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BIOTECHNOLOGY

Molecular modeling and docking approach to predict the potential interacting partners of AtMAPK3P with the members of bZIP transcription factor family in *Arabidopsis*

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Abstract

Protein-protein interaction plays key role in predicting the protein function of target protein thus the identification of PPIs is of primary importance. The in vitro and in vivo methods have their own limitations, thus *in silico* methods which include structure-based approaches were developed. In this research work, we endeavor to identify the downstream interaction partners of (AtMAPK3P) in *Arabidopsis thaliana* using the docking approach. The results of our study revealed that out of 73 bZIP members of *Arabidopsis thaliana* 47 members are showing interaction with AtMAPK3P. Elucidation of protein interaction networks also contributes greatly to the analysis of signal transduction pathways. Recent developments along with the results obtained essentially enhance our knowledge of the MAPK interacting protein network and provide a valuable research resource for developing a nearly important link between pathogen-activated MAPK signaling pathways and downstream transcriptional programming.

Highlights

- Out of 73 bZIP members of *Arabidopsis thaliana* 47 members are showing interaction with AtMAPK3P.

Keywords: Protein -protein interactions (PPIs), transcription factor (TF), docking, MAPK, bZIP

Plants have very well developed integrated signaling networks that mediate the perception of and responses to the hormones, nutrients, and environment cues and stresses that govern plant growth and development. These Signal transduction pathways are extremely complex to reveal all the cross talks. Out of many signaling pathways involved in abiotic and biotic stress response in plants, mitogen activated protein kinase (MAPK) cascade is one of the major and evolutionary conserved pathway. It minimally consist of three sequentially activated MAPK family modules MAP3Ks (for MAPK kinase kinase), MAP2Ks (for MAPK kinase), and MAPKs. These signaling modules via a phosphorelay mechanism amplify developmentally regulated or environmental signals and pass them on to MAPK-phosphorylated proteins, culminating in an appropriate response (Singh *et al.*, 2012). Extensive studies revealed that MAPKs are known to regulate many physiological and developmental responses (Joshi *et al.*, 2011). Although very little is known about MAPK's downstream targets but despite this gap in our knowledge it is clear that MAPKs interact with the transcription factors (Popescu *et al.*, 2008).

Transcription factors are master regulators of gene expression at the transcriptional level. Controlling the activity of these factors alters the transcriptome of the plant, leading to metabolic and phenotypic changes in response to stress. For elucidating the role of these transcriptional regulators in different signaling cascades it is very important to functionally analyse the interactions between transcription factors and other proteins. In plants there are mainly six families involved in plant defense: bZIP, WRKY, MYC, MYB, AP2/EREBP, NAM, ATAF and NAC (Alves *et al.,* 2014). bZIPs and WRKYs are two important plant transcription factor (TF) families regulating diverse developmental and stress related processes. WRKYs are strongly regulated at the transcriptional level by each other whereas bZIPs are regulated predominantly at the post-translational level via the formation of heterodimers. The name of the family is derived from the basic region/leucine zipper (bZIP) domain present in all its members. This domain consists of an uninterrupted α-helix comprising a basic region (BR) which is necessary and sufficient to bind the DNA, followed by a C-terminal leucine zipper (LZ) motif responsible for the dimerization (Llorca *et al.,* 2014). MAPK-substrate interactions are very transient and unstable therefore it is difficult to identify the downstream interacting partners or the substrates of MAPKs. There are many *in vitro* and in *vivo* methods have been developed to study the protein-protein interactions including yeast two hybrid systems, affinity purification followed by mass spectrometry and the phage display libraries, but these methods have its own limitations and suffer from high false positive rate (Deane *et al.,* 2002). Therefore these limitations highlight the need of *in silico* interaction predictions. ''Docking'' strategy is used extensively in mitogen-activated protein kinase (MAPK) signaling (Bardwell 2006 and - Sharrocks *et al.,* 2000).

Regulation of protein activity is required for functional signaling pathways and metabolism.

Besides expression regulation, post-translational modification is a common mechanism to regulate the activity of transcription factors. Phosphorylation/ dephosphorylation through mitogen-activated protein kinase (MAPK) cascades is a conserved post-translational modification in eukaryotes. The phosphorylation of serine, threonine and tyrosine residues can affect protein structure, enzymatic activity and subcellular localization, interaction with other proteins as well as it is crucial in signal transduction**.** In eukaryotes MAPKs are catalytically inactive in their base state and require phosphorylation. The dual-specificity MAP2Ks phosphorylate MAPKs on both serine/threonine and tyrosine residues in the activation loop**.** Once activated, MAPKs can phosphorylate functionally divergent substrates on serine or threonine residues within a minimal S/T-P motif (Giri *et al.,* 2013).

Table 1. bZIP transcription factor family genes showing interaction and non-interaction with AtMAPK3P.

Gene loci Id Showing Interaction	47	ABF1, AtbZIP11, AtbZIP12, AtbZIP13, AtbZIP15, AtbZIP17, AtbZIP2, AtbZIP20, AtbZIP21, AtbZIP23, AtbZIP24, AtbZIP25, AtbZIP26, AtbZIP28, AtbZIP29, AtbZIP3, AtbZIP30, AtbZIP33, AtbZIP34, AtbZIP37, AtbZIP40, AtbZIP41, AtbZIP42, AtbZIP43, AtbZIP44, AtbZIP46, AtbZIP47, AtbZIP48, AtbZIP49, AtbZIP5, AtbZIP50, AtbZIP51, AtbZIP52, AtbZIP53, AtbZIP54, AtbZIP56, AtbZIP57, AtbZIP58, AtbZIP59, AtbZIP60, AtbZIP61, AtbZIP67, AtbZIP68, AtbZIP70, AtbZIP7, AtbZIP8, AtbZIP9
Gene loci Id Showing Non-Interaction	20	ABF2, AtbZIP1, AtbZIP6, AtbZIP10, AtbZIP16, AtbZIP18, AtbZIP19, AtbZIP22, AtbZIP31, AtbZIP38, AtbZIP39, AtbZIP4, AtbZIP45, AtbZIP55, AtbZIP62, AtbZIP63, AtbZIP66, AtbZIP69, AtbZIP72, AtbZIP74

Supplementary Data:

To mimic this regulation activity we phosphorylated the AtMAPK3 protein at threonine (196) and tyrosine (198) residue in TEY motif located in the activation loop (T-loop). In an effort to better understand

Table 2. bZIP TF Description

Locus ID	Protein Name
At1g49720	ABF1
At1g45249	ABF2
At5g49450	AtbZIP1
At4g02640	AtbZIP10
At4g34590	AtbZIP11
At2g41070	AtbZIP12
At5g44080	AtbZIP13
At4g35900*	AtbZIP14
At5g42910	AtbZIP15
At2g35530	AtbZIP16
At2g40950	AtbZIP17
At2g40620	AtbZIP18
At4g35040	AtbZIP19
At2g18160	AtbZIP2
At5g06950	AtbZIP20
At1g08320	AtbZIP21
At1g22070	AtbZIP22
At2g16770	AtbZIP23
At3g51960	AtbZIP24
At3g54620	AtbZIP25
At5g06960	AtbZIP26
At2g17770*	AtbZIP27
At3g10800	AtbZIP28
At4g38900	AtbZIP29
At5g15830	AtbZIP3
At2g21230	AtbZIP30
At2g13150	AtbZIP31
At2g12980*	AtbZIP32
At2g12900	AtbZIP33
At2g42380	AtbZIP34
At4g34000	AtbZIP37
At3g19290	AtbZIP38
At2g36270	AtbZIP39
At1g59530	AtbZIP4
At1g03970	AtbZIP40
At4g36730	AtbZIP41
At3g30530	AtbZIP42

Locus ID	Protein Name
At5g38800	AtbZIP43
At1g75390	AtbZIP44
At3g12250	AtbZIP45
At1g68640	AtbZIP46
At5g65210	AtbZIP47
AT2g04038	AtbZIP48
At3g56660	AtbZIP49
At3g49760	AtbZIP5
At1g77920	AtbZIP50
At1g43700	AtbZIP51
At1g06850	AtbZIP52
At3g62420	AtbZIP53
At4g01120	AtbZIP54
At2g46270	AtbZIP55
At5g11260	AtbZIP56
At5g10030	AtbZIP57
At1g13600	AtbZIP58
At2g31370	AtbZIP59
At2g22850	AtbZIP6
At1g42990	AtbZIP60
At3g58120	AtbZIP61
At1g19490	AtbZIP62
At5g28770	AtbZIP63
At3g56850	AtbZIP66
At3g44460	AtbZIP67
At1g32150	AtbZIP68
At1g06070	AtbZIP69
At4g37730	AtbZIP7
At5g60830	AtbZIP70
At2g24340*	AtbZIP71
At5g07160	AtbZIP72
At2g13130*	AtbZIP73
At2g21235	AtbZIP74
At1g68880	AtbZIP8
At5g24800	AtbZIP9
At3g17610*	HYH

*Structures not drawn by MOE software

the protein-protein interactions, we applied the docking approach to predict the potential downstream interacting bZIP proteins in *Arabidopsis* with AtMAPK3P.

Methodology

The sequences for bZIP transcription factors were downloaded from DATF (Database of *Arabidopsis* Transcription factor) (http://datf.cbi.pku.edu.cn/.) and the sequence of *Arabidopsis thaliana* (AtMAPK3) is downloaded from TAIR *Arabidopsis* genome database (http://www.arabidopsis.org/) in FASTA format. Homology modeling of the AtMAPK3 and bZIP transcription factors was done with the help of MOE (Molecular Operating Environment). For constructing the structures a template for homology modeling were searched with PDB search Program of MOE. The final structures were done after constructing and evaluating 3D models. Structure of AtMAPK3 was phosphorylated (AtMAPK3P) with MOE as phosphorylation is essential for its enzymatic activity. Structural refinement through energy minimization were performed using energy minimization tool keeping parameter value constant for all structures. The minimized structures were finally saved as *.pdb files and were analyzed and validated by Ramachandran plot. After structure formation the refined structure of AtMAPK3P was taken as receptor and the structures of bZIP transcription factor family were taken as ligand for the docking studies on the on line server ROSETTA DOCK. After docking, the results were analyzed with the help of MOE.

Results and Discussion

Protein-protein interactions constitute the signaling network that coordinates diverse cellular

Functions and provide a framework for understanding the biological processes. Mitogen activated protein kinase is a conserved link between cell receptor and cell response and is mediated through gene expression which is regulated by transcription factors. There is a very little work in the literature regarding prediction of AtMAPK3 interaction with transcription factors in *Arabidopsis thaliana.* As bZIPs transcription factor reported to

regulate diverse developmental and stress related processes (Llorca *et al.,* 2014). Therefore, the paper focuses on identifying the interacting bZIPs transcription factors with AtMAPK3P of *Arabidopsis thaliana* which is involved in disease signaling process. The docking studies performed, predicted that out of 73 members of bZIP transcription factor, 47 members are showing interaction with AtMAPK3P while the rest are showing non-interaction. Table 1. Docking studies are based on geometric and steric considerations. The more stable the complex structure (less global energy) higher the probability of their interaction. A similar kind of study was carried out to predict the downstream interacting partners of MAPK3 in *Arabidopsis thaliana* through molecular modeling and docking approach (Giri *et al.,* 2013 and Sharma *et al.,* 2013). The need for bioinformatics methods to find out protein partners is being driven by the generation of sequences at a rate far beyond our ability to carry out experimental functional analysis. The results of present study further need to validate by physiochemical features.

Conclusion

The PPI networks can give insights into the mechanisms of diseases and provide a spectrum for the understanding of biological processes. Interaction networks can aid in designing signal transduction pathway and help to find the disease suppressive agents as well as uncover the key genes those are responsible for senescence and defense responses against pathogens.

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