14-3-3 protein; Phylogenetic and protein interaction, An Insilico analysis

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Abstract

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14-3-3 proteins are a family of conserved regulatory molecules that have the ability to bind a multitude of functionally diverse signaling including kinases, phosphatases, and transmembrane proteins, receptors.14-3-3 proteins are conserved among many eukaryotic organisms and interact with other target proteins to trigger the transcriptional regulation of genes that generate specific modifications. 14-3-3 proteins are important components in signal transduction pathways affecting multiple plant functions by mediating protein-protein interactions through post-translational modification via phosphorylation .This protein family is involved in a wide range of cellular functions including the regulation of hormonal induction in response to stress stimuli, mediation of symbiotic relationships between the plant host and symbionts, as well as the mediation of functions between cellular components and enzymes. 14-3-3 proteins are an important component in biological pathways involved in the mitigation of biotic and abiotic stresses. This study has been undertaken to study the phylogenetic relationship of the 14-3-3 protein in different plants like Vitis vinifera (Grape), Solanum tuberosum (Potato), Populus tricocarpa (Poplar) and Glycine max (Soyabean). It was observed that 14-3-3 protein is highly conserved across all the different plant species and that the 14-3-3 protein of Solanum tuberosum is closest to Vitis vinifera. The minimum evolutionary distance was observed for Glycine that shows that the14-3-3 protein sequence of Glycine has least diverged from its ancestor. We also studied the protein-protein interactions of the 14-3-3 protein from Solanum tuberosum. The protein is interacting with protein kinases, ATP binding proteins and elongation factors as shown in literature. These interactions are probably helping the protein play a role in combating stress.

Keywords: Phosphorylation, Kinases, Phosphatases, Stress, Solanum tuberosum, Glycine max, Vitis vinifera, Populus tricocarpa.

Introduction

Protein phosphorylation is the key to the regulation of many proteins. Altered protein activity often requires the interaction of the phosphorylated protein with a class of 'adapters' known as 14-3-3 protein. 14-3-3 proteins were identified as abundant, acidic, soluble brain proteins with a molecular weight of 25±32 KDa [1] .Their enigmatic name according was assigned to fractionation on DEAE cellulose and gel electrophoretic mobility on starch electrophoresis during the purification of a number of bovine brain proteins of unknown function.

14-3-3 proteins are a family of conserved regulatory molecules that have the ability to bind a multitude of functionally diverse signaling proteins, including kinases, phosphatases,

and transmembrane receptors [2]. 14-3-3 proteins are conserved among many eukaryotic organisms and interact with other target proteins to trigger the transcriptional regulation of genes that generate specific modifications. 14-3-3 proteins are important components in signal transduction pathways affecting multiple plant functions by mediating protein-protein interactions through post-translational modification via phosphorylation .This protein family is involved in a wide range of cellular functions including the regulation of hormonal induction in response to stress stimuli, mediation of symbiotic relationships between the plant host and symbionts, as well as the mediation of functions between cellular components and enzymes. 14-3-3 proteins are an important component in pathways involved in biological the mitigation of biotic and abiotic stresses [3].

Plant 14-3-3 isoforms, like their highly conserved homologues in mammals, function by binding to phosphorylated client proteins to modulate their function. Through the regulation of a diverse range of proteins including kinases, transcription factors, structural proteins, ion channels and pathogen defense-related proteins, they are being implicated in an expanding catalogue of physiological functions in plants. 14-3-3 themselves affected, are both transcriptionally and functionally, by the extracellular and intracellular environment of the plant. They can modulate signaling pathways that transduce inputs from the environment and also the downstream elicit physiological proteins that the response. The key emerging roles for plant 14-3-3s includes role in the response to the plant extracellular environment, particularly environmental stress, pathogens and light conditions. It also plays potential key roles in primary metabolism, hormone signaling, growth and cell division.

There is involvement of 14-3-3 protein in signal transduction pathways with emphasis on the regulation of plant metabolism. Originally 14-3-3 proteins were thought to be found only in brain tissue [4]. However, it became clear that 14-3-3 proteins belong to a family of proteins present in all the eukaryotic organisms investigated.

14-3-3 proteins mainly exist as saddleshaped homo- or hetero dimers in which a broad central groove is able to bind to target proteins [5]. Thus, 14-3-3 proteins can function as 'adapters', leading specific target proteins to each other or to a specific location.

Materials and methods

Protein sequence: Protein sequences of 14-3-3 protein of *Solanum tuberosum* having accession no. P93785 was retrieved from Uniprot (<u>www.uniprot.org</u>).

BLASTP: The similar protein sequences to the query were retrieved using BLASTP. (<u>https://blast.ncbi.nlm.nih.gov</u>).The 14-3-3protein sequences of the different plants *Populus tricocarpa* (Poplar) XP 002301071.1, *Vitis vinifera* (Grape) NP 001267852.1, *Glycine*

max (Soyabean) NP 001238389.1 were saved in the notepad and were used to multiple sequence alignment using Clustal Omega.

Clustal Omega: Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo /) was used to generate multiple sequence alignment for all the above protein sequences mentioned. Phlyogenetic tree analyzed generated was to see the evolutionary relationship between the different above mentioned protein sequences.

STRING: The interactions network of the 14-3-3 protein with other protein was found using STRING database (<u>http://string-db.org/</u>). The sequence used as a query was the 14-3-3 protein of *Solanum tuberosum* and the results were analysed.

Results and Discussion

14-3-3 proteins are a family of conserved regulatory molecules that have the ability to bind a multitude of functionally diverse proteins, including signaling kinases, phosphatases, membrane and trans receptors. 14-3-3 proteins are conserved among many eukaryotic organisms and interact with other target proteins to trigger the transcriptional regulation of genes that generate specific modifications (Fig. 1). 14-3-3 proteins are important components in signal transduction pathways affecting multiple plant functions by mediating protein-protein interactions through posttranslational modification via phosphorylation. This protein family is involved in a wide range of cellular functions including the regulation of hormonal induction in response to stress stimuli, mediation of symbiotic relationships between the plant host and symbionts, as well as the mediation of functions between cellular components and enzymes. 14-3-3 proteins are an important component in biological pathways involved in the mitigation of biotic and abiotic stresses.

In this project the phylogenetic study 14-3-3 protein from Solanum tuberosum was done which is playing a role in combating the biotic and abiotic stress in plants with different plant like Populus tricocarpa (Poplar) Vitis vinifera (Grape), Glycine max (Soyabean) whose sequences were retrieved using BLAST P (Fig. 2). The phylogram shows that 14-3-3 protein is highly conserved across all the different plant species and that the 14-3-3 protein of Solanum tuberosum is closest to Vitis vinifera. The minimum evolutionary distance was observed for Glycine max that shows that the14-3-3 protein sequence of Glycine max has least diverged from its ancestor.

Tools > Multi	ple Sequence Alignment > Clustal Omega
Results for	r job clustalo-I20161010-092257-0665-88705867-pg
Alignments	Result Summary Phylogenetic Tree Submission Details
Download Al	lignment File Hide Colors Send to ClustalW2_Phylogeny
CLUSTAL	O(1.2.3) multiple sequence alignment
Solanum	MASPREENVYMAKLAEQAERYEEMVEFMEKVVAALNGEELTVEERNLLSVAYKNVIG
Populus	MAVTPSAREENVYMAKLAEQAERYEEMVEYMEKVSASLENEELTVEERNLLSVAYKNVIG
Glycine	MAAAPSPREENVYMAKLAEQAERYEEMVEFMEKVSAAADNEELTVEERNLLSVAYKNVIG
Vitis	MAAAPSAREENVYMAKLAEQAERYEEMVEFMEKVSAAVDSEELTVEERNLLSVAYKNVIG
Solanum	ARRASWRIISSIEQKEESRGNEDHVASIKKYRSQIENELTSICNGILKLLDSKLIGSAAT
Populus	ARRASWRIISSIEQKEESRGNEDHVSVIRDYRAKIETELSSICDGILKLLDSRLIPTASA
Glycine	ARRASWRIISSIEQKEESRGNEDHVSVIRDYRSKIESELSNICDGILKLLDSRLIPSASS
Vitis	ARRASWRIISSIEQKEESRGNDHHVAMIRDYRSKIESELSSICDGILKLLDSRLIPSASS

Solanum	GDSKVFYLKMKGDYYRYLAEFKTGTERKEAAENTLSAYKSAQDIANGELAPTHPIRLGLA
Populus	GDSKVFYLKMKGDYHRYLAEFKTGAERKEAAESTLTAYKAAQDIANAELAPTHPIRLGLA
Glycine	GDSKVFYLKMKGDYHRYLAEFKTGAERKEAAESTLSAYKAAQDIANAELPPTHPIRLGLA
Vitis	GDSKVFYLKMKGDYHRYLAEFKTGAERKEAAESTLTAYKSAQDIANAELAPTHPIRLGLA

Solanum	LNESVEYYEILNSPDRACNLAKOAEDEAIAELDTLGEESYKDSTLIMOLLRDNLTLWTSD
Populus	LNFSVFYYEILNSPDRACSLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLTLWTSD
Glycine	LNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNLTLWTSD
Vitis	LNFSVFYYEILNSPDRACNLAKQAFDEAIAELDTLGEESYKDSTLIMQLLRDNHTLWTSD

Solanum	MODDGTDEIKEPSKADNE
Populus	MODDGADEIKEAAPKPGDEQQ
Glycine	MQDDGADEIKEAAPKQDDQ
Vitis	MQDDGADEIKEAPKRDDEQQ-

Fig 1: Multiple sequence alignment using Clustal Omega



Fig 2: Phylogram showing the evolutionary relationship of 14-3-3 protein

The interaction of 14-3-3 protein with other proteins was found using STRING database. The 14-3-3 protein sequence of *Solanum tuberosum* was used as a query (Fig. 3). The

results showed the protein interaction network with 10 different proteins that includes kinases, ATP binding proteins, Elongation factor 1-gamma, Minichromosome maintenance protein MCM etc (Fig. 4).



Fig 3: The interactions of 14-3-3 protein from Solanum tuberosum

🖶 30G	14-3-3 protein (254 ав)	oorho usio urenu ressi ment ining ining
Predicted Functional Partners:		Neighl Gene f Coococ f Experii Databu I Partab I Honton Score
PGSC0003DM	1400073771 Phosphatidylinositol 4-kinase (546 aa)	• • 0.927
PGSC0003DM	1400065617 Elongation factor 1-gamma (414 aa)	• 0.894
PGSC0003DM	1400040683 Elongation factor 1-gamma (413 aa)	• 0.894
PGSC0003DM	1400079686 Kinase family protein (288 aa)	 0.885
PGSC0003DM	1400079685 ATP binding protein (873 aa)	 0.885
PGSC0003DM	1400069308 Protein kinase (364 aa)	 0.885
PGSC0003DM	T400069307 ATP binding protein (876 aa)	 0.885
PGSC0003DM	1400068117 Protein kinase family protein (422 aa)	 0.885
PGSC0003DM	1400043636 Hypothetical Ser-Thr protein kinase (654 aa)	 0.885
PGSC0003DM	T400032740 Minichromosome maintenance protein MCM (581 aa)	 0.809

Fig 4: The interacting proteins of 14-3-3 protein using STRING

Conclusions

The study showed the evolutionary relationship of 14-3-3 protein of Solanum tuberosum which plays a role in combating the biotic and abiotic stress in plants with different plant like. Populus tricocarpa (Poplar) Vitis vinifera (Grape), Glycine max (Soyabean). The phylogram shows that that 14-3-3 protein is highly conserved across all the different plant species and that the 14-3-3 protein of Solanum tuberosum is closest to Vitis vinifera. The minimum evolutionary distance was observed for Glycine max that shows that the14-3-3 protein sequence of Glycine max has least diverged from its ancestor. The 14-3-3 protein intereacts with 10 different proteins that includes kinases, ATP binding proteins, Elongation factor 1-Minichromosome gamma, maintenance protein MCM etc.

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