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Binding of Myosin Essential Light Chain to the Cytoskeleton-Associated Protein IQGAP1

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The 190 kD human IQGAP1 protein, by virtue of its N-terminal calponin-homology domain, is found associated with the actin cytoskeleton, and is capable of cross-linking actin filaments. IQGAP1 complexes with several proteins, including the Rho family GTPases Cdc42 and Rac, as well as calmodulin. It was previously noted that one of the IQ motifs of IQGAP1 displays significant similarity to a myosin heavy chain IQ motif responsible for binding the calmodulin-related myosin essential light chain (ELC). Employing the yeast two-hybrid methodology as well as in vitro binding experiments, we present evidence that a truncated version of IQGAP1 can interact with the myosin ELC. This interaction may have significant consequences for various cellular processes that involve actomyosin contractility, and suggests that the biological targets of the ELC may not be restricted to the myosin heavy chain.

IQGAP1 and -2 are actin- and calmodulin-binding proteins that appear to act as effector molecules for Cdc42 and Rac, two members of the Rho family of p21 small GTPases (1-6). The 190 kD IQGAP1 molecule is composed of multiple domains, including an N-terminal calponin homology region (CH), six repeats of 50-60 amino acids designated as IQGAP repeats (IR) that have no known function, a central region consisting of a WW domain and four IQ motifs, and a C-terminal half containing a segment resembling the catalytic domains of Ras-GTPase activating proteins (GAP-related domain or GRD)(1, 2, 7). The CH domain is similar to a Drosophila muscle protein as well as N-terminal actin-binding segments in various cytoskeleton and signaling proteins, including the smooth muscle-specific molecule calponin, α-actinin, and the proto-Vav protein, which is a nucleotide exchanger for certain Rho family members (8, 9). The WW domain, present in a host of proteins including dystrophin, the membrane-associated protein that is mutated in Duchenne and Becker muscular dystrophy, represents a 38-40 amino acid block interspersed with conserved residues, including two tryptophans (10-12). The targets for WW domains are short proline-rich motifs, either PPLP or PPXY (X is any amino acid, L is leucine, and Y is tyrosine). IQ motifs, found in many cytoskeleton and signaling molecules, are typically 20-25 amino acids in length and possess several conserved residues including adjacent isoleucine and glutamine residues. IQ motifs have been shown to bind molecules classified as members of the EF-hand consortium of polypeptides, including calmodulin and the homologous myosin light chains (13, 14). IQGAPs are characterized as non-myosin-like proteins that contain IQ motifs, similar to calmodulin-binding Ras exchange factors Ras-GRF1 and -2, neuromodulin, and endosome-associated polypeptide EEA1 (15-18). Interestingly, calmodulin binding to the Ras-GRFs is calcium-dependent, in contrast with the binding to neuromodulin and IQGAPs (15, 16, 18, 19).

Mammalian IQGAPs do not appear to associate with or directly affect in any way the activity of Ras. However, modulation of Ras-mediated pathways via Rho-related GTPases is likely, given the fact that IQGAPs have been shown to interact with Cdc42 and Rac via their C-terminal half, including the GRD and flanking regions (1, 2), and that Rho-related proteins are involved in Ras-mediated transformation (20, 21). Studies on the binding avidity of IQGAP1 for Cdc42 and Rac1 has revealed that it exhibits selectivity towards the GTP-bound forms of Cdc42 and Rac1, although the affinity for Cdc42 is 10-fold greater (2-4, 22). This observation led to the proposal that IQGAP1 functions as an effector for these two GTPases rather than as a GAP (2-4). Consistent with this hypothesis, a known effector of Cdc42, mPAK-3, competes for IQGAP1 binding to Cdc42 while a Cdc42 GAP does not (4). Recent data

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suggest that constitutive activation of Rho GTPases may be involved in tumorigenesis and mental retardation (23-25).

IQGAP1 mRNA is expressed in a multitude of human cells[3] and tissues (7) and is one of the most abundant calcium-independent calmodulin-binding proteins in normal and malignant breast cancer cell lines (19). Results from several laboratories have demonstrated that IQGAP1 associates with the actin cytoskeleton, an interaction mediated by its N-terminal calponin-homology domain and regulated in part by calmodulin (5, 26, 27). In fact, highly purified IQGAP1 is capable of cross-linking actin filaments in vitro (26, 27). The finding that Cdc42 and Rac are also intimately involved in governing the formation of various intracellular actin-containing structures such as lamellipodia and filopodia suggests a further complexity regarding the role of IQGAP1 in cytoskeleton reorganization (28, 29). Recent data have also implicated IQGAP1 as a regulator of cell-cell adhesion (30).

These findings for the IQGAPs suggest that this class of RasGAP-related proteins conspire with cytoskeleton elements, directly or indirectly, to affect particular cellular pathways involving reorganization of actin-rich structures. Other circumstantial evidence further supports this proposal. For example, the activities of other effectors of Rac1 and Cdc42 impinge on cytoskeleton dynamics. The Wiskott-Adlrich syndrome protein and Genghis Khan, two Cdc42 binding molecules, can trigger actin polymerization, and p140Sra-1, a Rac1-interacting polypeptide, binds actin (31-33). To gain further information concerning the role of IQGAP1 in cytoskeleton function, we explored the hypothesized connection between IQGAP1 and myosin, the molecular motor that converts chemical energy into directed movement of actin-containing thin filaments, and is involved in several physiological processes including muscle contraction, cell motility, and cytokinesis (34-35). Inactivation of any of the three subunits of type II myosin in Dictyostelium leads to aberrant cytokinesis (36-39), similar to the results obtained for IQGAP homologs studied in lower organisms (40-45). Interestingly, both Cdc42 and calmodulin, two binding partners of mammalian IQGAPs, are also required for cytokinesis (46-48). We previously observed that one of the four IQ motifs present in the IQGAP1 sequence bears a striking similarity to the conventional type II myosin heavy chain (MHC) IQ segment pinpointed as the recognition site for the essential light chain (ELC), one of two different calmodulin and troponin C-related EF-hand-containing proteins that associate with the MHC (7, 49, 50). We present evidence obtained from yeast two-hybrid experiments and in vitro binding studies that a truncated form of IQGAP1 can associate with the myosin ELC, thus demonstrating that the ELC may be promiscuous in its binding. Possible implications of this interaction are discussed in the context of cellular regulation of actin-based movements.

[3] Weissbach, unpublished results.

MATERIALS AND METHODS

Yeast and bacterial reagents. A human T-cell unidirectional cDNA library constructed in the yeast/bacteria shuttle vector pPC86 (51), the yeast strain MaV103, and control yeast harboring plasmids encoding interacting proteins were generous gifts of Mark Vidal, MGH Cancer Center. MaV103 is auxotrophic for leucine, tryptophan, and uracil, and contains chromosomal insertions of three reporter genes, URA3, HIS3, and lacZ (52).The pPC97 plasmid (bait vector) contains the GAL4 DNA binding domain and permits growth in the absence of leucine. The pPC87 plasmid prey vector harbors the GAL4 transcriptional activation domain and confers growth capability in the absence of tryptophan. Three-aminotriazole was obtained from Sigma Corp. Reagents for yeast media were purchased form Difco Corp. Pfu polymerase and SURE bacterial cells were procured form Stratagene, Corp., and bacterial strain DH5α was from Gibco/Life Sciences. Reagents for expressing and purifying a polyhistidine-tagged version of IQGAP1 were obtained from Qiagen.

Cloning into plasmid expression vectors. A cDNA fragment encompassing the WW and four IQ motifs in IQGAP1 for use in both yeast two-hybrid experiments (pPC97 plasmid vector) and bacterial expression (pQE-30 plasmid vector, Qiagen) was prepared by PCR. Oligonucleotide primers containing appropriate restriction sites for insertion in-frame with the N-terminal fusion polypeptide in both vectors (GAL4 DNA binding domain in pPC97 and hexahistidine tag in pQE-30) were employed. The sense primer was (5′ → 3′ direction): AACATTGGTACCAAGTCGACCAGTGATCTTGCTGAAGCC, corresponding to nucleotides 2462 - 2480 in the IQGAP1 cDNA (GenBank accession number L33075). The antisense primer was (5′ → 3′ direction): GTTAGAAGATCTAAGCTTTTGGTCCAGCAGGTGGAC, corresponding to nucleotides 3084-3102 of the IQGAP1 cDNA. The 678 bp PCR product was cleaved either with Sal I/Bgl II for insertion into complementary ends in pPC97, or Kpn I/HdIII for cloning into corresponding restriction sites in pQE-30. The digested fragments were ligated into the appropriate plasmid vector with T4 DNA ligase, transformed into SURE cells, and isolated plasmid DNA sequenced by the dideoxychain termination technique, employing sequencing primers that bind upstream of the insert: MaV103 - 5′ GGCTTCAGTGGAGACTGATATGCCTC; pQE30 - 5′ CGGATAACAATTTCACACAG, which lies 27 bp upstream from the initiating methionine codon. The desired clones containing the IQGAP1 cDNA sequence in frame with the vector-encoded N-terminal tags were used to transform either the yeast strain MaV103 or M15 bacterial cells (see below).

A bait construct for the WW domain and first two IQ motifs of IQGAP2 was also constructed. PCR was performed, using the following oligonucleotide primers: (sense, 5′ → 3′) GCAAAAGAGTCGACATCTGAAAGA, corresponding to nt 1978-2001 of the IQGAP2 cDNA (Accession No. U51903), and antisense: TCGGAACCACTAGTGAATCTTCAC, corresponding to nt 2395-2418. The 441 bp PCR product was cleaved with Sal 1 and Spe 1 and subcloned into the corresponding sites within pPC97 as described above for IQGAP1.

Yeast two-hybrid procedure. Yeast containing the IQGAP1/GAL4 DNA binding domain fusion construct in pPC97 were transformed with a human T-cell cDNA library fused to the GAL4 transcriptional activation domain in the pPC86 plasmid vector (prey vector), and plated onto agar plates lacking leucine and tryptophan, to select for transformants possessing both plasmids. Colonies were replica plated onto plates lacking leucine, tryptophan, and histidine, and containing 50 mM 3-aminotriazole, which represses the activity of the HIS3 reporter gene product, creating a more stringent selective environment for protein interactor pairs. Yeast colonies that thrived under these conditions were isolated and tested for β-galactosidase

activity by an overlay assay. Yeast isolates that were able to activate both reporter genes were used for plasmid DNA isolation, which in turn was transformed into SURE bacterial cells for large scale isolation. Both the IQGAP1 bait vector DNA and the putative interactor-containing prey vector were transformed into yeast to test for reconstitution of interaction, as judged by growth of the yeast in the absence of leucine, tryptophan, and histidine (+ 50 mM 3-aminotriazole) as well as stimulation of β-galactosidase activity. T-cell library sequences in pPC86 that reconstituted reporter gene activity in the presence of the truncated IQGAP1 bait were sequenced to determine their identity.

Recombinant protein isolation. Expression and purification of the truncated IQGAP1 recombinant protein from bacteria were accomplished under denaturing conditions, employing Ni-NTA agarose to bind the hexahistidine-tagged fusion protein. Ligated DNA was transformed into M15 cells containing the pREP4 plasmid, which expresses high levels of the lac repressor, ensuring tight control over transcription of inserted genes. One DNA isolate containing the correct sequence in frame with the polyhistidine tag was used for large scale purification of the IQGAP1 recombinant fusion protein. The DNA sequence predicted a fusion protein consisting of a 213 amino acid partial IQGAP1 sequence (amino acids 666-878) fused to a 21 amino acid N-terminal tag and a 3 amino acid C-terminal tag, with a composite molecular weight of 27,967 daltons and an isoelectric point of 10.7.

Purification of the fusion protein under denaturing conditions was accomplished as follows: 10 ml of an overnight culture of the M15 cells harboring the recombinant plasmid were added to 500 ml of LB media containing ampicillin and kanamycin, and shaken at 37°C until the OD_{600} was 0.8-0.9. IPTG was added to a final concentration of 2 mM, and the culture was shaken for an additional 3-4 hr. The cells were harvested by centrifugation, and the cell pellet was dissolved in 5 ml of Buffer A per gram wet weight (Buffer A is 8 M urea, 0.1 M NaH_2PO_4, 0.01 M imidazole, 0.01 M β-mercaptoethanol, 0.01 M Tris, pH 8.0). The solution was stirred for 1 hr at room temperature, and then centrifuged at 20,000 × g for 15 min. Four ml of a 50% slurry of Ni-NTA resin, previously equilibrated in Buffer A, were added to the supernatant, and the solution again stirred at room temperature for 1 hour. The mixture containing the resin and the bacterial extract was loaded into an empty 6 ml syringe column (2 ml resin volume) and washed sequentially with 10 column volumes Buffer A and 10 column volumes of Buffer B (8 M urea, 0.1 M NaH_2PO_4, 0.01 M imidazole, 0.01 M β-mercaptoethanol, 0.01 M Tris, pH 6.25). Material bound to the resin was eluted with 6 column volumes of Buffer C (8 M urea, 0.1 M NaH_2PO_4, 0.25 M imidazole, 0.01 M β-mercaptoethanol, 0.01 M Tris, pH 6.25). Fractions of 0.5 ml were collected and 5 μl of each fraction analyzed by SDS-PAGE for the presence of the IQGAP1 fusion protein. Fractions containing the peak of protein were pooled, dialyzed extensively against Buffer D (0.05 M Tris/HCl, pH 7.5, .05 M NaCl, 0.001 M β-mercaptoethanol), and frozen at -80°C.

In vitro binding and immunoblot analysis. In vitro binding experiments were performed using IQGAP1 bound to Nickel-NTA agarose. Initially, 50 μg recombinant IQGAP1 protein was preloaded onto 250 μl Nickel-NTA agarose beads in the presence of Buffer A (see above) and 1 mg bovine serum albumin (BSA). As a control, beads were incubated with Buffer A and BSA in the absence of IQGAP1 fusion protein. The beads were shaken for 12 h at room temperature. The beads were pelleted and washed three times with phosphate-buffered saline (PBS), and resuspended in 200 μl PBS. A 20 μl aliquot of the beads was then analyzed by SDS-PAGE for binding of the recombinant protein, and binding of IQGAP1 polypeptide was estimated at approximately 20%. A binding reaction consisting of 90 μl IQGAP1-bound beads (approximately 10 μg protein) and 30 μg bovine muscle myosin light chains (Sigma) in a total volume of 4 mls buffer (PBS containing 0.05% Triton X-100 and 0.5 mM EDTA) was shaken for 4 hr at room temperature. As a control, the beads that were not exposed to recombinant IQGAP1 protein were also incubated with the same components. The beads from each reaction were then washed three times with PBS, and bound protein eluted by boiling and analyzed by SDS-PAGE and coomassie blue staining. For Western Blot analysis, the eluted protein was separated by SDS-PAGE and transferred to PVDF-Plus membrane (MSI, Inc.) and probed with a mixture of four myosin light chain monoclonal antibodies. One antibody, MF5, a generous gift of Donald Fishman, Cornell University, was generated against the regulatory (LC2) light chain of chicken myosin and cross-reacts with the ELC (53). The other three antibodies, S21, T14, and F310, a generous gift of Frank Stockdale, Stanford University, recognize chicken skeletal fast and slow muscle ELC isoforms (54).

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                          * *       *
IQGAP1          LITRLQARCRGYLVRQEFRSRM
H. Myo.         LITRTQAVCRGFLMRVEFQKMV
R. Myo.         LITRTQAVCRGFLMRVEFQKMM
C. Myo          LITRTQARCRGFLMRVEFKKMM
M. VIIa         RIIQFQARCRAYLVRKAFRHRL
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FIG. 1. Homology of myosin ELC binding site to the N-terminal IQ motif in human IQGAP1. The region encompassing amino acids 748-769 in IQGAP1 is aligned with the protein segments in various myosin heavy chains implicated in ELC binding as well as one unconventional myosin, myosin-VIIa (56). H. Myo: human embryonic fast skeletal muscle myosin heavy chain; R. Myo: rat embryonic fast skeletal muscle myosin heavy chain; C. Myo: chicken embryonic fast skeletal muscle myosin heavy chain. The underlined amino acids in the IQGAP1 sequence are identical to at least three of the four corresponding aligned amino acids in the other vertebrate myosin sequences shown, while amino acids in bold are identical in all five sequences. Amino acids with an asterisk above them are conserved between IQGAP1 and all four myosin polypeptides.

RESULTS AND DISCUSSION

Previous experiments investigating binding partners for IQGAP1 and -2 in yeast two-hybrid analysis, employing clones that encompassed the entire coding regions for both cDNAs, yielded only calmodulin as an associated protein (1). To search for proteins that interacted with the WW domain of IQGAP1, we constructed a truncated bait sequence for use in the yeast two-hybrid method, consisting of the WW domain and the four IQ motifs of IQGAP1, reasoning that as an internal positive control we should detect calmodulin binding to the IQ motifs (1, 2). WW domains normally recognize short proline-enriched peptides such as PPLP or PPxP (12). However, we had noted previously that the most N-terminal of the IQ motifs in IQGAP1 bore a strong resemblance to the IQ peptide segment in skeletal muscle myosin heavy chain that anchors the myosin ELC, as well as to an IQ motif in the non-muscle myosin-VIIa, which is mutated in Usher syndrome 1B (55) (Figure 1).

Thus, it was not completely unanticipated that a screening of a human T-cell interactor cDNA library with the truncated IQ1 bait construct yielded three calmodulin clones and four myosin ELC clones, all of

which included the entire coding region for these two cDNAs. The ELC clones were derived from two genes, designated as MLC3nm and MLC1sa (57, 58). We feel it is likely that the binding of the ELC clones is mediated by the most N-terminal IQ motif, although we can not rule out that the WW domain is responsible for this interaction, especially in light of the proline-rich N-terminal appendage present in certain isoforms of the ELC (see below). In many muscle and non-muscle tissues, there are two versions of the ELC, referred to as L17/L23 (for 17 and 23 kD) or MLC1/MLC3 (with MLC1 corresponding to the 23 kD isoform and MLC3 corresponding to the 17 kD variant).The MLC3nm gene is alternatively spliced and encodes 17 kD polypeptides that diverge at their C-terminus. The isoform containing exon 6 is expressed in smooth muscle, but exon 6 is absent in the mRNA found in non-muscle cells (57). Alternative splicing also accounts for the two tissue specific ELC versions present in chicken gizzard and non-muscle cells, respectively, as well as the two ELC isoforms expressed in fast skeletal muscle of a variety of vertebrate species (reviewed in ref. 59). The slow skeletal muscle ELC isoform MLC1sa, which is expressed in both skeletal and non-muscle tissues (apparently in contrast to the MLC1sb isoform, which is restricted in its synthesis to slow skeletal muscle), contains a 41 amino acid proline-rich extension at its N-terminus, similar to other MLC1 isoforms (59). These proline-rich segments, absent in the MLC3 alternatively spliced variants, mediate actin binding, and may be important for myosin function (see below for discussion).

To demonstrate specificity for ELC binding to IQGAP1, we performed a reconstitution experiment, comparing IGGAP1 and IQGAP2 for their ability to activate the β-galactosidase reporter gene in the presence of various prey vector constructs, including the MLC3nm cDNA-containing plasmid. The results are shown in Figure 2. Row 1 contains five controls, with B to E harboring plasmids encoding interacting proteins of increasing strength, and A corresponding to the two parent vectors (52). Each row beneath the control row represents 10 steaks derived from individual yeast colonies from a single co-transformation of two plasmids. The 10 streaks in each row should be identical in their behavior, but because of plasmid instability or mutation in the yeast, we frequently observe some heterogeneity, as seen in rows 4 and 7. Therefore, we routinely analyze five or more streaks to make determinations about interacting pairs. The truncated IQGAP1 bait activates the reporter gene (as demonstrated by the blue color) only in the presence of calmodulin or MLC3nm, (rows 4 and 5, respectively), but not when combined with an empty prey vector or HAX-1 (rows 2 and 3, respectively). HAX-1, a ubiquitously-expressed protein, was found to interact with a truncated IQGAP2 protein containing its WW domain and two N-terminal IQ motifs[4] (60). In contrast, a construct containing an IQGAP2 partial cDNA encomapassing its WW domain and two of four IQ motifs did not interact with the ELC (row 8), but did reconstitute β-galactosidase reporter gene activity in the presence of HAX-1 (row 7). These data argue that a truncated version of IQGAP1 can associate with the ELC in the context of the yeast two-hybrid system.

Experiments aimed at confirming this interaction in vitro were undertaken. A recombinant polyhistidine tagged form of the truncated IQGAP1 was produced in bacteria and purifed with a nickel affinity agarose resin. This protein was identical to the IQGAP1 polypeptide fused to the GAL4 DNA binding domain for use in the yeast two-hybrid experiments except for the N-terminal epitope tag. A commercially available preparation of bovine skeletal muscle myosin light chains was also used, and the contents of the recombinant IQGAP1 and the myosin light chains preparations are shown in lanes 1 and 2 of figure 3A, respectively. The light chains of approximate Mr 25 and 17 kD in lane 2 correspond to the two isoforms (L1 and L3) of the ELC, while the 20 kD polypeptide represents the regulatory light chain. Nickel-agarose beads were preincubated in the presence or absence of recombinant IQGAP1, and both bead suspensions were subsequently mixed with the myosin light chains in the presence of 0.5 mM EGTA, eliminating any effects of calcium on the binding (higher levels of EGTA would chelate the nickel ions). Lane 3 demonstrates that IQGAP1-nickel-agarose beads, when incubated with an excess of myosin light chains, is found associated in approximately a 1:1 molar ratio with a protein of 25 kD that matches the L1 ELC isoform, while lane 4 shows that in the absence of IQGAP1 preincubation, none of the myosin light chains binds, ruling out non-specific binding to the nickel-agarose resin. To confirm that the IQGAP1-associated protein corresponds to the myosin ELC, we performed a Western blot analysis, testing for reactivity of the proteins in lanes 1-3 of figure 3A, employing a mixture of myosin light chain antibodies that are immunoreactive with the L1 isoform of the ELC. As shown in figure 3B, the L1 myosin ELC isoform is detected both in the purified myosin light chain prep (lane 2) as well as in the IQGAP1-associated proteins (lane 3), but IQGAP1 itself (lane 1) does not react with the antibody mix. Because this antibody mixture does not recognize the ELC L3 isoform or the RLC, we can not definitively say that these two light chains have no affinity for the IQGAP1 polypeptide, and the yeast results suggest that the non-muscle MLC3nm variant also interacts with IQGAP1. However, the coomassie blue staining of the released proteins in lane 3 argue that the skeletal L1 ELC isoform appears to preferentially associate with IQGAP1 relative to the

[4] Weissbach, unpublished results.

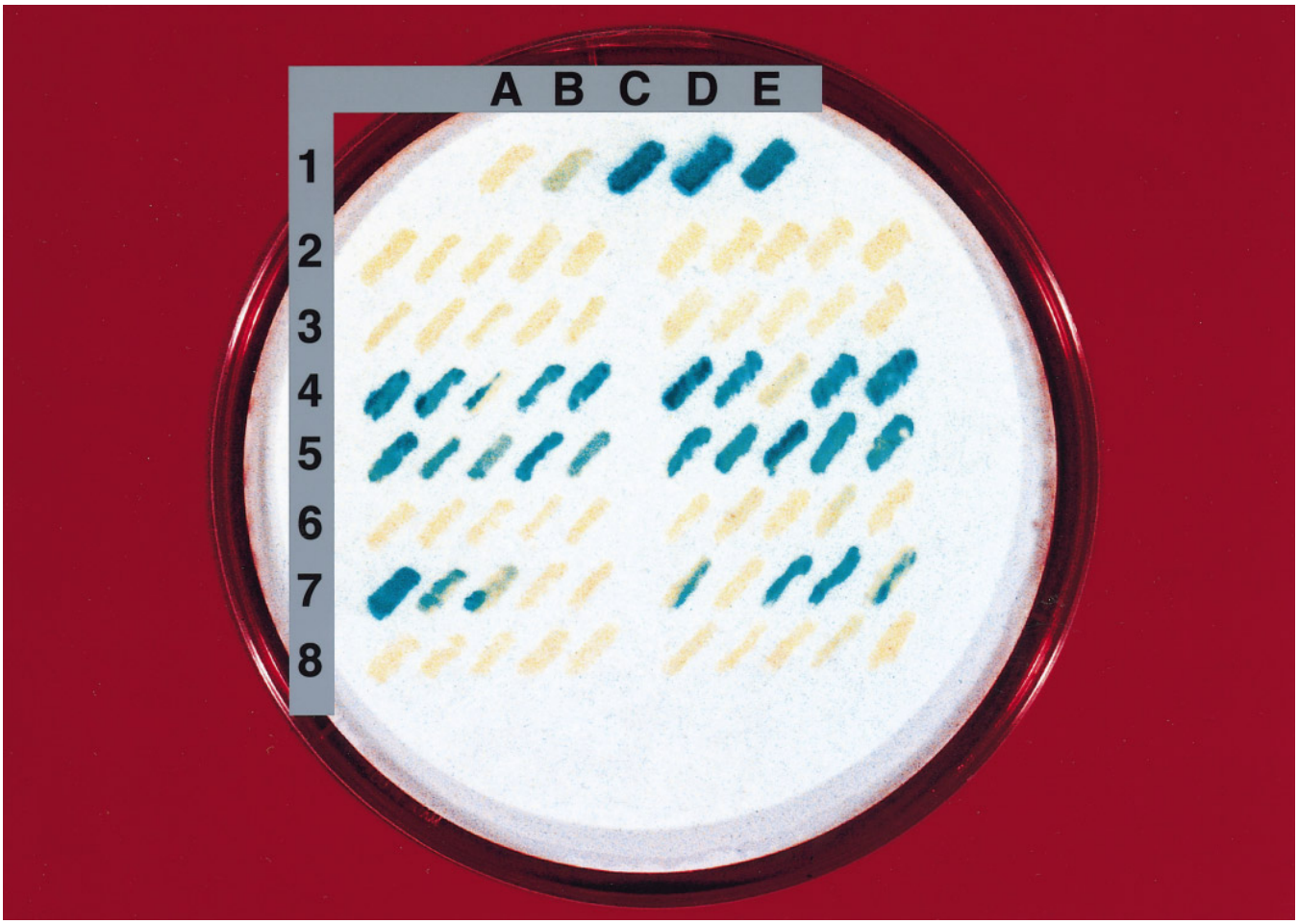

FIG. 2. Reconstitution of myosin ELC with truncated IQGAP1 and IQGAP2 by the yeast two-hybrid method. Bait vector containing either no cDNA insert (DB) or IQGAP1 cDNA encompassing the WW and four IQ domains (DB-IQ1) or IQGAP2 cDNA covering the WW and two IQ motifs (DB-IQ2) were co-transformed with different prey vector constructs into the MaV103 yeast strain, and an X-GAL overlay assay performed to detect protein-protein interaction. The prey constructs were: no cDNA insert (AD); plus HAX-1 cDNA (AD-HAX); plus calmodulin (AD-CAL); or plus myosin ELC (AD-ELC). Row 1: Five controls containing interacting protein pairs of different avidity (Vidal et al., 1996). From left to right: DB+AD (negative control); DB-pRB+AD-E2F1 (RB=retinoblastoma); DB-Fos+AD-Jun; Gal4+AD;DB-dDP1+AD-dE2F. Rows 2-8 contain 10 streaks/row of individual yeast transformants that should contain identical plasmid constructs. Row 2: DB-IQ1+AD; Row 3: DB-IQ1+AD-HAX; Row 4: DB-IQ1+AD-CAL; Row 5: DB-IQ1+AD-ELC; Row 6: DB-IQ2+AD; Row 7: DB-IQ2+AD-HAX; Row 8: DB-IQ2+AD-ELC.

skeletal muscle L3 isoform. These results demonstrate that a truncated version of IQGAP1 covering the WW domain and four IQ motifs is capable of specifically interacting with the skeletal L1 isoform of the myosin ELC in vitro.

The function of vertebrate myosin ELC has been studied in great detail, but for many years was the least understood of the three myosin subunits. Recent biochemical and genetic data have begun to link the ELC to specific effects on myosin functioning. The two myosin light chains, the ELC and the regulatory light chain (RLC), are structurally related to various calcium-binding proteins such as calmodulin and troponin C. These light chains bind to a hinge or regulatory region (also called neck region) located towards the C-terminus of the globular head area in the MHC which contains the motor domain, responsible for the ATPase and actin binding necessary for sliding of the myosin-laden thick filaments past the actin-containing thin filaments within the actomyosin complex (61). In skeletal muscle, the actin-activated MHC ATPase does not seem to rely on the presence of the two light chains (62-64). However, the light chains are thought to provide a scaffold that stabilizes an extended α-helix, which may be crucial for myosin's ability to generate a fully active power stroke and concomitant actin movement (65).

The fact that of the two light chains, the ELC is in closer proximity to the enzymatic active site within the motor domain, suggests that the ELC may have a greater impact on modulating conformational perturbations of myosin during filament movement. Recent

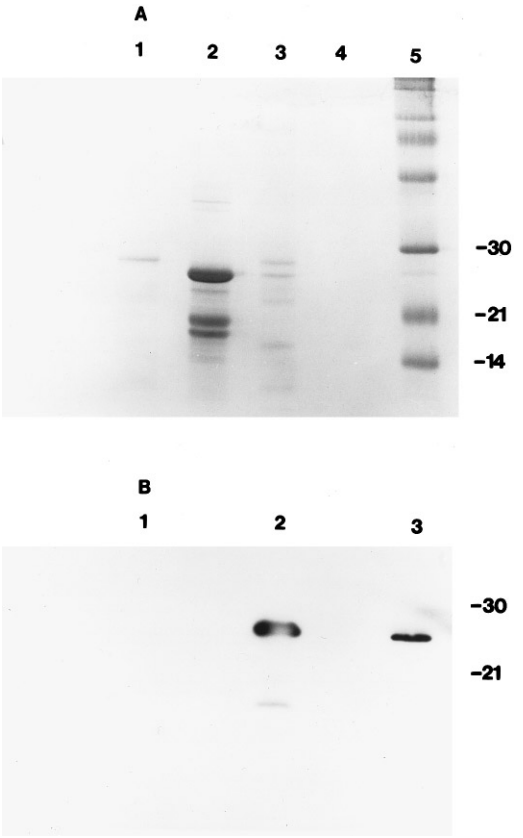

FIG. 3. In vitro binding studies with IQGAP1 and myosin ELC. Nickel-agarose beads were preincubated with either truncated IQGAP1 recombinant protein or buffer alone, and both bead suspensions were then incubated with an excess of myosin light chains. **(A)** Bound proteins were released from the beads and analyzed by SDS-PAGE and coomassie blue staining. Lane 1: Purified IQGAP1 recombinant protein; Lane 2: Purified myosin light chains, consisting primarily of the two ELC isoforms (17 and 25 kD) and the RLC (20 kD); Lane 3: proteins that bound the nickel-agarose beads preincubated with recombinant IQGAP polypeptide; Lane 4: proteins that bound the nickel-agarose beads preincubated with buffer. **(B)** Proteins electrophoresed in lanes 1-3 in (A) were transferred to PVDF membrane and probed with a mixuture of monoclonal antibodies that recognize skeletal muscle myosin ELC and visualized by a chemiluminescent procedure. Lanes 1-3 correspond to lanes 1-3 in (A).

studies have provided evidence that the ELC may in fact assist the propagation of myosin conformational alterations in response to ATP hydrolysis (66). Genetic data have reinforced the notion that the ELC is a critical component for myosin functioning. Various mutations in the cardiac β-myosin heavy chain situated in the vicinity of the ELC binding site have been linked to hypertrophic cardiomyopathy (HCM)(67). Moreover, a rare variation of HCM that affects both cardiac and skeletal muscle has been traced to mutations in either of the myosin light chains, thus strongly suggesting a connection betwwen the ELC, force generation, and myosin integrity (68). The effect of ELC on IQGAP1 activity is unclear at the present time, for IQGAP1 does not appear to be an ATPase , although it does bind to actin through its CH region. The potential effects of the ELC on the binding of actin, rac, and Cdc42 to IQGAP1 as well as on the ability of IQGAP1 to crosslink actin filaments need to be addressed. The observation that rho can modulate myosin activity through the action of a 164 kD Rho-assciated kinase (also called ROK) raises an additional signaling connection between myosin, Rho GTPases and their effectors (such as IQGAP1). Rho bound to GTP activates this serine/threonine kinase, which in turn inhibits myosin phosphatase but also triggers phosphorylation of the myosin RLC (69, 70). This sequence of reactions yields phosphorylated RLC, culminating in enhanced myosin ATPase activity (similar to the outcome carried out by the enzymatic activity of calmodulin-dependent myosin light chain kinase in smooth muscle and non-muscle cells)(71). The enhanced myosin ATPase activity promulgates muscle contraction in smooth muscle cells, and various actin-dependent movements in non-muscle cells (71, 72).

It may be noteworthy that the L1 rather than the L3 isoform of the ELC binds to IQGAP1 (figure 3), for the proline-rich N-terminal extension unique to this isoform is capable of mediating binding to the C-terminal region of actin (73-75). Paradoxically, the L1 ELC isoform has been linked to a decrease in actin filament movement (76-77). The exact mechanism underlying this inhibition is unclear, but could be due to a decreased actin-activated ATPase activity, a destabilization of the actin-myosin interface, or to a "clamping" of the actomyosin filament cross-bridges, resulting in impaired motion (76, 78-80). The implications of this binding for myosin and IQGAP1 activity are not clear, but the fact that both the MHC and IQGAP1, when complexed to the ELC, harbor multiple actin-binding domains (i.e., when included with the actin binding motifs in the N-terminal regions of these proteins), suggests a commonality of function.

Attempts to detect the ELC in complex with full-length IQGAP1 have been unsuccessful, employing co-immunoprecipitations from human non-muscle cells (Weissbach and Bernards, unpublished data). It seems unlikely that a conformational state unique to the full-length IQGAP1 can explain this result, for this procedure easily detects calmodulin in association with full-length IQGAP1, and the IQ motifs responsible for this binding are adjacent to the one presumably responsible for ELC binding. One possible explanation for this observation relates to the specificity of the antibodies, which were generated against chicken skeletal ELC. Although they recognize bovine muscle ELC (Figure 3), these antibodies may not cross-react with human non-muscle ELC. We plan to address this possibility by performing immunoprecipitations with a polyclonal antiserum that will be generated against human non-muscle ELC.

We speculate that under normal conditions, calmodulin binding, either to adjacent IQ motifs or to the myosin-like IQ segment itself in IQGAP1, may interfere with ELC binding to IQGAP1. Possibly, a signal that releases calmodulin from IQGAP1, such as phosphorylation, might allow the ELC to complex with IQGAP1, leading to as yet unknown effects on IQGAP1-mediated signaling. The ELC may be analogous to calmodulin in that it complexes with a variety of proteins, thereby affecting in some as yet undetermined manner a wide spectrum of intracellular signaling pathways.

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