Cerebral cavernous malformations (CCMs) affect 0.1–0.5% of the population resulting in leaky vasculature and severe neurological defects. KRIT1 (Krev interaction trapped-1) mutations associate with ~40% of familial CCMs. KRIT1 is an effector of Ras-related protein 1 (Rap1) GTPase. Rap1 relocalizes KRIT1 from microtubules to cell membranes to impact integrin activation, potentially important for CCM pathology. We report the 1.95 Å co-crystal structure of KRIT1 FERM domain in complex with Rap1. Rap1–KRIT1 interaction encompasses an extended surface that encompasses both Switch I and Switch II loops in Rap1 and F1 and F2 lobes of KRIT1 FERM domain. Point mutagenesis confirms the interaction. High similarity between KRIT1-F2/F3 and talin is revealed. Additionally, the mechanism for FERM domains acting as GTPase effectors is suggested. Finally, structure-based alignment of each lobe suggests classification of FERM domains as ERM-like and TMFK-like (talin-myosin-FAK-KRIT-like) and that FERM lobes resemble domain “modules.”

Cerebral cavernous malformation (CCM) disease is a neurovascular dysplasia found in 0.1–0.5% in the human population (1), with >1 million people estimated to be affected in the United States (2). The disease occurs as both familial and sporadic forms, but both result in leaky endothelial cell junctions and mulberry-shaped lesions that are prone to hemorrhage. Approximately 20% of strokes are hemorrhagic but are responsible for >half of stroke-related deaths (3). CCMs result in 50–70% lifetime risk of hemorrhage and other focal neurological defects including seizure and epilepsy (2). The first gene to be associated with CCM was KRIT1 (Krev interaction trapped 1; CCM1, cerebral cavernous malformation 1) (4), the other two associated genes are CCM2 and PDCD10 (2). In the familial form of CCM >40% of cases are associated with mutations in KRIT1, most truncating the protein KRIT1 (1). In these patients it is thought that acquisition of a “second-hit” acquired somatic mutation results in complete loss of functional KRIT1 and CCM onset (5, 6). Indeed, expression of KRIT1 is required for development, and its loss is incompatible with life (7), a finding that highlights the importance of understanding the molecular mechanisms of KRIT1 function.

There are three defined regions in the 736-amino acid protein KRIT1, an N-terminal NPXY motif-rich region, an ankyrin repeat domain, and a C-terminal band four-point-one, ezrin, radixin, moesin (FERM) domain (Fig. 1A). KRIT1 associates with microtubules (8), but on release from microtubules translocates to cell-cell junctions (9). At cell-cell junctions KRIT1 sequesters the integrin activation repressor, ICAP1 (integrin cytoplasmic-associated protein 1; ITGB1BP1, integrin β1-binding protein 1) (10) from β1 integrin cytoplasmic tails to allow integrin activation (11). The ICAP1-bound KRIT1 can translocate to the nucleus where it performs an unknown role (12–14). There is now substantial evidence that the key mechanism that allows release of KRIT1 from microtubules and translocation to the membrane is direct interaction of the KRIT1 FERM domain with the small GTPase Rap1 (10) (Fig. 1B). Indeed, KRIT1 was originally named Krev-Rap1 interaction trapped 1 because it binds Rap1 (also known as Krev-1) (15), a finding that was controversial for a time (1, 11), but which has since been validated by multiple groups (9, 10, 16, 17).

Rap1 is a Ras-like small G protein with two isoforms (A and B) of ~95% sequence identity. As is typical for small G proteins, Rap1 cycles between an active GTP-loaded form and an inac-
Co-crystal Structure of Rap1 in Complex with KRIT1

FIGURE 1. Schematic and overall structure of KRIT1 in complex with Rap1. A, schematic domain diagram of KRIT1 and Rap1. KRIT1 contains a N-terminal region of unknown fold followed by three NPXY/F motifs, an ankyrin repeat domain, and a FERM domain. In this paper we show that Rap1 interacts with the F1 and F2 lobes of the KRIT1 FERM domain. B, schematic diagram illustrating that the function of Rap1 binding to KRIT1 is to relocalize KRIT1 from microtubules to the membrane. C, ribbon diagram showing the crystal structure of KRIT1 in complex with Rap1B. The KRIT1 FERM domain lobes F1, F2, and F3 are colored pink through purple. Rap1 is colored green. Bound GTP$\gamma$S is shown in stick format. Structure figures are generated using CCP4 mg (59).

dograms for the FERM domain lobes, which suggest the FERM lobes resemble protein domain “modules.”

EXPERIMENTAL PROCEDURES

Expression and Purification of Rap1 and KRIT1—Human KRIT1 FERM domain (420–736) was subcloned into pGEX6p-1 expression vector and then overexpressed in BL21(DE3) Escherichia coli. Protein expression was induced by 0.2 mM isopropyl 1-thio-β-d-galactopyranoside at 16 °C overnight. Cells were then harvested at 4 °C and lysed in lysis buffer (20 mM Tris, 500 mM NaCl, 20 mM imidazole, 10% glycerol, pH 8.0) by freeze/thaw using dry ice and ethanol and warm water followed by three cycles of sonication on ice. The supernatant was then loaded onto glutathione-Sepharose 4B beads (GE Healthcare) and cleaved with PreScission protease overnight at 4 °C on beads. Eluted proteins were then loaded onto a Resource-S ion-exchange chromatography column (GE Healthcare) with 20 mM Hapes, pH 7.0, and eluted over a 5–500 mM NaCl gradient. The fractions were then further purified by size-exclusion chromatography on a Superdex 200 column (GE Healthcare) using 20 mM Hapes, pH 7.0, buffer. Human Rap1B cDNA was purchased online from Thermo Scientific Open Bio-systems. Rap1B(1–167) was subcloned into a modified pET-32 vector with an N-terminal His$_6$ tag and a tobacco etch virus cleavage site. Mutation of Gly-12 to valine was conducted using the QuikChange Lightning site-directed mutagenesis kit (Stratagene). Recombinant Rap1-G12V protein was overexpressed in E. coli Rosetta (Novagen) host cells by induction with 0.2 mM isopropyl 1-thio-β-d-galactopyranoside at 16 °C, $A_{600}$ 0.6. Cell pellets were resuspended in lysis buffer and then lysed by freeze/thaw followed by three cycles of sonication on ice. The supernatant was applied to HisTrap HP (GE Healthcare) initially and then eluted with 200 mM imidazole in lysis buffer. The fusion protein was cleaved by tobacco etch virus protease at 4 °C overnight dialyzing against dialysis buffer (20 mM Tris, 500 mM NaCl, 10% glycerol, pH 8.0). The sample was then reloaded onto over a HisTrap HP column to remove the uncleaved fusion proteins. The elution fractions were then concentrated and loaded onto a size-exclusion chromatography Superdex 75 column with 20 mM Hapes, pH 7.0, buffer. Proteins were then concentrated using Millipore concentrators. Both KRIT1 and Rap1B proteins were tested by SDS-polyacrylamide gels and found to be higher than 95% purity.

Co-crystallization of Rap1–KRIT1—The FERM domain of KRIT1 was concentrated to 15 mg/ml in 200 mM NaCl, 20 mM Hapes, pH 7.0, 2 mM DTT. G12V mutant Rap1 was concentrated to 10 mg/ml in 150 mM NaCl, 20 mM Hapes, pH 7.0, 1 mM DTT. The two proteins were then mixed by carefully evaluating the molar ratio as 1:1. The protein mixture was then incubated on ice for more than 2 h with 1 mM GTP$\gamma$S and 5 mM MgCl$_2$. Initial co-crystallization screens were performed using the JCSG+ sparse matrix crystallization kit (Qiagen). We observed initial hits in conditions containing 0.2 mM potassium nitrate, 20% PEG3350. Optimization was then performed by carefully screening the concentration of these two chemicals in the crystallization drop. The optimized KRIT1–Rap1 co-crystals were grown using hanging-drop methodology at room temperature.
(drop size, 2 μl). Crystals grew after 3 days in the crystallization conditions of 0.1–0.2 mM KNO₃, 15–20% PEG3350. Crystals were flash frozen in liquid nitrogen using the same crystallization conditions as cryoprotectant. Crystallographic data were collected at beamline 24-ID-C (NE-CAT) at Argonne National Laboratory, Advanced Photon Source. All data were processed using HKL2000 (22).

**Structure Determination**—The KRIT1-Rap1 complex co-crystallized into space group P2₁, with unit cell parameters of a = 57.5 Å, b = 78.0 Å, c = 59.8 Å, α = γ = 90°, β = 90.9°. We obtained initial phases by conducting molecular replacement using a crystal structure of the GTP-loaded form of Rap1 (Protein Data Bank (PDB) ID 1C1Y) (23) and the FERM domain of radixin (PDB ID 1G6C) (24). The Rap1 model was stripped of ligands, the radixin model was reduced to a polyalanine-serine/glycine trace for each of the subdomains, using the program Chainsaw (25), and GTPase and each subdomain (F1, F2, and F3) were used as molecular replacement search models. Phaser (26) yielded translation Z-scores of 17.1 for Rap1 and 6.5 and 5.9 for two of the subdomains (F2 and F3) of radixin. The solution was then input into ARP/wARP (27), and following 200 cycles 254 residues were built with 128 in sequence and R_{free} factors of 31.4 and 54.4%, respectively. This model was then input into the Phenix phase_and_build module (28) for two cycles, and 250 residues were built with 218 placed in sequence and R and R_{free} factors of 41.7 and 44.6%, respectively. This model was then input into ARP/wARP using parameters identical to those of the previous round (200 cycles, add atoms above 3.1 σ, remove atoms below 0.9 σ, density modification at the start of autobuilding). This time ARP/wARP built a total of 473 residues in sequence and yielded R and R_{free} factors of 24.8 and 30.9%, respectively. Standard refinement was then conducted in Refmac (29) and Phenix with TLS (28). Model building was conducted in COOT (30). The final model was validated by MolProbity (31). The final structure has been deposited in the Protein Data Bank under accession code 4DXA.

**KRIT1-Rap1 Pulldown Assays**—GST-KRIT1 and His-Rap1 were expressed in *E. coli* as described above. Glutathione-Sepharose 4B (GE Healthcare) resin was equilibrated in PBS with 1% Triton X-100 (PBST). 1.5 ml GST-KRIT1 lysate was added to 120 μl of equilibrated resin in a 2-ml microcentrifuge tube and then incubated at 4°C with shaking for 1 h. The resin bound with fusion protein was washed three times with 1 ml of PBST. 10 μg of pulldown resin was then incubated with 20 μg of purified protein in 400 μl of buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 5 mM MgCl₂, 1 mM GTPγS tetralithium salt) at 4°C with shaking for 1.5 h. After incubation, the resin was washed three times with 1 ml of buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Triton X-100). Following washing, 30 μl of 1× SDS sample buffer was added to the resin, and samples were heated to 95°C for 5 min. The samples were then briefly centrifuged and loaded on a 15% SDS-polyacrylamide gel. The expression, purification, and pulldown protocols were identical for all KRIT1 and Rap1 mutant proteins. Mutations were generated using the QuickChange site-directed mutagenesis kit (Agilent Technologies).

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**RESULTS AND DISCUSSION**

**Overall Structure**

To determine the co-crystal structure of Rap1 GTPase and KRIT1 FERM domain, we expressed and purified Rap1B-G12V (residues 1–167) and KRIT1 (residues 420–736) in the *E. coli* expression system as N-terminally tagged His, and GST fusion proteins, respectively. Following proteolytic removal of the affinity tags and separate purification, the two proteins were mixed and co-crystallized together. We determined the 1.95 Å resolution co-crystal structure of KRIT1 in complex with Rap1 (Fig. 1C and Table 1).

**Structure of Rap1**

In the co-crystal structure of KRIT1 FERM domain in complex with Rap1B-G12V, the structure of Rap1 is highly similar to those of other monomeric GTPases in the PDB. The Rap1 structure includes the G-domain, G3, and G1, and these domains are highly similar to those of other monomeric GTPases. The G-domain contains the GTP-binding site, and the G3 and G1 domains are involved in the activation and GDP/GTP exchange reactions, respectively. The structure of Rap1 also shows that the activation of Rap1 is regulated by GTP binding, and the G-domain is involved in the activation of Rap1. The Rap1 structure also shows that the G-domain is highly similar to those of other monomeric GTPases, and the G3 and G1 domains are involved in the activation and GDP/GTP exchange reactions, respectively. The structure of Rap1 also shows that the G-domain is involved in the activation of Rap1, and the G3 and G1 domains are involved in the activation and GDP/GTP exchange reactions, respectively.
Co-crystal Structure of Rap1 in Complex with KRIT1

Co-crystal Structure of Rap1 in Complex with KRIT1

to previously determined active-state Rap1 structures, an expected result because the G12V mutation in Rap1 is a mutation that results in an activated GTPase. The structure superposes with an r.m.s.d. between 0.7 and 0.9 Å over 167 aligned residues (36) with the active-state Rap1 structures of Rap1 in complex with RAF-RBD (PDB IDs 1C1Y, 1GUA, and 3KUC) (23, 37, 38) and Rap1 in complex with RapGAP (PDB ID 3BRW) (39). Good electron density is observed throughout the structure, and the GTPyS moiety binds in the manner expected for this nonhydrolyzable nucleotide. The regions of Rap1 with highest B-factors are the Switch I and Switch II loops. This illustrates the conformational flexibility inherent in these important functional parts of the protein.

Structure of KRIT1

KRIT1 is expected to contain a band 4.1, ezrin, radixin, moesin (FERM) domain at the C terminus (40). Our structure confirms this and reveals that, as in other FERM domain structures, the KRIT1 has three subdomains, F1, F2, and F3 (Fig. 1C). These lobes fold in similar fashion to ubiquitin-like (F1, residues 420–514), acyl-CoA-binding protein (F2, residues 515–635), and phosphotyrosine binding/pleckstrin homology (F3, residues 636–729) domains (41). From the crystal structure we find that KRIT1 FERM domain is most similar to myosin-X FERM domain, with a Dali Z-score of 23.3, r.m.s.d. of 4.2 Å over 273 aligned residues, and a sequence identity of 14% (36). Indeed, the top 53 hits from the Dali server are FERM domains from myosin-X, band 4.1, radixin, merlin, ezrin, moesin, and FAK. These display a range of Dali Z-scores between 15.9 and 23.3 and r.m.s.d. values between 4.0 and 7.2 Å, over 221–273 aligned residues, with sequence identities between 12% (FAK) and 21% (radixin). The KRIT1 FERM domain does not display the extended conformation observed recently for talin (42), but instead folds as a classical trefoil FERM domain.

Interaction of Rap1 and KRIT1

The KRIT1 FERM domain can interact with the KRIT1 N terminus (10, 43), the Heart-of-Glass adhesion receptor (44), and Rap1 (9, 16, 17). The KRIT1-Rap1 interaction described here is the first of these to be investigated crystallographically. Broadly speaking, this interaction utilizes the whole Rap1 Switch region (Switches I and II) to interact with sites on both the F1 and F2 lobes of KRIT1. The interaction is in the plane of the FERM domain trefoil, thus creating a diamond shape, or four-leaved clover. A total of 1750 Å² (849 Å² in KRIT1 and 901 Å² in Rap1) of surface area is buried, with ~1100 Å² (~63%) being contributed by the Rap1-F1 interaction and ~650 Å² (37%) being contributed by the Rap1-F2 interaction (Fig. 2A) (as defined by the PISA server) (45). The shape complementarity of the KRIT1-Rap1 interaction is 0.66 (46). We suggest that the intricate and extended nature of the Rap1-KRIT1 interaction may be the reason for the reported unusual specificity of KRIT1 for Rap1 but not for Ras (9, 47). Interestingly, analysis by the PISA server finds no examples of similar GTPase domain interactions with acyl-CoA-binding protein (F2)-like fold proteins. As described below, GTPase interactions with ubiquitin (F1)-like fold proteins are frequently observed and can be compared with the KRIT1-Rap1 complex.

In detail, the interaction of Rap1 with KRIT1 encompasses 11 hydrogen-bonding interactions and 90 nonbonded interactions (PDBsum) (48). These interactions cluster into two sites that we term the “Switch” site and the “Distal” site (Fig. 2B). The Switch site buries a greater surface area and encompasses both Rap1 Switch I and Switch II, and regions from both KRIT1 F1 and F2 lobes. The Distal site is smaller and is distal from the GTP binding site in Rap1, and it encompasses residues from the Rap1 β2-turn-β3 hairpin and KRIT1 F2 (Fig. 2C). We mapped sequence conservation of KRIT1 over 29 species onto the surface of KRIT1 FERM domain using the CONSURF server (49). It is striking that the surface of KRIT1, which interacts with Rap1, is almost completely conserved through evolution (Figs. 2D and 3). Furthermore, this complete surface conservation is contiguous between both the Switch and Distal sites and extends past these regions.

The Switch site can be broadly subdivided into three interconnecting regions that encompass Switch I-F2 interactions, the β-sheet, and Switch II-F1 interactions (Fig. 4, A and B).

Switch I-F2—At the N terminus of the Rap1 Switch I loop a broadly hydrophobic interaction is found centered around Ile-25RAP which stacks against KRIT1 F2 residues Pro-525KRIT and Leu-526KRIT. This surface is extended to the aliphatic region of Gln-25RAP which interacts with Leu-526KRIT. Gln-25RAP also hydrogen bonds to Arg-452KRIT, one of two KRIT1 arginine residues critical for the Rap1-KRIT1 complex.

β-Sheet Region—The second critical arginine residue, Arg-432KRIT, hydrogen bonds to the backbone carboxyl of Switch I residue Pro-34RAP. It also takes part in an intricate hydrophobic bonding network between Arg-432KRIT, Arg-452KRIT, Asp-38RAP, Tyr-40RAP, and multiple water molecules (Fig. 4A).

Switch II-F1—For the Rap1 Switch II loop, hydrophobic interactions are observed between Met-67RAP and Lys-421KRIT, and Phe-64RAP and Phe-419KRIT. We note that although Phe-419KRIT is vector-derived, it probably packs in a fashion similar to the wild-type KRIT1 residue, which is a tyrosine (Fig. 4A).

Distal site—The Distal site is centered at the F2 lobe residue Tyr-563KRIT. Tyr-563KRIT hydrogen bonds to Gln-43RAP and stacks against Gln-50RAP. These residues are at the C terminus of the β2 strand and the N terminus of the β3 strand in Rap1. An intricate water-mediated hydrogen bonding network is formed between these residues, Gln-45RAP and the C terminus of KRIT1 helix α8 (Fig. 4A).

KRIT1-Rap1 Pulldown Assays

To validate the crystallographically observed interaction, we generated point mutations in KRIT1 and Rap1 and conducted pulldown assays. We generated KRIT1 mutations S430E,
R432E, and R452E, which we expected to compromise the interaction with Rap1, and Rap1 mutations Q25A and D38A, which we expected to compromise the interaction with KRIT1 (Fig. 4B). We also generated mutations outside of the binding interface, which we expected not to alter KRIT1-Rap1 binding but which are crystal-packing interactions in the structure (R501E in KRIT1 and Q137R in Rap1). We found that the KRIT1 mutations S430E, R432E, and R452E did indeed compromise the interaction with Rap1 (Fig. 4C) and that the Rap1 mutations Q25A and D38A compromised the interaction with KRIT1 (Fig. 4D). The control mutations did not affect the interaction by pulldown. These pulldown assays therefore confirm the interaction surface between KRIT1 and Rap1 and confirm the previous finding that R452E mutation in KRIT1 compromises KRIT1-Rap1 interaction by reducing the binding $K_d$ from 1.8 μM to 67 μM (9).

Comparison of Rap1-KRIT1 Complex with Other GTPase Complex Structures

The Rap1 GTPase domain interacts with the F1 and F2 lobes of KRIT1. These lobes fold in the manner of ubiquitin-like (F1) and acyl-CoA-binding protein (F2) domains. Because ubiquitin-like fold proteins are frequently shown to interact with GTPases, we investigated whether the mode of binding between the KRIT1 F1 lobe and Rap1 was similar to that observed previously for ubiquitin-like fold GTPase-binding proteins. Indeed, as was first observed for Raf in complex with Rap1 (23), the ubiquitin fold β sheet of KRIT1 F1 extends through the GTPase Switch I loop in an antiparallel fashion (Fig. 5). The F1-Rap1 interaction is the larger surface, but shows lower shape complementarity (0.64) than the F2-Rap1 interaction (0.69) (46). We do not find example crystal structures in the

FIGURE 2. Overview of KRIT1-Rap1 interaction surface. A, surface of KRIT1-Rap1 complex. Residues that interact are colored purple (KRIT1) and green (Rap1). B, open book format for A. The locations of KRIT1 F1 and F2 lobes are indicated. The Switch and Distal sites are labeled. C, same orientation as B in schematic format. Helices and strands that are important for the interaction interface are labeled. GTPγS is shown in stick format. The Switch I and Switch II loops of Rap1 are indicated. D, surface conservation for KRIT1. Dark blue indicates complete conservation. The F1-F2 surface of KRIT1 is completely conserved over 29 species. The three images are rotated through 90° and 180°.
Co-crystal Structure of Rap1 in Complex with KRIT1
Co-crystal Structure of Rap1 in Complex with KRIT1

**FIGURE 3. Sequence alignment for KRIT1.** Secondary structure is shown as cylinders for α helices and arrows for β strands. Rap1-binding residues are indicated with green triangles. Junctions of the F1–F2 and F2–F3 lobes of the KRIT1 FERM domain are indicated. Uniprot or GenBank accession number are followed by the protein data bank of F2 (acyl-CoA-binding protein)-like fold proteins interacting with GTPases, therefore we believe that the Rap1–F2 interaction defines a new GTPase interaction surface. Because KRIT1 displays unusual specificity for Rap1 over Ras (9, 47) we propose that the mode of interaction between Rap1 and KRIT1 represents a multidentate mechanism for GTPases to obtain high effector specificity.

**Comparison of KRIT1 FERM Subdomains**

As discussed above, the KRIT1 FERM domain is most similar to myosin-X; however, to investigate whether the subdomains of KRIT1 are similar to other FERM domain lobes we submitted F1, F2, and F3 individually to the Dali server. Similar to the whole KRIT1 FERM domain, the F1 lobe has highest structural
Similar results were obtained using GST-tagged specific interaction between KRIT1 and integrin similarities between KRIT1 and talin, we could not detect a described protocols (32, 33). However, despite the structural His-tagged integrin cytoplasmic tail constructs and previously recombinant KRIT1 FERM, well characterized recombinant FERM domain using F1 and F2 lobes. Because Rap1 has previously been shown to release KRIT1 from microtubules (10), the finding that KRIT1 is extremely conserved on the contiguous surfaces of the F1 and F2 lobes (Fig. 2D) potentially makes sense from an evolutionary standpoint; it is harder for the sequence of a protein to wander through evolution if there is a requirement to bind multiple partners. We therefore propose that the F1 and F2 surface is utilized by multiple binding partners, including Rap1, and that Rap1 directly competes with a binding partner that localizes KRIT1 to the microtubule machinery.

FERM domain intermolecular recognition and targeting have predominantly been shown to be mediated by the F3 lobe, as illustrated for radixin (Fig. 6A). In contrast, intramolecular mechanisms of regulation for FERM domains so far seem to be mediated by the F1 and F2 lobes, as illustrated for FAK (52) and moesin (53) (Fig. 6A). The KRIT1-Rap1 complex represents the first description of intermolecular recognition by a FERM domain using F1 and F2 lobes. Because Rap1 has previously been shown to release KRIT1 from microtubules (10), the finding that KRIT1 is extremely conserved on the contiguous surfaces of the F1 and F2 lobes (Fig. 2D) potentially makes sense from an evolutionary standpoint; it is harder for the sequence of a protein to wander through evolution if there is a requirement to bind multiple partners. We therefore propose that the F1 and F2 surface is utilized by multiple binding partners, including Rap1, and that Rap1 directly competes with a binding partner that localizes KRIT1 to the microtubule machinery.

FERM domain interactions with GTPases may be more common than previously thought. Recent work has shown that several sorting nexin endosomal trafficking proteins (SNX17, SNX27, and SNX31) contain FERM domains. These FERM domains can bind activated Ras in vitro and may be involved in endosomal Ras signaling processes (54). Furthermore, the atypical FERM domain of talin has also been shown to bind GTPases by its F0 lobe, a ubiquitin-like fold domain similar to the F1 lobe (55). We therefore propose that the KRIT1-Rap1 interaction represents a template to understand FERM domain containing protein-GTPase interactions. It is interesting to note that the KRIT1-Rap1 interaction surface significantly overlaps with the FAK kinase-FERM autoinhibitory interaction surface (Fig. 6A) (52). Demonstration of this mode of FERM-GTPase domain
interaction raises the possibility that GTPases may play undiscovered functionally important roles in FDCP conformational rearrangements, which could impact regulation of the FERM domain-containing enzymes. It will therefore be interesting to discover whether FERM domain-containing kinases, (including the JAKs, FAK, and PYK2) and FERM domain-containing phosphatases (including PTPN3, PTPN4, and PTPN13) can bind and be regulated by GTPases.

**Considerations for FERM Domain Classification**

Primary sequence alignment analysis has classified FDCPs into three subgroups named for their prominent members: kindlin and talin; ERM proteins, GEFs, kinases, and phosphatases; and myosin and KRIT1 proteins (56). Based on our structure-based Dali analysis that shows highest similarity to myosin-X, the overall structure of KRIT1 would support the previous classification. In contrast, however, our structure-based analysis of the F2 and F3 sublobes of KRIT1 supports the notion that KRIT1 could potentially also be included in the talin and kindlin FDCP group or may represent a crossover FDCP with similarities to both talin/kindlin and to myosin. To investigate the classification of FERM domains further, we therefore constructed structure-based cladograms using each of the F1, F2, and F3 lobes for examples of all FERM domains that exist in the PDB (Fig. 6B). The cladograms suggest two groups of FERM domain in the current structural library, which we term ERM-like and TMFK-like (talin-myosin-FAK-KRIT1-like). The pairwise similarity of the TMFK-like FERMs varies per lobe. For example, talin clusters with FAK in the F1 cladogram, but with myosin-VII when analyzing F2 and F3. Although these results are somewhat different from the previous classification based only on primary sequence for whole FERM domains (56), together both studies raise the interesting prospect that FERM domain lobes resemble domain modules within the FDCP family of proteins. Structure-based classification of the FERM domain lobes may be particularly useful as the functional similarities and differences of FERM domains are dissected.

**CONCLUSIONS**

The loss of KRIT1 is associated with acquisition of CCMs and is incompatible with life (7). The functions of this protein are, however, only now becoming understood. One of these functions is to provide a binding partner and sink for ICAP1; KRIT1 sequesters this regulator of integrin activation away from integrin β1 tails, thus allowing talin- and kindlin-mediated integrin activation (57). Clearly, the localization of KRIT1 to the membrane must therefore be under tight regulation to maintain proper balances of integrin activation. The small GTPase Rap1 has been suggested to be the primary player in relocalizing KRIT1 from microtubules to the membrane (9). Interestingly, Rap1 also interacts with RIAM (Rap1-GTP-interacting adaptor molecule). As one of the roles of RIAM is to attract talin to integrin tails to facilitate integrin activation (58), an elegant system is now revealing itself. In this model, Rap1 facilitates KRIT1 relocalization to the membrane where it releases an integrin suppressor, ICAP1, from binding integrin cytoplasmic tails, concomitantly Rap1 binds RIAM which recruits talin to integrin. Thus, Rap1 plays a key role in regulating proper spatio-temporal regulation of β1 integrin activation, and to do this it requires its binding partner, KRIT1. The mechanisms revealed here for Rap1 and KRIT1 interaction are therefore an important step toward understanding molecular basis for this elegant system for integrin regulation.

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