Functions of IQD proteins as hubs in cellular calcium and auxin signaling: A toolbox for shape formation and tissue-specification in plants?

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**ABSTRACT**

Calcium (Ca$^{2+}$) ions play pivotal roles as second messengers in intracellular signal transduction, and coordinate many biological processes. Changes in intracellular Ca$^{2+}$ levels are perceived by Ca$^{2+}$ sensors such as calmodulin (CaM) and CaM-like (CML) proteins, which transduce Ca$^{2+}$ signals into cellular responses by regulation of diverse target proteins. Insights into molecular functions of CaM targets are thus essential to understand the molecular and cellular basis of Ca$^{2+}$ signaling. During the last decade, IQ67-domain (IQD) proteins emerged as the largest class of CaM targets in plants with mostly unknown functions. In the March issue of Plant Physiology, we presented the first comprehensive characterization of the 33-membered IQD family in Arabidopsis thaliana. We showed, by analysis of the subcellular localization of translational green fluorescent protein (GFP) fusion proteins, that most IQD members label microtubules (MTs), and additionally often localize to the cell nucleus or to membranes, where they recruit CaM Ca$^{2+}$ sensors. Important functions at MTs are supported by altered MT organization and plant growth in IQD gain-of-function lines. Because IQD proteins share structural hallmarks of scaffold proteins, we propose roles of IQDs in the assembly of macromolecular complexes to orchestrate Ca$^{2+}$ CaM signaling from membranes to the nucleus. Interestingly, expression of several IQDs is regulated by auxin, which suggests functions of IQDs as hubs in cellular auxin and calcium signaling to regulate plant growth and development.

The founding member of the IQD family, IQD1 from Arabidopsis thaliana, was found in a screen of T-DNA activation tagged lines for novel regulators of glucosinolates, a class of defense-related metabolites, and overexpression of AtIQD1 in transgenic Arabidopsis plants was reported to stimulate glucosinolate accumulation and plant defense.1 Subsequently, IQD families have been systematically annotated in the genomes of Arabidopsis and rice (Oryza sativa),2 and more recently in several additional angiosperms, including tomato (Solanum lycopersicum)3 and maize (Zea mays),4 where they are encoded by multigene families of 23 to 67 members. The protein family is defined by the central conserved IQ67-domain, which spans 67 amino acids and contains up to three IQ motifs required for binding to CaM Ca$^{2+}$ sensors.4,5 On both sites, the IQ67-domain is flanked by large regions of predicted intrinsic disorder, interspersed only by short IQD-specific motifs of unknown functions.2,6 Phylogenetic analyses of IQD families from angiosperms indicate the presence of four subgroups that mostly differ in the distribution of IQD-specific motifs. Despite the large family sizes and their proposed implications in cellular Ca$^{2+}$ signaling however, functions of IQD proteins are mostly elusive. We thus initially focused our analyses on AtIQD1, and, in a previous study, demonstrated localization of GFP-AtIQD1 to MTs and the nucle(ol)us.5 Intriguingly, we observed AtIQD1-dependent recruitment of RFP-AtCaM2 to the MT cytoskeleton, which raised our particular interest as it suggested roles for AtIQD1 in Ca$^{2+}$-CaM-dependent regulation of MT organization and dynamics, an active area of current research.7 Functions of IQDs at the MT cytoskeleton and in growth regulation are supported indirectly by genetic studies in tomato, which identified SIIQD12/SUN as a key regulator of fruit shape.8

**IQD proteins control MT organization, cell shape and plant growth**

To study systematically IQD functions, we initiated a comprehensive characterization of the 33-membered Arabidopsis IQD family using reverse genetics approaches. Analysis of the subcellular localization of translational GFP fusion proteins in transient expression assays in tobacco (Nicotiana benthamiana) leaves revealed that most IQDs aligned uniformly along the MT lattice, in patterns reminiscent of proteins with MT bundling functions.9 For a subset of IQDs, we validated that subcellular localization is independent of the position of the GFP tag.

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**CONFIDENTIALITY STATEMENT**

The authors confirm that no information is presented that is confidential or which could cause a conflict of interest.

**AUTHOR DISCLOSURE**

The authors confirm that they have no conflicts of interest related to the content of this manuscript.

**ETHICAL APPROVAL**

The authors confirm that all procedures involving animals were performed in accordance with institutional guidelines and regulations, and that the welfare of the animals was a primary concern.

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**DATA AVAILABILITY**

The authors confirm that the data generated during this study are available upon request from the corresponding author.

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(N- and C-terminal), of expression levels (cauliflower mosaic virus (CaMV) 35S promoter and endogenous promoter), or of the experimental system (tobacco and Arabidopsis). In agreement with potential MT organizing functions, we observed differential effects of IQD overexpression on MT patterns in tobacco and in transgenic Arabidopsis plants, and phenotypes of Arabidopsis IQD gain-of-function lines resembled mutants defective in MT function, such as longifolia\textsuperscript{10} or tortifolia\textsuperscript{11} mutants.

Functions of IQD proteins, however, are not limited to MTs, but additionally include membranes and the cell nucleus, as evidenced by analysis of GFP-IQD fluorescence (Fig. 1A). Intriguingly, we also observed altered cell shape and plant growth in transgenic Arabidopsis plants overexpressing the exclusively plasma membrane (PM) localized IQD member, IQD25. Possible explanations are functions of IQD25 in PM tethering of MTs, in regulation of, e.g., cellulose deposition, in the establishment of cellular polarity or symmetry breaking, or in the regulation of, e.g., endocytotic cycling of PM-localized receptors, as has been reported for the PM subdomain localized scaffold protein AtFlotilin1 and the brassinosteroid receptor BRASSINOSTEROID-INSENSITIVE 1 (BRI1).\textsuperscript{12}

To gain insights into spatio-temporal expression patterns of IQD genes, we generated a cellular expression map for a subset of Arabidopsis IQD members in transgenic promoter (Pro)IQD:GFP-GUS reporter lines. Promoter activity was largely restricted to meristematic and actively elongating tissues, e.g., in the root and shoot meristem, or in the stomata lineage.\textsuperscript{9} Analysis of transgenic ProIQD:n3GFP (nuclear 3x GFP) lines during embryo development in a recent study from Möller, et al.\textsuperscript{13} further indicates high tissue-specificity of IQD gene expression in actively dividing tissues. Together, we propose that IQDs control meristem functions by integrating Ca\textsuperscript{2+} signaling at the PM and MTs to shape tissues and organs.
**IQD-CaM signaling pathways**

Interestingly, our findings from coexpression analyses and bimolecular fluorescence complementation assays indicated IQD-specific recruitment of CaM Ca$^{2+}$ sensors to the distinct sites of subcellular IQD localization, including MTs, cell nuclei and PM subdomains. PM attachment of IQDs, either directly or possibly stabilized via S-acylation, could serve to anchor IQD-CaM complexes at sites where Ca$^{2+}$ enters cells via PM-localized ion channels (Fig. 1B). Upon activation of the channels, steep concentration gradients in free Ca$^{2+}$ are generated around entry sites, which can give rise to activation of Ca$^{2+}$ sensors. Notably, the two other main families of Ca$^{2+}$ sensors in plants, i.e. Ca$^{2+}$-dependent protein kinases (CPKs) and calcineurin B-like (CBL) proteins are directly localized to the PM through lipid modification by myristoylation and S-acylation. With few exceptions, CaM/CMLs lack conserved lipidation motifs, and transient expression of RFP-CaM2 fusions under the CaMV35S promoter results in cytosolic accumulation. Thus, we speculate that IQD-dependent preassembly of CaM signaling modules at sites of Ca$^{2+}$ influx enables CaM/CML sensors to compete with CPK and CBL-CBL interacting protein kinase (CIPK) signaling modules for incoming Ca$^{2+}$ signals.

Functions of IQD proteins as platforms in cellular signaling are supported by the large content of regions with predicted intrinsic disorder, which is a hallmark of scaffold proteins that assemble macromolecular complexes involved e.g., in the same signaling cascade. Candidate constituents of IQD signaling complexes are members of the kinesin light chain-related (KLCR)/cellulose-microtubule uncoupling (CMU) protein family, which interact with IQD proteins in yeast and in plants. Work by Liu, et al.19 revealed localization of GFP-CMUn1 to MTs and the PM. Loss-of-function mutants of cmu1cmu2 display defects in MT dynamics and MT-related phenotypes, such as increased epidermal cell file rotation. Disruption of cellulose synthase attachment to MTs by introduction of the pom2/cellulose synthase interacting1 (csil) mutation rescues cmu1cmu2 phenotypes, and points to functions of KLCR/CMUs in stabilization of MTs against the pushing force of cellulose synthase complexes (CSCs) at the PM-MT nexus. Interplay of IQD function and auxin signaling

Meristem identity and activity is controlled tightly during development, and the plant hormone auxin plays an instrumental role in the regulation of cell division, cell expansion, and cell identity establishment. Local auxin maxima activate transcriptional reprogramming by release of Auxin/Indole-3-acetic acid (Aux/IAA) dependent repression of auxin response factor (ARF) transcription factors, which regulate the expression of numerous target genes. Interestingly, a recent study aimed at elucidating functions of ARF5/Monopteros (MP) during embryo development identified several IQD genes as potential ARF5/MP targets. Auxin-dependent regulation of IQD gene expression may serve to fine-tune and to restrict locally IQD protein abundance in specific cells or tissues to orchestrate shape formation and cellular growth via IQD-mediated control of the MT cytoskeleton (Fig. 1A). In addition, auxin treatment induces an increase in cytosolic Ca$^{2+}$ concentrations, and thus may regulate IQD function posttranslationally by activation of Ca$^{2+}$-CaM signaling. Ca$^{2+}$-CaM, on the other hand, affects auxin output by direct interaction with components of the auxin transport and signaling machinery, such as PINOID (PID) or small auxin upregulated RNA 19 (SAUR19). However, the molecular mechanism of auxin and Ca$^{2+}$ interplay remain largely obscure. The emerging evidence for important functions of IQD proteins in cellular Ca$^{2+}$ signaling during development, and the indications for auxin-dependent regulation of IQD output make them promising candidates for functions as a hub in cellular auxin and Ca$^{2+}$ signaling. Elucidating their precise functions and molecular mechanisms of action will thus be an interesting aspect of future research.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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