



Human DNA polymerases ι and η interact with ubiquitin; a mechanism for controlling their sub-cellular localization

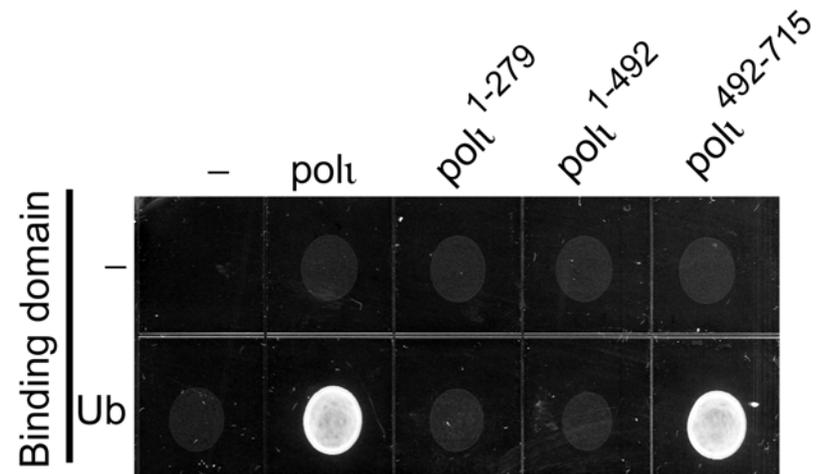
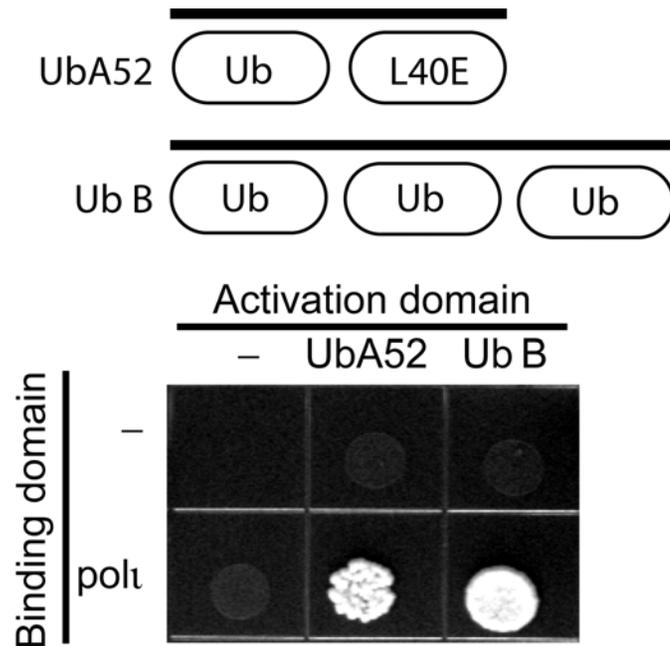
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**Goal: Identify proteins that
interact with pol₁**

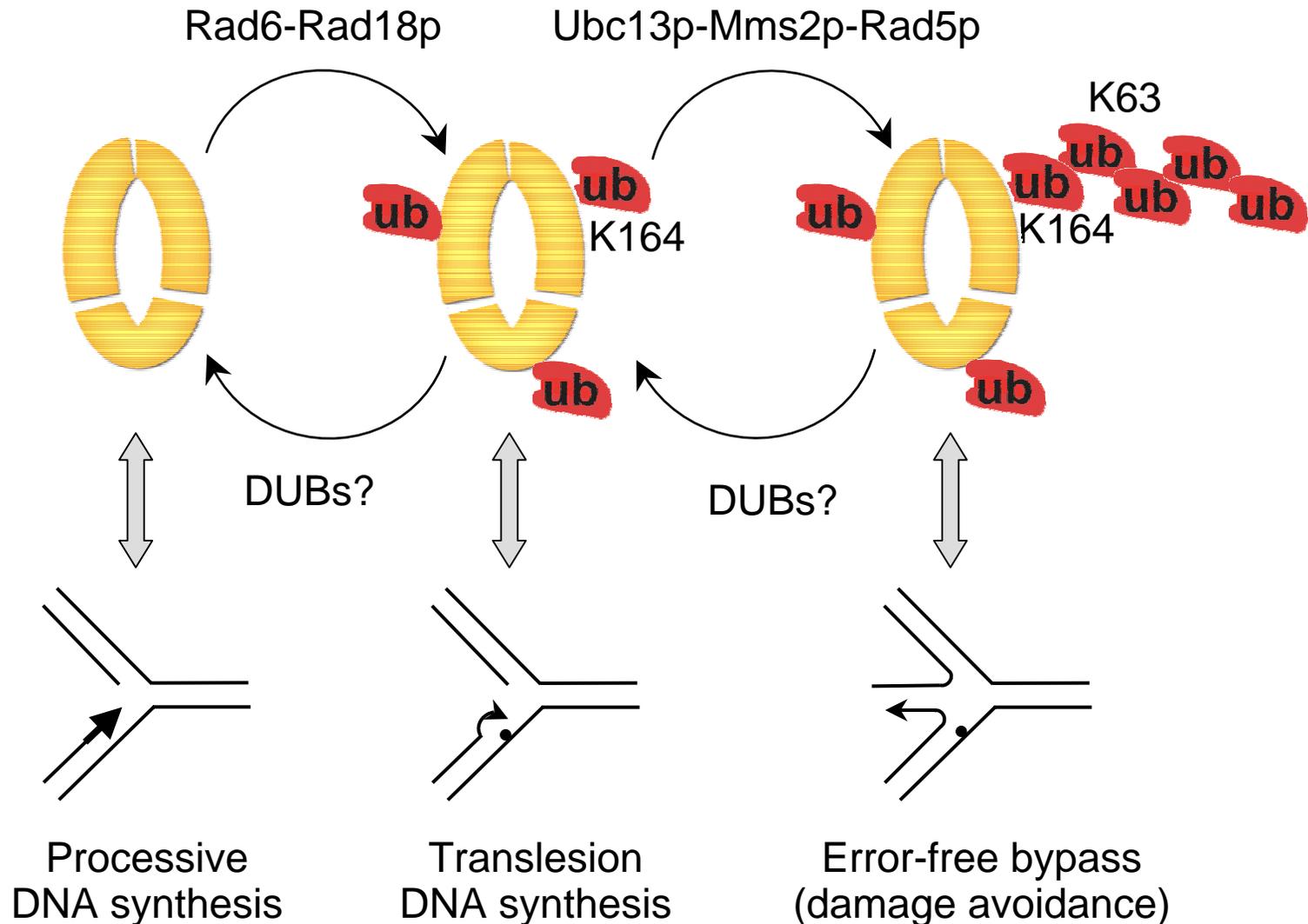
**Approach: Perform yTH screen
using full-length pol₁ as the bait**

**Result: ~30% of positive interactions
were ubiquitin!**

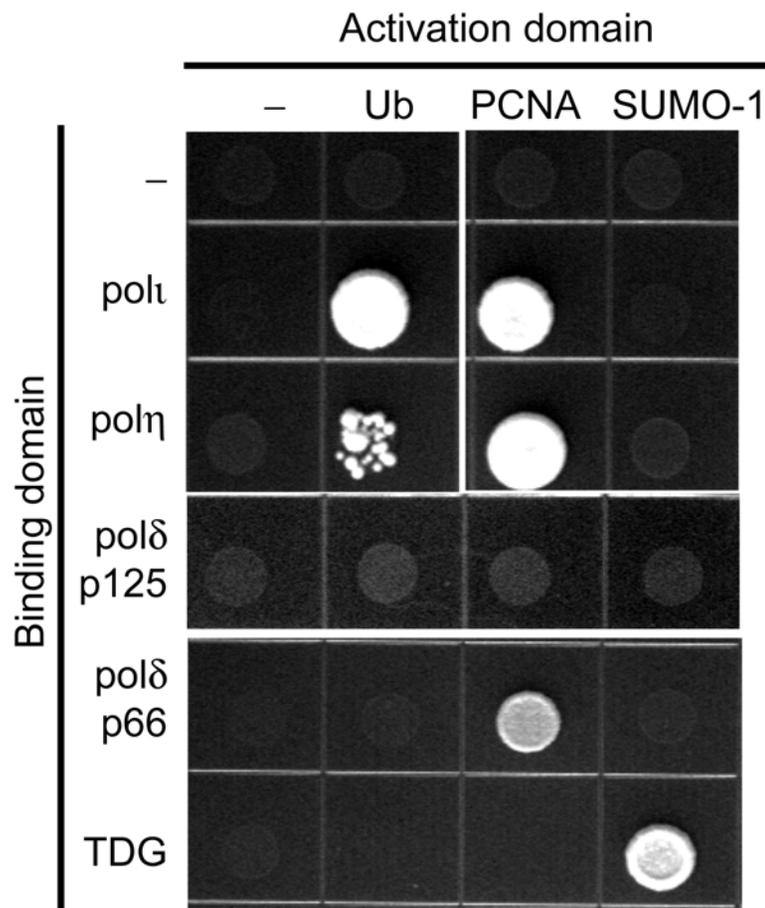
The c-terminus of pol_I interacts with ubiquitin



PCNA is mono- and poly-ubiquitinated after DNA damage!

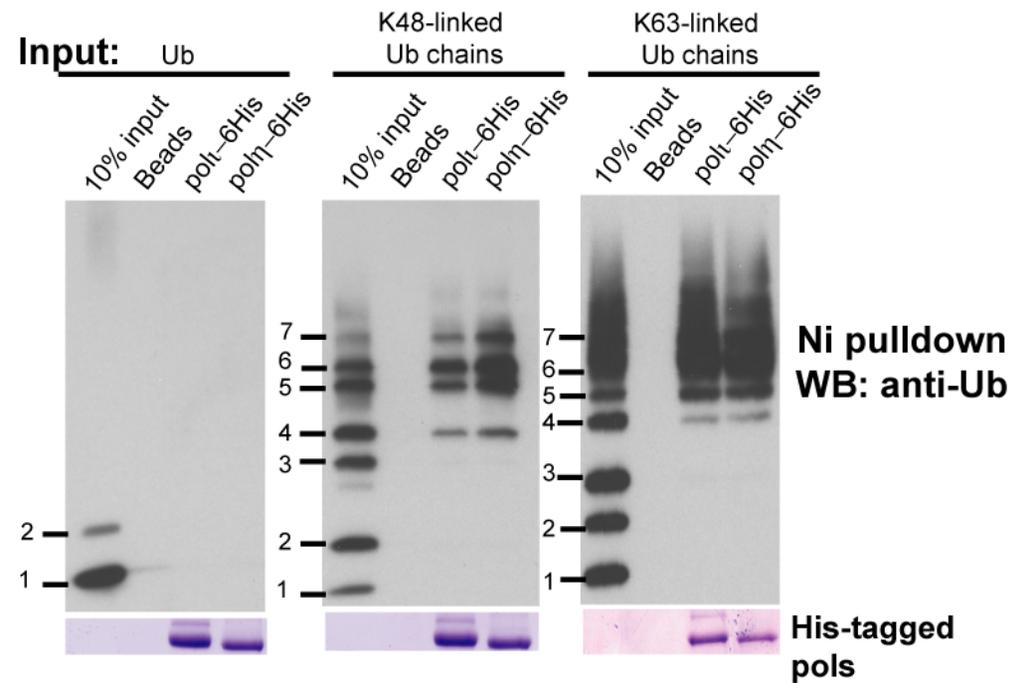
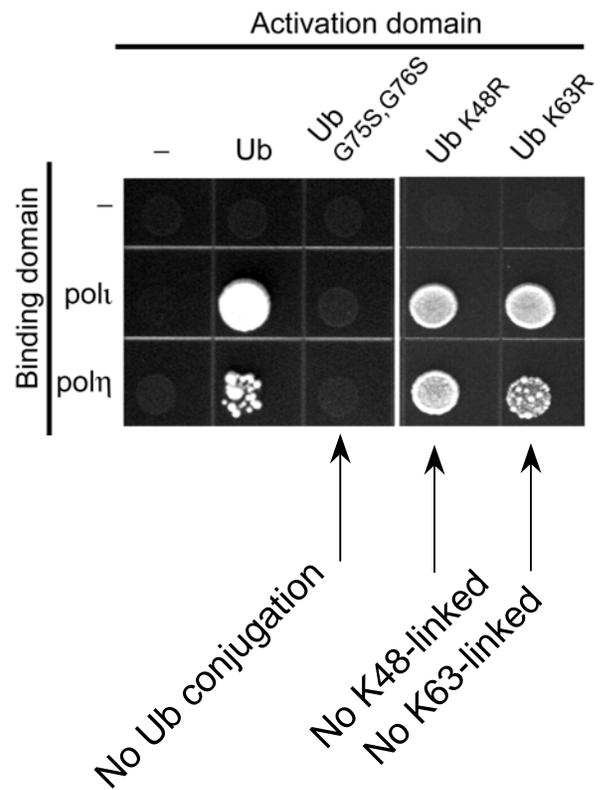


Pols η and ι interact with ubiquitin while $\text{pol}\delta$ does not

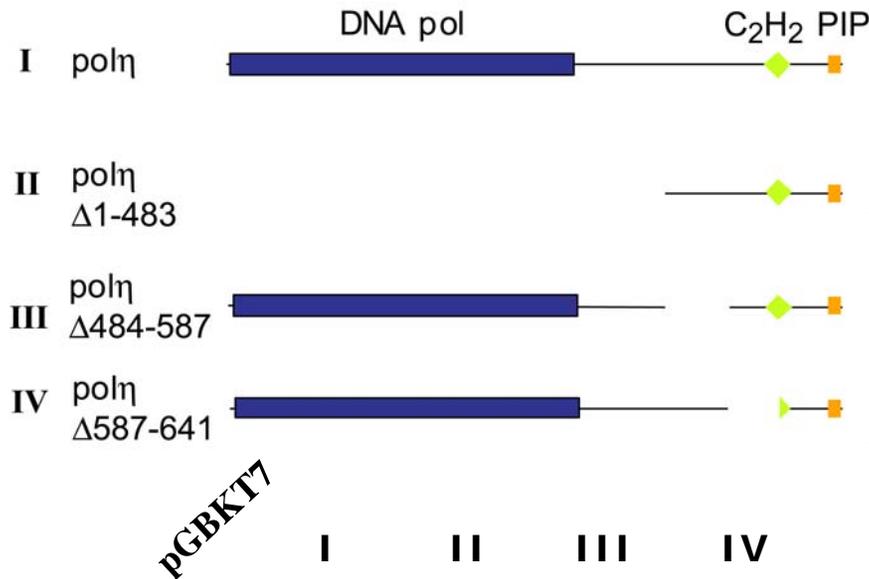


- Both Y-family polymerases bind Ub and PCNA
- They do not bind to SUMO-1, a Ub-like protein
- Subunits of $\text{pol}\delta$, the cells main replicase, do not bind Ub

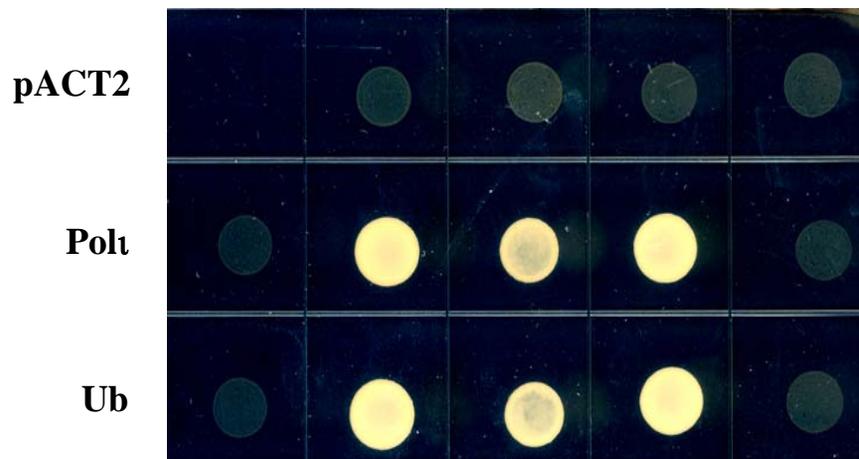
The interactions are noncovalent, but require ubiquitin conjugation



Protein interactions mediated by pol η 's Zn-finger

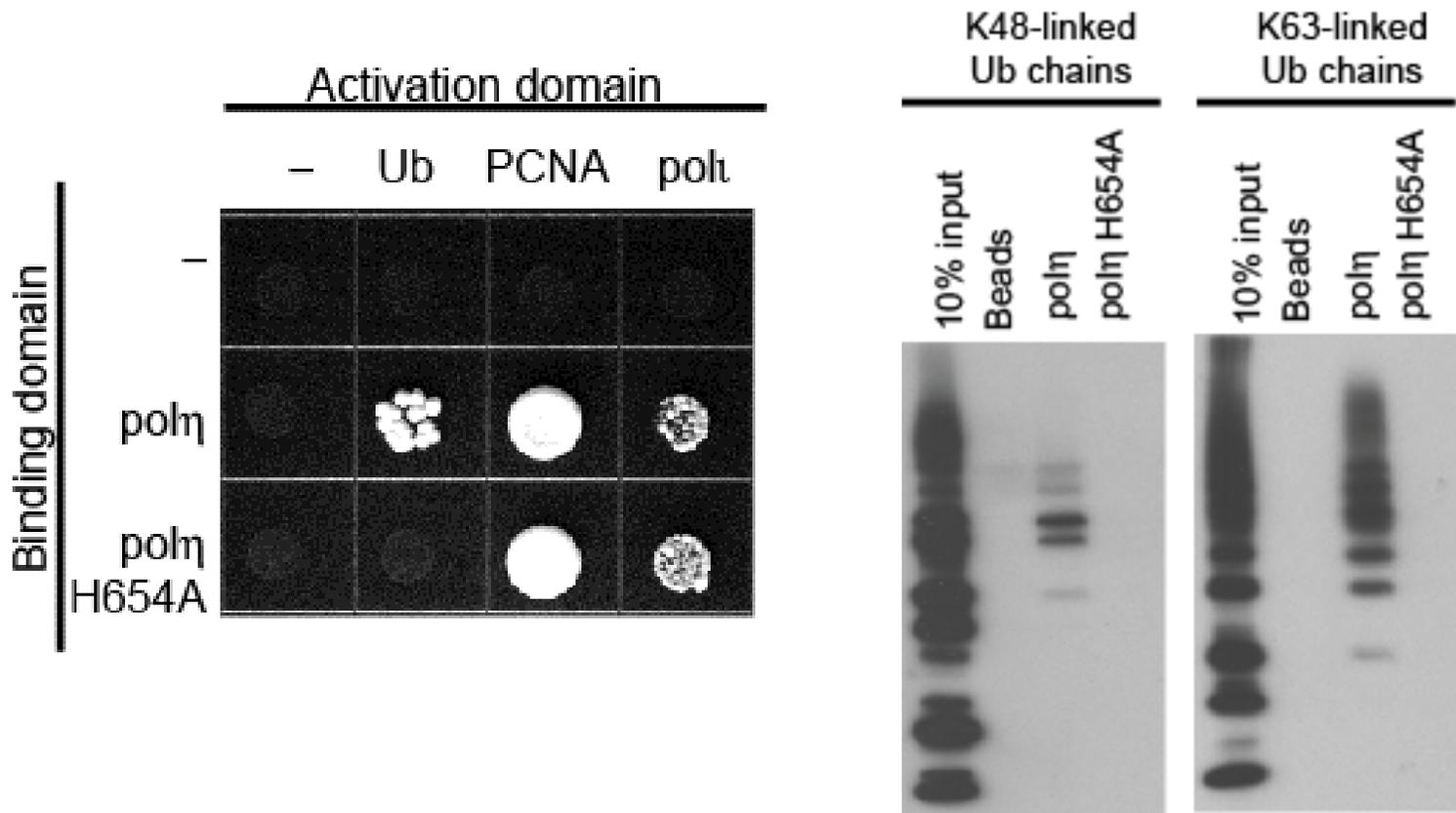


- Zn-fingers are known to mediate protein/protein and protein/DNA interactions
- It appears that pol η 's Zn-finger is involved in the interaction with pol ι and Ub



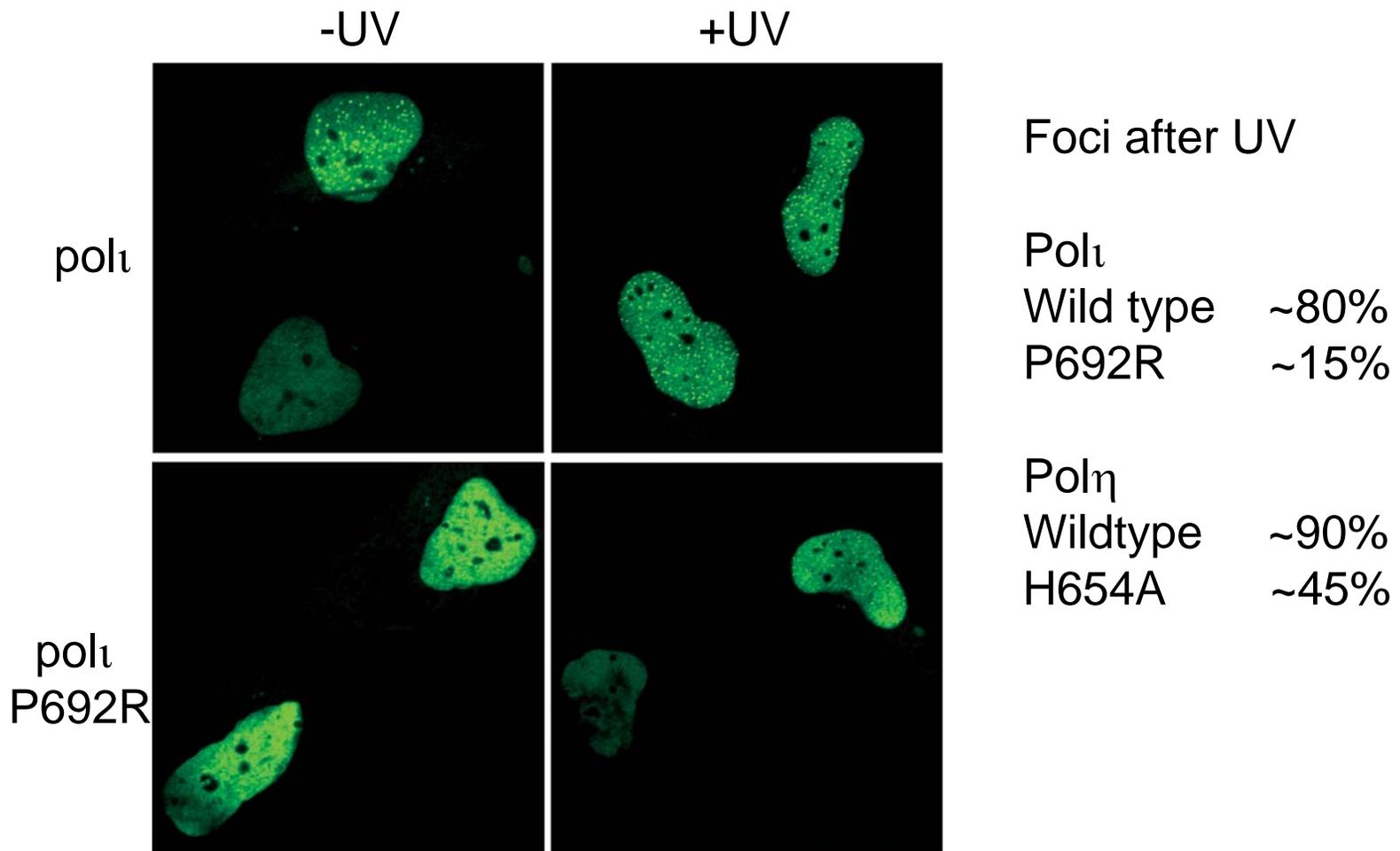
Identification of the region in $pol\text{I}$
required for the interaction with
 $pol\eta$ and Ub: the power of genetics
and a case of serendipity!

The zinc-finger of pol η is also a novel ubiquitin binding motif.

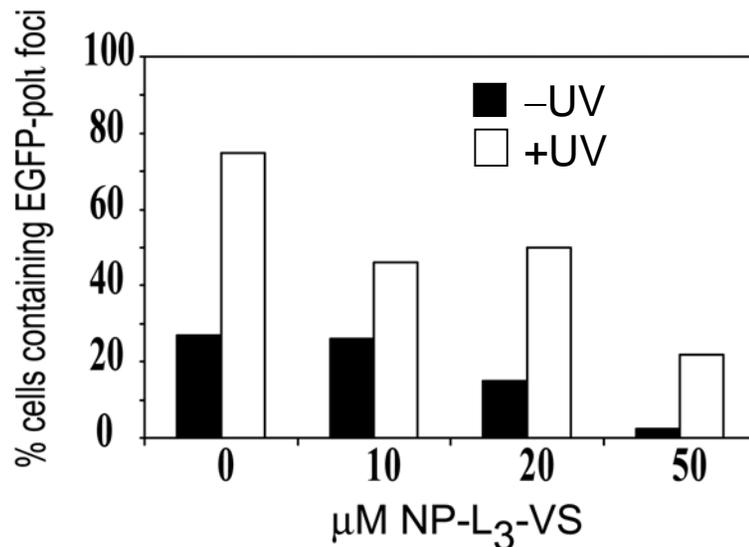
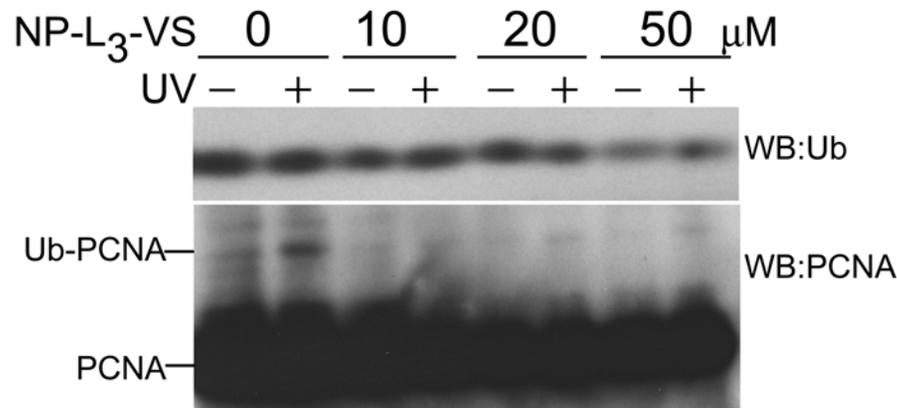


UBZ-DQVP**CEK**CGSLVPVWDMPE**H**MDY**H**FALEL
 ↓
 A

Ub binding mutants exhibit reduced foci formation after UV

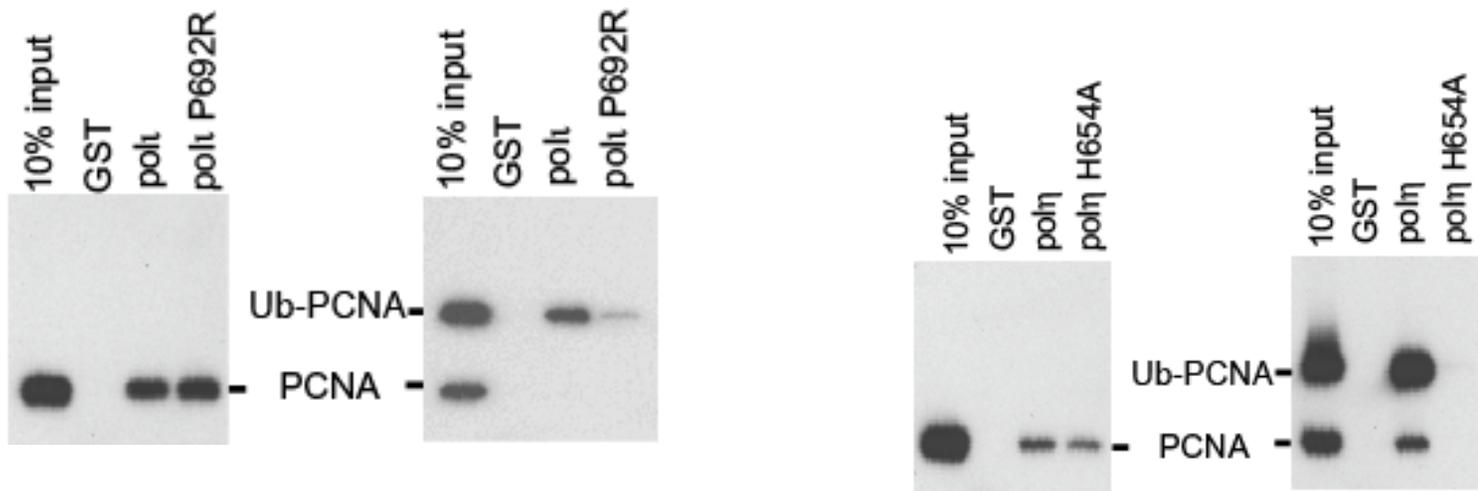
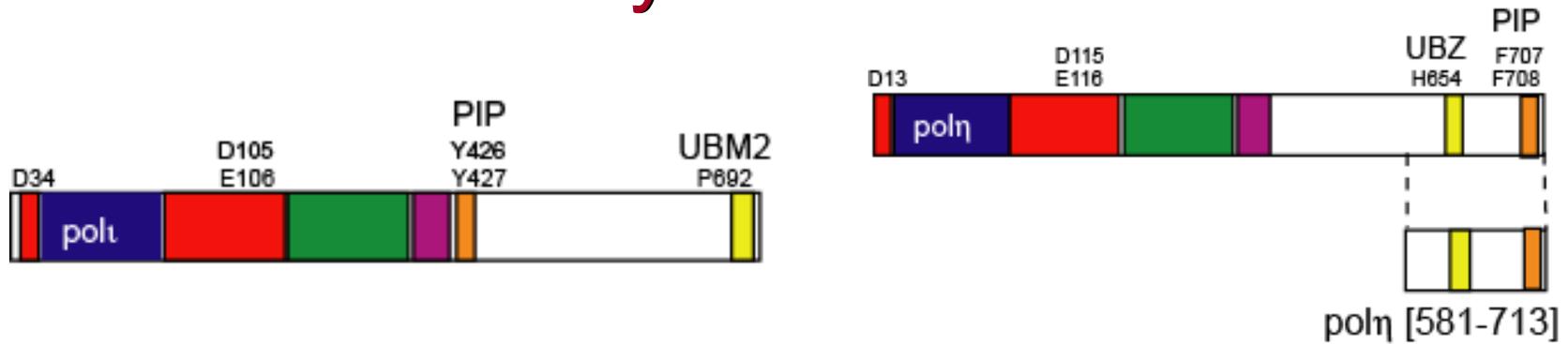


Proteasome inhibition also reduces foci formation

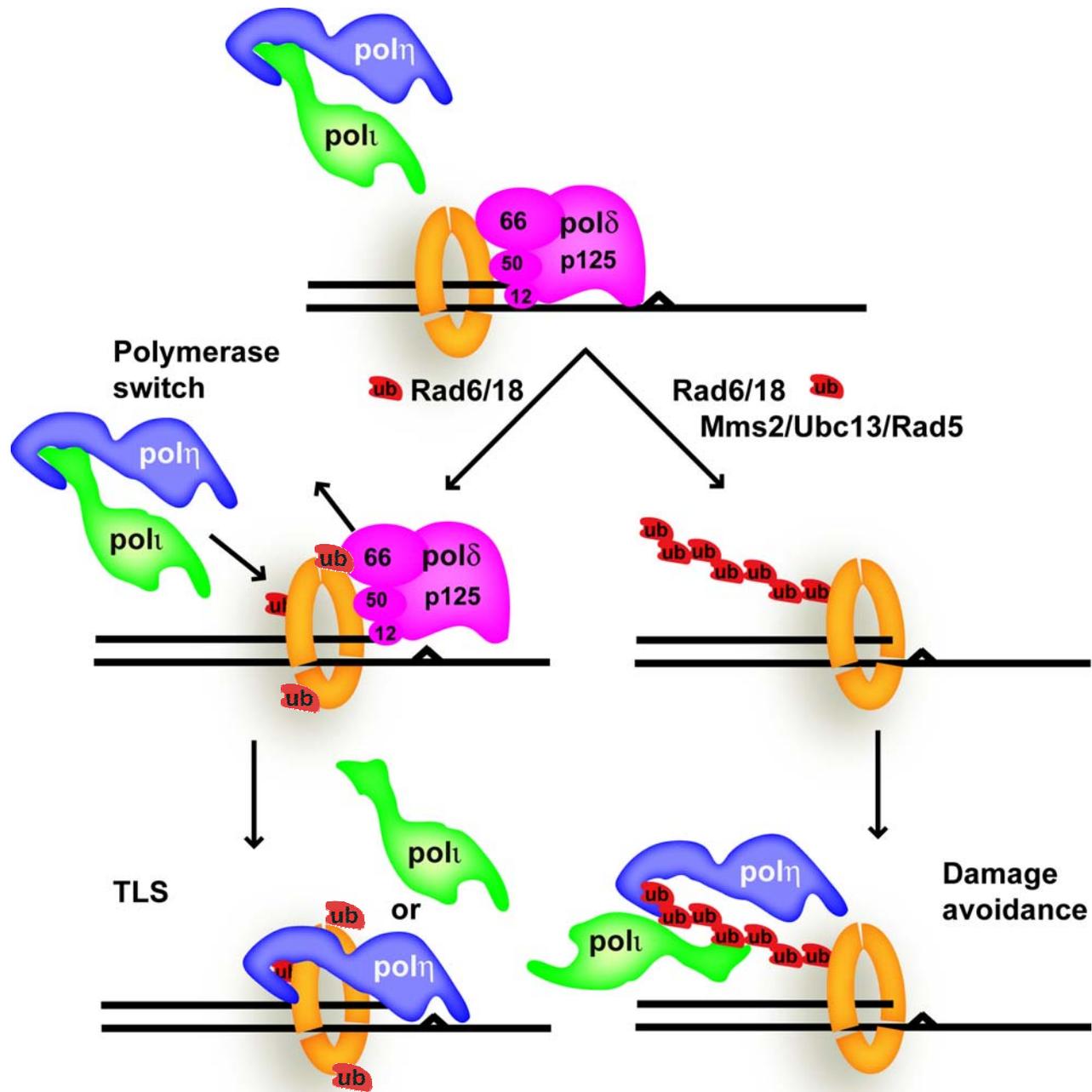


- Blocking the proteasome prevents recycling of Ub
- Therefore, there is limited Ub for *de novo* ubiquitination
- Levels of Ub-PCNA after UV are reduced
- Foci are reduced

Ub binding mutants have reduced affinity for Ub-PCNA



GST-pulldown
WB: anti-PCNA



Conclusions

- Both pol ι and pol η bind Ub.
- Both enzymes bind Ub-PCNA.
- Pol δ binds PCNA but not Ub.
- Ub-binding mutants have a reduced ability to form foci after DNA damage.
- Are there are other ubiquitinated targets to which either pol ι or pol η bind?

Acknowledgments

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