Current Plant Biology 5 (2016) 36–44

Contents lists available at ScienceDirect

Current Plant Biology

journal homepage: www.elsevier.com/locate/cpb

A protein–protein interaction network linking the energy-sensor kinase SnRK1 to multiple signaling pathways in *Arabidopsis thaliana*☆

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ARTICLE INFO

Article history:
Received 30 September 2015
Accepted 28 October 2015

Keywords:
Arabidopsis
SnRK1
Protein–protein interaction
Stress signaling

ABSTRACT

In plants, the sucrose non-fermenting (SNF1)-related protein kinase 1 (SnRK1) represents a central integrator of low energy signaling and acclimation towards many environmental stress responses. Although SnRK1 acts as a convergent point for many different environmental and metabolic signals to control growth and development, it is currently unknown how these many different signals could be translated into a cell-type or stimulus specific response since many components of SnRK1-regulated signaling pathways remain unidentified. Recently, we have demonstrated that proteins containing a domain of unknown function (DUF) 581 interact with the catalytic α subunits of SnRK1 (AKIN10/11) from *Arabidopsis thaliana* and could potentially act as mediators conferring tissue- and stimulus-type specific differences in SnRK1 regulation. To further extend the SnRK1 signaling network in plants, we systematically screened for novel DUF581 interaction partners using the yeast two-hybrid system. A deep and exhaustive screening identified 17 interacting partners for 10 of the DUF581 proteins tested. Many of these novel interaction partners are implicated in cellular processes previously associated with SnRK1 signaling. Furthermore, we mined publicly available interaction data to identify additional DUF581 interacting proteins. A protein–protein interaction network resulting from our studies suggests connections between SnRK1 signaling and other central signaling pathways involved in growth regulation and environmental responses. These include TOR and MAP-kinase signaling as well as hormonal pathways. The resulting protein–protein interaction network promises to be effective in generating hypotheses to study the precise mechanisms SnRK1 signaling on a functional level.

1. Introduction

Robustness is an inherent and essential property of all biological systems that enables to maintain phenotypic stability in the face of diverse perturbations arising from e.g., changing environmental conditions or genetic variation [1]. Due to their sessile lifestyle, plants have evolved a particular complex cellular signaling network that enables them to respond to a wide range of environmental signals in order to accommodate stress conditions leading to phenotypic robustness. Protein kinases are central to these signaling networks because through the phosphorylation of various substrates they relay extra and intracellular signals into a cellular response which could be e.g., transcriptional reprogramming, modulation of enzyme activity through phosphorylation and eventually mediating adjustment of the metabolic network to the novel conditions. In plants, the sucrose non-fermenting related kinase 1 (SnRK1) has emerged as a key regulator of cellular metabolism through activation of signaling cascades that are protective against various stresses. SnRK1 becomes activated by energy deprivation and abscisic (ABA) signals, and is inactivated by sugars that restore energy balance [2,3]. In addition, SnRK1 coordinates stress induced responses, including antiviral defense, and fundamental developmental processes, from germination and sprouting to reproduction and senescence [3]. Some of the known SnRK1 substrates are key metabolic enzymes such as sucrose phosphate synthase, nitrate reductase, and 3-hydroxy-3-methyl-glutaryl-CoA reductase [4]. Furthermore, activation of SnRK1 triggers extensive reprogramming of transcription, affecting over a thousand genes in *Arabidopsis,* that contributes to restoring homeostasis, promotes cell survival and long-term stress adaptation [2]. In general, SnRK1-mediated transcriptional reprogramming results in the

☆ This article is part of a special issue entitled "Protein networks – a driving force for discovery in plant science".

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http://dx.doi.org/10.1016/j.cpb.2015.10.004

down-regulation of energy consuming processes and the induction of catabolic pathways to provide alternative energy sources [5]. Although the broad effect on transcription and the specificity that is required to respond to a particular stress is likely to require modulation of a range of different downstream target proteins, only a few direct SnRK1 substrates have yet been identified [2,6–9]. In addition to the direct interactions between protein kinases and their substrates, sometimes the two proteins interact through the intermediacy of adaptors or scaffolds, which act as organizing platforms that recruit both the kinase and the substrate to the same complex [10]. We have previously demonstrated that proteins containing a domain of unknown function (DUF) 581 interact with the catalytic α subunits of SnRK1 (AKIN10/11) from *Arabidopsis thaliana* and could potentially act as mediators conferring tissue- and stimulus-type specific differences in SnRK1 regulation by recruitment of potential substrate proteins and the kinase into the same complex [11]. This hypothesis was based on the observation that expression of DUF581—containing proteins is highly responsive to hormones and various stress conditions and that one DUF581 protein tested had a common interaction partner with SnRK1 in the yeast two-hybrid system [11]. In the present study, we used the yeast two-hybrid system to assemble a comprehensive protein–protein interaction network comprising the two catalytic α subunits of *Arabidopsis* SnRK1, AKIN10 and AKIN11, and members of the DUF581-protein family with additional interaction partners that potentially could play a role in SnRK1 signaling in plants. The network architecture provides clues for connections between SnRK1 signaling and other central signaling pathways involved in growth regulation and environmental responses. These include TOR and MAP-kinase signaling as well as hormonal pathways and transcription factors. The resulting protein–protein interaction network promises to be effective in generating hypotheses to study the precise mechanisms of SnRK1 signaling in plants on a functional level.

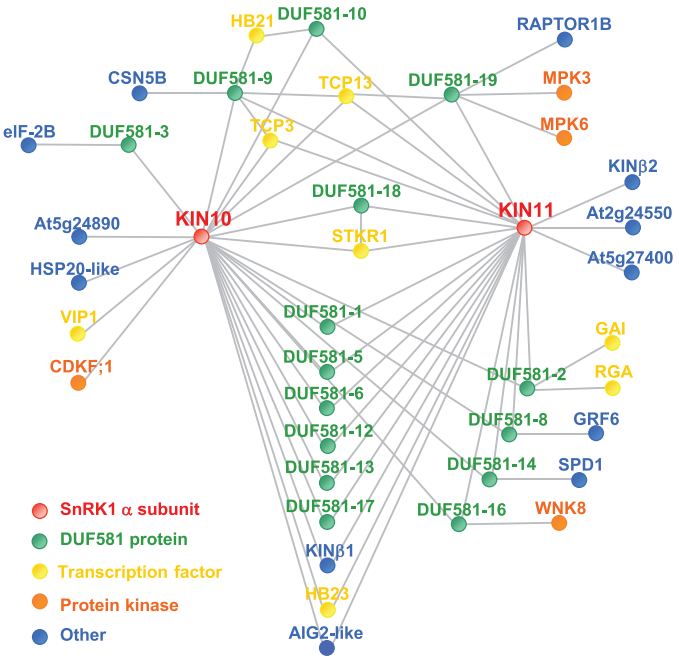

Fig. 1. *Arabidopsis* yeast two-hybrid protein–protein interaction network surrounding SnRK1 a subunits (KIN10/11) and DUF581-domain containing proteins. Nodes in the network represent proteins and are colored according to their functional class (see color key). The protein–protein interactions are indicated by lines ("edges"). The network was produced using Pajek software (http://vlado.fmf.uni-lj.si/pub/networks/pajek/). TAIR annotations of network nodes are listed in Supplementary Table S1.

2. Results and discussion

In order to construct a comprehensive SnRK1/DUF581-protein interaction network we conducted exhaustive yeast two-hybrid (Y2H) screenings of two different cDNA libraries from *Arabidopsis* using 16 of the 18 *Arabidopsis* DUF581-proteins as baits. Only DUF581-1 and DUF581-11 were excluded from the screen, the first because of auto-activation of the yeast reporter genes when fused to the GAL4 DNA binding domain and the latter because we were not able to amplify a corresponding cDNA fragment likely owing to its low expression level. To identify additional direct interaction partners of SnRK1 the two α subunits AKIN10 and AKIN11 were also used as baits. All potential DUF581-protein and AKIN10/11 interaction partners identified during the library screenings were retested in direct Y2H interaction assays including appropriate negative and positive controls. Furthermore, we mined publicly available protein–protein interaction data derived from high-throughput Y2H screens [12] to identify additional potential DUF581–protein interactors with a known function. These were then used in direct Y2H interaction assays to confirm their binding to a given DUF581-protein. Selected candidate interaction partners of individual DUF581-proteins identified during the library screens were also tested in direct assays against other DUF581-protein isoforms as well as against the SnRK1 subunits AKIN10 and AKIN11, respectively. Collectively, the interaction data reveal a highly interconnected network (41 nodes with 65 edges), with some proteins identified as interaction partners of two or more of the bait proteins (Fig. 1). For 6 of the DUF581-proteins (DUF581-1, -5, -6, -12, -13, -17) no novel interaction partners apart from SnRK1 could be identified. This could either be due to the absence of a potential interactor from the libraries used for the screening or because binding partners are not functional in the yeast assay (e.g., not localizing to the nucleus, integral membrane proteins). Three out of the 18 DUF581-proteins from *Arabidopsis* (DUF581-3, -8, -15) did not bind to AKIN10/11 when the SnRK1 subunits were used as bait proteins in direct yeast assays [11]. The screening identified AKIN10 as an interaction partner for BD-DUF581-3 and BD-DUF581-8 indicating that orientation of the BD/AD-fusion proteins in yeast can affect the outcome of the Y2H assay. For DUF581-15 no binding partners could be identified.

A comparison of the gene ontology annotations of the newly identified AKIN/DUF581-interacting proteins to the *Arabidopsis* genome indicated enrichment in these categories: nucleus, other cytoplasmic components, protein binding, kinase activity, transferase activity, and transcription factor activity (Supplementary Table S1). We have previously shown that several DUF581-proteins tested displayed a nucleo-cytoplasmic localization when transiently expressed as GFP fusion proteins in *Nicotiana benthamiana* and co-expression with SnRK1 shifted the fluorescence signal into sub-nuclear speckles [11]. Thus, the enrichment for interaction partners with a suspected nuclear localization can serve as a criterion for high-quality and biologically significant interactome data sets [13].

According to our hypothesis the DUF581 serves as a generic SnRK1 interaction domain while the non-conserved part of the DUF581-containing proteins mediates binding of additional partners specific for individual DUF581-protein isoforms. Thus, while all DUF581 proteins should interact with SnRK1, binding of additional proteins should display a certain degree of specificity. To test this, we analyzed the specificity of interaction between certain DUF581-proteins and some of the newly identified interactors. The experiment revealed that for instance TCP3 (TEOSINTE BRANCHED1, CYCLOIDEA, PROLIFERATING CELL FACTORS), which had been identified as an interaction partner of DUF581-9, did not