Uncovering mechanisms of BER regulation in human cells

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NIH DNA Repair Videoconference
WebEx Edition
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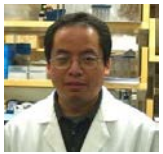
Acknowledgments



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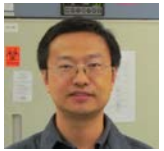

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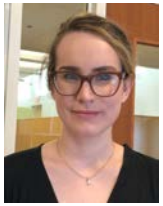

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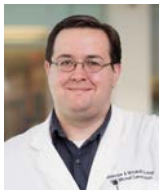

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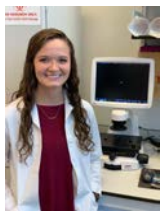

Bailey Manning

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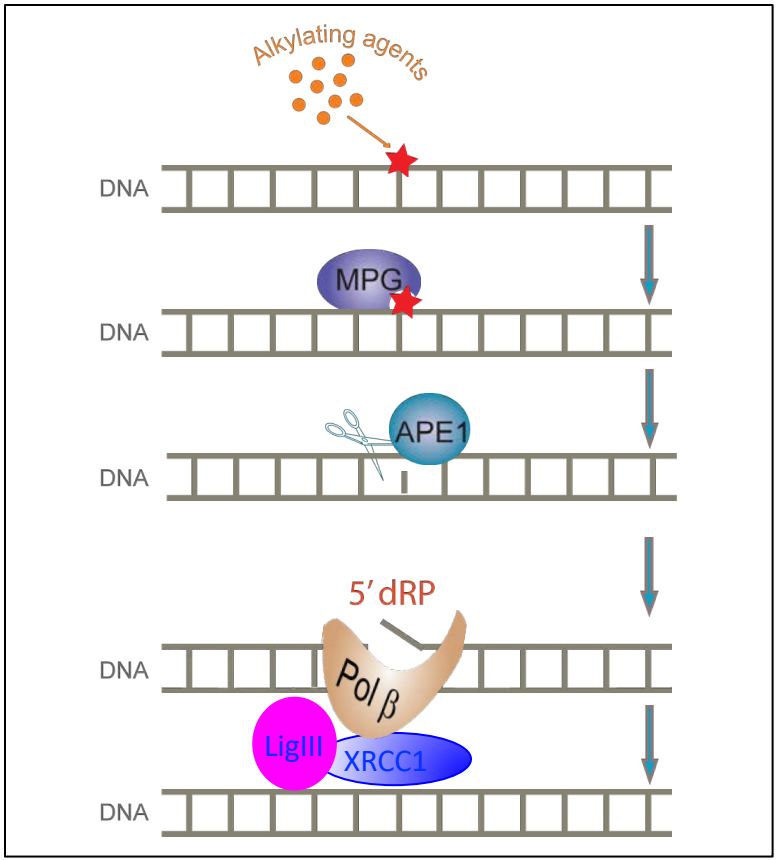
PARP1, SSBR, BER and Cancer

- Both BER and SSBR play critical roles in genome stability and genome mutation avoidance.
- BER/SSBR proteins such as some glycosylases, PNKP, POLB and XRCC1 shown to be altered in cancer, driving genome instability.
- The BER protein NEIL3 essential for DNA crosslink repair.
- BER/SSBR factors APE1, APE2, PNKP and Lig3 considered novel & druggable targets for synthetic lethality.
- There are effective small molecule inhibitors to the poly-ADP-ribose synthesis and degradation enzymes PARP1 and PARG that show preferential killing (synthetic lethality) in some cancer backgrounds (BRCA1, BRCA2 etc).
- Our long-term goals are to uncover novel BER/SSBR protein and metabolic factors that may be regulated or targeted for effective/enhanced treatment response in cancer.

Simple base excision repair (BER) Model



Base damage induced by exogenous or endogenous sources

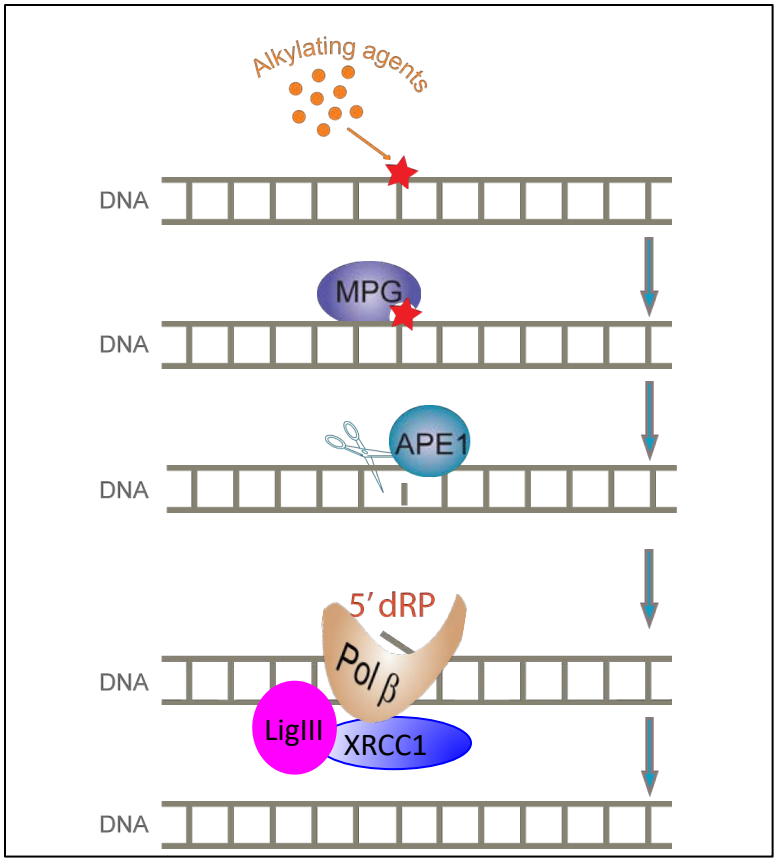
Base damage removed by a DNA glycosylase

DNA cleaved at the abasic site by an AP endonuclease

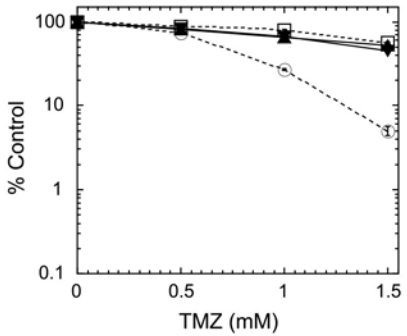
DNA polymerase β recruited to the site to:

1) Tailor the gap (5'dRPase)
2) Synthesize DNA (one base insertion)

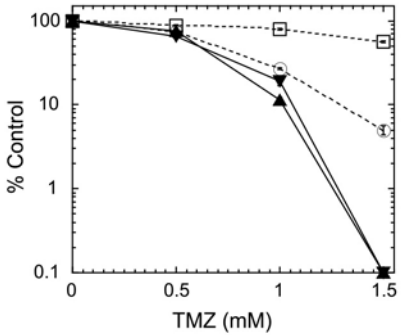
DNA polymerase β is essential for repair and cell survival in response to base damage, especially the gap tailoring activity



Polβ WT

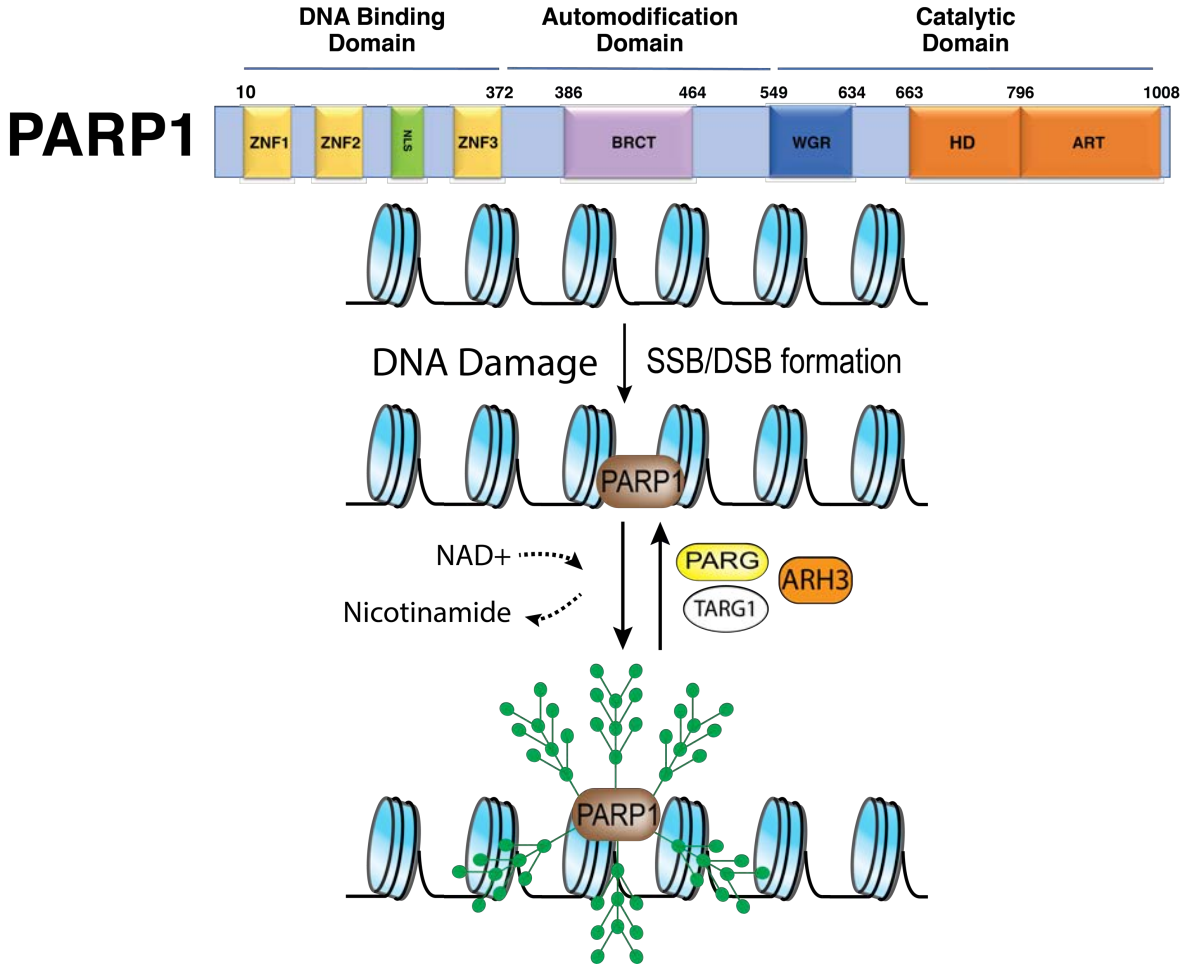


Polβ Lyase-dead

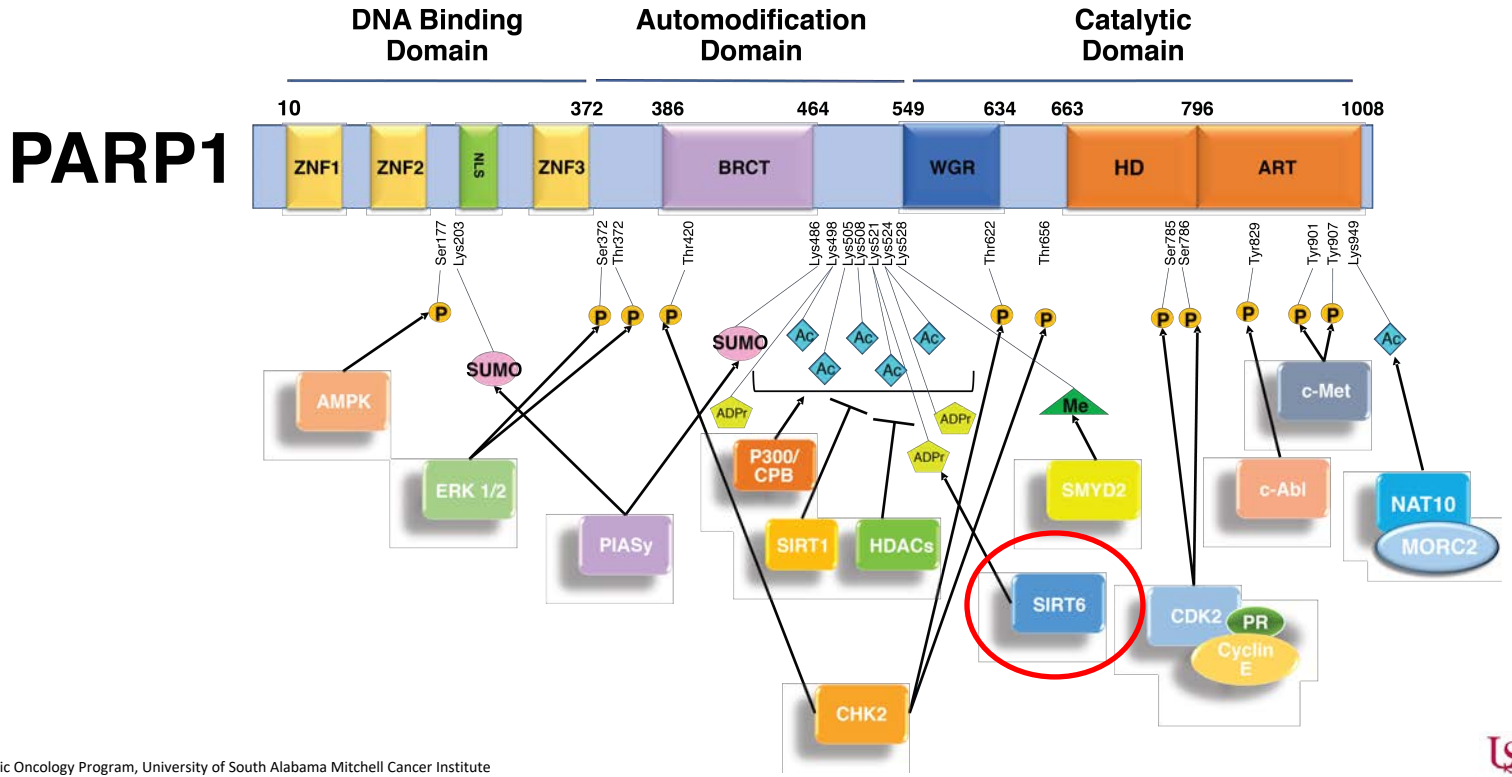


Nature 1996; Nature 2000; Cancer Res 2007, MCB 2010

PARP1 is a critical BER factor in mammalian cells



PARP1: highly regulated by post-translational modifications

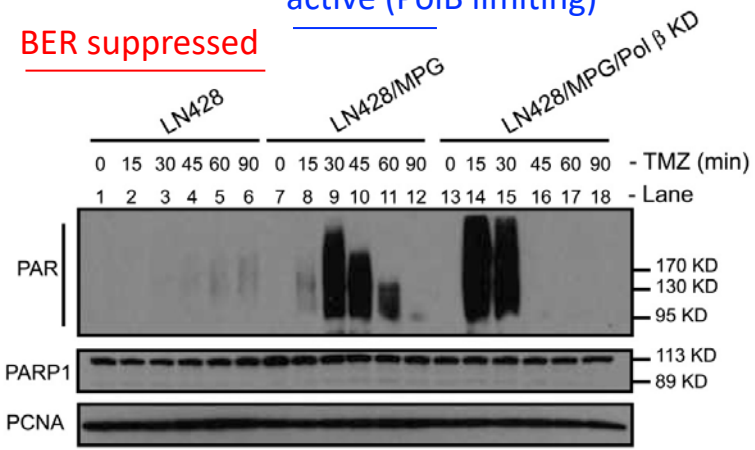


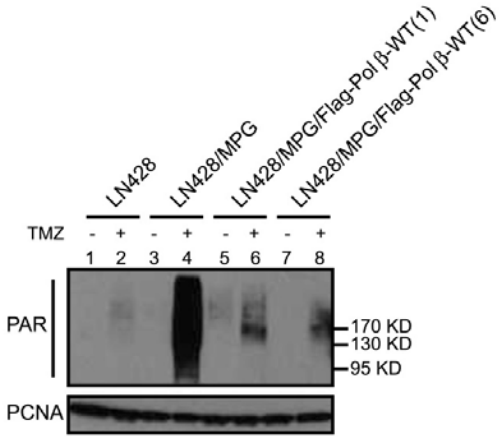
Base Excision Repair (BER) defect triggers PARP-activation dependent cell death

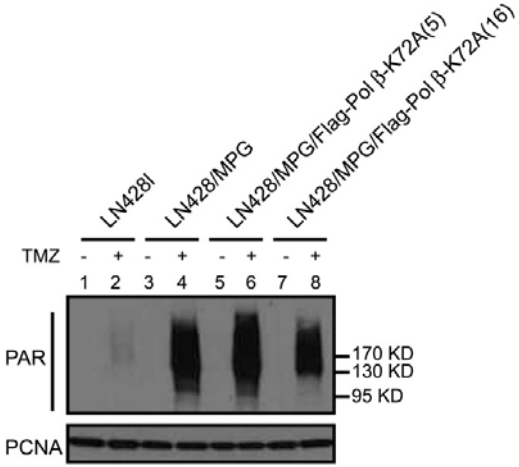
BER hyper-active
+ PolB suppressed

BER hyper-
active (PolB limiting)

BER suppressed

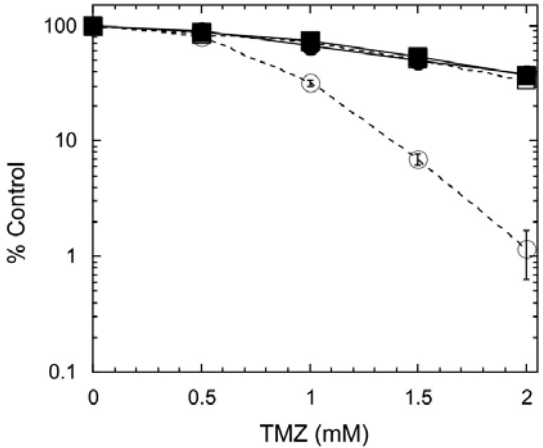


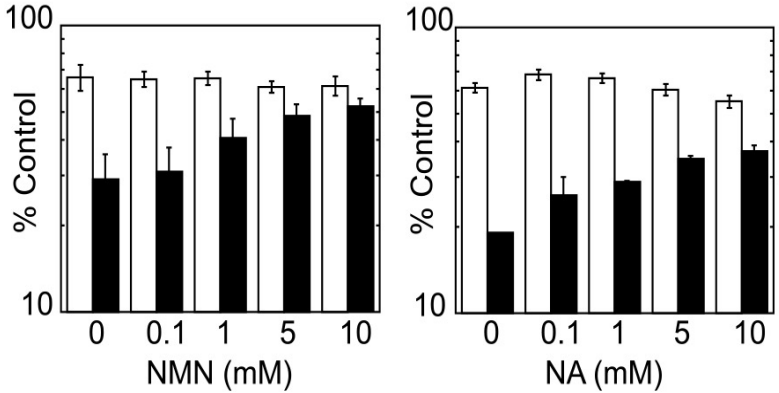




Tang JB et al.
Mol Cancer Res. 2010 Jan;8(1):67-79.

Base Excision Repair (BER) defect triggers PARP-activation dependent cell death -rescue by PARPi or NMN supplementation





Tang JB et al.
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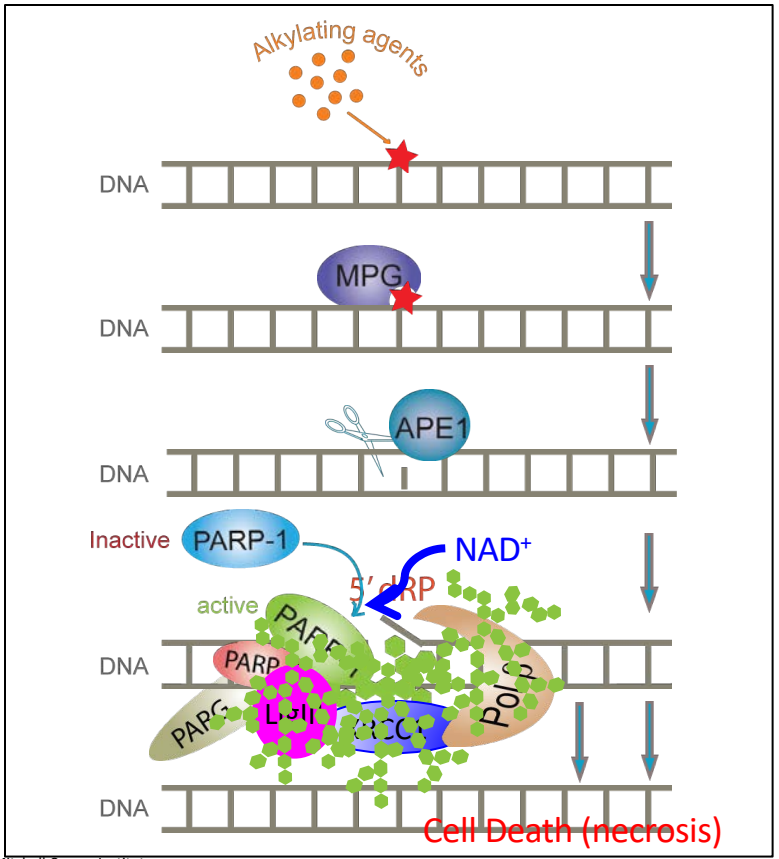
Mammalian BER Model

⇒ Support for this model:
Polß loss triggers damage-induced PARP1 hyperactivation and likely dependence on NAD⁺ levels



This model places PARP1 upstream of Polβ

The implication is that the PARP1 substrate NAD⁺ is an essential BER co-factor.

Fornsaglio JL, et al.
Mutat Res. (2010)
Apr 1;686(1-2):57-67.

Tang JB, et al.
Mol Cancer Res. (2010)
Jan;8(1):67-79.

Tang JB, et al.
Neuro Oncol. (2011)
May;13(5):471-86.

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Mar 15;71(6):2308-17.

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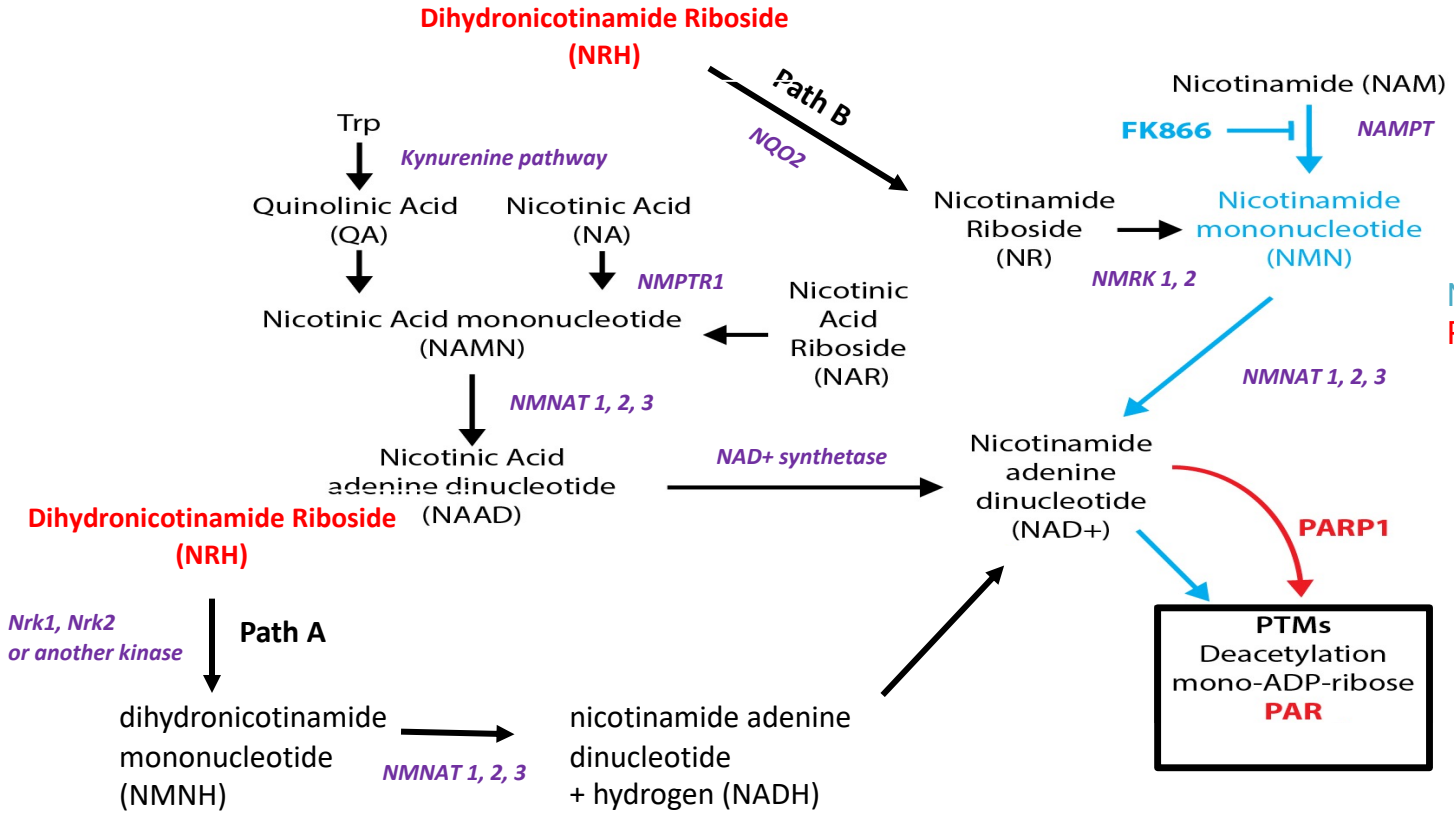
Genes Dev. (2013)
May 15;27(10):1089-100.

Calvo JA, et al.
PLoS Genet. (2013)
Apr;9(4):e1003413.

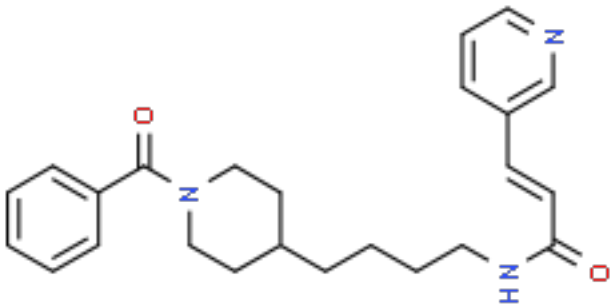
Differential metabolic alterations mediated by PARP1 activation or NAD+ depletion by FK866



NAD depletion by FK866
PARP1 activation

Sobol, DNA Repair November 2014, DOI:10.1016/pmic.201410007

NAD Biosynthesis inhibition



FK866

Nicotinamide
(NAM)

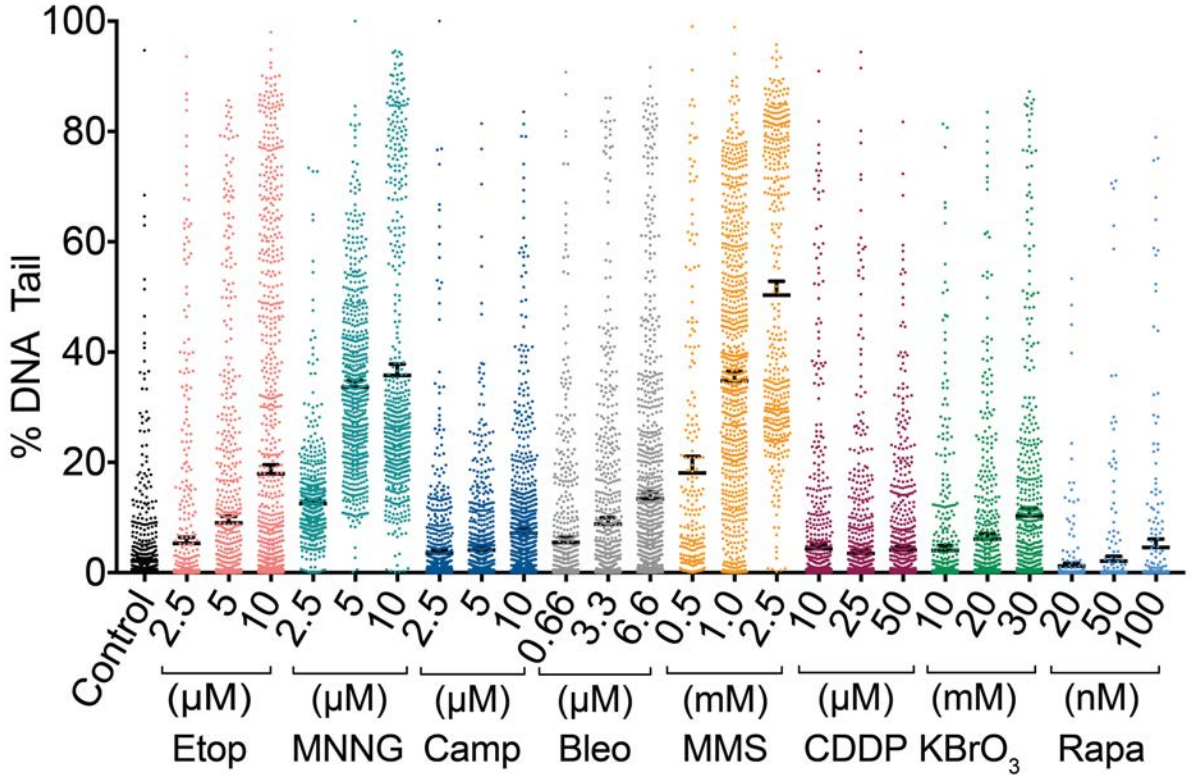
FK866 ⊣ NAMPT ↓

Nicotinamide mononucleotide
(NMN)

NMNAT ↓

Nicotinamide adenine dinucleotide
(NAD^+)

Dose response for Chemical-induced genomic DNA damage



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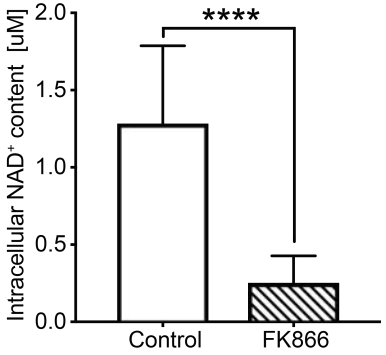
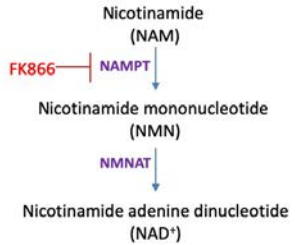
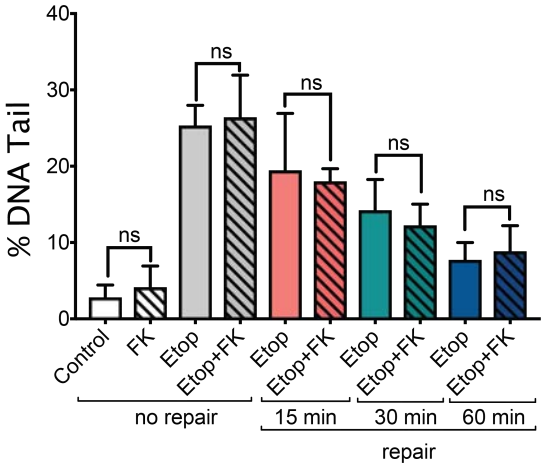
DNA repair of genomic DNA damage from some chemicals is suppressed when NAD⁺ is deficient

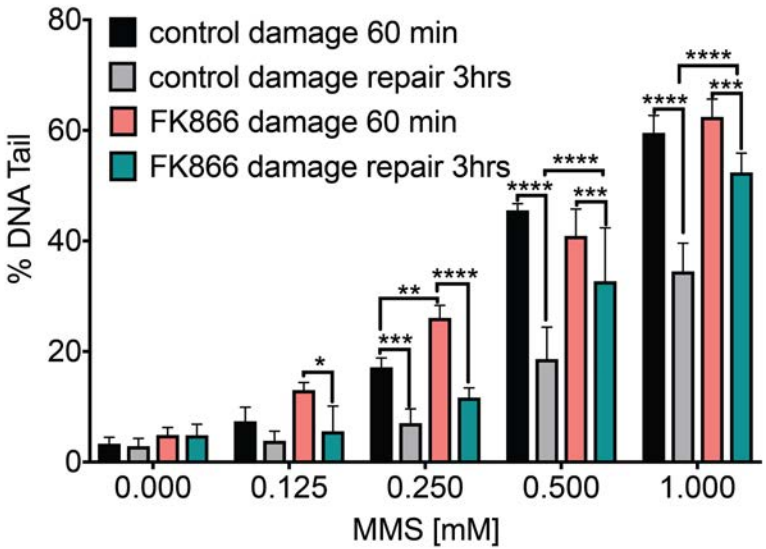




Etoposide



MMS



➔ Supports a role for NAD⁺ as a critical BER factor

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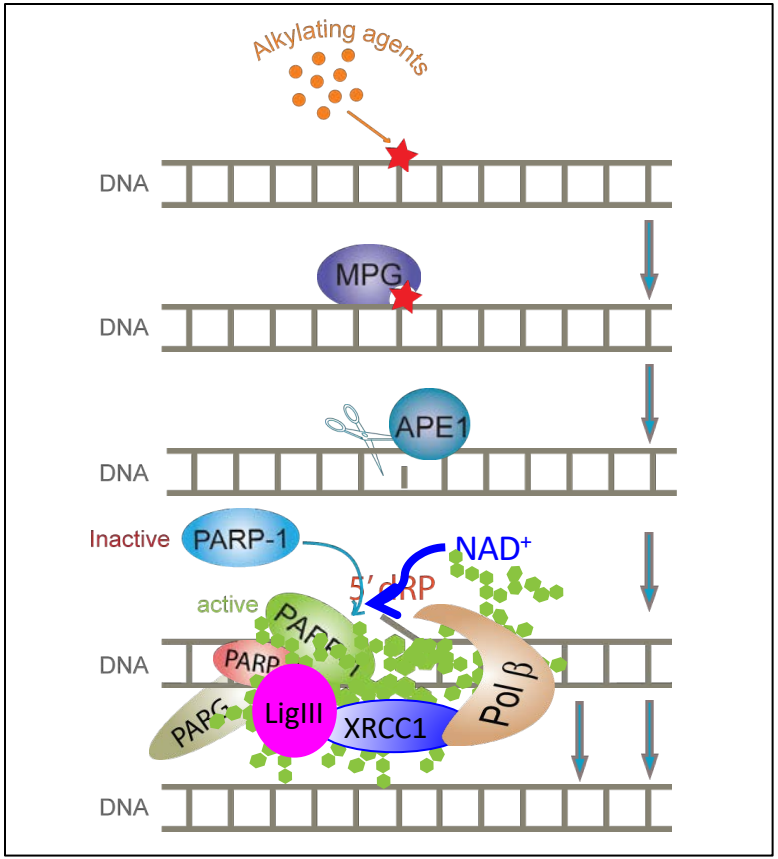
Mammalian BER Model

⇒ Can we modulate BER complex formation with alterations in NAD^+ levels ?



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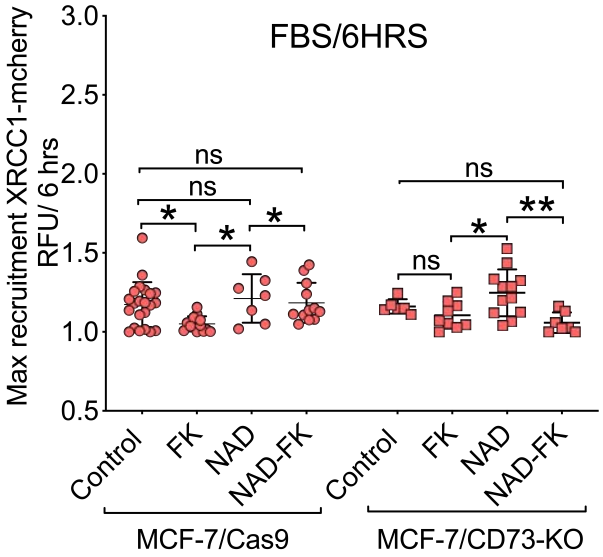
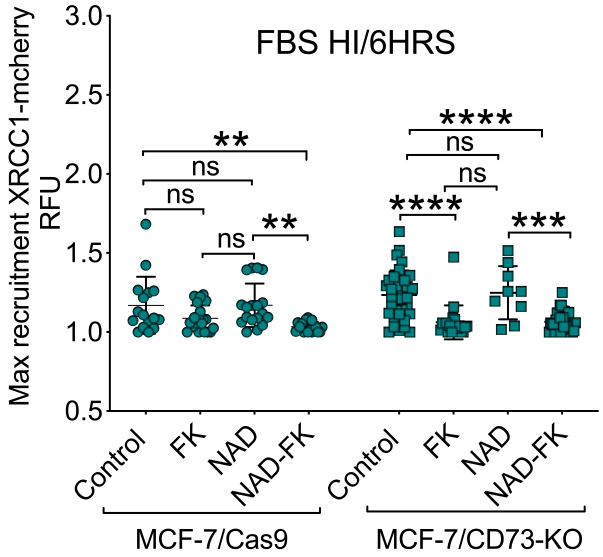
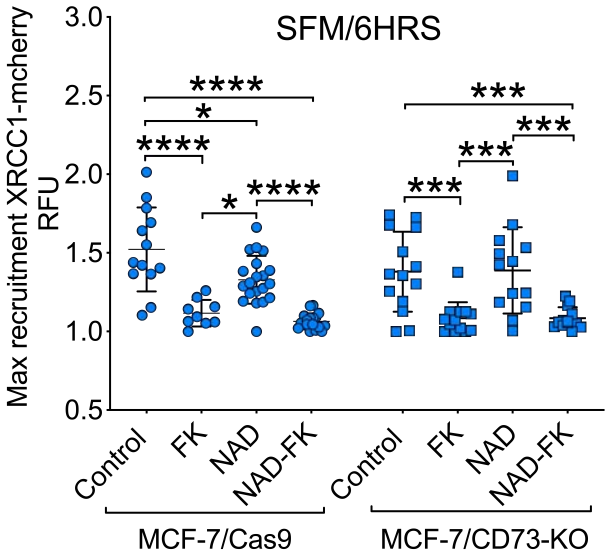
XRCC1 complex formation in cells following reduction (FK) and supplementation of NAD^+







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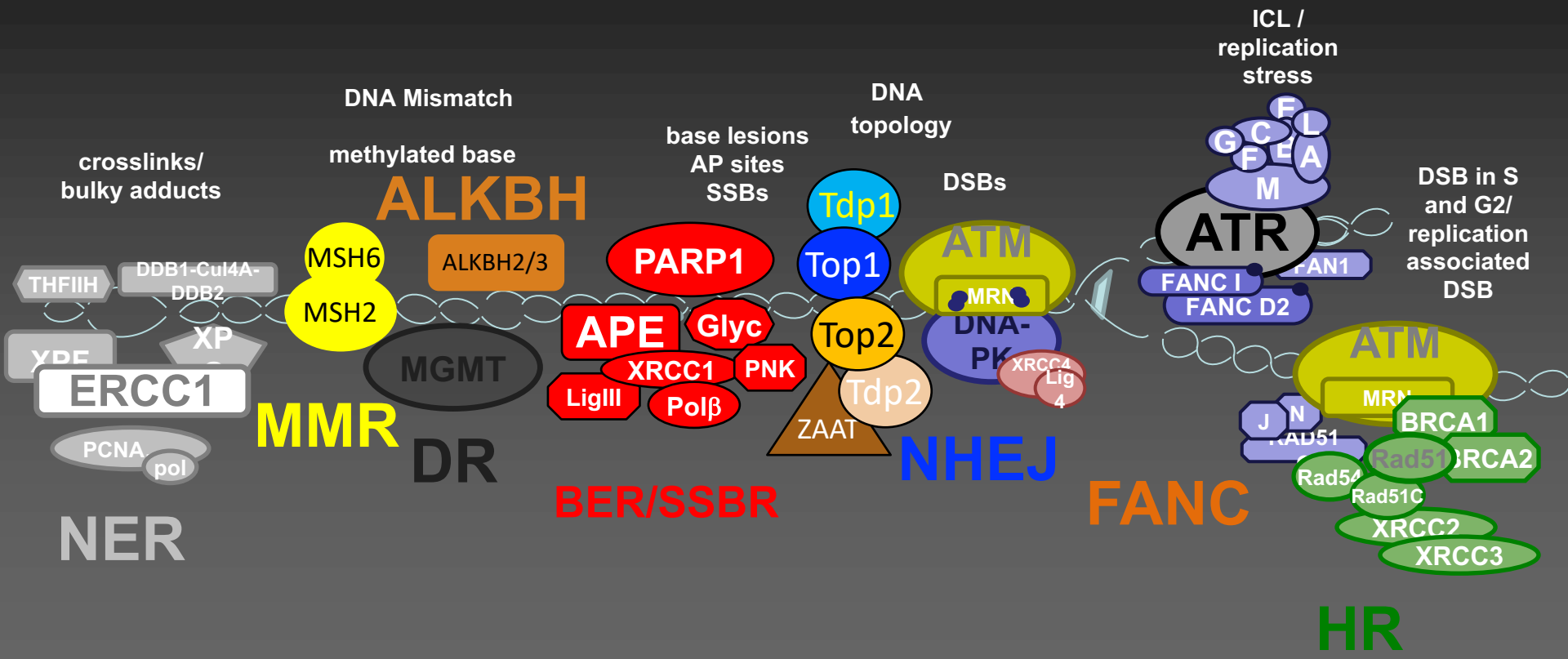
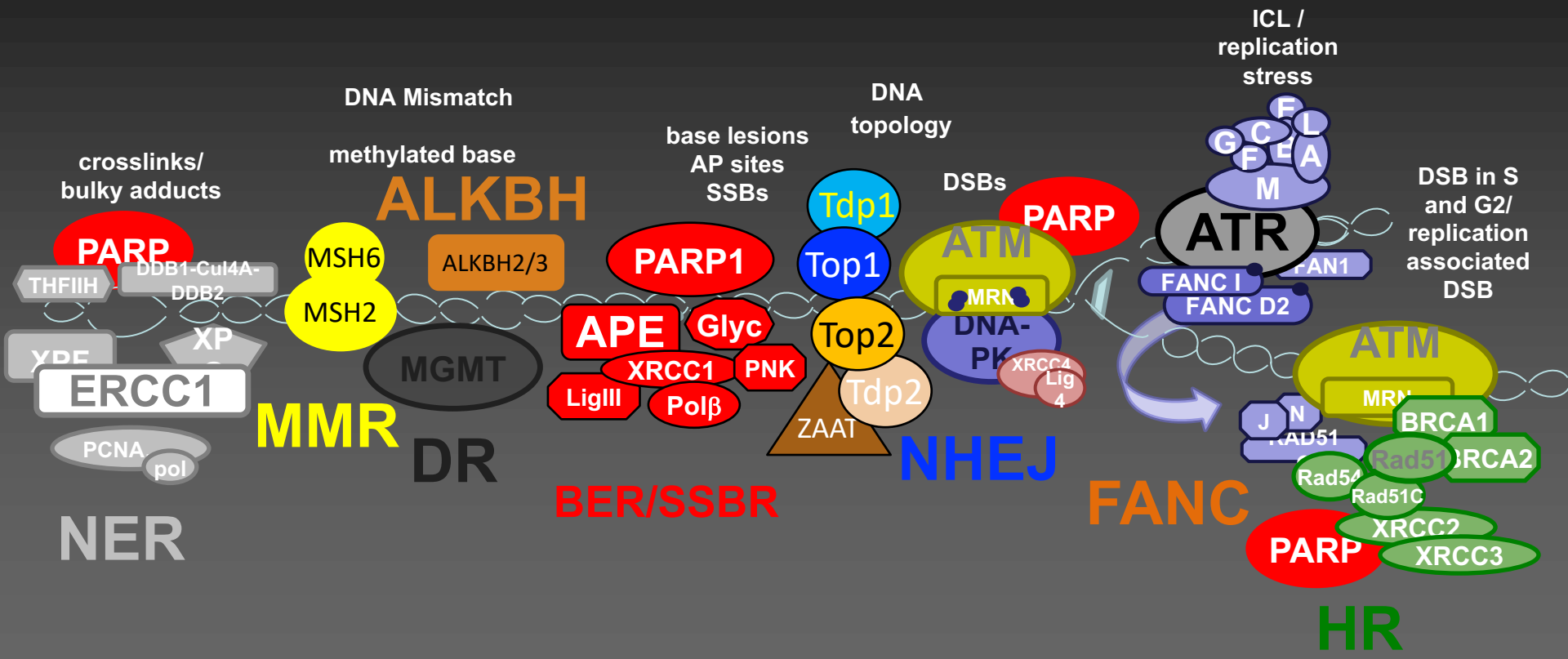
DNA Repair Pathways mediating genotoxin/chemo/radiation resistance



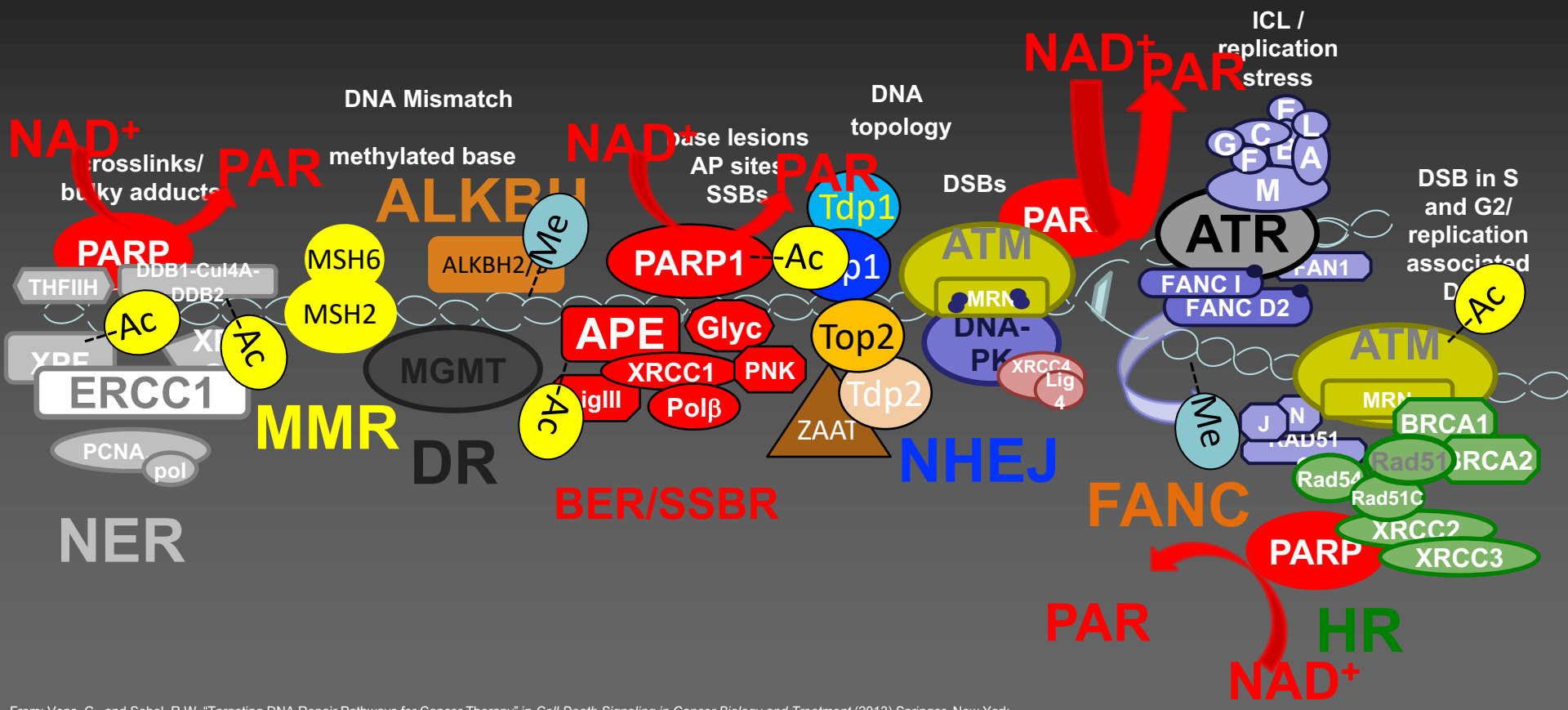
From: Vens, C., and Sobol, R.W. "Targeting DNA Repair Pathways for Cancer Therapy" in *Cell Death Signaling in Cancer Biology and Treatment* (2013) Springer, New York.

PARP1 and other signaling factors play a critical role in the DDR, transmitting the signal via PTMs



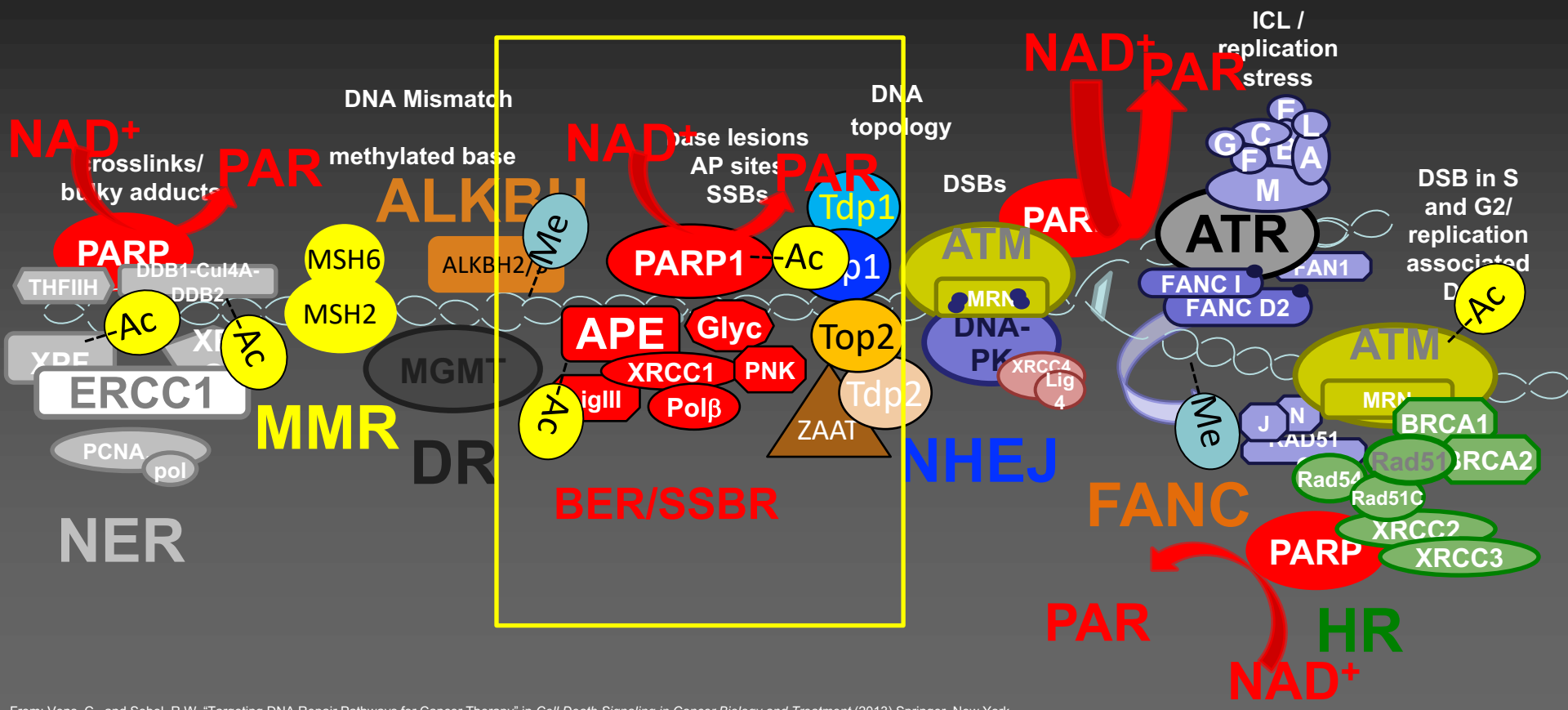
From: Vens, C., and Sobol, R.W. "Targeting DNA Repair Pathways for Cancer Therapy" in *Cell Death Signaling in Cancer Biology and Treatment* (2013) Springer, New York.

Many of these pathways require post-translational modifications that are regulated by NAD+ or related small metabolites



From: Vens, C., and Sobol, R.W. "Targeting DNA Repair Pathways for Cancer Therapy" in *Cell Death Signaling in Cancer Biology and Treatment* (2013) Springer, New York.

Our lab's focus has been primarily on the BER pathway



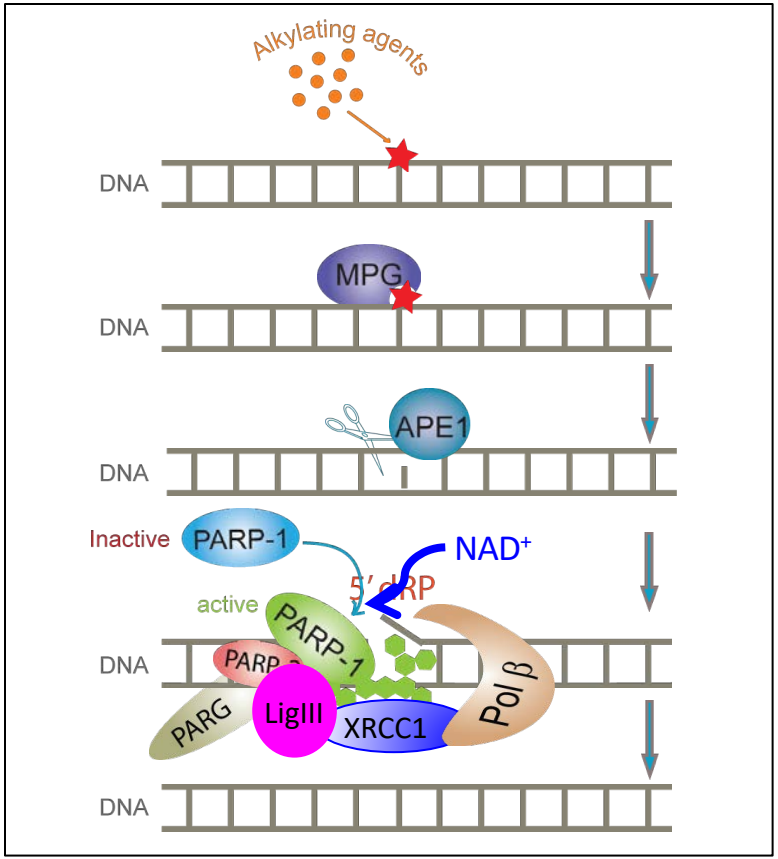
From: Vens, C., and Sobol, R.W. "Targeting DNA Repair Pathways for Cancer Therapy" in *Cell Death Signaling in Cancer Biology and Treatment* (2013) Springer, New York.

Mammalian base excision repair (BER/SSBR) Model

Glycosylases (11)

Poly-ADP-ribose polymerases (2)

PARG



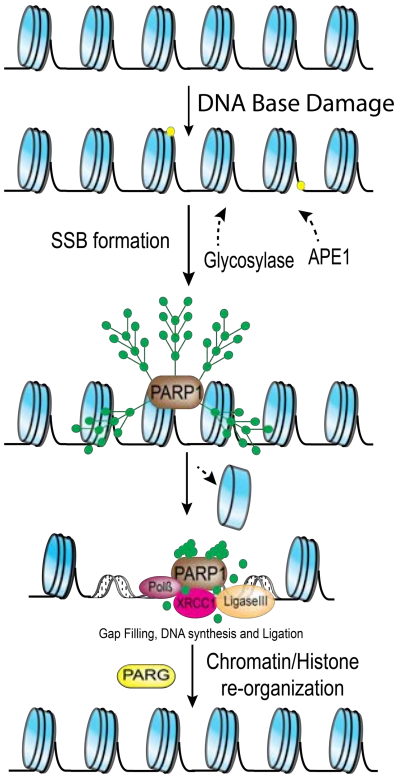
What about when in chromatin?

AP endonucleases (2)

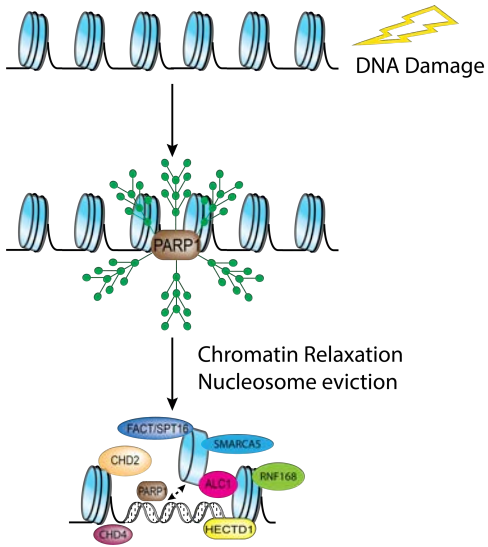
DNA polymerases in BER (4)

DNA ligases (2)

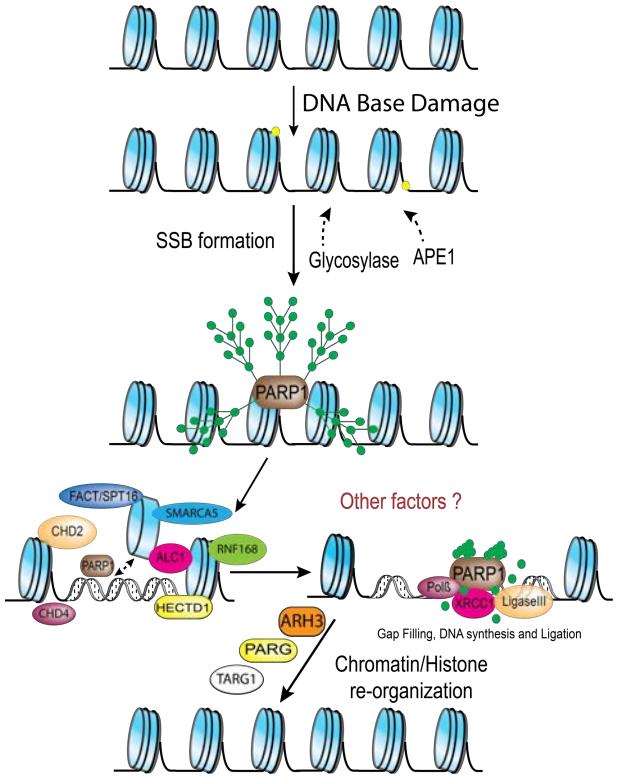
SSBR/BER



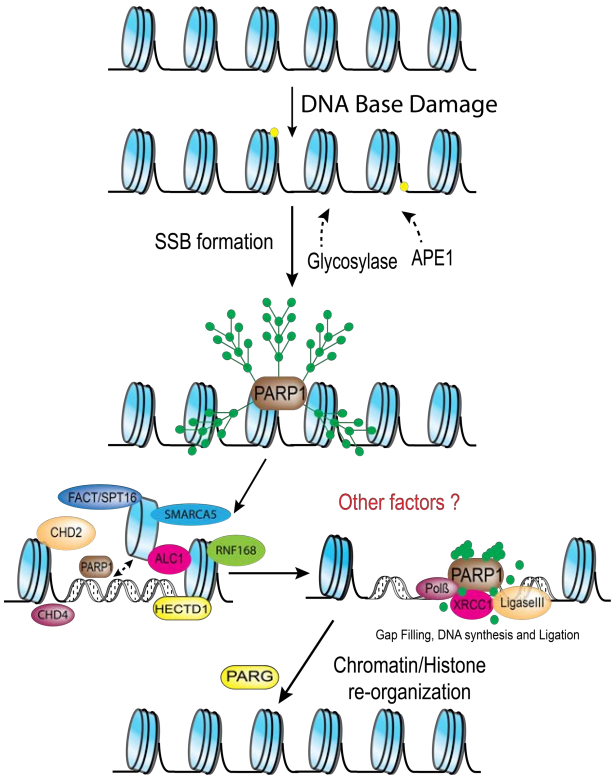
PARP1 & Chromatin relaxation



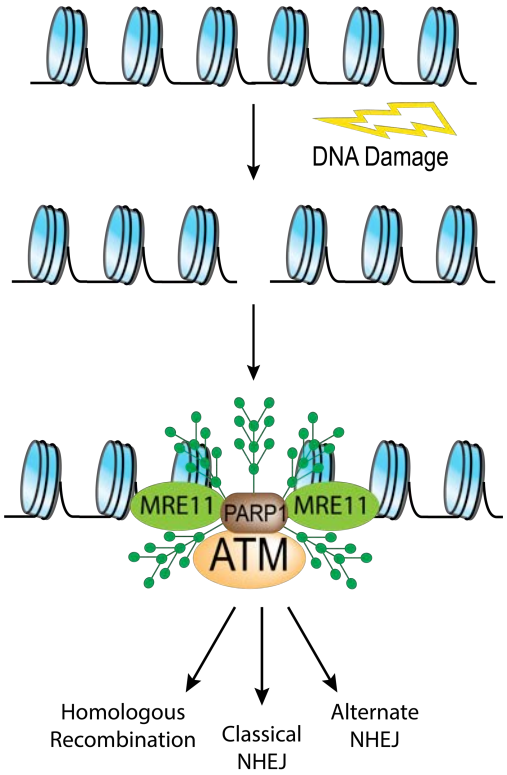
SSBR/BER & chromatin factors



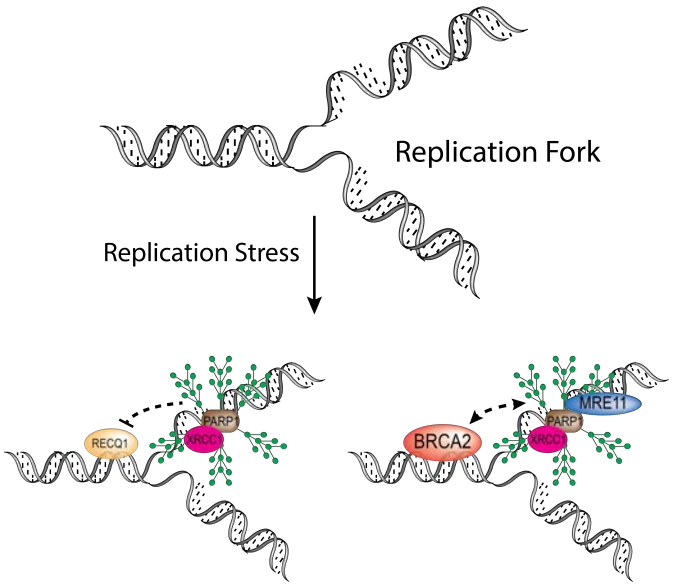
PARP1 in SSBR/BER



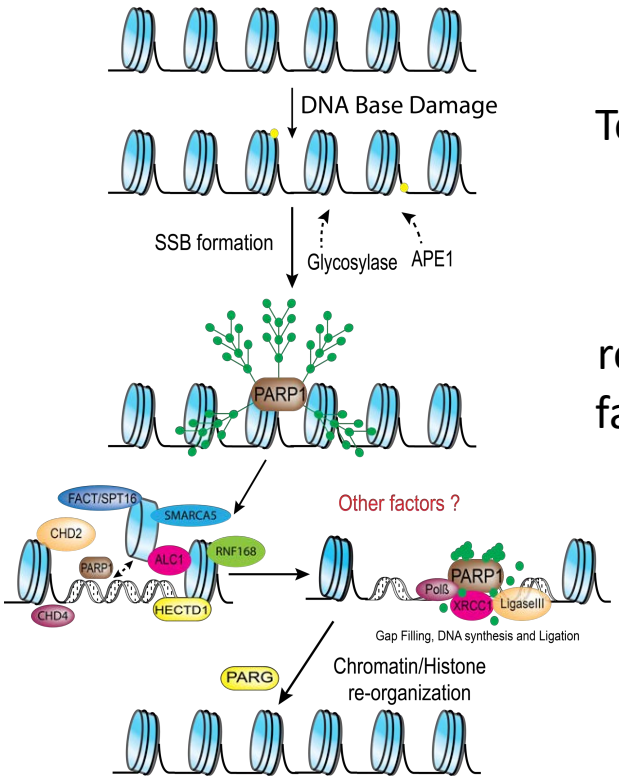
PARP1 in DSBR



PARP1 in SSBR at Replication forks



PARP1 in SSBR/BER

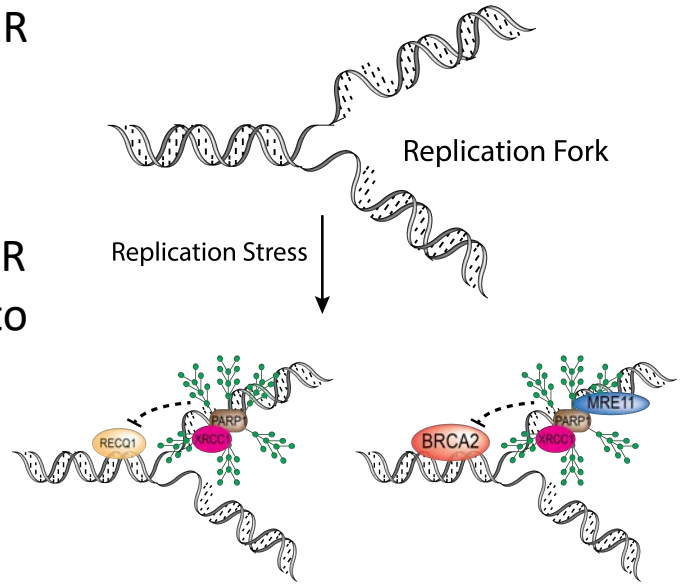


Ongoing/future plans

To temporally map the SSBR/BER interactome in cancer.

Goal: To discover novel regulatory or essential BER/SSBR factors that can be considered to enhance cancer treatment response.

PARP1 in SSBR at Replication forks



Uncovering novel BER/SSBR factors

- Temporal map protein factors by BioID, Split-BioID and classical protein interactome analysis.
- Uncover the protein and histone acetylation/ADP-ribosylation code that regulates classical BER/SSBR.
- Define the role of PARP1, XRCC1 and the PARP1/PAR interactome in SSBR at replication forks that govern the intra S-phase checkpoint.

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- Temporal map protein factors by BioID, Split-BioID and classical protein interactome analysis.
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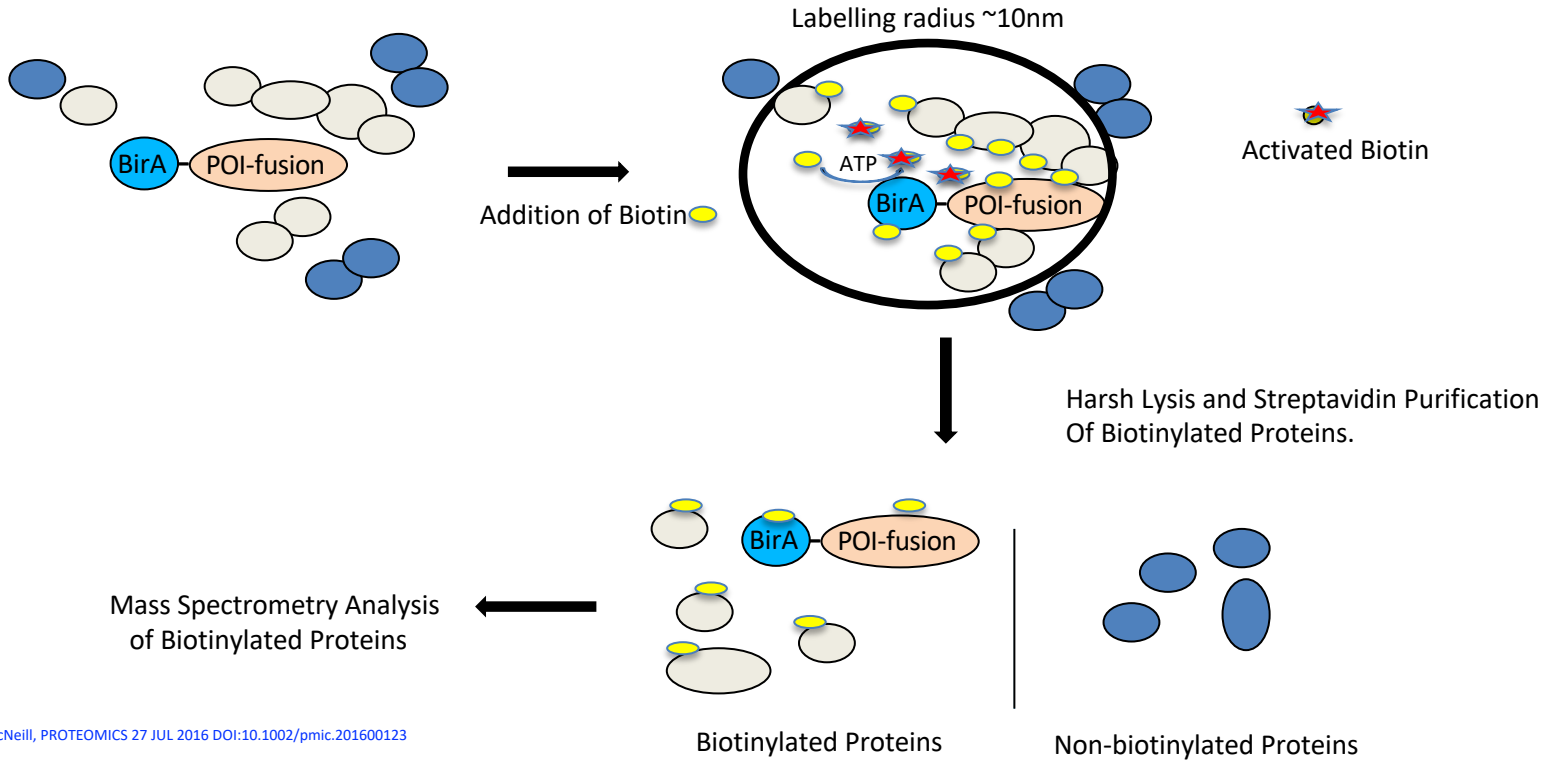
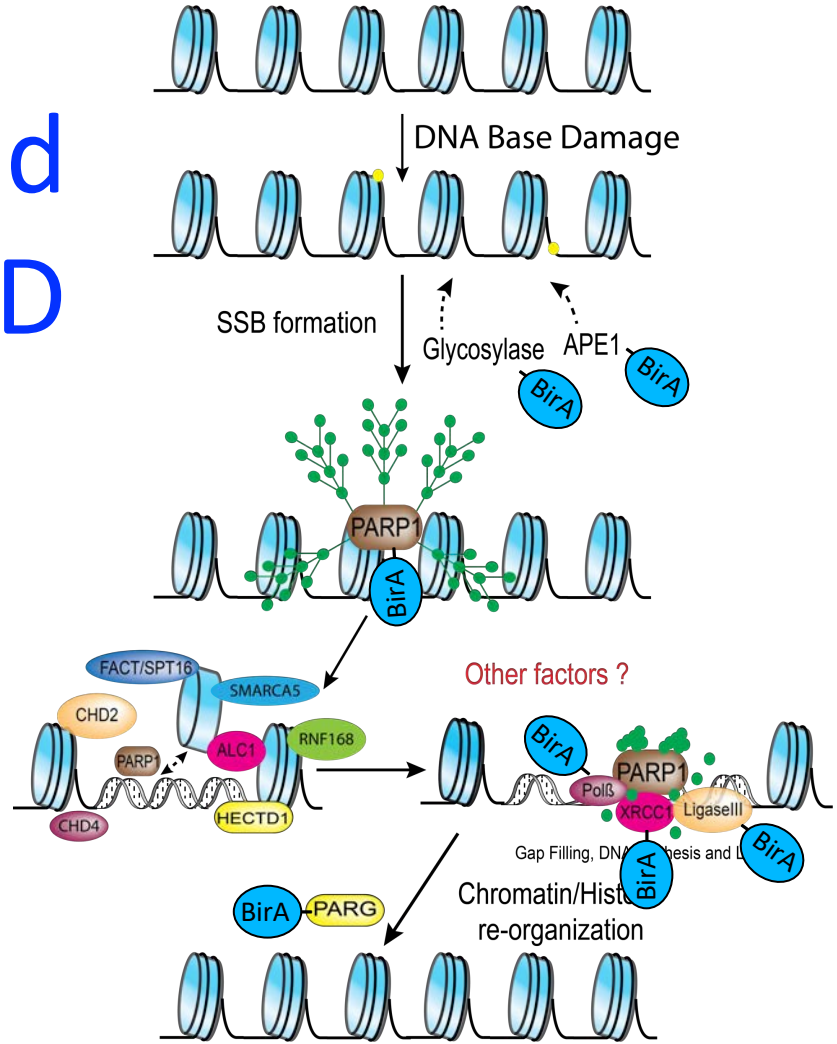
Proximity Protein Identification-BioID

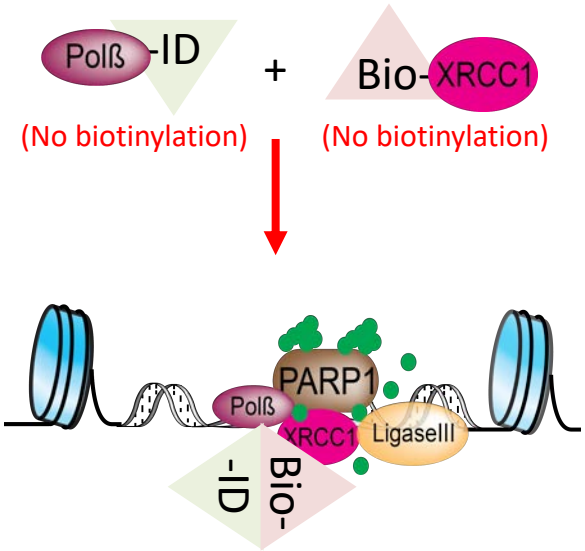


Varnaite and MacNeill, PROTEOMICS 27 JUL 2016 DOI:10.1002/pmic.201600123

Jenn Clark – In preparation

Bio-ID and Split-BioID scheme

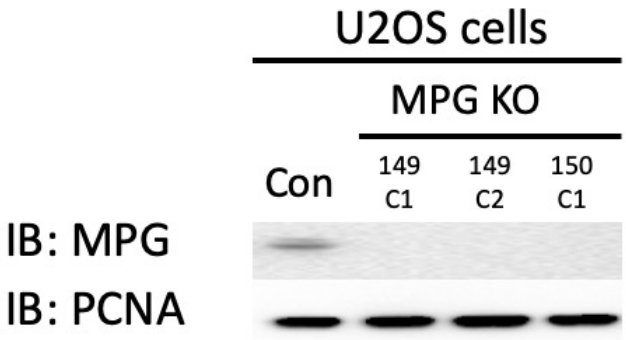


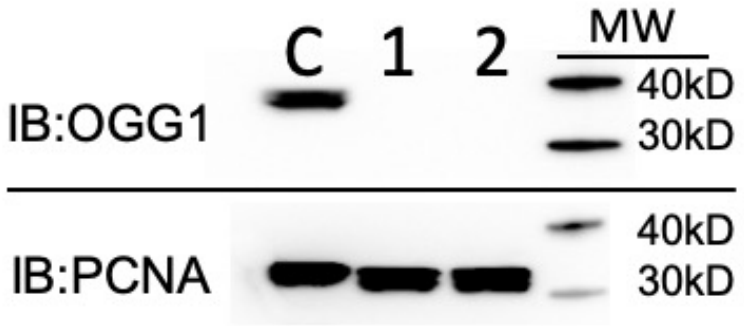


Overall Glycosylase BioID scheme

- KO DNA glycosylase of interest by CRISPR/cas9 (MPG, OGG, etc).
- Express BioID fused MPG (MPG-BirA) or OGG1 (OGG1-BirA) in KO cells using Tet-regulated (Dox-on) lentiviral vector.
- Identify biotinylated proteins +/- Dox and +/- DNA damage (MMS, H_2O_2, etc).

KO DNA glycosylase of interest by CRISPR/cas9 (MPG, OGG, etc).



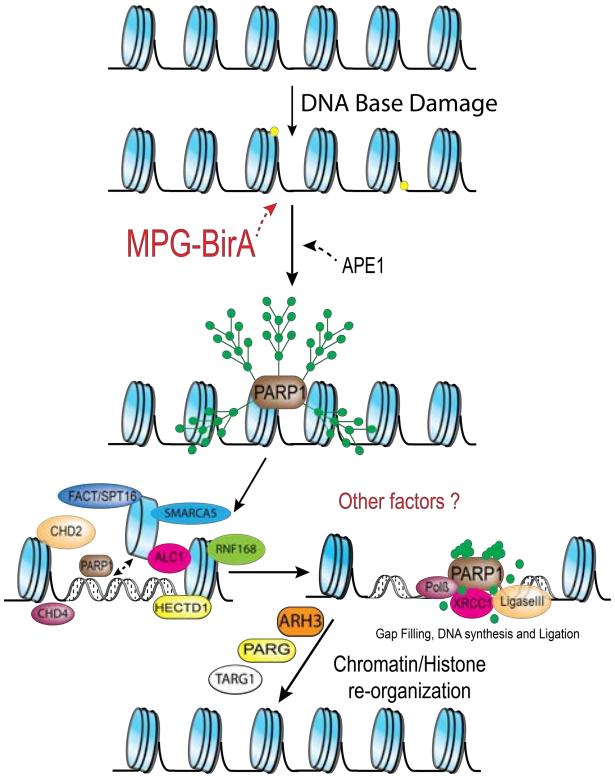


For each, express BioID fused glycosylase in U2OS cells.

Jenn Clark – In preparation

Repair of N-alkylated bases

BER



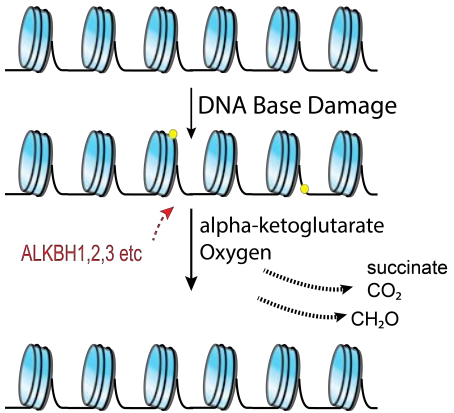
Damage sensor role of UV-DDB during base excision repair.
Nat Struct Mol Biol. 2019 Aug;26(8):695-703.
(Van Houten group)

MPG-Bio-ID

+/- MMS

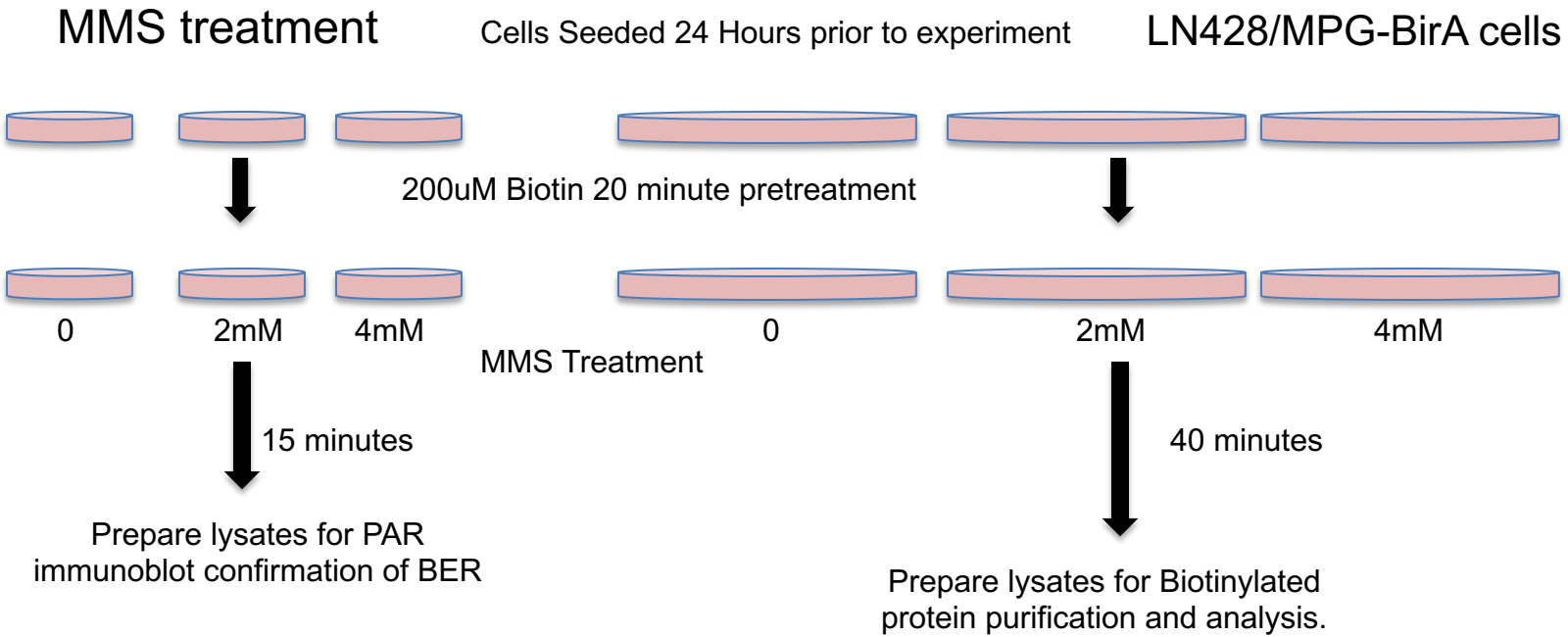
Repair of N-alkylated bases

ALKB-family



Direct repair of 3,N(4)-ethenocytosine by the human ALKBH2 dioxygenase is blocked by the AAG/MPG glycosylase.
DNA Repair (Amst). 2012 Jan 2;11(1):46-52.
(Samson group)

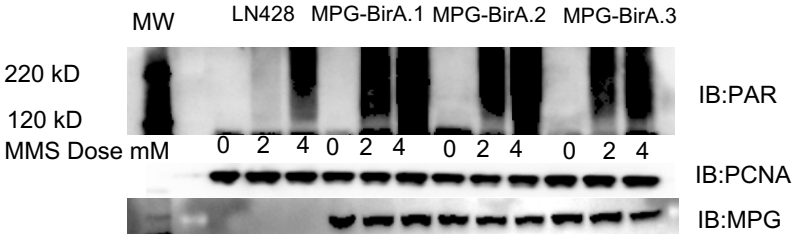
MMS treatment schedule



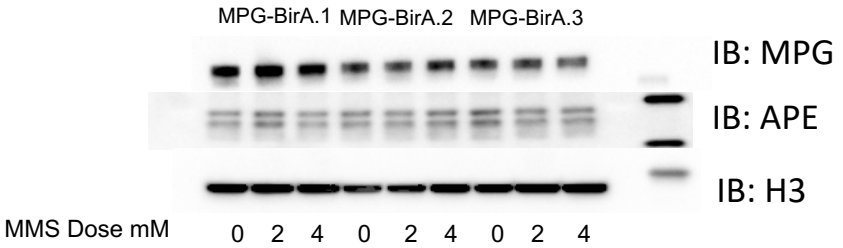
Methyl methanesulfonate (MMS) is an alkylating agent that methylates N7-deoxyguanosine and N3-deoxyadenosine.

Jenn Clark – In preparation

PAR analysis confirming MMS-induced DNA damage



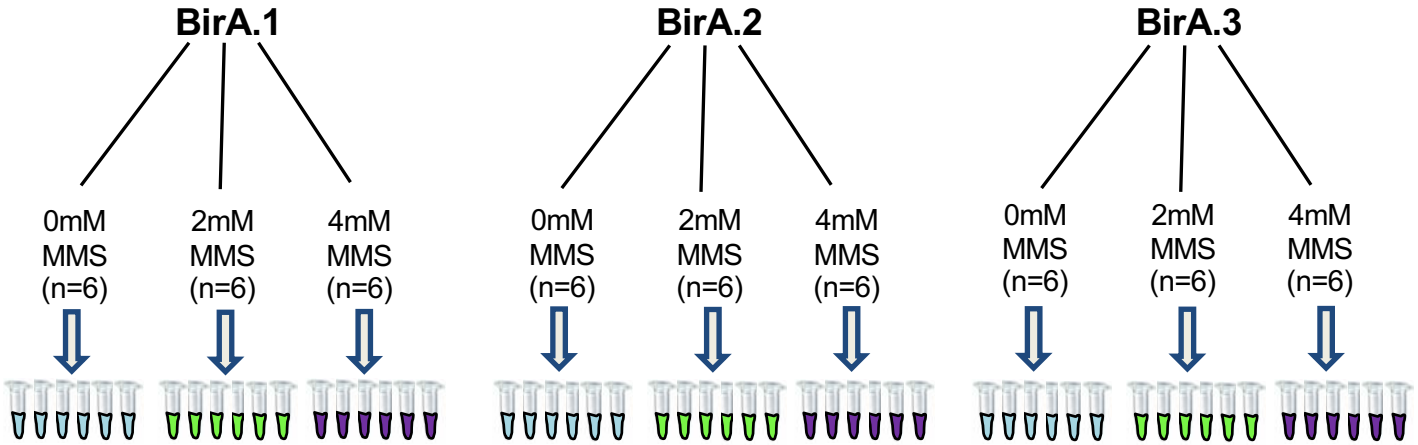
PAR blot- 15 min time-point



Streptavidin Pull-Down 40 minutes post-MMS

Jenn Clark – In preparation

Mass Spectrometry analysis sample prep

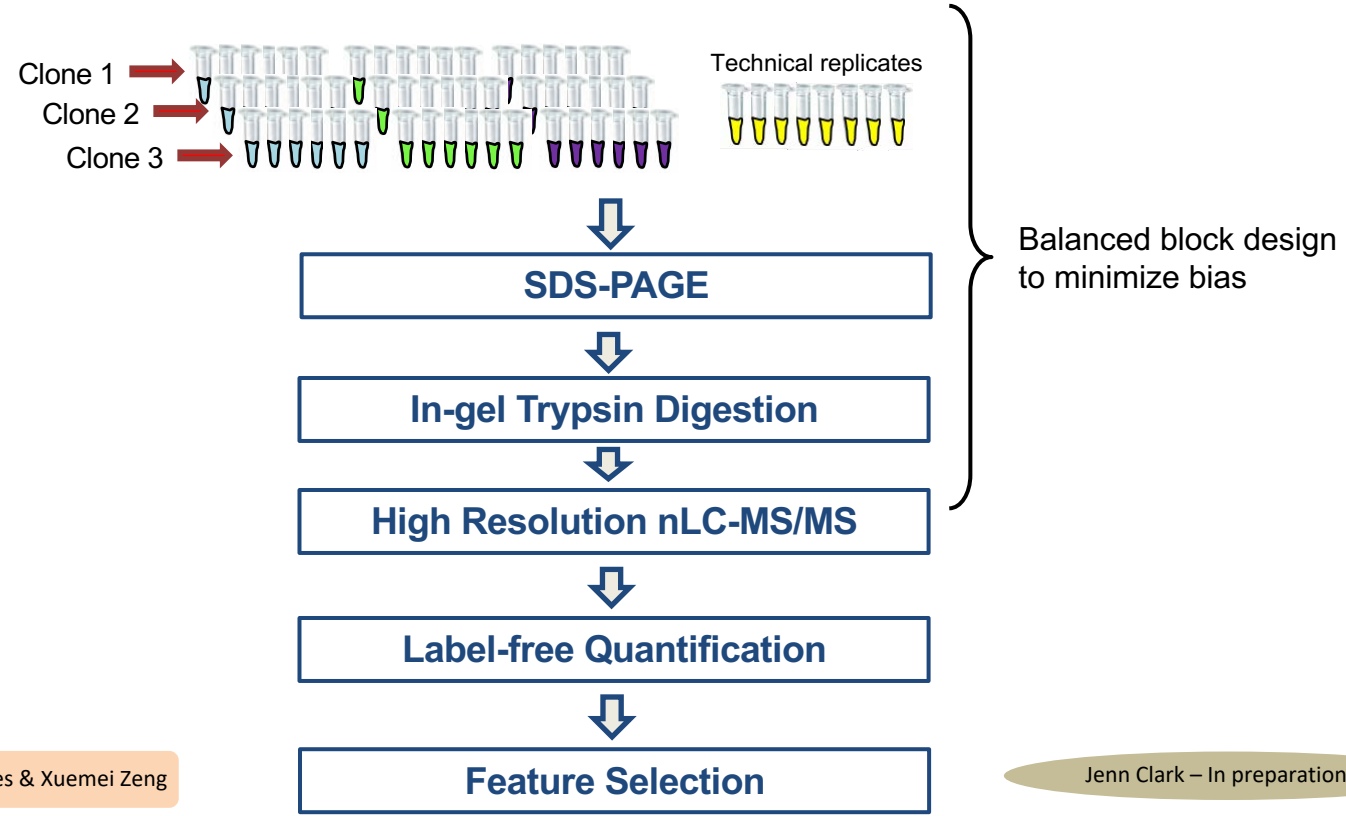


- Three different clones (BirA1, BirA2, and BirA3) of cell line LN428 were treated with media or methyl methane sulfonate (MMS) at 0, 2, and 4mM for 40mins.
- All cells were spiked with 200μM biotin at 20mins before damage.
- Biotin-labeled proteins were isolated by streptavidin-magnetic beads. A total of 54 streptavidin IP samples were received.

Proteomics w/Nathan Yates & Xuemei Zeng

Jenn Clark – In preparation

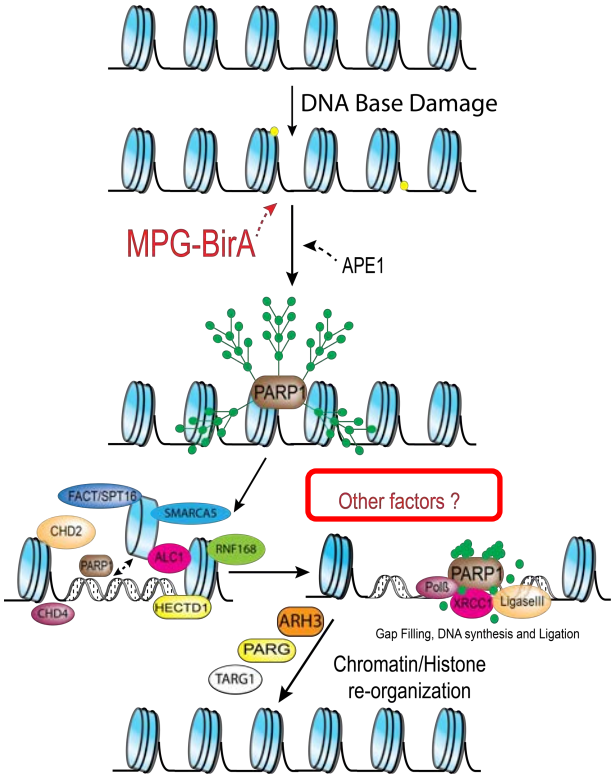
Label-free Differential Mass Spectrometry Workflow



Proteomics w/Nathan Yates & Xuemei Zeng

Jenn Clark – In preparation

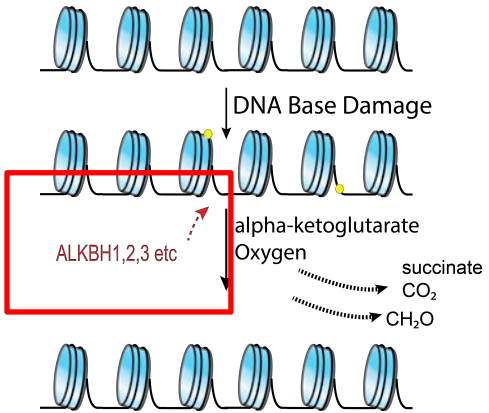
Repair of N-alkylated bases
BER



MPG-Bio-ID

+/- MMS

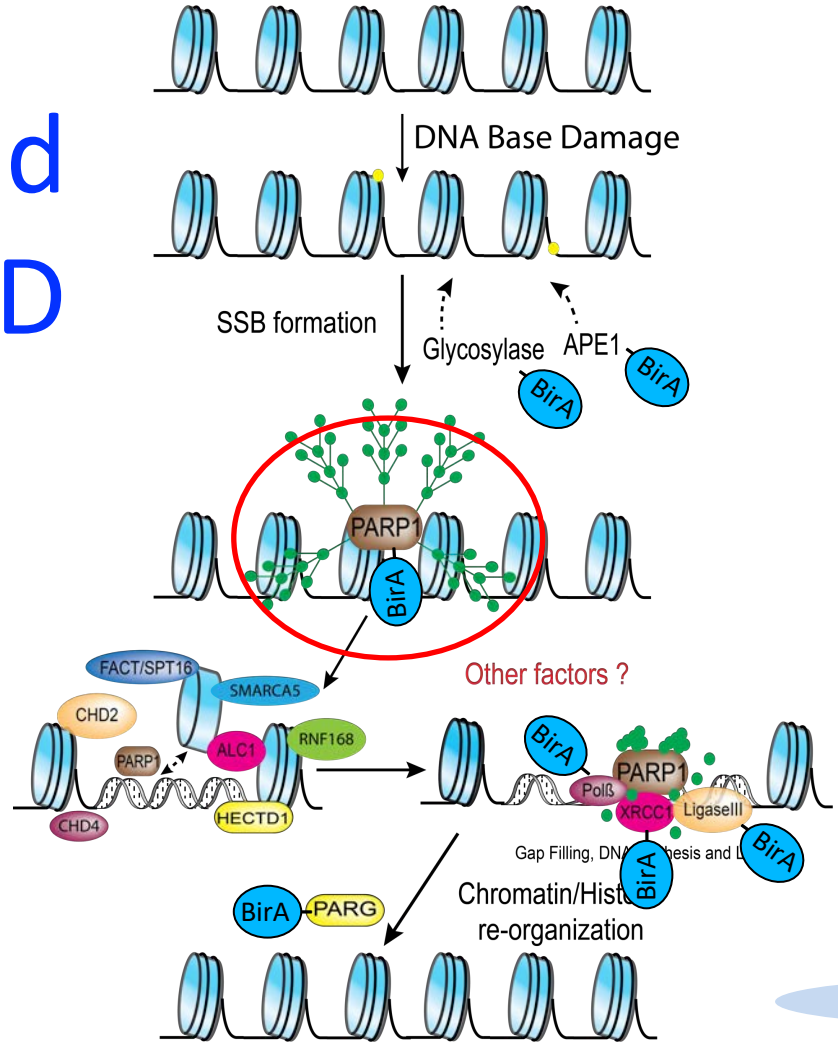
Repair of N-alkylated bases
ALKB-family

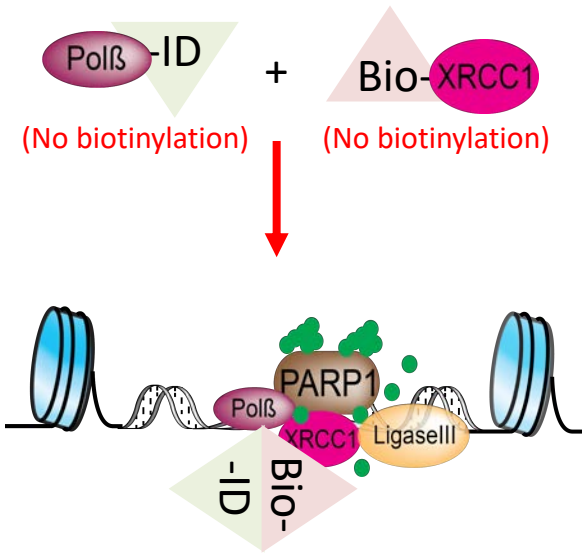


Jenn Clark – In preparation

Bio-ID and Split-BioID scheme

PARP-Bio-ID

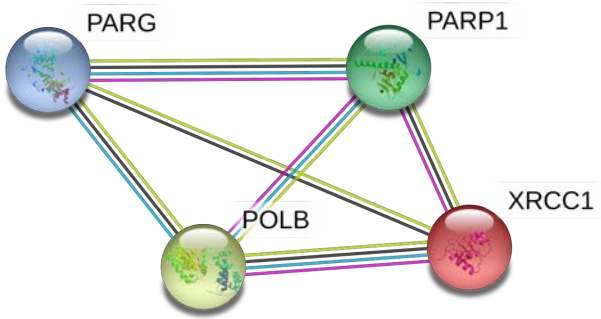




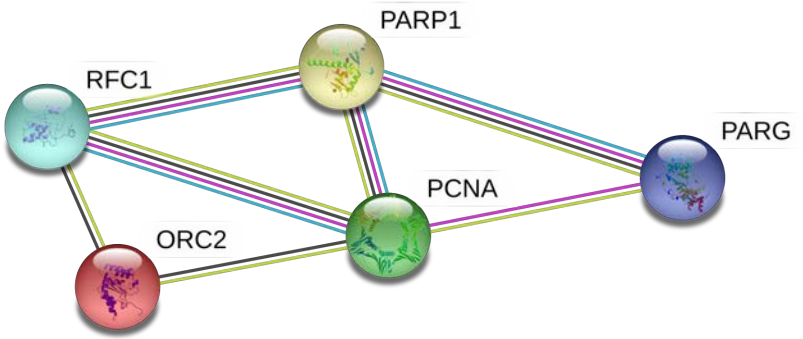
Kate Seville – In preparation

PARP1-BirA expressed in glioma cells (LN428)

Immunoblot / MS ID of biotinylated proteins



The ID of XRCC1 and PARG supports the role of PARP1 in BER/SSBR



The ID of replication factors PCNA, ORC2 and RFC1 is more in line with the role of PARP1 in controlling replication stress

Kate Seville – In preparation

Uncovering novel BER/SSBR factors

- Temporal map protein factors by BioID, Split-BioID and classical protein interactome analysis.
- Uncover the protein and histone acetylation/ADP-ribosylation code that regulates classical BER/SSBR.
- Define the role of PARP1, XRCC1 and the PARP1/PAR interactome in SSBR at replication forks that govern the intra S-phase checkpoint.

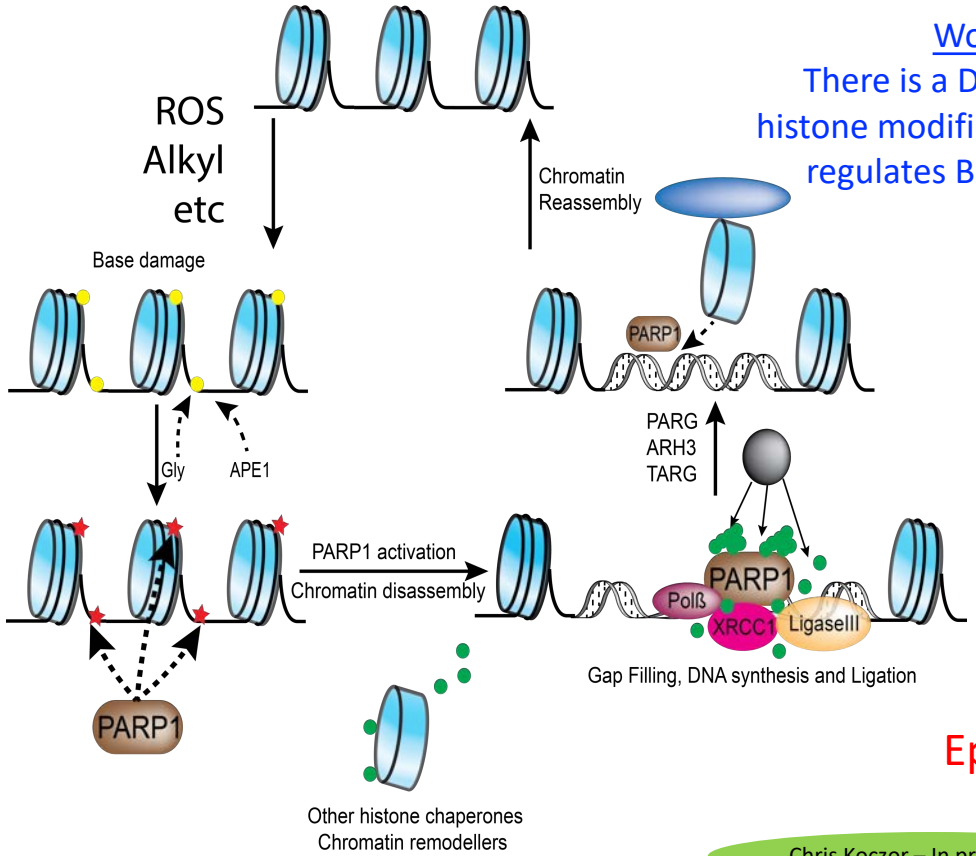
BER in chromatin

PARP1-mediated chromatin unwinding via recruitment of modifying complexes

DDR Factors

SIRTuins

Histone chaperones



Working Hypothesis

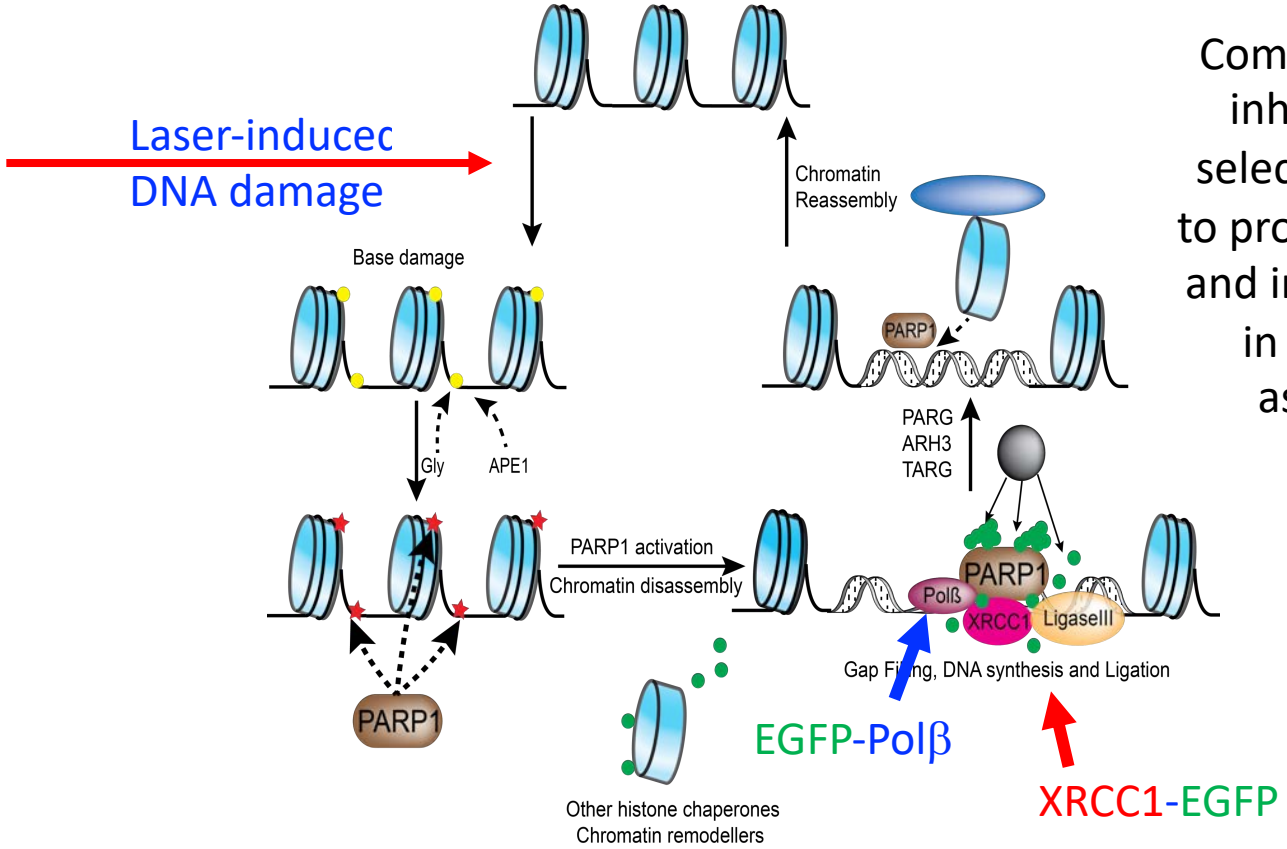
There is a DNA damage response and histone modification code that dictates and regulates BER complex assembly and disassembly.

Chromatin modifiers

Epigenetic modulators

Chris Koczor – In preparation

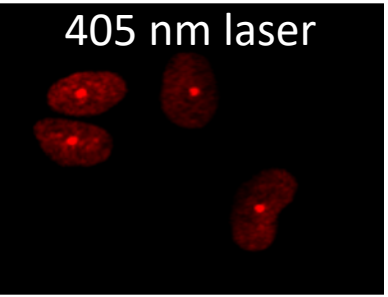
Tracking BER complex assembly / disassembly



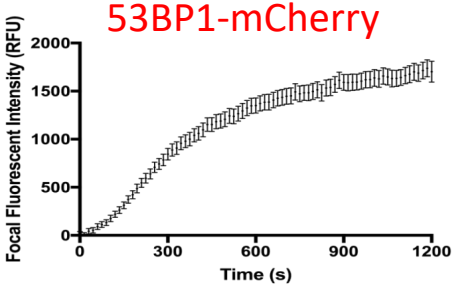
Combined with inhibitors or select gene KOs to probe function and involvement in complex assembly

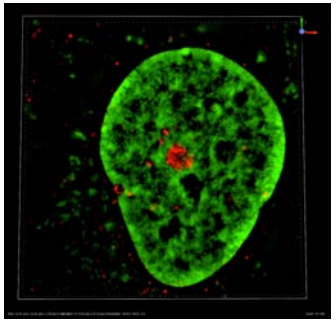
Chris Koczor – In preparation

Analysis of protein recruitment following laser-induced DNA damage

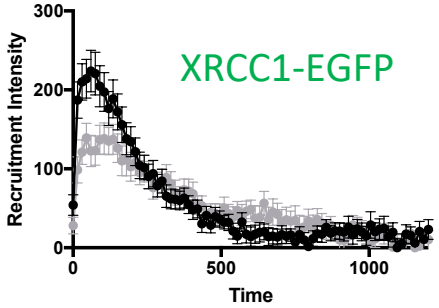


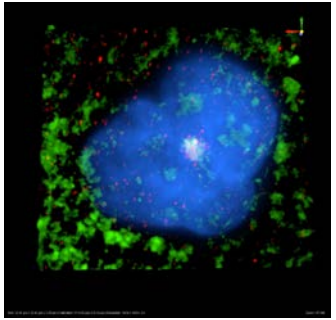
MIDAS





MIDAS

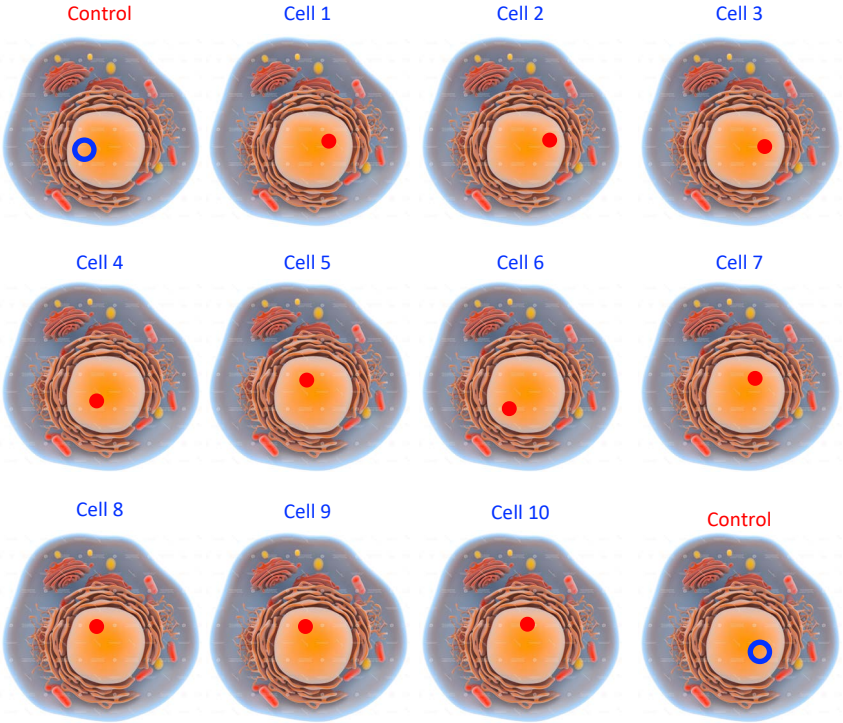


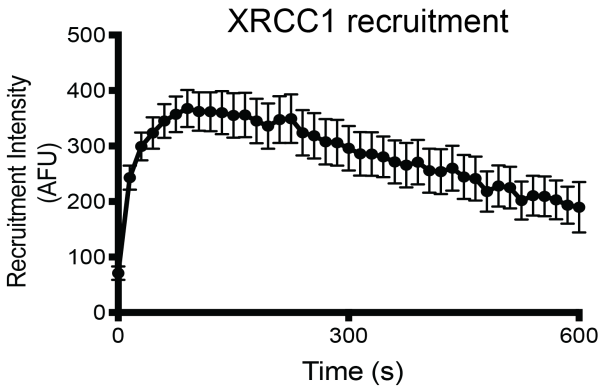


Chris Koczor – In preparation

MIDAS platform developed by Joel Andrews (MCI)

MIDAS - Parallel

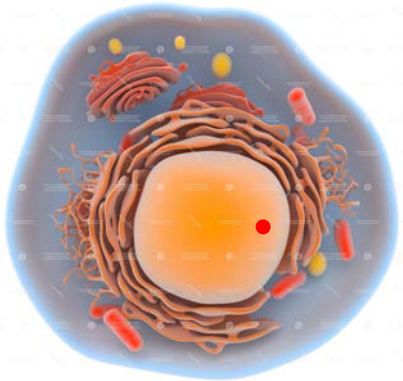


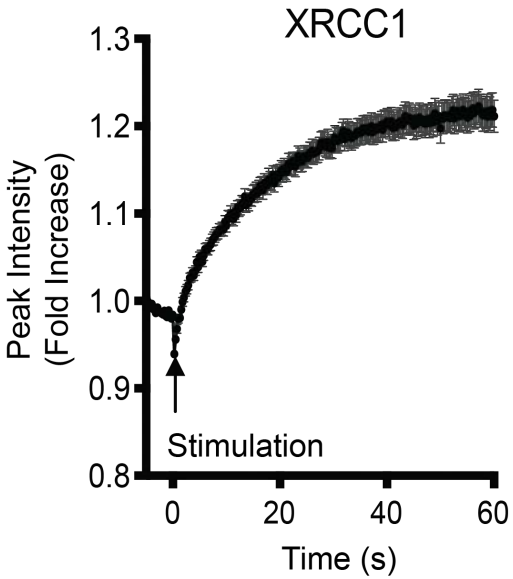


Result summary of >40 cells

Parallel analysis provides quantification of repair protein recruitment in 10 cells per exp.

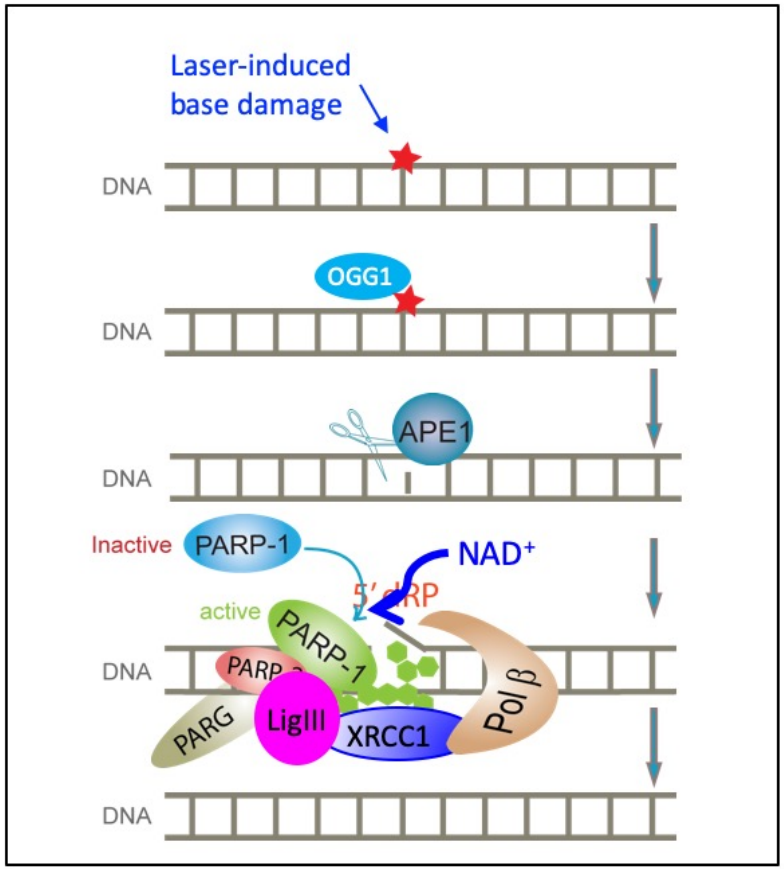
MIDAS - Serial





Serial analysis provides rapid, single cell quantification of repair protein recruitment

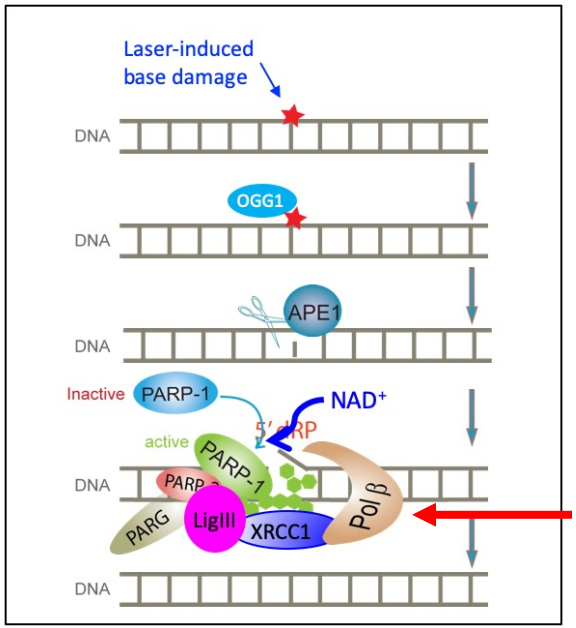
Polβ steps in BER



5'dRP lesion removal (K72)

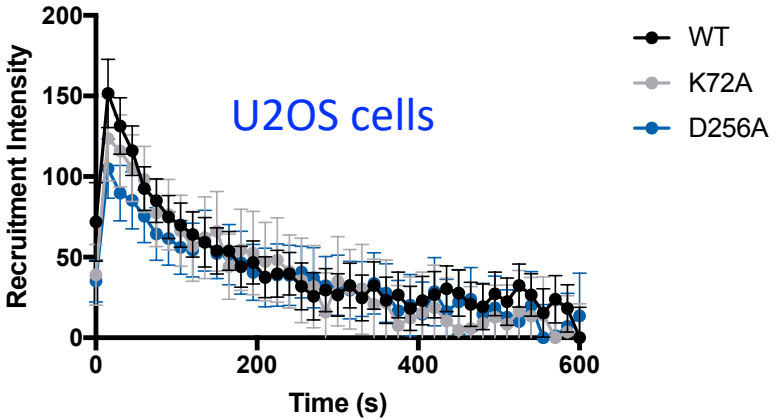
DNA synthesis (D256)

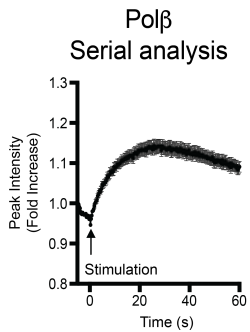
Recruitment of EGFP-POLB is not dependent on function



Complex assembly = Peak recruitment

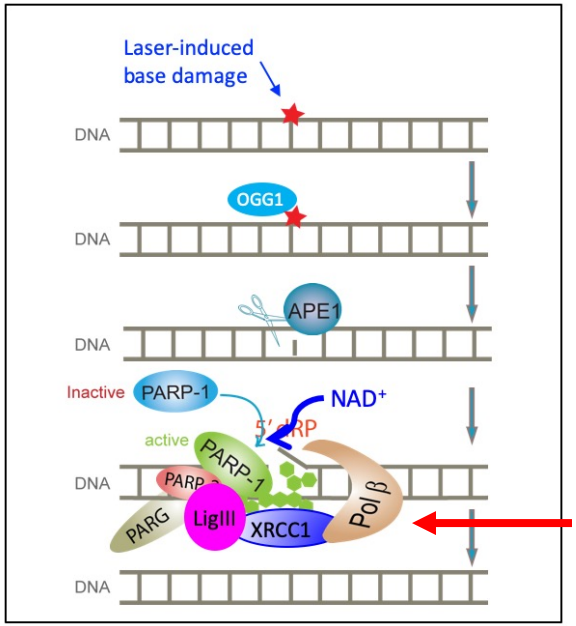
Complex disassembly = Half-life

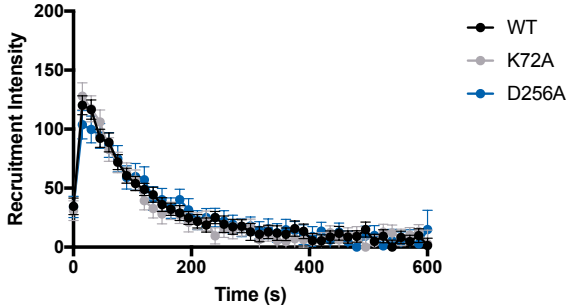




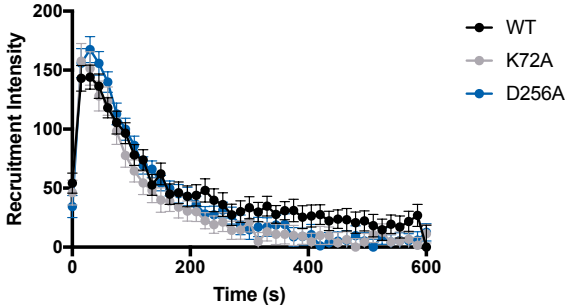
Chris Koczor – In preparation

Recruitment of EGFP-POLB is not effected by endogenous POLB

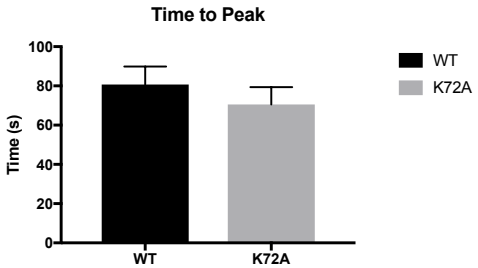


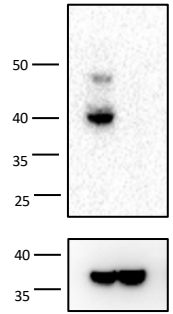


U2OS/cas9 cells



U2OS/POLB-KO cells

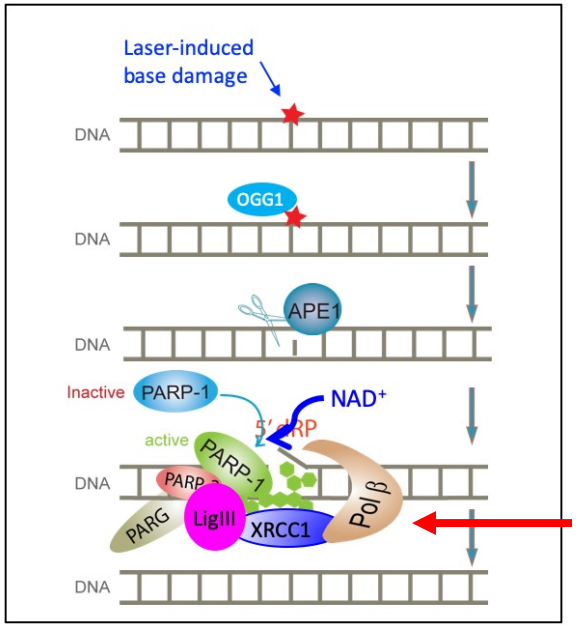


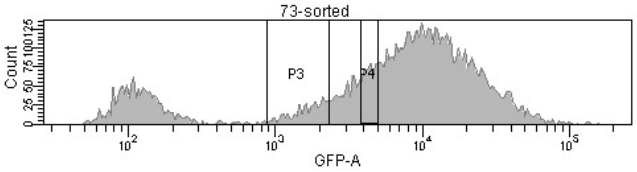


No change in peak recruitment time or half-life

Chris Koczor – In preparation

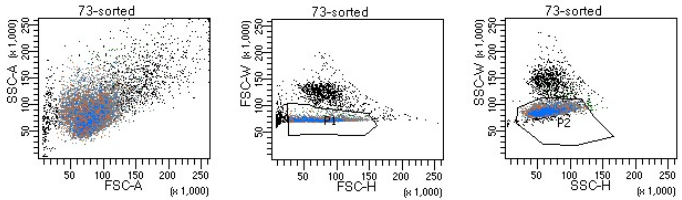
Is recruitment of EGFP-POLB is effected by transgene expression level?

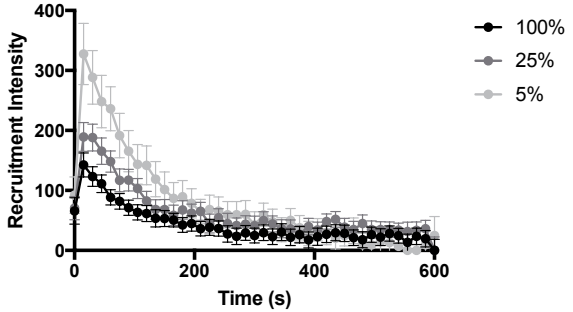




Tube: sorted

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	8,781	87.8	87.8
P2	8,723	99.3	87.2
P3	519	5.9	5.2
P4	563	6.5	5.6

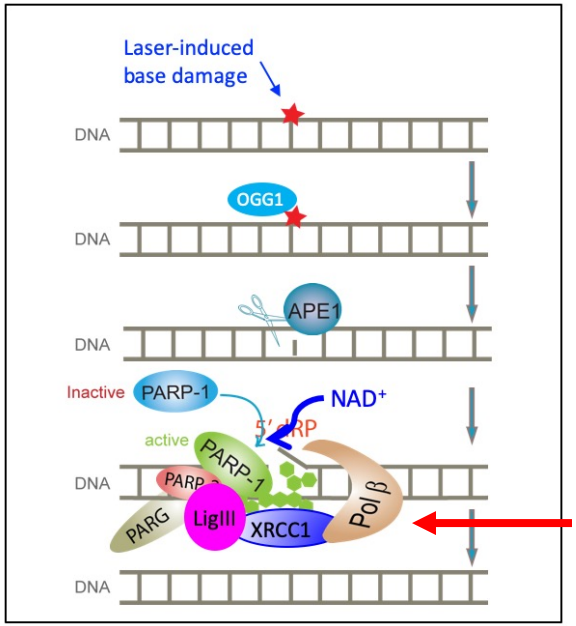


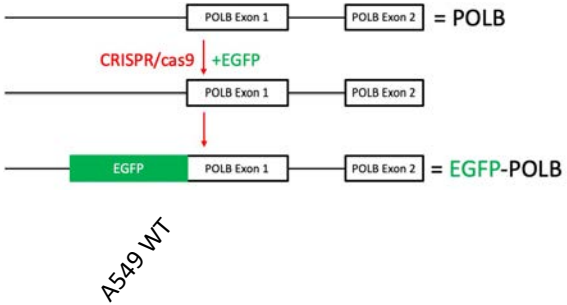


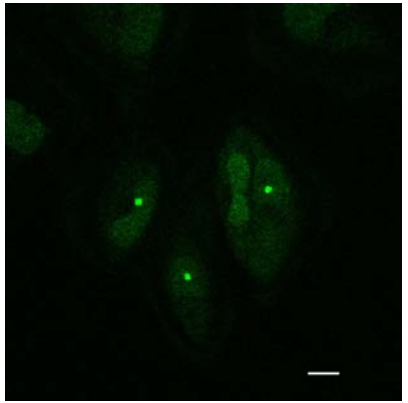
Recruitment of PolB is not effected by level of expression (peak time; half-life)

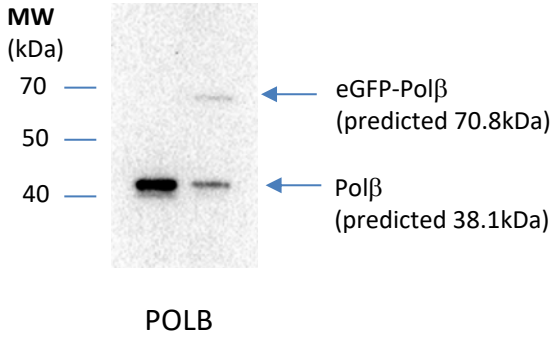
Chris Koczor – In preparation

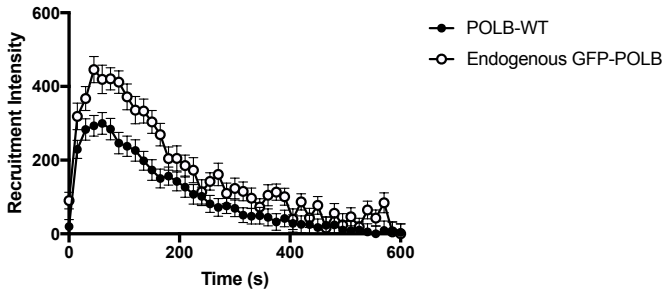
Does tagging the endogenous POLB gene improve analysis?







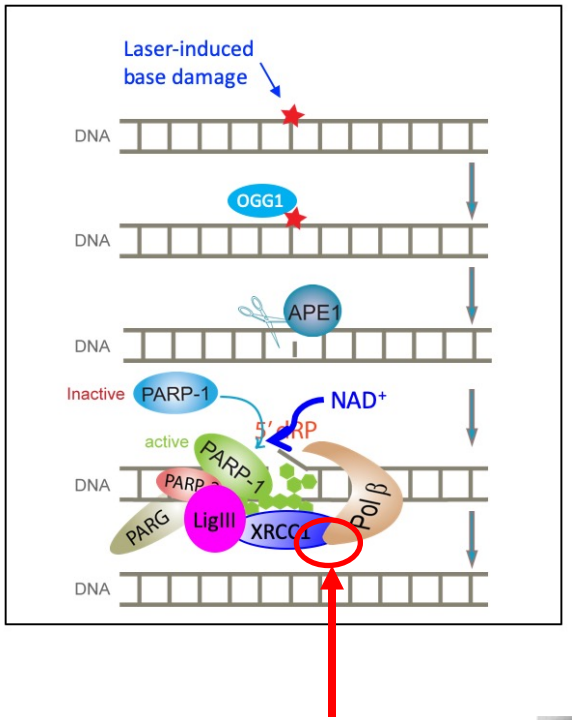


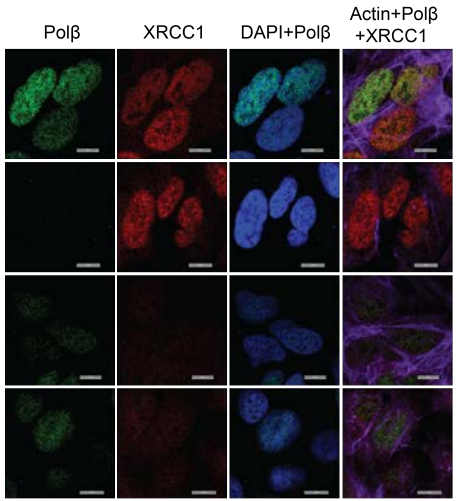


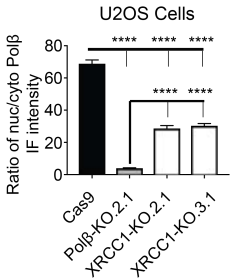
Higher signal to noise level of endogenous EGFP-POLB and no change in peak recruitment time or half-life

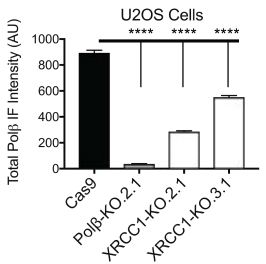
Chris Koczor – In preparation

Is recruitment of EGFP-POLB dependent on XRCC1?



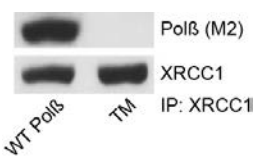




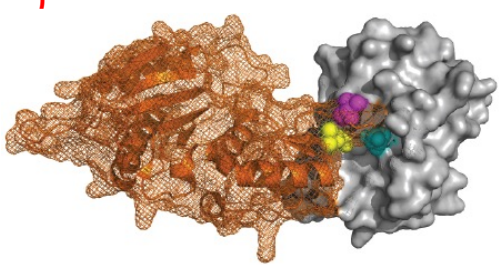


XRCC1 promotes chromatin/nuclear accumulation of Polβ

Fang, Andrews, … Sobol (2019) NAR, 47(12):6269-6286

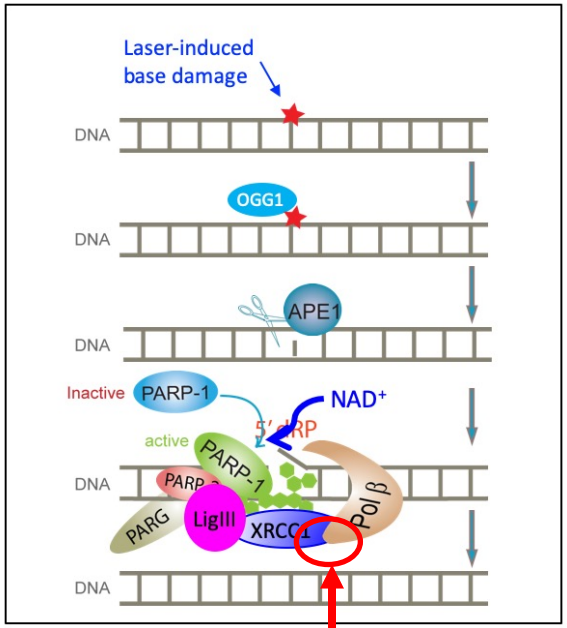


Fang et al (2014) Nat Comm, Nov 26;5:55.

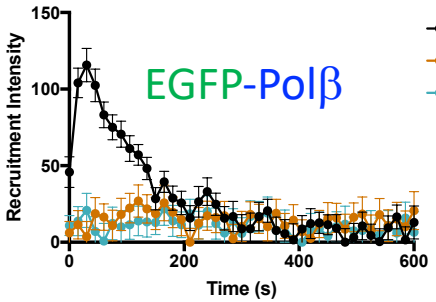


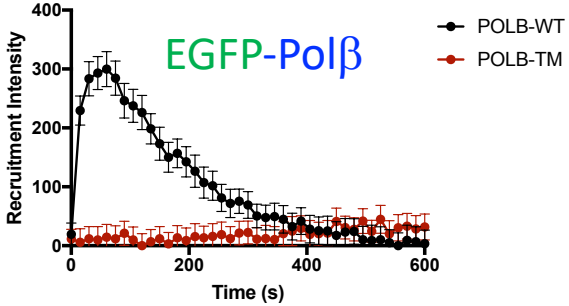
Proc Natl Acad Sci U S A. 2010 Apr 13;107(15):6805-10

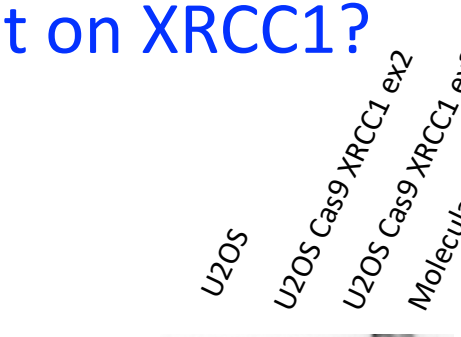
Is recruitment of EGFP-POLB dependent on XRCC1?

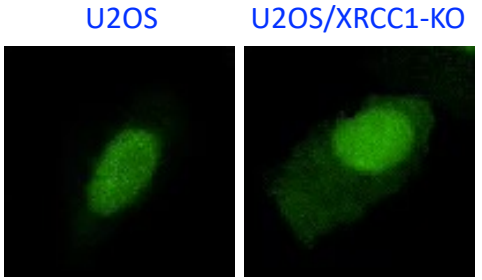


Recruitment requires XRCC1 presence and an interaction between PolB and XRCC1







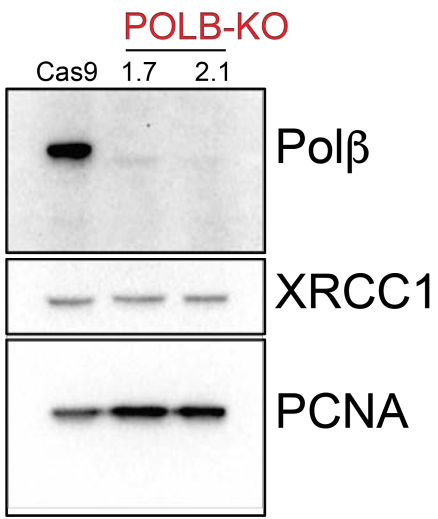


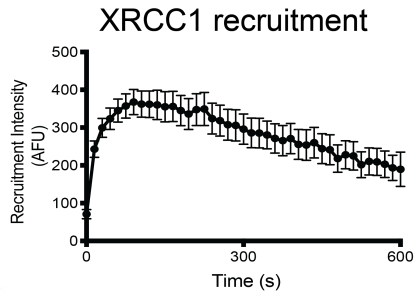
Still unknown if the proteins are recruited as a heterodimer

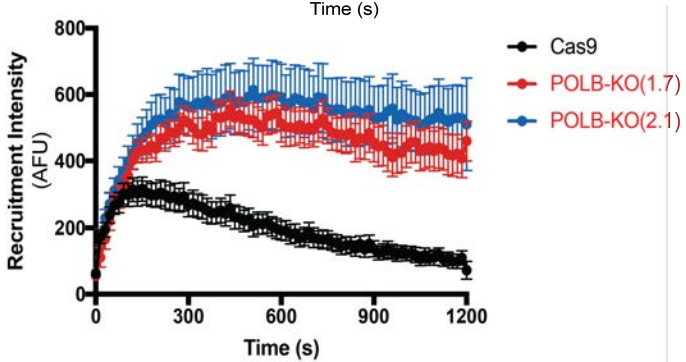
Chris Koczor – In preparation

Both the PolB-XRCC1 interaction and the expression of each is essential for completion of BER complex assembly - what about disassembly?

POLB-KO
Validation by immunoblot

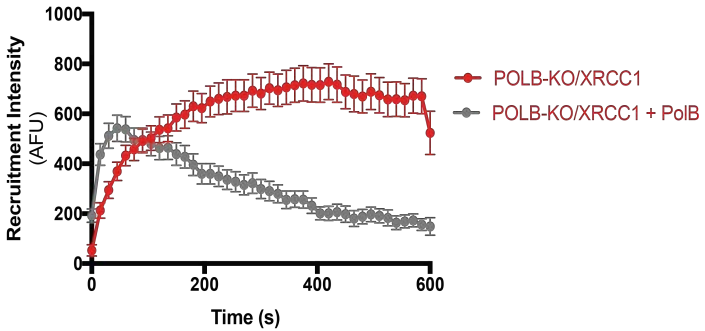


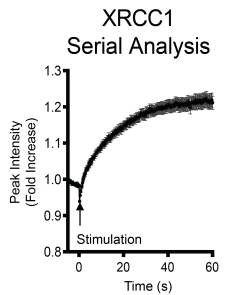




Recruitment of XRCC1-EGFP in U2OS/POLB-KO cells.
XRCC1 demonstrates slower disassembly from the repair complex in the absence of Polβ.

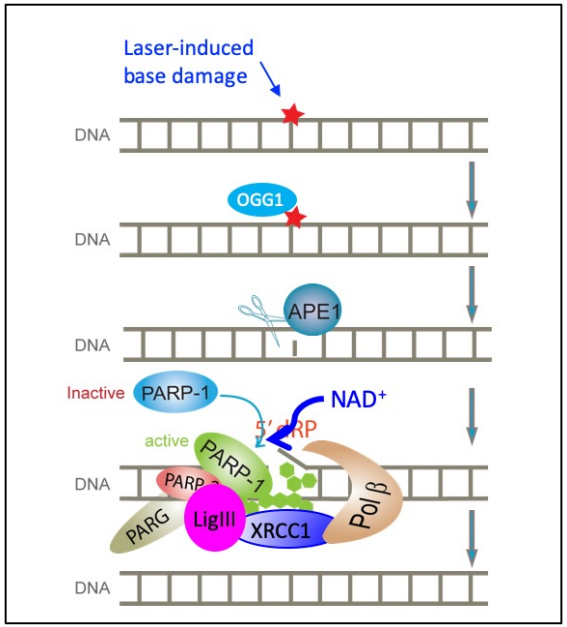
Recruitment of XRCC1-EGFP in U2OS/POLB-KO cells with POLB restored.
Re-expression of Polβ was able to restore the disassembly of XRCC1 from sites of DNA damage.

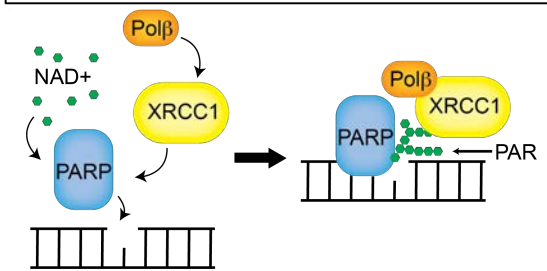


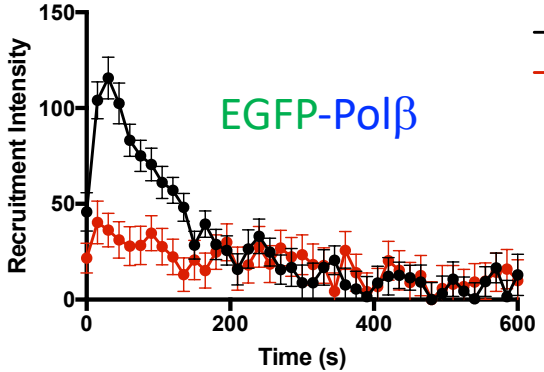


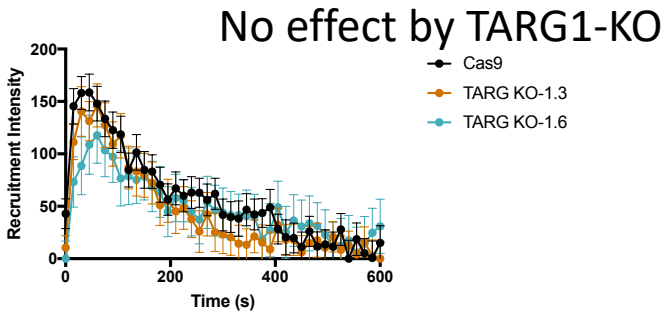
Chris Koczor – In preparation

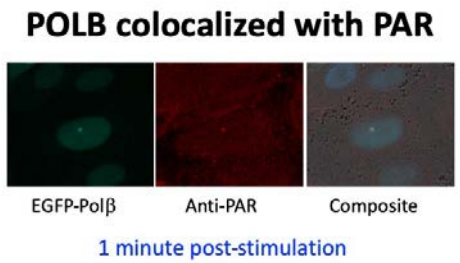
Is POLB AND XRCC1 complex assembly and dis-assembly regulated by poly-ADP-ribose?

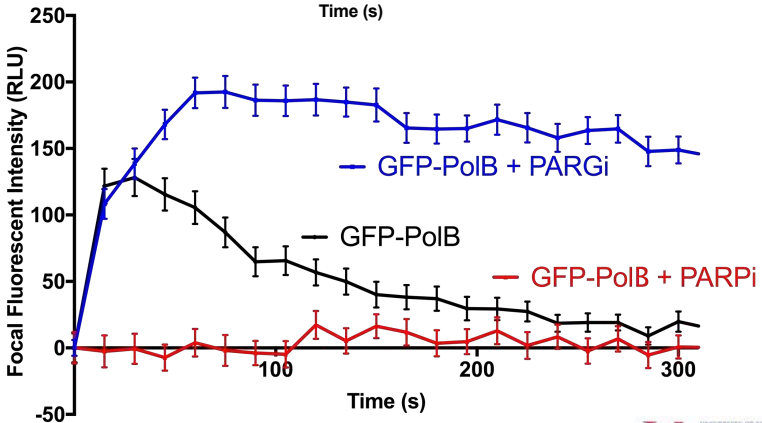






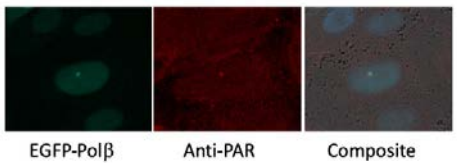




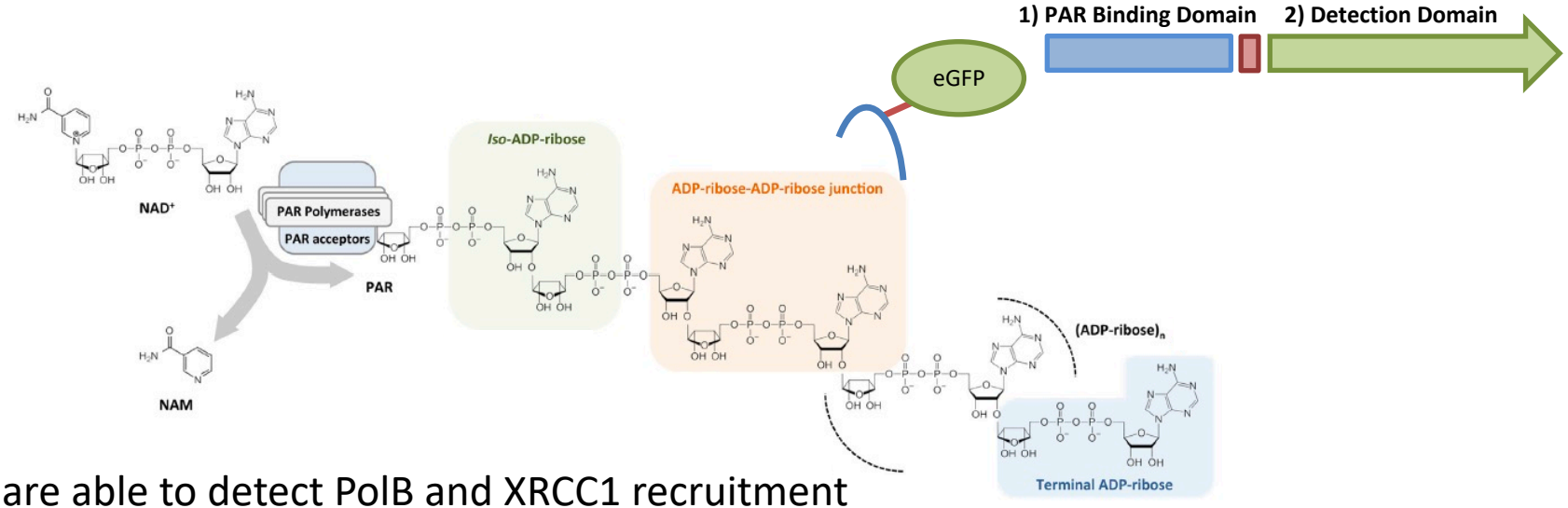


Chris Koczor – In preparation

Can we enhance our mechanistic analysis by tracking PAR?



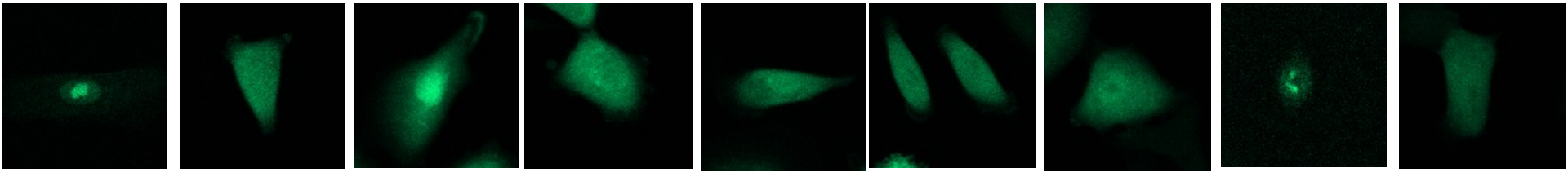
RealPAR



- We are able to detect PolB and XRCC1 recruitment in real time, but similar options were not available for PAR.
- We generated a PAR binding domain fused to eGFP to measure PAR in real time. We termed it "RealPAR".

Teloni and Altmeyer, *Nuc. Ac. Res, 2016*

Expression of PBD-eGFP in A549 cells



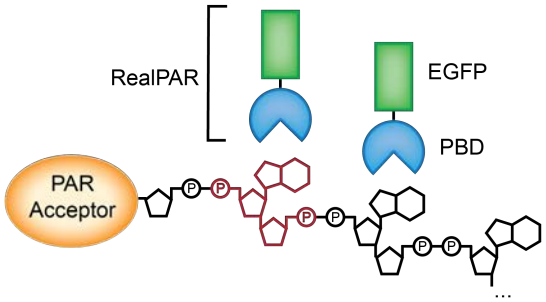
PBM-eGFP PBZ/C-eGFP RG-eGFP WWE-eGFP OB-eGFP BRCT-eGFP FHA-eGFP KR-eGFP Macro-eGFP

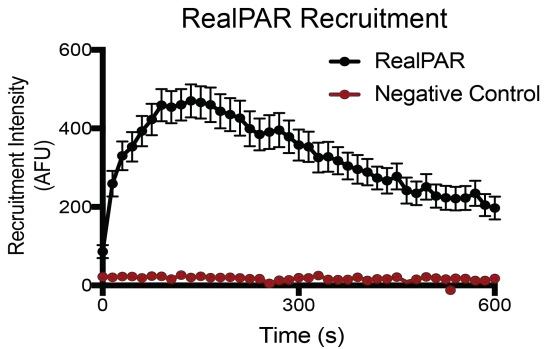
- All cells were tested for 405nm and 355nm recruitment.
- Only 1 recruited to laser-induced damage.

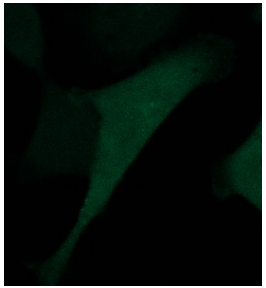
	Readers of poly(ADP-ribose)		
	Module	**Module Size**	**Interaction Type**
1	PBM	~20 residues	unknown
2	PBZ	~30 residues	consecutive ADP-ribose moieties
3	Macrodomain	~130-190 residues	terminal ADP-ribose
4	WWE	~80-100 residues	iso-ADP-ribose
5	FHA/BRCT	~80-100 residues	ADP-ribose or iso-ADP-ribose
6	RRM	~60-80 residues	unknown
7	SR/KR-rich	variable	unknown
8	OB-fold	~70-150 residues	iso-ADP-ribose
9	PIN domain	~130-150 residues	unknown
10	RG/RGG domain	variable	unknown

Chris Koczor – In preparation

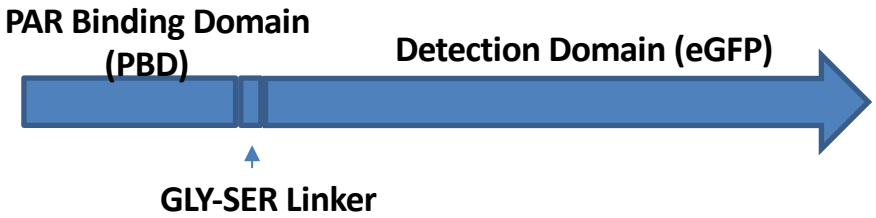
Development of REAL-PAR, a live-cell probe of DNA damage-induced PAR formation

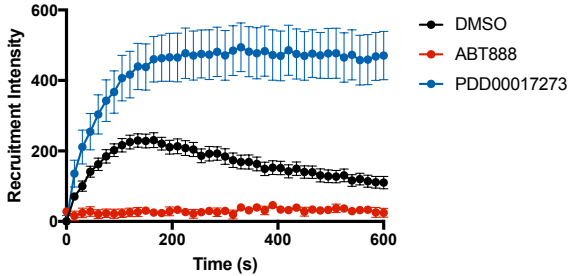






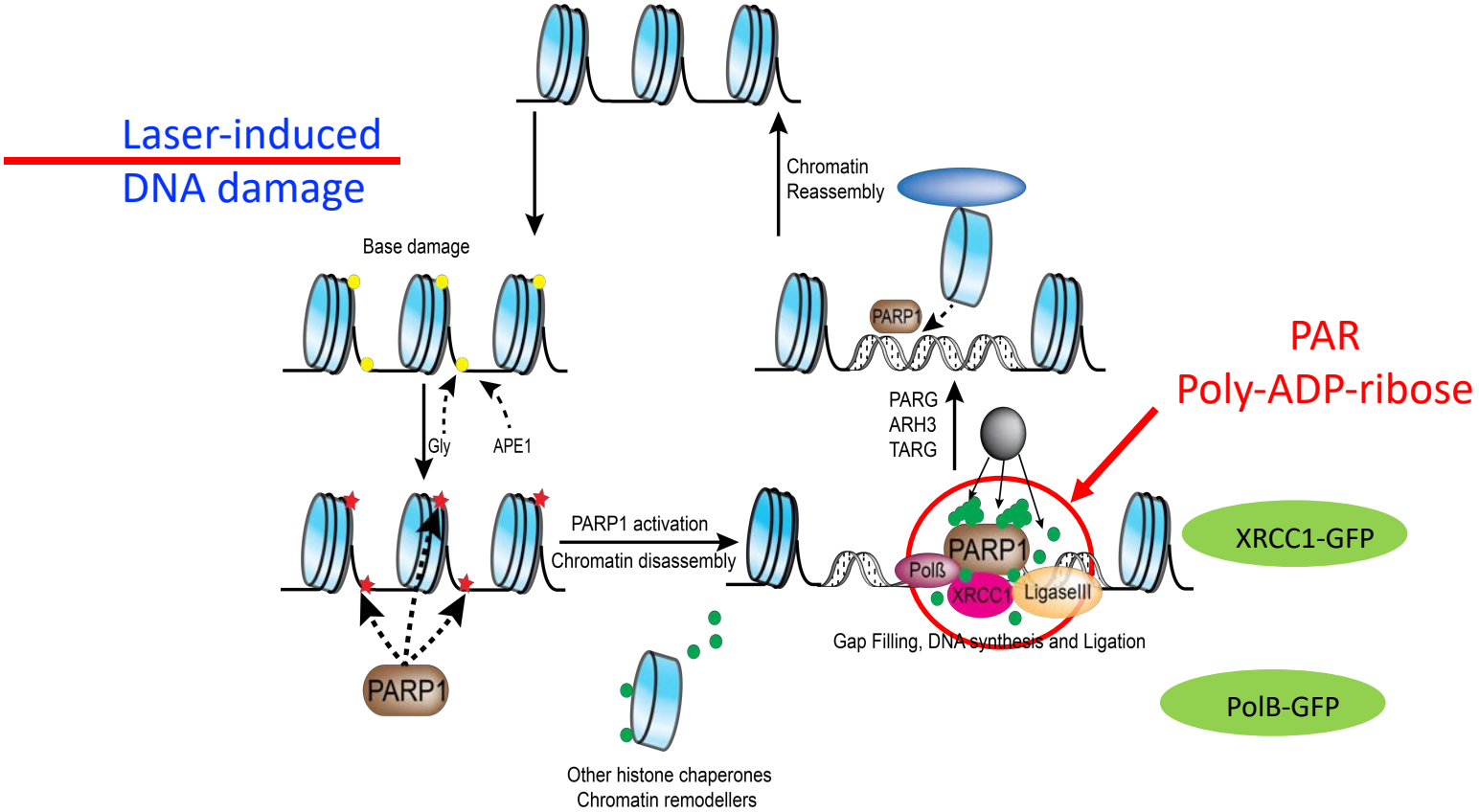
A549 cells
1727 construct (PBD-eGFP)
405nm laser, 1/8sec stimulation



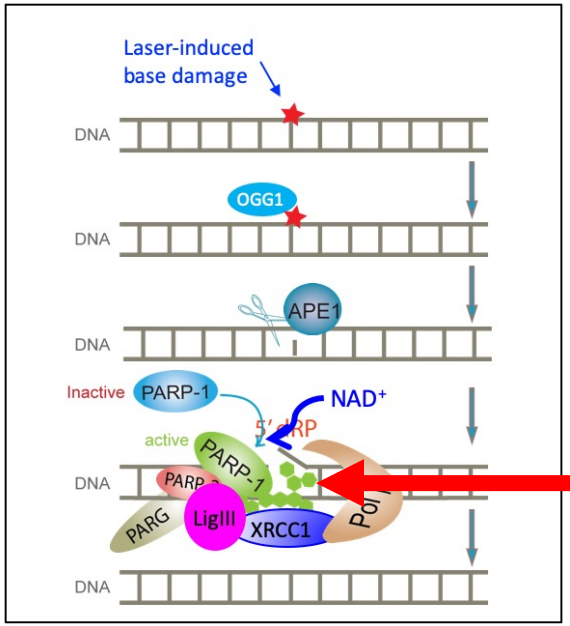


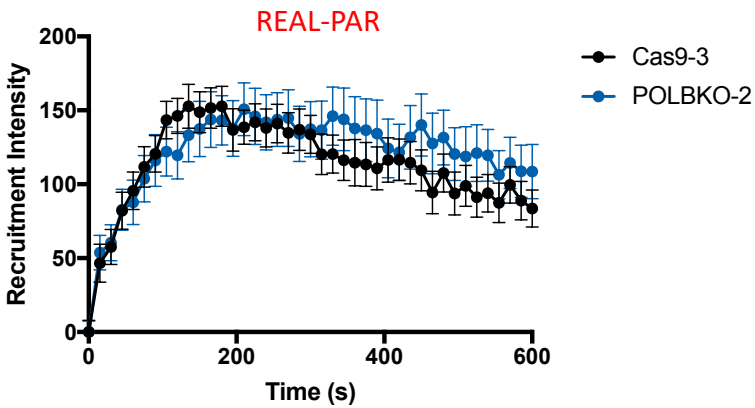
Chris Koczor – In preparation

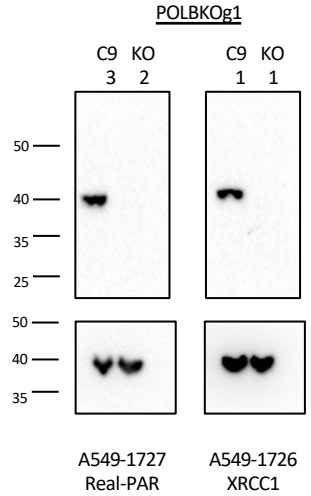
REAL-PAR can now be used to probe PAR formation kinetics along with BER complex assembly / disassembly

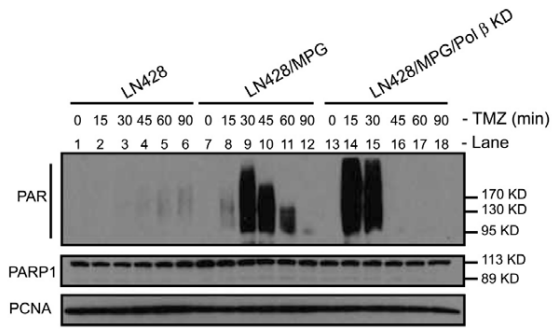


Does loss of POLB impact damage-induced PAR formation?







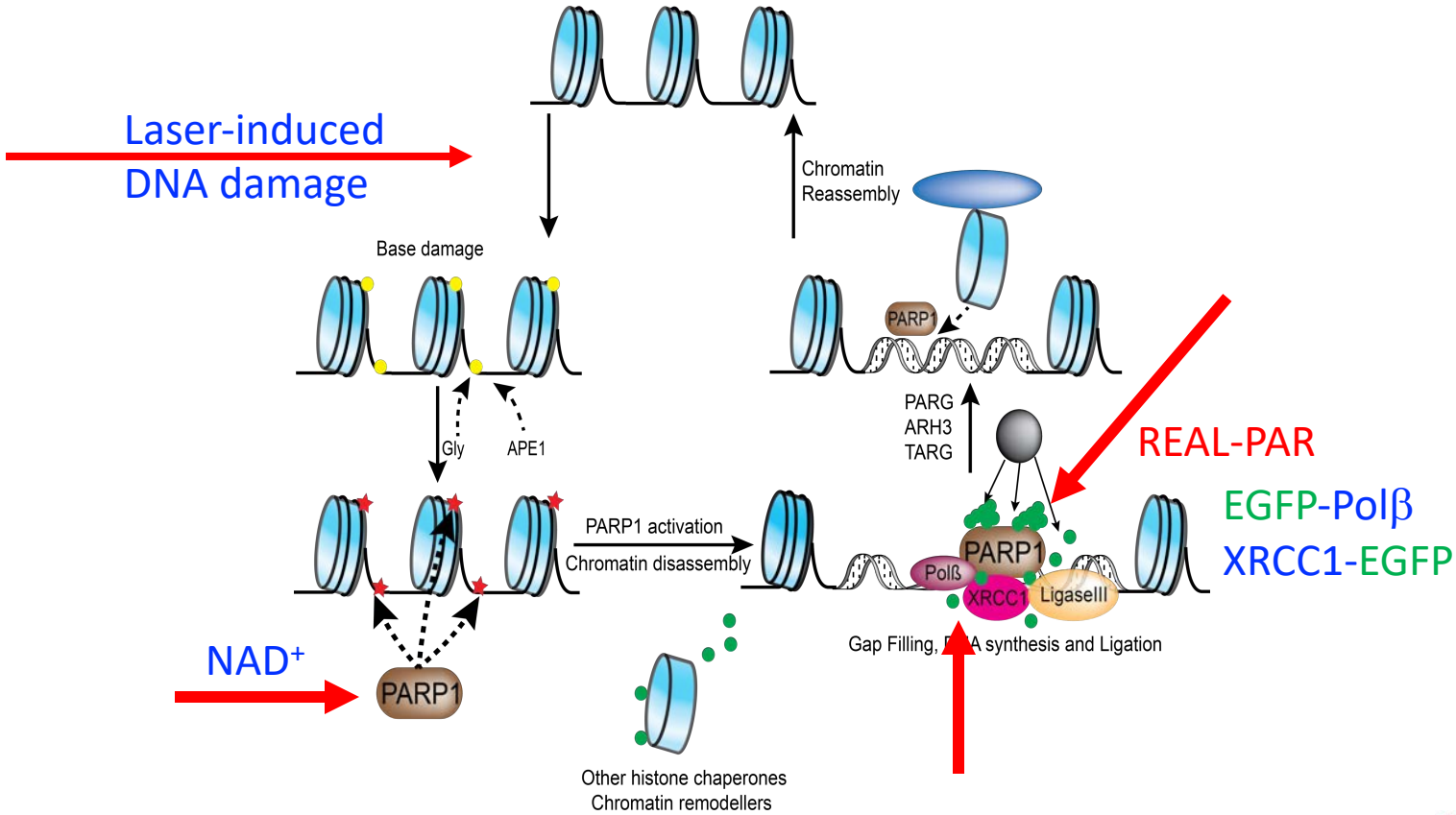


Tang JB et al.
Mol Cancer Res. 2010 Jan;8(1):67-79.

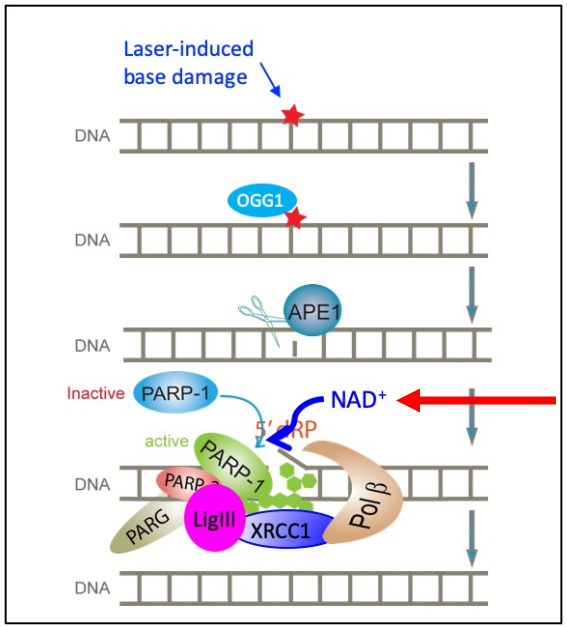
No change in PAR when PolB is absent – a backup? (PolQ, Poli, other?) PARG?

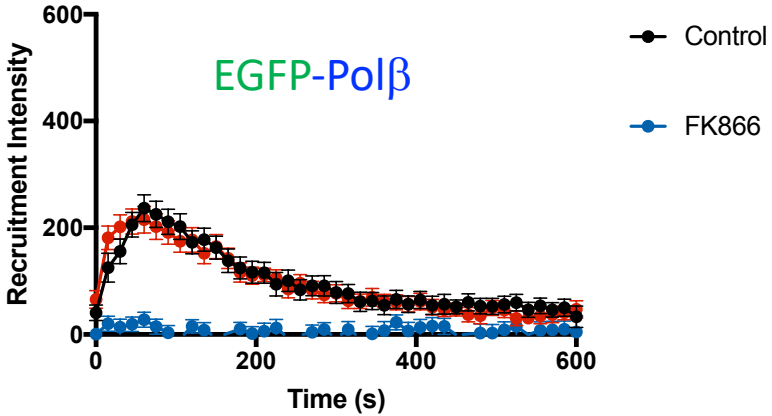
Chris Koczor – In preparation

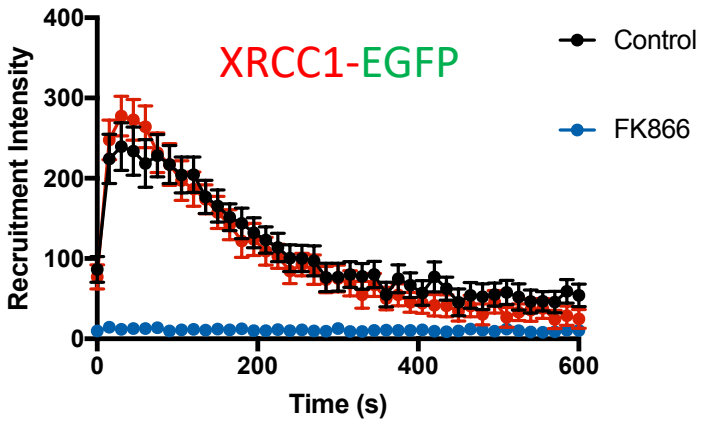
Factors affecting BER complex assembly / disassembly ?

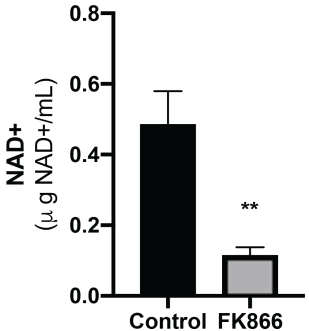


Recruitment of EGFP-POLB or XRCC1-EGFP is lost when NAD⁺ is deficient









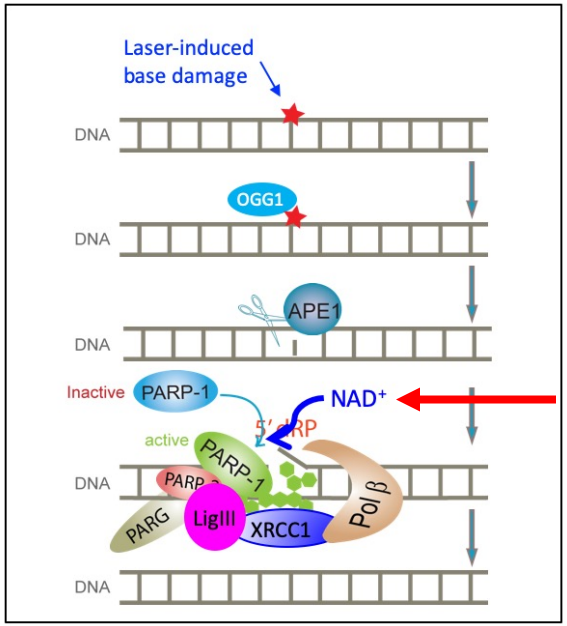
Chris Koczor – In preparation

Kate Seville – In preparation

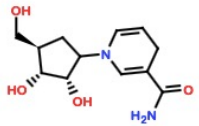
What about alterations in NAD^+ bioavailability?

- We should consider co-factors or enzyme substrates such as NAD^+ as BER regulatory factors.
- The biosynthesis of NAD^+, an essential substrate for both sirtuins and PARPs, is compartmentalized and highly regulated.
- Fluctuations in cellular levels of NAD^+ have been linked to the aging process.
- Cancer-related DNA repair defects may be, in part, the result of known variations in NAD^+ biosynthesis in different tissues.
- Increasing NAD^+ bioavailability may provide an opportunity to increase cellular DNA repair capacity.
- However, it remains to be determined if too much of a good thing (NAD^+) can also be detrimental.

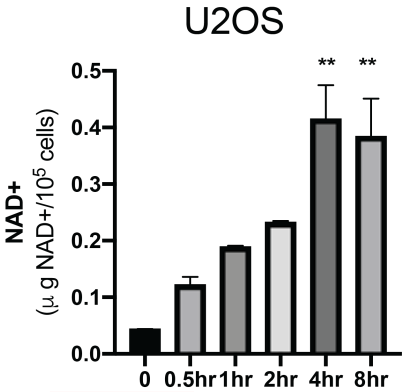
Cellular NAD+ levels can be significantly elevated by supplementation of NRH
This leads to enhanced PARP1 activation in cells with elevated PARP1 levels

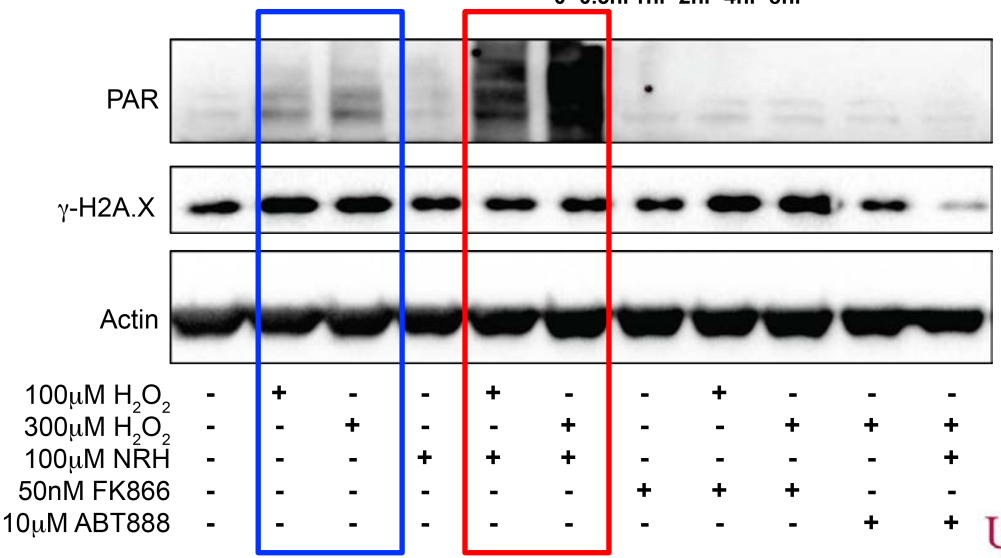


Kate Seville – In preparation



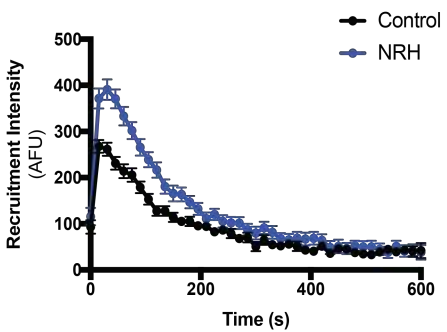
Dihydronicotinamide Riboside (NRH)



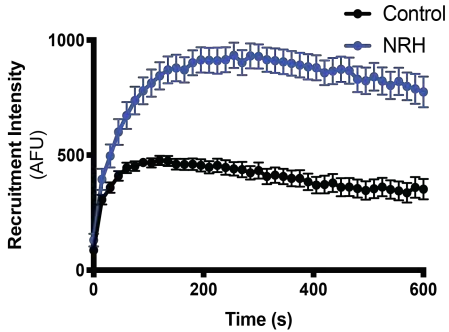


Recruitment of EGFP-POLB, XRCC1-EGFP and RealPAR is enhanced when NAD^+ is elevated

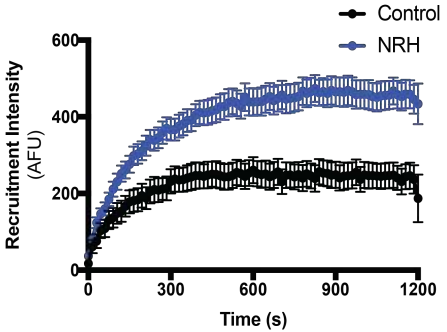
Polβ recrutiment



XRCC1 recrutiment



RealPAR recrutiment

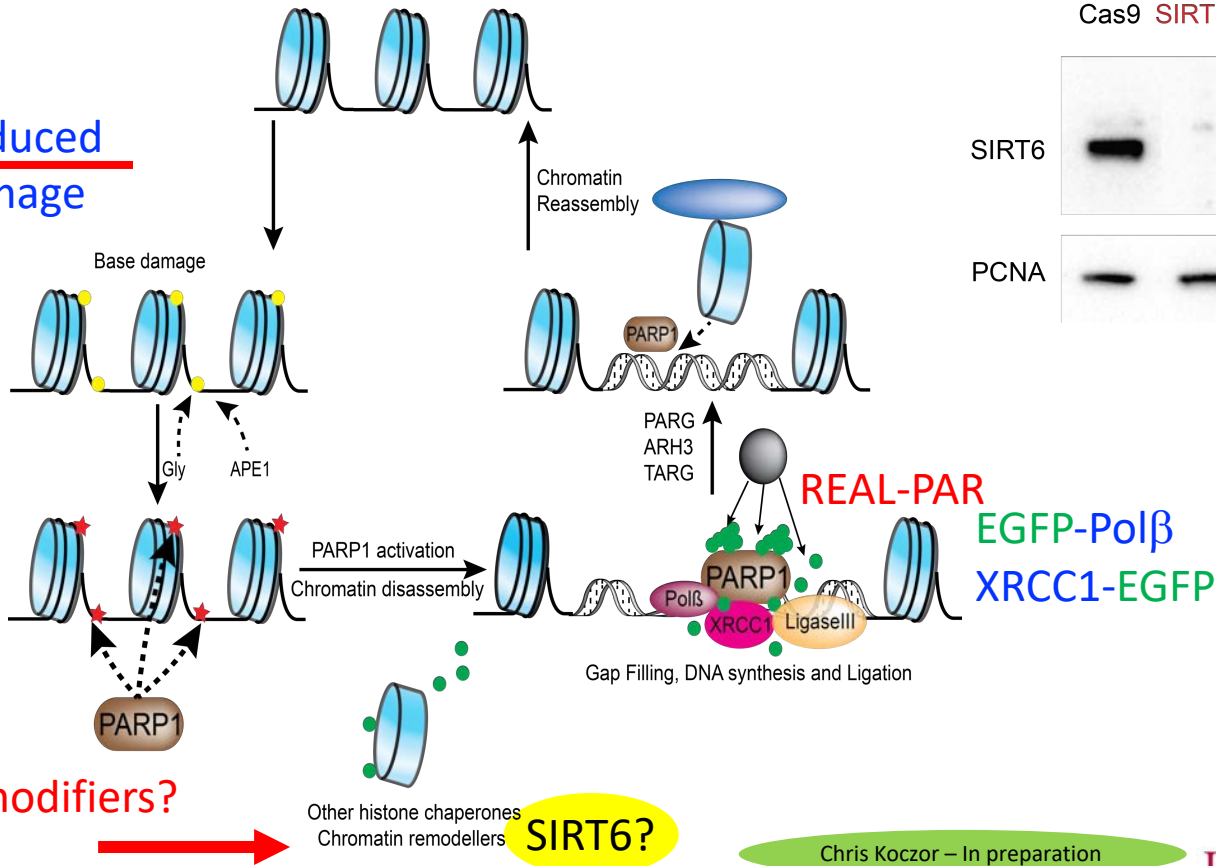


Kate Seville – In preparation

Chris Koczor – In preparation

Factors affecting BER complex assembly / disassembly ?

Laser-induced DNA damage



Changes in NAD impacts BER complex formation.
Is this all via PAR/PARP1?

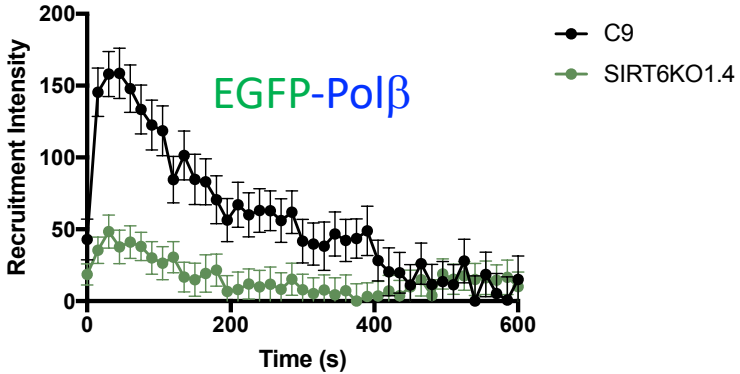
What about Chromatin modifiers?

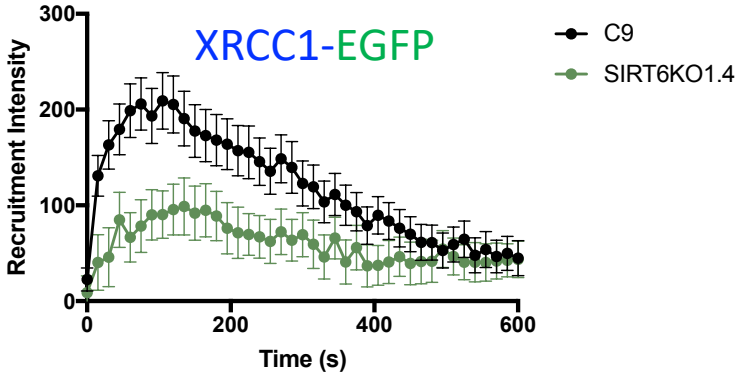
Chris Koczor – In preparation

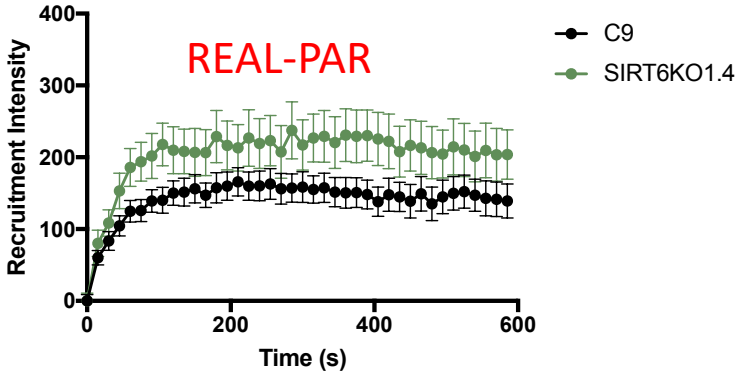
How can SIRT6 affect repair?

- SIRT6 first suggested to have a role in BER:
  - Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Mostoslavsky et al Cell. 2006 Jan 27;124(2):315-29.
- SIRT6 can modify BER proteins:
  - SIRT6 protein deacetylase interacts with MYH DNA glycosylase, APE1 endonuclease, and Rad9-Rad1-Hus1 checkpoint clamp. BMC Mol Biol. 2015 Jun 11;16:12.
- SIRT6 deacetylates H3K65-Ac and H3K9-Ac
  - Chromatin Regulation and Genome Maintenance by Mammalian SIRT6; Trends Biochem Sci . 2011 Jan;36(1):39-46.
- SIRT6 can affect chromatin relaxation in response to DSB formation:
  - SIRT6 coordinates with CHD4 to promote chromatin relaxation and DNA repair. Nucleic Acids Res. 2020 Apr 6;48(6):2982-3000 & SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. Mol Cell. 2013 Aug 22;51(4):454-68.
- SIRT6 activates PARP1 in response to DSBs by mono-ADP-ribosylation:
  - SIRT6 promotes DNA repair under stress by activating PARP1. Science. 2011 Jun 17;332(6036):1443-6.

SIRT6 alters POLB/XRCC1 recruitment but not PARP1 activation



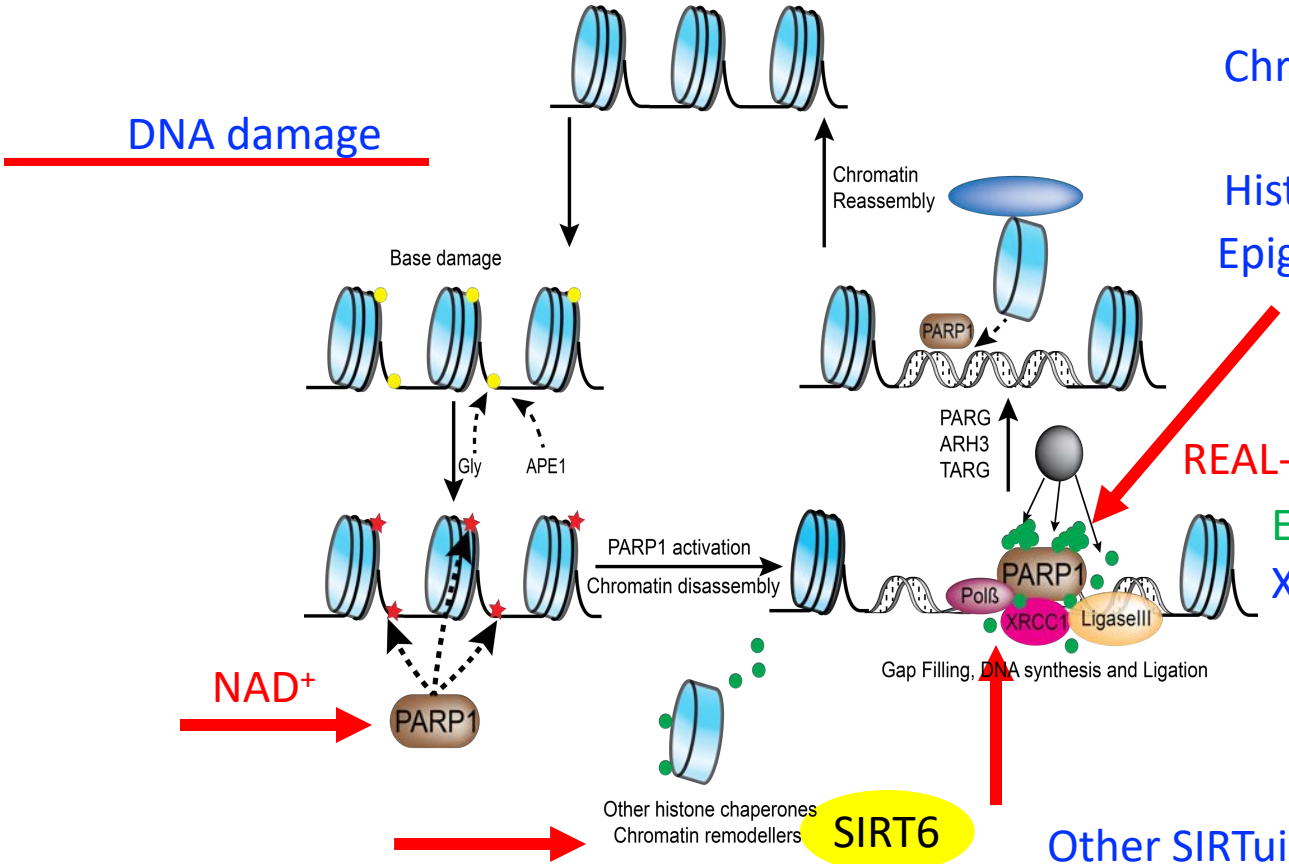




➔ SIRT6 affects recruitment by modulating the PARP1/XRCC1 complex or the chromatin state (deacetylation of H3K9 or H3K56?) to impact recruitment but does not impact laser-induced PAR formation (PARP1 activation).

Chris Koczor – In preparation

Factors (metabolites, proteins) affecting DNA repair complex assembly / disassembly, PARP1 activation and DNA Repair



Chromatin modifiers?

DDR Factors?

Histone chaperones?

Epigenetic modulators?

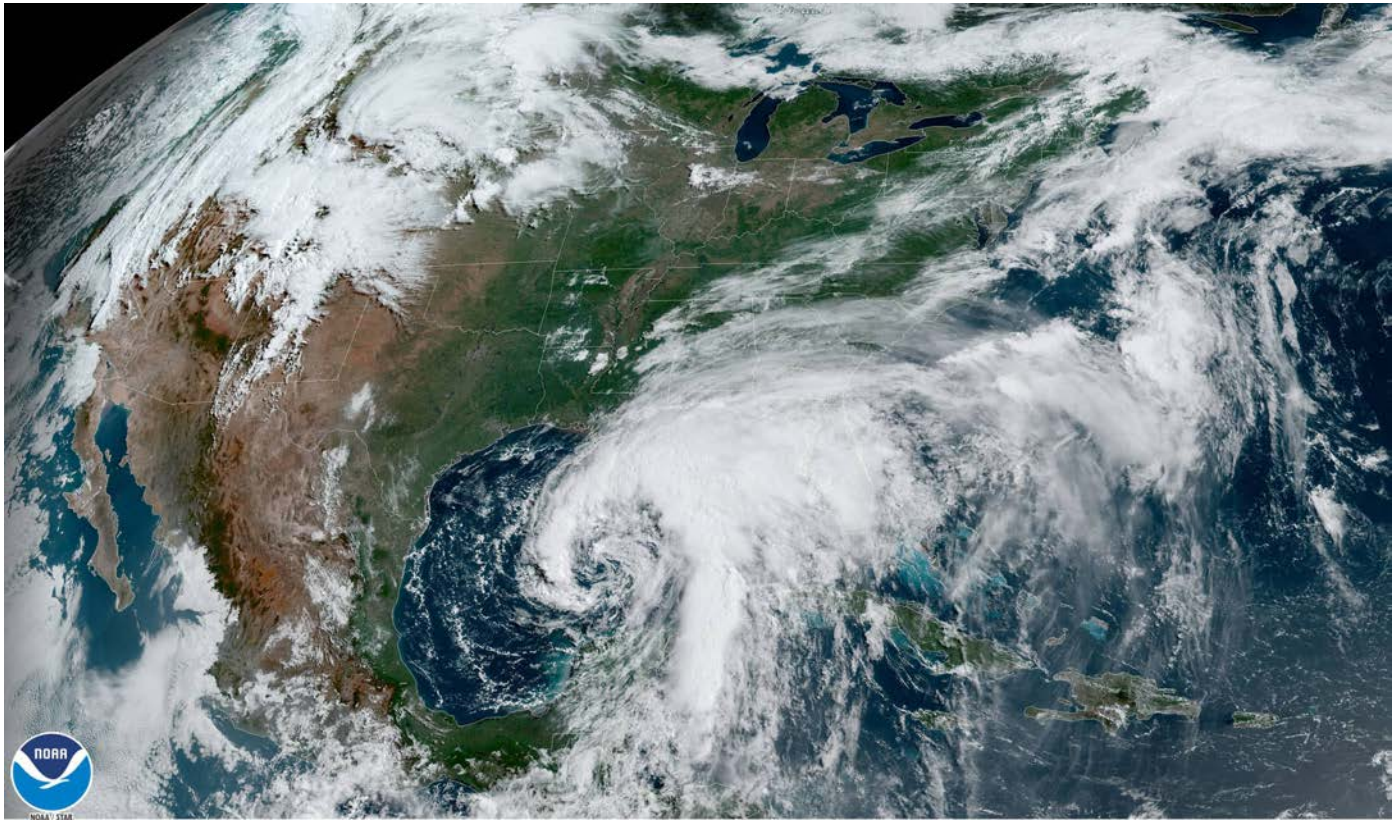
REAL-PAR

EGFP-Polβ

XRCC1-EGFP

Other SIRTuins?

Thank you – Questions?



*Postdoctoral positions available