DNA strand break repair and genetic disease

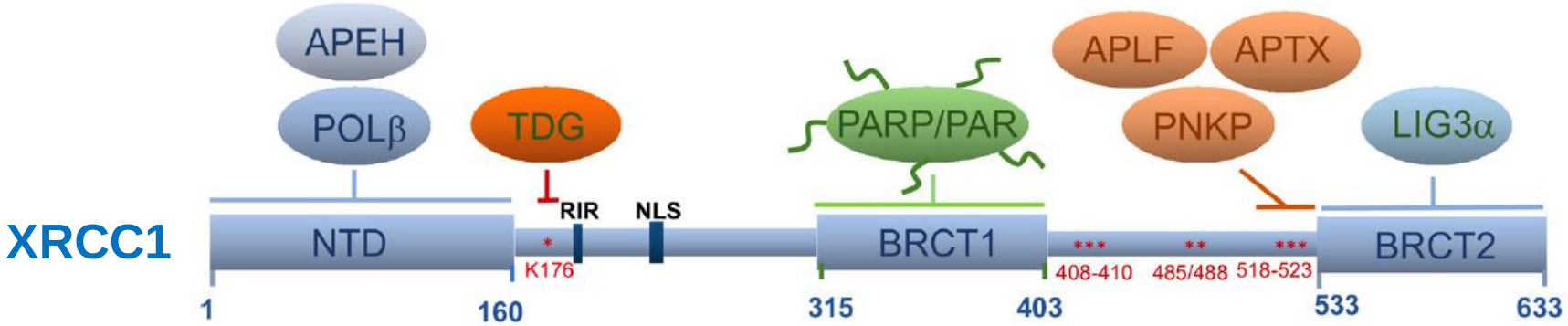
(The role of XRCC1 in mammalian DNA base excision repair)

aka good monkeys and bad monkeys

Annie Demin

(Shunichi Takeda, Kouji Hirota, Masataka Tsuda, Hiroyuki Sasanuma)

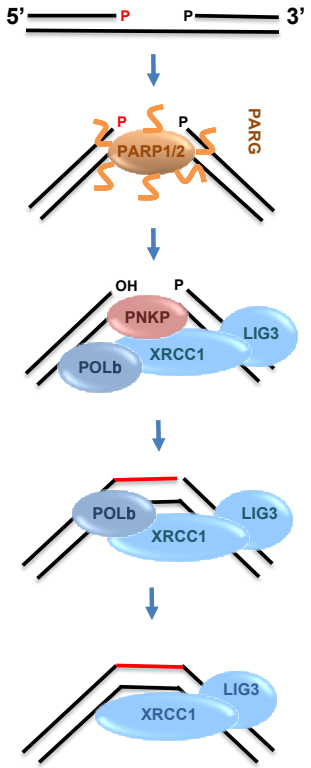
XRCC1 Scaffold Protein



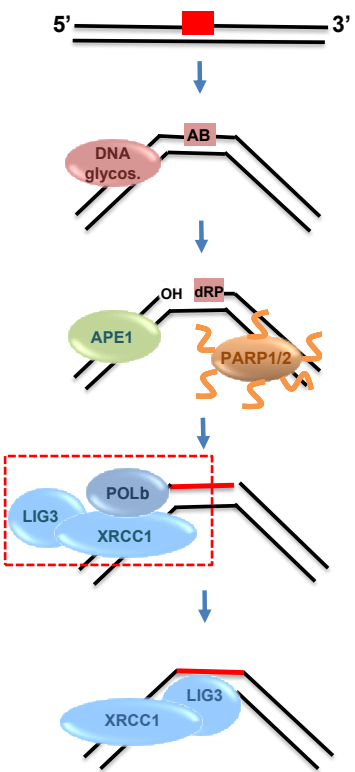
Caldecott KW, *DNA Repair*, 2019. XRCC1 Protein Form and Function

XRCC1 and SSB repair

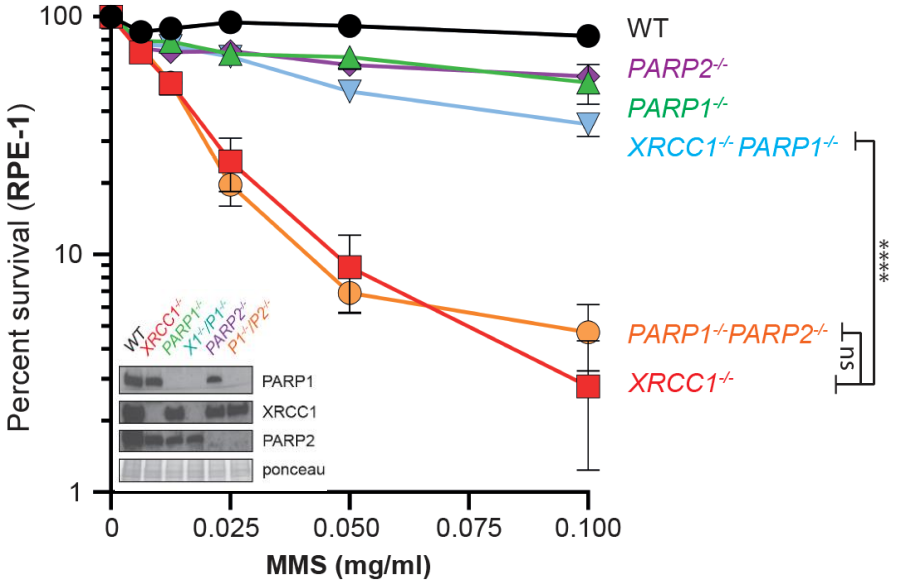
'Direct' SSBs
e.g. IR/H_2O_2-induced sugar fragmentation

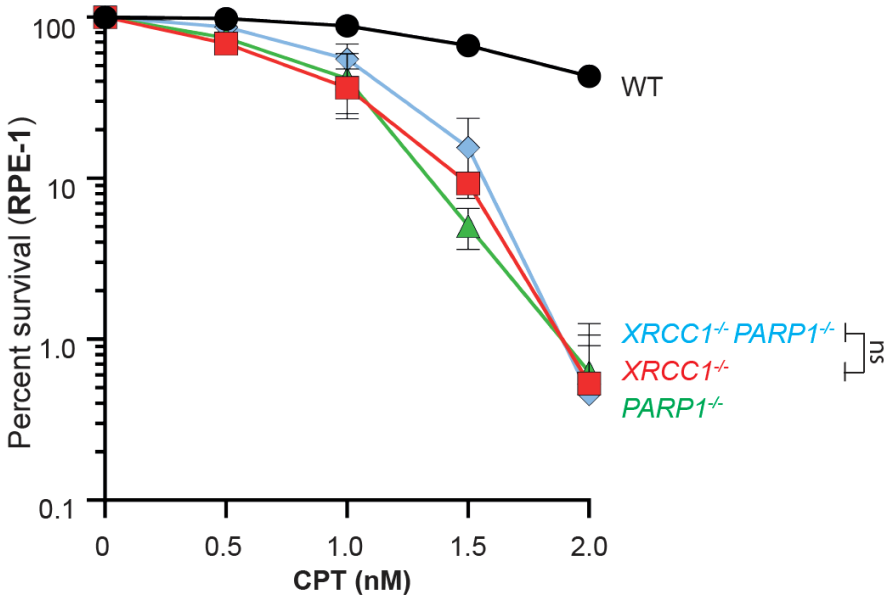


Base Excision Repair

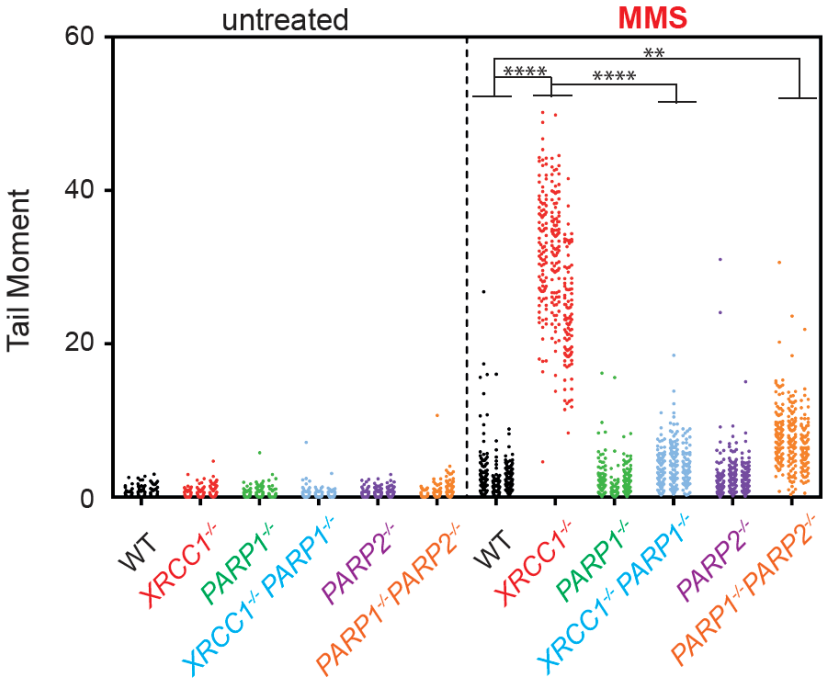


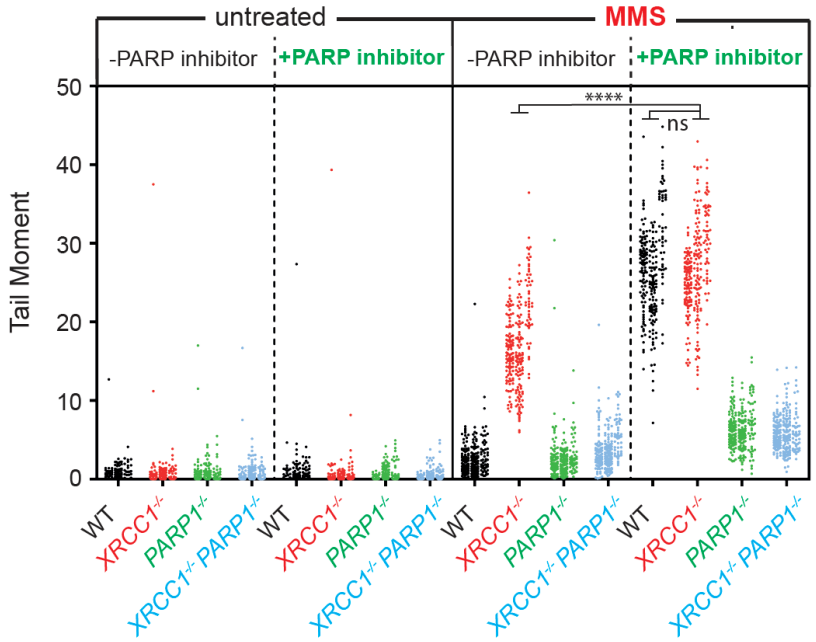
XRCC1 prevents PARP1-induced toxicity during BER





XRCC1 prevents PARP1-dependent SSB accumulation during BER

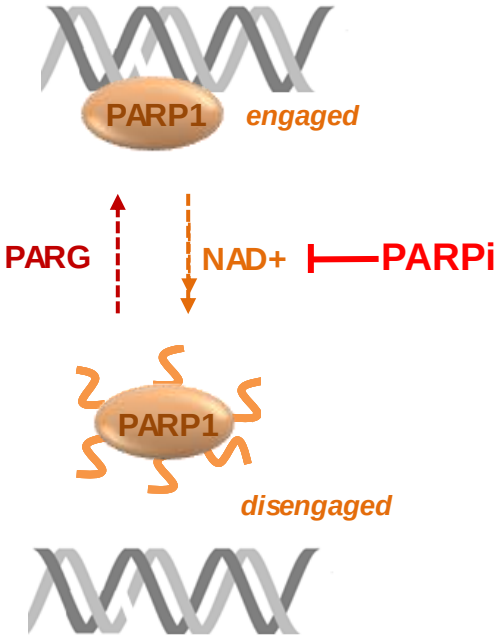


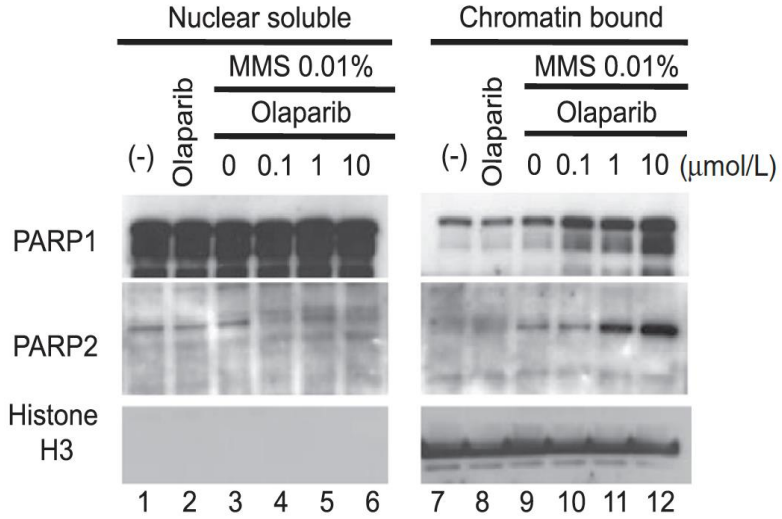


XRCC1 loss phenocopies PARP inhibitor.....does PARP1 become 'trapped' during BER (in the absence of XRCC1)?

The PARP1 auto-ribosylation cycle and 'trapping'

'trapping'= increased engagement at SSBs

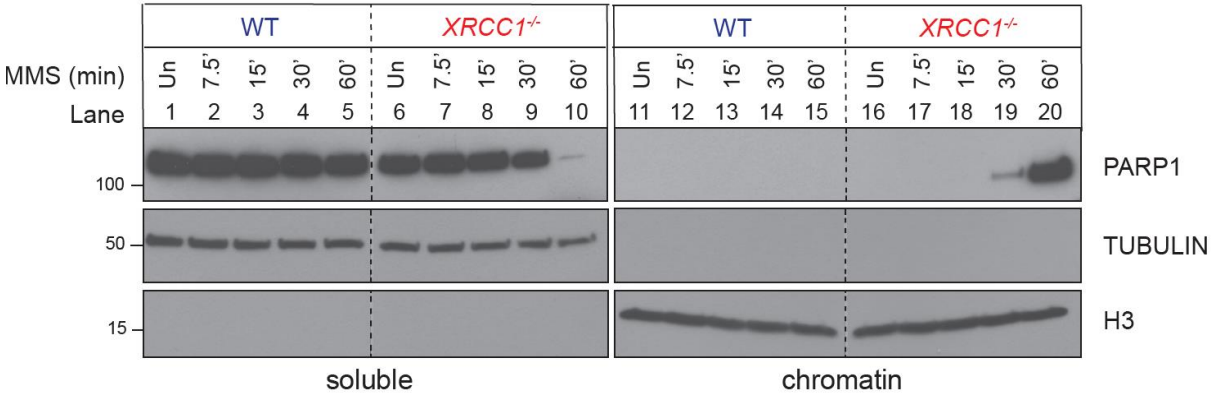


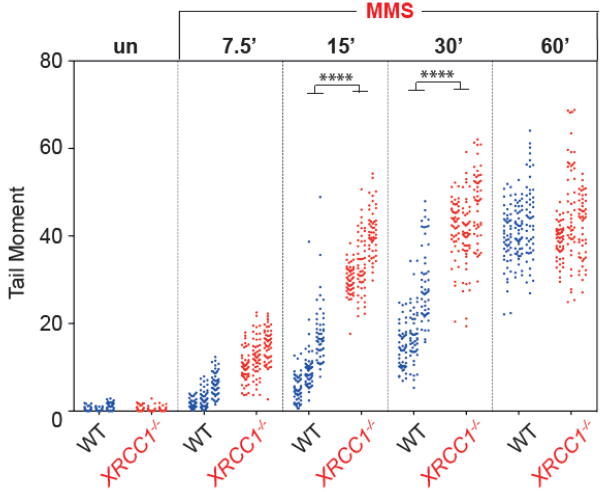


Murai et al, Cancer Research 2012

XRCC1 loss phenocopies PARP inhibitor.....does PARP1 become 'trapped' during BER (in the absence of XRCC1)?

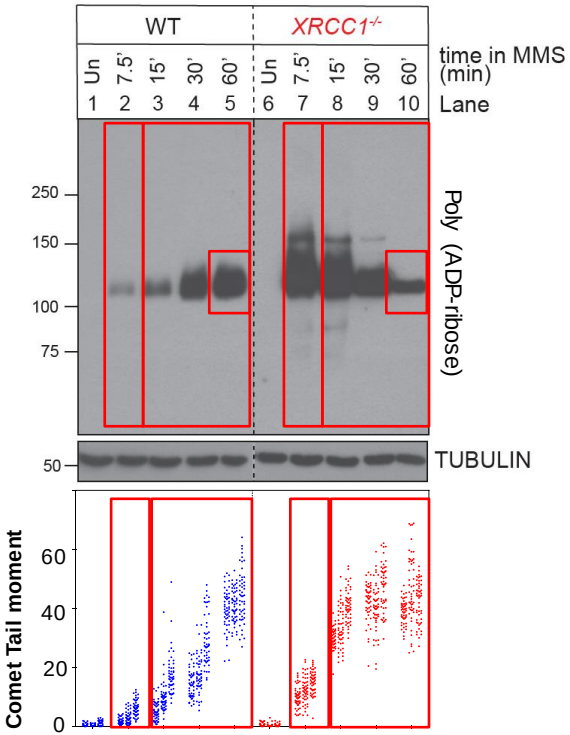
PARP1 accumulates in chromatin during BER in *XRCC1*$^{-/-}$ cells

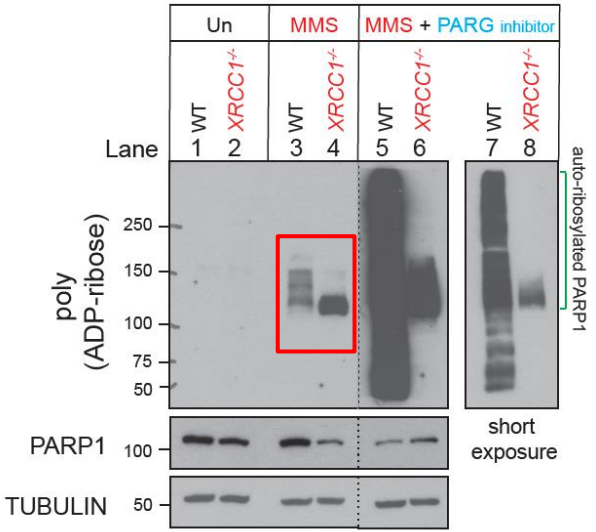




PARP1 'trapping' by PARP inhibitors is associated with reduced PARP activity/auto-ribosylation……

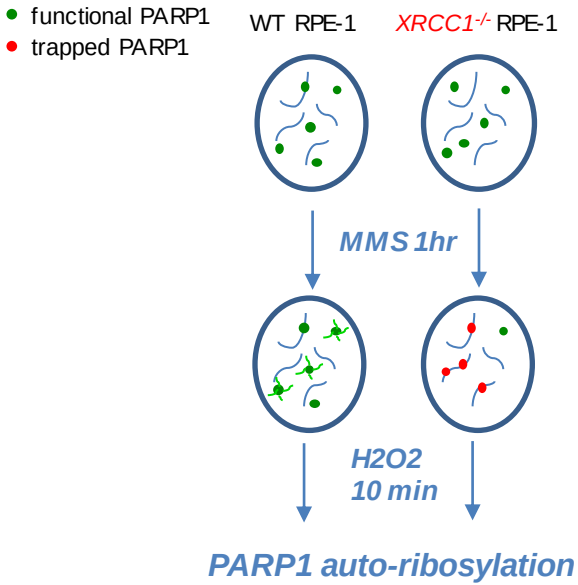
PARP1 is *hyper*active and then *hypo*active in *XRCC1*$^{-/-}$ cells, during BER

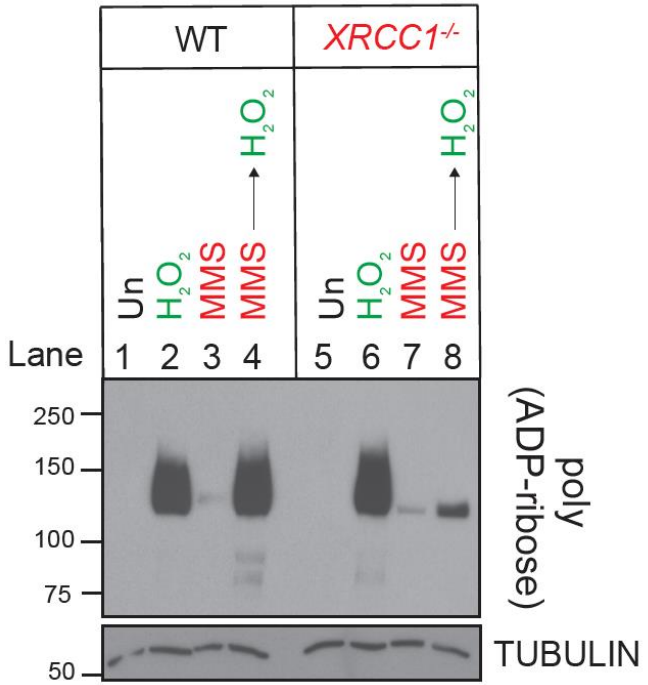




Can the 'inactive' (chromatin-trapped) PARP1 in XRCC1$^{-/-}$ *cells be re-activated by a second burst of SSBs?*

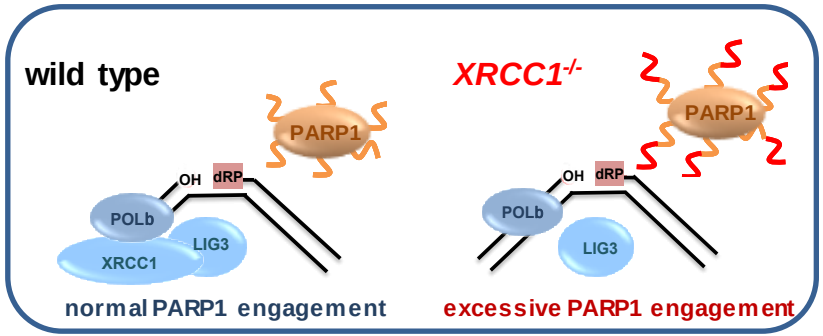
The chromatin-trapped PARP1 in *XRCC1*$^{-/-}$ cells cannot be reactivated by a second burst of SSBs



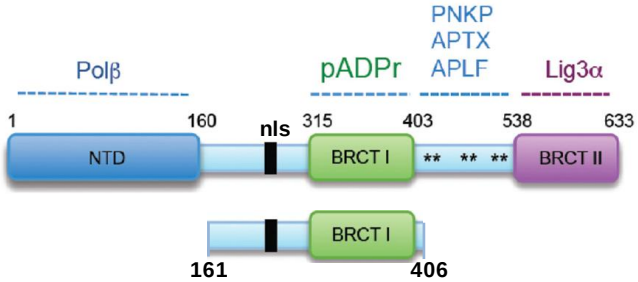


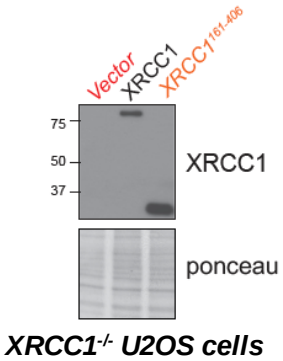
What is the cause of the hyperactivity and subsequent inactivity of PARP1, and are they connected?

A model for the suppression of PARP1 hyperactivity during BER by XRCC1 protein complexes



XRCC1 has a modular structure

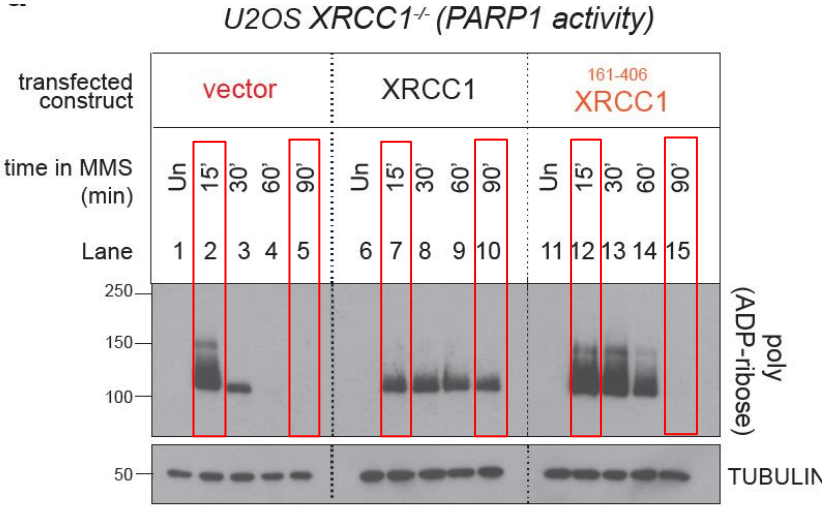


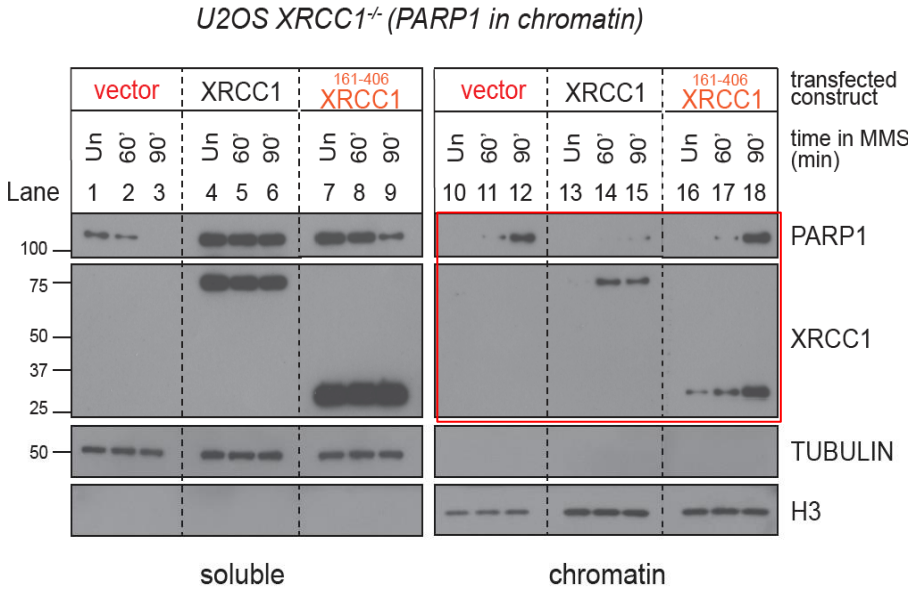


XRCC1-/- U2OS cells

Is the scaffolding function of XRCC1 required to suppress PARP1 hyperactivity during BER?

XRCC1 protein complexes regulate PARP1 activity and chromatin trapping during BER





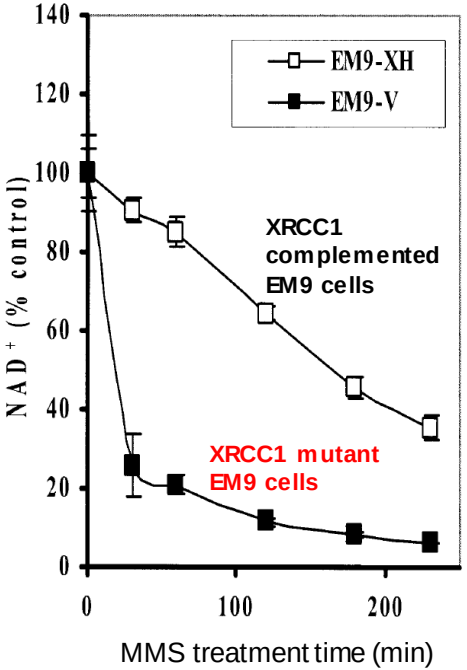
What is the cause of the progressive decline in activity of PARP1?

Rapid NAD+ depletion in XRCC1-mutant cells during BER

Nucleic Acids Research, 2003, Vol. 31, No. 17 ***e104***
DOI: 10.1093/nar/gng105

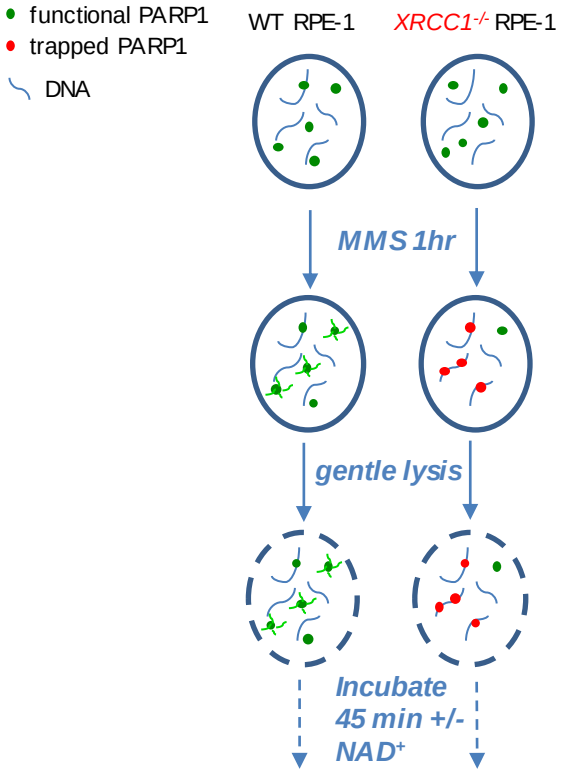
Quantitation of intracellular NAD(P)H can monitor an imbalance of DNA single strand break repair in base excision repair deficient cells in real time

Jun Nakamura[1,*], Shoji Asakura[1], Susan D. Hester[3], Gilbert de Murcia[4], Keith W. Caldecott[5] and James A. Swenberg[1,2]

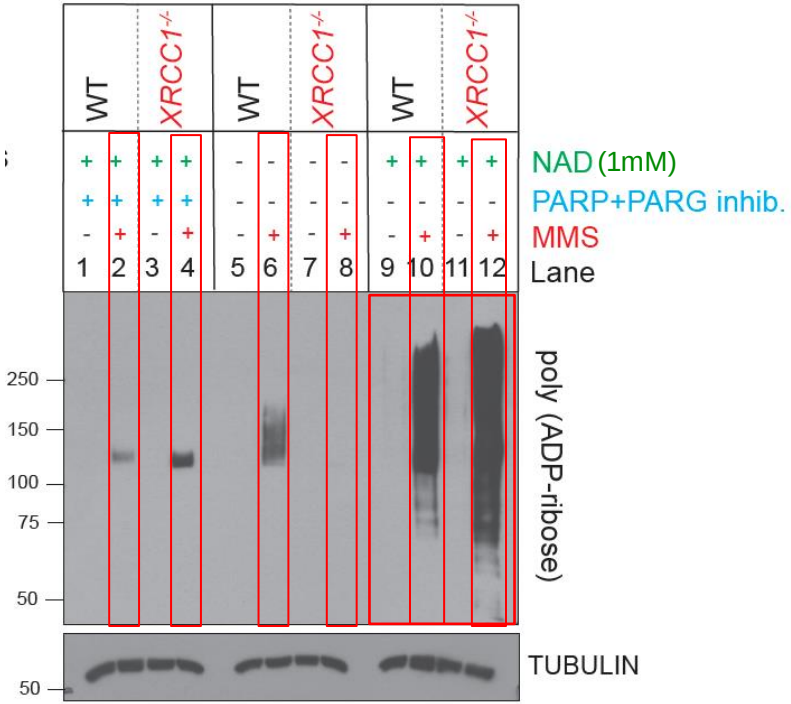


Does PARP1 activity decline during BER in XRCC1$^{-/-}$ cells because of NAD+ exhaustion?

The activity of trapped PARP1 in *XRCC1*$^{-/-}$ cells is rescued by NAD+ supplementation

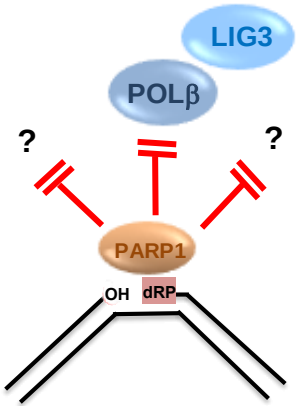


PARP1 auto-ribosylation

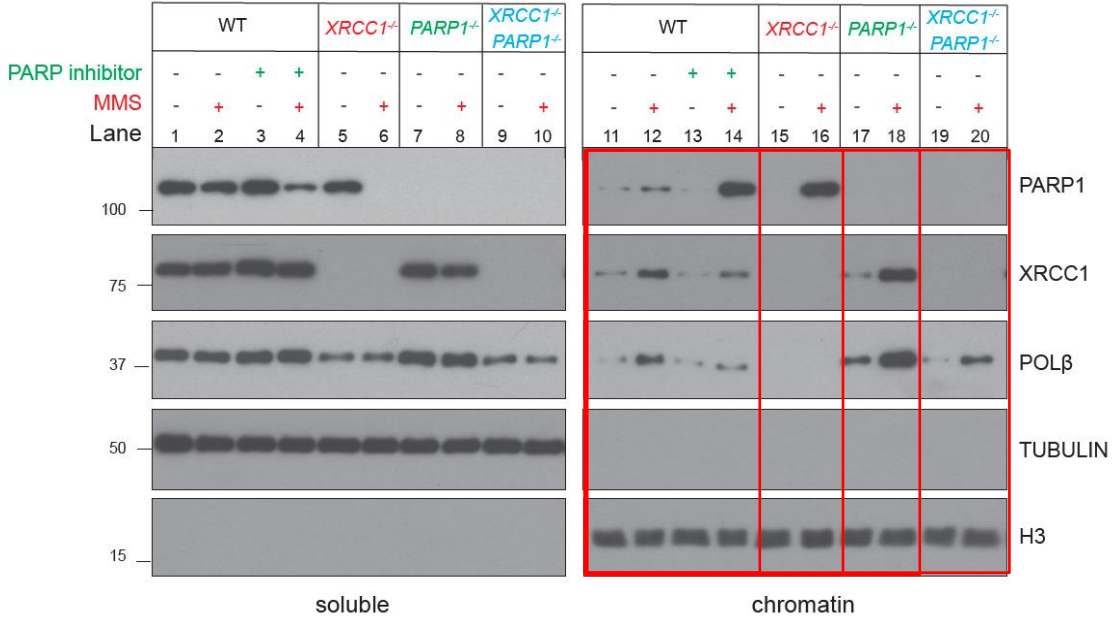


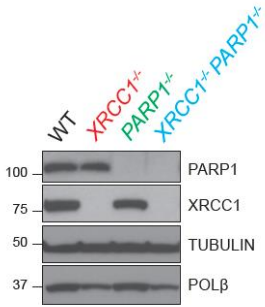
How does trapped PARP1 block BER ?

Endogenous PARP1 trapping in *XRCC1*$^{-/-}$ cells during BER blocks POLβ recruitment

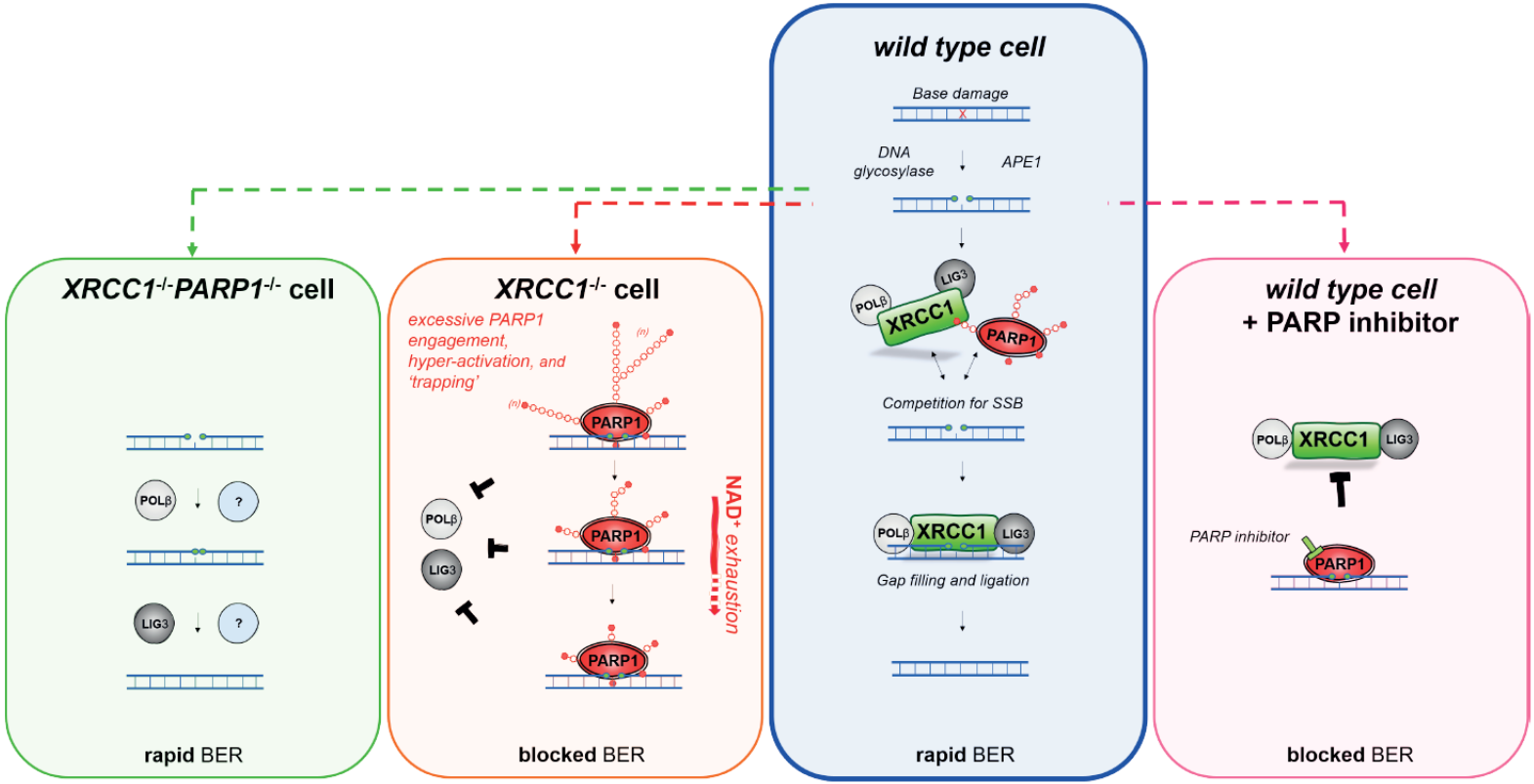


excessive PARP1 engagement

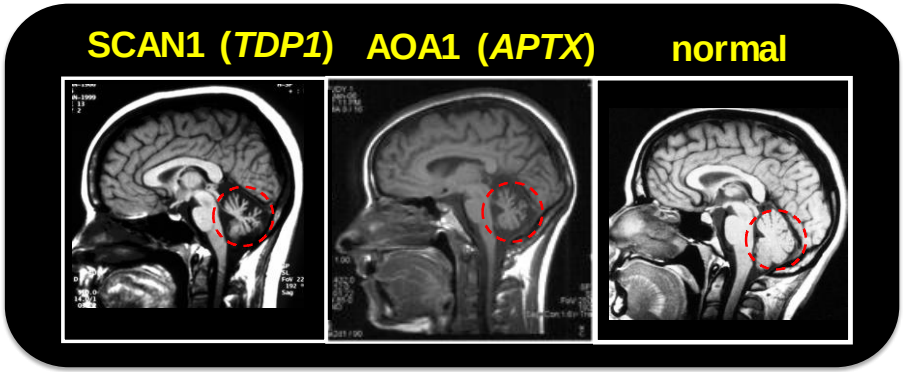


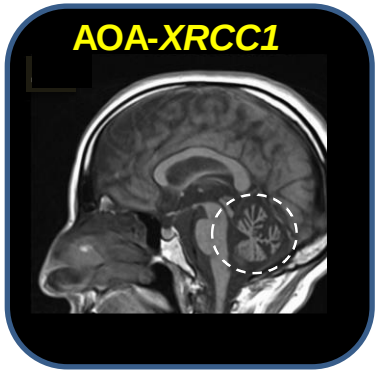


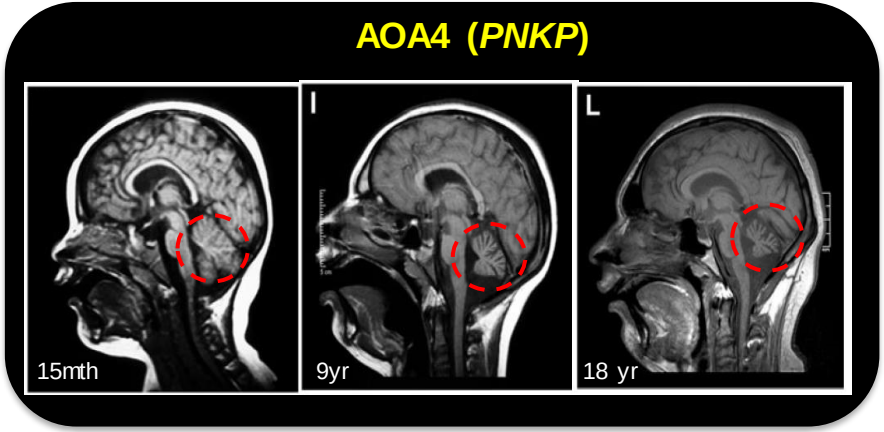
XRCC1 is an endogenous PARP “anti-trapper”



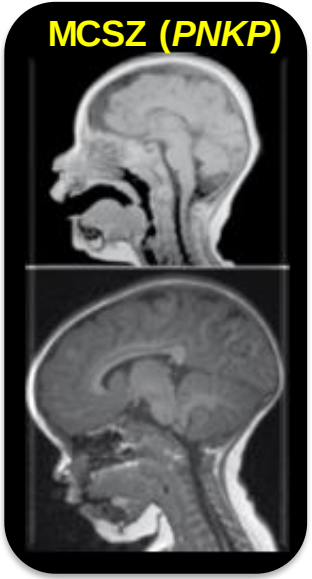
SSBR-Associated Diseases, Cerebellar Degeneration, & Microcephaly



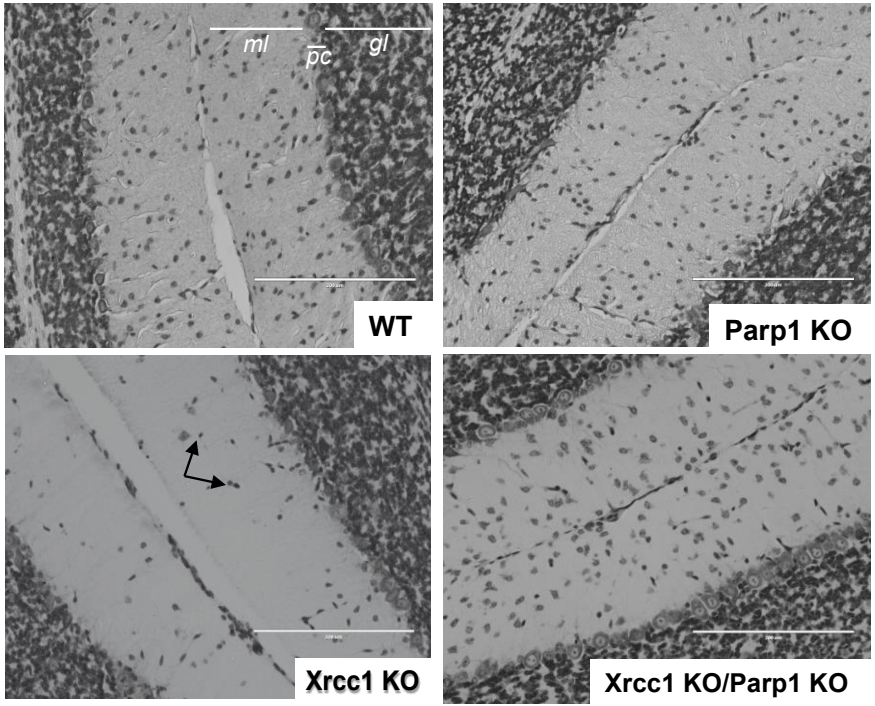




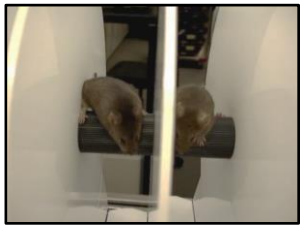
disease spectrum

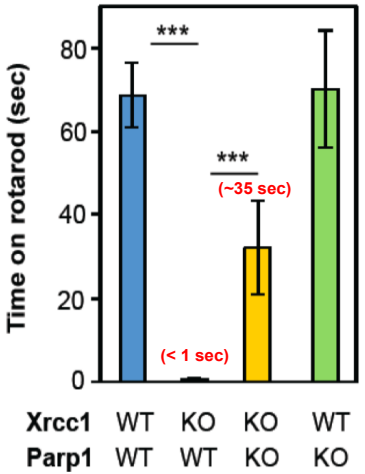


Parp1 deletion rescues cerebellar interneurons and suppresses ataxia in *XRCC1*$^{Nestin\text{-}Cre}$ mice (3-wk)



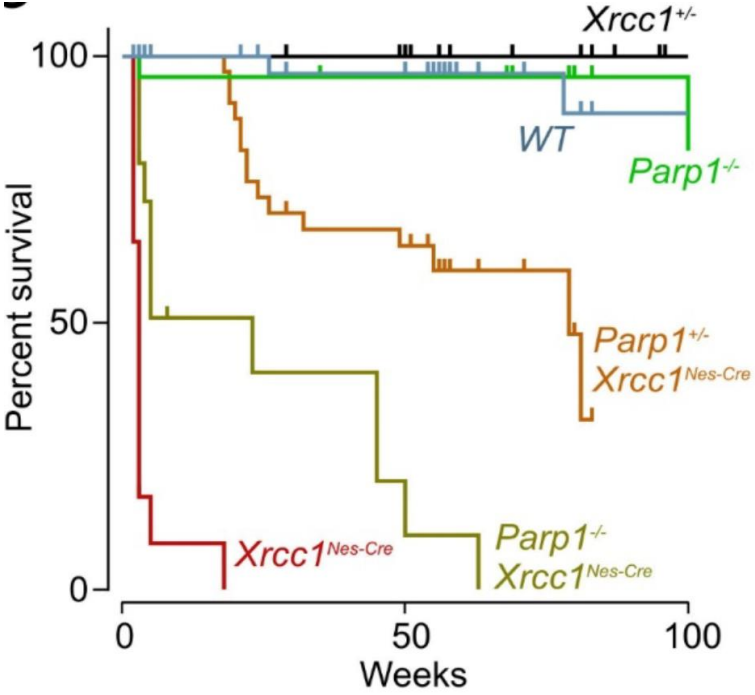
Nissl staining





Hoch et al Nature, 2017

Parp1 Deletion Prevents Lethal Seizures and Juvenile Mortality in *XRCC1*$^{Nestin\text{-}Cre}$ mice



Genotype	n	Median survival	p value vs *WT*	p value vs *Xrcc1*$^{Nes\text{-}Cre}$
WT	48	Und	-	<0.0001
Xrcc1$^{+/-}$	18	Und	ns	<0.0001
Parp1$^{-/-}$	25	Und	ns	<0.0001
Xrcc1$^{Nes\text{-}Cre}$	23	3	<0.0001	-
Parp1$^{+/-}$ *Xrcc1*$^{Nes\text{-}Cre}$	35	79	<0.0001	<0.0001
Parp1$^{-/-}$ *Xrcc1*$^{Nes\text{-}Cre}$	15	23	<0.0001	<0.0001

Komulainen et al BioRxiv 2020

Summary

- The essential role of XRCC1 during BER is to assemble BER protein complexes **that can compete effectively with PARP1**, thereby limiting excessive PARP1 engagement and activity.

- Excessive **PARP1 engagement** and activity can lead to NAD+ exhaustion and PARP1 accumulation in chromatin, blockage of BER intermediates from access by other DNA repair enzymes (e.g. POLβ), and consequently the accumulation of BER intermediates and cellular toxicity.

- XRCC1 is the an endogenous PARP1 “anti-trapper”.

GDSC
Genome Damage & Stability Centre

- **Annie Demin**
- Marek Adamowicz
- Jan Brazina
- Richard Hailstone
- Will Gittens
- Hana Hanzlikova

MRC Medical Research Council

CANCER RESEARCH UK

- Limei Ju
- Emilia Komulainen
- Jack Badman

erc
European Research Council

- **Shunichi Takeda**
- **Kouji Hirota**
- **Masataka Tsuda**
- **Hiroyuki Sasanuma**

Kyoto