The *Mycobacterium tuberculosis* DNA-repair helicase UvrD1 is activated by redox-dependent dimerization via a 2B domain cysteine conserved in other Actinobacteria.

Ankita Chadda [a], Drake Jensen [a], Eric J. Tomko [a], Ana Ruiz Manzano [a], Binh Nguyen [a], Timothy Lohman [a], and Eric A. Galburt [a]*

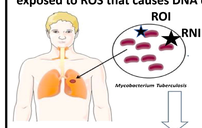
[a] Department of Biochemistry and Molecular Biophysics, Washington University in Saint Louis, 660 South Euclid Avenue, Saint Louis, MO, 63110, USA
*egalburt@wustl.edu

Washington University in St. Louis

Slide 1

Abstract

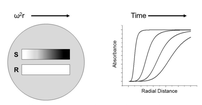
Mtb **a causative agent of Tuberculosis is exposed to ROS that causes DNA damage**



Dua et al 2018

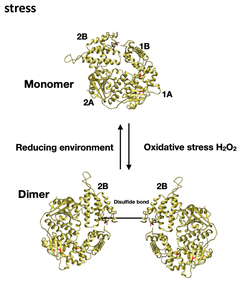
Methods

AUC

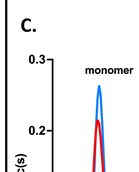


https://www.biorxiv.org/content/10.1101/359356v1.full

Mtb **UvrD1 is activated via cysteine dependent dimer formed in oxidative stress**

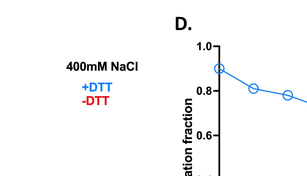


UvrD1 uses energy from ATP hydrolysis to unwind damaged DNA strand



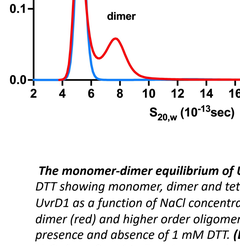
Raney et al 2013

Stopped flow



Background

UvrD plays a role in multiple DNA repair pathways

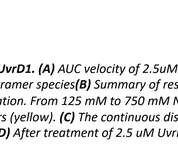


https://www.flickr.com/photos/agathman/4932657815

Mismatch repair

Ordabayev et al 2018

E. coli **UvrD helicase exist in monomer-dimer and tetramer equilibrium and unwind as dimers**

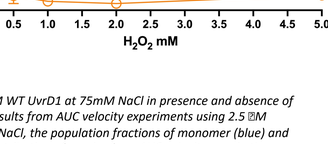


Maluf et al 2003

Transcription-coupled repair

https://phys.org/news/2014-01-molecular-star-dna-html

Mtb **UvrD1 has been shown to be a monomer by equilibrium sedimentation and size exclusion**

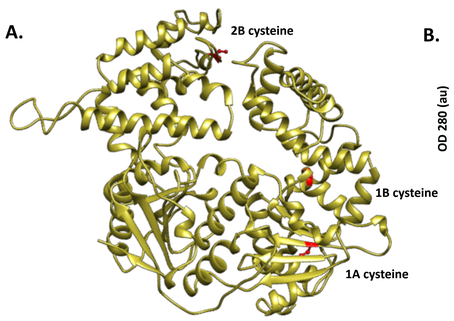


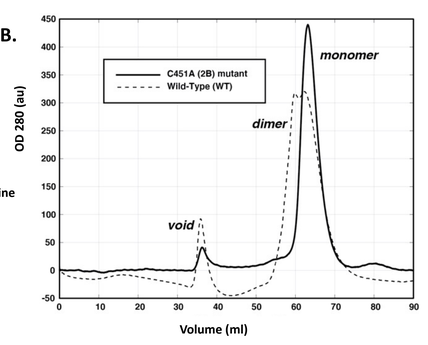
Mtb **UvrD1 has been shown to unwind as a monomer**

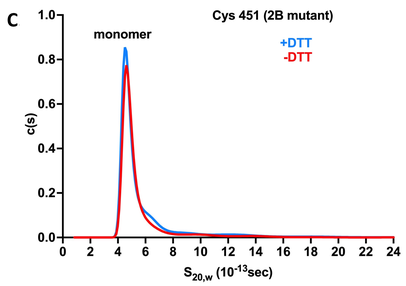
Curti et al 2007

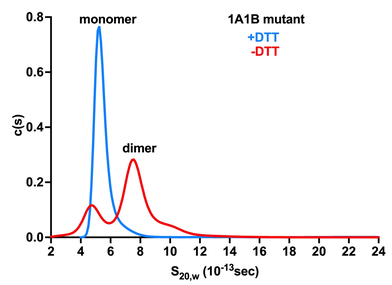
Slide 3

2B-2B domain disulfide bond is responsible for redox-dependent dimerization of UvrD1





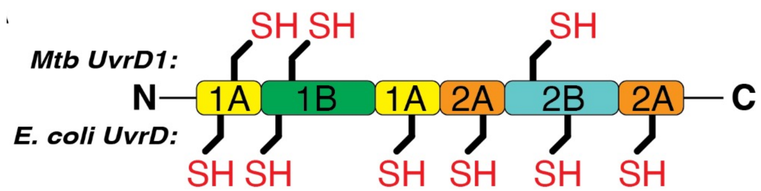




Redox-Dependence of UvrD1 dimerization is due to Cys451 in the 2B domain. (A) *Predicted structure of UvrD1 from threading UvrD1 sequence on the E. coli UvrD structure (PDB: 3LFU). Domain organization is indicated as well as the position of the three cysteine residues.* ***(B)*** *Size exclusion chromatography (S300) of C451A mutant (solid) as compared to WT (dashed).* ***(C)*** *AUC velocity experiments on C451A mutant in presence (blue) and absence (red) of DTT indicate that the mutant loses the ability to dimerize. Each trace is an average of two runs.* ***(D)*** *In contrast, AUC velocity of the C107T/C269T double mutant in the presence (blue) and absence (red) of 1 mM DTT indicates that this mutant retains and even enhances dimer formation. Each trace is an average of two runs.*

Slide 5

The 2B-cysteine is conserved in families of largest order Corynebacteriales of Phylum and Class Actinobacteria

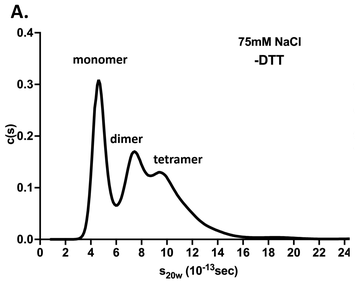


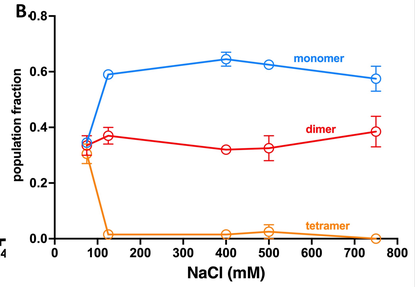
E. coli UvrD	EIKDALSYLRLIANRNDDAAFERVVNTPTRGIGDRTLDVVRQTSR 431
E. coli Rep	EIKDLLAYLRVLTNPDDDSAFLRIVNTPKREIGPATLKKLGEWAM 426
B. subtilis PcrA	EIKDILAYLRLVSNPDDDISFTRIVNVPKRGVGATSLEKIASYAA 435
M. tuberculosis UvrD1	EIRDIVAYLRVLDNPGDAVSLRRILNTPRRGIGDRAEACVAVYAE 457
C. difficile PcrA	EVRDVVAYLRVVANPDDSVSLRRILNTPRRGIGDRAEACVAVHSE 473
G. bronchialis UvrD	EVRDVVAYLRVVANPDDAVSLRRILNTPRRGIGDRAEACVAVHSE 468
D. cinnamea UvrD	EVRDVVAYLKVLNNPDDAVAIRRILNTPRRGIGDRAEACVVVHAE 462
T. paurometabola PcrA	EVRDLVAYLRAIDNPNDTVSLQRIVNTPRRGIGDRAQACLTVHAE 471
H. rhizosphaerae PcrA	EVRDIVAYLRVLANPDDAVSLRRILNTPRRGIGDRAEACVSVHAE 495
T. cavernea UvrD	EIRDVVAYLRVLLNEEDTVSVRRILNTPRRGIGDRAEACVSVHAE 502
M. ulcerans PcrA	EIRDIVAYLRVLDNPGDAVSMRRILNTPRRGIGDRAEACVAVYAE 452
R. hoagii PcrA	EVRDIVAYLRVLANEDDTVSLRRILNTPRRGIGDRAEACVSVYAE 470
S. regensis PcrA	EVRDIVAYLRVLENPDDAVSMRRILNTPRRGIGDRAEACVAVHAE 509
S. nassauensis PcrA	EVRDALAYLRLTVNEDDIVSGRRVVNVPKRGIGDRAVACVDALAD 453
A. thailandica PcrA	EVRDALAYLRAIANPDDVVSVRRILNTPRRGIGERAEACVDALAS 480
L. tulufanense PcrA	EVRDALAYLRVLDNPEDTVSLRRILNVPKRGIGDRAEACVAAHAE 478
A. pittospori PcrA	EVRDALAYLRLLANPEDTVSLRRILNVPKRGIGDRAEACVEAFAQ 456
T. lapilli PcrA	EVKDALAYLRVISNPTDTVNLRRILNTPKRGIGDRAEACVAALAE 441
A. logoneensis PcrA	EVRDLLAYLRVLANPEDTVSLRRILNVPKRGIGDRAEACVEAYAS 441

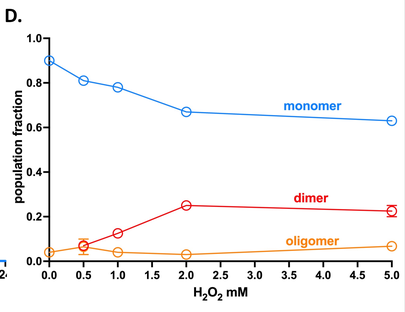
Sequence distribution across bacterial species. (A) *Distribution of cysteine residues in Mtb UvrD1 compared to* E. coli *UvrD.* ***(B)*** *Sequence containing 2B cysteine residue is distinct from* E. coli *and* B. subtilis *UvrD family enzymes but is conserved in Actinomycetes and Closterioides difficile.*

Slide 2

The dimer of *Mtb* UvrD1 is redox dependent



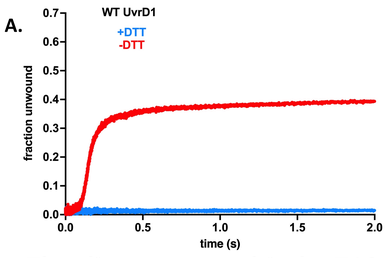


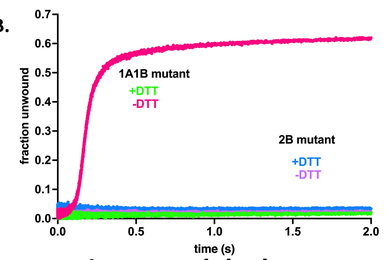


The monomer-dimer equilibrium of UvrD1. (A) *AUC velocity of 2.5uM WT UvrD1 at 75mM NaCl in presence and absence of DTT showing monomer, dimer and tetramer species* ***(B)*** *Summary of results from AUC velocity experiments using 2.5 ⍰M UvrD1 as a function of NaCl concentration. From 125 mM to 750 mM NaCl, the population fractions of monomer (blue) and dimer (red) and higher order oligomers (yellow).* ***(C)*** *The continuous distribution of species from AUC velocity runs in the presence and absence of 1 mM DTT.* ***(D)*** *After treatment of 2.5 uM UvrD1 in 75 mM NaCl with 1 mM DTT, a titration series of H_2O_2 from 0 mM to 5 mM was run in AUC velocity experiments. Increasing concentrations of H_2O_2 result in a decreasing monomer population (blue) and an increasing dimer population (red). Higher order oligomers (yellow) represented less than 10% under all conditions.*

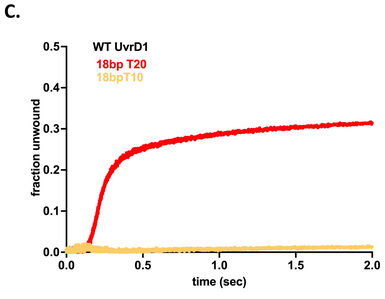
Slide 4

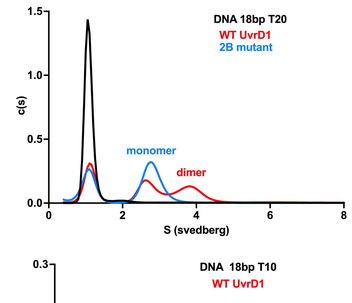
The dimer of *Mtb* UvrD1 unwinds the DNA in oxidized conditions





The dimer cannot bind a T10 tail suggesting a model when both subunits need to be engaged with ss DNA for unwinding

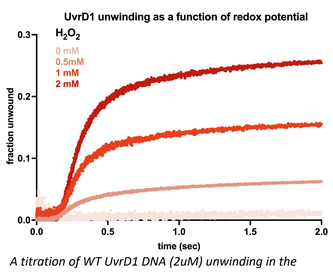




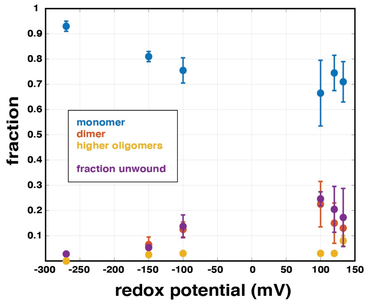
Unwinding activity is dependent on redox-dependent dimer. *Stopped-flow assay for monitoring DNA unwinding. Schematics shown in Slide 1* ***(A)*** *Fraction of DNA template unwound with 200 nM wild-type UvrD1 in the presence (blue) and absence (red) of DTT.* ***(B)*** *Fraction of DNA template unwound with 200 nM 1A1B and 2B UvrD1 mutants in the presence (green and cyan) and absence (pink and purple) of DTT.* ***(C).*** *Fraction of DNA unwound with WTUvrrD1 using T20 and T10 tail.* ***(D).*** *AUC in the first panel with T20 tailed DNA shows both monomers and dimers bound to DNA in case of WT UvrD1 and only monomer bound in case of 2B mutant. Second panel shows that in the case of T10 tail only monomers are able to bind the DNA and hence no unwinding as shown in figure C*

Slide 6

DNA unwinding activity is titrated by redox potential through dimer formation



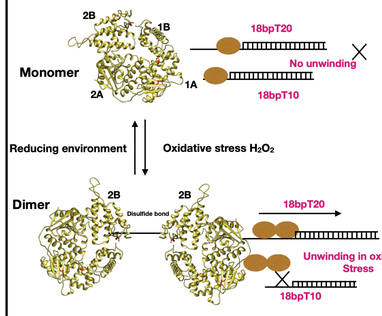
A titration of WT UvrD1 DNA (2uM) unwinding in the presence of 2 mMH_2O_2. A control in the absence of oxidizing agent is shown for comparison (-H_2O_2).



UvrD1 (2 uM 1A1B mutant)was treated with 1 mM DTT and then different concentrations of H_2O_2 (0-5 mM). Redox potential was measured and the samples were subject to AUC and used for DNA unwinding assays. The fraction of monomer present in different oligomeric states and fraction DNA unwound are plotted as a function of redox potential as follows: monomer (blue), dimer (orange), higher oligomers (yellow), and fraction DNA unwound (purple).

Model

Mtb **UvrD1 is activated in oxidative stress via 2B-2B disulfide bond . DNA binding using T20 and T10 tailed oligos suggest a mechanism of dimer unwinding where both subunits need to engage with ssDNA for unwinding**



Future directions

1) To determine if KU an NHEJ repair factor increases the processivity of a *Mtb* UvrD1 monomer and a dimer.
2) To determine if *Mtb* UvrD1 functions as a redox sensor *in-vivo*
3) Cryo-em structure of *Mtb* UvrD1 with DNA to understand the mechanism of dimerization.
4) To study the role of *Mtb* UvrD1 in transcription-coupled repair

References

1) E. Curti, S. J. Smerdon, E. O. Davis, Characterization of the Helicase Activity and Substrate Specificity of Mycobacterium tuberculosis UvrD ▾ . *J Bacteriol* **189**, 1542–1555 (2007).
2) https://www.flickr.com/photos/agathman/4932657815
3) N. K. Maluf, T. M. Lohman, Self-association Equilibria of Escherichia coli UvrD Helicase Studied by Analytical Ultracentrifugation. *J Mol Biol* **325**, 889–912 (2003).
4) Raney et al, Structure and Mechanisms of SF1 DNA Helicases. Adv Exp Med Biol 2013;767:17-46.
5) Y. A. Ordabayev, B. Nguyen, A. Niedziela-Majka, T. M. Lohman, Regulation of UvrD Helicase Activity by MutL. *J Mol Biol* **430**, 4260–4274 (2018).
6) Dua et al 2018. Multi-drug resistant *Mycobacterium tuberculosis* & oxidative stress complexity: Emerging need for novel drug delivery approaches.
7) https://phys.org/news/2014-01-molecular-star-dna-html
8) https://www.biorxiv.org/content/10.1101/359356v1.full

Acknowledgement

IDT32 infectious training grant fellowship (2020-2022)
NIH grant (R01GM134362))