NIH National Institutes of Health
Turning Discovery Into Health

NIH DNA Repair Interest Group Video Conference

UNIVERSITY OF NORTH CAROLINA CHARLOTTE

Functional studies of APE1/2 in genome integrity and cancer etiology

Shan Yan, Ph.D., Professor
Associate Chair for Research

Department of Biological Sciences
UNC Charlotte

June 13, 2023

Outline

- ❖ **Introduction**
- ❖ **Functional studies of APE2 in ATR DDR and cancer biology**
  1. APE2 in OS-induced ATR DDR
  2. APE2 in SSB-induced ATR DDR
  3. APE2 in cancer biology and therapeutics
- ❖ **Molecular mechanisms of APE1 in SSB response, global and nucleolar DDR**
  1. APE1 in SSB-induced ATR DDR
  2. APE1 in ATRIP recruitment to ssDNA gaps
  3. APE1 in nucleolar DDR

Outline

- **Introduction**
- **Functional studies of APE2 in ATR DDR and cancer biology**

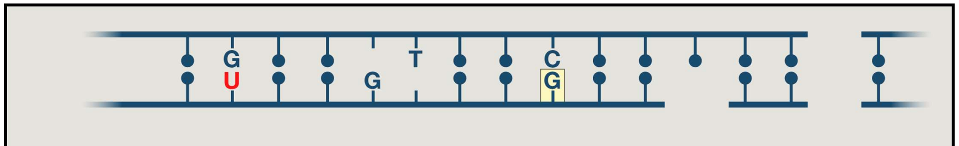
  1. APE2 in OS-induced ATR DDR
  2. APE2 in SSB-induced ATR DDR
  3. APE2 in cancer biology and therapeutics

- **Molecular mechanisms of APE1 in SSB response, global and nucleolar DDR**

  1. APE1 in SSB-induced ATR DDR
  2. APE1 in ATRIP recruitment to ssDNA gaps
  3. APE1 in nucleolar DDR

DNA lesions generated by endogenous and exogenous sources

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	**Dose Exposure (mSv)**	**DNA Lesions Generated**	**Estimated Number Lesions/Cell**
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

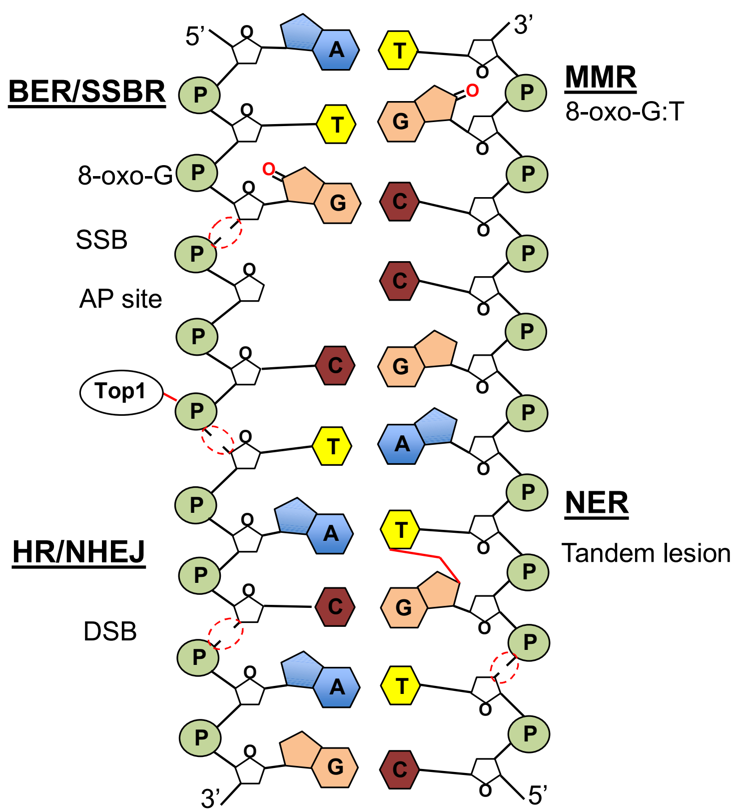


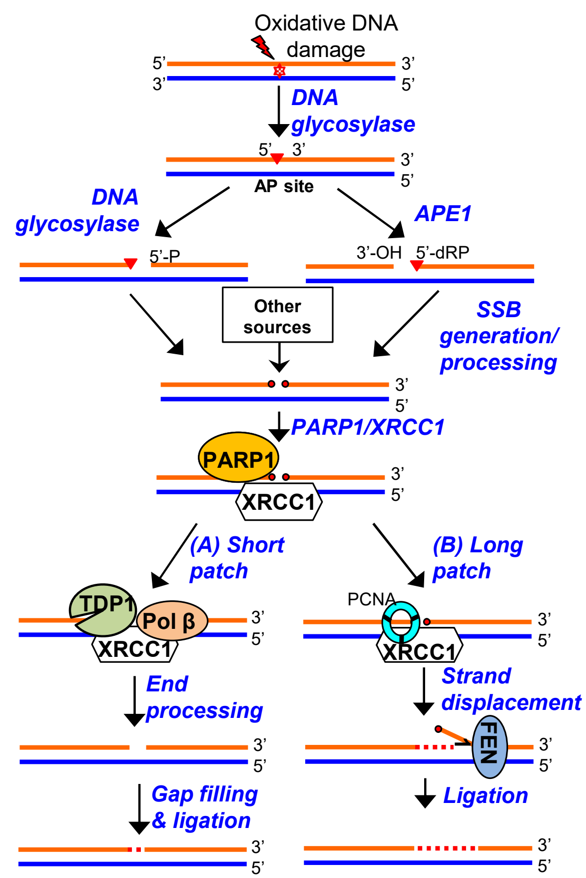
Damage	Cytosine deamination	Depurination/ depyrimidination	8-oxoG	SSB	DSB
Estimated frequency (per cell per day)	192	12,000/600	2,800	55,000	25
Predominant mutation	C>T	Substitutions	G>T	Substitutions, DBSs	Chromosome rearrangements

- Oxidative DNA damage is inevitable endogenous DNA lesions
- ~12,000 AP sites and ~55,000 SSBs per mammalian cell each day

Ciccia and Elledge, *Molecular Cell*, 2010;
Tubbs and Nussenzweig, *Cell*, 2017

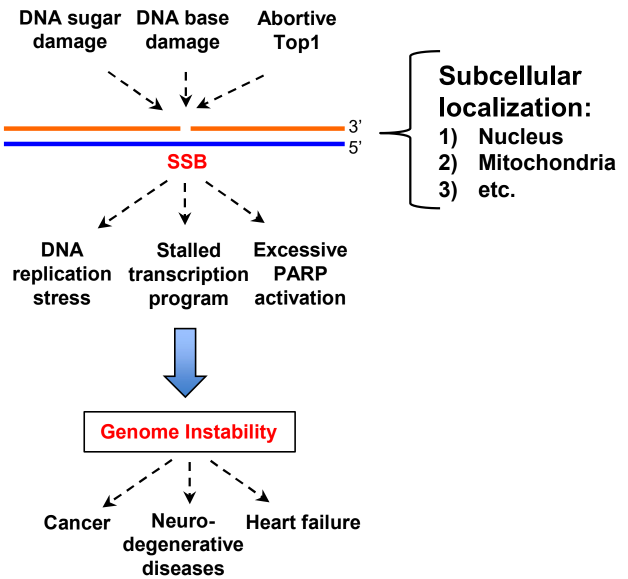
Oxidative DNA damage and DNA repair (BER & SSBR) pathways





Yan et al., *CMLS*, 2014

DNA single-strand break (SSB)



- How is SSB repair and signaling regulated?

Nassour et al., *Nat Commun*, 2015;
Higo et al., *Nat Commun*, 2017;
Caldecott, *Nat Rev Genetics*, 2008;
Hossain et al., *Int J Mol Sci*, 2018

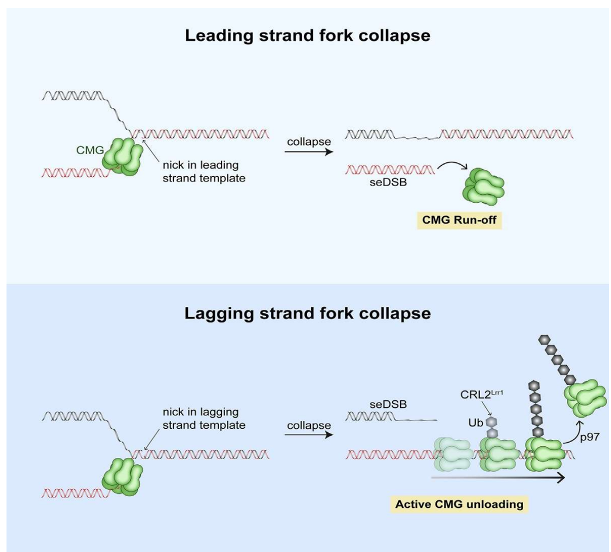
Molecular Cell
CellPress

Short Article

Single-strand DNA breaks cause replisome disassembly

Kyle B. Vrtis,[1] James M. Dewar,[1,3] Gheorghe Chistol,[1,4] R. Alex Wu,[1] Thomas G.W. Graham,[1,5] and Johannes C. Walter[1,2,6,*]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Blavatnik Institute, Boston, MA 02115, USA
[2]Howard Hughes Medical Institute, Boston, MA 02115, USA
[3]Present address: Department of Biochemistry, Vanderbilt University, Nashville, TN 37205, USA
[4]Present address: Chemical and Systems Biology Department, Stanford School of Medicine, Stanford, CA 94305, USA
[5]Present address: Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720, USA
[6]Lead contact
*Correspondence: johannes_walter@hms.harvard.edu

https://doi.org/10.1016/j.molcel.2020.12.039



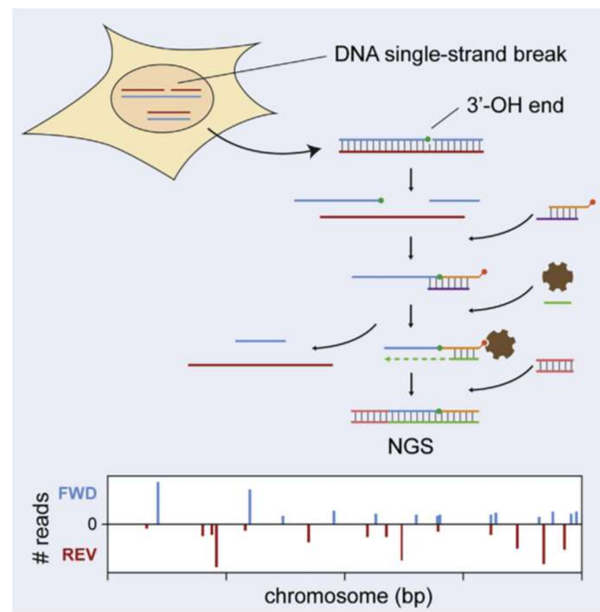
Molecular Cell
CellPress

Technology

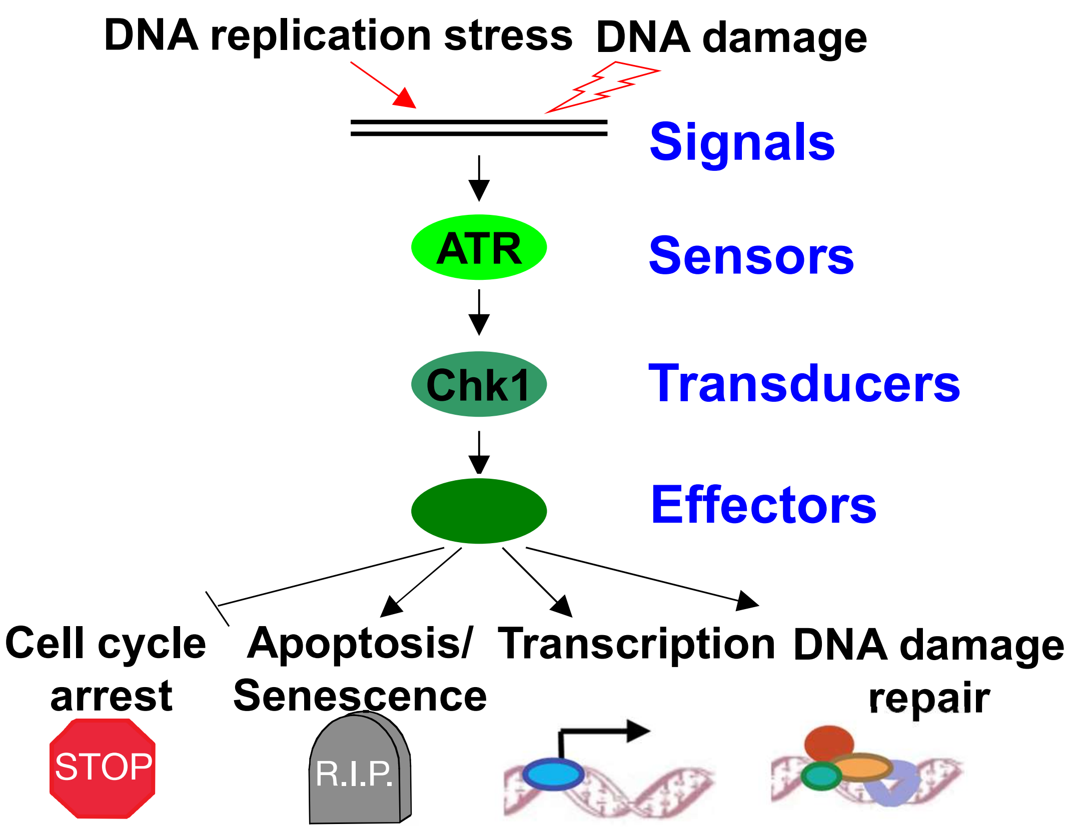
Genome-wide Nucleotide-Resolution Mapping of DNA Replication Patterns, Single-Strand Breaks, and Lesions by GLOE-Seq

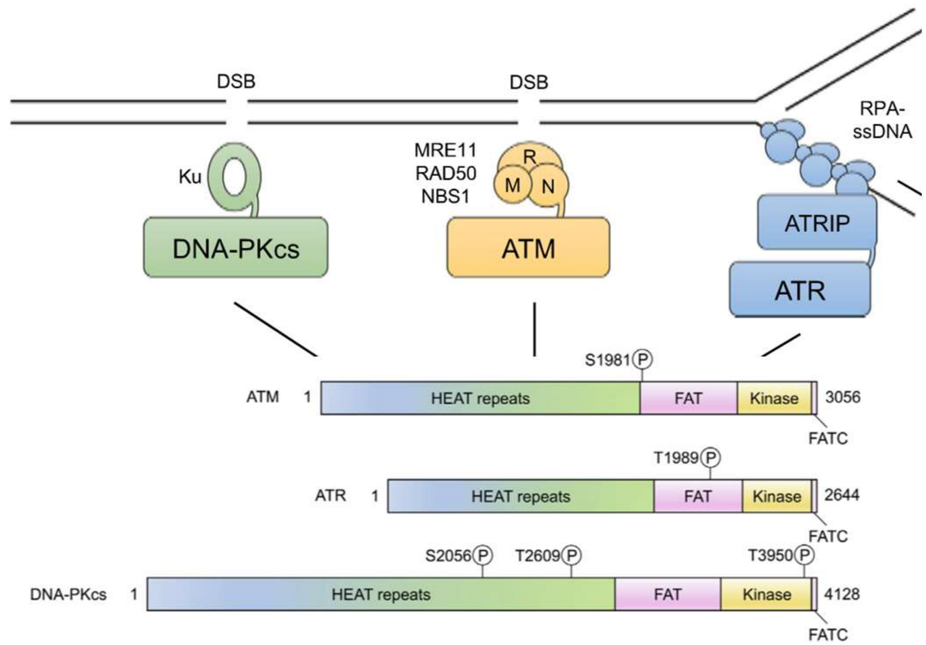
Annie M. Sriramachandran,[1] Giuseppe Petrosino,[1] María Méndez-Lago,[1] Axel J. Schäfer,[1] Liliana S. Batista-Nascimento,[1] Nicola Zilio,[1,*] and Helle D. Ulrich[1,2,*]
[1]Institute of Molecular Biology (IMB), Ackermannweg 4, 55128 Mainz, Germany
[2]Lead Contact
*Correspondence: n.zilio@imb-mainz.de (N.Z.), h.ulrich@imb-mainz.de (H.D.U.)

https://doi.org/10.1016/j.molcel.2020.03.027



The DNA damage response (DDR) pathway



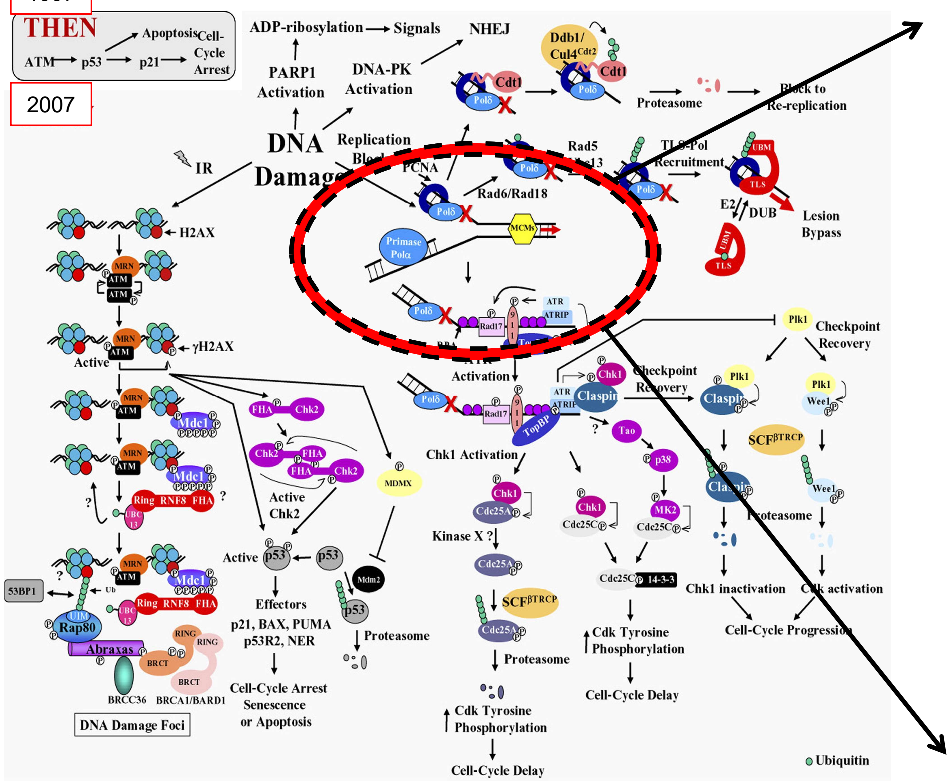


Adapted from Zhou and Elledge, *Nature*, 2000;
Blackford and Jackson, *Mol Cell*, 2017

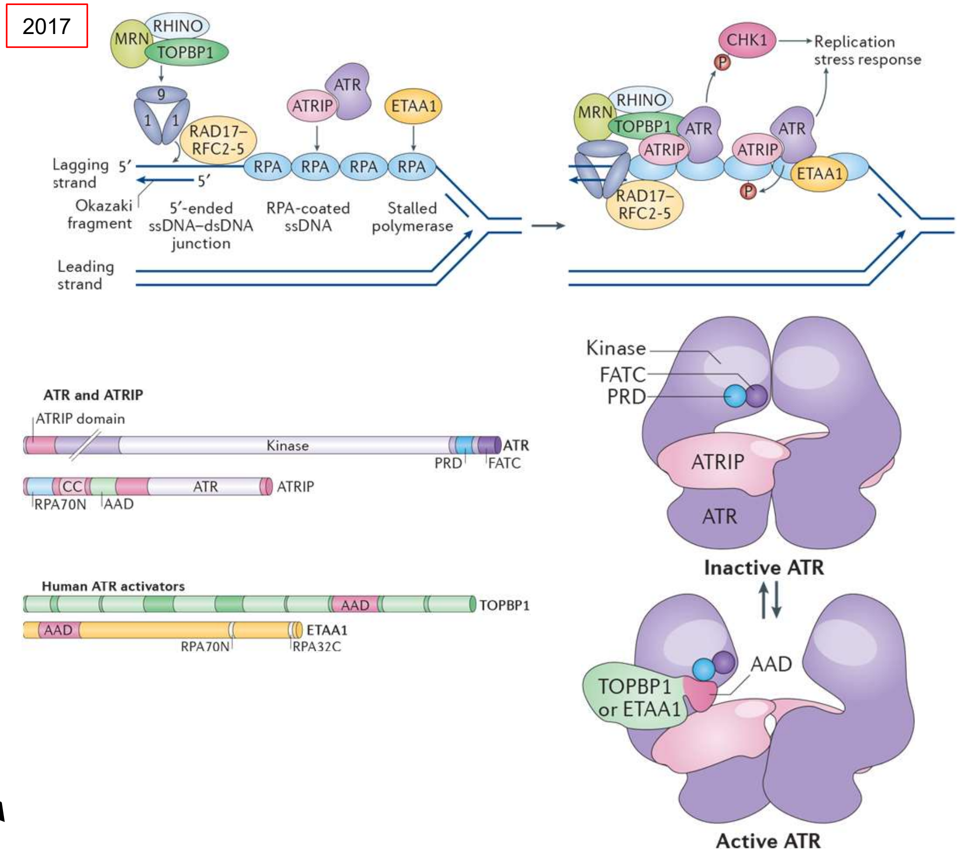
The complexity of DDR

1997

2007



2017



Haper and Elledge, *Mol Cell*, 2007;

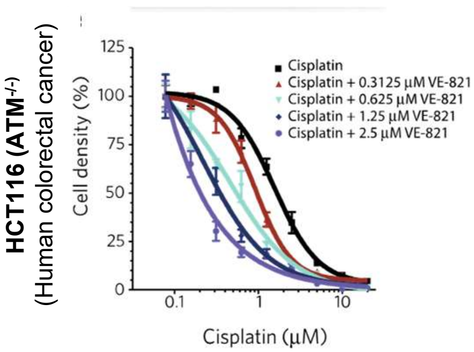
Saldivar, Cortez, and Cimprich, *Nat Rev Mol Cell Biol*, 2017

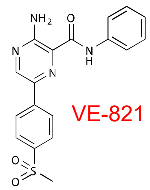
Targeting ATR DDR for cancer therapies

BRIEF COMMUNICATION
PUBLISHED ONLINE: 13 APRIL 2011 | DOI: 10.1038/NCHEMBIO.573
nature chemical biology

Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR

Philip M Reaper, Matthew R Griffiths, Joanna M Long, Jean-Damien Charrier, Somhairle MacCormick, Peter A Charlton, Julian M C Golec & John R Pollard*

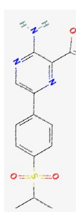



VE-821

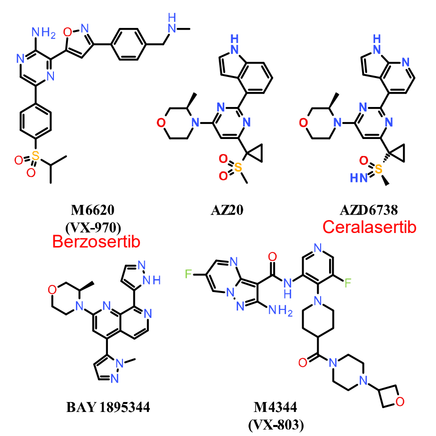
original reports

Phase I Trial of First-in-Class ATR Inhibitor M6620 (VX-970) as Monotherapy or in Combination With Carboplatin in Patients With Advanced Solid Tumors

Timothy A. Yap, MBBS, PhD, FRCP[1,2]; Brent O'Carrigan, MBBS, PhD[1]; Marina S. Penney, PhD[3]; Joline S. Lim, MBBS, MRCP, MMed, MCI, FAMS[1]; Jessica S. Brown, MD, PhD[1]; Maria J. de Miguel Luken, MD, PhD[1]; Nina Tunariu, FRCR, MRCP, MDRes, MBBS[1]; Raquel Perez-Lopez, MD, PhD[1]; Daniel Nava Rodrigues, MD, PhD[2]; Ruth Riisnaes, FIBMS[2]; Ines Figueiredo, BSc Hons[2]; Suzanne Carreira, PhD[2]; Brian Hare, PhD[3]; Katherine McDermott, MA[3]; Saira Khalique, MBChB[4]; Chris T. Williamson, PhD[4,5]; Rachael Natrajan, PhD[4]; Stephen J. Pettitt, PhD[4,5]; Christopher J. Lord, DPhil[4,5]; Udai Banerji, MD, PhD[1,2]; John Pollard, BSc Hons, PhD[6]; Juanita Lopez, MB, BChir, MRCP, PhD[1]; and Johann S. de Bono, MB, ChB, FRCP, MSc, PhD, FMedSci[1,2]

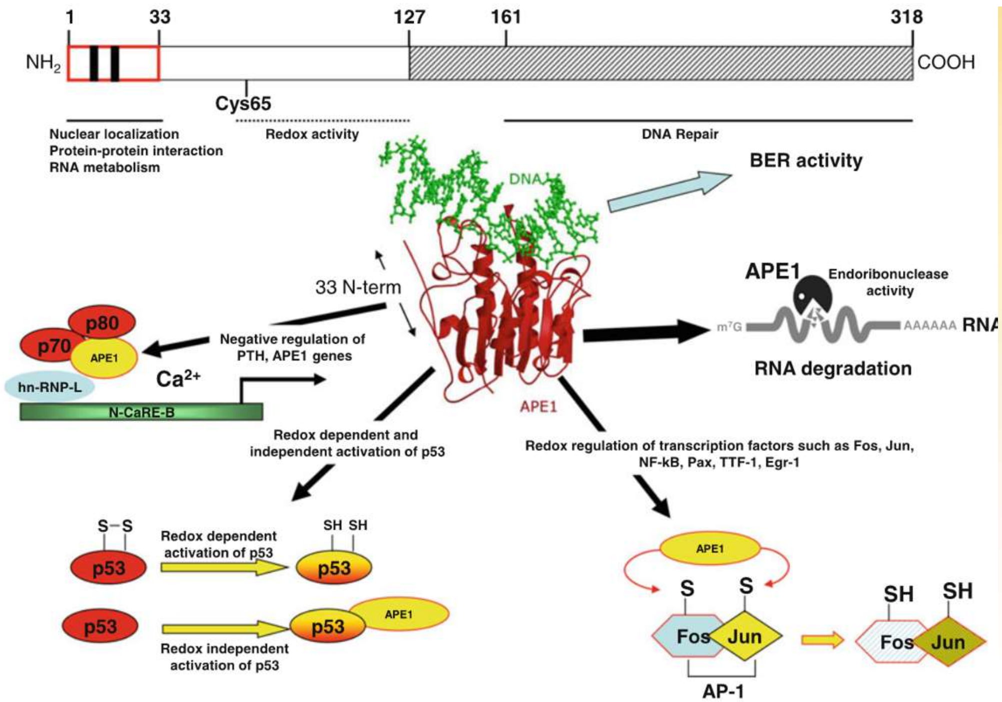

VE-822 (M6620/VX-970/ Berzosertib)

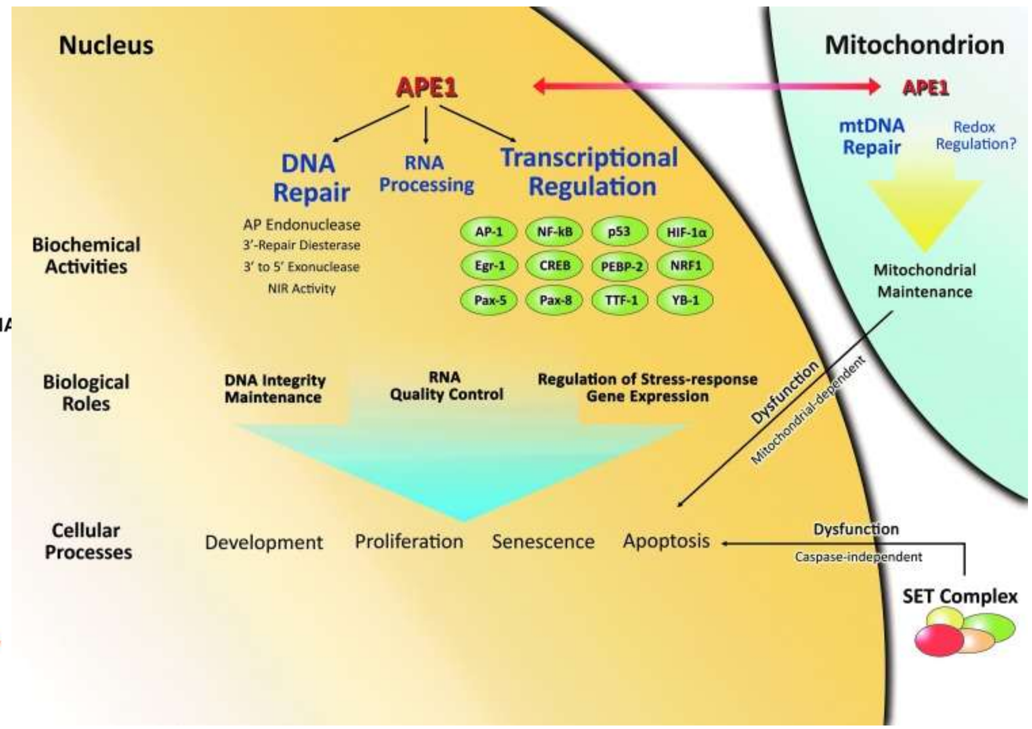
Compound	Target	Additional Treatment	Condition	Inclusion Criteria	Phase	Identifier
Berzosertib	ATR	Radiation therapy	Breast cancer		1	NCT04052555
Berzosertib	ATR	Carboplatin	mCRPC		2	NCT03517969
Ceralasertib	ATR	Olaparib	mCRPC		2	NCT03787680
Ceralasertib	ATR	Olaparib	TNBC		2	NCT03330847
Ceralasertib	ATR	Olaparib	Advanced breast cancer	Germline BRCA mutation	2	NCT04090567
M4344	ATR	Chemotherapy	Advanced solid tumors		1	NCT02278250
BAY 1895344	ATR		Advanced solid tumors and lymphomas		1	NCT03188965
BAY 1895344	ATR	Chemotherapy	Advanced solid tumors		1	NCT04491942
BAY 1895344	ATR	Niraparib	Advanced solid tumors		1	NCT04267939
BAY 1895344	ATR	Pembrolizumab	Advanced solid tumors		1	NCT04095273



Reaper et al., *Nat Chem Biol*, 2011;
Yap et al., *J Clin Oncol*, 2020;
Wengner et al., *IJMS*, 2020;
Lucking et al., *J Med Chem*, 2020

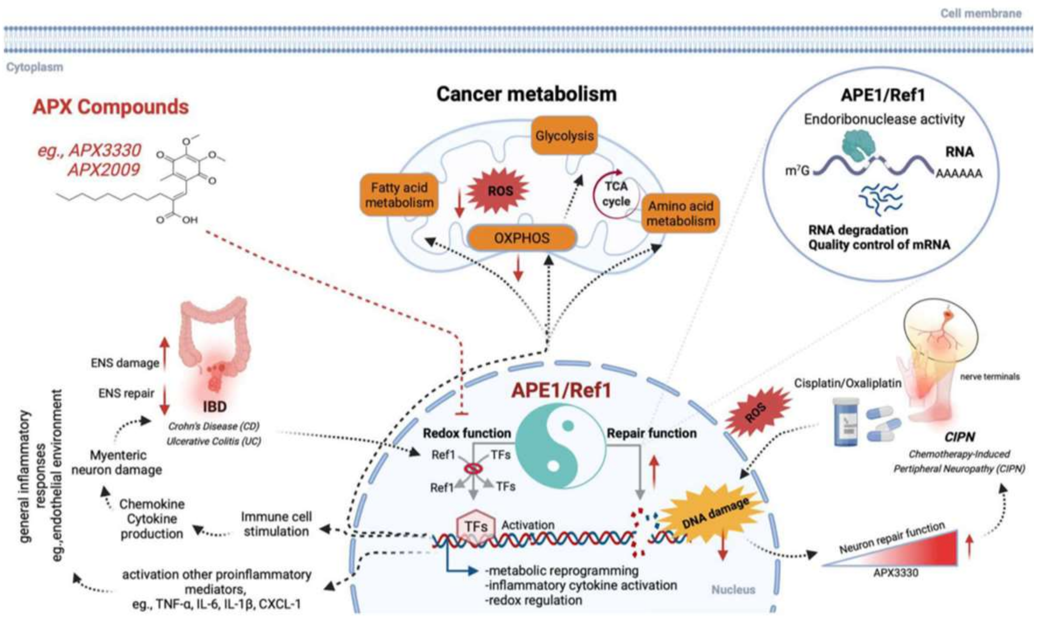
Functions of APE1 in genome integrity





Tell et al., *Cell Mol Life Sci*, 2010;
Li and Wilson, *Antioxid Redox Signal*, 2014

Targeting APE1 in the treatments of human diseases



- Redox function
- DNA repair
- RNA metabolism?
- CIPN?
- DDR?

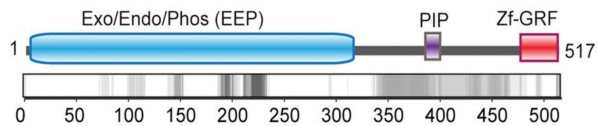
~1,500 APE1 papers in PubMed

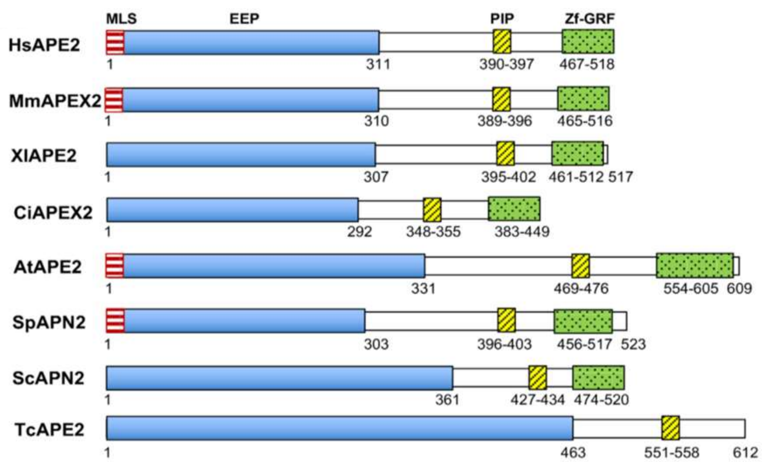
- Phase I (NCT03375086): A Study of APX3330 in Patients With Advanced Solid Tumors (APX3330). 19 participants, open-label, dose-escalation to determine safety and tolerability.
- Phase II (NCT04692688): Study of the Safety and Efficacy of APX3330 in Diabetic Retinopathy (ZETA-1). Placebo-controlled double-masked, randomized in 100 participants

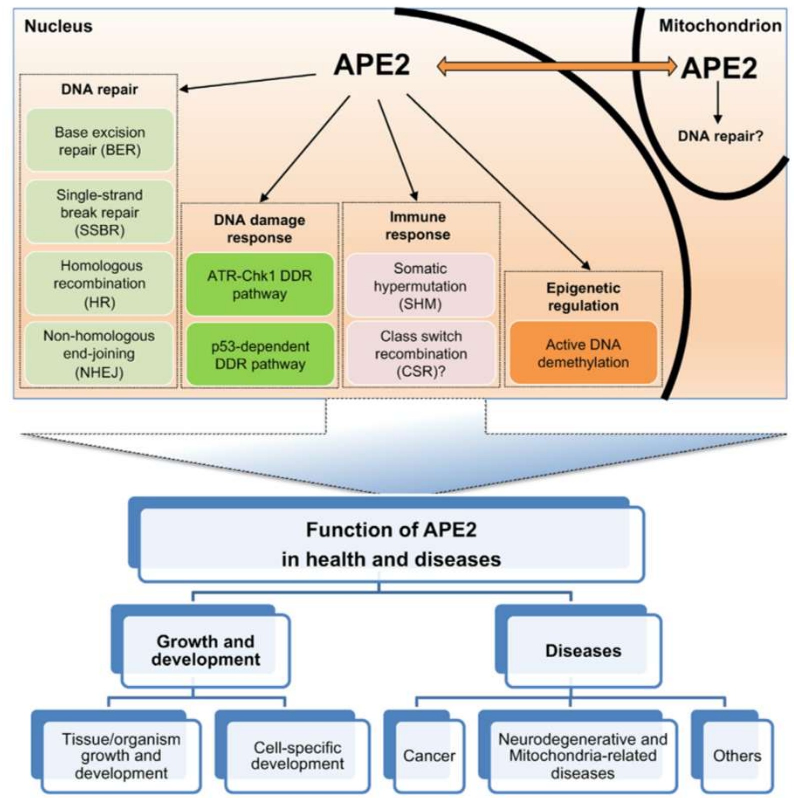
Qian et al., *Drug Des Dev Ther*, 2014;
Wang et al., *Invest New Drugs*, 2020;
Mijit et al., *J Cell Signal*, 2021;
Caston et al., *Drug Discovery Today*, 2021

Function and mechanisms of APE2 in genome/epigenome integrity

APE2 (AP endonuclease 2, APEX2, APN2)
~73 APE2 papers in PubMed

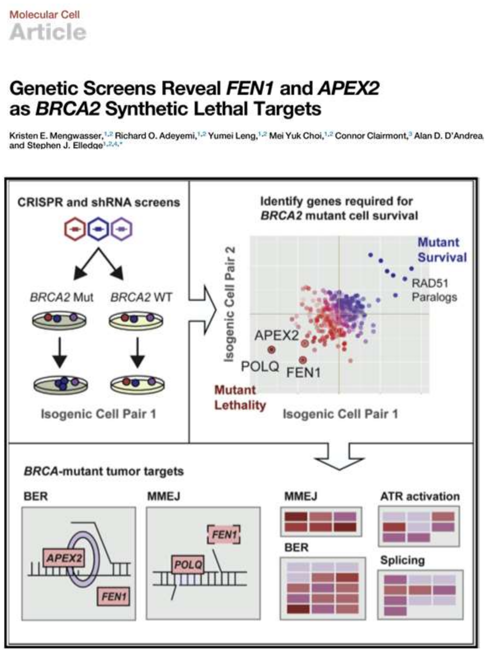


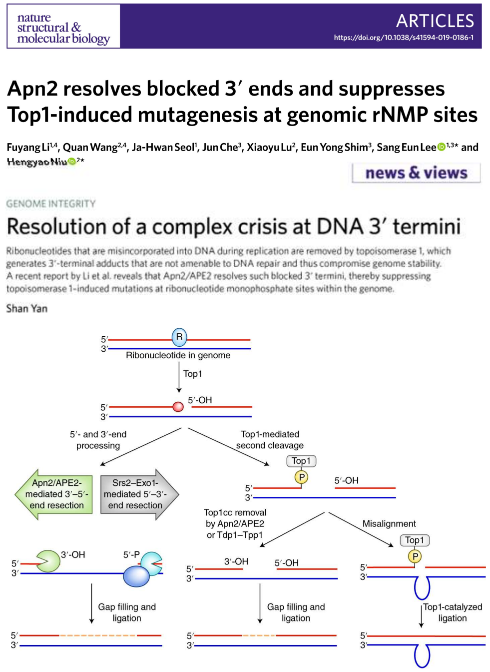


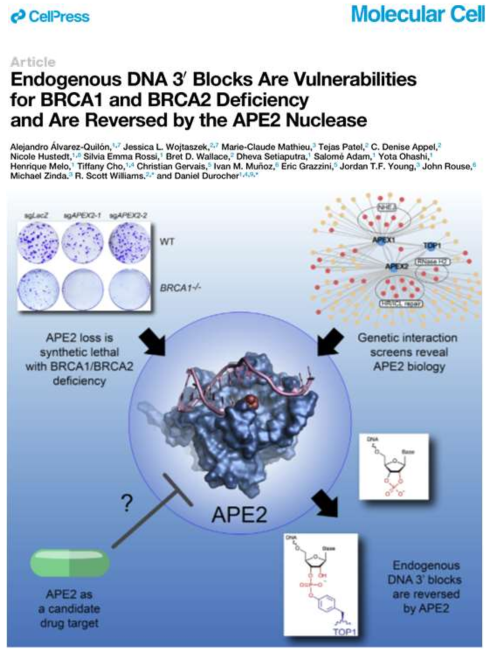


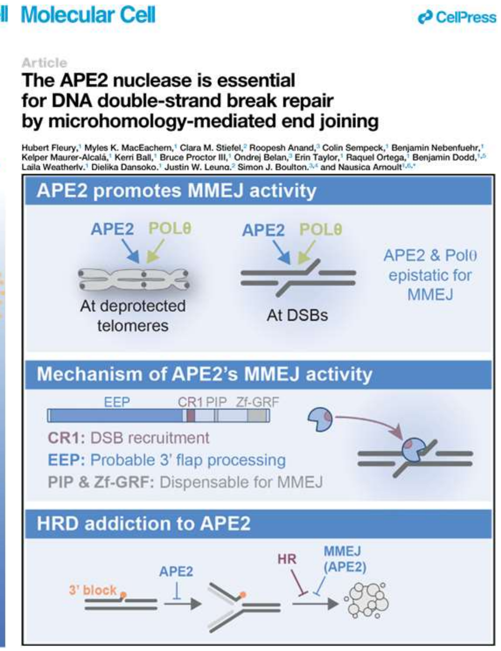
Johnson et al., *Genes & Dev*, 1998; Hadi and Wilson, *Environ Mol Mutagen*, 2000; Tsuchimoto et al., *Nucleic Acids Res*, 2001; Ide et al., *Blood*, 2004; Burkovics et al., *Nucleic Acids Res*, 2009; Guikema et al., *J Immunol*, 2011; Willis et al., *PNAS*, 2013; Stavnezer et al., *PNAS*, 2014; Wallace et al., *PNAS*, 2017; Lin et al., *Mut Res*, 2021

APE2 is a synthetic lethal target in BRCA1/2-deficient cells









Mechanism:
Base excision repair (BER)?

Mechanism:
Resolution of 3'-blocked ends?

Mechanism:
Resolution of 3'-flap structures?

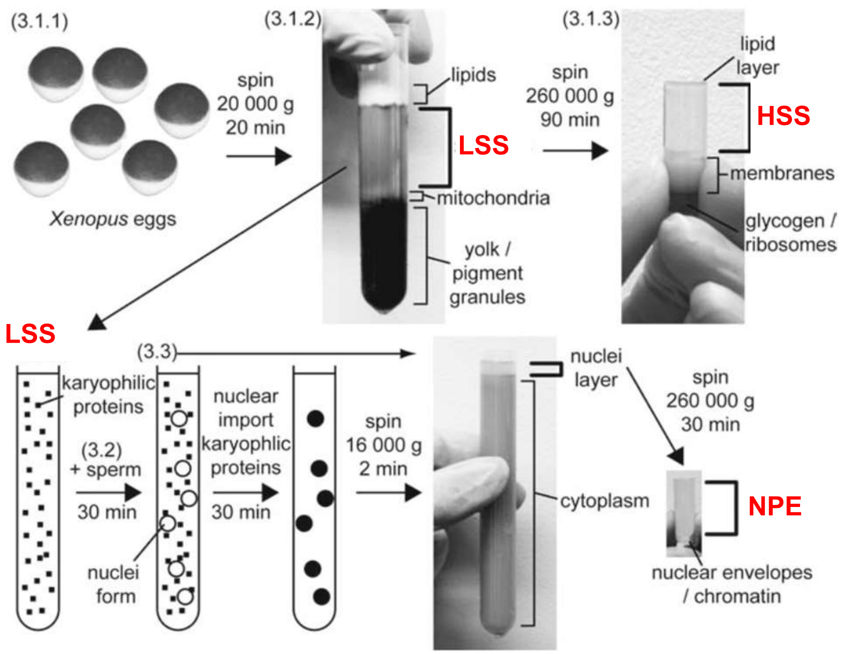
Li…Lee, and Niu, *Nat Struct Mol Biol*, 2019;
Yan *Nat Struct Mol Biol*, 2019;
Mengwasser…Elledge, *Mol Cell*, 2019;
Álvarez-Quilón...Williams, and Durocher, *Mol Cell*, 2020;
Fleury…Arnoult, *Mol Cell*, 2023

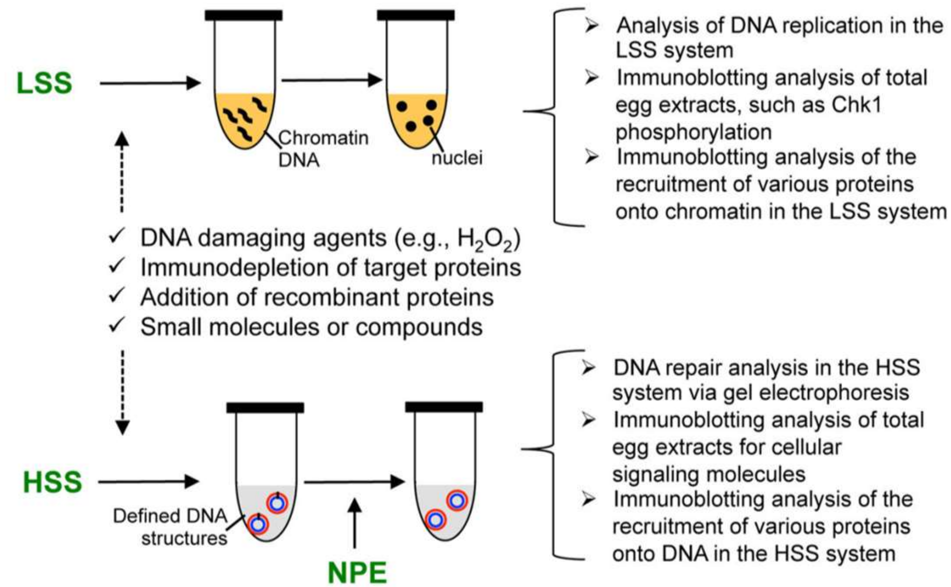
AP endonuclease	3'-5' exonuclease	Embryonic lethal (KO)
Strong/fast	Weak/slow	Yes
Weak/slow	Strong/fast	No

APE1: Bro, I was discovered first. I can do everything (~1,500 papers in PubMed).

APE2: Really? Maybe I can do something that you don't (~70 papers in PubMed).

The *Xenopus* LSS and HSS/NPE systems





LSS: Low-speed supernatant
HSS: High-speed supernatant
NPE: Nucleoplasmic extracts

Lebofsky et al., *Methods Mol Biol*, 2009;
Willis et al., JoVE, 2012;
Cupello et al., *Int J Dev Biol*, 2016

Outline

- **Introduction**
- **Functional studies of APE2 in ATR DDR and cancer biology**

  1. APE2 in OS-induced ATR DDR
  2. APE2 in SSB-induced ATR DDR
  3. APE2 in cancer biology and therapeutics

- **Molecular mechanisms of APE1 in SSB response, global and nucleolar DDR**

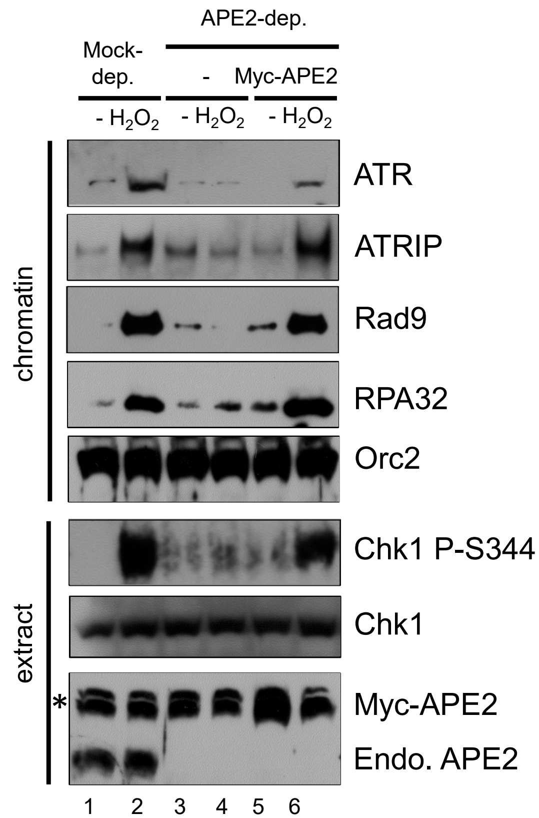
  1. APE1 in SSB-induced ATR DDR
  2. APE1 in ATRIP recruitment to ssDNA gaps
  3. APE1 in nucleolar DDR

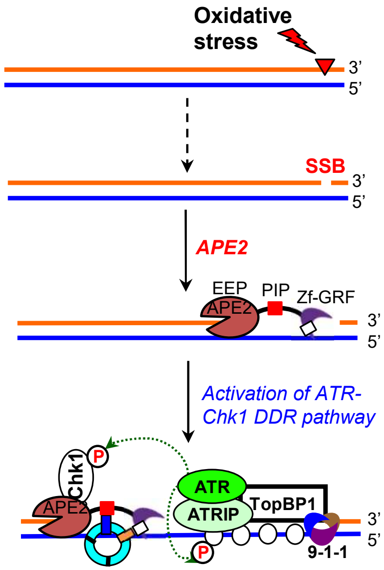
APE2 is required for the hydrogen peroxide-induced Chk1 phosphorylation in Xenopus LSS system

Jeremy Willis
MS student

Yogin Patel
Honors student

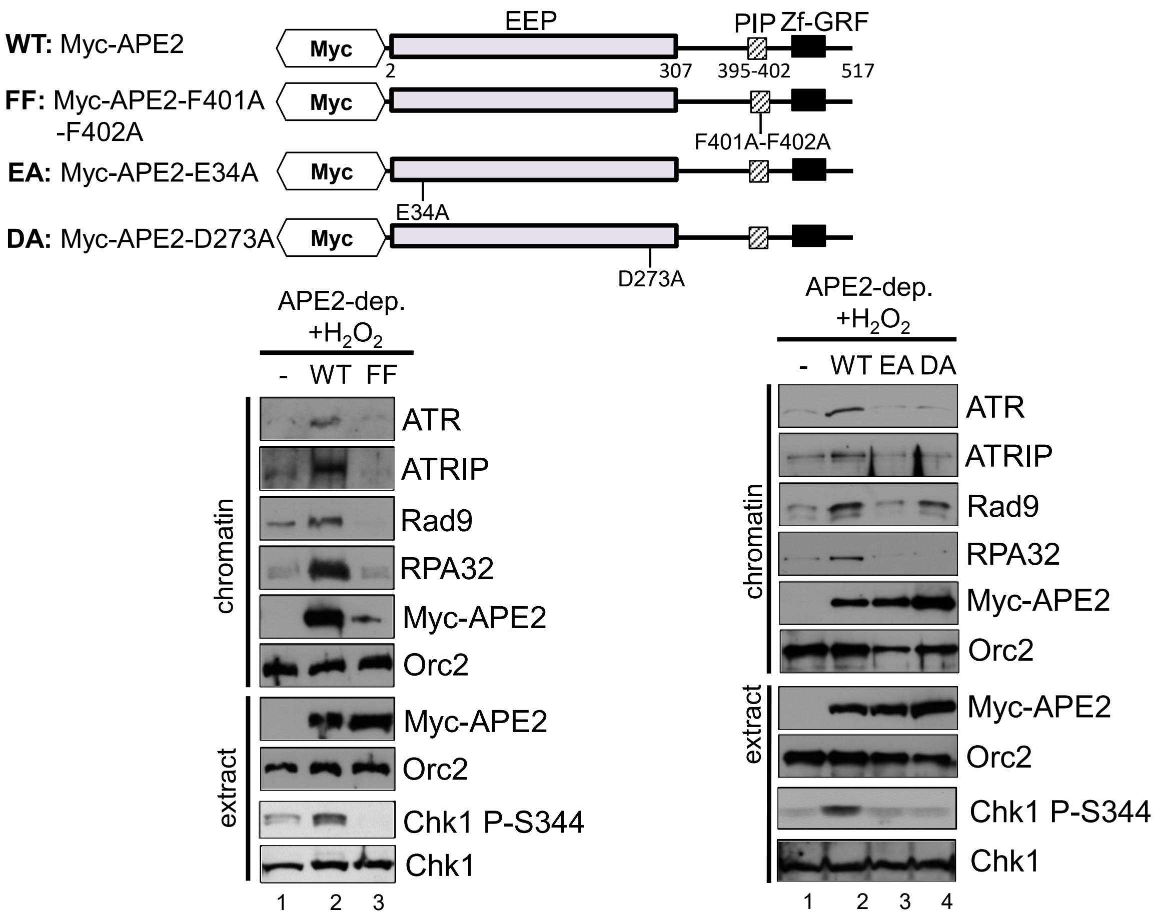
Barry Lentz
Honors student

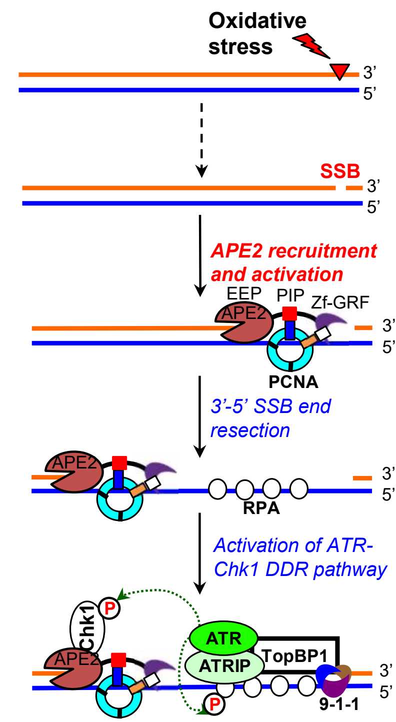




Willis et al., *PNAS*, 2013

APE2's PCNA interaction and exonuclease activity are important for its role in OS-induced ATR DDR





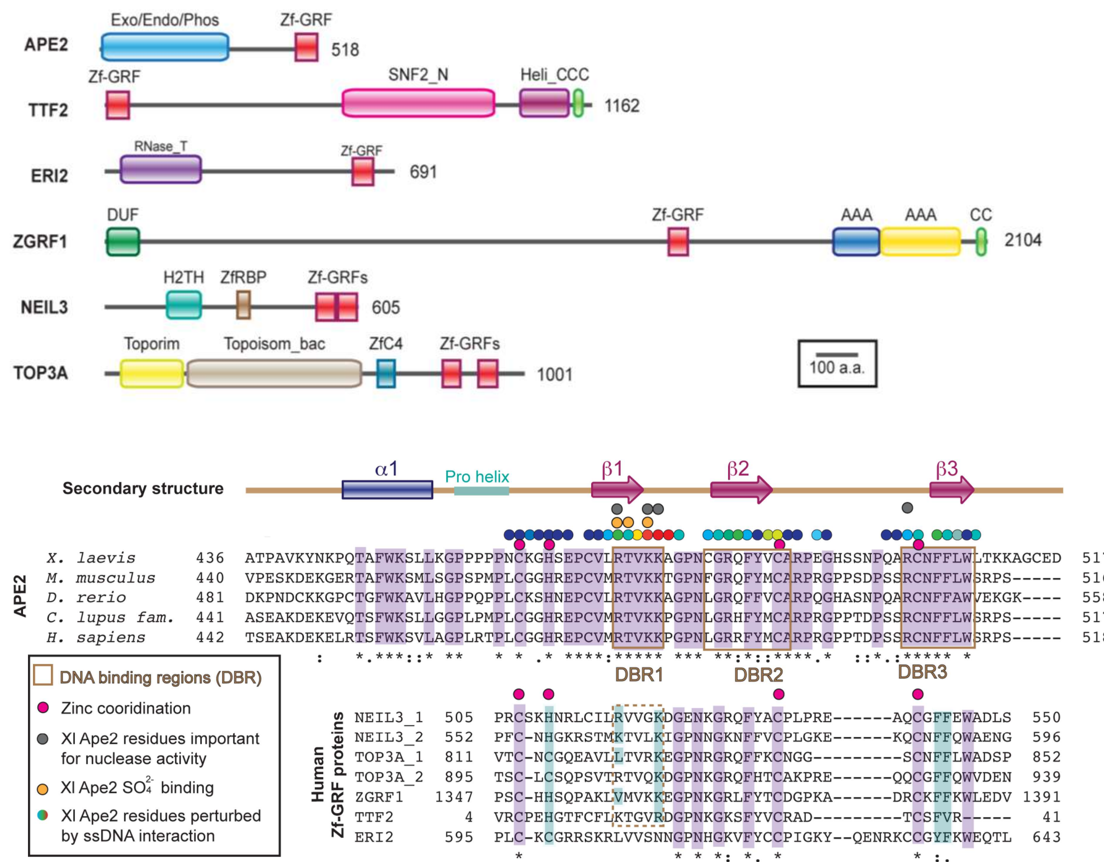
Willis et al., *PNAS*, 2013

Zf-GRF is a unique motif in DNA/RNA metabolism proteins

Dr. Scott William
NIH/NIEHS

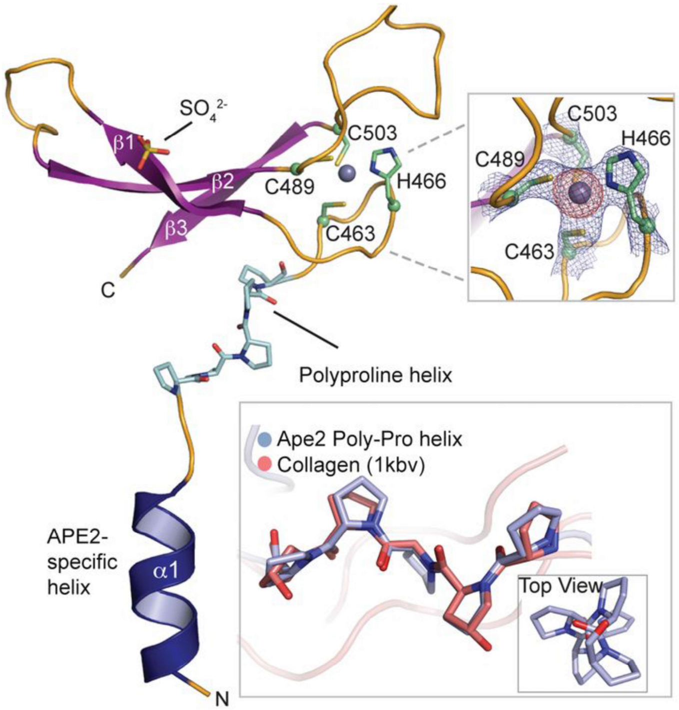
Zack Berman
Graduate student

Yunfeng Lin
Postdoc/Research Assistant Professor

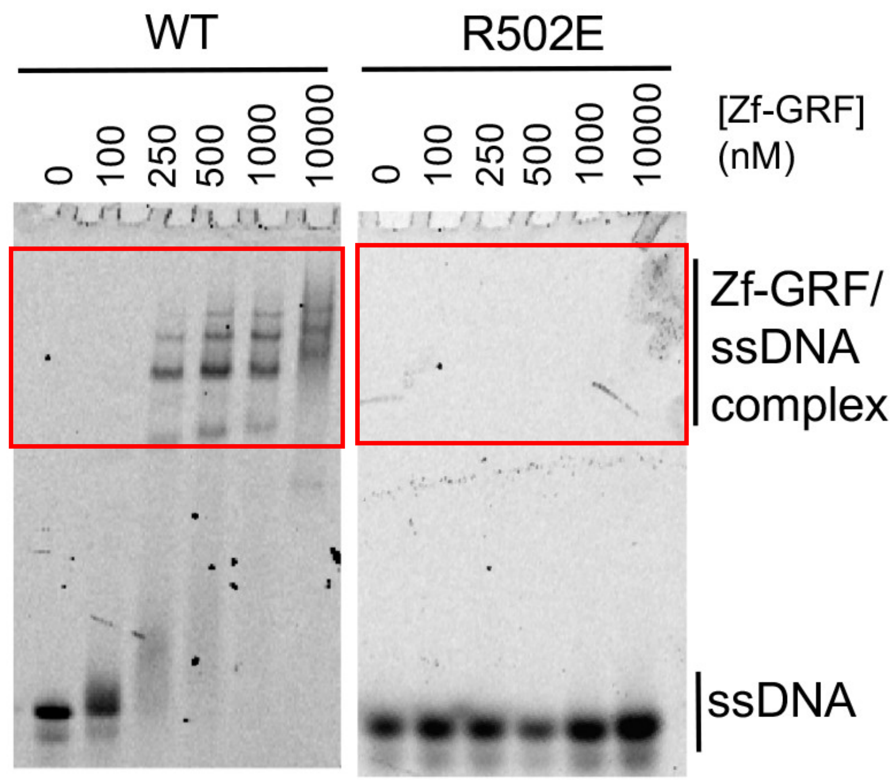


Wallace et al., *PNAS*, 2017

Crystal structure of APE2 Zf-GRF motif and its ssDNA association

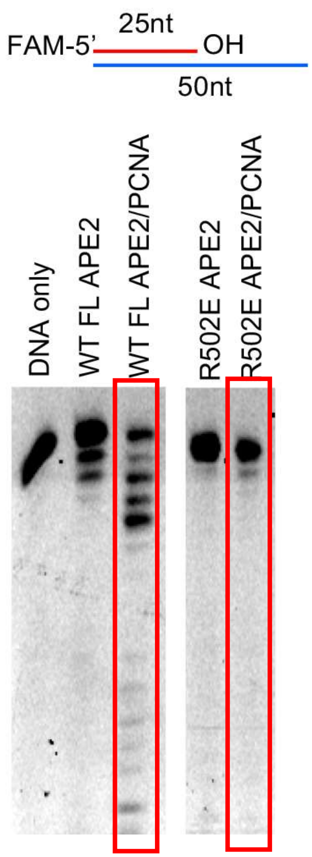


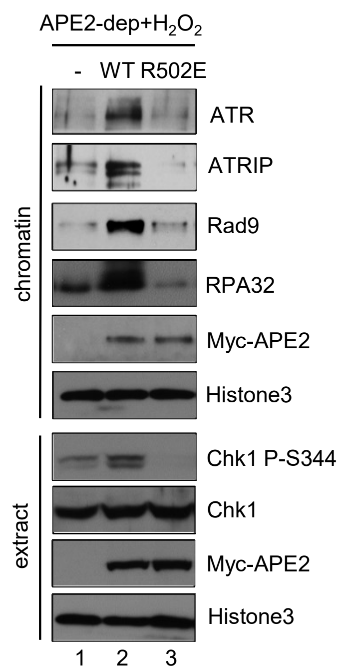
RCSB PDB: 5U6Z

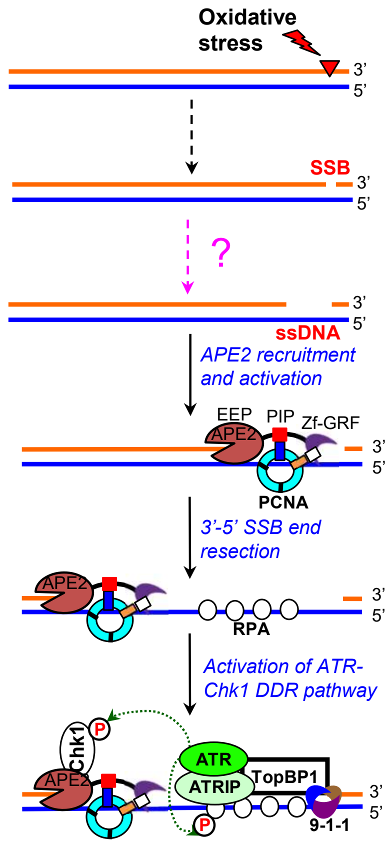


Wallace et al., *PNAS*, 2017

APE2 Zf-GRF motif is important for its exonuclease activity and OS-induced ATR DDR







Two critical questions:

1. Hydrogen peroxide may generate different types of oxidative DNA damage. What happens for any defined SSB structures?
2. How is ssDNA generated at SSB site in the first place? How is APE2 recruited to SSB site?

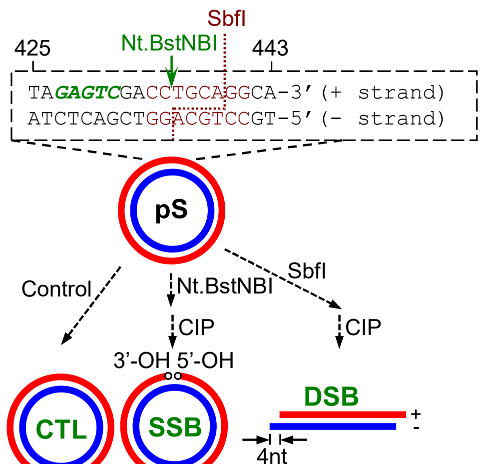
Wallace et al., *PNAS*, 2017

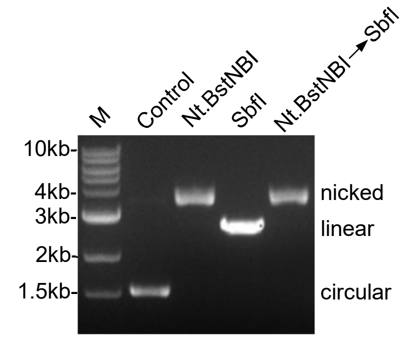
Establishment of a cell-free DNA SSB repair and signaling technology

- Does a single SSB trigger a DDR pathway activation?
- What is the molecular mechanism of SSB-induced DDR pathway?

Yunfeng Lin
Postdoc/
Research
Assistant
Professor

Liping Bai
Postdoc



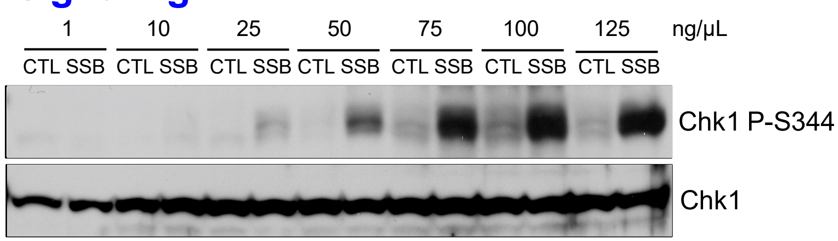


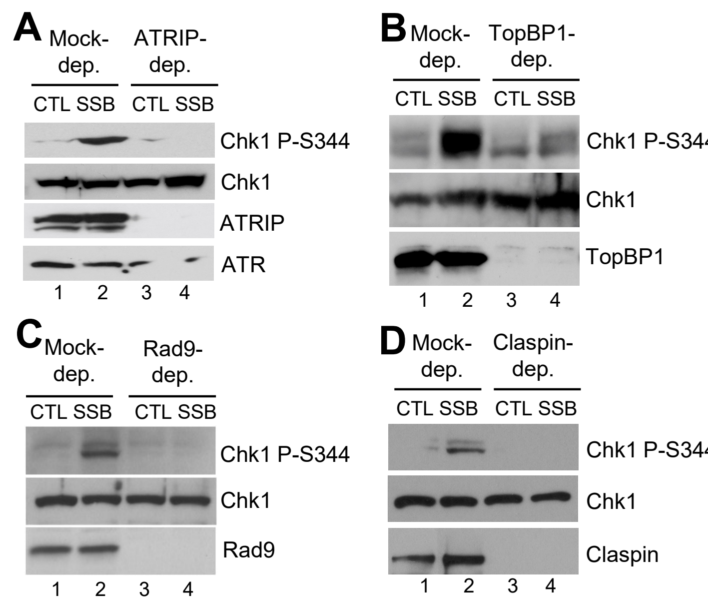
As expected, the SSB plasmid is resistant to further SbfI treatment

Lin et al., *Nucleic Acids Res*, 2018

A defined SSB structure triggers Chk1 phosphorylation in the HSS system

SSB signaling:

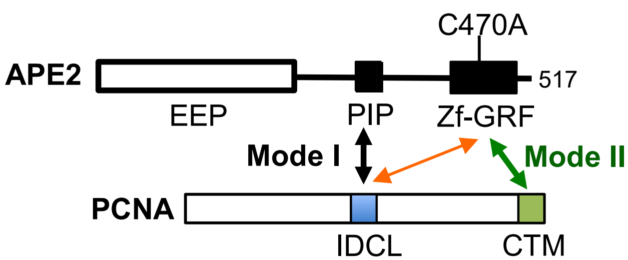


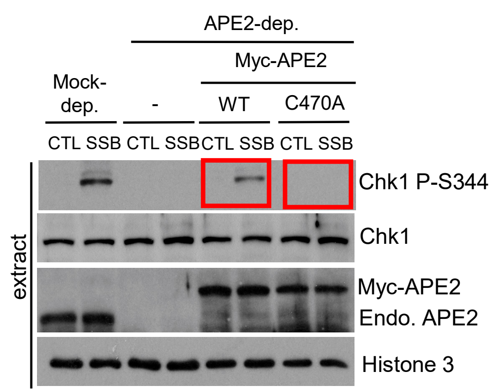


* ATRIP, TopBP1, Rad9, and Claspin are canonical checkpoint protein!

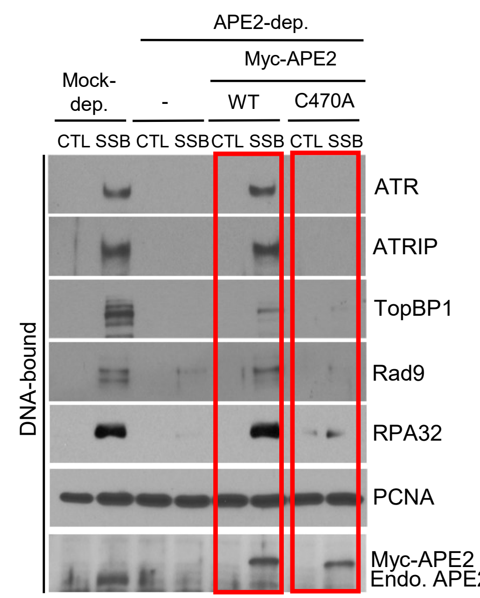
Lin et al., *Nucleic Acids Res*, 2018

APE2 Zf-GRF interaction with PCNA C-terminus is critical for SSB-induced ATR DDR in HSS system





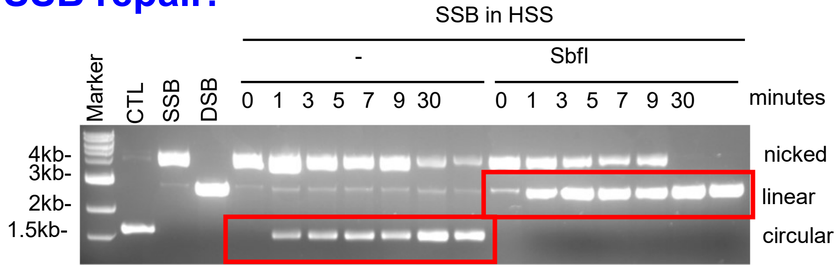
* C470A APE2 Zf-GRF fails to interact with PCNA CTM

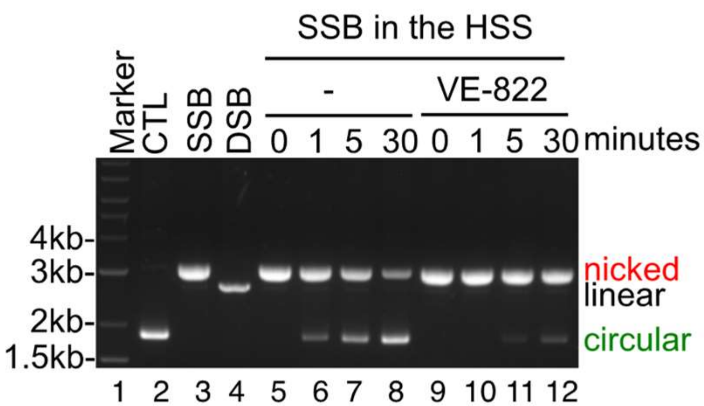


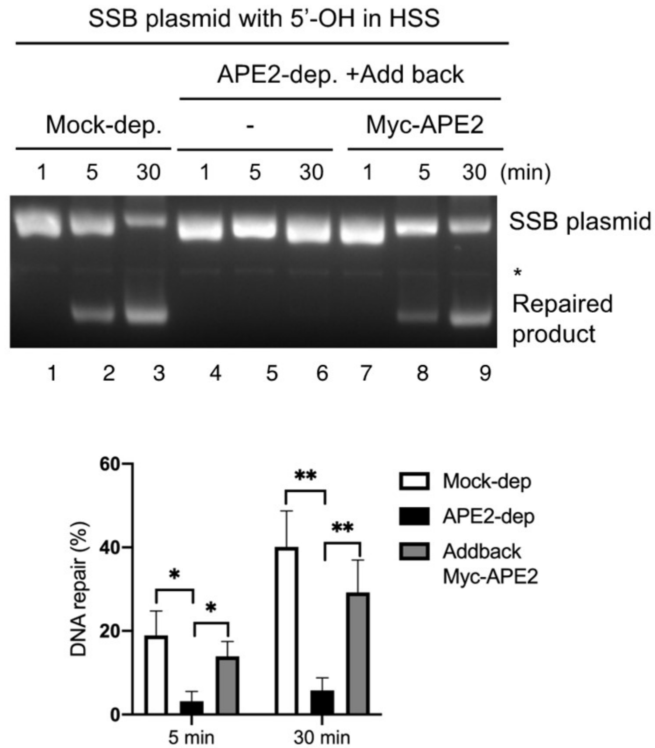
Lin et al., *Nucleic Acids Res*, 2018

ATR and APE2 are essential for SSB repair

SSB repair:

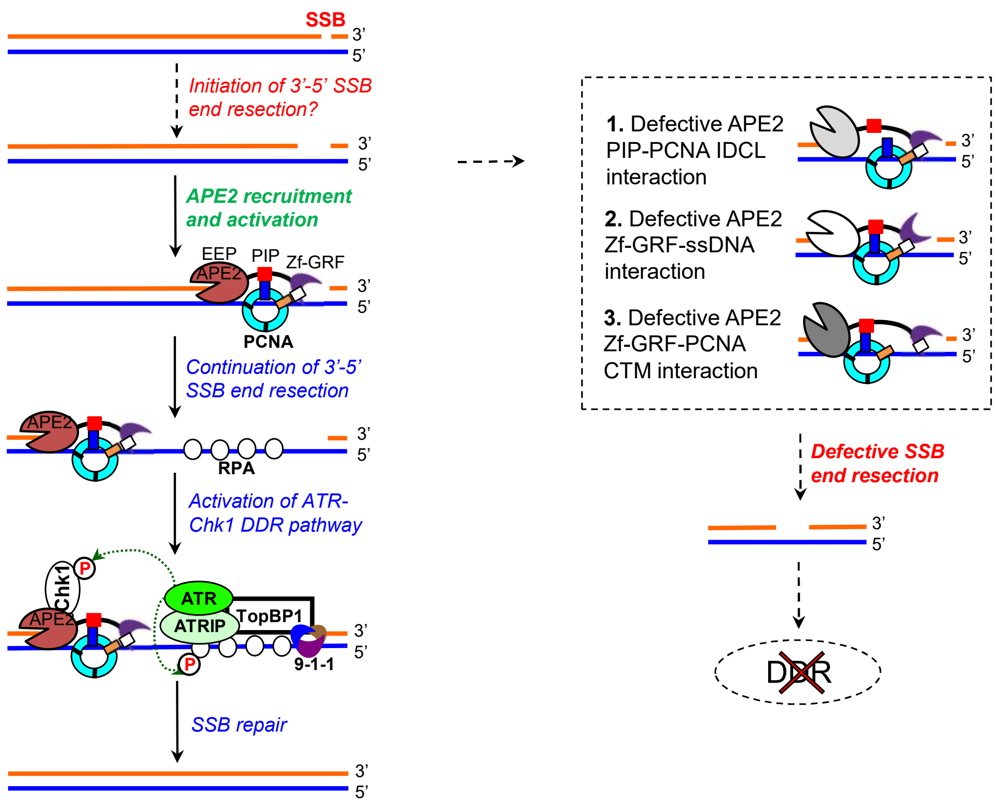






Lin et al., *Nucleic Acid Res*, 2018;
Cupello et al., *Biochem J*, 2019

Molecular mechanisms of DNA SSB repair and signaling by APE2

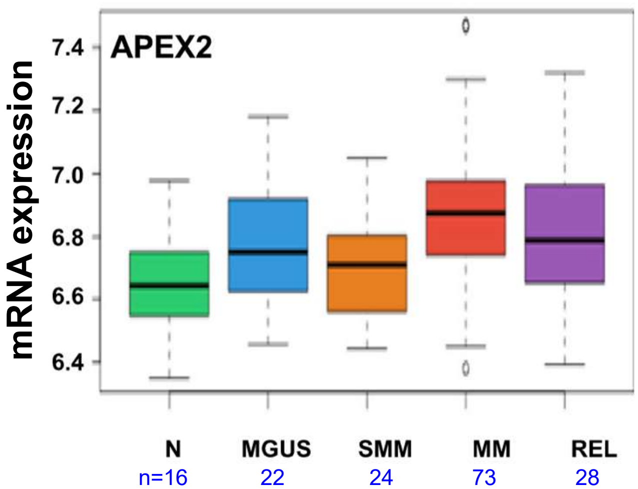


Next questions:

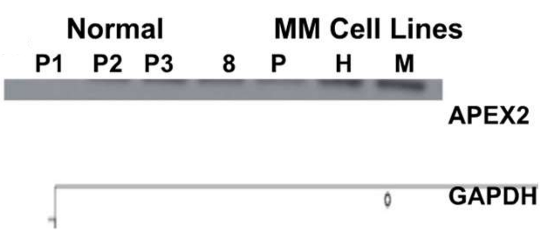
1. Bioinformatics analysis of APE2 expression in cancer vs normal cells?
2. How does APE2 contribute to cancer etiology? Is APE2 a driver or passenger of cancer?
3. Can APE2 be targeted for cancer treatment?

Willis et al., *PNAS*, 2013;
Wallace et al., *PNAS*, 2017;
Lin et al., *Nucleic Acids Res*, 2018;
Cupello et al., *Biochem J*, 2019

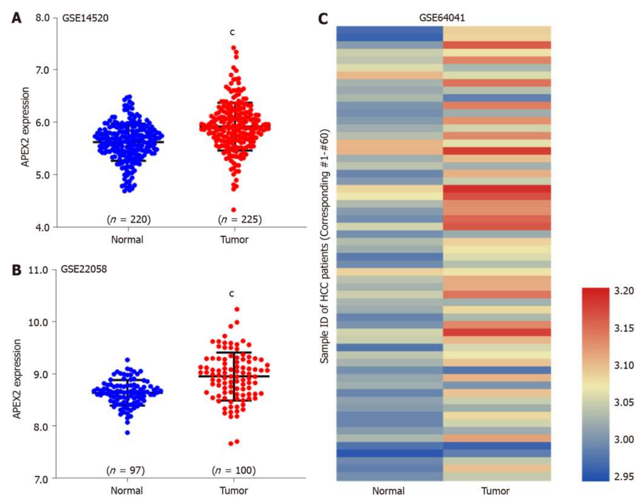
APE2 overexpression in multiple myeloma (MM) and liver cancer

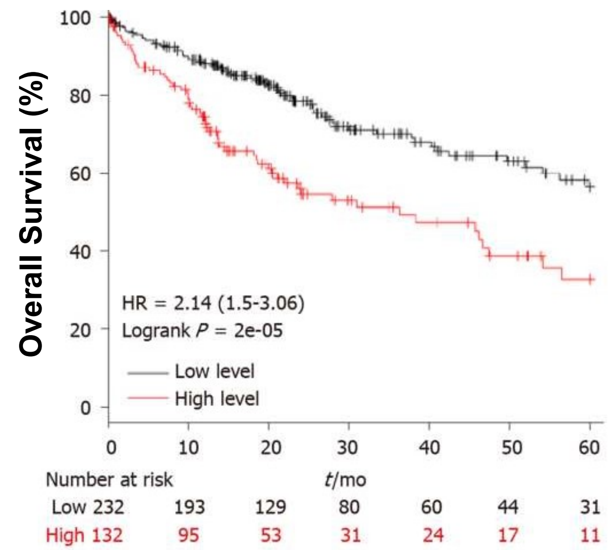


N: normal; **MGUS:** monoclonal gammopathy of undermined significance; **SMM:** smoldering multiple myeloma; **MM:** multiple myeloma; **REL:** relapsed



APE2 is an oncogene of liver cancer?





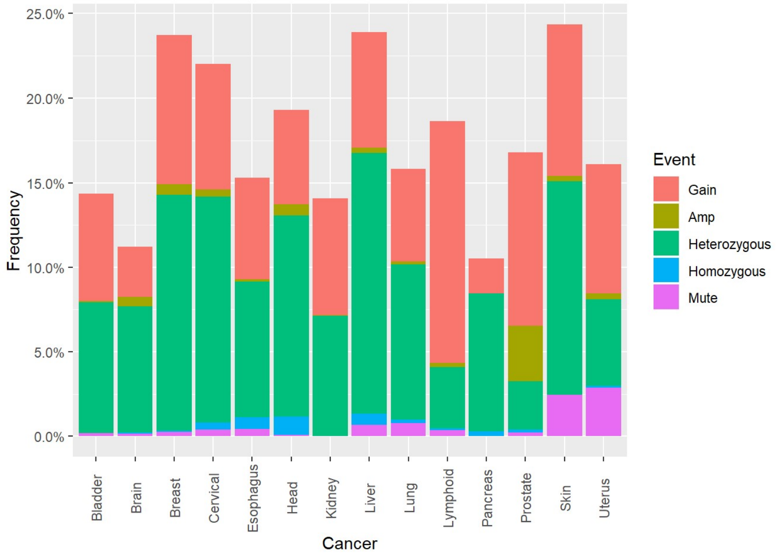
Kumar…**Munshi**, Shammas, *Blood Cancer J*, 2018;
Zheng et al., *World J Clin Cases*, 2020

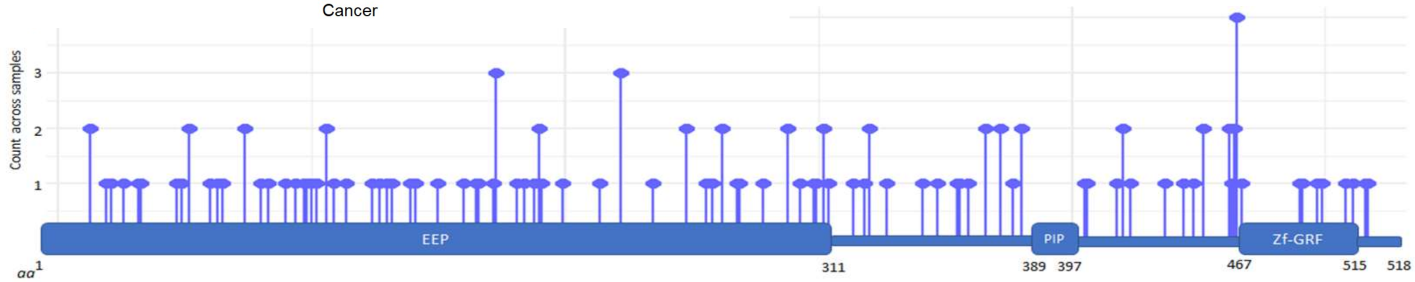
Physiological significance of APE2 in genome integrity and cancer etiology

Dr. Xinghua Shi
Temple University

Katie Jensen
Graduate student

Akram Hossain
Graduate student

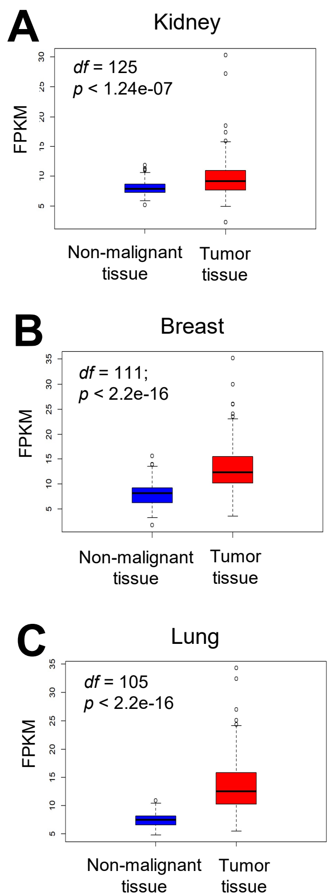


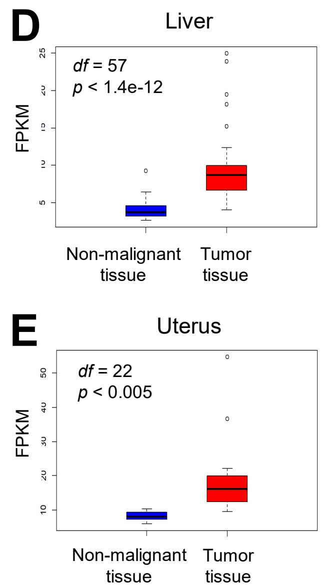


- ~17% frequency of genomic alterations in APE2 in 14 cancer types (n=21,769, TCGA)
- 117 somatic mutations in 12 cancer types

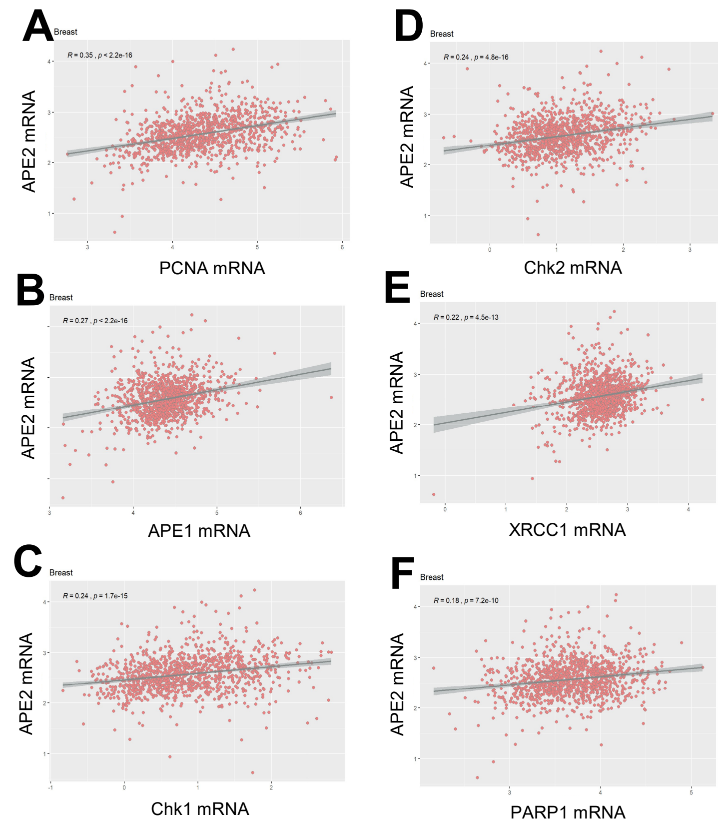
Jensen et al., *Sci Rep*, 2020

APE2 mRNA is overexpressed in tumor tissue and correlates with other DNA repair/DDR proteins





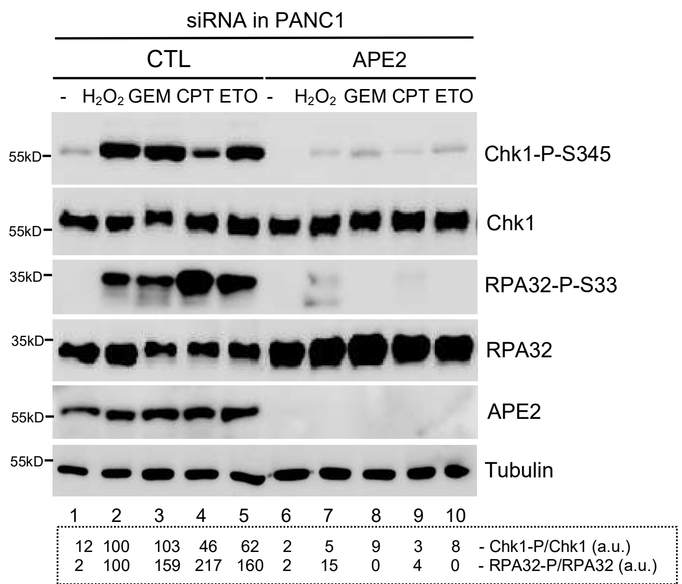
Note: From matched tissue samples



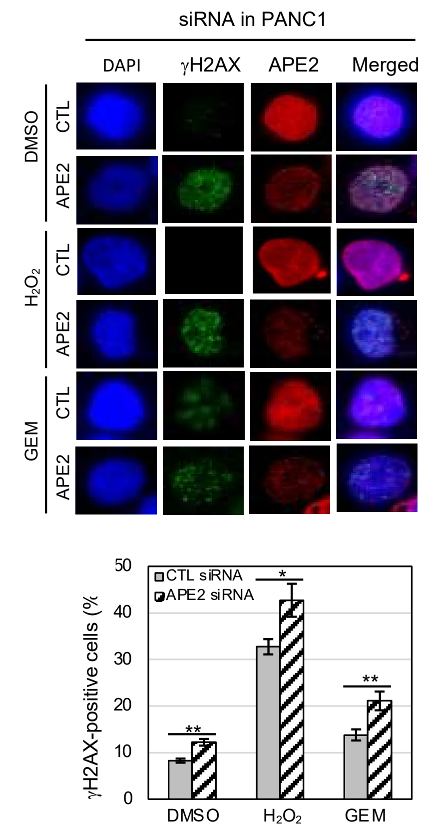
In breast cancer

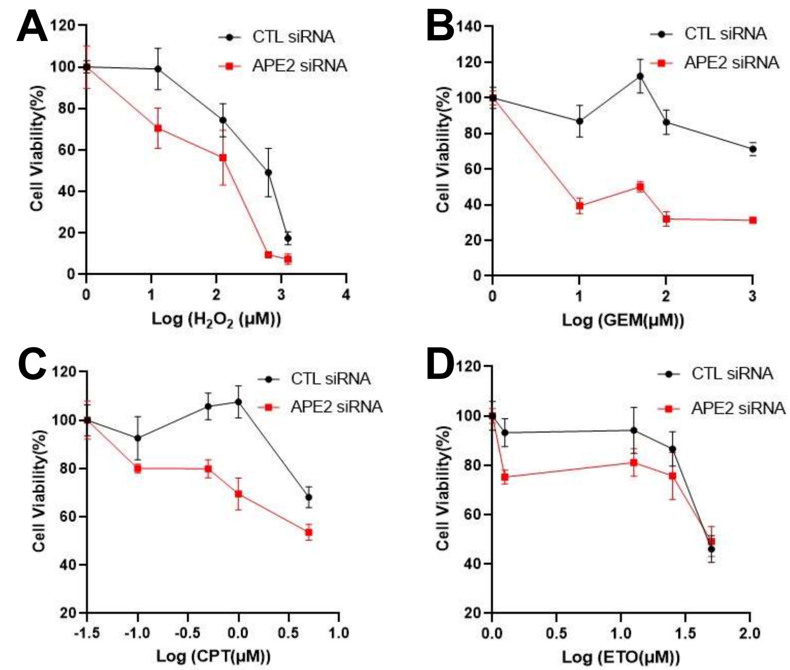
Jensen et al., *Sci Rep*, 2020

APE2-KD led to decreased ATR DDR, increased gamma-H2AX, and decreased cell viability in pancreatic cancer cells



GEM: Gemcitabine
CPT: Camptothecin
ETO: Etoposide





Hossain et al., *Frontiers Cell Dev Biol*, 2021

Targeting APE2 for cancer therapy via first-known compound inhibitor Celastrol

Therapeutics, Targets, and Chemical Biology

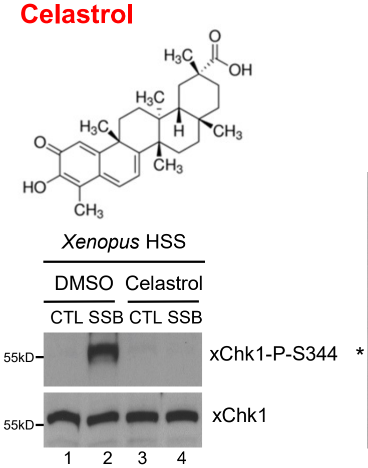
Cancer Res; 74(24) December 15, 2014

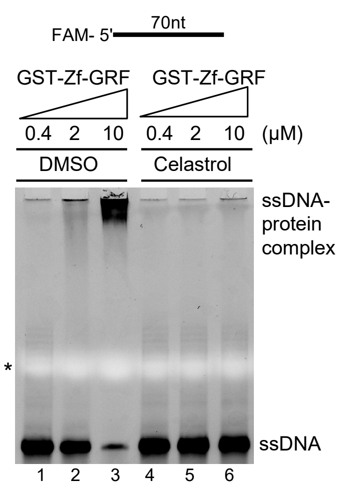
Cancer Research

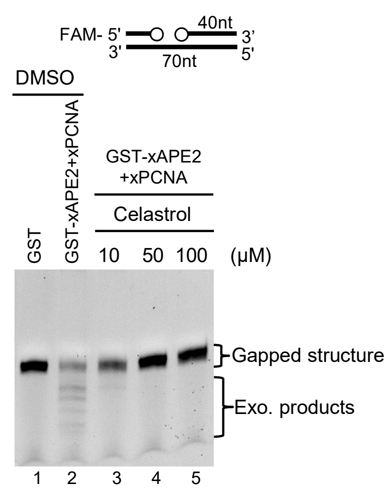
Identification of ATR–Chk1 Pathway Inhibitors That Selectively Target p53-Deficient Cells without Directly Suppressing ATR Catalytic Activity

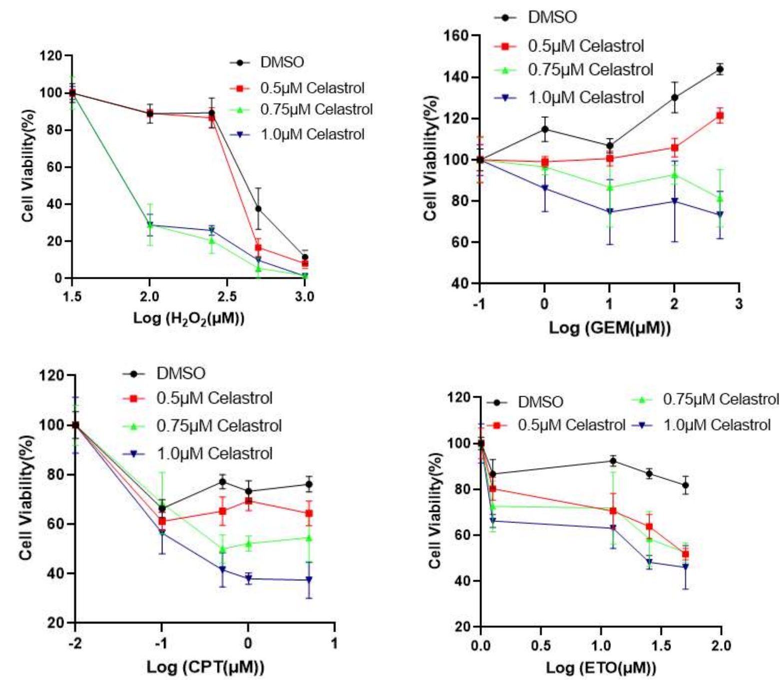
Masaoki Kawasumi[1], James E. Bradner[2,3,4], Nicola Tolliday[2], Renee Thibodeau[1], Heather Sloan[1], Kay M. Brummond[5], and Paul Nghiem[1,6]

Celastrol









Hossain et al., *Frontiers Cell Dev Biol*, 2021

Cisplatin-induced APE2 upregulation leads to acute kidney injury (AKI)

Dr. Jianjun Zhao
Cleveland Clinic

CANCER RESEARCH

Home About Articles For Authors Alerts News COVID-19 Webinars Search

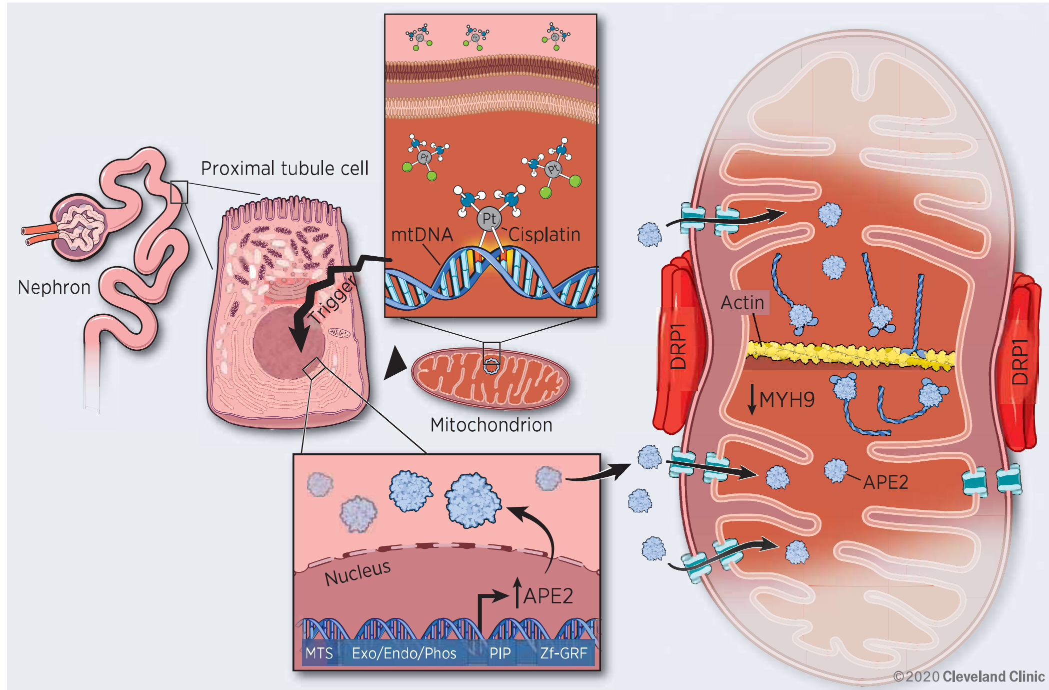
Research Article

Cisplatin-mediated upregulation of APE2 binding to MYH9 provokes mitochondrial fragmentation and acute kidney injury

Yi Hu, Chun Yang, Tania Amorim, Mohsin Maqbool, Jenny Lin, Chen Li, Chuanfeng Fang, Li Xue, Ariel Kwart, Hua Fang, Mei Yin, Allison J. Janocha, Daisuke Tsuchimoto, Yusaku Nakabeppu, Xiaofeng Jiang, Alex Mejia-Garcia, Faiz Anwer, Jack Khouri, Xin Qi, Qing Y. Zheng, Jennifer S. Yu, Shan Yan, Thomas LaFramboise, Kenneth C. Anderson, Leal C. Herlitz, Nikhil C. Munshi, Jianhong Lin, and Jianjun Zhao

Add to Cart ($50)

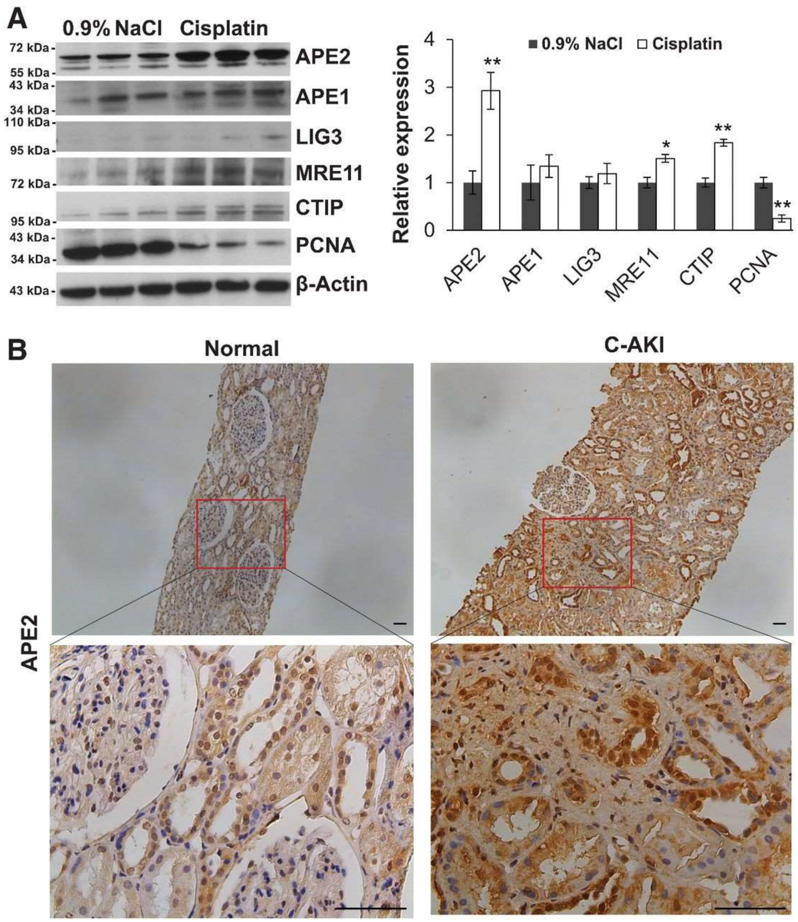
DOI: 10.1158/0008-5472.CAN-20-1010 Check for updates

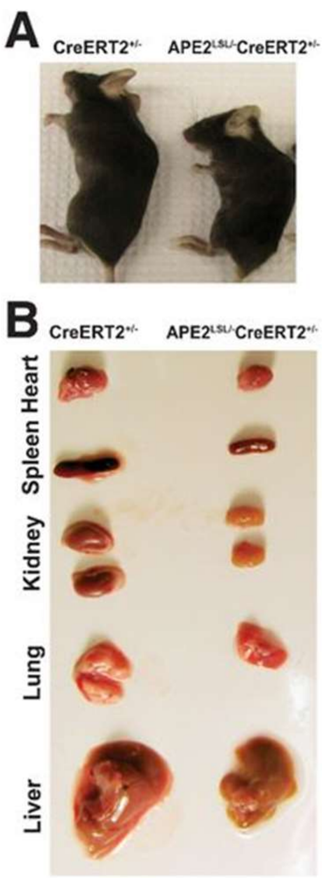


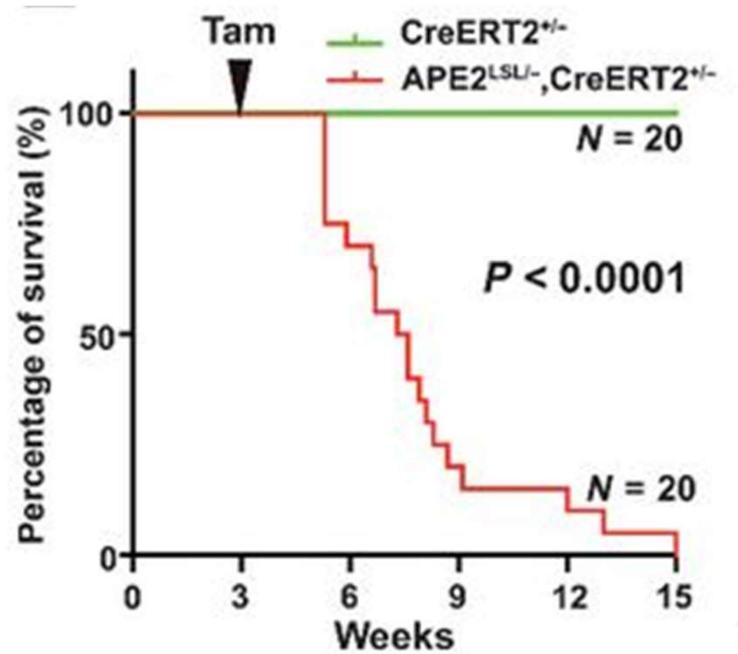
Cisplatin promotes acute kidney injury by upregulating APE2, which leads to subsequent MYH9 dysfunction in the mitochondria of proximal tubule cells.

Hu et al., *Cancer Res*, 2021

Cisplatin induces APE2 upregulation and AKI, which is recapitulated by APE2-OE transgenic mice

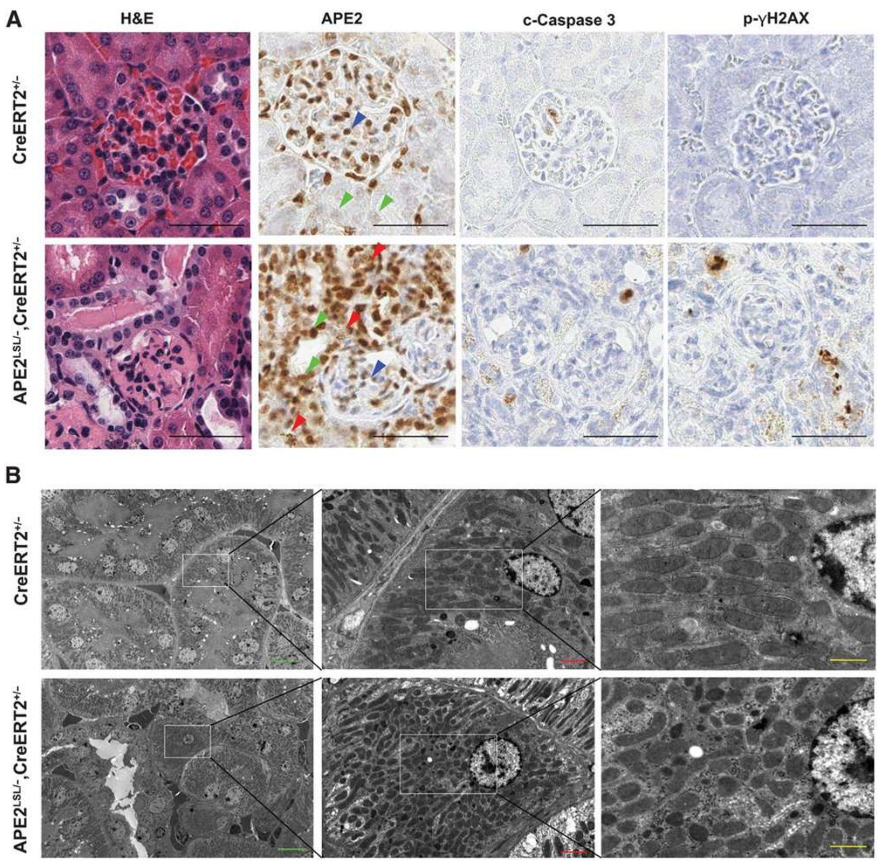


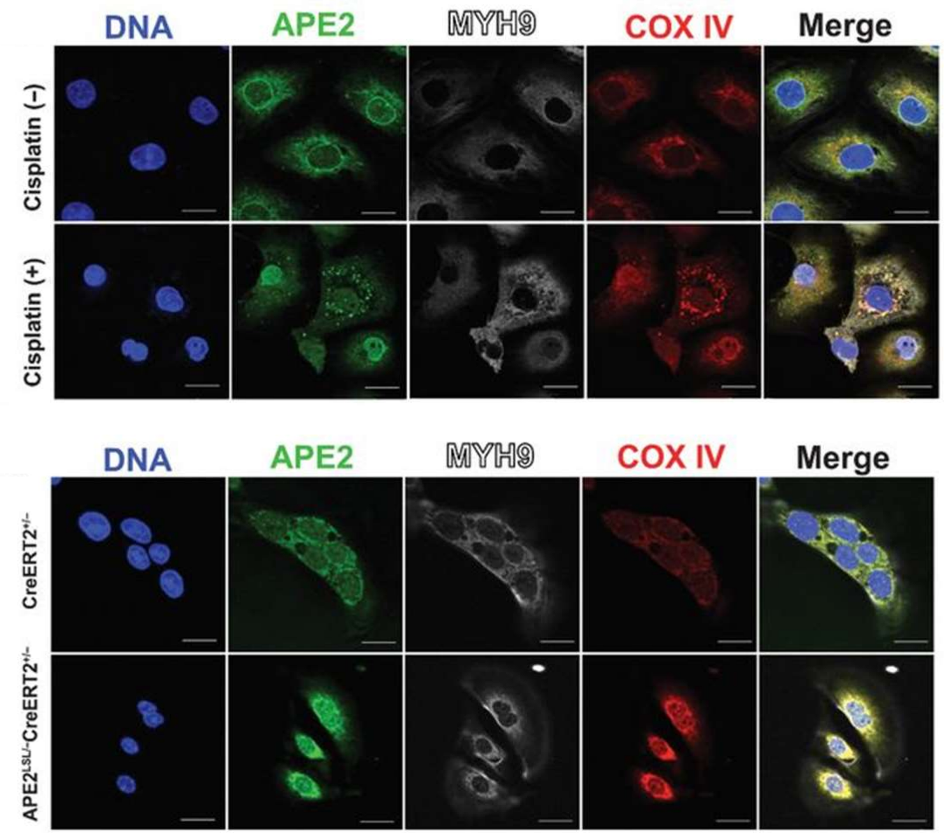




Hu et al., *Cancer Res*, 2021

APE2 transgenic mice lead to cell death and mitochondrial fragmentation and instability





Hu et al., *Cancer Res*, 2021

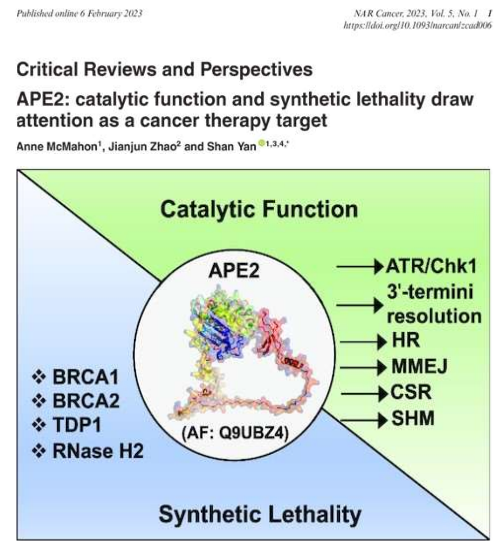
Summary 1 – APE2

APE2

✓ 1. APE2 and its nuclease activity and Zf-GRF motif are required for the OS-induced ATR DDR.

✓ 2. APE2 and its exonuclease activity is required for the SSB-induced ATR DDR and SSB repair.

✓ 3. Function of APE2 in ATR DDR and AKI can be targeted for cancer treatment.


Published online 6 February 2023

NAR Cancer, 2023, Vol. 5, No. 1 1
https://doi.org/10.1093/narcan/zcad006

Critical Reviews and Perspectives

APE2: catalytic function and synthetic lethality draw attention as a cancer therapy target

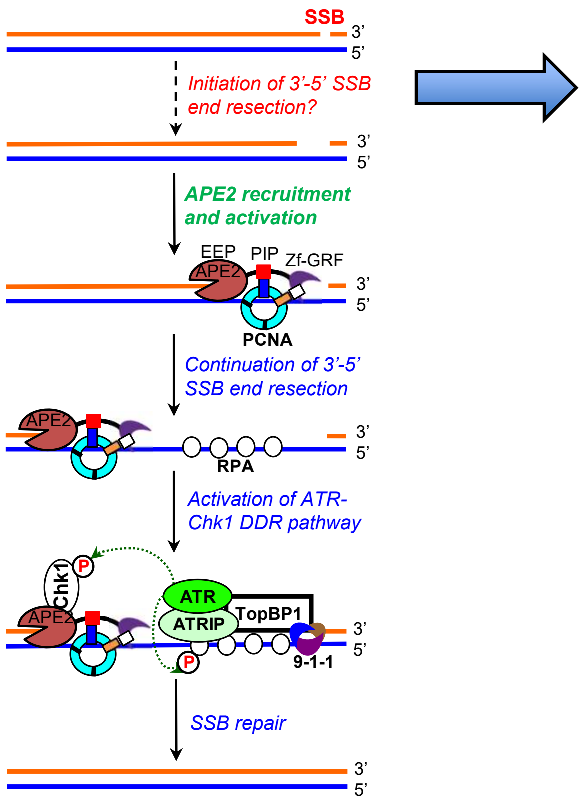
Anne McMahon[1], Jianjun Zhao[2] and Shan Yan[1,3,4,*]

Willis et al., *PNAS*, 2013;
Wallace et al., *PNAS*, 2017;
Lin et al., *Nucleic Acids Res*, 2018;
Hossain et al., *In J Mol Biol*, 2018;
Cupello et al., *Biochem J*, 2019;
Yan, *Nat Struct Mol Biol*, 2019;
Jensen et al., *Sci Rep*, 2020;
Hossain et al., *Frontiers Cell Dev Biol*, 2021;
Hu et al., *Cancer Res*, 2021;
Lin et al., *Mutat Res Rev*, 2021;
McMahon, Zhao, Yan, *NAR Cancer*, 2023

Outline

- **Introduction**
- **Functional studies of APE2 in ATR DDR and cancer biology**
  1. APE2 in OS-induced ATR DDR
  2. APE2 in SSB-induced ATR DDR
  3. APE2 in cancer biology and therapeutics
- **Molecular mechanisms of APE1 in SSB response, global and nucleolar DDR**
  1. APE1 in SSB-induced ATR DDR
  2. APE1 in ATRIP recruitment to ssDNA gaps
  3. APE1 in nucleolar DDR

Molecular mechanisms of DNA SSB repair and signaling



Question:
How is ssDNA generated at SSB site for APE2 activation?

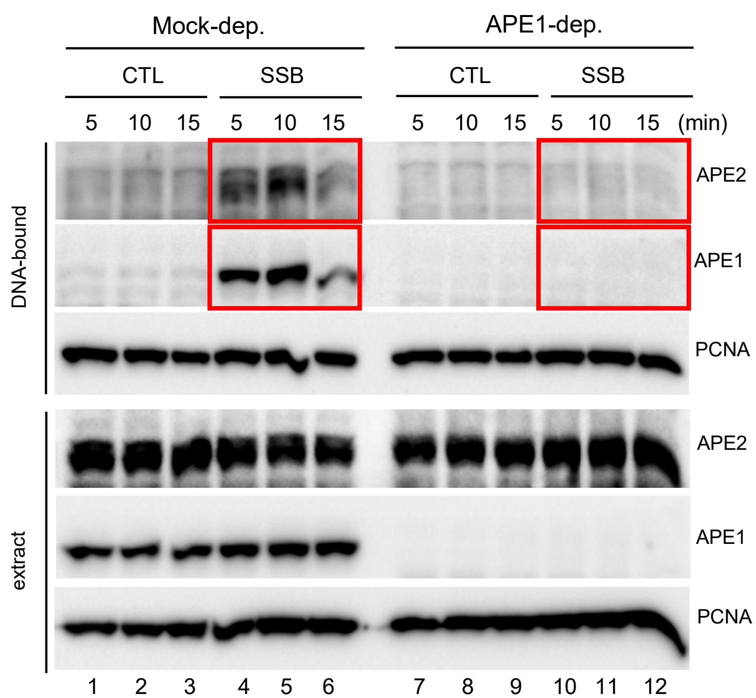
Hypothesis:
APE1 may play a direct role in SSB repair and signaling?

Willis et al., *PNAS*, 2013;
Wallace et al., *PNAS*, 2017;
Lin et al., *Nucleic Acids Res*, 2018;
Cupello et al., *Biochem J*, 2019

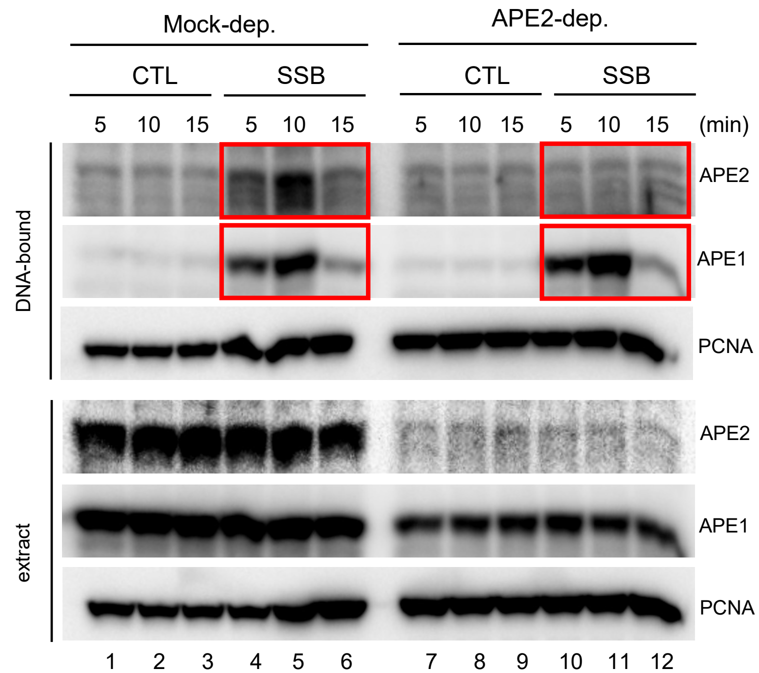
APE1 is required for APE2 recruitment to SSB sites but not *vice versa*

Dr. Yunfeng Lin
Postdoc/
Research Assistant Professor

Jude Raj
Honors student
Now @Duke MD/PhD



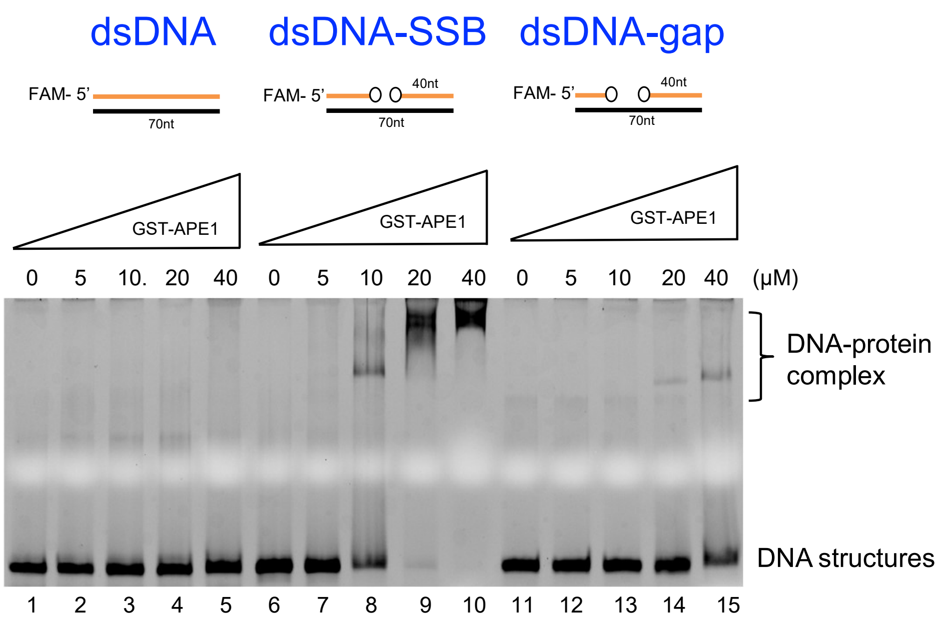
Recruitment of APE2 to SSB sites requires APE1.



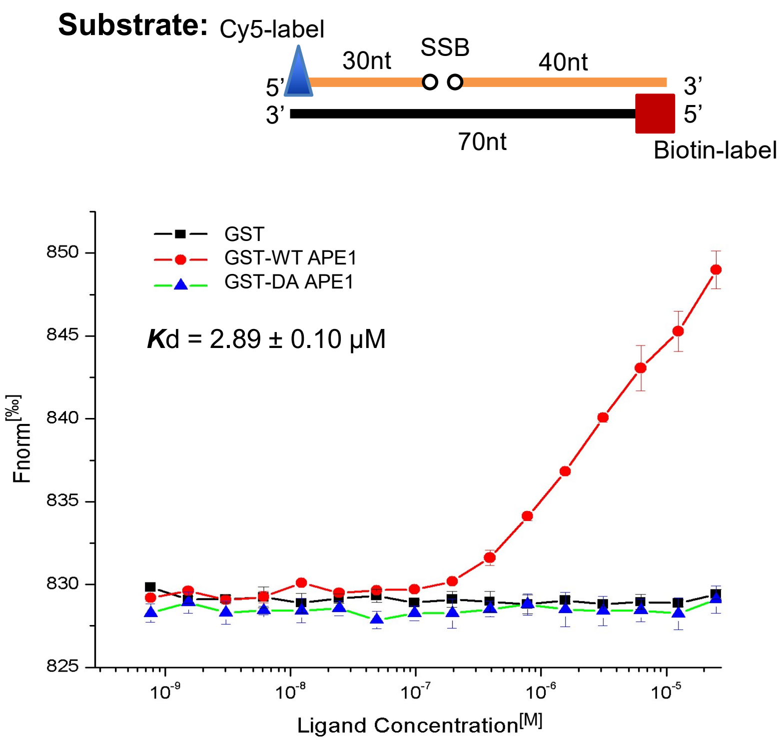
However, APE2 is dispensable for APE1 recruitment to SSB sites.

Lin et al., *Nucleic Acids Res*, 2020

APE1 senses and binds to SSB structures



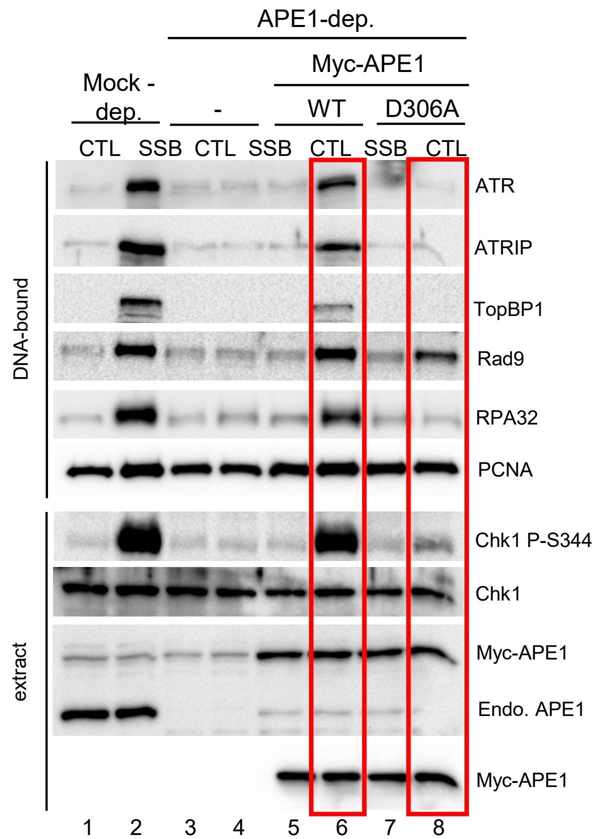
APE1 recognizes SSB structure preferentially and may dissociate from gap (~1-3nt) structure.



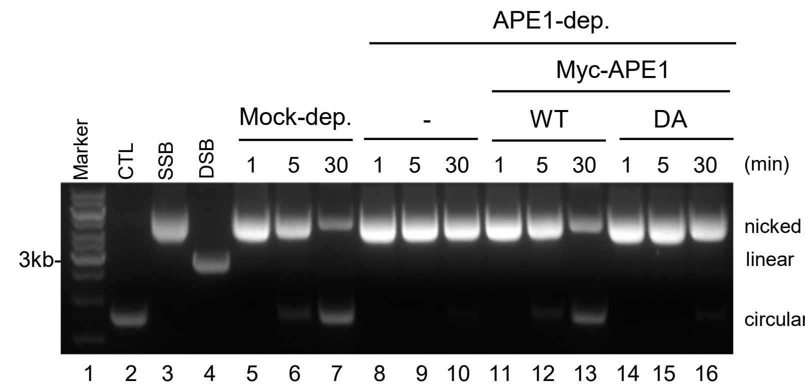
Microscale Thermophoresis (MST) experiments

Lin et al., *Nucleic Acids Res*, 2020

APE1 exonuclease activity is important for SSB-induced DDR pathway and SSB repair



APE1 and its exo activity are important for SSB-induced DDR pathway.



APE1 and its exo activity are important for SSB repair in the HSS system.

D306A

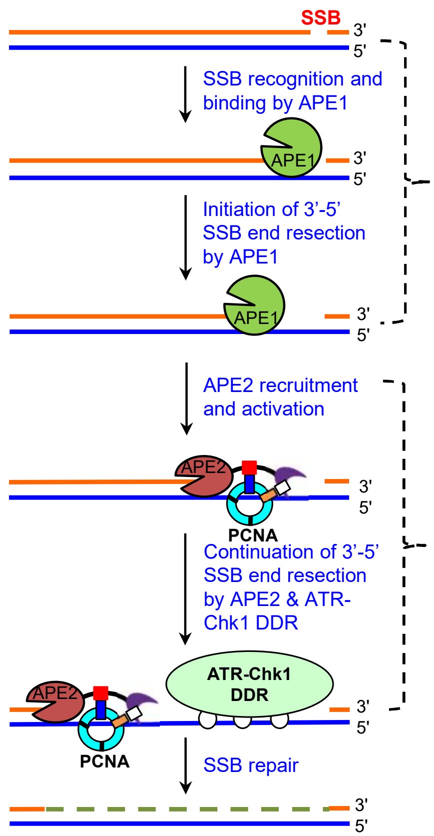
```
Xl APE1 297-KIRSKVMGSDHCPITLLMAI-316
Hs APE1 299-KIRSKALGSDHCPITLYLAL-318
Mm APE1 298-KIRSKALGSDHCPITLYLAL-317
            ****.:*********  :*:
```

D306A: exo-deficient but endo-proficient

Lin et al., *Nucleic Acids Res*, 2020

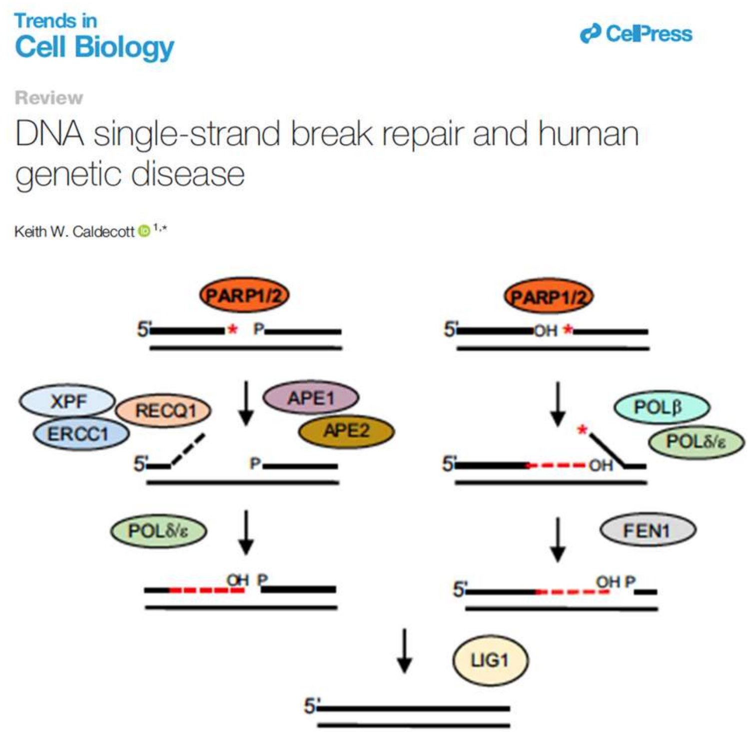
What are the molecular mechanisms of SSB-induced ATR-Chk1 DDR?

A two-step mechanism of SSB repair and signaling



Step 1: APE1-mediated initiation of SSB end resection

Step 2: APE2-mediated continuation of SSB end resection

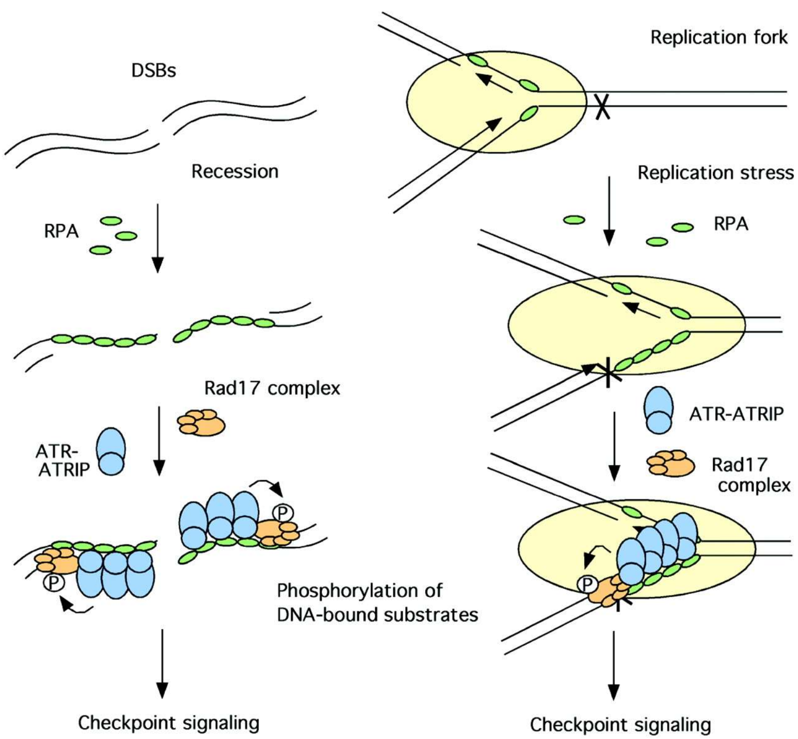


Lin et al., *Nucleic Acids Res*, 2018;
Lin et al., *Nucleic Acids Res*, 2020;
Caldecott, *Trends Cell Biol*, 2022

The ATR/ATRIP complex is recruited to ssDNA via the RPA-ATRIP interaction

Science AAAS

Sensing DNA Damage Through ATRIP Recognition of RPA-ssDNA Complexes
Lee Zou and Stephen J. Elledge
Science **300**, 1542 (2003);
DOI: 10.1126/science.1083430

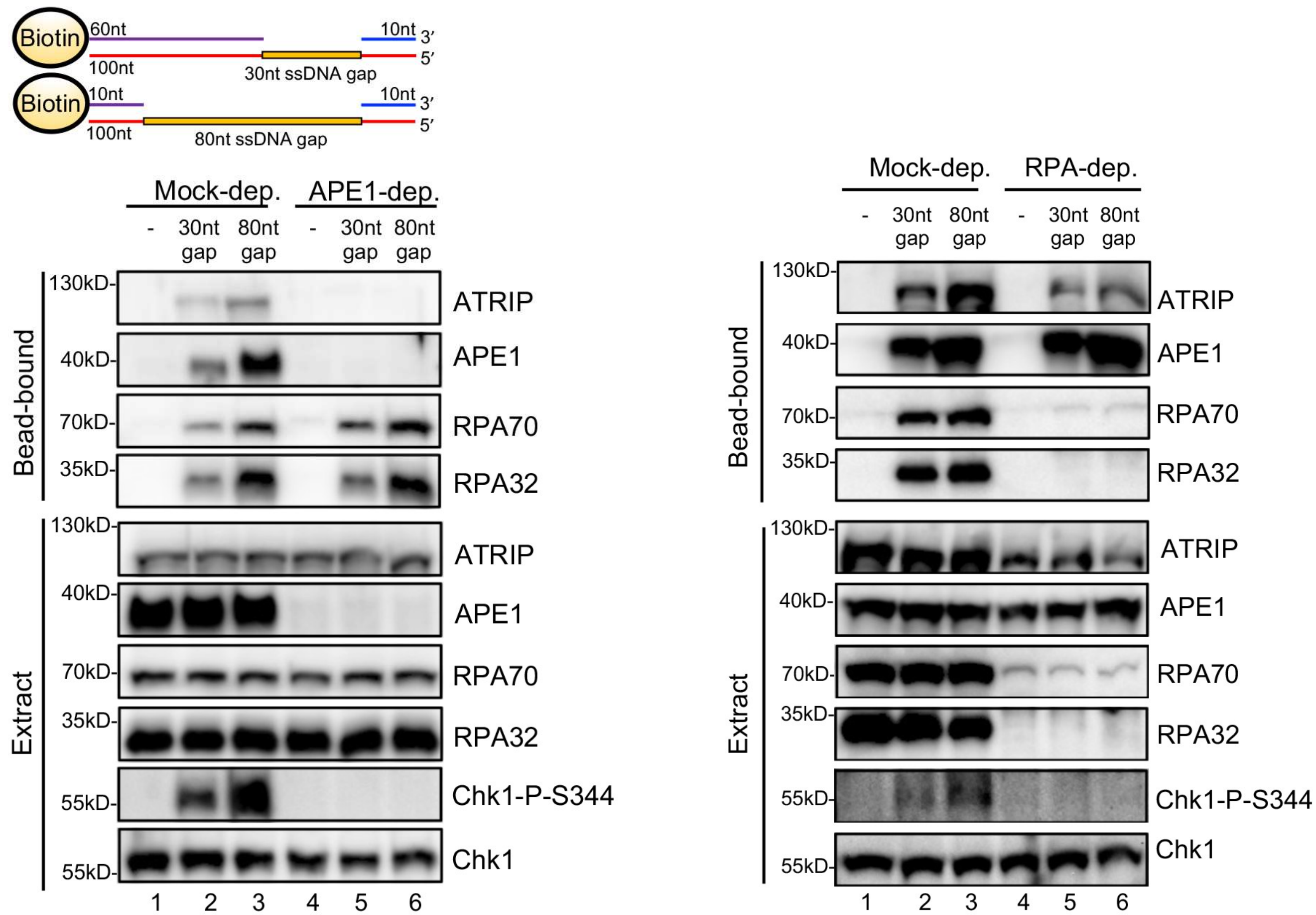


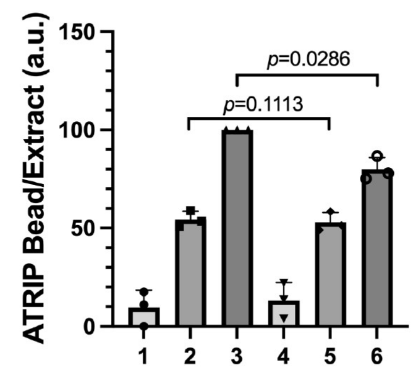
Question:
What protein brings ATRIP to ssDNA in an RPA-independent manner for ATR DDR?

Hypothesis:
APE1 plays a critical role in ATRIP recruitment to ssDNA via a non-catalytic function.

Zou and Elledge, *Science*, 2003;
Lee, Kumagai, Dunphy, *Mol Cell*, 2003;
Unsal-Kaqmaz and Sancar, *Mol Cell Biol*, 2004;
Bomgarden…Cimprich, *J Biol Chem*, 2004;
Kim, Kumagai, Dunphy, *J Biol Chem*, 2005;
Ball, Mayers, Cortez, *Mol Biol Cell*, 2005;
Namiki and Zou, *PNAS*, 2006

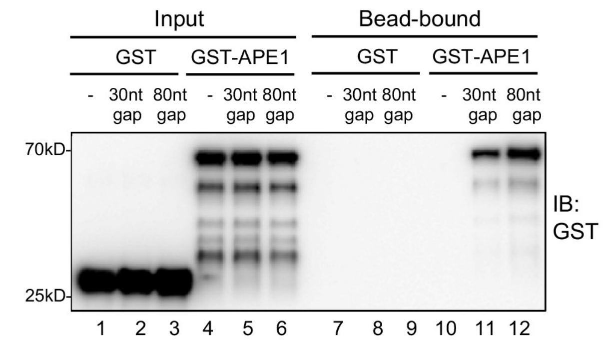
APE1 is required for the recruitment of ATRIP onto RPA-ssDNA in *Xenopus*

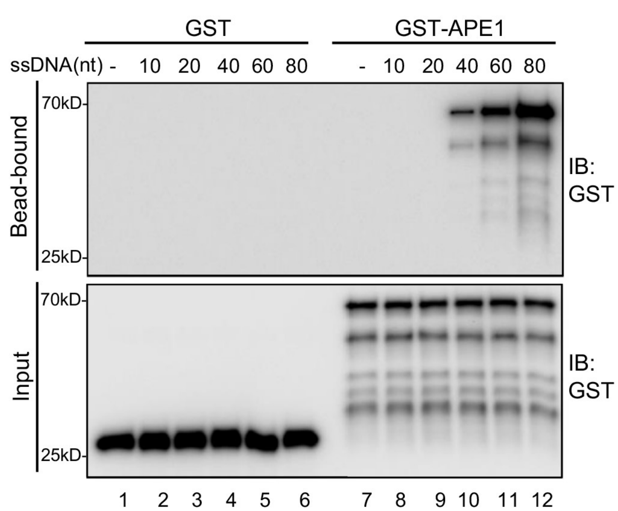




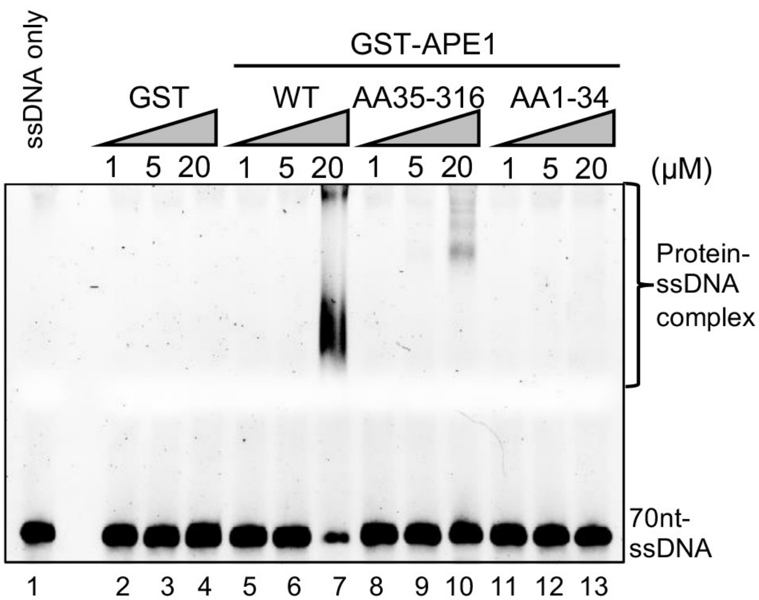
Lin et al., *eLife*, 2023

APE1 protein interacts with ssDNA in a length-dependent manner



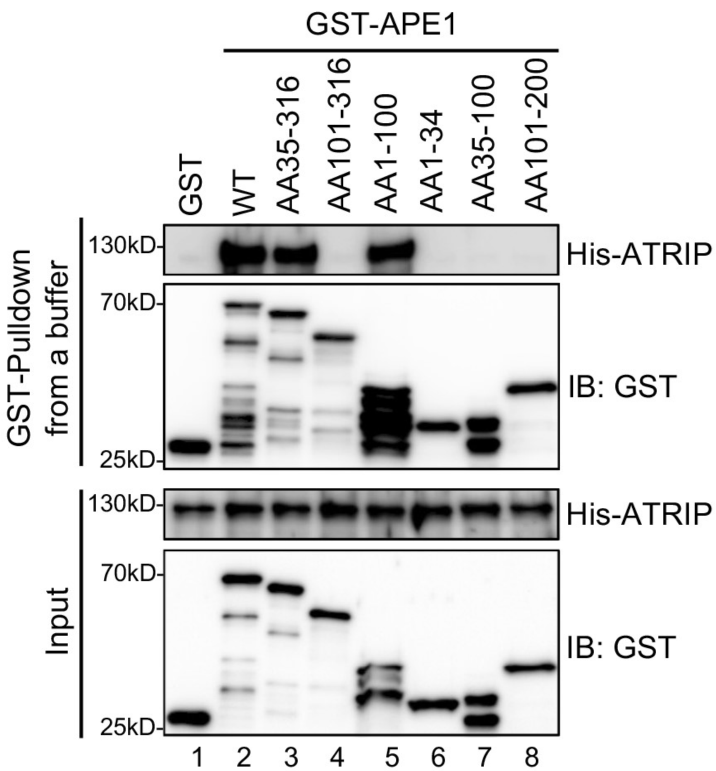


ssDNA association	ATRIP interaction	RPA interaction
+++	+++	+++
+/-	+++	+++
+++	-	+

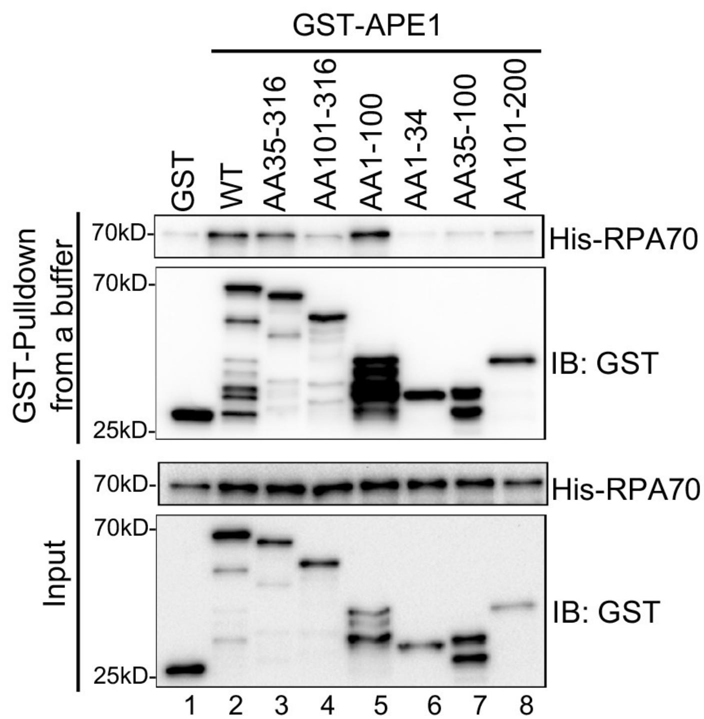


Lin et al., *eLife*, 2023

APE1 interacts with ATRIP protein and RPA complex

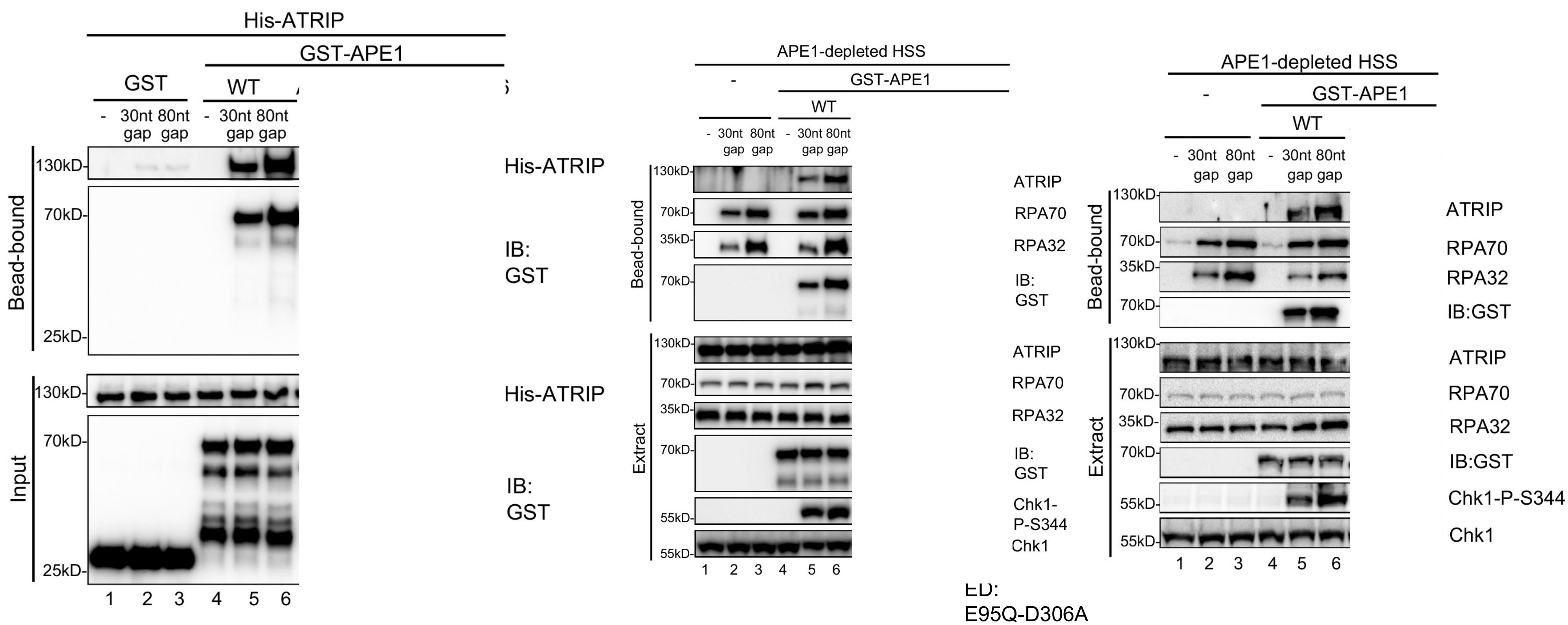


ssDNA association	ATRIP interaction	RPA interaction
+++	+++	+++
+/-	+++	+++
+++	-	+



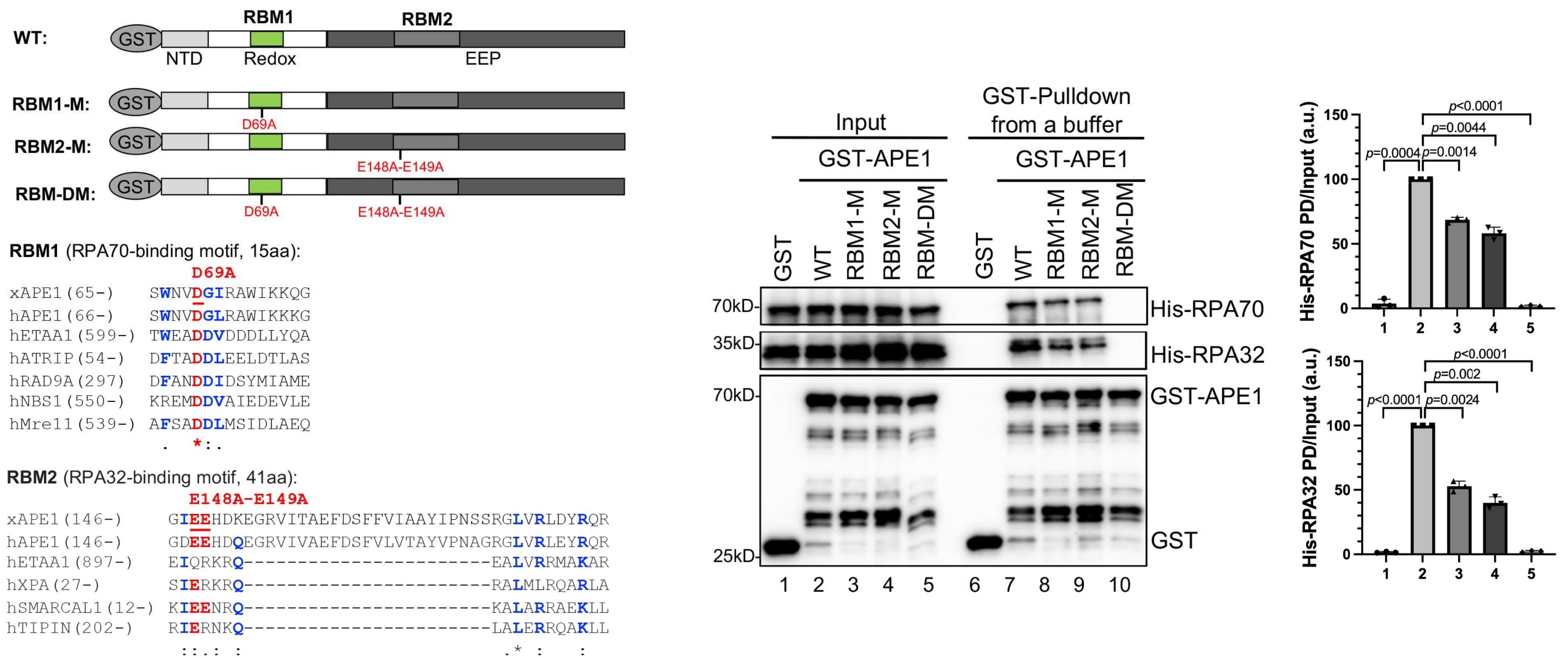
Lin et al., *eLife*, 2023

APE1 is sufficient for ATRIP recruitment to ssDNA in vitro, and required for ATRIP recruitment to ssDNA in *Xenopus* egg extracts



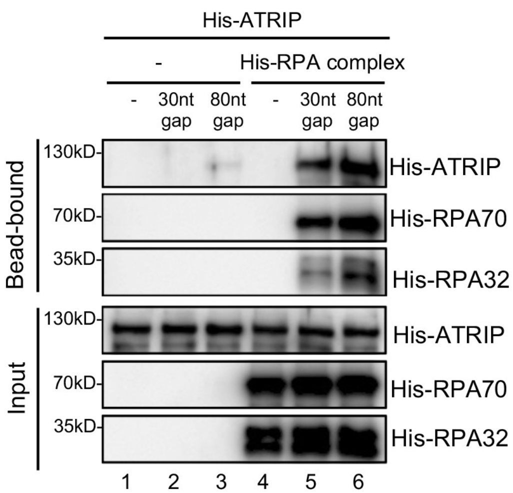
Lin et al., *eLife*, 2023

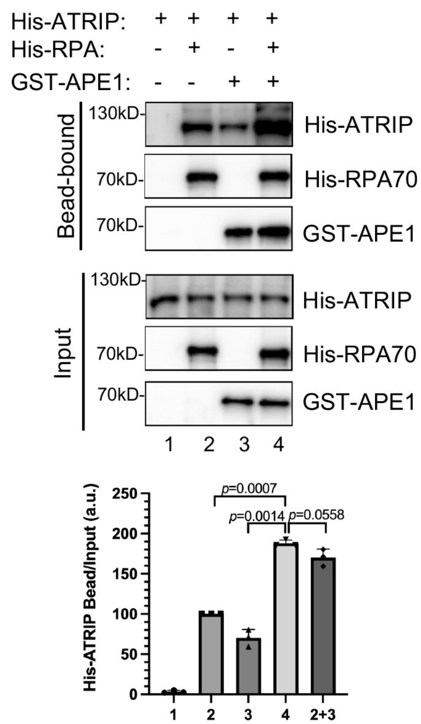
APE1 displays two distinct distinct binding sites for RPA complex

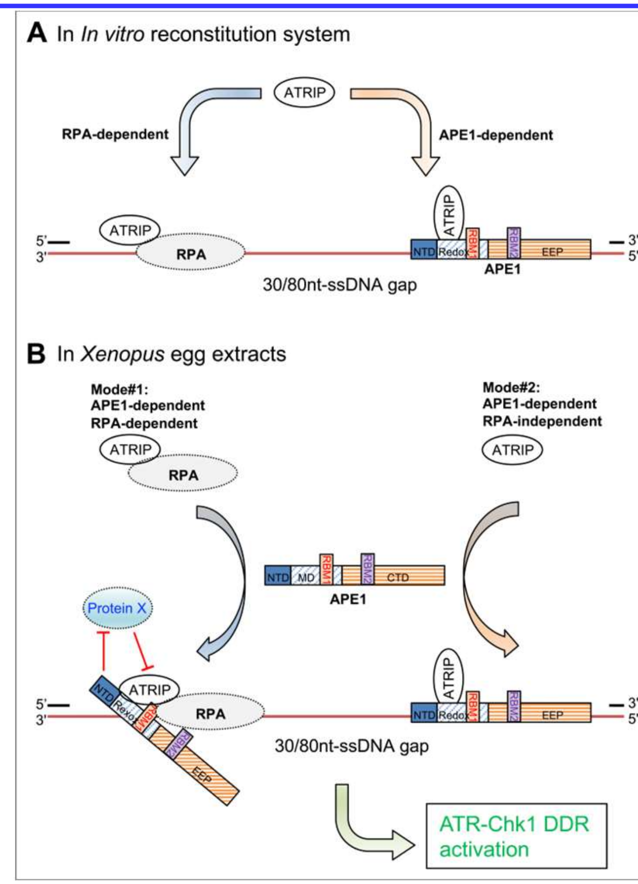


Lin et al., *eLife*, 2023

APE1 recruits ATRIP to ssDNA in an RPA-dependent and -independent manner for ATR DDR

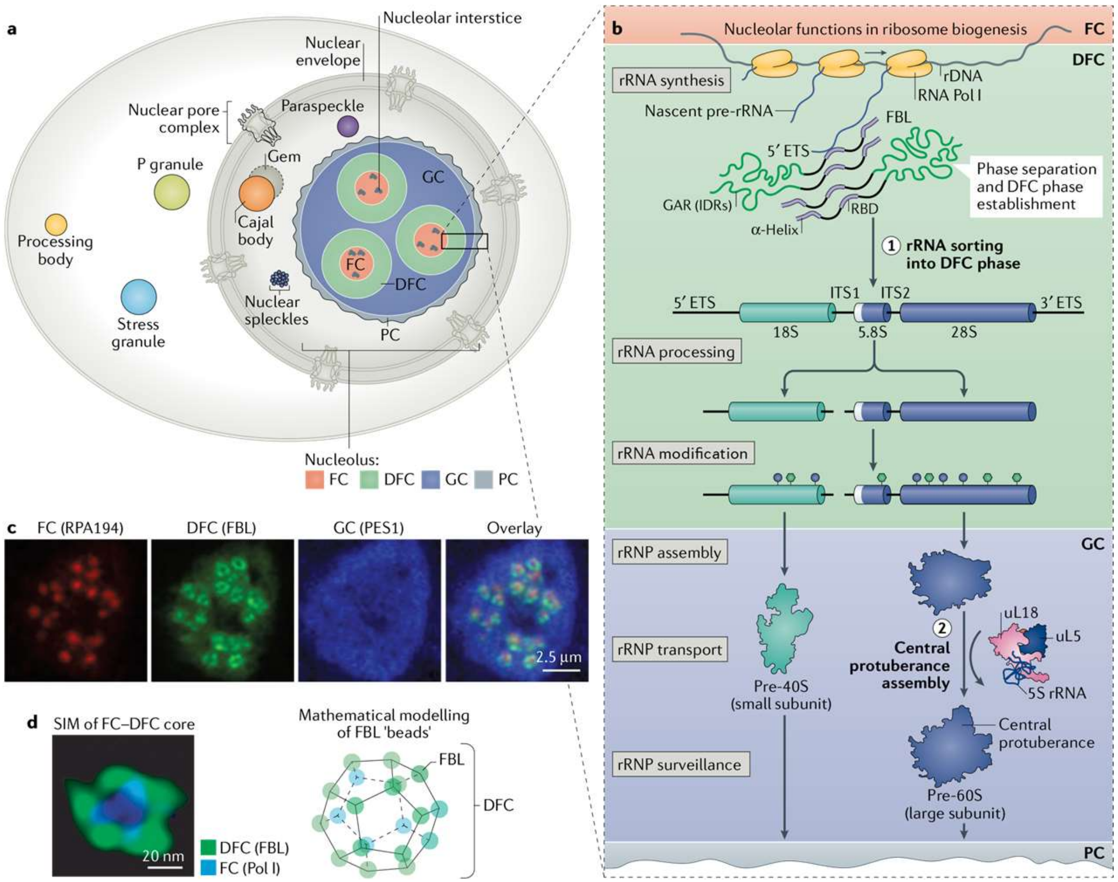


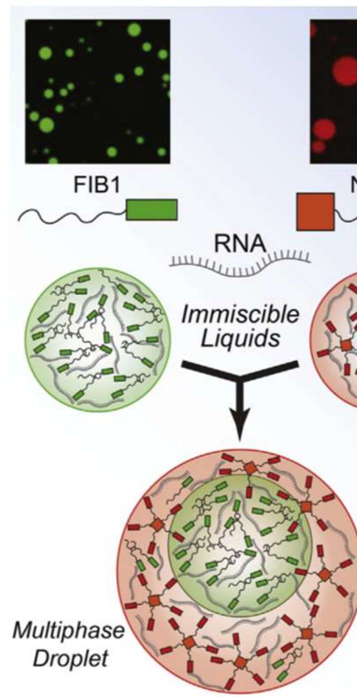




Zou and Elledge, *Science*, 2003
Lin et al., *eLife*, 2023

Biomolecular condensates by liquid-liquid phase separation (LLPS) in nucleolus





- **FC:** Fibrillar Center
- **DFC:** Dense Fibrillar Component (FIB1)
- **GC:** Granular Component (NPM1)

Feric,…Brangwynne, *Cell*, 2016;
Lafontaine ,…Brangwynne, *Nat Rev Mol Cell Biol*, 2021;
https://breakthroughprize.org

Molecular mechanisms of nucleolar DNA damage response

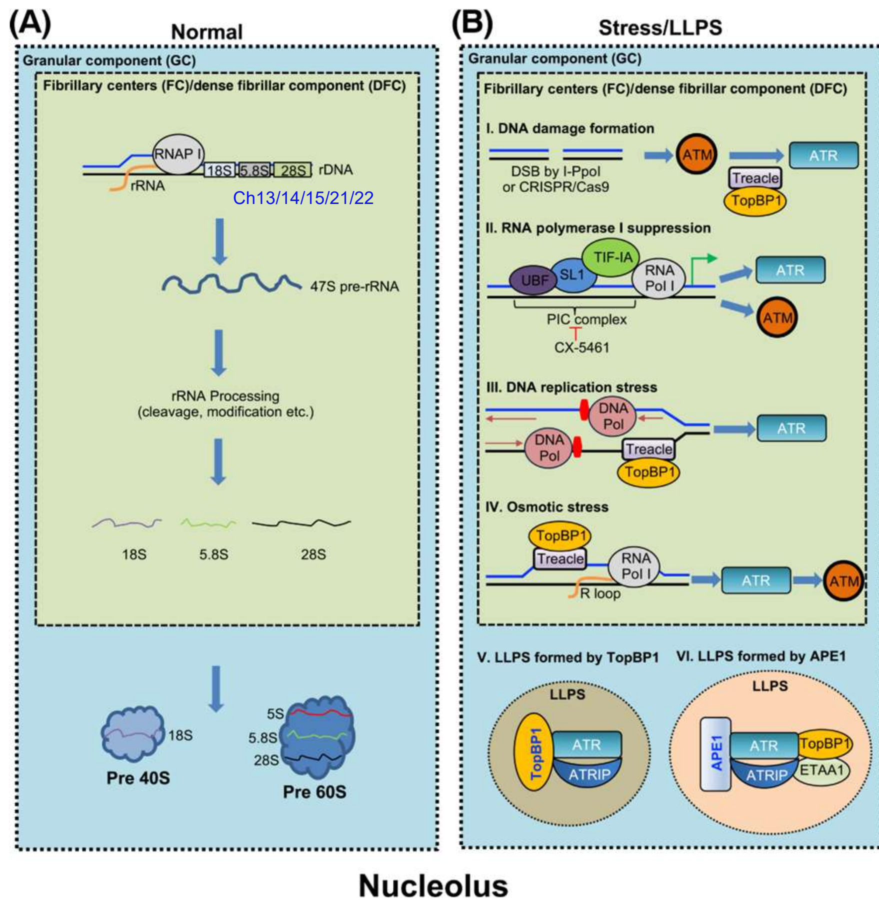
Trends in Cell Biology

Forum

Molecular mechanisms of nucleolar DNA damage checkpoint response

Jia Li[1] and Shan Yan[1,2,3,*]

Ribosomal DNA (rDNA) is transcribed into RNA in the nucleolus and is often challenged by different stress conditions. However, the underlying mechanisms of nucleolar DNA damage response (DDR) pathways remain elusive. Here, we provide distinct perspectives on how nucleolar DDR checkpoint pathways are activated by different stresses or by liquid–liquid phase separation (LLPS).

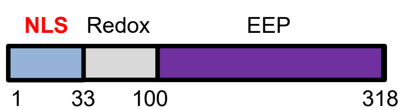


Kruhlak et al., *Nature*, 2007;
Kumar et al., *Cell*, 2014;
Sokka et al., *Nucleic Acids Res*, 2015;
Sanji et al., *Nat Commun*, 2020;
Velichko et al., *J Cell Biol*, 2021;
Frattini et al., *Mol Cell*, 2021;
Li et al., *Nucleic Acids Res*, 2022;
Li and Yan, *Trends Cell Biol*, 2023

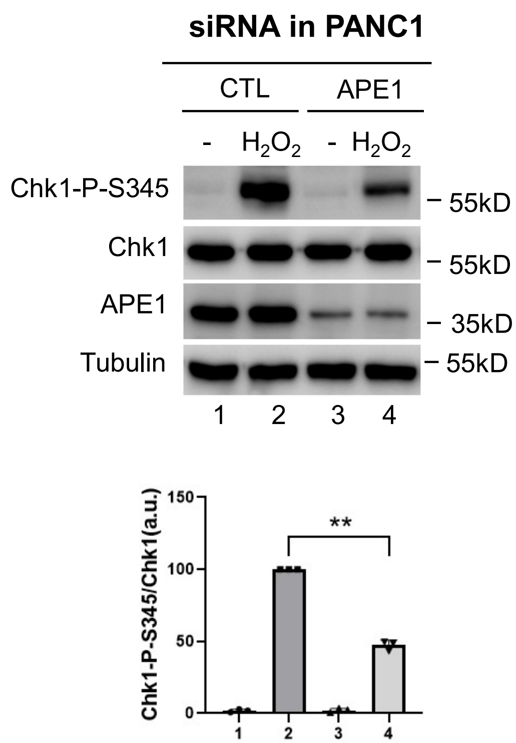
APE1 is important for the oxidative stress-induced ATR DDR in mammalian cells

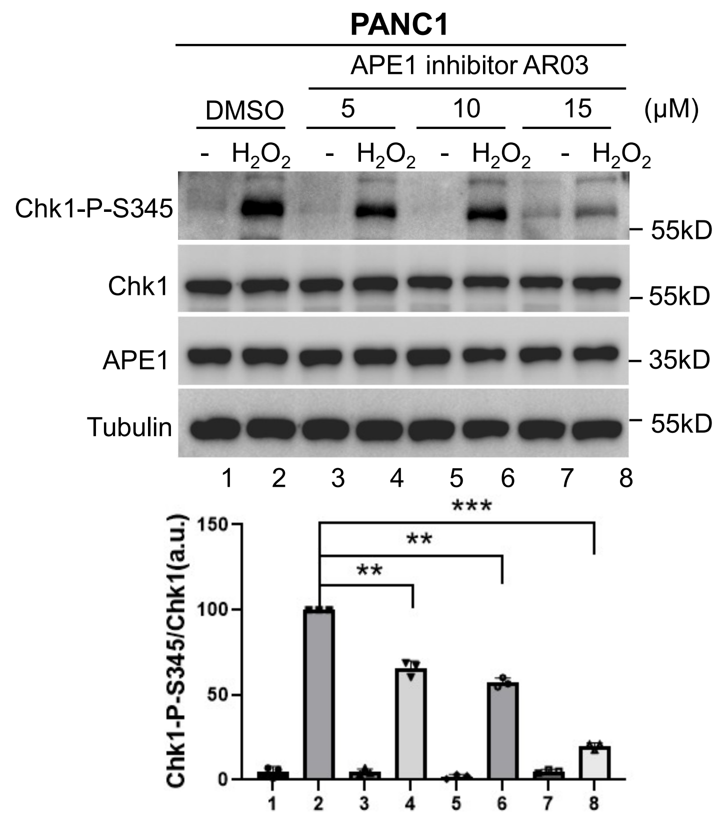
What is the role and mechanism of APE1 in DDR in the nucleoli?

- Intrinsically disordered motif
- RNA interaction



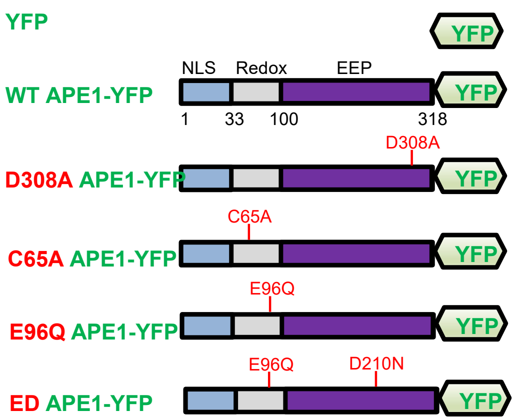
Dr. Jia Li/Postdoc



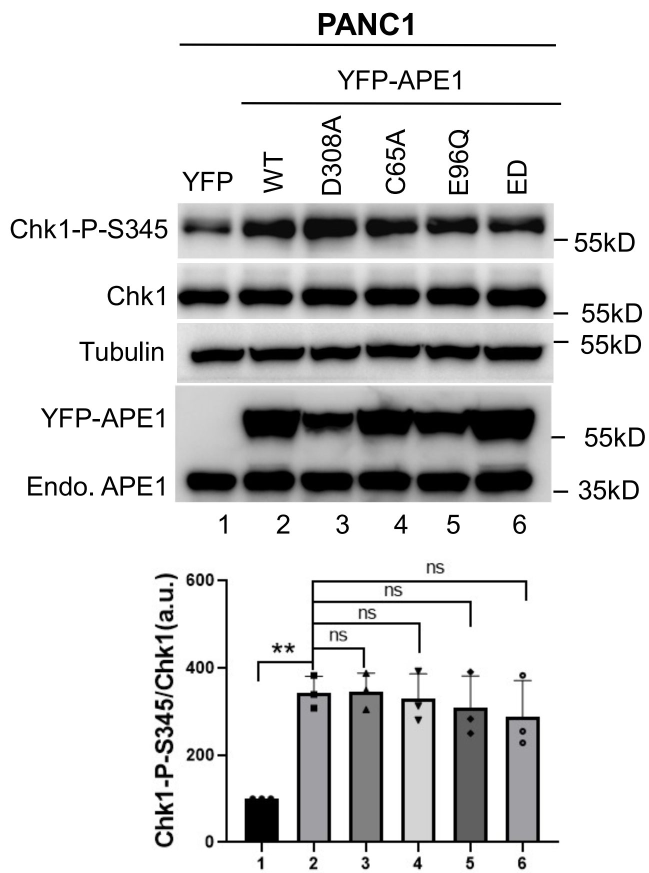


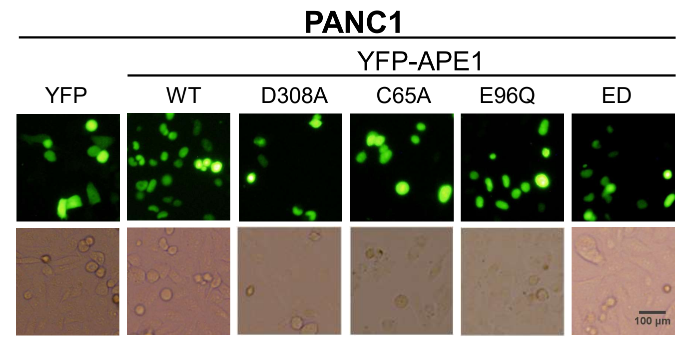
Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

Overexpression of YFP-APE1 but not YFP triggers ATR DDR in mammalian cells



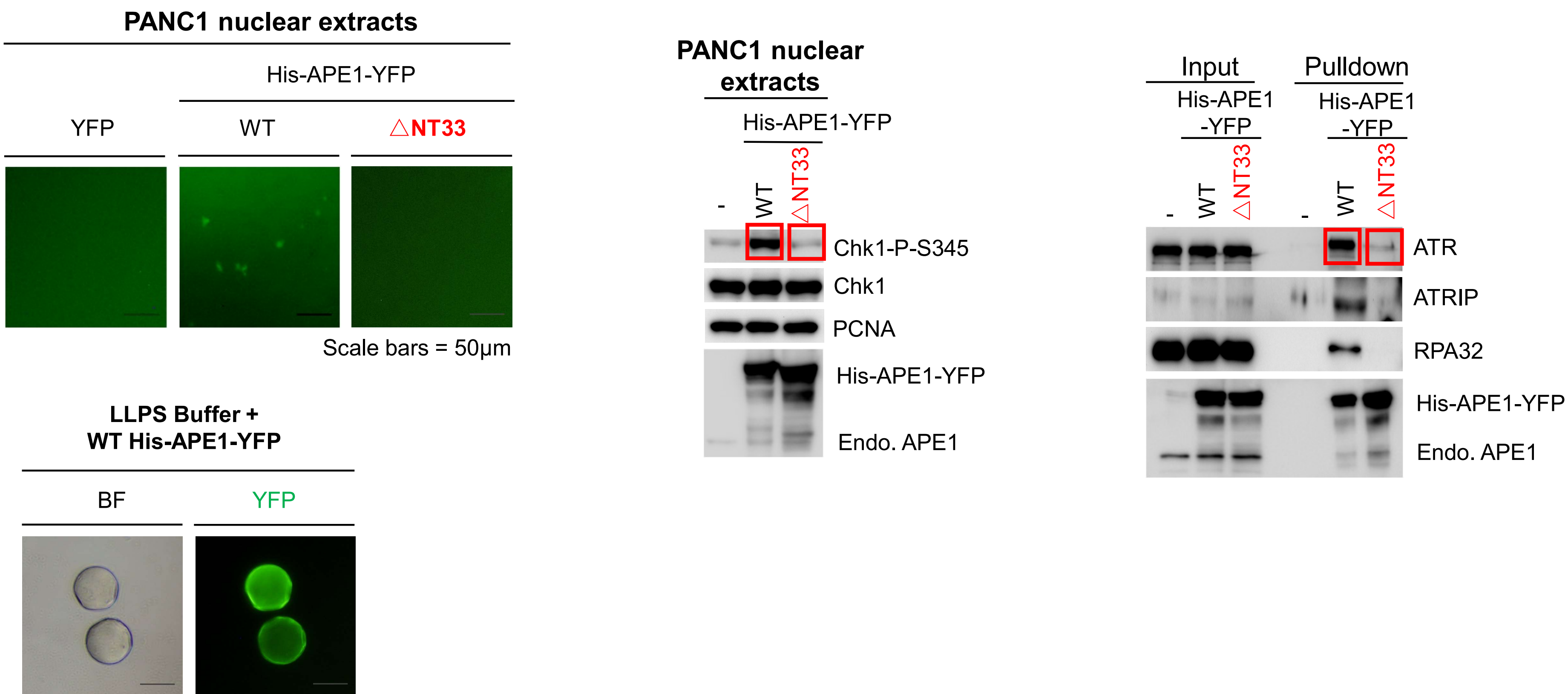
D308: exo-deficient, endo-proficient,
C65A: redox-deficient, nuclease-proficient
E96Q: endo/exo-deficient
ED(E96Q/D210N): endo/exo-deficient





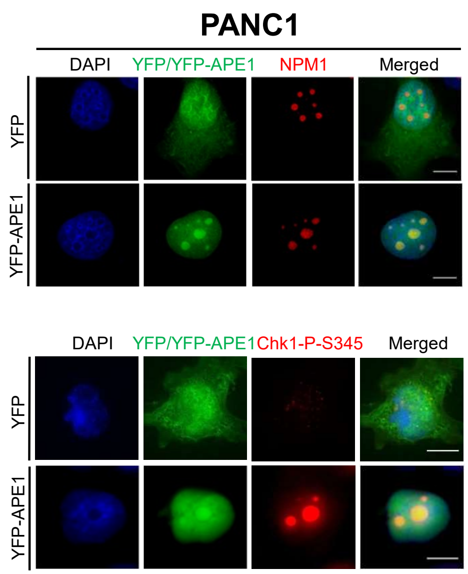
Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

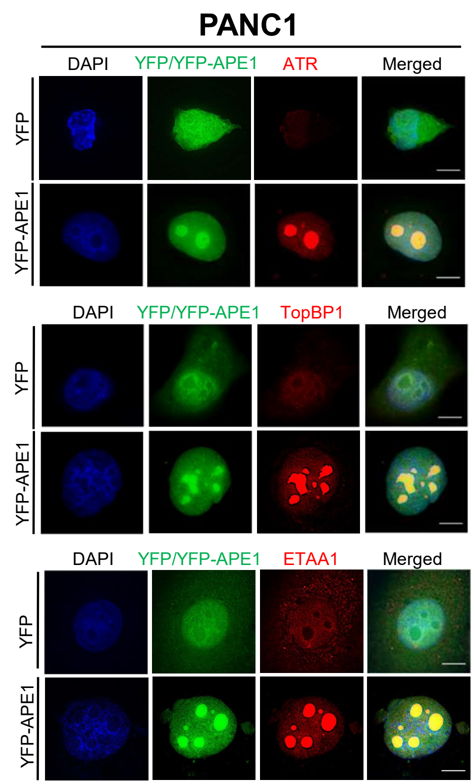
APE1 forms liquid-liquid phase separation (LLPS) *in vitro* to activate ATR

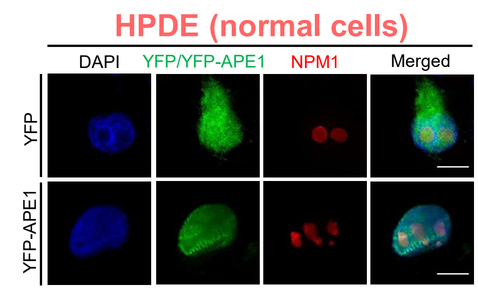


Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

Overexpressed APE1 is translocated to the nucleoli to activate ATR DDR in cancer but not normal cells



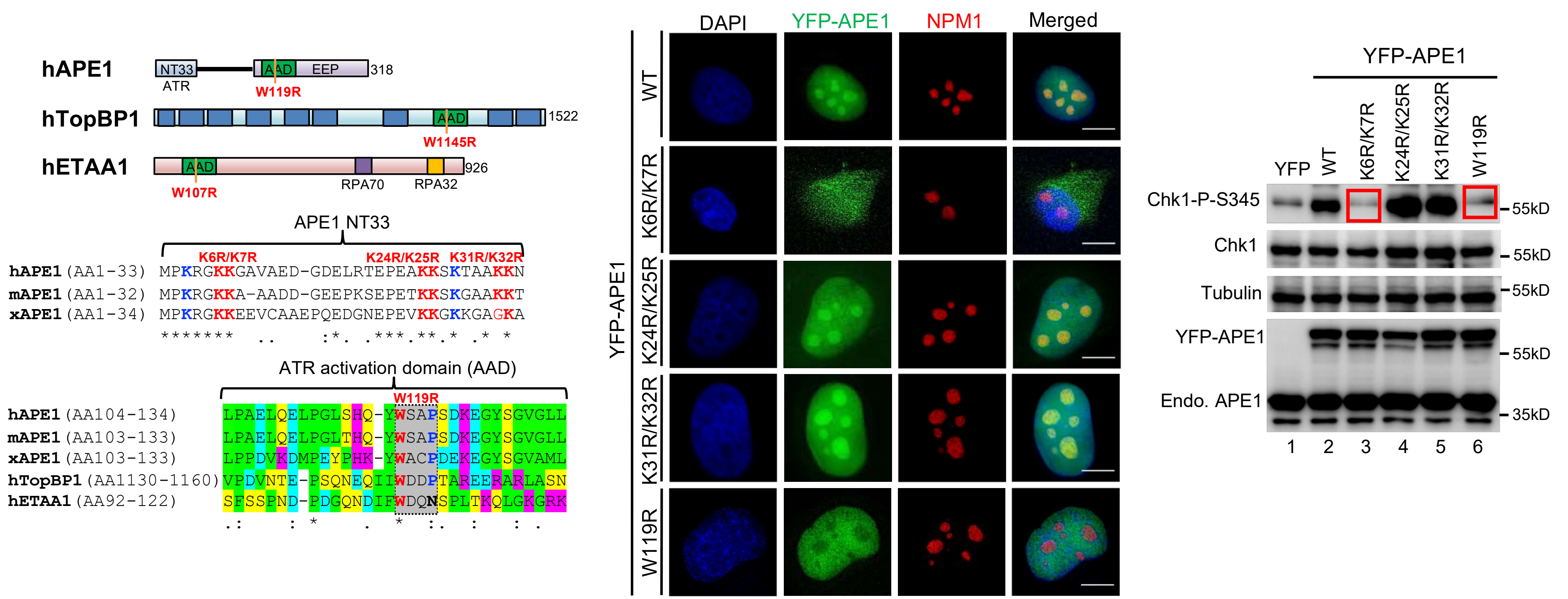




Scale bars = 10μm

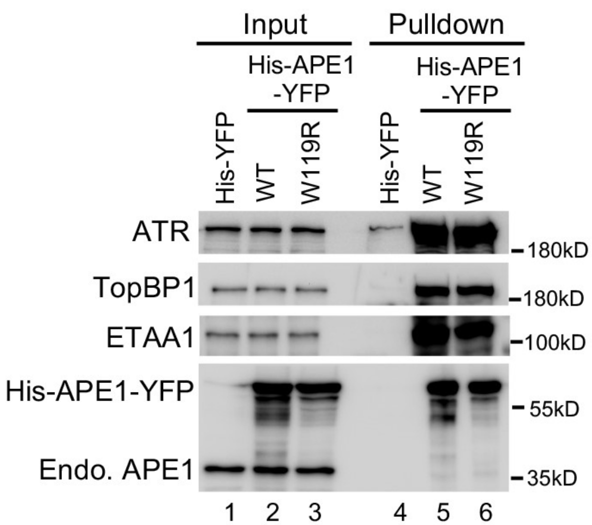
Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

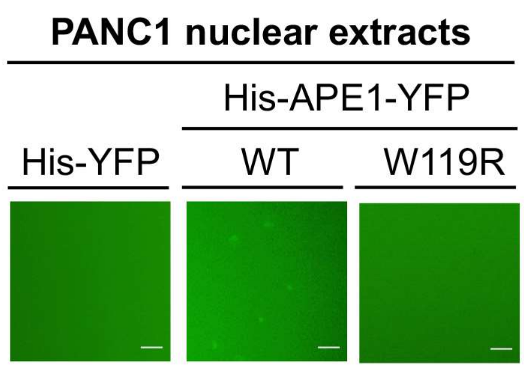
APE1 is a previously unidentified direct activator of the ATR kinase

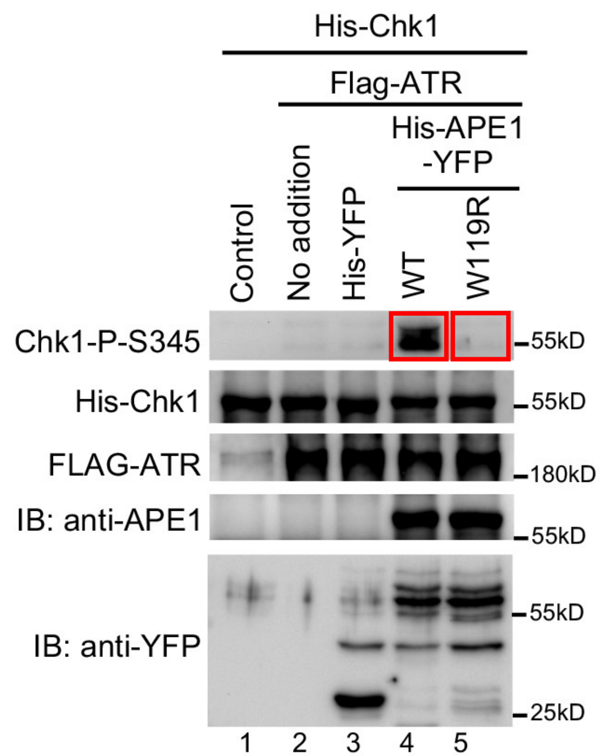


Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

W119 in hAPE1 is critical for direct activation of ATR kinase by APE1

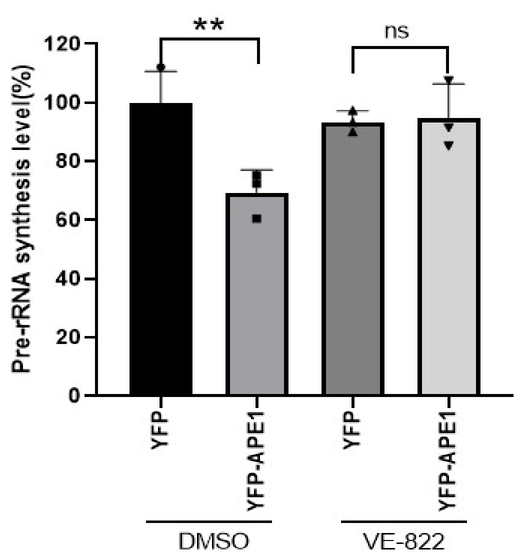


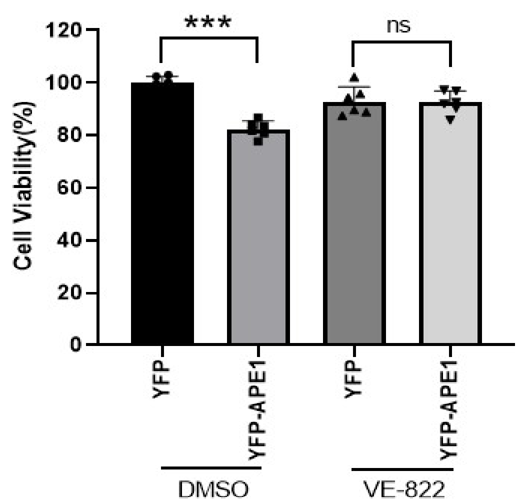


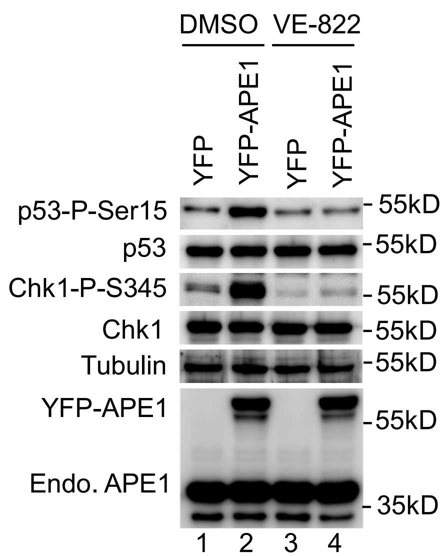


Li et al., *bioRxiv,* 2022;
Li et al., *Nucleic Acids Res*, 2022

APE1-OE-induced nucleolar ATR DDR compromises pre-rRNA transcription and impairs cell viability

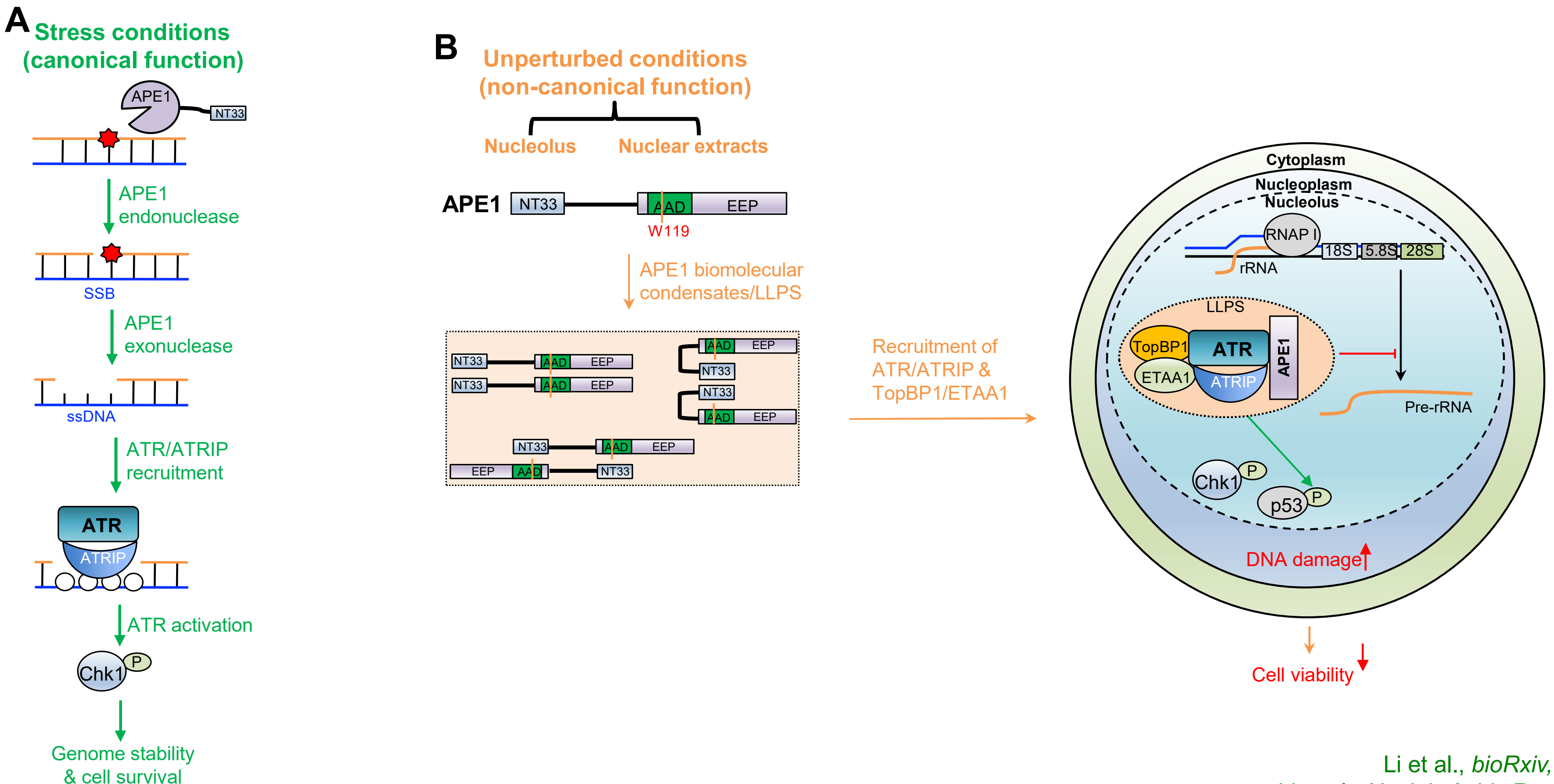






Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

A working model for the multiple mechanisms of APE1 in the ATR DDR in cancer cells

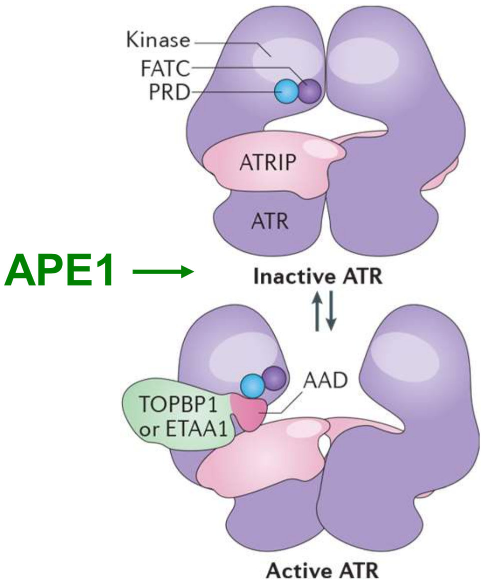


Li et al., *bioRxiv*, 2022;
Li et al., *Nucleic Acids Res*, 2022

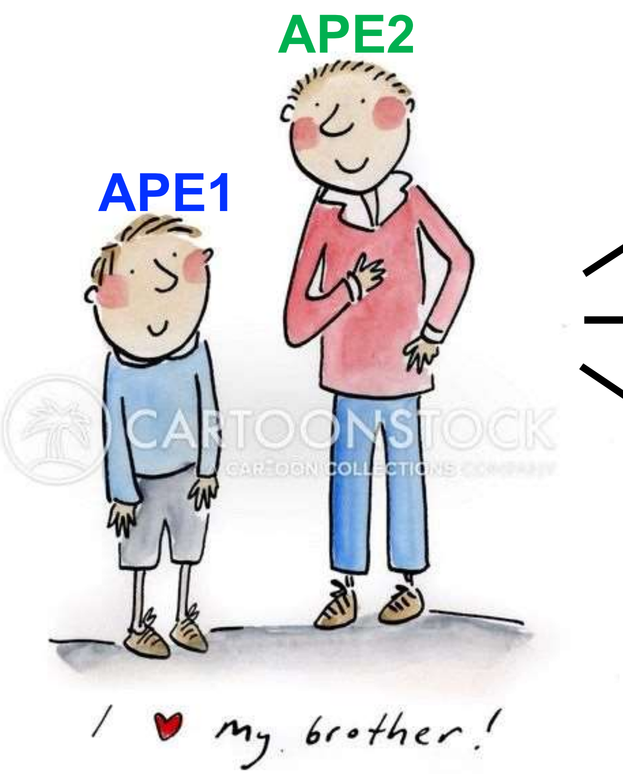
Summary 2 – APE1

APE1

- ✓ 1. APE1 and its exonuclease activity is required for SSB repair and signaling.
- ✓ 2. APE1 interacts with and recruits ATRIP to ssDNA in an RPA-dependent/-independent manner for ATR DDR via a non-catalytic function.
- ✓ 3. APE1 assembles biomolecular condensates to promote the ATR-Chk1 DDR in nucleolus in cancer but not normal cells.



Lin et al., *Nucleic Acids Res*, 2020;
Ha et al., *J Biol Chem*, 2020;
Lin et al., *eLife*, 2023;
Li et al., *Nucleic Acids Res*, 2022;
Li and Yan, *Trends Cell Biol*, 2023



BER

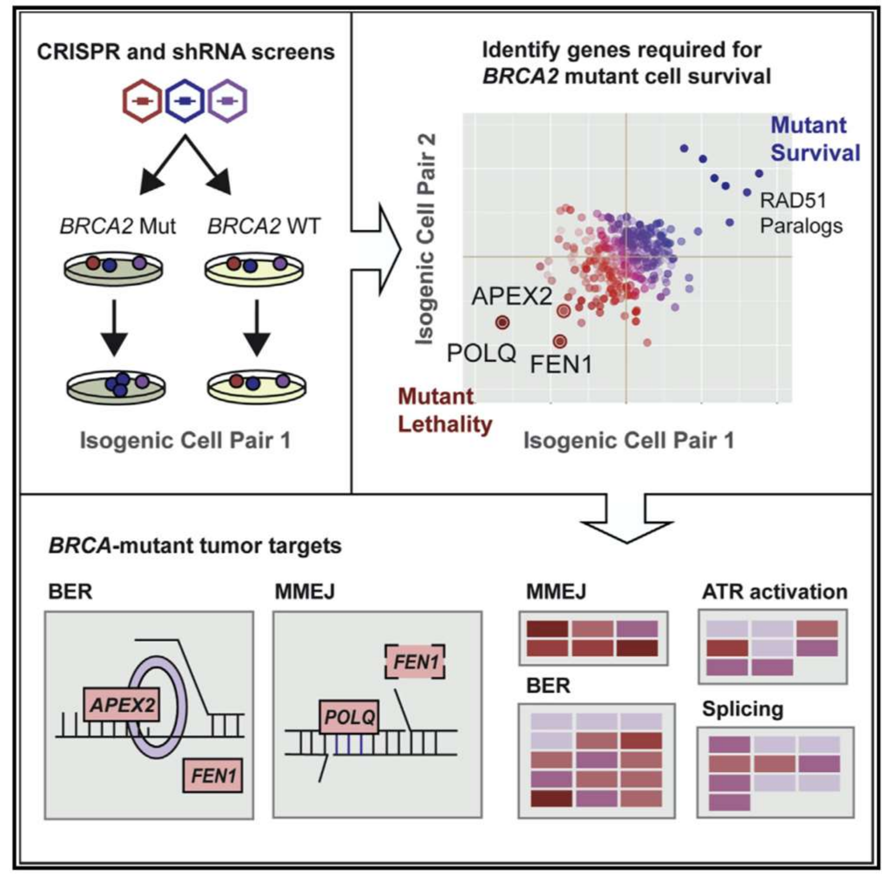
MMEJ

ATR DDR

Molecular Cell
Article

Genetic Screens Reveal *FEN1* and *APEX2* as *BRCA2* Synthetic Lethal Targets

Kristen E. Mengwasser,[1,2] Richard O. Adeyemi,[1,2] Yumei Leng,[1,2] Mei Yuk Choi,[1,2] Connor Clairmont,[3] Alan D. D'Andrea, and Stephen J. Elledge[1,2,4,*]



Acknowledgements

Research Assistant Professor:

- ➤ **Yunfen Lin**, PhD

Postdoctoral Fellows:

- ➤ **Jia Li**, PhD
- ➤ Haichao Zhao, PhD
- ✓ Liping Bai, PhD, Professor, CAMS

Graduate students:

- ➤ **Garrett Driscoll**
- ➤ **Anne McMahon**
- ➤ **Josh Matos**
- ➤ **Kelly McGhee**
- ✓ **Md. Akram Hossain**
- ✓ **Katie Jensen**
- ✓ **Steven Cupello**
- ✓ **Zachary Berman**
- ✓ **Jeremy Willis**
- ✓ Megan Mitchem
- ✓ Anh Ha
- ✓ Melissa McLeod
- ✓ Bradley Deem
- ✓ Darla DeStephanis

Undergraduate student:

- ✓ **Katie Jensen**
- ✓ **Jude Raj**
- ✓ **Yogin Patel**
- ✓ **Barry Lentz**
- ✓ Brianna Bush
- ✓ Amogh Bandekar
- ✓ Amit Thot
- ✓ Naya Kayali
- ✓ Anh Ha
- ✓ Mary Tess Overton
- ✓ Krystal Carmichael
- ✓ Vrushab Gowda
- ✓ Victoria Fitts
- ✓ Alexa R. Campbell
- ✓ Dafe Omerhi
- ✓ Harleen Kaur
- ✓ Peyman “Alex” Gharavizadeh
- ✓ Jagat Patel
- ✓ James Hooper
- ✓ Mallorie Hartmann
- ✓ Mohammad Ahmad
- ✓ John Dukes

Collaborators

- ➤ Matt Michael, University of Southern California
- ➤ Jianjun Zhao, Cleveland Clinic
- ➤ Scott Williams, NIH/NIEHS
- ➤ Xiaoqi Liu, Purdue University
- ➤ Xinghua Shi, Temple University
- ➤ Karlene Cimprich, Stanford University
- ➤ Zhongsheng You, Washington University St. Louis
- ➤ Howard Lindsay, Lancaster University, U.K.
- ➤ Pinku Muhkerjee
- ➤ Christine Richardson
- ➤ Juan Vivero-Escoto
- ➤ Andrew Truman
- ➤ Junya Tomida
- ➤ Jun-tao Guo
- ➤ Ian Marriott

UNIVERSITY OF NORTH CAROLINA CHARLOTTE

North Carolina Biotechnology Center

NATIONAL CANCER INSTITUTE

R01CA225637
R03CA270663
R01CA251141
R01CA263897
R15CA263784

NIH NIEHS

R21ES032966

R01GM139885
R15GM101571
R15GM114713