The interaction of IQGAPs with calmodulin-like proteins

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Abstract
Since their identification over 15 years ago, the IQGAP (IQ-motif-containing GTPase-activating protein) family of proteins has been implicated in a wide range of cellular processes, including cytoskeletal reorganization, cell-cell adhesion, cytokinesis and apoptosis. These processes rely on protein–protein interactions, and understanding these (and how they influence one another) is critical in determining how the IQGAPs function. A key group of interactions is with calmodulin and the structurally related proteins myosin essential light chain and S100B. These interactions occur primarily through a series of IQ motifs, which are α-helical segments of the protein located towards the middle of the primary sequence. The three human IQGAP isoforms (IQGAP1, IQGAP2 and IQGAP3) all have four IQ motifs. However, these have different affinities for calmodulin, myosin light chain and S100B. Whereas all four IQ motifs of IQGAP1 interact with calmodulin in the presence of calcium, only the last two do so in the absence of calcium. IQ1 (the first IQ motif) interacts with the myosin essential light chain Mlc1sa and the first two undergo a calcium-dependent interaction with S100B. The significance of the interaction between Mlc1sa and IQGAP1 in mammals is unknown. However, a similar interaction involving the Saccharomyces cerevisiae IQGAP-like protein Iqg1p is involved in cytokinesis, leading to speculation that there may be a similar role in mammals.

Introduction: the IQGAP (IQ-motif-containing GTPase-activating protein) family of proteins
The first member of the IQGAP family of proteins was identified in 1994 by Weissbach et al. [1]. Initial characterization of this 180 kDa multidomain protein showed that it interacted with actin, calmodulin and the small GTPases Cdc42 (cell division cycle 42) and Rac1 [1–6]. This immediately pointed to a role at the interface between diverse cellular signalling pathways and the actin cytoskeleton. Interestingly, despite sequence similarities to GAPs (GTPase-activating proteins), members of the IQGAP family appear to inhibit, rather than activate, the rate of GTP hydrolysis by the small GTPases they interact with [2]. The discovery of the first mammalian IQGAP was followed by the identification of a second one, IQGAP2, and similar proteins in a wide range of eukaryotic organisms including the budding yeast Saccharomyces cerevisiae [7–9]. Following the completion of the human genome sequence, a third mammalian isoform was postulated, namely IQGAP3 [10].

IQGAP family members are multidomain proteins (Figure 1). At the N-terminus is a single CHD (calponin homology domain). This region binds to actin [3,6,11,12] and possibly also to calmodulin [3]. Towards the middle of the primary sequence is a series of IQ motifs. This motif is commonly found in proteins that interact with calmodulin and related proteins such as myosin light chains [13]. In the three human IQGAPs, there are four IQ motifs, except in a testes-specific alternatively spliced form of IQGAP2, which has only three [14]. These IQ motifs have been reported to interact with calmodulin [15,16], myosin essential light chain [17,18] and S100B [18,19]. They may also be a site for the homodimerization of IQGAP [20] although the CHD has also been implicated in this interaction [6]. Towards the C-terminus, there is a GAP-related domain that mediates interaction with Cdc42 and Rac1 [2–4,7]. Over the last 5 years, the catalogue of proteins that have been shown to interact directly with human IQGAP1 has expanded considerably. This has led to a commensurate expansion in the number of cellular functions that it may be involved in. Interaction partners include protein kinases (e.g. extracellular-signal-regulated kinase 2 [21]), the kinase and phosphatase scaffolding protein AKAP79 (A-kinase-anchoring protein 79) [22], the cytoskeletal organizer N-WASP (neuronal Wiskott–Aldrich syndrome protein) [23], the microtubule-organizing protein CLIP-170 (cytoplasmic linker protein 170 kDa) [24], RNase H [25] and E-cadherin [26]. Taken together, these results on the various protein–protein interactions implicate human IQGAP1 in processes as diverse as kinase signalling, microtubule regulation, cell–cell adhesion and apoptosis [21,24–27]. The S. cerevisiae IQGAP-like protein Iqg1p is also relatively well studied.

Key words: calmodulin, Iqg1p, IQ-motif, IQ-motif containing GTPase-activating protein (IQGAP), myosin light chain, S100B.

Abbreviations used: APC, adenomatous polyposis coli; Cdc42, cell division cycle 42; CHD, calponin homology domain; GAP, GTPase-activating protein; IQ1, first IQ motif; IQGAP, IQ-motif-containing GAP.

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Domain structure of a typical IQGAP family member

The location of the domains in the linear sequence is shown below examples of the three-dimensional structures of these domains (where known). The CHD at the N-terminus interacts with actin (and possibly calmodulin) [3,6,11,12]. The NMR structure of this domain from human IQGAP1 (PDB code 2RR8) [33] is shown here. The WW domain (protein–protein interaction domain containing two conserved tryptophan residues) is not present in the fungal IQGAPs [9,31]. The structure is not known. The IQ motifs interact with calmodulin, S100B and myosin light chains [15–17,19,30,46]. Although there is no experimentally determined structure for this region, it is assumed that the motifs themselves mostly form α-helices. This hypothesis is supported by molecular modelling [46] (see also Figure 3). The GRD (GAP-related domain) interacts with the small GTPases Cdc42 and Rac1 [2–4,7]. IQGAPs do not function as GAPs and actually stabilize the active GTP-bound state of the molecule, thus prolonging signalling from the GTPase [2]. The crystal structure of this region from human IQGAP1 is shown (PDB code 3FAY) [34]. The extreme C-terminus is folded into a RasGAP C-terminal domain (RGC). Here the NMR structure from human IQGAP1 is shown (PDB code 1X0H). It is currently unknown how these various domains fold relative to each other in the intact molecule.

IQGAPs in disease

Given the wide range of roles of IQGAP family members in normal cellular function, it is not surprising that they are also implicated in a number of diseases. Their role in collecting signals from various cellular signalling pathways, integrating these and passing on messages to the actin and microtubular cytoskeletons, means that they are likely to be involved in changes in cell shape and in cell motility. This, combined with their role in cell–cell adhesion, means that it is not surprising that abnormal expression levels are associated with a number of different types of cancer [37,38]. Only one cancer-associated mutation has been identified, M1231I, which lies in the GTPase-binding region [39]. Structural analysis shows that the residue is found in the interior of the domain and is not part of the postulated GTPase-binding site. This suggests that this alteration will result in general structural instability rather than a failure to interact with a specific partner [34]. Altered interactions with the APC (adenomatous polyposis coli) protein may be involved in the generation of a cancerous state. Several of the cancer-associated mutations in APC are located in the putative IQGAP1 interaction site [40,41]. However, the functional consequences of these sequence variations on APC–IQGAP1 affinity are currently unknown. In general, the precise role(s) of IQGAPs in cancer is hard to assess. In particular, knowing whether up-regulation of expression is a cause or effect of cancerous behaviour is uncertain. Cells such as metastasing cancer cells change shape and migrate. These processes require changes in the actin cytoskeleton. An increase in the amount (or actin-modulating activity) of IQGAP may stimulate this and help cause metastasis. However, if other processes are causing metastasis to occur, then these may cause the up-regulation of IQGAP's actin-modulating activity.
Figure 2 | Interaction between IQ1 of human IQGAP1 and the myosin essential light chain Mlc1sa

Mlc1sa (35 μM) runs as a single band (first lane, left-hand side arrow). A synthetic peptide (IQ1; sequence: LITRLQARCRGYLVRQEFR) was not visualized on the gel at a concentration of 35 μM (second lane). However, when it was added in increasing concentrations to a constant concentration (35 μM) of Mlc1sa, an additional, discrete band, corresponding to an IQ1–Mlc1sa complex was observed (right-hand side arrow, lanes 3–8) [18].

Figure 3 | Predicted structures of the IQ motifs from human IQGAP1

Each sequence was submitted to the online homology modelling tool Phyre (http://www.sbg.bio.ic.ac.uk/~phyre/) [49]. The highest ranked model was then computationally solvated and energy minimized using YASARA (http://www.yasara.org/minimizationserver.htm) [50]. The resulting models can be viewed at http://www.biochemsoctrans.org/bst/039/bst0390694add.htm. All four IQ motifs are predicted to form α-helices. For clarity, they are shown here without the solvating water molecules. The conserved residues that permit the identification of these sequences as IQ motifs are shown in bold in both the peptide sequences and the models. Residues shown in lower-case letters in the sequences were present in the synthesized peptides used in the experiments [18], but were not represented in the final modelled structures.

IQGAP1 has also been implicated in the internalization of pathogens. It acts as a receptor for the Moloney-murine leukaemia virus matrix protein [42] and promotes the internalization of various bacteria, including Salmonella serovar Typhimurium [43].

Interactions of IQGAP family proteins with calmodulin

The four IQ motifs in mammalian IQGAPs provide at least four potential interaction sites for calmodulin. However, it is unlikely that all these are occupied under the same conditions. This occupancy is controlled by the intracellular calcium ion concentration and the binding of other proteins to IQGAP. Site-directed mutagenesis studies on human IQGAP1 suggested that, while all four IQ motifs are capable of interacting with calmodulin in the presence of low millimolar concentrations of calcium ions, only the third and fourth are able to interact in the absence of calcium ions [15]. Cdc42 and calmodulin influence each other’s binding to IQGAP: the association of Ca2+-calmodulin with IQGAP1 displaces Cdc42 [3,44]. The distance between the GTPase- and calmodulin-binding sites in the primary sequence (Figure 1) suggests that either there must be folding of the overall structure to permit contact between the two regions or that conformational changes transmit information through the three-dimensional structure. The biochemical analysis of the inter-relationship between calcium concentrations, GTPase binding and IQ motif occupancy provides some indication of the complexity of IQGAP’s interactions with targets and of the molecular mechanisms of signal integration.

Calmodulin has also been shown to interact with the CHD of IQGAP1 in vitro [3]. Although there are other examples of CHD–calmodulin interactions in the literature (e.g. [45]), the physiological significance of this interaction in IQGAPs (if any) is not known.

Interaction of IQGAPs with calmodulin-like proteins

Yeast two-hybrid analysis demonstrated that a construct containing the IQ motifs from human IQGAP1 interacts with the myosin essential light chain Mlc1sa [17]. It was hypothesized that this interaction occurs through IQ1, since this is the IQ motif most similar to that found in myosin heavy chains [17]. This hypothesis has been confirmed by the use of synthetic peptides and native gel electrophoresis [18]. The addition of a peptide containing the IQ1 sequence to recombinant Mlc1sa retards the mobility of this protein on native gels (Figure 2). That a discrete band is observed suggests that the complex is stable under the conditions of the experiment and does not decay rapidly. No complexes were observed with the remaining IQ motifs from human IQGAP1 [18]. All four IQ motifs from IQGAP1 are predicted to form α-helices (Figure 3) and thus the differences in binding specificity must arise from the sequence variations in the individual motifs. The yeast two-hybrid experiments also showed no interaction between human IQGAP2 and myosin light chains. This has also been confirmed by native gel electrophoresis experiments [46]. Similar experiments suggest that IQ1 from human IQGAP3 may also interact with Mlc1sa, albeit to form a less stable and long-lasting complex. The in vivo significance of these mammalian IQGAP–myosin...
light chain interactions is not known. Indeed for many years the myosin essential light chain was believed to function as a structural and (sometimes) a modulatory component of myosins. These interactions with IQGAPs suggest that it has further roles as a signalling molecule.

In *S. cerevisiae* the interaction between Iqg1p and Mlc1p is well characterized [28,29]. Overexpression of either component leads to the disruption of cytokinesis; however, the overexpression of both proteins in the same cell restores its ability to complete division [30]. This suggests that not only the complex between the two molecules is vital for cytokinesis, but also the ratio between the cellular concentrations, is crucial [30]. The Iqg1p–Mlc1p interaction is required for the assembly of the actin–myosin ring, which contracts to cause the splitting of the daughter cell from the parent [29–31].

Human IQGAP1 also interacts with the calcium- and zinc ion-binding protein S100B [18,19]. This interaction may be involved in membrane rearrangement [19]. Native gel electrophoresis studies demonstrated that the first and second IQ motifs interact in a Ca$^{2+}$/ion-dependent manner [18]. No interaction was observed with the third and fourth IQ motifs or with any of the IQ motifs in human IQGAP2 and IQGAP3 [46]. Interestingly, zinc ions were unable to promote interaction in the studies with peptides corresponding to individual IQ motifs [18], whereas they could when intact IQGAP1 was studied [19]. This may indicate that there are additional factors that control binding when the four IQ motifs are present compared with the isolated peptides.

**A role for IQGAPs in mammalian cytokinesis?**

Recent work has suggested that human IQGAP1 is involved in cell division [47]. However, these experiments implicated the interaction between IQGAP1 and Cdc42 and not, as in *S. cerevisiae*, between IQGAP and myosin essential light chain. Nevertheless, it is interesting to speculate that the mammalian IQGAP1–Mlc1sa interaction (and perhaps also the corresponding interaction with IQGAP3) may be involved in cytokinesis [48]. Although the processes are not identical in fungi and mammals, there are some common features, including the formation of an actin–myosin ring that contracts to separate the cells. Therefore it would be interesting to know whether an IQGAP1–Mlc1sa complex is localized to, and involved in the formation of, this ring. If it is, then it would offer an interesting, and novel, therapeutic target for the prevention of unwanted cell division in (for example) cancerous cells.

**Future perspectives and unanswered questions**

There is no doubt that the three-dimensional structure of an IQGAP family member would greatly accelerate our understanding of how these proteins function as integrators of cellular signalling and how they pass on messages to the cytoskeleton. The structure would enable us to see how the various domains arrange themselves relative to each other and thus provide clues to how information is transmitted within the molecule. This information would need to be considered alongside the growing amount of biochemical data about the sites of interactions and the effects of one IQGAP–target interaction on another. Even more challenging would be the solution of structures of an IQGAP with one (or more) of these targets bound. This would permit direct observation of some of the conformational changes that this protein almost certainly undergoes in its role as a signal integrator. Such structural biology would need to be coupled with further biochemical and biophysical studies of this protein and its isolated domains.

To gain further understanding of how IQGAP family members interact with calmodulin (and related proteins), it is necessary to move beyond studies of the individual IQ motifs and to attempt to understand how binding at one IQ motif influences interactions at the other three. It is also necessary to resolve whether (or not) interaction with calmodulin also occurs through the CHD, what is the significance of this and how it affects interactions occurring at the IQ motifs.

Consequently, the only way to understand this signal integrating protein is to combine knowledge and experiments from different disciplines including structural biology, biochemistry and cell biology with clinical studies of diseases in which the IQGAP family proteins are implicated.

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