

Homology Modeling, Structure and Active Site Prediction of Stem Rust Resistant Protein in Wheat

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Short Running Title: Bioinformatics analysis of RPG1 protein

ABSTRACT

Homology modeling is very helpful tool which produces 3D models when the target and template are closely related. The stem rust resistance gene *Rpg1* encodes RPG1 protein, which plays a complex role in stem rust resistance. Homology modeling of RPG1 is important to study its role and pathway. The homology modeling of this protein was done using MODELLER 9.11. Then models of same proteins were superimposed to check the validation. NIH server containing tools, ERRAT was used to evaluate the 3D structure of protein RPG1, ERRAT score for this model was 77%. This score was further improved by doing loop optimization of RPG1 using similar tool. It was improved upto 80%. 3D model of stem rust resistance was further used to analyze the activity of protein. Active sites of RPG1 were detected by using ADDS (Automated active sites detection, docking and scoring). Total of 15 active sites were identified and active sites with highest score were used in further studies. The information achieved may be useful to understand the biology of stem rust disease in wheat which may further help to manipulate the disease mechanism.

Key words: Stem Rust, *Triticum aestivum*, Homology Modeling, RPG1 protein, active site prediction

Introduction

The plants have ability to defend themselves from different pathogens. The defense mechanism is diverse in nature. It may be physiological as well as molecular. Among molecular defense mechanism, various genes are involved which are regulated in response to pathogen attack. The involved genes initiate the proper signaling process to accelerate defense responses (Nirmala *et al.*, 2010). *Rpg1* gene barley gene is well characterized to confer resistance against various races of rust disease causing fungi for over six decades (Steffenson, 1992; Kleinhofs *et al.*, 2009). The *Rpg1* gene was successfully cloned (Brueggeman *et al.*, 2002 & 2008), transformed and resulted in complete resistance (Horvath *et al.*, 2003). It encodes a kinase domain that is involved in signal pathway regulation (Nirmala *et al.*, 2006). The respective protein is involved in autophosphorylates (Nirmala *et al.*, 2006, 2007). The protein gets autophosphorylated just after exposure to fungal spores and initiates the signaling pathway involved in defense mechanism but the effectors of the proteins are not well characterized yet (Nirmala *et al.*, 2010). So, it is very important to identify the effector molecules for RPG1 protein. The present project was designed to model the protein and predict its active binding sites *in silico*. The information achieved will be used to identify the appropriate effectors for RPG1 protein first *in silico* and then *in vitro*.

Materials and Methods

Sequence Retrieval

Wheat RPG1 protein (UniProt Code: Q70AJ9) was retrieved from UniProt (<http://www.uniprot.org/>) which is a biggest repository of proteins.

Protein Homology Modeling

Homology modeling of proteins was done by using MODELLER 9.11 version. It is a computer program used for the homology modeling of protein three-dimensional structure (SALI AND BLUNDELL, 1993; FISER *et al.* 2000).

Model Evaluation

The 3D model of RPG1 protein was evaluated by ERRAT. This model helps in analyzing the statistics of non-bonded interactions among between atom types. This model also facilitate plotting the value of the error function versus position of a 9-residue sliding window. All these results were obtained by comparing values with statistics originated from highly refined structures (Colovos & Yeates, 1993).

Active Site Prediction

ADDS (Automated active sites detection, docking and scoring) was used to predict active sites (Tanya *et al.*, 2011) of RPG1 protein. The accuracy of this software was checked by the molecules of known active sites.

Results

Protein Model

The homology modeling of RPG1 protein of wheat (*Triticum aestivum*), which is encoded by stem rust resistance gene *Rpg1* was done by MODELLER 9.11.

The model was constructed using the 4fmzA as template for RPG1 protein. Sixty models were generated and the model with minimum pdf energy was selected as best model (**Table-1 & Figure-1**).

Model Evaluation

The selected best model was evaluated by ERRAT. The best model score of wheat RPG1 protein was 77 %. The score was further improved up to 80%

by doing loop optimization of RPG1 using similar tool (**Figure-2**).

Active Site Prediction

Active sites of wheat RPG1 proetin was predicted using ADDS (Automated active sites detection, docking and scoring). Total of 15 pockets were predicted initially but the only one pocket with highest score was selected for further analysis (**Figure-3**).

Table 1. Models of wheat RPG1 protein with their pdf energy

S. No.	Models Name	Pdf Energy	GA341 Score
1.	pkcdelta.B99990003	1686.99463	1.00000
2.	pkcdelta.B99990004	1688.86621	1.00000
3.	pkcdelta.B99990016 (Best model)	1677.76526	1.00000
4.	pkcdelta.B99990017	1741.86536	1.00000
5.	pkcdelta.B99990027	1709.98364	1.00000
6.	Pkcdelta.B99990029	1737.03186	1.00000
7.	Pkcdelta.B99990037	1715.08716	1.00000
8.	Pkcdelta.B99990038	1682.47205	1.00000
9.	Pkcdelta.B99990039	1701.55872	1.00000
10.	Pkcdelta.B99990054	1710.77869	1.00000

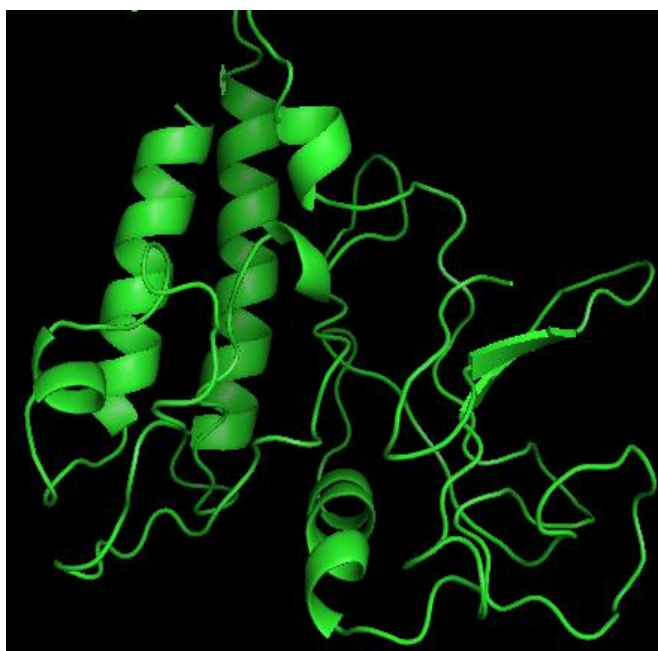


Figure 1. The 3D image of wheat RPG1 Protein, image depicts the helix and beta plated tertiary structure of proteins

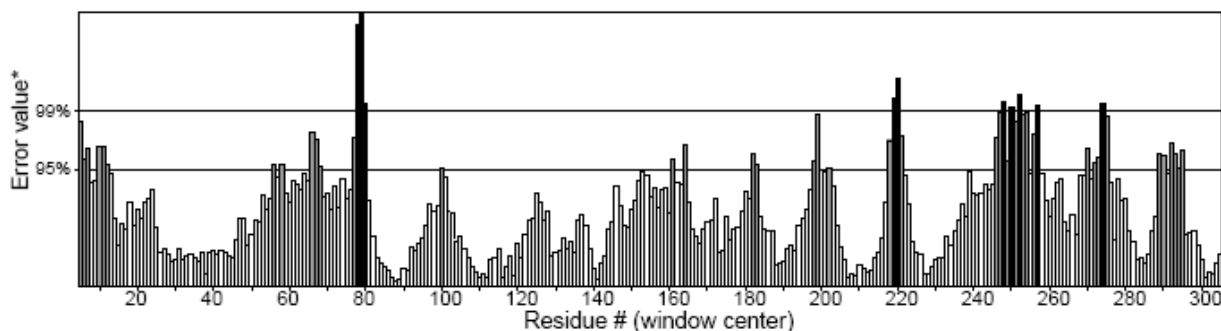


Figure 2. The ERRAT result for wheat RPG1 protein.

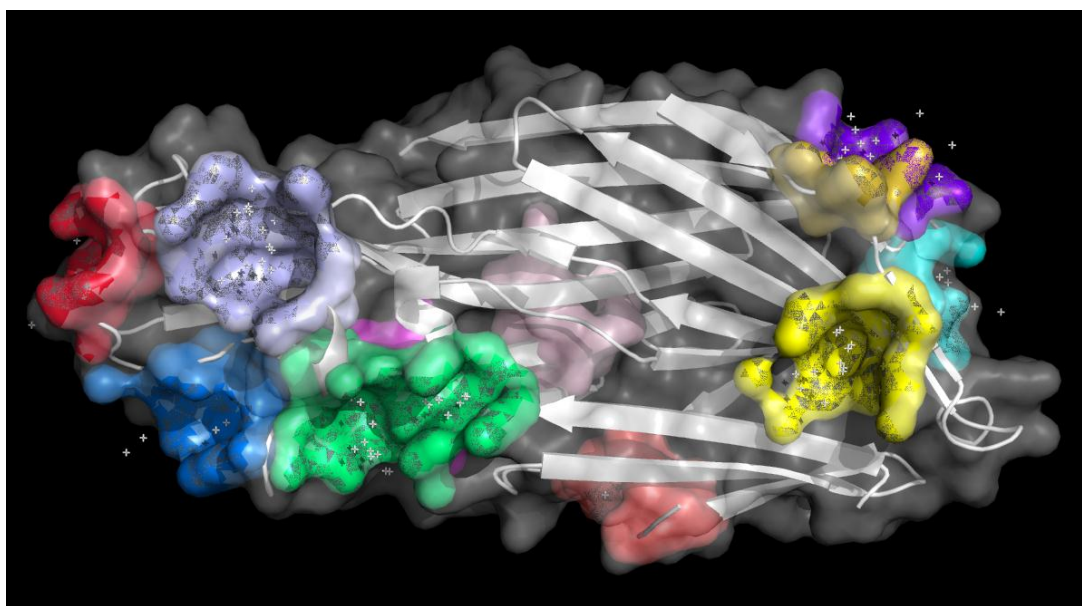


Figure3. Active Sites of wheat RPG1 protein

Discussion

The RPG1 protein is highly important for plant defense mechanism against fungal pathogens (Brueggeman *et al.*, 2002). It has been characterized that RPG1 protein conferred resistance against stem rust causing fungus in barley (Brueggeman *et al.*, 2002). RPG1 protein causes phosphorylation in disease defense signaling pathway. It is phosphorylated within 5 minutes after inoculation of the leaves with spores of pathogenic fungi. The phosphorylation state of RPG1 is maintained 20 hours of inoculation. After its phosphorylation, it is degraded (Nirmala *et al.*, 2007). It is evident by the robust response of the protein against pathogen that RPG1 has very pivotal role in the host disease resistance mechanism. The 5 minute time period is the very short time for defense response. This time is well before the germination of pathogen spores and the defense pathway starts before the spore germination (Nirmala *et al.*, 2010). The 3D model of RPG1 protein may be used to study the downstream effector

molecules which are taking part in disease resistance mechanism. The information achieved in the study may help to find out effector molecules by using *in silico* approach.

Conclusions

The stem rust resistant protein RPG1 was modeled and its active sites were predicted. The RPG1 protein plays very important role in stem rust resistance pathway and is well studied molecule, however, the exact mechanism is still to be explored. In this study, 3D model of the RPG1 protein was predicted which will be used to identify downstream effector molecules.

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References

- Brueggeman, R., A. Druka, J. Nirmala, T. Cavileer, T. Drader, N. Rostoks, A. Mirlohi, H. Bennypaul, U. Gill, D. Kudrna, C. Whitelaw, A. Kilian, F. Han, Y. Sun, K. Gill, B. Steffenson and A. Kleinhofs. 2008. The stem rust resistance gene *Rpg5* encodes a novel protein with nucleotide binding site, leucine-rich and protein kinase domains. *Proc. Natl. Acad. Sci. U.S.A.*, 105:14970-14975.
- Brueggeman, R., N. Rostoks, D. Kudrna, A. Kilian, F. Han, J. Chen, A. Druka, B. Steffenson and A. Kleinhofs. 2002. The barley stem rust resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. *Proc. Natl. Acad. Sci. U.S.A.*, 99:9328-9333.
- Colovos, C. and T. O. Yeates. 1993. Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci.*, 2(9):1511-9.
- Fiser, A., R. K. Do, and A. Sali. 2000. Modeling of loops in protein structures. *Protein Sci.*, 9: 1753-1773.
- Kleinhofs, A., R. Brueggeman, J. Nirmala, L. Zhang, A. Mirlohi, A. Druka, N. Rostoks and B. J. Steffenson. 2009. Barley stem rust resistance genes: Structure and function. *Plant Genome*, 2:109-120.
- Nirmala, J., R. Brueggeman, C. Maier, C. Clay, N. Rostoks, C. G. Kannangara, D. von Wettstein, B. Steffenson and A. Kleinhofs. 2006. Subcellular localization and functions of the barley stem rust resistance receptor-like serine/threonine-specific protein kinase Rpg1. *Proc. Natl. Acad. Sci. U.S.A.*, 103:7518-7523.
- Nirmala, J., S. Dahl, B. J. Steffenson, C. G. Kannangara, D. von Wettstein, X. Chen and A. Kleinhofs. 2007. Proteolysis of the barley receptor-like protein kinase RPG1 by a proteasome pathway is correlated with *Rpg1*-mediated stem rust resistance. *Proc. Natl. Acad. Sci. U.S.A.*, 104:10276-10281.
- Nirmala, J., T. Drader, X. Chen, B. Steffenson and A. Kleinhofs. 2010. Stem Rust Spores Elicit Rapid RPG1 Phosphorylation. *Mol. Plant Microbe Interact.*, 23(12): 1635–1642.
- Sali, A. and T. L. Blundell. 1993. Comparative protein modelling by satisfaction of spatial restraints. *J. Mol. Biol.*, 234: 779-815.
- Steffenson, B. J. 1992. Analysis of durable resistance to stem rust in barley. *Euphytica*, 63:153-167.