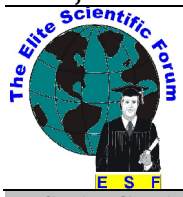
Pak. j. life soc. Sci. (2013), 11(1): 65-69 E-ISSN: 2221-7630;P-ISSN: 1727-4915



Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Characterization of *Oryza sativa* Rad51 for Phosphorylation using *in silico* Approach

Rana Salman Anjum[1,*], Muhammad Ashraf[2], Munir Ahmad Sheikh[1] and Amer Jamil[1,*]
[1]Molecular Biochemistry Laboratory, Department of Chemistry and Biochemistry, University of Agriculture Faisalabad, Pakistan
[2]Department of Botany, University of Agriculture Faisalabad, Pakistan

ARTICLE INFO
Received: Feb 04, 2013
Accepted: Feb 14, 2013
Online: Mar 14, 2013

Keywords
Bioinformatics
Kinase
Oryza sativa
Phosphorylation
Rad51

***Corresponding Authors:**
amerjamil@yahoo.com
rsa389@gmail.com

ABSTRACT

Repair of DNA double strand breaks by homology mediated DNA repair is crucial for accurate transmission of genetic information to the daughter cells or organisms. Rad51 is central to recombination reaction whose regulation is attributed to different proteins. Moreover, post-translational modifications such as phosphorylation also play a part to modulate its activity. In the present study, we have identified highly important phosphorylation sites in Rad51, a eukaryotic orthologue from *Oryza sativa* Indica (OsInd-Rad51). Overall the protein has been found highly conserved among different organisms especially involving residues predicted for phosphorylation. Therefore, it is tempting to speculate an important role of these phosphorylation sites in regulating recombination via Rad51. In future, these studies will have an impact in breast cancer control.

INTRODUCTION

Cellular DNA is under continuous stress from exogenous and endogenous agents. These include ultra violet rays, ionizing radiations or cellular metabolites such as reactive oxygen species (Jackson and Bartek, 2009). As a result of DNA damage, a suitable DNA repair mechanism is chosen. There exists a multitude of DNA repair mechanisms for appropriate type of damage (Bray and West, 2005). Of them all, the most complex and crucial is DNA double strand break repair (DSB). DNA DSB can occur even when a DNA polymerase encounters a mismatch leading to single strand break and ultimately to DSB. DNA DSBs are repaired via non homologous end joining (NHEJ) or homologous recombination (HR). NHEJ is error prone while HR is efficient in repairing the DNA accurately. However, HR occurs in the S phase of the cell cycle (Filippo et al., 2008).

At the core of HR lies the interaction between two evolutionarily conserved proteins namely Rad51 and BRCA2. Rad51 is a eukaryotic orthologue of bacterial RecA and performs homology search and recombination of DNA. It consists of two globular domains; the N-terminus which is mostly disordered and a highly conserved C-terminal ATPase domain (Lo et al., 2003; Sung, 1994). The core recombination activity is attributed to the ATPase domain which contains a pocket to accommodate ATP and performs its hydrolysis similar to F1 ATPases (Conway et al., 2004). BRCA2 interacts with Rad51 through its conserved BRC repeats and C-terminal domain to load Rad51 onto ss-dsDNA junction (Carreira and Kowalczykowski, 2011; Pellegrini et al., 2002). The disruptive function of BRC repeats is protected by the C-terminal domain which has a cyclin dependent kinase (CDK) phosphorylation site to regulate the function of Rad51 (Davies and Pellegrini, 2007).

Phosphorylation plays a central role in DNA damage response (DDR). Protein kinases such as ataxia telangiectasia mutated and ataxia telangiectasia related are the main signaling kinases which upon activation by DNA lesions successively activate many subsequent proteins (Jackson and Bartek, 2009; Somyajit et al., 2013). As a consequence, Rad51 is also phosphorylated at particular serine whose mutation increases the hypersensitivity to genotoxic agents (Flott et al., 2011).

Oryza sativa is a cereal plant with the shortest genome known so far and it can be used as a fine model plant for biochemical and genetic studies (Song et al., 2009). In the present study we have investigated a eukaryotic Rad51 orthologue from *O. sativa* for the presence of potential phosphorylation sites. Moreover, we relate our findings to the implication of Rad51 in the process of homology mediated DNA repair.

MATERIALS AND METHODS

Collection of osind-Rad51 orthologues

The amino acid sequence of Rad51 from *O. sativa* Indica (query) was retrieved from The National Centre for Biotechnology Information (NCBI) under GenBank accession number ABI58231.1. The query sequence was submitted as a FASTA format to protein BLAST with default parameters for error value, gap penalty and score.

Multiple sequence alignment

The protein BLAST resulted in a range of othologues for OsInd-Rad51 (data not shown) of which a few were selected for amino acid sequence comparison. Multiple sequence alignment was performed using CLUSTAL OMEGA (Sievers et al., 2011). To do this, each sequence was submitted as a FASTA format to CLUSATAL OMEGA. The resulting alignment was coloured, edited and analyzed using Jalview 2.7.

Prediction of protein parameters and potential phosphorylation sites

For calculating protein physical and chemical parameters, the OsInd-Rad51 sequence was submitted to ProtParam tool at ExPASy Bioinformatics Resource Portal (Gasteiger et al., 2005). And for phosphorylation sites prediction, OsInd-Rad51 sequence was submitted as FASTA format to NetPhos 2.0 server at ExPASy Bioinformatics Resource Portal (Blom et al., 1999). Moreover, the program was asked to provide the graphical representation of predicted phosphorylation sites for serine, threonine and tyrosine, individually.

RESULTS

OsInd-Rad51 is a highly conserved protein

The multiple sequence alignment of OsInd-Rad51 with different eukaryotes indicates that most of the key residues are conserved throughout; however, the N-terminus is not so conserved (Rajanikant et al., 2008) as revealed by Fig. 1. Most of the residues predicted for potent phosphorylation (Table 1 and Fig. 2) are located in the core ATPase domain (Wu et al., 2004) which is in fact the C-terminus of Rad51 while only two residues Ser 11 and Ser 49 belong to highly diverse N-terminus of Rad51. Moreover, the structural motifs belonging to different functions of Rad51 such as DNA binding domains and oligomerization interface have been found conserved (Shin et al., 2003).

OsInd-Rad51 can be phosphorylated at different sites

OsInd-Rad51 was tested for the presence of potential phosphorylation sites. For this, the full length protein sequence was submitted as a FASTA format to ExPASy Proteomics Server NetPhos 2.0 (Blom et al., 1999). The predicted phosphorylation sites are presented in tabulated (Table 1) as well as graphical forms (Figure 2). Table 1 shows the overall motif for each residue

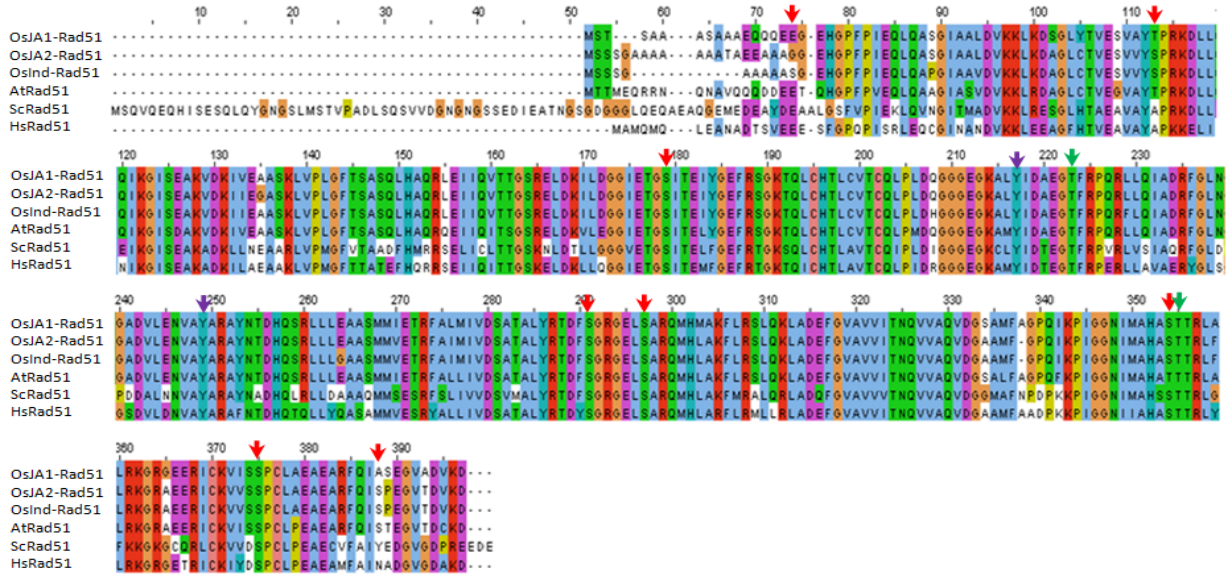


Fig. 1: Multiple sequence alignment of OsInd-Rad51 orthologues. Highly conserved residues are coloured accordingly. Residues predicted for phosphorylation are indicated by coloured arrows. Key to arrowed residues: Red, serine; green, threonine; purple, tyrosine

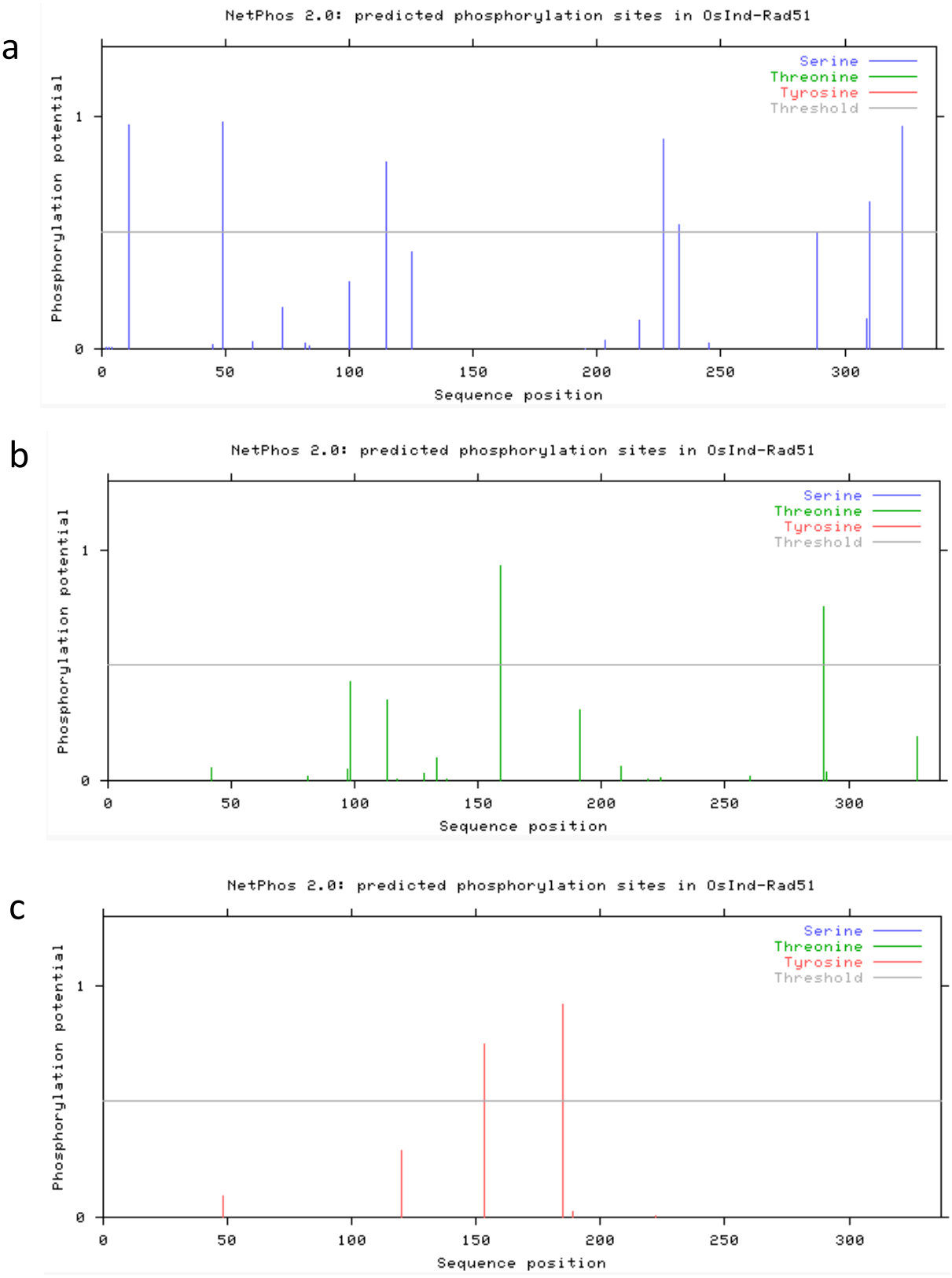


Fig. 2: Graphical representation of the predicted phosphorylation sites. (a) Indicates all the possible serine predicted for phosphorylation. (b) Shows all the threonine while (c) presents all the predicted tyrosine residues. However, residues above the threshold are more prone to phosphorylation

Table 1: Motif sequence and score of each predicted site for phosphorylation. Potential residue in its motif is bold coloured accordingly. Key to coloured residues: Red, serine; green, threonine; purple, tyrosine. Score values close to 1 indicate more potency for phosphorylation

Serial number	Residue name	Motif sequence	Residue number	Score
1	Serine	AAAA**S**GEHG	11	0.960
		SVVY**S**PRKD	49	0.975
		IETG**S**ITEI	115	0.801
		RTDF**S**GRGE	227	0.904
		RGEL**S**ARQM	233	0.533
		MAHA**S**TTRL	289	0.502
		KVVS**S**PCLA	310	0.633
		RFQI**S**PEGV	323	0.955
2	Threonine	DAEG**T**FRPQ	159	0.933
		AHAS**T**TRLF	290	0.757
3	Tyrosine	GKAL**Y**IDAE	153	0.746
		ENVA**Y**ARAY	185	0.922

within which the phosphorylation site is located alongwith its position in the protein. For the reliability of the potency of phosphorylation, the scores belonging to each phosphorylation site are indicated. Higher the number of score, higher is the chance for phosphorylation at that particular site and vice versa. There are present a total of 23 serine, 18 threonine and 6 tyrosine residues in OsIn-Rad51 as revealed by ExPASy ProtParam tool (data not shown). Eight serine residues have been predicted for potent phosphorylation belonging to different positions in the protein. However, five of them show very good scores. The same can be interpreted from the graphical representation of the serine phosphorylation (Figure 3a). The horizontal line in the middle shows the threshold while vertical lines indicate the score. Residues whose scores (vertical lines) are below or around the threshold, are not supposed to get phosphorylated while residues which show values high above the threshold have better chances for phosphorylation. Only two residues have been predicted for phosphorylation for threonine (Table 1 and Figure 2b) and same is the case for tyrosine (Table 1 and Figure 2c). Thr 290 is located next to Ser 289 and both have been predicted for phosphorylation but their scores are different (Table 2). Thr 290 shows a higher score value 0.757 while Ser 289 has a score value of 0.502. All the potential residues belonging to threonine and tyrosine phosphorylation have been predicted to have higher score values. The graphical representation of threonine and tyrosine phosphorylation (Figure 2b and c) indicates that multiple threonine or tyrosine have been predicted for phosphorylation but only two of them could cross the threshold limit which reveals that only two of them have the potential for phosphorylation.

DISCUSSION

In the present study, we have explored a eukaryotic Rad51 orthologue from *O. sativa* Indica for the presence of potential phosphorylation sites in the context of how phosphorylation can work to regulate the functions of Rad51 in homologous recombination. The multiple sequence alignment (Figure 1) reveals OsInd-Rad51 as an evolutionary conserved protein. Its core ATPase domain has been found more conserved among eukaryotes as compared to its N-terminus. We employed NetPhos 2.0 server (Blom et al., 1999) for phosphorylation site prediction. It works as an artificial neural network mechanism for substrate recognition and has sensitivity between 69-96%. The protein was predicted for the presence of phosphorylation sites as we found eight serine and two threonine and tyrosine residues potent for phosphorylation (Table 1 and Figure 2). Of eight serine residues predicted, five show good scores (Table 1) which indicates that they belong to some important function. Phosphorylation can happen at multiple sites in a protein (Lees et al., 1991). Serine phosphorylation is most common of all. To a mechanistic point of view, the serine/threonine specific kinases phosphorylate the hydroxyl group of the residue. In the cell cycle as well as during the recombination reaction, kinases play a crucial role (Jackson and Bartek., 2009). From sensing of the DNA damage to the completion of recombination, different kinases are phosphorylating their substrate proteins for their activation and deactivation, thereby regulating the complex events. Recently, a Rad51 from budding yeast has been phosphorylated in a Mec1 mediated manner and characterized how phosophorylation can control its normal functions. Rad51 phosphorylation at Ser 192 inactivated the ATP hydrolysis and interaction with DNA (Flott et al., 2011). Mutation of this Ser 192 led to the hypersensitivity to genotoxic agents which imparts the importance of Rad51 phosphorylation. Figure 1 indicates the presence of threonine in OsInd-Rad51 at site of Ser 192 in the yeast Rad51. This threonine is found highly conserved and it will be tempting to speculate that phosphorylation of this threonine may happen in a Mec1 mediated manner (Baroni et al., 2004). The phosphorylation of this residue may ultimately lead to the disassembly of Rad51 from nucleoprotein filaments suggesting to the termination of recombination event. Indeed, biochemical and structural approaches will be required in future to get deeper insights into the understanding of OsInd-Rad51 phosphorylation.

Acknowledgments

Authors are highly grateful for the financial support by Higher Education Commission of Pakistan.

REFERENCES

Baroni E, V Viscardi, H Cartagena-Lirola, G Lucchini and MP Longhese, 2004. The functions of budding yeast Sae2 in the DNA damage response require Mec1-and Tel1-dependent phosphorylation. Molecular and Cellular Biology, 24: 4151-4165.

Blom N, S Fammeltoft and S Brunak, 1999. Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites. Journal of Molecular Biology, 294: 1351-1362.

Bray CM and CE West, 2005. DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. New Phytologist, 168: 511-528.

Carreira A and SC Kowalczykowski, 2011. Two classes of BRC repeats in BRCA2 promote RAD51 nucleoprotein filament function by distinct mechanisms. Proceedings of the National Academy of Sciences, 108: 10448-10453.

Conway AB, TW Lynch, Y Zhang, GS Fortin, CW Fung, LS Symington and PA Rice, 2004. Crystal structure of a Rad51 filament. Nature Structural and Molecular Biology, 11: 791-796.

Davies OR and L Pellegrini, 2007. Interaction with the BRCA2 C terminus protects RAD51–DNA filaments from disassembly by BRC repeats. Nature Structural and Molecular Biology, 14: 475-483.

Filippo JS, P Sung and H Klein, 2008. Mechanism of eukaryotic homologous recombination. Annual Reviews of Biochemistry, 77: 229-257.

Flott S, Y Kwon, YZ Pigli, PA Rice, P Sung and SP Jackson, 2011. Regulation of Rad51 function by phosphorylation. European Molecular Biology Organization reports, 12: 833-839.

Gasteiger E, C Hoogland, A Gattiker, SE Duvaud, MR Wilkins, RD Appel and A Bairoch, 2005. Protein identification and analysis tools on the ExPASy server. The proteomics protocols handbook, pp: 571-607.

Jackson SP and J Bartek, 2009. The DNA-damage response in human biology and disease. Nature, 461: 1071-1078.

Lees JA, KJ Buchkovich, DR Marshak, CW Anderson and E Harlow, 1991. The retinoblastoma protein is phosphorylated on multiple sites by human cdc2. The European Molecular Biology Organization, 10: 4279-4290

Lo T, L Pellegrini, AR Venkitaraman and TL Blundell, 2003. Sequence fingerprints in BRCA2 and RAD51: implications for DNA repair and cancer. DNA repair, 2: 1015-1028.

Pellegrini L, SY David, T Lo, S Anand, M Lee, TL Blundell and AR Venkitaraman, 2002. Insights into DNA recombination from the structure of a RAD51–BRCA2 complex. Nature, 420: 287-293.

Rajanikant C, M Melzer, BJ Rao and JK Sainis, 2008. Homologous recombination properties of OsRad51, a recombinase from rice. Plant Molecular Biology, 68: 479-491.

Shin DS, L Pellegrini, DS Daniels, B Yelent et al, 2003. Full-length archaeal Rad51 structure and mutants: mechanisms for RAD51 assembly and control by BRCA2. The European Molecular Biology Organization Journal, 22: 4566-4576.

Sievers F, A Wilm, D Dineen, TJ Gibson et al, 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology, 7: 539

Somyajit K, S Basavaraju, R Scully and G Nagaraju, 2013. ATM-and ATR-mediated phosphorylation of XRCC3 regulates DNA double-strand break-induced checkpoint activation and repair. Molecular and Cellular Biology, (DOI:10.1128/MCB.01228-12), In press.

Song X, L Liu, Z Wang and S Qiang, 2009. Potential gene flow from transgenic rice (*Oryza sativa* L.) to different weedy rice (*Oryza sativa* f. spontanea) accessions based on reproductive compatibility. Pest Management Science, 65: 862-869.

Sung P, 1994. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science, 265: 1241-1243.