Co-expression and purification of the RadA recombinase with the RadB paralog from *Haloferax volcanii* yields multimeric ring structures.

Authors:

Bushra B. Patoli[a, 1,*], Jody A. Winter[a, 2] Atif A. Patoli[a, 1], Robin M. Delahay[b] and Karen A. Bunting[a,3]

Affiliations:

[a]School of Biology, Queens Medical Centre, University of Nottingham, Nottingham, UK, NG7 2UH

[b]School of Medicine, University of Nottingham, Nottingham, UK, NG7 2UH. Email: rob.delahay@nottingham.ac.uk.

[1]Institute of Microbiology, University of Sindh, Jamshoro, Pakistan (Present address). Email: bushrapatoli@gmail.com

[2]Nottingham Trent University, Nottingham, UK, NG11 8NS (Present address). Email: jody.winter@ntu.ac.uk

[3]Albumedix Ltd, Nottingham, UK, NG7 1FD (Present address). Email: karen.bunting@slidingclamp.com

[*]**Corresponding author :**

Dr. Bushra B. Patoli, Institute of Microbiology, University of Sindh, Jamshoro, Pakistan. Tel.: +92 3113005252. E.mail.bushrapatoli@gmail.com

Supplementary Methods and Figures

Hvo RadA-RadB complex did not form a complex with DNA in different trials

DNA binding of Hvo RadB over-expressed in *E. coli* is already established (GUY *et al.* 2006). However under the same reaction conditions, the RadA-RadB complex did not show detectable protein-DNA complex formation with either single stranded (ELB 40 (70 mer)) or a partial duplex (ELB 40/ELB 41 (33 mer)) previously used analysis with the Hvo RadB protein. The assay was also performed with salt concentrations ranging from 0.1 M to 1 M NaCl or KCl in the binding buffer. Replacing magnesium with an EDTA-based buffer and adding ATP in the binding buffer with increasing concentration of the RadA-RadB protein complex (0-8 μM) did not result in protein-DNA complex formation on either 10% or 7% polyacrylamide.

During this study, *E. coli* RecA (2 μM) was also used as a positive control in the same binding buffer used for Hvo RadA-B complex with the exclusion of salt (i.e. containing 20 mM Tris pH 7.5, 15 mM $MgCl_2$, 2 mM DTT, 0.05 mg/ml BSA, 6% glycerol, 2 mM non-hydrolyzable ATP analog). Hvo RPA3 at concentrations ranging from 3-9 μM was assayed for DNA binding in varying NaCl/KCl (0.1-1 M) and $MgCl_2$ (15-30 mM) concentrations. Addition of salt is generally considered unfavorable for agarose gel electrophoresis as this may cause overheating due to the high conductivity of salt-containing buffers. Limiting NaCl concentration up to 0.4 M was found to be compatible when gels and buffers were pre-cooled at 4^0C; a distinct nucleoprotein complex was observed for Hvo RPA3 in the given conditions. Electrophoretic Mobility Shift Assay (EMSA) of Hvo RPA3 using ΦX174 ssDNA in a horizontal agarose gel (0.6%) was also optimized to a NaCl/KCl concentration ranging from 0.4 M to 1 M KCl. Protein-DNA complexes were not observed for the RadA-B complex in contrast with Hvo RPA3 for which protein-DNA interaction was detected under similar conditions (WINTER *et al.* 2012).

Fluorescence polarisation anisotropy was attempted to obtain quantitative analysis of the Hvo RadA-RadB complex as this assay had previously been used successfully to demonstrate Hvo RPA3 DNA binding in high salt concentrations up to 3 M KCl (WINTER et al. 2012). The assay

was performed using an 18mer oligonucleotide labelled at the 5' end with a Cy5 fluorophore in the presence of 1-3 M KCl. No DNA binding to the Hvo RadA-B complex was detected.

DNA binding assay	**DNA substrate**	**Protein concentration**	**Binding buffer components**	**Incubation time and temperature**	**Running Buffer**
EMSA: Agarose gel (0.6%) retardation assay	500 ng of PhiX174 ssDNA (NEB)	0-10 μM	20mM Tris, 15mM or 30 mM MgCl2, 2mM DTT, 50 μg/mL BSA, 6% glycerol, 2 mM non-hydrolyzable ATP, and KCl concentration (0.2, 0.4, 0.5 or 1 M)	10 minutes at 37 ^{0}C or 40 ^{0}C.	1x TBE buffer
EMSA: Polyacrylamide TBE gel (10%)	0.2 nM (ELB40) 70-mer ssDNA or a partial duplex of ELB 40/ELB 41 (33 mer)	0-8 μM	20 mM Tris pH7.5, 15 mM $MgCl_2$, 2 mM DTT, 50 μg/mL BSA, 6% glycerol, 2 mM non-hydrolyzable ATP, and 0.4 M or 1 M NaCl.	10 minutes at 37 ^{0}C or 40 ^{0}C.	1x TBE buffer
Fluorescence polarisation anisotropy	20nM 18mer Cy5-fluorophore labelled oligonucleotide at the 5' end	0-8 μM	50mM HEPES pH 7.0, 10% glycerol, 0.03% BSA, 2 mM non-hydrolyzable ATP. KCl concentration either 1, 2 or 3 M.		

Table S1. Different methods employed for DNA binding assay using purified RadA-RadB complex

Supplementary Figure legends

Figure S1. Sequence alignment of recombination proteins in different domains of life.

S1A. A cartoon representation showing the structural comparison of *P. furiosus* (Pfu) RadA (1pzn) to eukaryotic Rad51 (1szp) and *E.coli* RecA (2reb). Pfu RadA is shown in blue, eukaryotic Rad51 in red and RecA is colored yellow. Pfu RadA shows similarity in the ATPase domain (AD) to both eukaryotic Rad51 and RecA. Pfu RadA possesses N-terminal domain (ND) like eukaryotic Rad51, with bacterial RecA instead contains C-terminal domain (CTD), colored yellow.

S1B. Amino acid sequence alignment of Hvo RadA with RadA homologues. Sequence alignment demonstrates the conservation of the ATP (Walker A and B) and DNA (L1 and L2) binding motifs, indicated in green and blue respectively. Highly conserved residues are highlighted in red. Abbreviations: *Methanococcus voltae* (Mvo), *Pyrococcus furiosus* (Pfu); *Archaeoglobus fulgidus* (Afu); *Sulfolobus solfataricus* (Sso); *Saccharomyces cerevisae* (Sc); *Homo sapiens* (Hs).

S1C. Comparison of RadA and RadB domain structure (adapted from (KOMORI *et al.* 2000)). Green boxes represent the Walker A and Walker B motifs, respectively, in the conserved ATPase domain (RadA-c). RadB lacks the N-terminal domain (RadA-n) and contains the conserved ATPase domain.

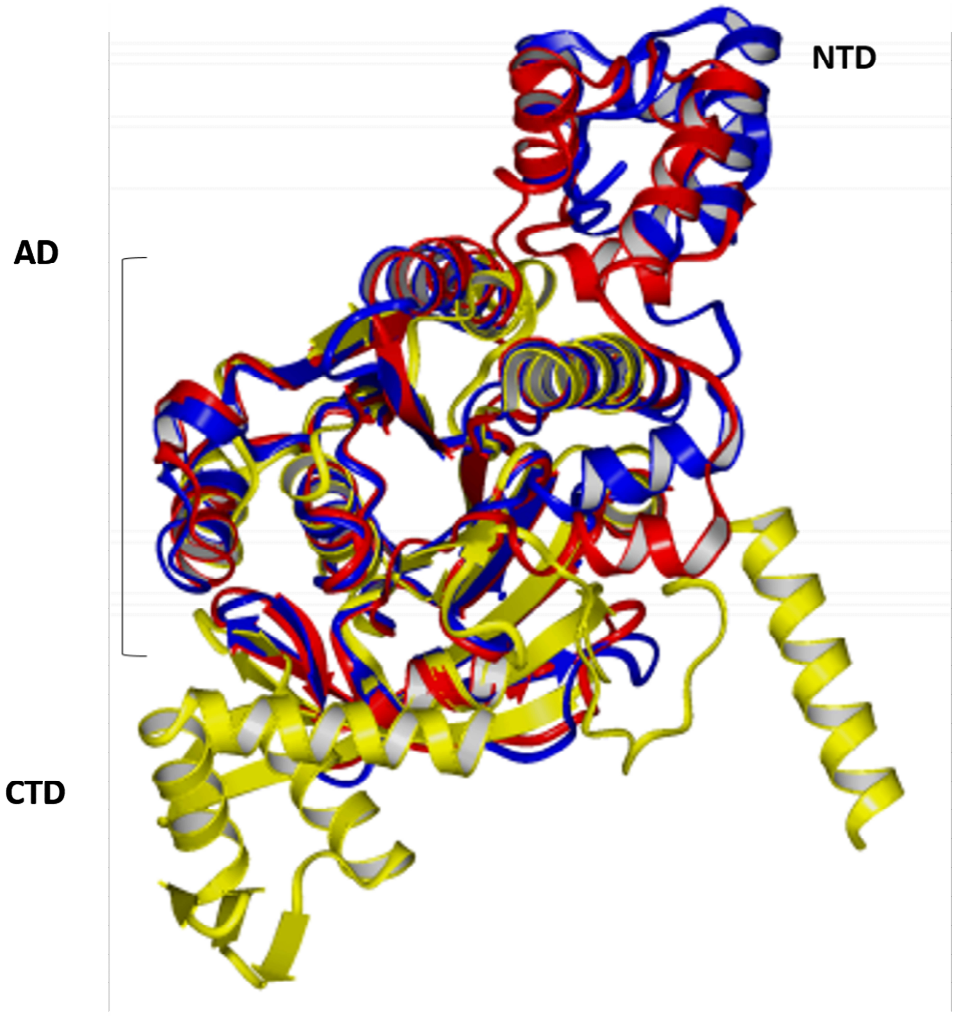
Figure S2. Selected amino acid usage in *H. volcanii* RadA compared with RecA/Rad51 homologs from other species. Sequence identification Accession numbers (NCBI) of each protein: Hvo RadA (ELY24318.1); Hs Rad51 (AFN04713.1); Mvo RadA (AAC23499.1); Afu RadA (KUK05438.1); Pfu RadA (AFN04713.1); Ec RecA (AFU91764.1). Amino acid quantitation of selected amino acids of RadA or homologues from different species was computed using ExPASy ProtParam and is presented as a relative percentage.

Figure S3. **EMSA at 0.4 M KCl shows DNA binding by Hvo RPA3 but not the RadA-B complex.** The Hvo RPA3 protein has been previously shown to form protein-DNA complexes

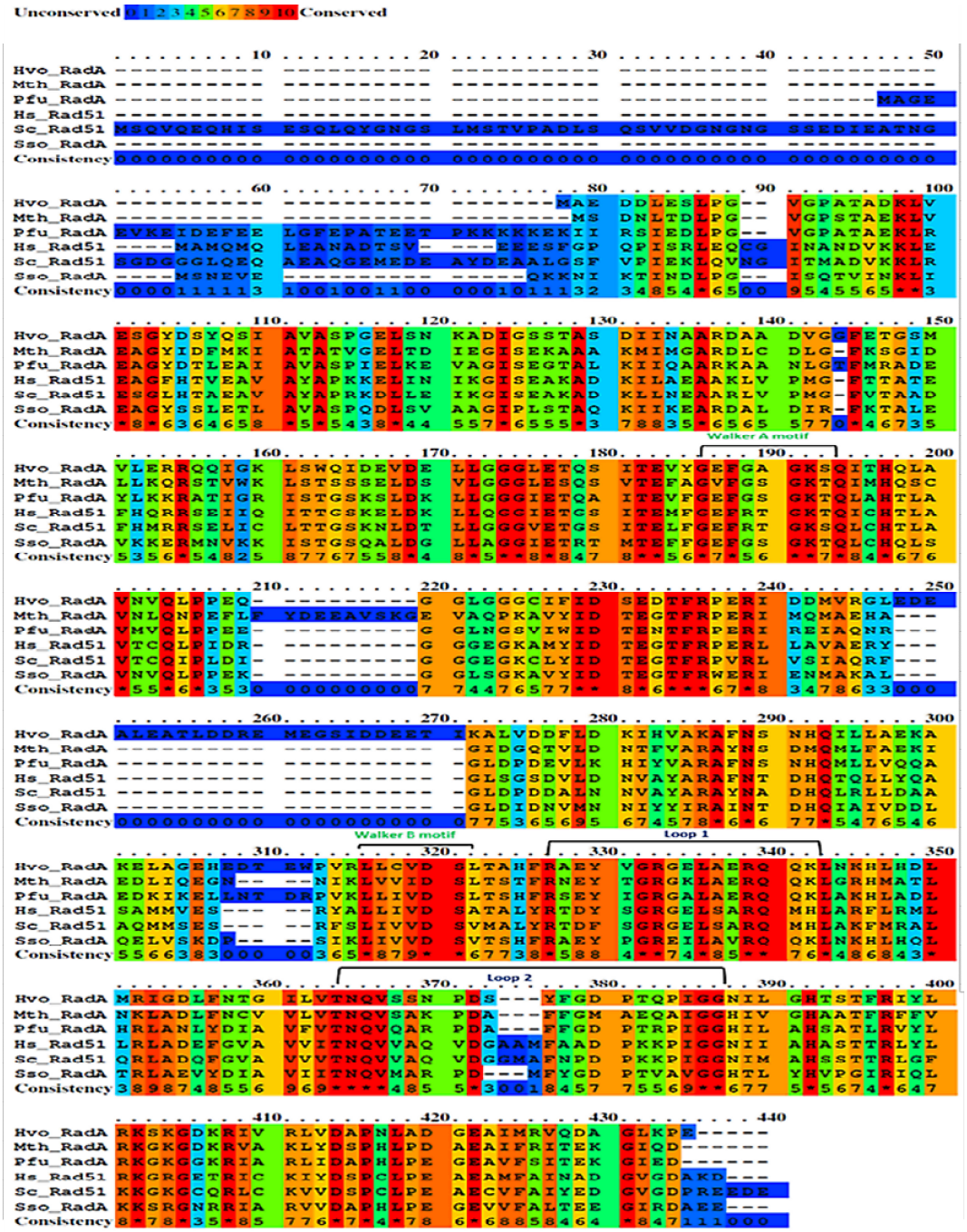
under *in vitro* conditions (Winter et al., 2012). Therefore RPA3 was used as a positive control. RPA3 and RadA-B complex samples were pre-incubated with ^{32}P-labeled DNA at 2 nM final concentration in binding buffer supplemented with 0.4 M NaCl at 42 ^{0}C, for 10 min. Protein-DNA complexes were analysed by 10% TBE gel in TBE buffer. Increasing protein concentration results in increased complex formation for RPA3, whereas no binding was observed for the RadA-B complex.

Figure S1

A.



B.


Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved
Hvo_RadA
Mth_RadA
Pfu_RadA
Hs_Rad51
Sc_Rad51
Sso_RadA
Consistency
Walker A motif
Walker B motif
loop 1
loop 2

C.

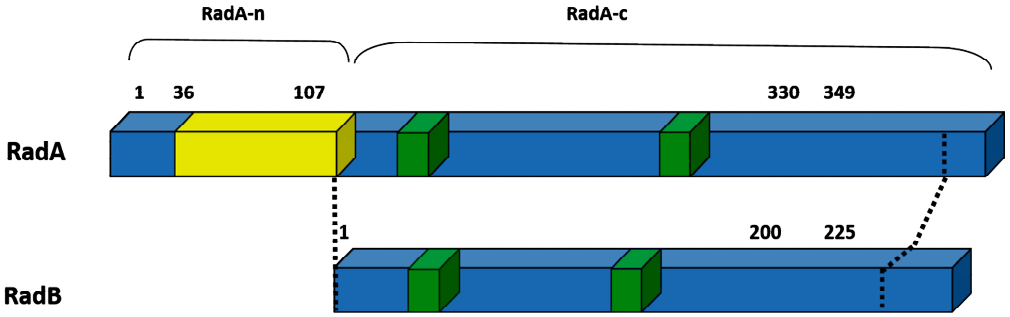


Figure S2

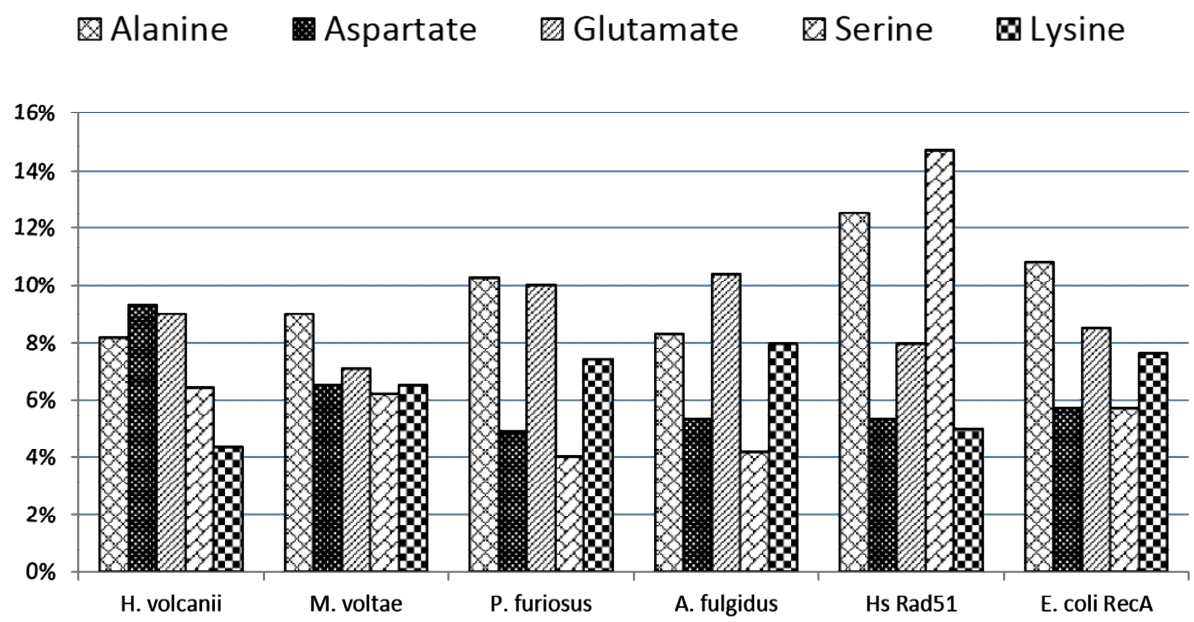
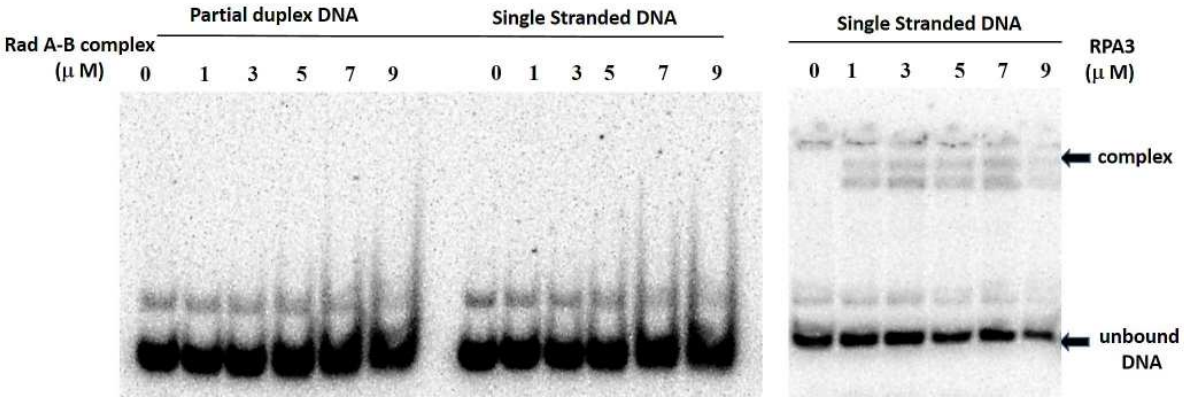

Alanine
Aspartate
Glutamate
Serine
Lysine
16%
14%
12%
10%
8%
6%
4%
2%
0%
H. volcanii
M. voltae
P. furiosus
A. fulgidus
Hs Rad51
E. coli RecA

Figure S3.



Supplementary References

Guy, C. P., S. Haldenby, A. Brindley, D. A. Walsh, G. S. Briggs *et al.*, 2006 Interactions of RadB, a DNA repair protein in archaea, with DNA and ATP. J Mol Biol 358**:** 46-56.

Komori, K., T. Miyata, J. DiRuggiero, R. Holley-Shanks, I. Hayashi *et al.*, 2000 Both RadA and RadB are involved in homologous recombination in Pyrococcus furiosus. J Biol Chem 275**:** 33782-33790.

Winter, J. A., B. Patoli and K. A. Bunting, 2012 DNA binding in high salt: analysing the salt dependence of replication protein A3 from the halophile Haloferax volcanii. Archaea 2012**:** 719092.