ATR enforces a fidelity-first hierarchy of translesion polymerases by regulating Pol ζ recruitment and activity

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Introduction

Unrepaired DNA lesions hinder replication fork progression, generating DNA gaps that activate ATR checkpoint signaling.

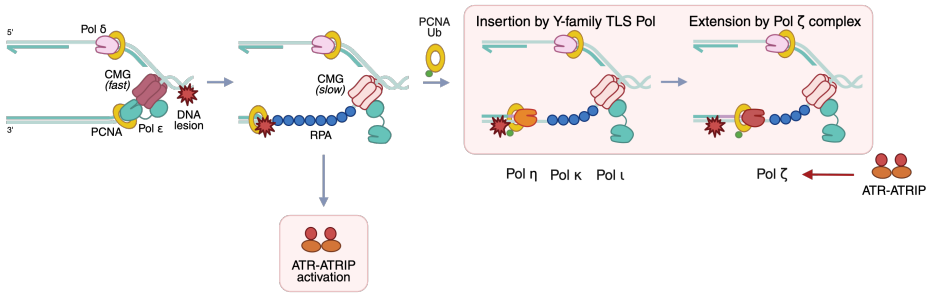
Cells can bypass the damage using specialized translesion synthesis (TLS) polymerases, some of which are highly error-prone.

Despite multiple links between ATR and individual TLS polymerases, it remains unclear whether ATR acts as a general regulator or whether its contribution is lesion-specific.

Aim

Investigate whether and how ATR modulates TLS damage bypass of site-specific DNA lesions using cell-free *Xenopus* egg extracts.

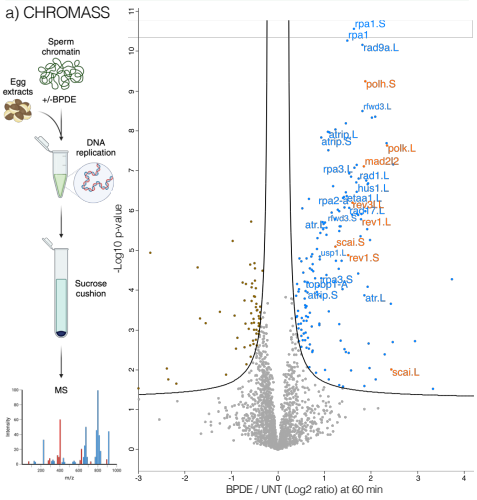
Model



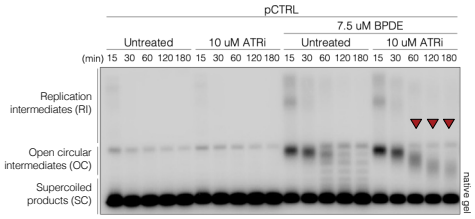
ATR enforces a fidelity-first hierarchy among TLS polymerases, allowing Y family polymerases to operate first, independently of ATR activity, and reserving the versatile and mutagenic Pol ζ as a backup choice to extend DNA synthesis past lesions that have not yet been bypassed.

1. Efficient bypass of BPDE-induced adducts depend on ATR activity

a) CHROMASS



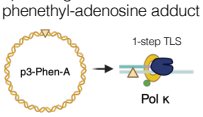
b) Replication of BPDE-damaged plasmids in egg extracts



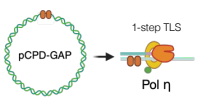
2. ATR activity is required for efficient 2-step TLS bypass

Replication of plasmid containing site-specific lesions:

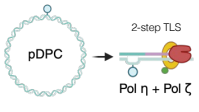
a) Minor groove 3-deaza-3-phenethyl-adenosine adduct

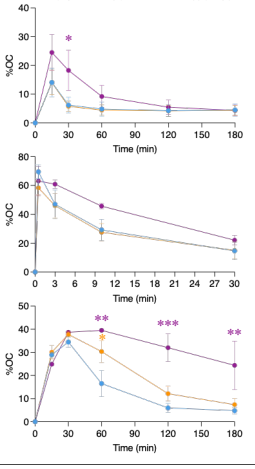


b) Cyclobutane pyrimidine dimer



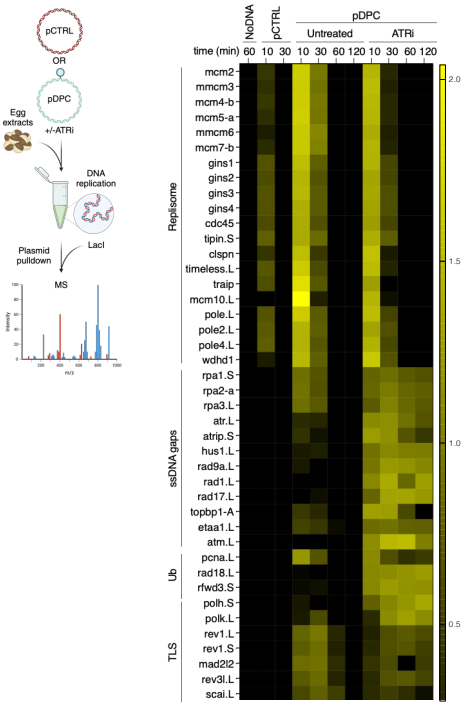
c) DNA-protein crosslink





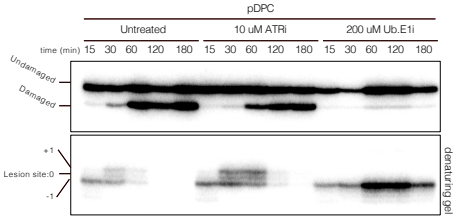
3. ATR activity promotes the recruitment of Pol ζ complex to damaged DNA

a) Plasmid pulldown coupled to mass spectrometry (PP-MS)

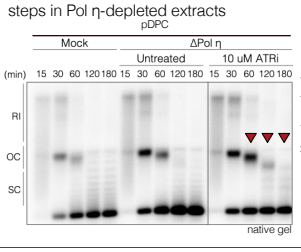


4. ATR activity is required for the Pol ζ-mediated bypass

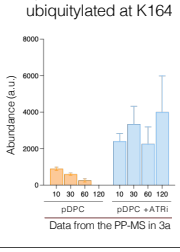
a) DPC bypass at nucleotide resolution



b) Pol ζ carries out insertion and extension steps in Pol η-depleted extracts

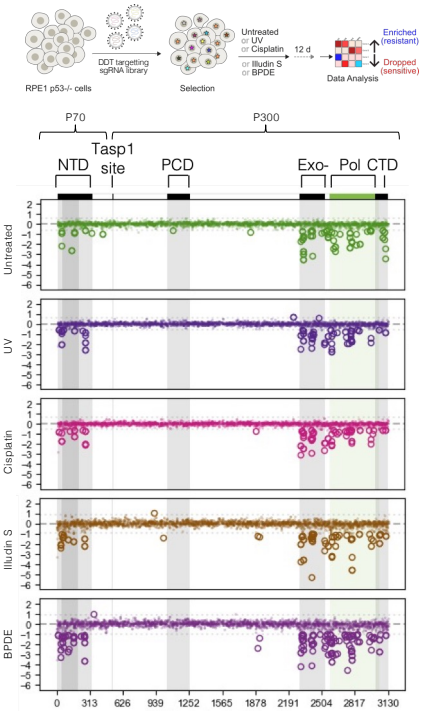


c) Levels of PCNA ubiquitylated at K164



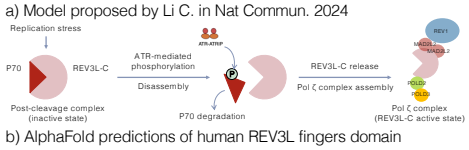
5. Both P70 and P300 REV3L subunits are key for DNA damage tolerance

a) CRISPR base-editing of hREV3L

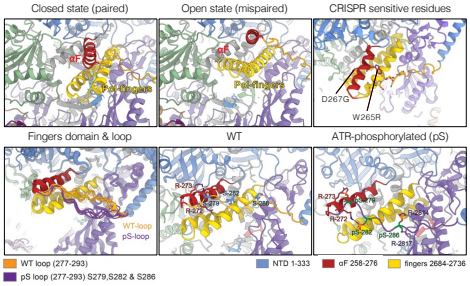


6. ATR phosphorylation could induce REV3L catalytic domain rearrangements

a) Model proposed by Li C. in Nat Commun. 2024



b) AlphaFold predictions of human REV3L fingers domain



References. DeStephanis D., Biochem Biophys Res Commun. 2016; Gallina I., Mol Cell. 2021; Göhler T., Cell Biol. 2011; Goulas J., Nat Commun. 2026; Hirano Y. and Sugimoto K., Curr Biol. 2006; Larsen N.B., Mol Cell 2019; Lee Y.S., PNAS 2014; Li C., Nat Commun. 2024; Malik R., Nat Struct Mol Biol. 2024; Selliés-Baiget S., Nat Struct Mol Biol. 2024; Wang F., Nucleic Acid Res. 2020; Yoon JH., Genes Dev. 2026.