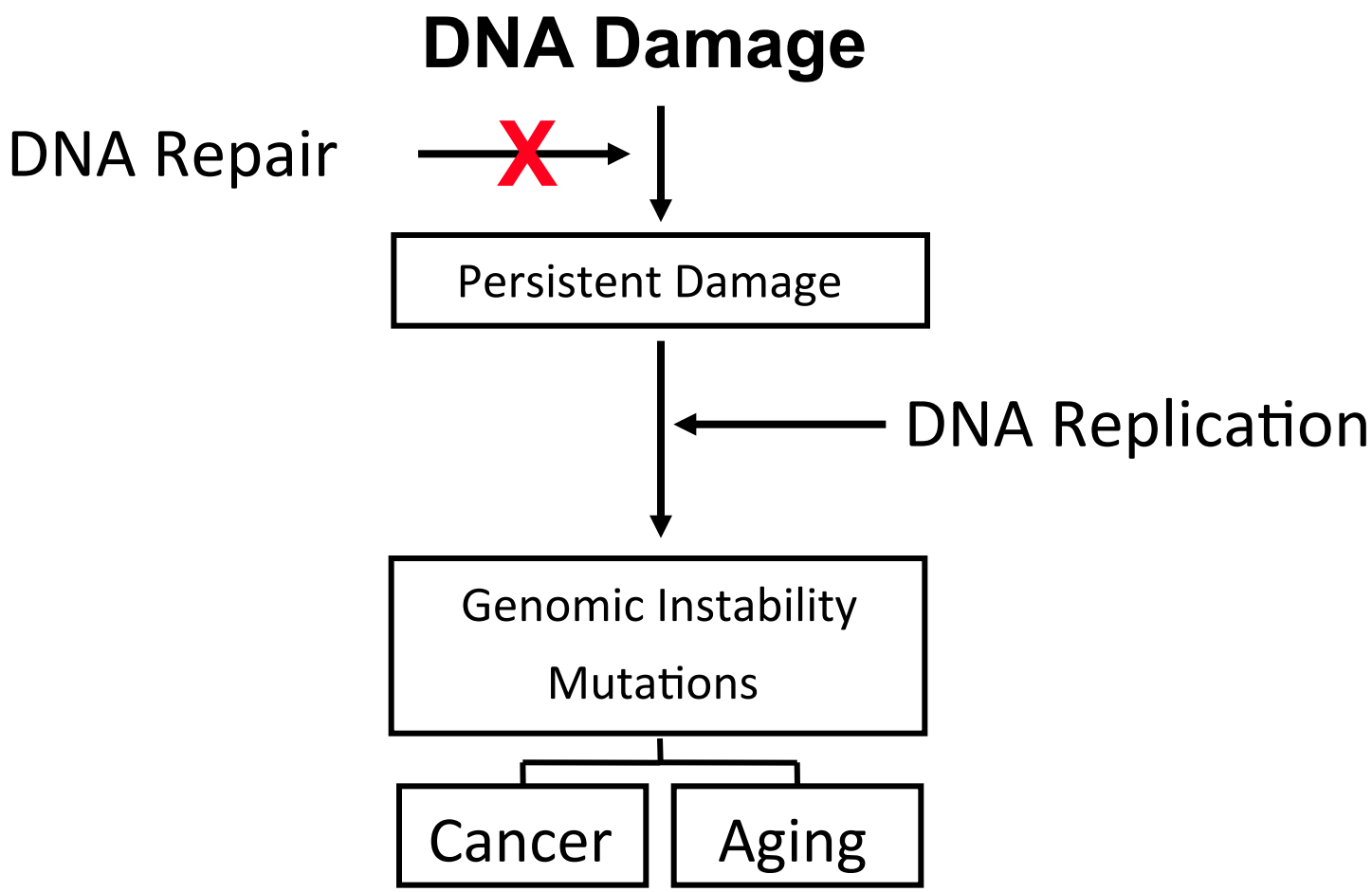
Regulation of translesion DNA synthesis: from transcriptional to posttranslational control

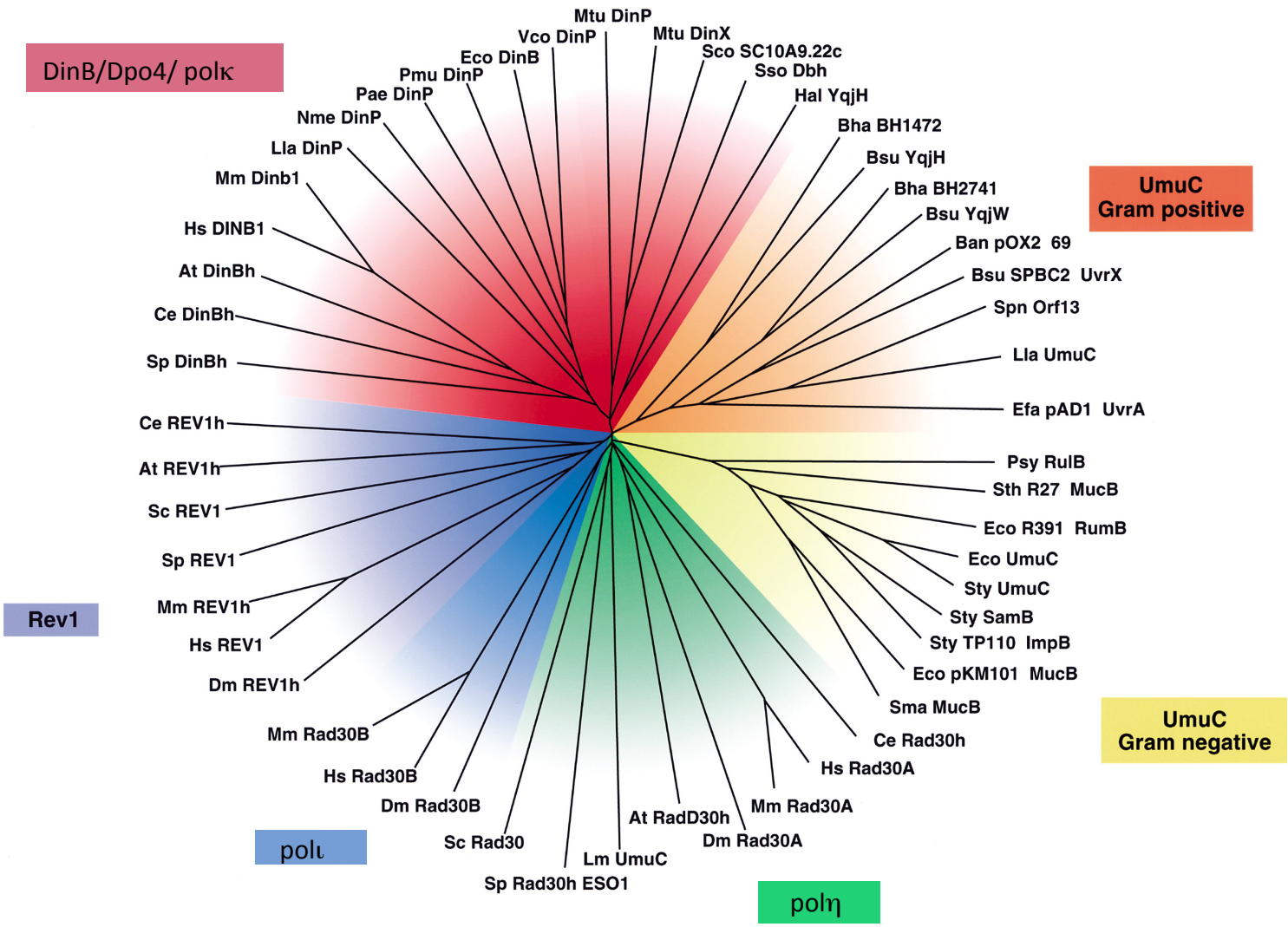
Roger Woodgate

National Institutes of Health

Consequences of DNA damage

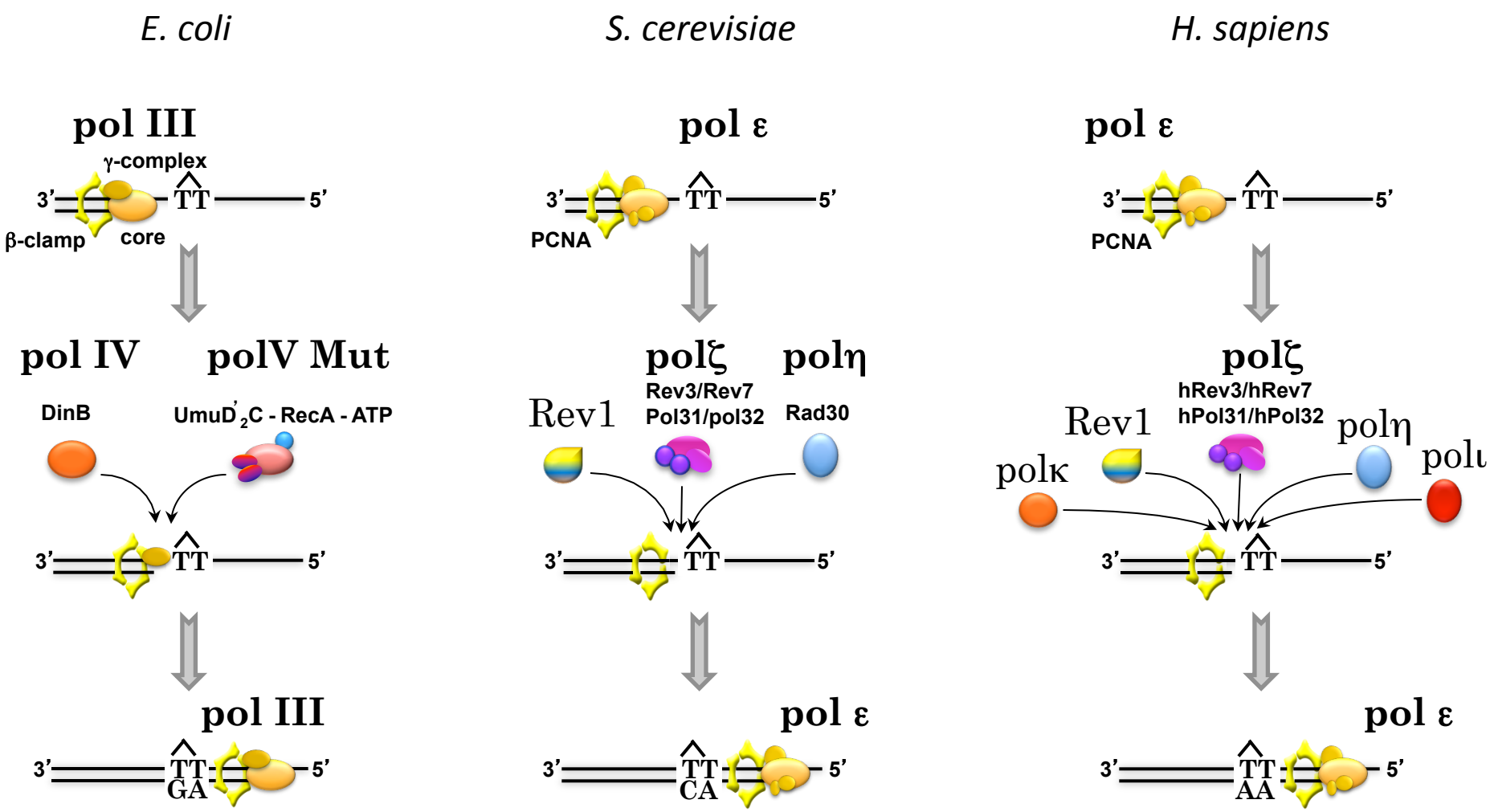


Unrooted Phylogenetic Tree of the Y-Family of Polymerases



Ohmori *et al.*, Molecular Cell (2001) 8; 7-8

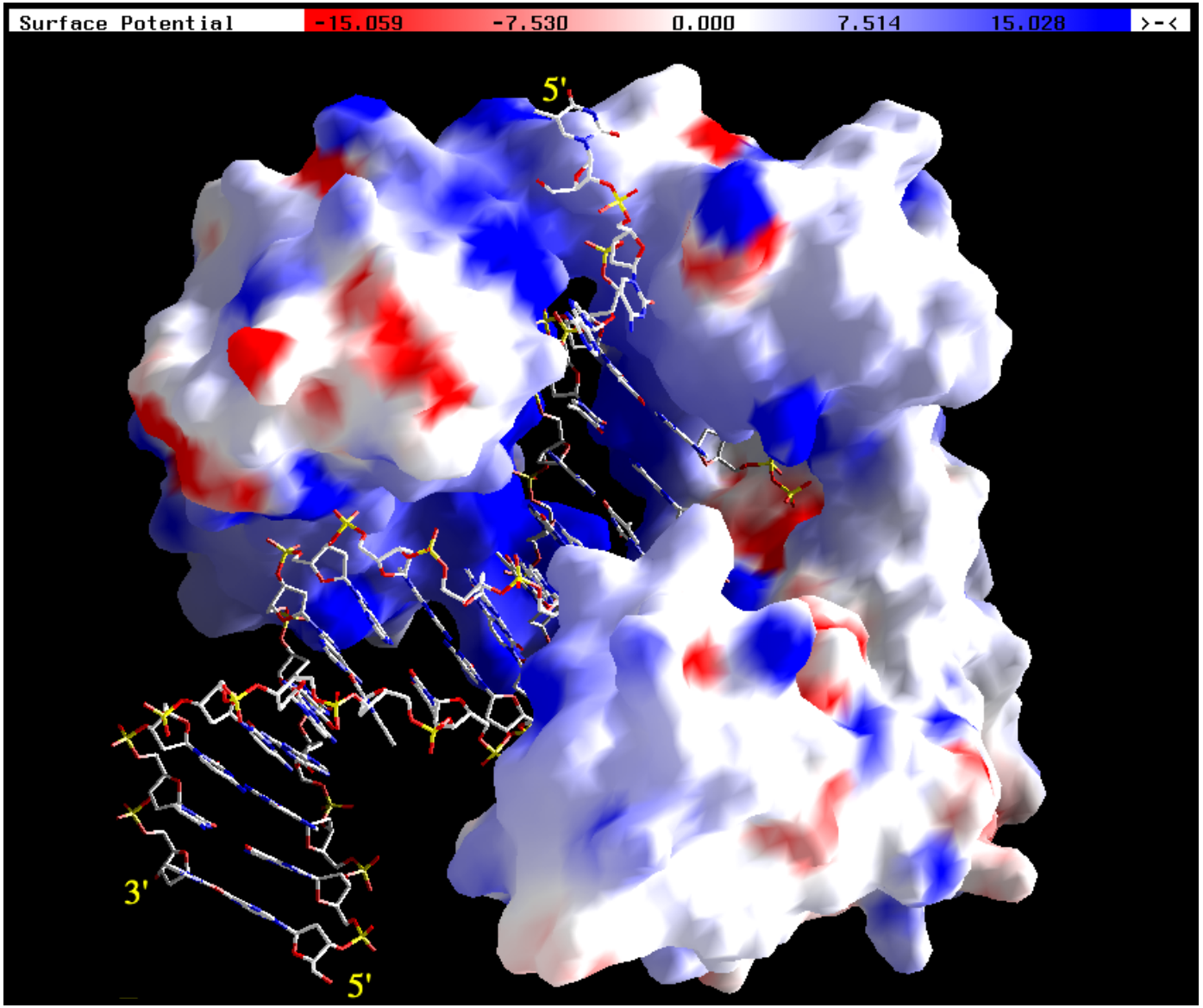
Conserved mechanism of translesion DNA synthesis



Error-free or error-prone replicative bypass

Woodgate, Genes and Dev (1999) 13; 2191-2195

Electrostatic surface of Dpo4



Ling *et al*., Cell (2001) 107; 91-102

Regulation of damage-induced mutagenesis in *E.coli*: the pol V story

Identification of the *umu* locus

Molec. gen. Genet. 156, 121–131 (1977)

MGG

Isolation and Characterization of Mutants of *Escherichia coli* Deficient in Induction of Mutations by Ultraviolet Light

Takesi Kato and Yukiko Shinoura

Department of Fundamental Radiology, Faculty of Medicine, Osaka University, Kita-ku, Osaka 530, Japan

Molec. gen. Genet. 165, 87–93 (1978)

MGG

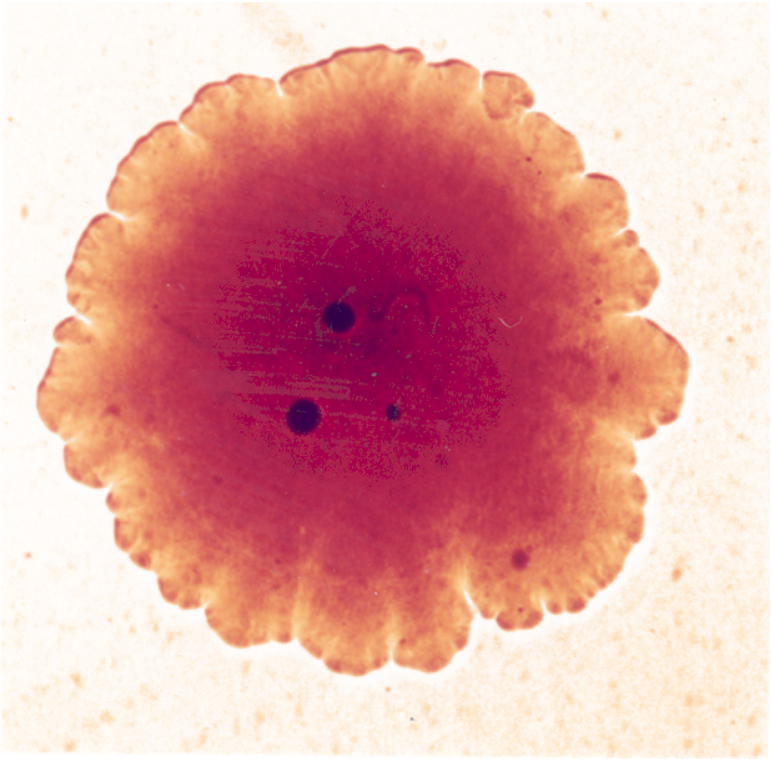
Uvm Mutants of *Escherichia coli* K12 Deficient in UV Mutagenesis

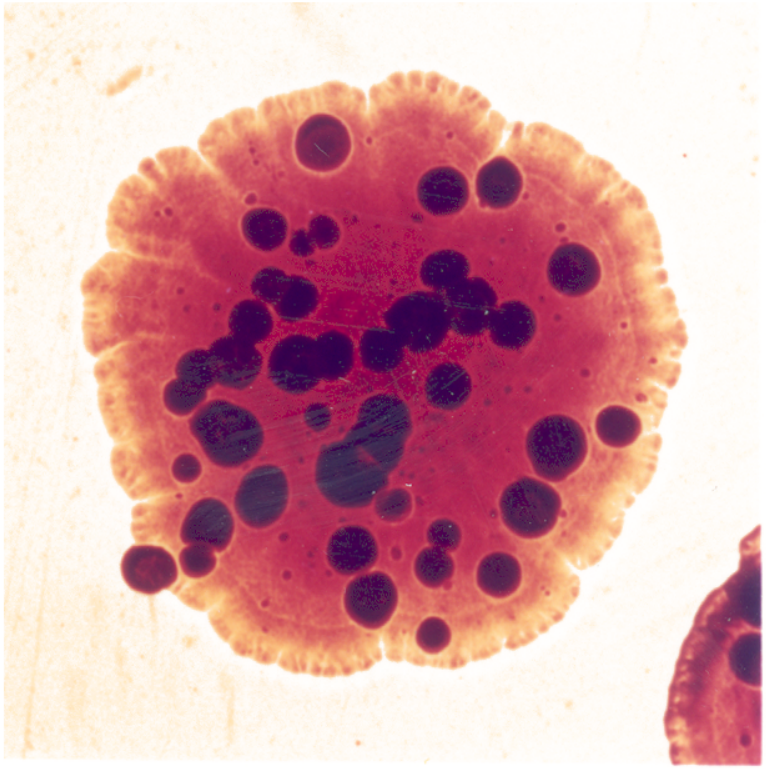
I. Isolation of *uvm* Mutants and Their Phenotypical Characterization in DNA Repair and Mutagenesis

Gerhard Steinborn

Zentralinstitut für Genetik und Kulturpflanzenforschung der ADW der DDR, 4325 Gatersleben, German Democratic Republic

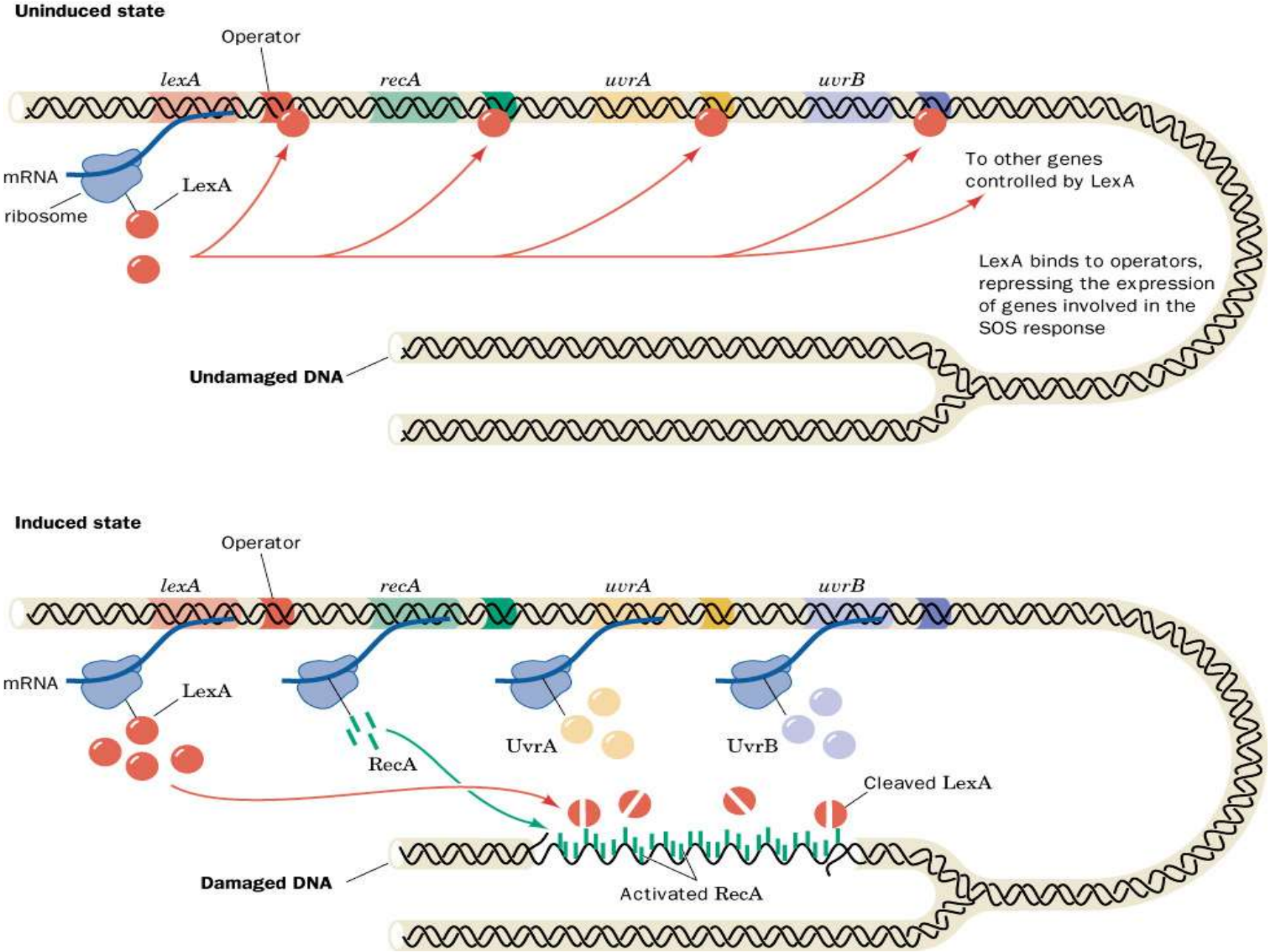
UmuDC-dependent mutagenesis





Ho *et al*., J. Bacteriol (1993) 175; 5411-5419

Regulation of the SOS response in *E. coli*



The *umuDC* operon is tightly regulated by LexA

Molecular Microbiology (2000) **35**(6), 1560–1572

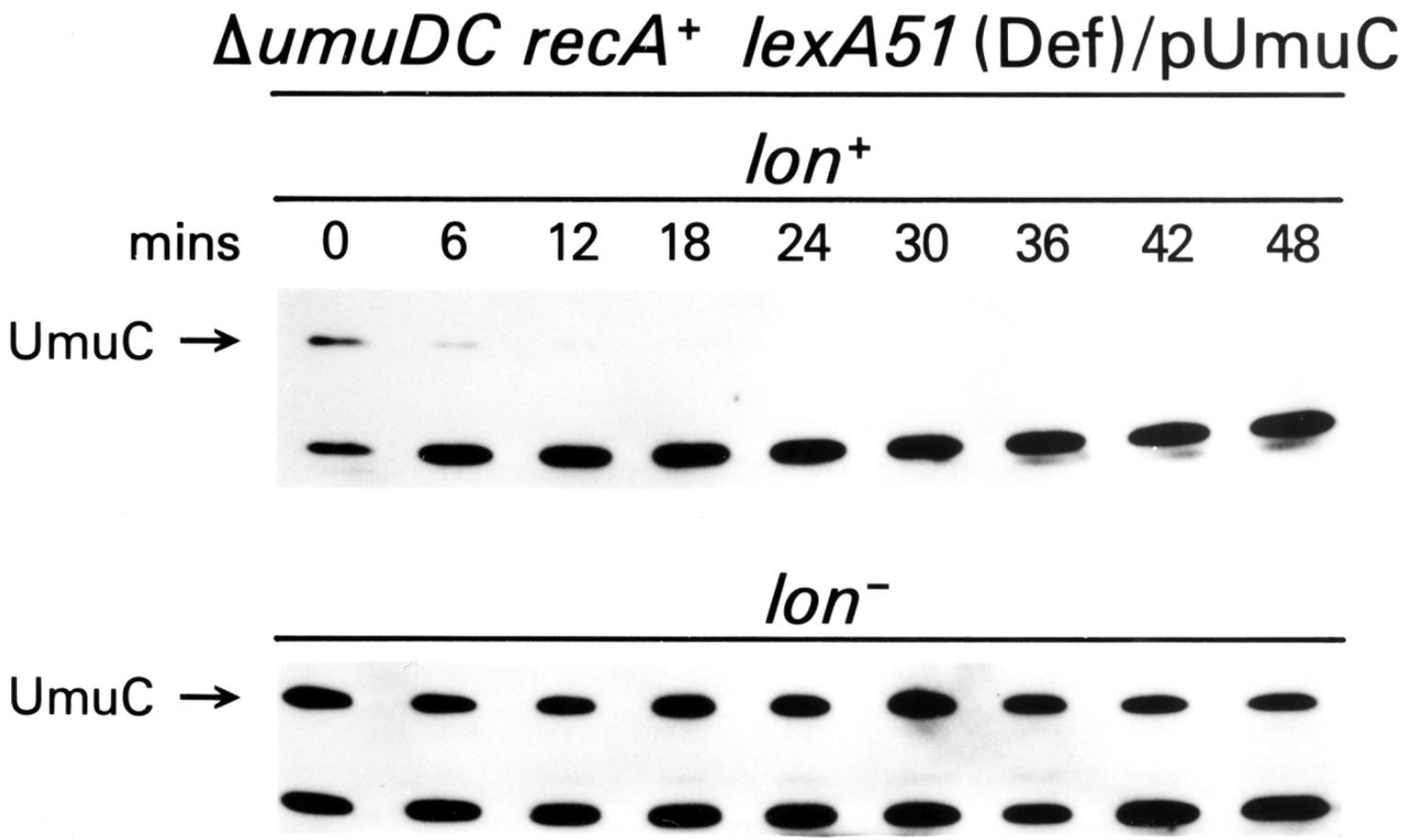
Identification of additional genes belonging to the LexA regulon in *Escherichia coli*

Antonio R. Fernández de Henestrosa,[1] Tomoo Ogi,[2] Sayura Aoyagi,[3] David Chafin,[3] Jeffrey J. Hayes,[3] Haruo Ohmori[2] and Roger Woodgate[1]*

Table 2. Potential LexA boxes in the *E. coli* chromosome[a].

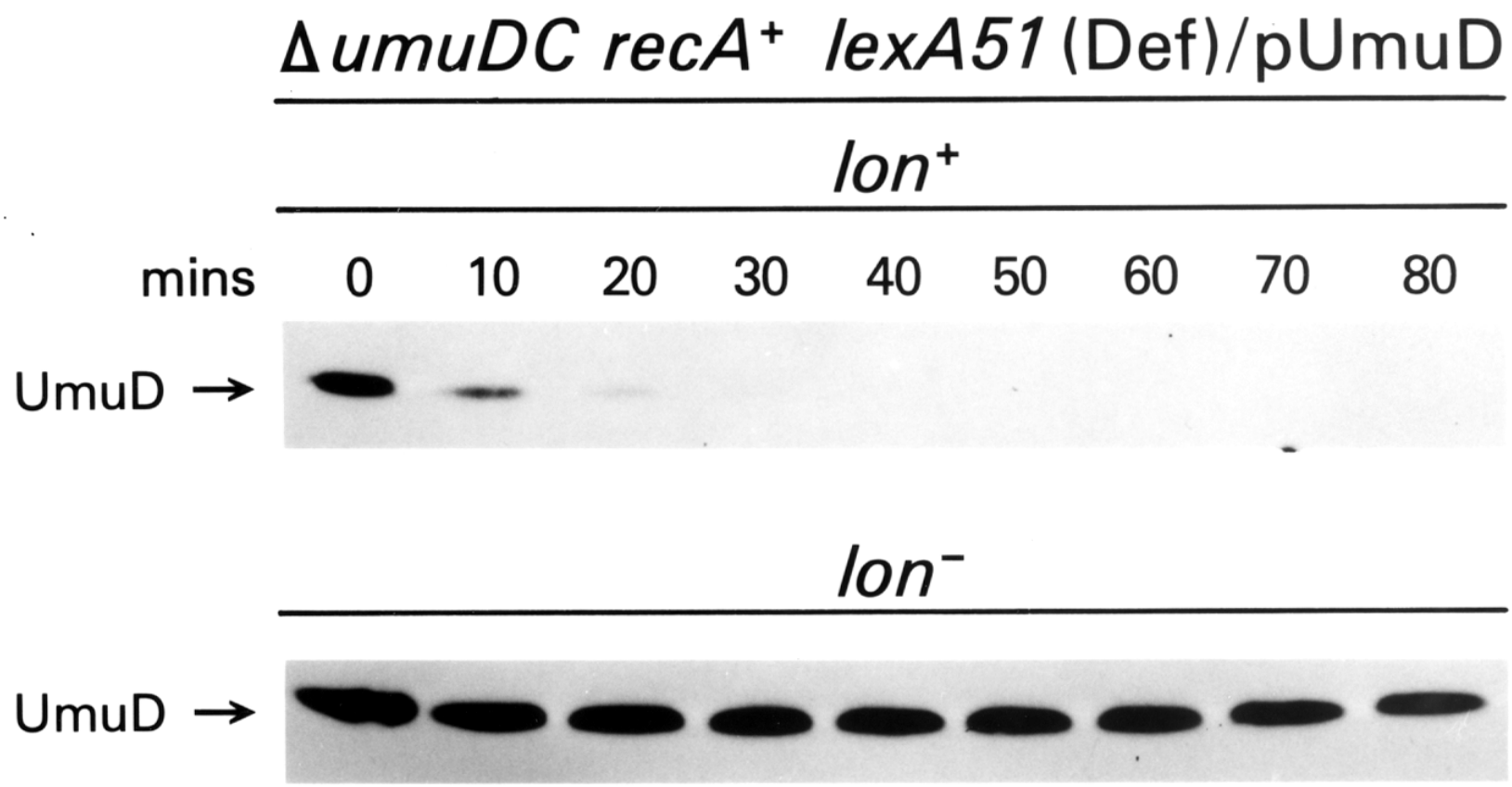
	Potential LexA box	Search pattern[b]	Gene[c]	Alternative name[d]	Distance[e]	HI
1.	TA**CTG**TTTATTTATA**CAG**TA	2, 3	*ysdAB*		−142	2.35
2[IR.]	TA**CTG**TATAAATAAA**CAG**TA	2, 3	*ilbL*	*b3672*	− 330	2.35
3.	TA**CTG**TATATAAAAA**CAG**TA	1, 2, 3	*umuDC*		−37	2.77*[f]
4.	TA**CTG**TATATAAAAA**CAG**TA	1, 2, 3	*sbmC*		−32	2.77*
5.	AA**CTG**TATATAAATA**CAG**TT	1, 2, 3	*pcsA*[g]	*dinD*	−61	3.34*
6.	TA**CTG**TATGCTCATA**CAG**TA	1, 2, 3	*recA*		−77	4.31*
7.	TA**CTG**TACATCCATA**CAG**TA	1, 2, 3	*sulA*		−42	4.65*
8.	TA**CTG**TATGATTATC**CAG**TT	2, 3	*dinQ*[h]		−139	4.83
9.	TA**CTG**TATATAAAAC**CAG**TT	1, 2, 3	*recN* (1)		−66	5.16*
10.	AA**CTG**TTTTTTTATC**CAG**TA	1, 2, 3	*uvrB*		−92	6.11*

UmuC is degraded by the Lon protease



Frank *et al*., PNAS (1996) 93; 10291-10296

UmuD is degraded by the Lon protease



Frank *et al*., PNAS (1996) 93; 10291-10296

Posttranslational modification of UmuD

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1806–1810, March 1988
Biochemistry

RecA protein-dependent cleavage of UmuD protein and SOS mutagenesis

(DNA damage/error-prone repair/posttranslational processing/protease/gene fusion)

HIDEO SHINAGAWA†, HIROSHI IWASAKI†, TAKESI KATO‡, AND ATSUO NAKATA†

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1811–1815, March 1988
Biochemistry

UmuD mutagenesis protein of *Escherichia coli*: Overproduction, purification, and cleavage by RecA

(SOS response/LexA cleavage/fidelity of DNA replication/protein self-cleavage)

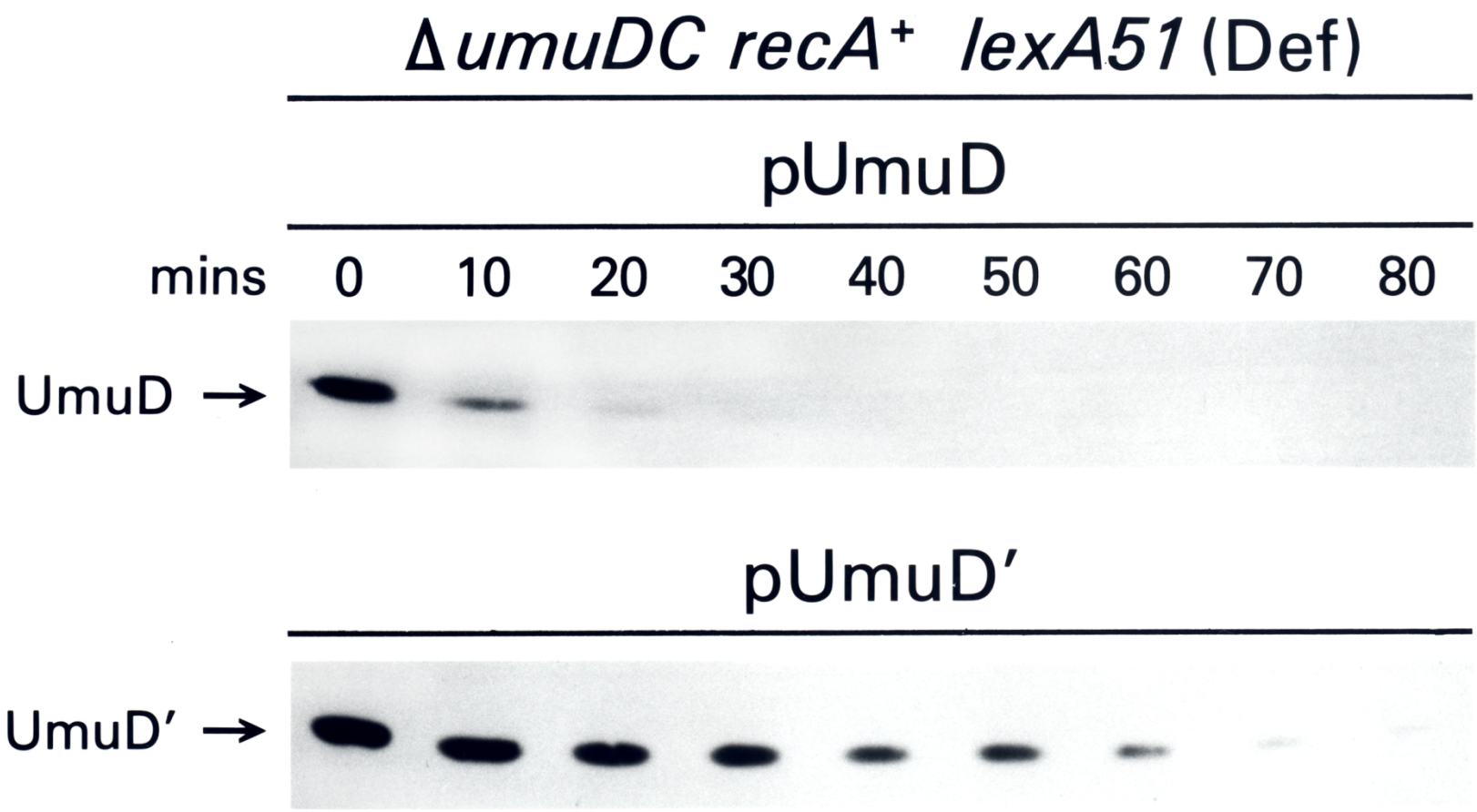
SABINE E. BURCKHARDT, ROGER WOODGATE, RICHARD H. SCHEUERMANN*, AND HARRISON ECHOLS

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1816–1820, March 1988
Biochemistry

RecA-mediated cleavage activates UmuD for mutagenesis: Mechanistic relationship between transcriptional derepression and posttranslational activation

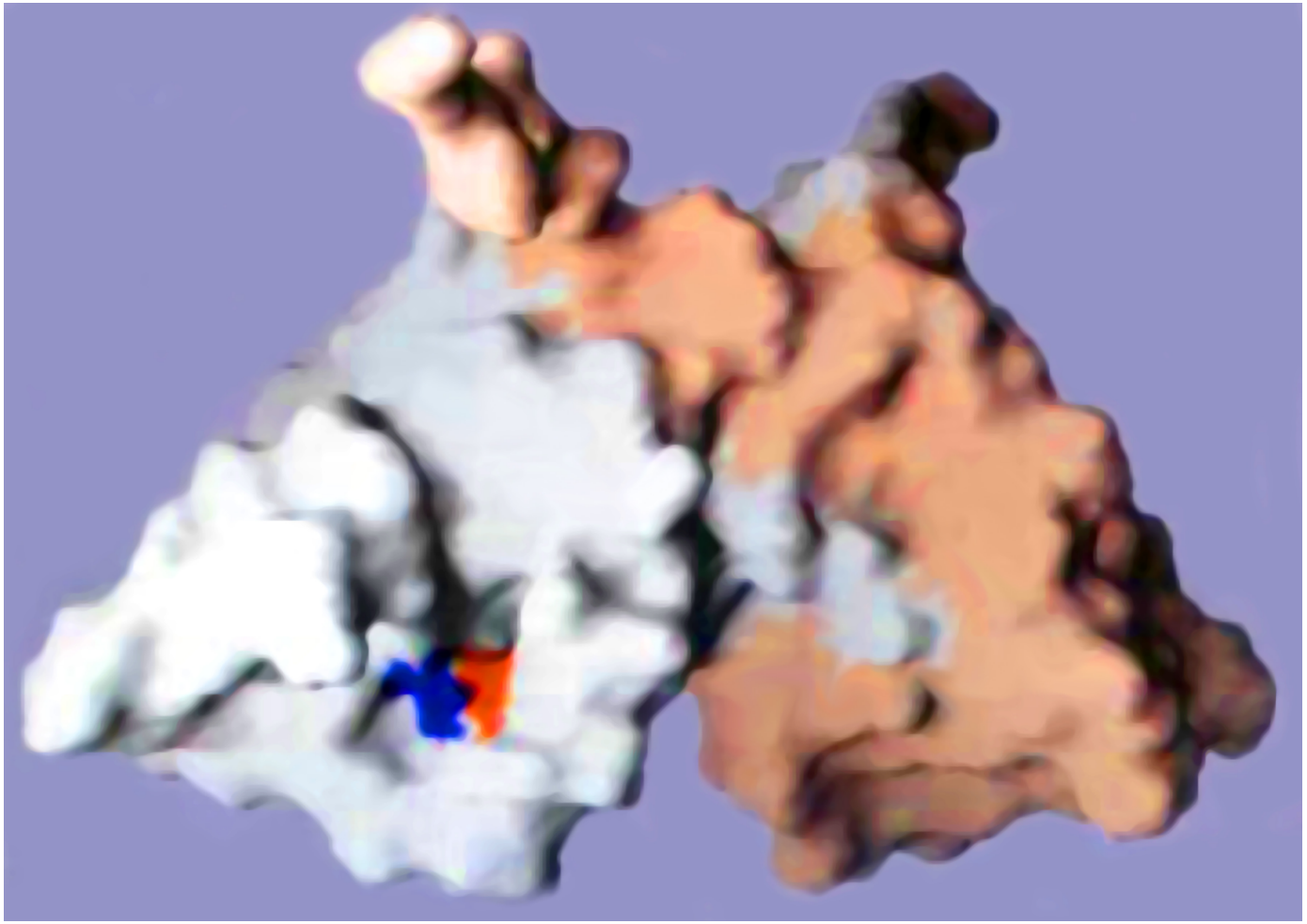
TAKEHIKO NOHMI, JOHN R. BATTISTA, LORI A. DODSON, AND GRAHAM C. WALKER

Stability of UmuD vs. UmuD'



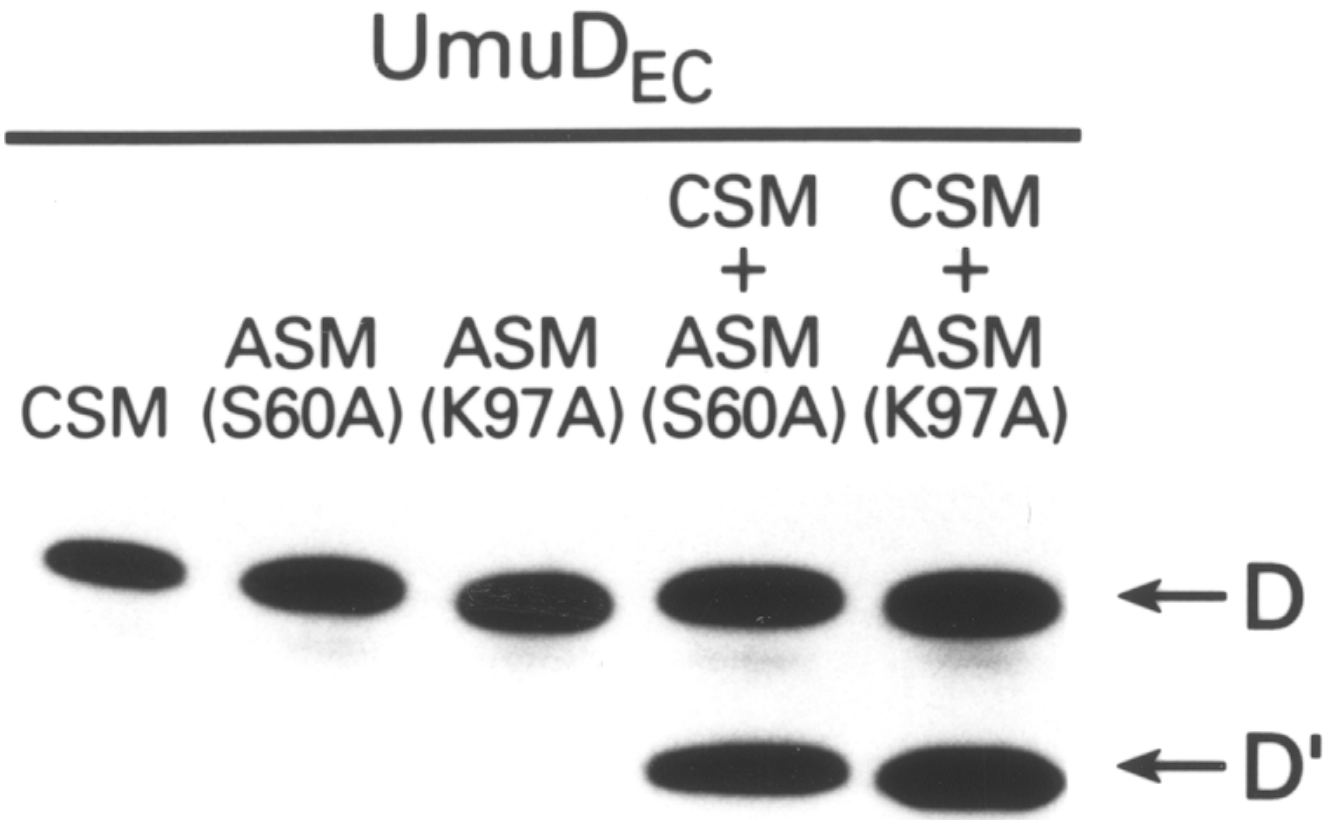
Frank *et al*., PNAS (1996) 93; 10291-10296

Crystal Structure of UmuD' homodimer



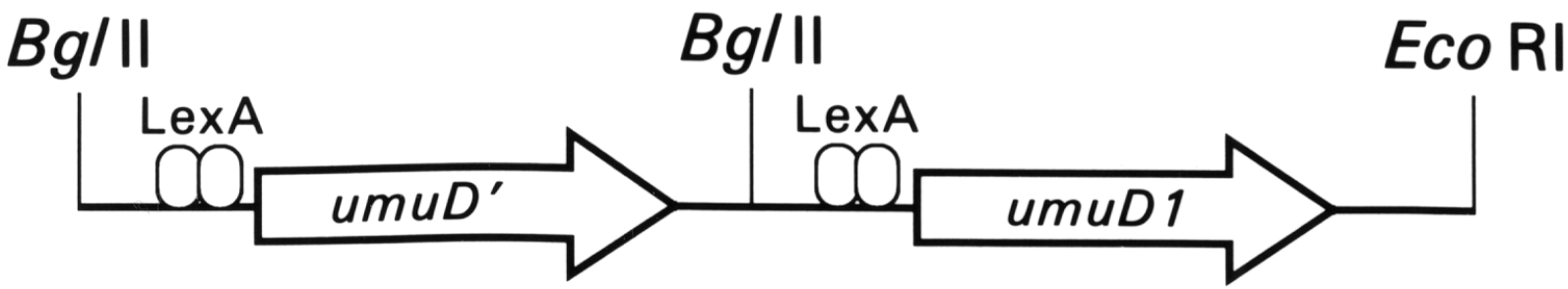
Gonzalez and Woodgate, BioEssays (2002) 24; 141-148

Intermolecular UmuD cleavage



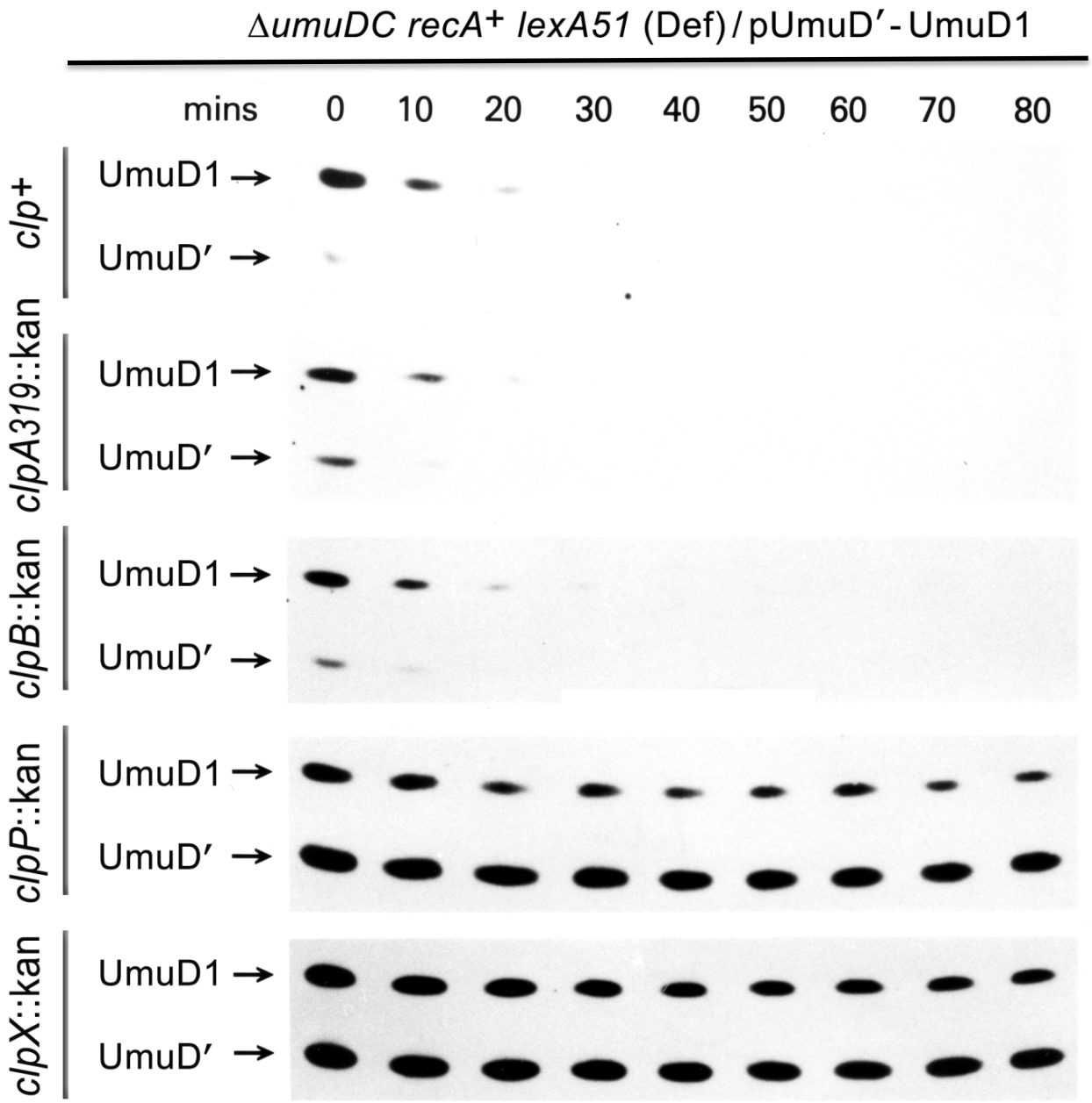
McDonald *et al*., PNAS (1998) 95; 1478-1483

Expression of UmuD/D' heterodimers



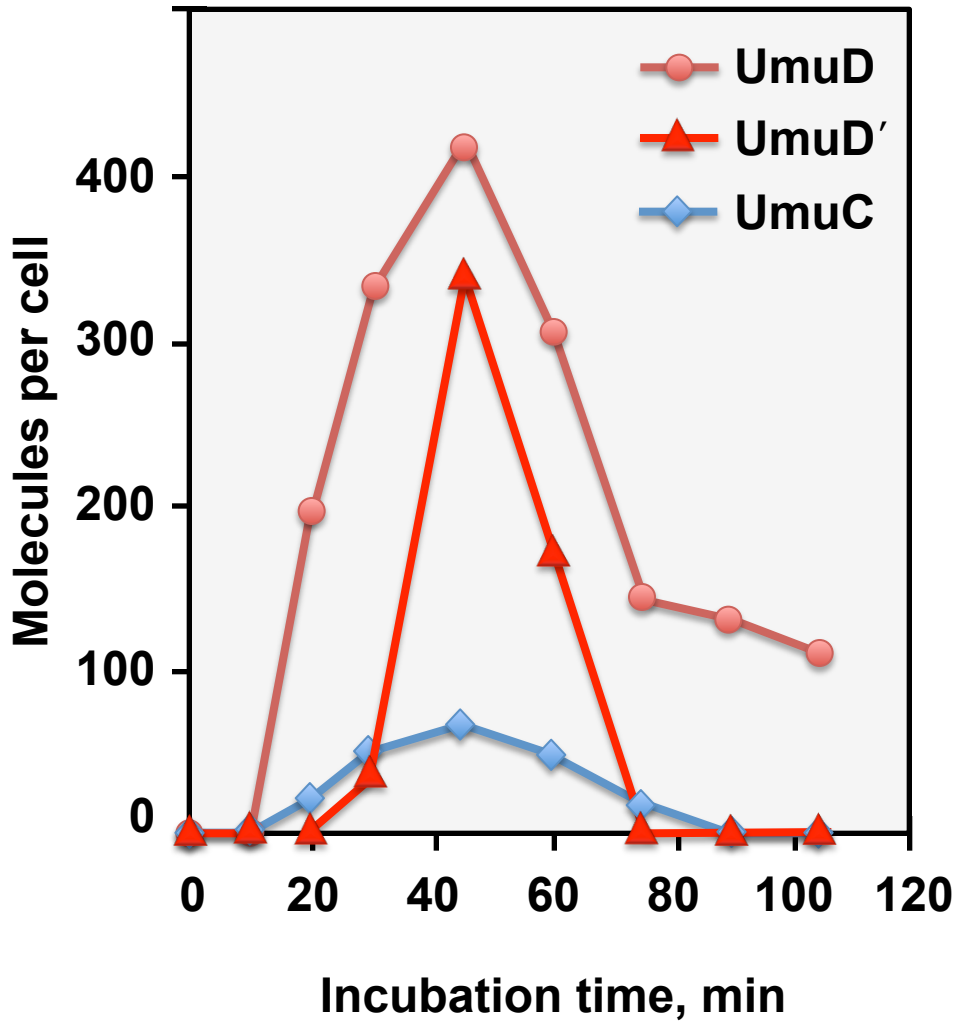
Frank *et al*., PNAS (1996) 93; 10291-10296

Stability of UmuD' in the context of a UmuD/D' heterodimer



Frank *et al.*, PNAS (1996) 93; 10291-10296

Umu proteins accumulate ~45 mins after DNA damage



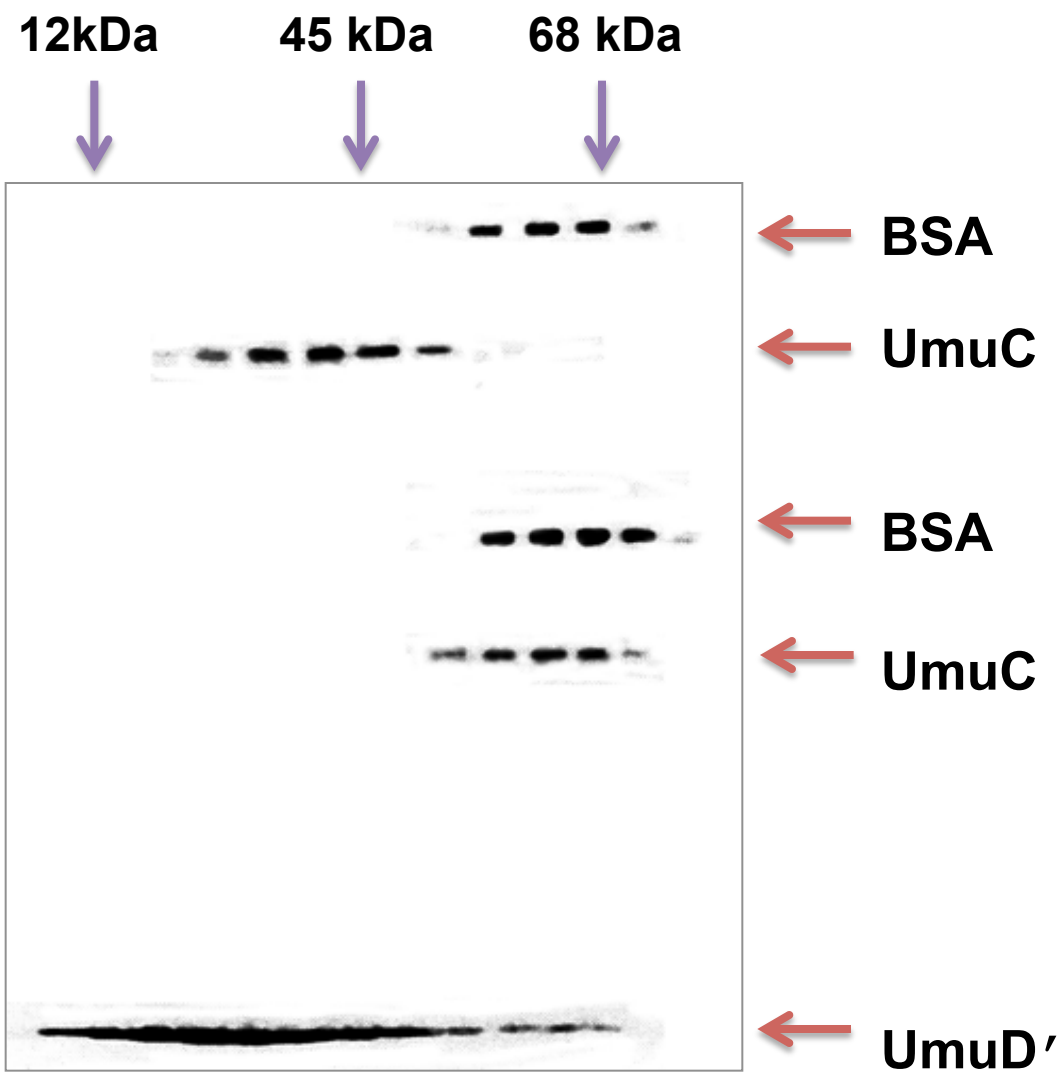
Sommer *et al.*, Mol Microbiology (1998) 28: 281-291

UmuC mutagenesis protein of *Escherichia coli*: Purification and interaction with UmuD and UmuD′

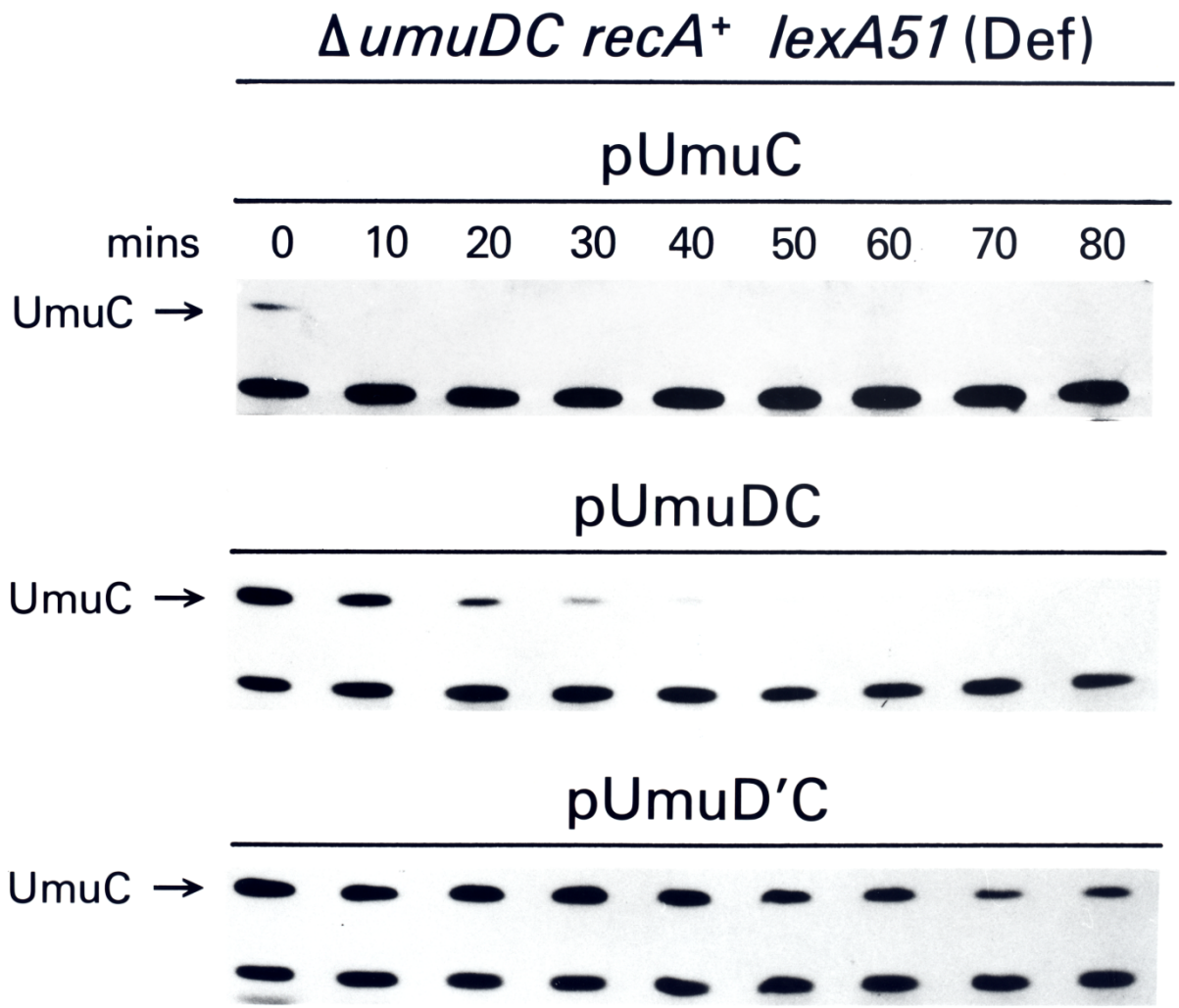
(SOS response/fidelity of DNA replication/protein–protein interaction)

ROGER WOODGATE, MALINI RAJAGOPALAN, CHI LU, AND HARRISON ECHOLS

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7301–7305, October 1989
Biochemistry

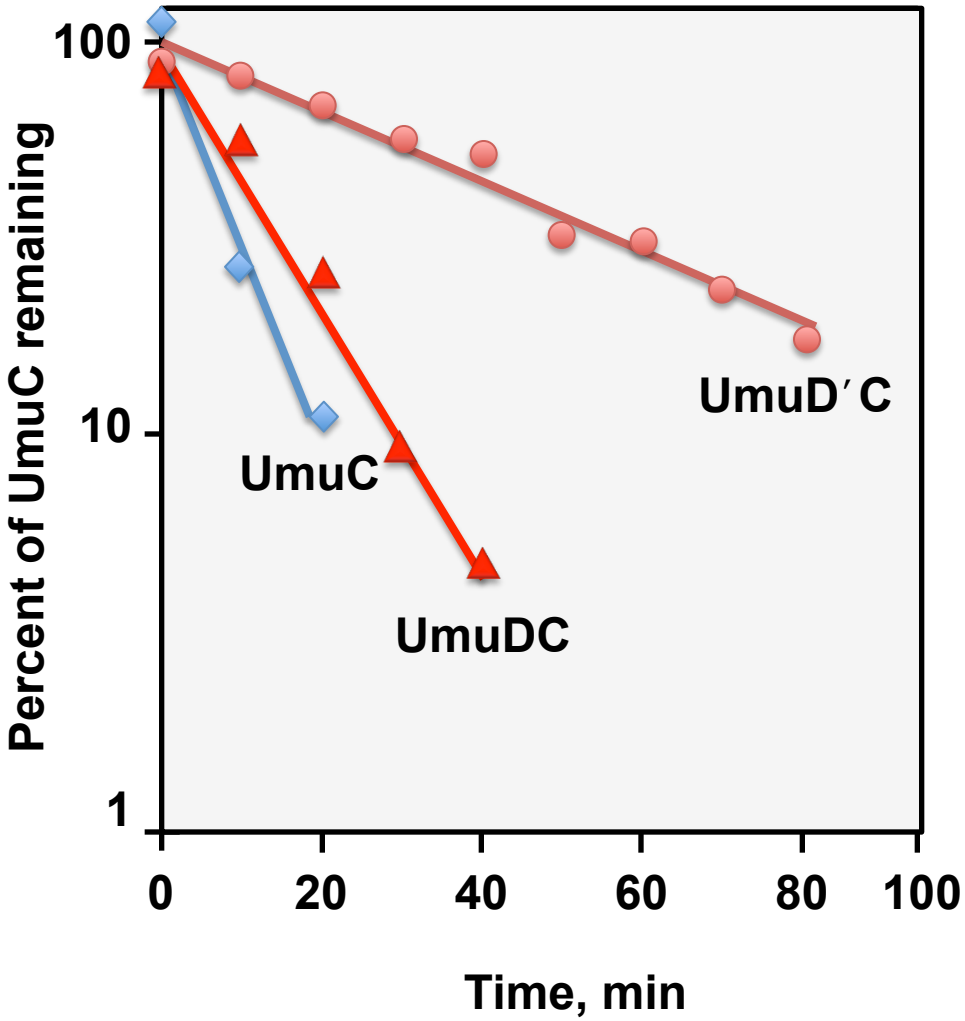


Stability of UmuC alone, coexpressed with UmuD or co-expressed with UmuD'



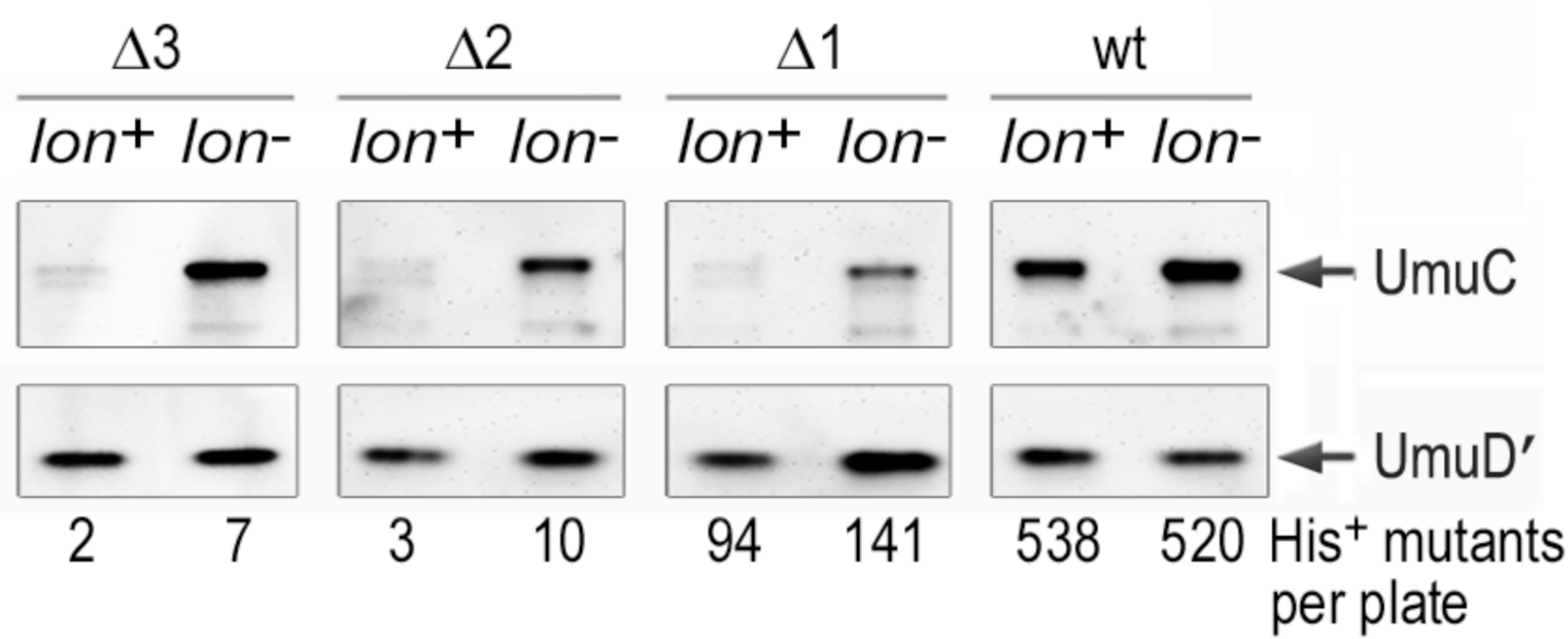
Frank *et al*., J. Bacteriol (1996) 178; 3550-3556

Stability of UmuC alone, coexpressed with UmuD or co-expressed with UmuD'



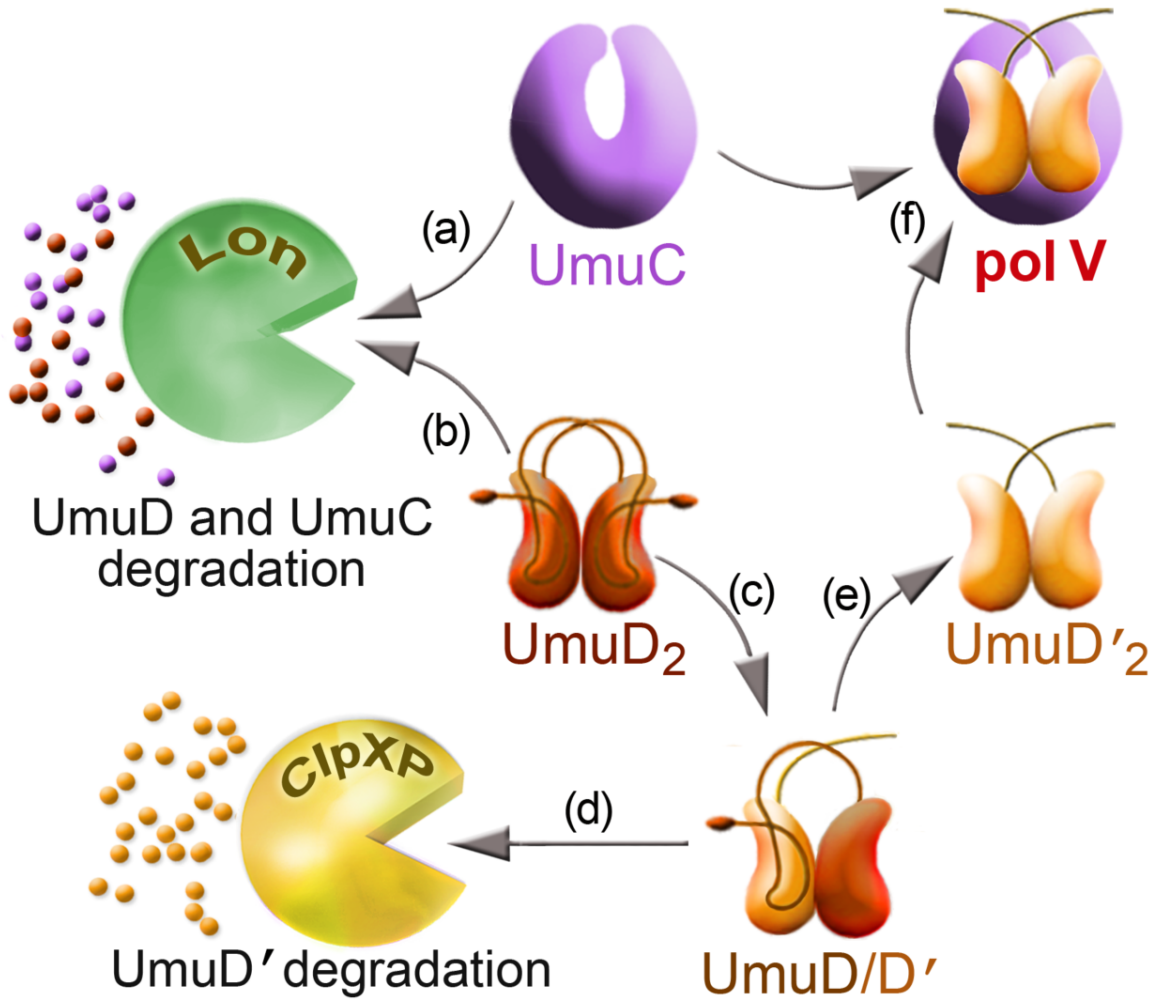
Frank *et al.*, J. Bacteriol (1996) 178; 3550-3556

Mapping the site of interaction between UmuC and UmuD'



Goodman *et al*., DNA Repair (2016) *In Press*

Posttranslational and proteolytic regulation of pol V



Goodman *et al*., DNA Repair (2016) *In Press*

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7190–7194, September 1990
Biochemistry

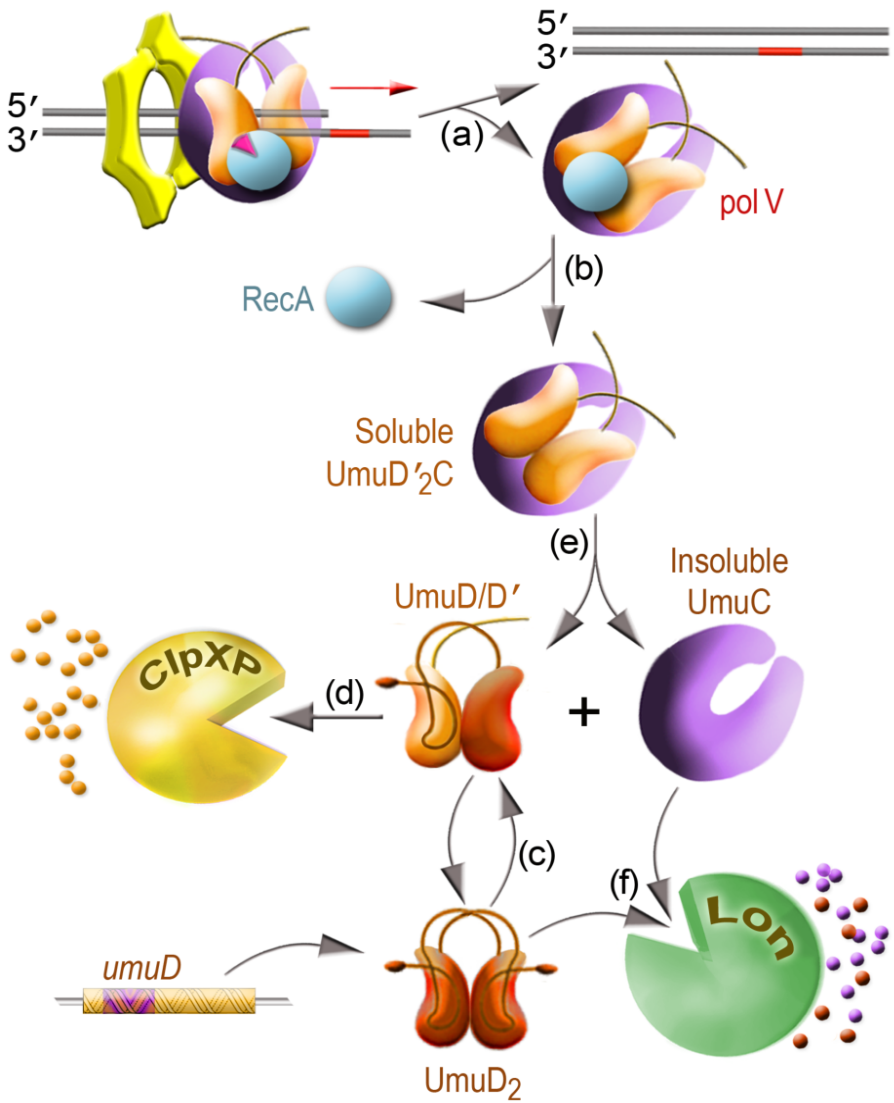
Dominant negative *umuD* mutations decreasing RecA-mediated cleavage suggest roles for intact UmuD in modulation of SOS mutagenesis

(SOS response/UV mutagenesis/*umuDC*/posttranslational regulation)

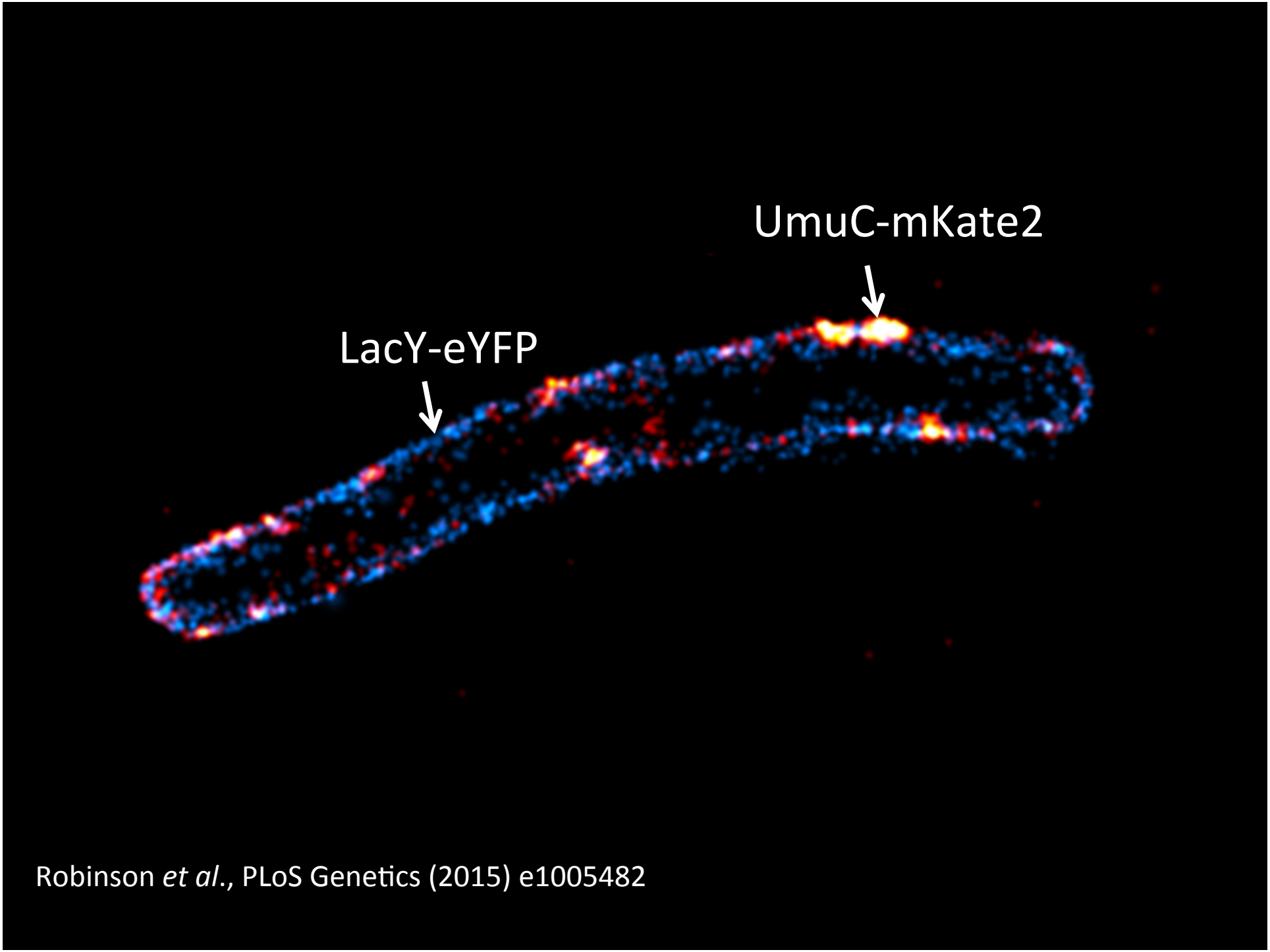
JOHN R. BATTISTA, TOSHIHIRO OHTA, TAKEHIKO NOHMI, WILLIAM SUN, AND GRAHAM C. WALKER

ABSTRACT The products of the SOS-regulated *umuDC* operon are required for most UV and chemical mutagenesis in *Escherichia coli*. The UmuD protein shares homology with a family of proteins that includes LexA and several bacteriophage repressors. UmuD is posttranslationally activated for its role in mutagenesis by a RecA-mediated proteolytic cleavage that yields UmuD′. A set of missense mutants of *umuD* was isolated and shown to encode mutant UmuD proteins that are deficient in RecA-mediated cleavage *in vivo*. Most of these mutations are dominant to $umuD^+$ with respect to UV mutagenesis yet do not interfere with SOS induction. Although both UmuD and UmuD′ form homodimers, we provide evidence that they preferentially form heterodimers. The relationship of UmuD to LexA, λ repressor, and other members of the family of proteins is discussed and possible roles of intact UmuD in modulating SOS mutagenesis are discussed.

Proteolytic regulation of pol V

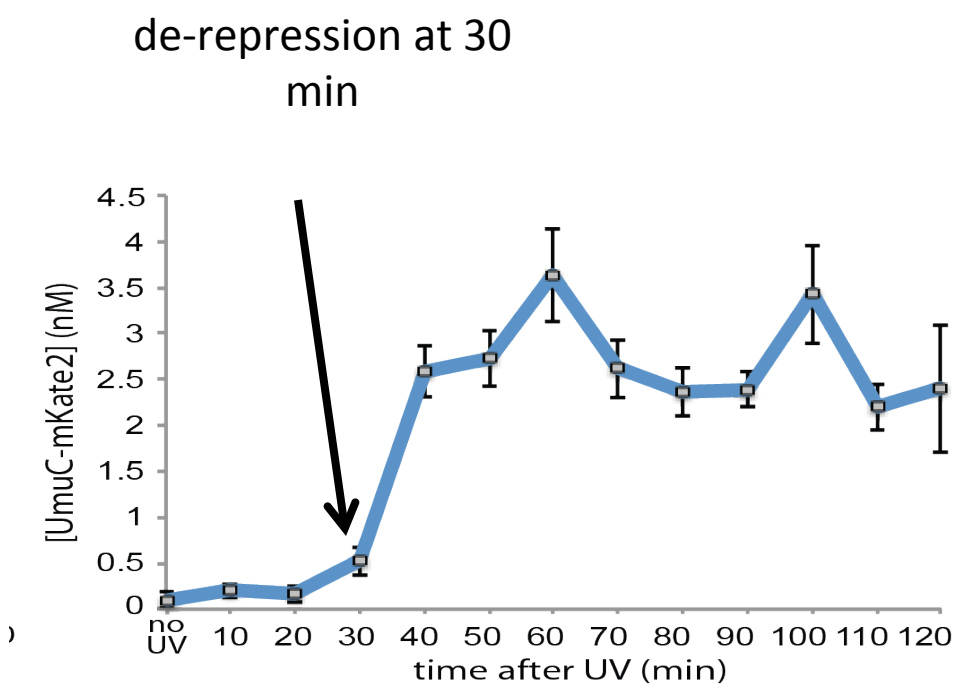


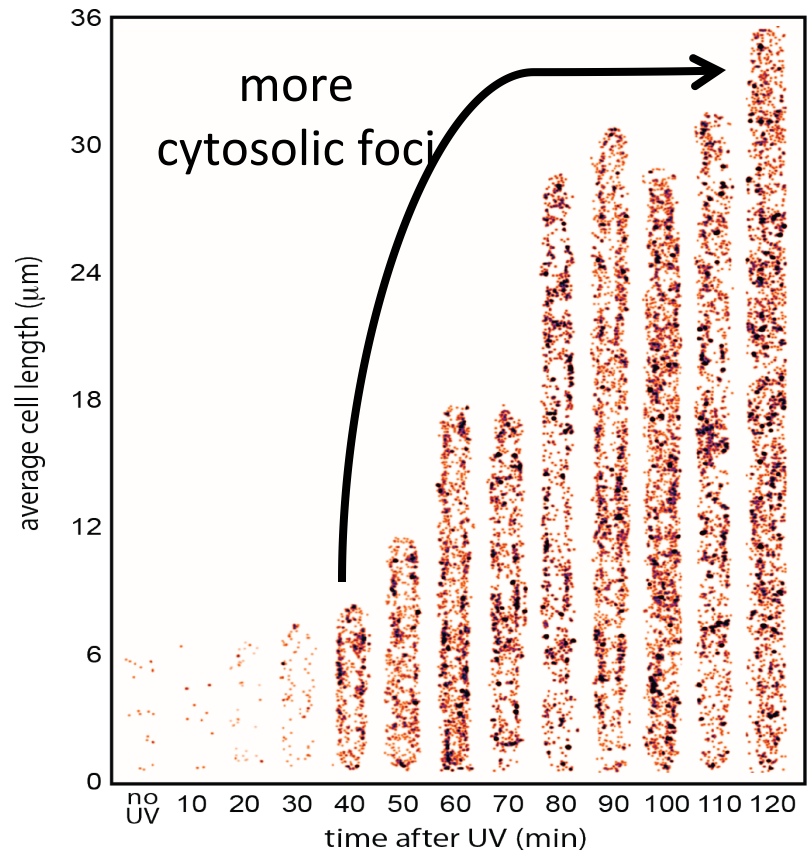
Goodman *et al.*, DNA Repair (2016) *In Press*



Robinson *et al*., PLoS Genetics (2015) e1005482

UmuC slowly redistributes to the cytosol after DNA damage

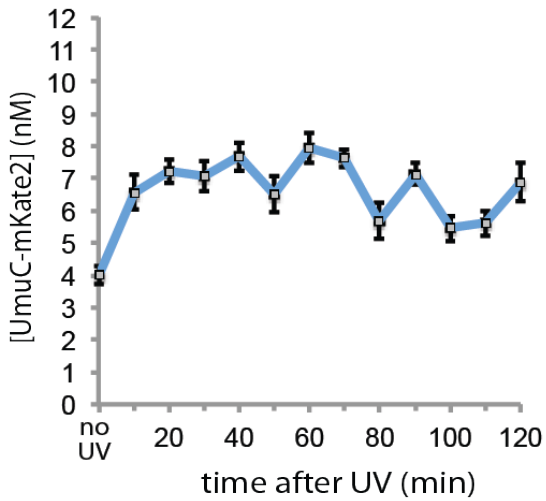




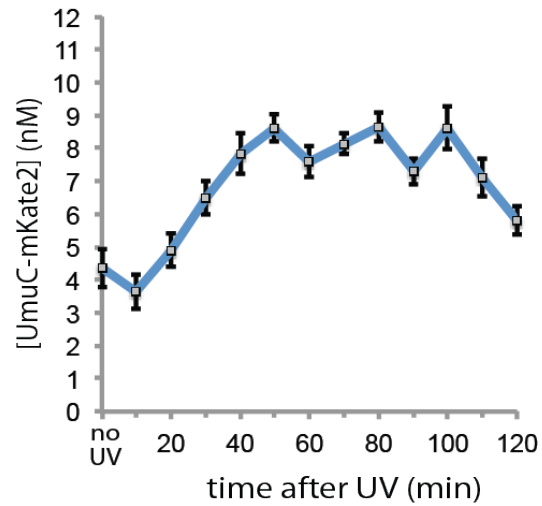
Robinson *et al.*, PLoS Genetics (2015) e1005482

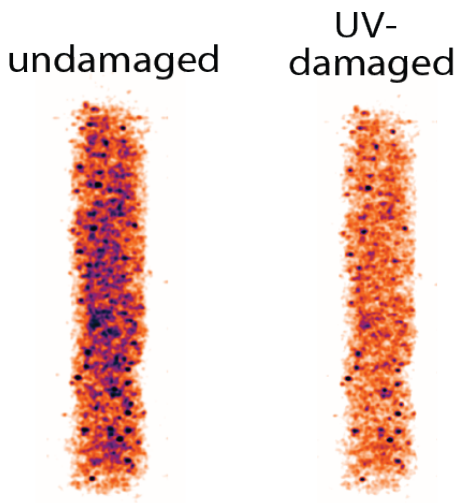
Membrane release requires cleavage of UmuD

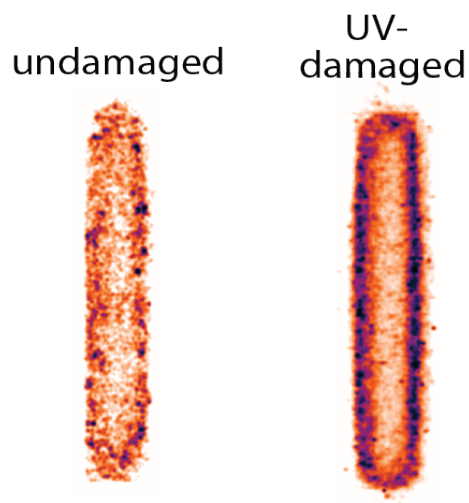
recA(E38K)



***recA*(E38K) *umuD*(K97A)**

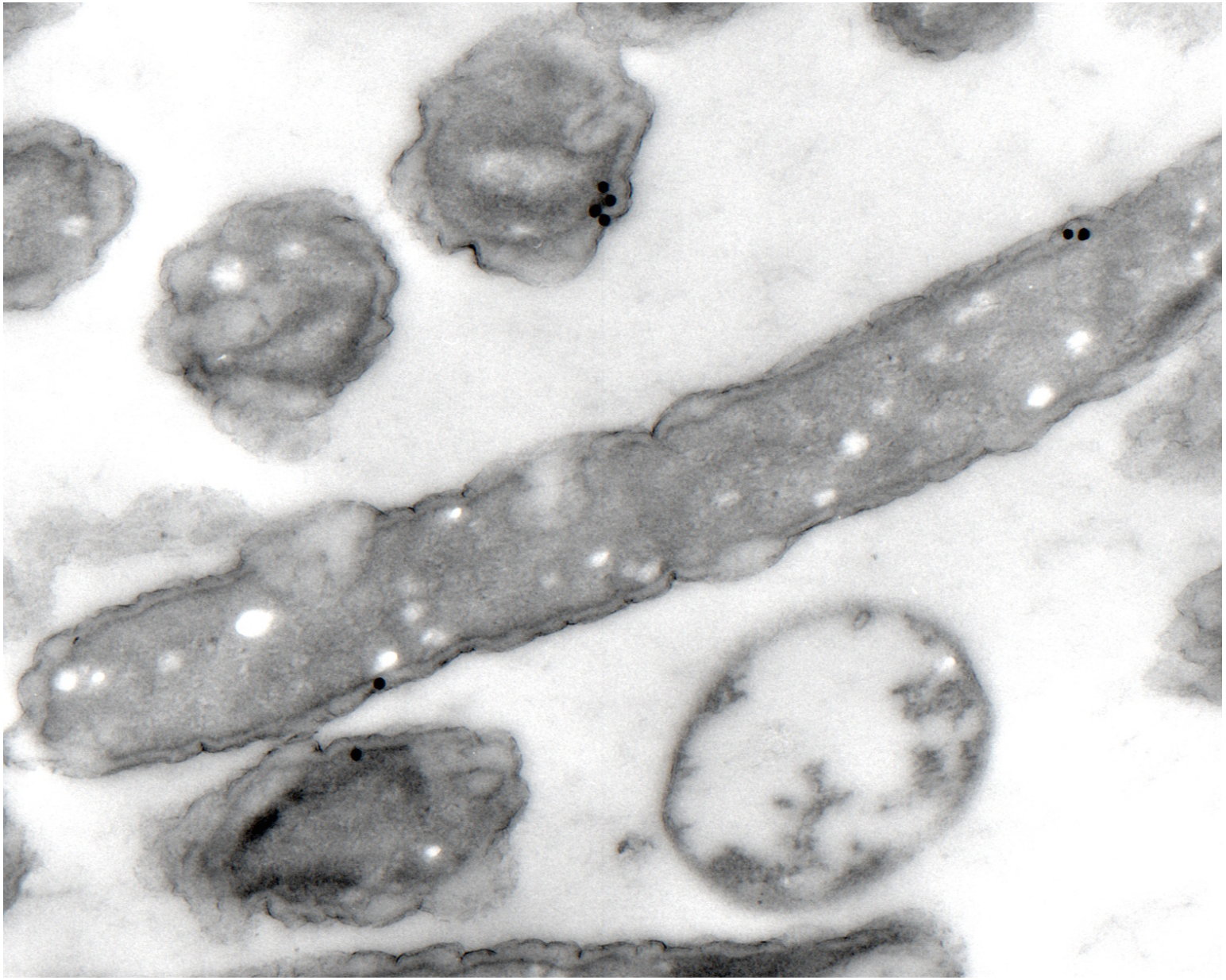






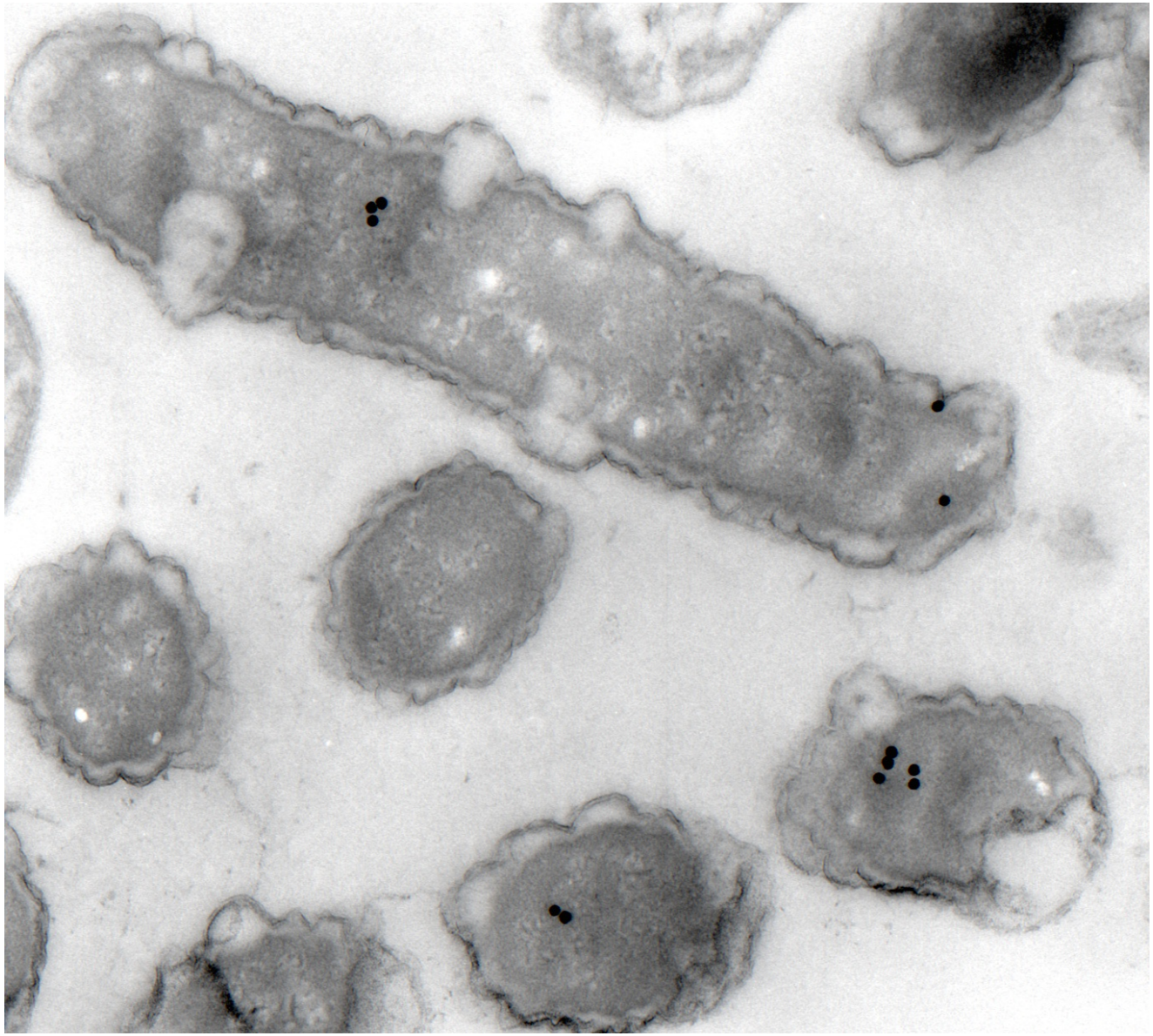
Robinson *et al*., PLoS Genetics (2015) e1005482

pUmuDC +UV



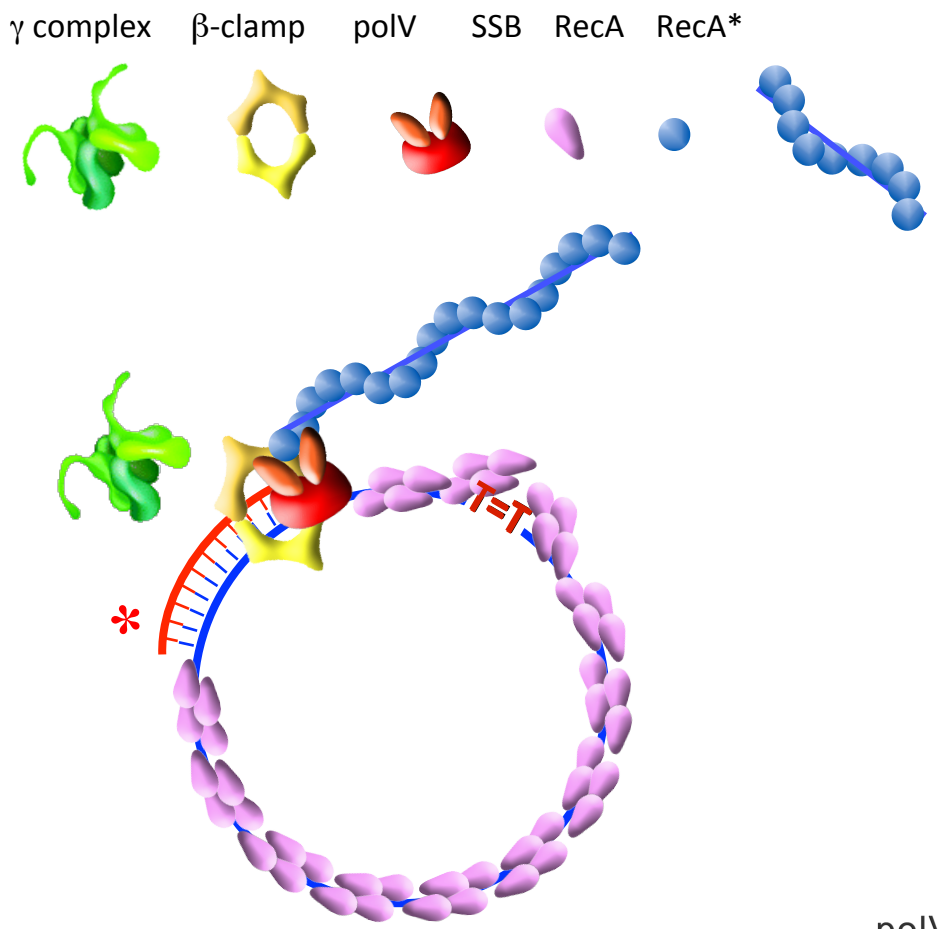
Robinson *et al.*, PLoS Genetics (2015) e1005482

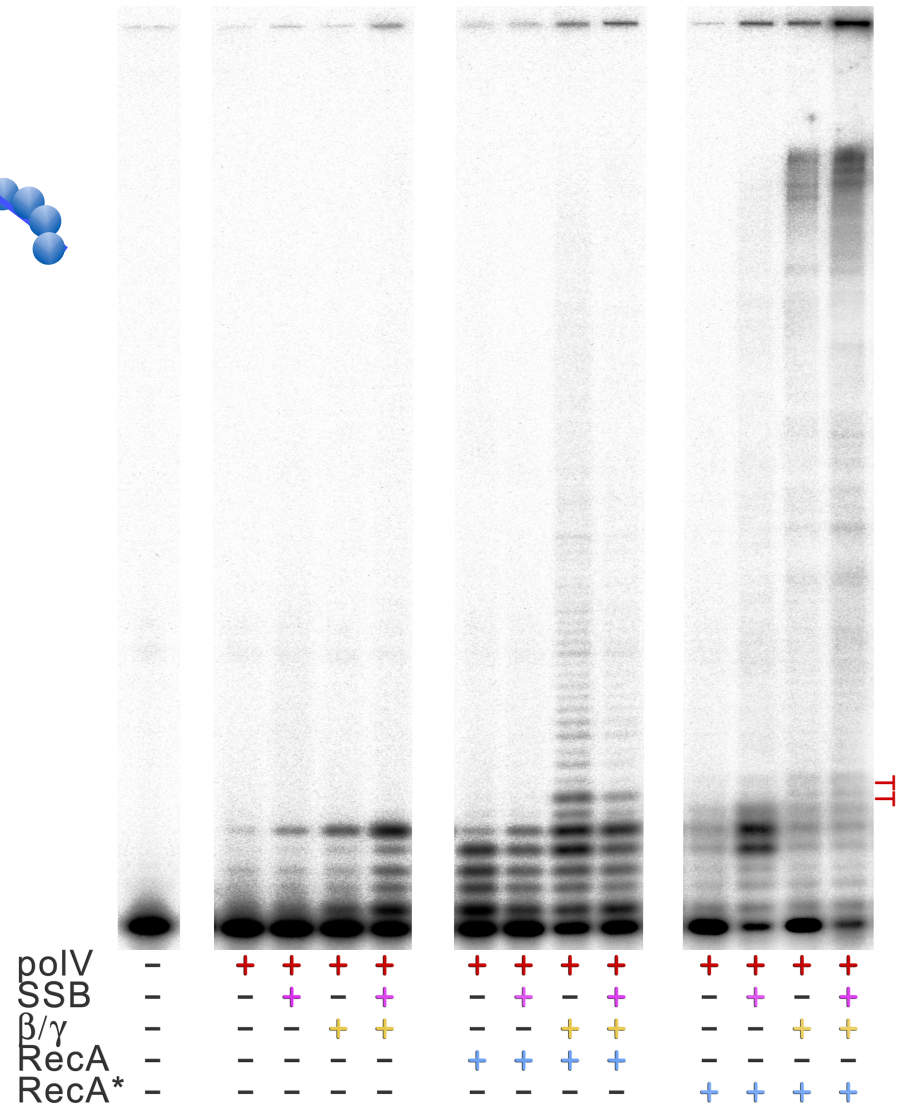
pUmuD’C +UV



Robinson *et al.*, PLoS Genetics (2015) e1005482

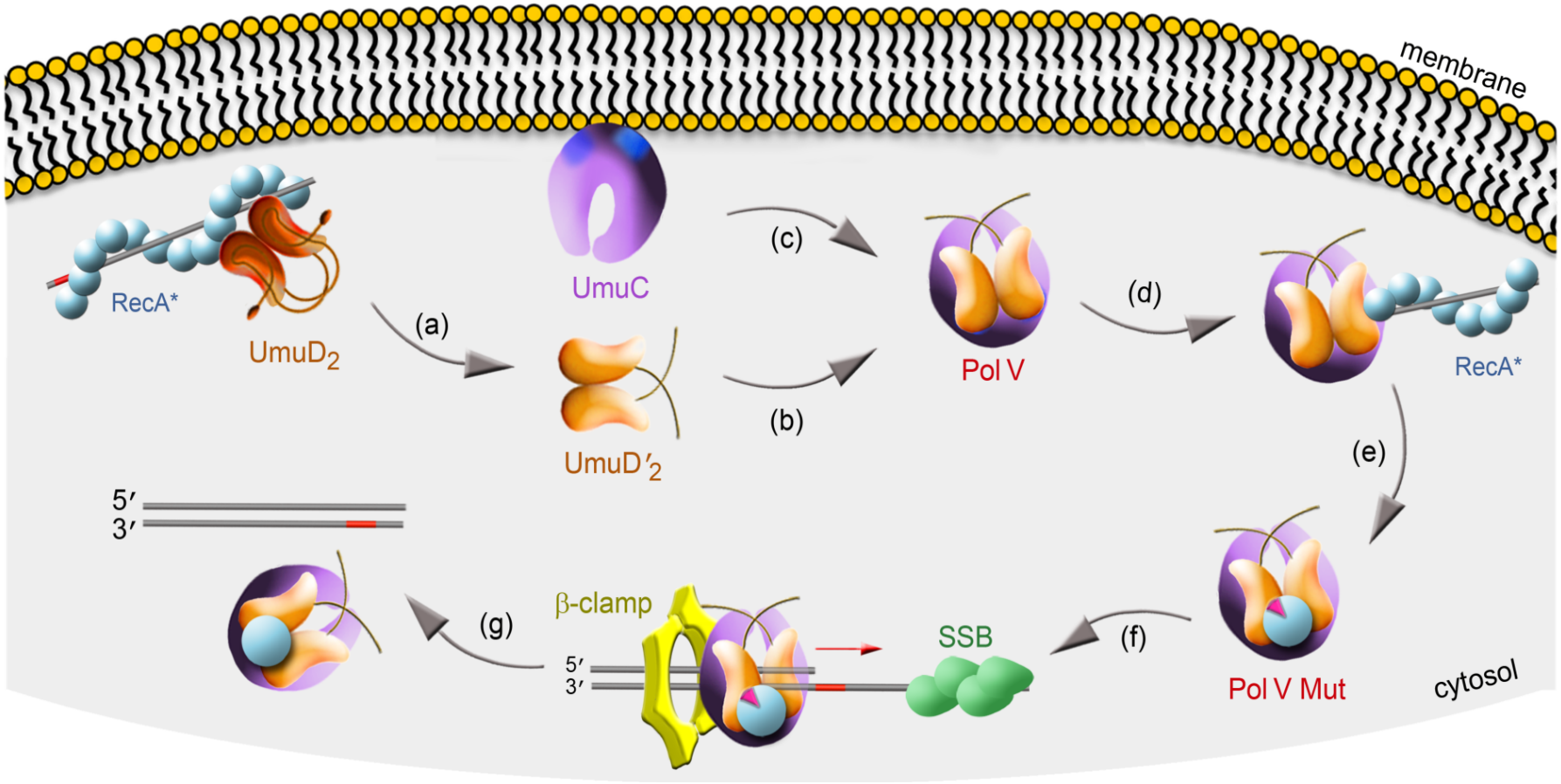
In vitro characterization of pol V





Karata *et al*., DNA Repair (2012) 11; 431-440

Protein Interactions that stimulate pol V



Goodman *et al.*, DNA Repair (2016) *In Press*

"Take home" messages from *E.coli* pol V

- *E.coli* UmuDC/ pol V is regulated at many levels so as to ensure the enzyme is utilized only when it's appropriate.

- Regulation is at the transcriptional level (LexA); the posttranslational level (RecA); by proteolysis (Lon/ClpXP); through protein-protein interactions (Beta-clamp/SSB)

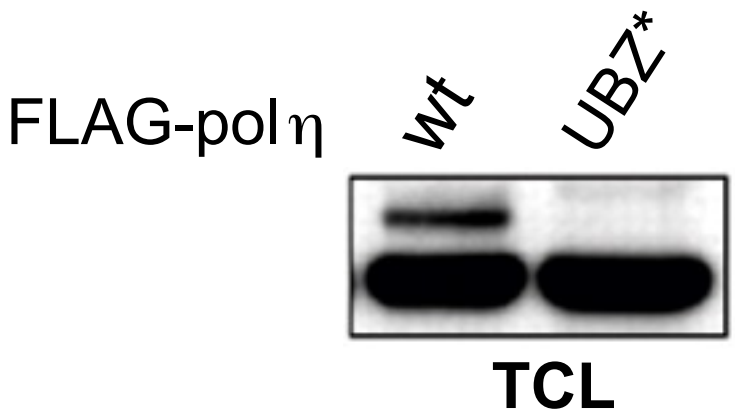
- pol V is regulated both temporally and spacially within the bacterium.

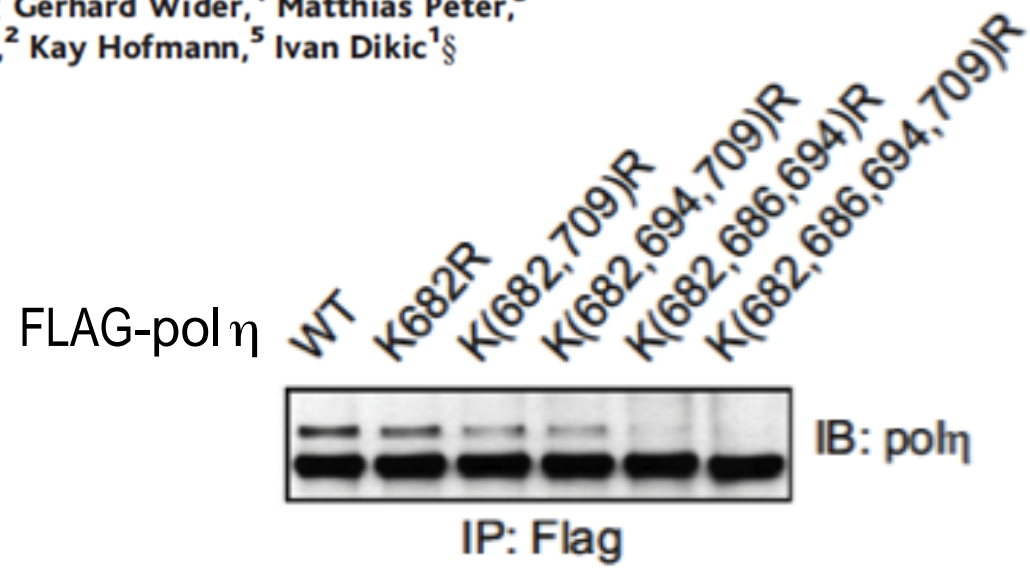
Regulation of human DNA TLS polymerases

Polη ubiquitination

Ubiquitin-Binding Domains in Y-Family Polymerases Regulate Translesion Synthesis

Marzena Bienko,[1] Catherine M. Green,[2] Nicola Crosetto,[1*]
Fabian Rudolf,[3*] Grzegorz Zapart,[1] Barry Coull,[2†]
Patricia Kannouche,[2‡] Gerhard Wider,[4] Matthias Peter,[3]
Alan R. Lehmann,[2] Kay Hofmann,[5] Ivan Dikic[1§]





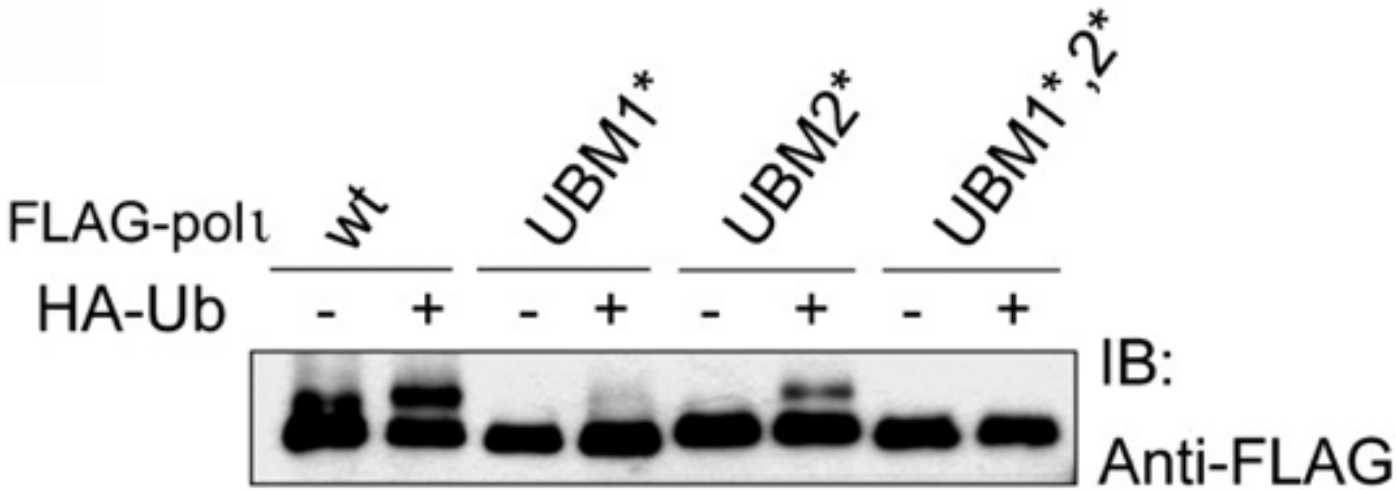
Science (2005) 310:1821-1824

Molecular Cell (2010) 37, 396-407

Polι ubiquitination

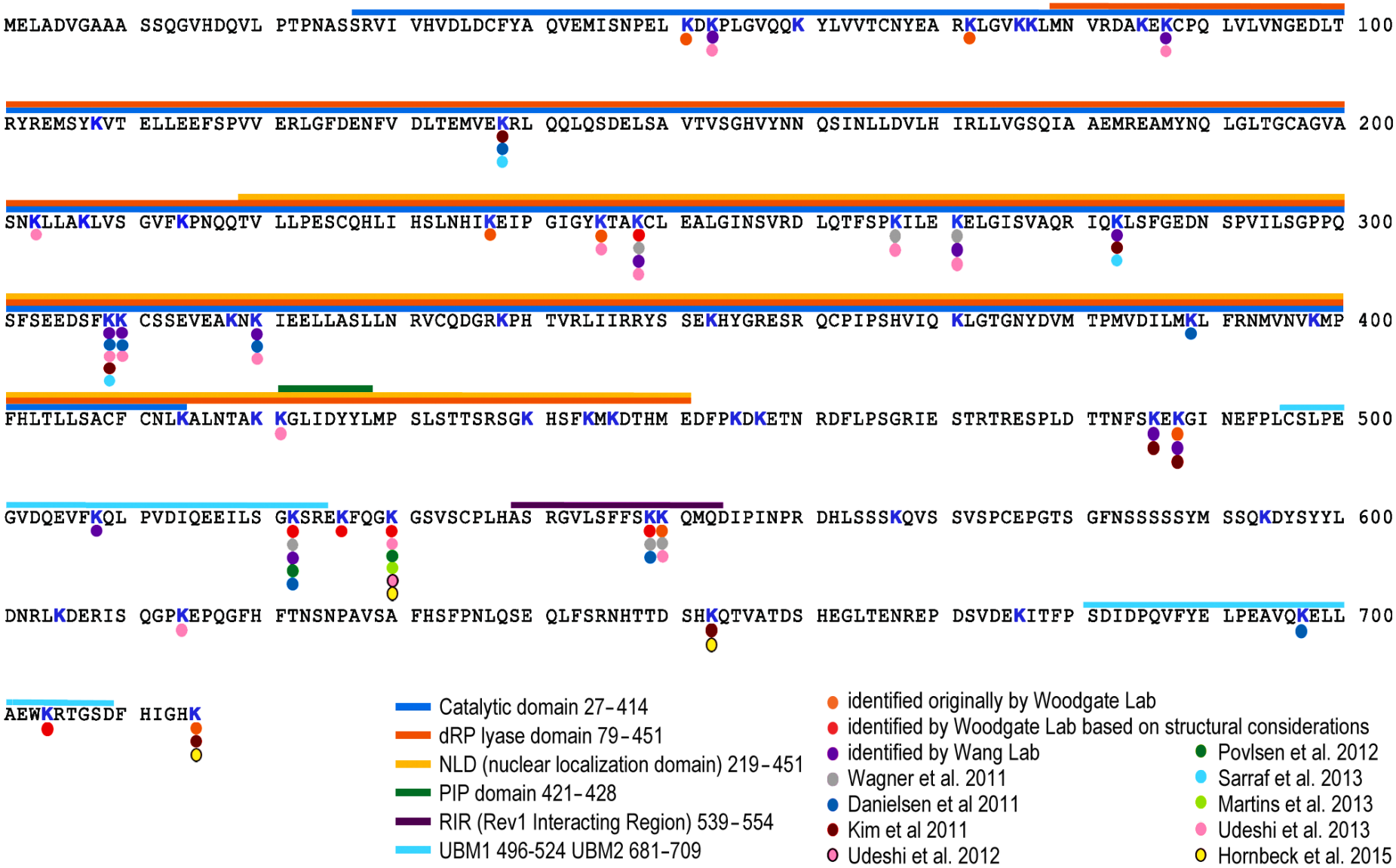
Ubiquitin-Binding Domains in Y-Family Polymerases Regulate Translesion Synthesis

Marzena Bienko,[1] Catherine M. Green,[2] Nicola Crosetto,[1*] Fabian Rudolf,[3*] Grzegorz Zapart,[1] Barry Coull,[2†] Patricia Kannouche,[2‡] Gerhard Wider,[4] Matthias Peter,[3] Alan R. Lehmann,[2] Kay Hofmann,[5] Ivan Dikic[1§]



Science (2005) 310:1821-1824

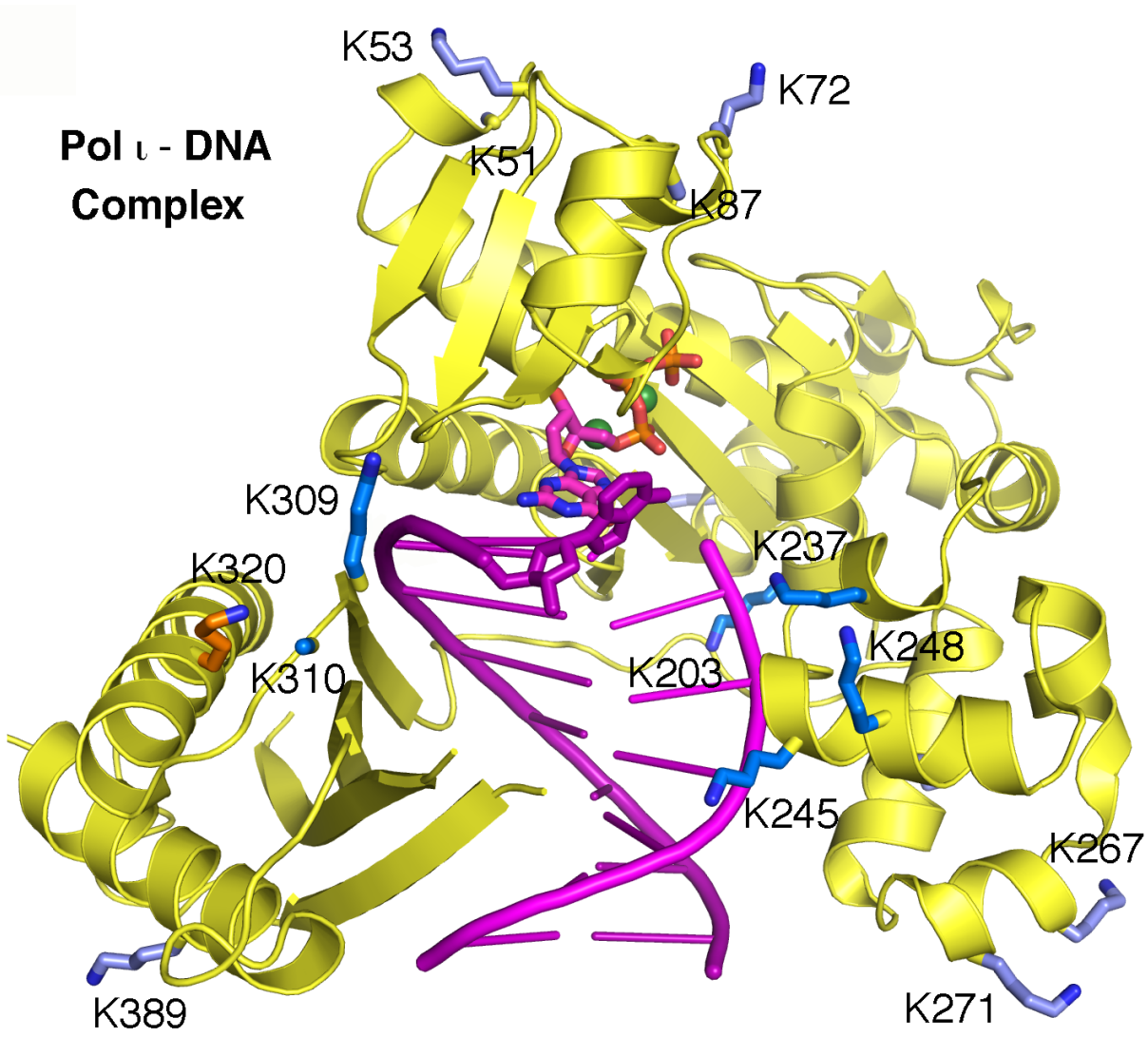
Multiple sites of pol ι's ubiquitination



McIntyre *et al*., J. Biol. Chem. (2015) 290; 27332-27344

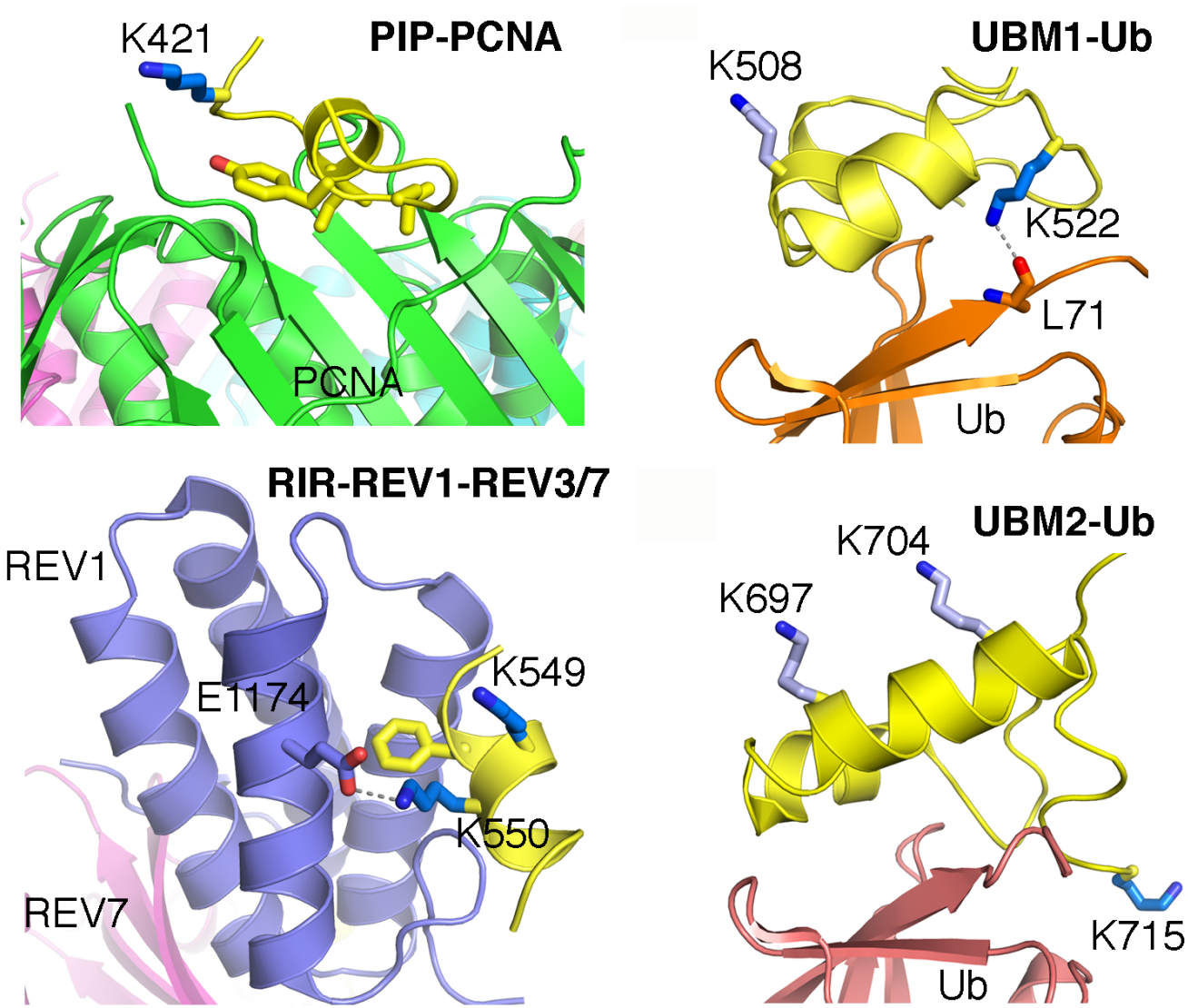
Location of ubiquitinated Lysines in the catalytic domain of Polι

McIntyre *et al.*, J. Biol Chem (2015) 290; 27332-27344



Location of ubiquitinated Lysines in various structural domains of Polι

McIntyre *et al.*, J. Biol Chem (2015) 290; 27332-27344



Posttranslational Regulation of Human DNA Polymerase ι

1. Unlike polη, which is monoubiquitinated at one primary site and a few secondary sites, polι can be ubiquitinated at >30 Lysines.

2. Polι is ubiquitinated in various structural domains, including catalytic, PCNA-interacting, Rev1-Interacting and UBM1 and UBM2. Ubiquitination at these sites may affect the activity and function of polι.

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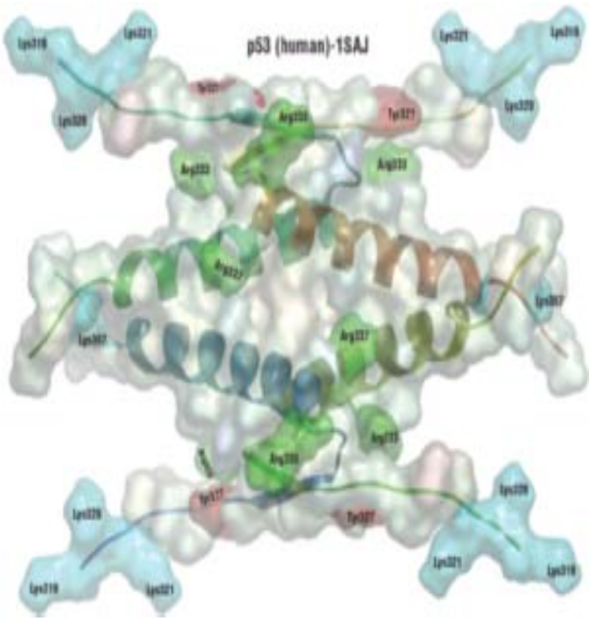


PhosphoSitePlus® is an online systems biology resource for information and tools related to the study of post-translational modifications (PTMs), including phosphorylation, ubiquitination, acetylation, and methylation.

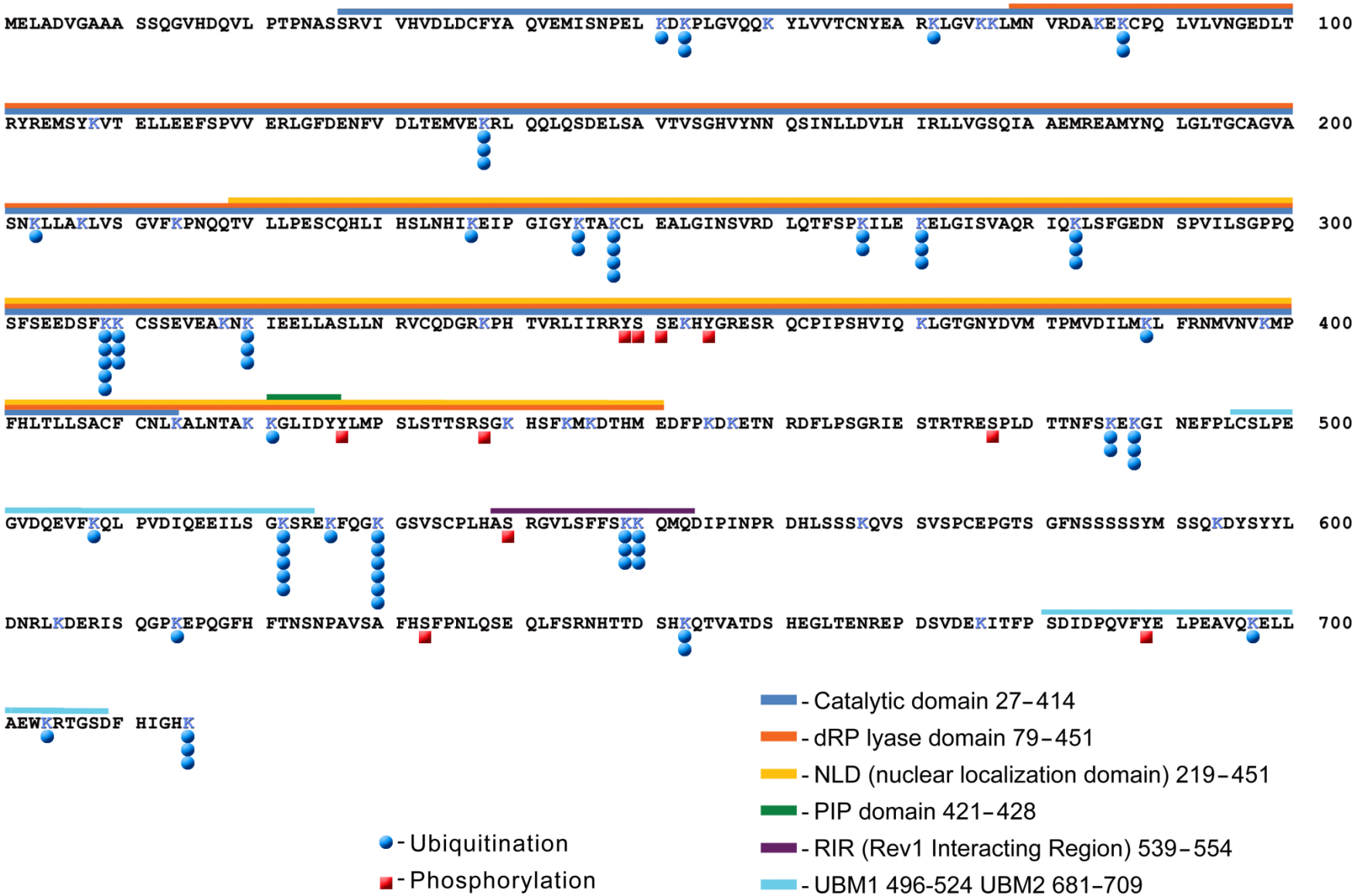
Created with grant support from the NIH and curated by CST scientists, this database is continuously updated and features a dynamic, open, and highly interactive format. It is designed to facilitate the study of the roles of PTMs in normal and pathological cellular processes, and to help accelerate the discovery of critical disease biomarkers and drug targets.

On the PhosphoSitePlus® database page you will be able to:

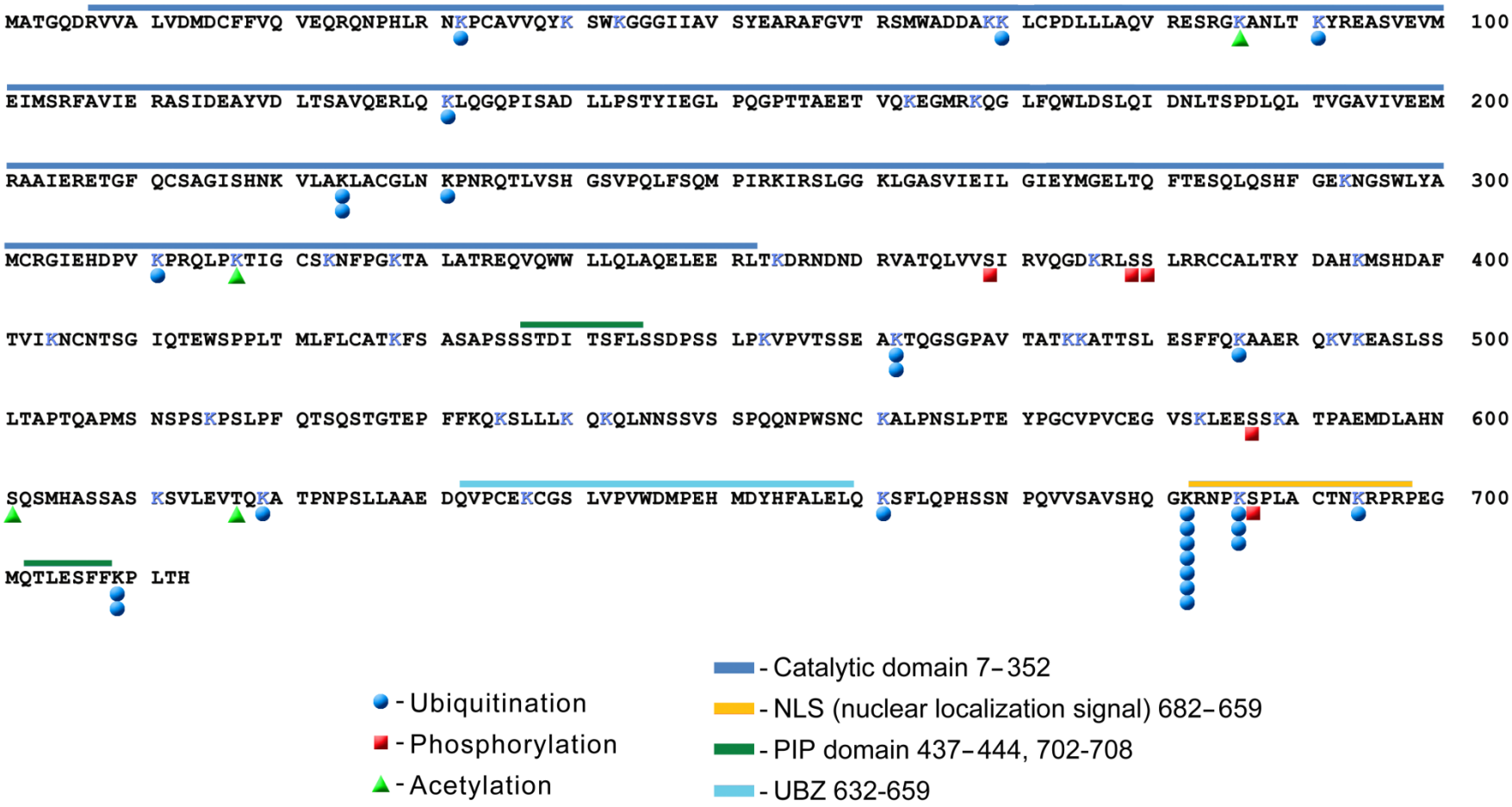
- Run simple searches for proteins or kinase substrates.
- Perform advanced searches for PTMs or proteins by domains, motifs or sequences, references, protein types, or GO terms.
- Browse MS/MS data and generate downloads of sets of PTMs associated with diseases, cell lines, or tissues.

Visit the PhosphoSitePlus® PTM Resource

Multiple sites of pol ι's modification



Multiple sites of pol η's modification



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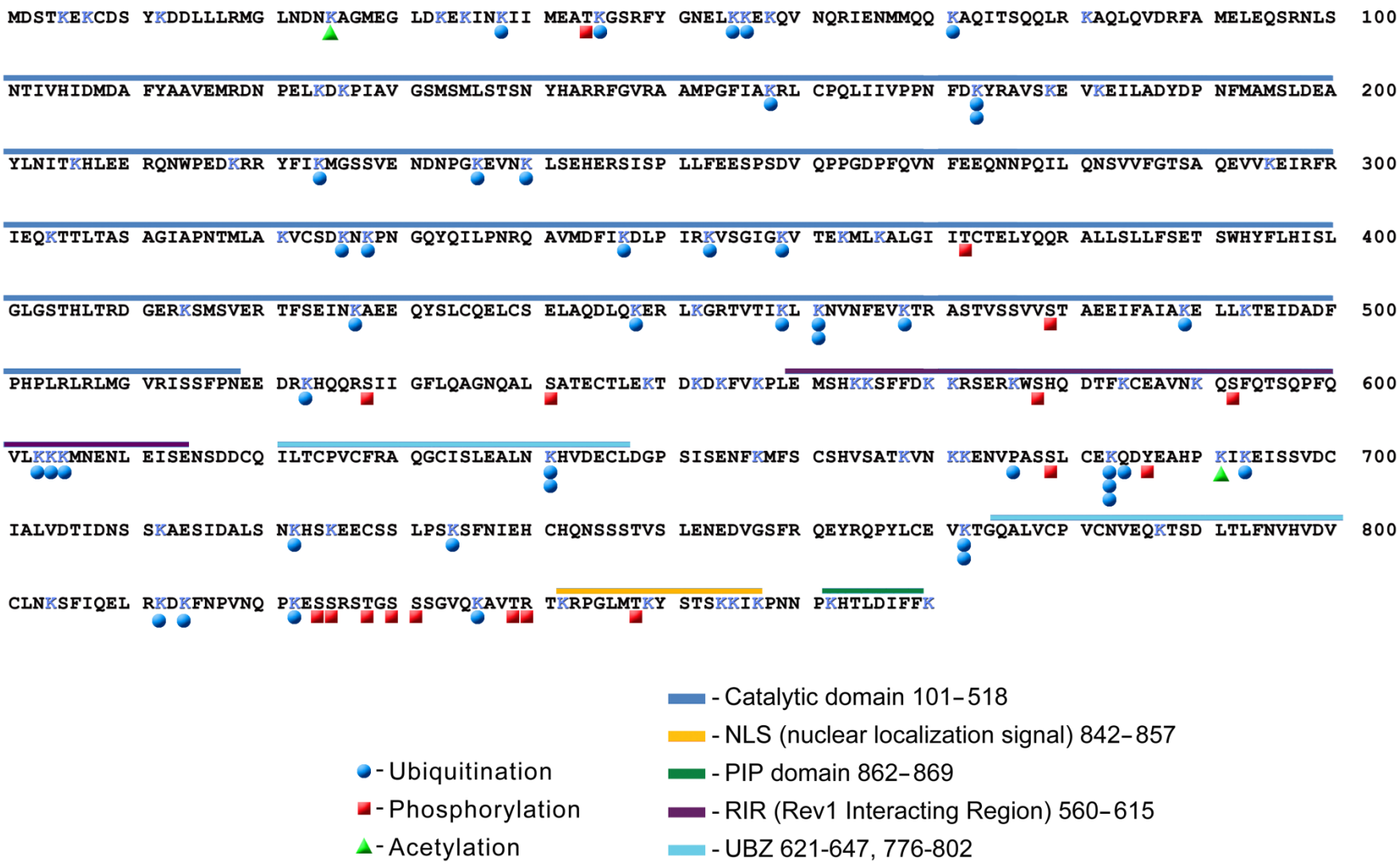
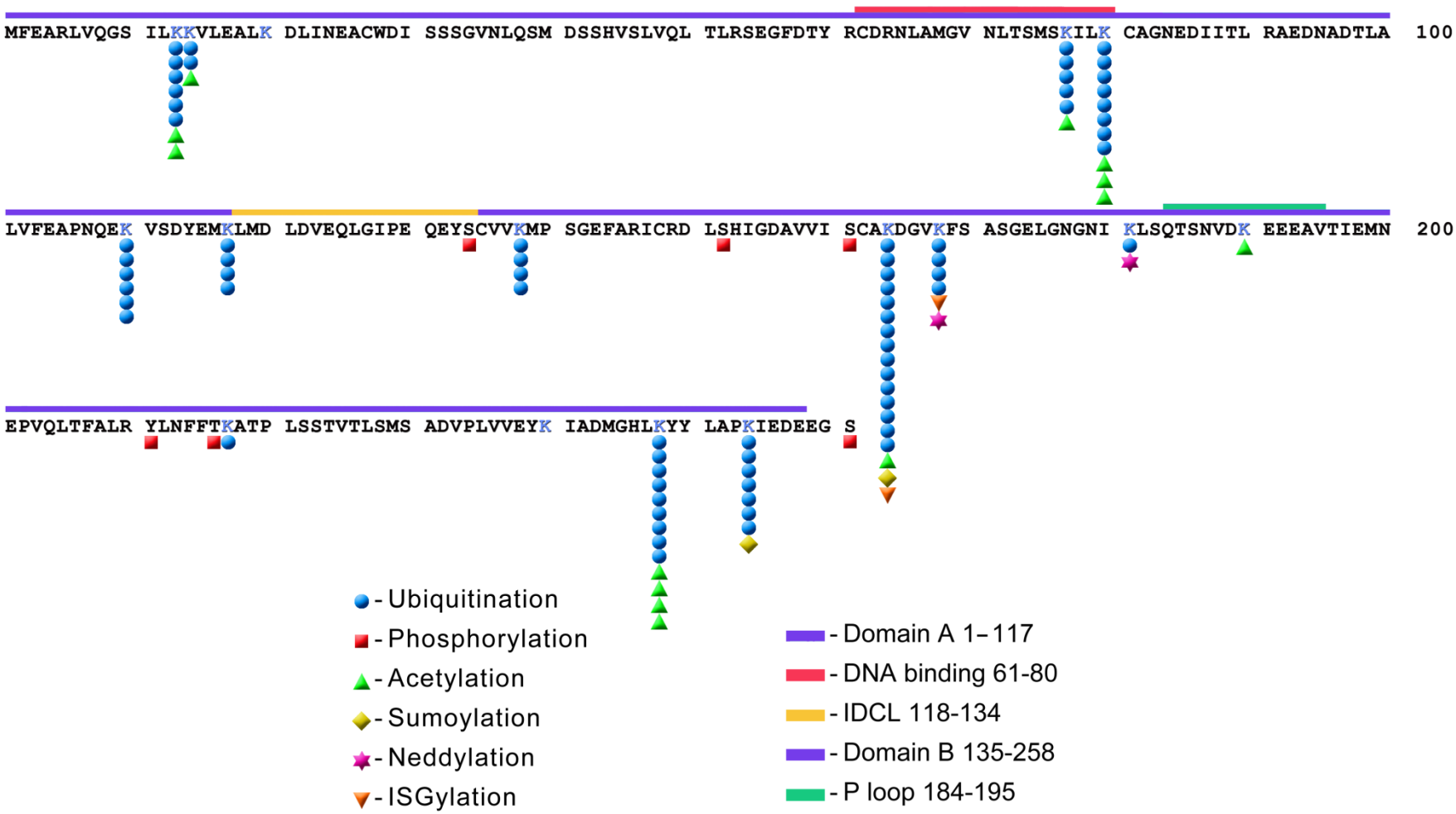


Multiple sites of PCNA's modification



McIntyre & Woodgate DNA Repair (2015) 29; 166-179

Acknowledgments

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