Regulation of Central Steps in Human Base Excision Repair

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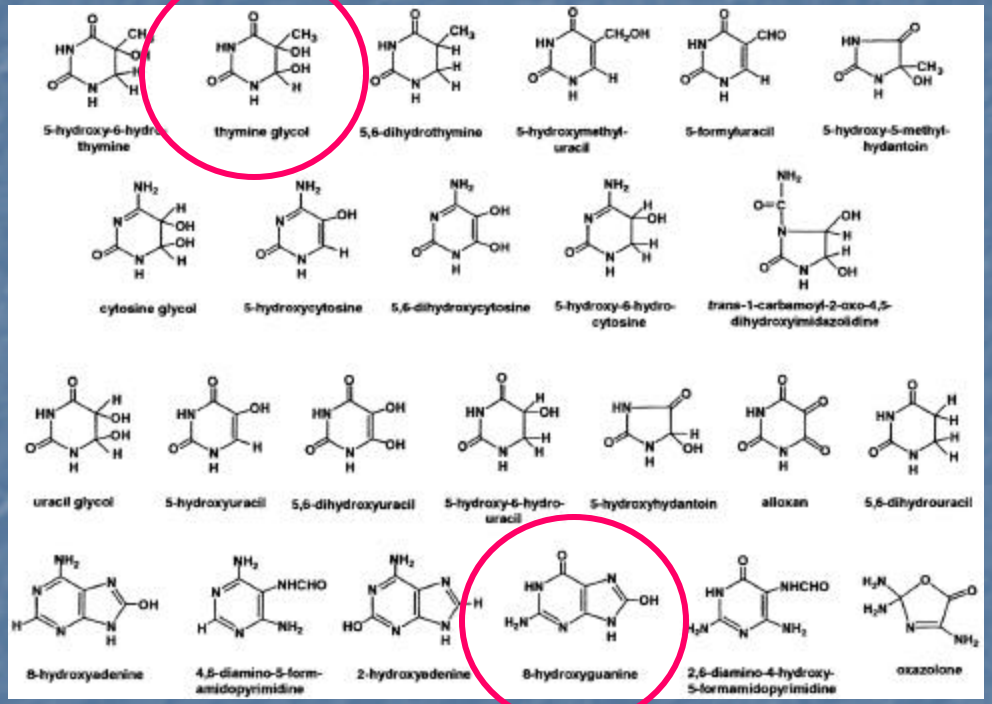
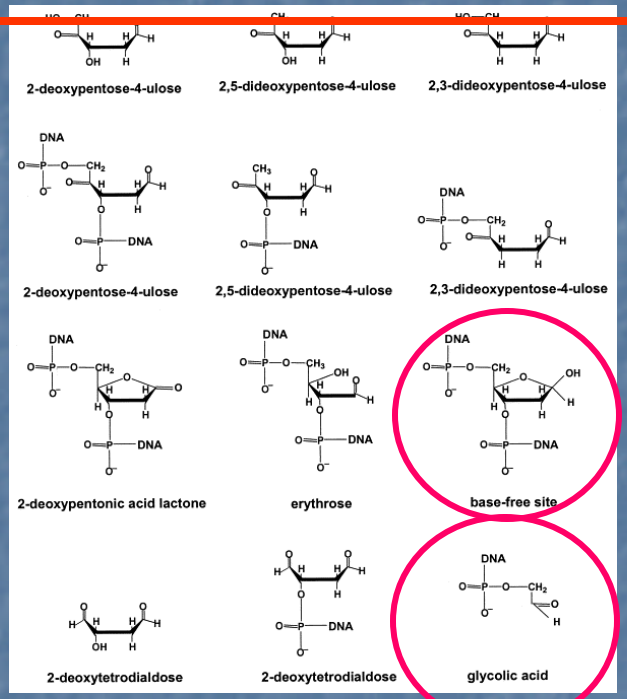
Oxidative DNA Damage

Base Damage



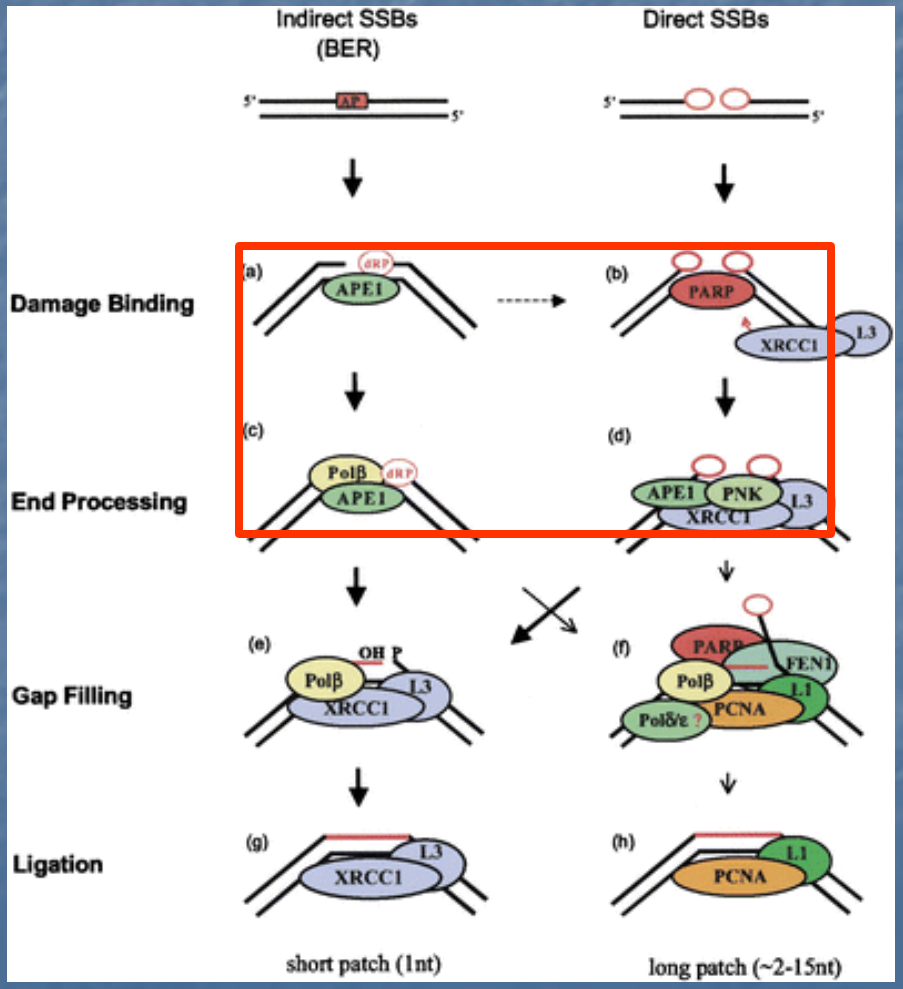
Sugar Damage



- can block replication or transcription progression.
- can induce mutagenesis and/or genetic instability.
- … …as a result, oxidative DNA damage can promote cell death or cellular dysfunction associated with cancer, neurodegeneration, and the aging process

Mammalian Base Excision Repair

Glycosylase-generated Spontaneous or damage-induced



The major pathway for spontaneous, oxidative, and alkylation DNA damage.

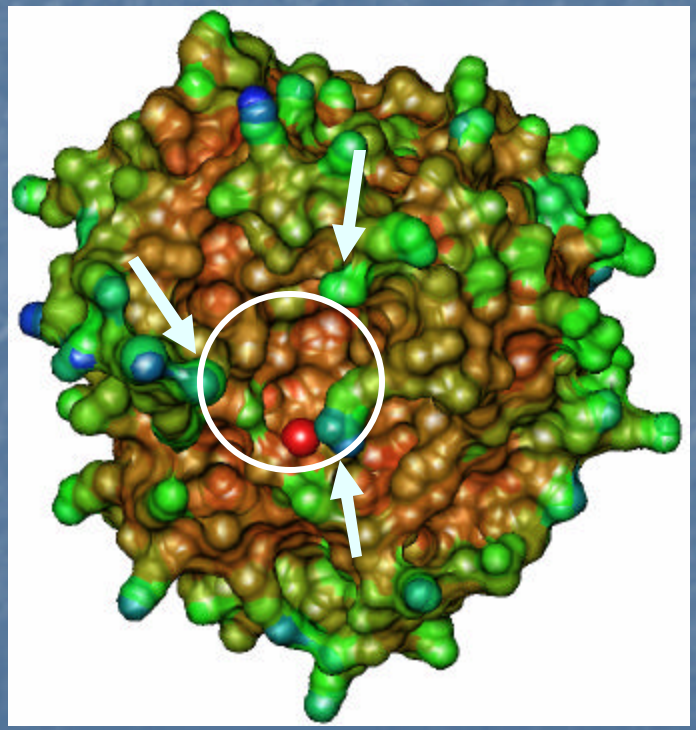
Defects are associated with cancer and premature aging characteristics.

We have focused primarily on defining the structure-function mechanisms of Ape1 and BER pathway coordination.

PRESENTATION OUTLINE

- Repair biochemistry of Ape1: its 3'-repair/nuclease function.
- Inactivation of Ape1 by environmental metals.
- A novel link between XRCC1 and DNA replication factories: XRCC1-PCNA interaction.

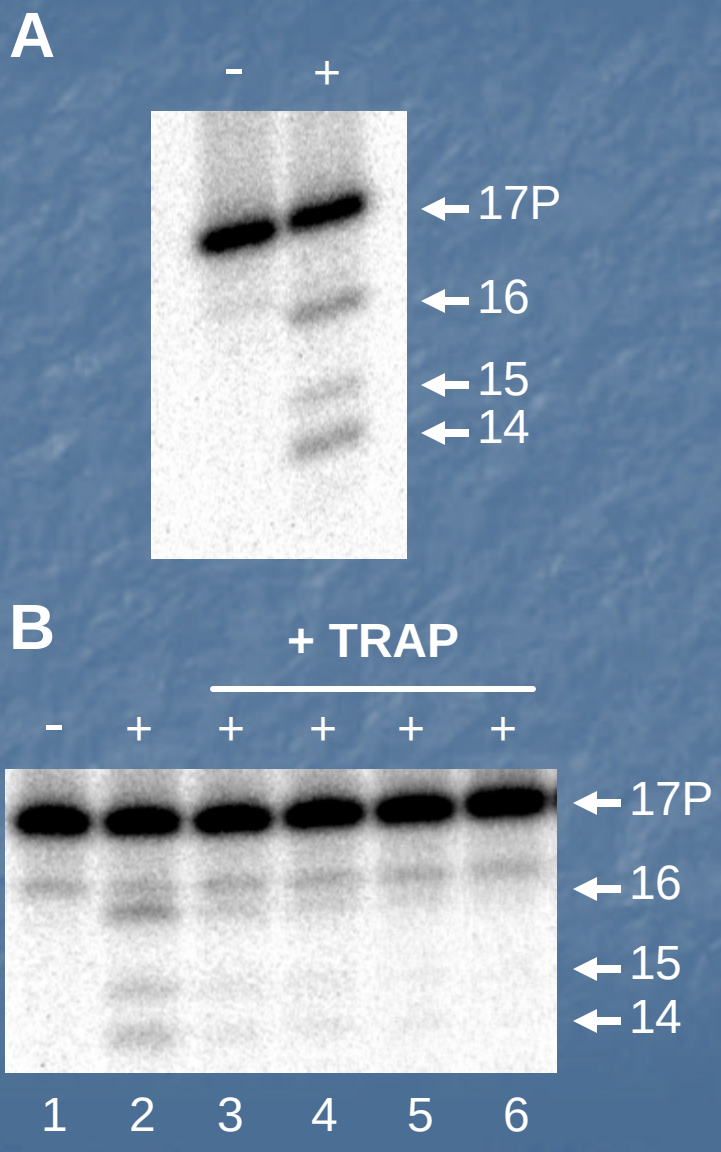
The Major Human AP Endonuclease, Ape1



- Accounts for >95% of the AP endonuclease activity in mammals
- Significant contributor to 3'-damage and 3'-mismatched nucleotide repair
- Has additional functions in gene regulation (e.g. Ref-1 activity)
- Member of the alpha/beta-fold superfamily of enzymes
- Employs unique loop regions and active site physiochemistry to target AP sites in DNA (protein-induced substrate kinking)
- Executes a metal-catalyzed hydrolytic reaction

Ape1 as a 3'-Nuclease

Ape1 is a Comparatively "Poor", Non-processive Exonuclease

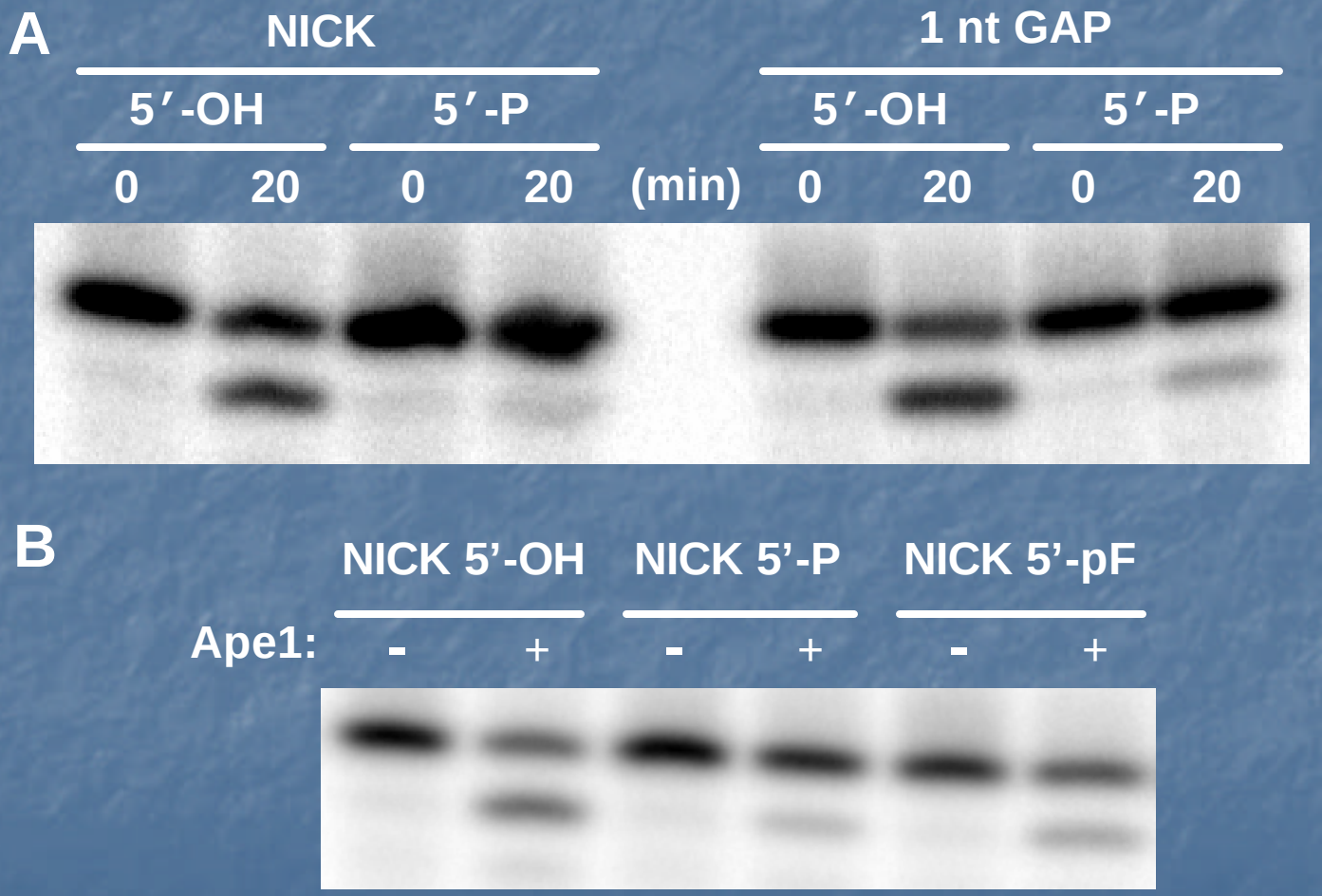


The General Exonuclease Profile for Ape1

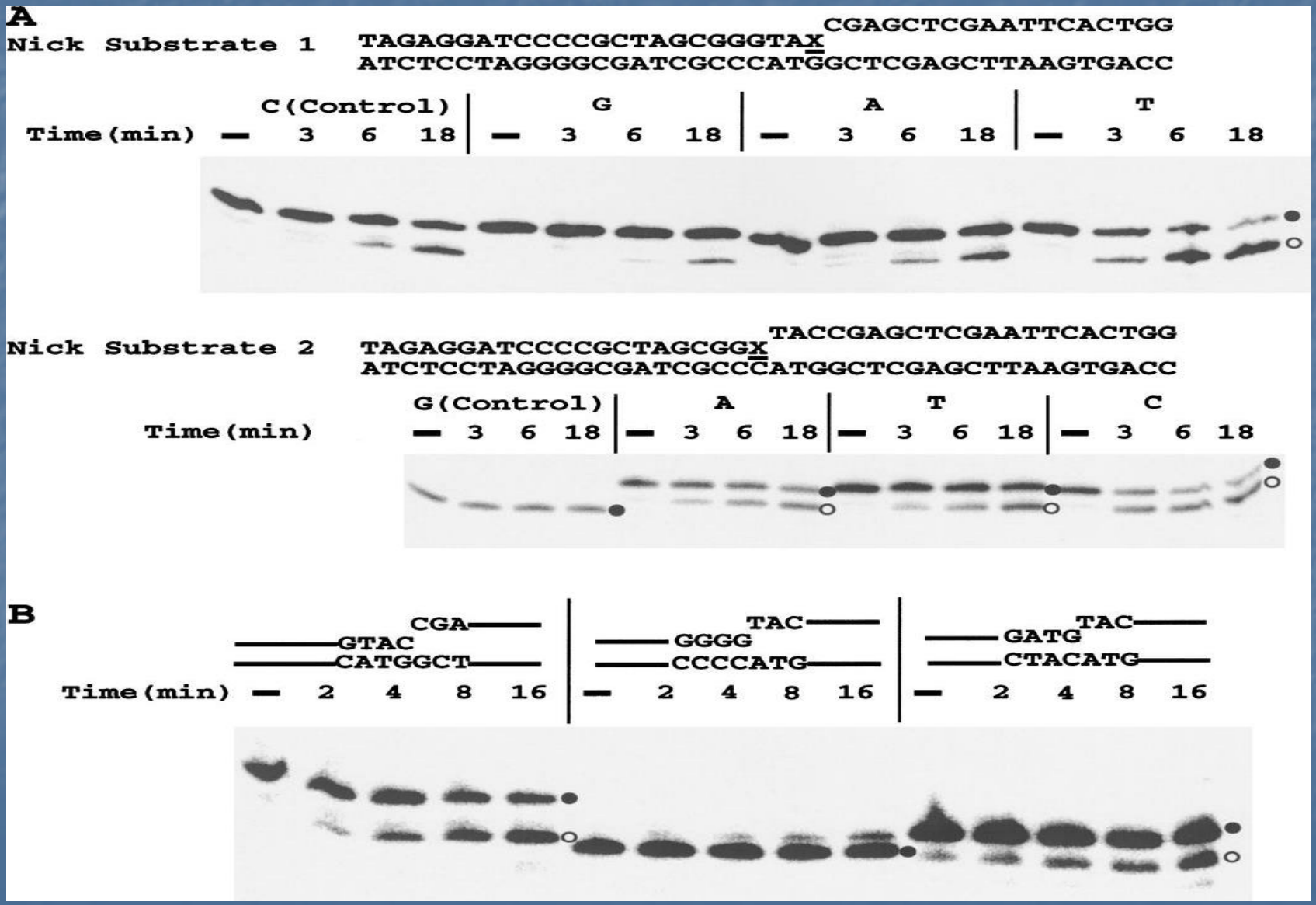
Maximal Velocities (pmolmin^{-1}) for Oligonucleotide Substrates

Oligonucleotide Substrate	Vmax
ssDNA	<0.0001
3′-REC	0.029 ± 0.004 (0.94)
NICK	0.021 ± 0.003 (0.68)
1 nt GAP	0.031 ± 0.002 (1)
2 nt GAP	0.0034 ± 0.0009 (0.11)

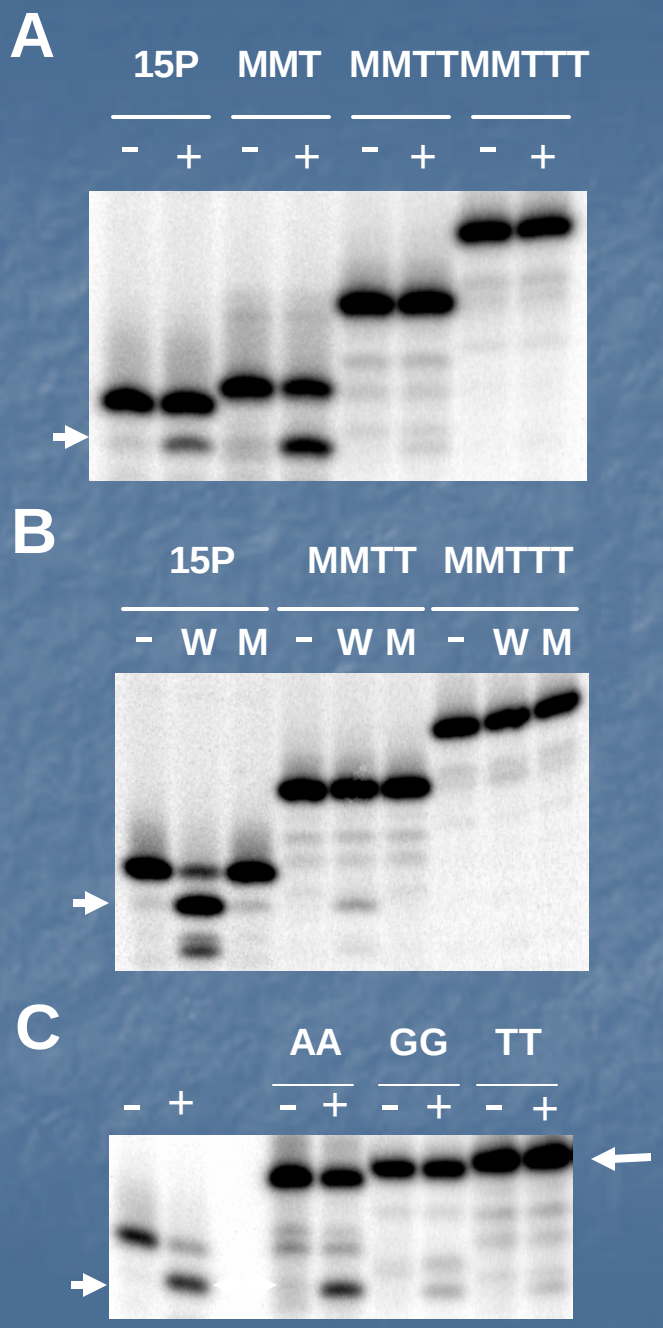
A 5′-Phosphate or a 5′-Abasic Terminus Hinders Ape1 Exonuclease Activity



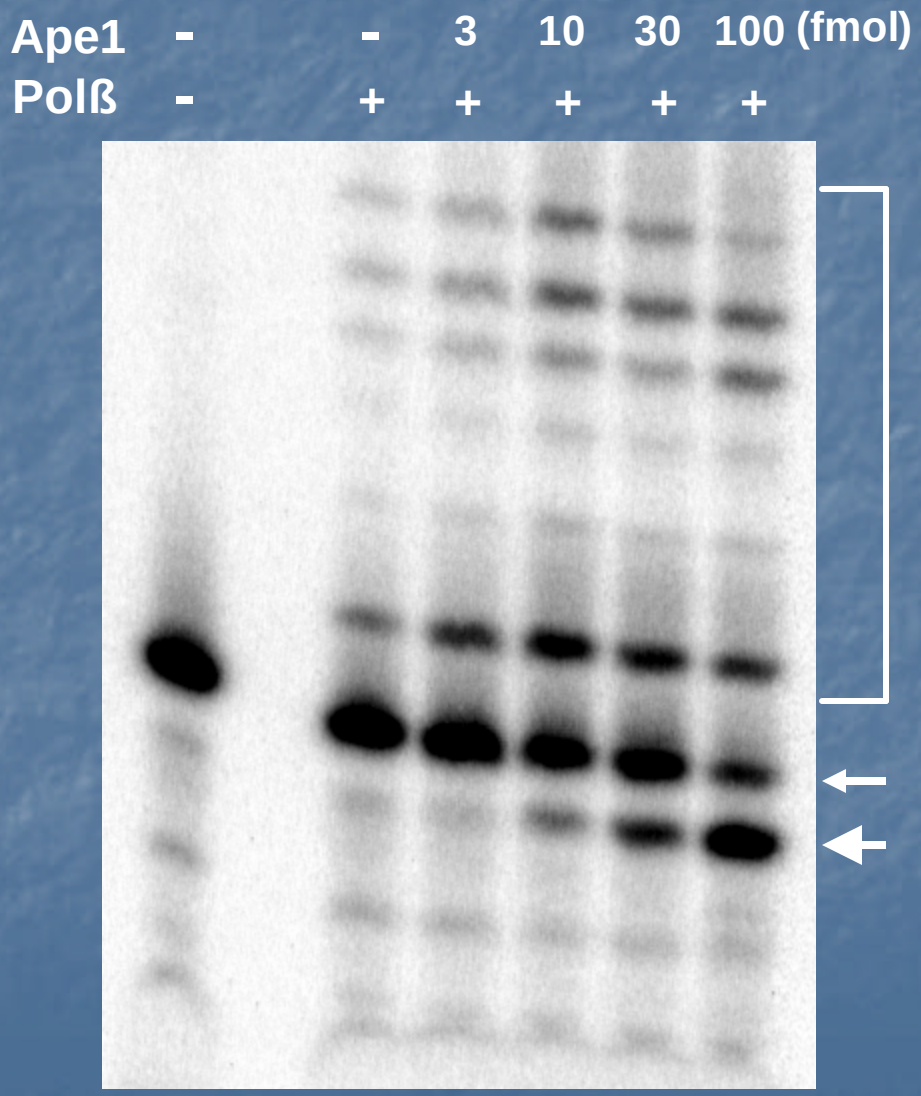
Ape1 exhibits preferential, yet substrate-selective, 3′-mismatch excision activity



Ape1 can excise double mismatched nucleotides, but not triple mismatches



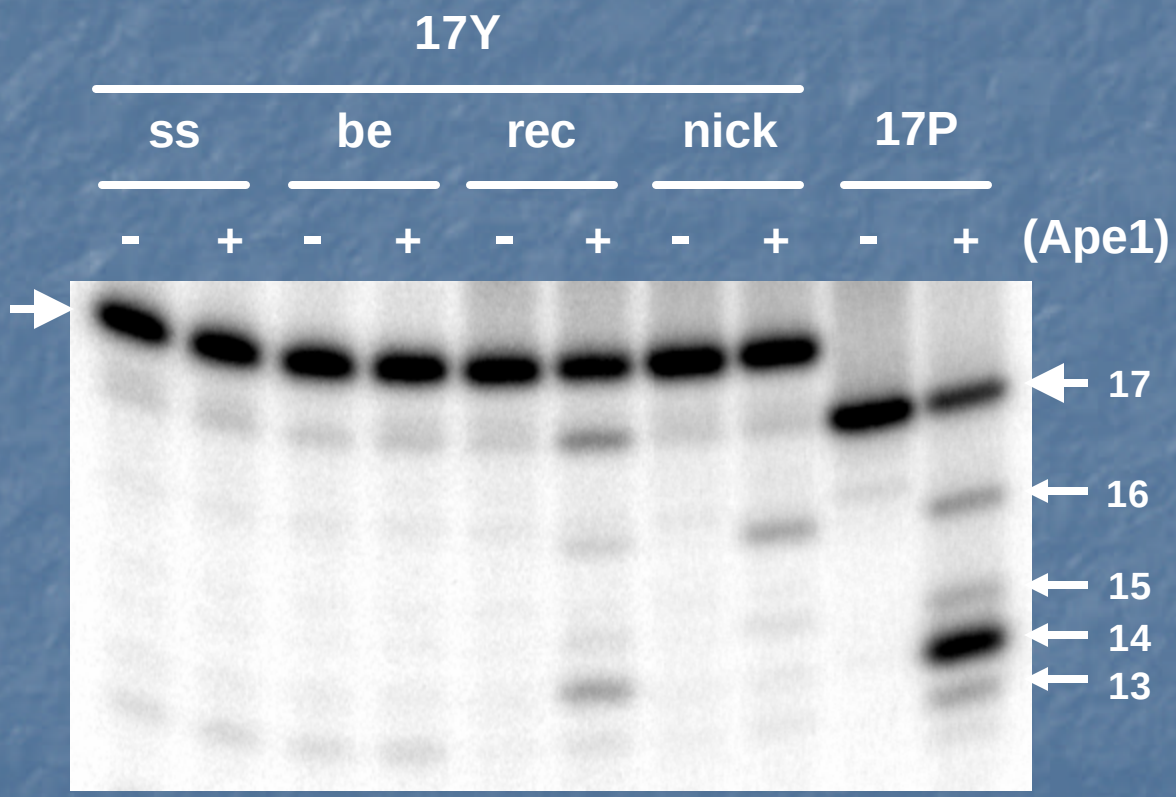
Ape1 can remove 3'-phosphate residues, activating DNA for Polβ extension



Ape1 displays substrate-selective 3'-phosphoglycolate excision activity

DNA SUBSTRATE	RELATIVE CATALYTIC EFFICIENCY (k_{cat}/K_m)
AP SITE	1
PG at INTERNAL GAP	0.01
PG at INTERNAL NICK	0.001
PG at 3'-RECESSED END	0.001
PG at BLUNT END	0.0005
PG at 3'-OVERHANG	NO ACTIVITY DETECTED

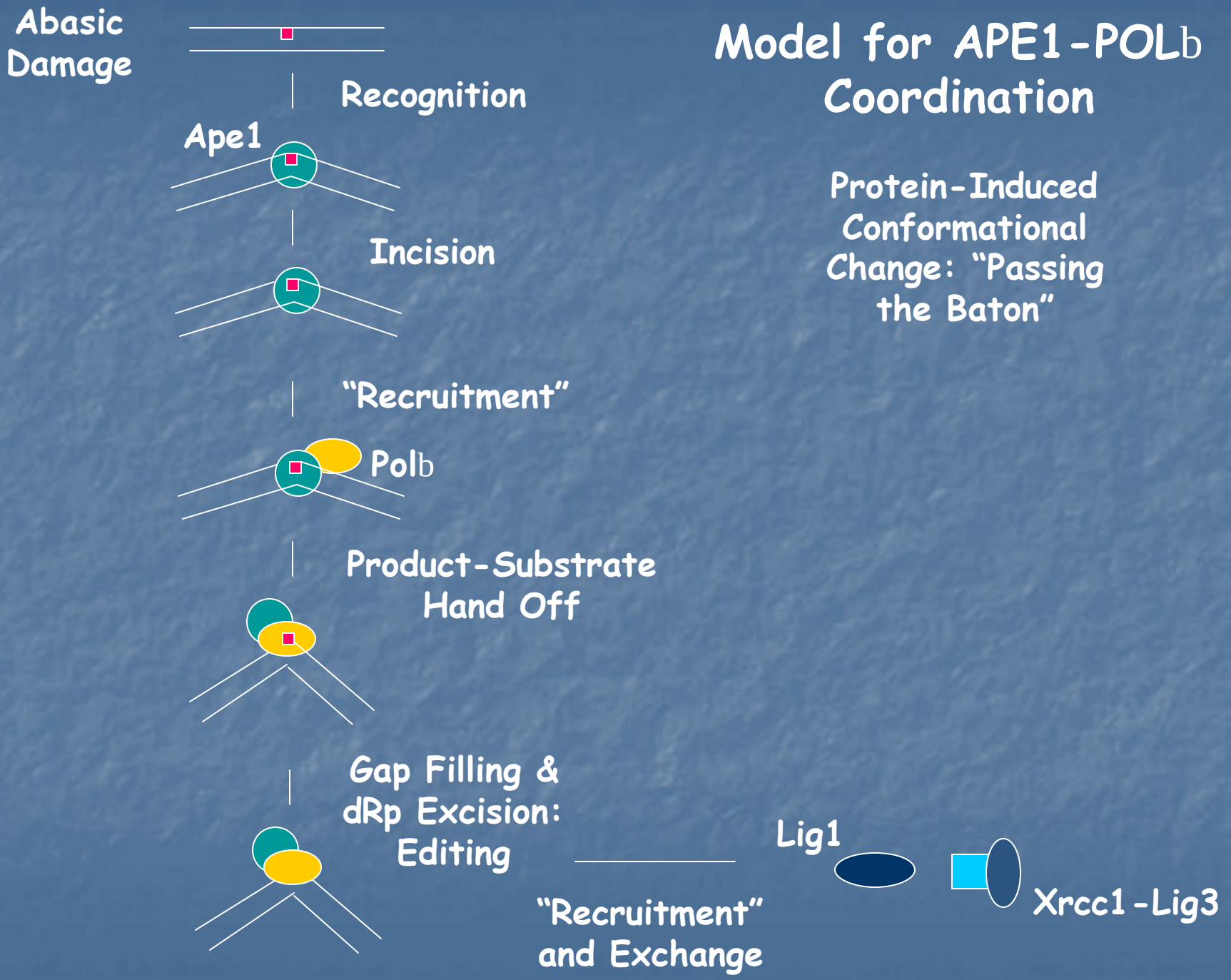
Ape1 can remove 3'-Tyr residues, which mimic the 3'-protein-DNA intermediates formed either by Topo I during DNA relaxation or by Camptothecin treatment



Summary

- Ape1 possesses a poor (but real), non-processive 3' to 5' exonuclease function.
- The 3' to 5' exonuclease and 3'-repair activities of Ape1 prefer gap, nick and 3'-recessed DNA substrates.
- Ape1 shows some selectivity, depending on sequence context, for 3'-mismatches (but should not be classified as a "proofreading" enzyme).
- Ape1 can excise double-mismatches (again, depending on sequence and structural context), L-configuration nucleoside analogs (Chou et al., JBC **275**:31009-31105), and Tyr residues from the 3'-terminus of DNA.

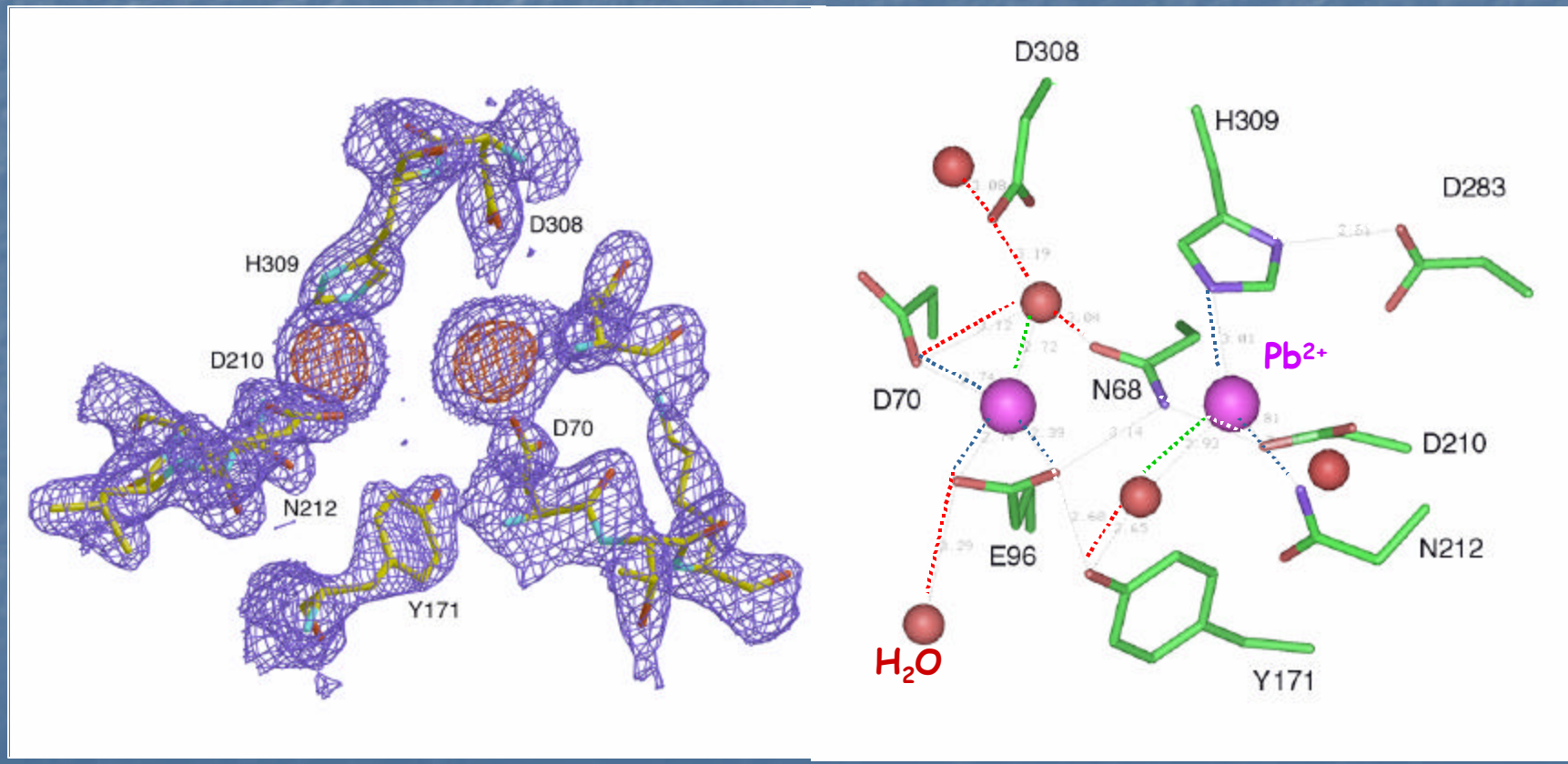
Model for APE1-POLb Coordination



Protein-Induced Conformational Change: "Passing the Baton"

Effects of Environmental Metals on Ape1 Incision Activity

X-ray Structure Reveals Two Lead Ions Bound within Ape1 Active Site



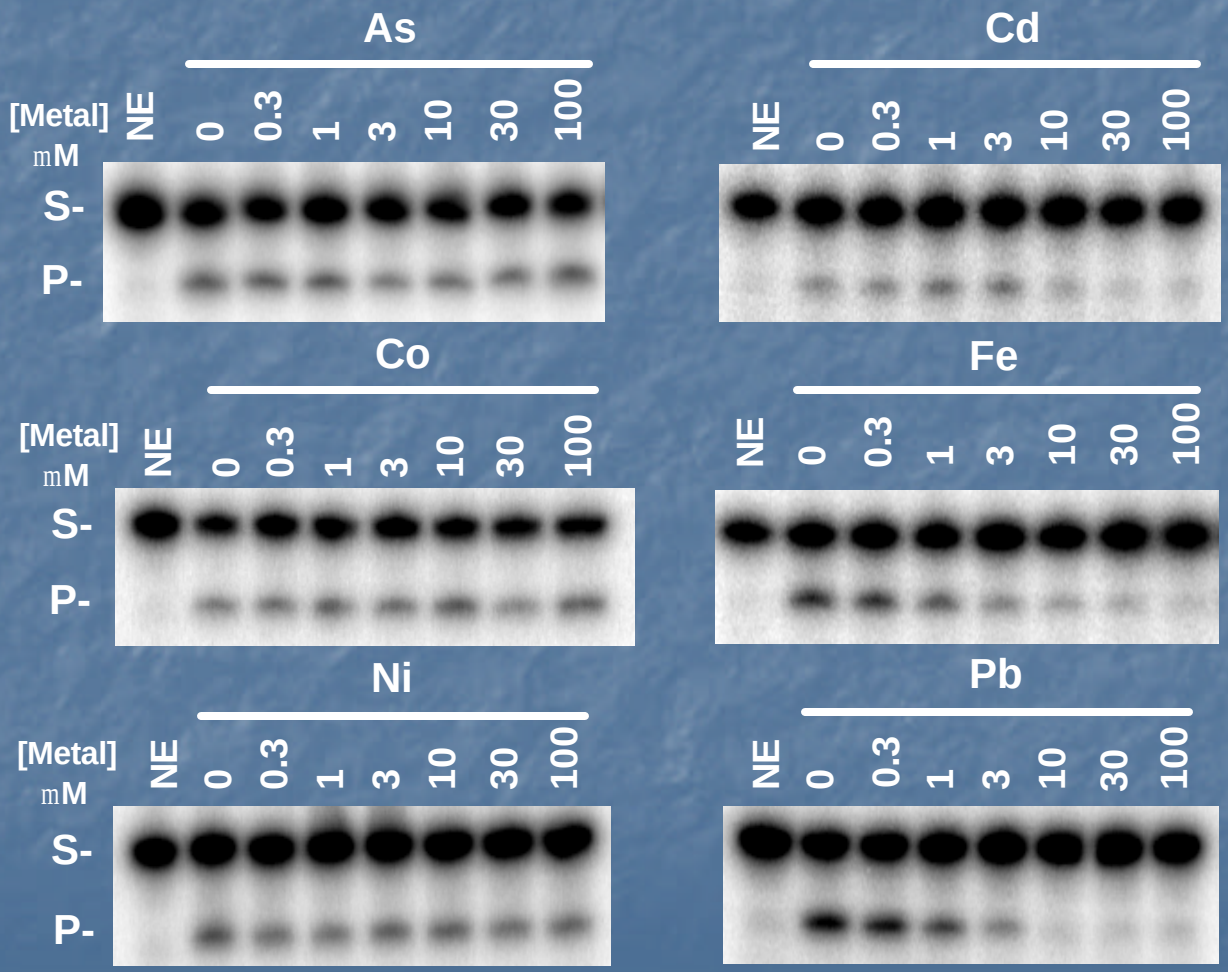
Potentially co-mutagenic environmental metals inhibit DNA repair

DNA Repair Target	Inhibitory Metal(s)	Cellular Outcome
Ogg1 (8-oxoguanine DNA glycosylase)	Cd(II) and Zn(II)	Reduced 8-oxoG repair
XPA (xeroderma pigmentosum group A protein)	Cd(II), Co(II), Cu(II), and Ni(II)	Reduced nucleotide excision repair
Parp-1 (poly[ADP-ribose] polymerase 1)	As(II), Cd(II), Co(II), Cu(II), and Ni(II)	Impaired strand break response
p53 (tumor suppressor protein)	Cd(II), Co(II), and Ni(II)	Impaired DNA damage response
Mismatch DNA Repair (**specific target unknown**)	Cd(II)	Elevated genetic instability

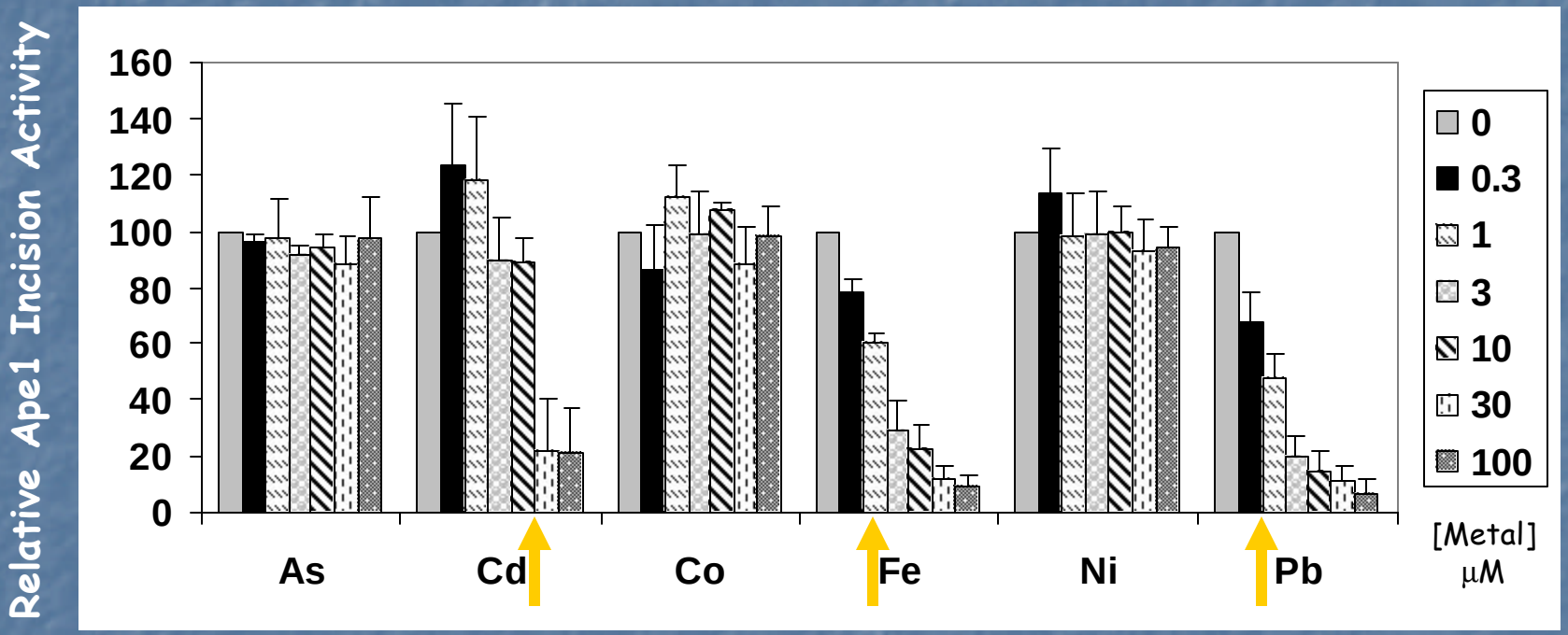
Hypothesis: environmental metals elicit their co-mutagenic and carcinogenic effects by inhibiting DNA repair processes.

We explored the effects of environmental metals on Ape1 activity.

Lead (Pb), Iron (Fe), and Cadmium (Cd) inhibit Ape1 endonuclease activity in the presence of 1 mM $MgCl_2$



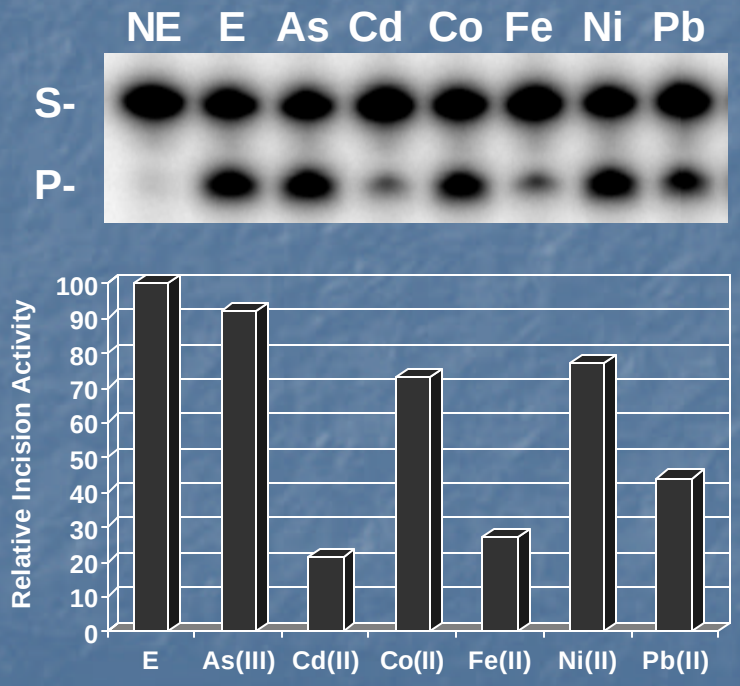
Pb, Fe, and Cd inhibit Ape1 endonuclease activity in the presence of 1 mM $MgCl_2$: Quantitation



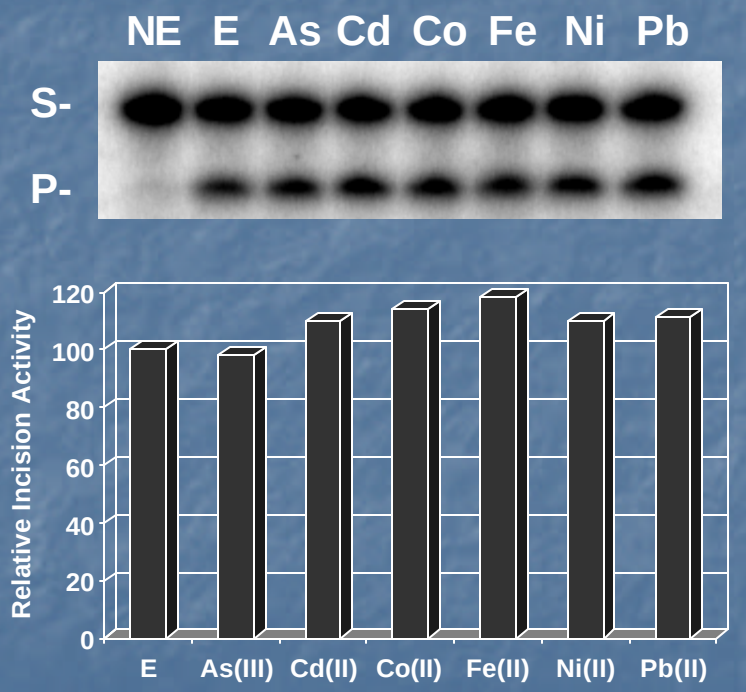
IC_{50} (mM): **26** **1** **0.6**

A similar pattern of inhibition is seen with the homologous protein, Exonuclease III, but not with the unrelated protein Endonuclease IV

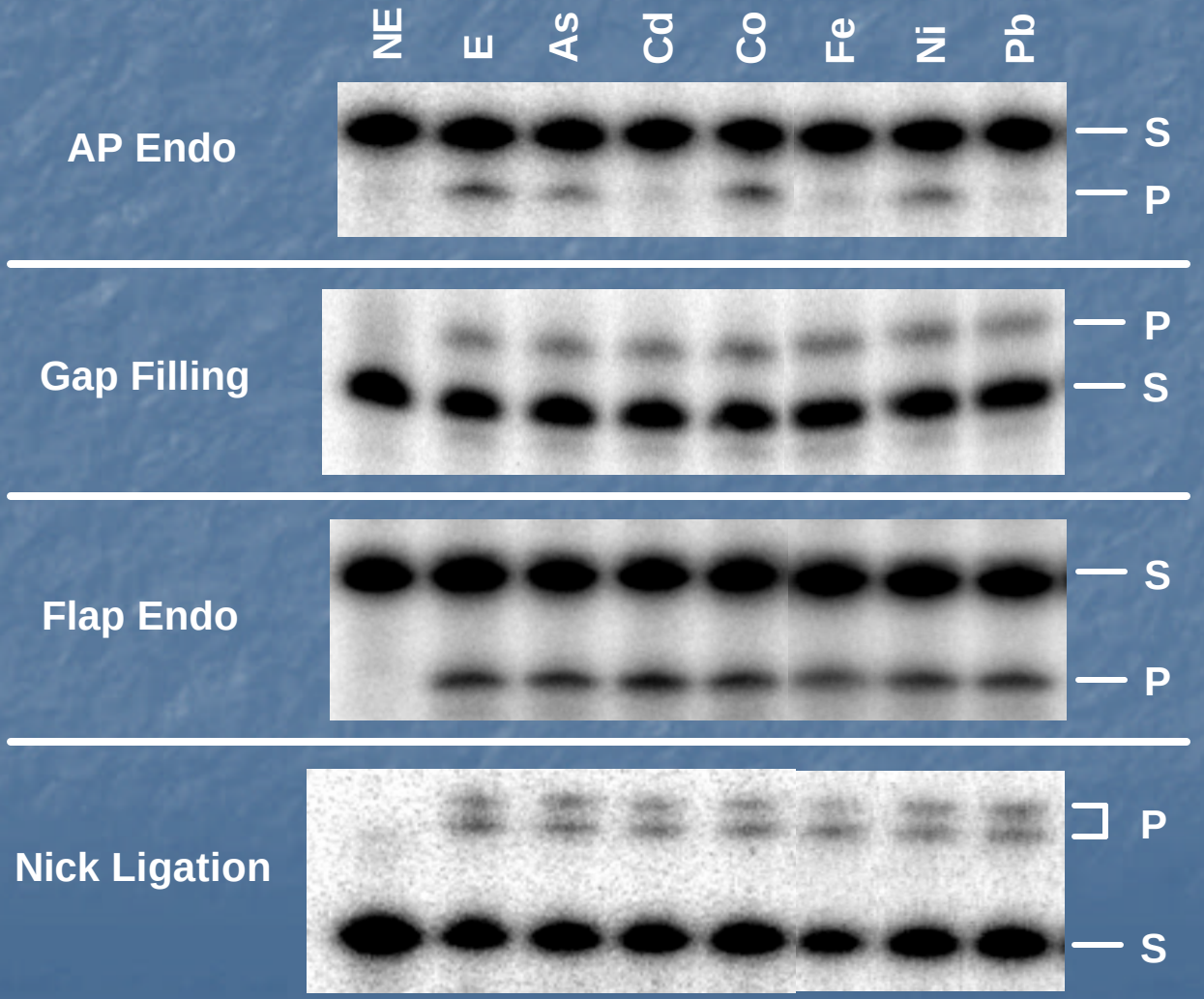
Exonuclease III



Endonuclease IV



Inhibition by lead, iron, and cadmium is specific for AP endonuclease activity in whole cell extracts

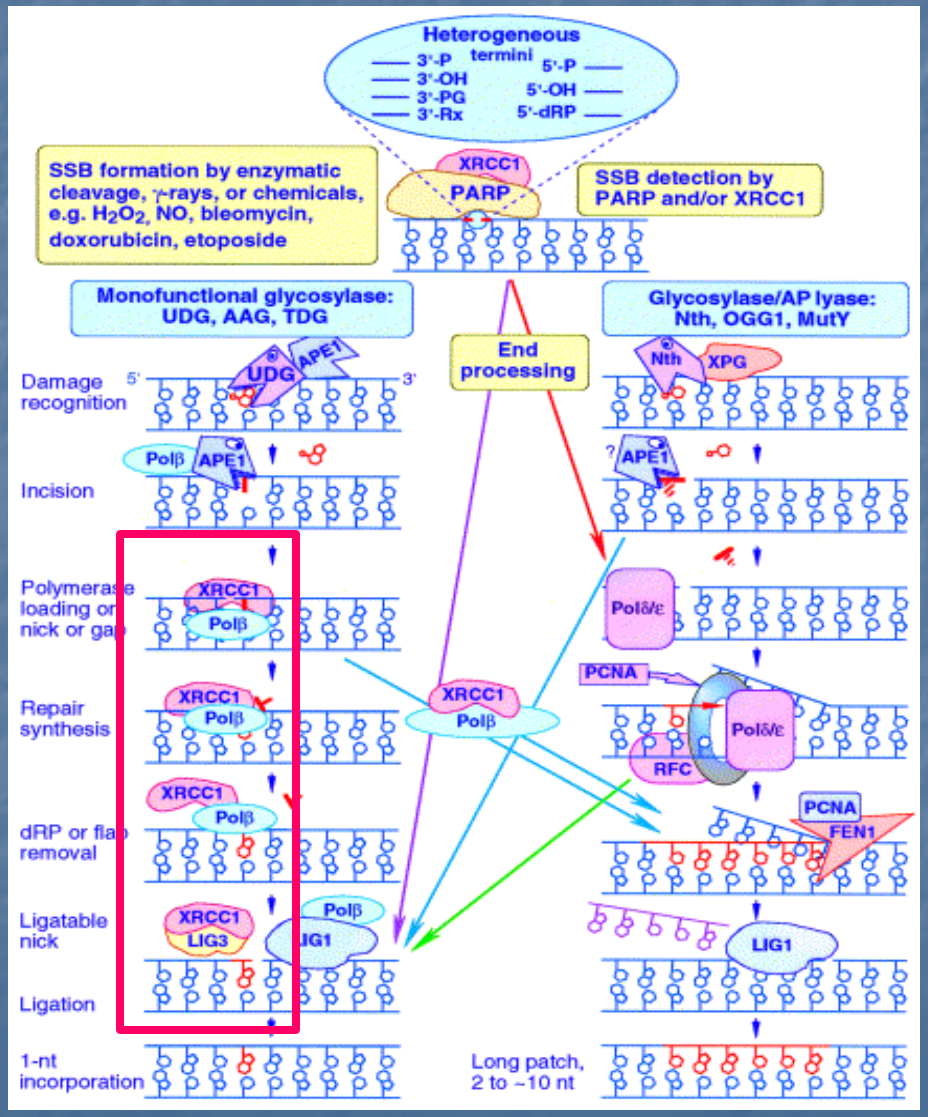


Summary

- Ape1 is **specifically** inhibited at physiological concentrations of environmental metals.
- Lead, iron, and cadmium may elicit their co-mutagenic and/or neurotoxic effects by inhibiting Ape1-specific DNA repair activities.
- Currently, we are determining the mechanism for Ape1 activation and the effect of Pb exposure on steady state AP site levels and mutagenesis in mammalian cells.

A Novel Link between DNA Repair and Replication: XRCC1-PCNA Interaction

XRCC1 (X-ray Cross Complementing 1):



No known enzymatic activity

May bind specifically to nick and gap DNA

Major scaffolding protein, facilitating interactions with many proteins contributing to BER and SSBR

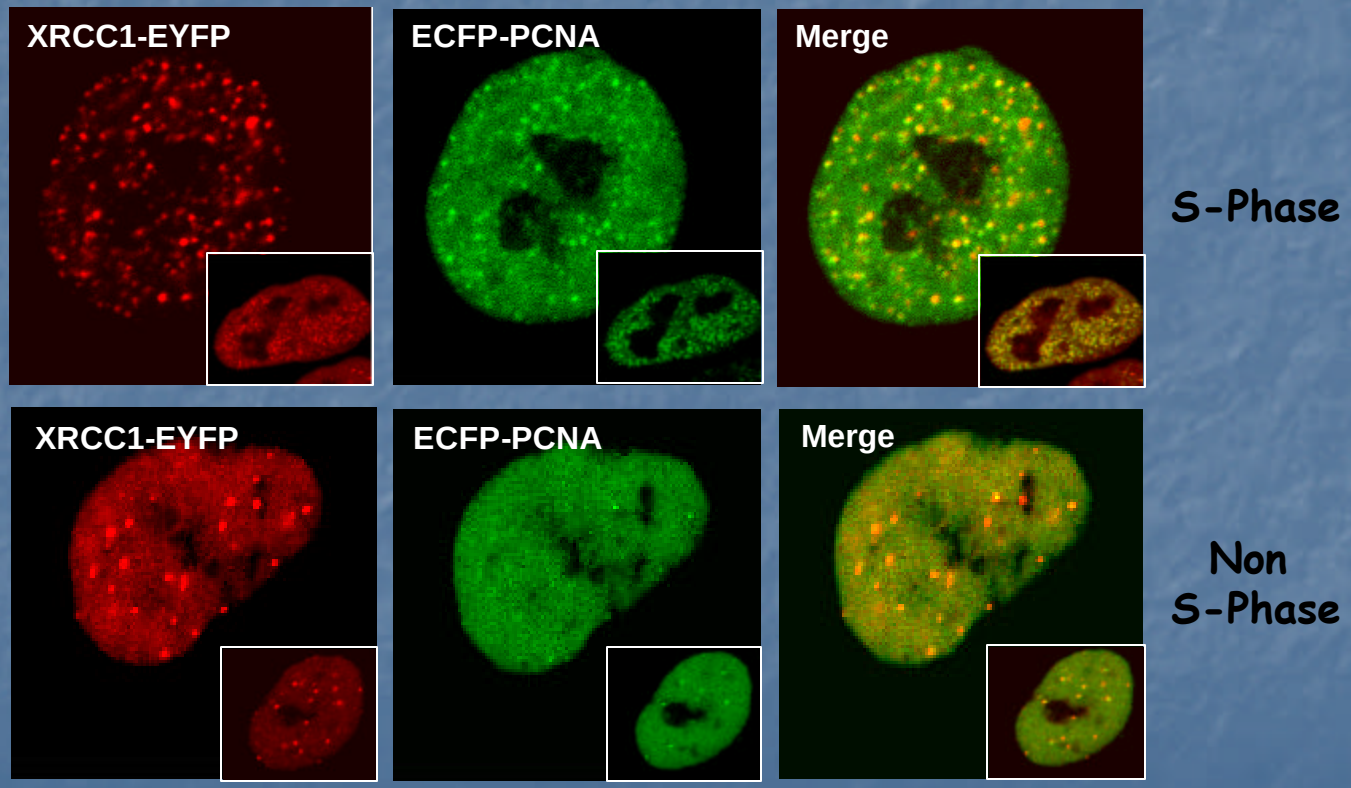
Cellular Phenotypes of XRCC1 Mutants

- Hypersensitive to alklyating agents (MMS and EMS), camptothecin, hydrogen peroxide, and ionizing radiation.
- Markedly elevated sister chromatid exchange (SCE) events.

Given the elevated SCE frequency, we were interested in the potential role of XRCC1 in coordinating repair/replication/recombination.

To explore further the contributions of XRCC1, we employed a fluorescently-tagged system to determine the in vivo localization pattern of the XRCC1 protein.

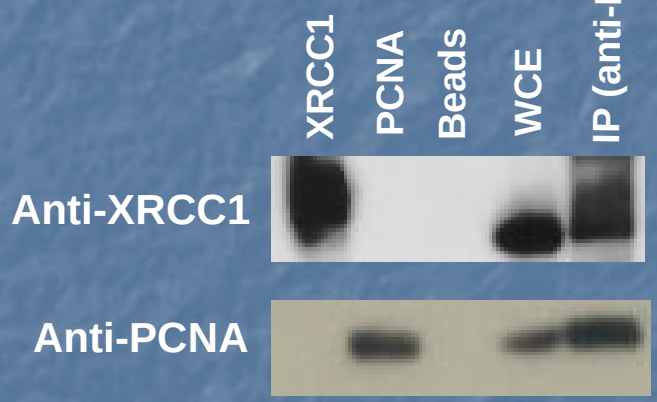
XRCC1 forms foci in undamaged human cells - XRCC1 Foci Coincide with PCNA Replication Factories



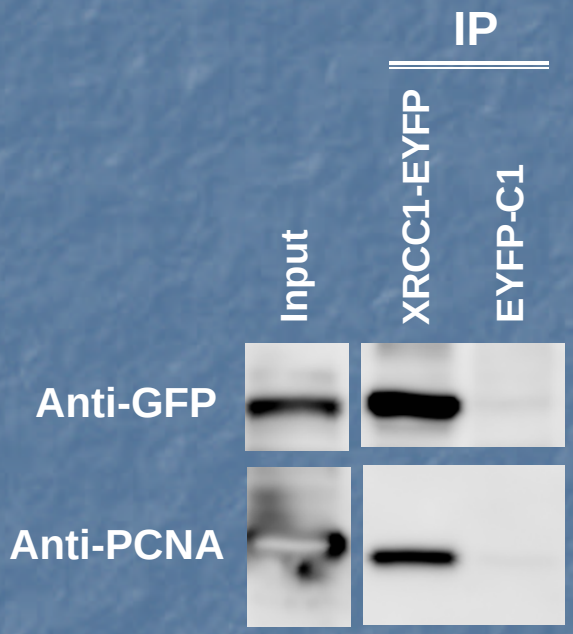
FRET analysis indicates that XRCC1 and PCNA are in close proximity in vivo.

Plasmid Constructs Co-Transfected	N_{FRET} = $FRET/(I_1 x I_3)^{1/2}$
XRCC1-ECFP and EYFP-PCNA#	0.16, 0.16, 0.15, 0.14, 0.12 0.06*
ECFP-PCNA and EYFP-PCNA	0.19, 0.16, 0.13, 0.12, 0.12 0.06*
UNG2-ECFP and UNG2-EYFP	0.05, 0.03 More than 95% of foci: ≤0.01*

XRCC1 and PCNA co-immunoprecipitate, and are therefore in a common protein complex.

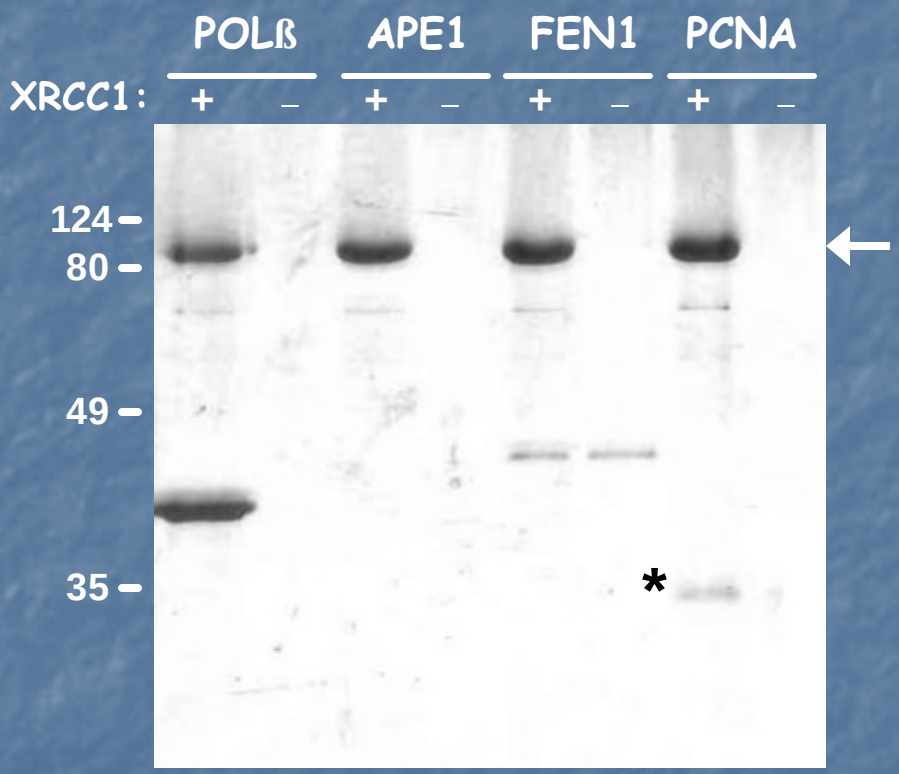


293T Whole Cell Extracts:
Anti-PCNA

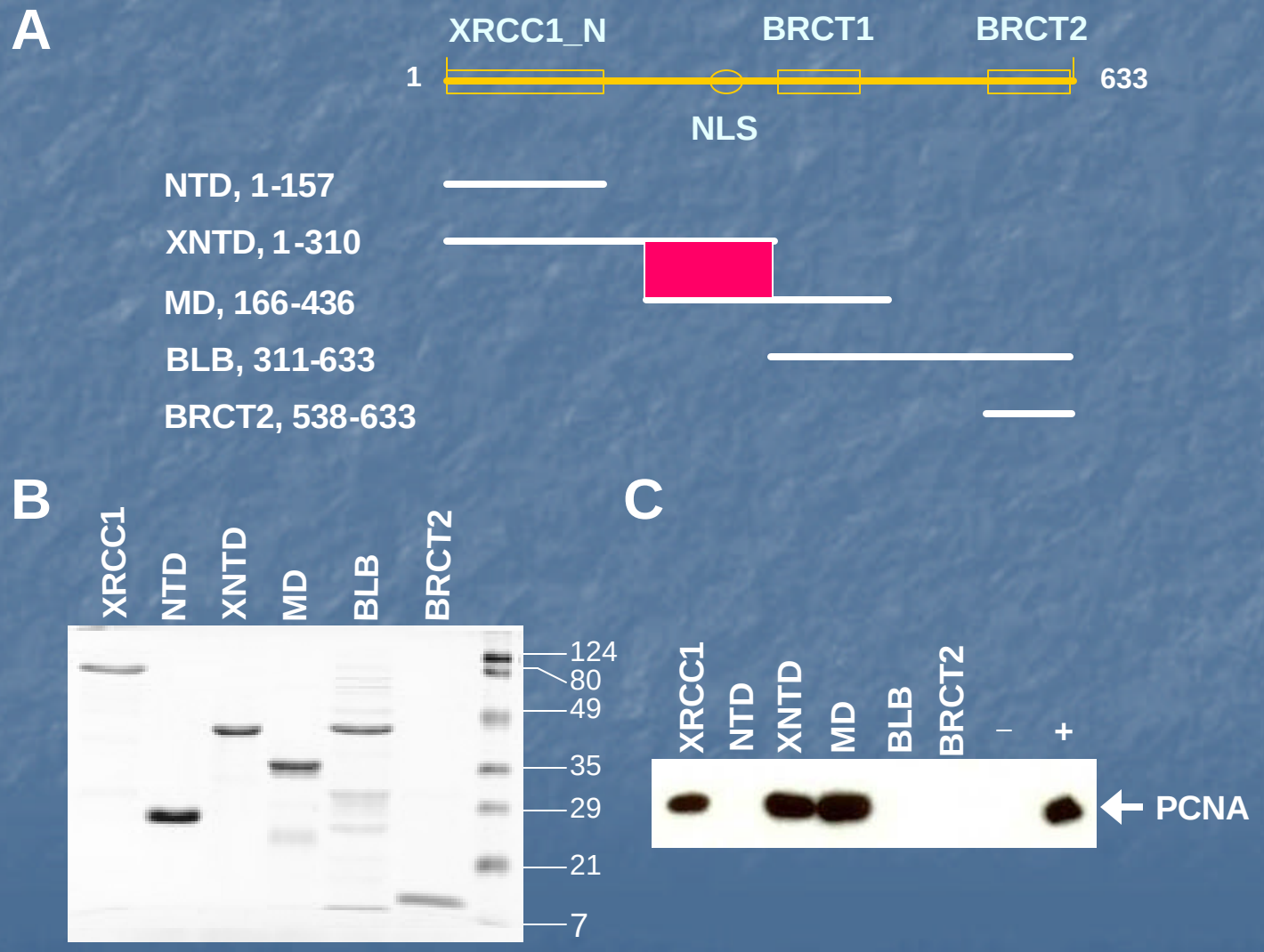


HeLa Cell Extracts from
Stably Transfected Line:
Anti-GFP

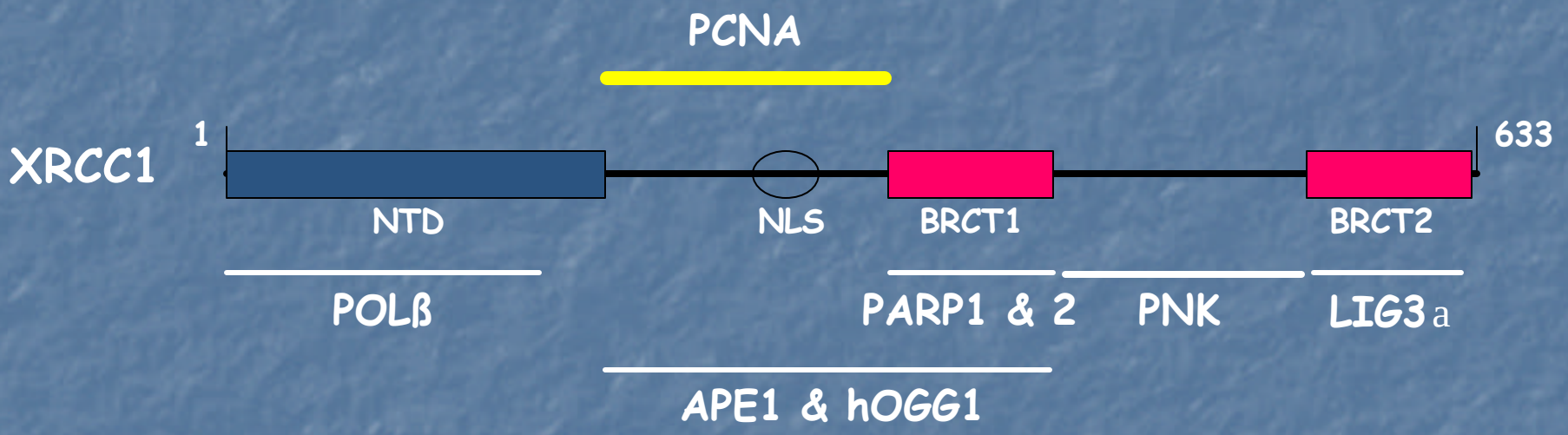
XRCC1 and PCNA Directly Interact



The XRCC1-PCNA Interaction is Mediated by Residues within 166-310 of XRCC1

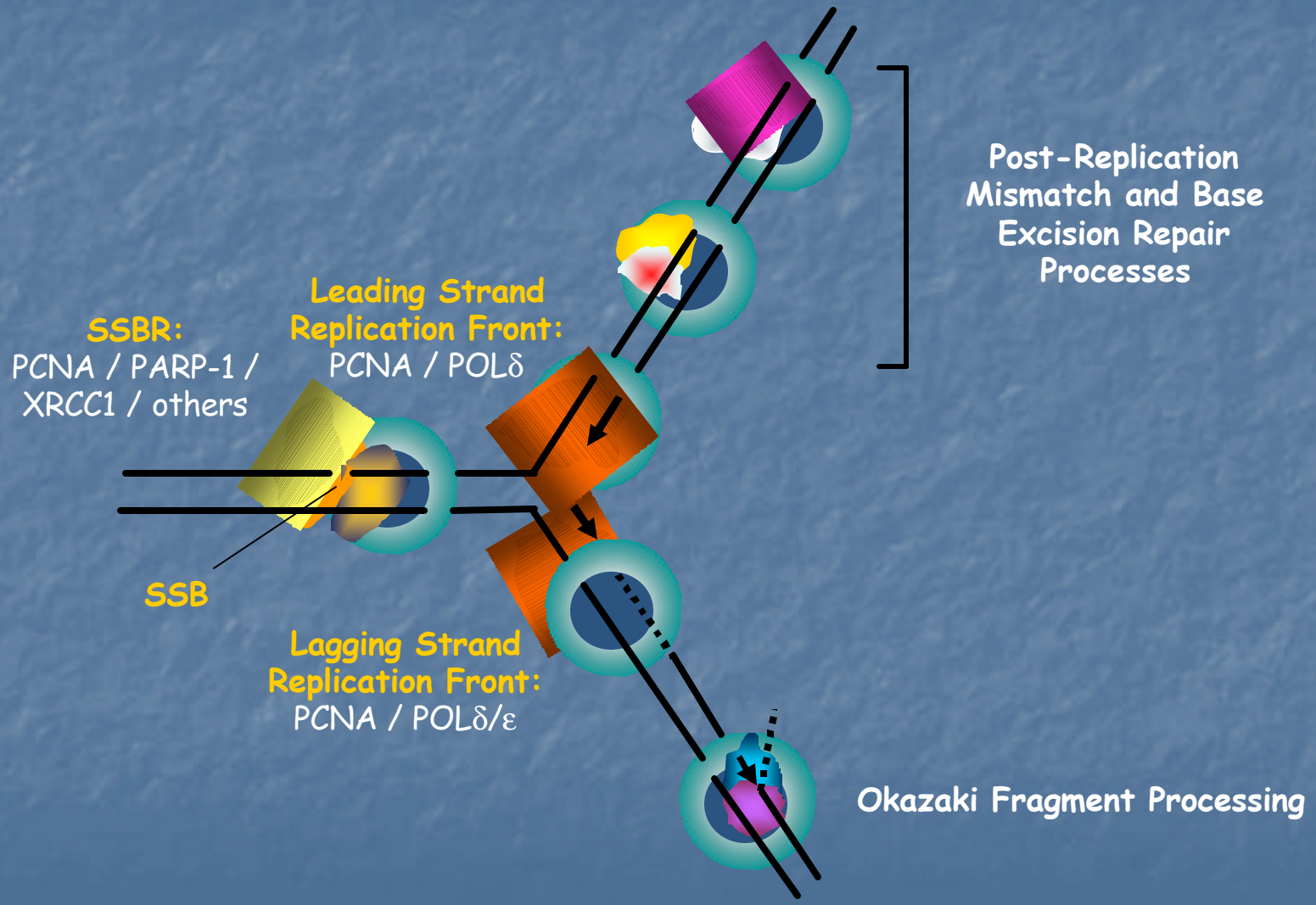


Currently Known XRCC1 Interactors



Not yet mapped on XRCC1 are TDP1 and Aprataxin

Our MODEL for "Replication-Coupled Repair"



Current Directions

- Determine which XRCC1interactions are biologically critical (site-specific mutants), and for which metabolic processes.
- Determine how the numerous interactions may be regulated (post-translational modification).

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