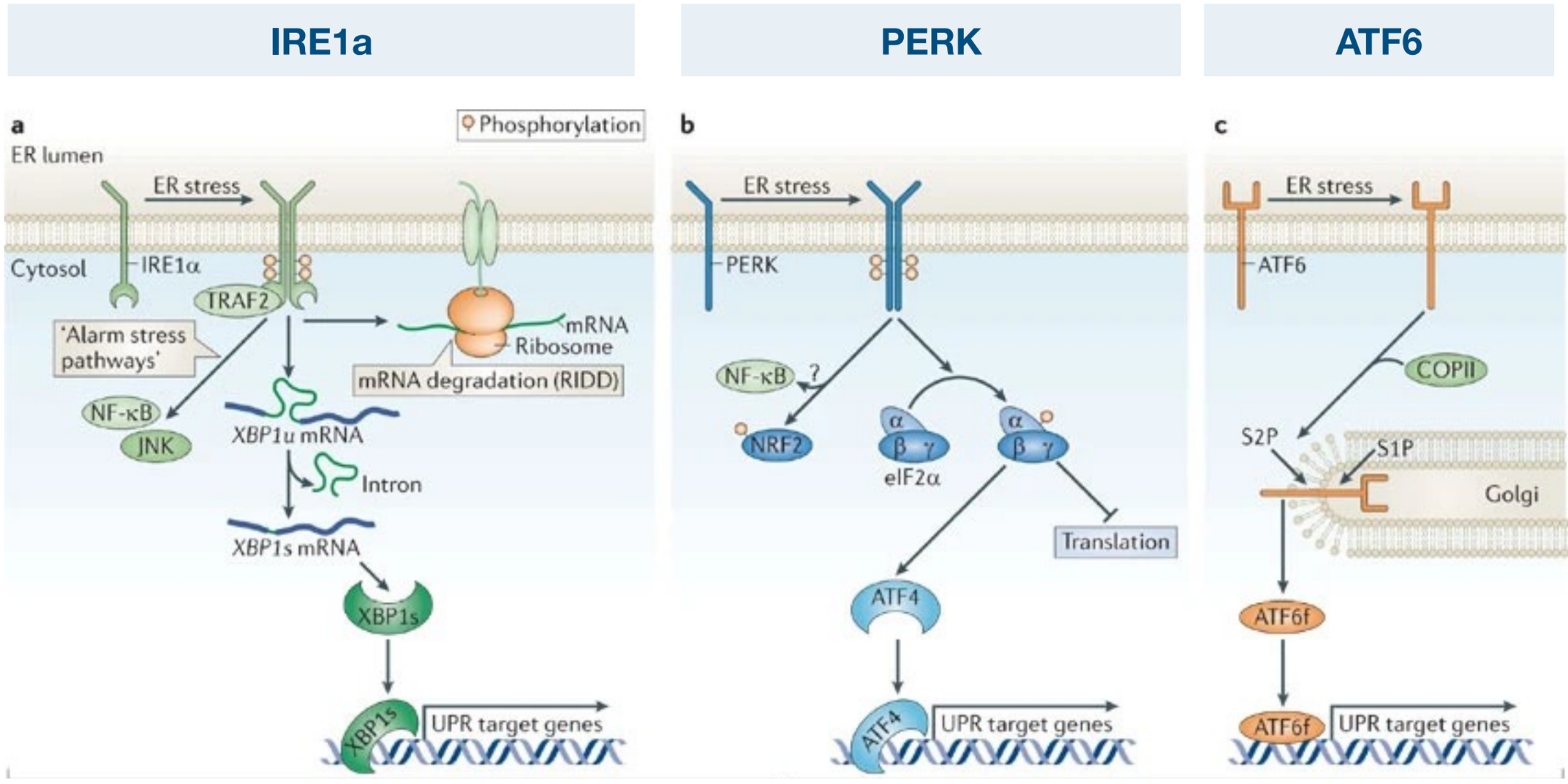
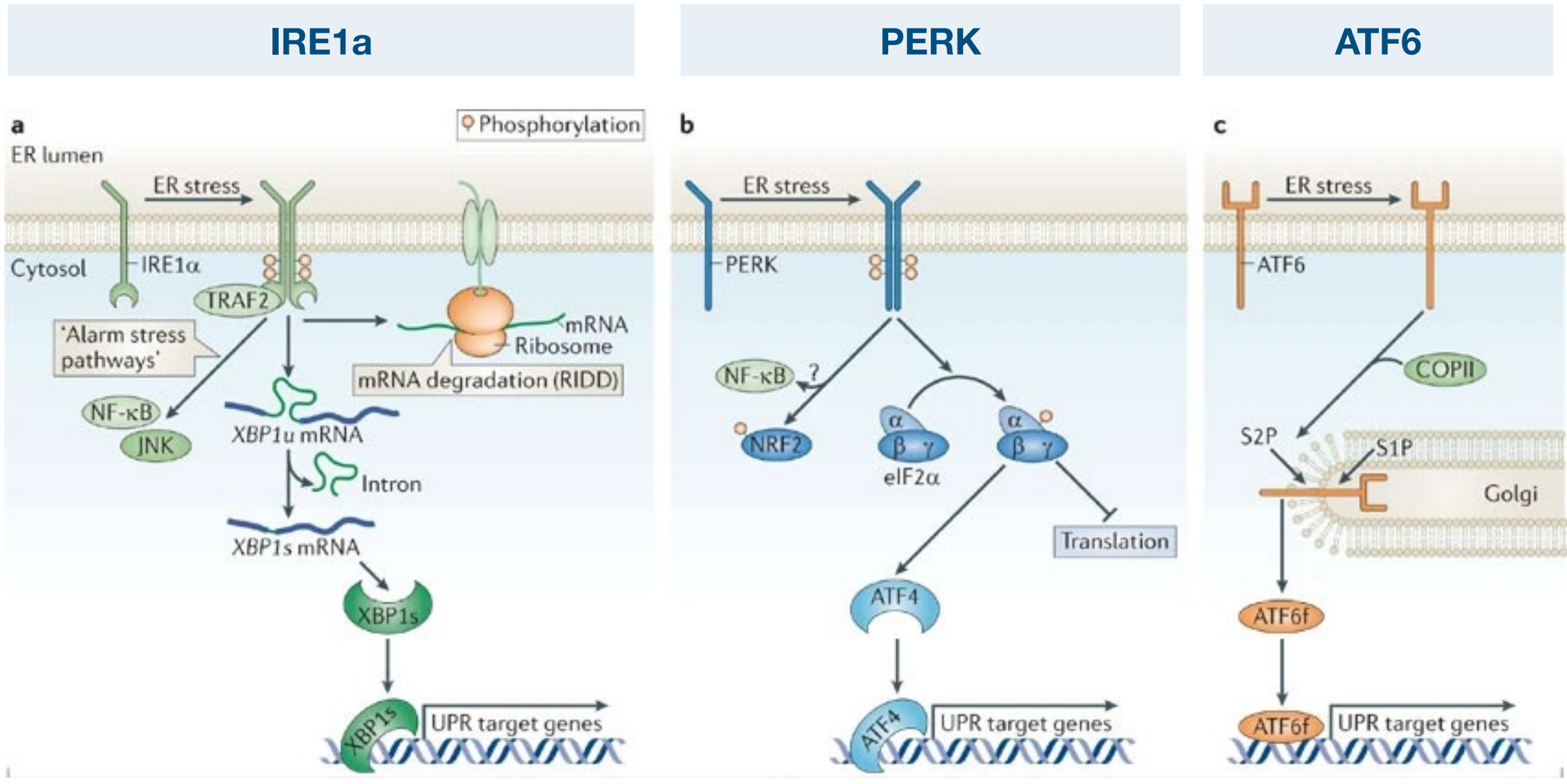
3 major pathways of unfolded protein response (UPR)



Degradation	***Folding***	***Lipid synthesis***	***Quality control***	***Protein secretion***

Hetz, C. *Nat. Rev. Mol. Cell. Biol.* **2012**, *13,* 89.

3 major pathways of unfolded protein response (UPR)



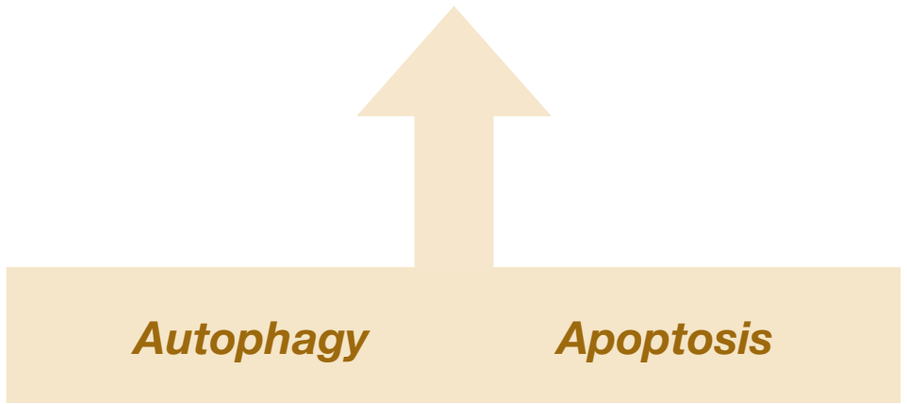
Degradation ***Folding*** ***Lipid synthesis*** ***Quality control*** ***Protein secretion***

Autophagy ***Apoptosis***

Hetz, C. *Nat. Rev. Mol. Cell. Biol.* **2012**, *13,* 89.

UPR in cancer

Cancer cells bypassed apoptotic signals and utilize UPR as pro-survival mechanism



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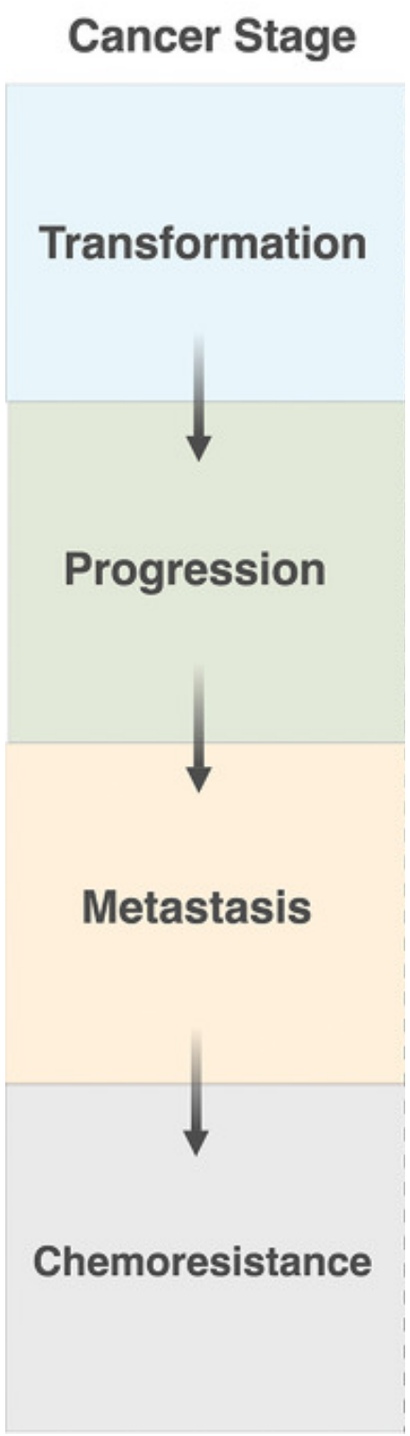
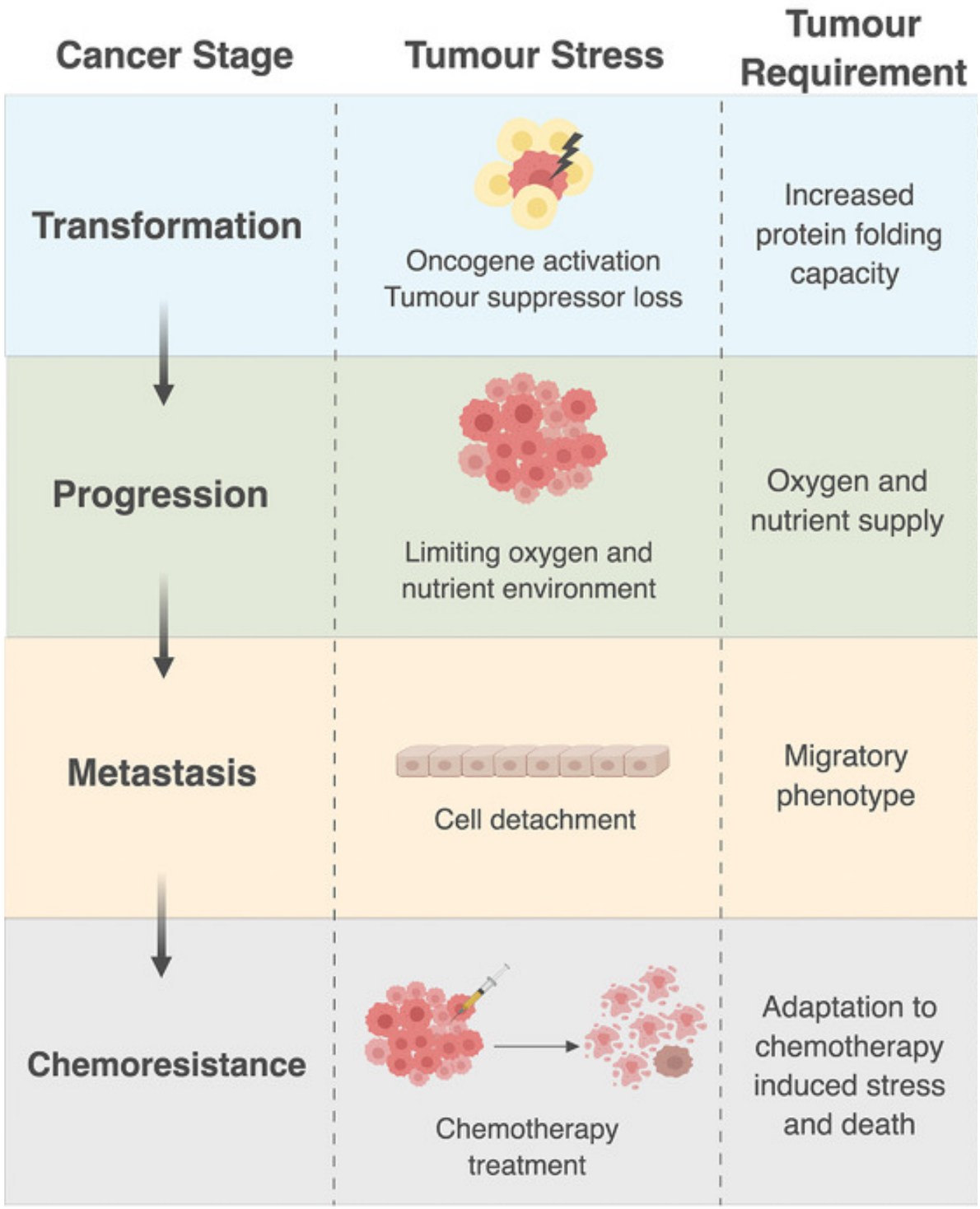
UPR in cancer



Madden, E. *et al*. *Biol. Cell.* **2019**, *111,* 1.

UPR in cancer



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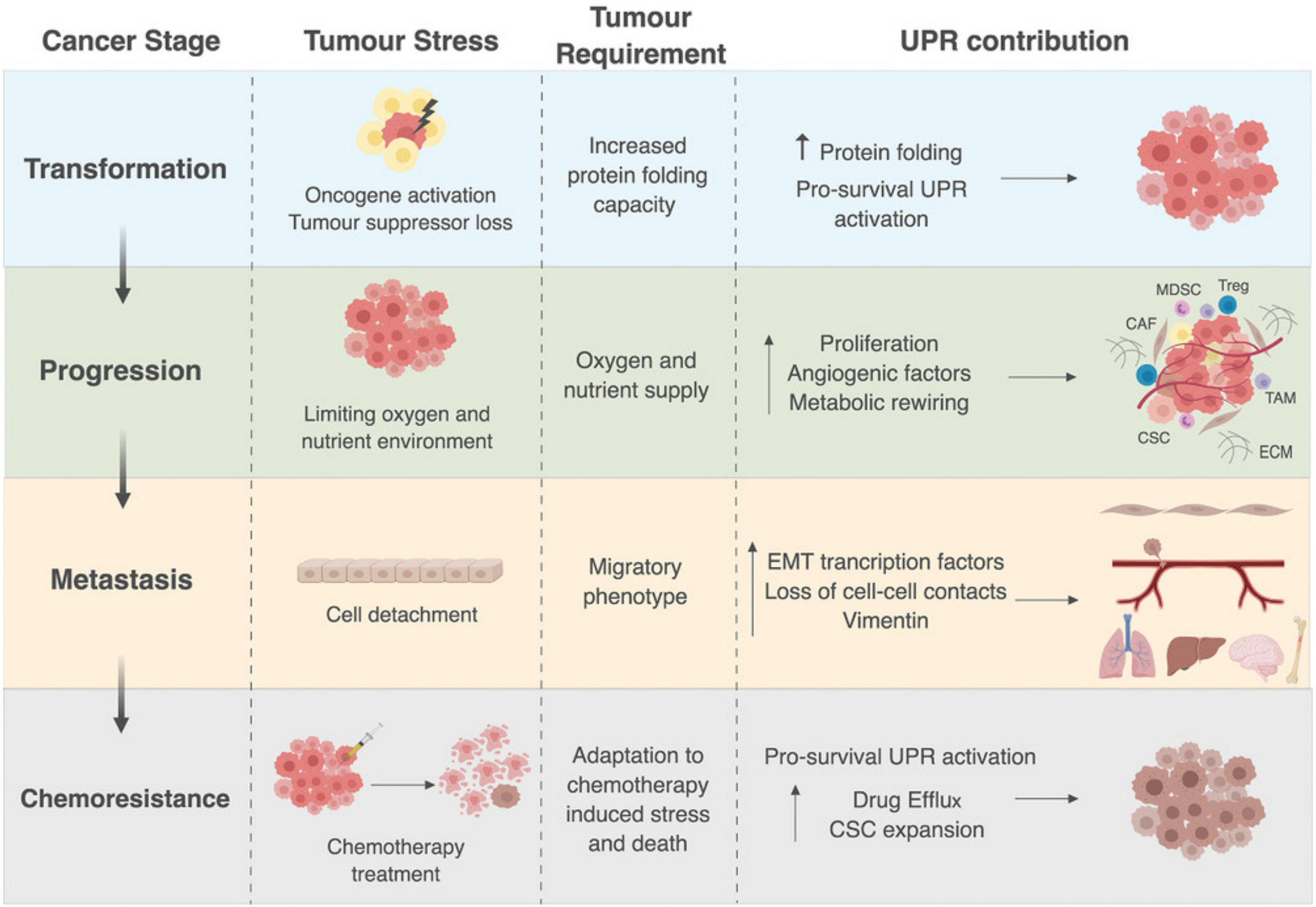
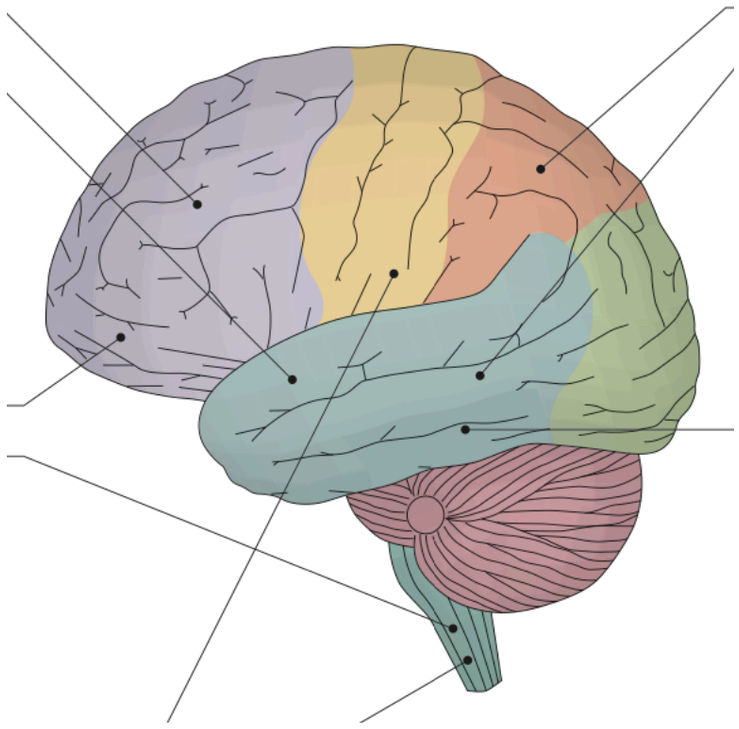


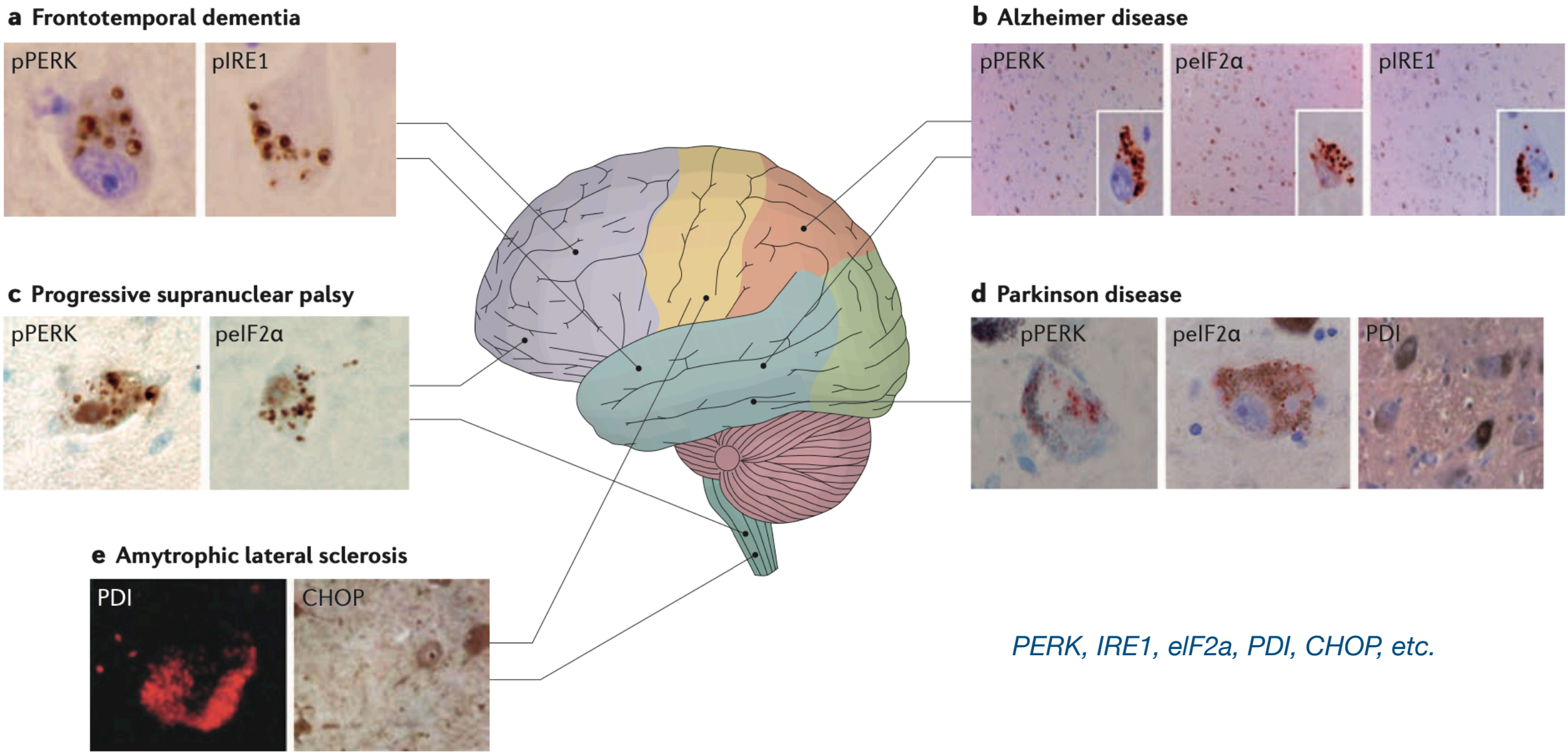
Madden, E. *et al. Biol. Cell.* **2019**, *111,* 1.

UPR involves in many neurological diseases



Hetz, C.; Saxena, S. *Nat. Rev. Neurol.* **2017**, *13,* 477.

UPR involves in many neurological diseases



PERK, IRE1, eIF2a, PDI, CHOP, etc.

Many key UPR players are founds in aggregates associated with neurodegenerative diseases

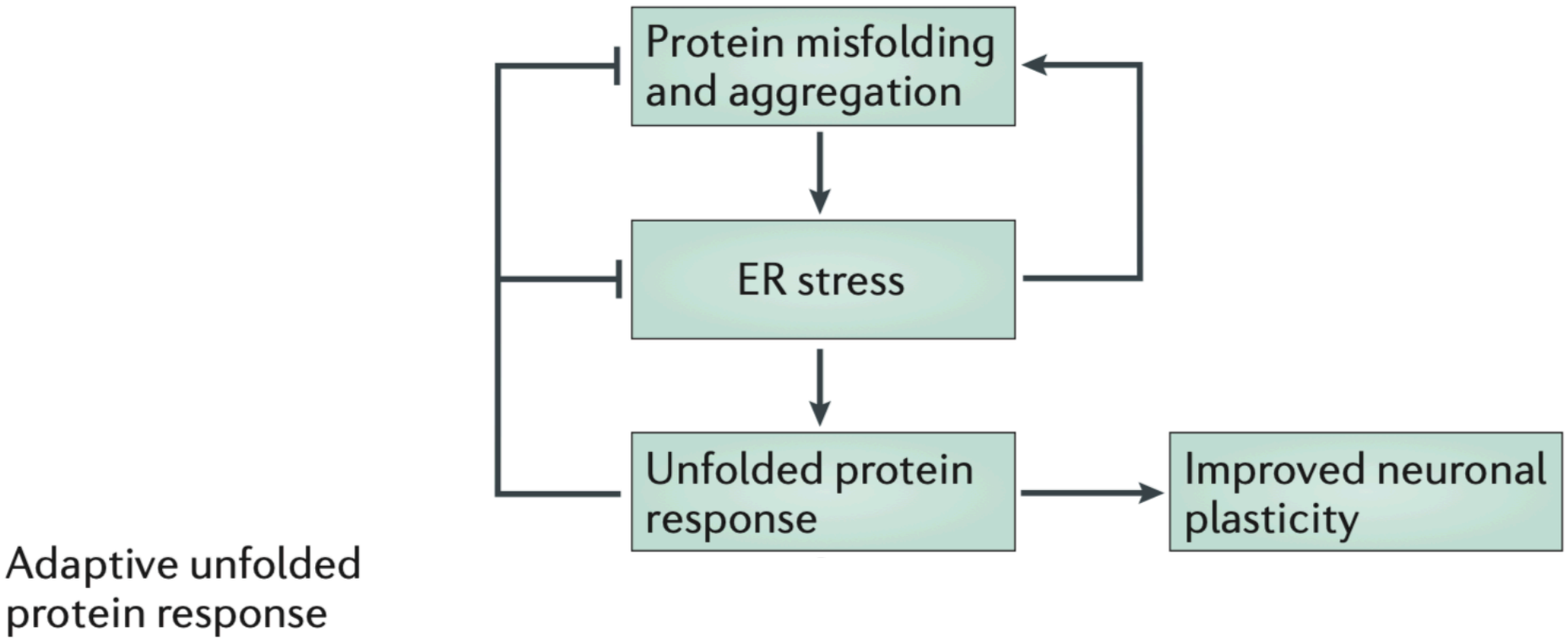
Hetz, C.; Saxena, S. *Nat. Rev. Neurol.* **2017**, *13,* 477.

UPR involves in many neurological diseases

Protein misfolding and aggregation

Hetz, C.; Saxena, S. *Nat. Rev. Neurol.* **2017**, *13,* 477.

UPR involves in many neurological diseases



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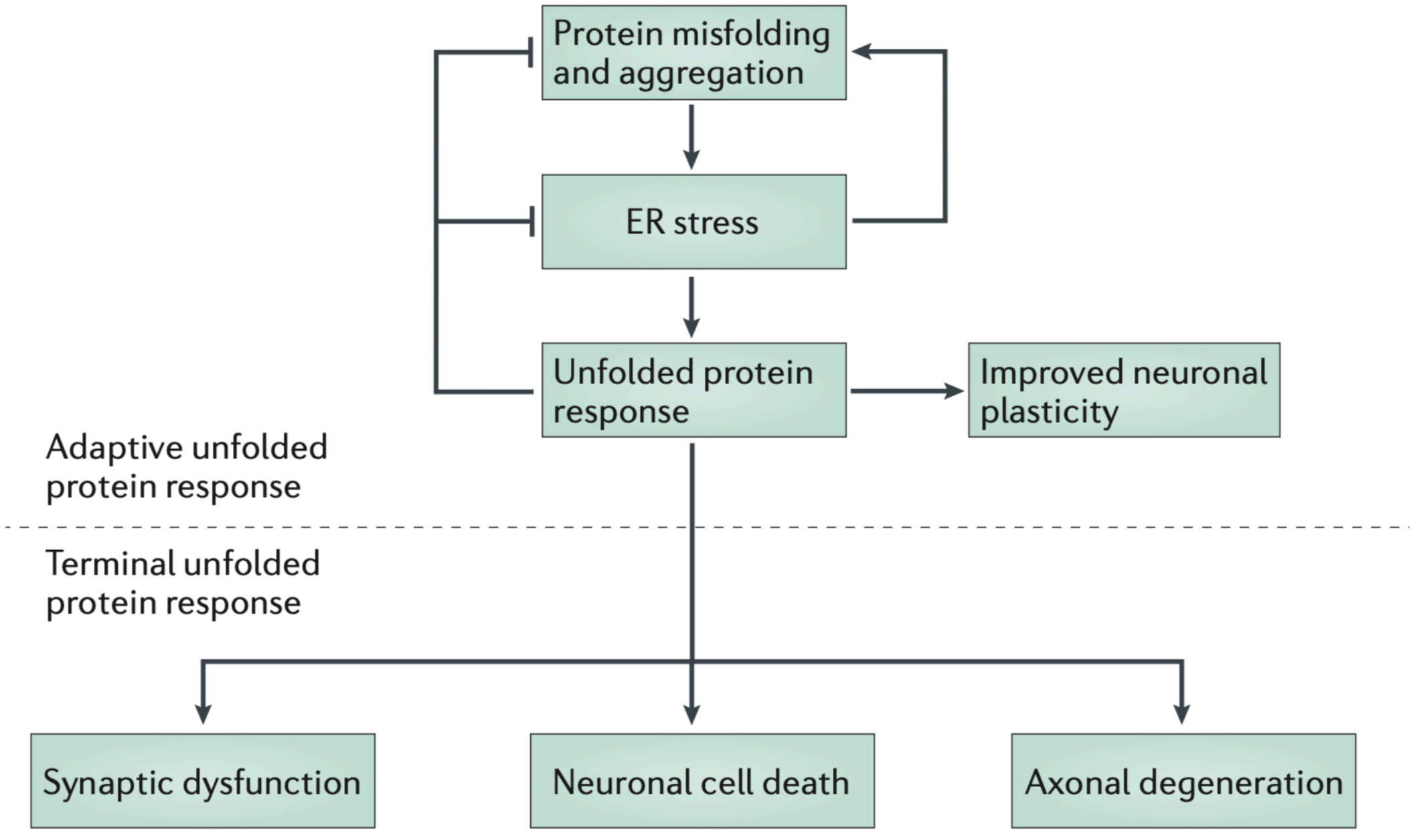
UPR involves in many neurological diseases



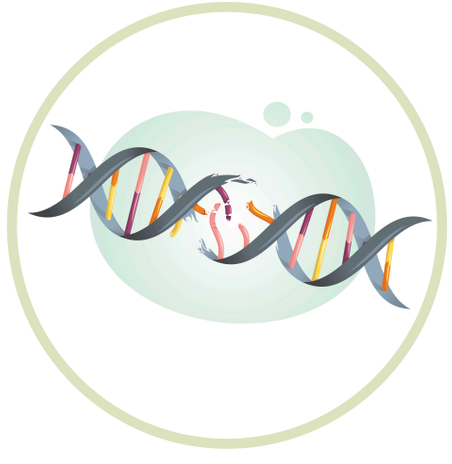
Hetz, C.; Saxena, S. *Nat. Rev. Neurol.* **2017**, *13,* 477.

What about macromolecules other than proteins?

Key types of stress response

Heat shock response

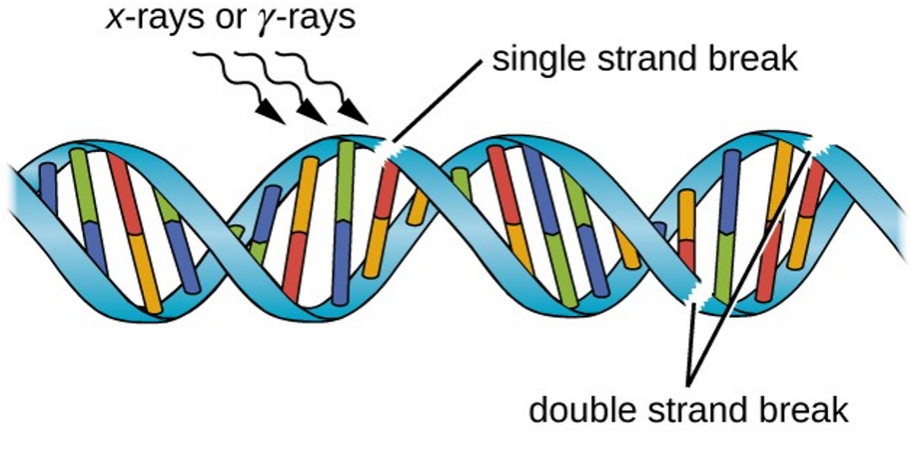
Unfolded protein response



DNA damage response

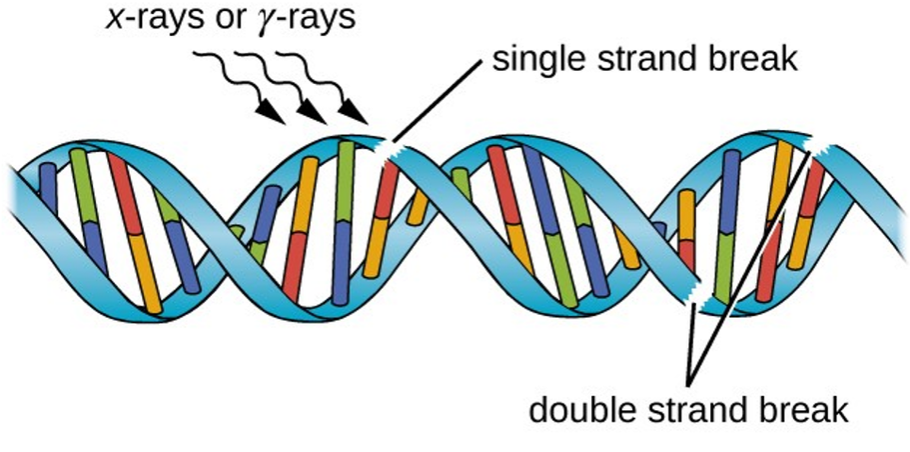
Response to oxidative stress

Types of DNA damage



Double/single strand break

Types of DNA damage



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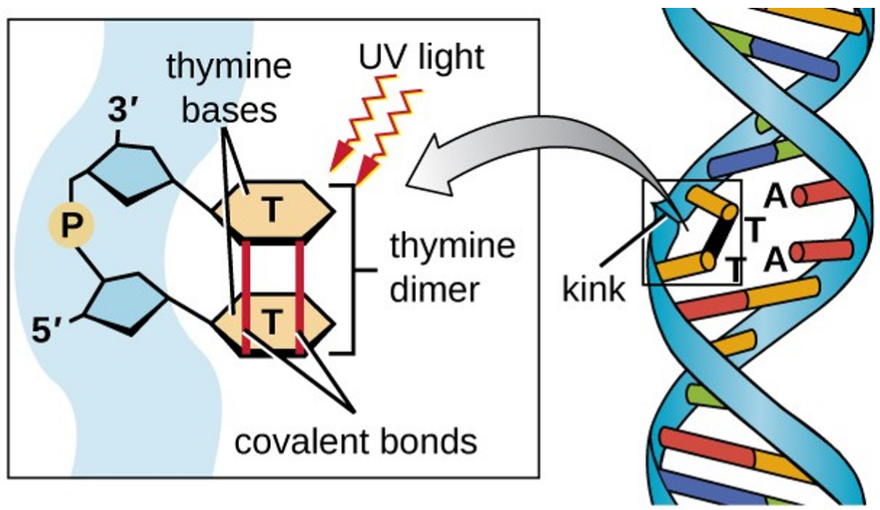
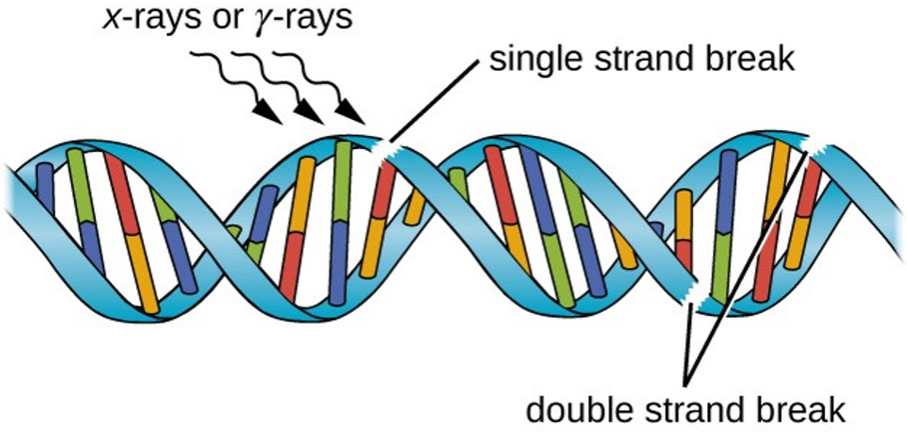
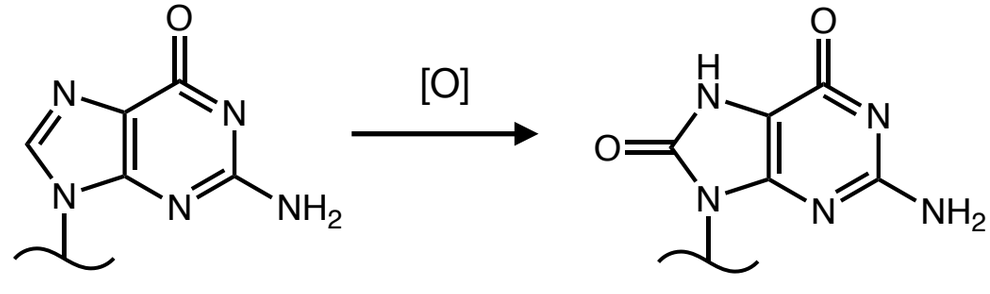


UV-crosslinking thymine dimers

Types of DNA damage

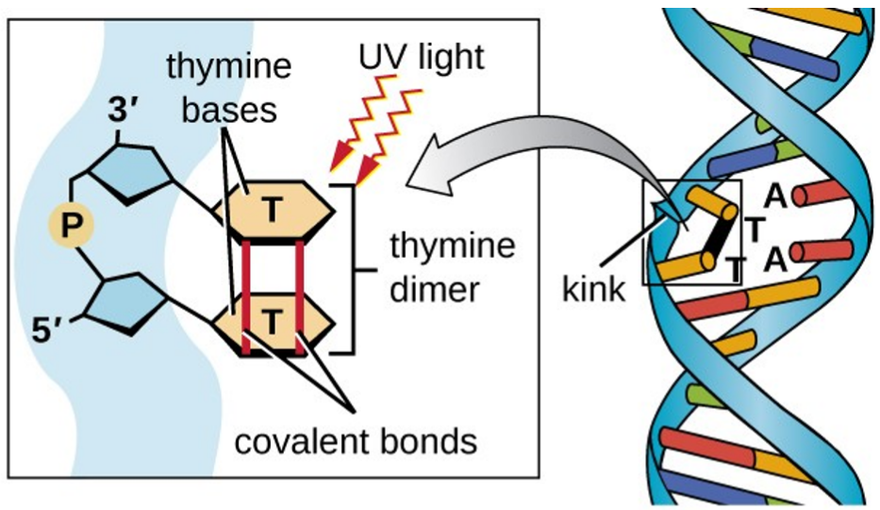


Double/single strand break



8-oxoG formation

Base alterations/oxidations



UV-crosslinking thymine dimers

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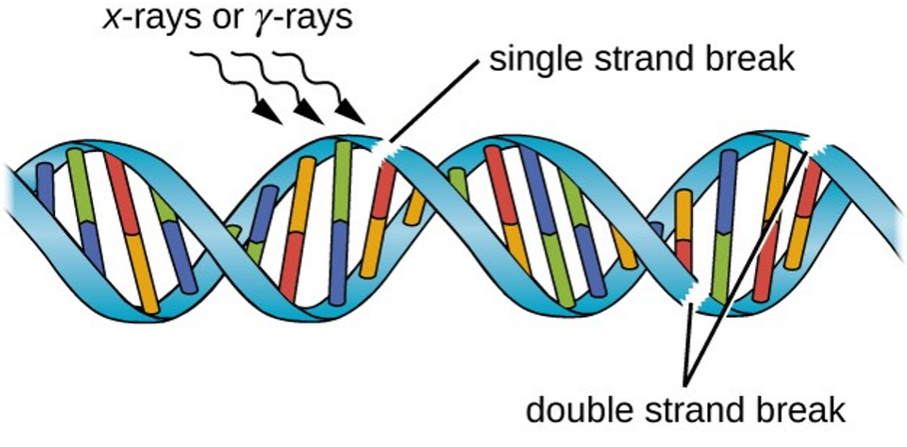
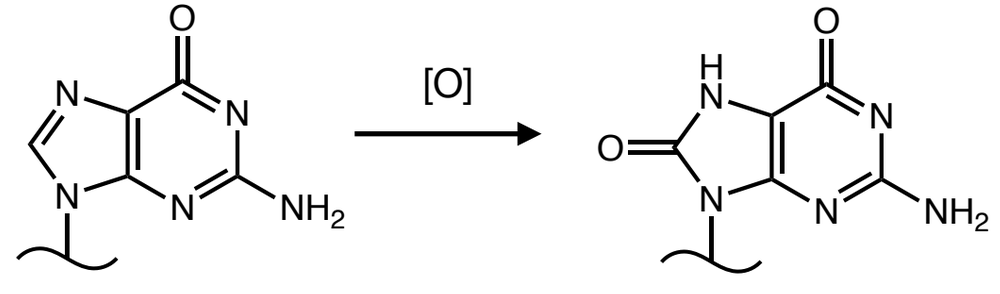


Double/single strand break



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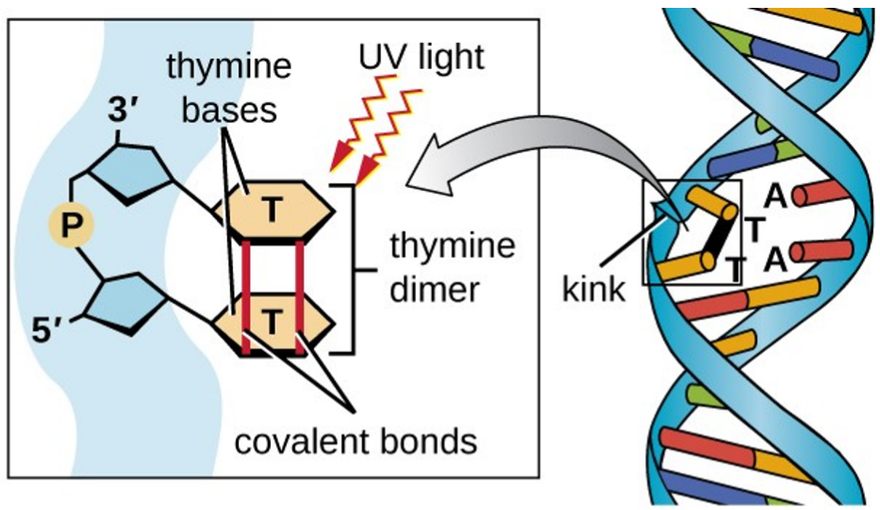
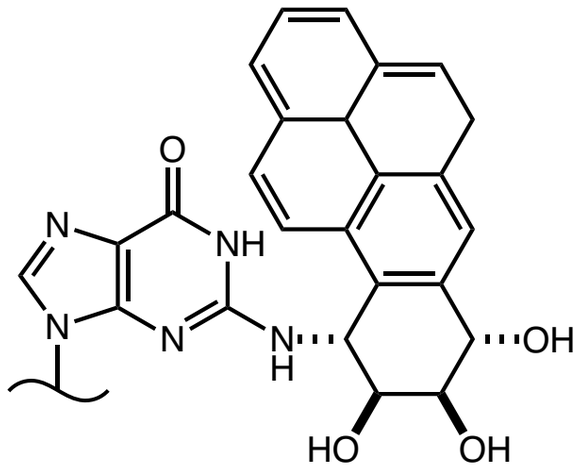


UV-crosslinking thymine dimers



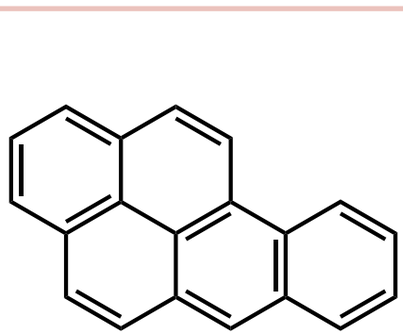
Bulky adduct formation

Bulky adduct formation

Benzopyrene

— Formed at 300-600C incomplete combustion

— found in forest fire, tobacco smoke, and food like grilled meats



Benzopyrene

Bulky adduct formation

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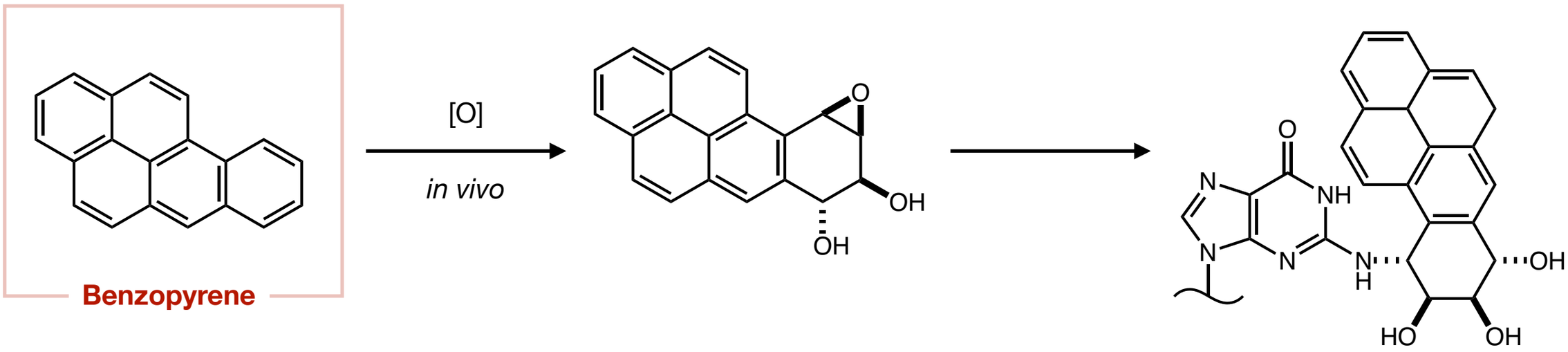


Wikipedia

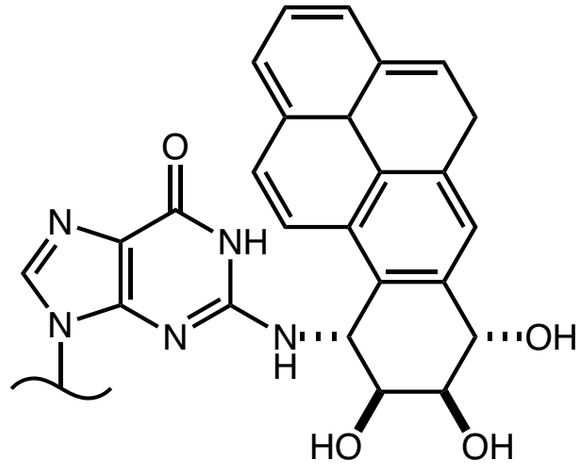
Bulky adduct formation

Benzopyrene

— Formed at 300-600C incomplete combustion

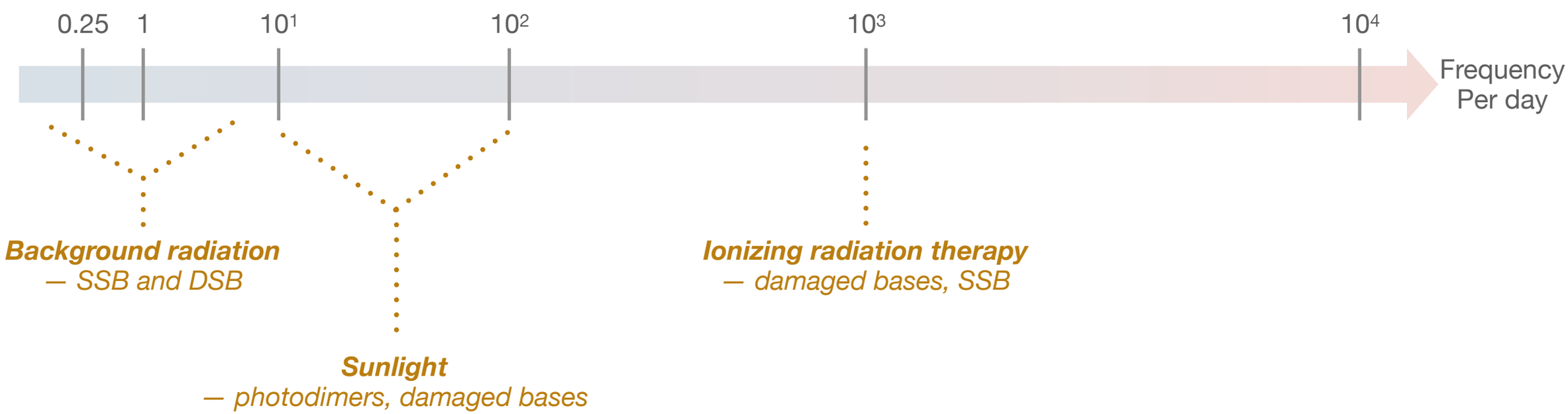
— found in forest fire, tobacco smoke, and food like grilled meats

During DNA replication, will commonly use A as complementary base pair — causing G —> T mutation



DNA damage happening DAILY

DNA damage happening DAILY



Environmental exposures

Yousefzadeh, M. *et al. eLife.* **2021**, *10,* e62852.

DNA damage happening DAILY

Endogenous DNA adducts

Depyrimidination

C deamination

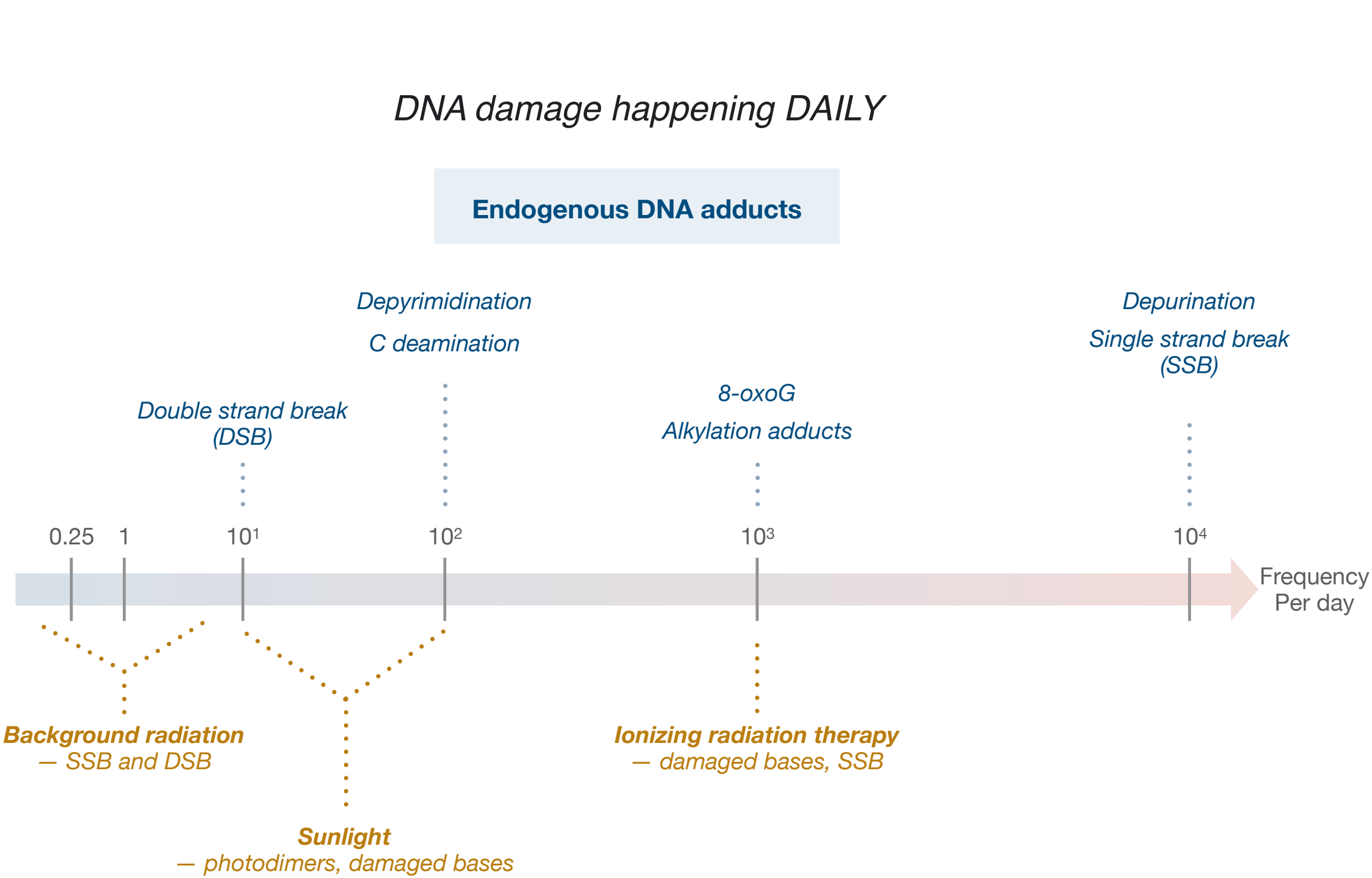
Depurination

Single strand break (SSB)

Double strand break (DSB)

8-oxoG

Alkylation adducts



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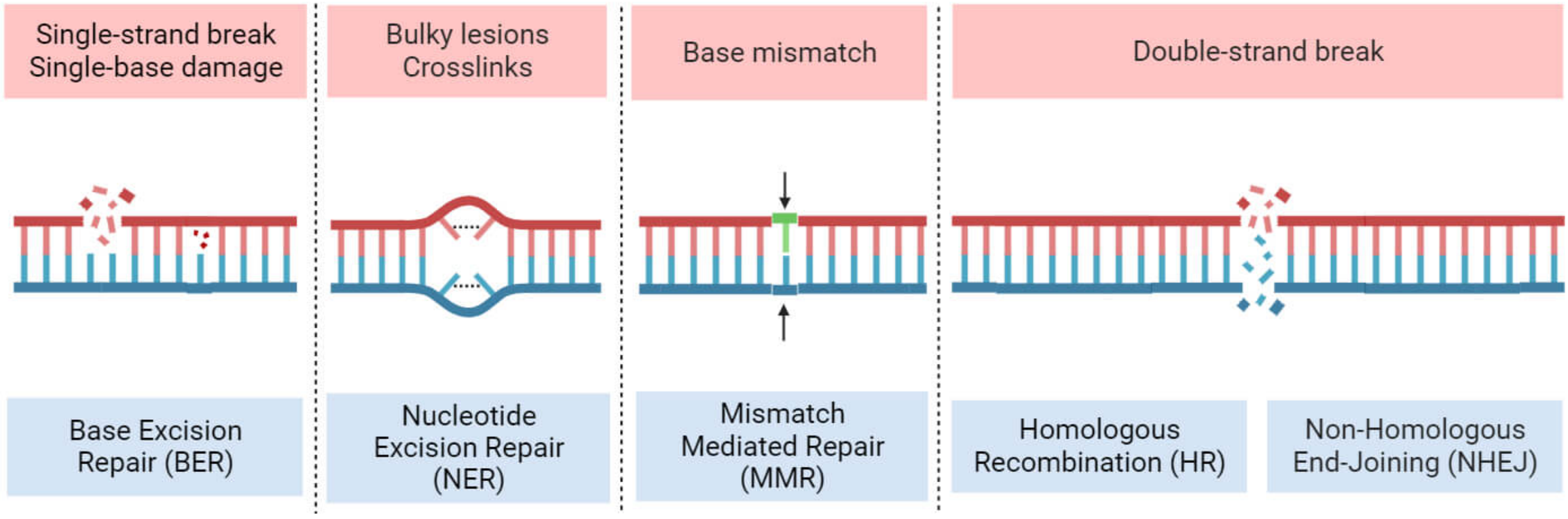
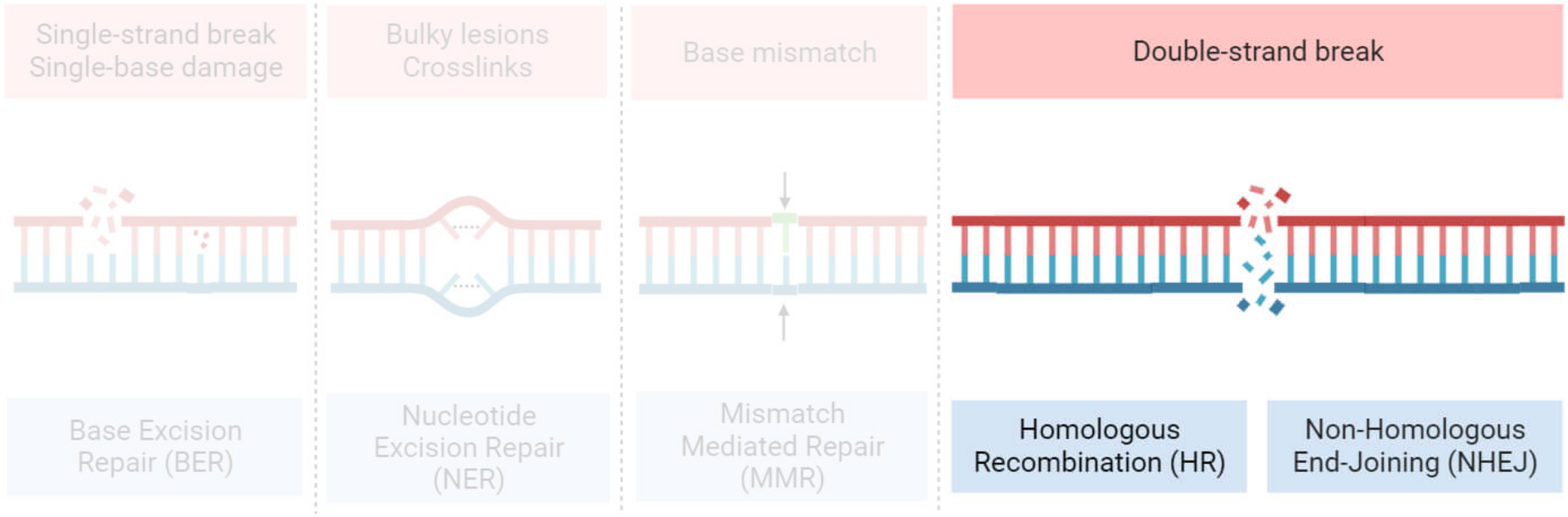
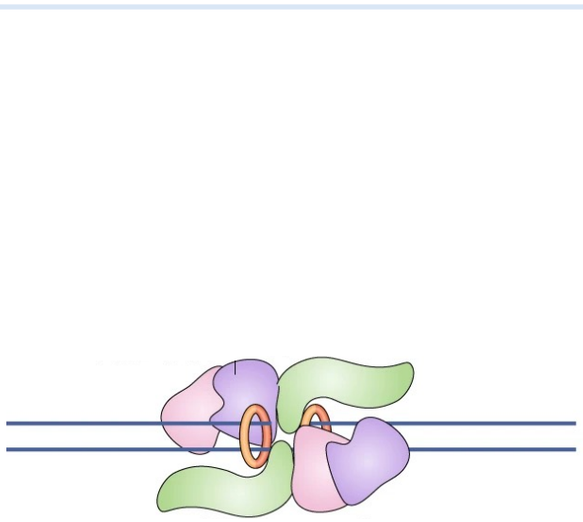
Different damage = different repair pathways



Different damage = different repair pathways



Double strand break repair



Non-homologous end joining (NHEJ)

Happens during G0/G1 phase of cell cycle

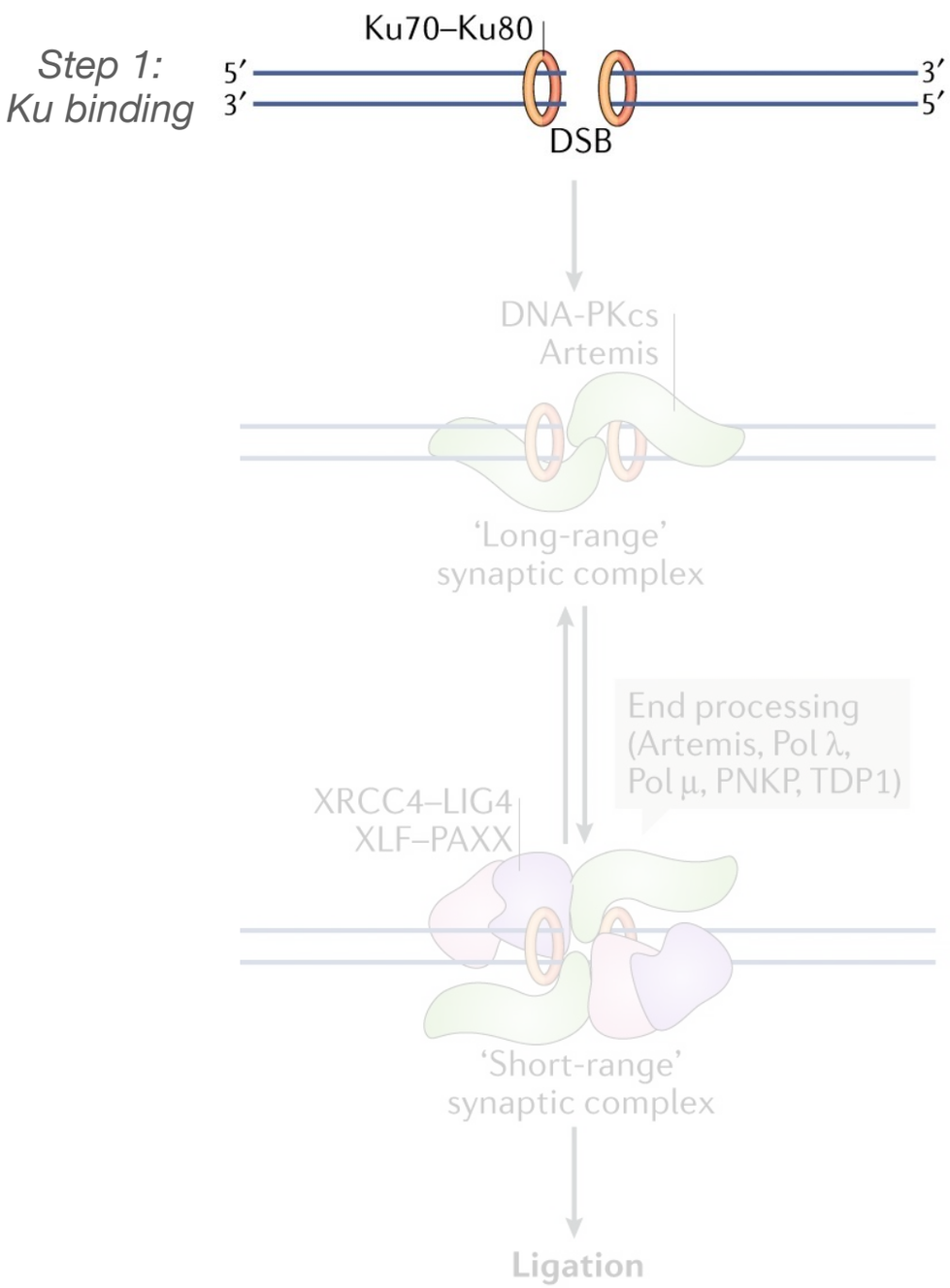
Error-prone repair

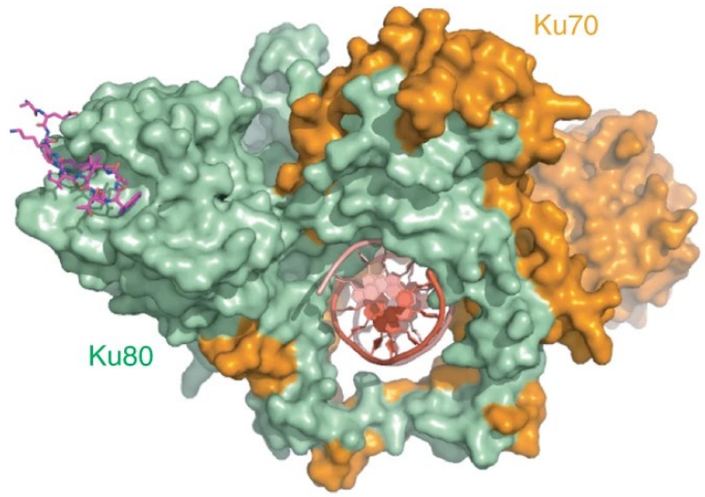
Homologous recombination (HR)

Happens during S/G2 phase of cell cycle

High-fidelity repair

Non-homologous end joining (NHEJ)





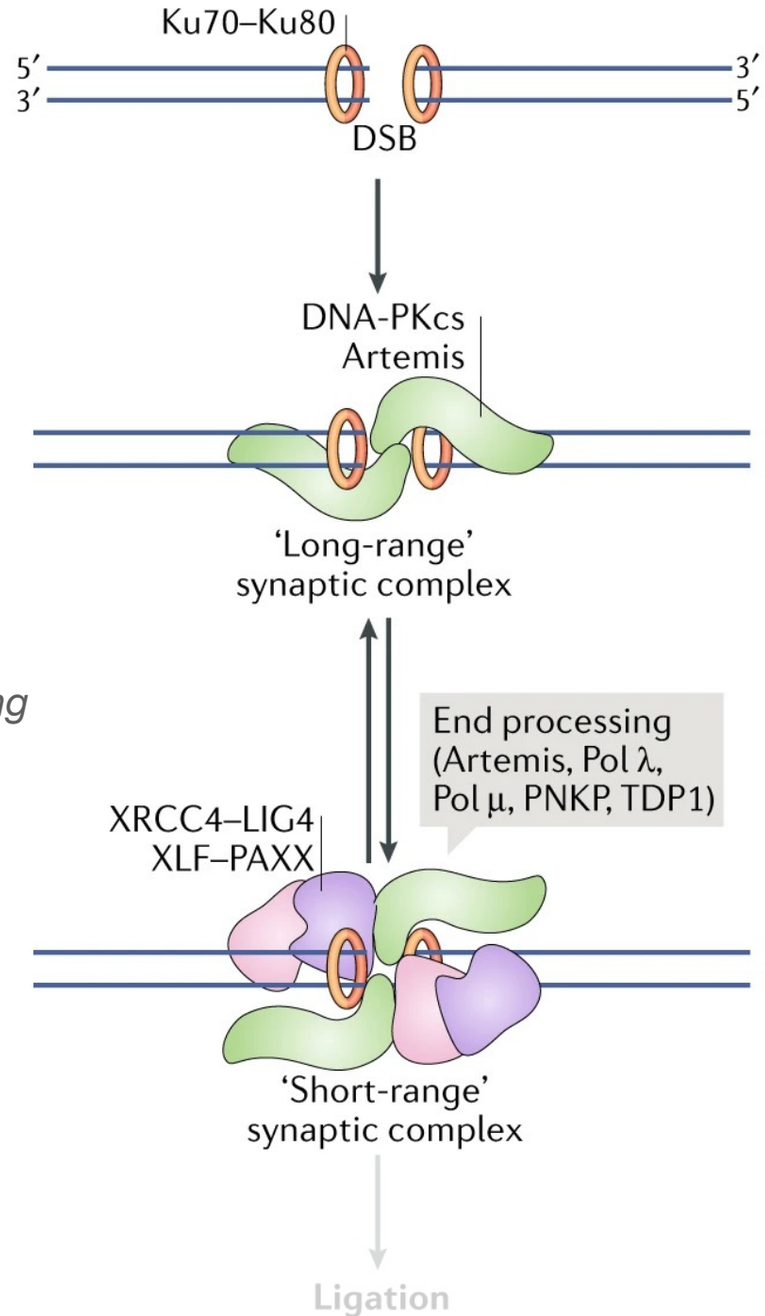
Ku70-Ku80 heterodimer *binds rapidly&tightly to ends DSB*
— recruit necessary NHEJ repair proteins

Scully, R. *et al*. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

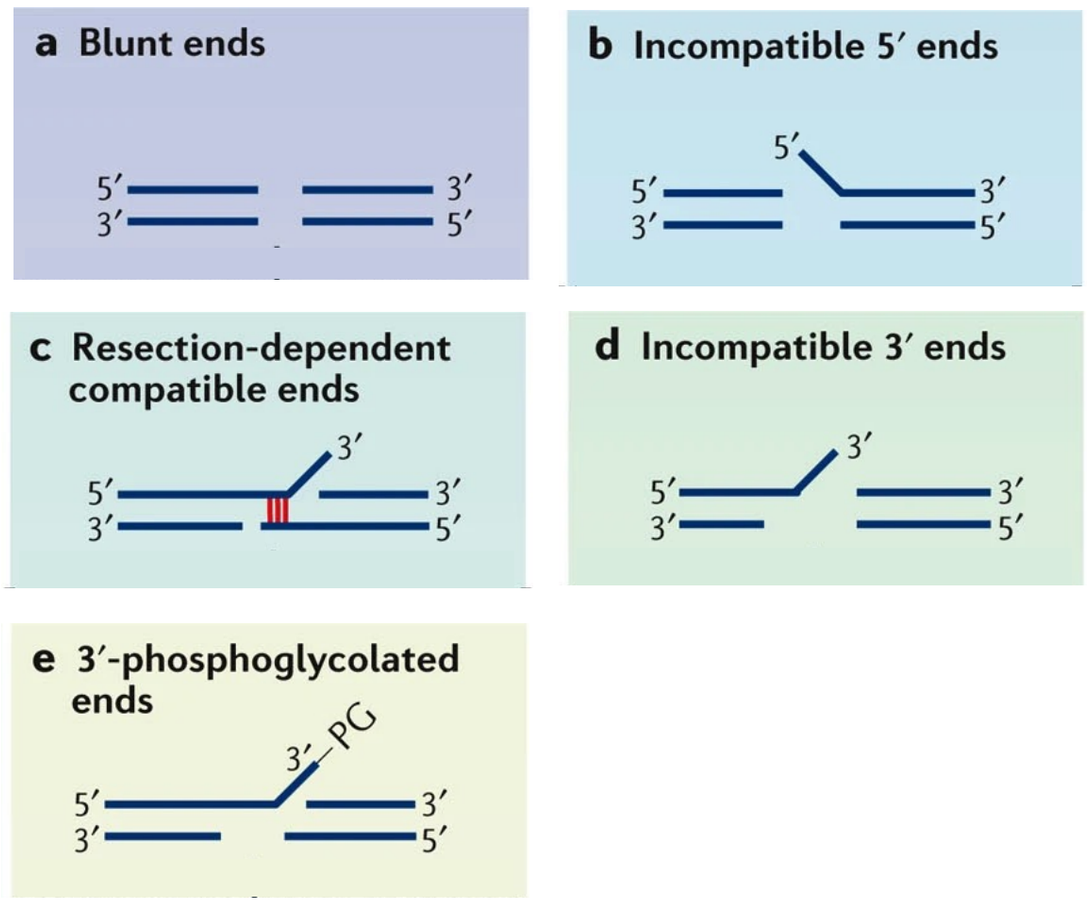
Nemoz, C. *et al*. *Nat. Struct. Mol. Biol.* **2018**, *25,* 971.

Non-homologous end joining (NHEJ)

Step 1:
Ku binding



Step 2:
End processing

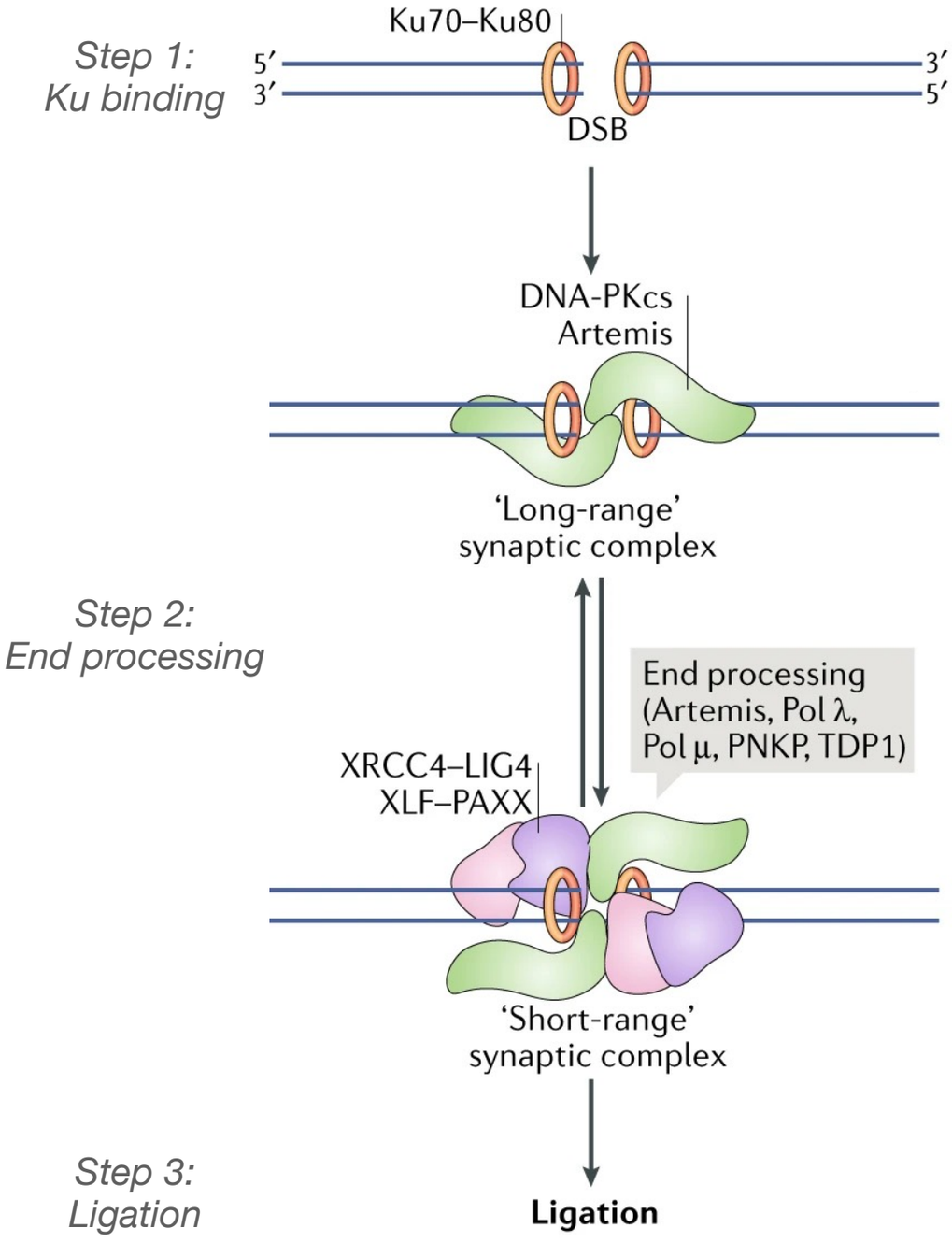


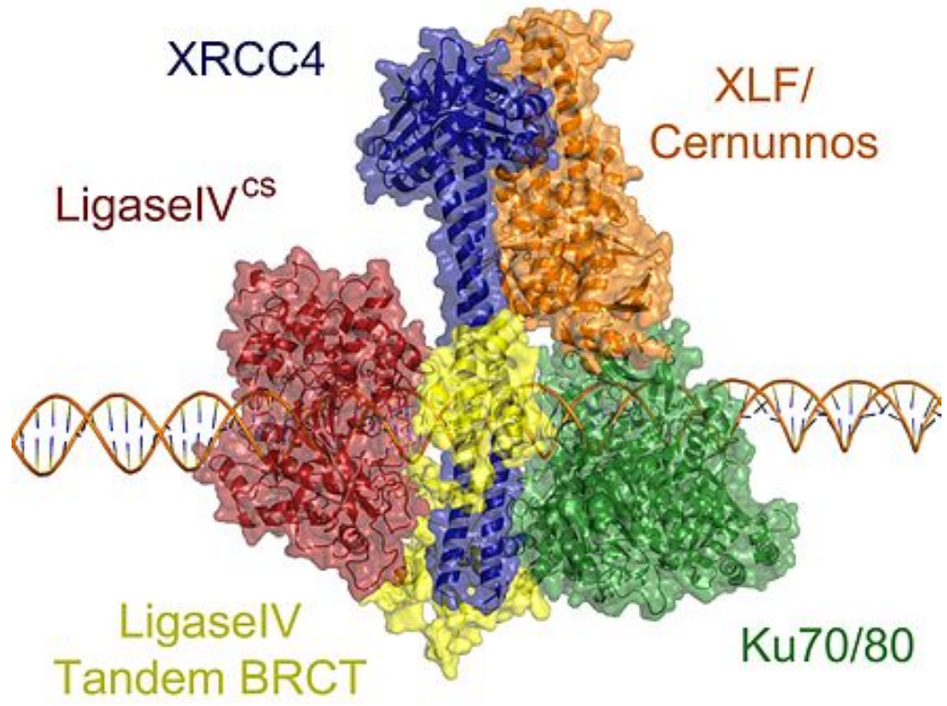
Many different types of DSB ends possible
— need to be processed to become substrates of DNA ligase IV

Scully, R. *et al. Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

Chang, H. H. Y. *et al. Nat. Rev. Mol. Cell. Biol.* **2017**, *18,* 495.

Non-homologous end joining (NHEJ)





Ligase IV *— specific ligase responsible for NHEJ repair; also participates in VDJ recombination*

XRCC4 and XLF *—structural components of Ligase IV complex*

Scully, R. *et al. Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

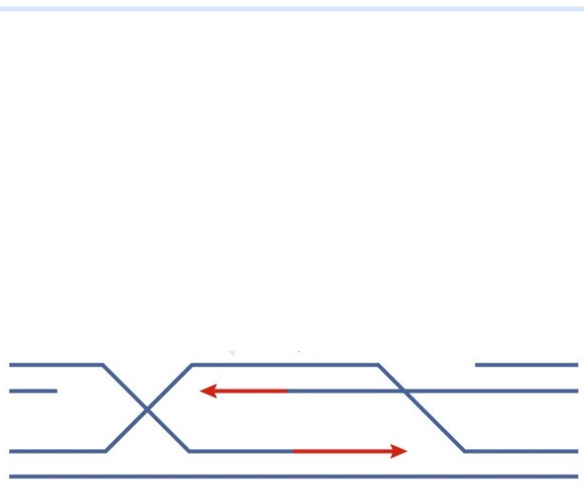
Chang, H. H. Y. *et al. Nat. Rev. Mol. Cell. Biol.* **2017**, *18,* 495.

Double strand break repair

Non-homologous end joining (NHEJ)

Happens during G0/G1 phase of cell cycle

Error-prone repair

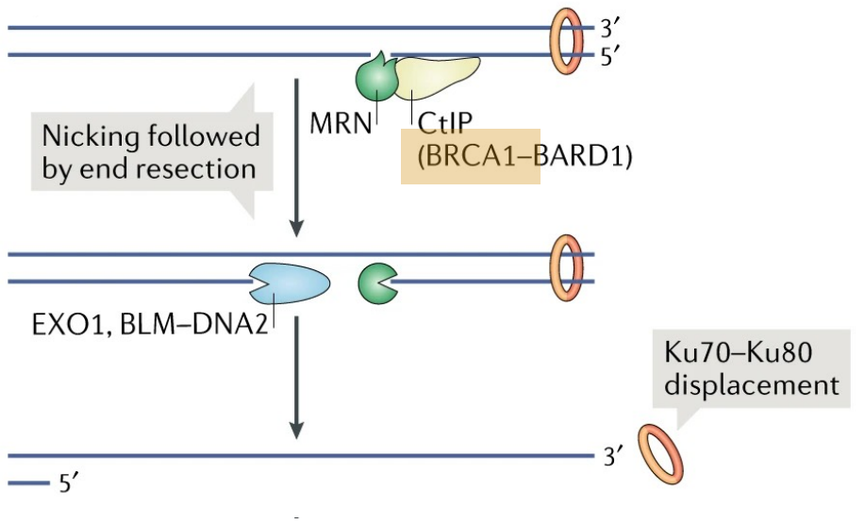


Homologous recombination (HR)

Happens during S/G2 phase of cell cycle

High-fidelity repair

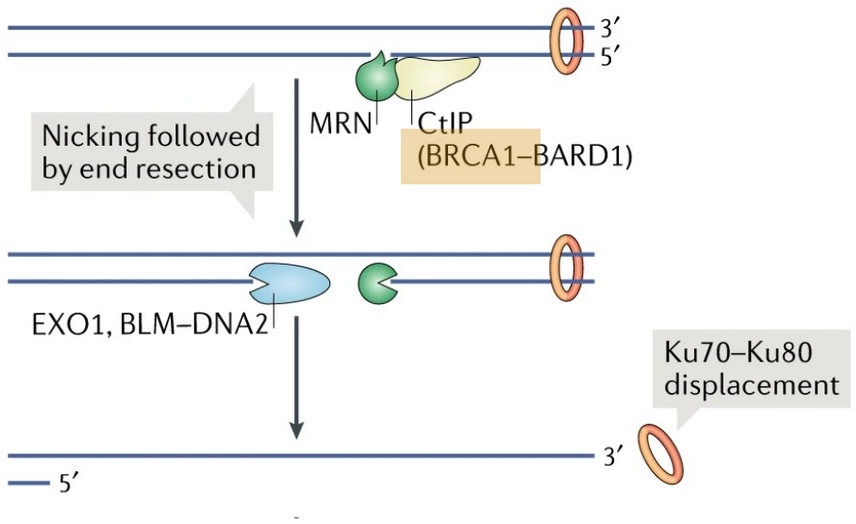
Homologous recombination (HR)



Step 1: 5' end resection

Scully, R. *et al*. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

Homologous recombination (HR)



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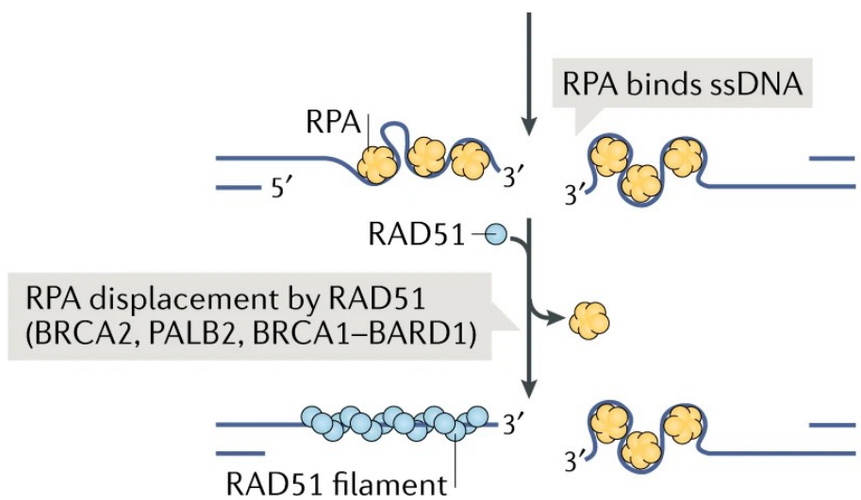
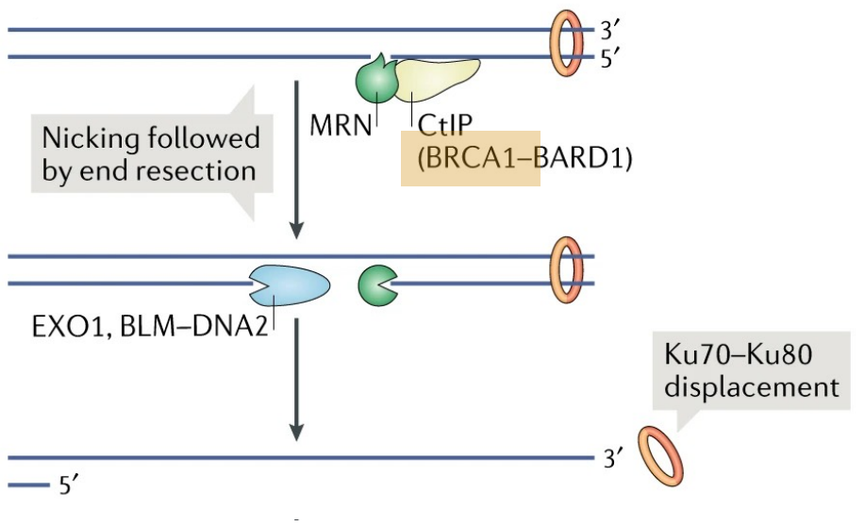


Step 2: 3' ssDNA binding

Scully, R. *et al*. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

Homologous recombination (HR)



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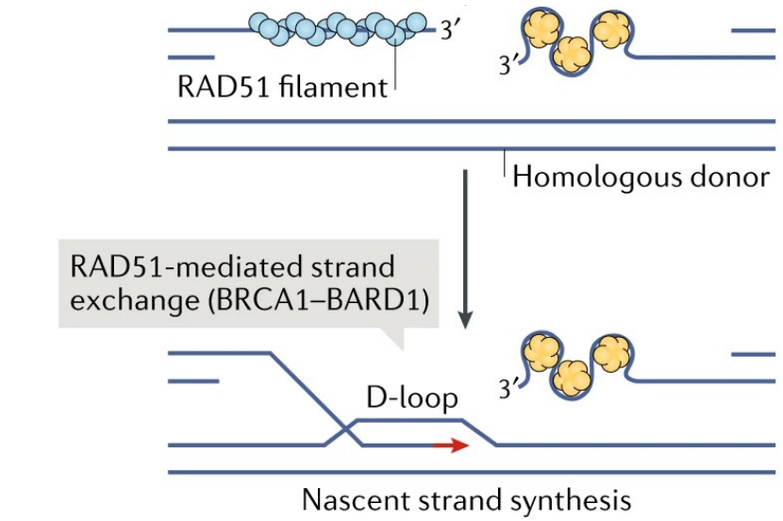
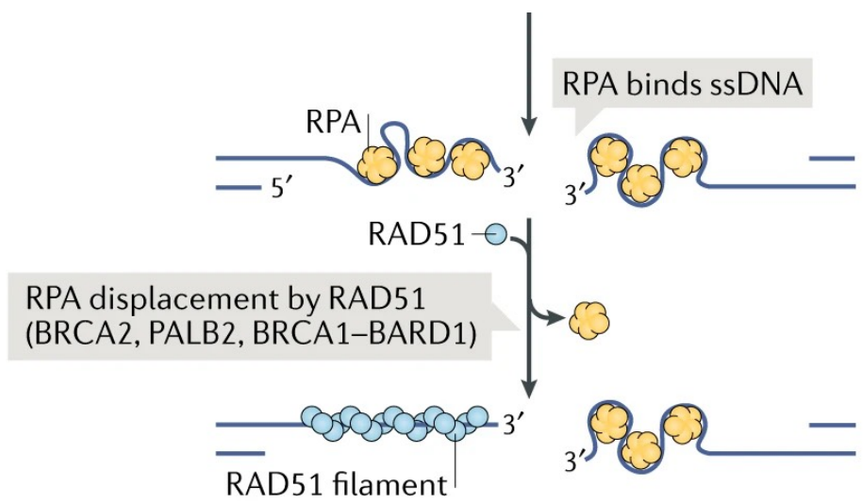


Step 3: template complementation



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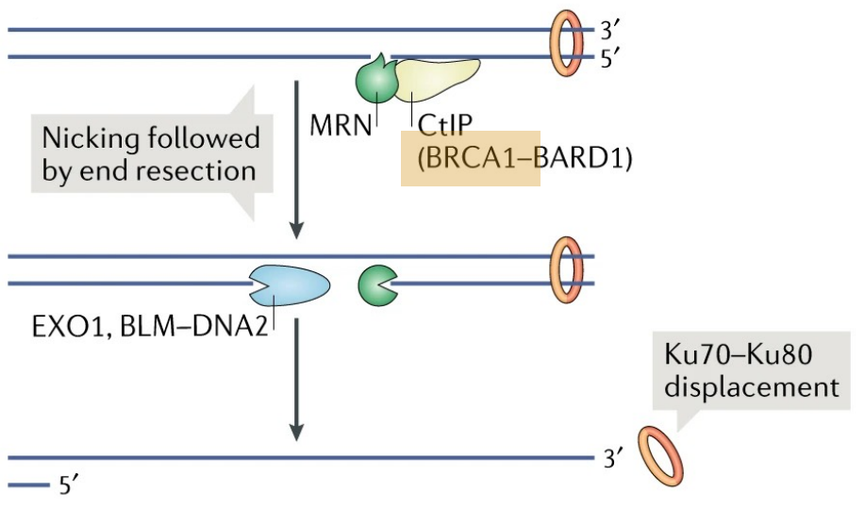
Homologous recombination (HR)



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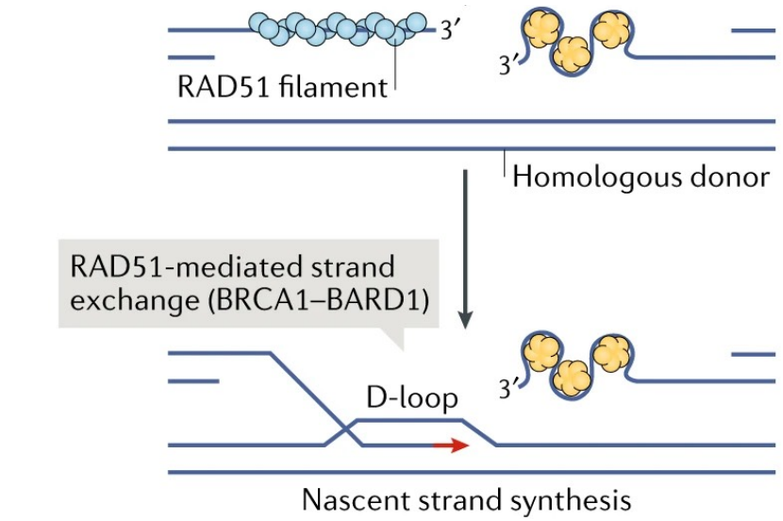

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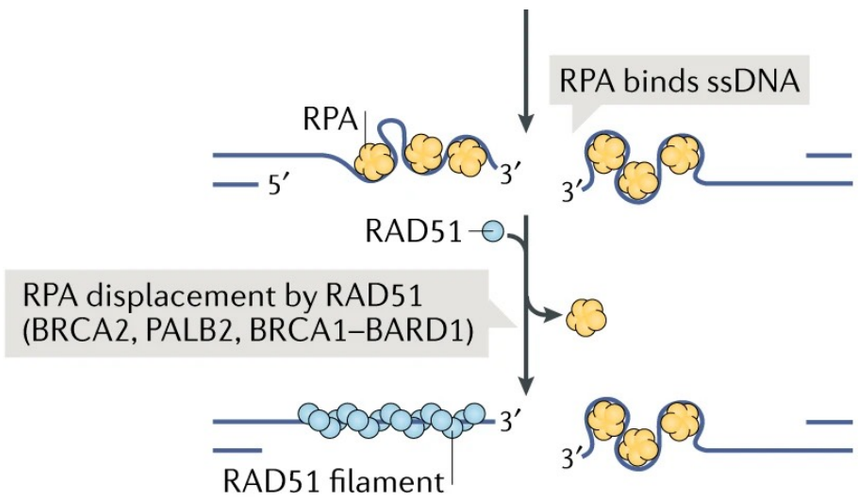
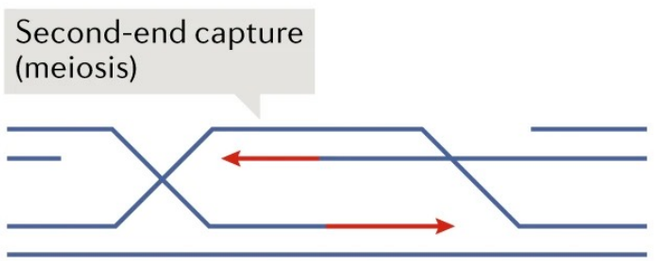


Step 2: 3' ssDNA binding



Step 4: further extension

Scully, R. *et al. Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

Recognizing DSB and other DNA damage — DNA damage response (DDR)

In order to perform DNA damage repair, early DDR is needed to recognize the need

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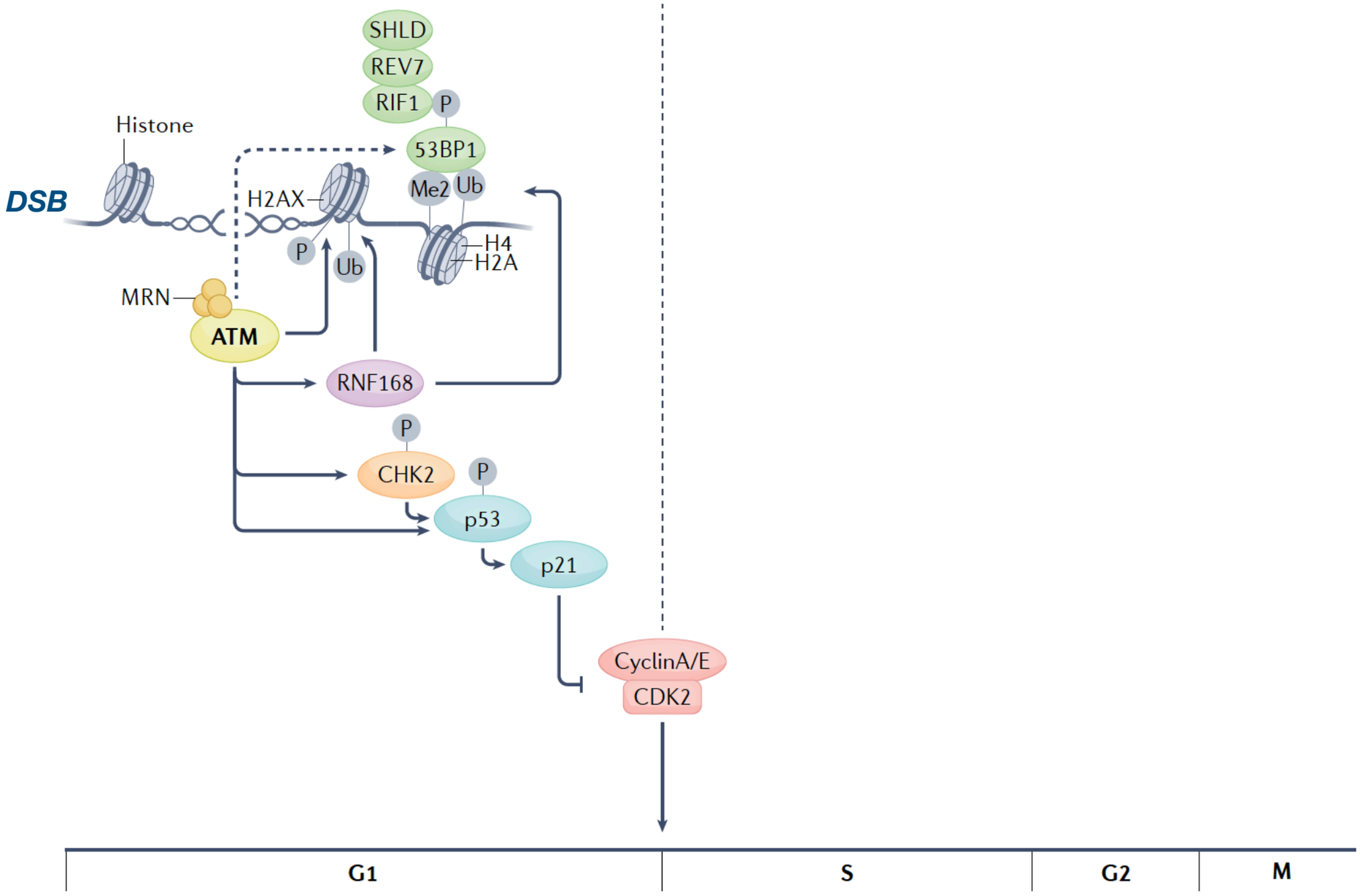

Non-homologous end joining (NHEJ)

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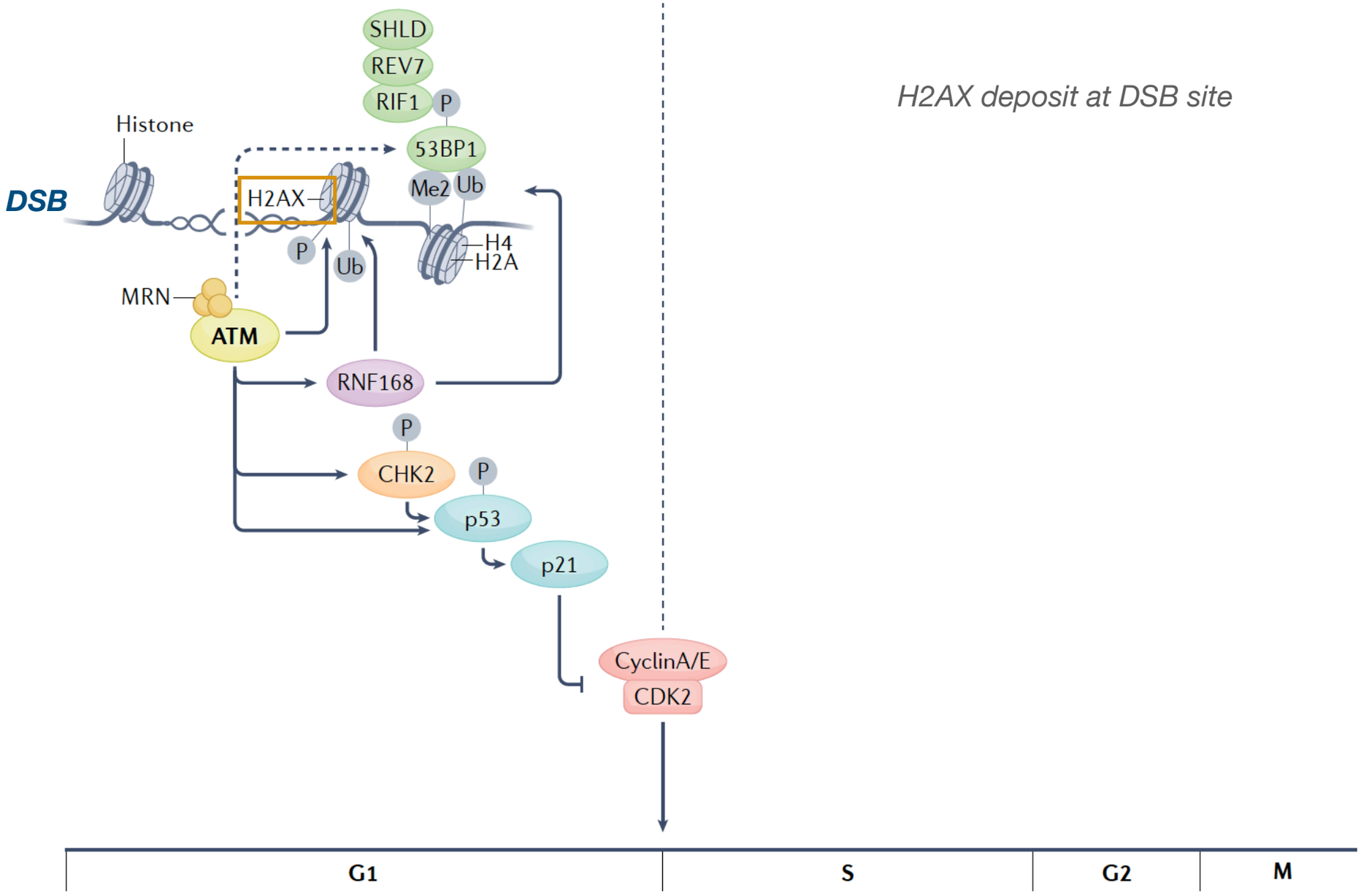

H2AX deposit at DSB site

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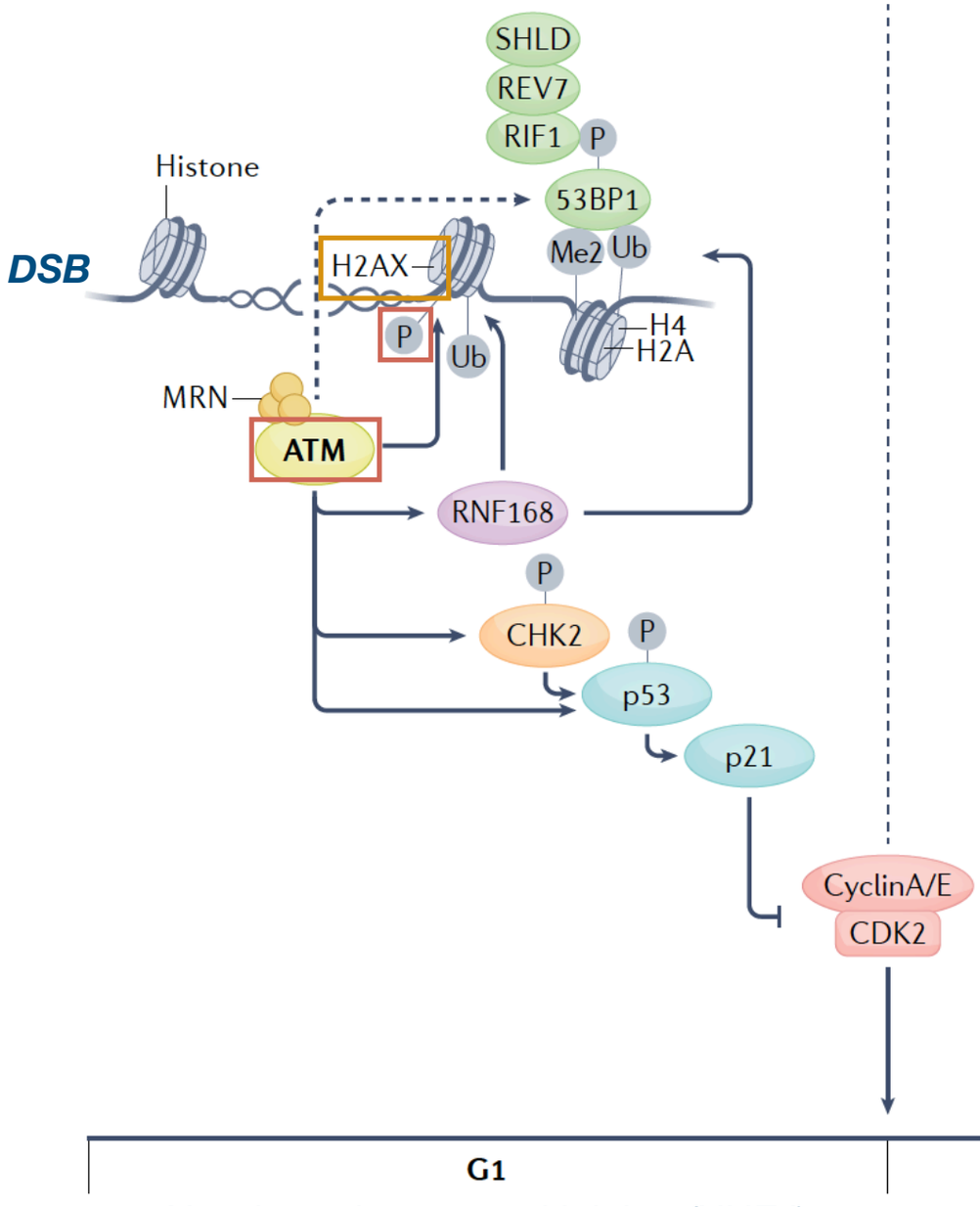

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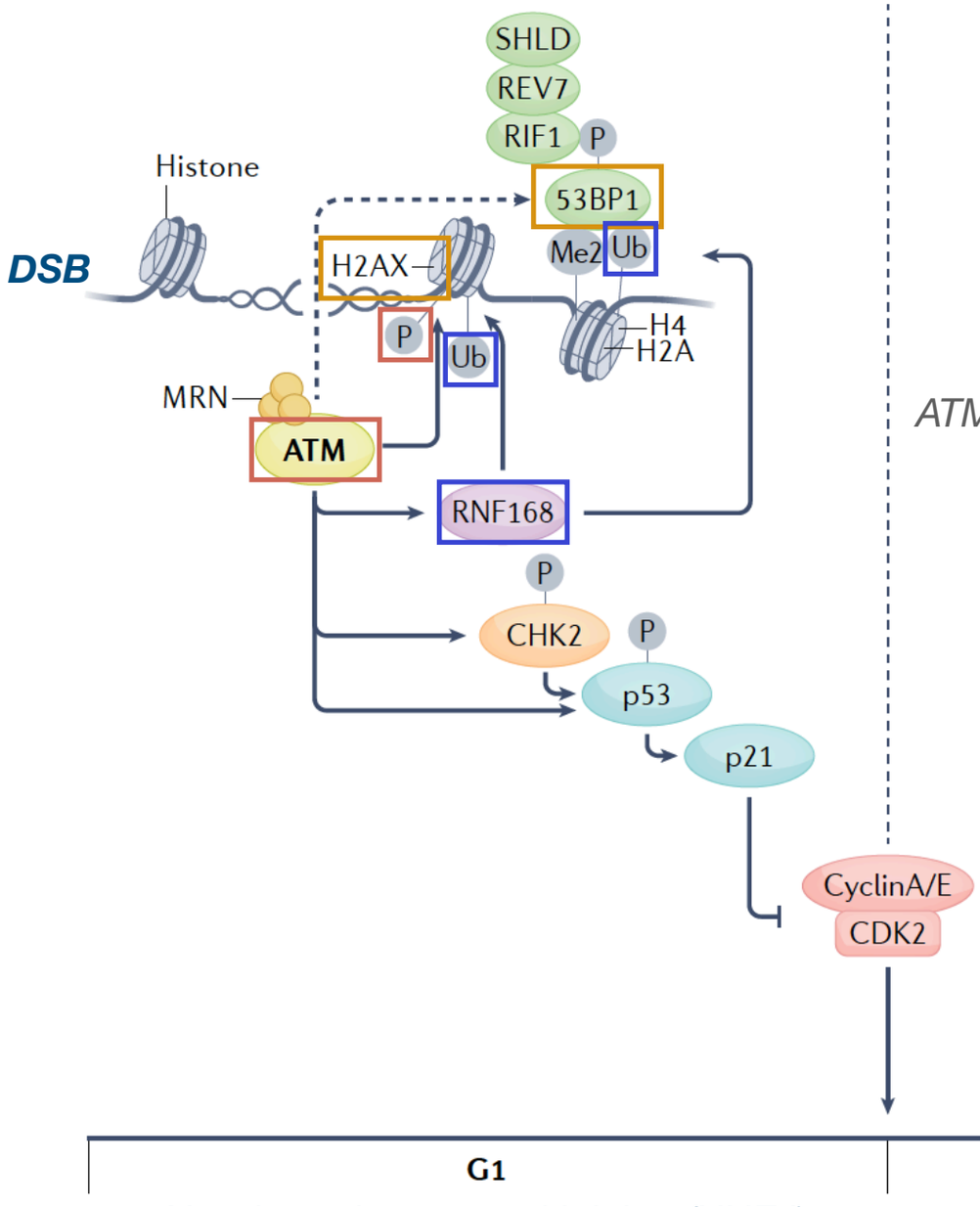

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ATM activates RNF168 — ubiquitinates H2A and providing docking platform for other DDR proteins

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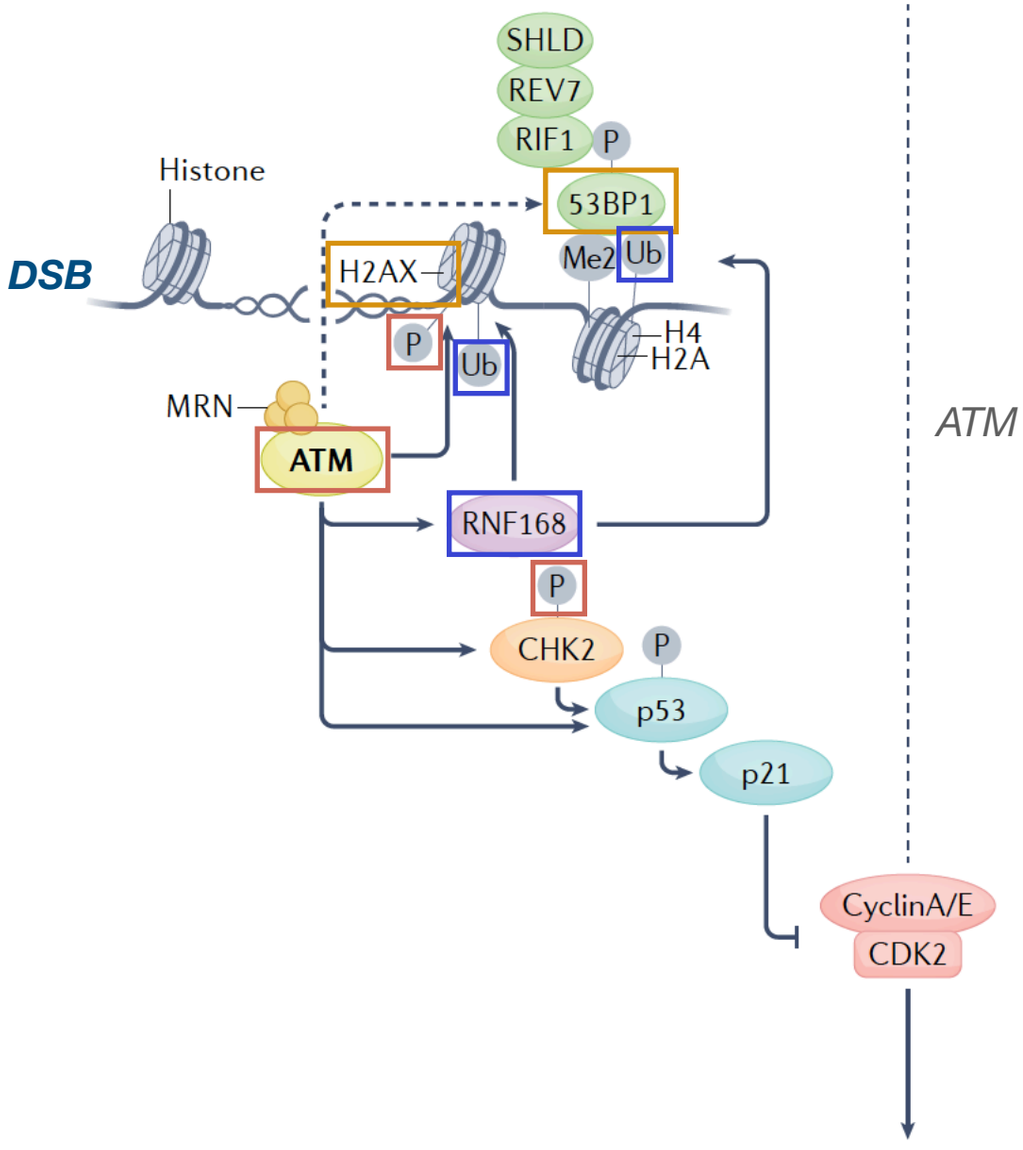

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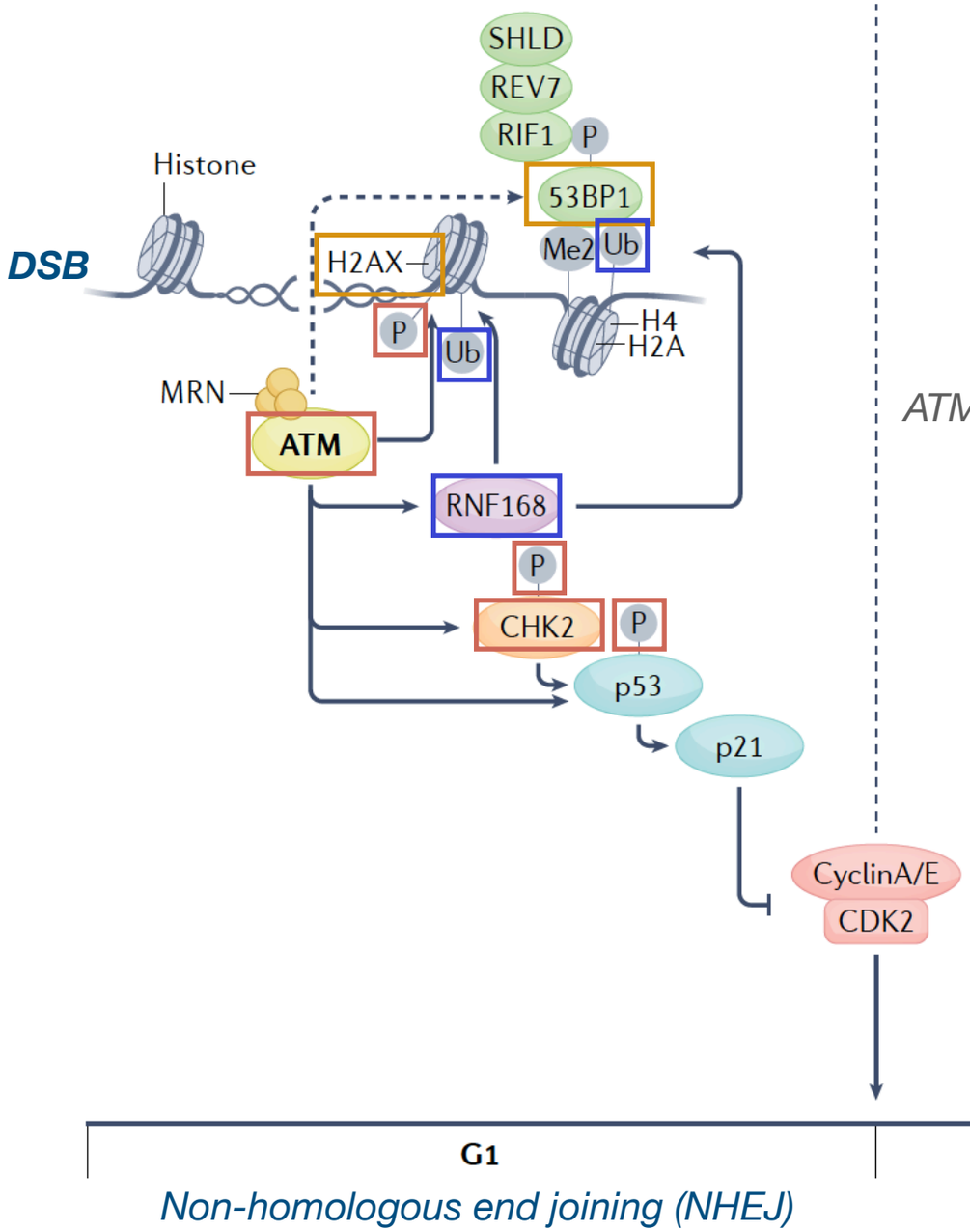

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Activated p-CHK2 phosphorylate p53

phospho-p53 activate p21 (CDKN1A)

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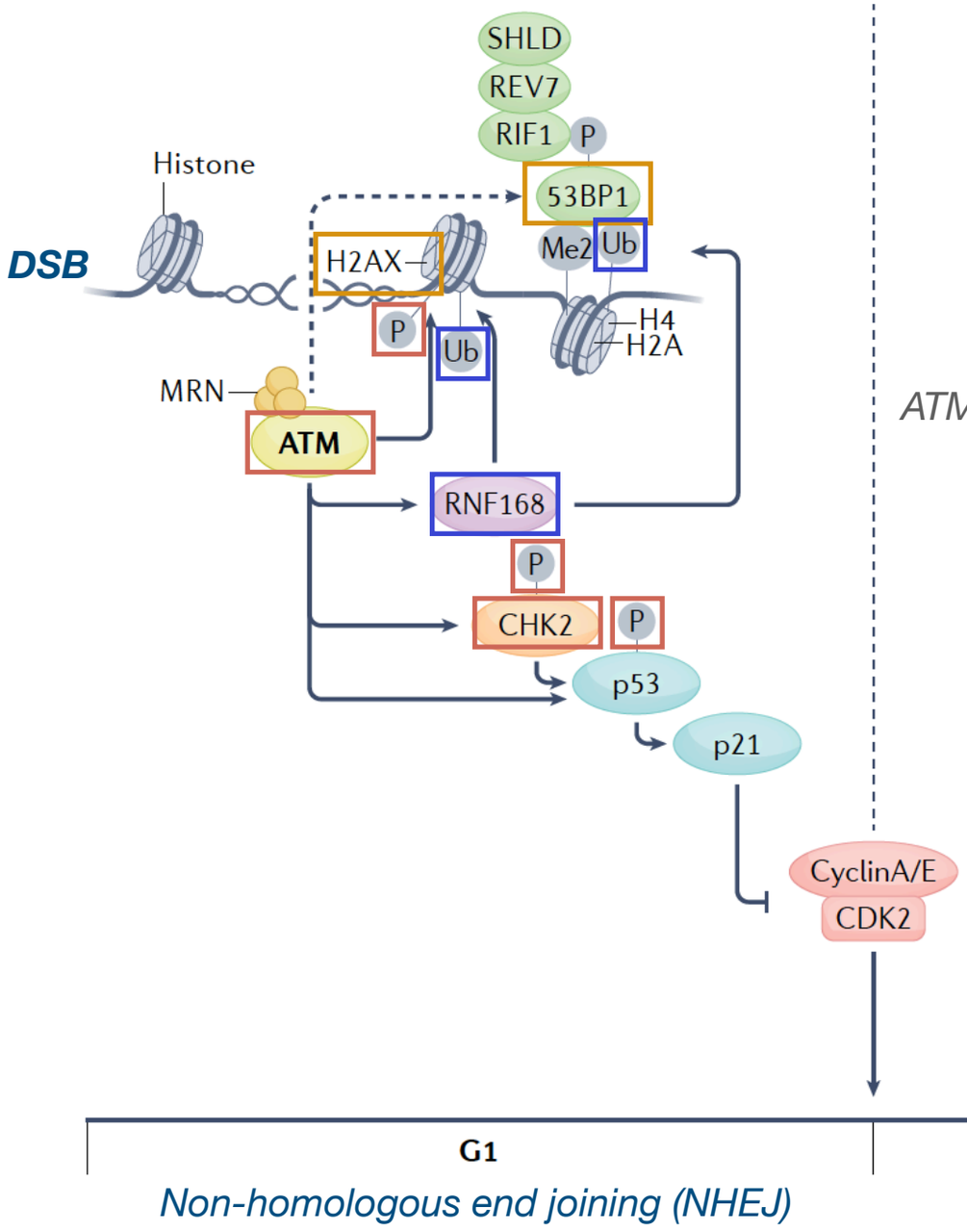

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ATM also phosphorylate CHK2

Activated p-CHK2 phosphorylate p53

phospho-p53 activate p21 (CDKN1A)

p21 inhibited CDK/cyclin to to arrest cell cycle
=Senescence

Non-homologous end joining (NHEJ)

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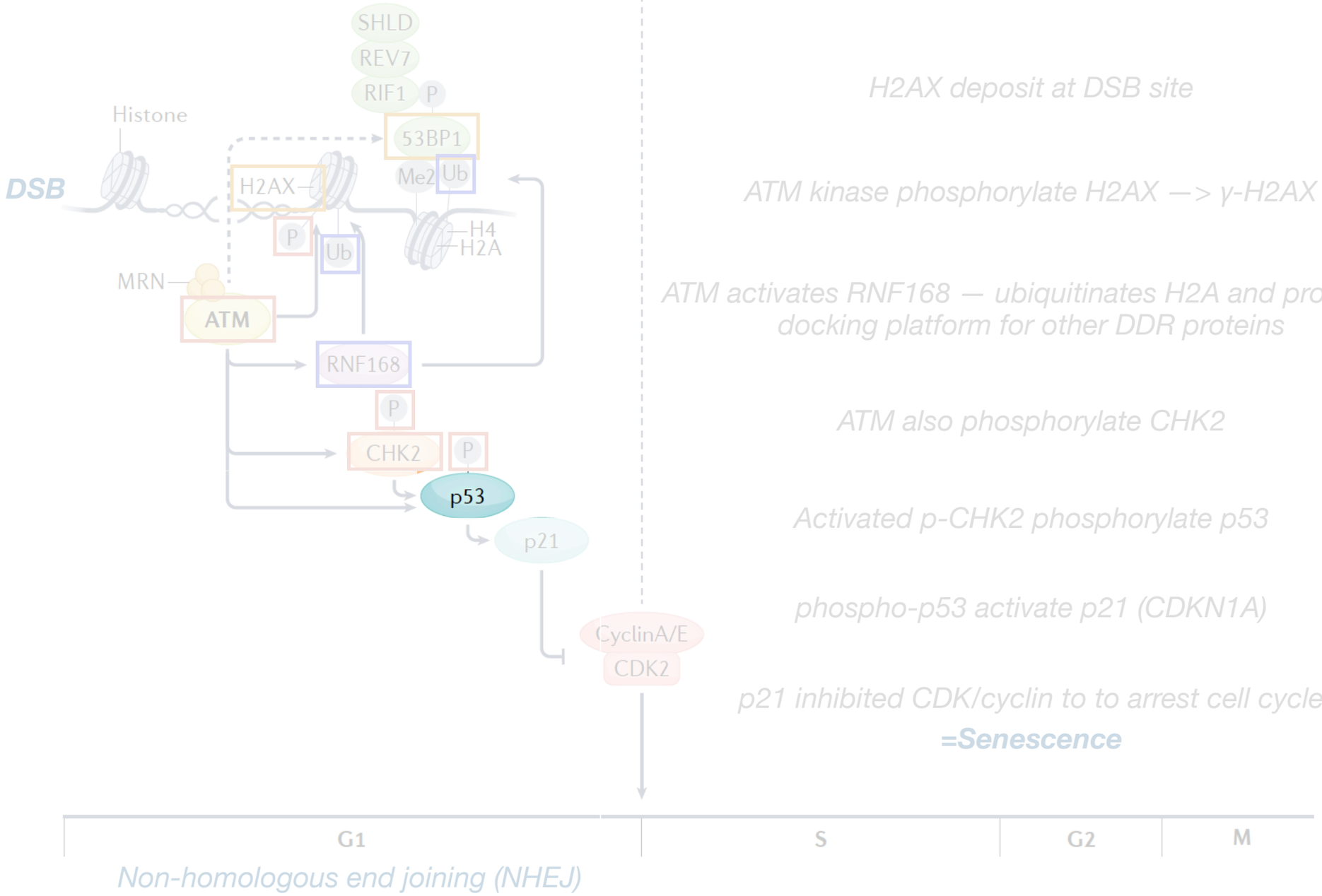


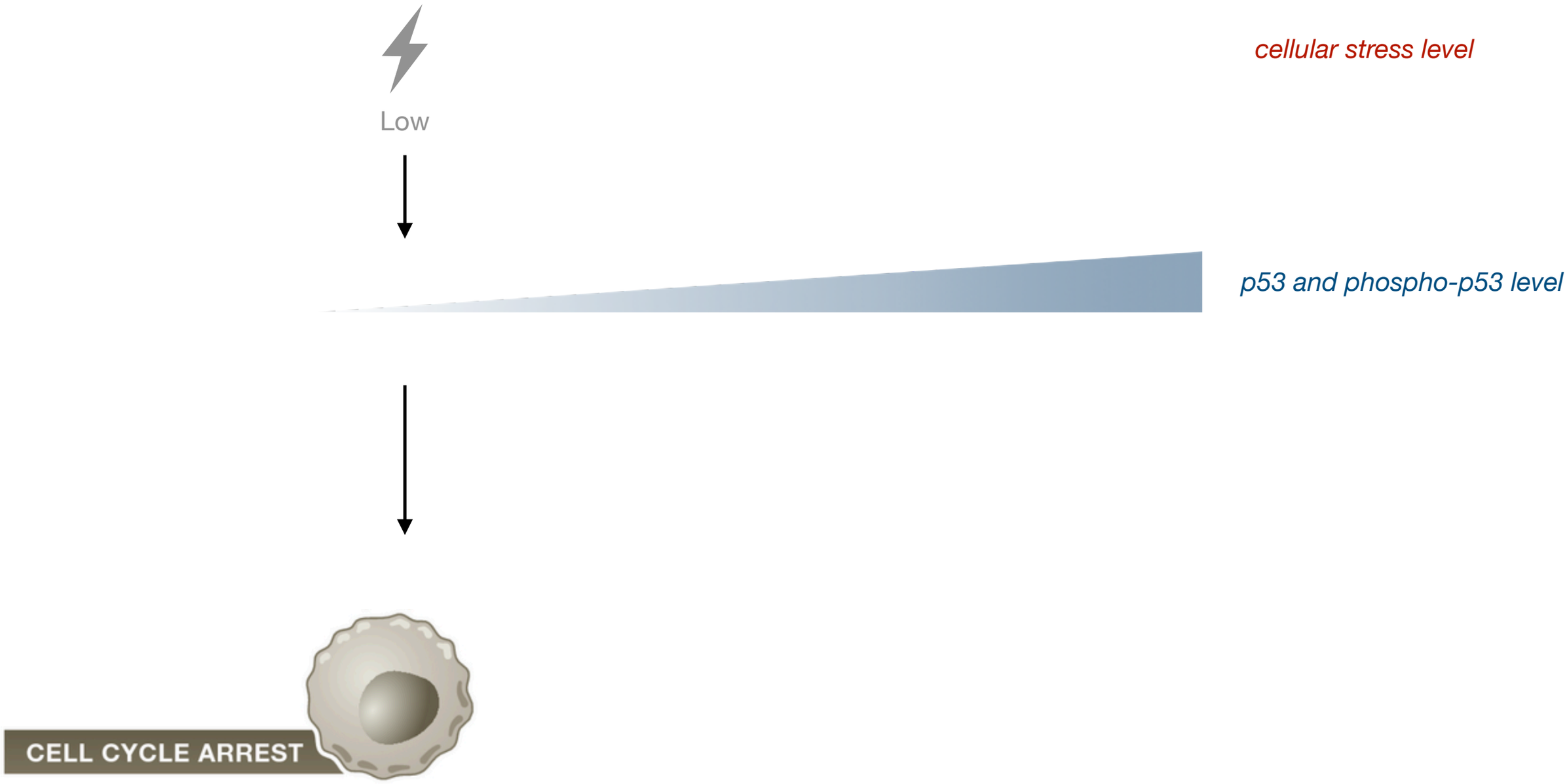
Scully, R. *et al*. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

p53 — determining between senescence and apoptosis

p53 and phospho-p53 level

Childs, B. G. *et al*. *EMBO Rep.* **2014**, *15,* 1139.

p53 — determining between senescence and apoptosis



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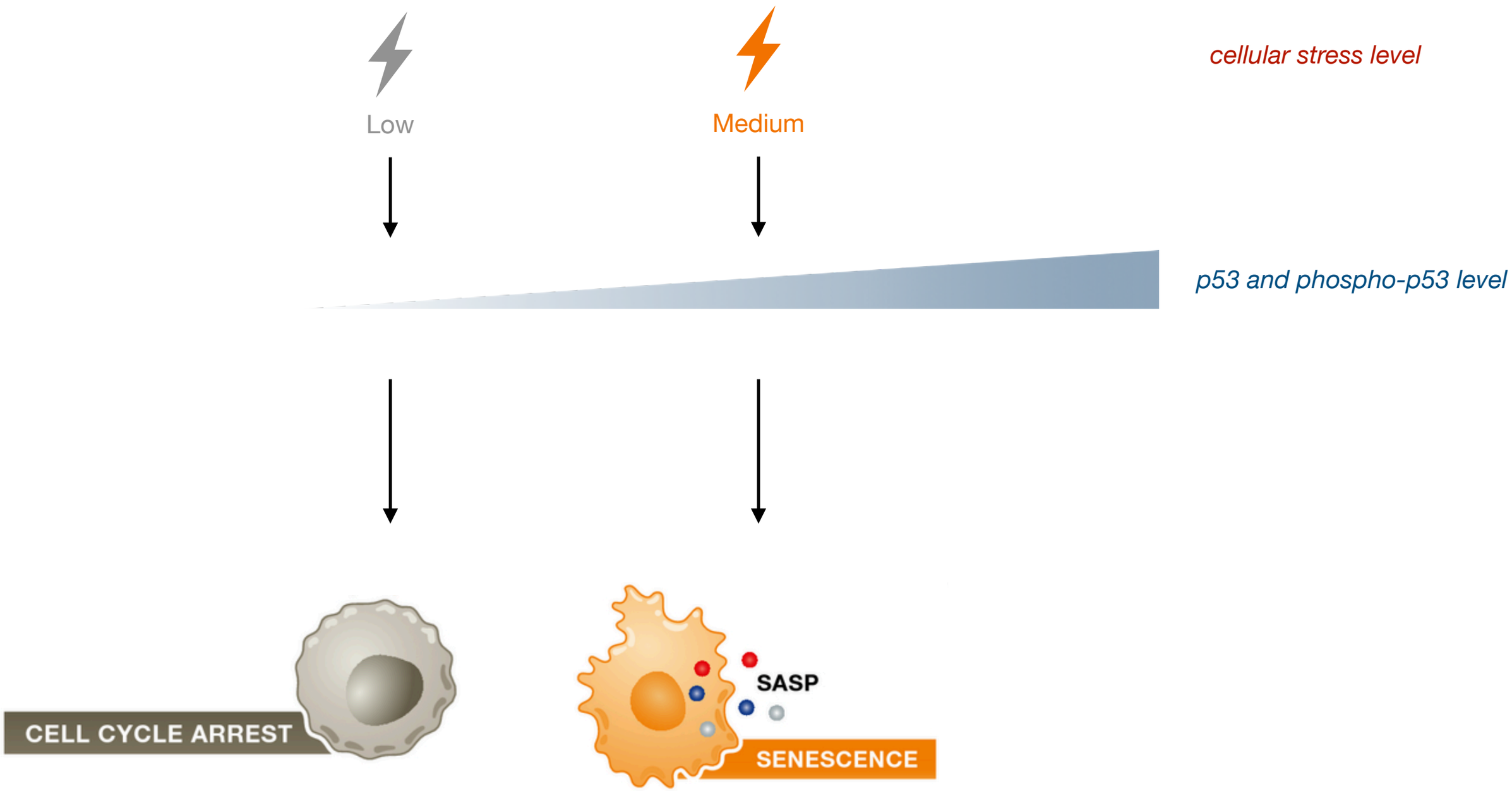

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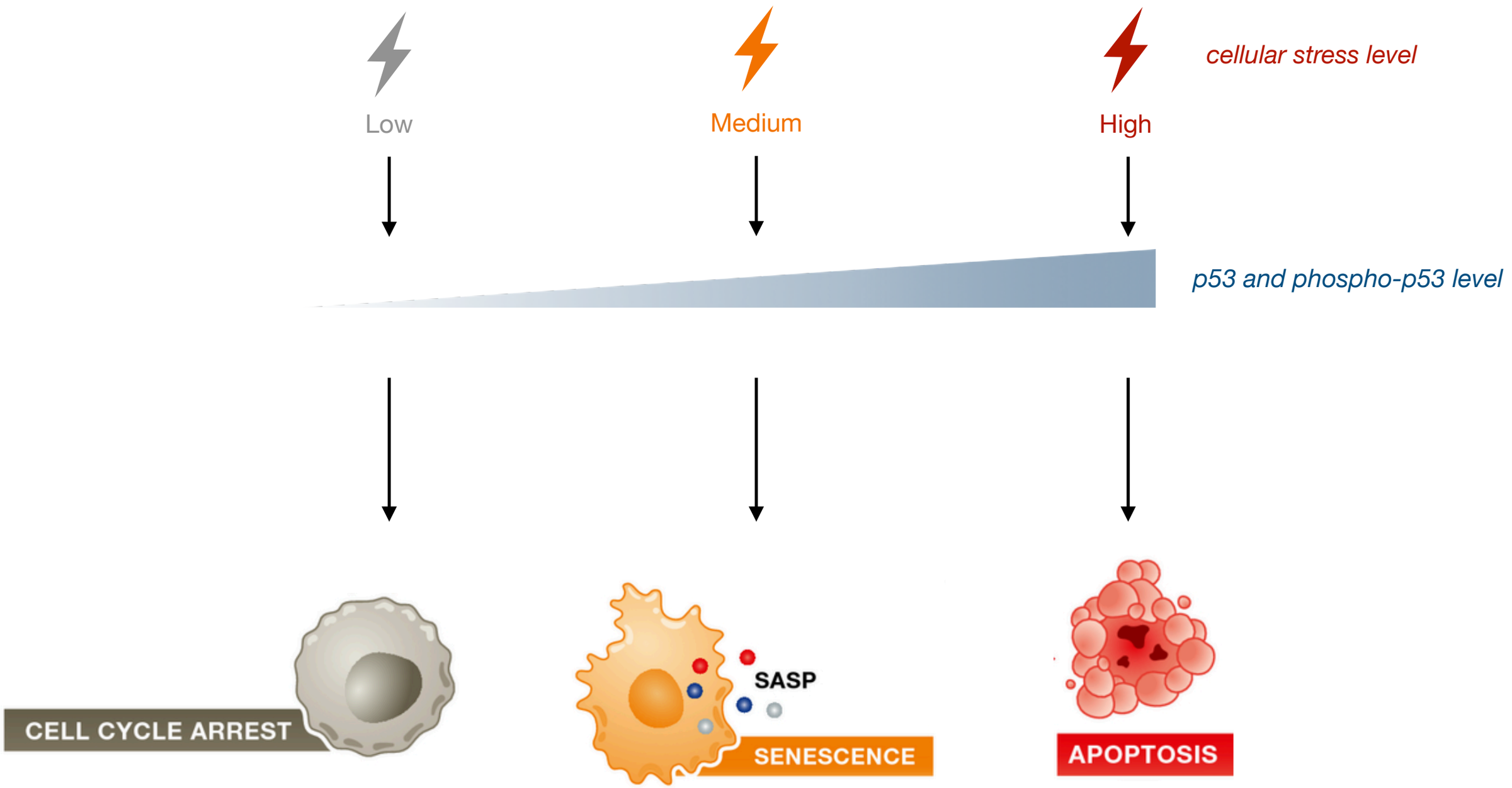

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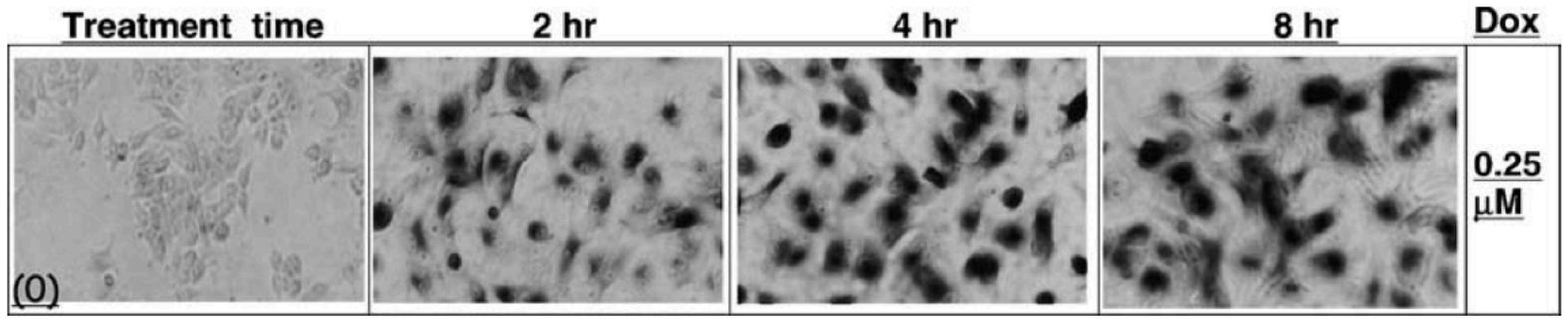

Senesence readout by β-gal staining (black)
Apoptosis readout by cell count

In MCF7 cells, low dose/short time of doxorubicin treatments leads to senescence;

Song, Y. S.; Lee, B. Y.; Hwang, E. S. *Mech. Ageing. Dev.* **2005**, *126,* 580.

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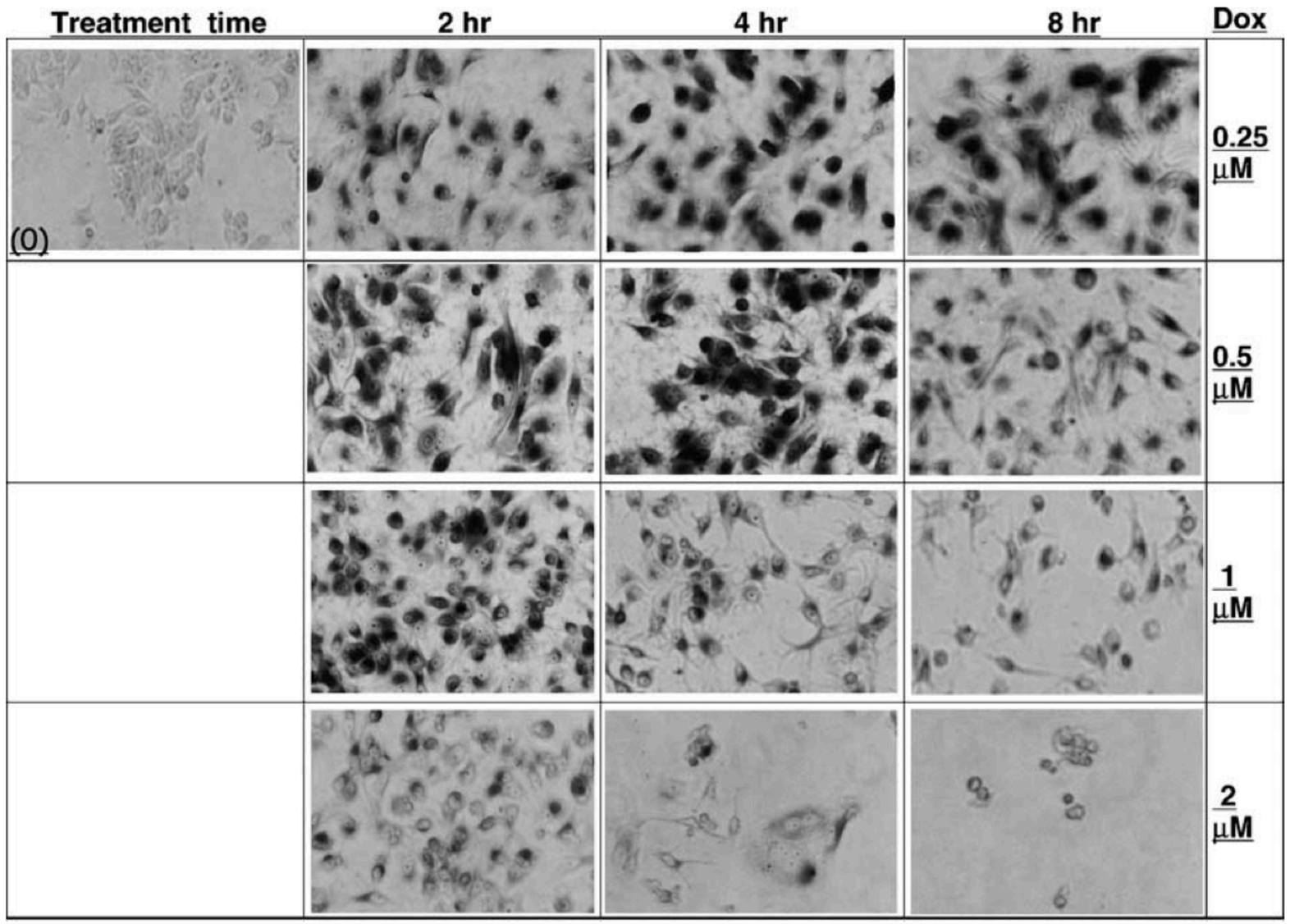

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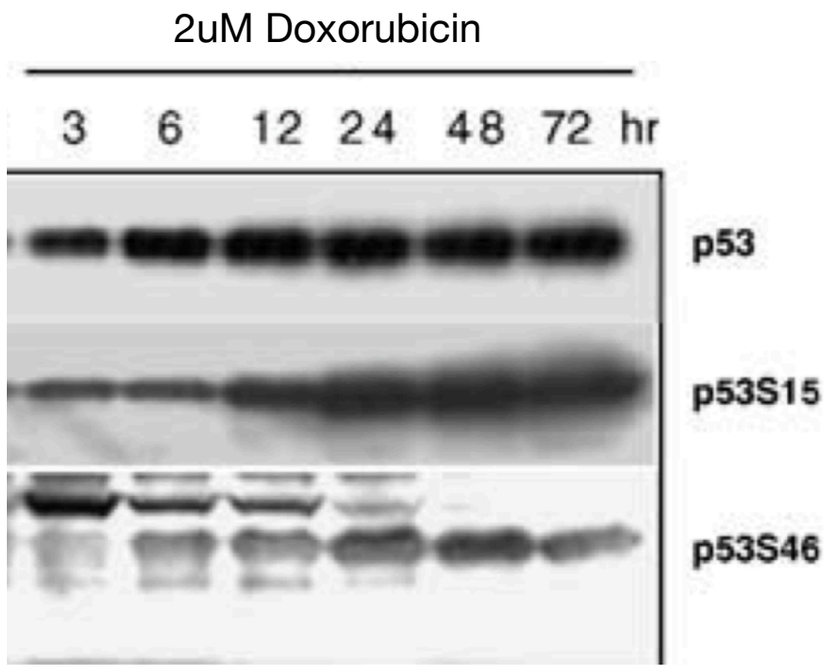

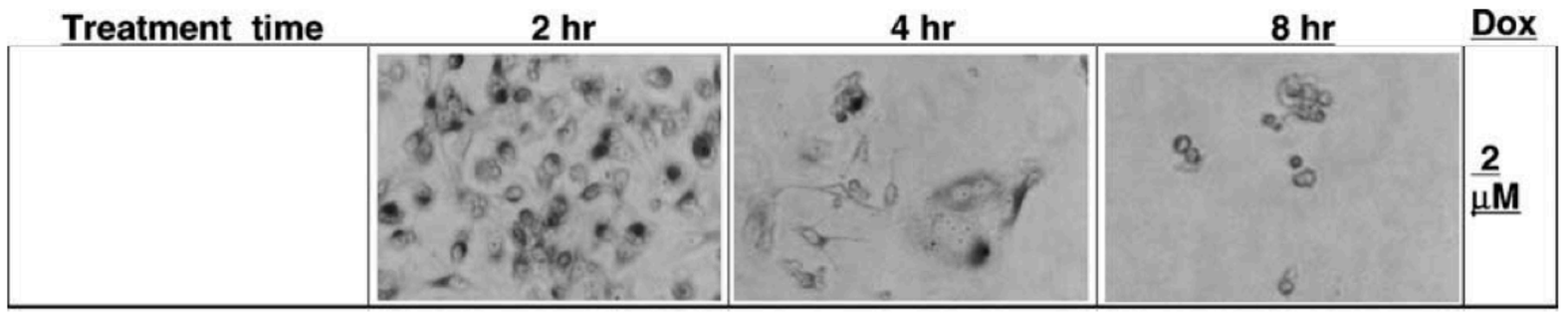

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Increased p53 and phospho-p53 levels observed in apoptotic conditions

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Recognizing DSB and other DNA damage — DNA damage response (DDR)

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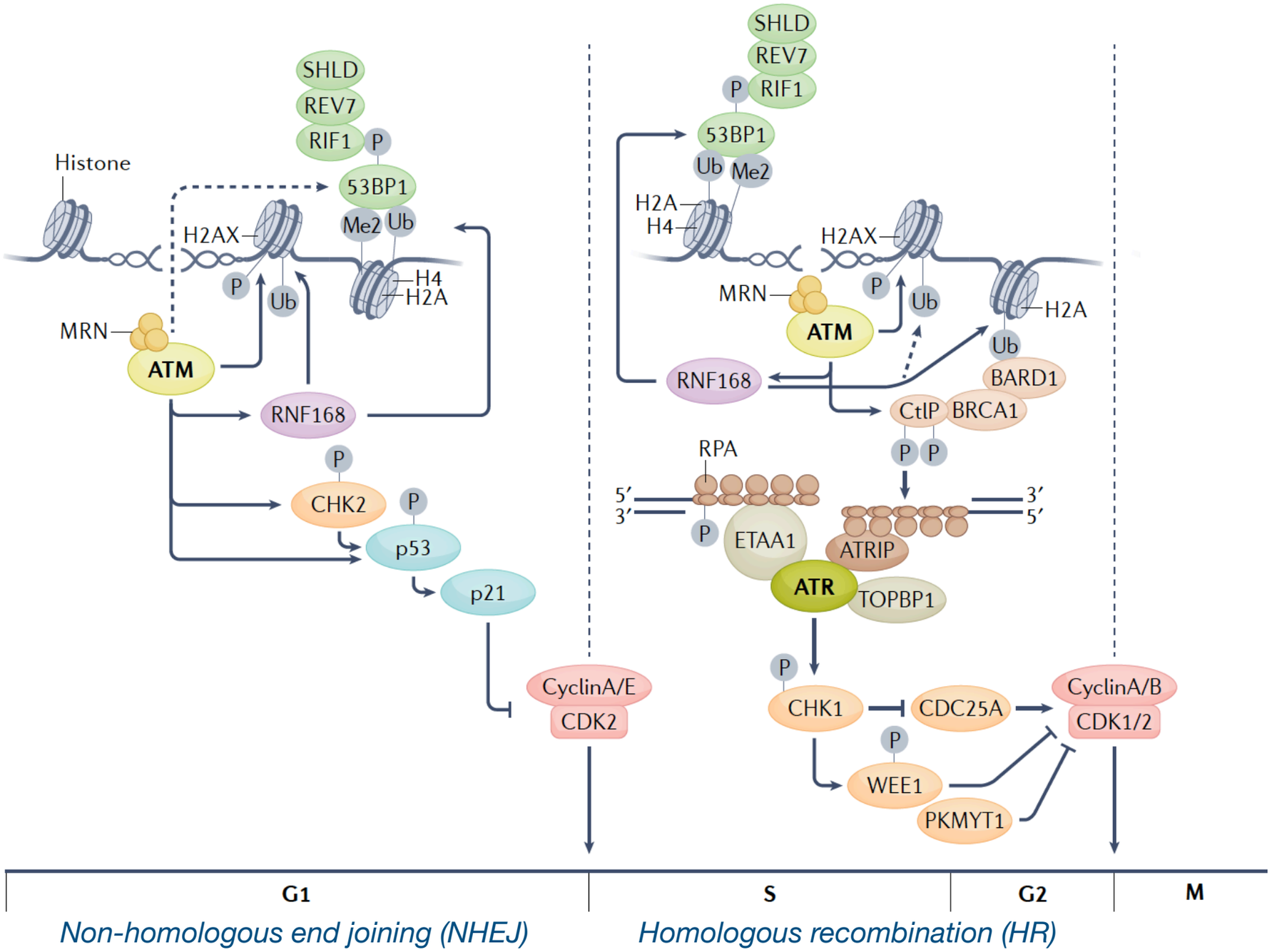

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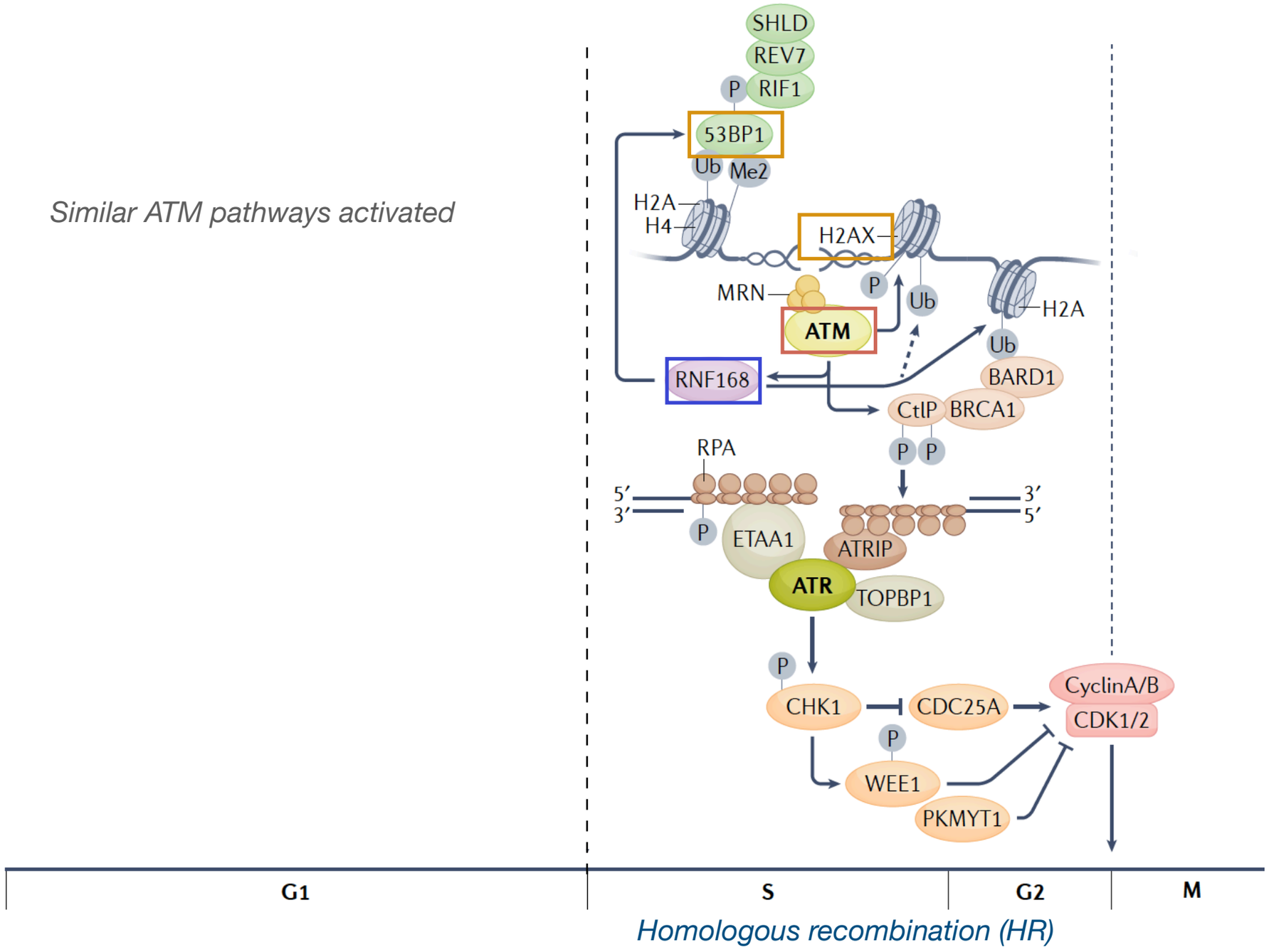
Similar ATM pathways activated



Homologous recombination (HR)

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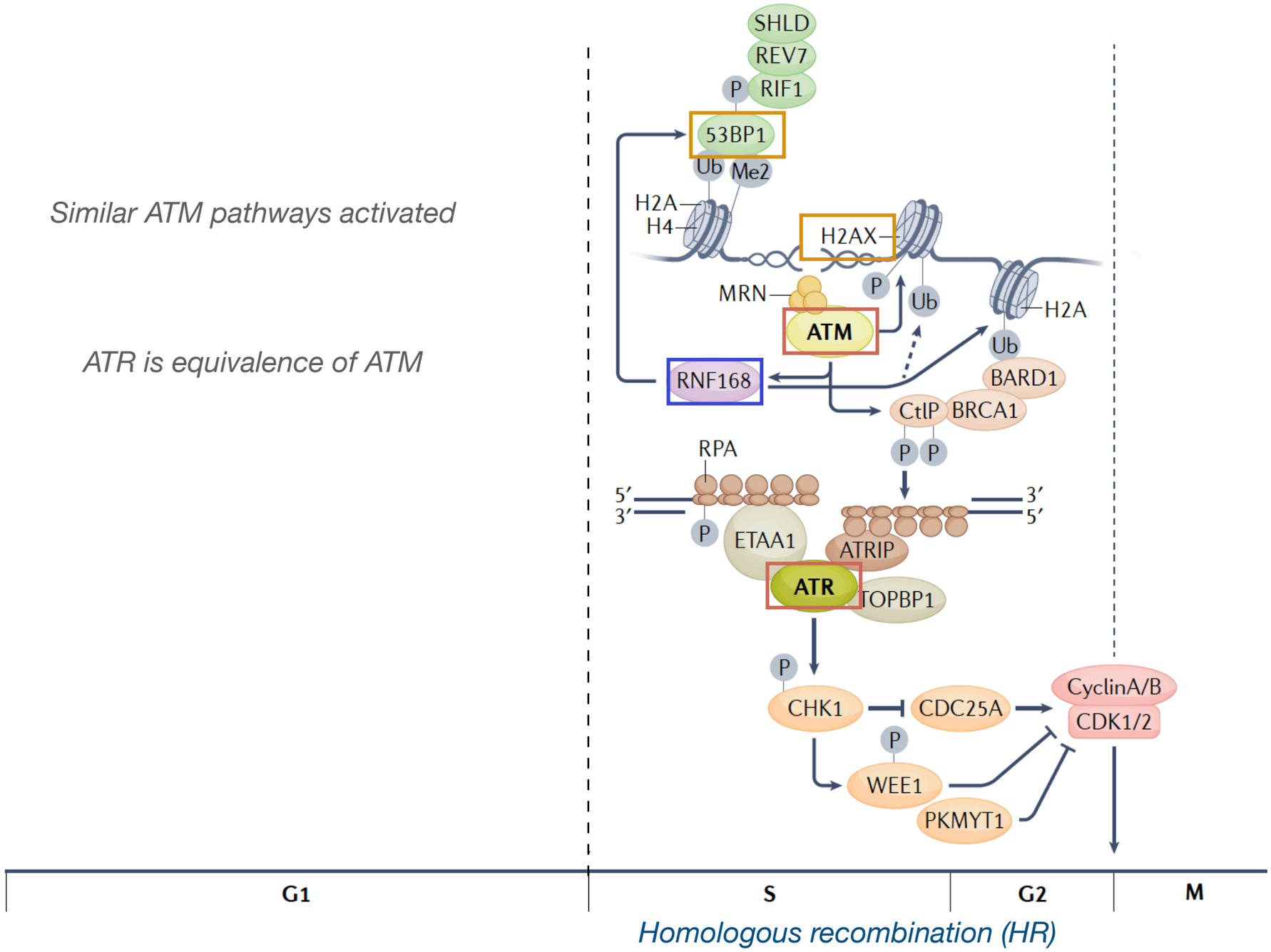
ATR is equivalence of ATM



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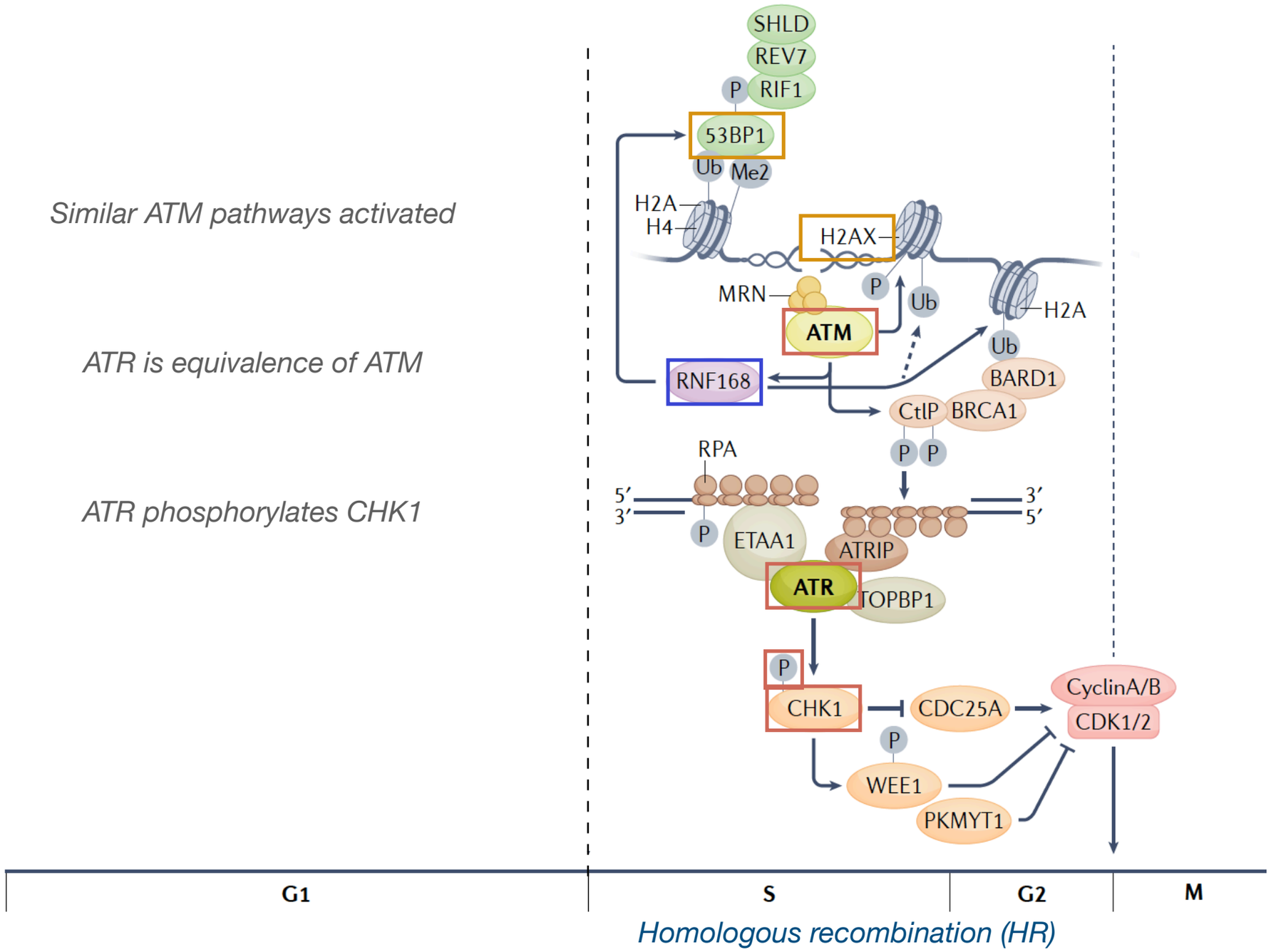
ATR phosphorylates CHK1



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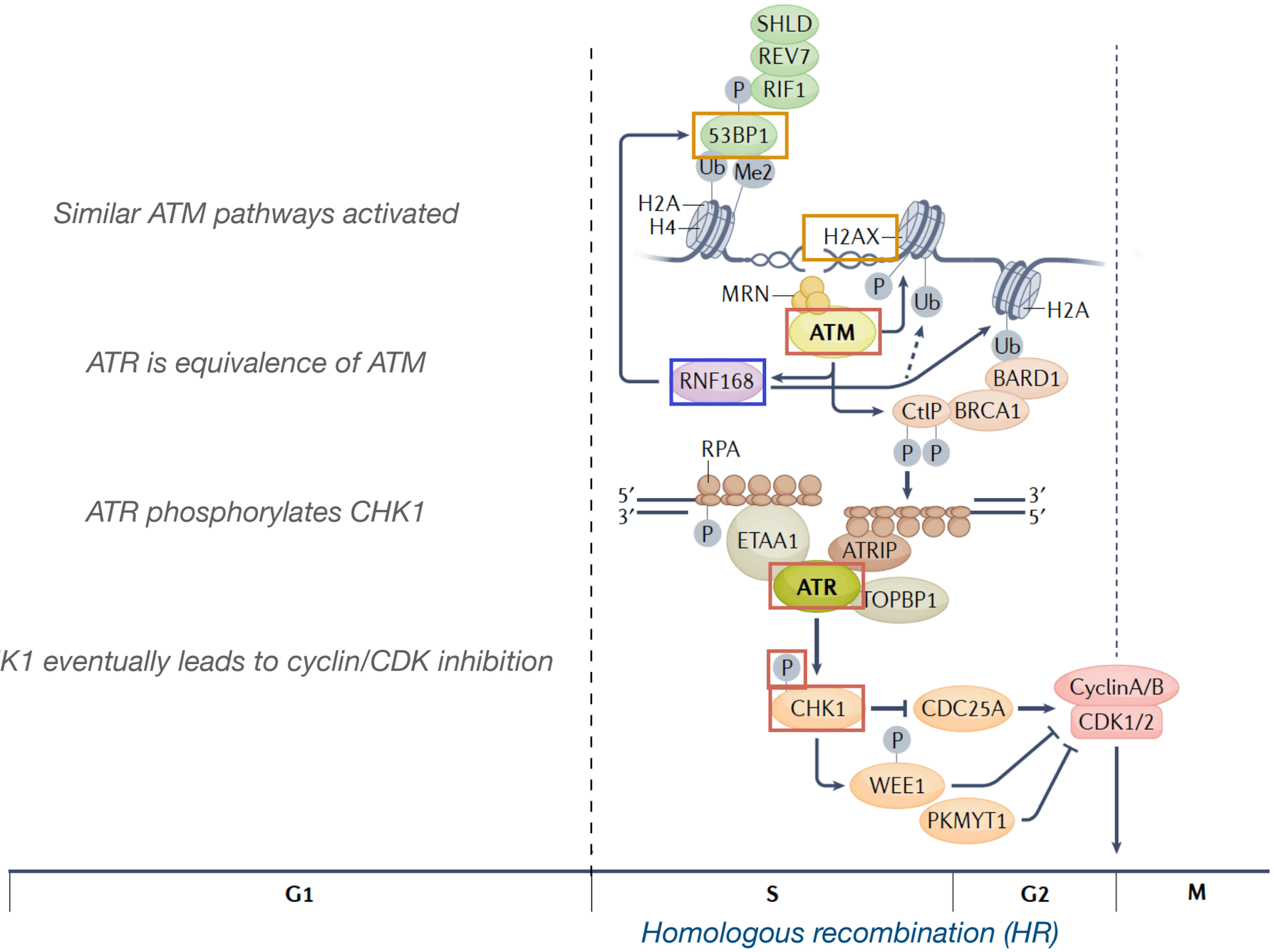
Similar ATM pathways activated

ATR is equivalence of ATM

ATR phosphorylates CHK1

CHK1 eventually leads to cyclin/CDK inhibition



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DDR and cancer

DDR dysfunction

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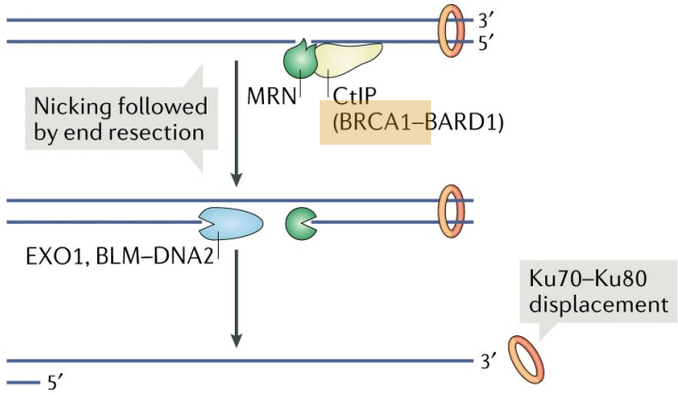
DDR and cancer

DDR dysfunction

eg. BRCA1 and breast cancer



Step 1 of HR

Scully, R. *et al*. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

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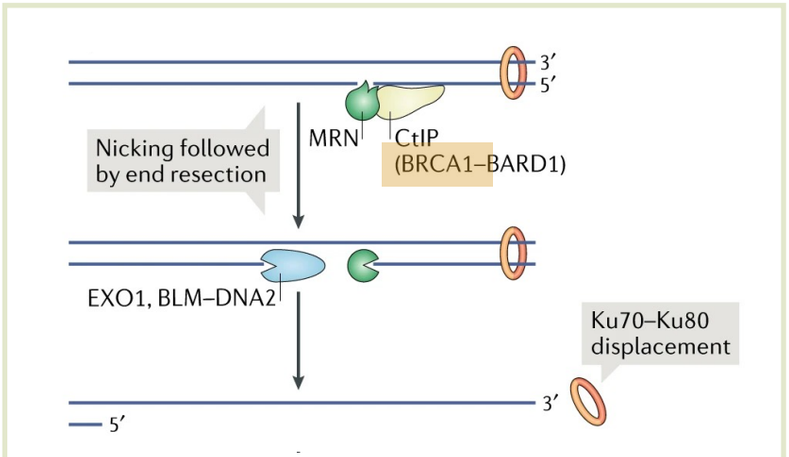


Step 1 of HR

Impaired high-fidelity DSB repair

Scully, R. *et al*. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20,* 698.

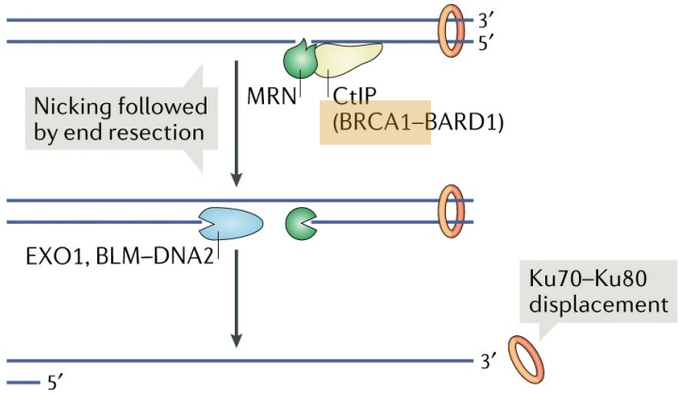
DDR and cancer

DDR dysfunction

Genomic instability

Cancer

eg. BRCA1 and breast cancer



Step 1 of HR

Impaired high-fidelity DSB repair

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Targeting DDR pathways for cancer treatment

Damage ↑

DNA damage inducing agent

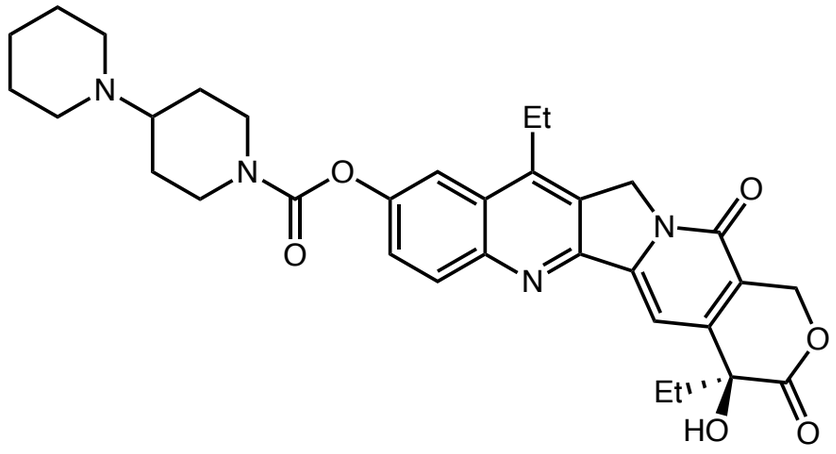
Maximize DNA lesions going into mitosis
— exceed the survival limit of cancer cell = apoptosis

Repair ↓

anti-DDR pathway

O'Connor, M. J. *Mol. Cell.* **2015**, *60,* 547.

Targeting DDR pathways for cancer treatment



Irinotecan, DNA topoisomerase I inhibitor

Maximize DNA lesions going into mitosis
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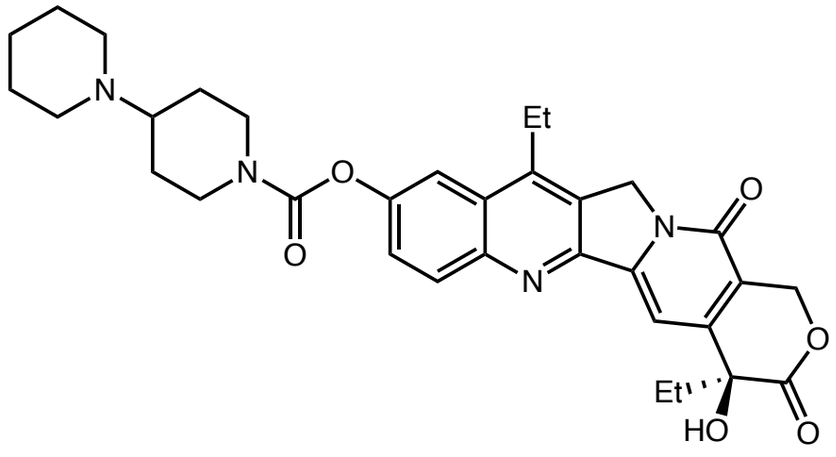

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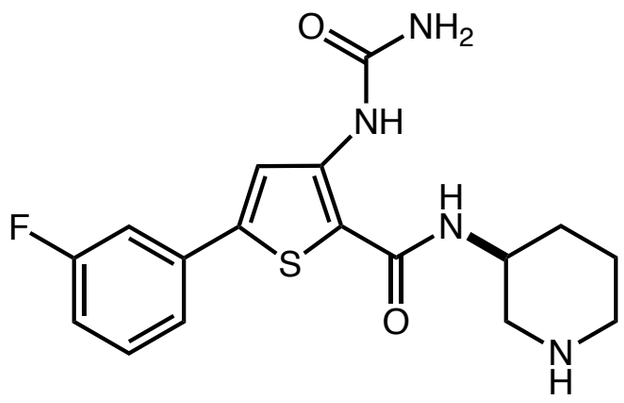

AZD7762, Chk1/2 inhibitor

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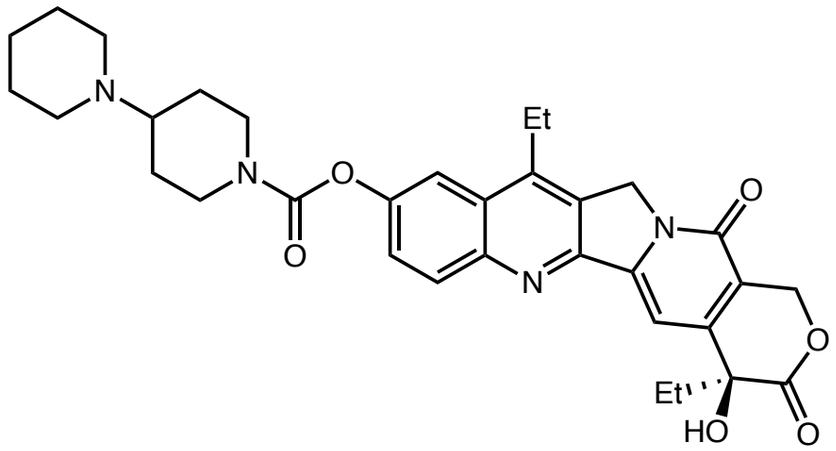

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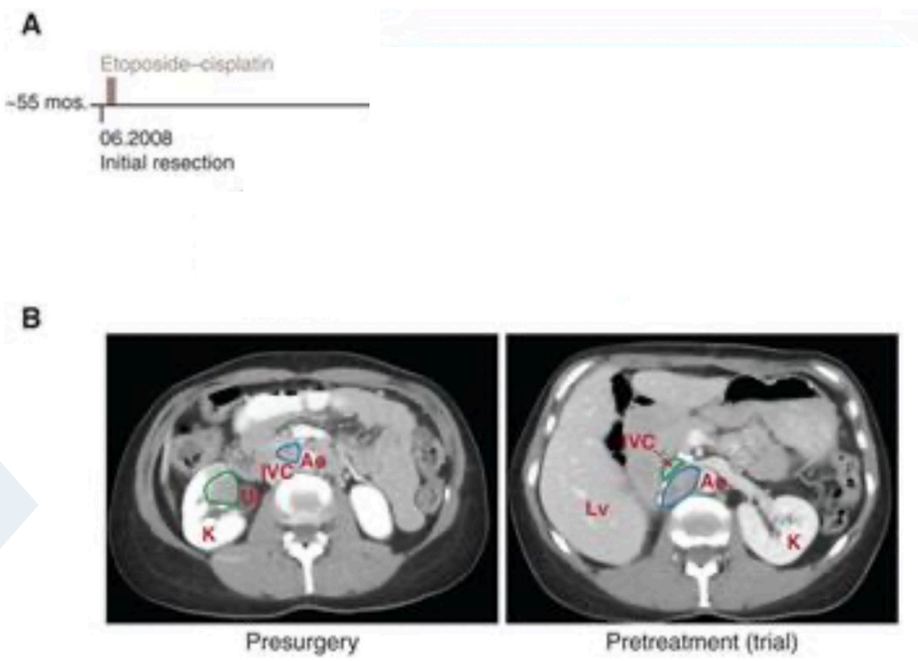
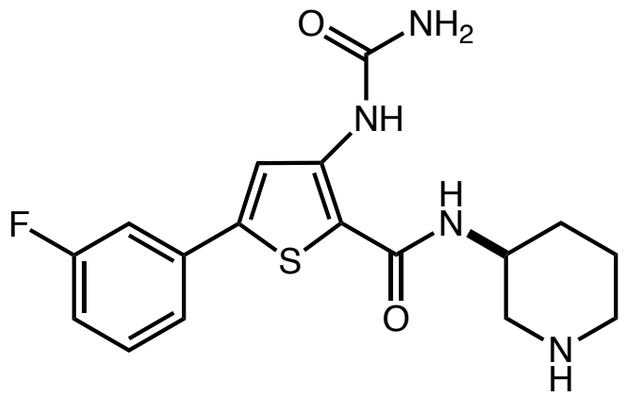


Metastatic kidney cancer

Recurred disease after Etoposide/cisplatin + surgery



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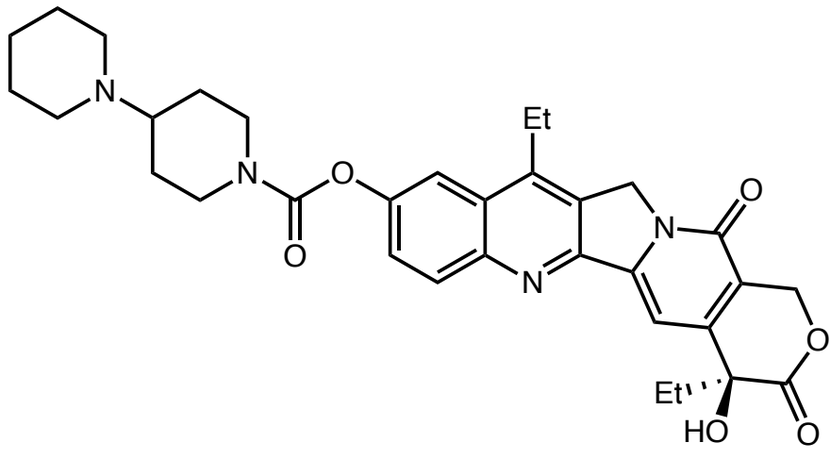

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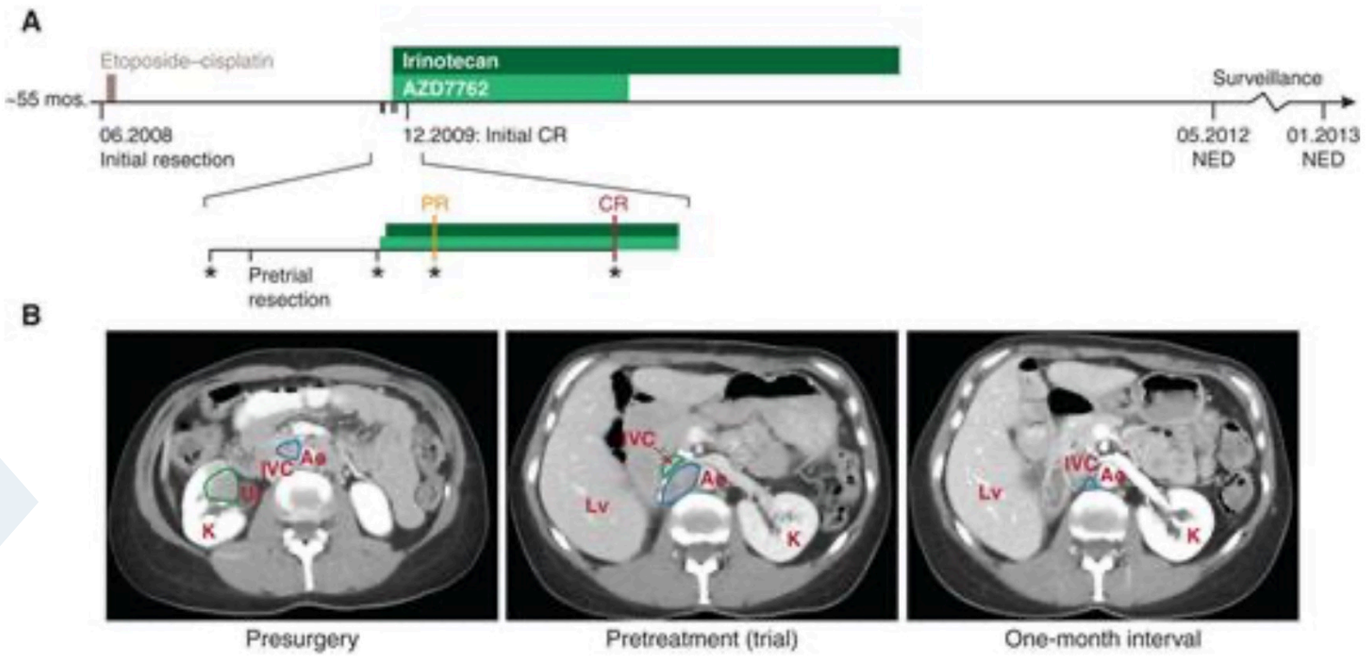

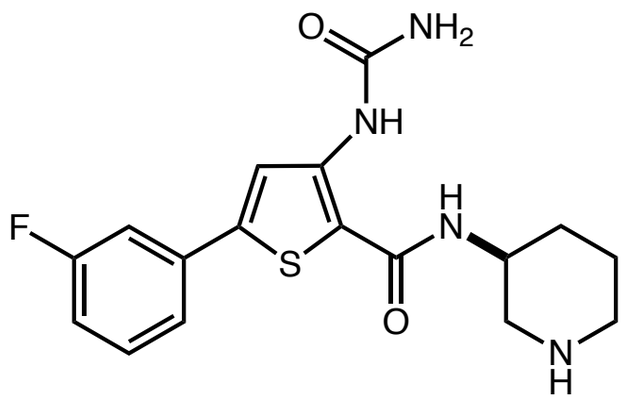

AZD7762, Chk1/2 inhibitor

Metastatic kidney cancer

Recurred disease after Etoposide/cisplatin + surgery

After combined DDR treatment, disease free for 3+ years

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Key types of stress response

Heat shock response

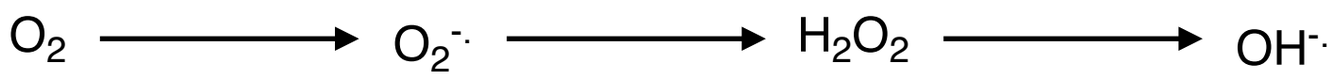
Unfolded protein response

DNA damage response

H_2O_2

Response to oxidative stress

Reactive oxygen species (ROS)



Sies, H.; Jones, D. P. *Nat. Rev. Mol. Cell. Biol.* **2020**, *21,* 363.

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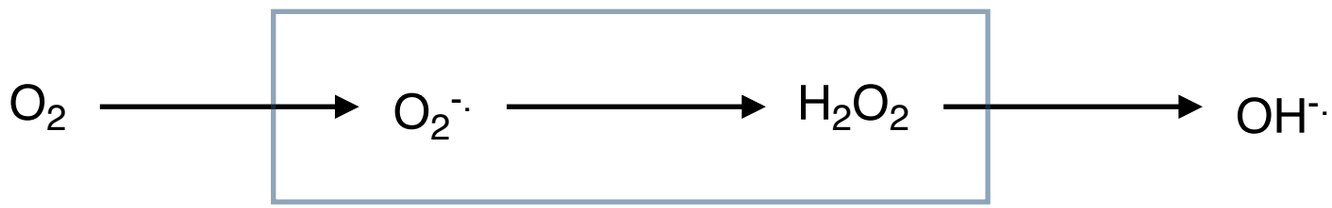

Low level reactive oxygen species are important for endogenous physiological signaling and activity

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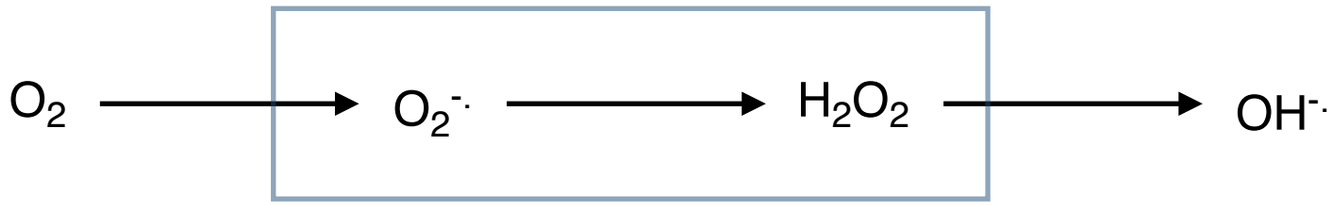

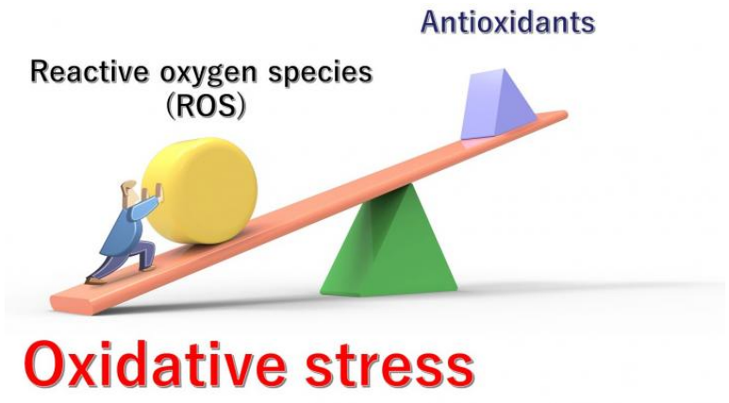

Low level reactive oxygen species are important for endogenous physiological signaling and activity

Supraphysiological concentration of ROS — reacts with DNA, proteins, lipids, etc.

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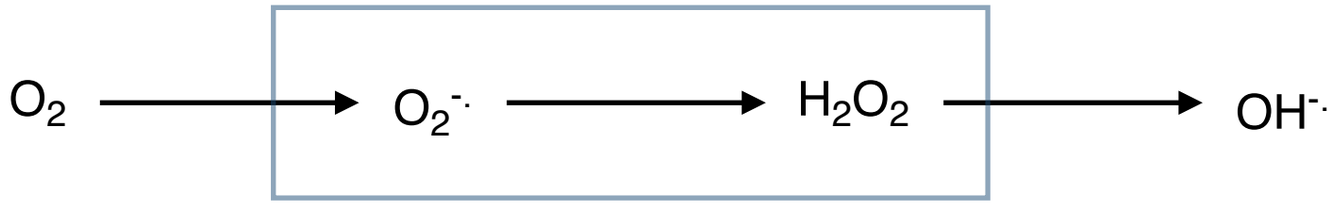
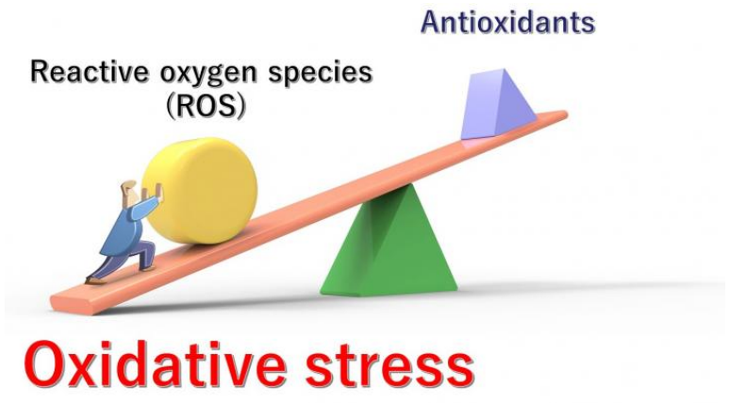
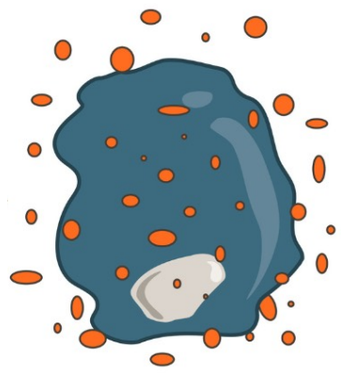






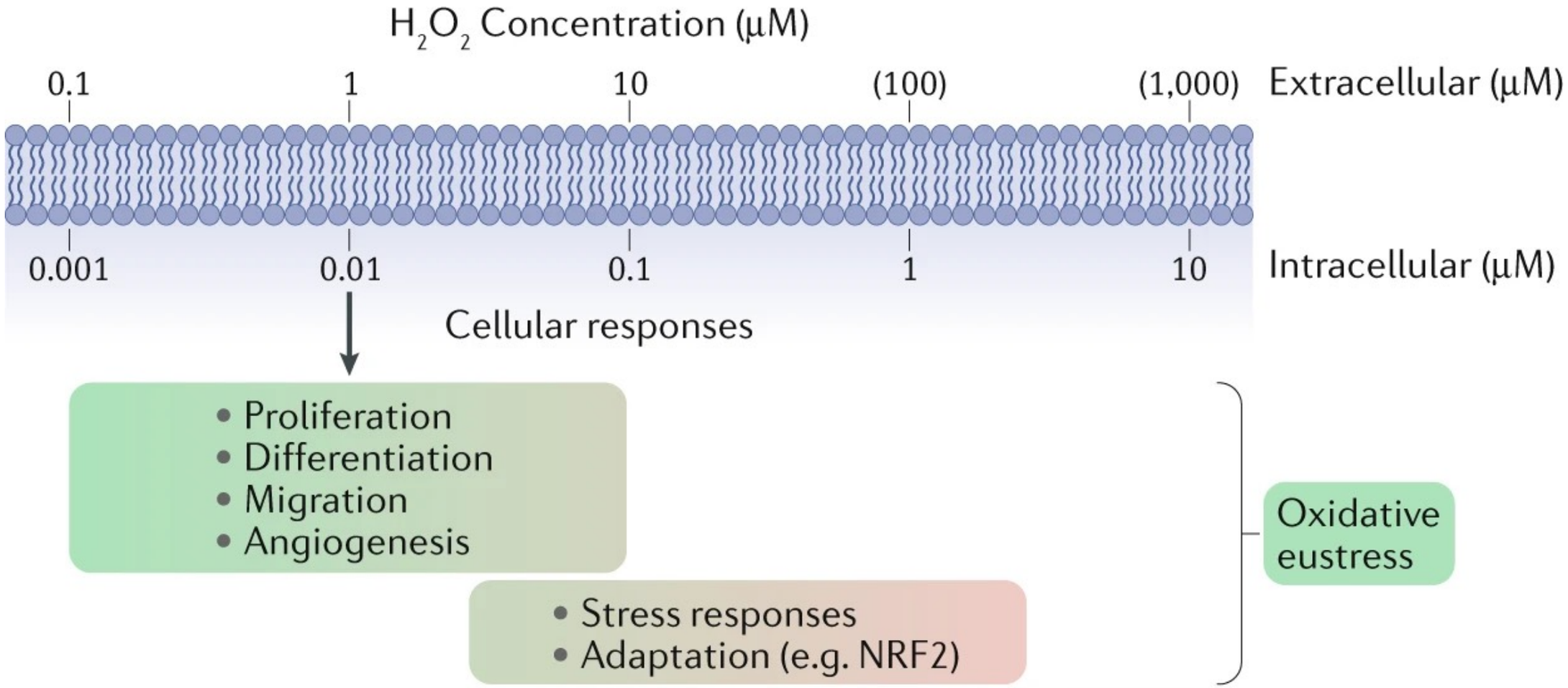
Low level reactive oxygen species are important for endogenous physiological signaling and activity

Supraphysiological concentration of ROS — reacts with DNA, proteins, lipids, etc.

Chronically oxidized cellular environment commonly associated with tumor, neurodegenerative diseases, aging, etc.

Sies, H.; Jones, D. P. *Nat. Rev. Mol. Cell. Biol.* **2020**, *21,* 363.

H_2O_2



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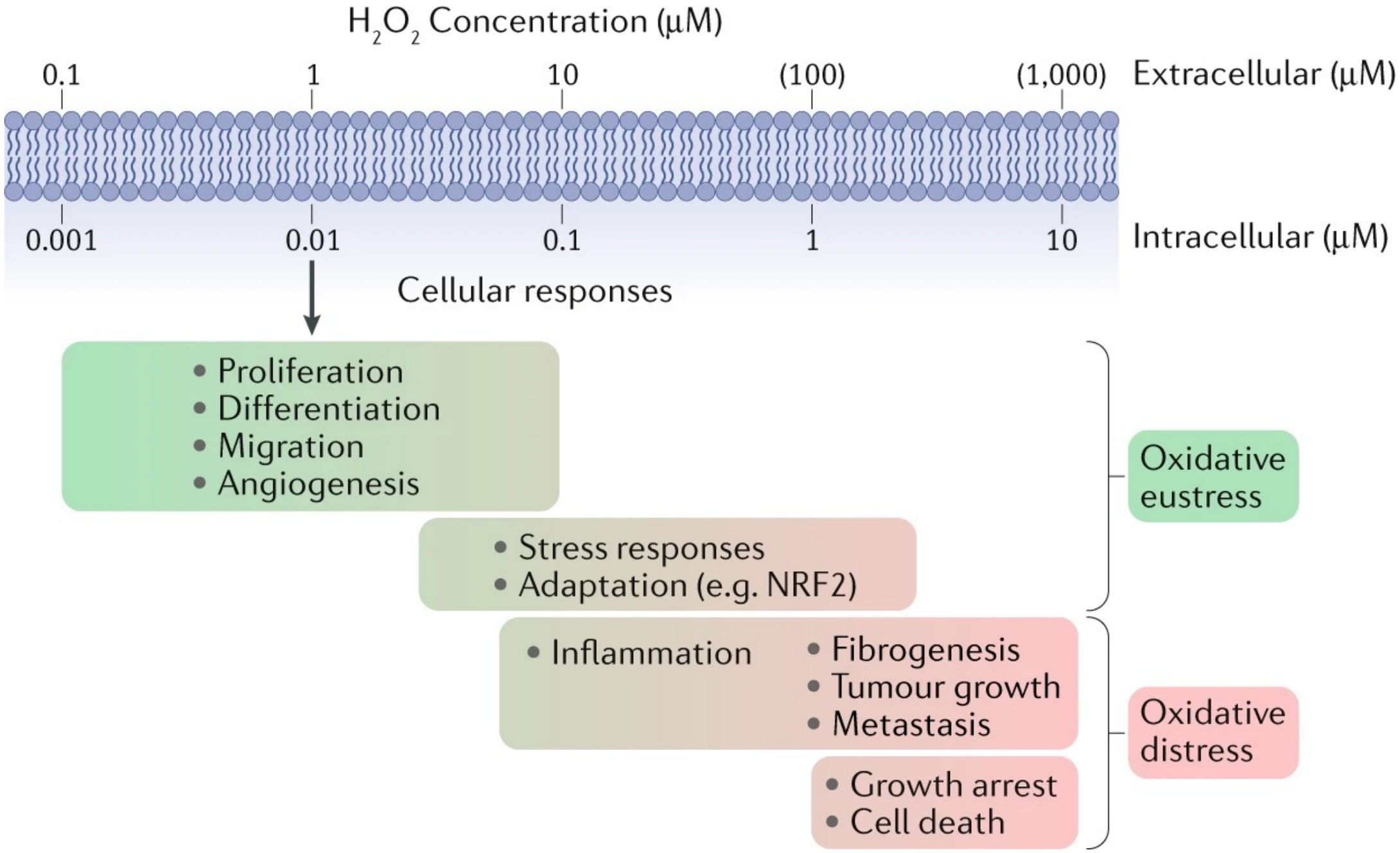

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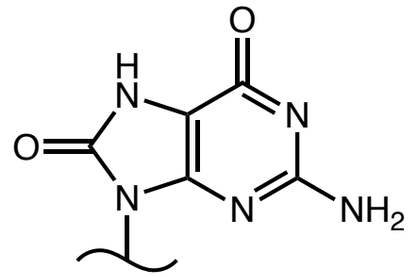
Examples of oxidative damage on macromolecules

DNA

DNA damage response (DDR)



8-oxoG formation

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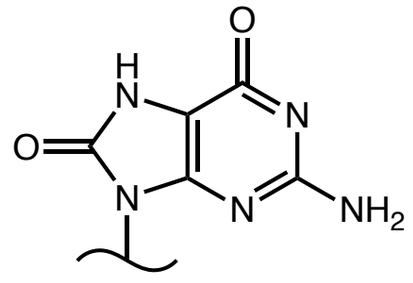
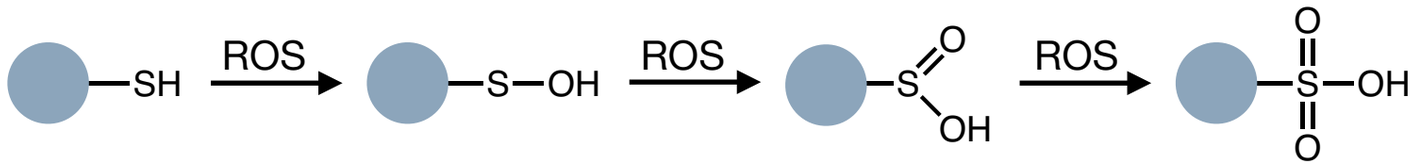


8-oxoG formation

Proteins → *Unfolded protein response (UPR)*

Cys oxidation



reversible *irreversible*

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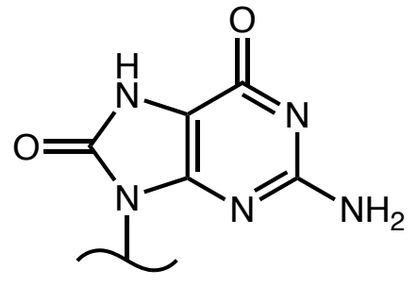

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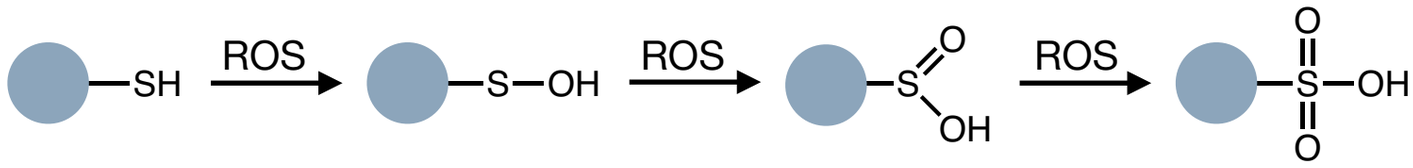
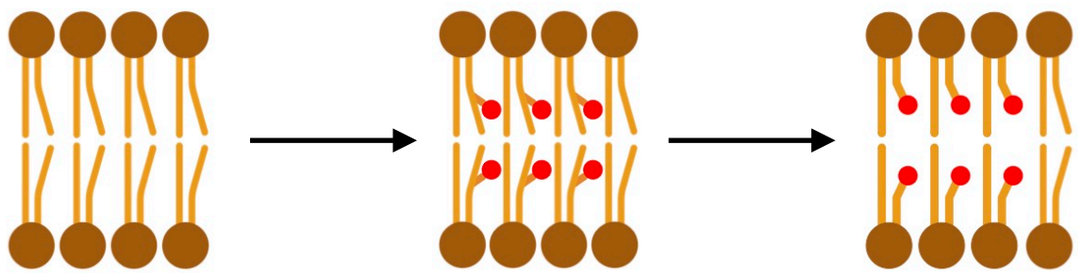


reversible *irreversible*

Lipids → *Ferroptosis*

Lipid peroxidation



oxidized lipids truncated lipids

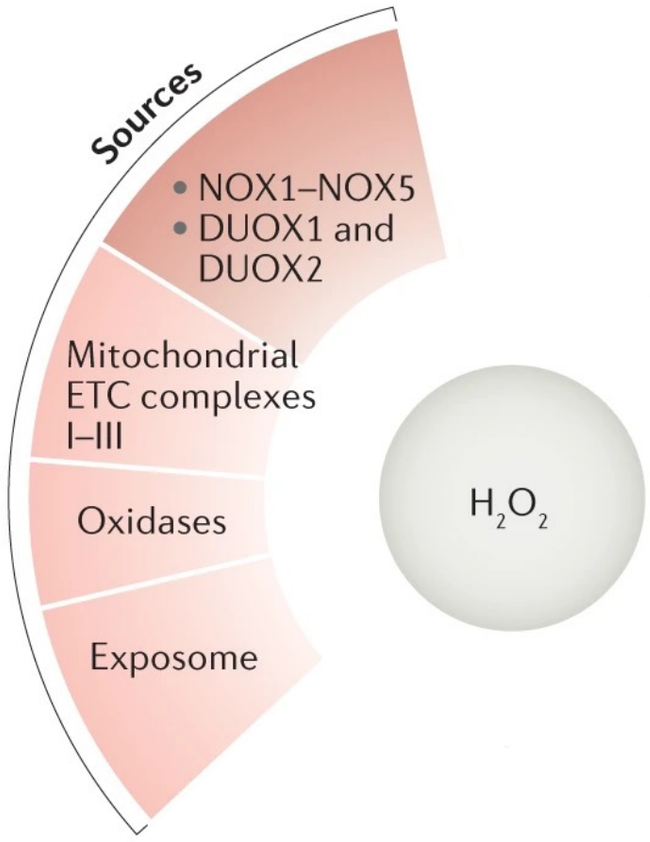
ROS generation and usage

NOX = NADPH oxidases

ETC = electron transport chain

Exposome = environmental exposure

Other locations include:
ER lumen
Peroxisomes
etc.



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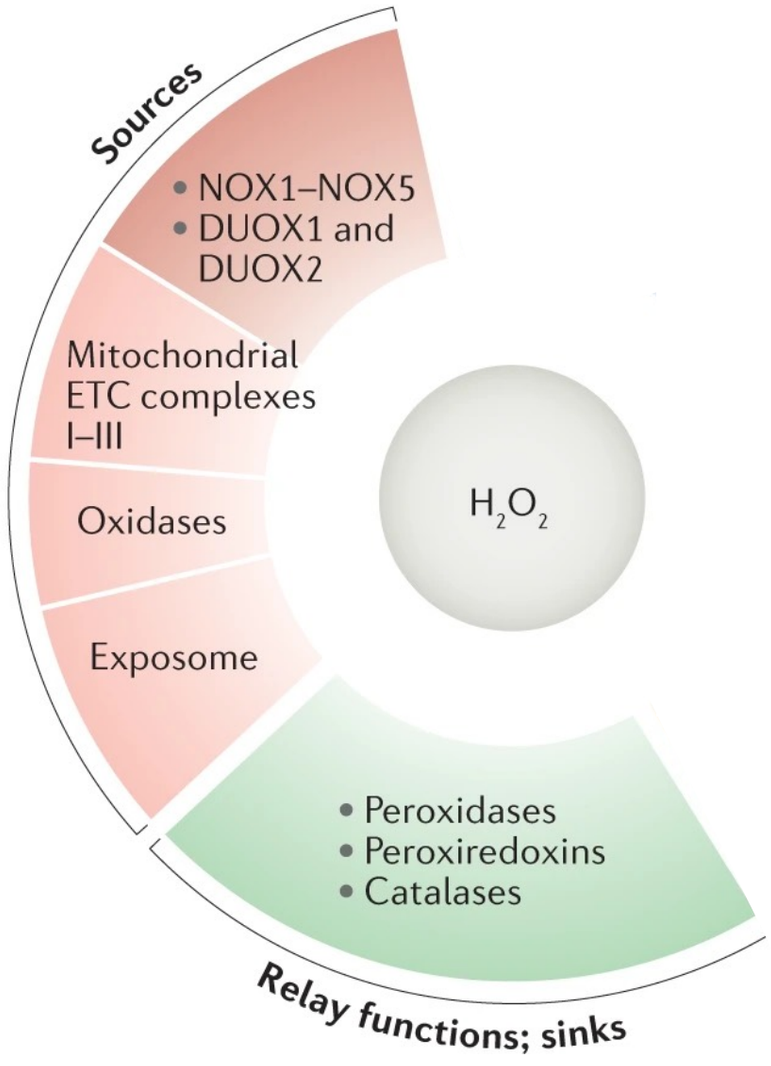

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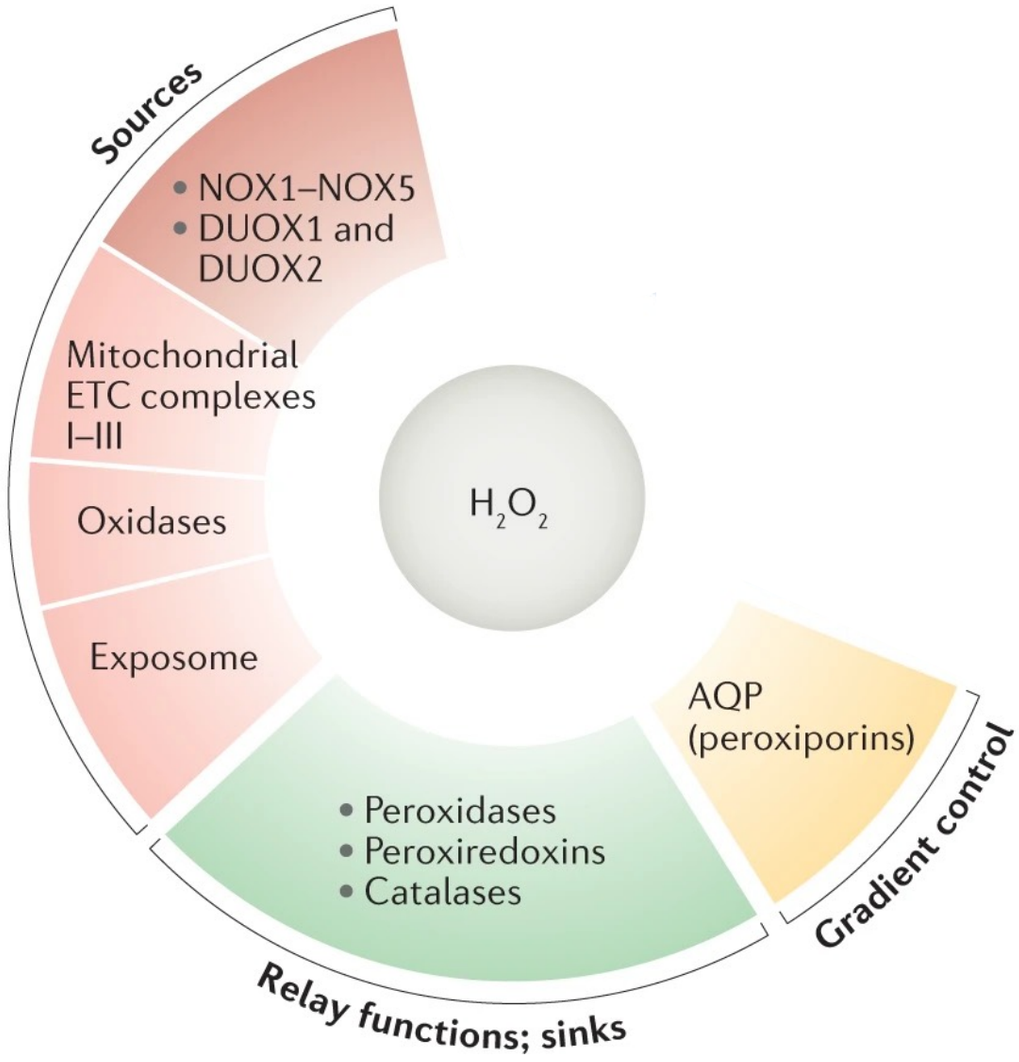

Peroxiporin = Aquaporins (AQP) that allows H_2O_2 transport

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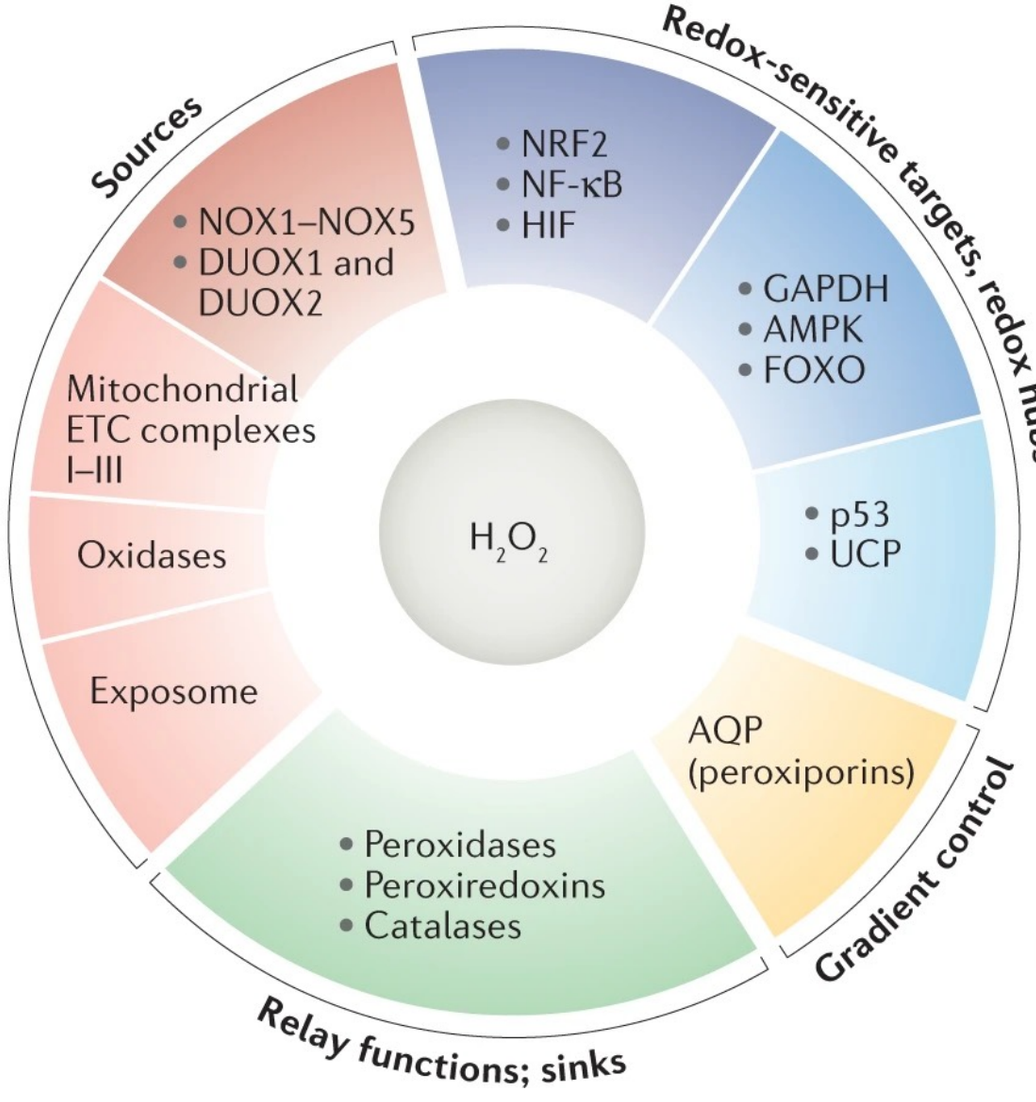

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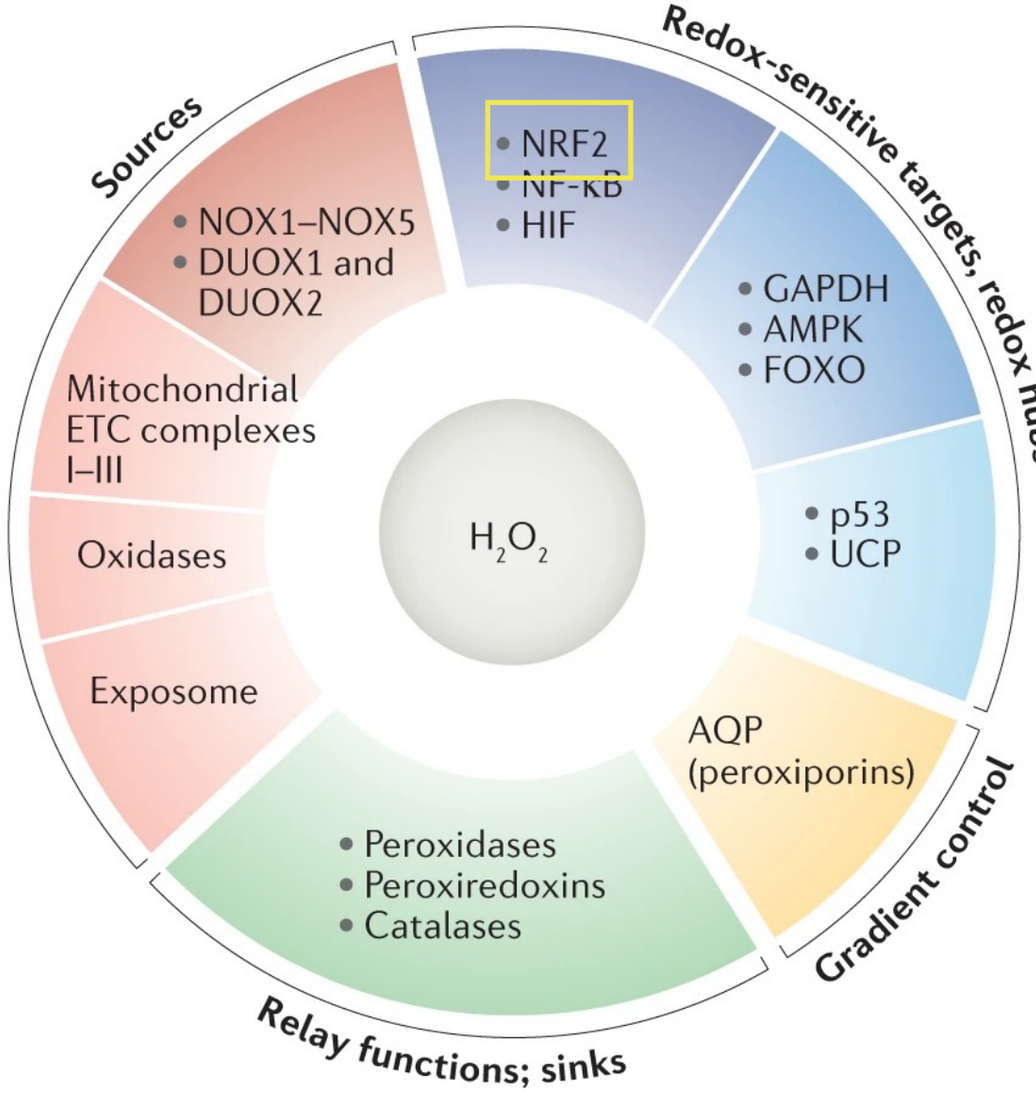

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NRF2(NFE2L2) acts as an oxidative stress sensor through KEAP1

Baird, L.; Yamamoto, M. *Mol. Cell. Biol.* **2020**, *40,* e0099.

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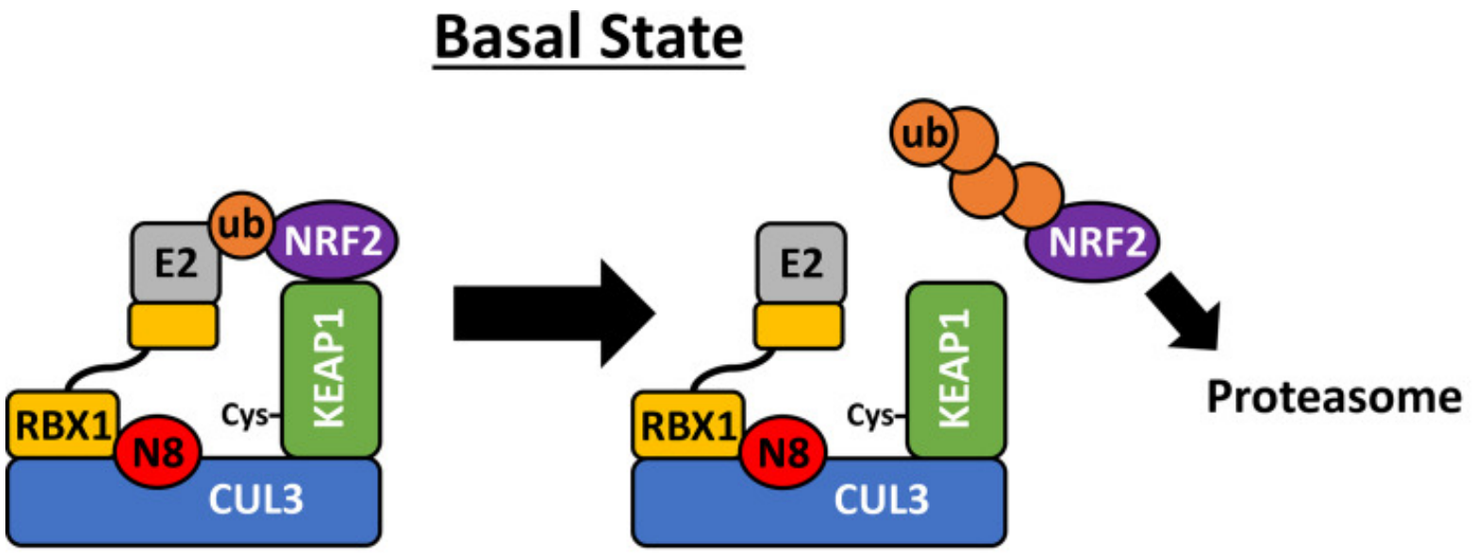
KEAP1 is an E3 ligase that negatively regulates NRF2 under normal conditions



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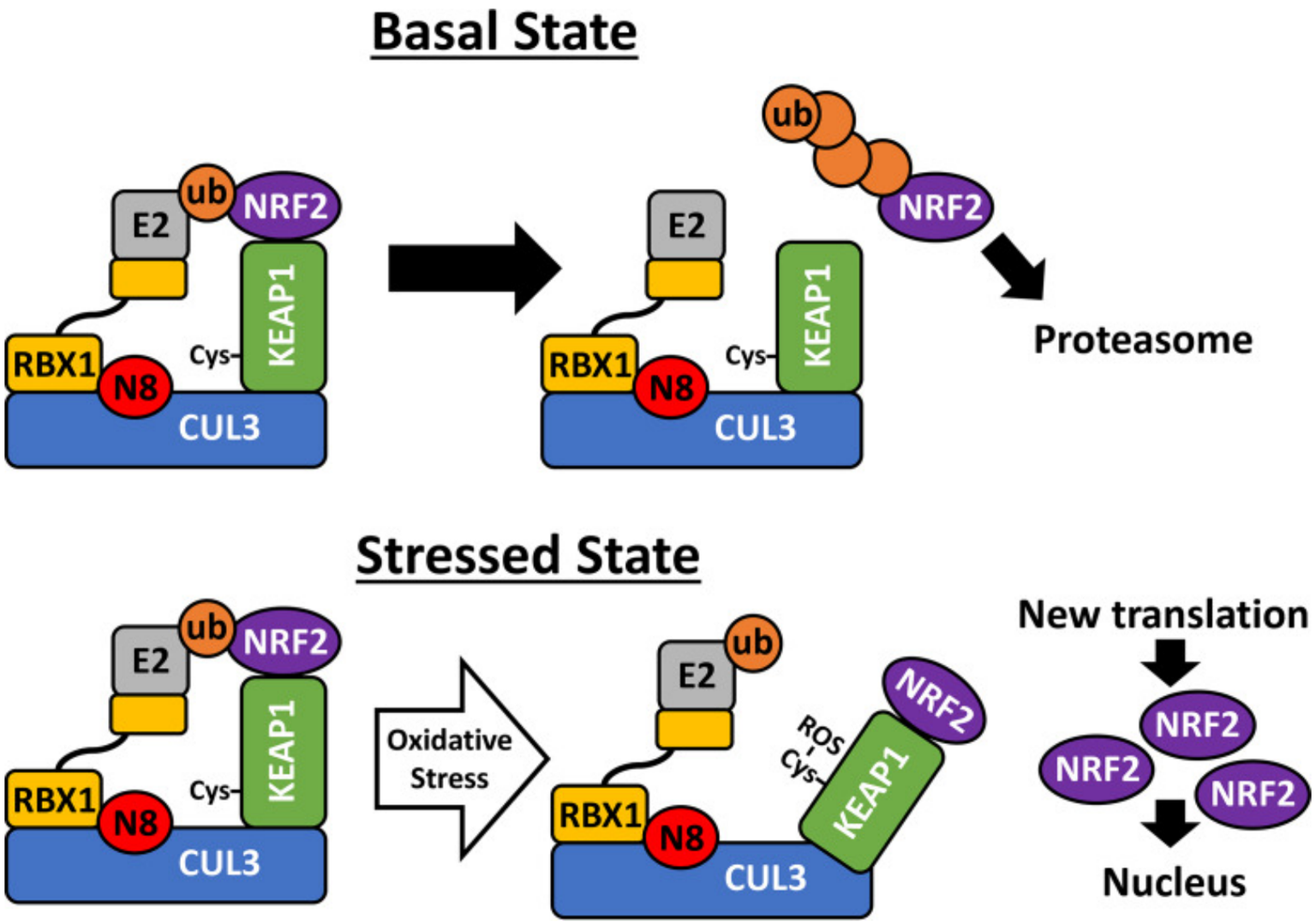
NRF2(NFE2L2) acts as an oxidative stress sensor through KEAP1

KEAP1 is an E3 ligase that negatively regulates NRF2 under normal conditions

KEAP1 has more than 20 Cys residues to "sense" oxidation



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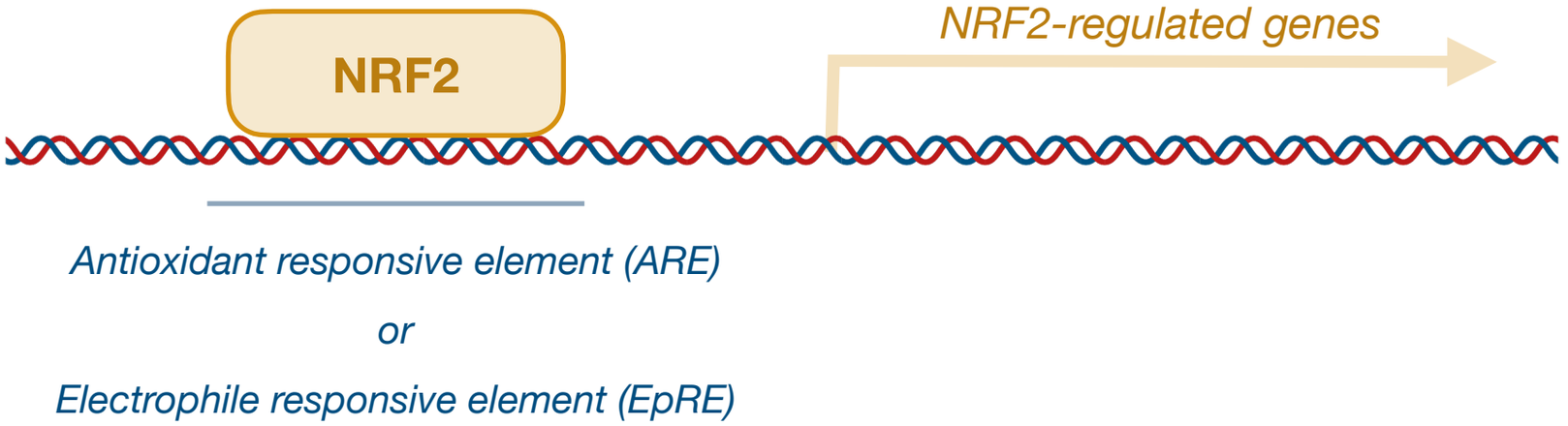
NRF2 effector function as a transcription factor



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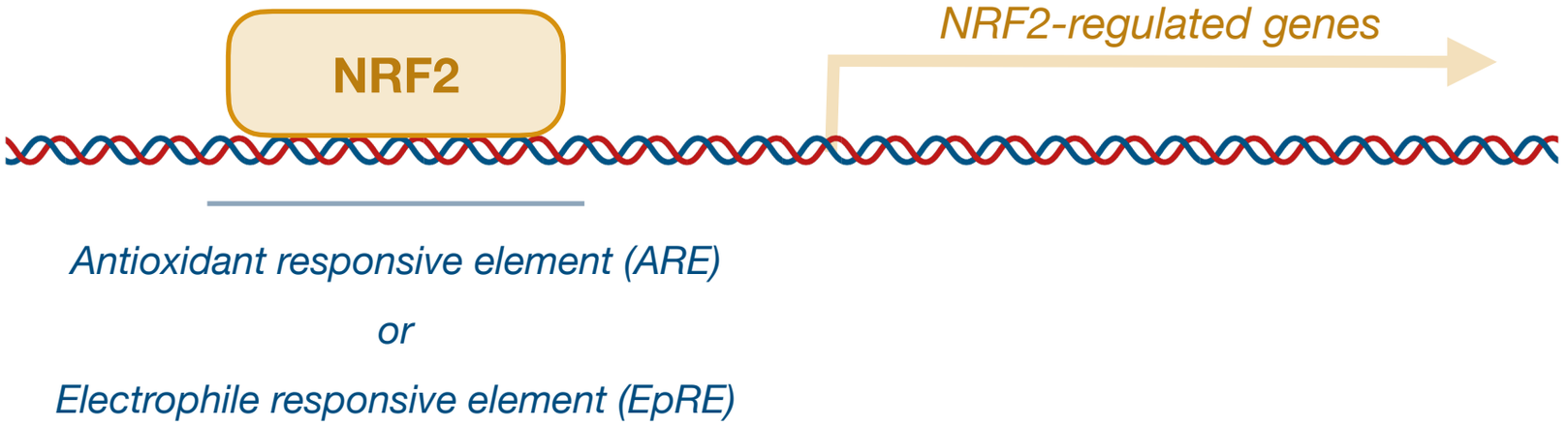

Purpose

short-term oxidative stress release, eg. catalase to destroy H2O2

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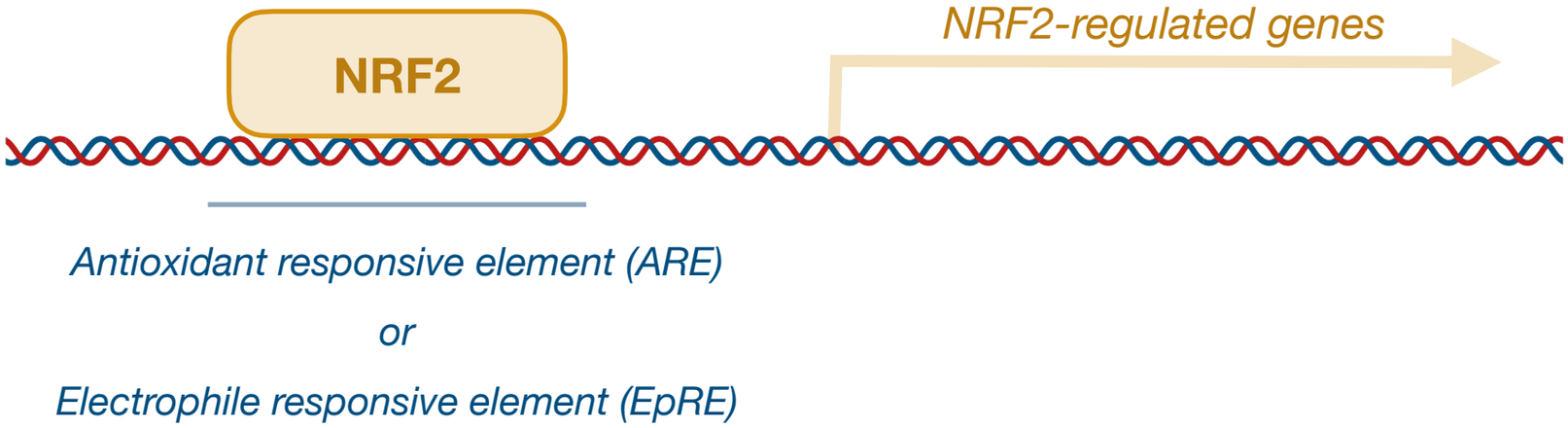

Antioxidant responsive element (ARE)

or

Electrophile responsive element (EpRE)

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Work with NF-κB, mTOR, p53, HSP, AP-1, and more for long-term cytoprotection and reprogramming

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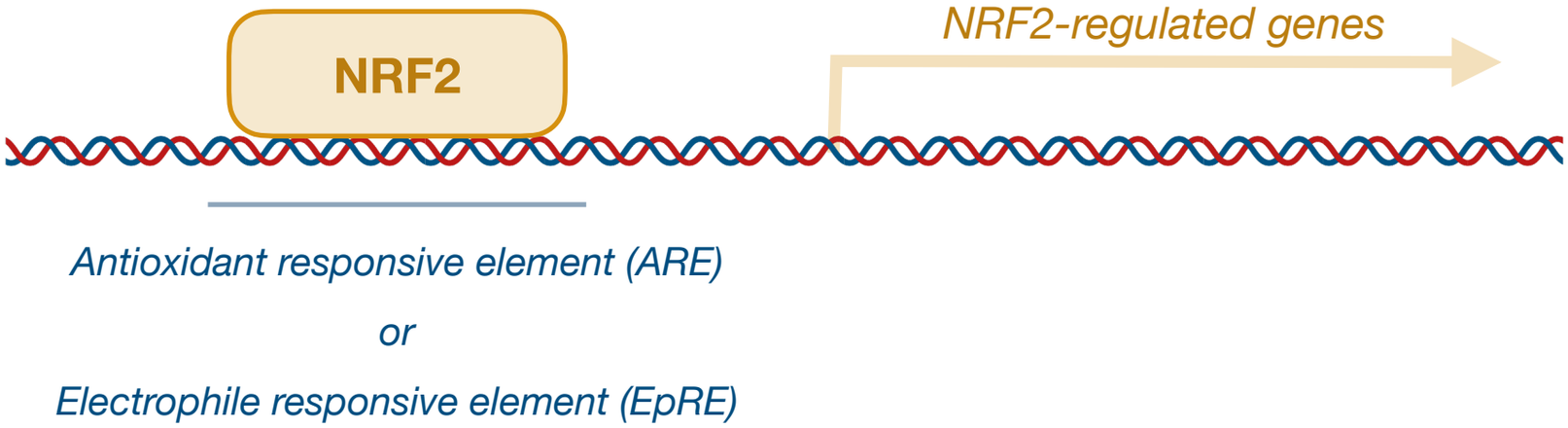

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Act as a tumor suppressor for early carcinogenesis

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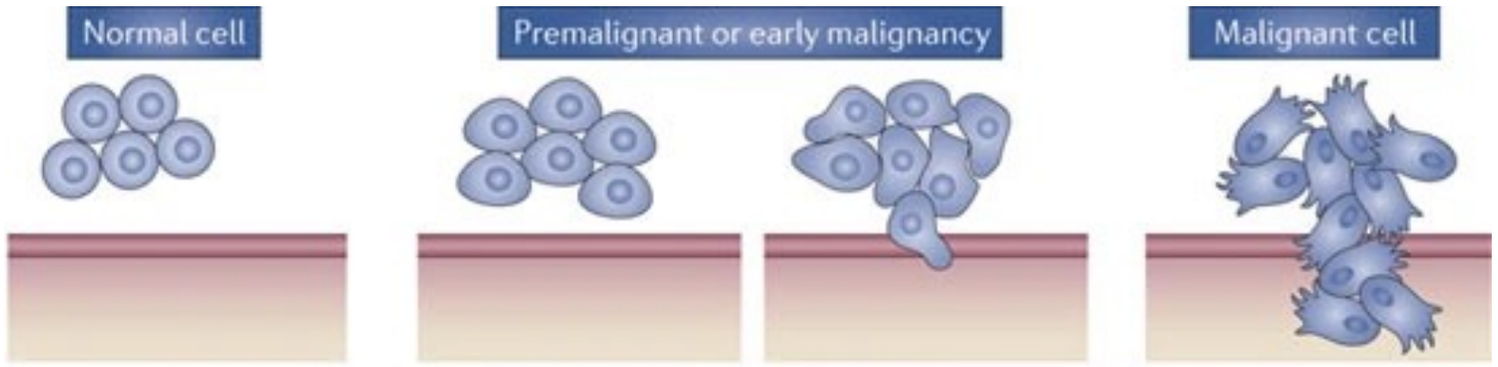
NRF2 role with cancer



NRF2 status **Turned on only upon stress**

Effect *Protect cells from oxidative damage*

Prevent cancer onset

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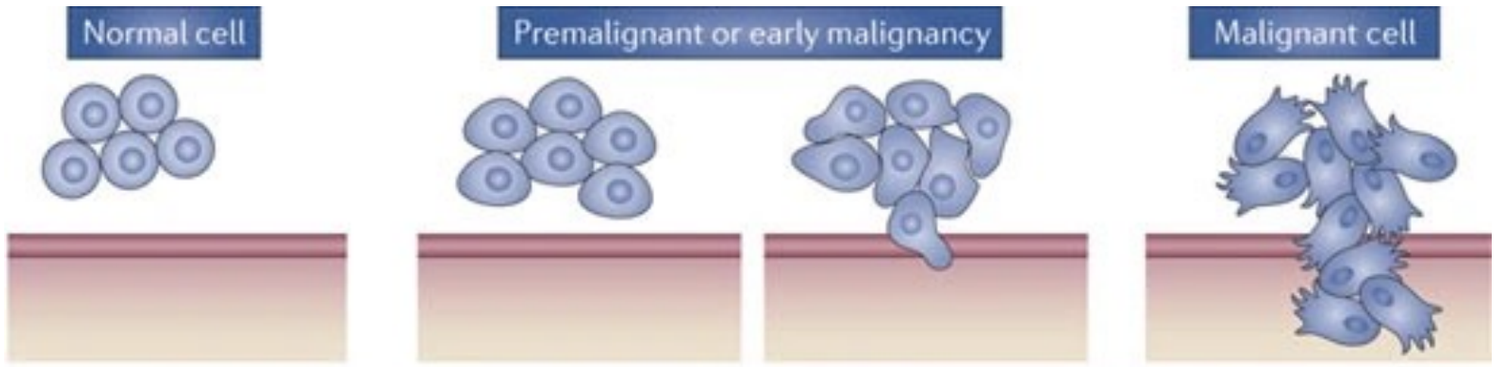
NRF2 role with cancer



Increased malignancy

NRF2 status	**Turned on only upon stress**	**Constitutively on with mutations**
Effect	*Protect cells from oxidative damage* *Prevent cancer onset*	*Protective against endogenous high level of ROS* *Increase resistance and survival of cancer cells — poor patient outcomes*

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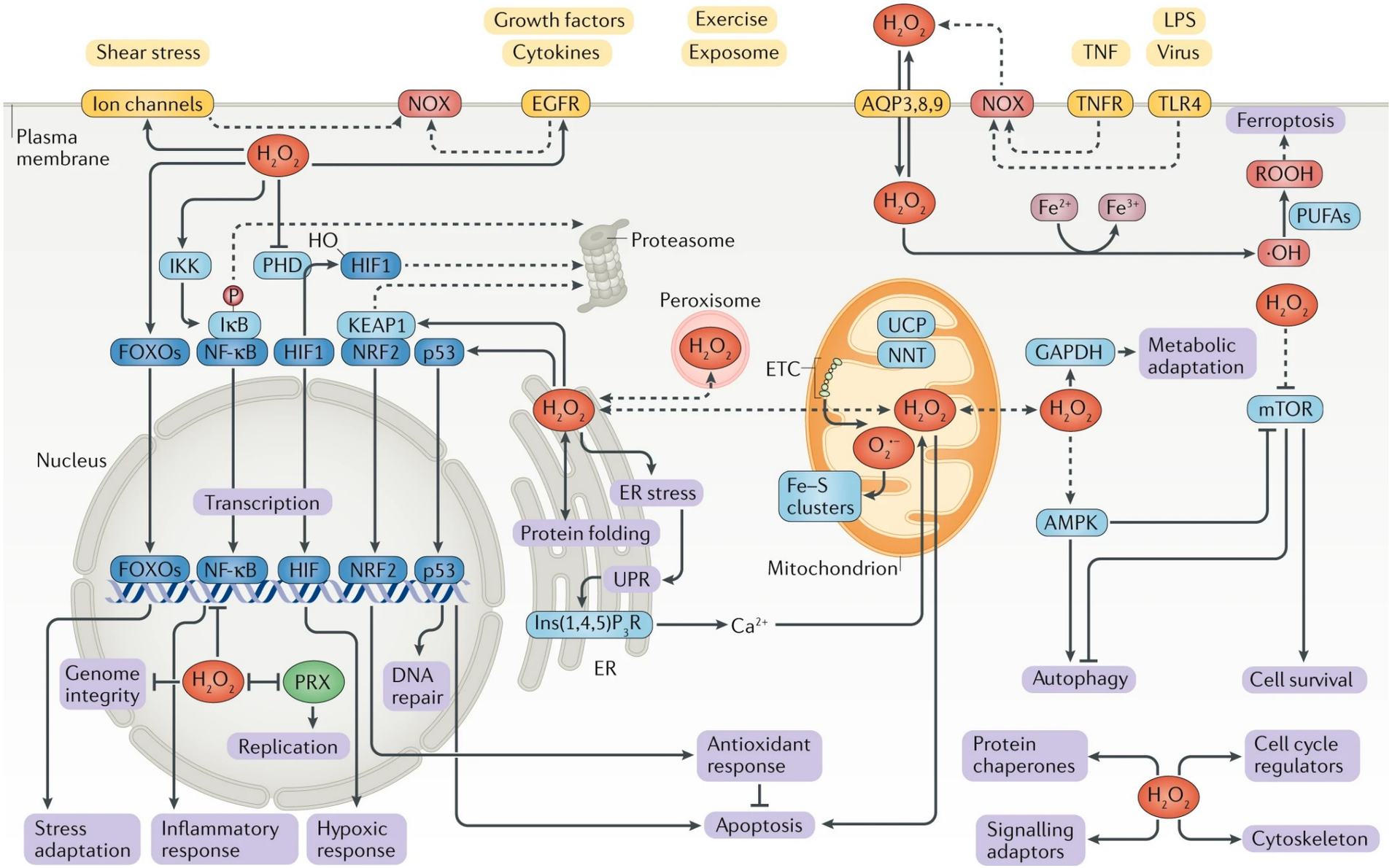
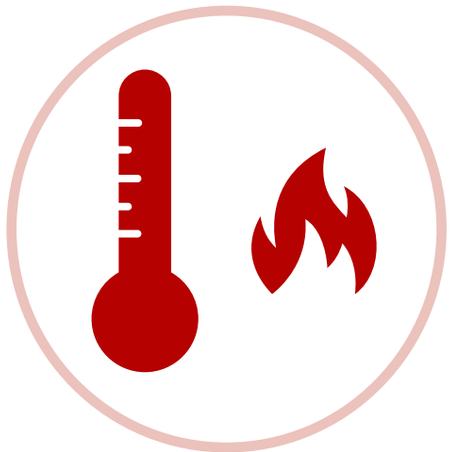
Pleiotropic response of ROS signaling



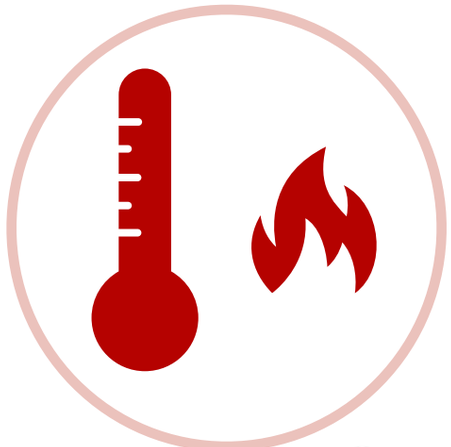
ROS physiological and pathological signaling are very complicated

Sies, H.; Jones, D. P. *Nat. Rev. Mol. Cell. Biol.* **2020**, *21,* 363.

Conclusion



Our cells are trying very hard to maintain cellular and organismal homeostasis



Questions?

H_2O_2