New model for the interaction of IQGAP1 with CDC42 and RAC1

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Abstract: The specific and rapid formation of protein complexes, involving IQGAP family proteins, is essential for diverse cellular processes, such as adhesion, polarization, and directional migration. Although CDC42 and RAC1, prominent members of the RHO GTPase family, have been implicated in binding to and activating IQGAP1, the exact nature of this protein-protein recognition process has remained obscure. Here, we propose a mechanistic framework model that is based on a multiple-step binding process, which is a prerequisite for the dynamic functions of IQGAP1 as a scaffolding protein and a critical mechanism in temporal regulation and integration of cellular pathways.

Abbreviations: CaM, calmodulin; CC, coiled-coil repeat region; CHD, calponin homology domain; CT, C-terminal domain; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor; GRD, GAP-related domain; GST, glutathion-S-transferase; GTPase, guanosintriphosphatase; IQ, four isoleucine/glutamine-containing motifs; IQGAP1, IQ-domain GTPase-activating protein 1; PAK1, p21- activated kinase 1; RGCT, RASGAP C-terminus; WASP, Wiskott-Aldrich syndrome protein; WW, tryptophan-containing proline-rich motif-binding region.

The RHO family GTPases, most prominently CDC42, RAC1, and RHOA, are known to play an important role in diverse cellular processes and progression of different diseases, such as cardiovascular diseases, developmental and neurological disorders, as well as in tumor invasion and metastasis [1]. RHO GTPases share two common functional characteristics, membrane anchorage and an on/off switch cycle [2]. Thus, membrane-associated RHO GTPases act, with some exceptions [3], as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state. This cycle underlies two critical intrinsic functions, GDP-GTP exchange and GTP hydrolysis [3] and is controlled by at least three classes of regulatory proteins, including guanine nucleotide dissociation inhibitors (GDIs), guanine nucleotide exchange factors (GEFs), and GTPase activating proteins (GAPs) [4] . The formation of the active GTP-bound state of RHO GTPases is accompanied by a conformational change in two regions, known as switch I and II (encompassing amino acids or aa 29–42 and 62–68, respectively) [4] , which provide a platform for the selective interaction with structurally and functionally diverse effectors, *e.g.*, PAK1, WASP, and IQGAP1. This class of proteins activates a wide variety of downstream signaling cascades [5-8] thereby regulating many important physiological and pathophysiological processes in eukaryotic cells [9, 10].

IQGAP1 is a ubiquitously expressed scaffold protein. It has been assigned to multiple subcellular sites and implicated in multiple functions by most probably safeguarding the strength, efficiency, and specificity of signal transduction (reviewed in [7, 8, 10-13]). Notably, IQGAP1 has been implicated as a drug target due to its vital regulatory roles in cancer development [14-17] although the molecular mechanism of its functions is unclear. IQGAP1 possesses distinct protein interaction domains and motifs to achieve its scaffolding functions. A prerequisite to understand its cellular properties is the dissection of its distinct domains and the analysis of their interactions with desired protein partners. It contains an N-terminal calponin homology domain (CHD), a coiled-coil repeat region (CC), a tryptophan-containing proline-rich motif-binding region (WW), four isoleucine/glutamine-containing motifs (IQ), a RASGAP-related domain (GRD), RASGAP C terminal domain (RGCT) and very C-terminal domain (CT) [6] as illustrated in Figure 1.
A multitude of IQGAP1 interacting partners have been reported [6, 18], among them RAC1 and CDC42 [7, 19, 20], which are crucial for the controlling of IQGAP's activities. For instance, it is well accepted that RAC1 and CDC42 facilitate the function of IQGAP1 in intercellular adhesion sites in epithelial cells, in actin-crosslinking, and determining its subcellular localization [7, 21]. IQGAP1 is localized in the sites of cell-cell contact [22] and its overexpression decreases E-cadherin mediated cell-cell adhesion by interacting with β-catenin and causing the dissociation of α-catenin from the cadherin-catenin complex in epithelial cells [22]. Active forms of RAC1 and CDC42 positively regulate E-cadherin-mediated cell-cell adhesion by inhibiting the interaction of IQGAP1 with β-catenin [23]. When the amount of active RAC1 and CDC42 increase, they interact with IQGAP1, thus crosslinking actin filaments. Under these conditions, IQGAP1 is not able to bind β-catenin and thus cannot dissociate α-catenin from the cadherin-catenin complex, leading to strong adhesion. On the other hand, when the amount of GDP-bound inactive RAC1/CDC42 increases, IQGAP1 is released from RAC1/CDC42 and then interacts with β-catenin to dissociate α-catenin from the cadherin-catenin complex and this result in weak adhesion.

Another example is in the case of IQGAP1 binding to actin filaments and crosslinking actin filaments [24]. This ability to crosslink depends on IQGAP1 dimerization and/or oligomerization [25]. In addition, it has been shown that binding of active forms of CDC42 or RAC1 enhance IQGAP1 dimerization or oligomerization [25, 26]. IQGAP1 directly binds to actin filaments through its N-terminal CHD. The IQGAP1 dimer or CDC42/RAC1-mediated IQGAP1 oligomer utilizes multiple CHDs to crosslink and bundle actin filaments.
While modulation of the cytoskeletal architecture was initially thought to be the primary function of the interaction of IQGAP1 with RHO proteins, it is now clear that they have critical physiological roles beyond the cytoskeleton [10]. One example is its neuronal function. IQGAP1 is observed throughout neuronal cells, along neuritis and the developing axon, and also at the growth cone [27]. It has been shown that an interaction between IQGAP1 and protein-tyrosine phosphatase PTPμ is necessary for

neurite outgrowth in ganglion cells [28]. PTPμ forms a complex with IQGAP1, N-cadherin, E-cadherin, and β-catenin [28]. Active GTP-bound CDC42 by interacting IQGAP1, promotes the interaction of PTPμ with IQGAP1 to stimulate actin remodeling and, eventually, neurite outgrowth [10, 27].

In spite of knowing the importance of these interactions, the molecular mechanisms are still obscure and remains to be clearly investigated *in vitro*. Physical interaction of IQGAP1 with these members of RHO family under cell-free conditions was first reported by Hart *et al*. [29]. They showed that the C-terminal half of IQGAP1, encompassing aa 915–1657 and containing the GRD, RGCT, and CT bound to CDC42 and RAC1 in a GTP-dependent fashion, but other deletion fragments did not, most probably due to their instability. Joyal *et al*. have shown a disruption of the interaction between CDC42 and full-length IQGAP1 in the presence of calmodulin (CaM) and calcium ions [30]. It has been suggested that CaM-bound IQGAP1 adopts a different conformation that is less accessible to CDC42 (see also [31]). Two interesting observations were made by Swart-Mataraza *et al*.: On the one hand, CDC42 binding was also shown with IQGAP1-ΔGRD (lacking aa 1122–1324) and on the other hand, IQGAP1 was still able to bind GDP-bound CDC42 although with a lower affinity [32]. This suggested that additional regions of IQGAP1, other than GRD, are responsible for CDC42 binding and that IQGAP1 is able to bind CDC42 outside the switch regions in a nucleotide-independent manner. Ho *et al*. later reported that increasing the calcium concentration enhanced the interaction between calmodulin and IQGAP1, with a concomitant decrease in the association of CDC42 with IQGAP1 [33]. Zhang *et al*. have determined an inhibitory constants of 0.4 and 2.1 μM for the inhibition of GTP hydrolysis of CDC42 and RAC1, respectively, in the presence of increasing concentration of the C-terminal half of IQGAP1 (GRD1-CT, aa 863-1657, Fig. 1) [34]. Li *et al*. have mapped the IQGAP1-binding regions on CDC42 [35]. Switch I and its surrounding regions (aa 29–55) as well as the insert region (aa 122–133) of CDC42 have been suggested as essential determinants for the IQGAP1 binding. Owen *et al*. have studied the interaction of GRD1-CT with a large panel of CDC42 and RAC1 variants with point mutations within and around switch I/II, α helices 1, 3 and 5, β stands 1, 2 and 3, and the insert region [36]. These analyses were conducted in the background of a constitutively active variant of both CDC42 and RAC1 (Gln-61 to leucine or Q61L). This comprehensive study has provided various suggestions for the IQGAP1 interaction with CDC42 *vs.* RAC1. Accordingly, IQGAP1 reveals only partially overlapping contact sites for CDC42 and RAC1. Despite a sequence identity of more than 70%, these GTPases apparently employ different regions to achieve high-affinity interactions with GRD1-CT. Tyr-32 and Val-36 of switch I, are critical for both CDC42 and RAC1, whereas Asp-63 and Arg-68 of switch II are critical for only RAC1 and optionally Asn-132 of the insert region only for CDC42. The impact of the latter maybe is insignificant since CDC42 lacking the insert region still efficiently binds GRD1-CT. However, Owen *et al*. have determined equilibrium dissociation constants (K_d) of 0.024 and 0.018 μM for the binding of GRD1-CT to CDC42 and RAC1, respectively [36]. Moreover, a shorter, mainly GRD-containing fragment, encompassing aa 950-1407, has shown a different binding behavior to the analyzed RHO GTPases. It binds CDC42 with significantly lower affinity (0.14 μM) as compared to GRD1-CT, but does not bind RAC1 [36].

The crystal structures of a GRD fragment (aa 962–1345; Fig. 1) alone and in complex with the constitutively active variant of CDC42 have been determined [37]. Kurella *et al*. have shown that the GRD adopts a RASGAP-like structure with a conserved central domain (GAPc) that is coupled to the variable flanking regions forming an extra domain (GAPex) [37-39]. IQGAP1 GRD, however, is functionally an inactive RASGAP due to the lack of critical catalytic and structural fingerprints [37, 40, 41]. Very recently, LeCour *et al*. have reported that IQGAP1 GRD dimerizes upon binding to CDC42 but not to RAC1 [26]. Accordingly, four CDC42 molecules interact differently with GAPc and GAPex domains of the GRD dimer by employing in both cases various regions, including switch I, II, α3, and the insert region [26]. The binding constants obtained for the CDC42 interaction with the GRD were 1.3 and 0.1 μM in the two studies by Kurella *et al*. and LeCour *et al*., respectively [26]. The CDC42-GAPc interaction, resembling the RAS-RASGAP binding mode [38], has previously been proposed by Owen *et al*. and Kurella *et al*. [36, 37]. Such a role of the GRD in associating with CDC42 is astonishing considering aforementioned studies on both GRD1-CT that binds CDC42 with a higher affinity as compared to

GRD and an IQGAP1 variant, lacking the GRD, which equally interacts with CDC42 as compared to IQGAP1 wild type. Consistently, Nouri *et al*. did not observe any fluorescence signal upon mixing CDC42 with the GRD1 or GRD2 which strongly suggest a binding of GRD adjacent to or outside the switch regions of CDC42 [42].
The studies by Elliot *et al*. and Nouri *et al*. have clearly disclosed the critical role of RGCT for the RHO GTPase interactions [42, 43]. They have shown that an IQGAP1 fragment, containing the GRD and RGCT (aa 877-1558, GRD1-RGCT), is significantly compromised in its ability to tightly bind CDC42 if using phosphomimetic mutations at Ser-1441 and Ser-1443 [42, 43], originally identified by Li *et al*. [27]. Nouri *et al*. have obtained a very low binding affinity for the interaction between the GRD and CDC42 (Fig. 1) that is 20- to 200-fold lower than that determined by Owen *et al*., Kurella *et al*. and LeCour *et al*. [26, 36, 37]. A principle explanation for this discrepancy is the use of the constitutive active $CDC42^{Q61L}$ by the latter groups that strongly increase the binding affinity for effector proteins, such as IQGAP1 [42]. Nouri *et al*. have shown, in addition, that GRD1-RGCT, lacking the CT, dissociates significantly faster from CDC42 as compared to GRD1-CT. This strongly indicates that CT itself may physically contact CDC42 as determined by equilibrium measurements (Fig. 1). Very similar data have been obtained by Nouri *et al*. for RAC1 interaction with GRD1-CT, GRD1-RGCT and CT (Fig. 1). However, RAC1 interaction with the GRD is different as compared to that of CDC42. All binding constants for the interaction between various IQGAP1 fragments and RHO GTPases along with the techniques applied are summarized in Nouri *et al*., Table 1 [42].

The switch regions of the RHO family proteins have been previously proposed as the first binding site for the downstream effectors and if this first contact is achieved then additional contacts outside the switch regions are required to fulfill effector activation [4]. It is still unclear how RHOGTPases, such as CDC42, activate IQGAP1. However, *in vitro* studies reported above clearly point to the importance of the C-terminal half of IQGAP1 to achieve the interaction with RAC1- and CDC42-like proteins. It utilizes at least three functionally distinct units, including GRD, RGCT, and CT [42] . GRD undergoes a low-affinity, GDP-/GTP-independent complex with RAC1 and CDC42 proteins outside their switch regions. RGCT only binds to the RAC1 and CDC42 proteins if they are active and exist in the GTP-bound forms, and the C-terminal region of IQGAP1 may potentiate the IQGAP1 interaction with RAC1 and CDC42 proteins by probably extending the resident time of the respective proteins complexes. The rationales underlying a multiple-step binding mechanism for the IQGAP1 interaction with CDC42 are summarized in Figure 2A. First, the RGCT recognize CDC42-GTP and associates with its switch regions with high affinity. Second, the GRD binds CDC42 with a very low affinity adjacent to and/or outside the switch regions and induces an active conformation of full-length IQGAP1 that is now accessible for additional downstream interactions. Third, the CT contacts, although with extremely low affinity, CDC42 outside the switch regions of the whole evolutionary process, which stabilizes this bimolecular interaction and prolongs residence time of IQGAP1 on CDC42. This scenario may be a prerequisite for the dynamic functions of IQGAP1 as a scaffolding protein and a critical mechanism in temporal regulation and integration of IQGAP1-mediated cellular responses [42]. In a different scenario, IQGAP1 acts as a scaffold protein upstream of the CDC42-WASP Pathway. The formation of such a ternary complex is likely initiated by phosphorylation of IQGAP1 at Ser-1443 leading to the release of its autoinhibited state that allows, as previously proposed [44, 45], the exposed C-terminal half to activate N-WASP in a CDC42-dependent manner and consequently leading to actin polymerization at the leading edge of the cells. The ability of GRD in binding outside the switch regions of CDC42 may facilitate the scaffolding function of IQGAP1 in synergistically localizing CDC42 and WASP at specific sites of the cell.

Despite a high sequence identity between RAC1 and CDC42, they obviously differ in regard to their IQGAP1 binding. RAC1 binds to GRD1-RGCT and CT as tight as CDC42 but exhibits different affinities for the GRD and GRD1-CT (Fig. 1). Unlike CDC42, an extremely low affinity was determined for the RAC1-GRD interaction [42]. This strongly suggests that the molecular nature of IQGAP1 interaction with CDC42 partially differs from that of RAC1. Given the hypothesis that the GRD does not play a

prime role in the recognition of CDC42 and its binding outside the switch regions induces a conformational change and activation of IQGAP1, the functional impact of the interaction of RAC1 remains rather ambiguous. If a direct impact of RAC1 on IQGAP1 activity does not follow the same trend as in the case of CDC42 then we need to think about an alternative regulatory mechanism. One possible scenario is that CaM potentiates RAC1-mediated activation of IQGAP1 by acting as a scaffold to assemble these accessory proteins (Fig. 2B). Dual roles of IQGAP1 as an effector downstream and a scaffold protein upstream of CDC42 and alternatively RAC1 remains to be experimentally explored.

Disclosure of potential conflicts of interest
The authors declare that they have no conflict of interest.

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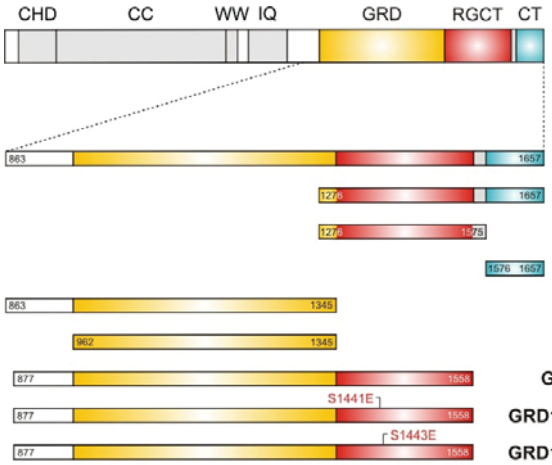
Figure legends

Figure 1. Domain organization of IQGAP1 and different constructs of C-terminal half (left panel) along with individual rate constants of interaction of corresponding proteins with active RAC1 and CDC42 (right panel). In the left panel IQGAP1 domain organization (adapted from White *et al*. [6] and different constructs used in our previous study is shown. Right panel shows the binding affinity of IQGAP1 proteins for RAC1/CDC42 proteins analyzed by stopped-flow fluorometry (kinetic measurements) and fluorescence polarization (equilibrium measurements) (adapted from Nouri *et al*. [42]). Kinetic conditions provide individual association and dissociation rate constants (k_{on} and k_{off}) and determine the dissociation constants (K_d) which is obtained from the ratio k_{off}/k_{on}. Equilibrium conditions determine the equilibrium dissociation constants (K_d) directly.

Figure 2. A proposed multiple-step mechanistic model of IQGAP1 interaction with CDC42 and RAC1. IQGAP1 harbors at least two distinct binding domains. It seems that the molecular nature of IQGAP1 interaction with CDC42 partially differs from that with RAC1 particularly with regard to the role of GRD. RGCT contributes with a high affinity binding to the switch regions of the GTP-bound, active (A) CDC42 and (B) RAC1. GRD more selectively recognizes active forms of CDC42 and RAC1 but also binds to other regions adjacent to the switch regions obviously in a nucleotide-independent manner. Equilibrium measurements using fluorescence polarization by Nouri *et al.*[42] demonstrated that GRD undergoes a low-affinity interaction with CDC42 but its binding in contrast to RGCT is partially nucleotide dependent. The very C-terminal domain (CT) of IQGAP1 may potentiate the IQGAP1 interaction with RAC1 and CDC42 proteins by probably extending the resident time of the respective proteins complexes. Lack of GRD interaction with RAC1 (B) may be compensated by calmodulin as an accessory protein [46], which has been reported to bind the polybasic region of RAC1 and IQ motifs of IQGAP1.

Nouri et al., Figure 1



	CDC42/RAC1			
	Kinetic measurements			Equilibrium measurements
	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	$K_d = k_{off}/k_{on}$ (μM)	K_d (μM)
GRD1-CT	1.3/0.71	0.39/0.66	0.30/0.94	3.4/5.5
RGCT-CT	not measurable			not measurable
RGCT	not measurable			not measurable
CT	no fluorescence signal observed			730/765
GRD1	no fluorescence signal observed			33.4/84.2
GRD2	no fluorescence signal observed			30.2/81.5
GRD1-RGCT	0.82/0.99	2.2/2.8	2.7/2.8	4.6/4.6
GRD1-RGCT^{S1441E}	0.89/0.84	1.8/2.7	2.0/3.2	3.6/6.7
GRD1-RGCT^{S1443E}	0.33/0.45	2.3/2.8	7.0/6.2	16.3/26.5

Nouri et al., Figure 2