Minireview

IQGAP1 as signal integrator: Ca^{2+}, calmodulin, Cdc42 and the cytoskeleton

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Abstract A family of proteins known as IQGAPs have been identified in yeast, amebas and mammals. IQGAPs are multidomain molecules that contain several protein-interacting motifs which mediate binding to target proteins. Mammalian IQGAP1 is a component of signaling networks that are integral to maintaining cytoskeletal architecture and cell–cell adhesion. Published data suggest that IQGAP1 is a scaffolding protein that modulates cross-talk among diverse pathways in complex regulatory circuits. These pathways include modulating the actin cytoskeleton, mediating signaling by Rho family GTPases and calmodulin, regulating E-cadherin and β-catenin function and organizing microtubules.

Key words: Cytoskeleton; Rho GTPase; Calcium; Calmodulin; Cytokinesis

1. The IQGAP family of proteins

IQGAP1 was first identified almost a decade ago as a cDNA clone predicted to encode a 1657-amino acid protein that contains a region with extensive sequence similarity to the catalytic domain of Ras GTPase activating proteins (RasGAPs) (Fig. 1) [1]. The Ras superfamily of GTPases act as molecular switches by cycling between inactive GDP- and active GTP-bound states [2,3]. This cycling is controlled by guanine nucleotide exchange factors, which activate Ras family members, and RasGAPs, which increase the intrinsic GTPase activity of Ras proteins, thereby inactivating them. The N-terminal half of IQGAP1 also contains four IQ motifs (Fig. 1), a sequence which mediates interactions with calmodulin and calmodulin-related proteins [4]. Therefore, IQGAP1 was hypothesized to represent a novel RasGAP-like protein that might link Ras signaling to some calmodulin-mediated processes [1]. Subsequently, IQGAP2, which is 62% identical to IQGAP1 [5], was identified and the IQGAP family of proteins was established.

The IQGAP family comprises a small group of eukaryotic proteins, with representatives in species as divergent as yeast and mammals. The *Saccharomyces cerevisiae* Iqg1p/Cyk1p [6,7] and *Schizosaccharomyces pombe* Rng2p [8] proteins have a domain structure similar to that of the entire IQGAP1 protein. Not all of the domains in mammalian IQGAP1 are conserved across species. For example, the GAPA and DGAP1 proteins of the soil ameba *Dictyostelium discoideum* are shorter and have considerable similarity to only the C-terminal half of mammalian IQGAPs [9,10]. Similarly, Sar1 in *S. pombe* shares domains with the C-terminal half of IQGAP1. The IQGAPs participate in regulating the actin cytoskeleton. *Saccharomyces* Iqg1p/Cyk1p and *Dictyostelium* GAPA regulate actin-dependent cellular morphogenesis; Iqg1p/Cyk1p by inducing actin-ring assembly and cytokinesis [6,7], and GAPA by severing the midbody connecting the daughter cells during cytokinesis [9]. Similarly, both endogenous and ectopically expressed human IQGAP1 co-localized with actin in lamellipodia and ruffling cell membranes [11]. The distantly related members of the eukaryotic IQGAP family (reviewed in [12]) therefore provide clues to the primordial function of the mammalian IQGAPs, and imply a fundamental role for IQGAP1 in modulating cytoskeletal architecture.

Notwithstanding these observations, mice lacking IQGAP1 were viable and developed gastric hyperplasia [13]. Several factors may contribute to the relatively mild phenotype, particularly functional redundancy with IQGAP2 and a third potential human IQGAP paralog, IQGAP3 [14]. This situation has been demonstrated in *Dictyostelium*. DGAP1 and GAPA, which exhibit 50% sequence identity, have overlapping functions. Elimination of DGAP1 does not impair cytokinesis, while $DGAP1^{-}/GAPA^{-}$ double-mutant cells have a severe defect in cytokinesis [15]. Because relatively little is known about IQGAP2 and virtually nothing about IQGAP3, the remainder of this review will focus on IQGAP1.

2. IQGAP1 and Cdc42 function

The Ras superfamily includes the Rho subfamily of GTPases. Over 20 members of the Rho subfamily have been identified, the best-characterized of which are RhoA, Rac1 and Cdc42 [2]. Both IQGAP1 [11,16–18] and IQGAP2 [5,19] specifically bind to Cdc42 and Rac1, but not to RhoA. IQGAP1 preferentially binds to active (GTP-bound) Cdc42 [11,17] and Rac1 [16]. Despite their similarity to part of the sequence of RasGAPs, neither IQGAP1 nor IQGAP2 interacts with Ras. Contrary to the prediction that it would function as a GAP, we and others have shown that IQGAP1 inhibits the intrinsic GTPase activity of Cdc42 in vitro, stabi-

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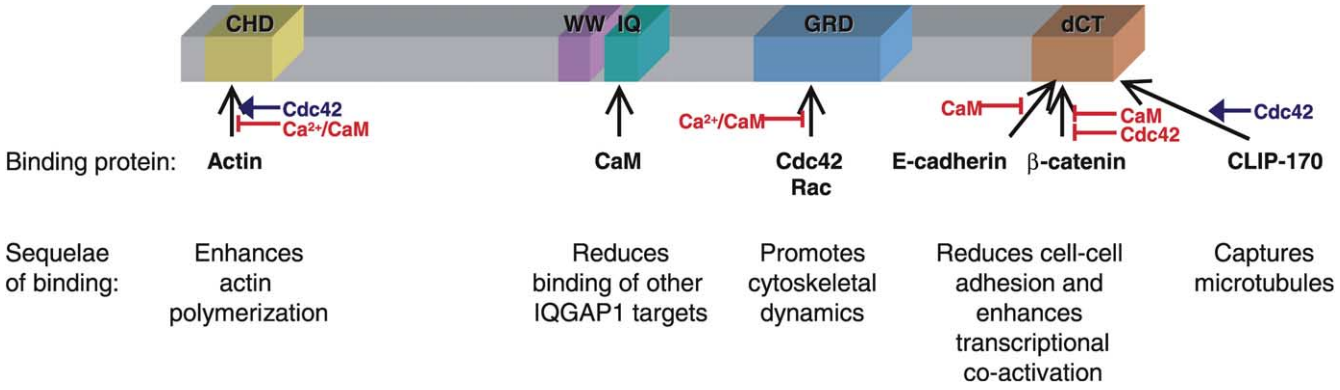

Fig. 1. Schematic diagram of IQGAP1 and its interactions with target proteins. A schematic diagram of IQGAP1 is indicated, with identified IQGAP1-binding proteins (bold) and their binding domains. Inhibitors (red) and enhancers (blue) of binding are depicted. Functional sequelae of the interaction of IQGAP1 with each of its targets are shown, highlighting the complex relationship among IQGAP1, calmodulin and the cytoskeleton (see text for details). CHD, calponin homology domain; WW, WW domain; IQ, four tandem calmodulin-binding motifs; GRD, RasGAP-related domain; dCT, distal C-terminus; CaM, calmodulin.

lizing Cdc42 in its active GTP-bound form [5,11,18,20,21]. Moreover, we demonstrated that IQGAP1 substantially increases the pool of GTP-bound Cdc42 in vivo [20,22]. Consistent with published evidence that active Cdc42 induces the formation of peripheral microspikes and filopodia [2,23], overexpressed IQGAP1 modulates cell morphology by stimulating filopodia formation [20]. In addition, IQGAP1 appears to be necessary for Cdc42 to localize to the plasma membrane [20]. Because translocation to the plasma membrane is believed to be essential for normal Cdc42 activity [24–26], our findings imply that IQGAP1 is required for Cdc42 function. We also documented that microinjecting IQGAP1 into early *Xenopus* embryos generates superficial ectoderm lesions in a Cdc42-dependent manner [22]. Collectively, these data strongly suggest that IQGAP1 provides a fundamental link between Cdc42 and the cytoskeleton.

3. IQGAP1 is a scaffolding protein

The IQGAP family contains several protein-interacting domains which mediate binding to a number of diverse target molecules (Fig. 1). Targets of IQGAP1 include calmodulin [11,17,18,27], actin [18,27,28], Cdc42 [11,16–18], Rac1 [11], E-cadherin [29,30], β-catenin [29,31] and CLIP-170 [32]. The diversity of IQGAP1 targets suggests that IQGAP1 functions as a scaffolding protein that can assemble multiprotein complexes. This hypothesis is supported by evidence from different groups who have documented that IQGAP1 can bind several proteins simultaneously. For example, a ternary complex of Cdc42, IQGAP1 and actin was immunoprecipitated using antibodies against Cdc42 [28]. Similarly, complexes of IQGAP1 containing Cdc42 and calmodulin [18], Rac1 and calmodulin [33] or Rac1/Cdc42 and CLIP-170 [32] have been identified. Analogous observations have been made in yeast. In contrast to mammalian IQGAPs, Iqg1p/Cyk1p lacks a WW domain and GAP motif [6]. Nevertheless, *S. cerevisiae* Iqg1p/Cyk1p forms a targeting patch that includes Bud4p, Cdc12p and Sec3p [34]. These findings reveal that Iqg1p/Cyk1p serves to interface proteins involved in axial budding (namely, Bud4p and Cdc12p) with proteins involved in exocytosis/secretion (Sec3p) and cytokinesis. Similarly, DGAP1 or GAPA forms with Rac1A, cortexillin I and cortexillin II a quaternary complex that is required for cytokinesis [15]. Thus, IQGAP1 functions as a scaffold that can co-localize several proteins to specific subcellular domains.

The ability of IQGAP1 to bind numerous molecules also raised the question of whether binding to one protein alters the ability of IQGAP1 to interact with (an)other protein(s). This concept was first addressed by Joyal et al. [17] who demonstrated that Ca^{2+}/calmodulin prevented IQGAP1 from binding Cdc42. Subsequent analysis revealed that calmodulin attenuates the interactions between IQGAP1 and all of its targets that have been investigated [18,27,30,31] (Fig. 1) (discussed in detail below). Similarly, Cdc42 regulates the association of IQGAP1 with other molecules. Binding of active Cdc42 prevents IQGAP1 from interacting with β-catenin, thereby attenuating the inhibitory effect of IQGAP1 on E-cadherin-mediated cell–cell attachment [29]. Conversely, Cdc42 has been reported to augment F-actin cross-linking produced by IQGAP1 in vitro [35] and to enhance the association of IQGAP1 with CLIP-170 [32] (Fig. 1).

4. Calmodulin regulates IQGAP1 function

4.1. The cytoskeleton

Ca^{2+} is a ubiquitous intracellular messenger responsible for controlling numerous cellular processes [36]. The primary mediator of Ca^{2+} signaling is calmodulin, which alters the function of multiple, diverse downstream targets [37,38]. A transient rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) induces a conformational change in calmodulin, allowing it to bind to specific domains on target proteins. These domains are predominantly amphiphilic α-helices [39] or IQ motifs [4]. Initially described in neuromodulin and unconventional myosin [4], examination of the Pfam database reveals IQ motifs in over 100 proteins. The IQ motif comprises 20–25 amino acids, with the core fitting the consensus IQXXXRGXXXR (where X is any amino acid) [4,40,41]. Calmodulin binds primarily to the four tandem IQ motifs of IQGAP1 [18,42], with an additional low-affinity site in the calponin homology domain [18]. Although it is generally believed that calmodulin targets that contain IQ motifs have a higher affinity for the Ca^{2+}-free form of calmodulin, IQGAP1 binds two- to three-fold more Ca^{2+}/calmodulin than Ca^{2+}-free (apo-) calmodulin [17]. Interestingly, IQGAP1 is the predominant calmodulin-binding protein in Ca^{2+}-free human breast epithelial cell lysates [17].

Moreover, immunodepletion analysis reveals that a significant proportion (at least 50%) of endogenous IQGAP1 is bound to endogenous Ca^{2+}/calmodulin [18]. Importantly, calmodulin abrogated the in vitro association of IQGAP1 with Cdc42 only in the presence of Ca^{2+} [17] and eliminated the effect of IQGAP1 on Cdc42-catalyzed GTP hydrolysis [18]. Increasing the Ca^{2+} concentration enhanced the interaction between calmodulin and IQGAP1 in cells, with a concomitant reduction in the association of IQGAP1 with Cdc42 [18]. Thus, IQGAP1 provides a molecular link integrating Ca^{2+}/calmodulin signaling with Cdc42 and the cytoskeleton.

IQGAP1 also regulates the cytoskeleton via a direct interaction with actin [18,27,35]. In vitro IQGAP1 stimulates cross-linking of F-actin [27,35]. As mentioned above, this cross-linking activity of IQGAP1 is enhanced by active Cdc42 [35], but is reduced by calmodulin in the presence of Ca^{2+} [27]. Together these findings suggest a model by which Ca^{2+} may regulate the actin cytoskeleton [18] (Fig. 2). In the absence of Ca^{2+}, IQGAP1 binds Cdc42 and stabilizes it in the active GTP-bound state. The N-terminal region of IQGAP1 binds actin, thereby localizing Cdc42 to cytoskeletal structures. By this mechanism, IQGAP1 couples Cdc42 to F-actin, enhancing cross-linking of F-actin. A localized increase in $[Ca^{2+}]_i$ increases the binding of calmodulin to IQGAP1. The resultant conformational change in IQGAP1 dissociates it from both Cdc42 and F-actin, thereby insuring a separation of Cdc42 from microfilaments (Fig. 2). In this model, IQGAP1 is both a regulator of Cdc42 localization and activity, as well as a target of Cdc42 function.

4.2. Cell–cell adhesion

IQGAP1 is also an important constituent of the E-cadherin cell–cell adhesion machinery [29,30]. E-cadherin is a Ca^{2+}-dependent adhesion molecule that is anchored to the cytoskeleton via catenins [43]. β-Catenin is both a central component of this cadherin cell–cell adhesion complex and has an essential role in the Wnt-1 signaling pathway [44] by binding to and co-activating members of the lymphocyte enhancer factor/T-cell factor family of transcription factors [45]. Overexpressed IQGAP1 dissociates α-catenin from the E-cadherin–β-catenin complex, resulting in a decrease in E-cadherin-mediated cell–cell adhesion; activated Cdc42 and Rac counteract the effect of IQGAP1 [29]. Moreover, calmodulin attenuates the binding of IQGAP1 to E-cadherin [30], providing a molecular mechanism by which $[Ca^{2+}]_i$ regulates E-cadherin function. When $[Ca^{2+}]_i$ is low, active Cdc42 and Rac1 bind IQGAP1, thereby stabilizing intercellular adhesion [29]. When $[Ca^{2+}]_i$ increases, calmodulin displaces Cdc42 from IQGAP1

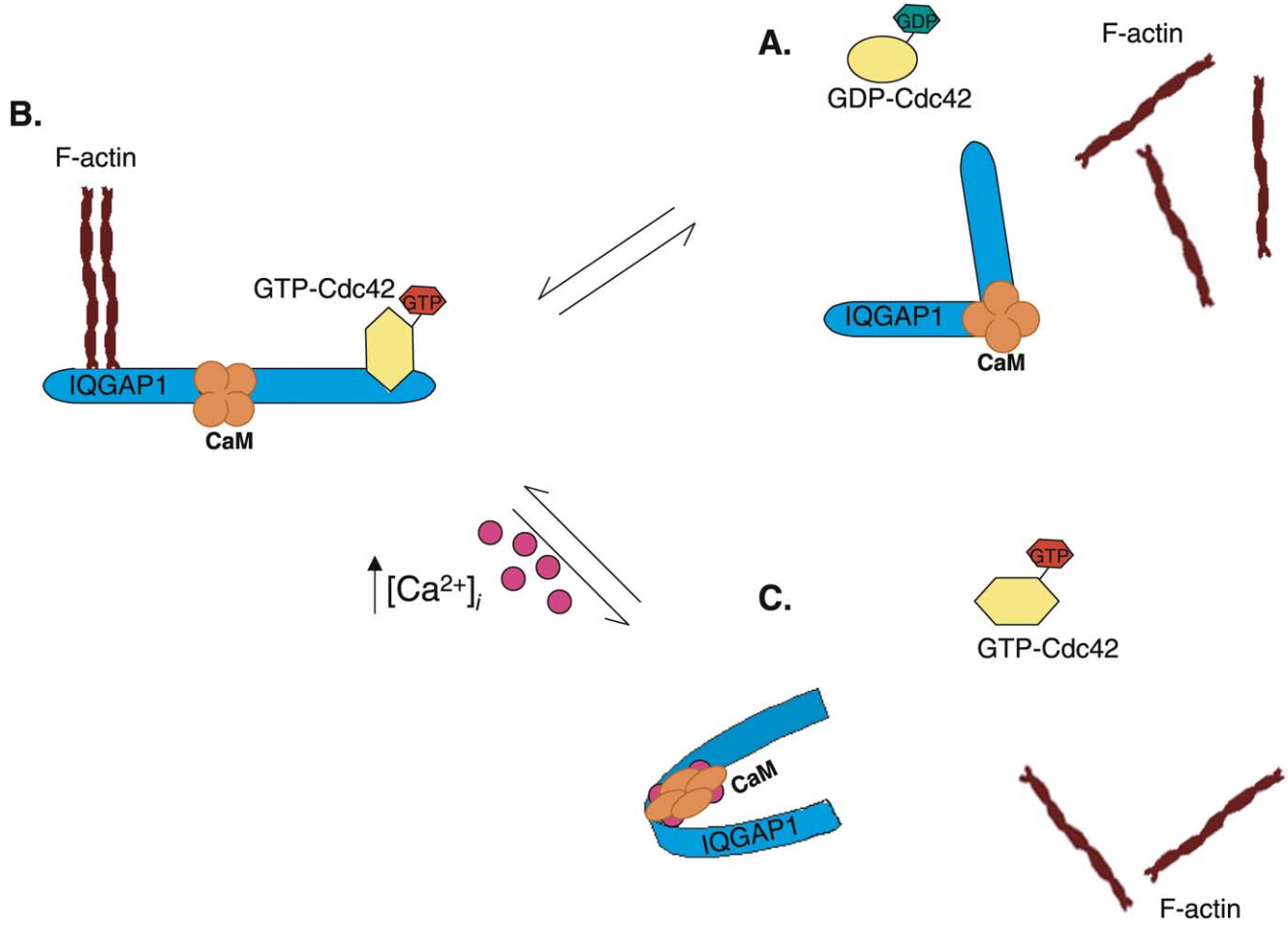

Fig. 2. Model of the interactions among IQGAP1, Cdc42, F-actin, Ca^{2+} and calmodulin. A: IQGAP1 is depicted bound to Ca^{2+}-free calmodulin (CaM) in the cell. B: On binding active Cdc42, IQGAP1 binds to F-actin and enhances cross-linking. C: A localized increase in $[Ca^{2+}]_i$ enhances the interaction of Ca^{2+}/calmodulin with IQGAP1, altering the tertiary conformation of IQGAP1. The change in shape of IQGAP1 results in dissociation of F-actin and Cdc42.

[17], allowing IQGAP1 to weaken E-cadherin-mediated cell–cell adhesion [46]. Inhibition of calmodulin binding to IQGAP1 (e.g. by calmodulin antagonists) further reduces cell–cell attachment [30]. Additional complexity is provided by the interaction of IQGAP1 with β-catenin and the effect of calmodulin. In contrast to its inhibitory effect on E-cadherin, IQGAP1 augments β-catenin-mediated transcriptional activity in the nucleus under certain conditions [31]. Unexpectedly, calmodulin binding was required for IQGAP1 to enhance the function of β-catenin in the nucleus [31].

4.3. Molecular mechanism

The molecular mechanism by which calmodulin regulates IQGAP1 function has not been identified. The interaction of Ca^{2+}/calmodulin with 'classic' target enzymes (e.g. myosin light chain kinases or Ca^{2+}/calmodulin-dependent protein kinases) via amphiphilic α-helices has been well characterized. Calmodulin binding induces a conformational change in the calmodulin-dependent protein kinase that relieves autoinhibition, leading to enzyme activation [37,47]. By contrast, the functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. The interaction of calmodulin – and the modulatory role of Ca^{2+} – with the four tandem IQ motifs of IQGAP1 is highly complex [42]. Ca^{2+}-free calmodulin binds to only the third and fourth IQ motifs of IQGAP1, while Ca^{2+}/calmodulin binds to all four IQ motifs [42]. The observation that Ca^{2+} is required for calmodulin to block the binding of Cdc42 to IQGAP1 [17] implies that Ca^{2+}/calmodulin induces a conformation in IQGAP1 different from that produced by apocalmodulin. Moreover, because calmodulin and Cdc42 bind exclusively to the N- and C-terminal halves of IQGAP1, respectively [11,18], the competition is presumably mediated by a change in the tertiary conformation of IQGAP1.

A second possible mechanism by which calmodulin may regulate IQGAP1 is by altering its subcellular location. An important feature of scaffolding proteins is that they provide a means by which signals can be spatially resolved within the cell [48]. Calmodulin, which regulates the subcellular location of several proteins [49,50], modulates IQGAP1 localization. Disrupting the association between calmodulin and IQGAP1 with a cell-permeable calmodulin antagonist induced translocation of IQGAP1 from the cytoplasm to cell–cell junctions, where it inhibited E-cadherin function [30]. Conversely, an increase in $[Ca^{2+}]_i$ (which enhances binding of calmodulin to IQGAP1) removed IQGAP1 from the cell cortex [27]. Thus, Ca^{2+}/calmodulin appears to regulate IQGAP1 function by modulating its ability to bind to other targets by two mechanisms: altering the conformation of IQGAP1 and influencing its subcellular location.

5. Summary

By interacting with a diverse array of proteins, IQGAP1 participates in multiple fundamental cellular functions. These include regulation of the cytoskeleton, cell–cell adhesion, embryogenesis and transcription. The ability of IQGAP1 to form multiprotein complexes that are regulated by interaction with several target molecules permits IQGAP1 to act as a scaffolding protein that integrates distinct signaling pathways. IQGAP1 couples Ca^{2+}/calmodulin signaling to cell structure and the cytoskeleton (via actin and Cdc42) and cell–cell adhesion (via the E-cadherin–β-catenin complex). Future studies are likely to yield additional targets and functions for the IQGAP family of proteins.

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