Integrating Genome Maintenance with Cell Cycle Progression via the E3 Ubiquitin Ligase Rad18

Cyrus Vaziri, Department of Pathology, UNC

1. Background: Responses to DNA Replication Fork Stalling

    (i) S-phase checkpoints
    (ii) Trans-Lesion Synthesis (TLS)

2. Regulation of TLS by Rad18 Phosphorylation

3. Replication Fork-Independent roles of Rad18 in DNA repair

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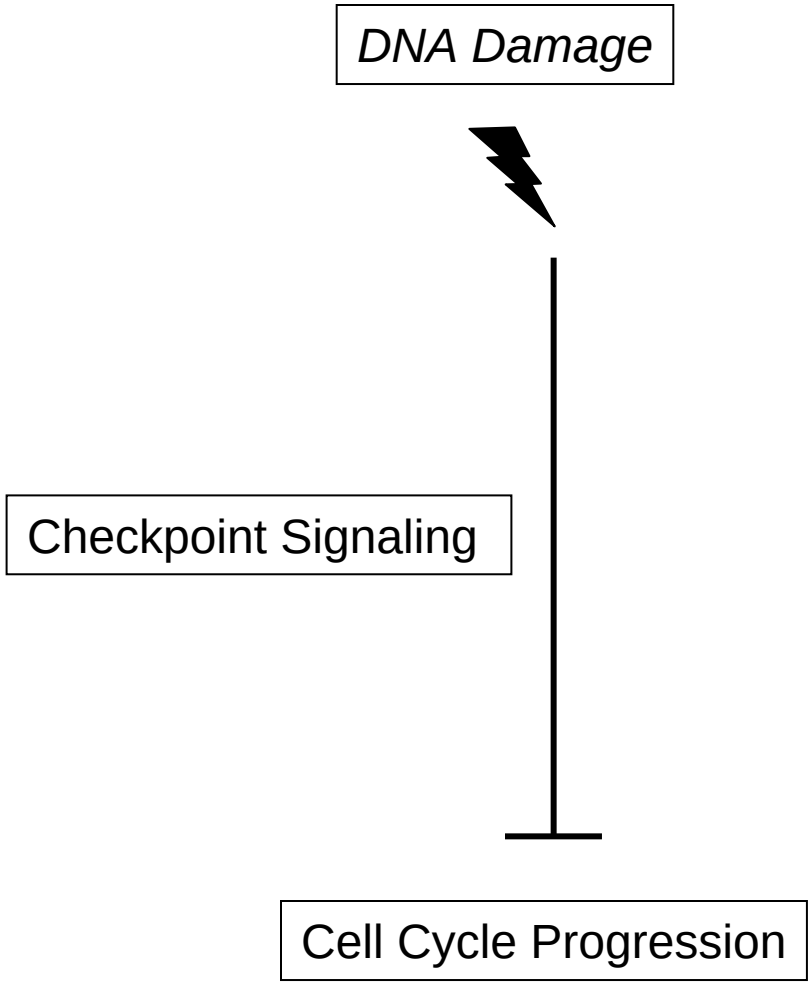
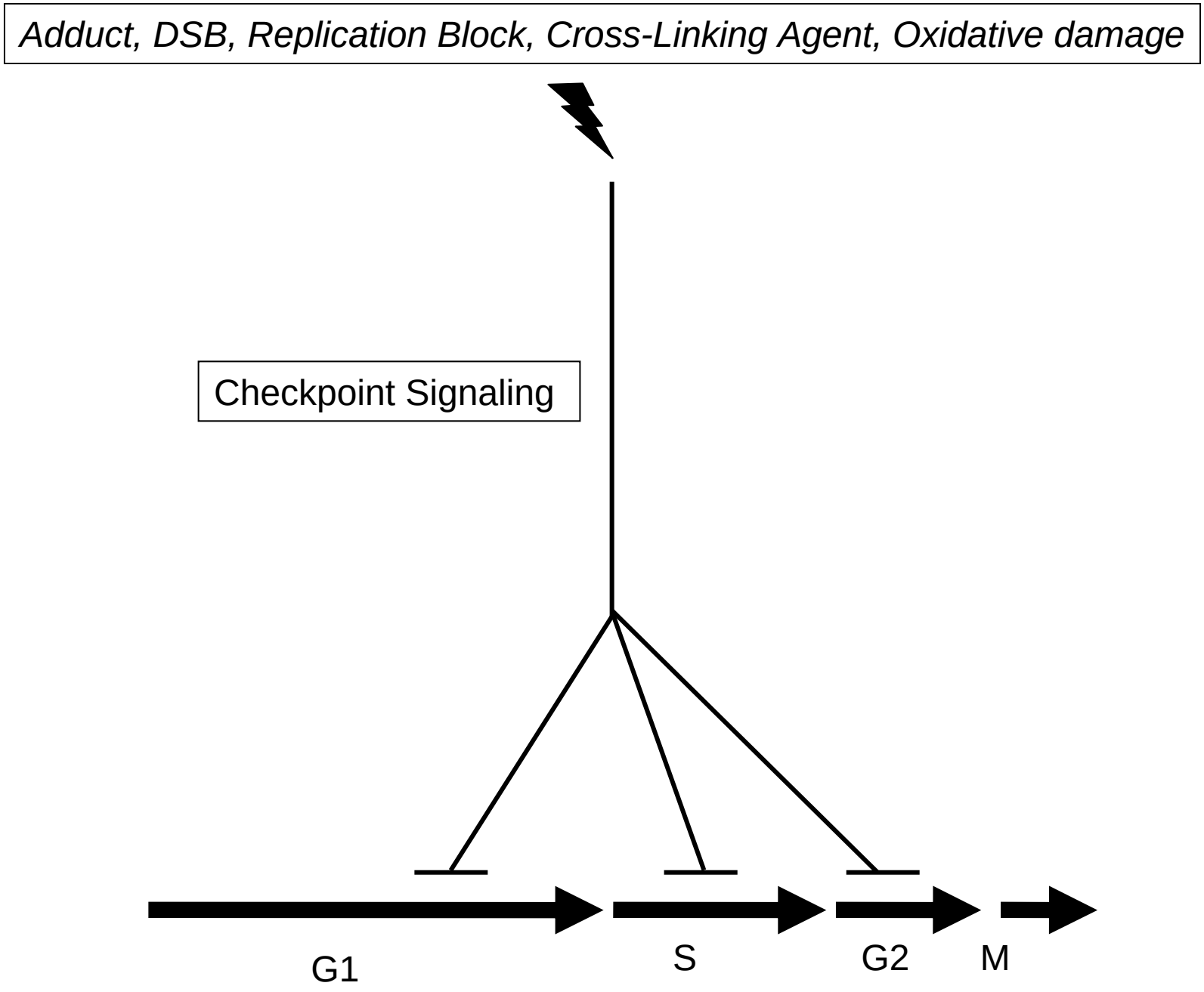
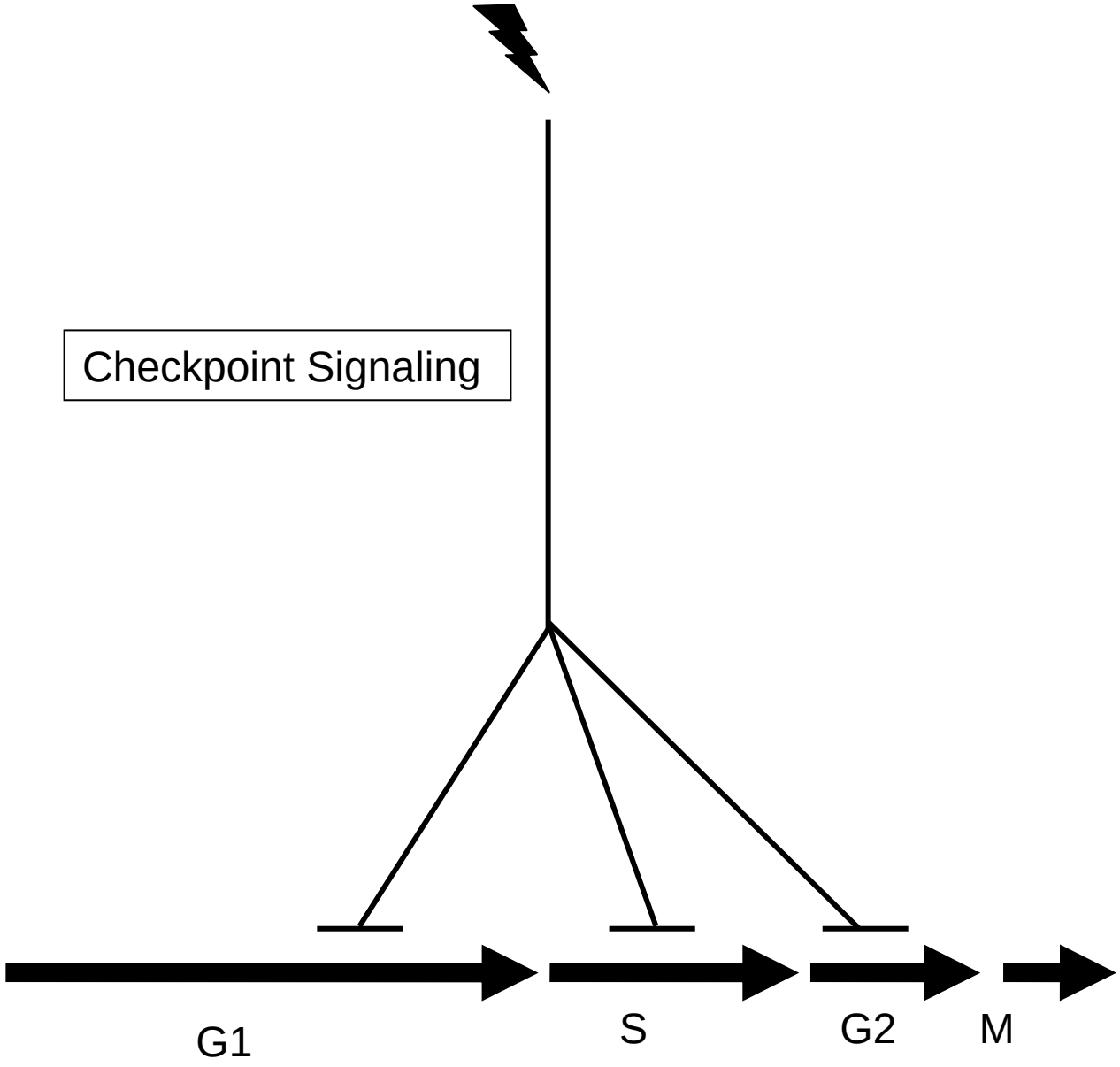
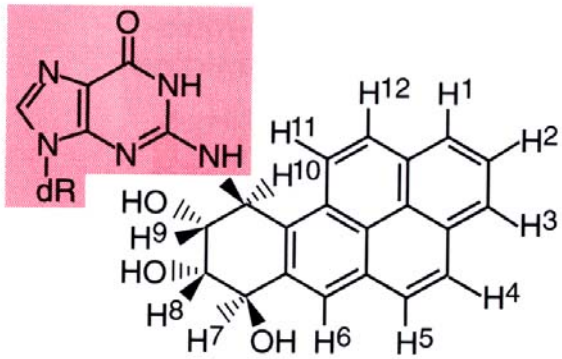

DNA Damage
Checkpoint Signaling
Cell Cycle Progression


Adduct, DSB, Replication Block, Cross-Linking Agent, Oxidative damage
Checkpoint Signaling
G1
S
G2
M

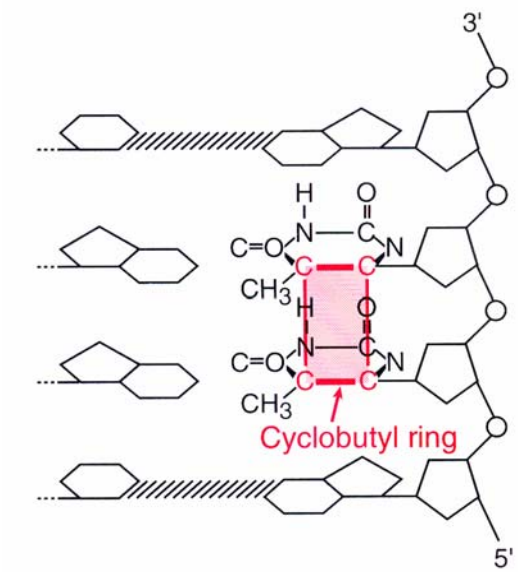
Adduct, *DSB, Replication Block, Cross-Linking Agent, Oxidative damage*



Environmentally-Induced Bulky DNA Lesions



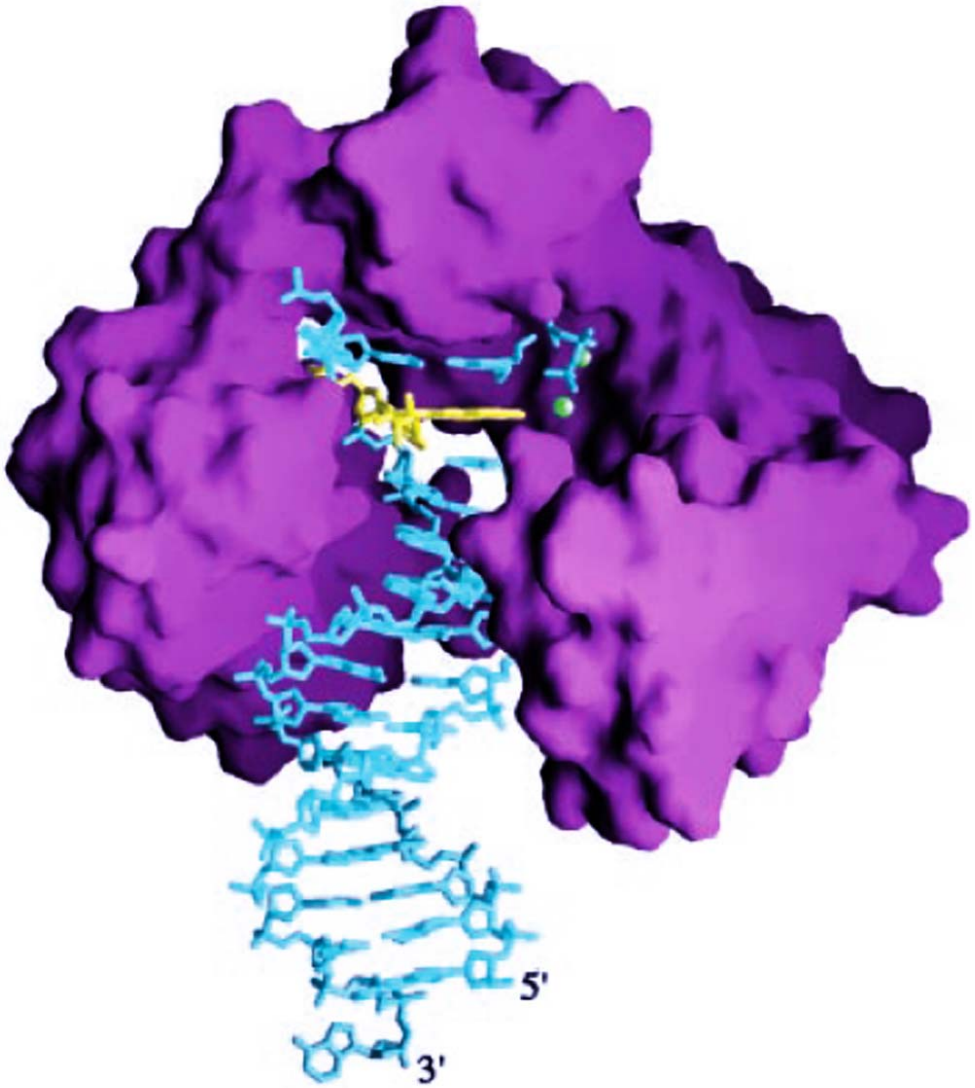
BPDE-DNA Adduct



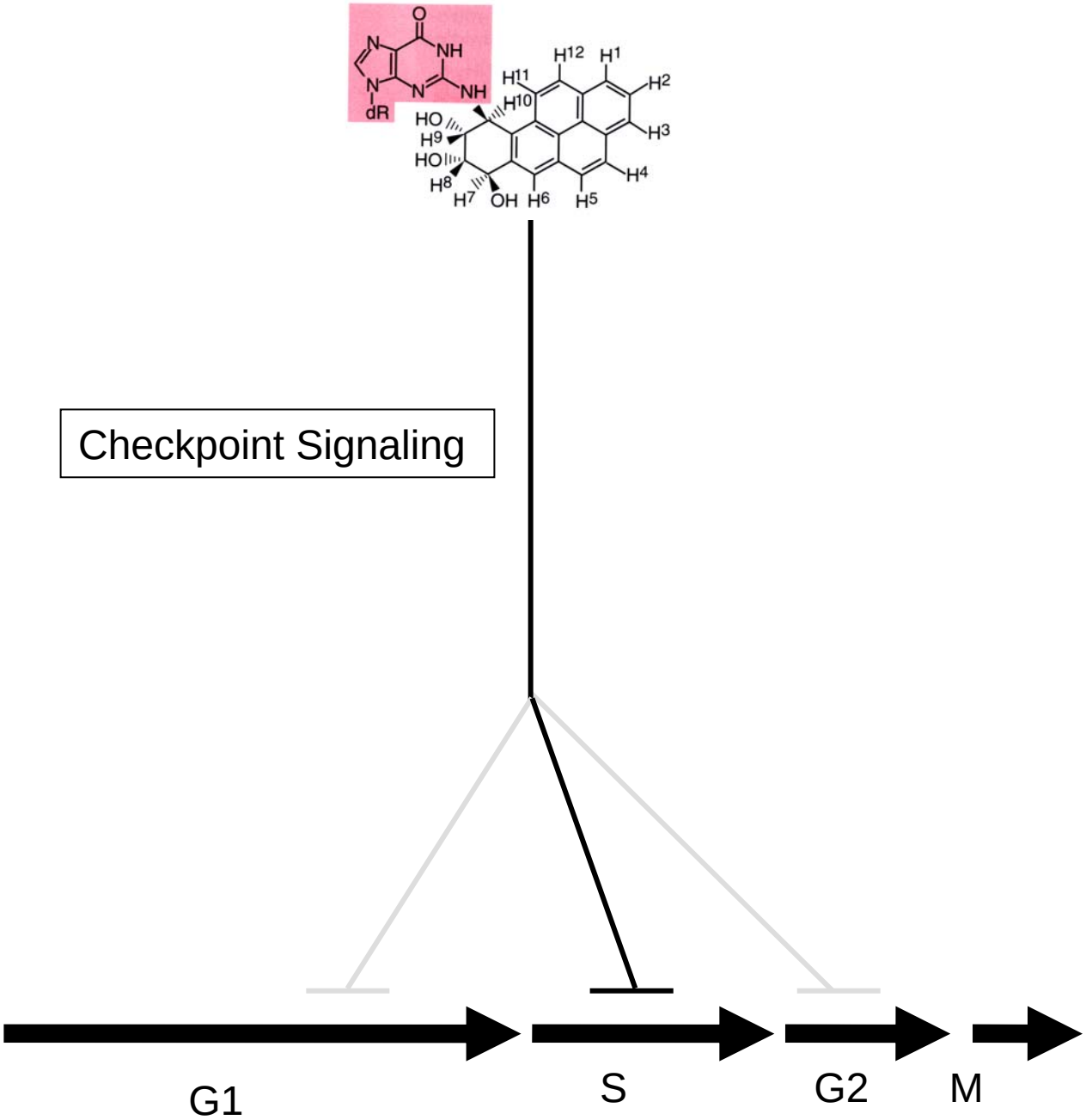
Cyclobutane Pyrimidine Dimer (CPD)

From *DNA Repair and Mutagenesis* , Friedberg, E. C. ed.

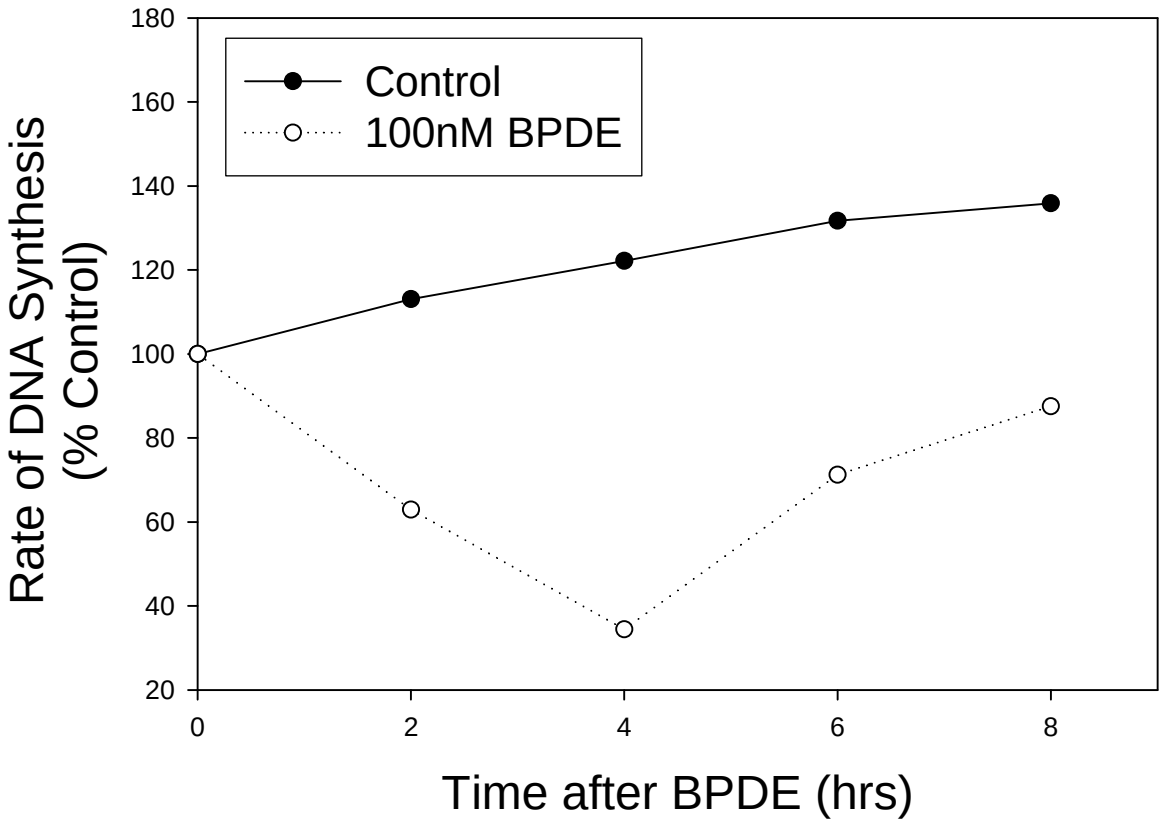
Crystal Structure of BPDE-Adduct in Ternary Complex with a DNA Polymerase



Ling et al., *PNAS* 101, p2265-2269 (2004)


Checkpoint Signaling
G1
S
G2
M

BPDE-induced Inhibition of DNA Synthesis



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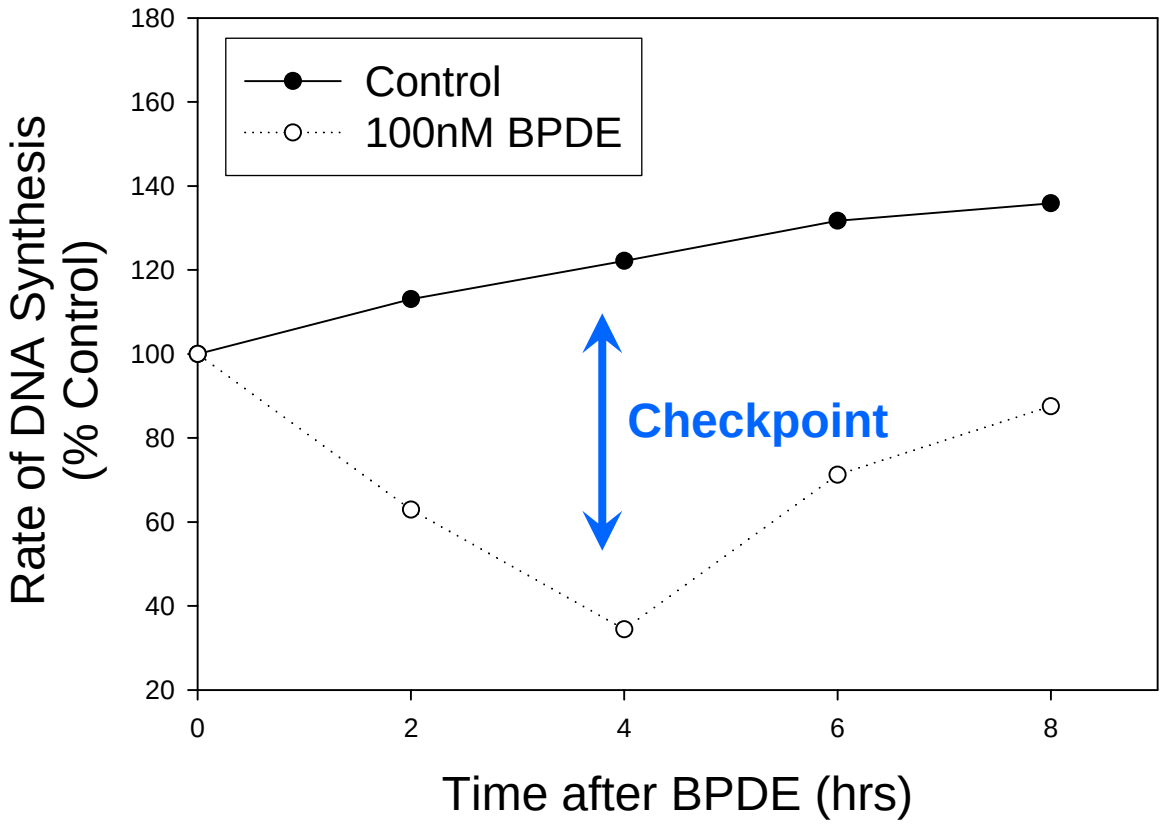

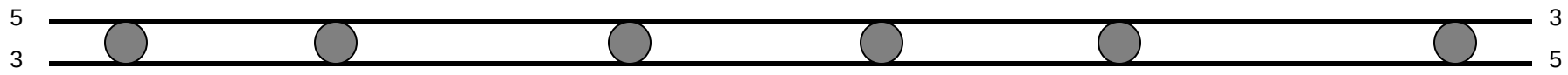
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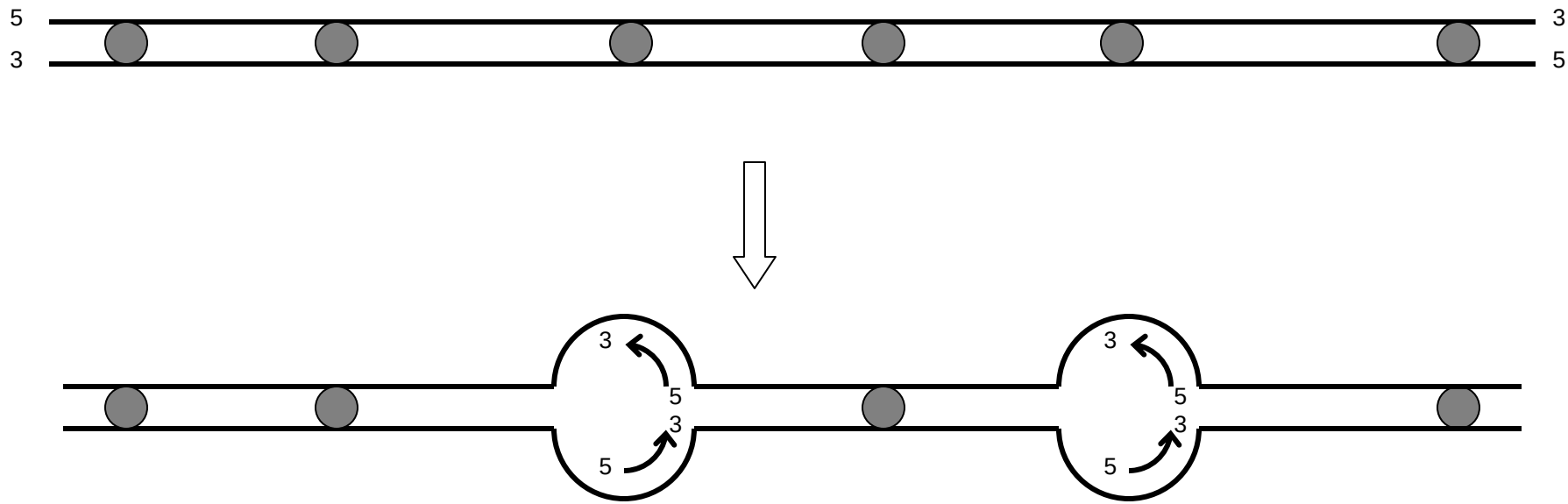
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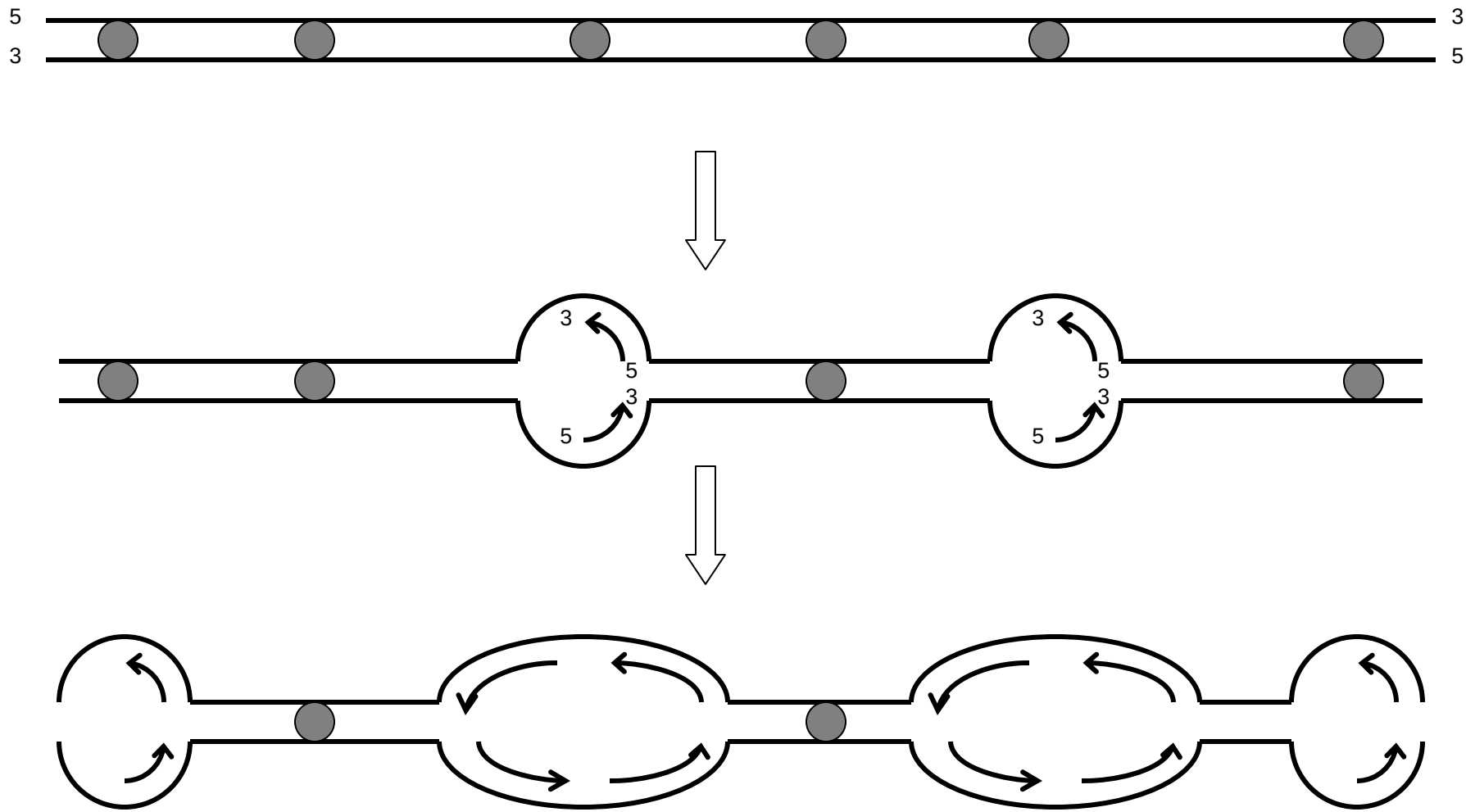
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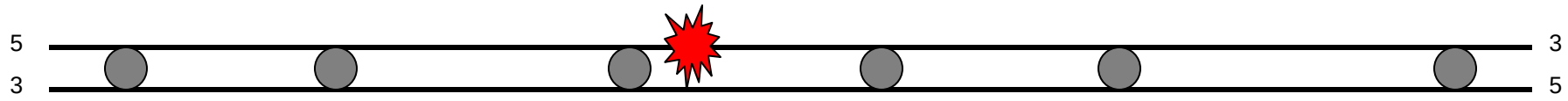
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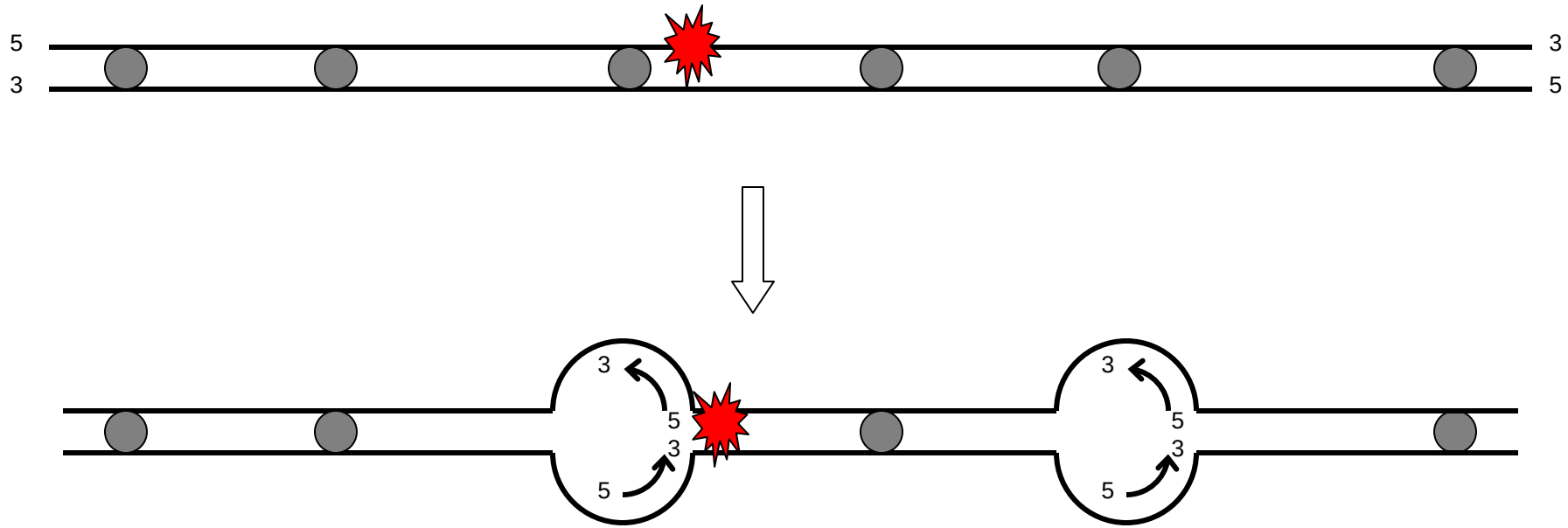
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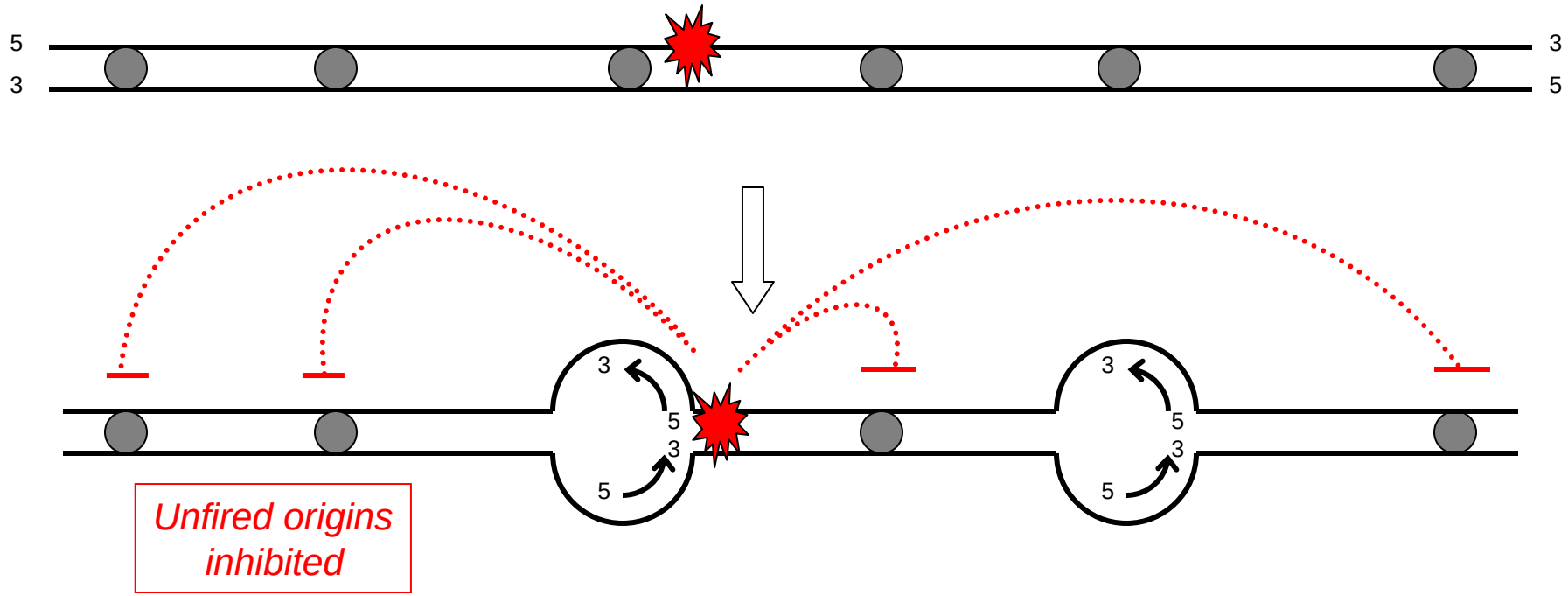
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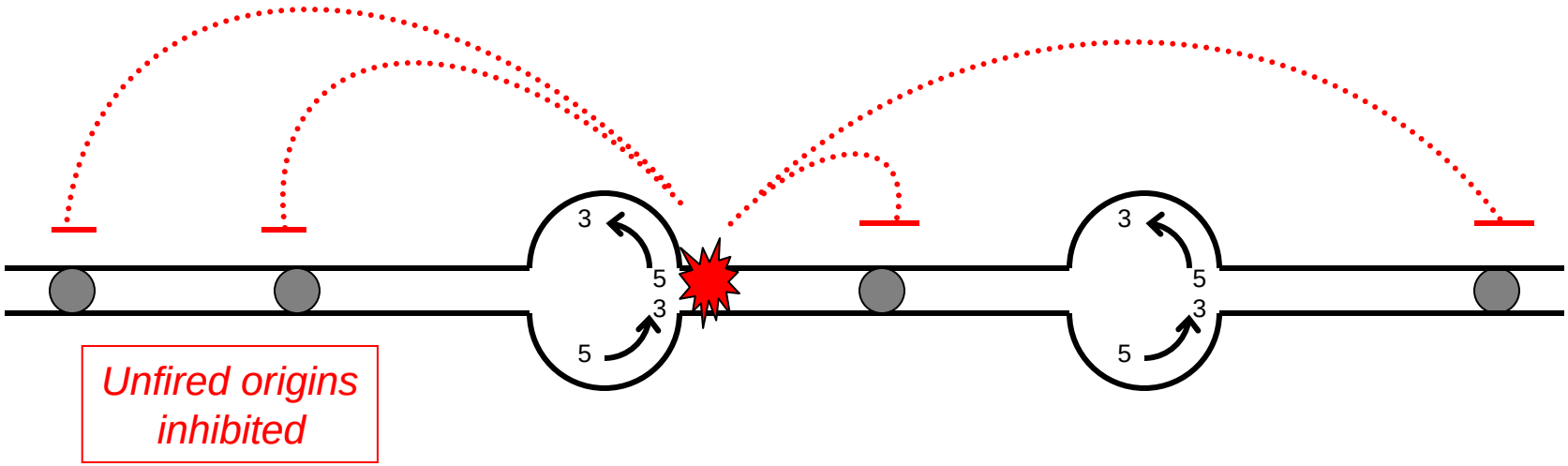
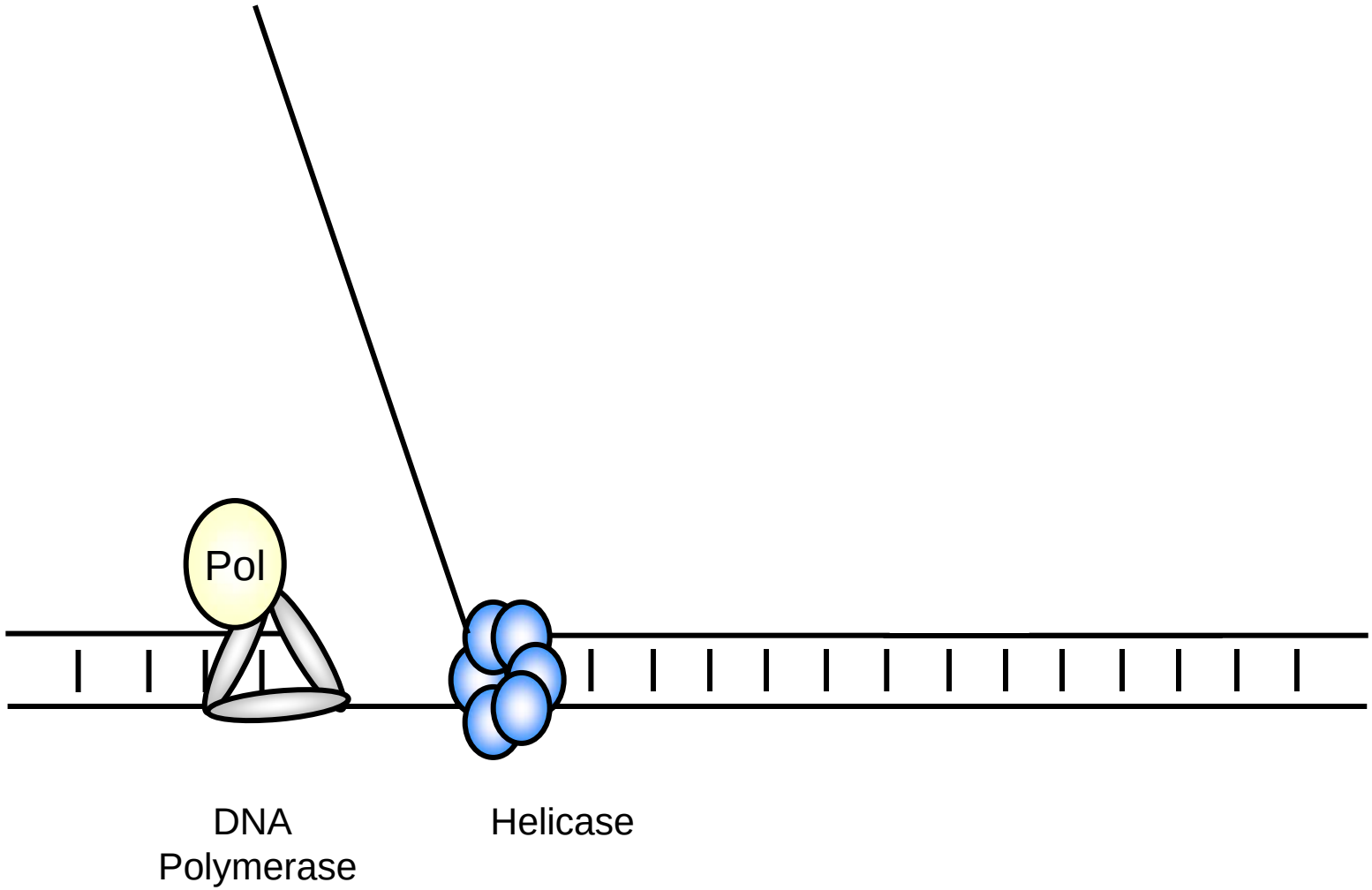
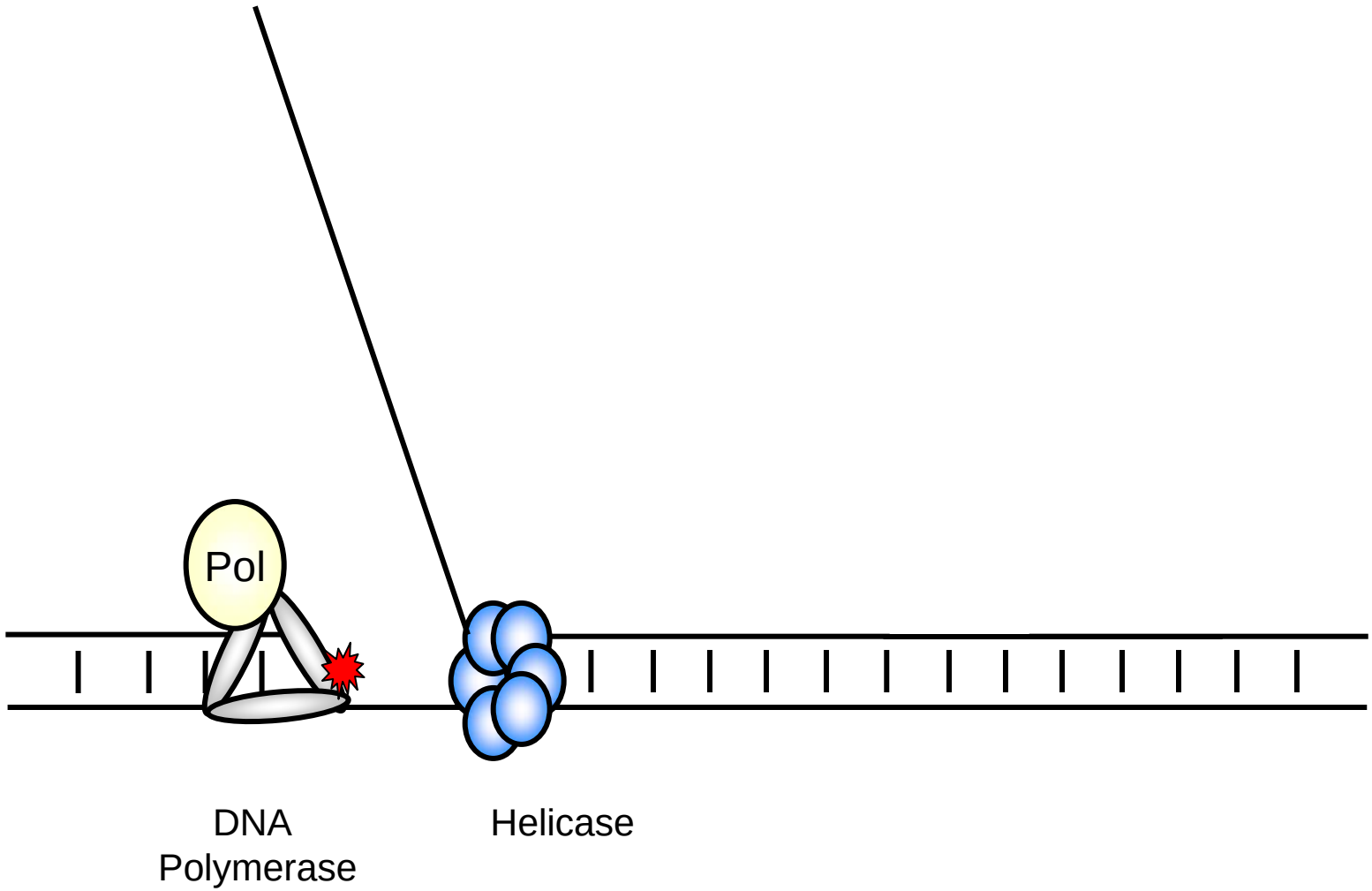
Unfired origins inhibited

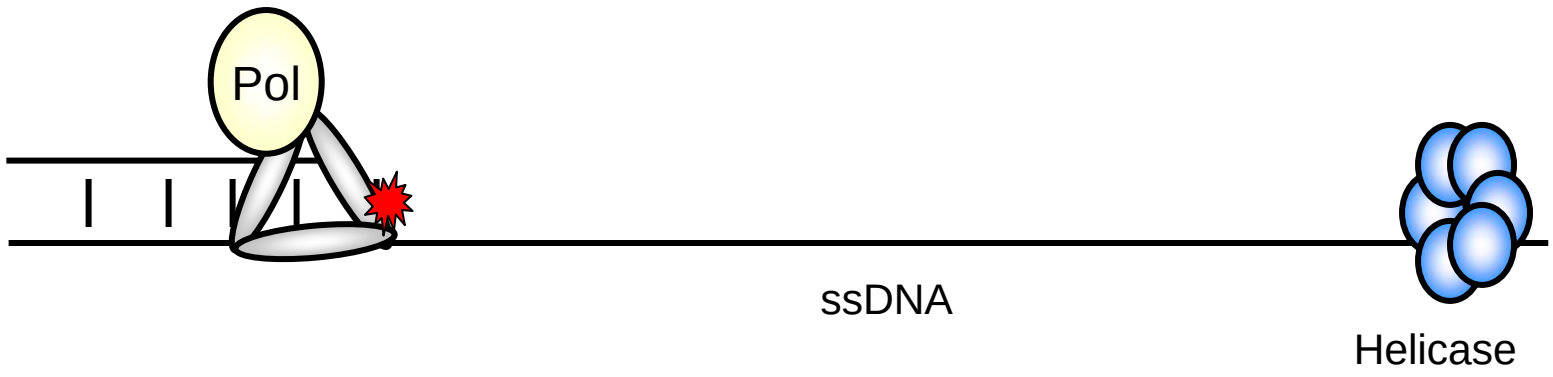
S-phase Checkpoint Signaling

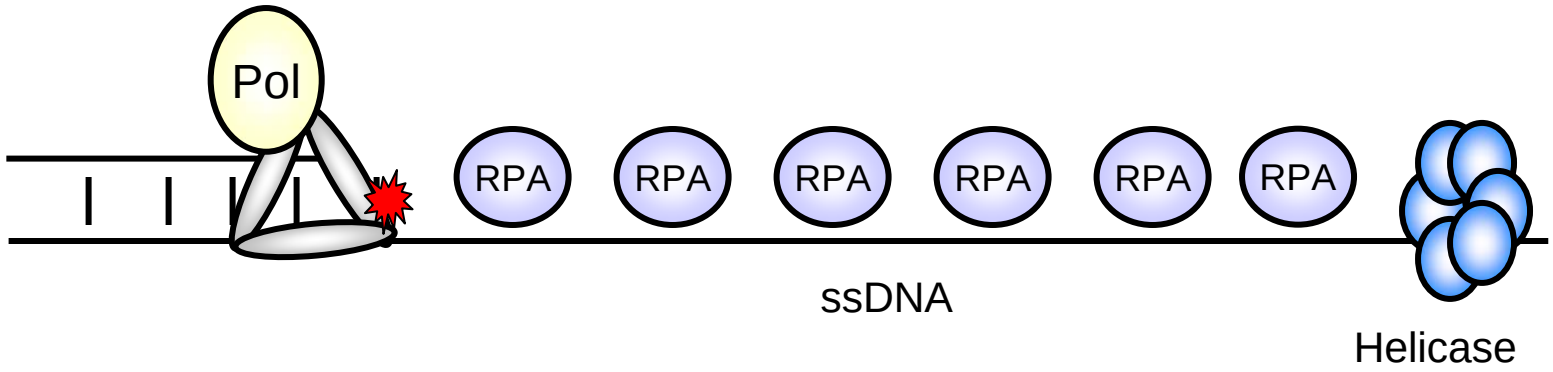


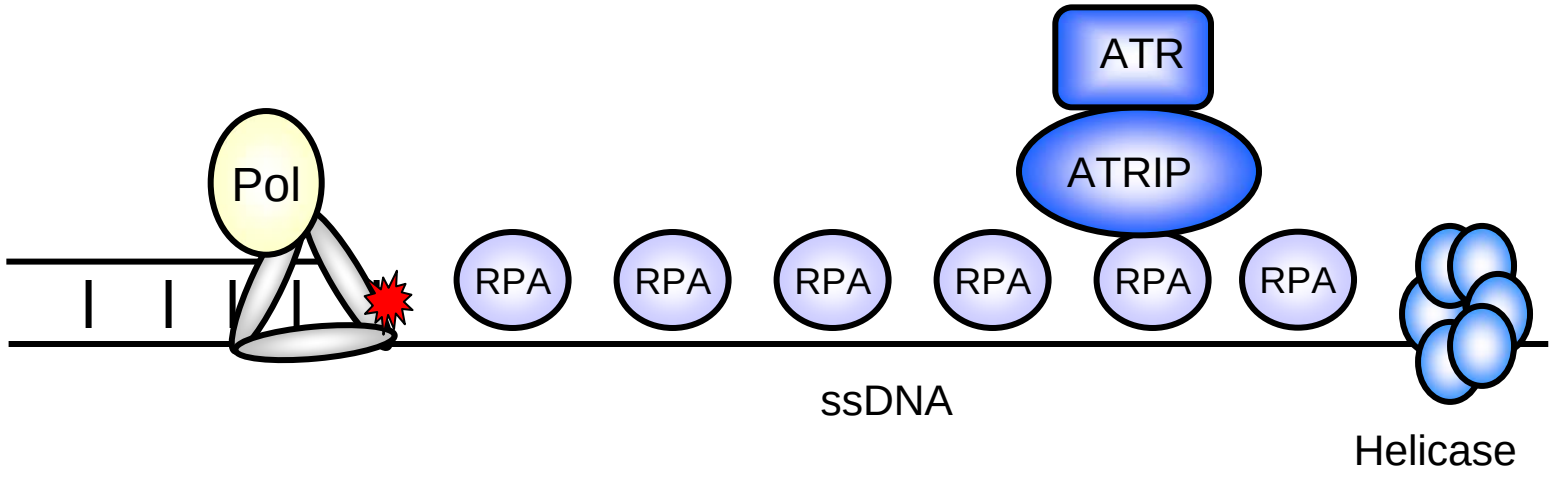
Unfired origins inhibited

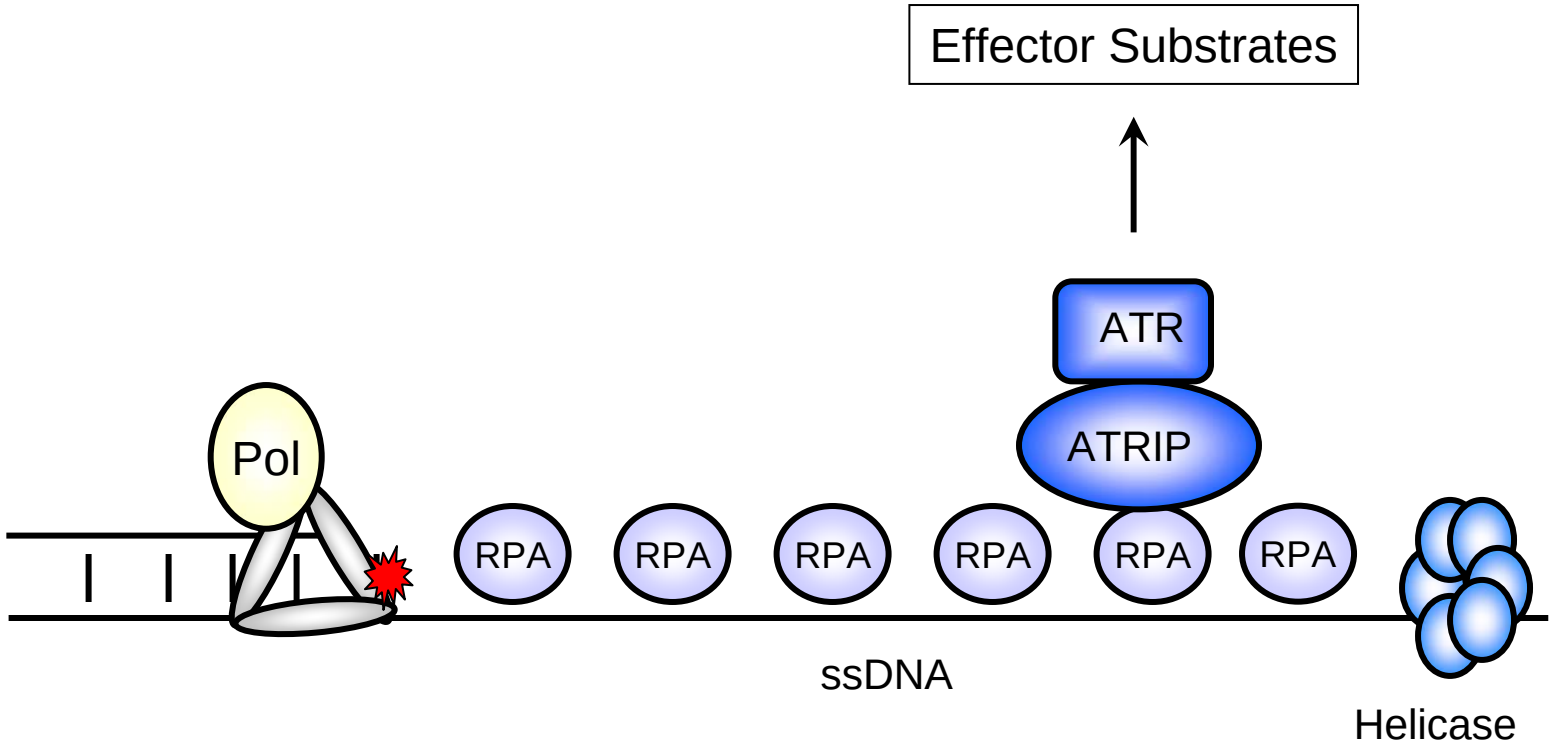

Pol
DNA
Polymerase
Helicase

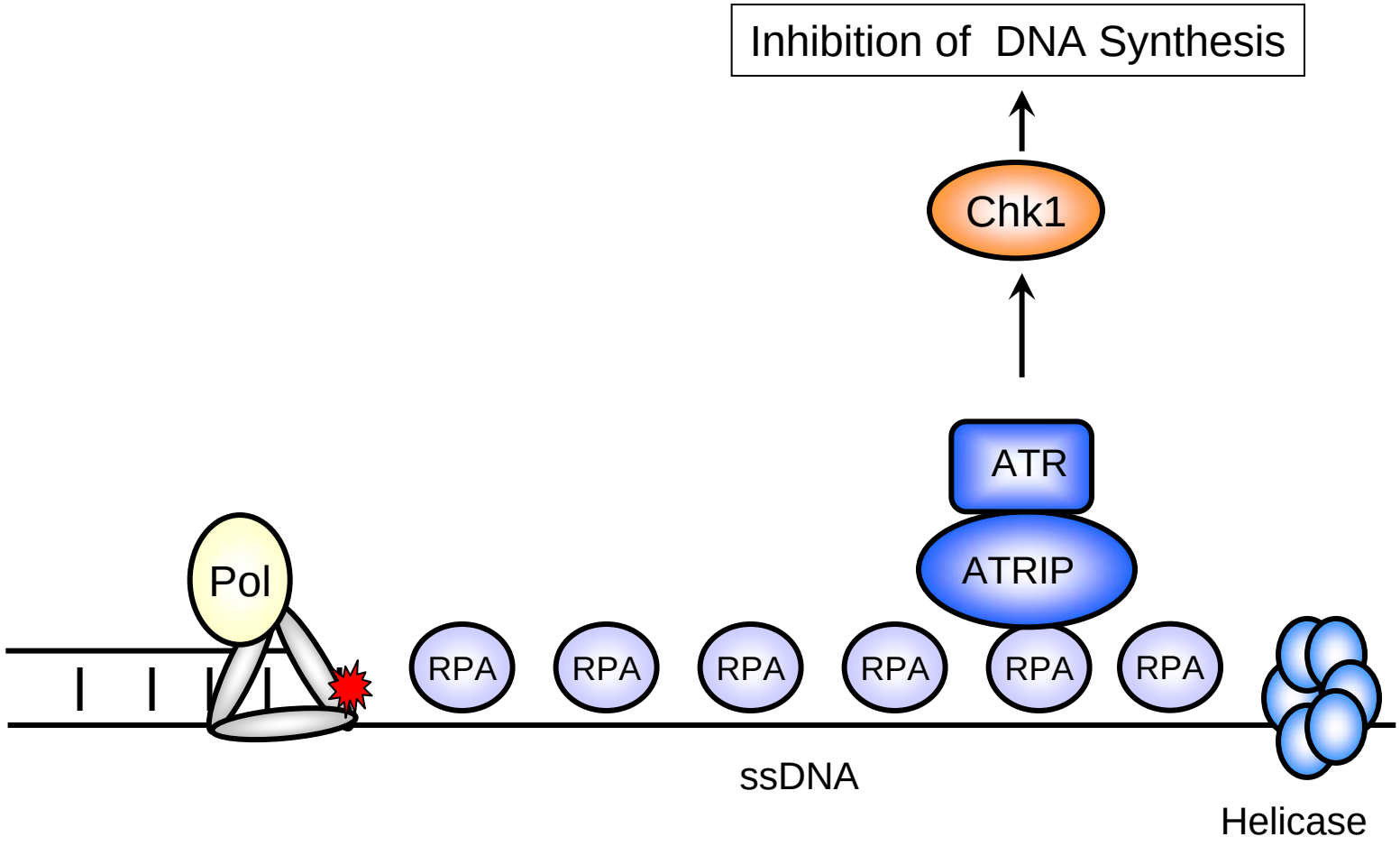

Pol
DNA
Polymerase
Helicase

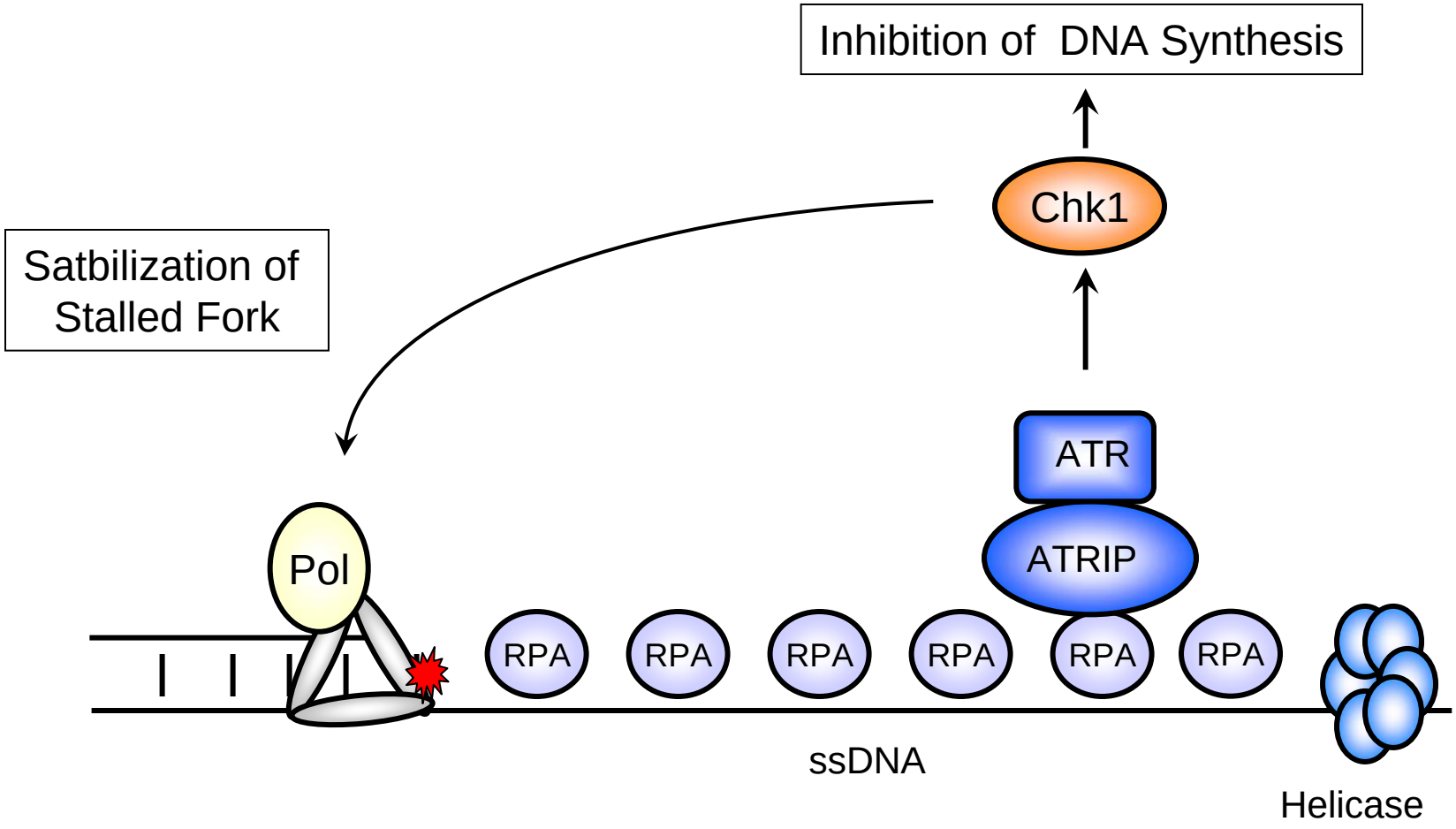

Pol
ssDNA
Helicase

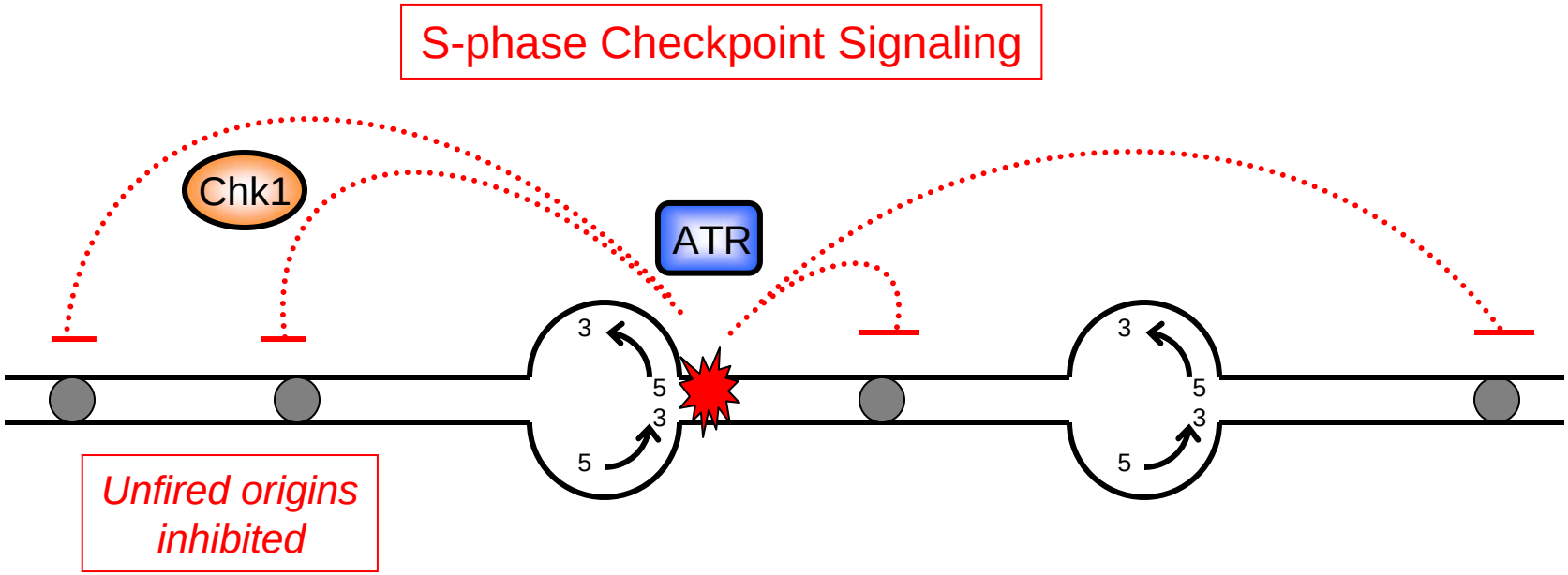

Pol
RPA
RPA
RPA
RPA
RPA
RPA
ssDNA
Helicase


ATR
ATRIP
Pol
RPA
RPA
RPA
RPA
RPA
RPA
ssDNA
Helicase


Effector Substrates
ATR
ATRIP
Pol
RPA
RPA
RPA
RPA
RPA
RPA
ssDNA
Helicase


Inhibition of DNA Synthesis
Chk1
ATR
ATRIP
Pol
RPA
RPA
RPA
RPA
RPA
RPA
ssDNA
Helicase


Inhibition of DNA Synthesis
Chk1
Satbilization of Stalled Fork
ATR
ATRIP
Pol
RPA
RPA
RPA
RPA
RPA
RPA
ssDNA
Helicase


S-phase Checkpoint Signaling
Chk1
ATR
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5
Unfired origins inhibited

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2. Regulation of TLS by Rad18 Phosphorylation

3. Replication Fork-Independent roles of Rad18 in DNA repair

Xeroderma Pigmentosum (XP)

The TLS polymerase Polη is defective in XP-Variant patients

From *DNA Repair and Mutagenesis* , Friedberg, E. C. ed.

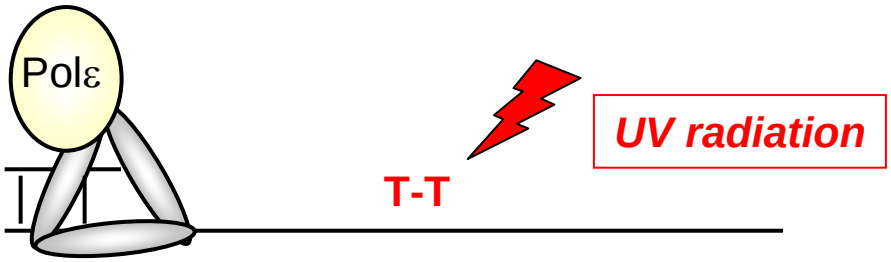
Replicative DNA Polymerases:

High fidelity on undamaged DNA template.

Polη, a Trans-Lesion Synthesis (TLS) DNA Polymerase:

Low fidelity on undamaged DNA, but can use UV-damaged DNA as template.

Role of Polη in Replicating UV-Induced Damage



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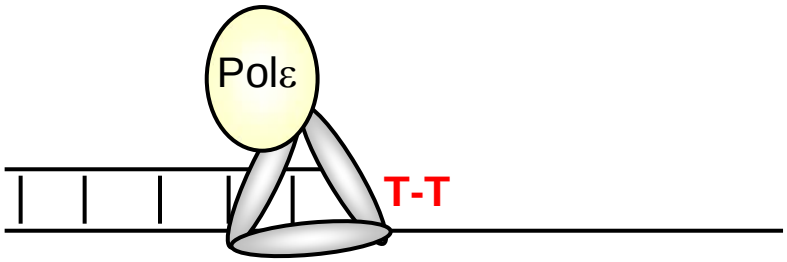

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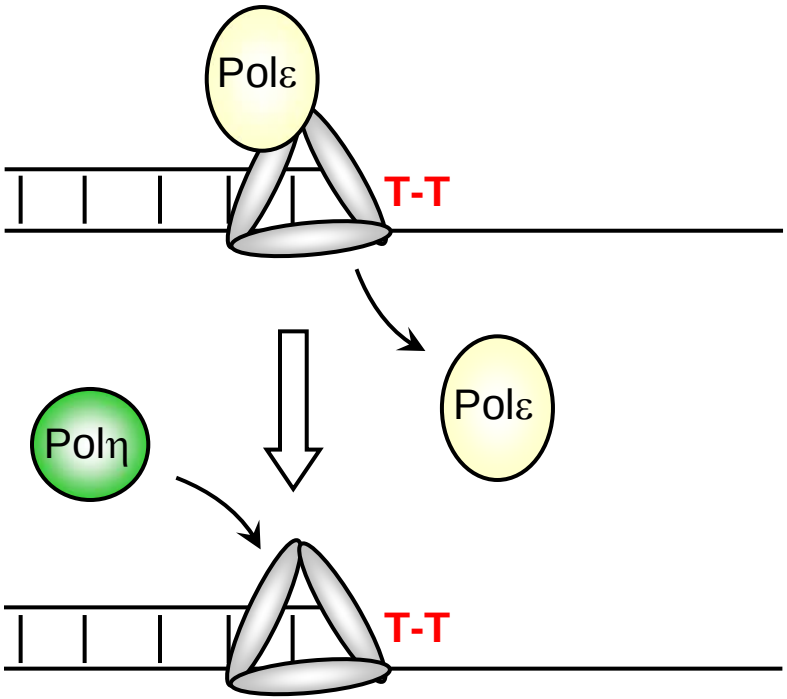
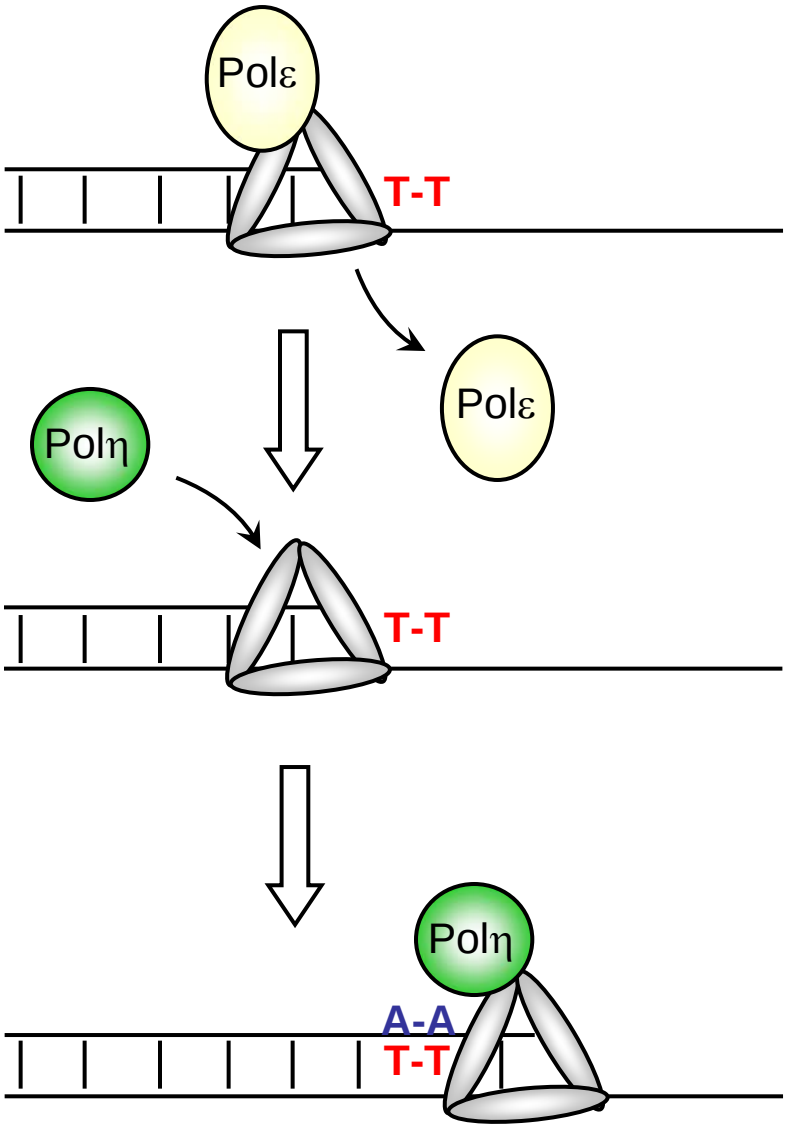
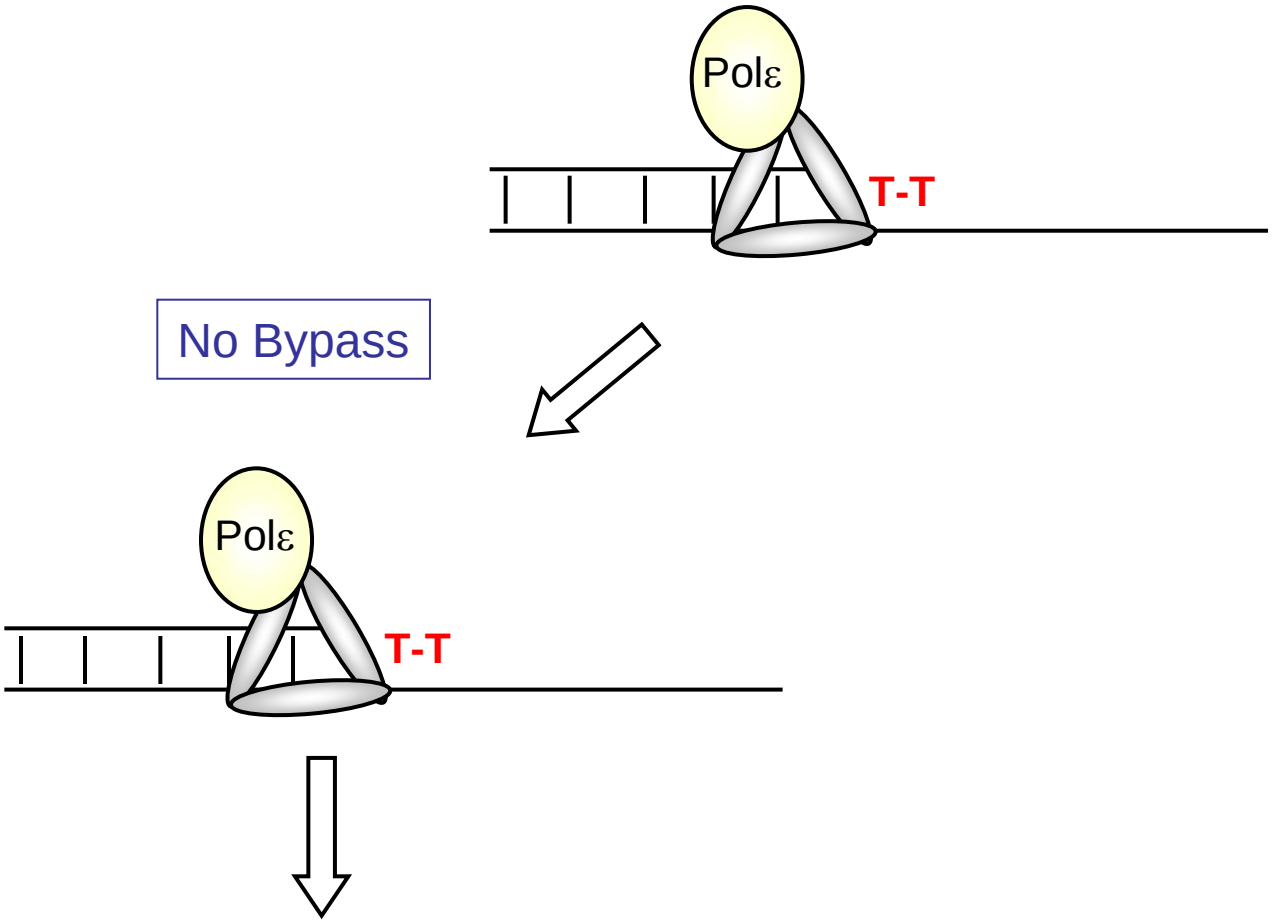


Role of Polη in Replicating UV-Induced Damage

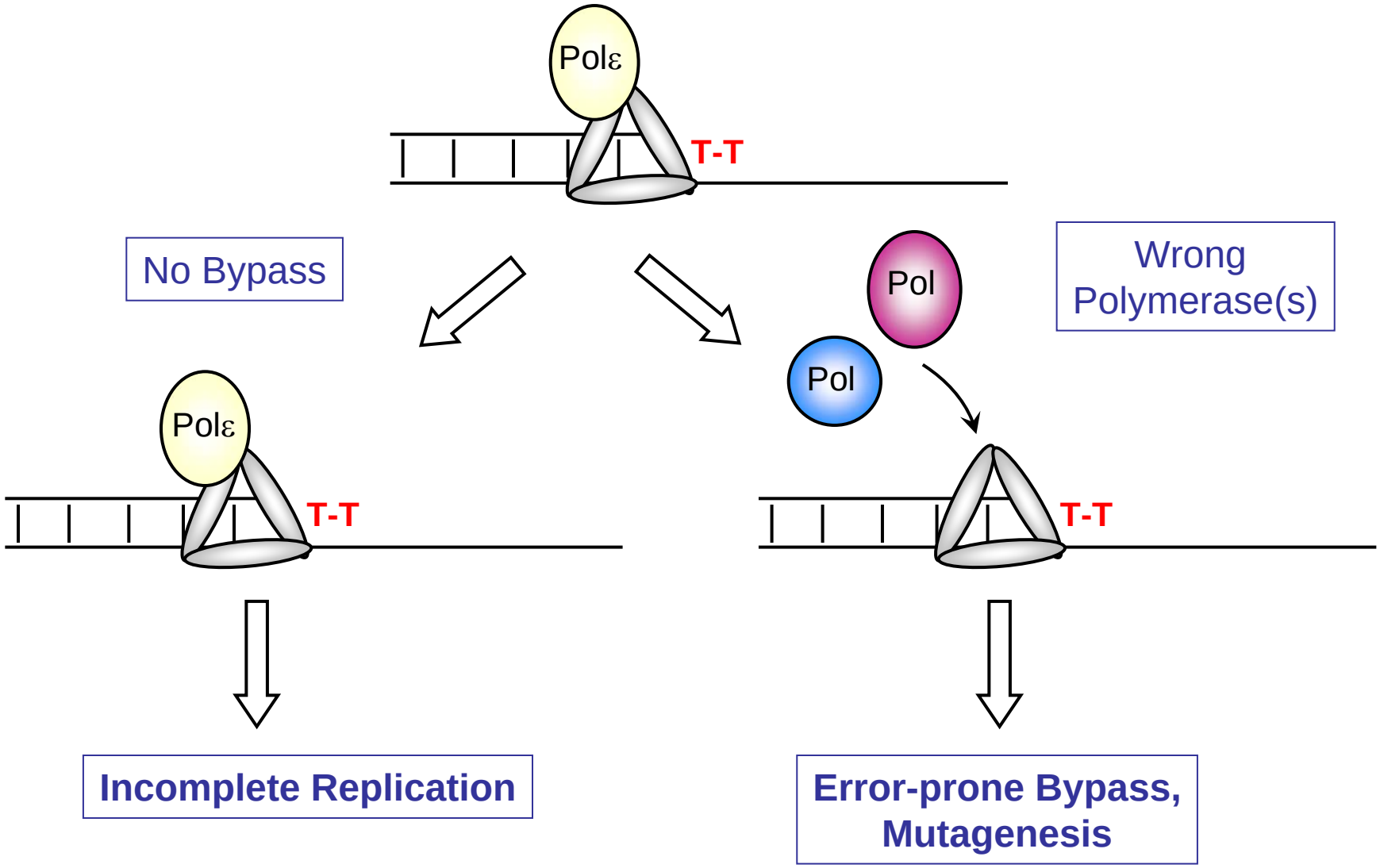


Effect of Polη-deficiency on Replication of UV-Damaged DNA



Incomplete Replication

Effect of Polη-deficiency on Replication of UV-Damaged DNA

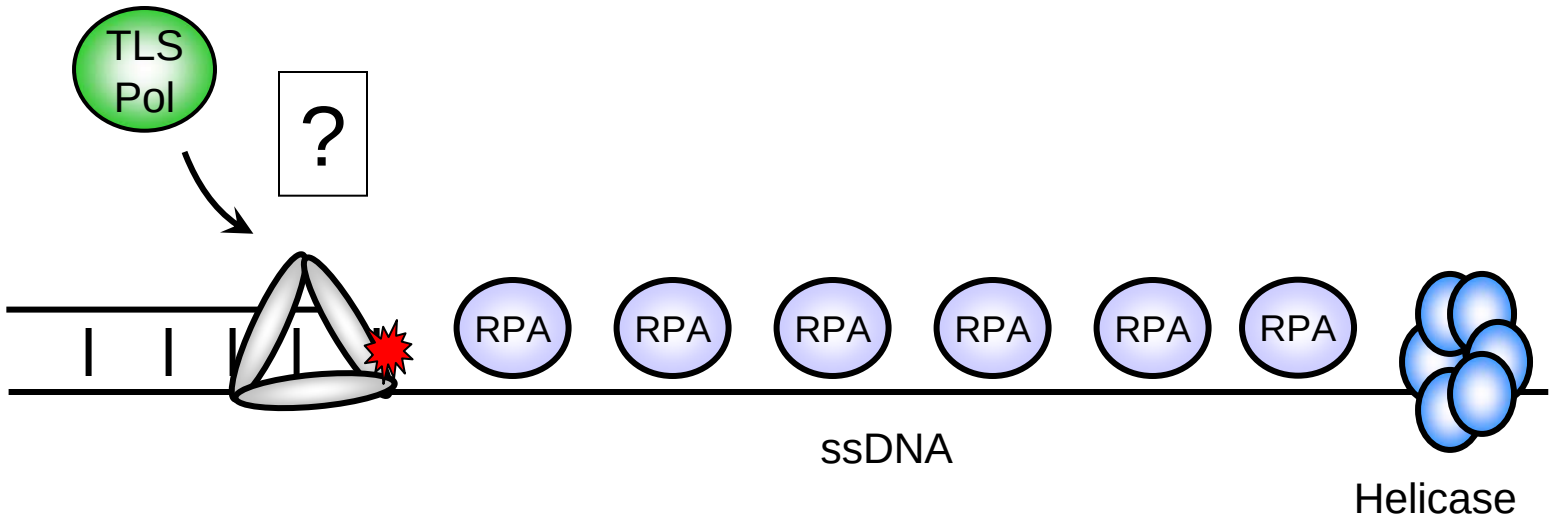


TLS Polymerases In Eukaryotes:

REV1, Polι, Polη, Polκ

Polη bypasses TT dimers (but not BPDE adducts) *in vitro*.

Polκ bypasses BPDE adducts *in vitro*.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Pol
?
RPA
RPA
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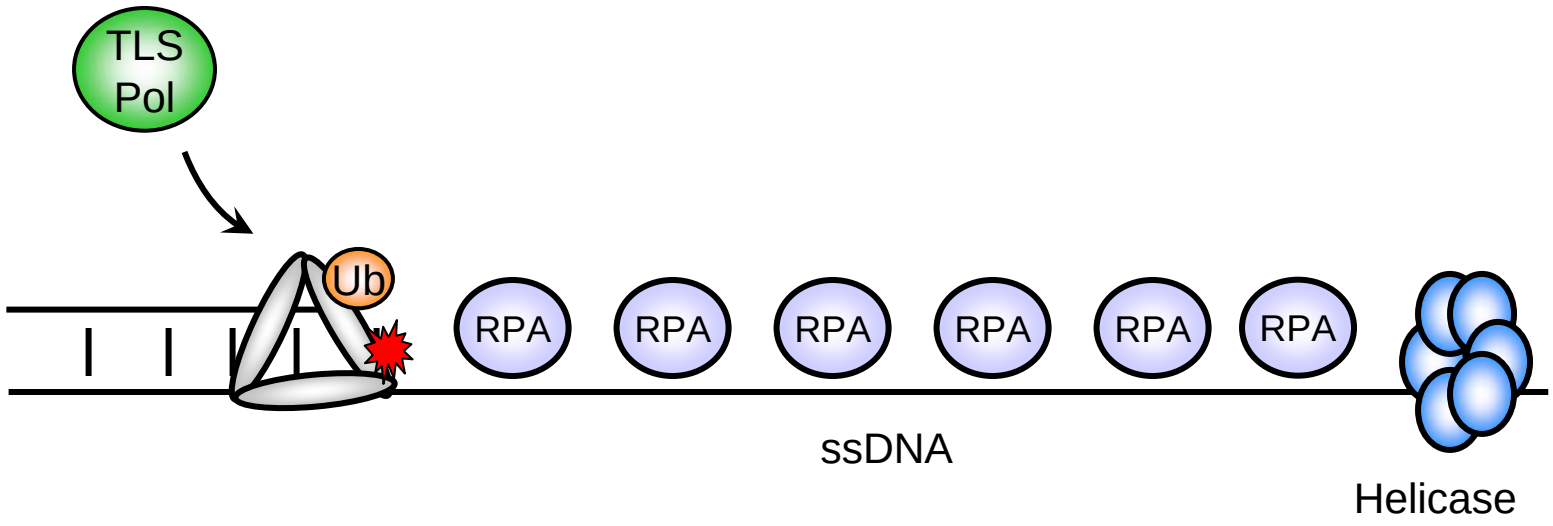
RPA
ssDNA
Helicase


TLS
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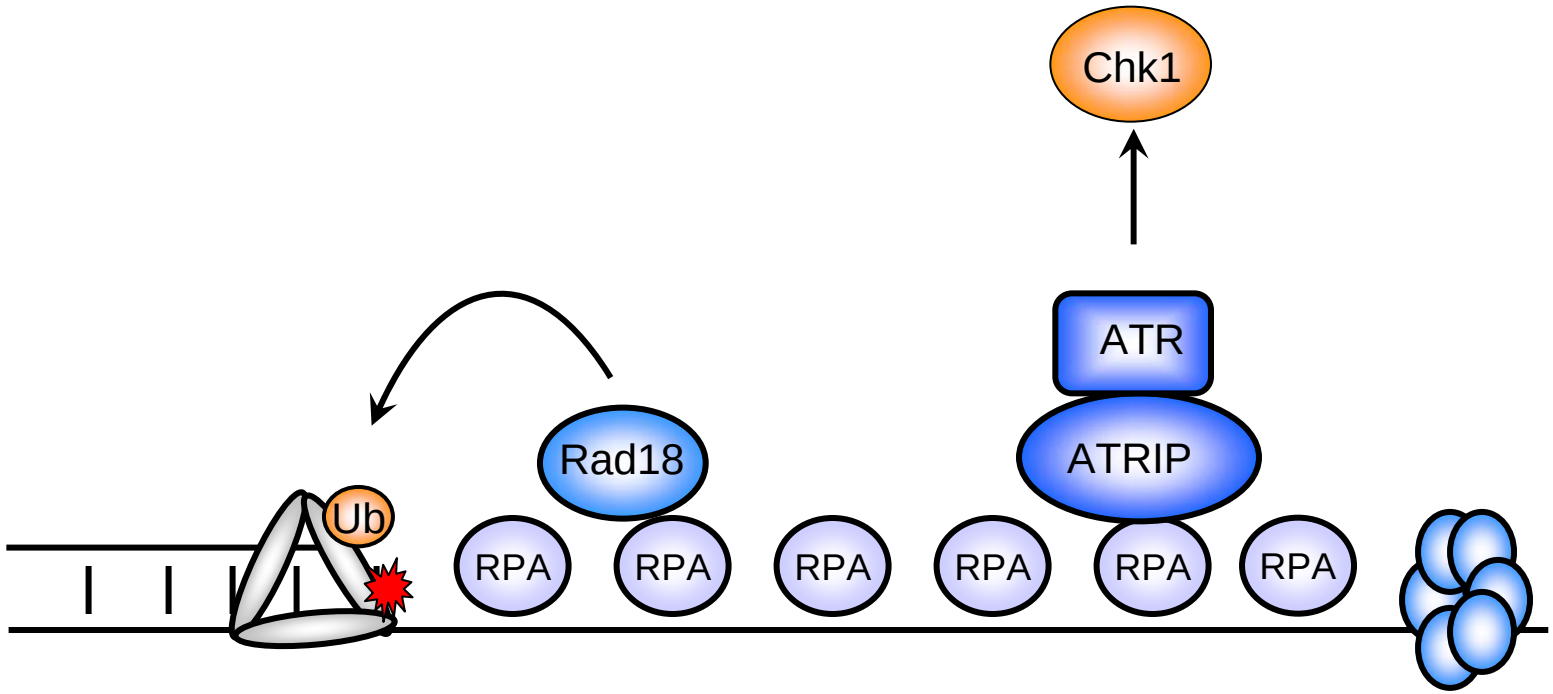
ssDNA
Helicase


Chk1
ATR
ATRIP
Rad18
Ub
RPA
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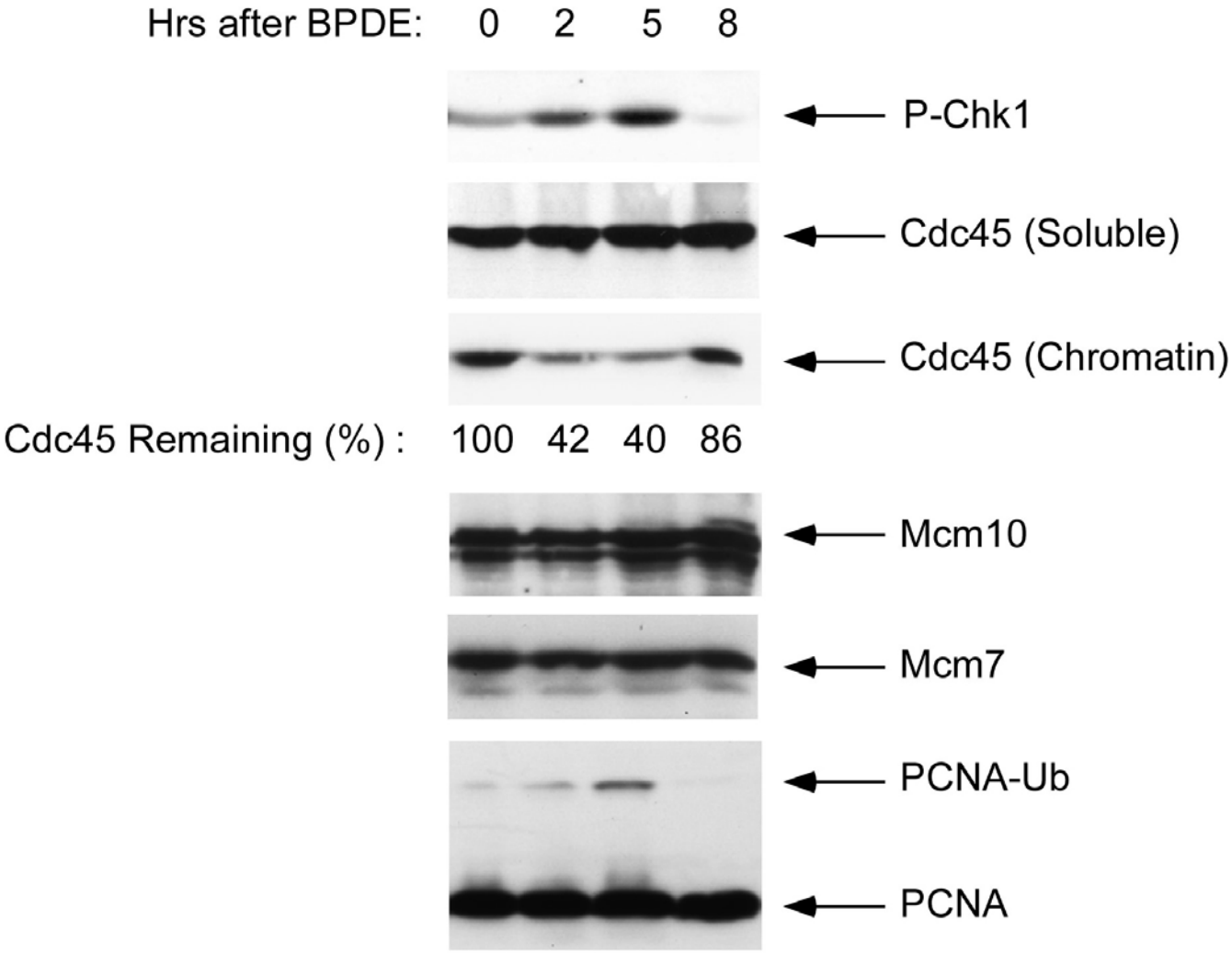
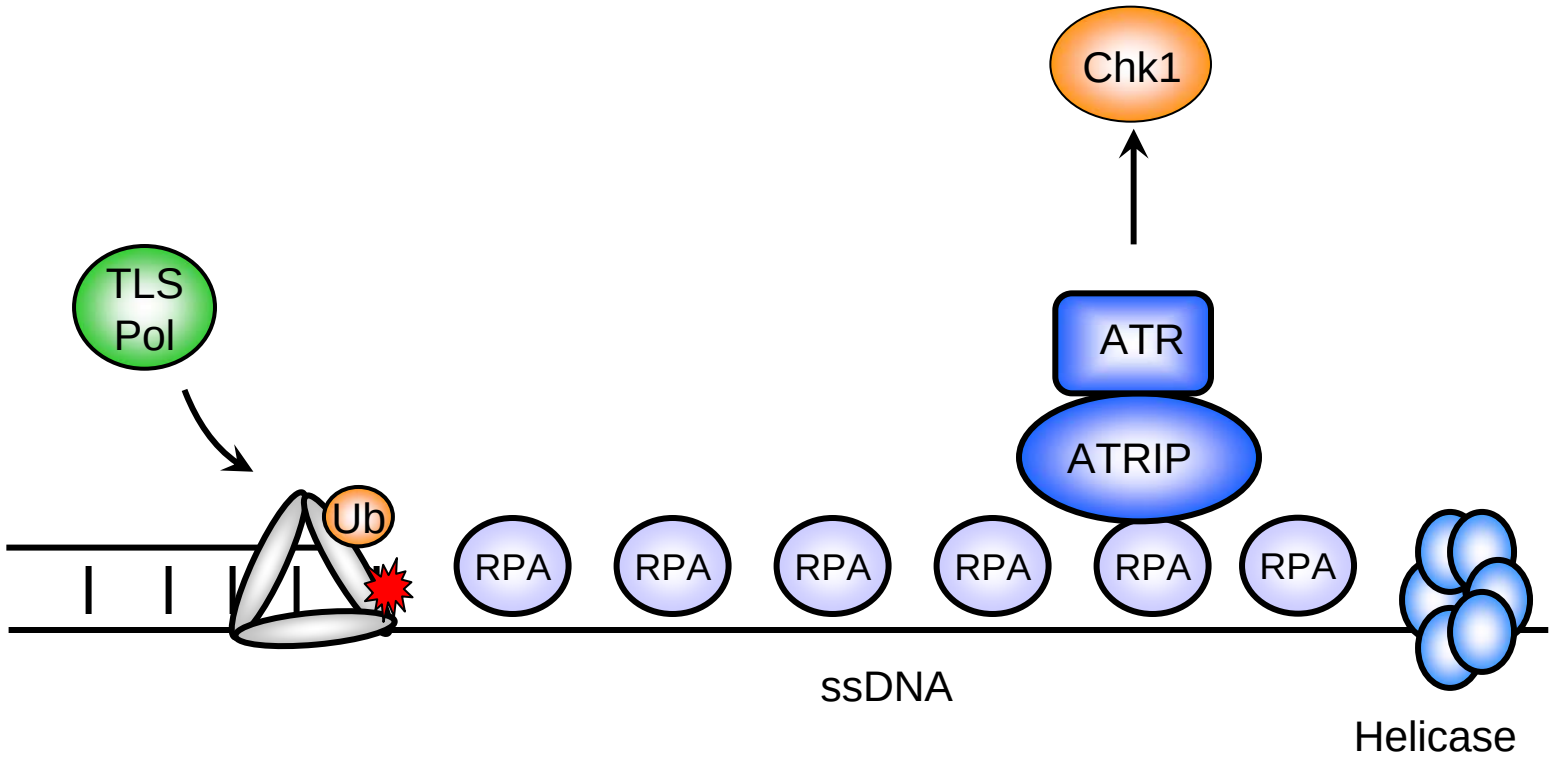
RPA

Chk1 Phosphorylation is Coincident with PCNA mono-ubiquitination




Chk1
TLS
Pol
ATR
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Ub
RPA
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RPA
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RPA
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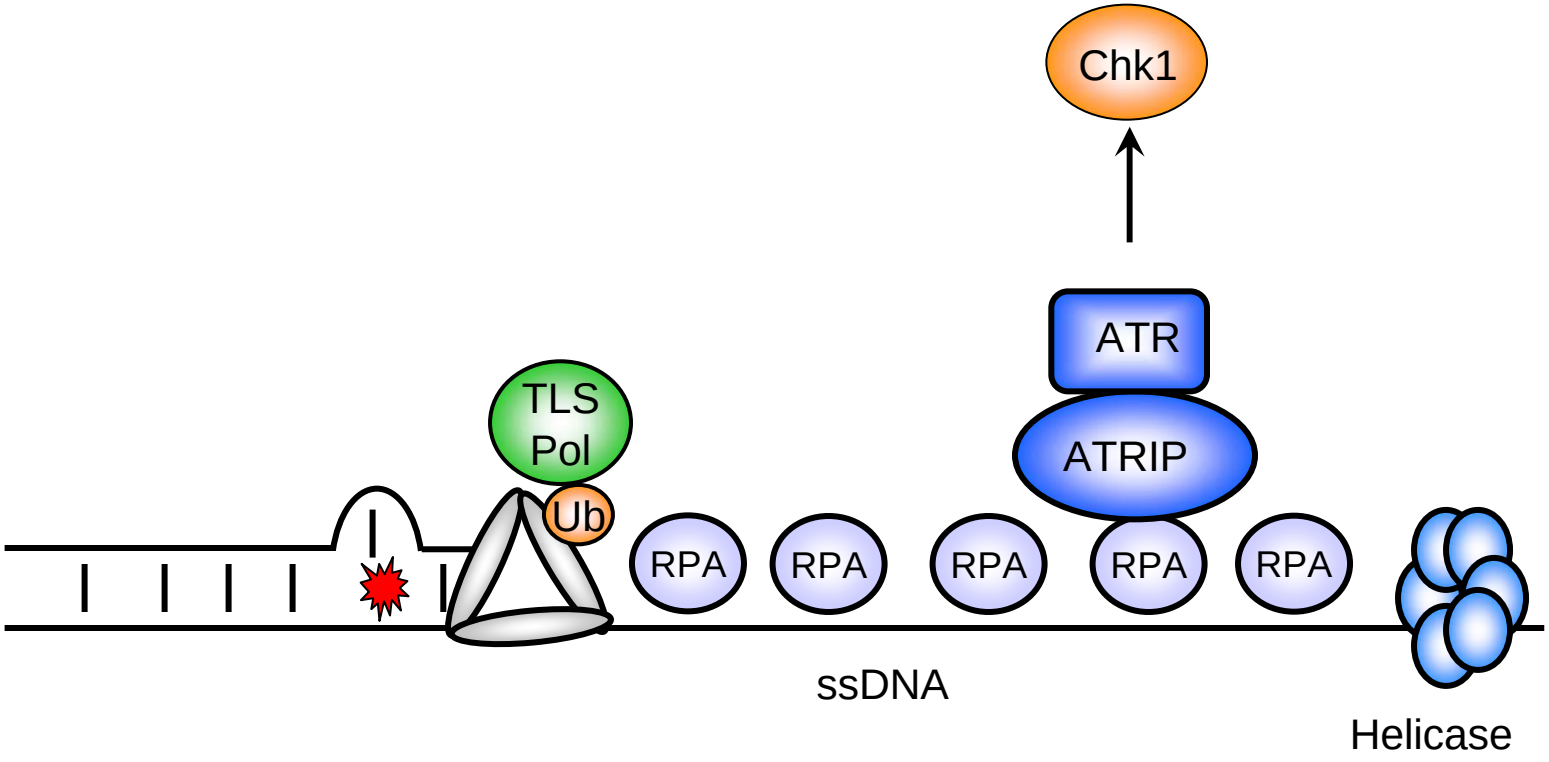
Helicase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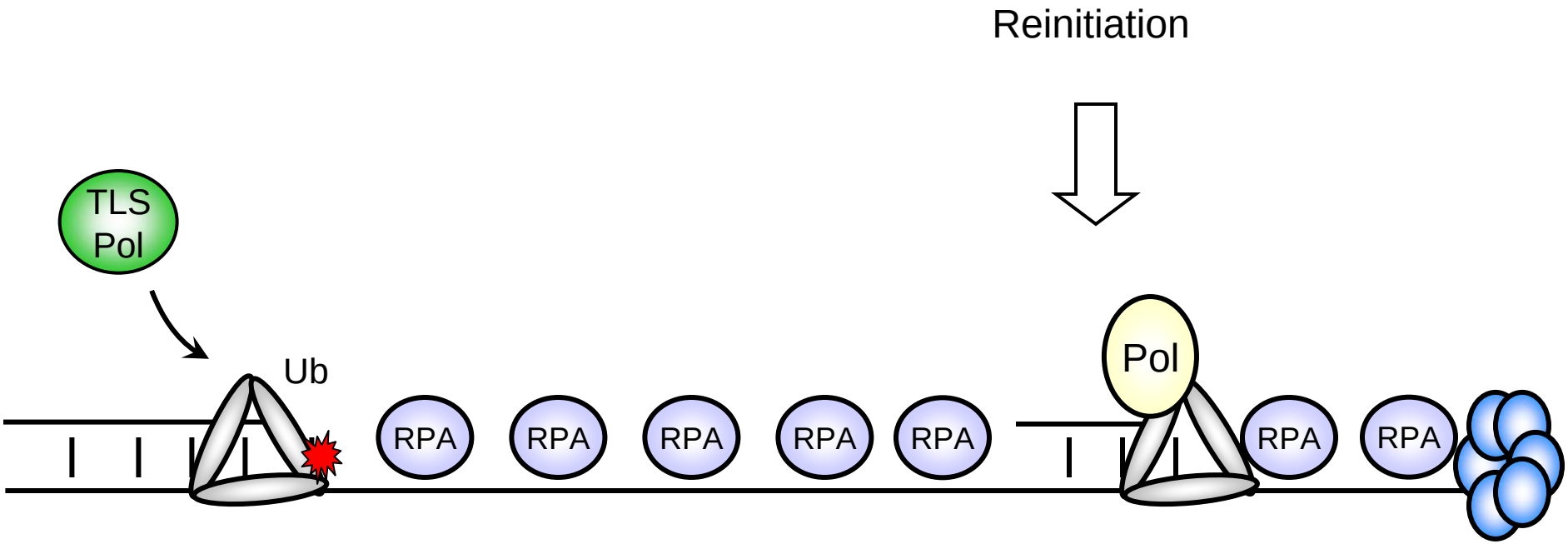
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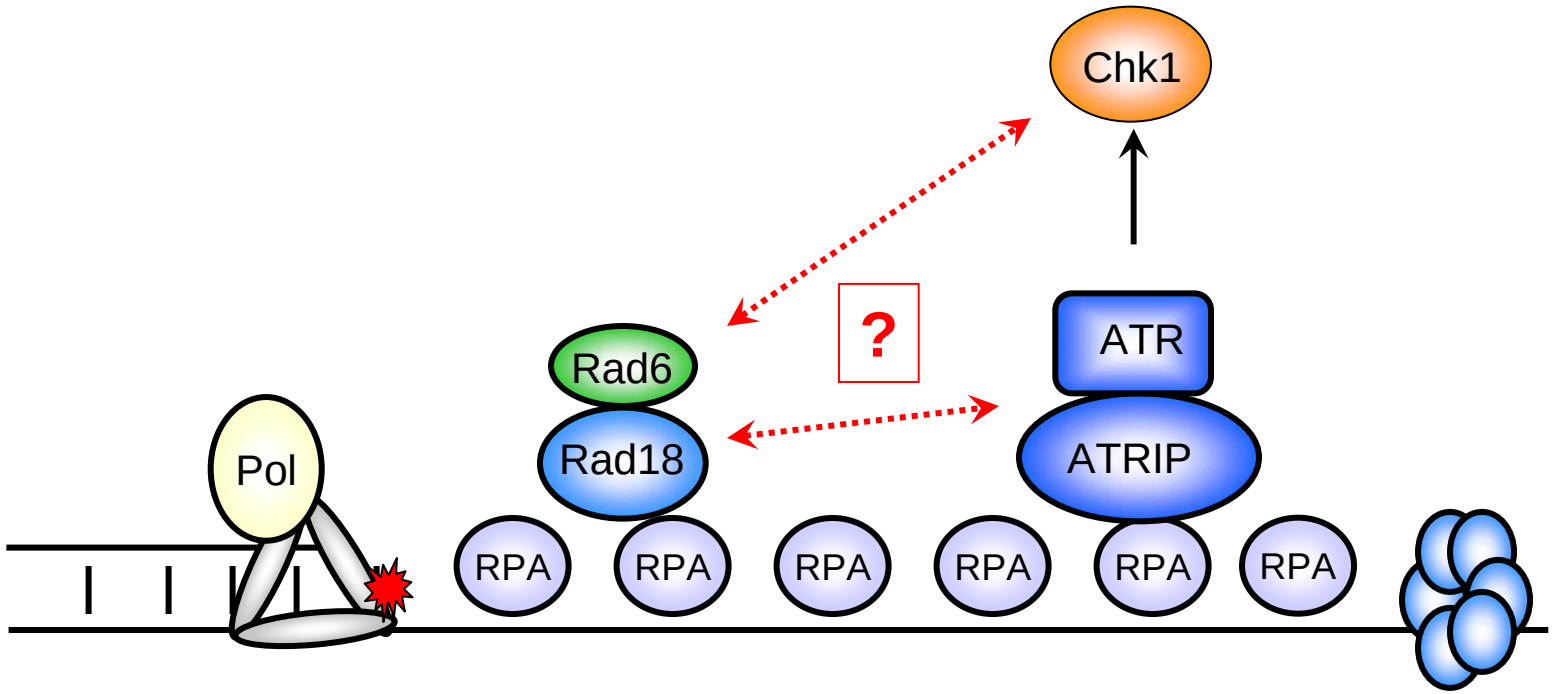
Chk1
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Rad6
Rad18
ATRIP
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RPA
RPA
RPA
RPA

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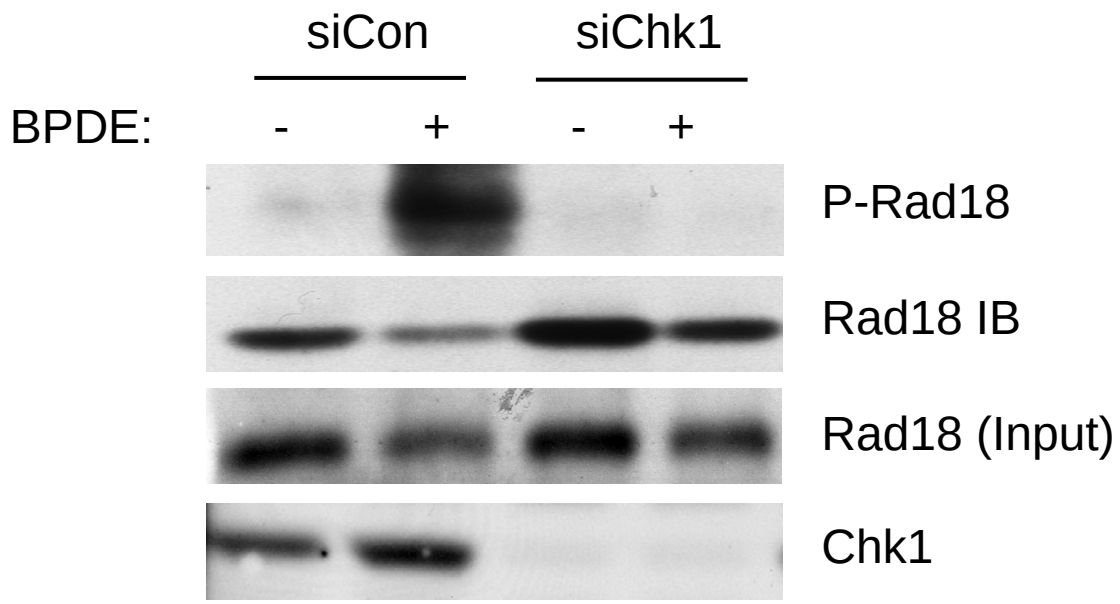
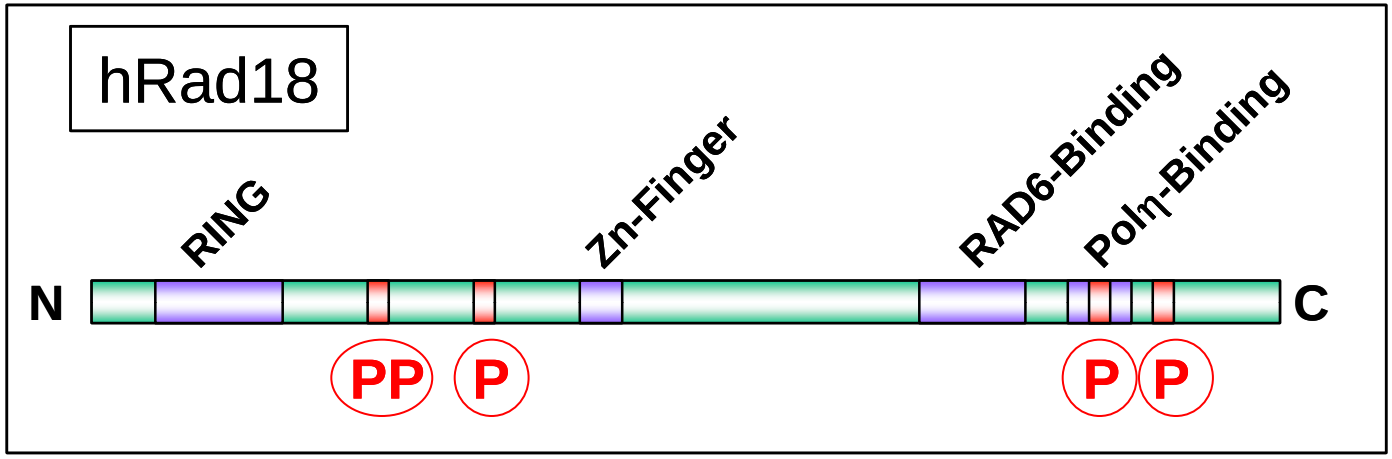
Role of Chk1 in Rad18 Phosphorylation



Rad18 Phosphorylation Sites Identified by Mass Spectrometry



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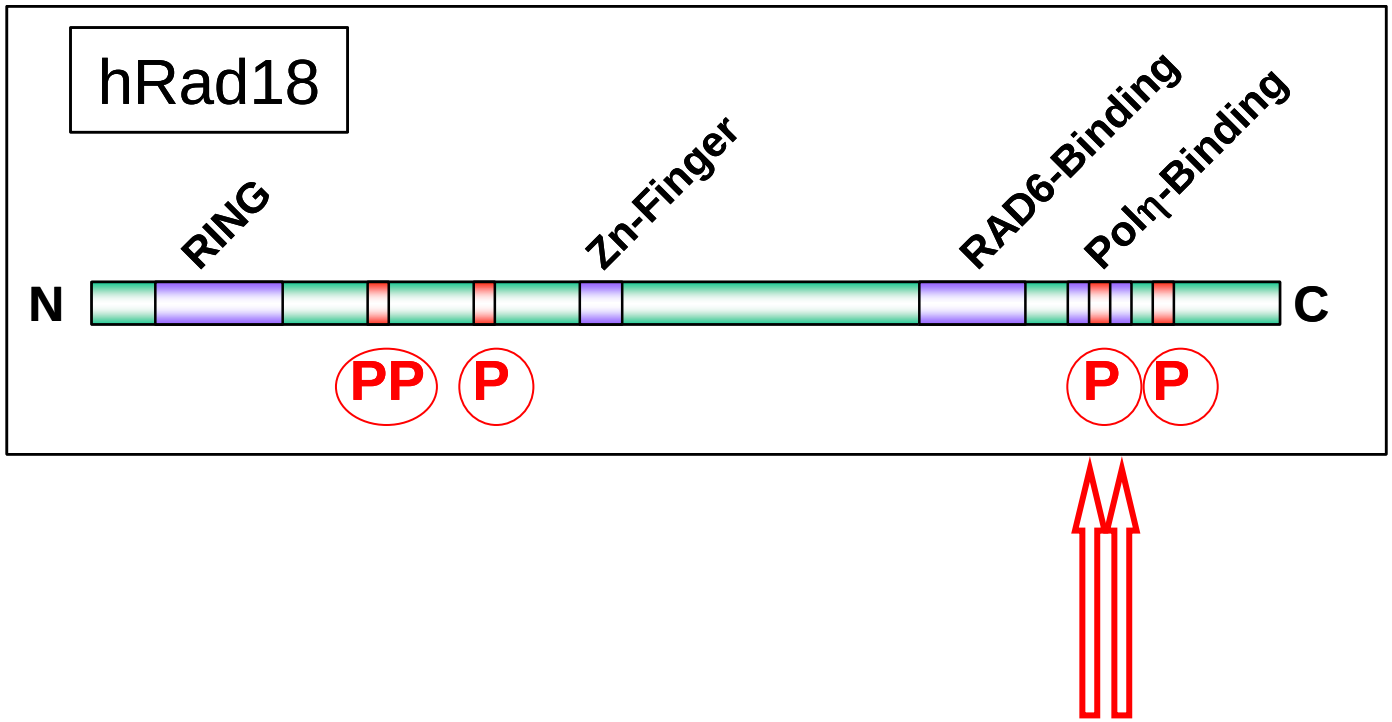

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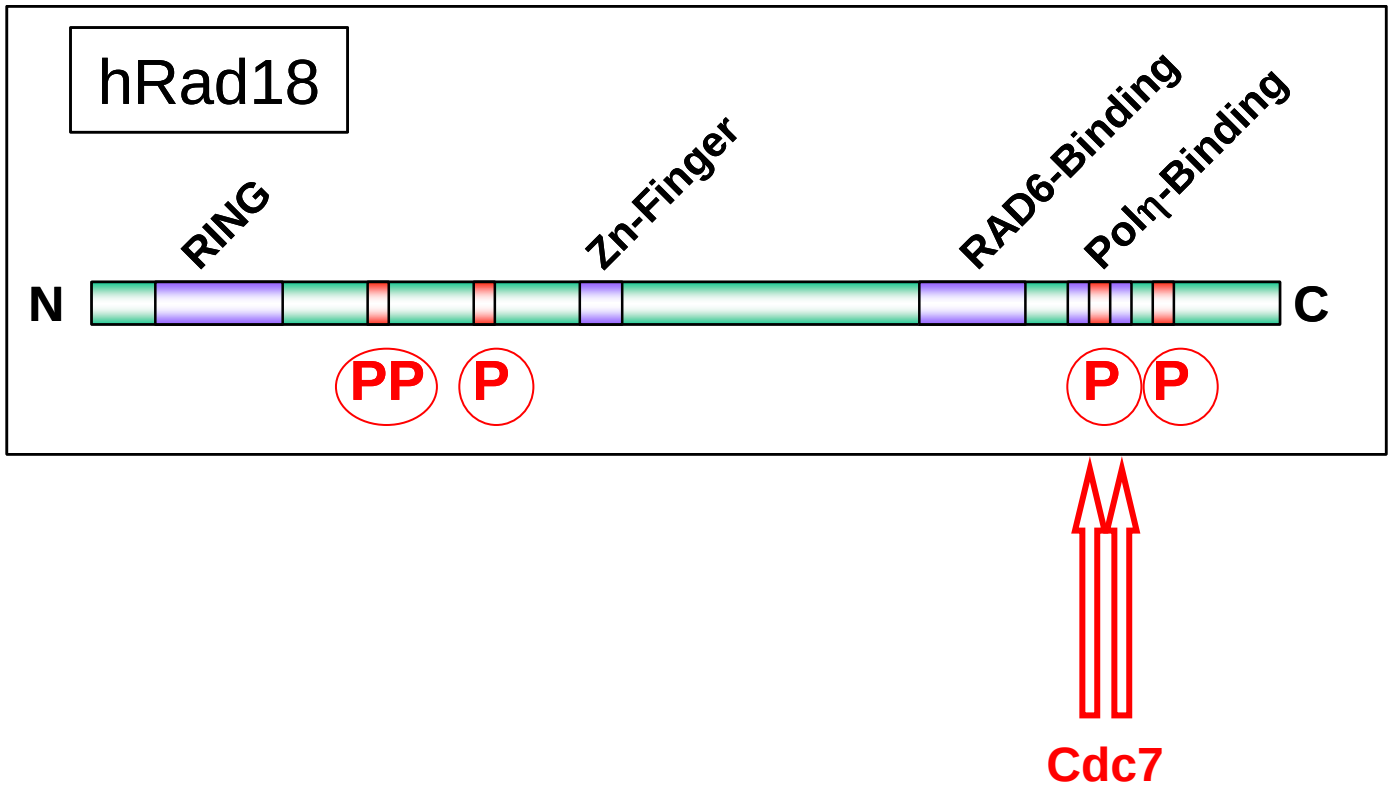

Cdc7 (Dbf4-Dependent Kinase, DDK)

Cdc7 involved in initiation.

Once considered a candidate target of S-checkpoint.

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Now, appears that Cdc7 remains active during rep stress.

Cdc7 Is an Active Kinase in Human Cancer Cells Undergoing Replication Stress*[S]

Received for publication, May 10, 2006, and in revised form, October 5, 2006 Published, JBC Papers in Press, October 24, 2006, DOI 10.1074/jbc.M604457200

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Role of Cdc7 in recovery?

A Fission Yeast Gene, *him1*$^{+}$/*dfp1*$^{+}$, Encoding a Regulatory Subunit for Hsk1 Kinase, Plays Essential Roles in S-Phase Initiation as Well as in S-Phase Checkpoint Control and Recovery from DNA Damage

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Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, [1] and CREST, Japan Science and Technology Corporation (JST),[2] Tokyo 108-8639, Japan

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Role of Cdc7 in recovery?

Role of Cdc7 in TLS?

CDC7/DBF4* Functions in the Translesion Synthesis Branch of the *RAD6* Epistasis Group in *Saccharomyces cerevisiae

Luis Pessoa-Brandão* and Robert A. Sclafani*,†,1

Genetics 167: 1597–1610 (August 2004)

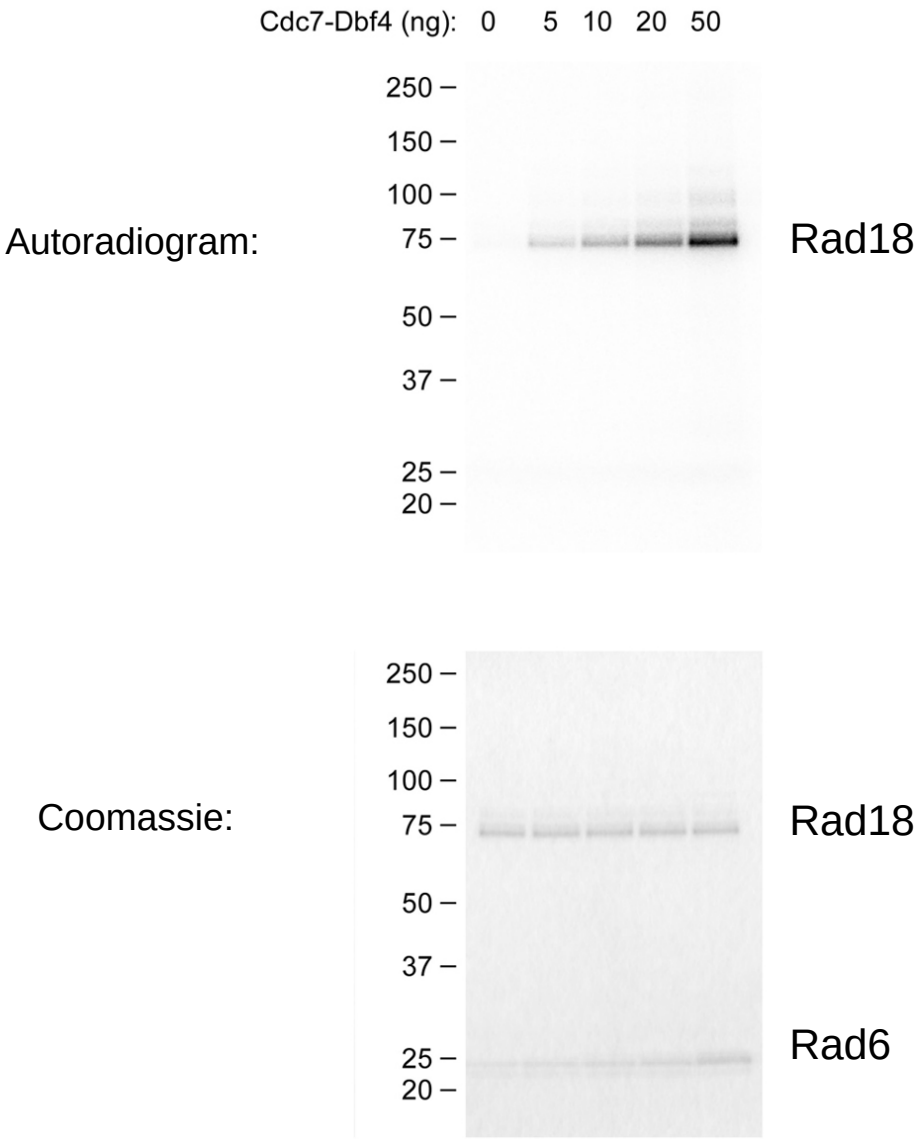
Rad18 regulated by phosphorylation.

Rad18 involved in recovery from replication stress.

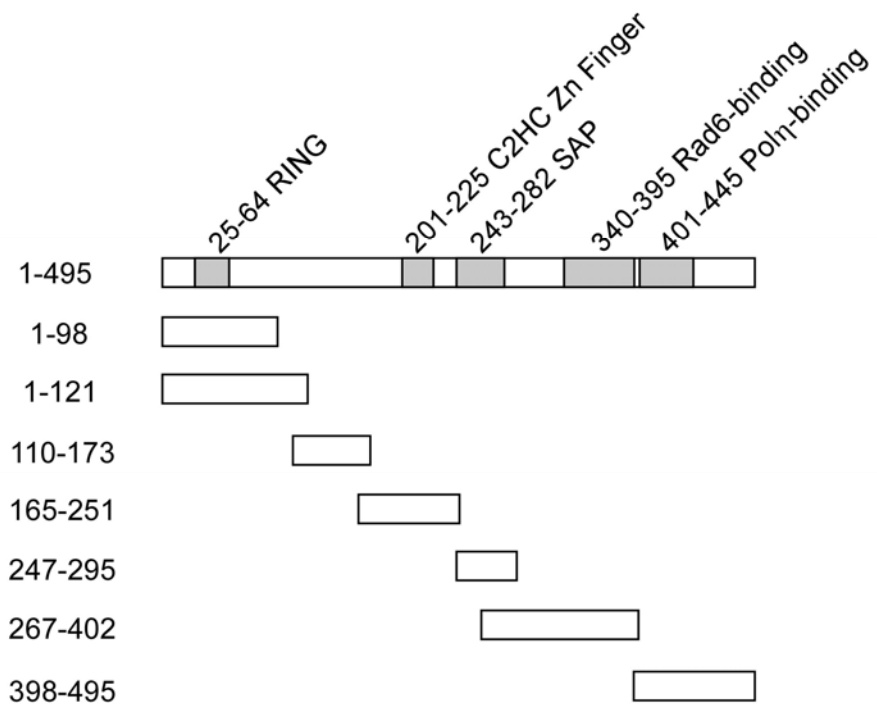
Epistatic relationship between Rad6 and Cdc7.

Is Cdc7 a Rad18-directed kinase?

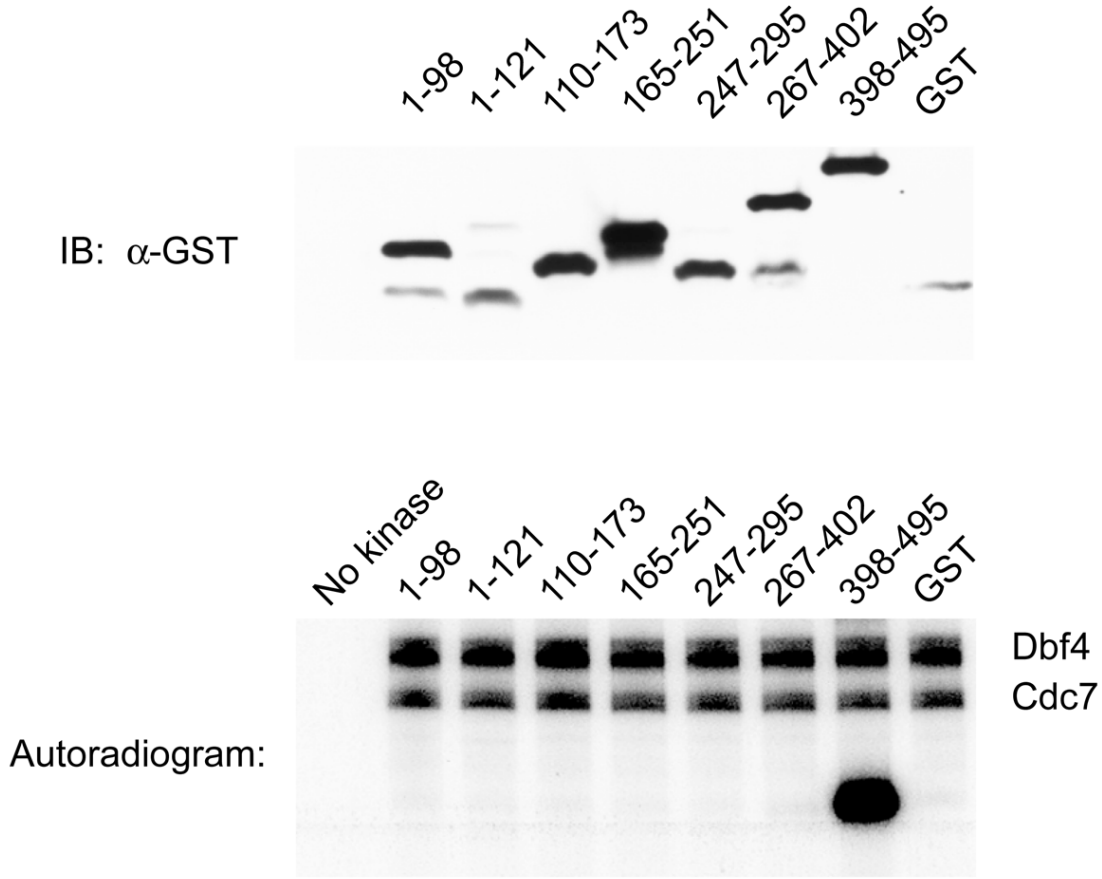
DDK Phosphorylates Rad18-Rad6 Complex



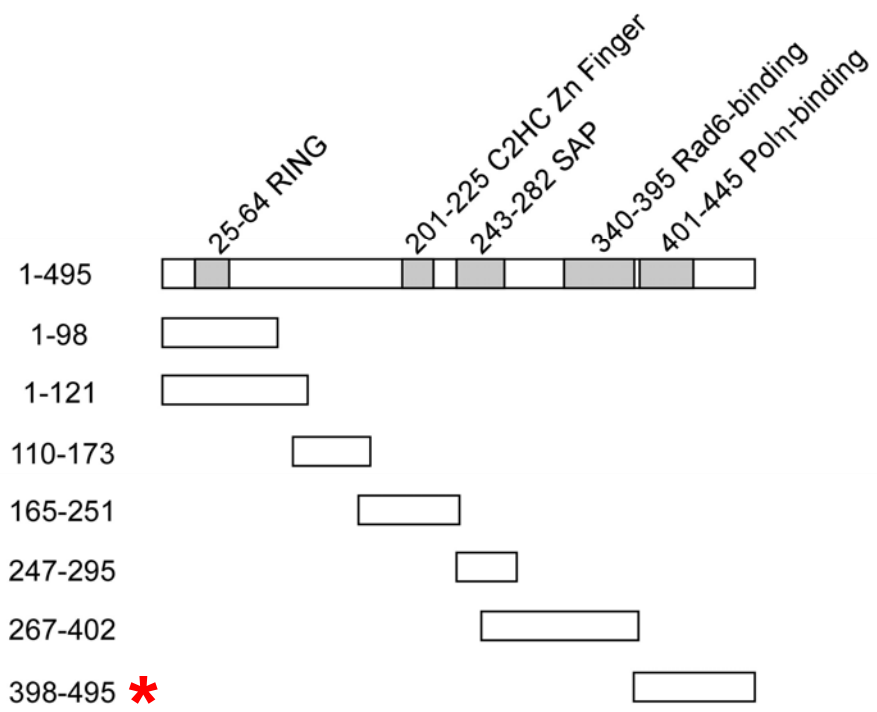
Recombinant Rad18 fragments



Dbf4-Cdc7 Phosphorylates Rad18 *in vitro*



Recombinant Rad18 fragments



Rad18 guides polη to replication stalling sites through physical interaction and PCNA monoubiquitination

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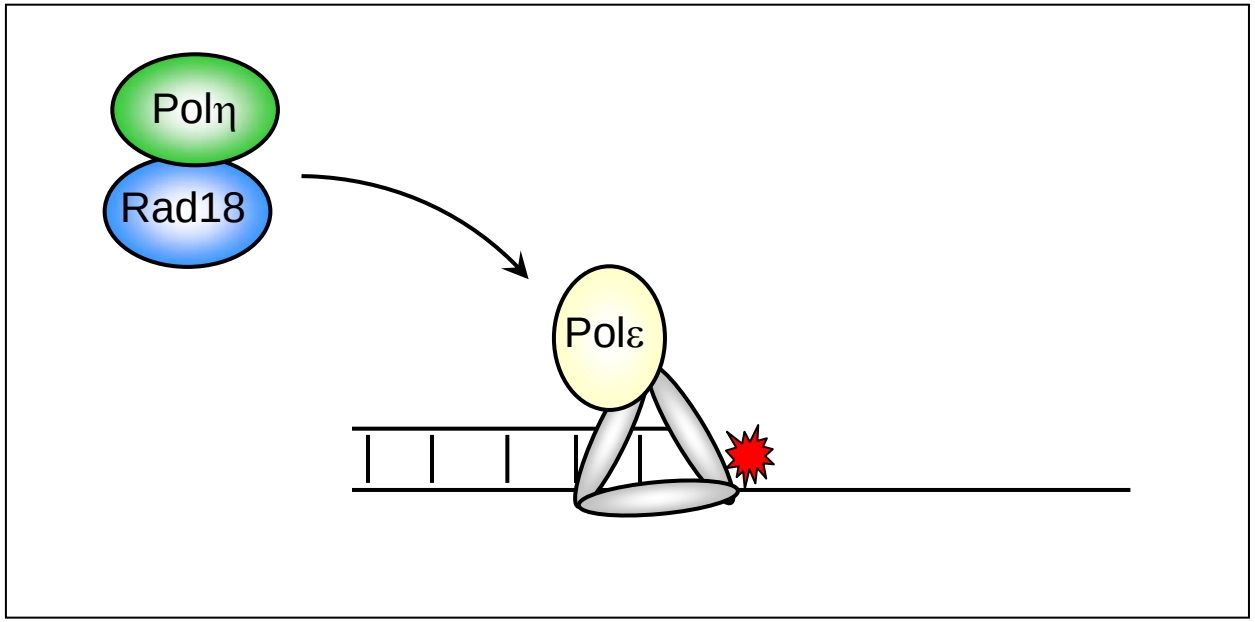
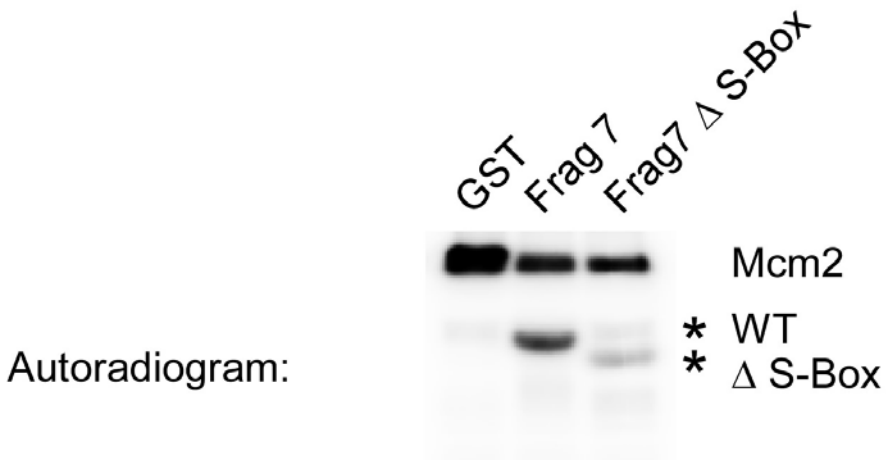
Kenji Watanabe[1,2,5], Satoshi Tateishi[1,5], Michio Kawasuji[2], Toshiki Tsurimoto[3], Hirokazu Inoue[4] and Masaru Yamaizumi[1,*]



Polη-binding motif of Rad18

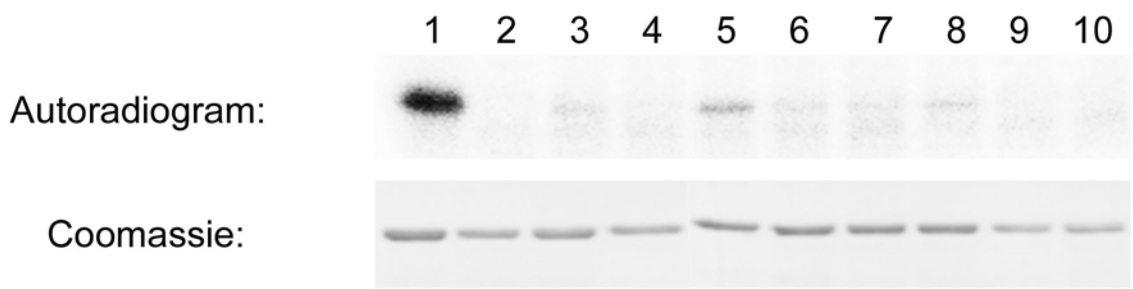
VTNHFSQSKLDSPEELEPDREEDSSSCIDIQEVLSSSESDSCNSSSSDIIRDLLEEEEAWE

403, 405, 409 | 421-423 | S-Box



Alanine-scanning of S-Box

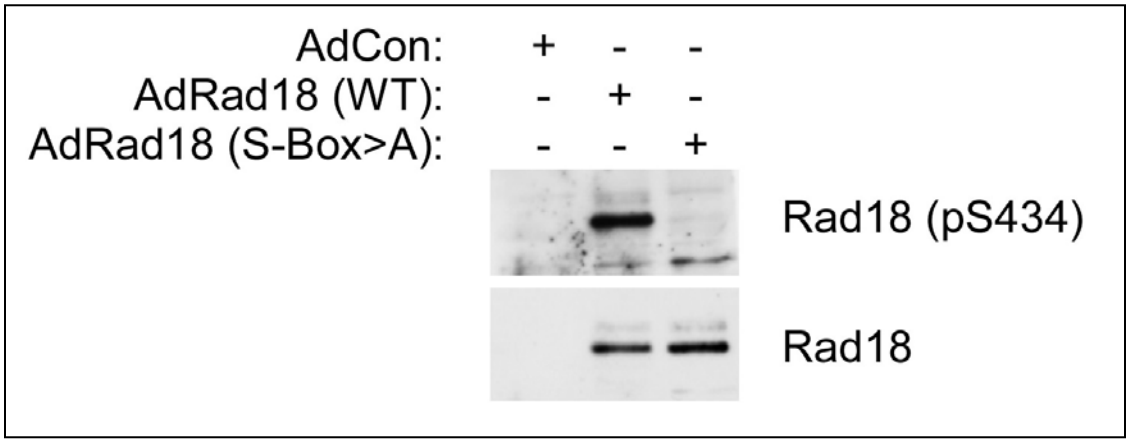
1. WT	LSSSESDSCNSSSSD
2. All A	LAAAEADACNAAAAD
3. 432S	LSAAEADACNAAAAD
4. 433S	LASAEADACNAAAAD
5. 434S	LAASEADACNAAAAD
6. 436S	LAAAESDACNAAAAD
7. 438S	LAAAEADSCNAAAAD
8. 442S	LAAAEADACNASAAD
9. 443S	LAAAEADACNAASAD
10. 444S	LAAAEADACNAAASD

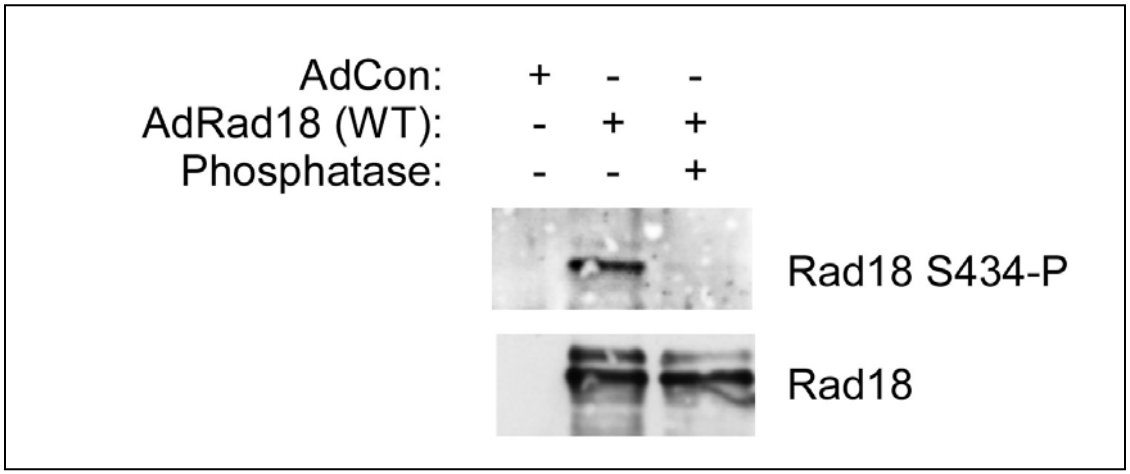


Species Conservation of Rad18 S434

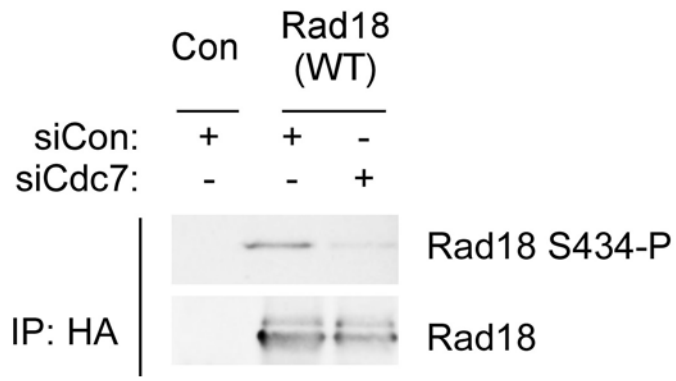
```
human        SSESDSCNSSSSDIIRDLLEEEEAWEASHKN--DLQDTEISPR---QNRRTRAAESAEIE
orangutan    SSESDSCNSSSSDIIRDLLEEEEAWEASHKN--DLQDTEISPR---QNRRTRAAESAEIE
dog          SSESDSFNSSSSDIIRDLLEEEEAWEASHKNDLDLQDAEMSPV---QNRRTRSSENAEIE
mouse        SSDSDSCN--------------------RND---QNREVSPQ---QTRRTRASECVEIE
rat          SSESDSCNSSSSDIIRDLLEEEEAWEAAHKND---QNKEVSPQ---QNRRTRATERAEIE
chicken      FSDSSGSSSSSSDILRDLEAEICSVSSDSN--CLLDLKASKR---KVCQSELSGGDRLL
zebrafish    ISTSLKVKS-------------------------EDTVIELP-----PRSSSPAISEVS
SpoRad18     NSQTGSDGPQS-----------------------------------------------
SceRad18     SDQGTEDQQMEKDQDTVINEDRVAGERNLPNE---DSTDADLS----RELMDLNEYSKDP
```

Rad18 S434 Phosphorylation in Intact Cells

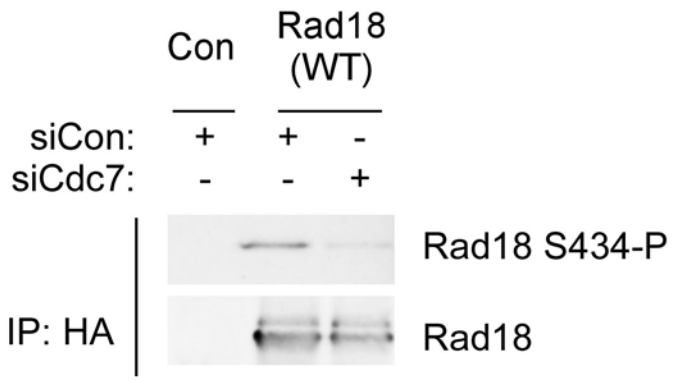


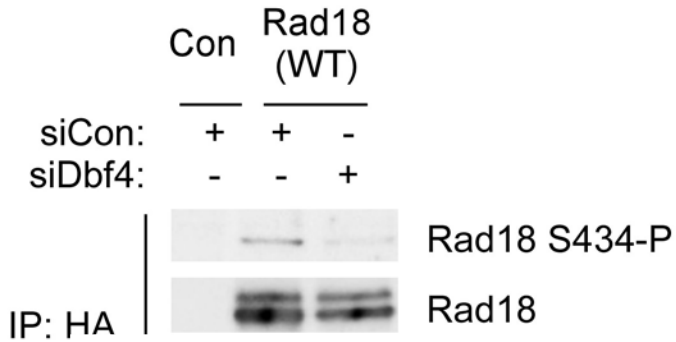


DDK Ablation inhibits S434 phosphorylation and Rad18-Polη association



DDK Ablation inhibits S434 phosphorylation and Rad18-Polη association





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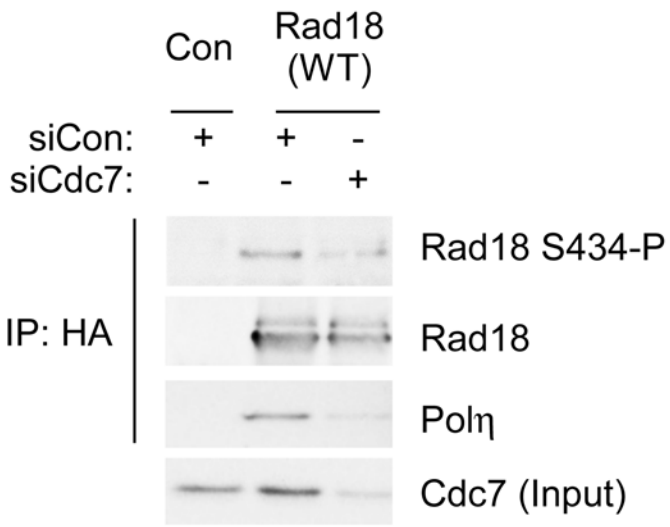

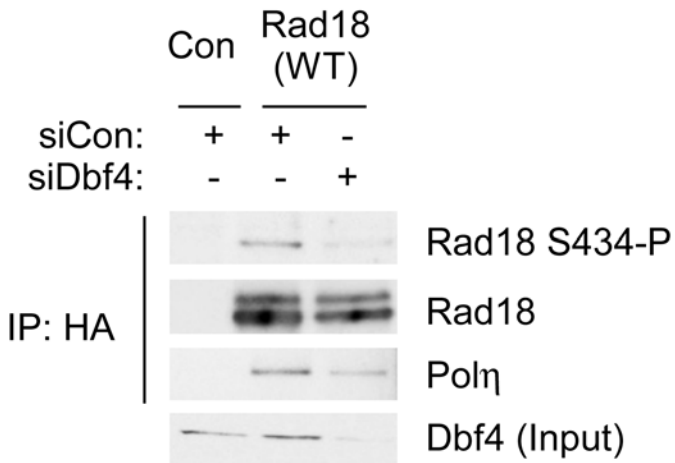

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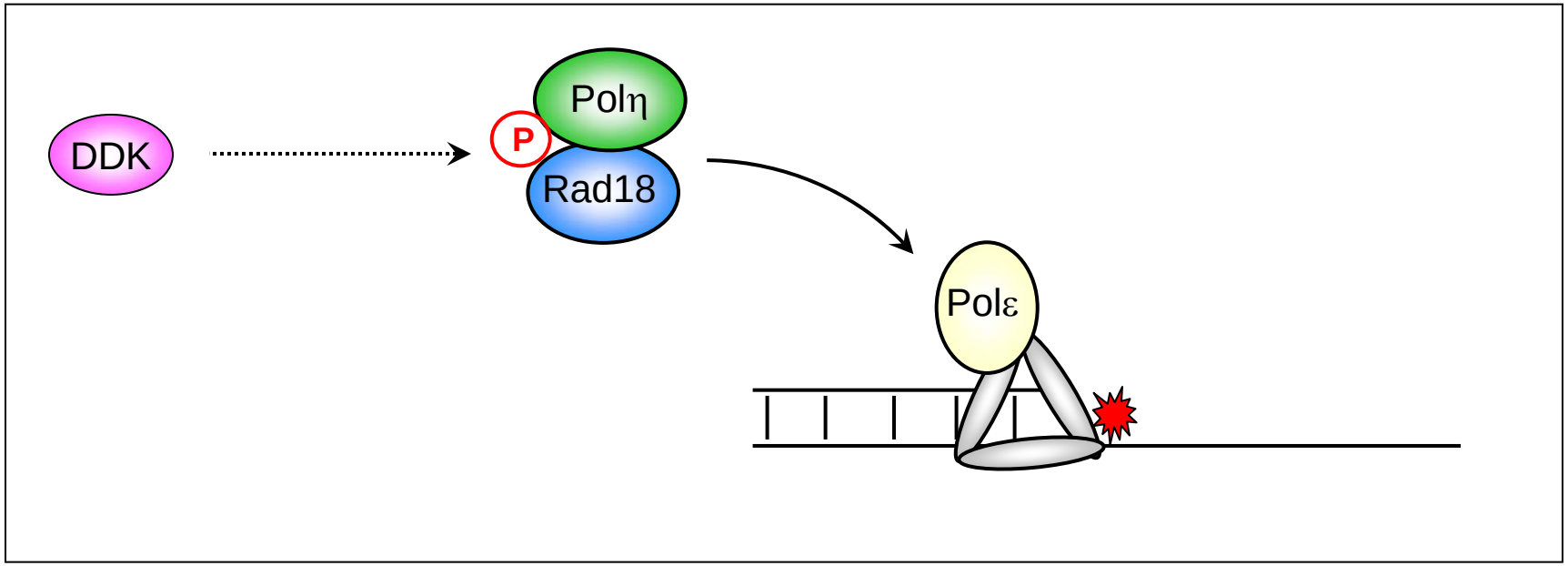
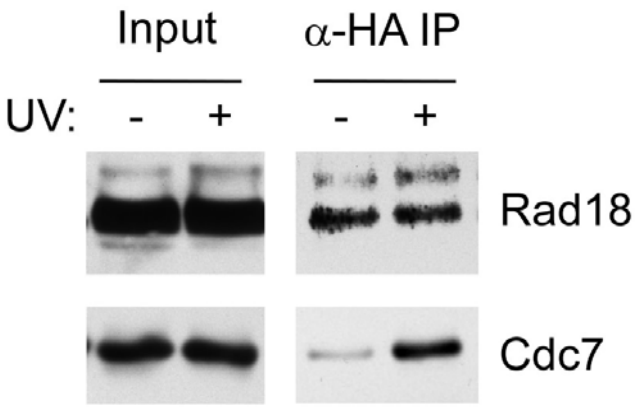
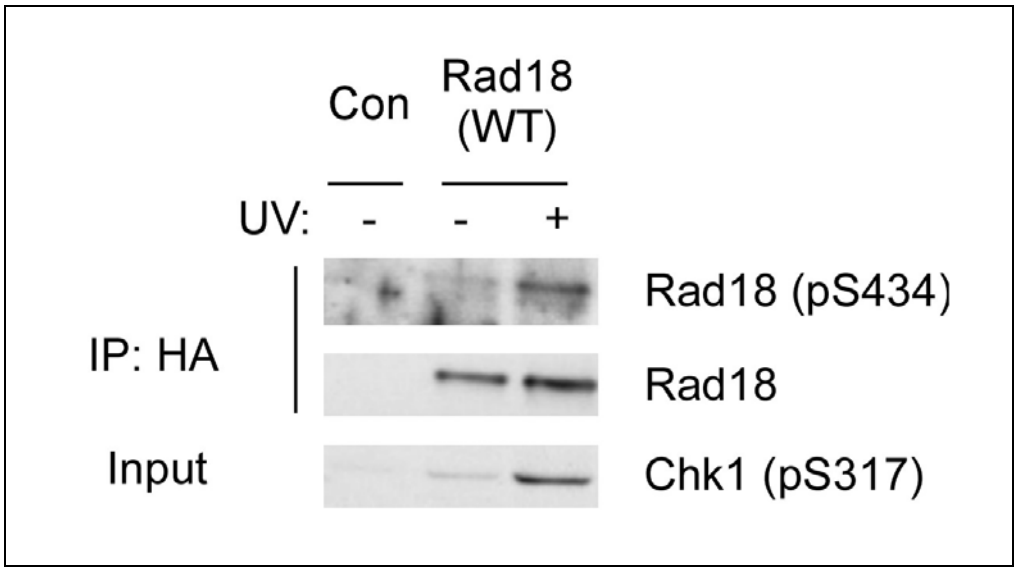


Relationship of Rad18-Cdc7 during genotoxin response?

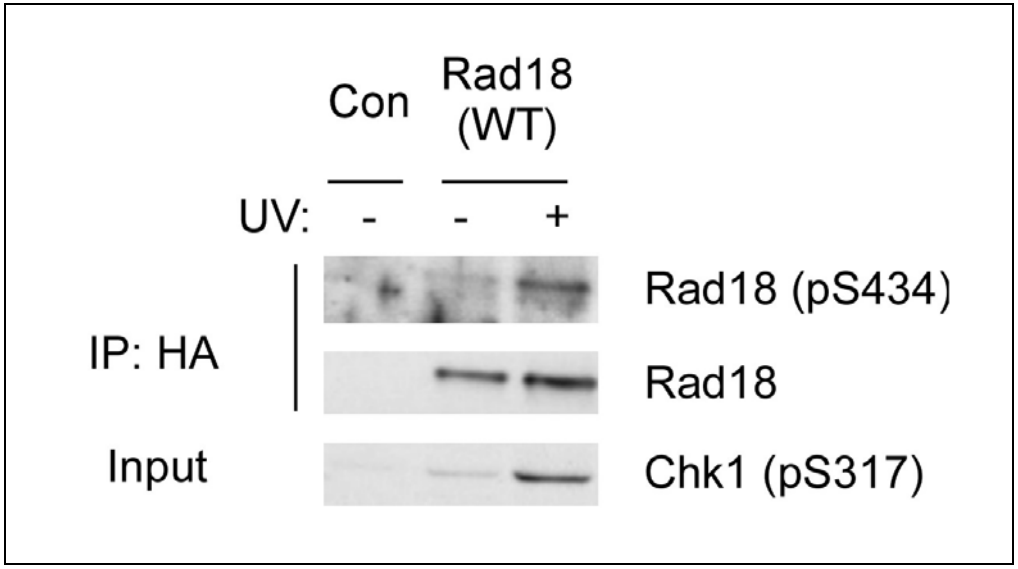
UV-Induced Association of Rad18 with Cdc7



UV-Induced Phosphorylation of Rad18 S434

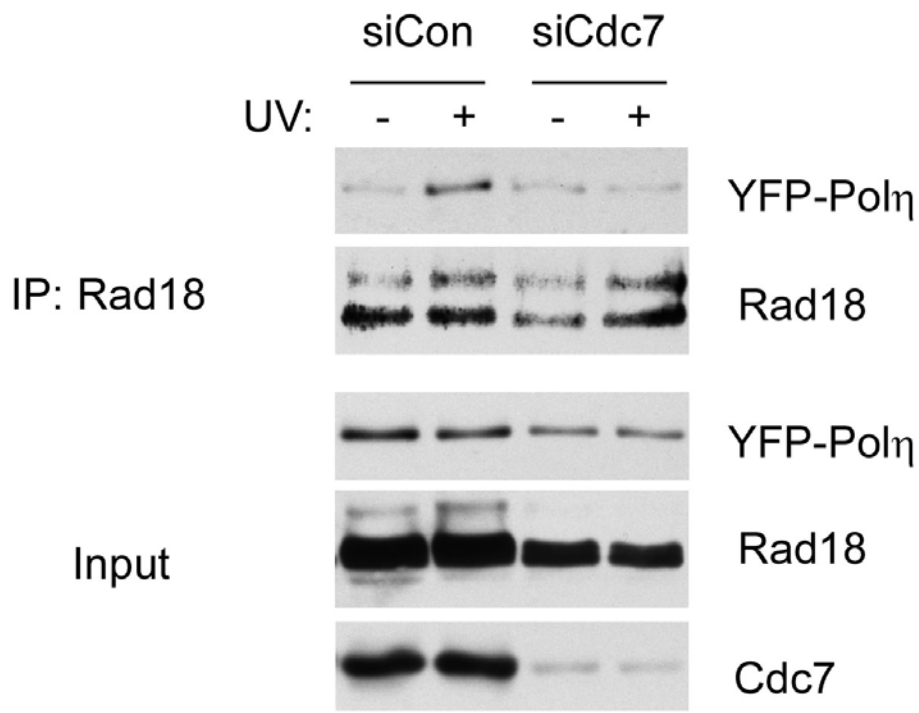


UV-Induced Phosphorylation of Rad18 S434

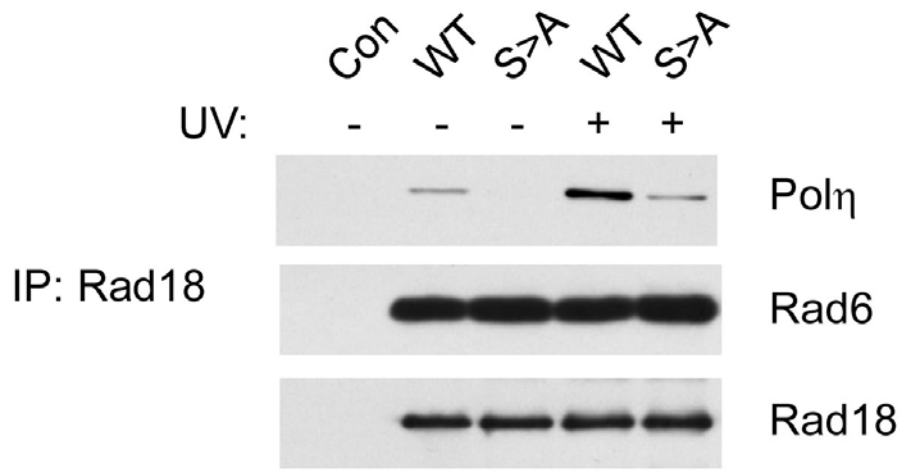


Is Cdc7 Necessary for DNA damage-induced Rad18-Polη Association?

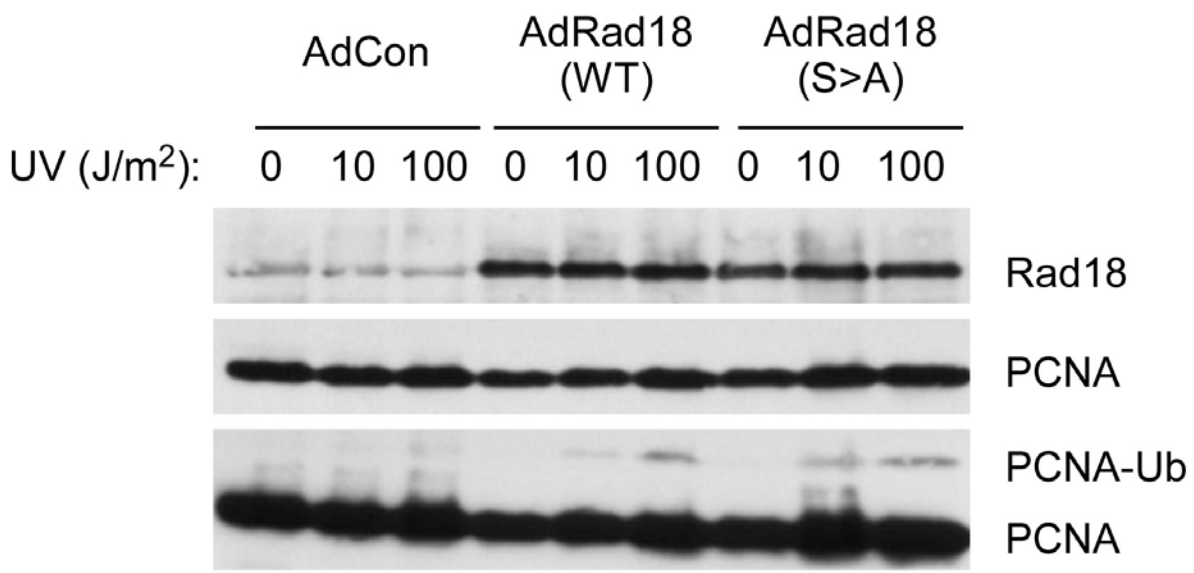
UV-induced Rad18-Polη interaction is Cdc7-dependent



S434 phosphorylation Required for Association of Rad18 with Polη but not Rad6



Rad18 S434 phosphorylation does not affect PCNA-directed E3 ligase activity



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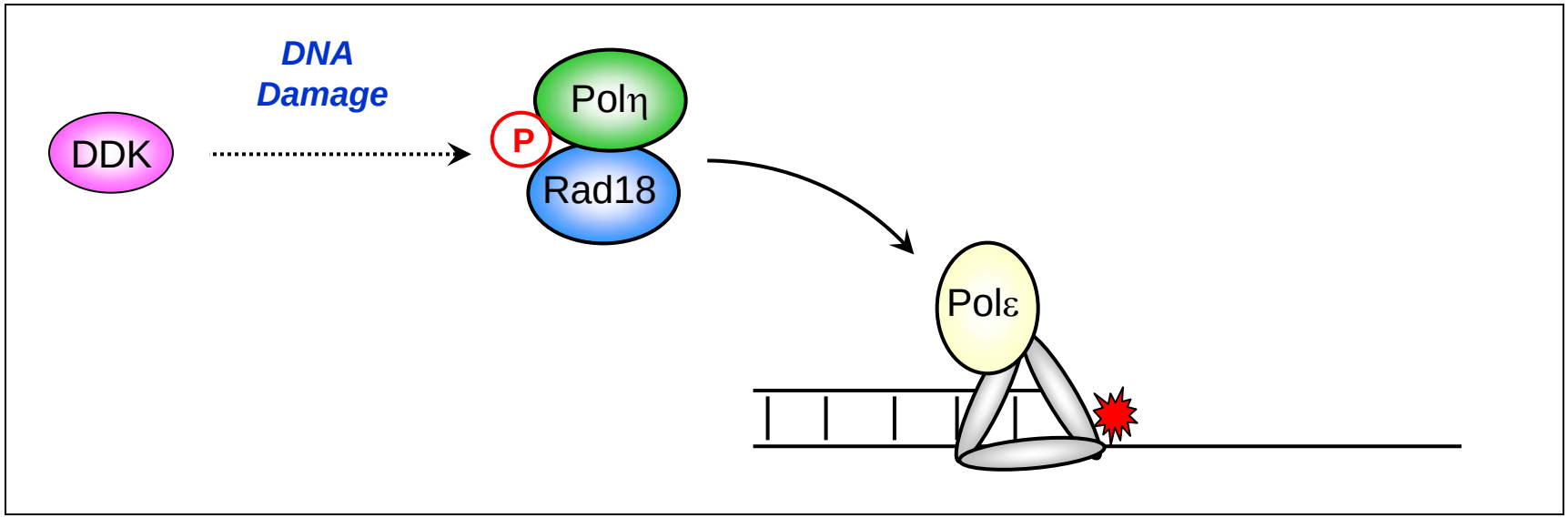
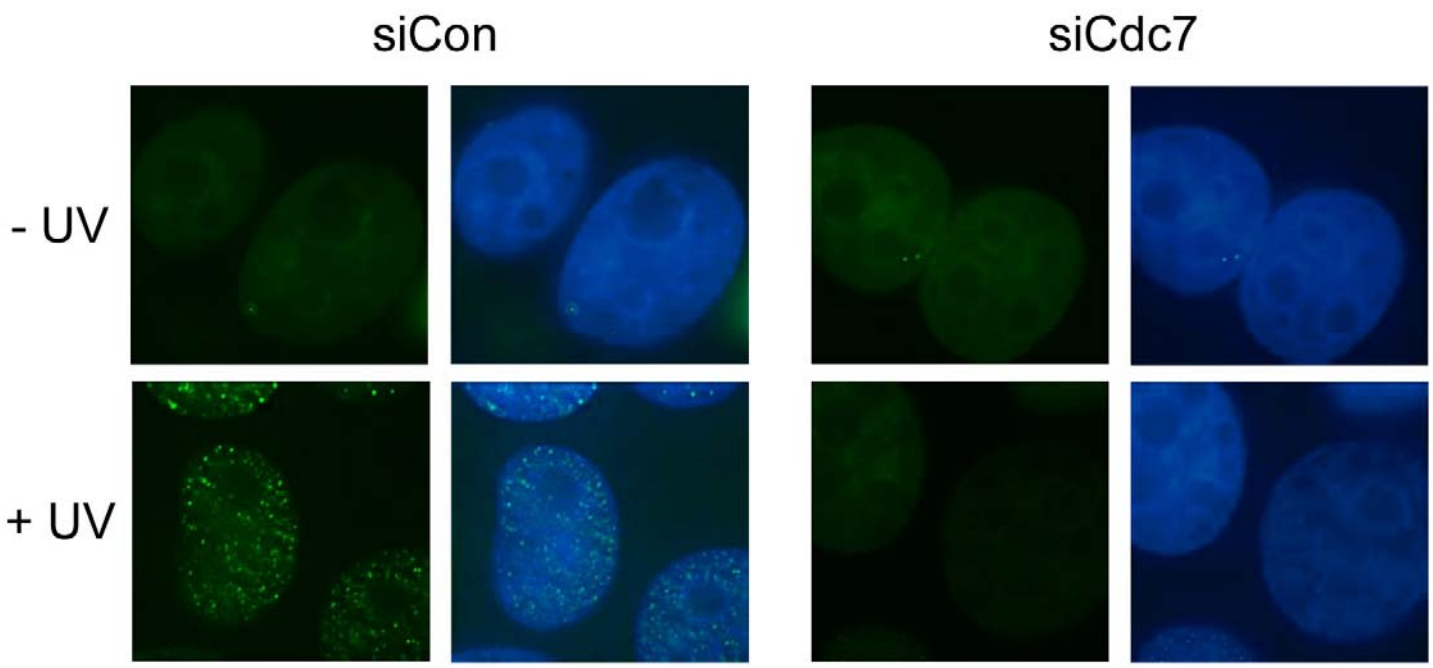
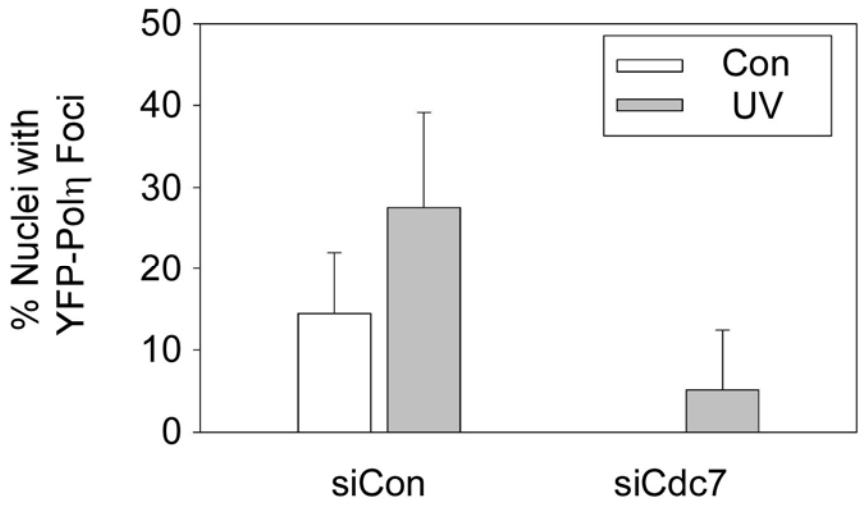


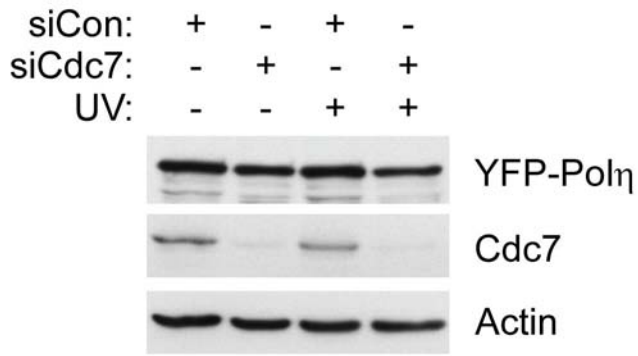
Prediction: DDK necessary for guiding of Polη to replication foci by Rad18

Effect of Cdc7 Depletion on Polη foci

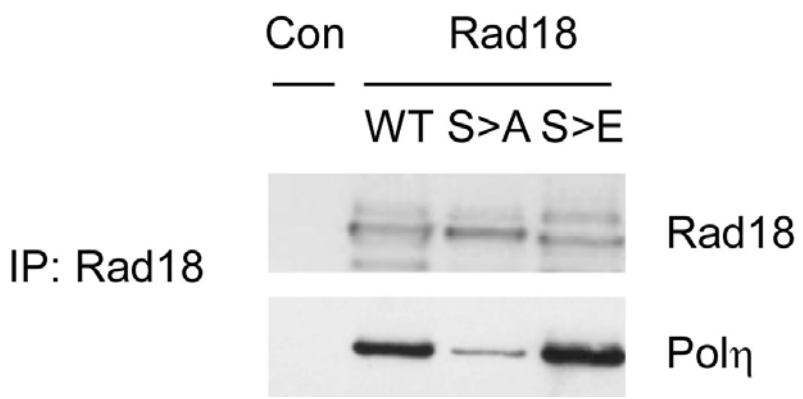


Effect of Cdc7 Depletion on Polη foci

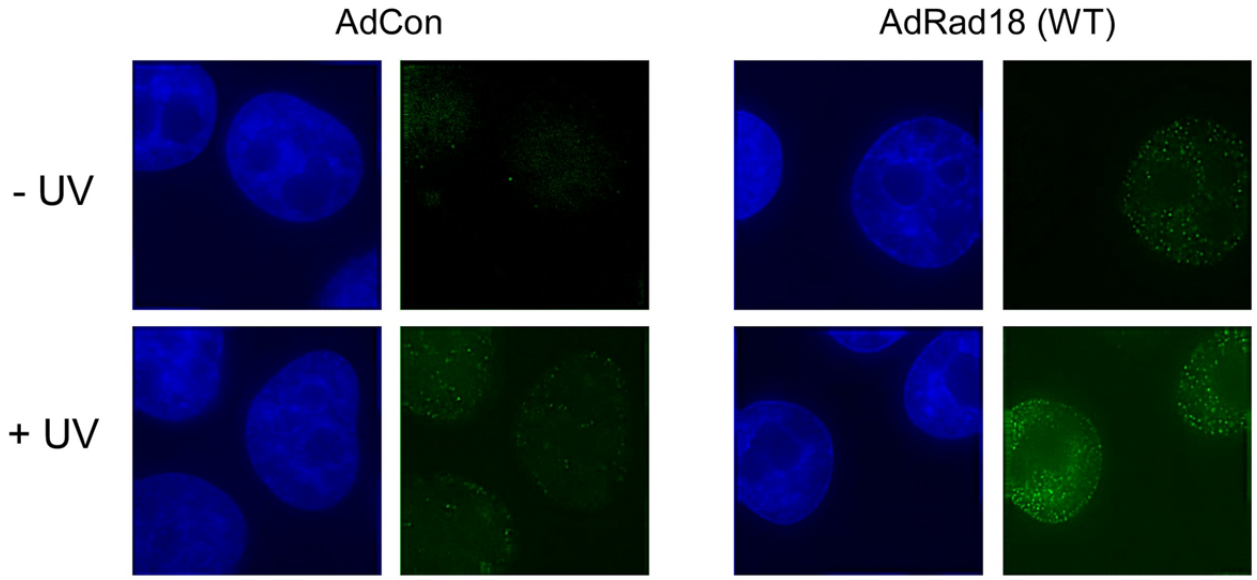




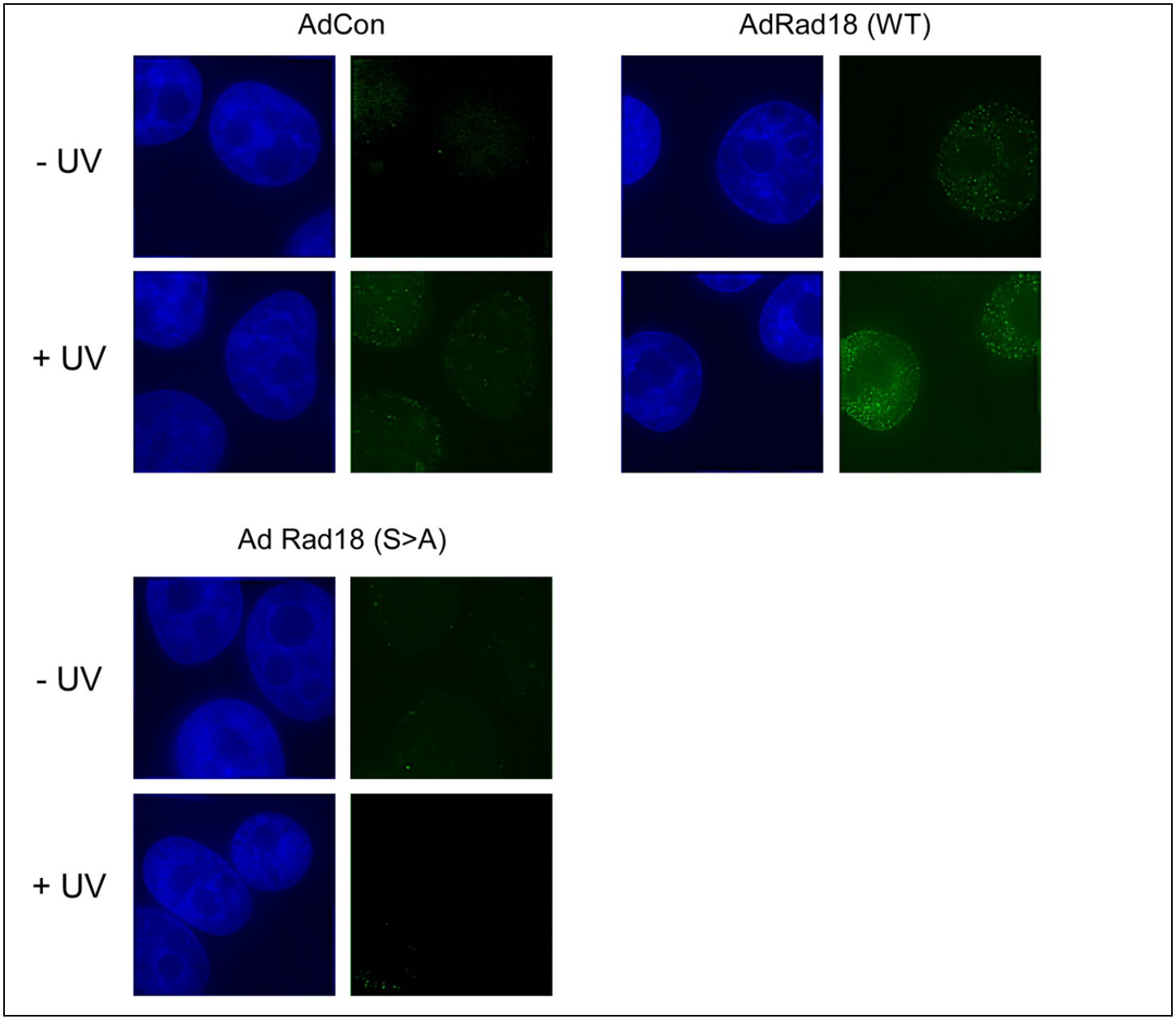
Effect of S434>A and S434>E substitutions on Rad18-Polη association



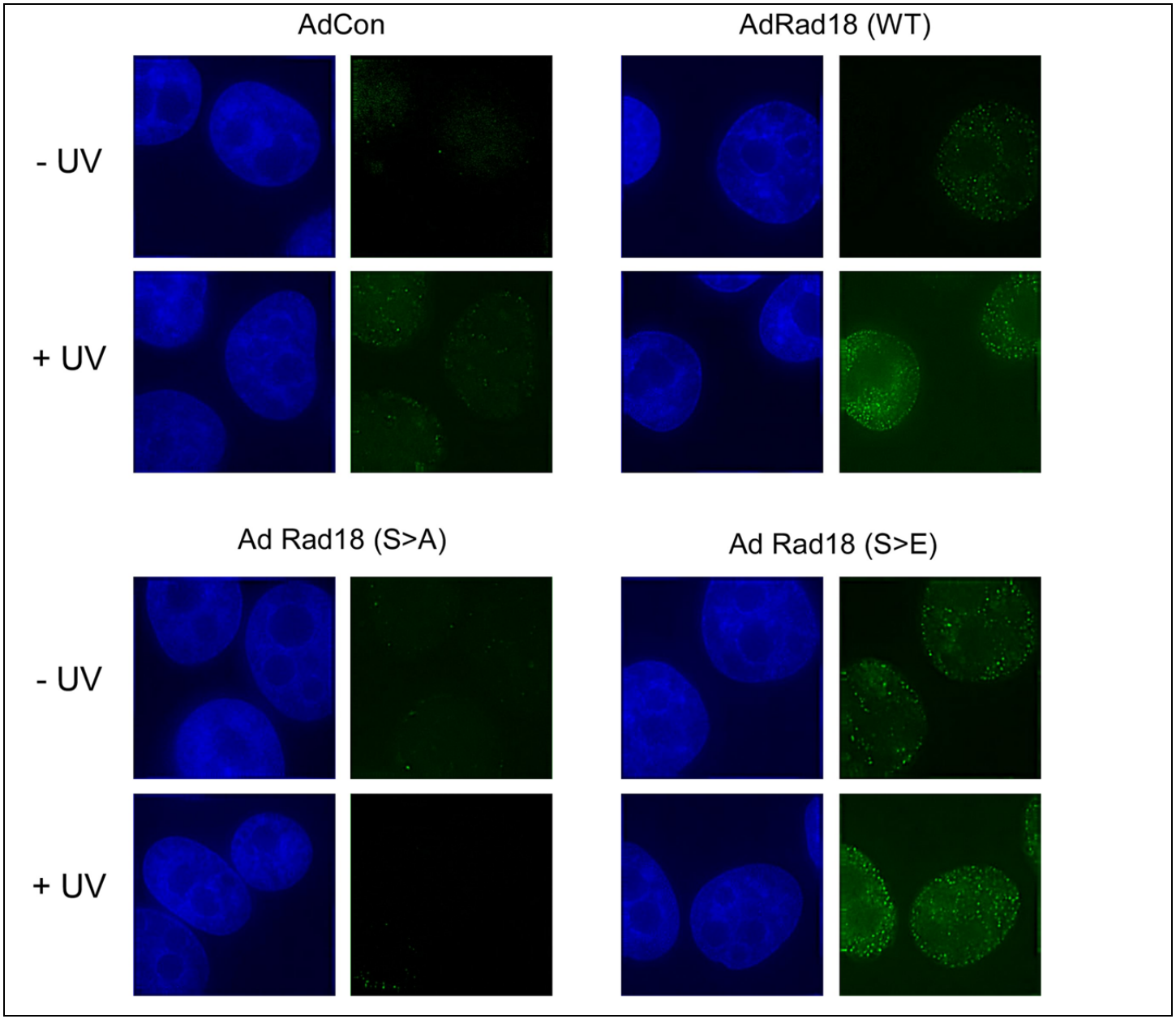
Effect of S434>A and S434>E substitutions on Polη foci



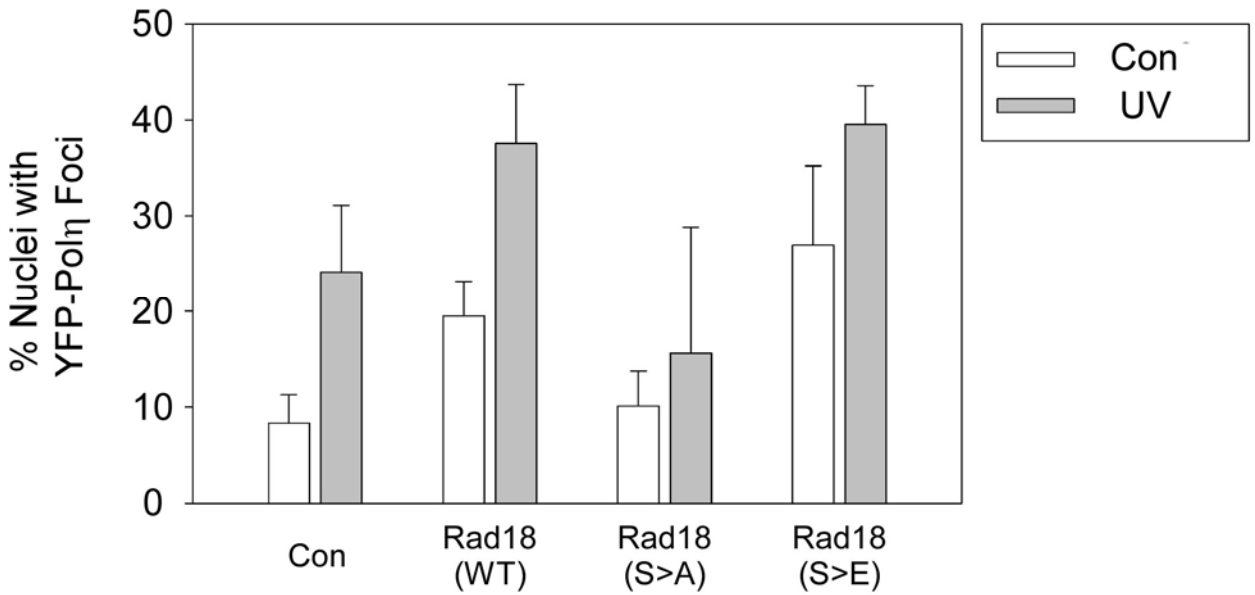
Effect of S434>A and S434>E substitutions on Polη foci

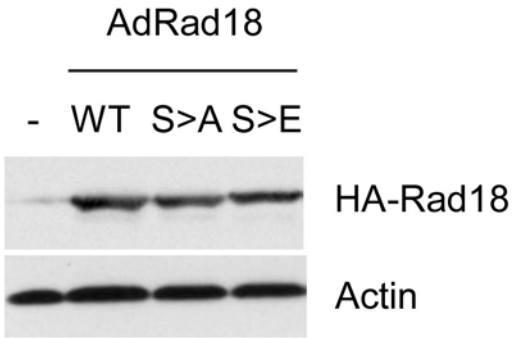


Effect of S434>A and S434>E substitutions on Polη foci



Effect of S434>A and S434>E substitutions on Polη foci



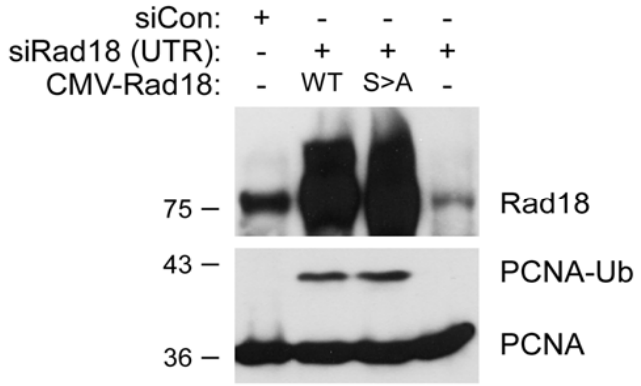


Biological Consequences of Failure to Recruit Polη Efficiently

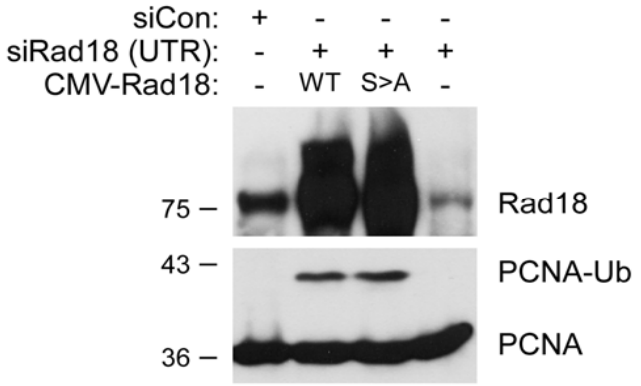
XPV cells are often UV-sensitive.

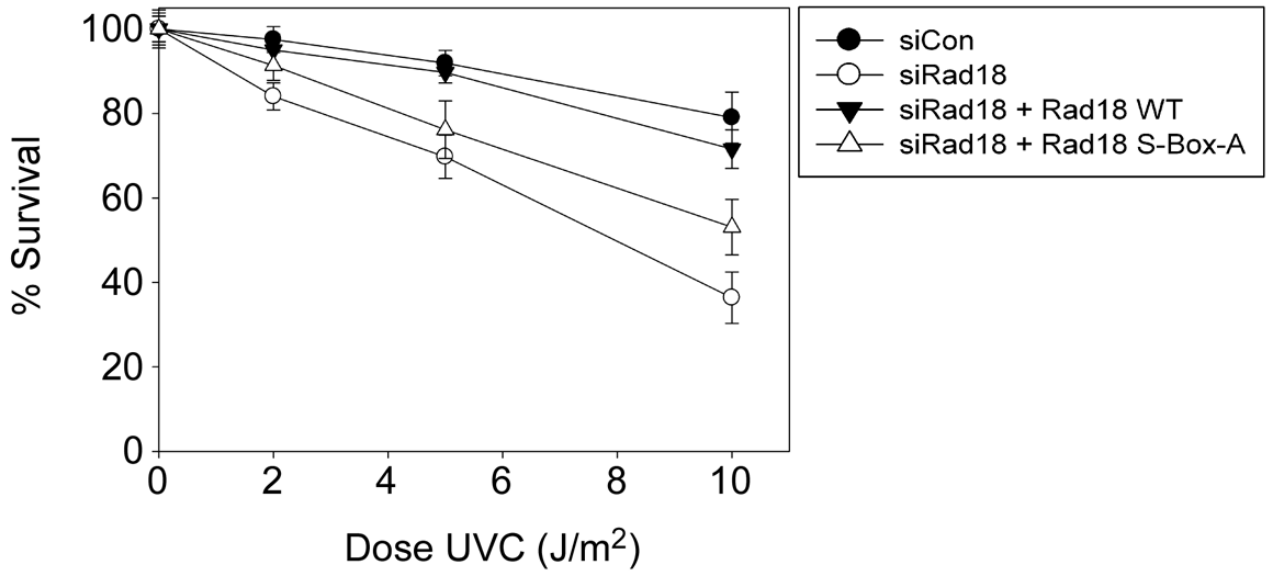
Does Phosphorylation-resistant Rad18 Phenocopy XPV?

Effect of Rad18 S434>A substitution on UV-tolerance

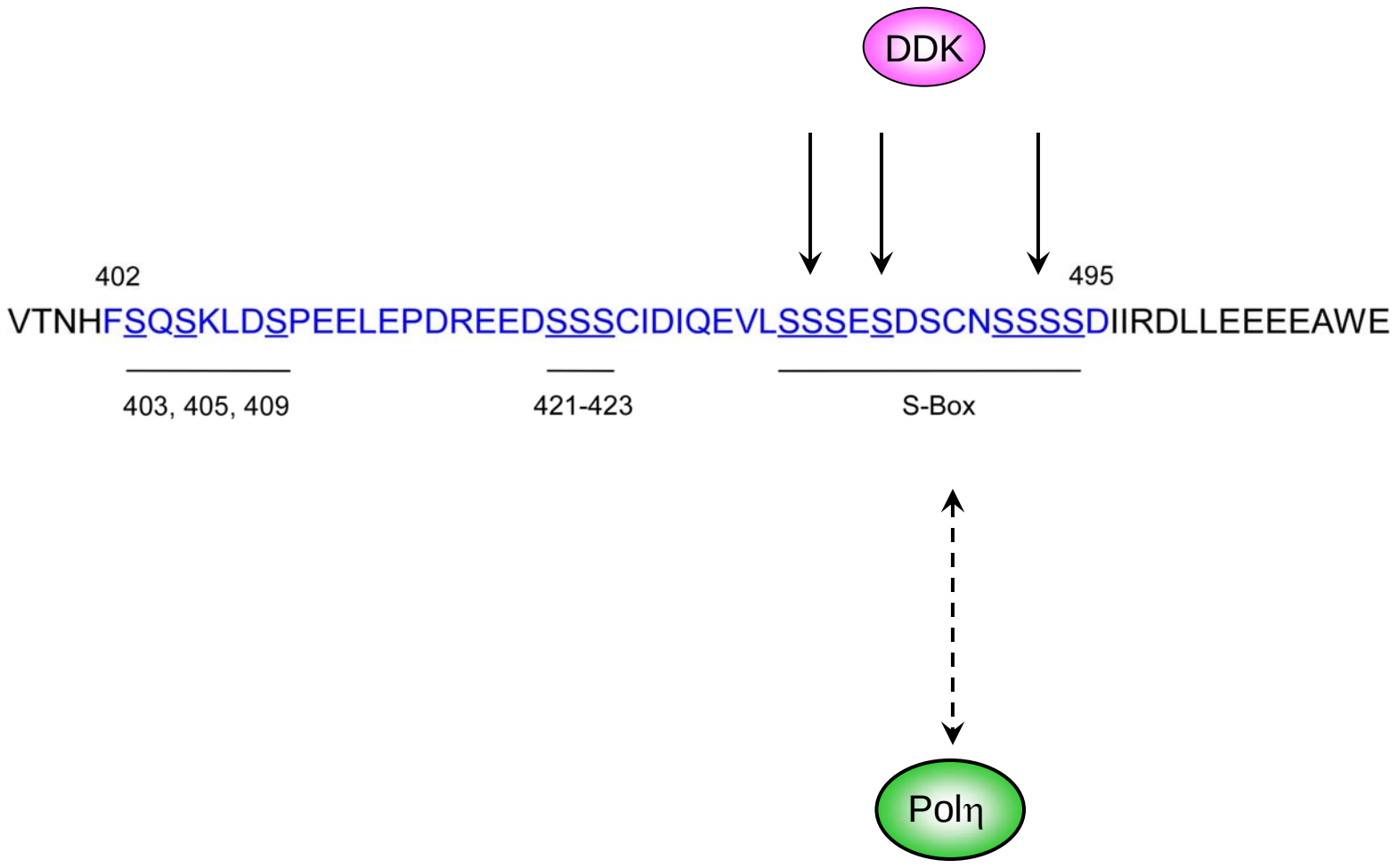


Effect of Rad18 S434>A substitution on UV-tolerance

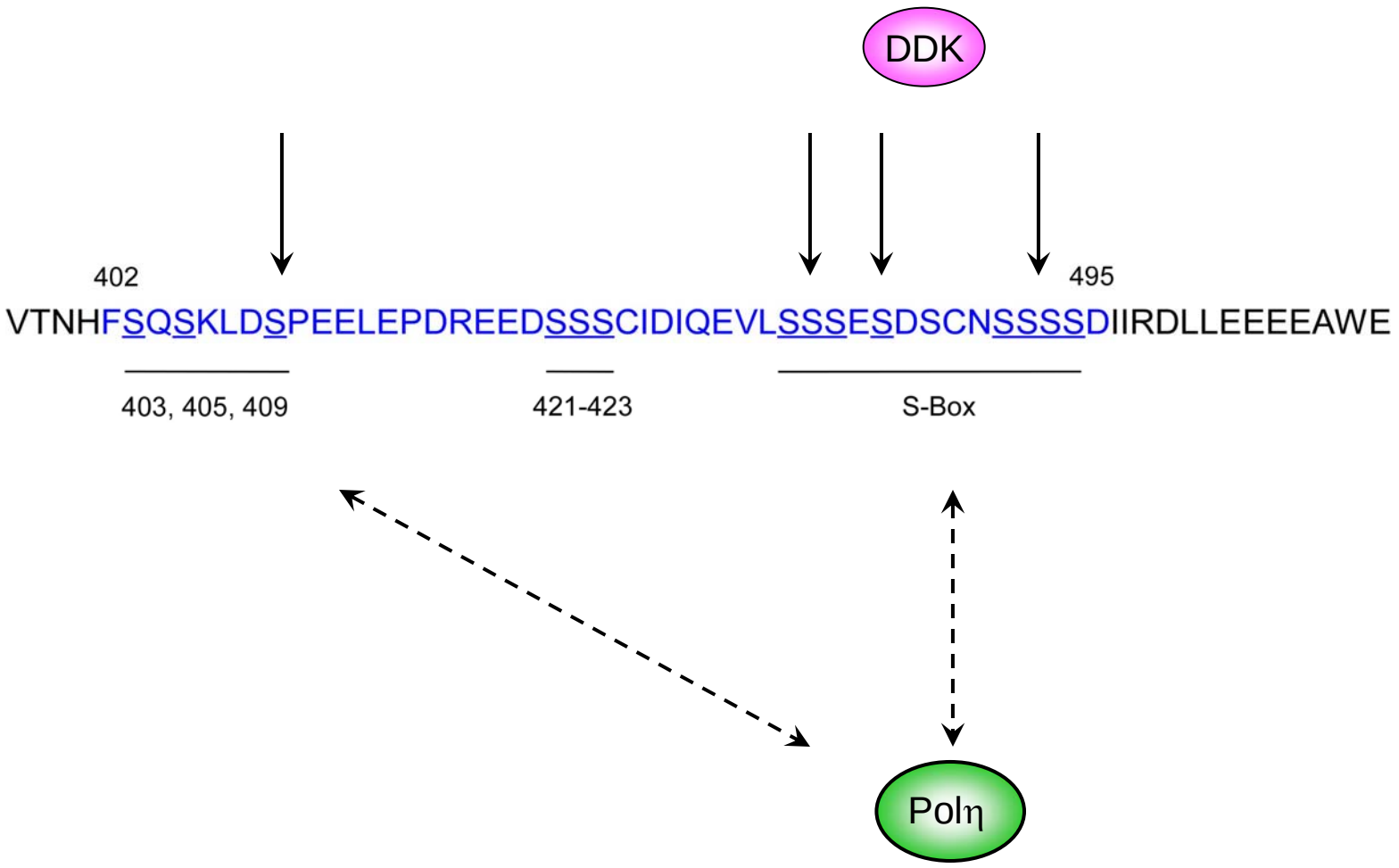




Additional Phosphorylation Sites in Polη-Binding Domain of Rad18



Additional Phosphorylation Sites in Polη-Binding Domain of Rad18



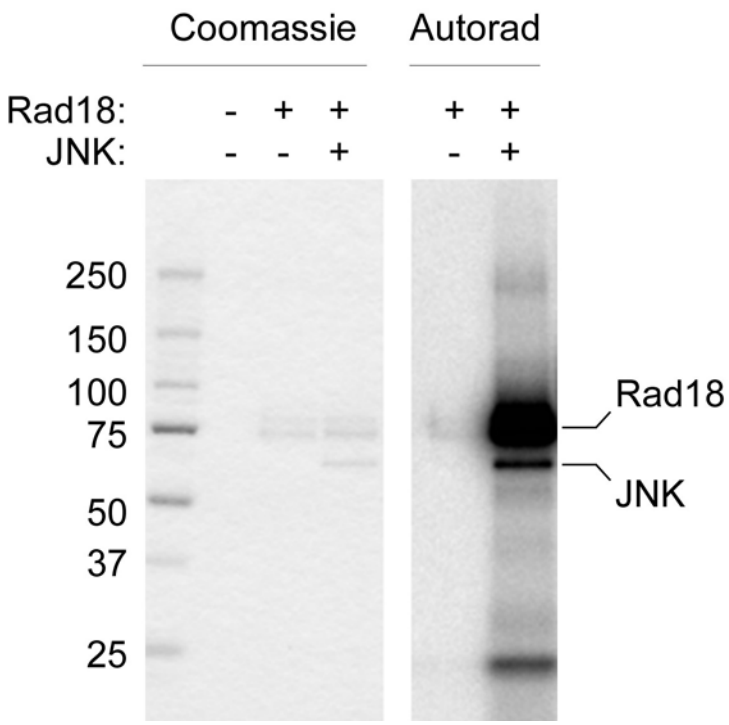
JNK Phosphorylates Rad18 *in vitro*

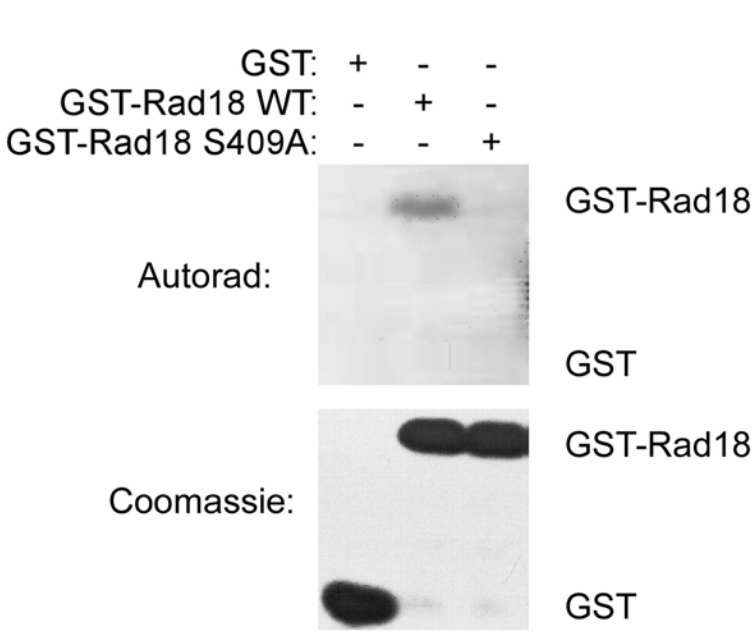
Rad18 (S409):	KLDSPEEL
AP-1, JunD, JunB, ATF-2:	KLASPELE

JNK Phosphorylates Rad18 *in vitro*

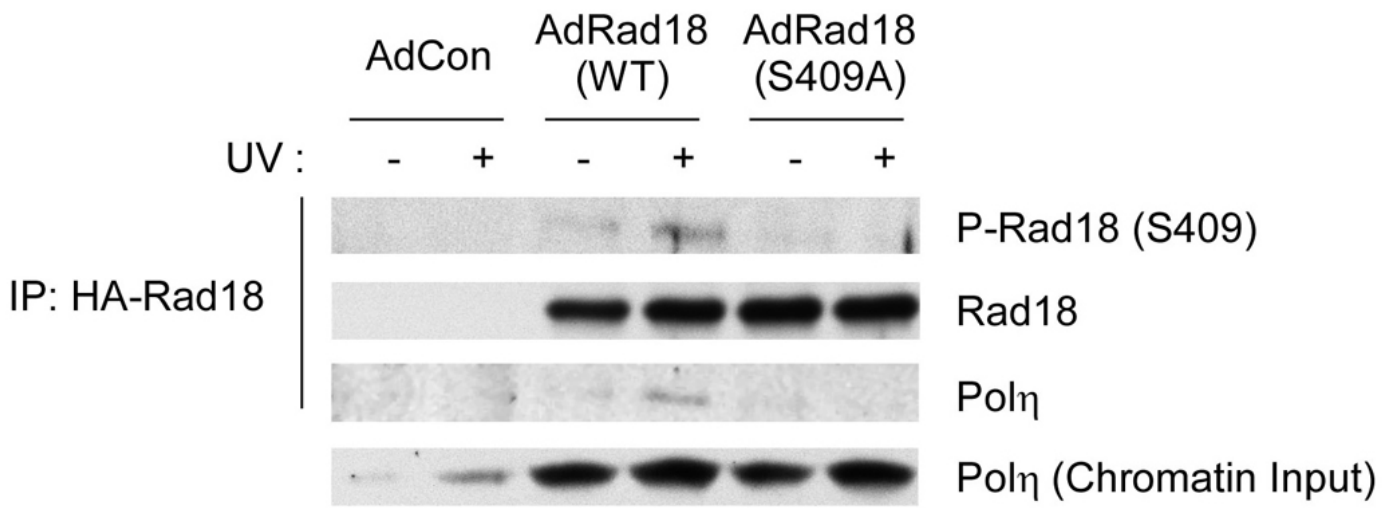
Rad18 (S409): KLDSPEEL

AP-1, JunD, JunB, ATF-2: KLASPELE

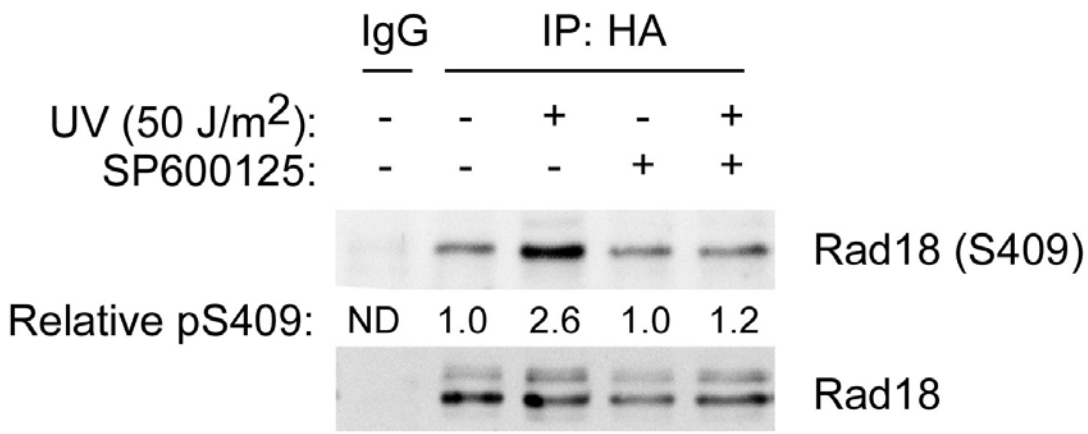


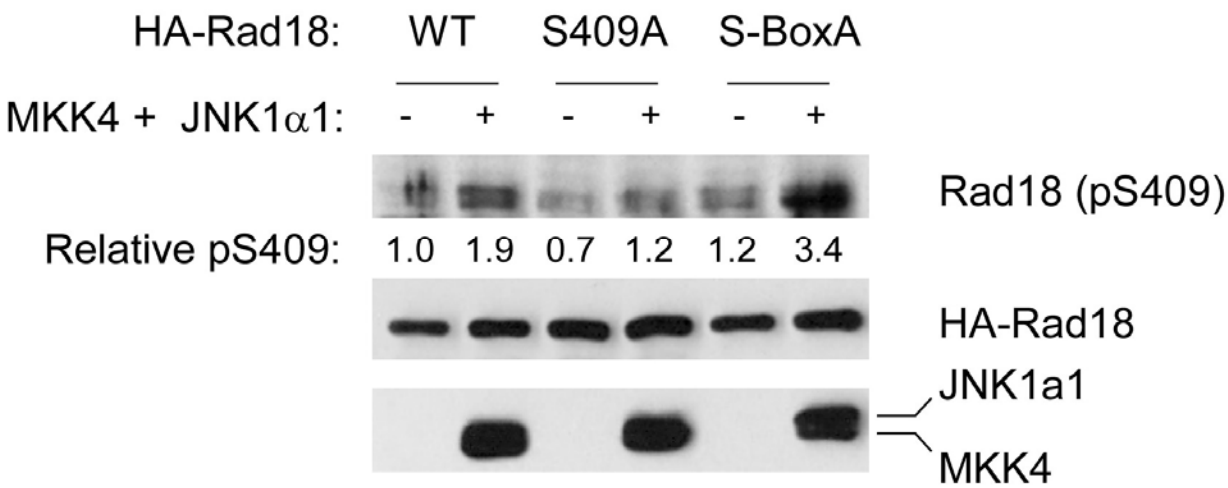


UV-inducible Rad18 S409 Phosphorylation in Intact Cells

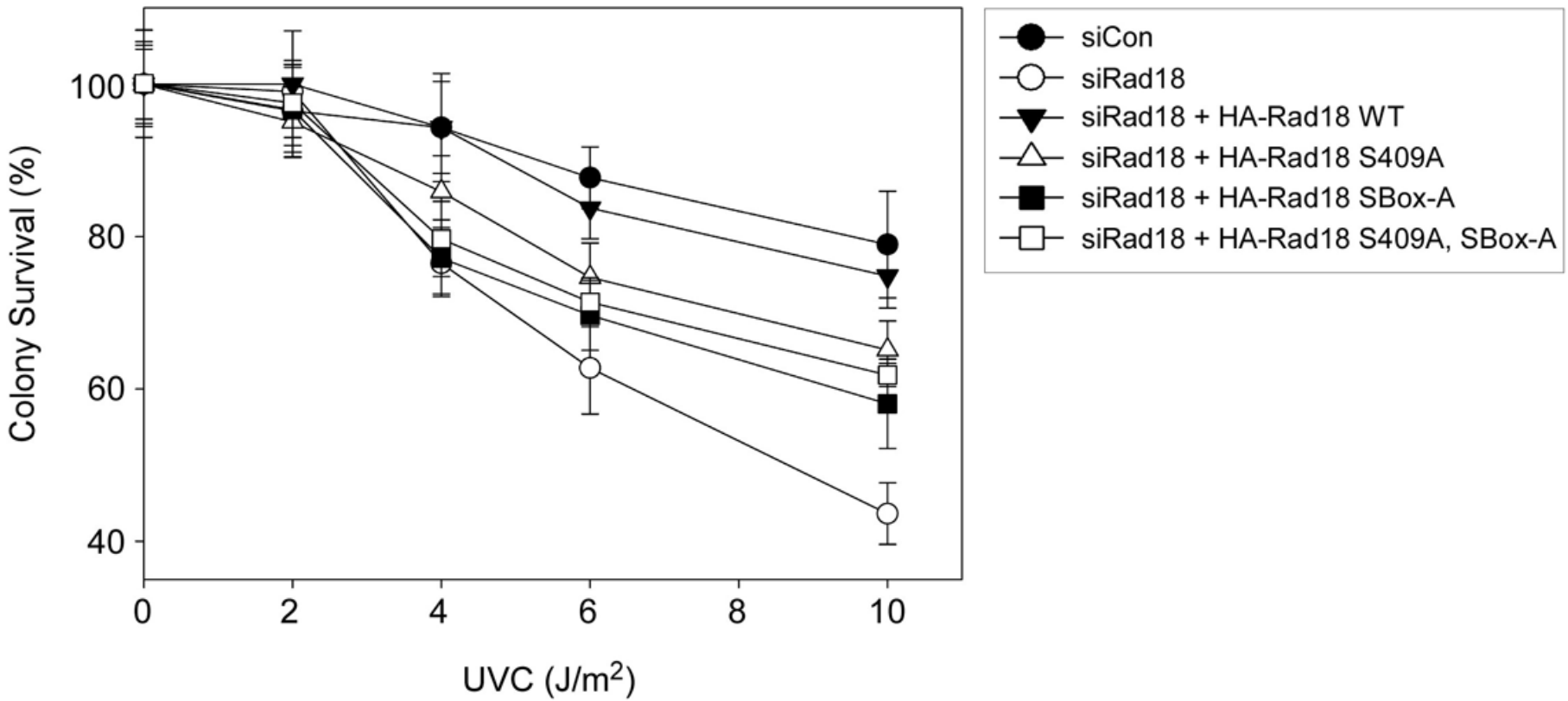


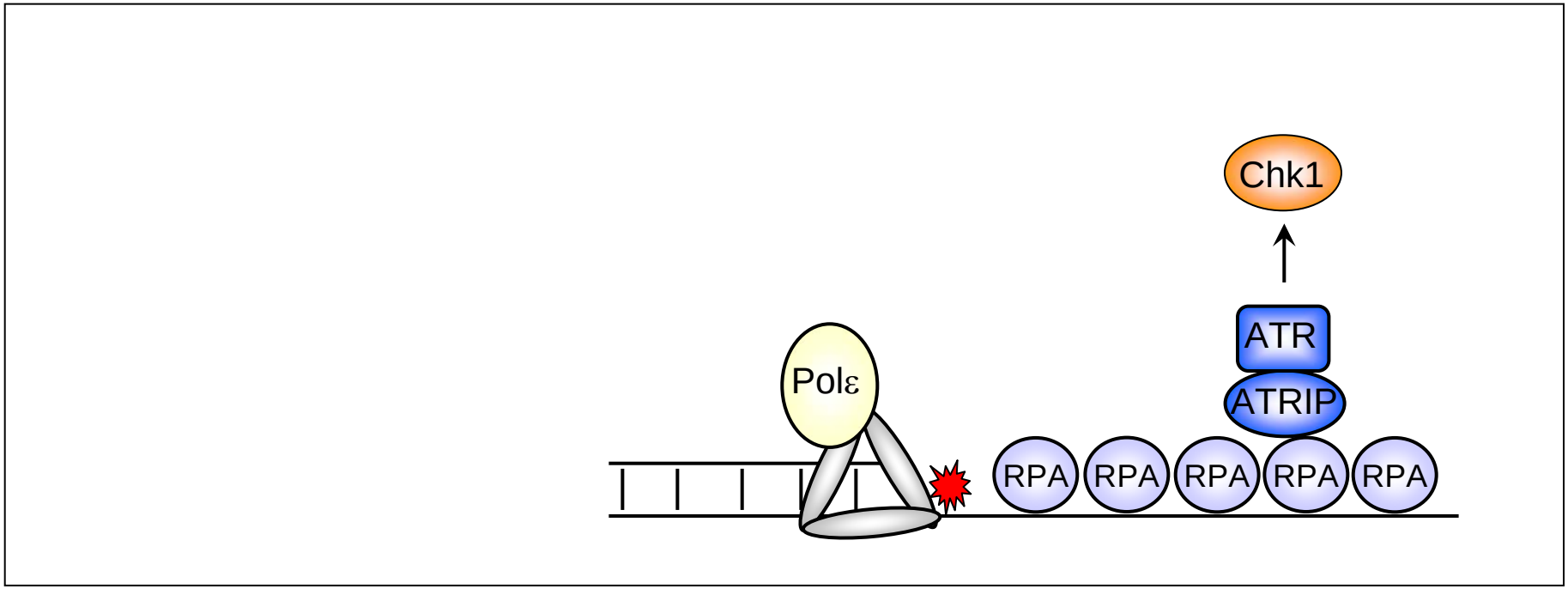
UV-Induced Rad18 S409 Phosphorylation is JNK-Dependent

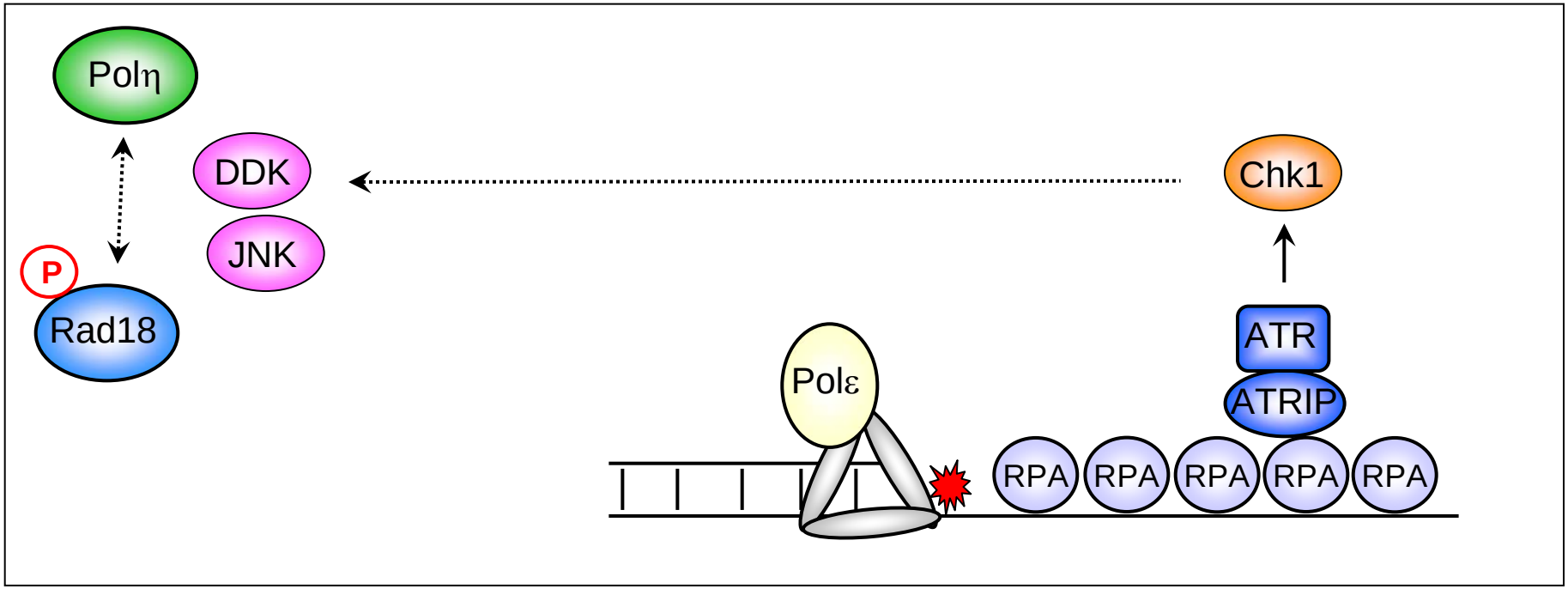


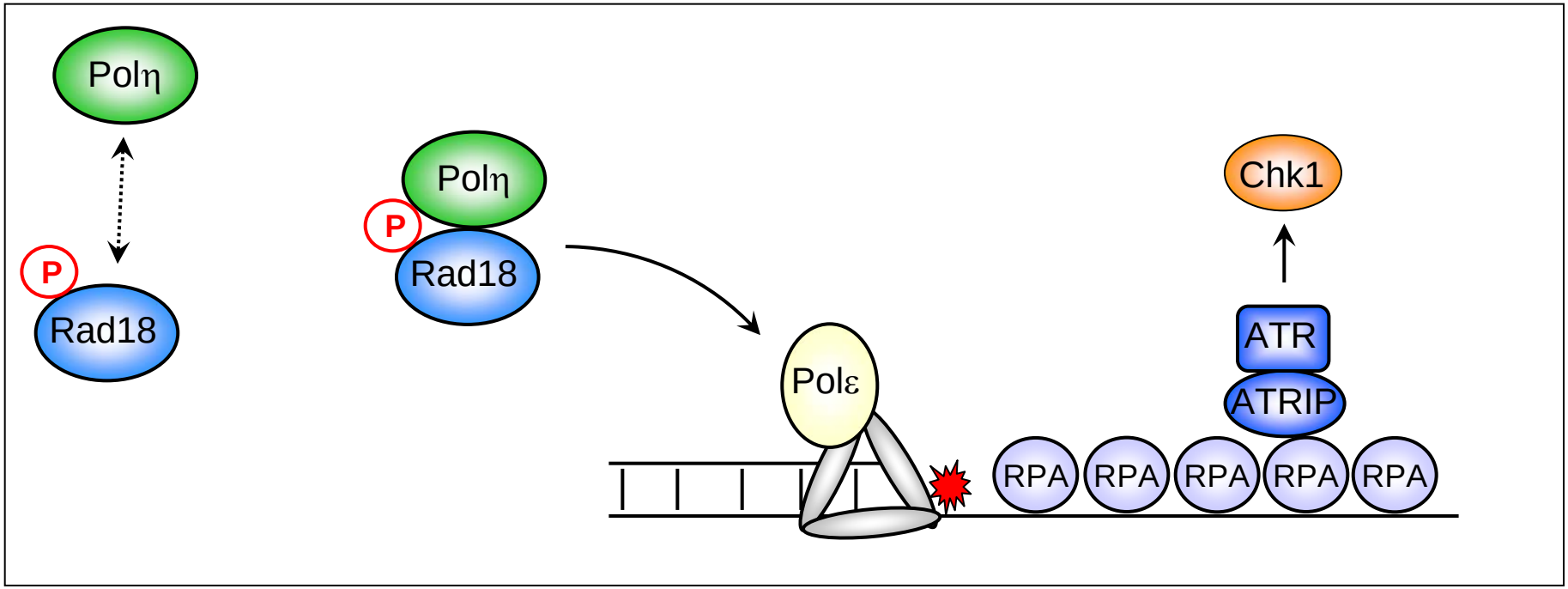


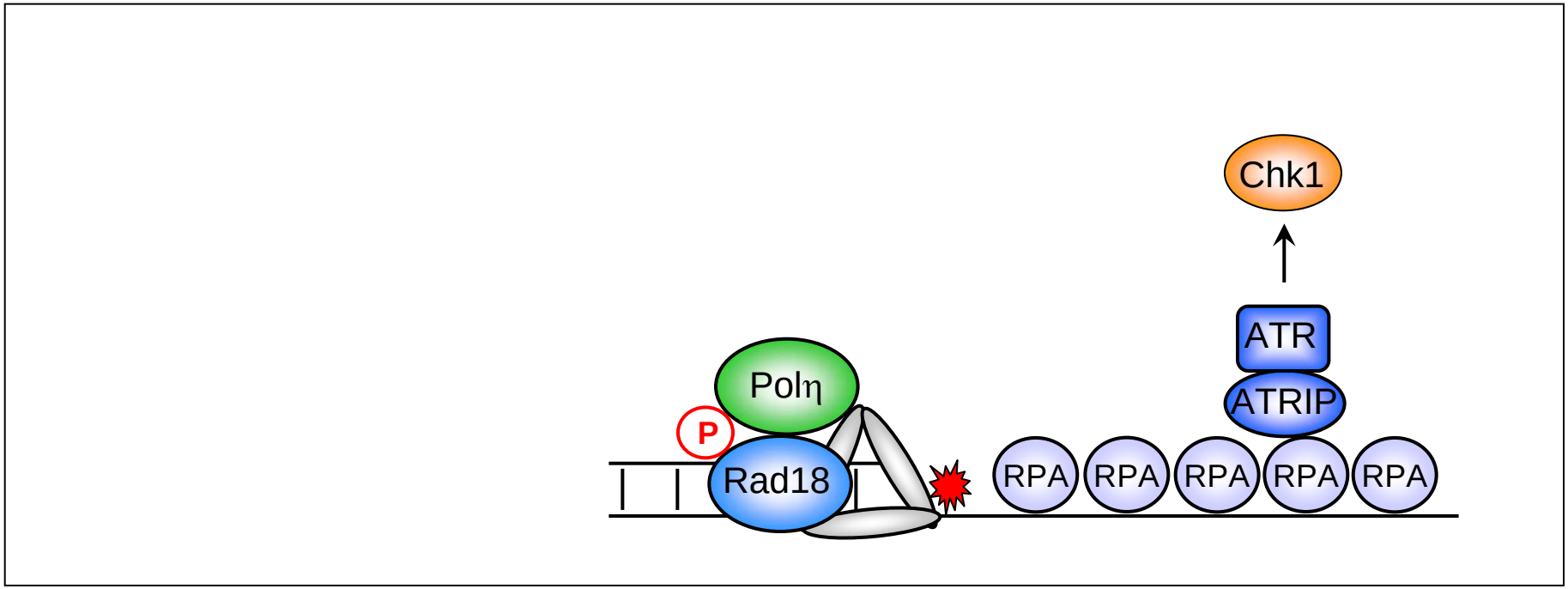
Rad18 S409A Expressing Cells are UV-sensitive




Chk1
ATR
ATRIP
Polε
RPA
RPA
RPA
RPA
RPA


Polη
DDK
JNK
P
Rad18
Chk1
ATR
ATRIP
Polε
RPA
RPA
RPA
RPA
RPA


Polη
P
Rad18
Polη
P
Rad18
Polε
Chk1
ATR
ATRIP
RPA
RPA
RPA
RPA
RPA


Chk1
ATR
ATRIP
Polη
P
Rad18
RPA
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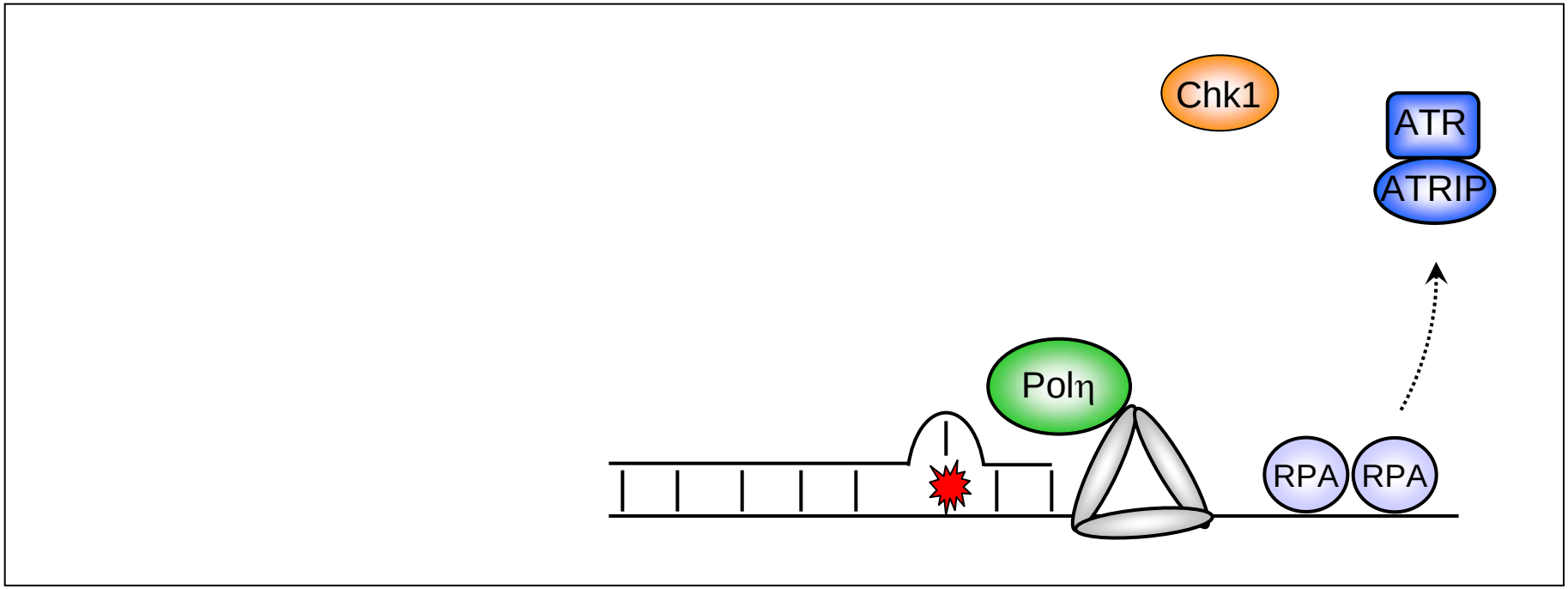

Chk1
ATR
ATRIP
Polη
RPA
RPA

Rad18 Phosphorylation – Further Questions:

Relationship between Chk1 signaling, DDK, JNK and Rad18?

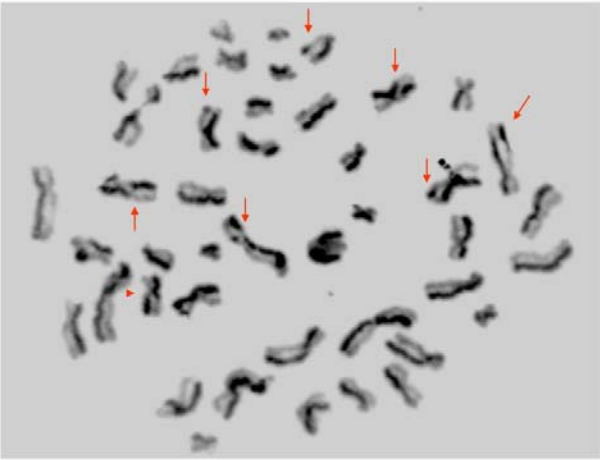
Mechanism of association between Rad18 and Polη

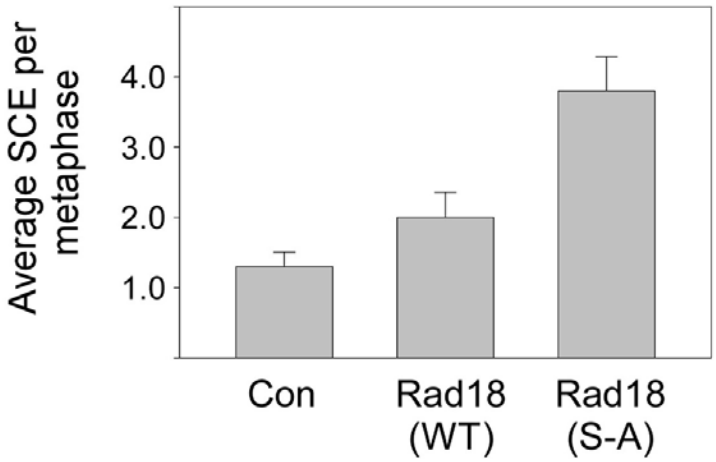
SIgnificance of Rad18 Phosphorylation *in vivo*?

Role of Rad18 Phosphorylation in regulating Polι, Polκ, Rev1?

Does Rad18 phosphorylation influence other (non-TLS)
DNA Repair pathways?

Complementation of *RAD18-/-* cells with Rad18 S434A induces SCE





1. Background: Responses to DNA Replication Fork Stalling

   (i) S-phase checkpoints
   (ii) Trans-Lesion Synthesis (TLS)

2. Regulation of TLS by Rad18 Phosphorylation

3. Replication Fork-Independent roles of Rad18 in DNA repair

Rad18 regulation as a function of the cell cycle?

Rad18 regulation by stresses that activate SAPK pathway:

UV, Reactive Oxygen Species (ROS)

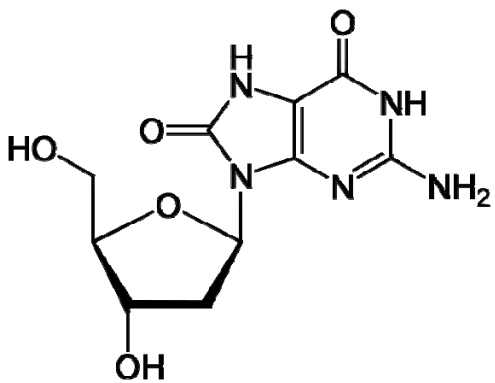
Reactive Oxygen Species (ROS)

ROS generated endogenously via aerobic metabolism.

Environmental Sources: UVA, B[a]P

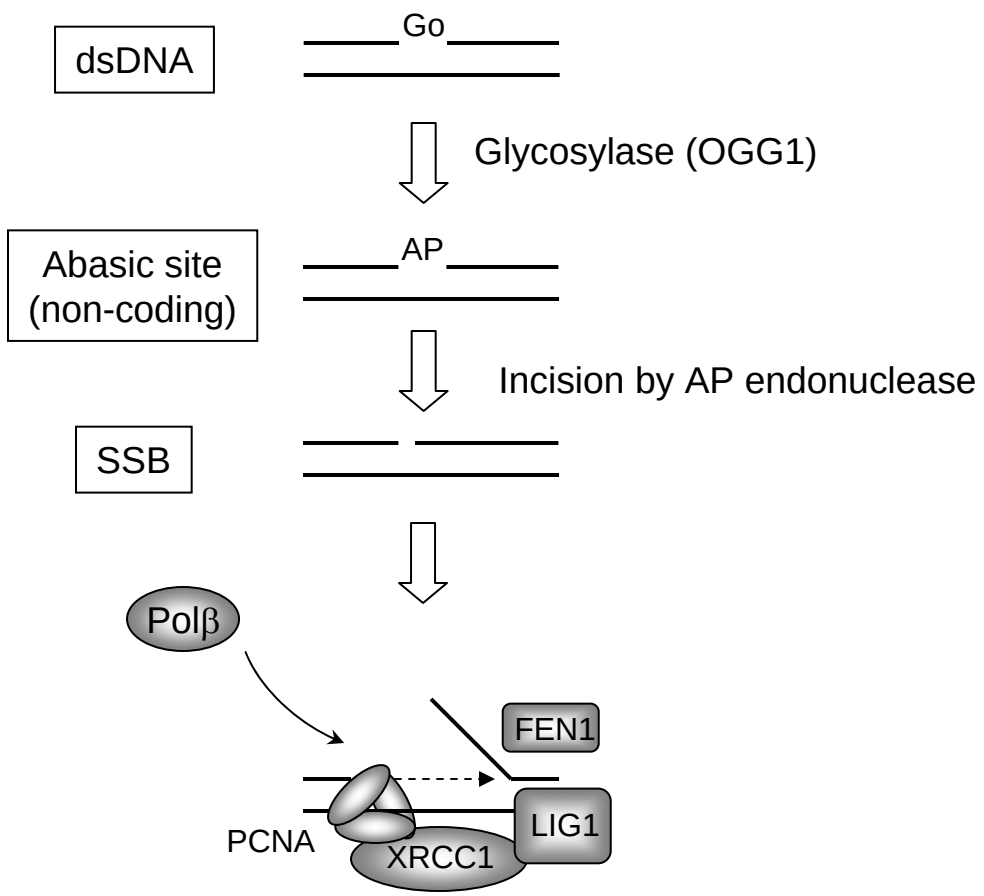
Oxidative DNA damage is implicated in cancer, aging

8-oxoG is one of most abundant DNA lesions induced by ROS



8-oxo-dG

Base Excision Repair



2,400 8-oxoG / cell
30,000 Abasic sites / cell
Swenberg et al., Tox Sci 120 130-145 (2011)

Relationship between TLS and ROS-Induced DNA Lesions

8-oxoG is highly mutagenic, bypassed accurately by Polη

Polη prevents mutagenesis due to 8-oxodG
Haracska et al., Nat Genetics 25, p458 (2000)
McCulloch et al., NAR 37, p2830 (2009)
Lee & Pfeifer Mut Res 641, p19 (2008)

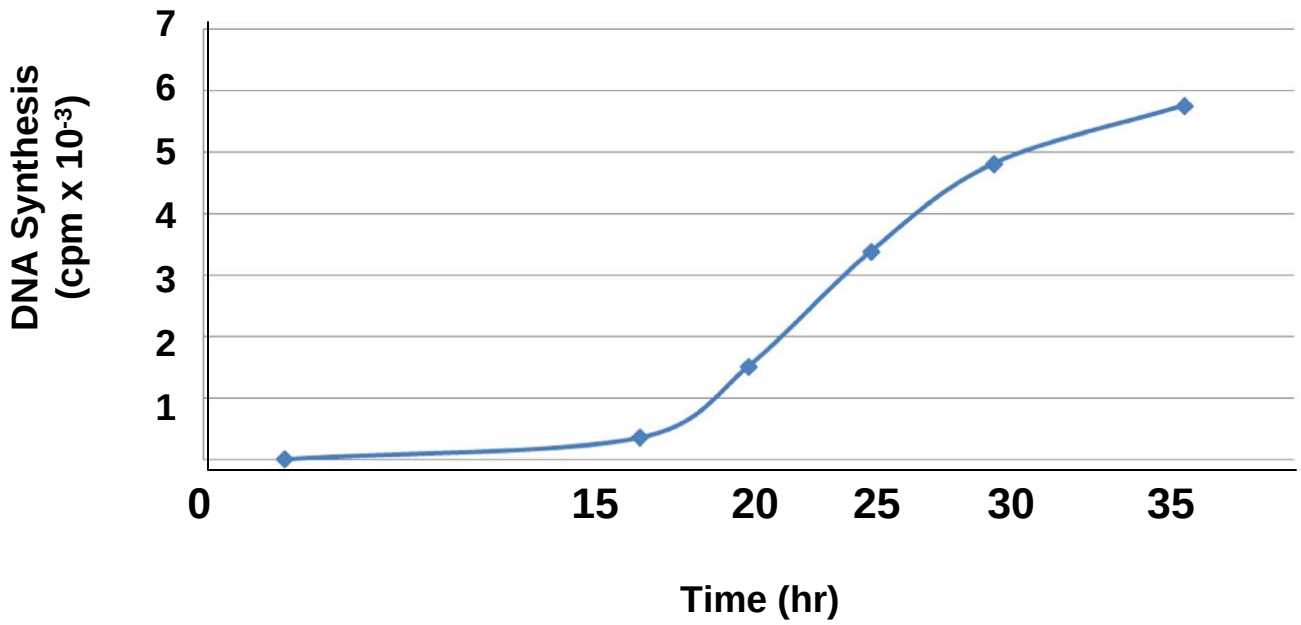
Polη mediates TLS across non-instructional abasic sites
Choi et al. J. Mol Biol 404, p34-44 (2010)

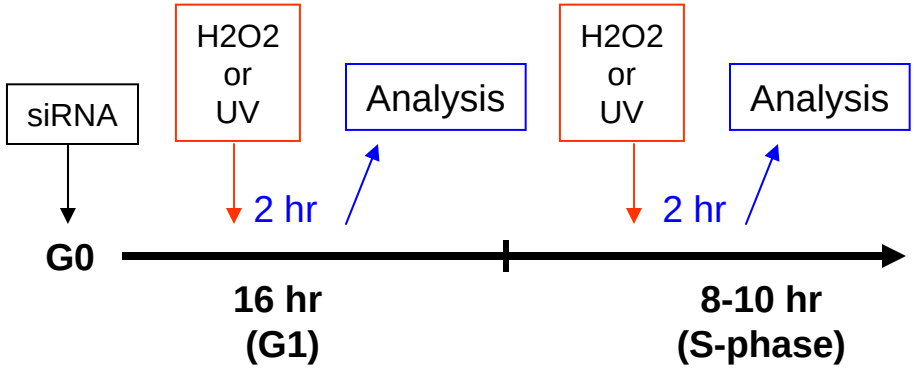
Rad18 regulation as a function of the cell cycle?

Rad18 regulation by stresses that activate SAPK pathway:

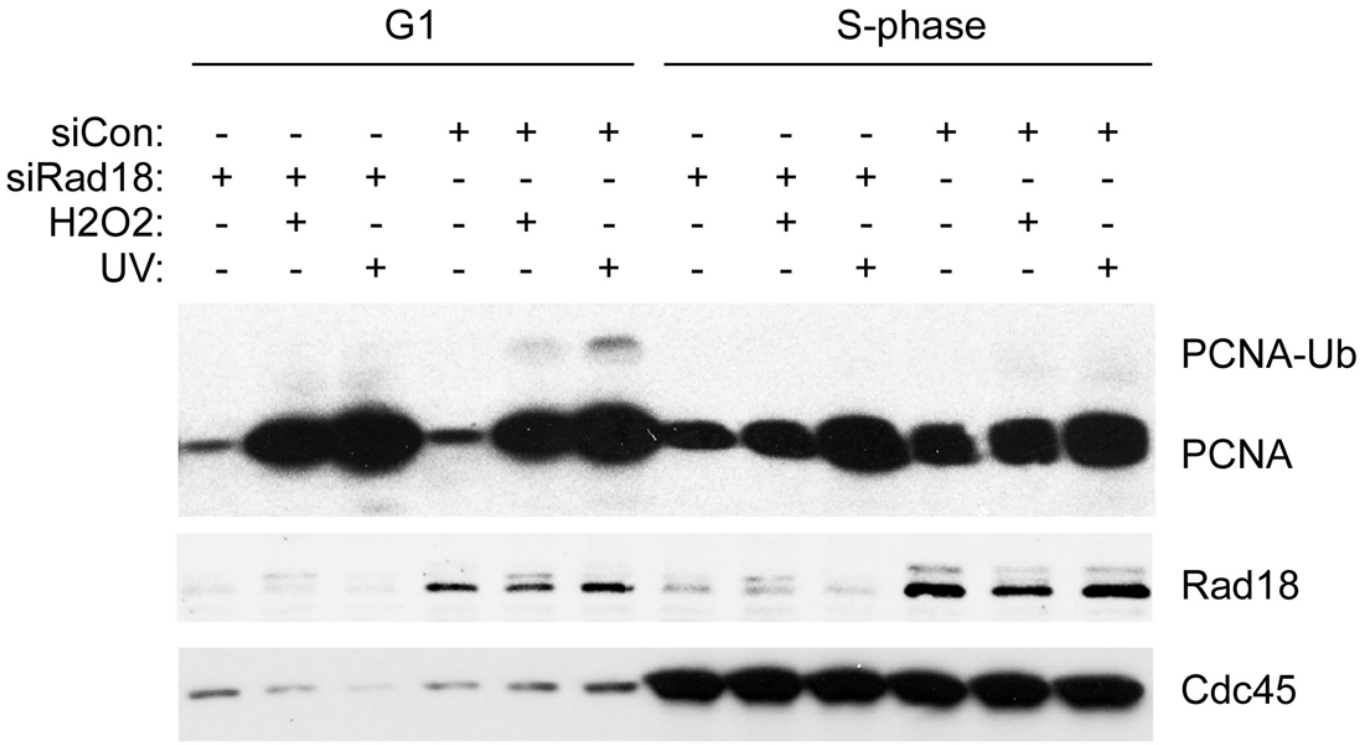
UV, Reactive Oxygen Species (ROS)

Cell Cycle Kinetics of Primary Human Fibroblasts

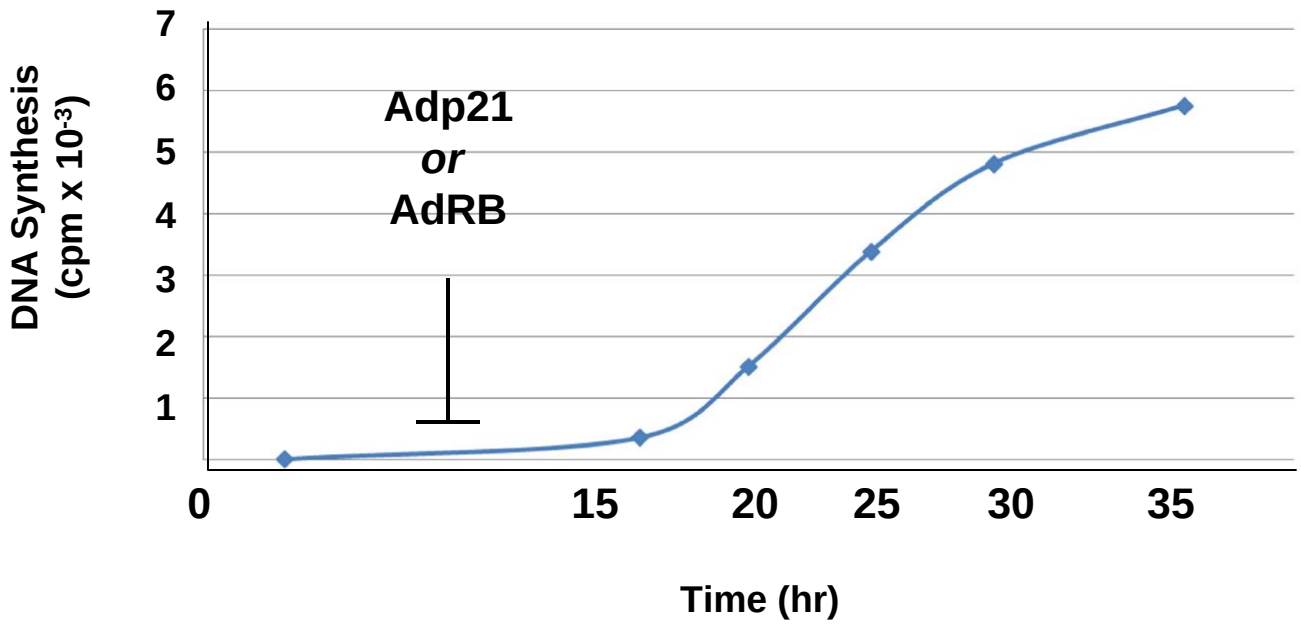


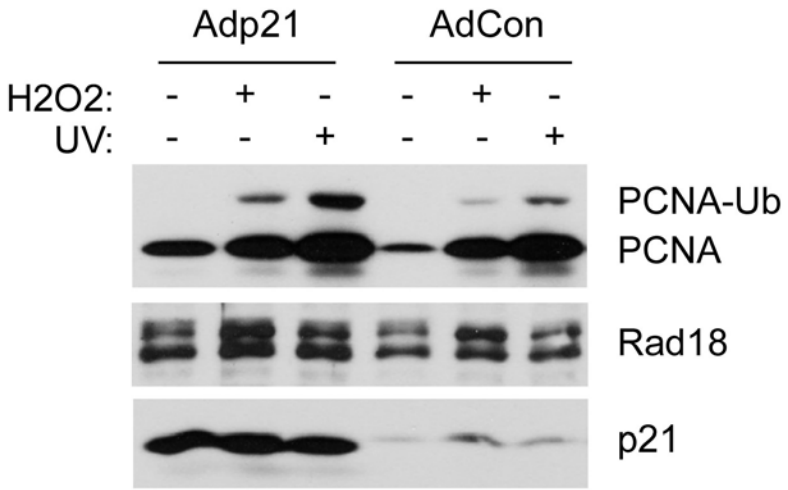


PCNA Mono-ubiquitination is DNA Replication-Independent

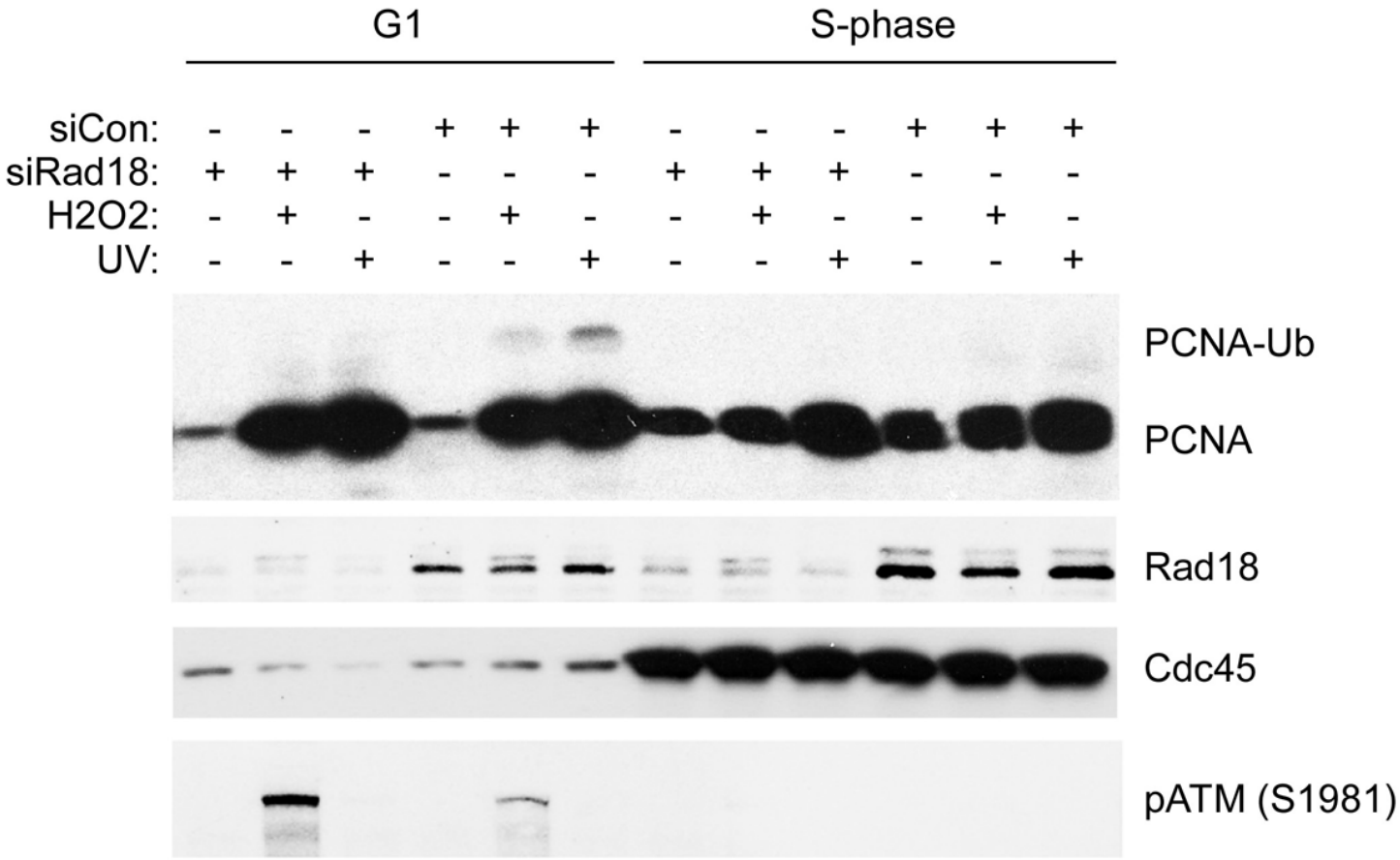


PCNA Mono-ubiquitination is DNA Replication-Independent

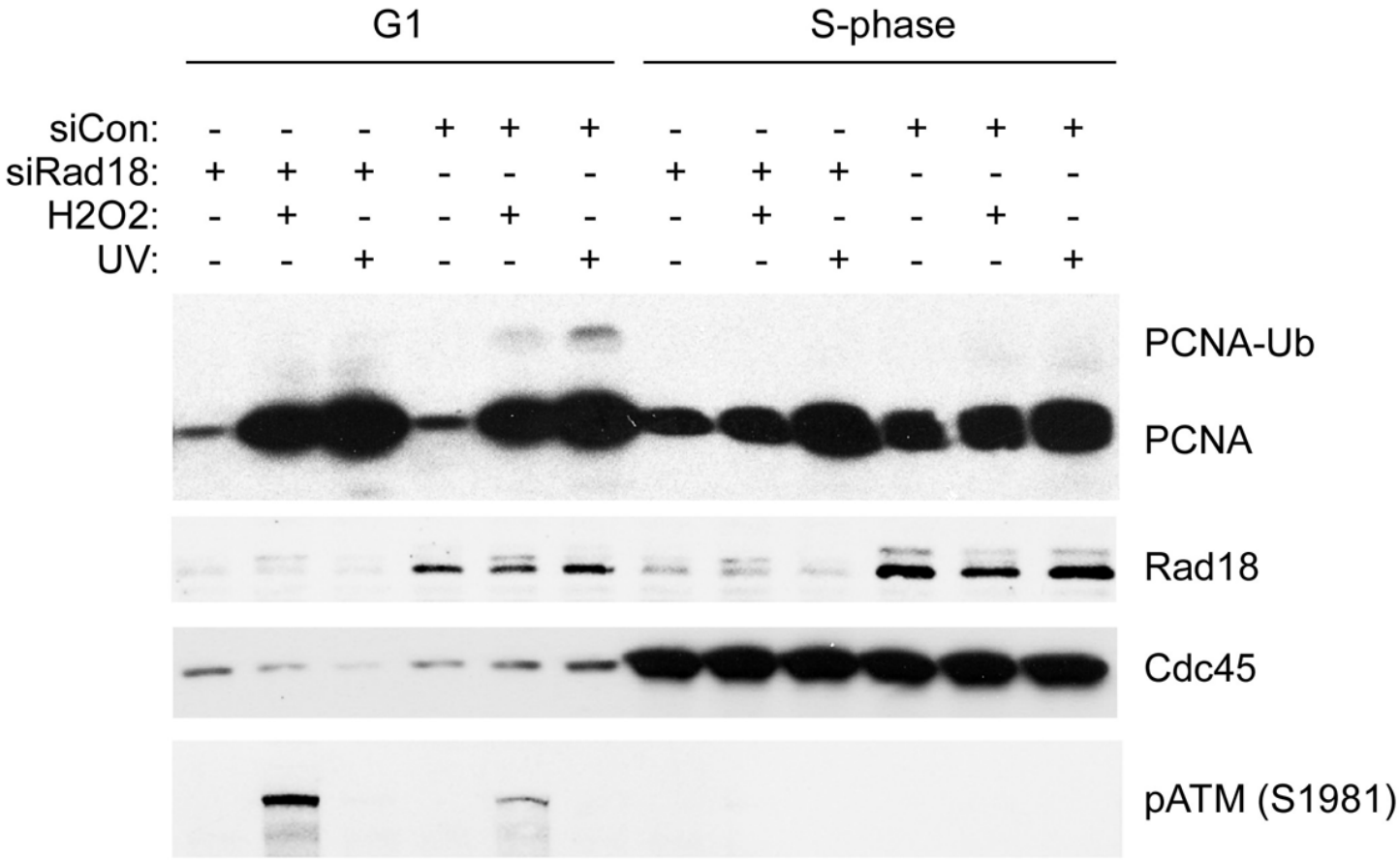




PCNA Mono-ubiquitination is DNA Replication-Independent



PCNA Mono-ubiquitination is DNA Replication-Independent

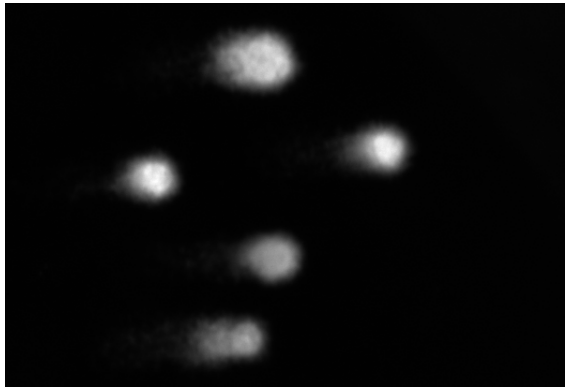


<u>H2O2-induced ATM hyper-phosphorylation:</u>

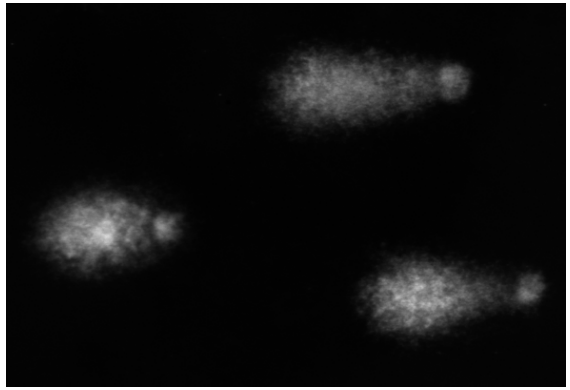
Phenocopied by Polη depletion,
Corrected by Rad18 or Polη over-expression (not Polκ)

Single Cell Gel Electrophoresis 'Comet' Assay

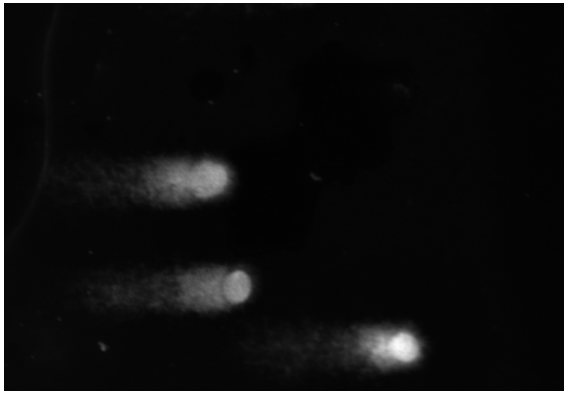
Untreated



0 hr + H_2O_2

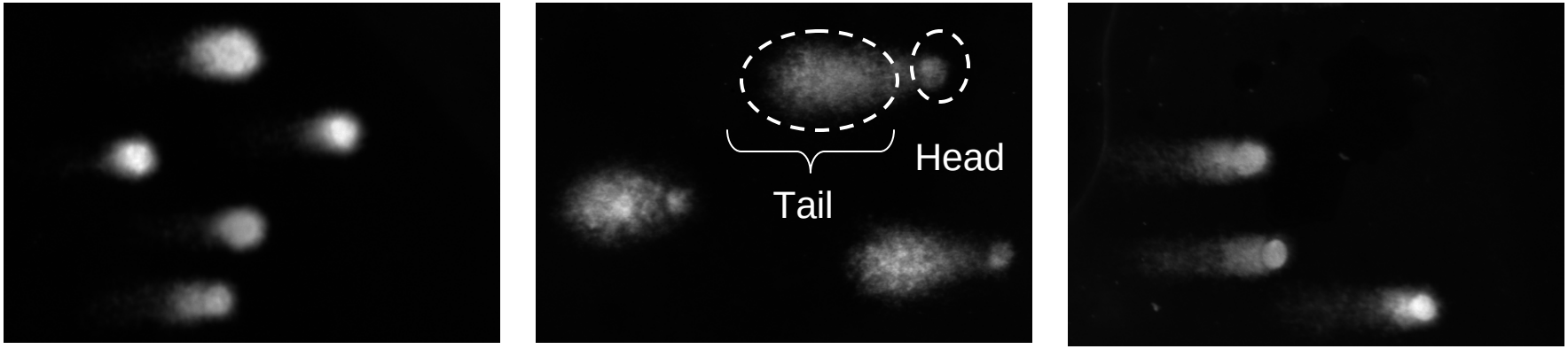


1 hr post-H_2O_2



Single Cell Gel Electrophoresis 'Comet' Assay

Untreated | 0 hr + H_2O_2 | 1 hr post-H_2O_2



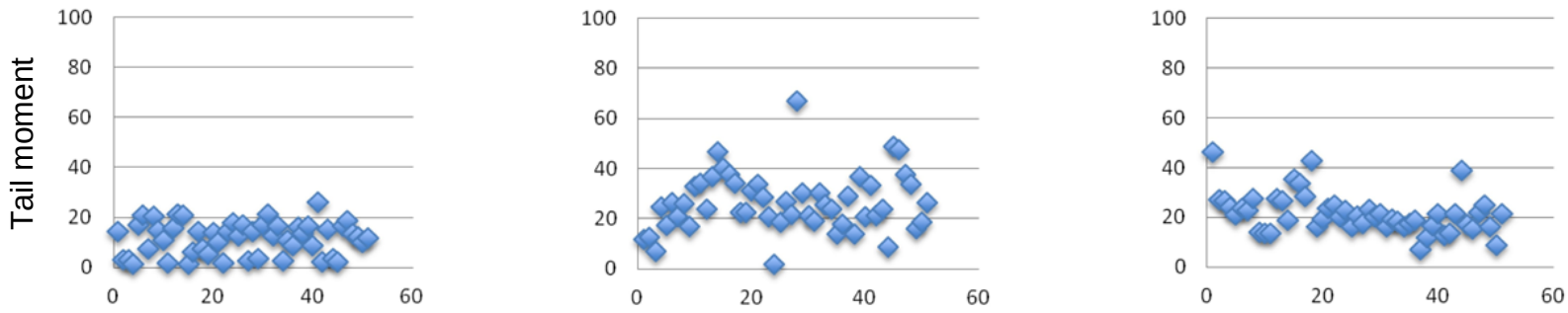
***'Tail moment'**: %DNA in tail x length of tail*

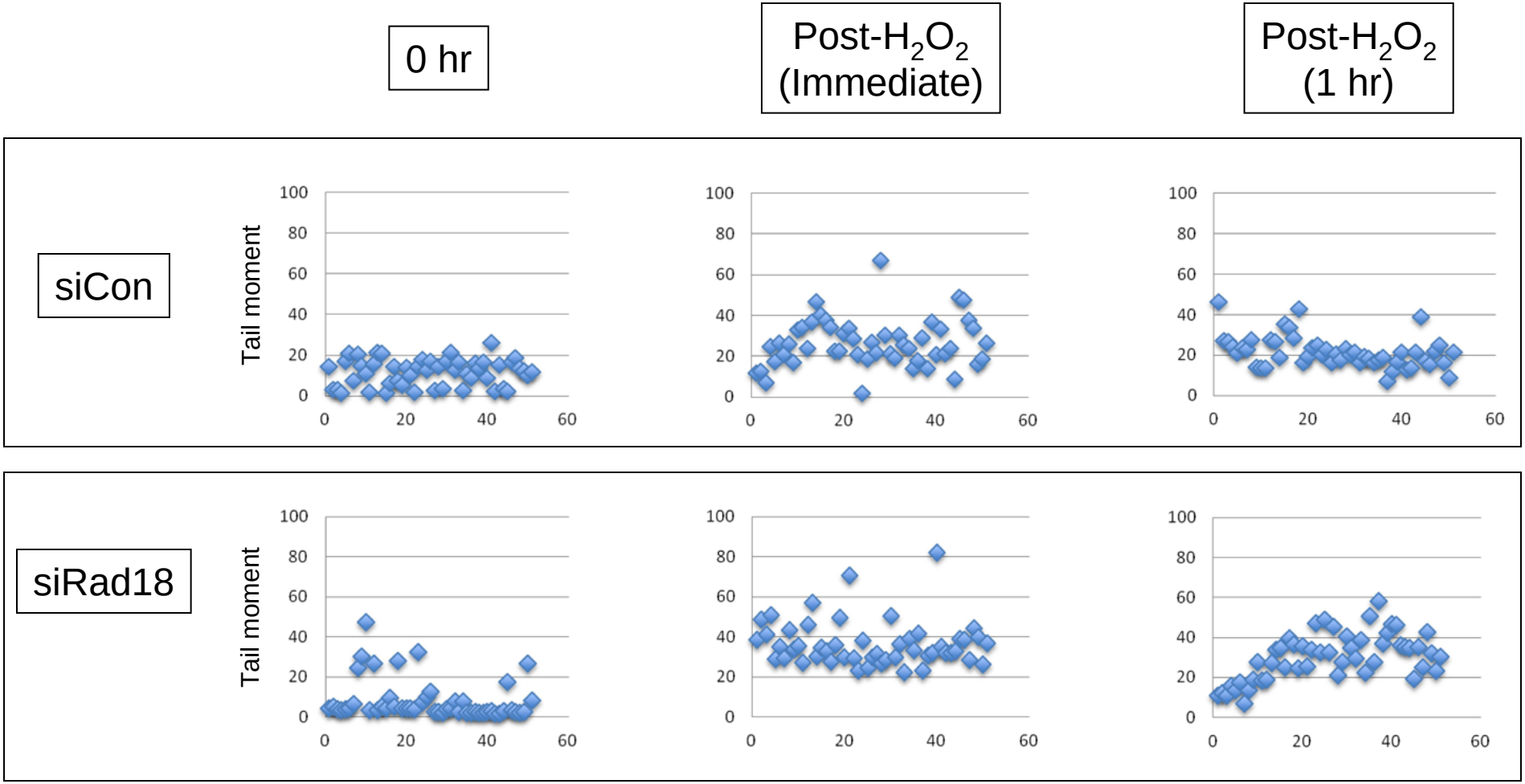
0 hr

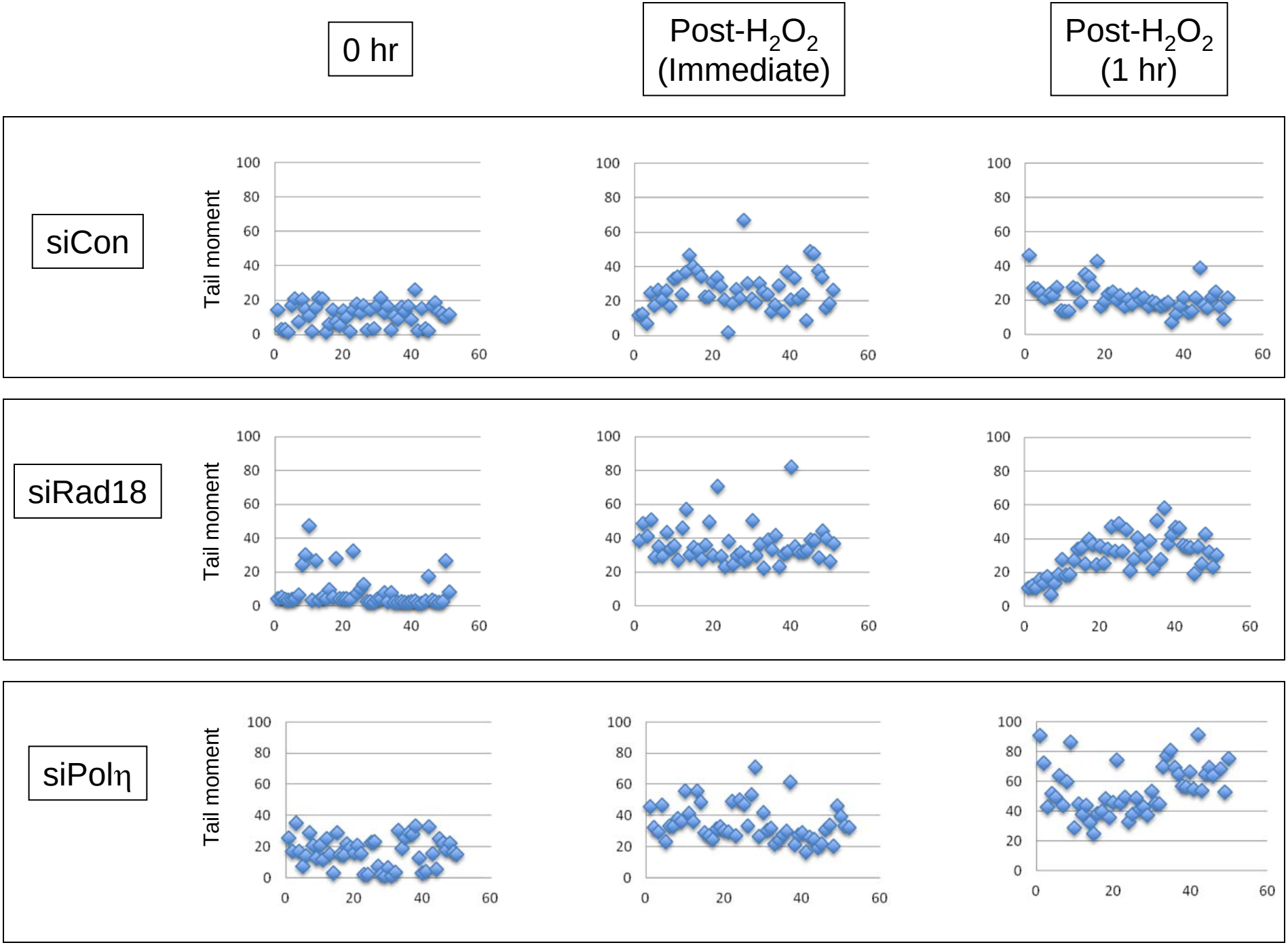
Post-H_2O_2 (Immediate)

Post-H_2O_2 (1 hr)

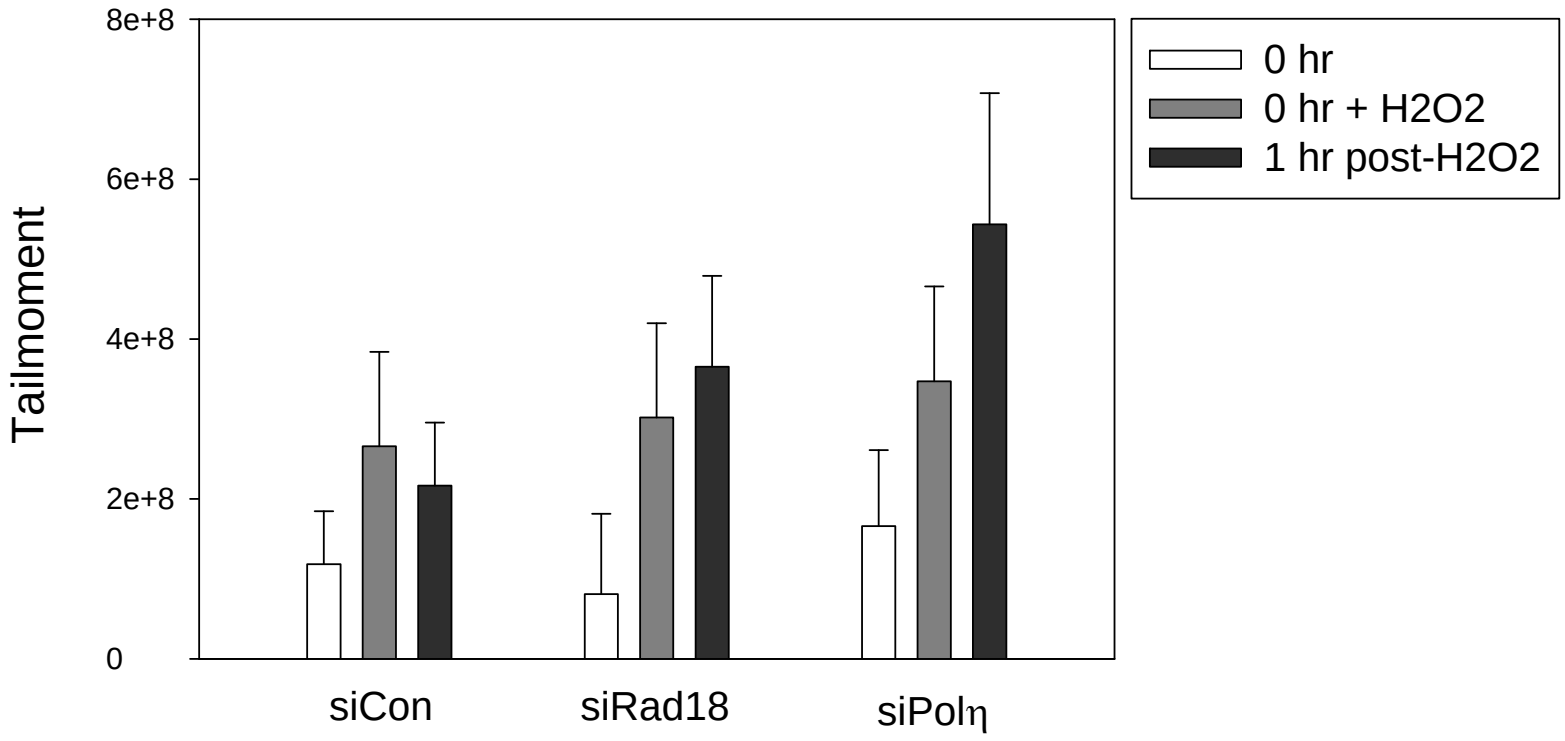
siCon



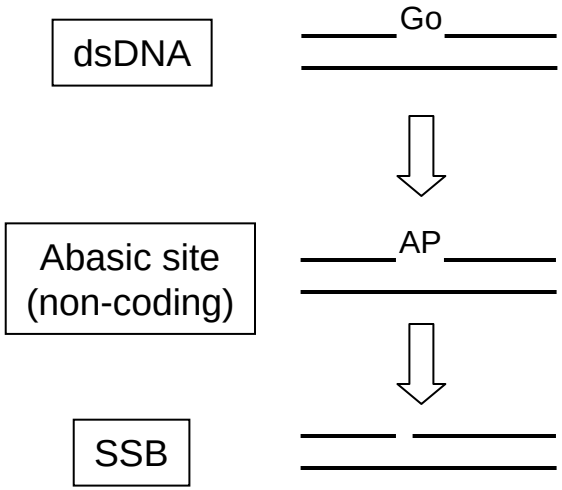

0 hr
Post-H_2O_2 (Immediate)
Post-H_2O_2 (1 hr)
siCon
siRad18
Tail moment
100
80
60
40
20
0
0
20
40
60


0 hr
Post-H_2O_2 (Immediate)
Post-H_2O_2 (1 hr)
siCon
siRad18
siPolη
Tail moment
0
20
40
60
80
100

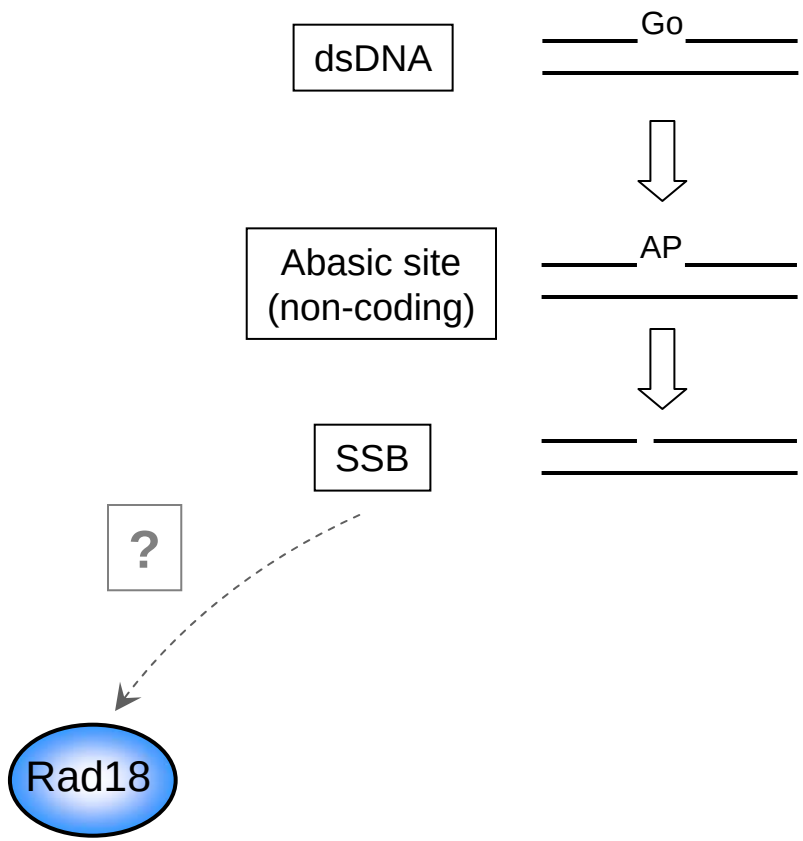
Kinetics of DSB Repair in TLS-defective Cells



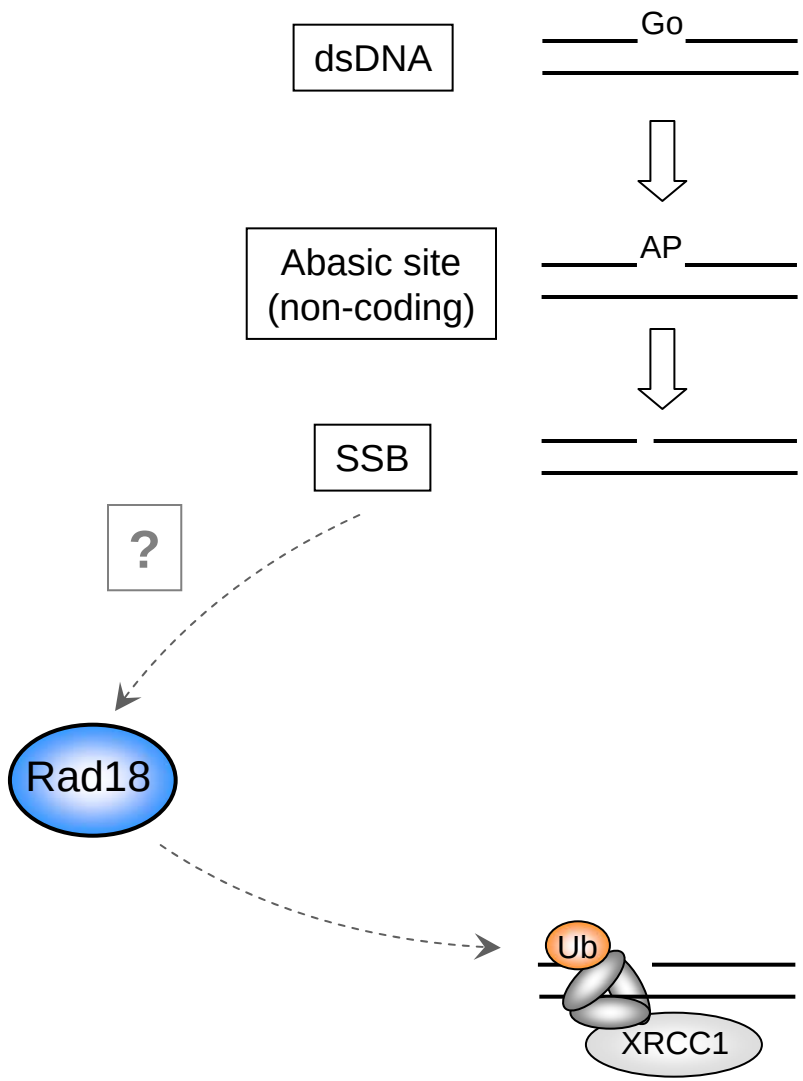
Hypothetical Role of Rad18 in SSB Repair



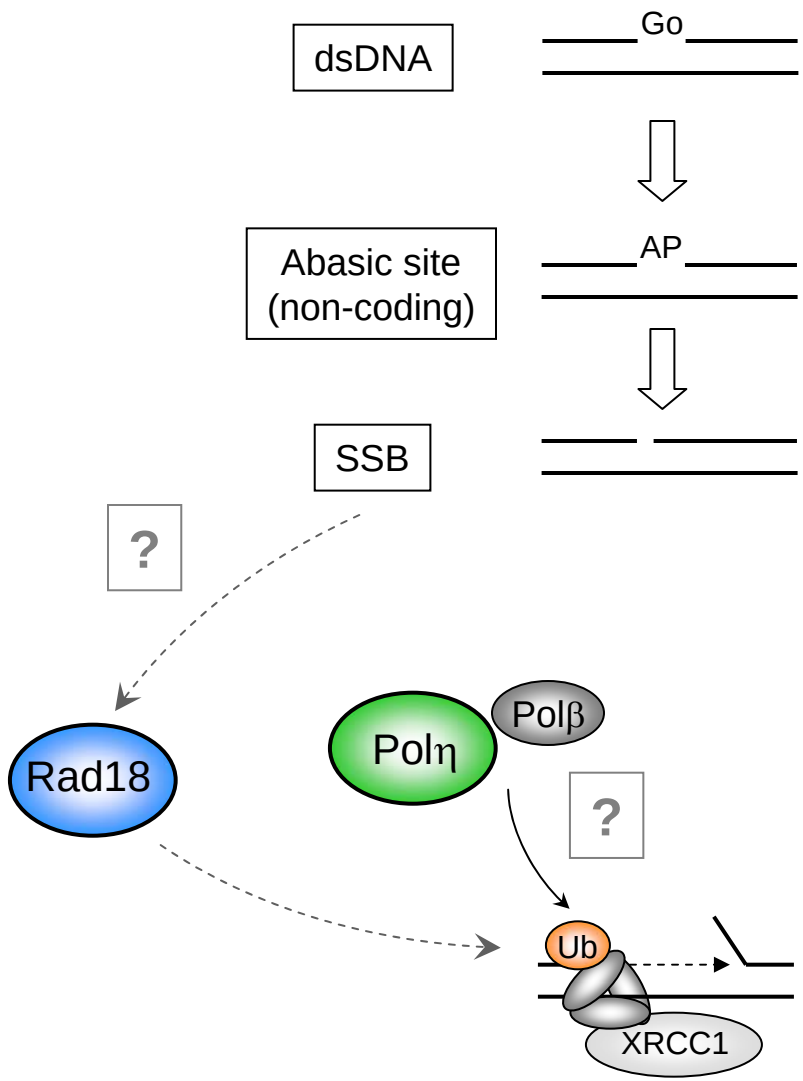
Hypothetical Role of Rad18 in SSB Repair



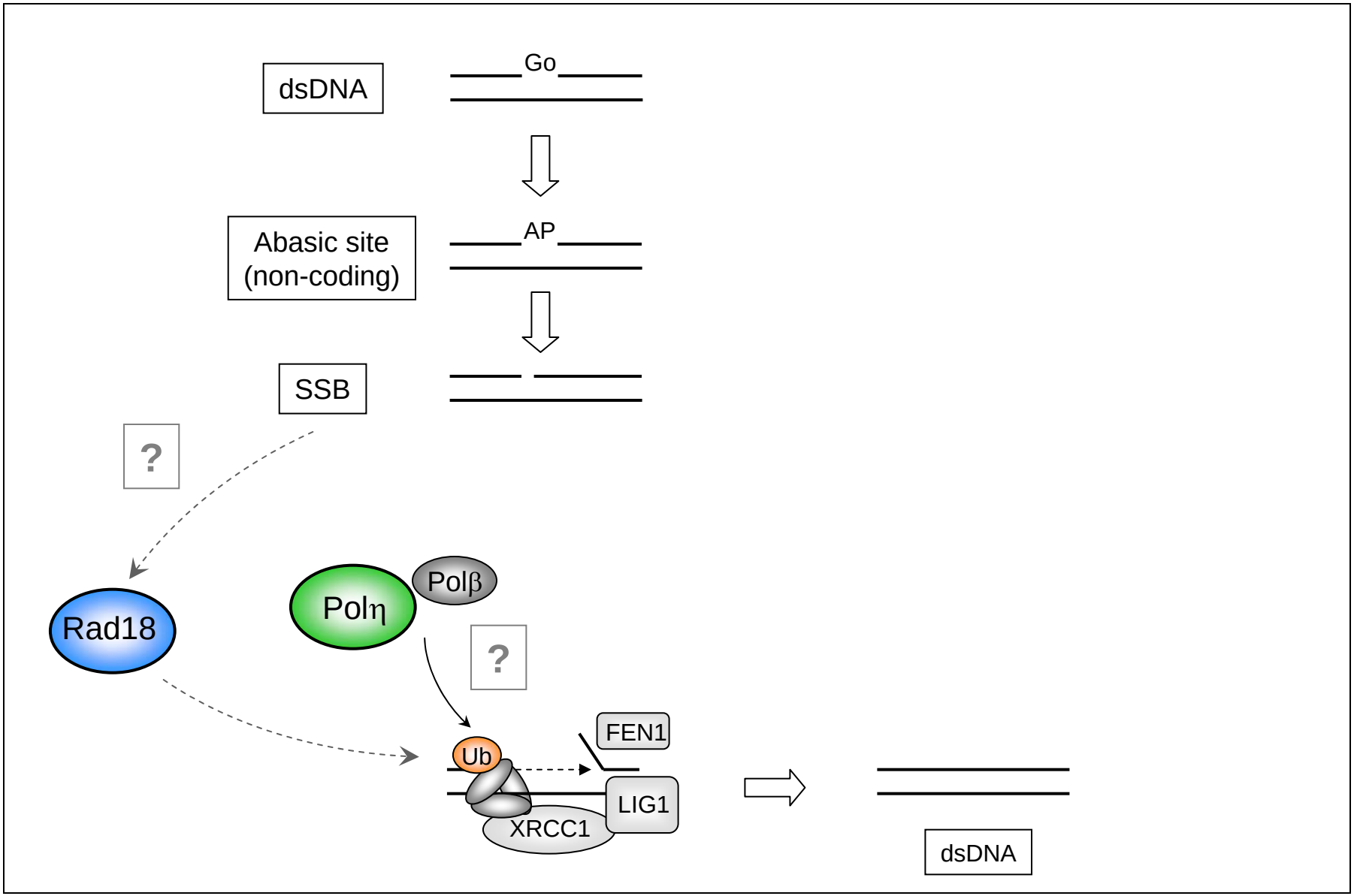
Hypothetical Role of Rad18 in SSB Repair



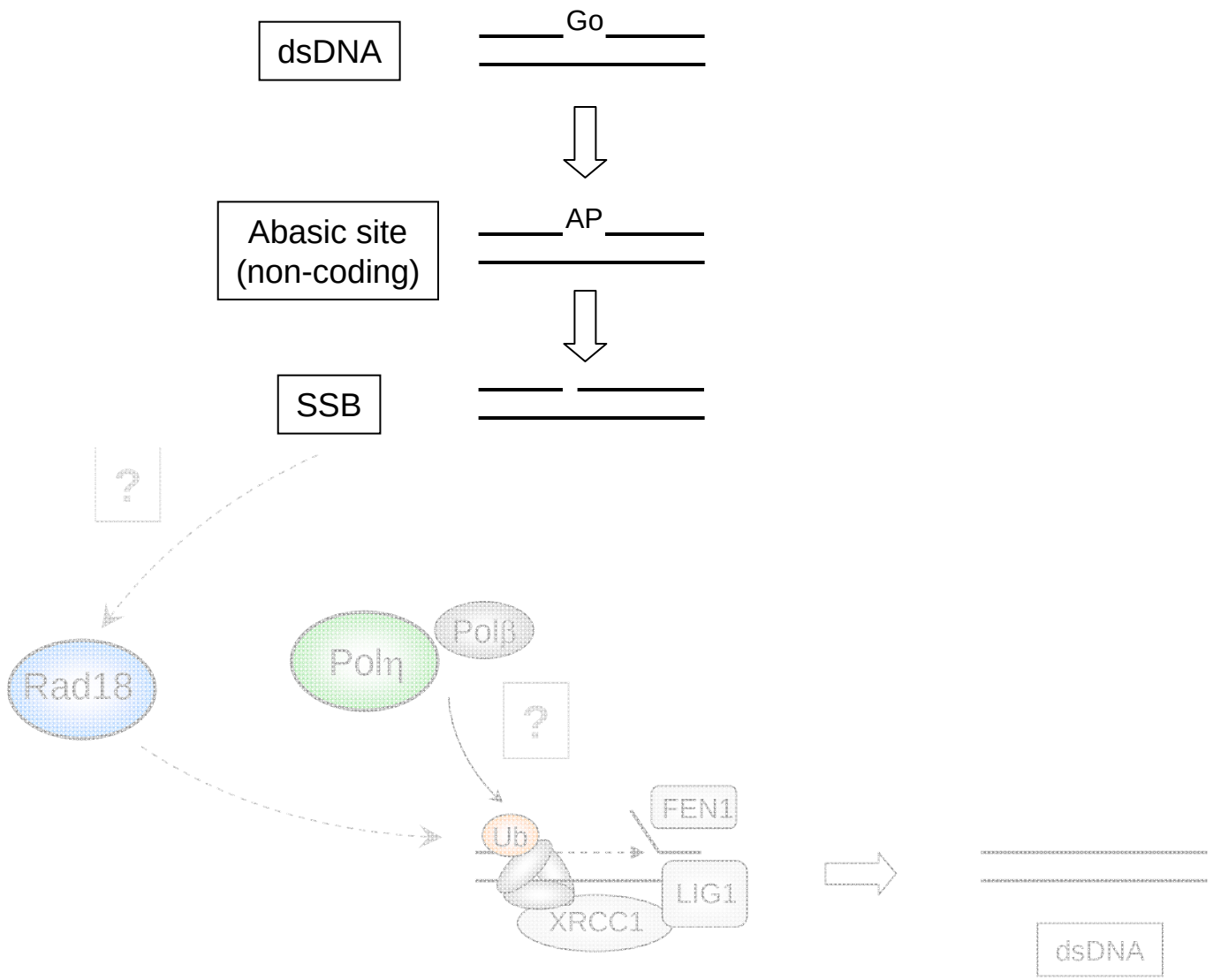
Hypothetical Role of Rad18 in SSB Repair



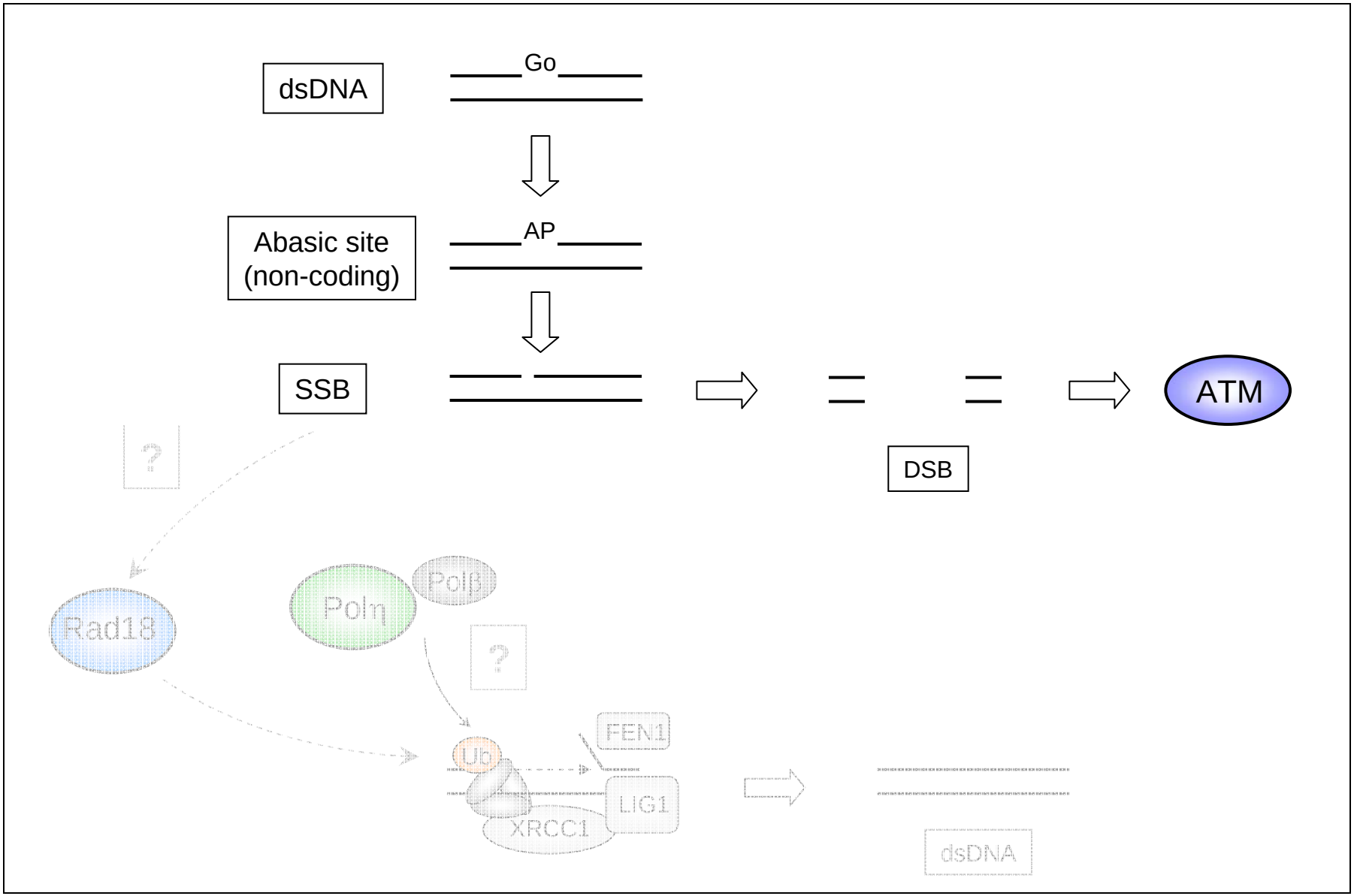
Hypothetical Role of Rad18 in SSB Repair



Hypothetical Role of Rad18 in SSB Repair



Hypothetical Role of Rad18 in SSB Repair



Three DNA Polymerases, Recruited by Different Mechanisms, Carry Out NER Repair Synthesis in Human Cells

Tomoo Ogi,[1,*] Siripan Limsirichaikul,[1,2,8] René M. Overmeer,[3,8] Marcel Volker,[4,8] Katsuya Takenaka,[5,8] Ross Cloney,[4,8] Yuka Nakazawa,[1,8] Atsuko Niimi,[1,4] Yoshio Miki,[5] Nicolaas G. Jaspers,[6] Leon H.F. Mullenders,[3,9] Shunichi Yamashita,[1,9] Maria I. Fousteri,[3,7,9] and Alan R. Lehmann[4,*]

Molecular Cell *37*, 714–727, March 12, 2010

The hMsh2-hMsh6 Complex Acts in Concert with Monoubiquitinated PCNA and Pol η in Response to Oxidative DNA Damage in Human Cells

Anastasia Zlatanou,[1,3] Emmanuelle Despras,[1] Tirzah Braz-Petta,[1] Imenne Boubakour-Azzouz,[1] Caroline Pouvelle,[1] Grant S. Stewart,[3] Satoshi Nakajima,[4,5] Akira Yasui,[4] Alexander A. Ishchenko,[2,*] and Patricia L. Kannouche[1,*]

Molecular Cell *43*, 649–662, August 19, 2011 ©2011

Conclusion:

Replication Fork-Independent Mechanisms of Rad18-Polη activation.

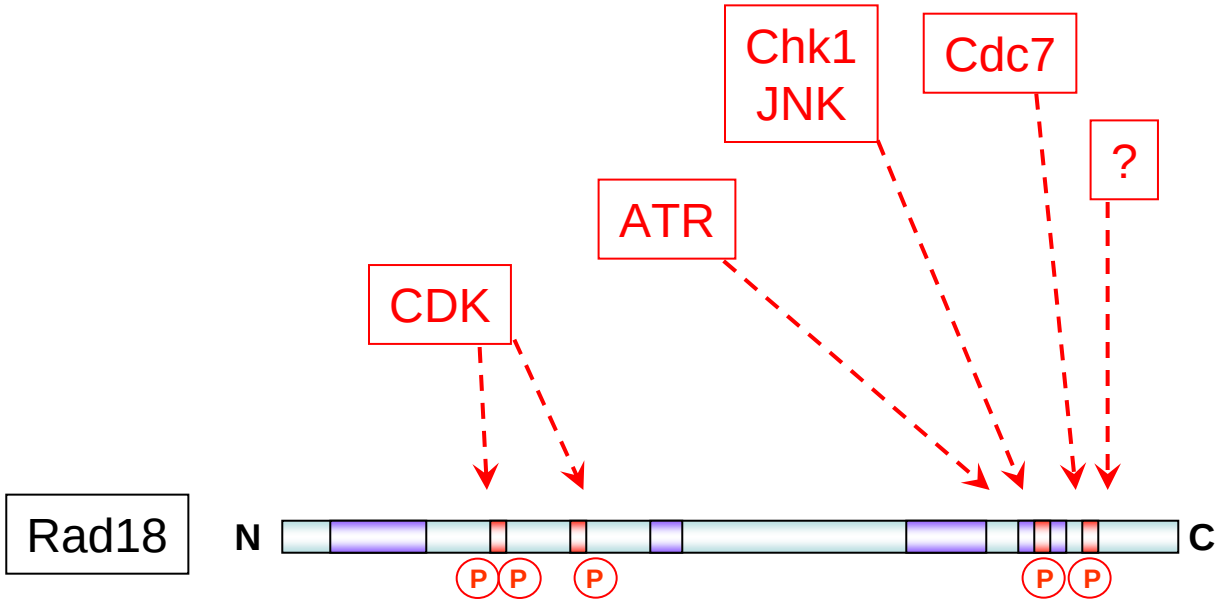
Ongoing:

Differential contribution of PCNA-Ub and Polη chaperone activities of Rad18 in G1 and S-phase?

Roles of Rad18 in Tolerance of ROS-Induced DNA Damage in Post-Mitotic Cells?

Synthetic Lethality between Rad18 and DSB Repair in Repair of ROS-Induced DNA Damage?

Role of Rad18 PTM in Cell Cycle-Specific Responses to DNA Damage?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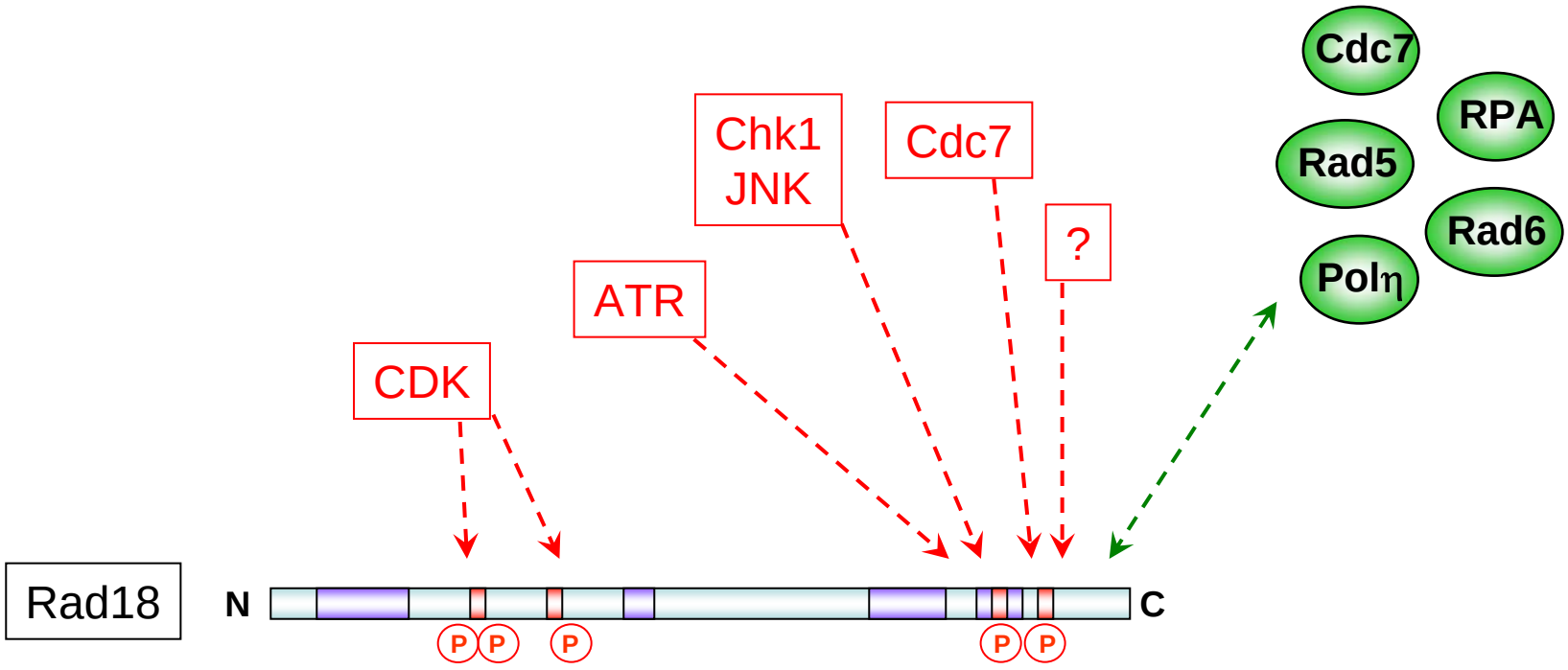
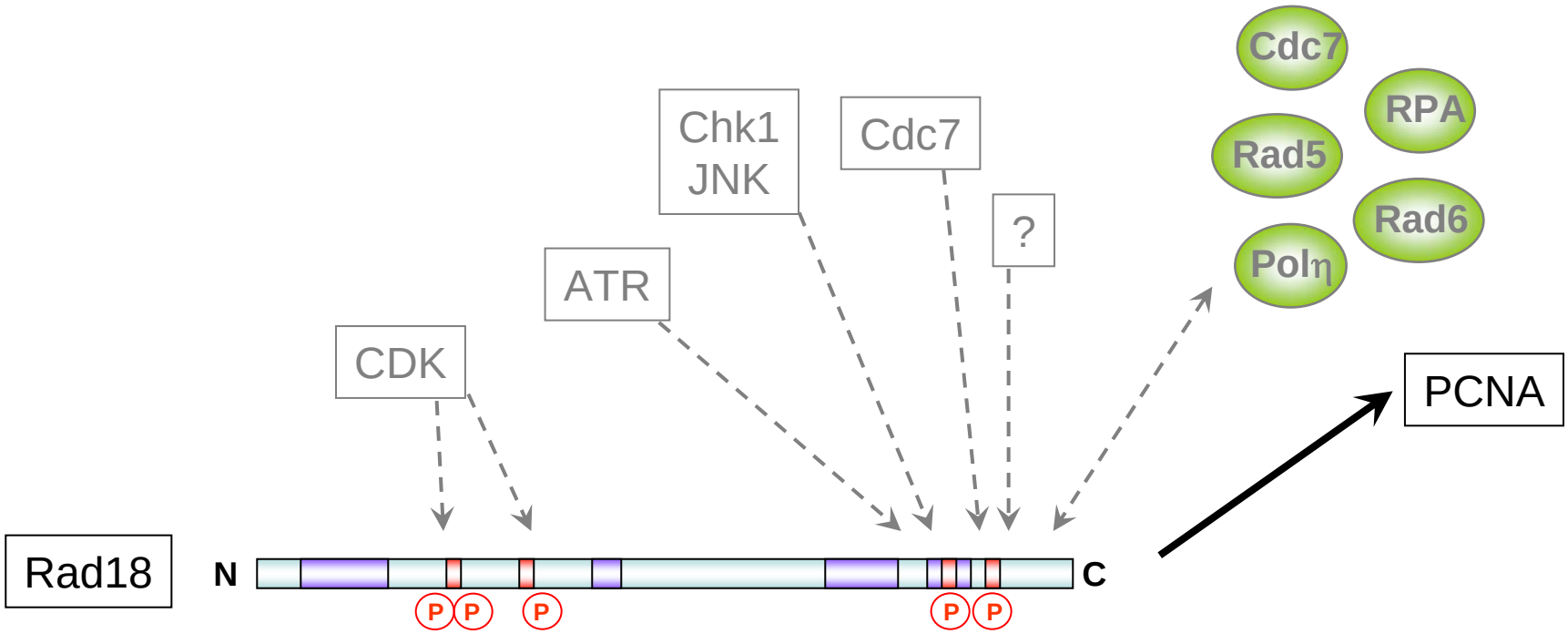
JNK
Cdc7
?
ATR
CDK
Rad18
N
C
P
P
P
P
P


Cdc7
RPA
Rad5
Rad6
Polη
Chk1
JNK
Cdc7
?
ATR
CDK
Rad18
N
C
P
P
P
P
P


Cdc7
RPA
Rad5
Rad6
Polη
Chk1
JNK
Cdc7
?
ATR
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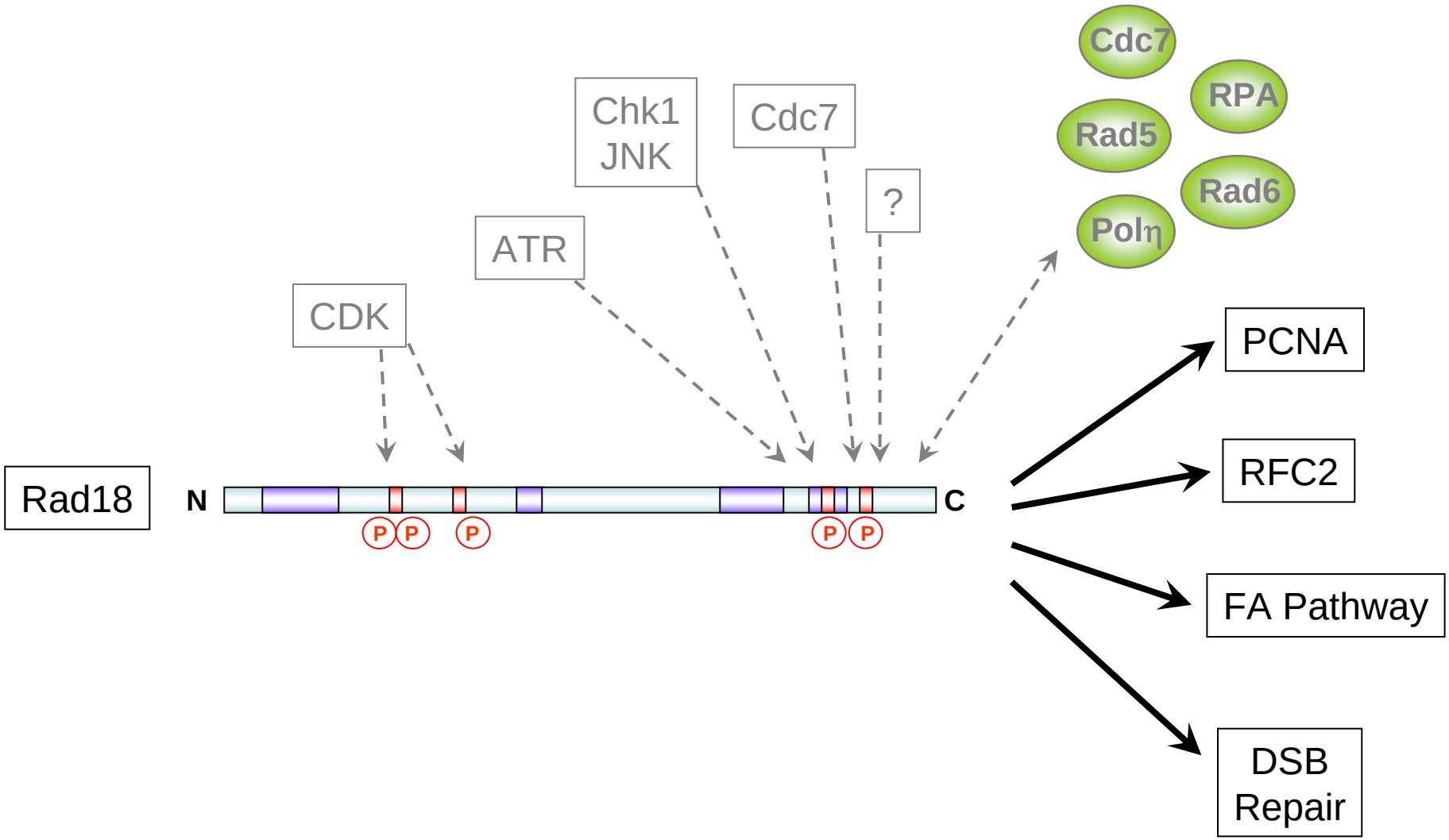
P
P
P
P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Rad6
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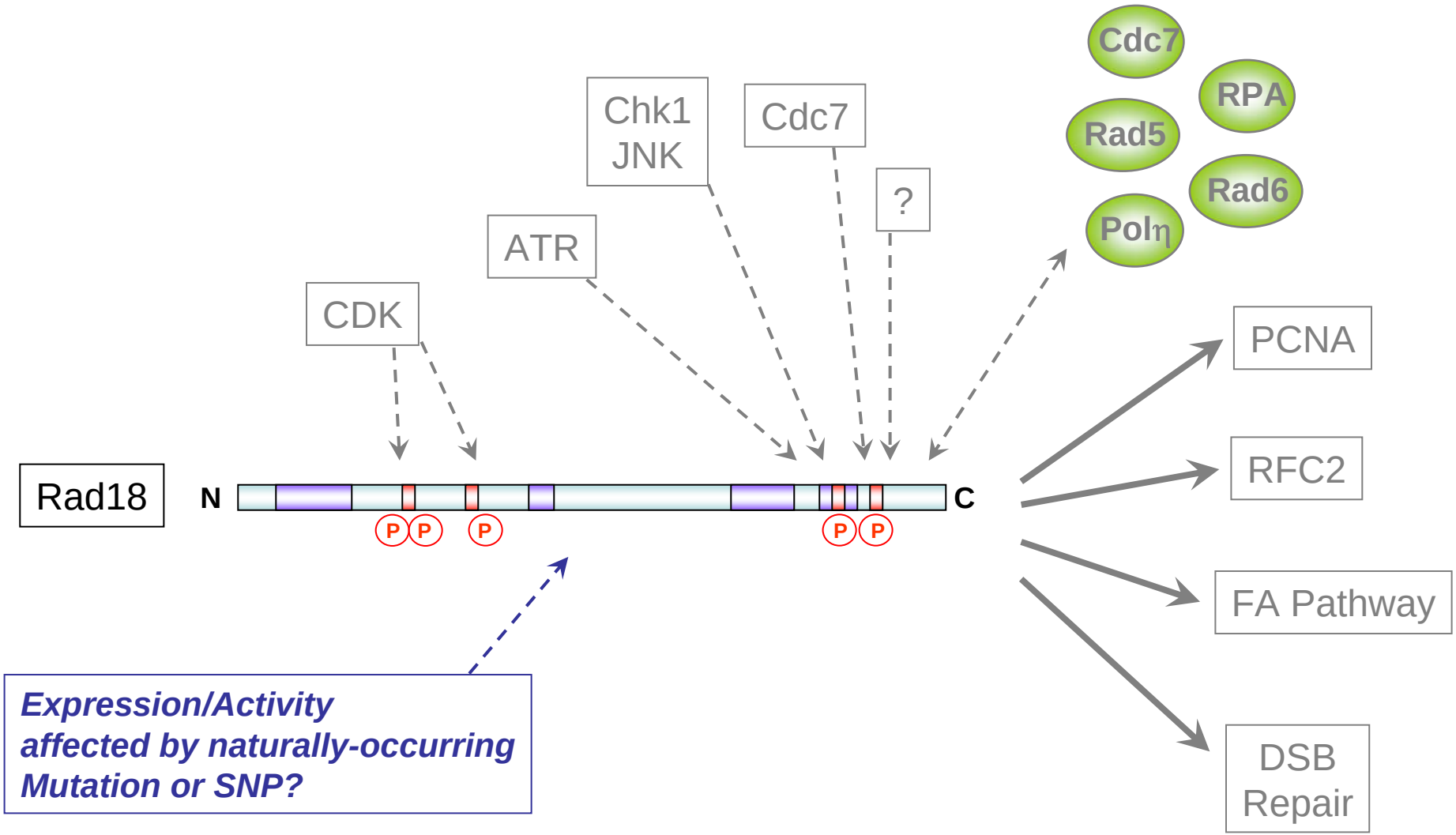
Rad18
N
C
P
P
P
P
P
FA Pathway
DSB
Repair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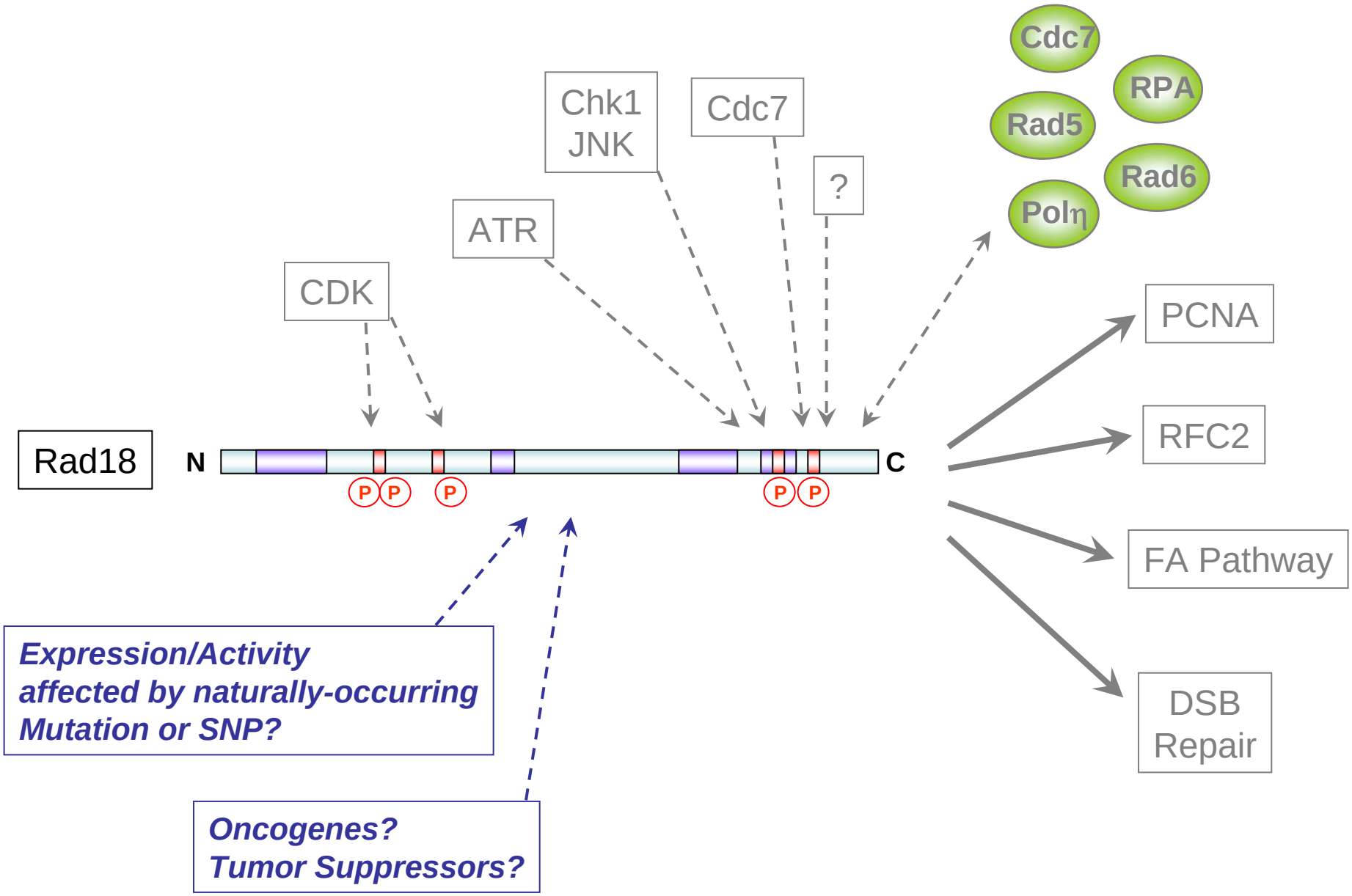
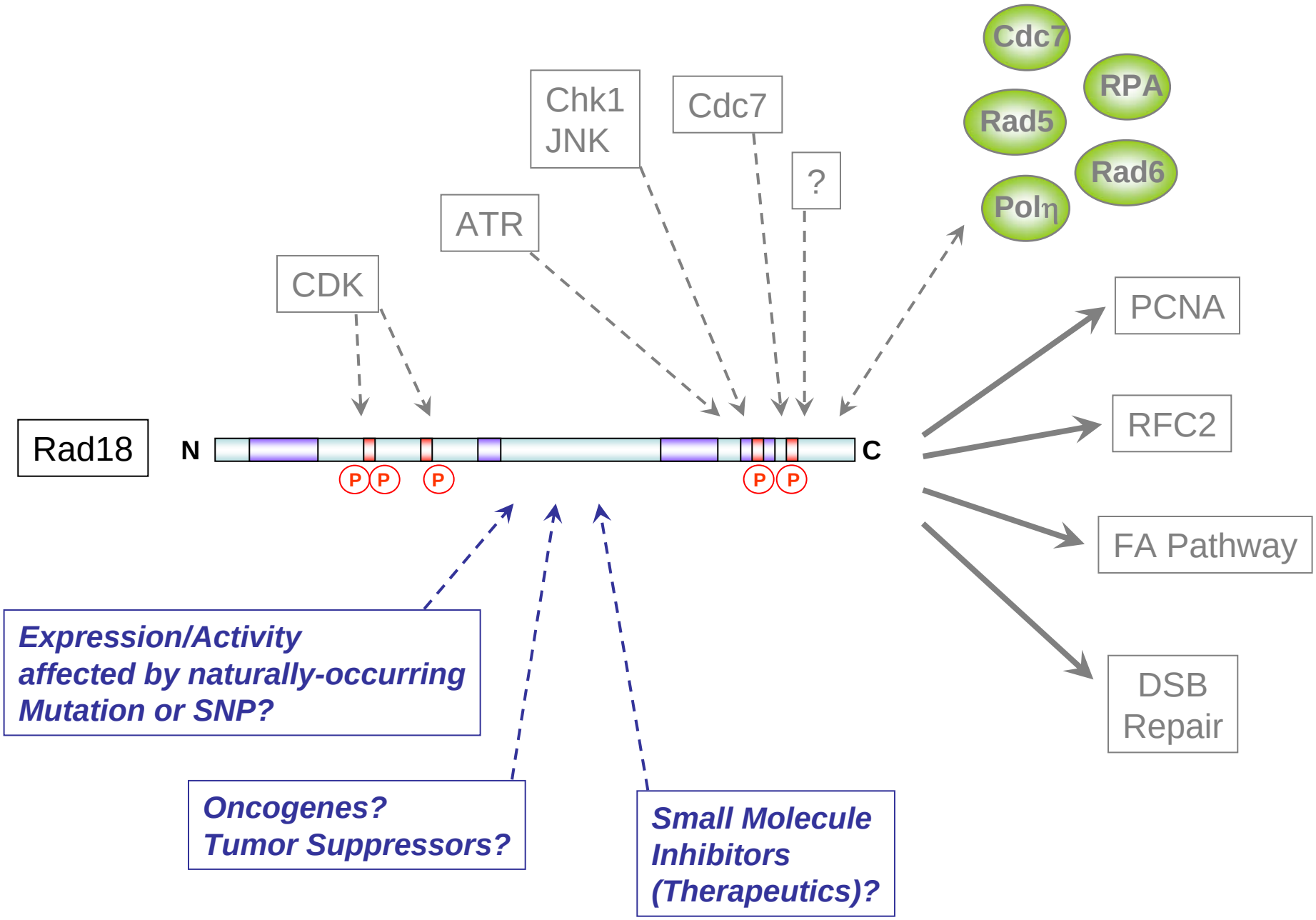
Rad5
Rad6
Polη
Chk1
JNK
Cdc7
?
ATR
CDK
PCNA
RFC2
Rad18
N
C
P
P
P
P
P
FA Pathway
Expression/Activity
affected by naturally-occurring
Mutation or SNP?
DSB
Repair

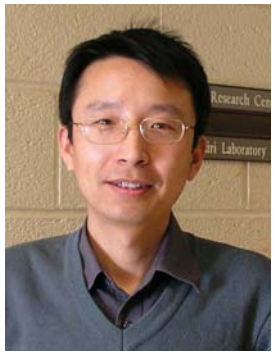

Cdc7
RPA
Rad5
Rad6
Polη
Chk1
JNK
Cdc7
?
ATR
CDK
PCNA
RFC2
Rad18
N
C
P
P
P
P
P
FA Pathway
DSB
Repair
Expression/Activity
affected by naturally-occurring
Mutation or SNP?
Oncogenes?
Tumor Suppressors?


Cdc7
RPA
Rad5
Rad6
Polη
Chk1
JNK
Cdc7
?
ATR
CDK
PCNA
RFC2
Rad18
N
C
P
P
P
P
P
FA Pathway
DSB
Repair
Expression/Activity
affected by naturally-occurring
Mutation or SNP?
Oncogenes?
Tumor Suppressors?
Small Molecule
Inhibitors
(Therapeutics)?

Acknowledgements

Xiaohui Bi

Aditi Gurkar

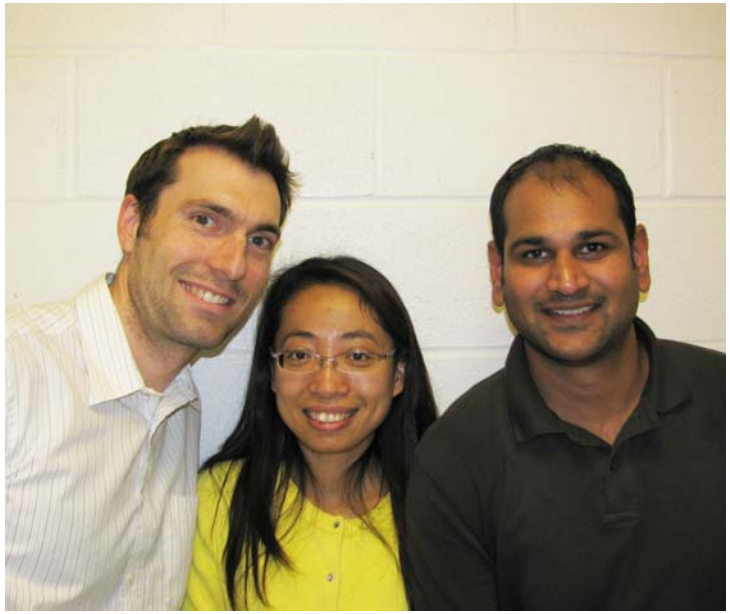


Peijun Liu

Laura Barkley

Ihnyoung Song

Tovah Day



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Dr. Beth Sullivan (Duke)

Dr. Satoshi Tateishi
(Kumamoto University)

Drs. Jim Swenberg, Natalie Kerr, Bill Kaufmann, Stephanie Smith-Roe, Dale Ramsden, Bill Janzen, Monte Willis (UNC)