Nucleotide Excision Repair in *Mycobacterium tuberculosis*

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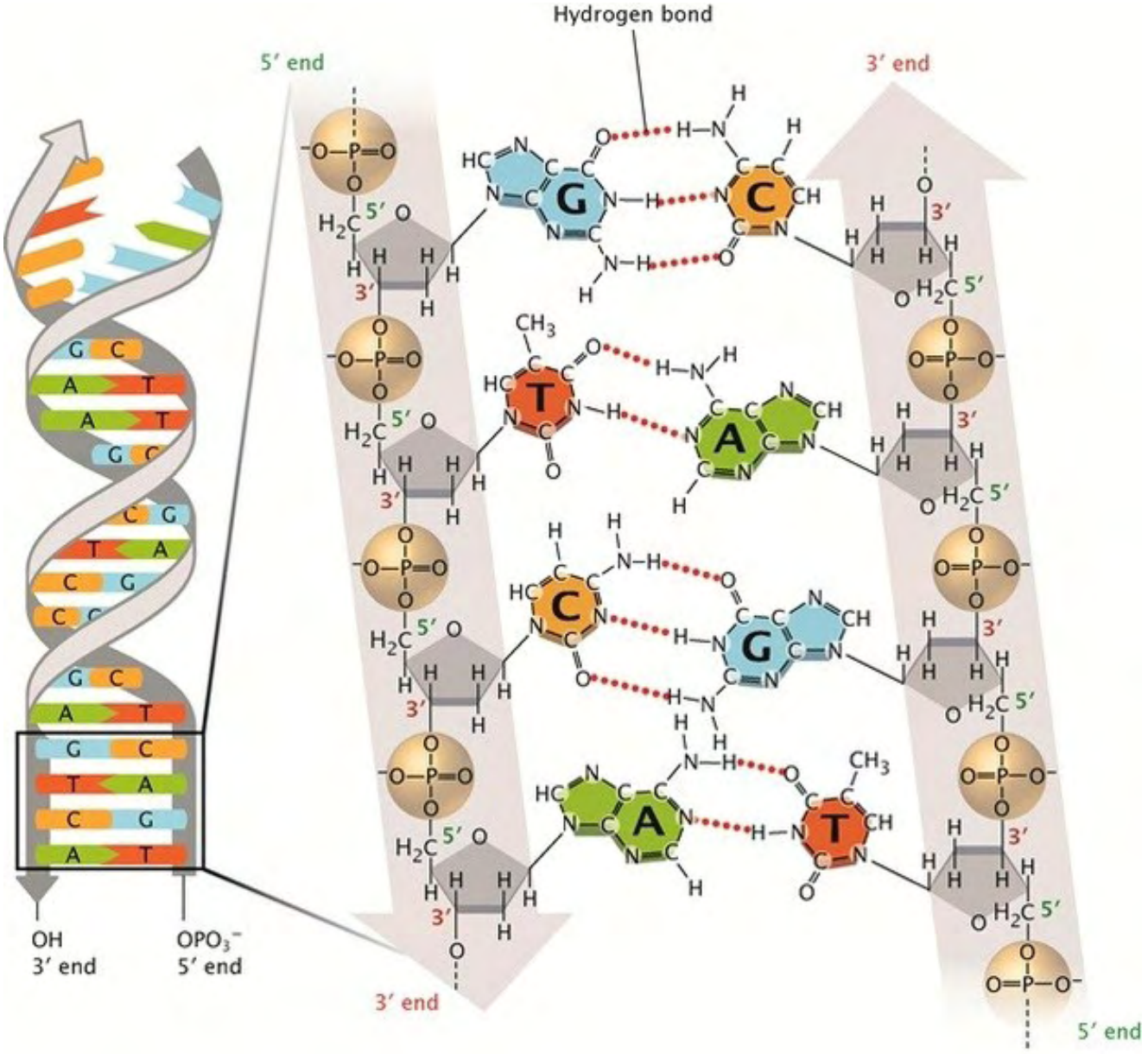
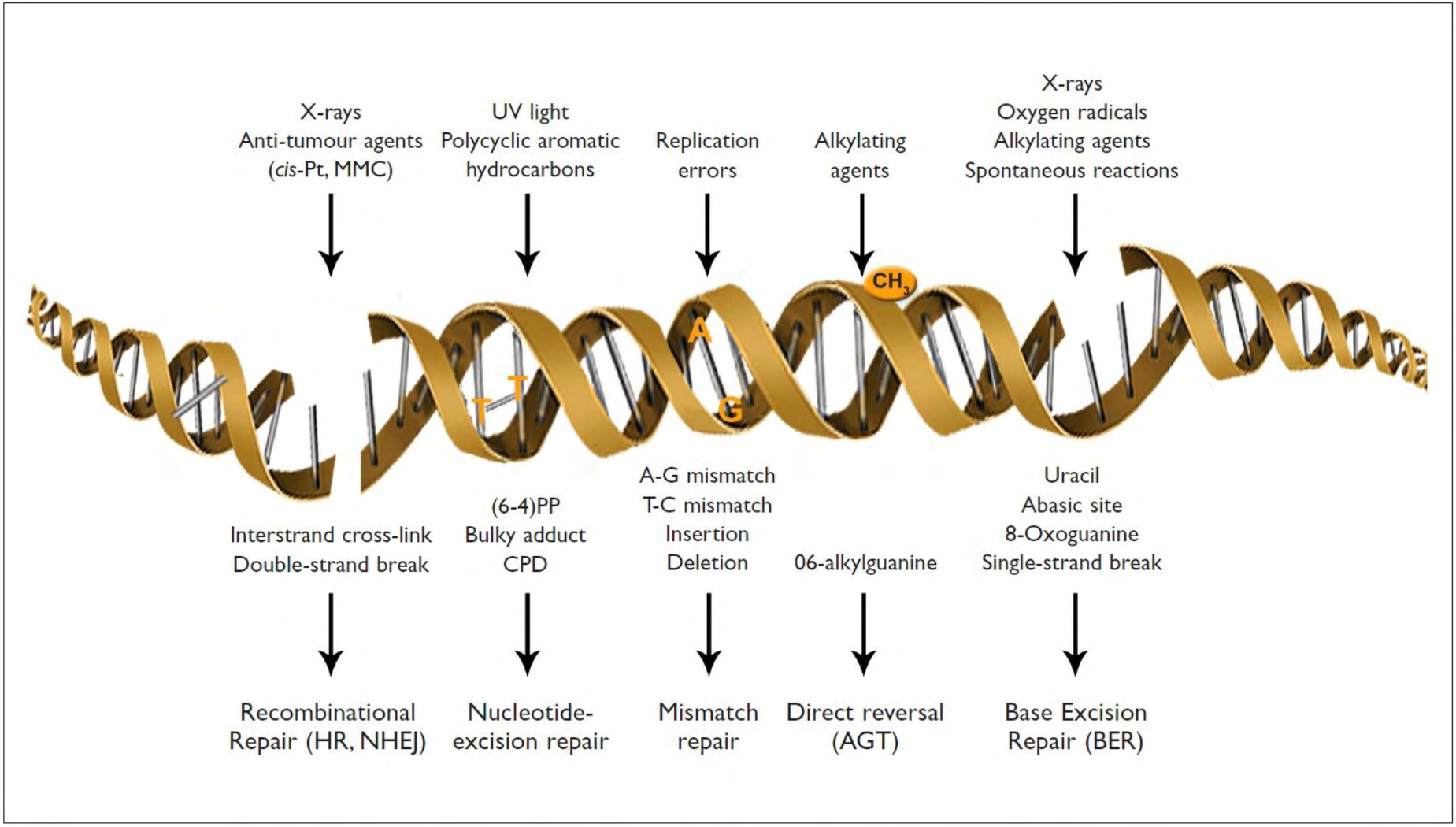
Manhattan, New York
05.09.2020

Chemical composition of DNA



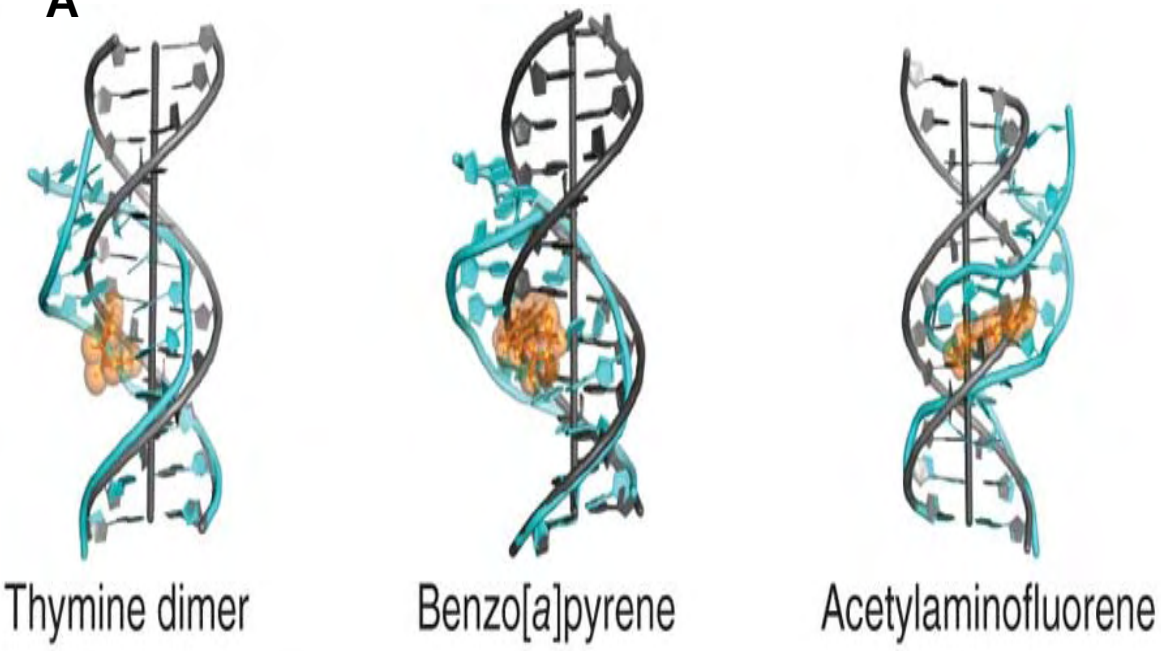
Types of DNA damages and DNA repair processes



Stephen P. Jackson and Cheryl L. Bishop, DDW Fall 2003 issue

Deformed DNA conformations processed by NER

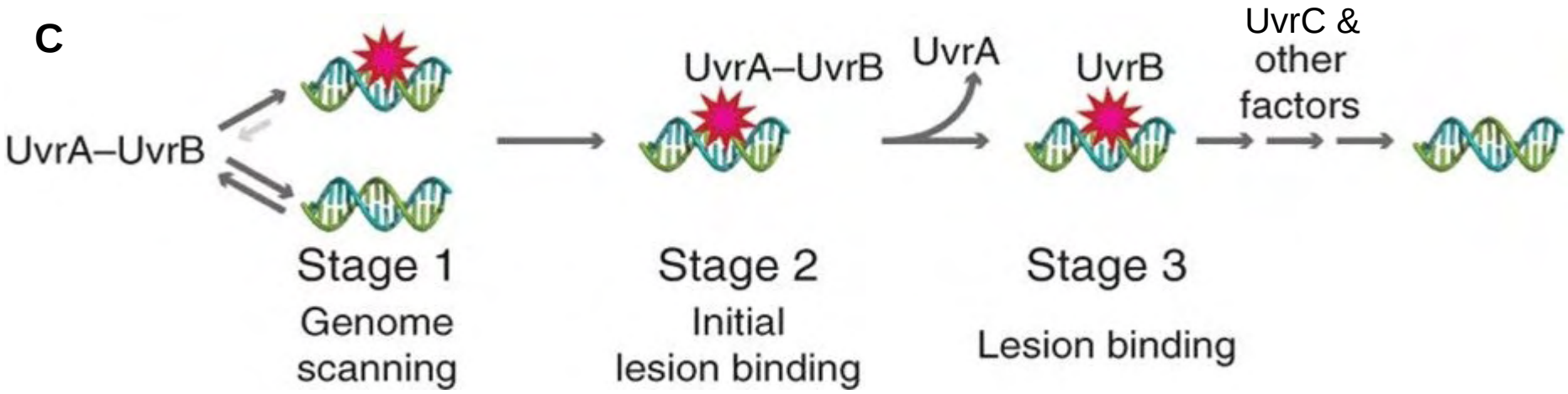
A



B

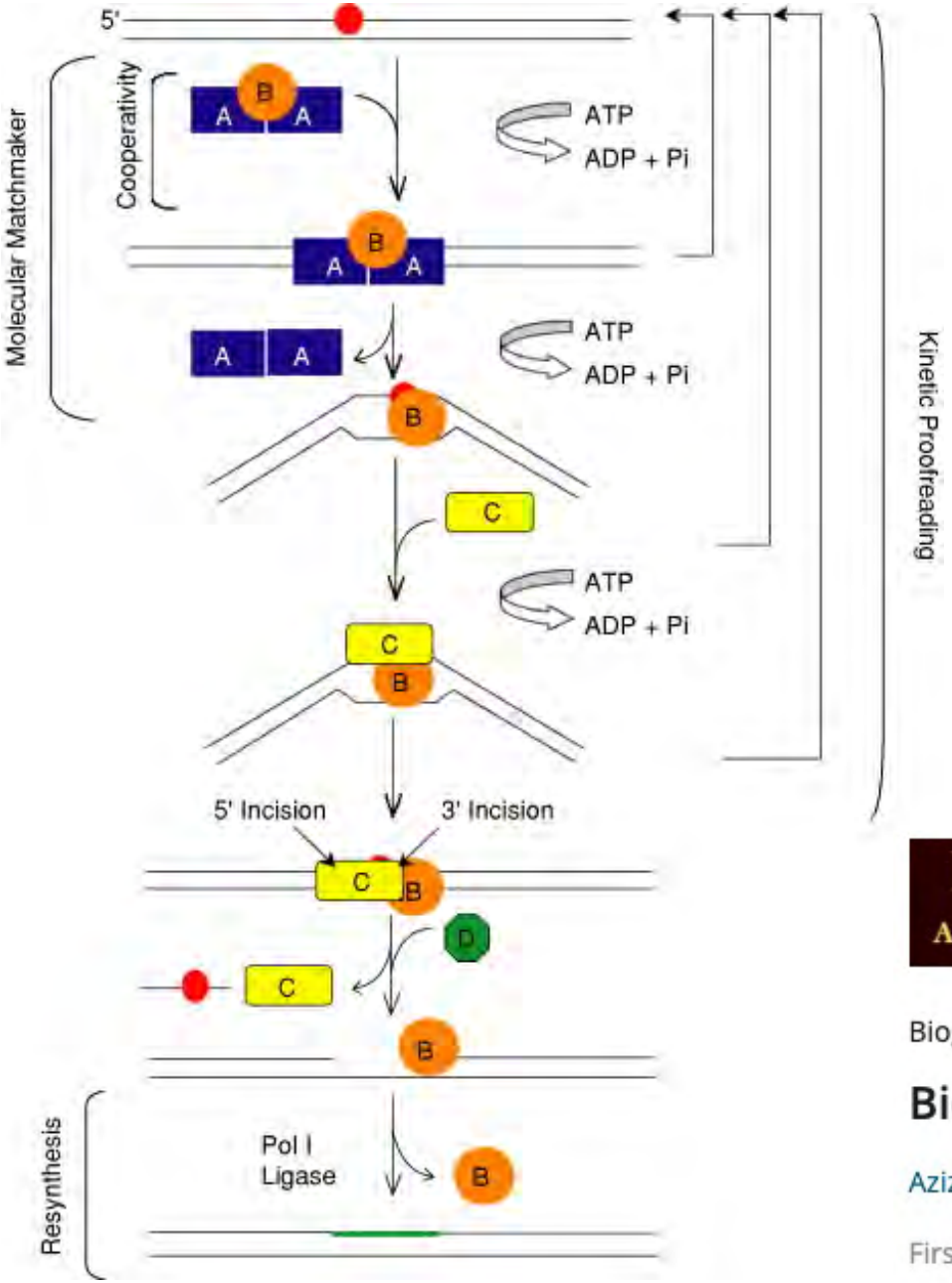
Type of lesion	Lesion
Single base modification	Thymine glycol
	Dihydrothymine
	Benzo[α]pyrene adduct
	Anthramycin adduct
	O^4-alkyl thymine
	O^6-methyl guanine
	N^6-methyl adenine
	Psoralen adduct
	Nitrogenous base removed (AP site)
Intra-DNA strand cross-links	*cis*-Platin adduct
	Pyrimidine dimer
	(6-4) photoproduct
Inter-DNA strand cross-links	*cis*-Platin adduct
	Nitrogen mustard adduct
Non-covalent modifications	Psoralen bisadduct
	Caffeine complex
	Ditercalinum complex

C



Pakotiprapha *et al*., (2012) *Nat. Struct. & Mol. Biol.*

Nucleotide excision repair in *Escherichia coli*



Sancar & Reardon (2004) Adv. Protein Chem.

Aziz Sancar
The Nobel Prize in Chemistry 2015
Prize share: 1/3: "for mechanistic studies of DNA repair."

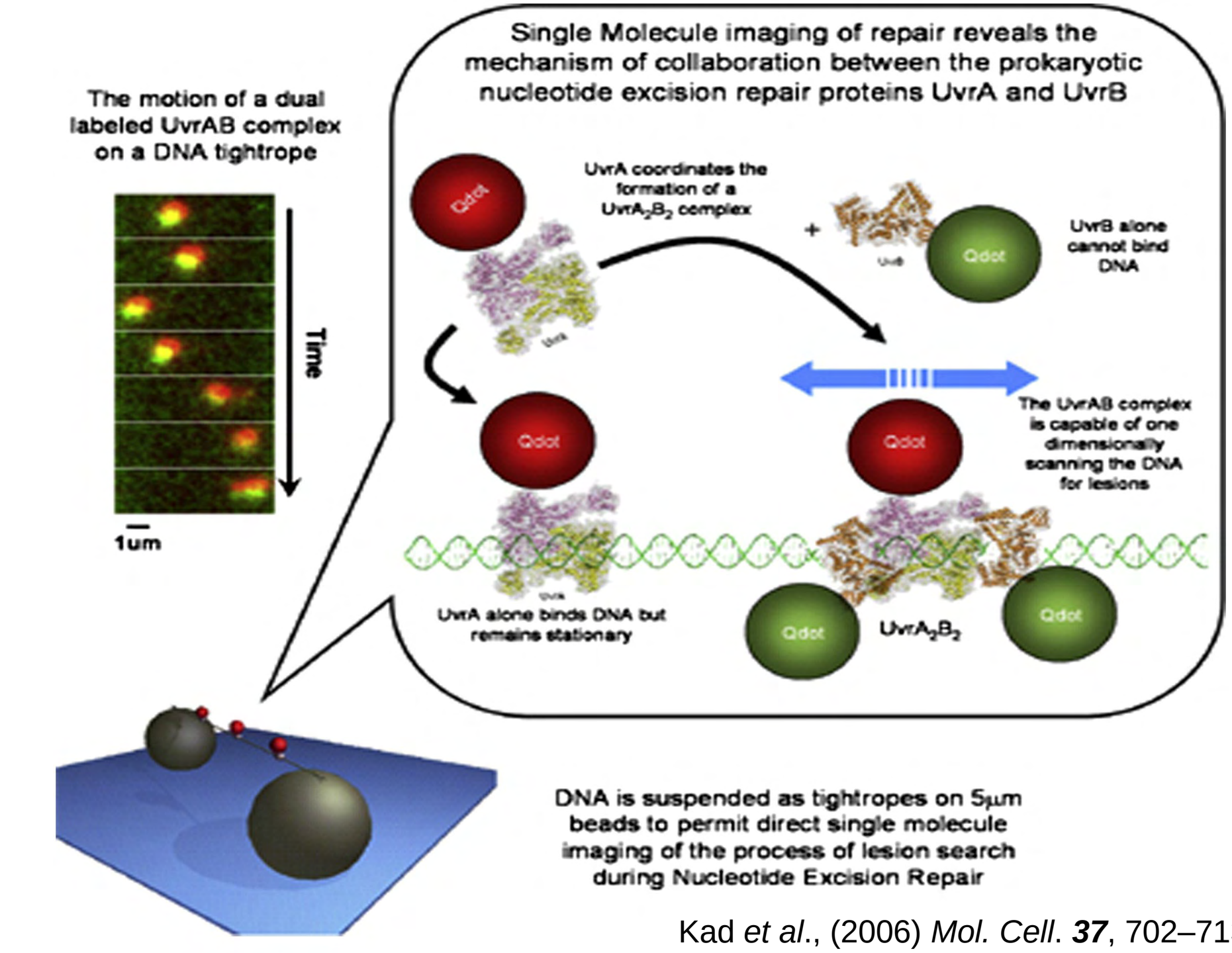
Photochemistry and Photobiology

Biography

Biography

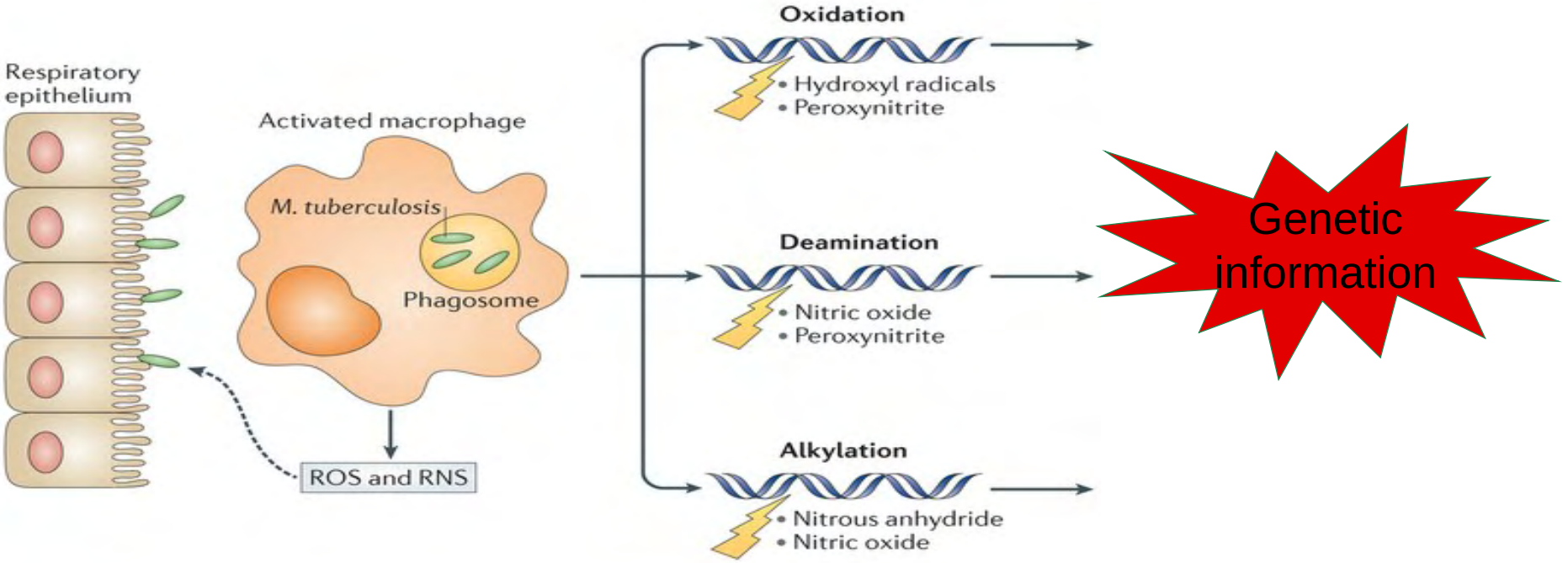
Aziz Sancar

First published: 17 February 2017 | https://doi.org/10.1111/php.12731



Kad *et al*., (2006) *Mol. Cell*. ***37***, 702–713.

DNA repair systems and *Mycobacterium tuberculosis*



Veen & Tang (2015) *Nat. Rev. Microbiol.*

- **However, in contrast to *Helicobacter pylori,* where the absence of a functional MMR system was correlated to a markedly high level of genetic diversity, *M. tuberculosis* genomes are very stable.**
  Kang & Blaser (2006) *Nat. Rev. Microbiol.* ***4***, 826–836.

Transcription of the *uvr*A gene has been shown to be up-regulated in human macrophage-grown *M. tuberculosis bacilli* hours post infection. Graham & Clark-Curtiss (1999) *Proc. Natl. Acad. Sci. U S A.* ***96***, 11554-11559

Gene inactivation and trans-complementation analyses demonstrated a crucial role for UvrB in mycobacterial resistance to nitrosative, oxidative and UV exposure-induced DNA damage. Darwin *et al*., (2003) *Science* ***302***, 1963-1966

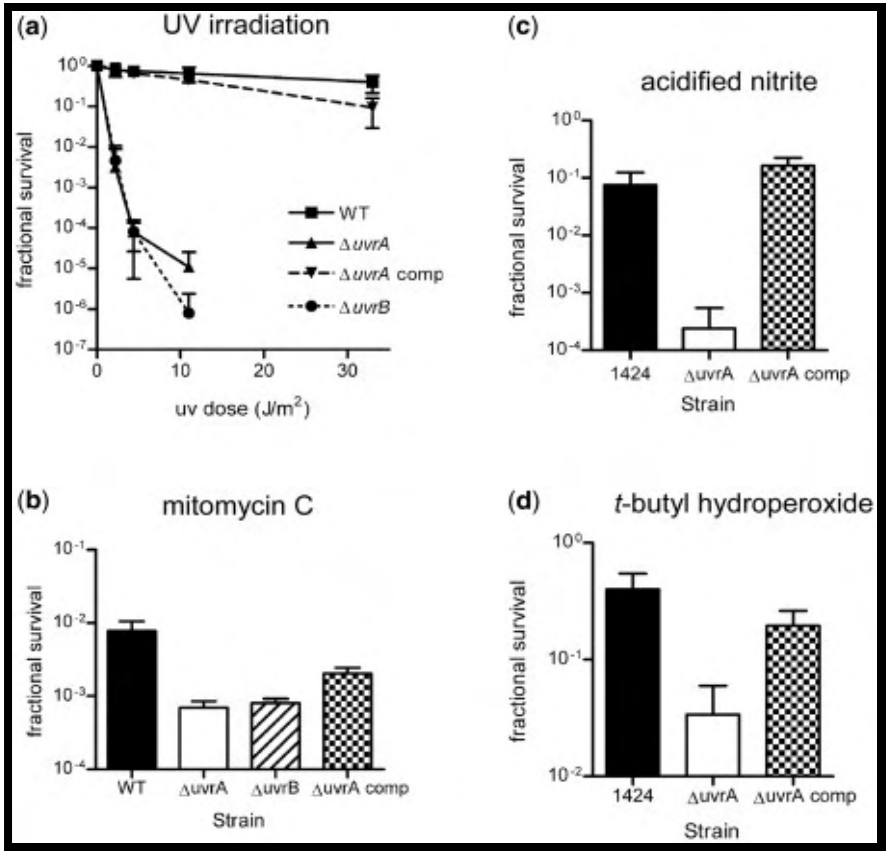
Importance of the *uvr*B gene for *M. tuberculosis* survival and virulence in the mouse model. Darwin & Nathan (2005) *Infect. Immun*. ***73***, 4581– 4587

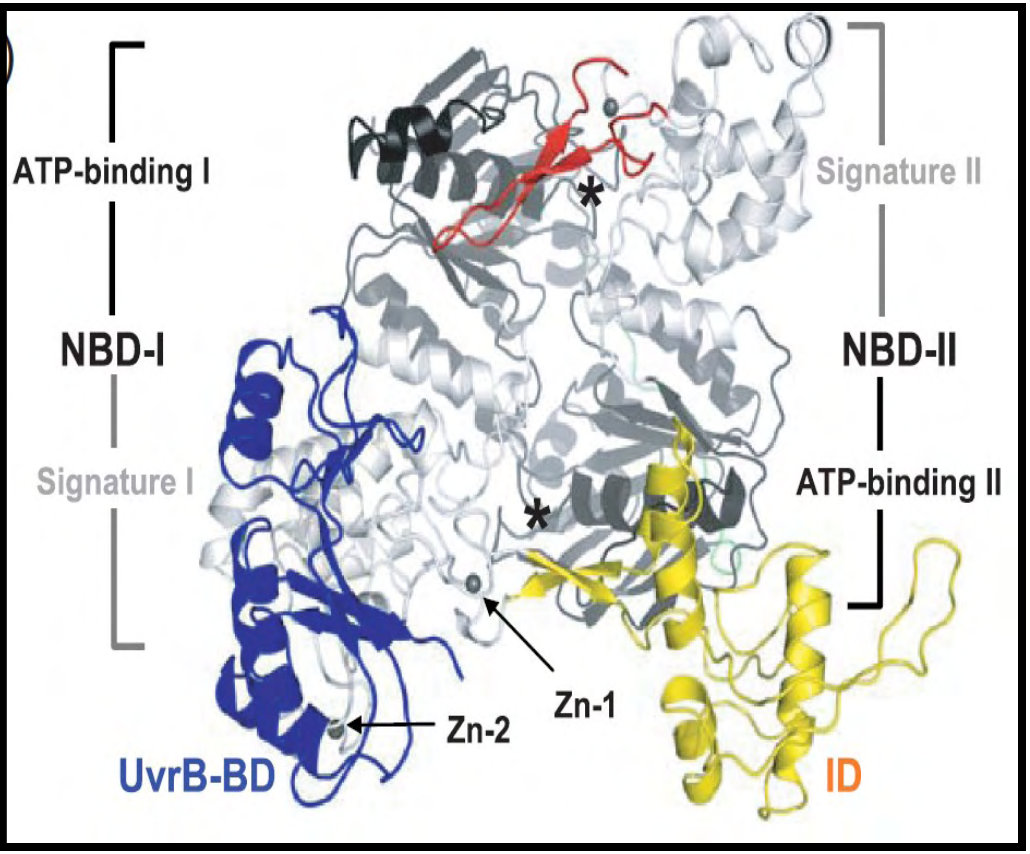
Identification of a chemical that inhibits the mycobacterial UvrABC complex in nucleotide excision repair. Mazloum *et al*., (2011) *Biochemistry* ***50***, 1329-1335

7316–7328 Nucleic Acids Research, 2011, Vol. 39, No. 16 Published online 27 May 2011
doi:10.1093/nar/gkr271

The biological and structural characterization of *Mycobacterium tuberculosis* UvrA provides novel insights into its mechanism of action

Franca Rossi , Jasbeer Singh Khanduja , Alessio Bortoluzzi , Joanna Houghton , Peter Sander , Carolin Güthlein , Elaine O. Davis , Burkhard Springer , Erik C. Böttger , Annalisa Relini , Amanda Penco , K. Muniyappa and Menico Rizzi *





Examples Using NER Proteins: MtUvrA, MtUvrB and MtUvrC

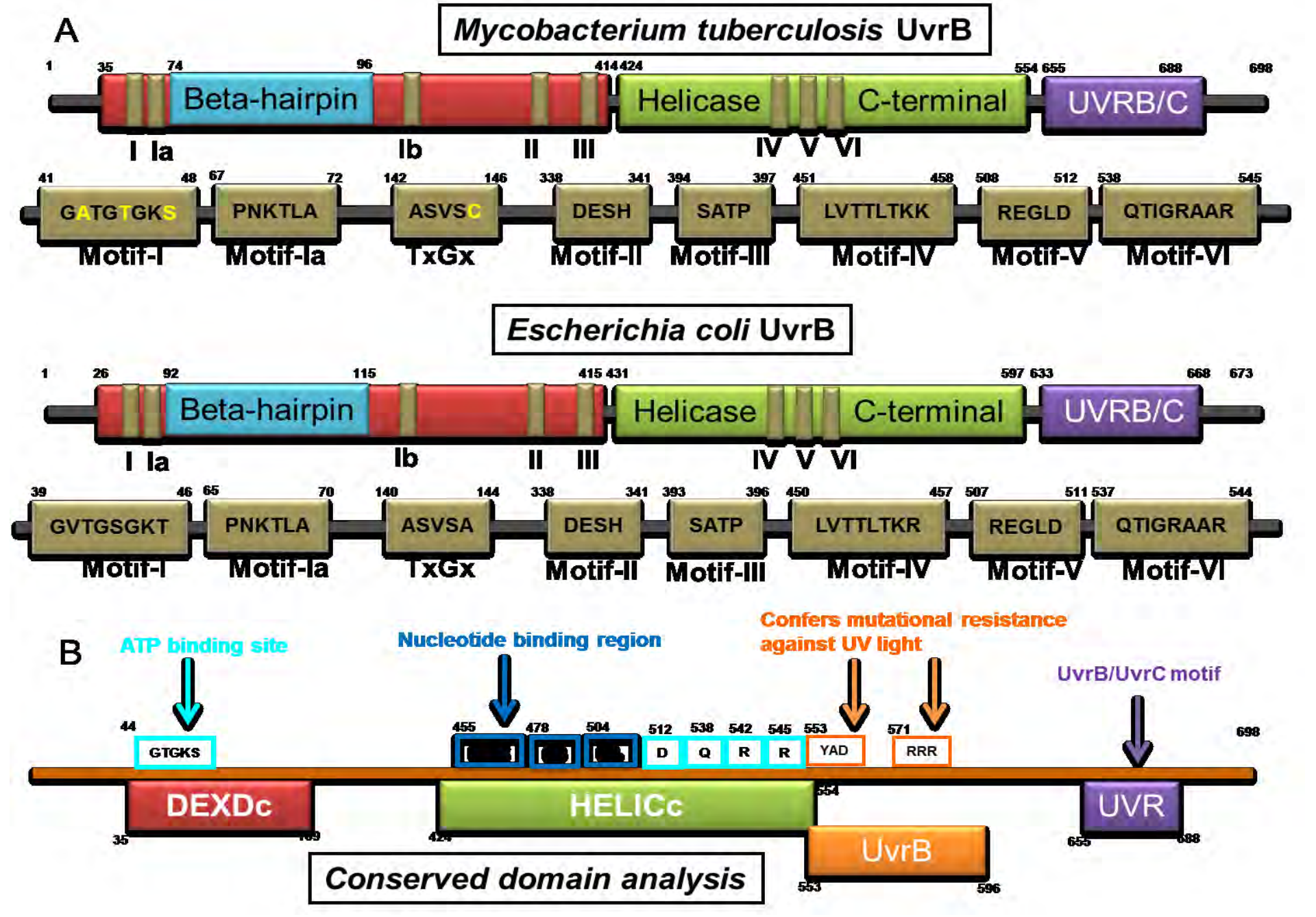
Strategies

- ✓ How to purify *M. tuberculosis* protein from the surrogate host, *E. coli* ?
- ✓ Site Directed Mutagenesis
- ✓ Understanding the role of Motif in the protein
- ✓ Techniques to study protein-protein interaction

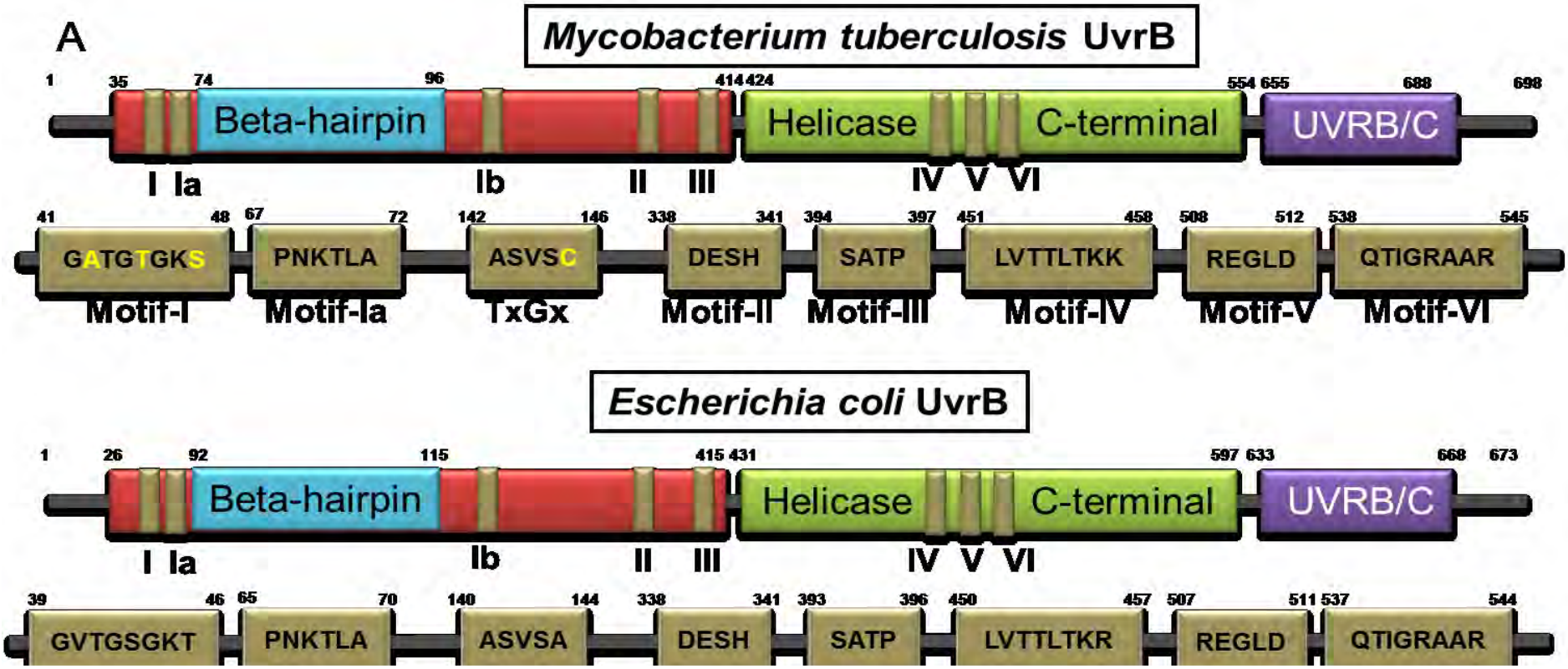
Comparison of *E. coli* and *M. tuberculosis* UvrB Protein

Organism	Similarity (%)	Identity (%)
Mycobacterium smegmatis	97.1	92.2
Mycobacterium leprae (Strain TN)	96.7	95
Mycobacterium bovis	100	100
Escherichia coli (Strain K12)	68.3	54
Thermotoga maritima	64.8	50
Thermus thermophilus	66.5	55
Bacillus subtilis (Strain 168)	70.1	58

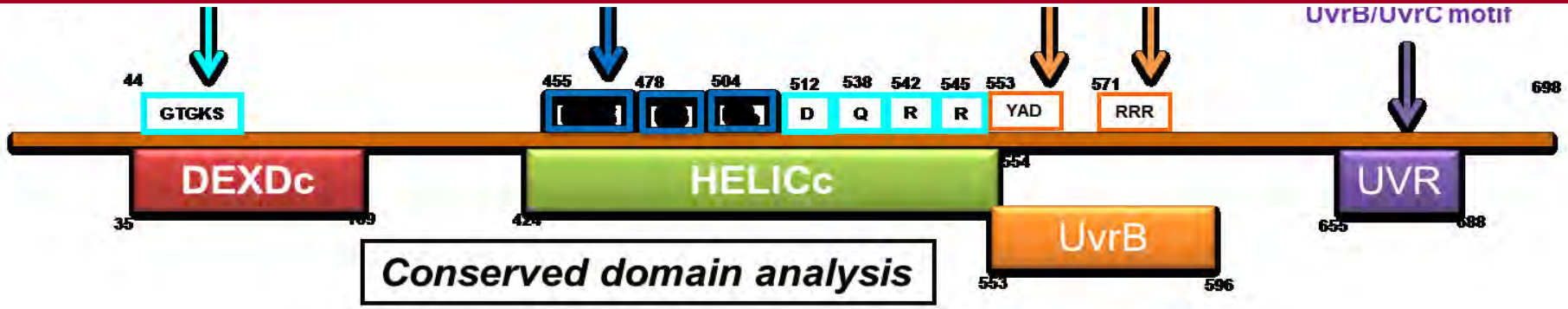
Comparison of *E. coli* and *M. tuberculosis* UvrB Protein



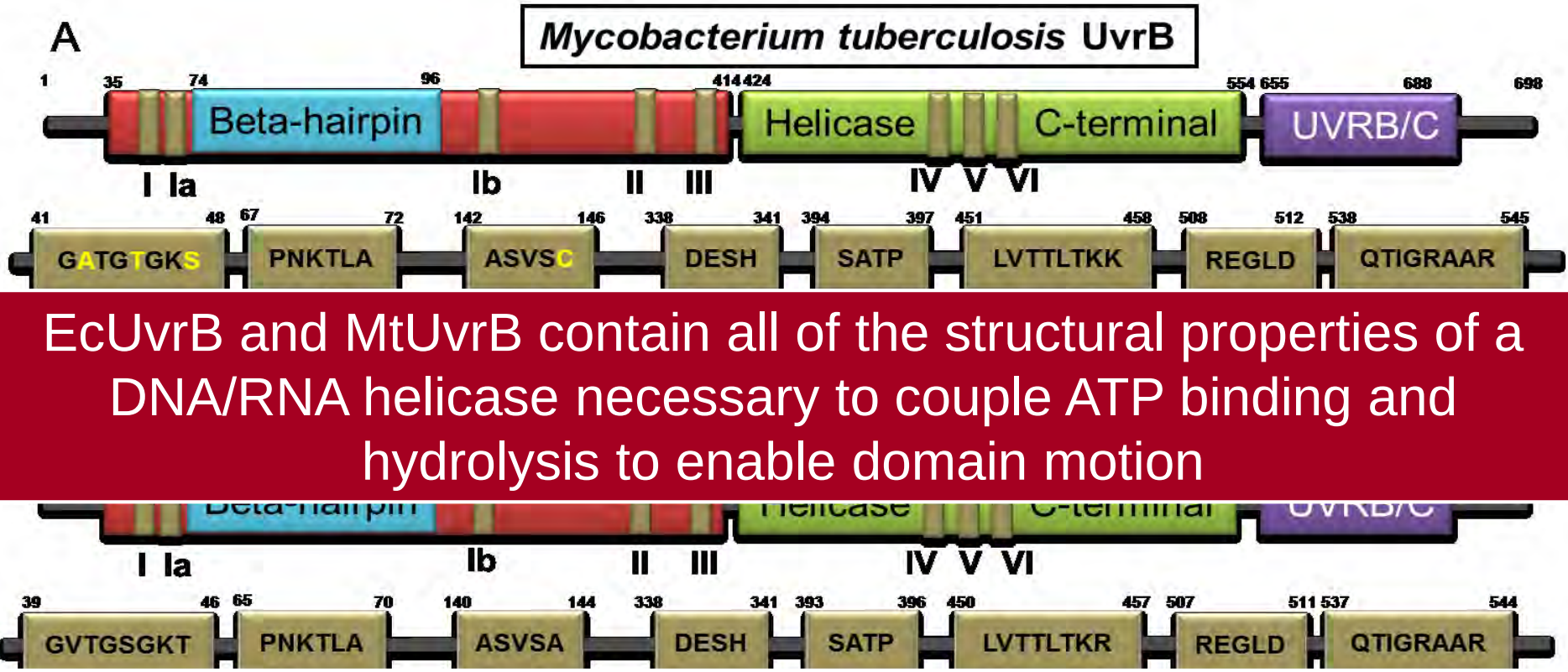
Comparison of *E. coli* and *M. tuberculosis* UvrB Protein



What is the role of these structural domains in the activity of MtUvrB?

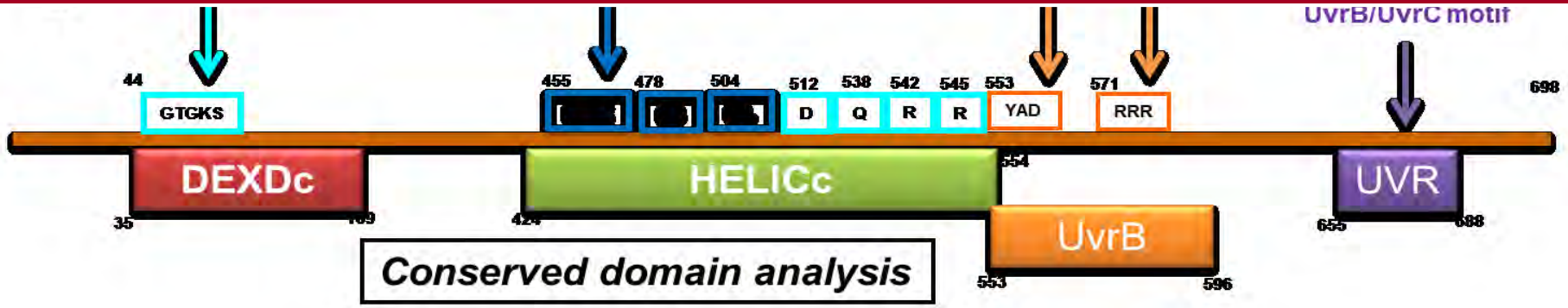


Comparison of *E. coli* and *M. tuberculosis* UvrB Protein

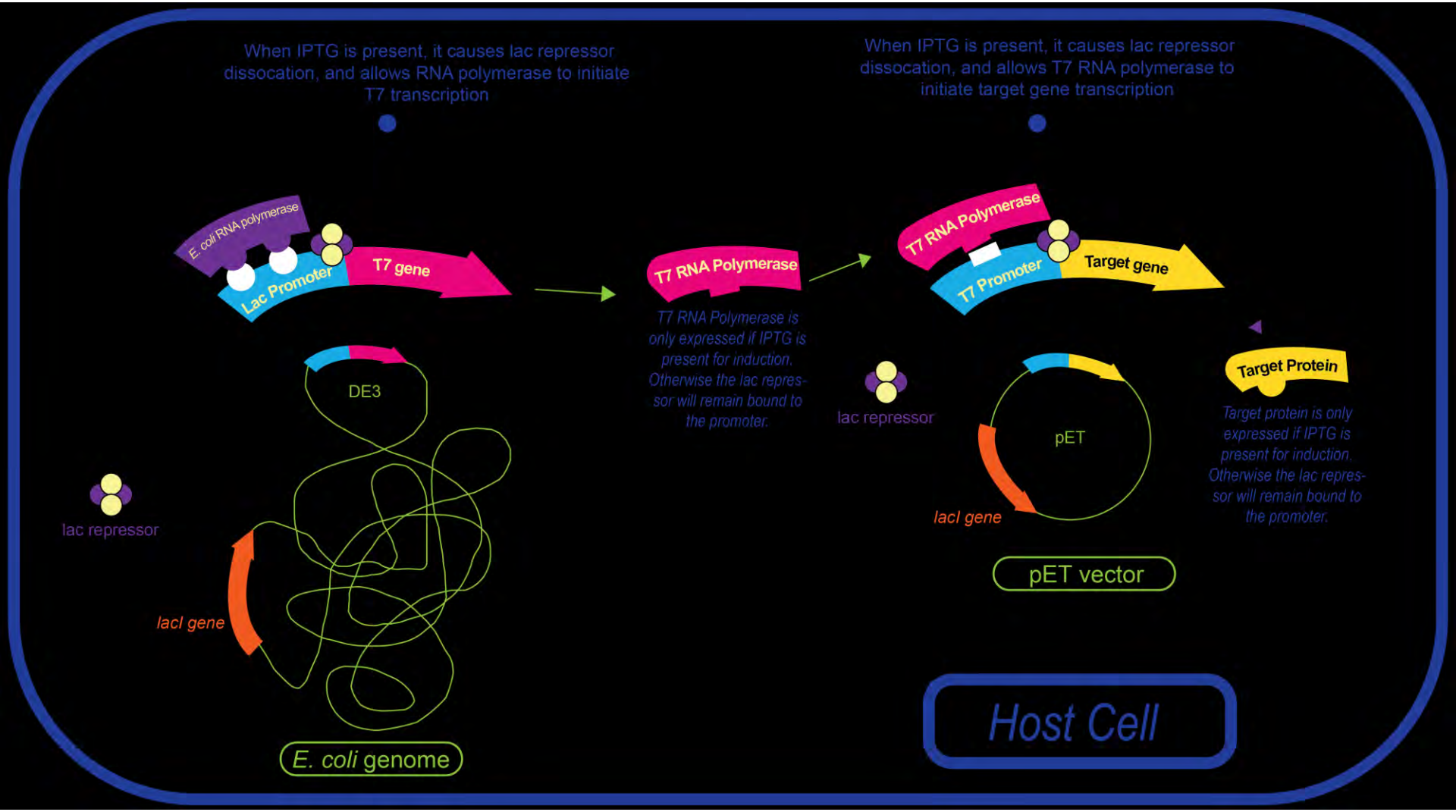


EcUvrB and MtUvrB contain all of the structural properties of a DNA/RNA helicase necessary to couple ATP binding and hydrolysis to enable domain motion

What is the role of these structural domains in the activity of MtUvrB?

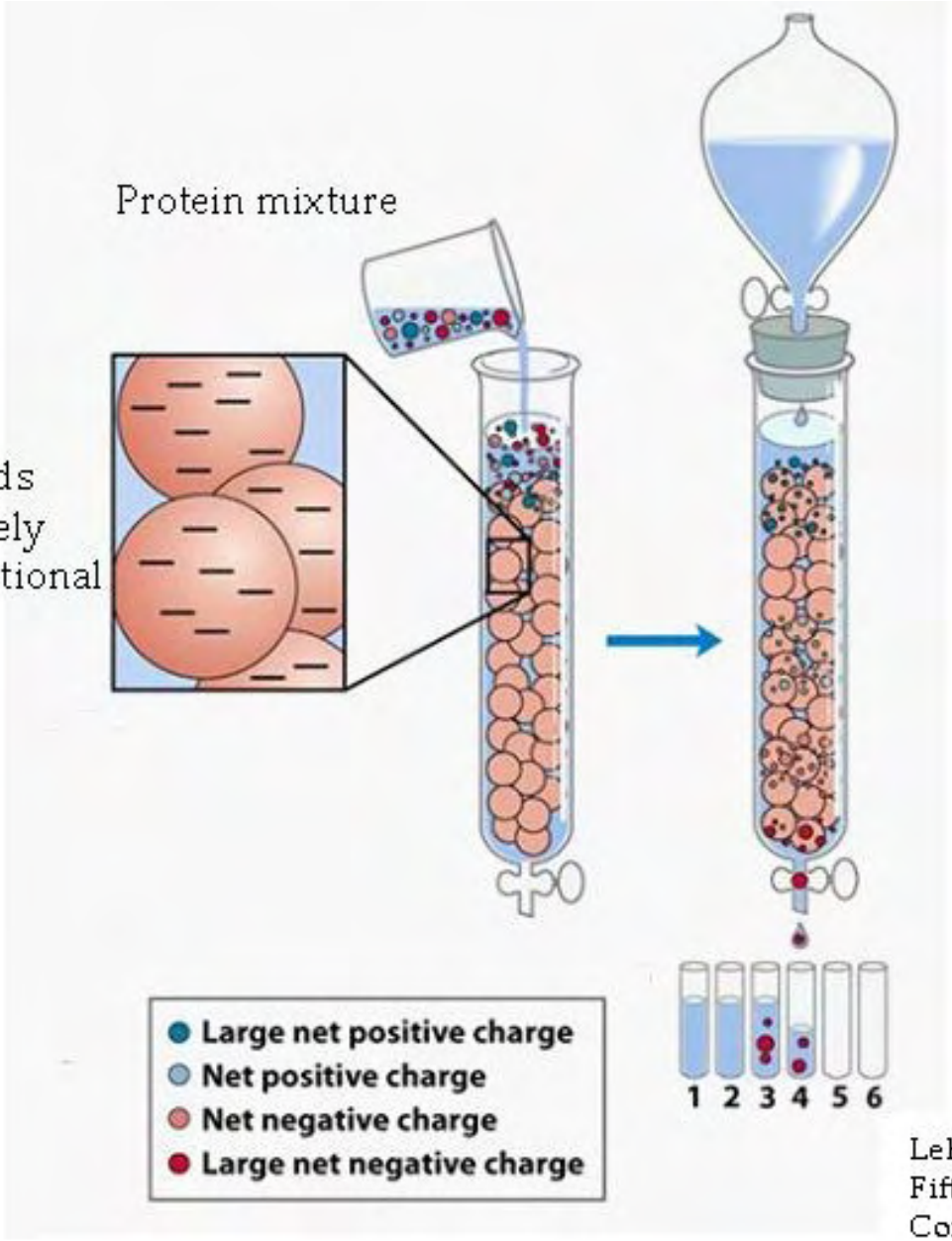


Overexpression of proteins using *E. coli* as a host



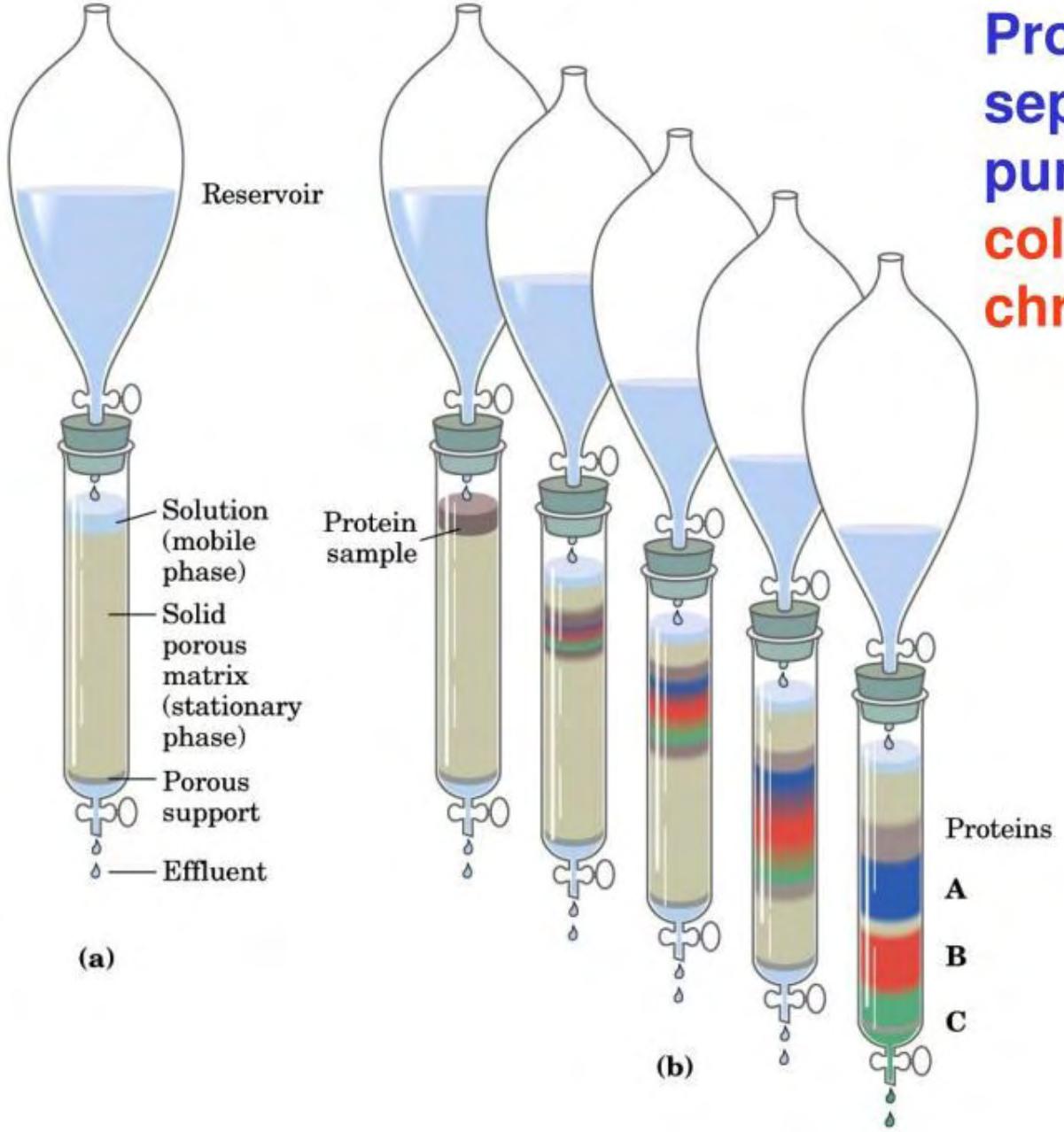
https://www.goldbio.com/articles/article/a-deep-dive-into-iptg-induction

Ion Exchange Chromatography



Lehninger Principles of Biochemistry, Fifth Edition, © 2008 W.H. Freeman and Company

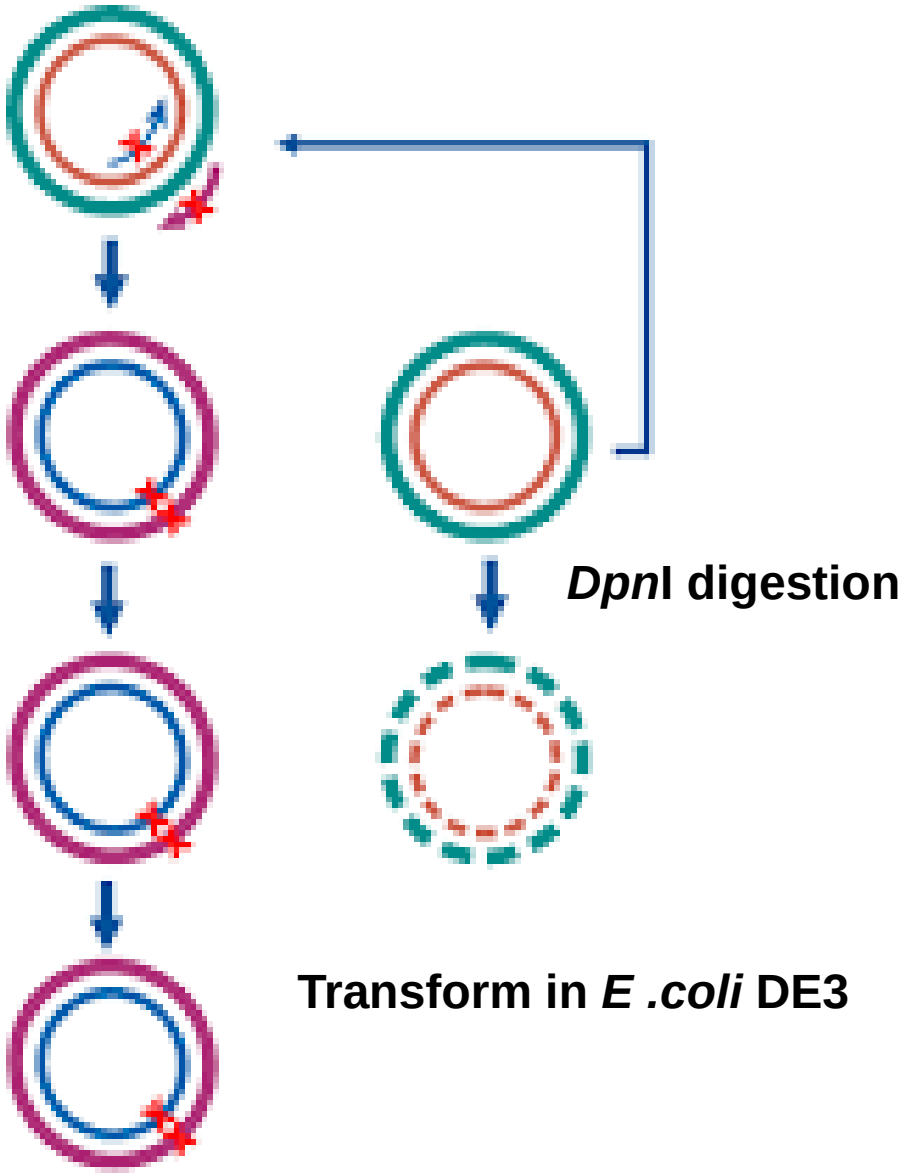
Chromatographic separations



Protein separation and purification by column chromatography

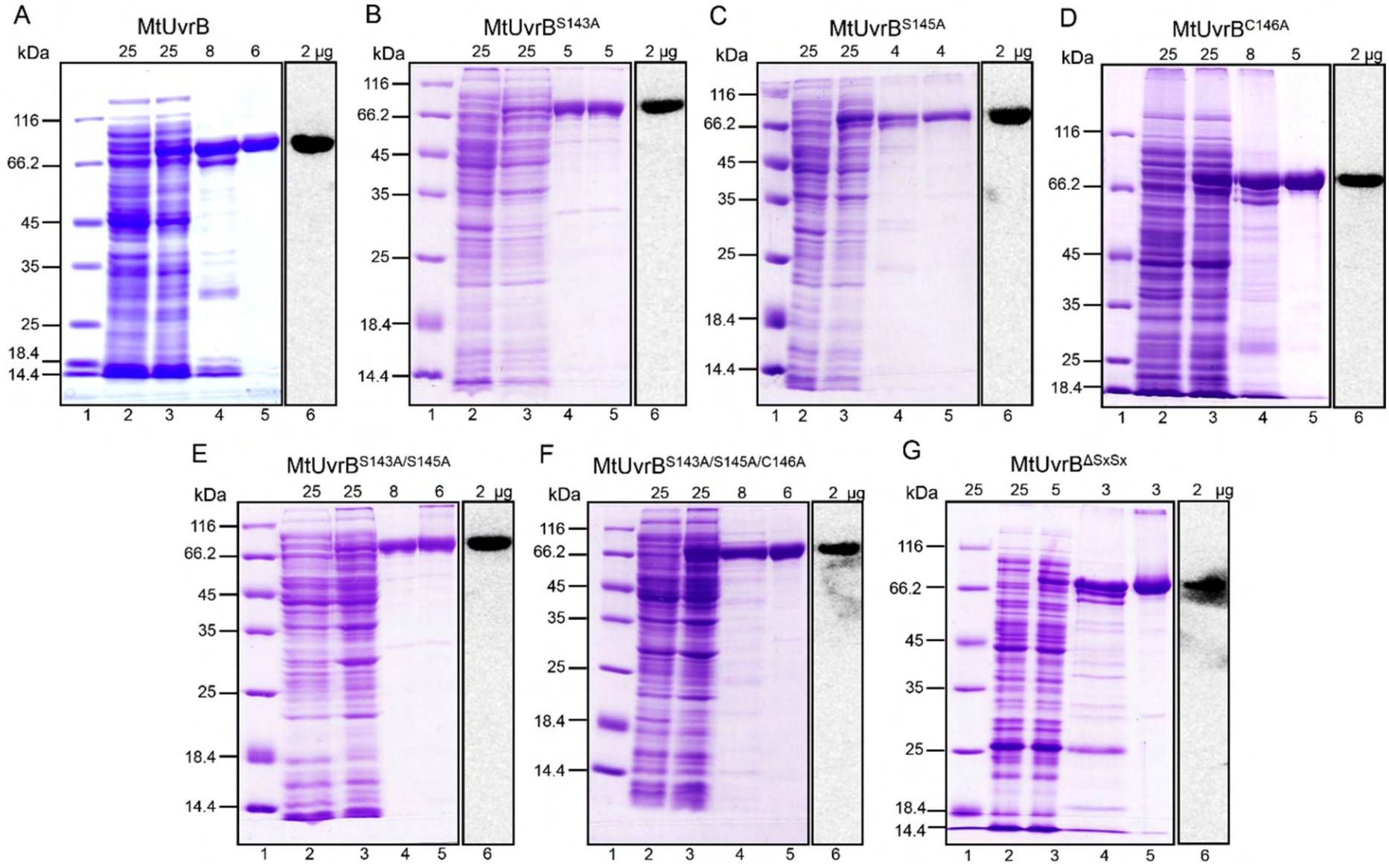
From Lehninger Principles of Biochemistry

Site Directed Mutagenesis Using Single/Complimentary Primers



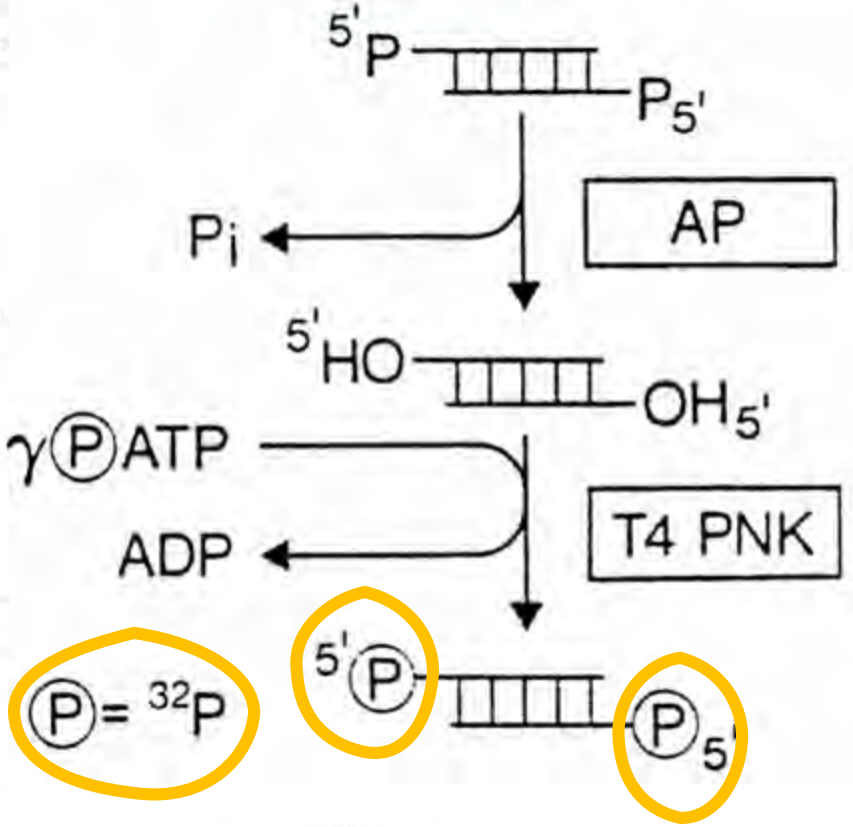
U.S. Patent Nos. 6,391,548, 5,932,419, 5,789,166, 7,132,265, and 7,176,004

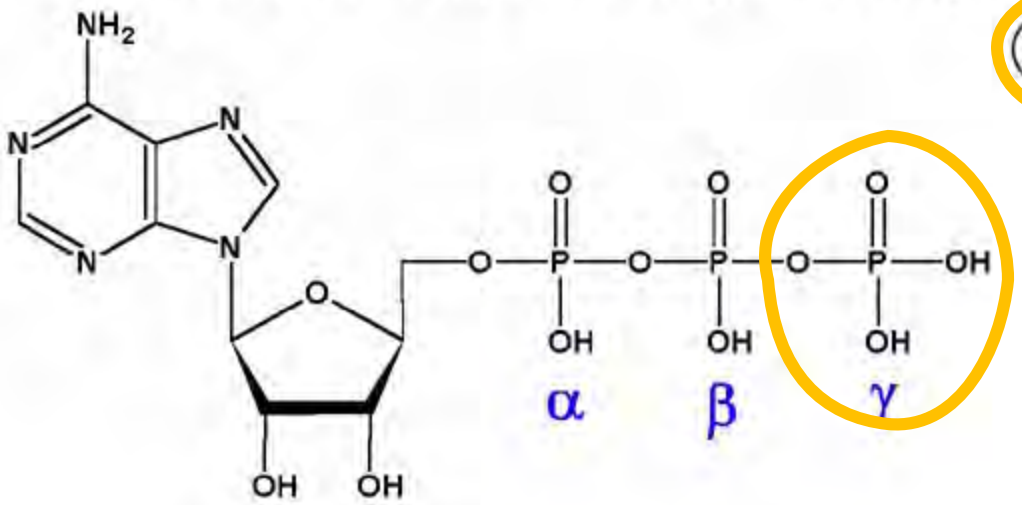
Expression and Purification of MtUvrB or its variants

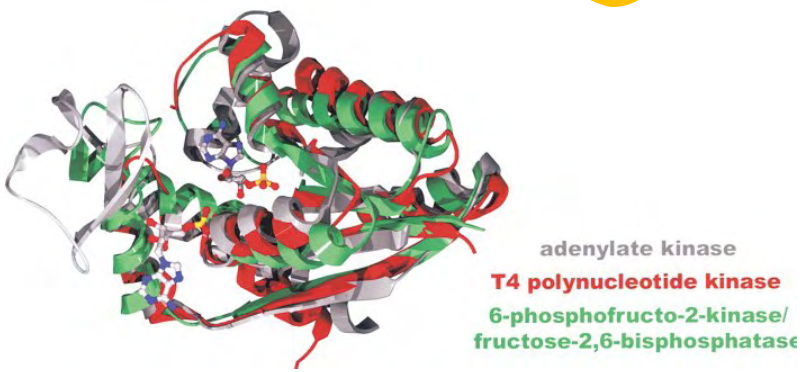


T4 Polynucleotide Kinase

- transfers γ-PO_4 from ATP to 5'-OH
- major uses:
  - end-labeling (dephosphorylate first if necessary)
  - phosphorylate synthetic oligonucleotides
  - Maxim-Gilbert sequencing



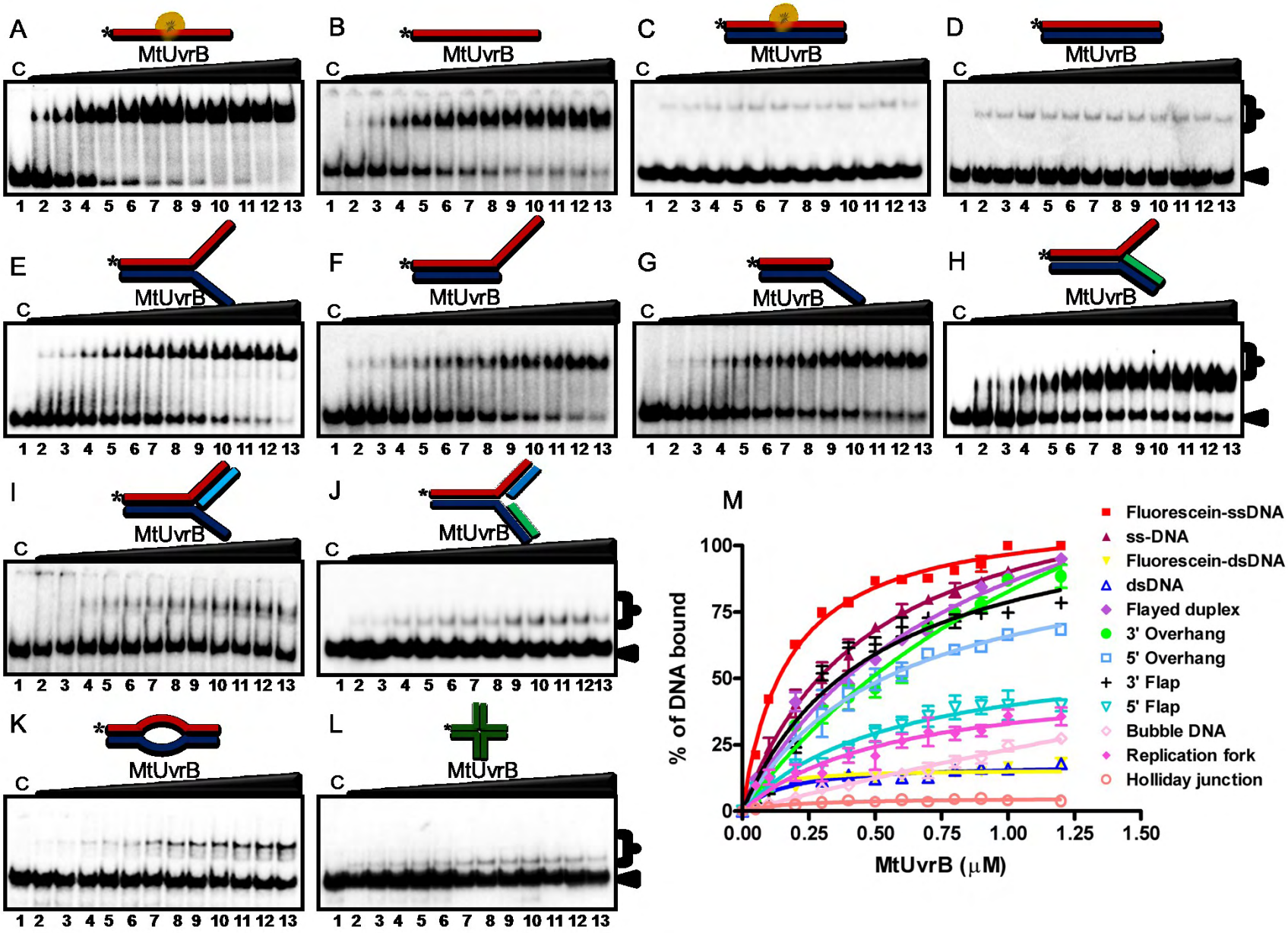




https://slideplayer.com/slide/759729/

Galburt et al., 2002 Structure

Characterization of DNA Substrate Specificity of MtUvrB



Characterization of DNA Substrate Specificity of MtUvrB

Substrate	K_d (nM)
Fluorescein-ssDNA	136.8 ± 4.46
Unmodified ssDNA	285.4 ± 3.19
3' flap	365.4 ± 2.69
Splayed duplex	389.5 ± 2.69
3' overhang	482 ± 2.51
5' overhang	530.5 ± 1.72
5' flap	1427.5 ± 2.17
Replication fork	1716.2 ± 3.30
Bubble-containing dsDNA	2247.9 ± 0.23
Fluorescein-dsDNA	ND
Unmodified dsDNA	ND
Holliday junction	ND

Volume 16 Number 20 1988 Nucleic Acids Research

Involvement of a cryptic ATPase activity of UvrB and its proteolysis product, UvrB* in DNA repair

Paul R.Caron+ and Lawrence Grossman

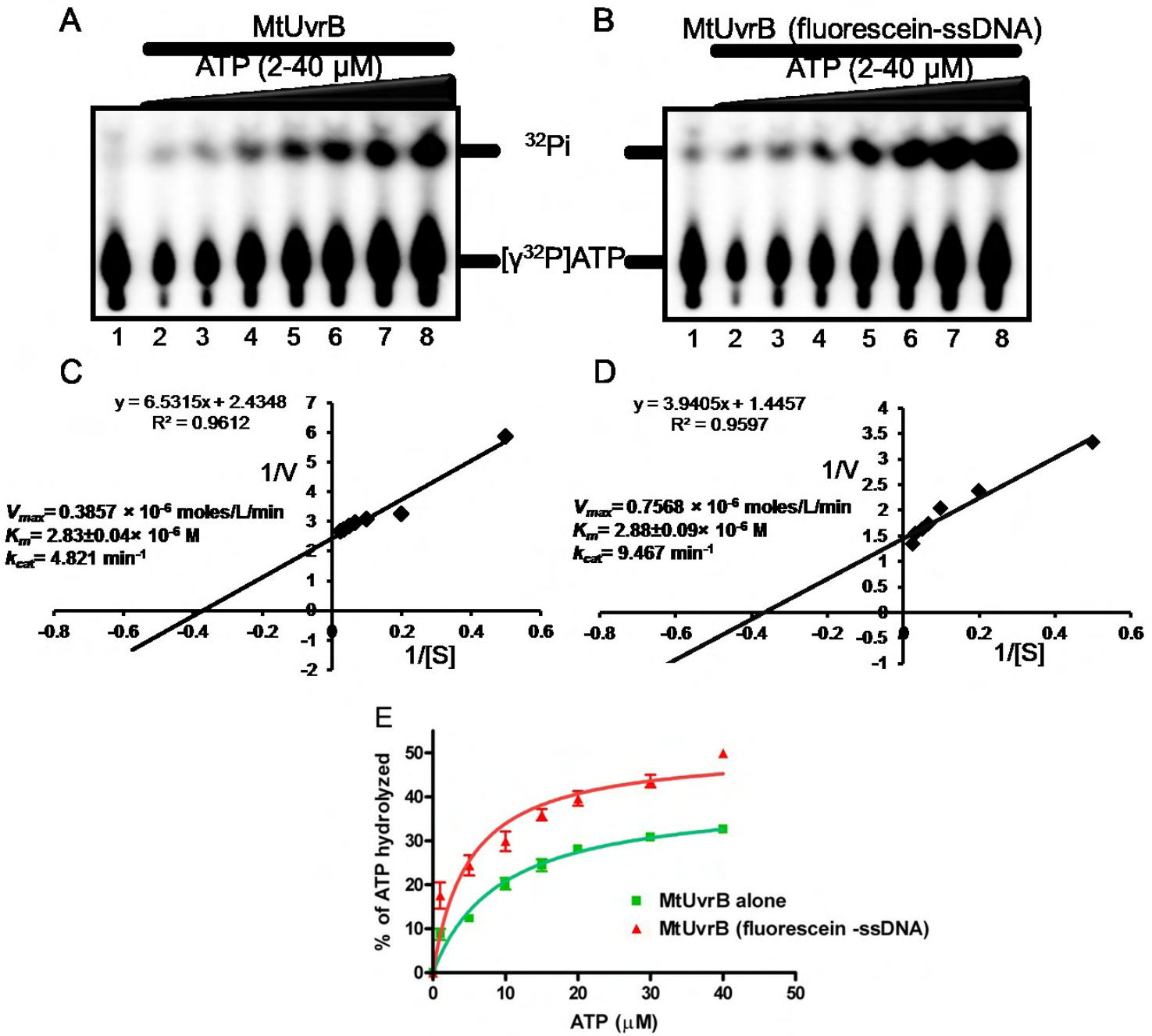
THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 271, No. 16, Issue of April 19, pp. 9612–9618, 1996

Printed in U.S.A.

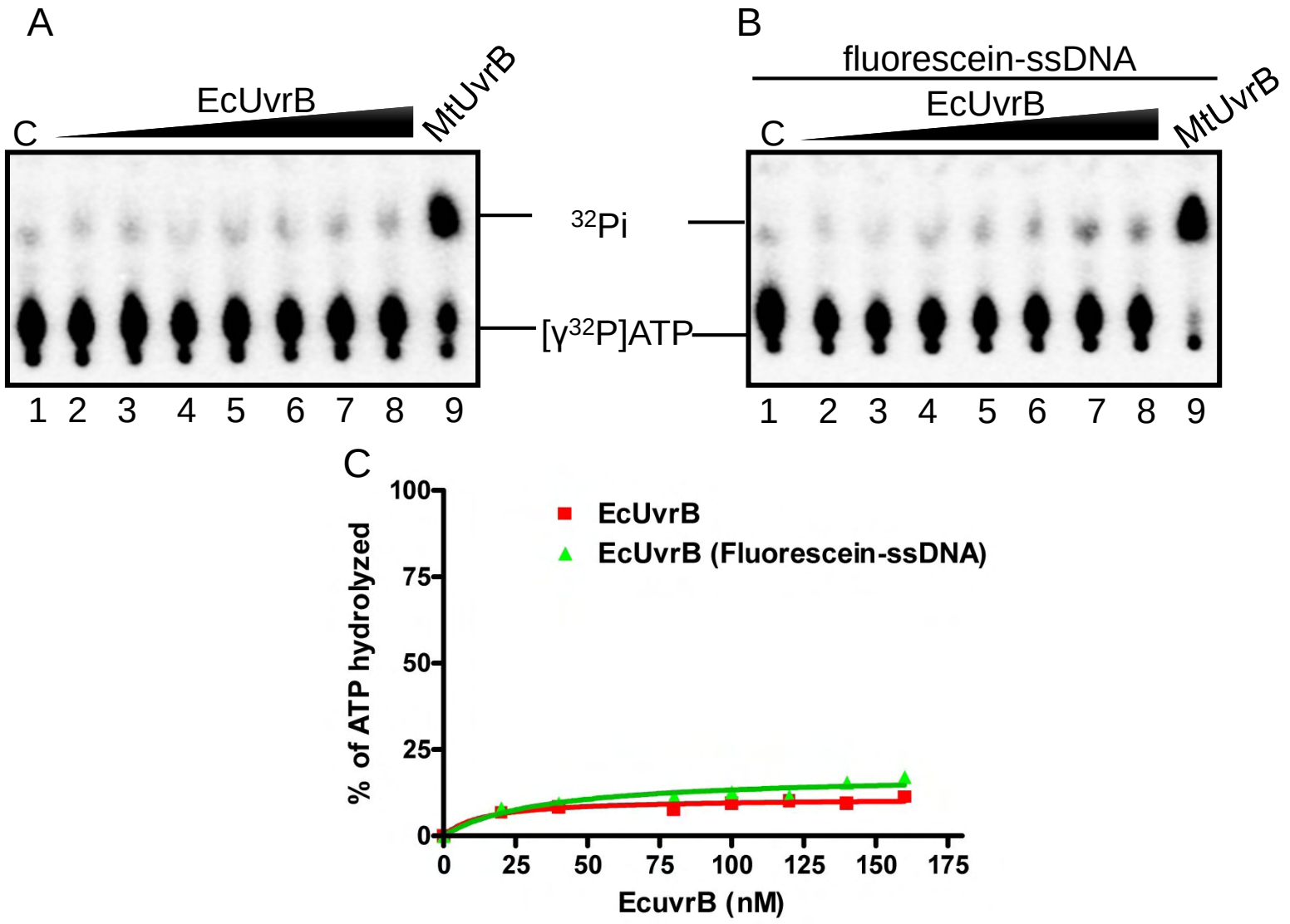
ATPase Activity of UvrB Protein from *Thermus thermophilus* HB8 and Its Interaction with DNA*

Ryuichi Kato, Noriko Yamamoto, Keiichi Kito, and Seiki Kuramitsu‡

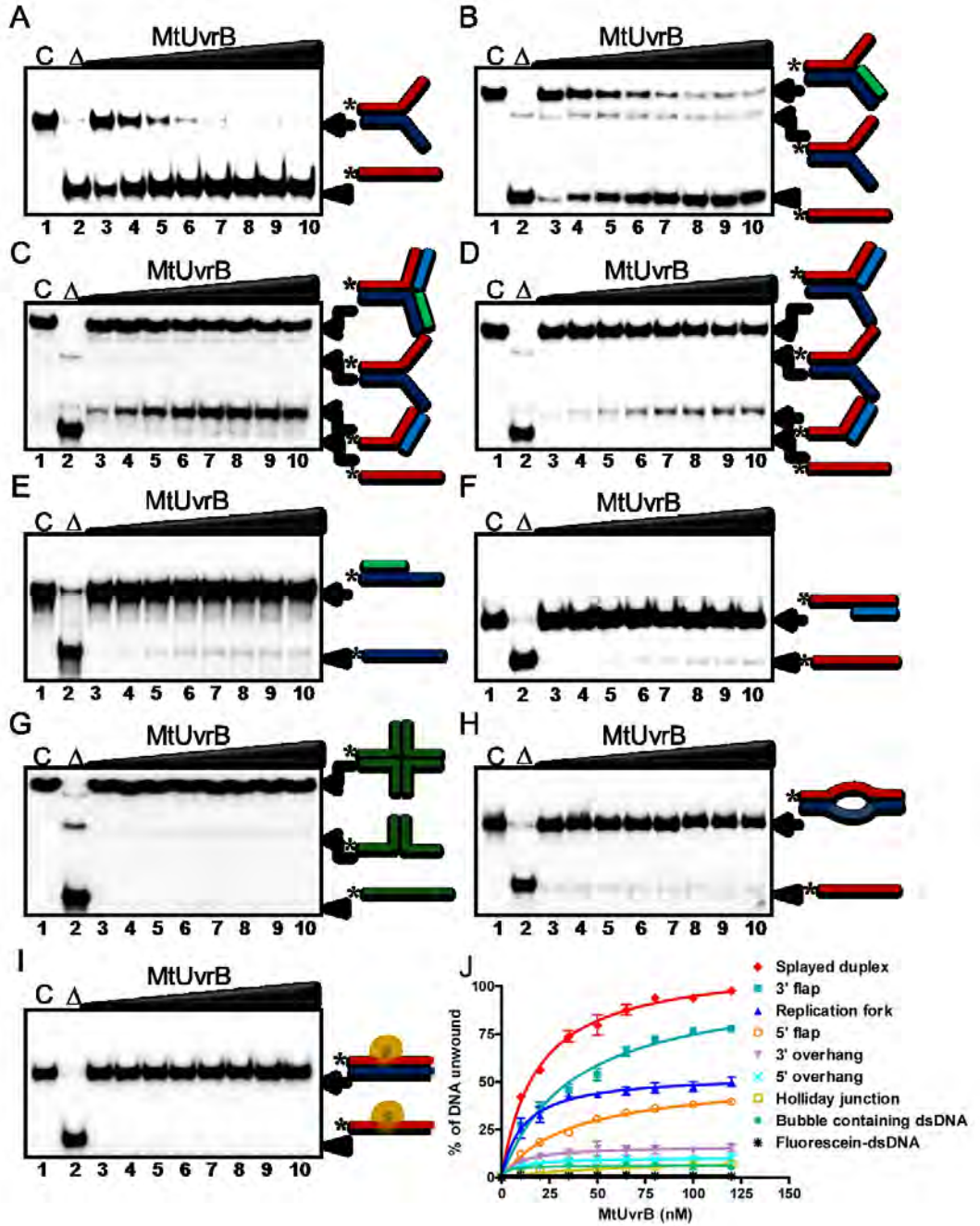
MtUvrB Possesses ATPase Activity Which Is Stimulated by DNA



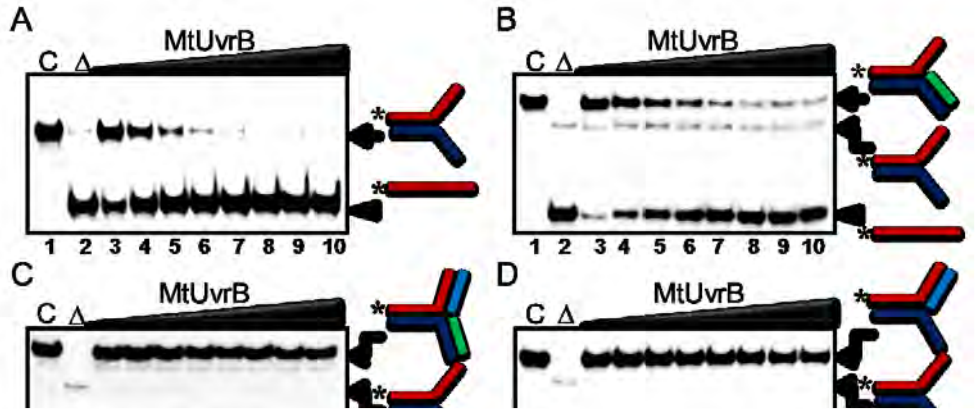
Evaluation of the ATPase Activity of EcUvrB in Absence and Presence of Fluorescein-ssDNA



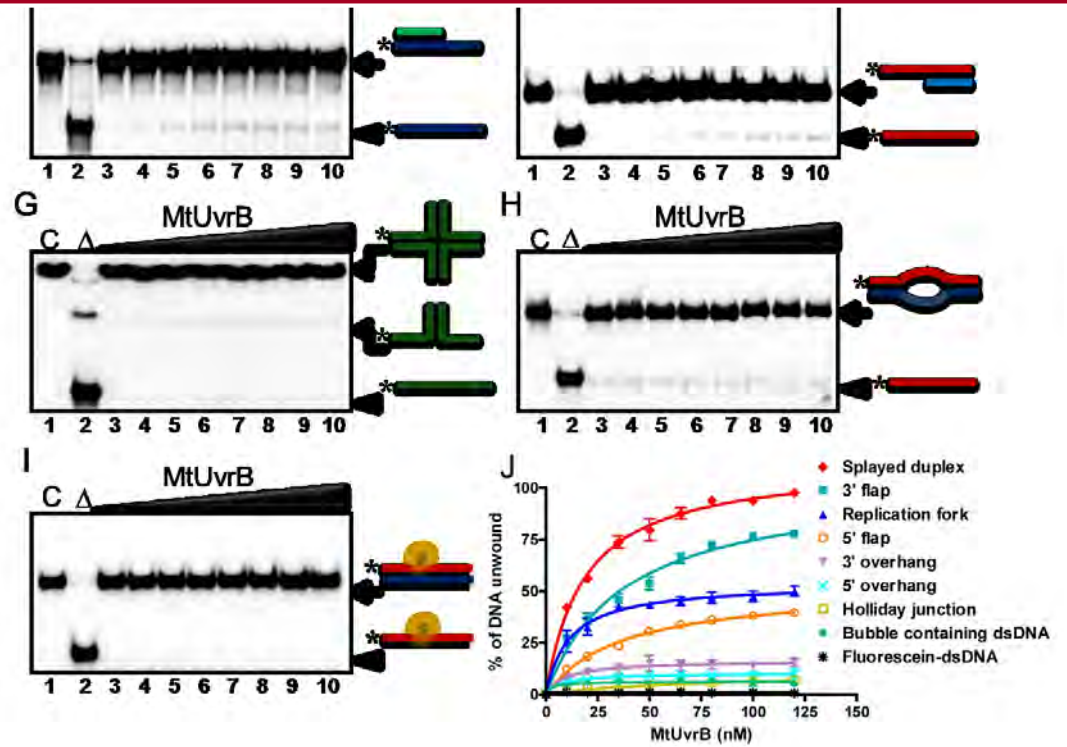
MtUvrB Unwinds DNA Replication/Repair Structures



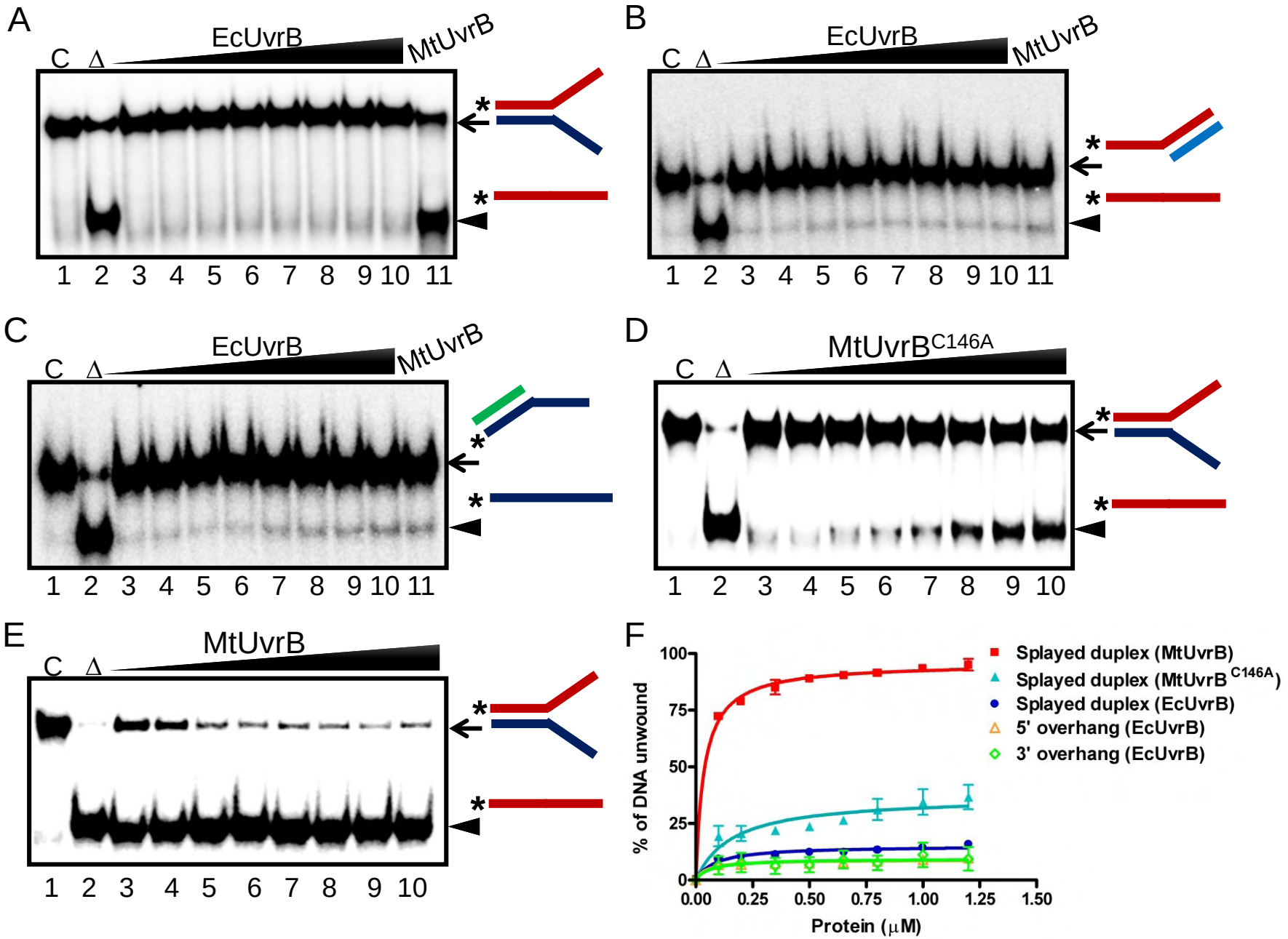
MtUvrB Unwinds DNA Replication/Repair Structures



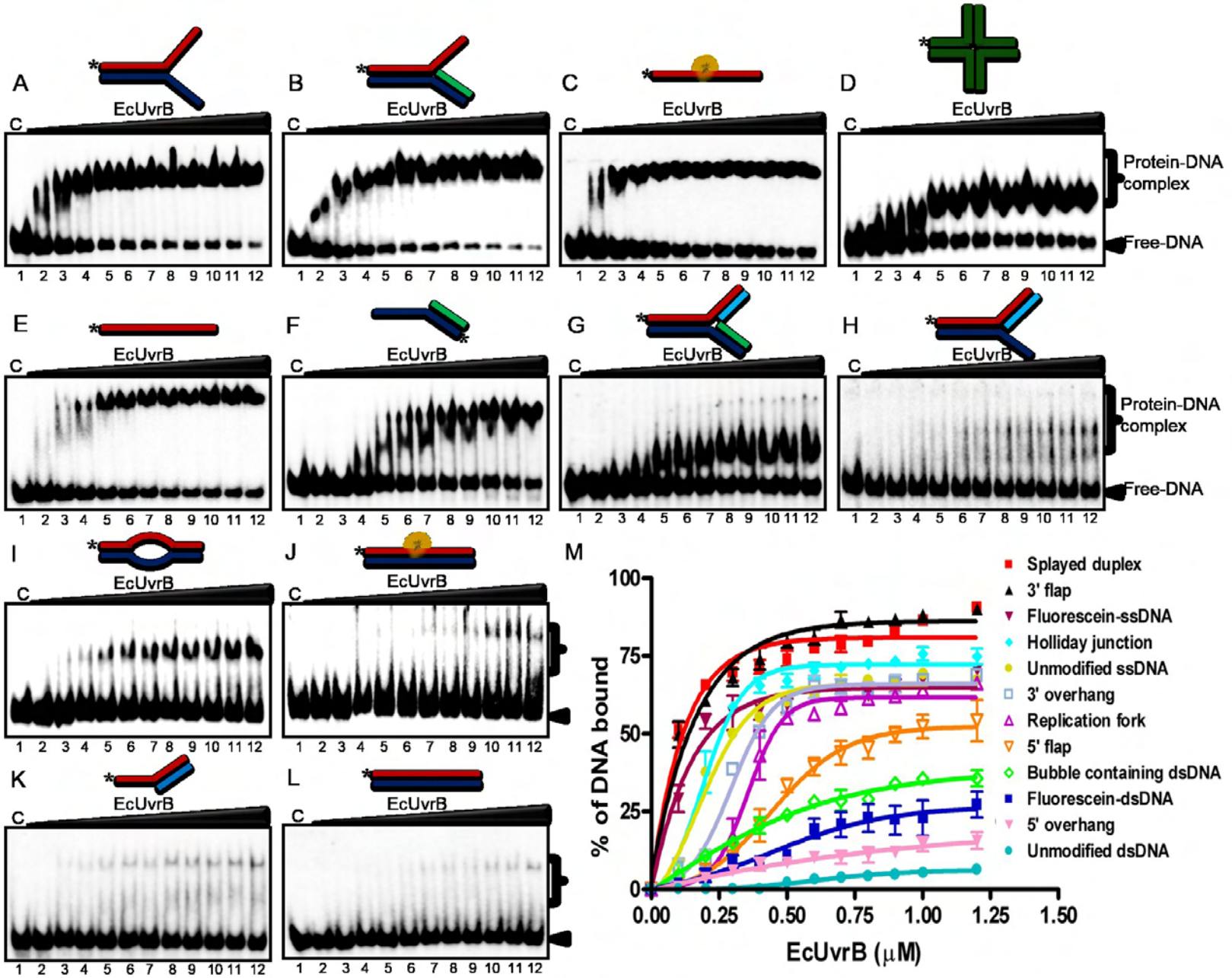
MtUvrB requires a single-stranded region for unwinding and proceeds in a directional manner

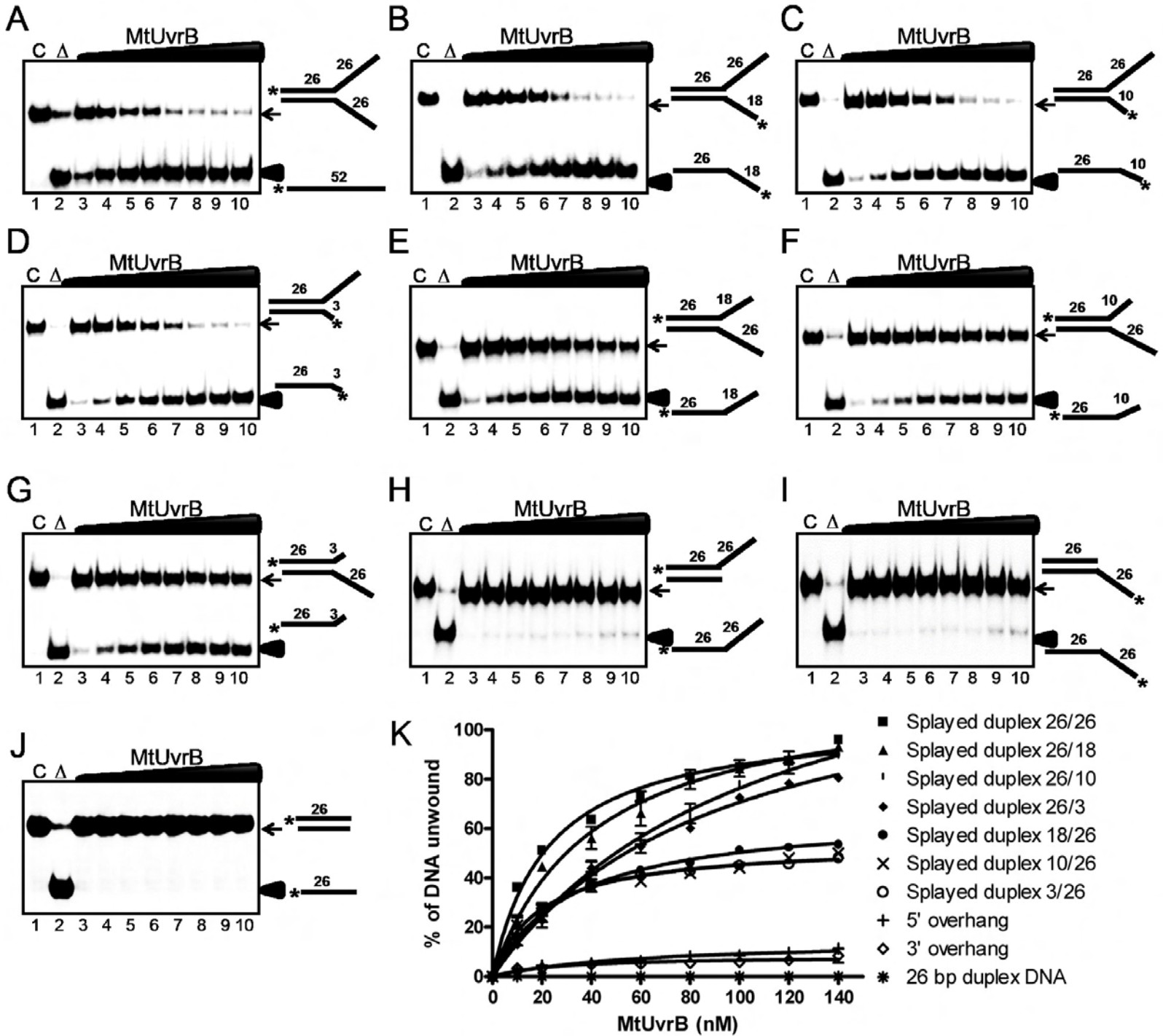


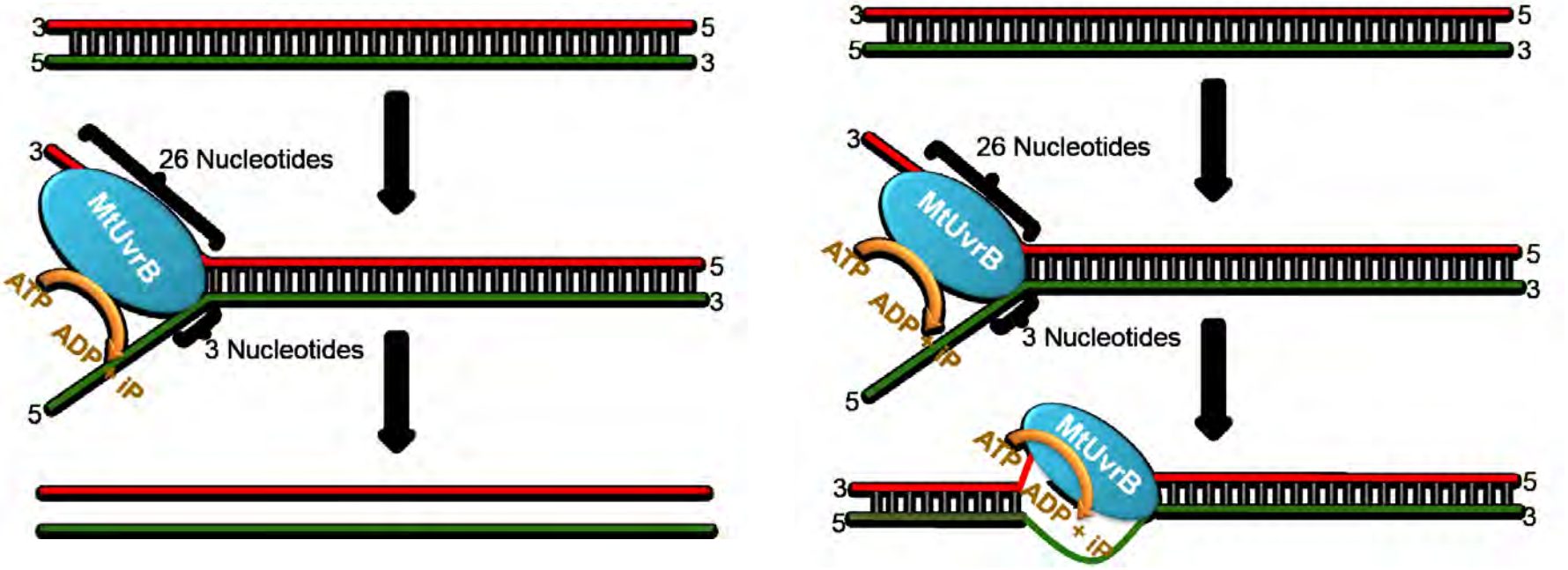
Helicase Activity of *E.coli* UvrB and MtUvrB^{C146A}



Characterization of DNA Substrate Specificity of EcUvrB

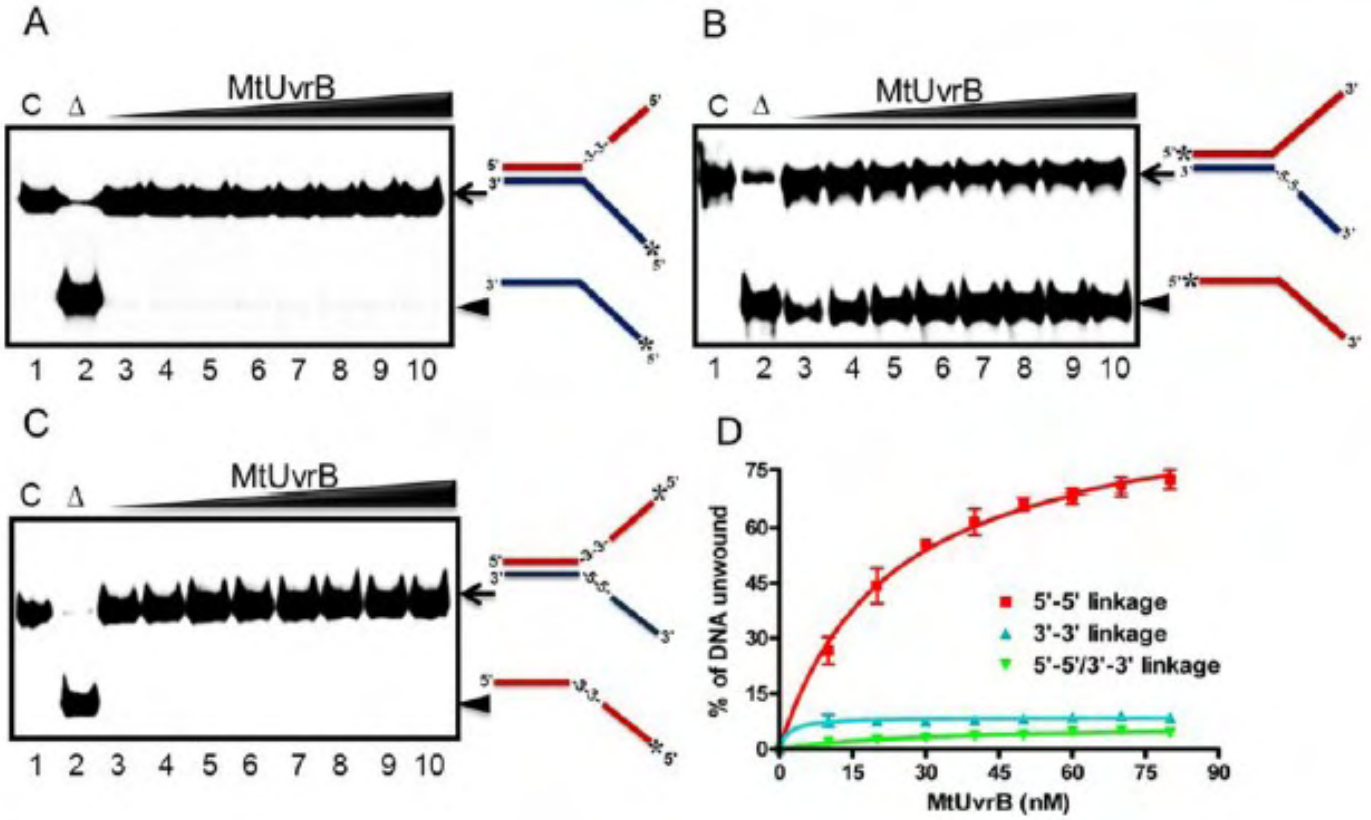


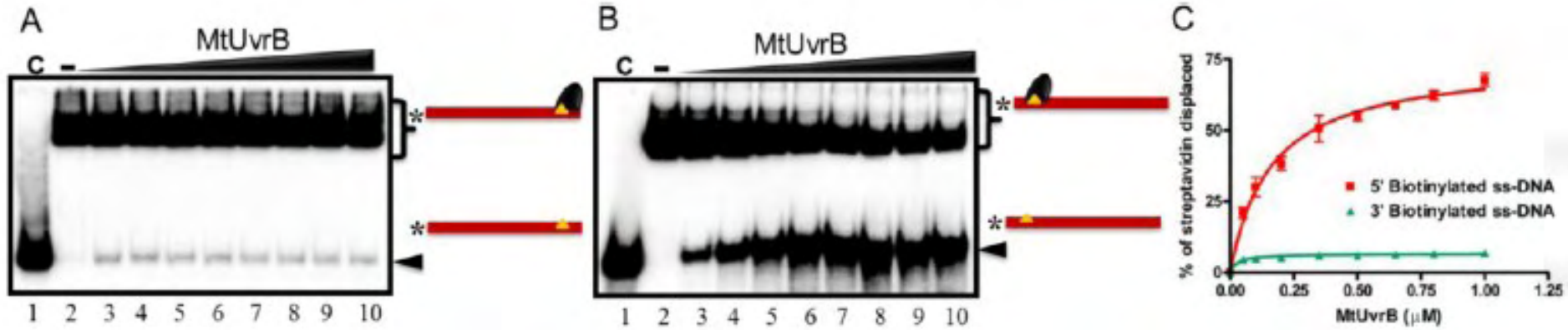

A
MtUvrB
C Δ
26
26
26
52
1 2 3 4 5 6 7 8 9 10
B
MtUvrB
C Δ
26
26
18
26
18
1 2 3 4 5 6 7 8 9 10
C
MtUvrB
C Δ
26
26
10
26
10
1 2 3 4 5 6 7 8 9 10
D
MtUvrB
C Δ
26
3
26
3
1 2 3 4 5 6 7 8 9 10
E
MtUvrB
C Δ
26
18
26
26
18
1 2 3 4 5 6 7 8 9 10
F
MtUvrB
C Δ
26
10
26
26
10
1 2 3 4 5 6 7 8 9 10
G
MtUvrB
C Δ
26
3
26
26
3
1 2 3 4 5 6 7 8 9 10
H
MtUvrB
C Δ
26
26
26
26
1 2 3 4 5 6 7 8 9 10
I
MtUvrB
C Δ
26
26
26
26
1 2 3 4 5 6 7 8 9 10
J
MtUvrB
C Δ
26
26
1 2 3 4 5 6 7 8 9 10
K
% of DNA unwound
100
80
60
40
20
0
0 20 40 60 80 100 120 140
MtUvrB (nM)
Splayed duplex 26/26
Splayed duplex 26/18
Splayed duplex 26/10
Splayed duplex 26/3
Splayed duplex 18/26
Splayed duplex 10/26
Splayed duplex 3/26
5' overhang
3' overhang
26 bp duplex DNA



- Fork junction is required for MtUvrB catalysed helicase activity
- 26 nucleotides on 3' ss-dna tails are required for loading MtUvrB to perform helicase activity
- 3 Nucleotides are required on 5' ss-DNA tails to perform helicase activity
- Hence, in addition to translocating strand, it also interacts with non-translocating strand
- The fraction of DNA unwound by MtUvrB decreases significantly as the length of the duplex increases
- Thus, reannealing of the strands starts occurring behind the translocating MtUvrB

MtUvrB Unwinds DNA with a 3′ to 5′ Polarity.





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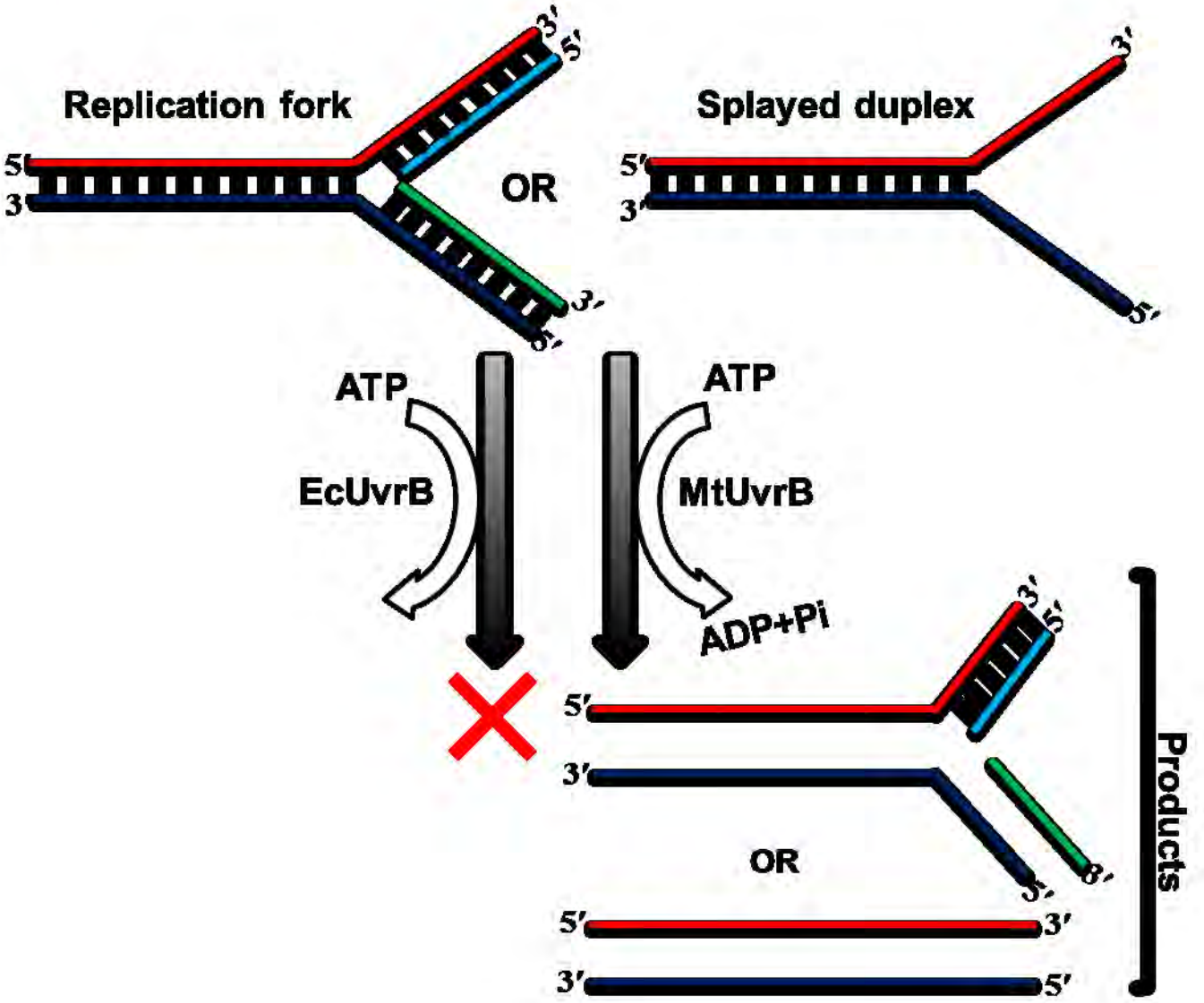
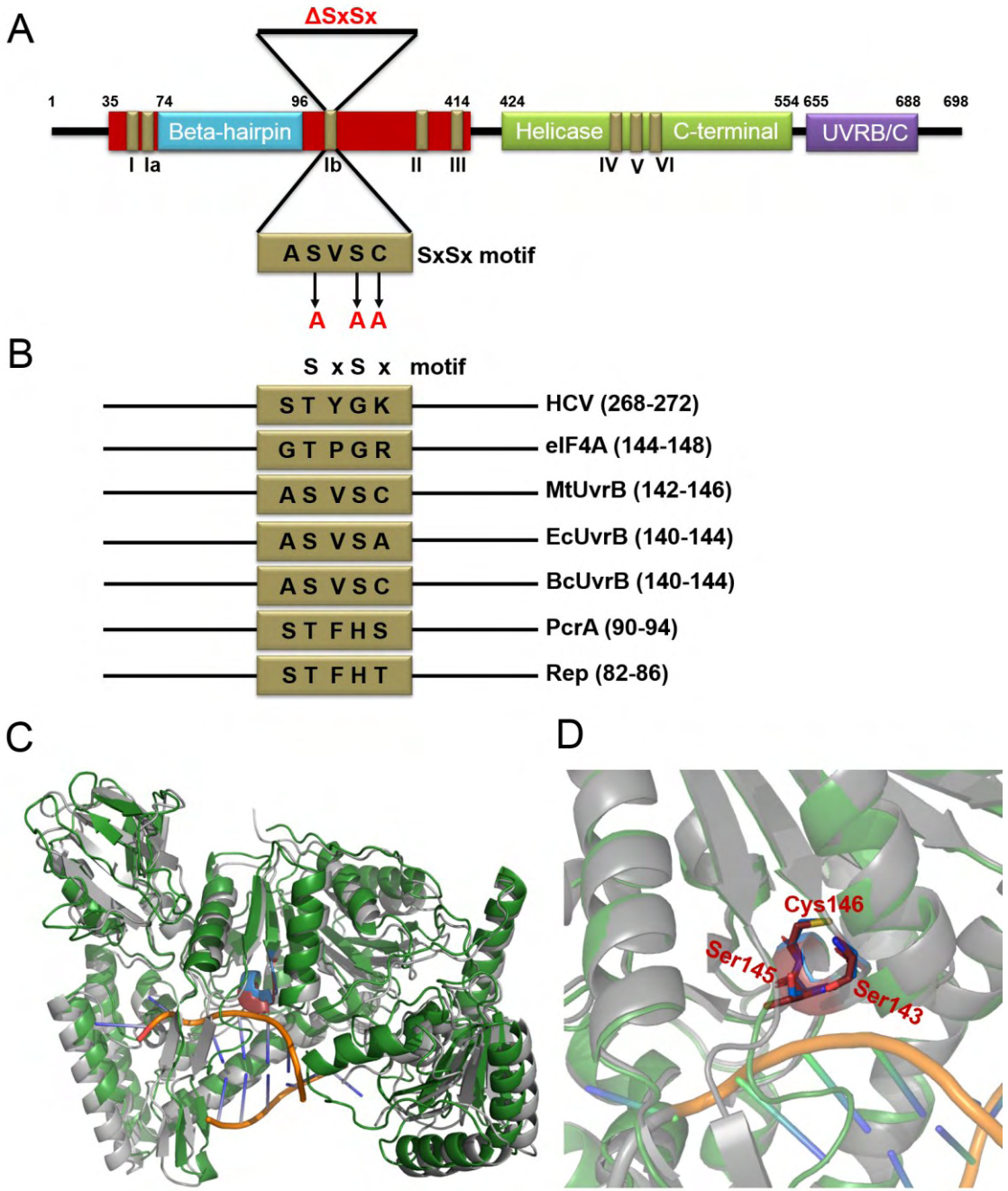
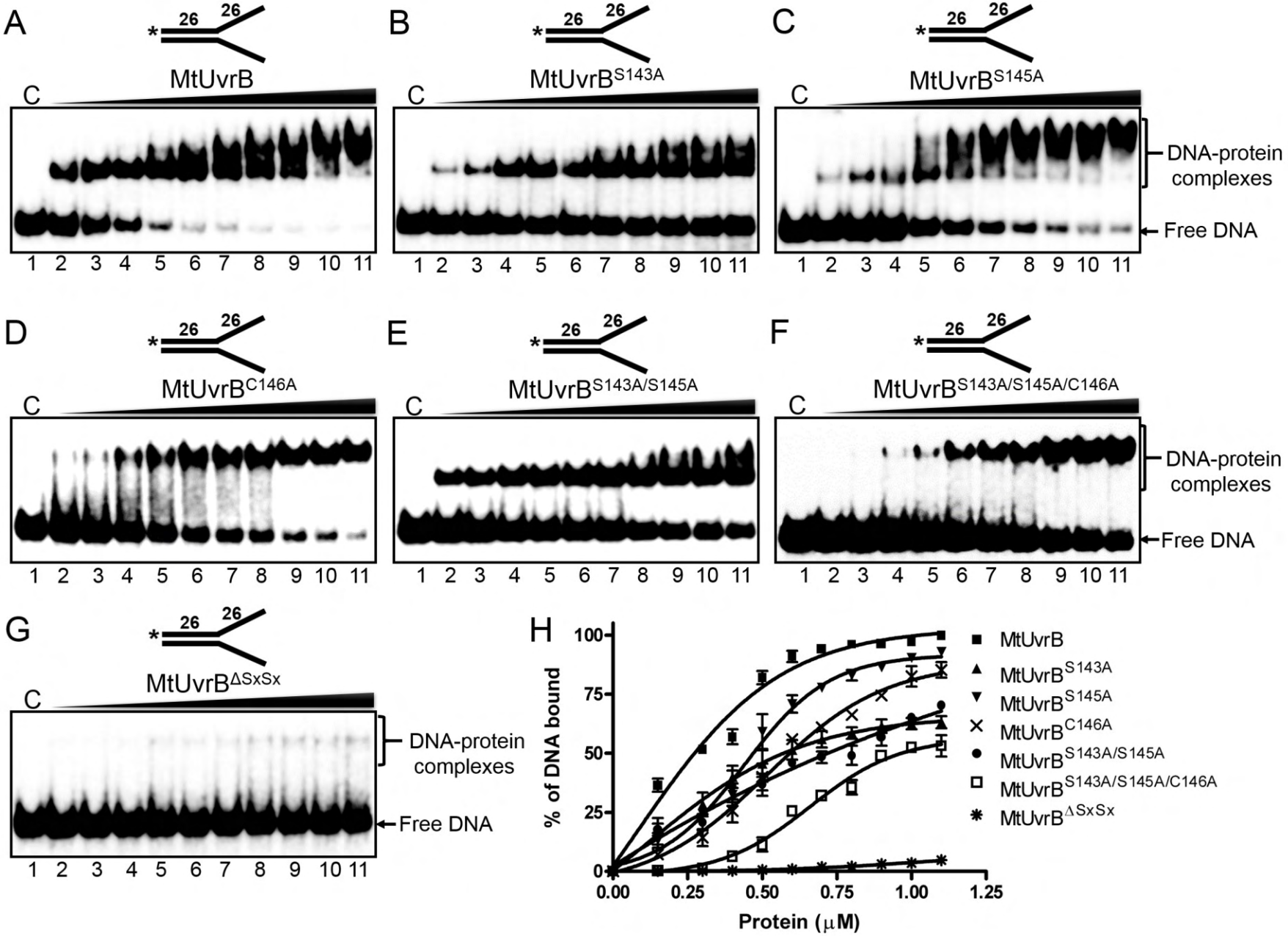
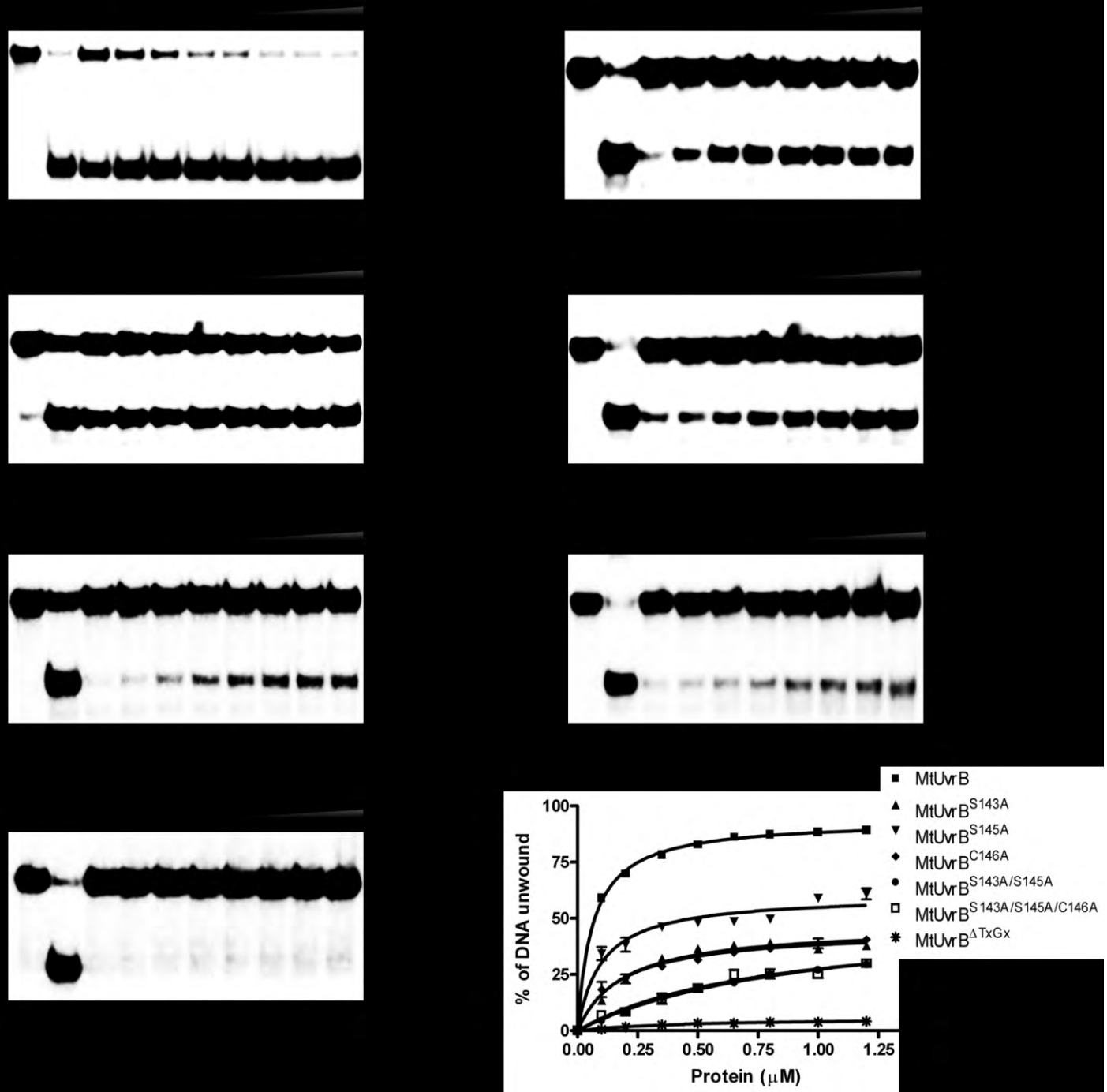
Mycobacterium tuberculosis UvrB Is a Robust DNA-Stimulated ATPase That Also Possesses Structure-Specific ATP-Dependent DNA Helicase Activity

Manoj Thakur, Mohan B. J. Kumar



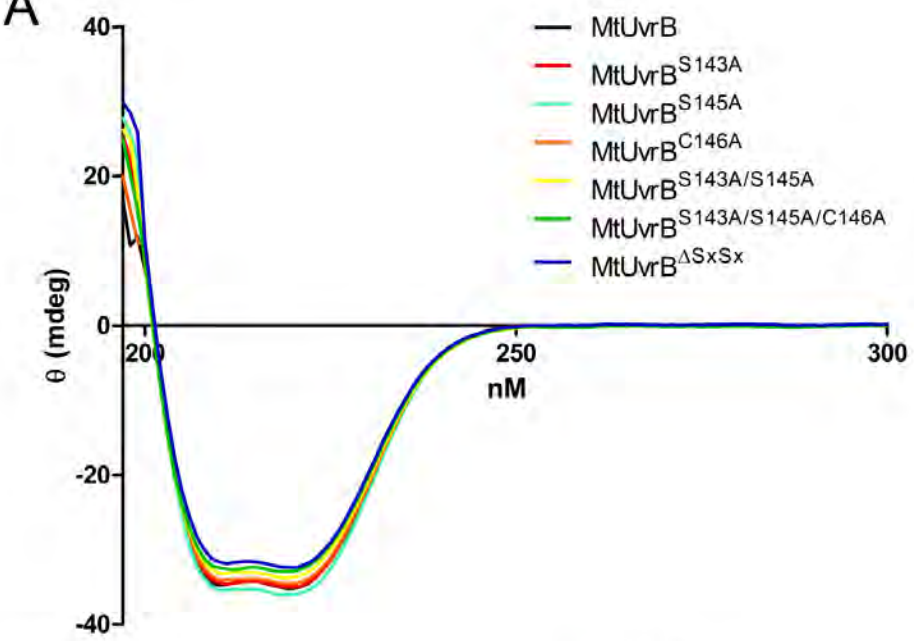

A
ΔSxSx
1
35
74
96
414
424
554
655
688
698
Beta-hairpin
Helicase
C-terminal
UVRB/C
I
Ia
Ib
II
III
IV
V
VI
A S V S C
SxSx motif
A
A A
B
S x S x motif
S T Y G K
HCV (268-272)
G T P G R
eIF4A (144-148)
A S V S C
MtUvrB (142-146)
A S V S A
EcUvrB (140-144)
A S V S C
BcUvrB (140-144)
S T F H S
PcrA (90-94)
S T F H T
Rep (82-86)
C
D
Cys146
Ser145
Ser143


A
B
C
D
E
F
G
H
26
26
*
C
MtUvrB
MtUvrB^S143A
MtUvrB^S145A
MtUvrB^C146A
MtUvrB^S143A/S145A
MtUvrB^S143A/S145A/C146A
MtUvrB^ΔSxSx
DNA-protein complexes
Free DNA
1 2 3 4 5 6 7 8 9 10 11
% of DNA bound
Protein (μM)
0.00
0.25
0.50
0.75
1.00
1.25
0
25
50
75
100


MtUvrB
MtUvrB^S143A
MtUvrB^S145A
MtUvrB^C146A
MtUvrB^S143A/S145A
MtUvrB^S143A/S145A/C146A
MtUvrB^ΔTxGx
% of DNA unwound
100
75
50
25
0
0.00
0.25
0.50
0.75
1.00
1.25
Protein (μM)

CD Spectroscopy and Thermal Shift Assay

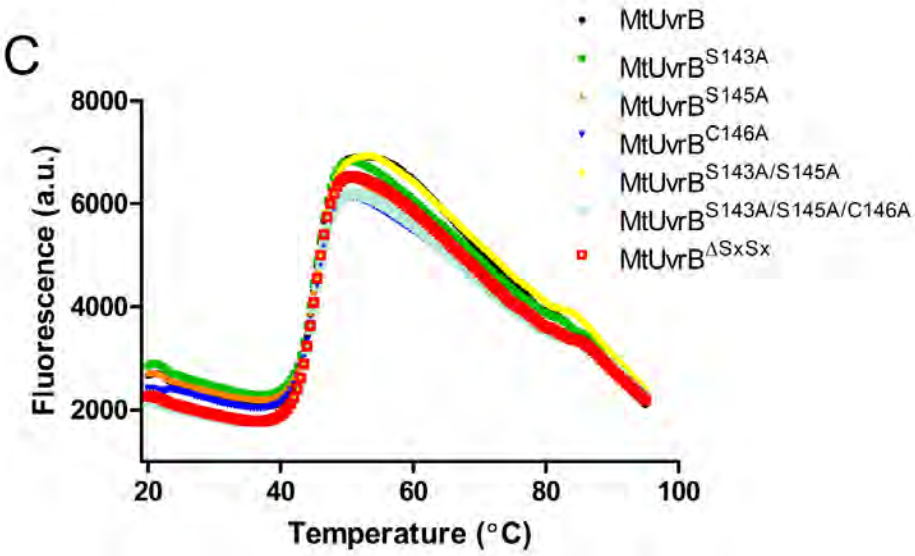
A



B

Protein	% α-helix	% β-sheets
MtUvrB	62.78	13.44
MtUvrB^{S143A}	62.77	13.44
MtUvrB^{S145A}	62.77	13.44
MtUvrB^{C146A}	62.77	13.44
MtUvrB$^{S143A/S145A}$	62.77	13.47
MtUvrB$^{S143A/S145A/C146A}$	59.85	13.48
MtUvrB$^{\Delta SxSx}$	58.68	13.44

C



D

Protein	T_m (°C)
MtUvrB	45.80 ± 0.17
MtUvrB^{S143A}	45.75 ± 0.11
MtUvrB^{S145A}	45.75 ± 0.11
MtUvrB^{C146A}	45.83 ± 0.11
MtUvrB$^{S143A/S145A}$	45.67 ± 0.10
MtUvrB$^{S143A/S145A/C146A}$	45.67 ± 0.10
MtUvrB$^{\Delta SxSx}$	45.66 ± 0.10

Biochimie 170 (2020) 94–105

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Research paper

Deciphering the essentiality and function of SxSx motif in *Mycobacterium tuberculosis* UvrB

Manoj Thakur, K. Muniyappa*

Department of Biochemistry, Indian Institute of Science, Bangalore, 560012, India

ARTICLE INFO

Article history:
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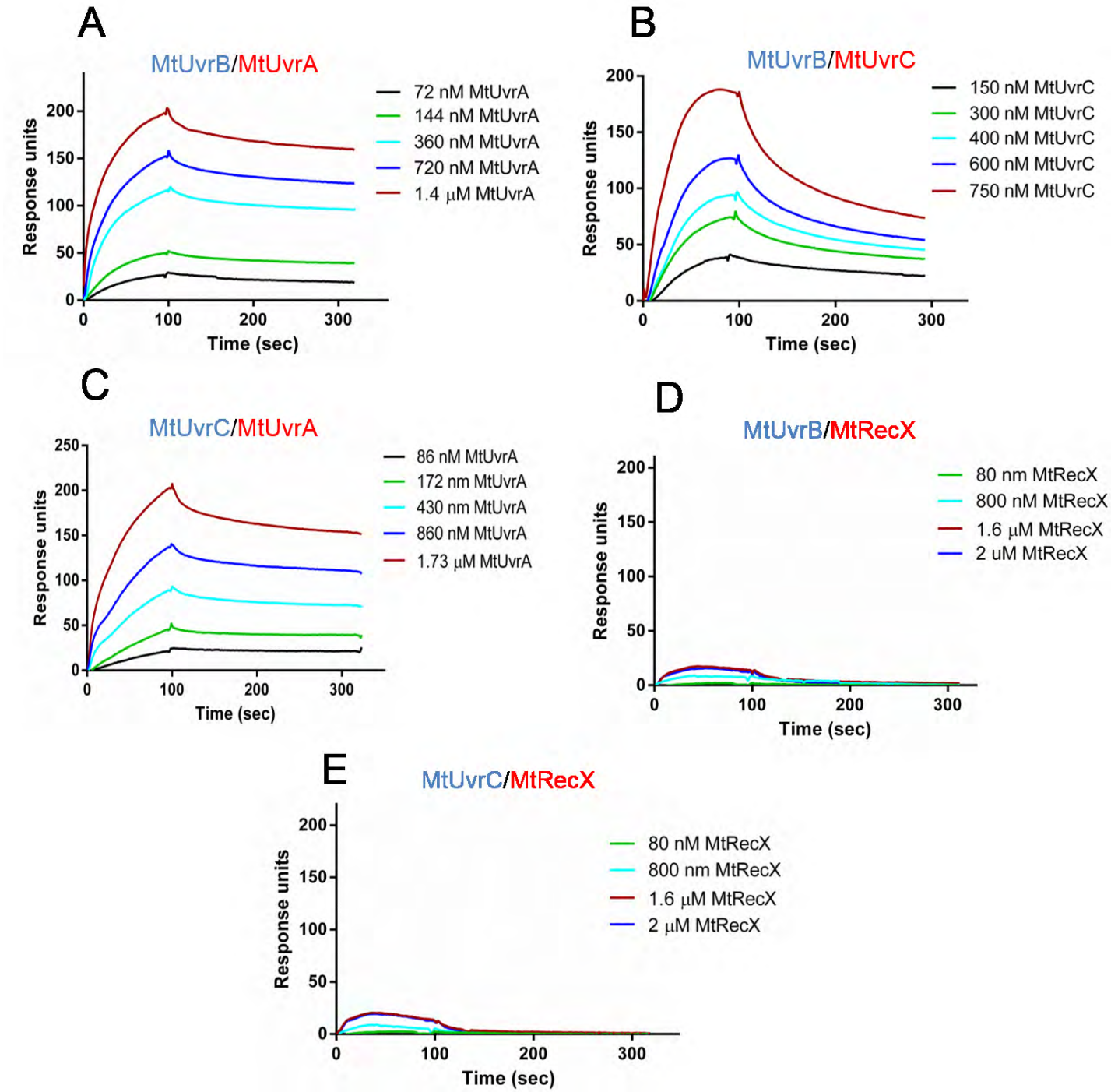
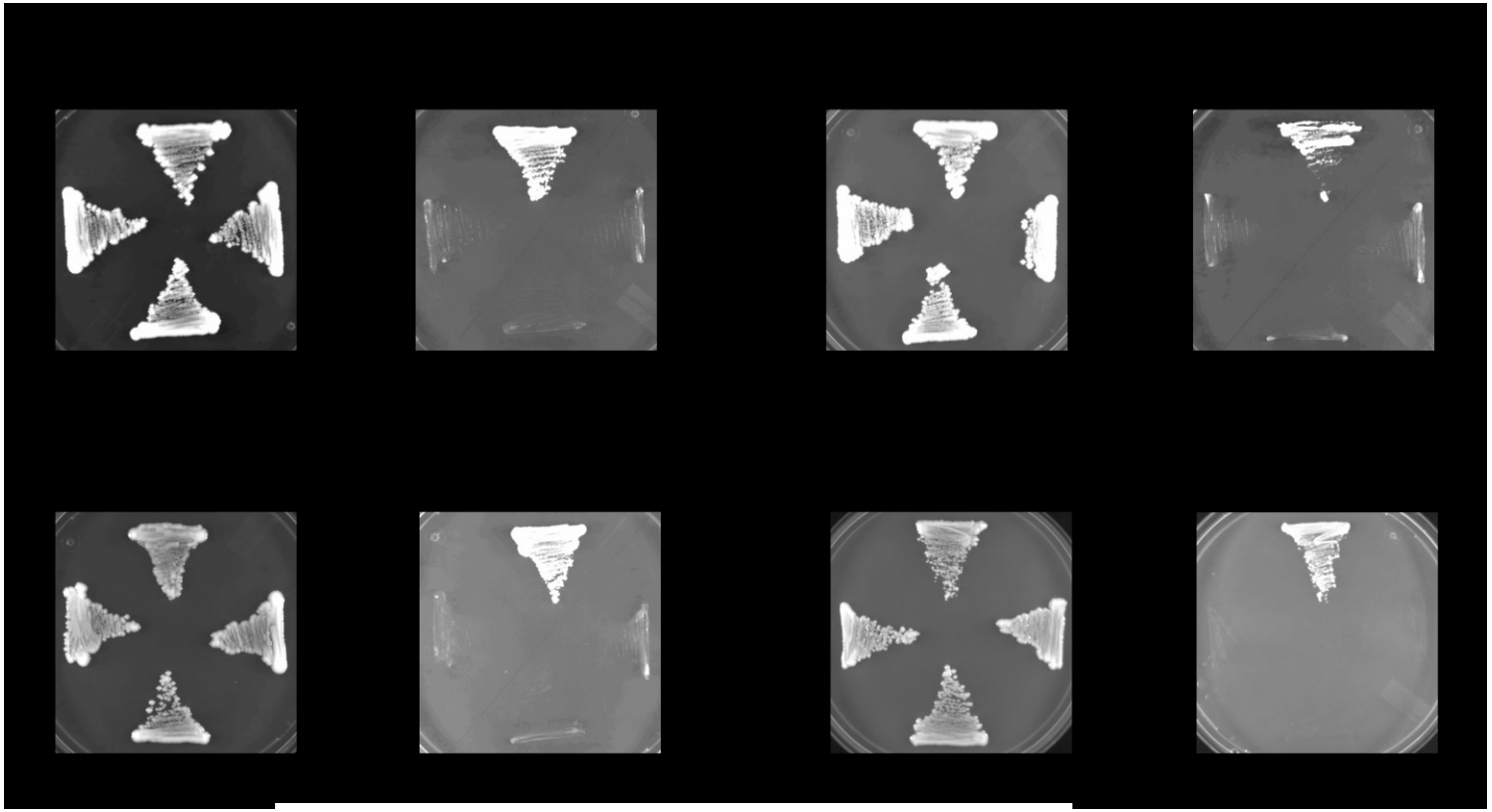
Keywords:
Mycobacterium tuberculosis
Nucleotide excision repair
SxSx motif
ATPase
DNA helicase
TxGx motif

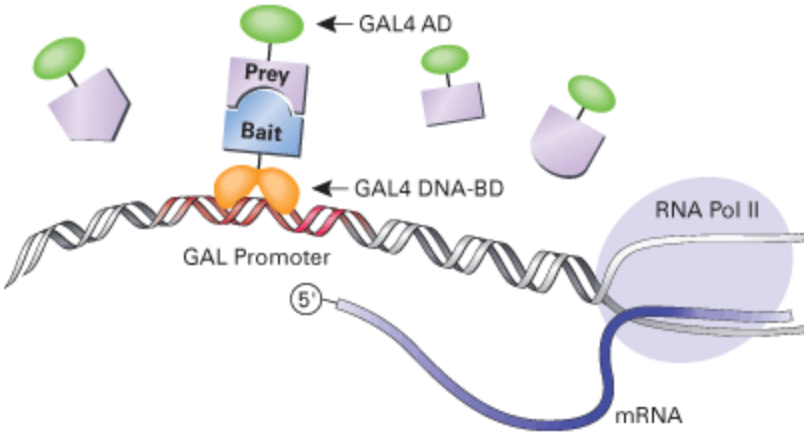
ABSTRACT

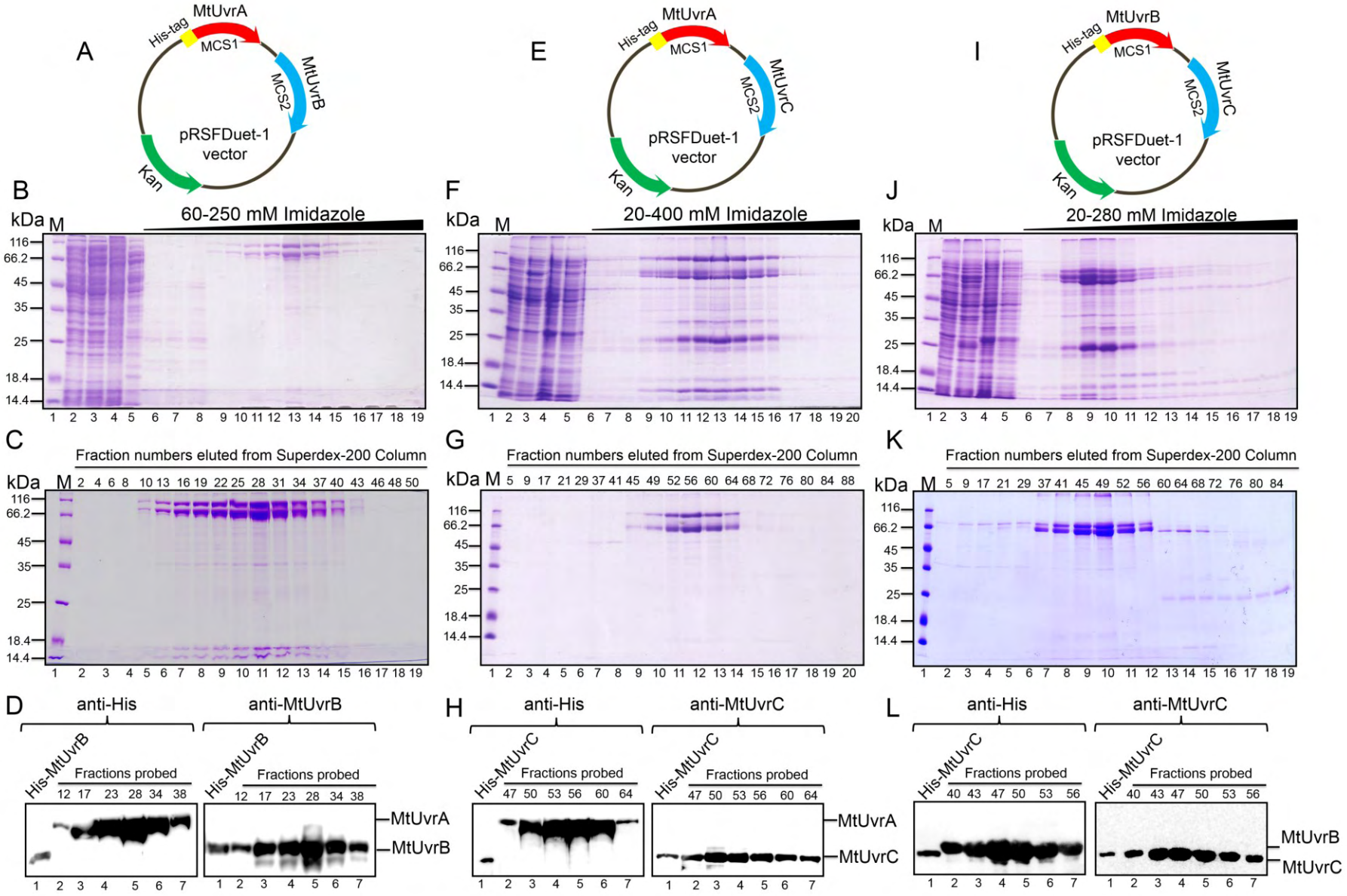
The UvrB subunit is a central component of the UvrABC incision complex and plays a pivotal role in damage recognition, strand excision and repair synthesis. A conserved structural motif (the SxSx motif) present in UvrB is analogous to a similar motif (TxGx) in the helicases of superfamily 2, whose function is not fully understood. To elucidate the significance of the SxSx (Ser143-Val144-Ser145-Cys146) motif in *Mycobacterium tuberculosis* UvrB (MtUvrB), different variants of MtUvrB subunit were constructed and characterized. The SxSx motif indeed was found to be essential for MtUvrB function: while Ser143 and Cys146 residues within this motif were crucial for MtUvrB function, Ser145 plays an important but less essential role. The SxSx motif-deleted mutant was drastically attenuated and three single (S143A, S145A and C146A) mutants and a double (S143A/S145A) mutant exhibited various degrees of severity in their DNA-binding, DNA helicase and ATPase activities. Taken together, these results highlight a hitherto unrecognized role for SxSx motif in the catalytic activities of UvrB.


A
MtUvrB/MtUvrA
Response units
Time (sec)
72 nM MtUvrA
144 nM MtUvrA
360 nM MtUvrA
720 nM MtUvrA
1.4 μM MtUvrA
B
MtUvrB/MtUvrC
Response units
Time (sec)
150 nM MtUvrC
300 nM MtUvrC
400 nM MtUvrC
600 nM MtUvrC
750 nM MtUvrC
C
MtUvrC/MtUvrA
Response units
Time (sec)
86 nM MtUvrA
172 nm MtUvrA
430 nm MtUvrA
860 nM MtUvrA
1.73 μM MtUvrA
D
MtUvrB/MtRecX
Response units
Time (sec)
80 nm MtRecX
800 nM MtRecX
1.6 μM MtRecX
2 uM MtRecX
E
MtUvrC/MtRecX
Response units
Time (sec)
80 nM MtRecX
800 nm MtRecX
1.6 μM MtRecX
2 μM MtRecX

MtUvrA interacts with MtUvrC independent of MtUvrB






A
His-tag
MtUvrA
MCS1
MCS2
MtUvrB
pRSFDuet-1
vector
Kan
B
kDa
M
60-250 mM Imidazole
116
66.2
45
35
25
18.4
14.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
C
Fraction numbers eluted from Superdex-200 Column
M 2 4 6 8 10 13 16 19 22 25 28 31 34 37 40 43 46 48 50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
D
anti-His
anti-MtUvrB
His-MtUvrB
Fractions probed
12 17 23 28 34 38
1 2 3 4 5 6 7
MtUvrA
MtUvrB
E
His-tag
MtUvrA
MCS1
MCS2
MtUvrC
pRSFDuet-1
vector
Kan
F
kDa
M
20-400 mM Imidazole
116
66.2
45
35
25
18.4
14.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
G
Fraction numbers eluted from Superdex-200 Column
kDa M 5 9 17 21 29 37 41 45 49 52 56 60 64 68 72 76 80 84 88
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
H
anti-His
anti-MtUvrC
His-MtUvrC
Fractions probed
47 50 53 56 60 64
1 2 3 4 5 6 7
MtUvrA
MtUvrC
I
His-tag
MtUvrB
MCS1
MCS2
MtUvrC
pRSFDuet-1
vector
Kan
J
kDa
M
20-280 mM Imidazole
116
66.2
45
35
25
18.4
14.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
K
Fraction numbers eluted from Superdex-200 Column
kDa M 5 9 17 21 29 37 41 45 49 52 56 60 64 68 72 76 80 84
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
L
anti-His
anti-MtUvrC
His-MtUvrC
Fractions probed
40 43 47 50 53 56
1 2 3 4 5 6 7
MtUvrB
MtUvrC

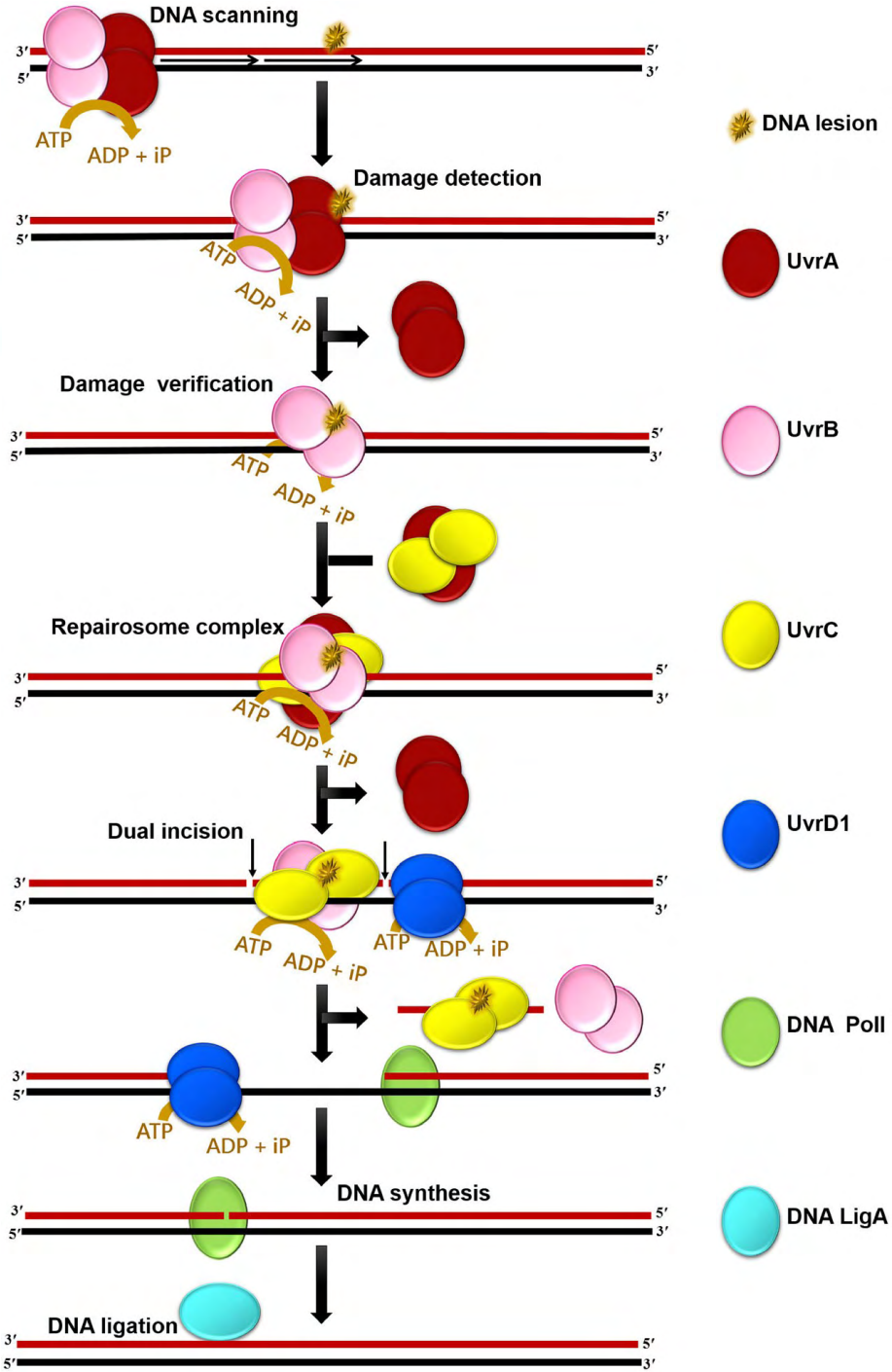
(2020) 594(5), 851-863.

FEBS Letters

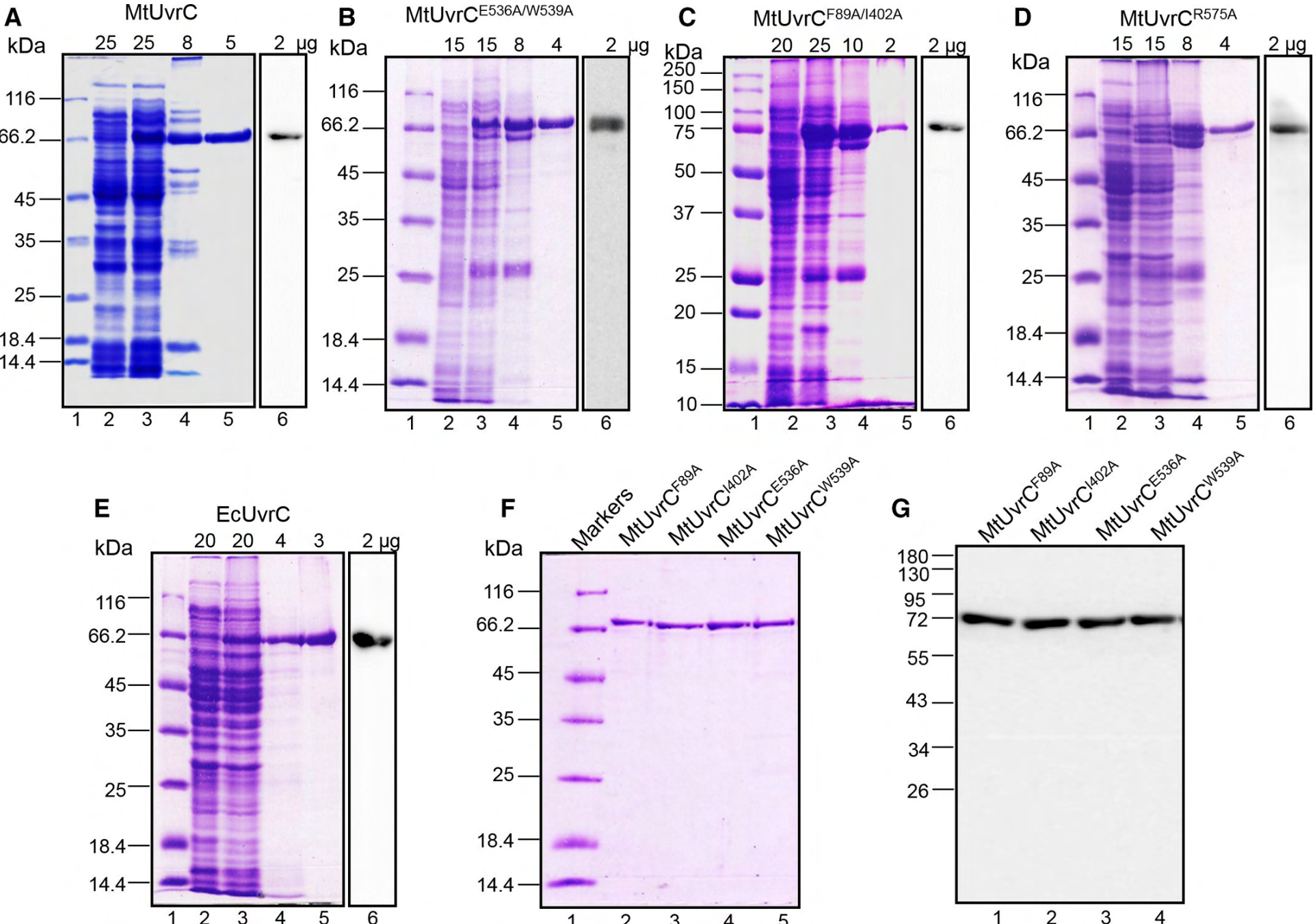
FEBS PRESS

UvrA and UvrC subunits of the *Mycobacterium tuberculosis* UvrABC excinuclease interact independently of UvrB and DNA.

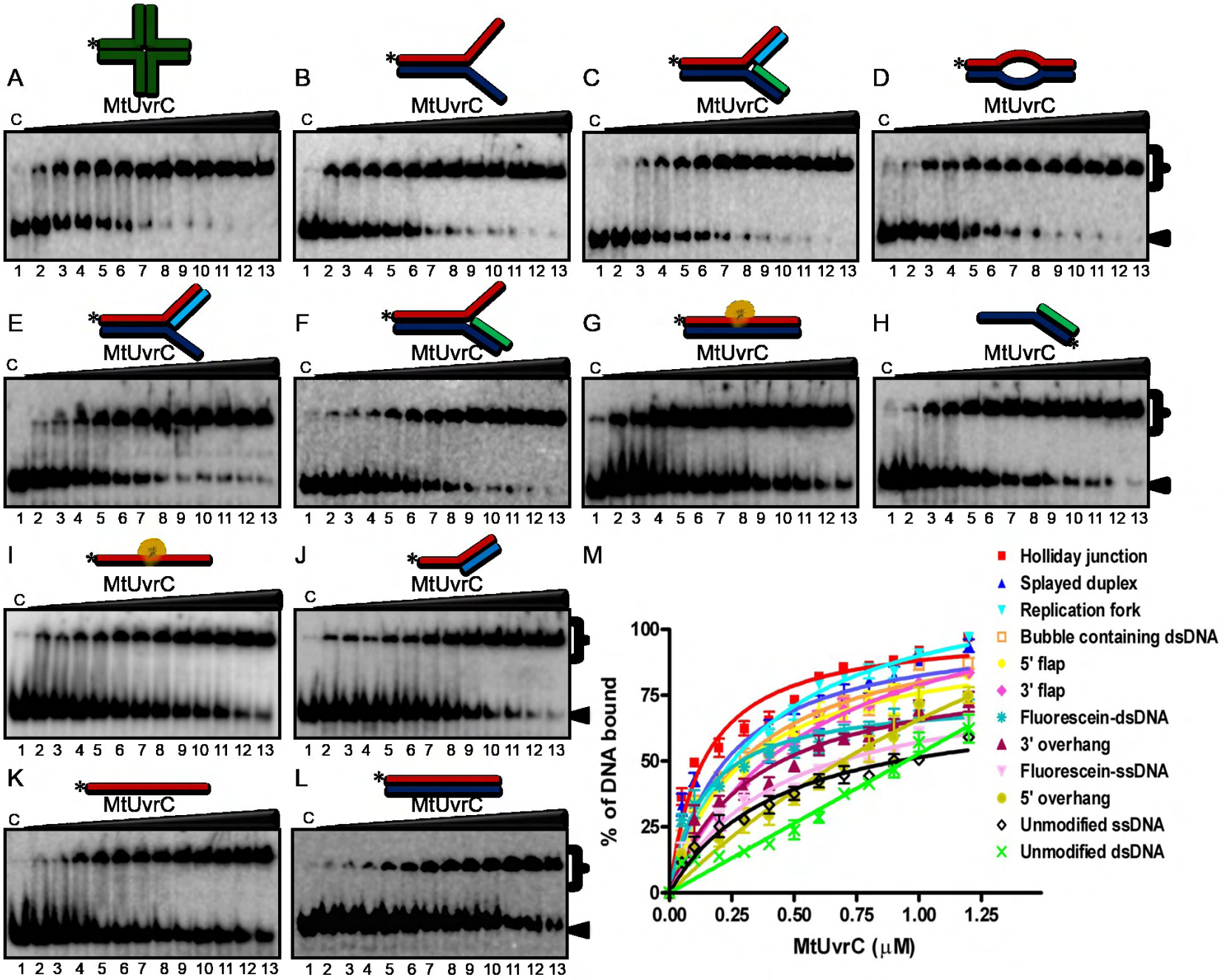
Thakur, M., Badugu, S and Muniyappa, K



Expression and Purification of MtUvrC or its variants

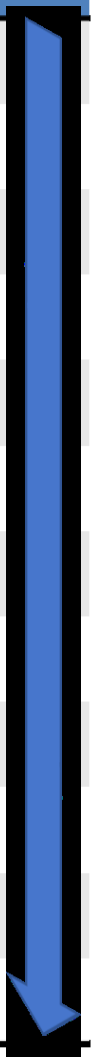


Characterization of DNA Substrate Specificity of MtUvrC



Characterization of DNA Substrate Specificity of MtUvrC

DNA substrate	k_d values of MtUvrC (nM)
Holliday junction	140 ± 1.32
Splayed duplex	225 ± 1.73
Replication fork	253 ± 1.86
Bubble-containing dsDNA	285 ± 3.52
5' flap	310 ± 2.54
3' flap	389 ± 5.04
Fluorescein-dsDNA	400 ± 0.04
3' overhang	453 ± 2.37
Fluorescein-ssDNA	682 ± 1.12
5' overhang	690 ± 0.88
Unmodified ssDNA	915 ± 1.29
Unmodified dsDNA	951 ± 3.69



Characterization of DNA Substrate Specificity of MtUvrC

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3' overhang	453 ± 2.37
Fluorescein-ssDNA	682 ± 1.12
Unmodified ssDNA	915 ± 1.29
Unmodified dsDNA	951 ± 3.69

These results support a model in which UvrC might bind and process various DNA intermediates that arise due to genotoxic stress from cellular processes.

Effect of DNA and UvrB and UvrC proteins on ATPase activity of UvrA protein

Additions[a]	ATPase	Relative activity[b]
	pmol hydrolyzed	
None	2020	100
ssDNA	1810	89.6
dsDNA	2120	105
UV-DNA	2010	99.5
B[c]	1510	74.7
B + dsDNA	3090	153
B + UV-DNA	5440	269
C[c]	1920	95.0
C + dsDNA	2050	101
C + UV-DNA	1880	93.1
B + C	1430	70.8
B + C + dsDNA	2810	139
B + C + UV-DNA	4950	245

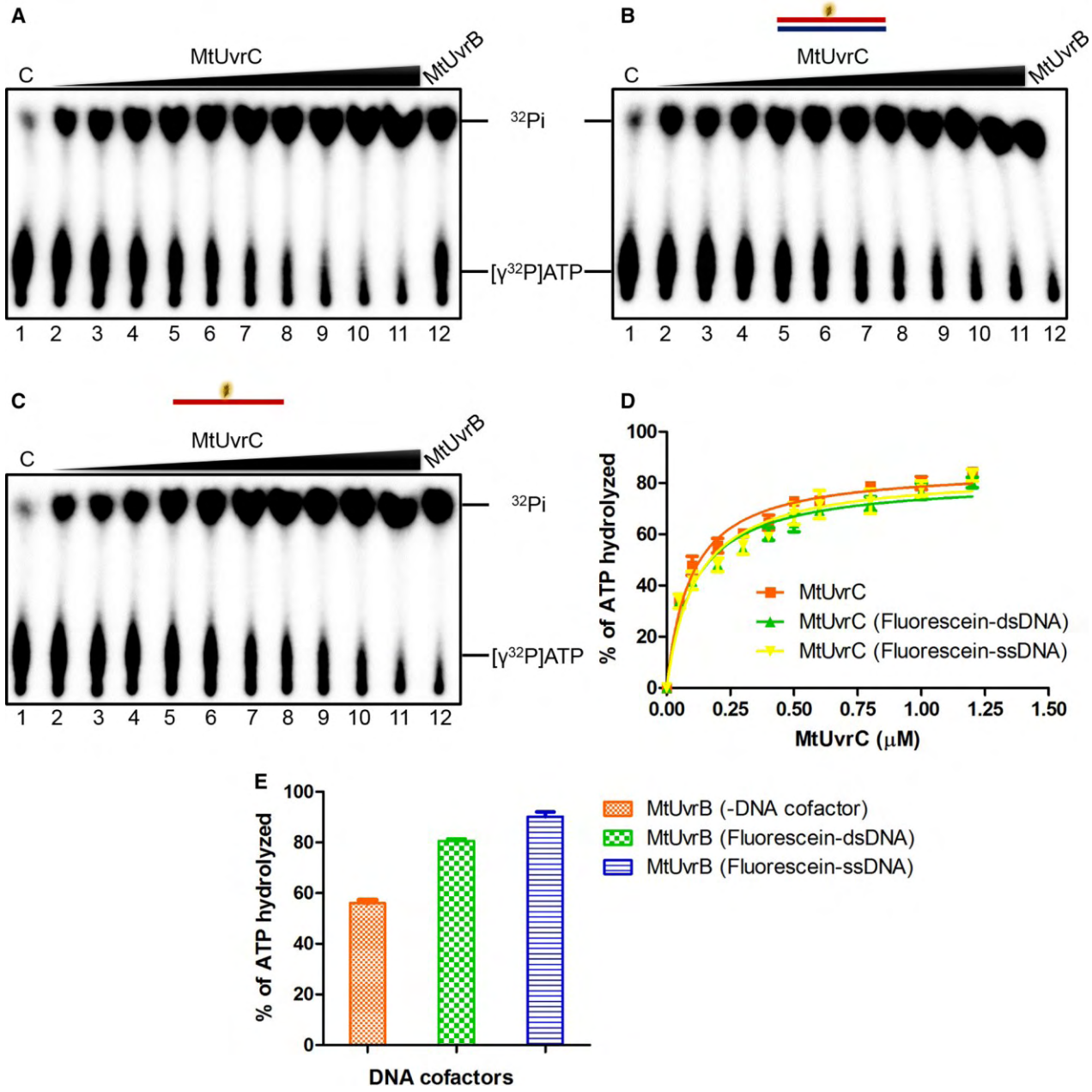
Thomas *et al*., (1985) *The Journal of biological chemistry* ***260***, 9875-9883

Effect of S8 on the ATP hydrolysis activity of UvrB and UvrC

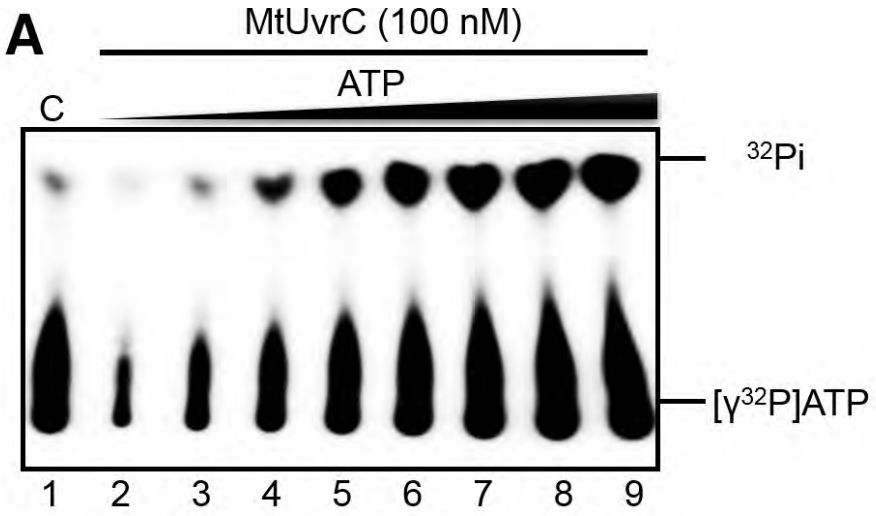
	Rate
	min^{-1}
UvrB[a]	b.d.[b]
UvrB + S8[c]	0.66
UvrC[d]	b.d.
UvrC + S8	b.d.
UvrB + UvrC	0.16
UvrB + UvrC + S8	2.5

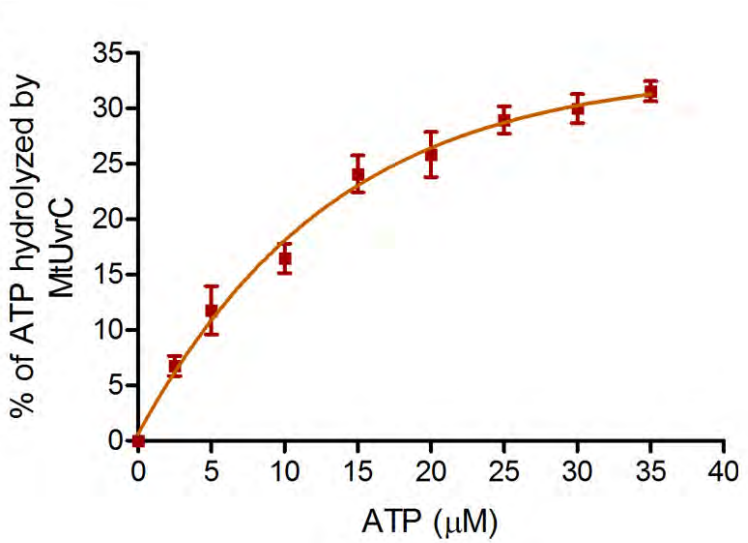
Zou *et al*., (1997) *The Journal of biological chemistry* ***272***, 4820-4827

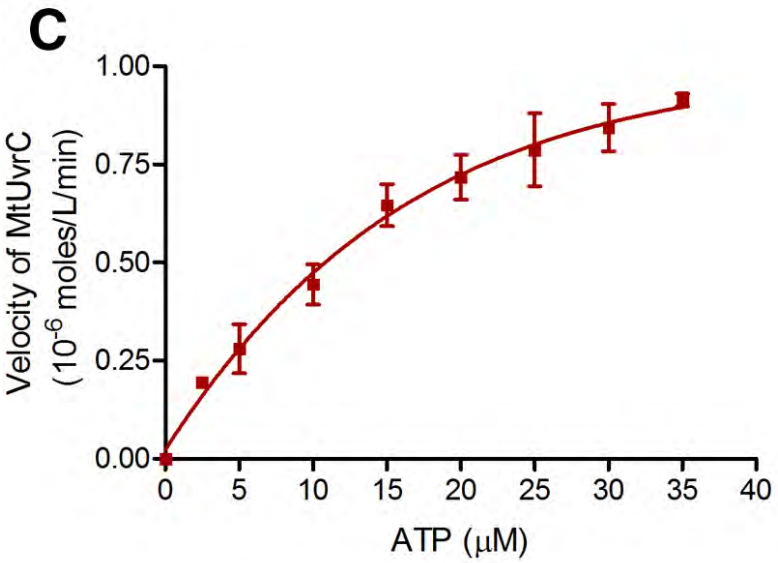
MtUvrC ATPase Activity Is Not Stimulated by DNA

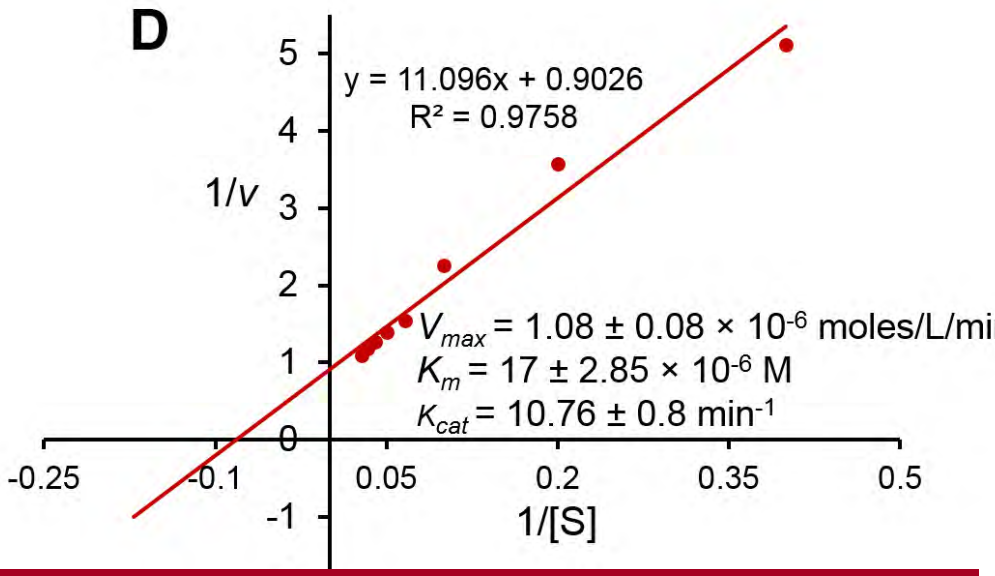


Determination of the Kinetic Parameters of MtUvrC ATPase Activity

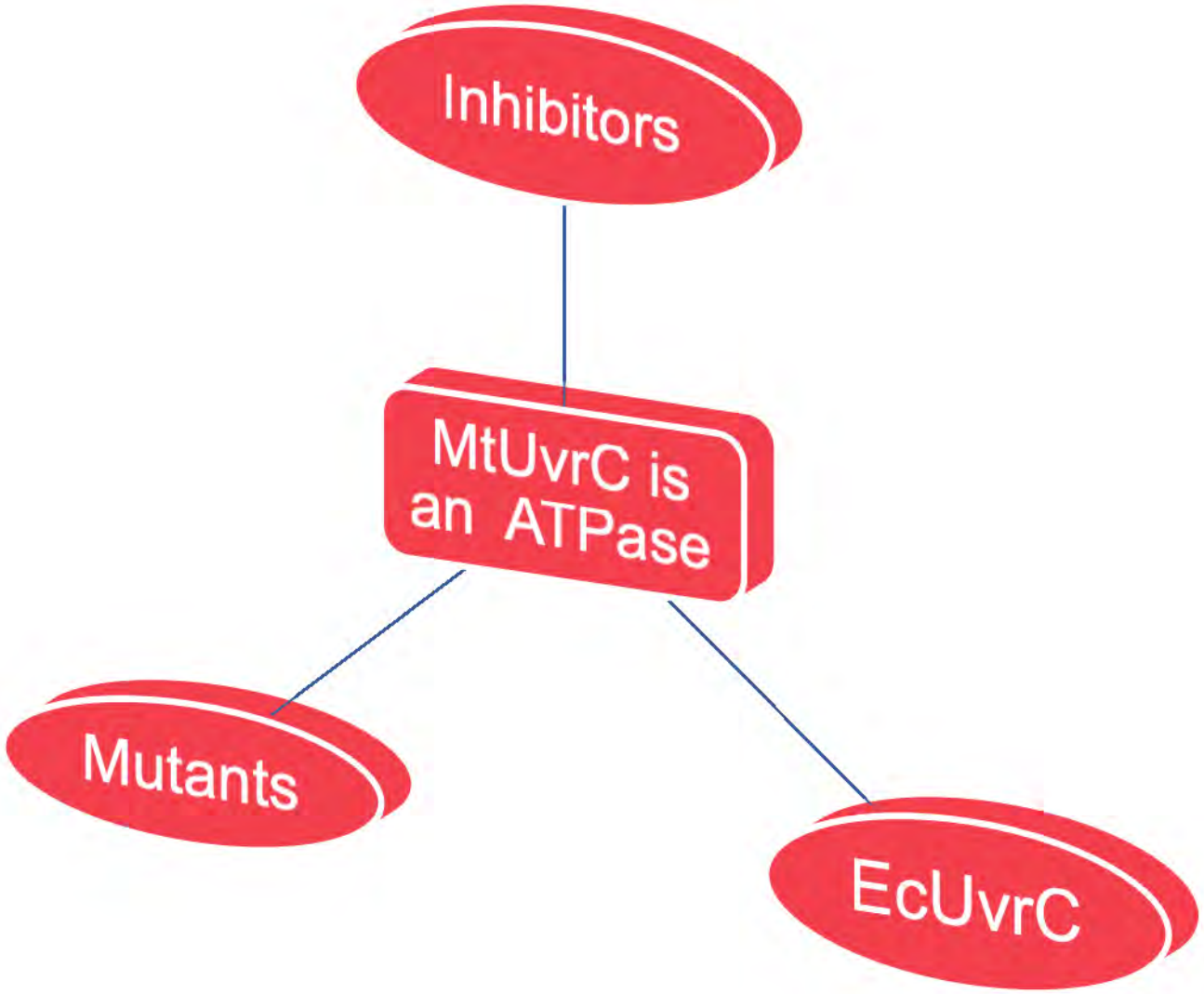




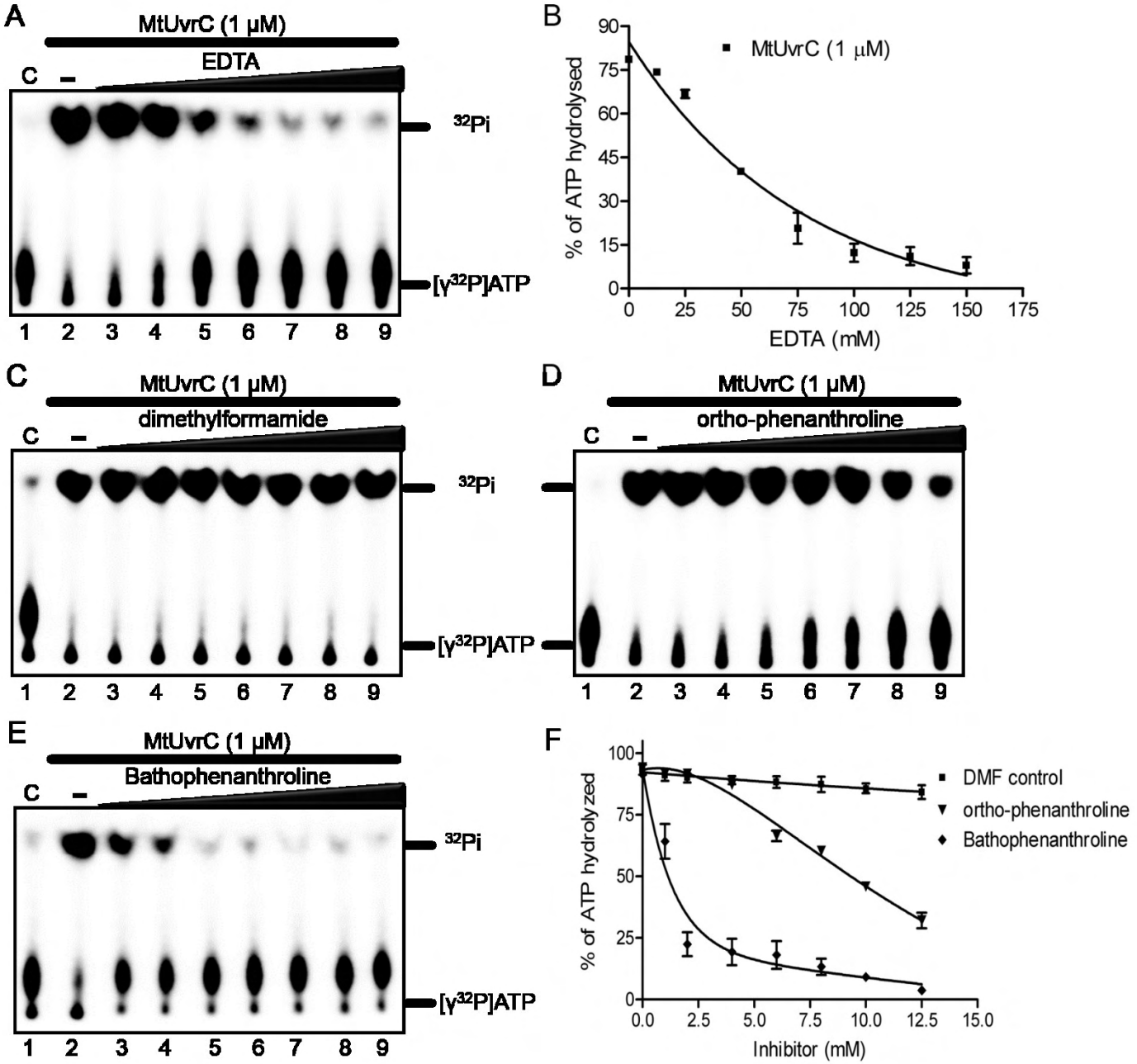




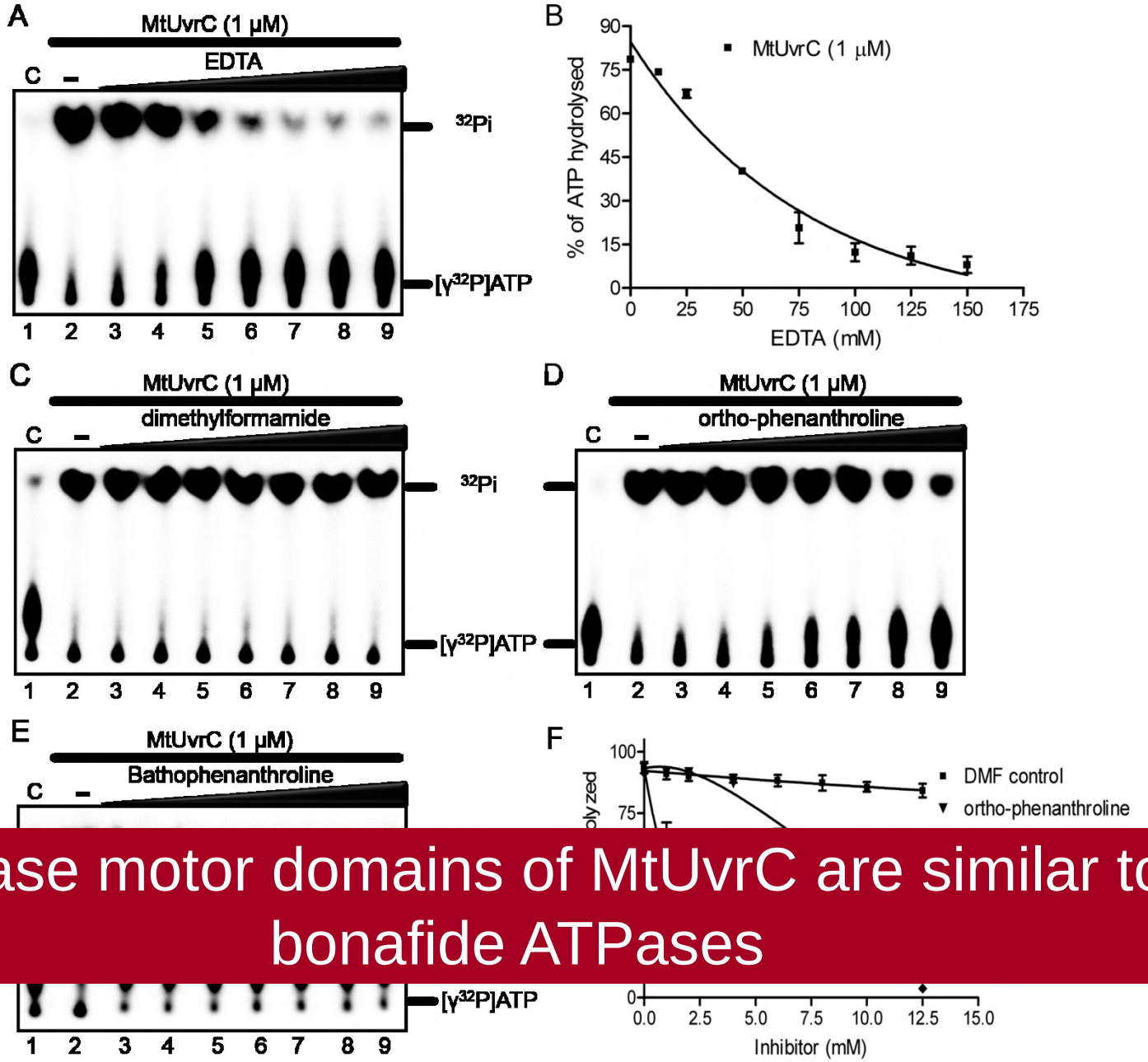
The similar value of k_{cat} of MtUvrB and MtUvrC suggests its significance in terms of mechanism of action of both the proteins in complex while performing its function.


Inhibitors
MtUvrC is an ATPase
Mutants
EcUvrC

ATPase Assay of MtUvrC in the presence of inhibitors

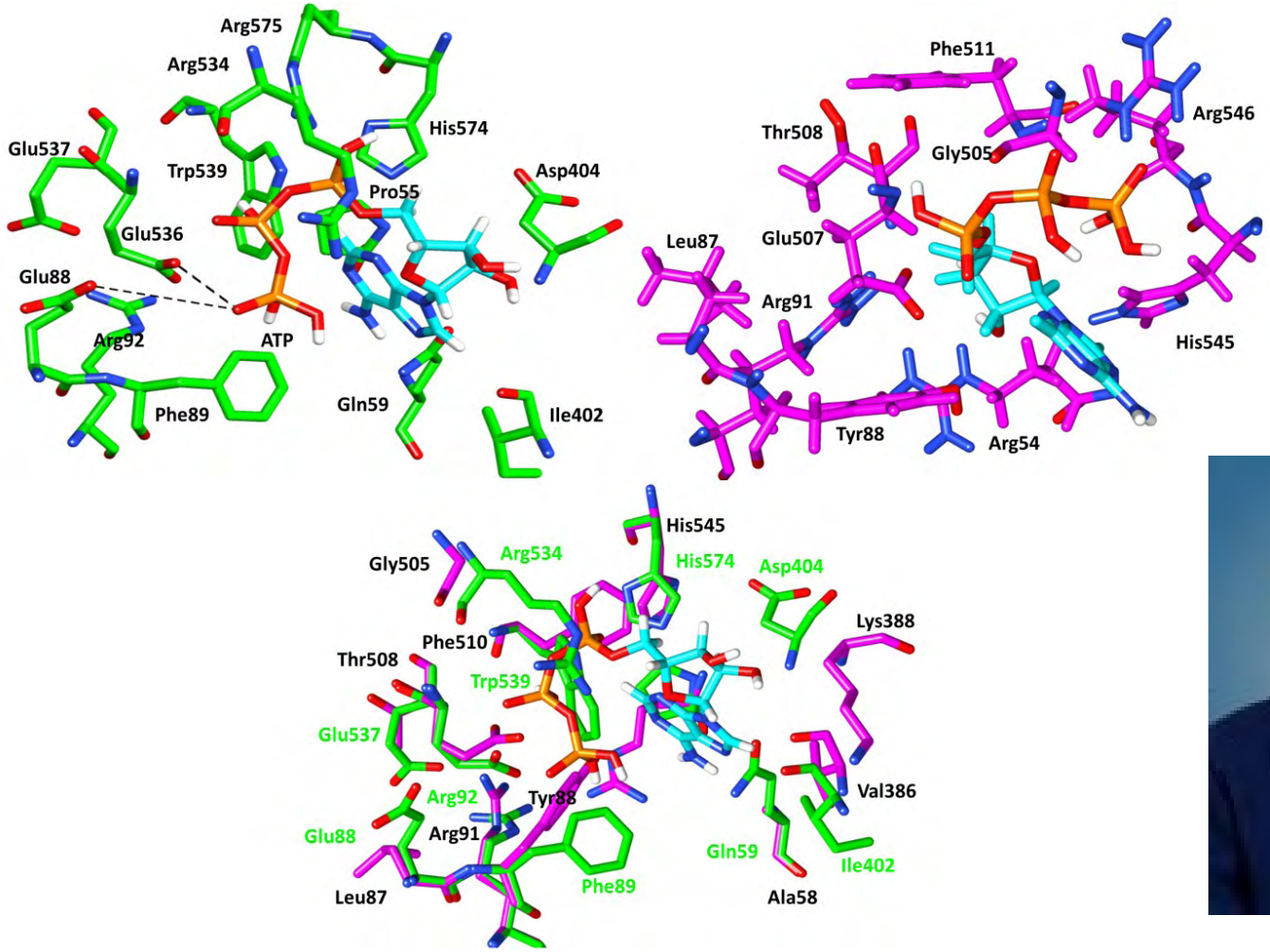


ATPase Assay of MtUvrC in the presence of inhibitors



ATPase motor domains of MtUvrC are similar to bonafide ATPases

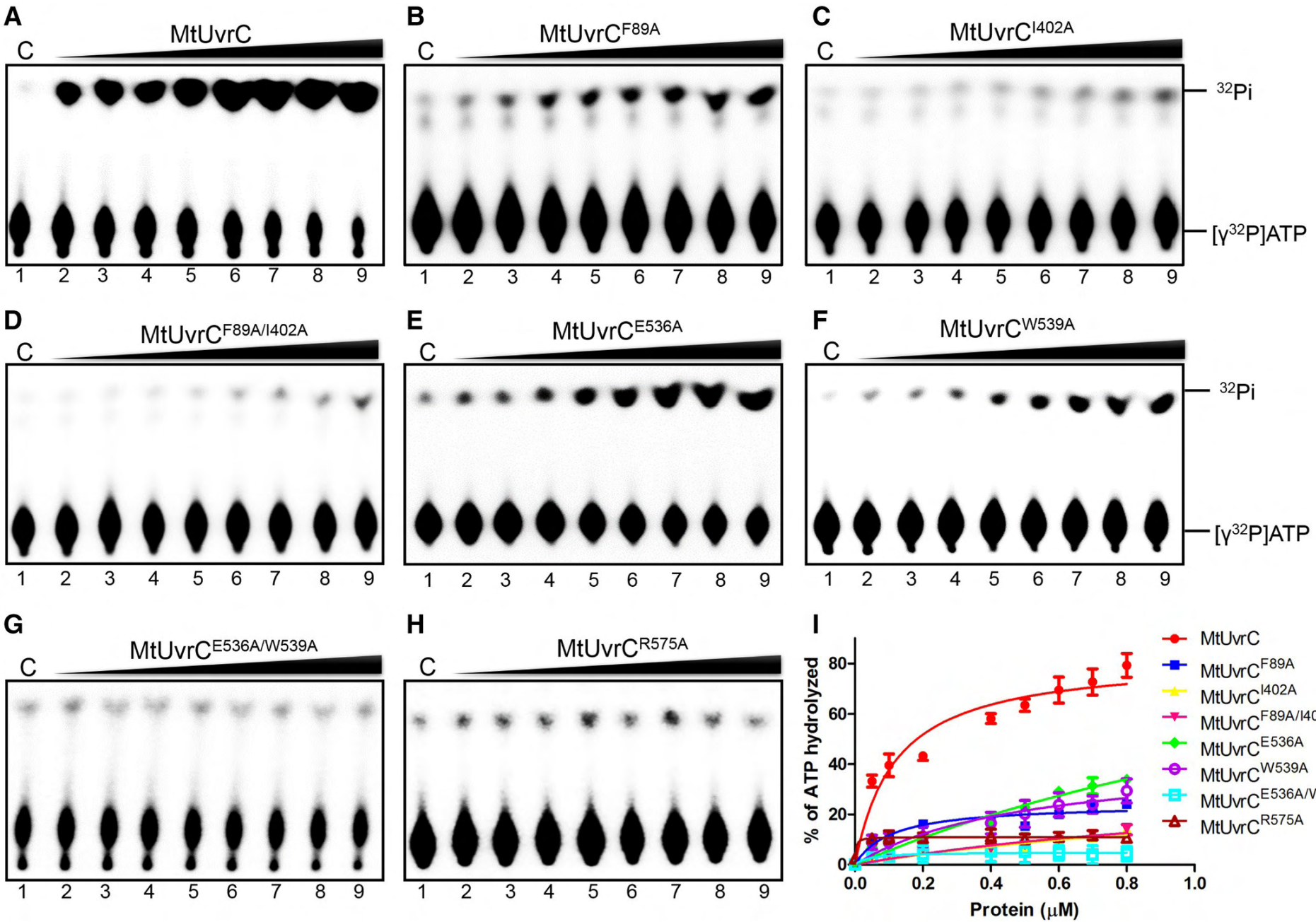
MtUvrC and EcUvrC binds ATP

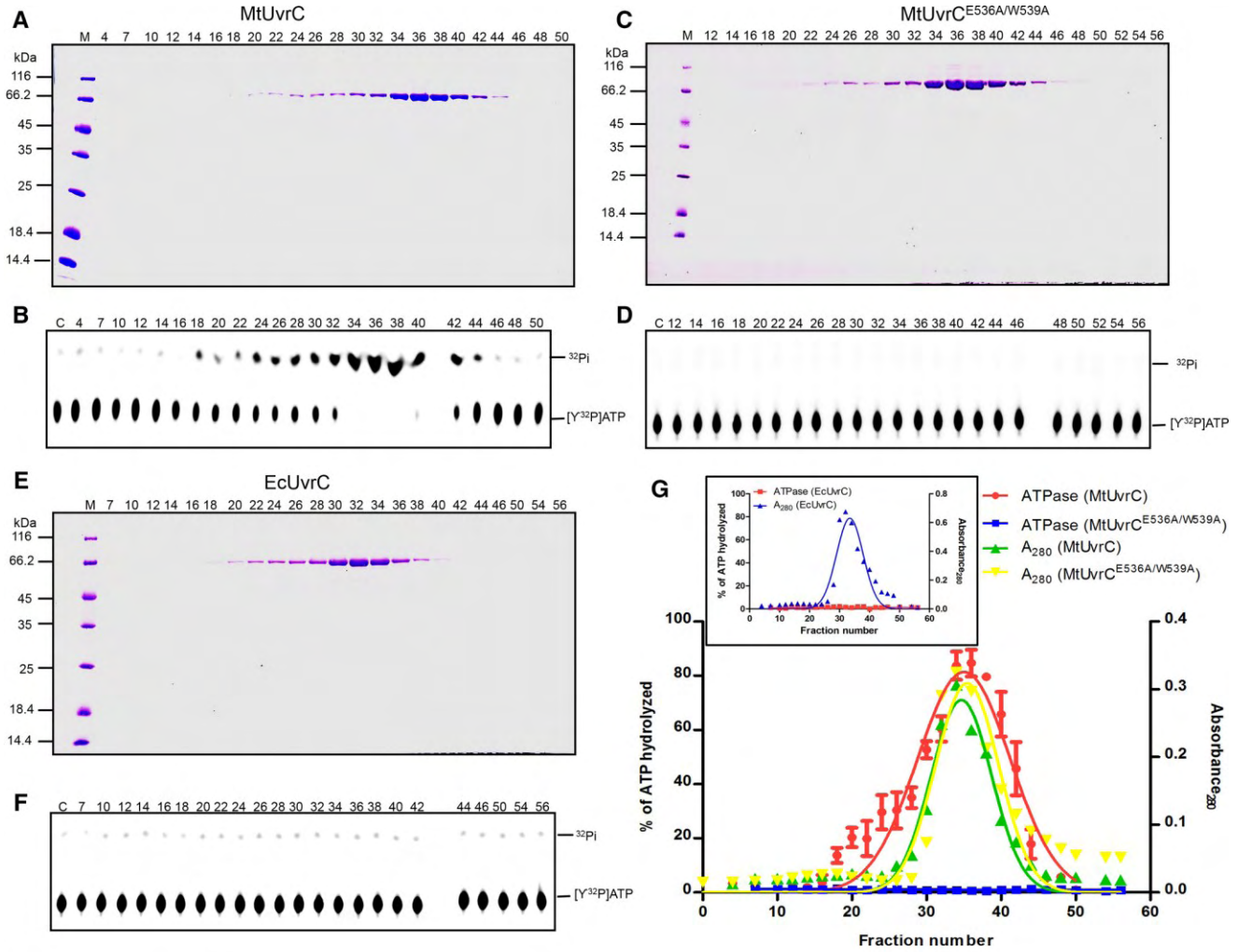


In collaboration with **Prof. K. D. Sonawane** (Shivaji University)
Structural Bioinformatics Unit and Department of Microbiology, Shivaji University, Maharashtra, India

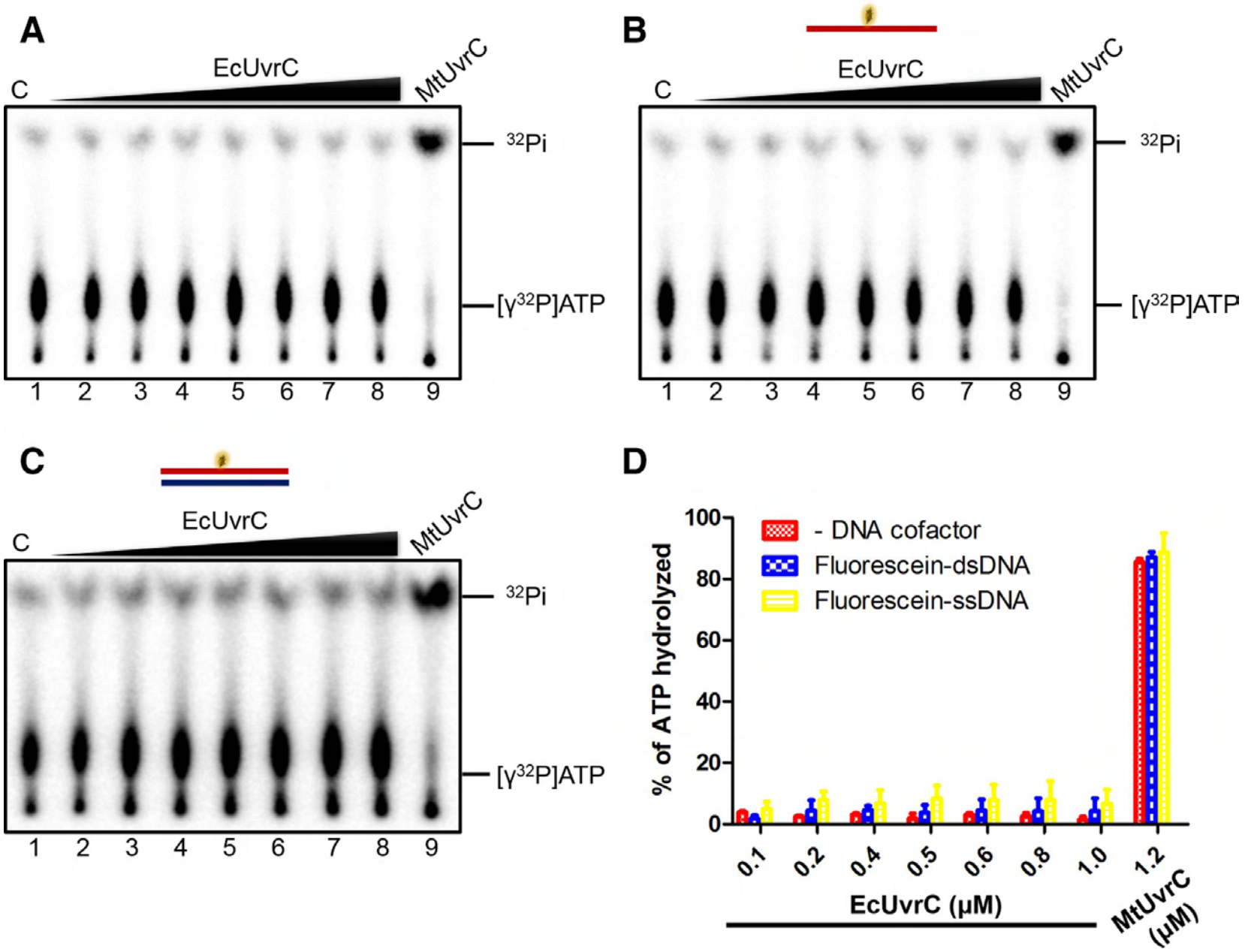
Rishikesh S Parulekar and **Sagar S Barale** are highly acknowledged

Mutations in the ATP binding pocket lead to the abrogation of ATP hydrolysis




A
MtUvrC
M 4 7 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
kDa
116
66.2
45
35
25
18.4
14.4
B
C 4 7 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
32Pi
[γ32P]ATP
C
MtUvrCE536A/W539A
M 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56
D
C 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56
E
EcUvrC
M 7 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 50 54 56
F
C 7 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 50 54 56
G
ATPase (MtUvrC)
ATPase (MtUvrCE536A/W539A)
A280 (MtUvrC)
A280 (MtUvrCE536A/W539A)
% of ATP hydrolyzed
Absorbance280
Fraction number
H
MtUvrCK206A
I
MtUvrCK206A
C
1 2 3 4 5 6 7 8 9
J
MtUvrC purified from ΔuvrAΔuvrB strain
K
MtUvrC purified from ΔuvrAΔuvrB strain
MtUvrB
1 2 3 4 5 6 7 8 9 10 11 12

Evaluation of the ATPase Activity of EcUvrC in Absence and Presence of DNA



- ✓ **According to our studies, MtUvrC possesses robust ATPase activity independent of MtUvrB and MtUvrA.**

These findings prompted us to ask following questions:

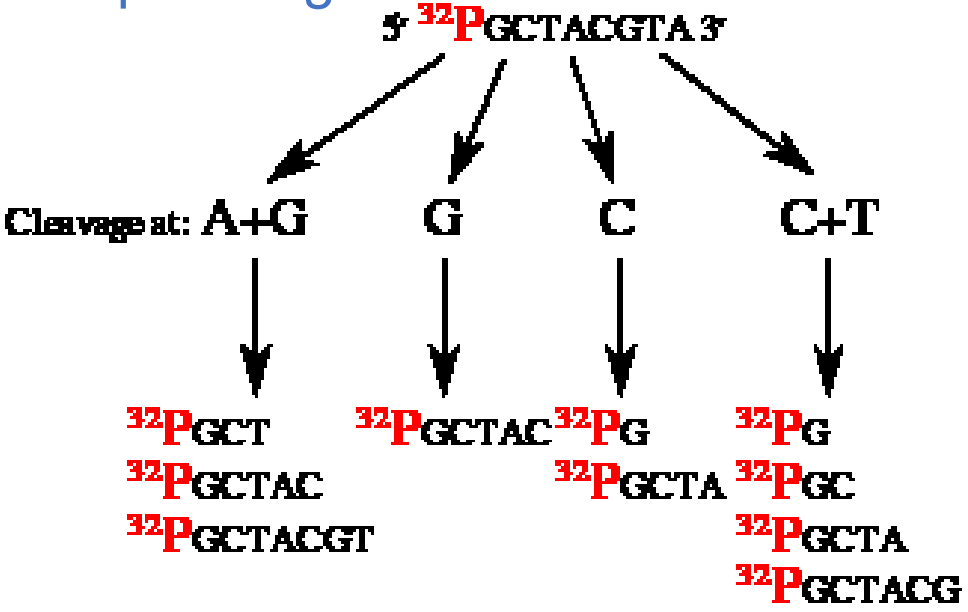
- ✓ **Can these two activities influence GIY-YIG and RNaseH endonuclease domains of MtUvrC to perform cleavage function?**

- ✓ **Besides harbouring these properties, does MtUvrC still require MtUvrA and MtUvrB for cleavage of damaged nucleotides on DNA?**

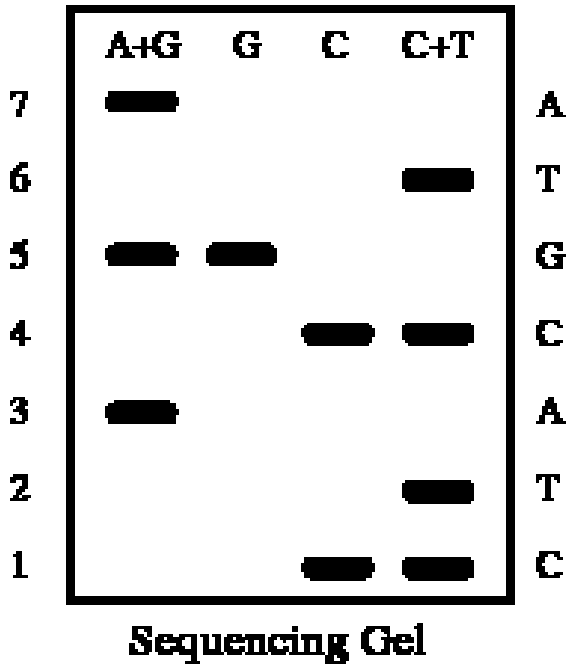
- ✓ **Does MtUvrC alone have the potential to discriminate between damaged and undamaged strands?**

Allan Maxam and Walter Gilbert's Sequencing

- ✓ 1977 paper "**A new method for sequencing DNA**" was honored by a Citation for Chemical Breakthrough Award from the Division of History of Chemistry of the American Chemical Society for 2017. It was presented to the Department of Molecular & Cellular Biology, Harvard University

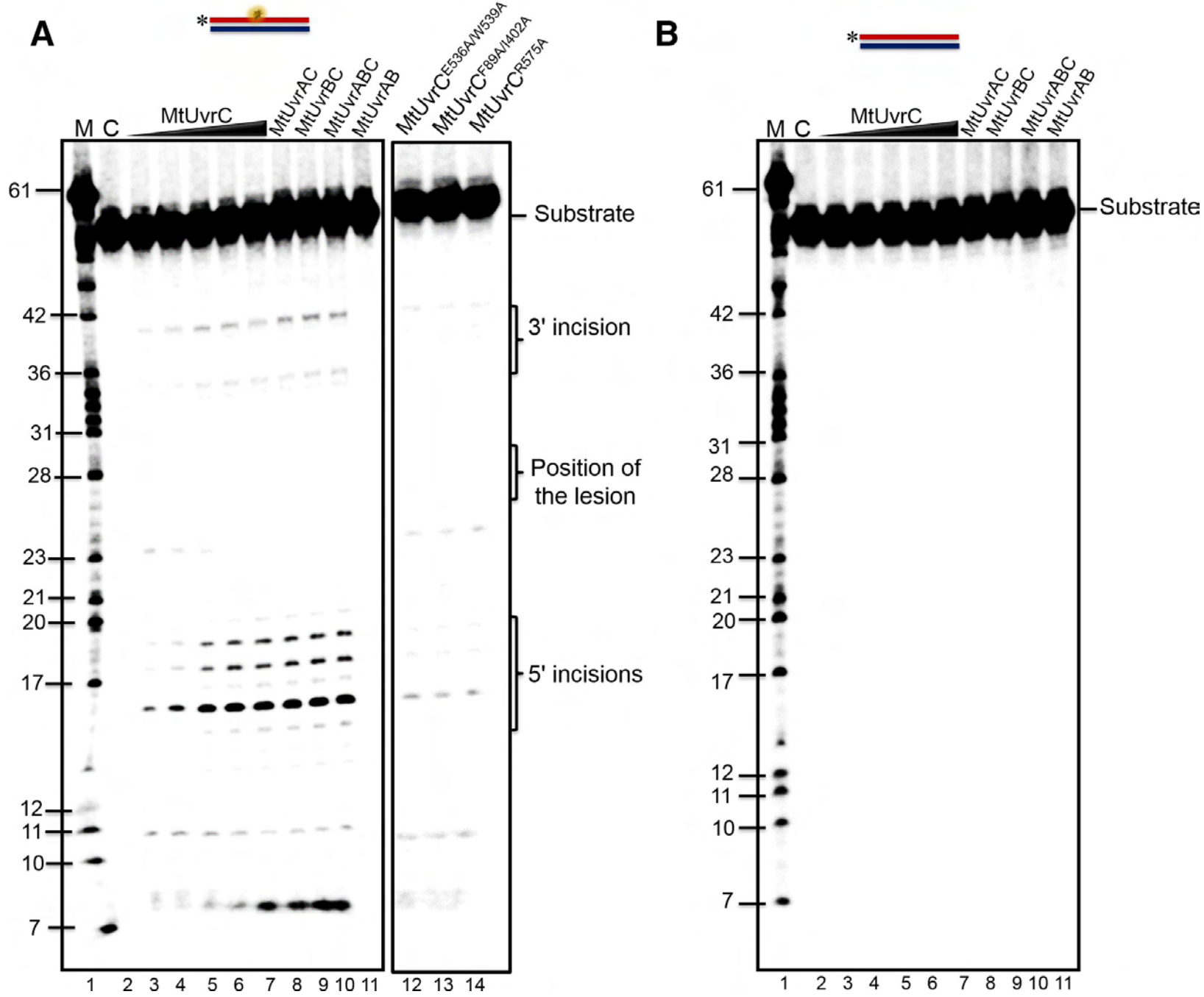


- ✓ purines (A+G) are depurinated using formic acid,
- ✓ the guanines are methylated by dimethyl sulfate,
- ✓ the pyrimidines (C+T) are hydrolysed using hydrazine.
- ✓ The modified DNAs may then be cleaved by hot piperidine; $(CH_2)_5NH$ at the position of the modified base.



Sequencing Gel

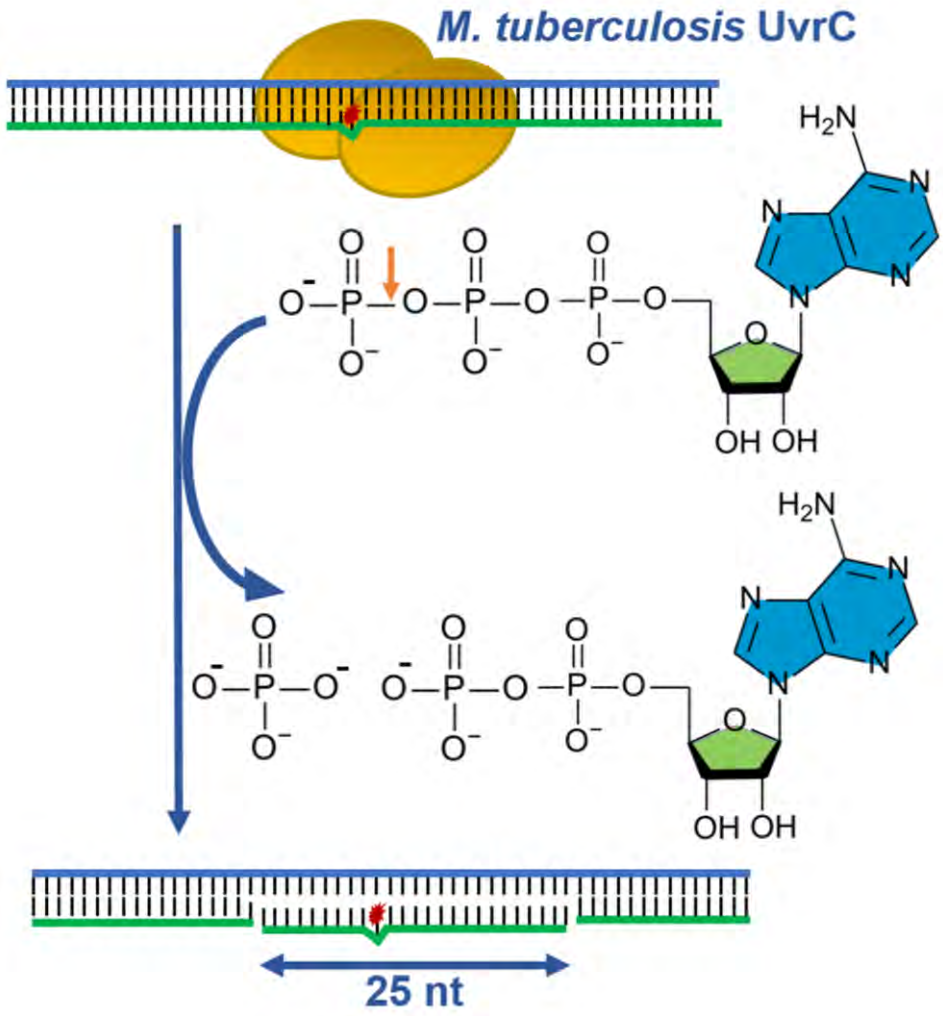
https://en.wikipedia.org/wiki/Maxam%E2%80%93Gilbert_sequencing


A
B
M
C
MtUvrC
MtUvrAC
MtUvrBC
MtUvrABC
MtUvrAB
MtUvrC^E536A/W539A
MtUvrC^F89A/I402A
MtUvrC^R575A
Substrate
3' incision
Position of the lesion
5' incisions
61
42
36
31
28
23
21
20
17
12
11
10
7
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FEBS J. 2020 Jun 29. doi: 10.1111/febs.15465. Online ahead of print.

The intrinsic ATPase activity of Mycobacterium tuberculosis UvrC is crucial for its damage-specific DNA incision function

Manoj Thakur [1], Ankit Agarwal [1], Kalappa Muniyappa [1]



Future perspectives

- Screening of small molecule inhibiters and the development of antitubercular agents against the proteins involved in the first steps of NER
- Crystallizations trials of MtUvrA, MtUvrB and MtUvrC proteins alone and with modified DNA
- SAXS analysis and cryoelectron microscopy of novel UvrAC complex
- Characterization of the domains involved in the interaction of MtUvrA and MtUvrC
- Analysis of MtUvrABC excinuclease or its individual subunit's crosstalk with other DNA related machinery

Seyed Ehtesham Hasnain
Nasreen Z. Ehtesham · Sonam Grover
Editors

Mycobacterium tuberculosis: Molecular Infection Biology, Pathogenesis, Diagnostics and New Interventions

Springer

Nucleotide Excision Repair Pathway in Mycobacteria

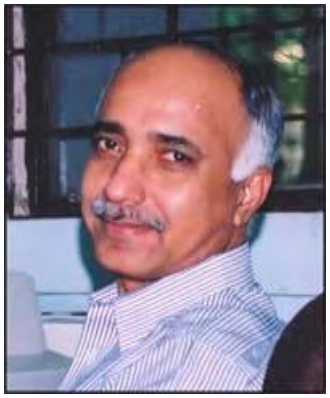
16

Manoj Thakur and K. Muniyappa

Abstract

Nucleotide excision repair (henceforth abbreviated as NER) plays a pivotal role in all organisms to protect their genetic material against radiations, toxic chemicals, and normal by-products of cellular metabolism. In humans, defects in excision repair causes inherited diseases, and NER-related human diseases are associated with cancer and aging. Much of our current understanding of NER has emerged from experimental evidence in model systems including *Escherichia coli*, yeast, and mammalian cells. Considering the importance of NER in the maintenance of genome integrity, it is surprising that only a few studies have investigated NER in mycobacteria. Here we provide a brief overview of the mechanism of the DNA repair in mycobacteria. A detailed understanding of structure-function relationship of DNA repair proteins in tubercle bacillus could facilitate the identification and development of novel therapeutic targets for tuberculosis therapy.

Acknowledgements



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IISc, Bangalore