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14-3-3 Proteins fine-tune plant nutrient metabolism

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ABSTRACT

14-3-3 Proteins regulate many cellular processes by binding to phosphorylated proteins. Previous findings suggest a connection between three 14-3-3 isoforms and plant nutrient signaling. To better understand how these 14-3-3s regulate metabolism in response to changes in plant nutrient status, putative new targets involved in nitrogen (N) and sulfur (S) metabolisms have been identified. The interactions between these 14-3-3s and multiple proteins involved in N and S metabolism and altered activity of the target proteins were confirmed in planta. Using a combination of methods, this work elucidates how 14-3-3s function as modulators of plant N and S metabolic pathways.

1. Introduction

14-3-3 Proteins are a large family of proteins found in all eukaryotes [1,2]. Functionally, these proteins act as phosphoserine binding proteins and modulate cellular processes such as signal transduction, cell cycle, metabolism, membrane trafficking, stress responses, and apoptosis in mammalian cells [1]. Relatively few proteins that interact with 14-3-3s have been characterized in plants to determine the effects on their biochemical function [3]. For example, 14-3-3s are known to regulate nitrate reductase, proteins related to plant growth and brassinolide signaling [2], and the proton ATPase [4]. More recently, the KAT1 potassium (K) channel [5] and a bZIP transcriptional activator of gibberellic acid [6] have been identified as being regulated by interactions with 14-3-3s. There are limited studies that show how 14-3-3s alter target protein activity and localization in plants and only a few of these interactions have been confirmed in planta [6,7]. The structural and functional effects of 14-3-3 binding to target proteins remain poorly characterized in vivo.

Recent reports indicate that 14-3-3s play important roles in regulating metabolic homeostasis under different nutrient conditions in plants. In Arabidopsis, the SnRK2.8 kinase is down-regulated by K, nitrogen (N), and phosphorus (P) deprivation and is linked to the regulation of growth and metabolism through phosphorylation of 14-3-3s and other proteins [8]. Previous work demonstrated that the phosphorylation of three 14-3-3 isoforms by SnRK2.8 occurs following nutrient deprivation [8]. In this paper, we present data supporting the interaction of these 14-3-3s with many proteins involved in K, N, and sulfur (S) metabolism and show that 14-3-3 protein interaction alters the activities of target proteins in planta. Overall, these results suggest a role for 14-3-3s in regulating multiple steps in N and S metabolism.

2. Materials and methods

2.1. Plant material

Arabidopsis plants were grown hydroponically under either a full nutrient or nutrient deficient condition and root growth assays performed, as described [9]. Knockout (KO) plants were isolated from ABRC Salk collection (Salk_142285c – 14-3-3χ; Salk_001375 – 14-3-3κ). RNAi lines for 14-3-3ψ were created using pART27 [10]. For overexpression (OX) plants, the N-terminal FLAG-tag pSHF vector was used [11]. Detailed Q-PCR procedures are described in the Supplementary methods.

Abbreviations: AGT, alanine-glyoxylate aminotransferase; CRC, cysteine regulatory complex; GS, glutamine synthetase; IP, immunoprecipitation; KO, knockout; N, nitrogen; OASTL, O-acetylserine(thiol)lyase; OX, overexpression; PEPC, phosphoenolpyruvate carboxylase; P, phosphorus; K, potassium; SAT, serine acetyltransferase; S, sulfur

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2.2. Identification of 14-3-3 interacting proteins

Plant total crude protein extract was incubated with N-terminal His-tagged 14-3-3 coated Daynabeads M270 (Invitrogen) at 4 °C and eluted using a 14-3-3-binding-phosphopeptide (ARAApSAPA) [12]. Glutathione-S-transferase-Dynabeads was analyzed as a negative control to ensure specificity of the 14-3-3 proteins bound to the Dynabeads. Half of the eluate was trypsin digested in solution and the other half was trypsin digested after running on 1D gels. Resulting peptides were sequenced using LC-MS/MS (see Supplementary methods).

2.3. Proteins interactions and enzyme activity

To confirm protein–protein interaction, yeast two-hybrid analysis (Invitrogen) and co-immunoprecipitation (IP) (FLAG IP kit, Sigma) were performed according to the manufacturer's instructions. For co-IP, the vector encoding HA-tagged target protein [13] was infiltrated into leaf tissue for transient expression in the FLAG::14-3-3 OX plants [14]. Anti-FLAG antibody and anti-HA antibody were used for detecting 14-3-3 and target proteins, respectively.

All enzyme assays were performed on crude protein extracts. Alanine-glyoxylate aminotransferase (AGT) was assayed by monitoring the change in A_{340nm} using a coupled-assay system [15]. Glutamine synthetase (GS) was measured by monitoring the absorbance change of NADH at A_{340nm} [16]. Phosphoenolpyruvate carboxylase (PEPC) was assayed with plant crude extracts in 110 mM Tris sulfate buffer (pH 8.5) and phosphoenolpyruvate as a substrate using a coupled-assay system (A_{340nm}) [17]. O-Acetylserine(thiol)lyase (OASTL) was assayed by colorimetrically measuring cysteine formation [18]. Serine acetyltransferase (SAT) was measured by monitoring the transacetylation reaction (A_{232nm}) [18].

3. Results and discussion

3.1. Roles of 14-3-3s in nutrient deprivation

In *Arabidopsis*, 14-3-3χ, κ, and ψ are targets of the SnRK2.8 kinase that is regulated by nutrient deprivation [8]. This previous work suggested a link between these 14-3-3s and nutrient metabolism in planta. To determine whether nutrient deprivation alters the expression of these 14-3-3s, q-PCR was performed. The expression of 14-3-3χ did not change significantly under nutrient deprivation (Fig. 1A and B); however, expression of 14-3-3κ increased after K-deprivation in leaves (Fig. 1C), but not in roots (Fig. 1D). Although transcript levels of 14-3-3ψ increased after N and P deprivation in leaves (Fig. 1E), they decreased in roots following K and N deprivation (Fig. 1G).

Because 14-3-3κ and 14-3-3ψ were differentially expressed in response to nutrient deprivation, we examined the effect of these isoforms on plant growth. Plants overexpressing 14-3-3κ had long-

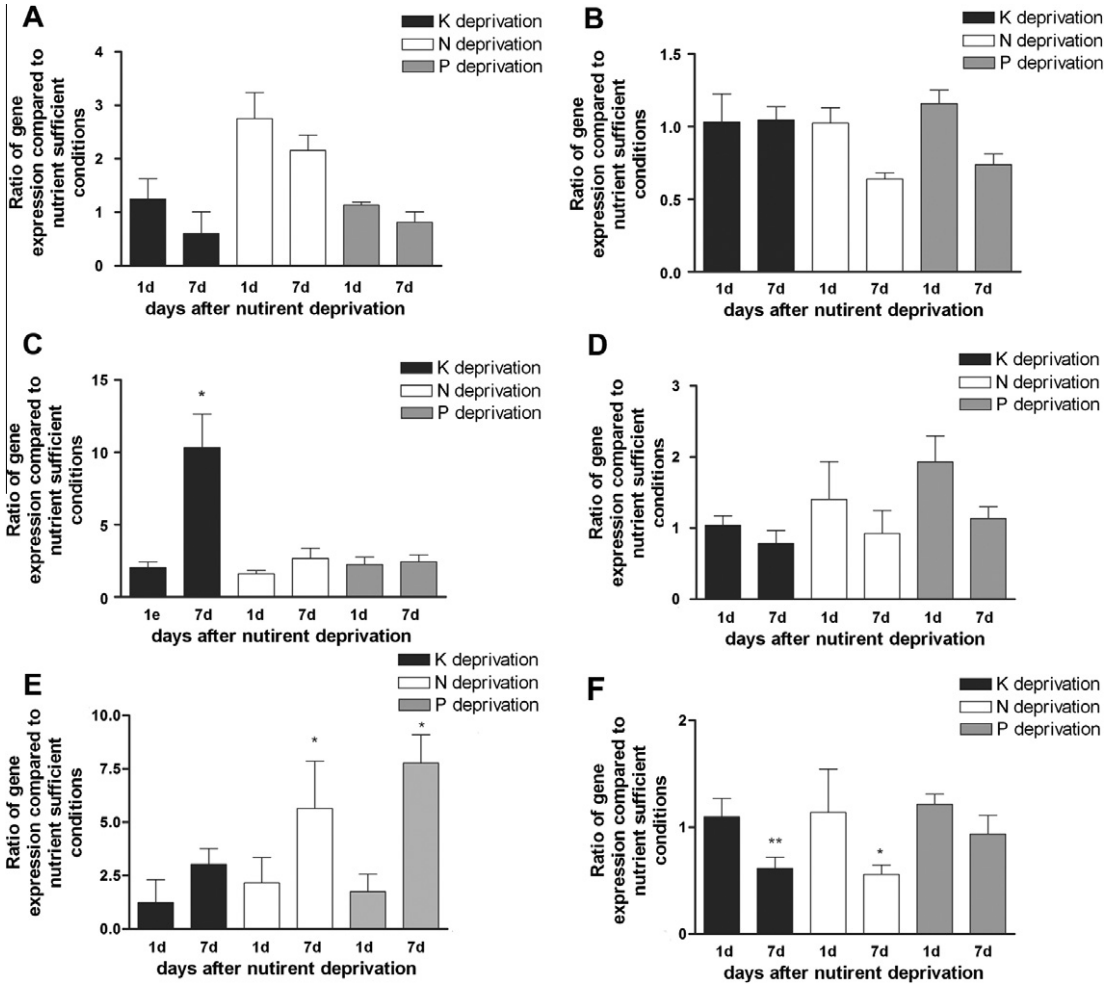

Fig. 1. q-PCR analysis of 14-3-3s under nutrient deprived conditions. Hydroponically-grown *Arabidopsis* were harvested 1 and 7 d after transferring to solutions deficient in K, N, or P. The qPCR data for leaves (A, C, and E), roots (B, D, and F) of 14-3-3χ (A, B), 14-3-3κ (C, D) and 14-3-3ψ (E, F) are shown. The asterisk indicates transcript levels that are significantly different from the control based on Student's *t*-test ($n = 3$, $P < 0.05$). Error bars indicate standard error.