The adhesion modulation domain of 
*Caenorhabditis elegans* α-catenin regulates actin binding during morphogenesis

Xiangqiang Shao,a,b,c,d,* Bethany Lucasb, Jared Strauchc, and Jeff Hardinda,b,c,*

aProgram in Genetics and bDepartment of Integrative Biology, University of Wisconsin–Madison, Madison, WI 53706; cDepartment of Biology, Regis University, Denver, CO 80221

**ABSTRACT** Maintaining tissue integrity during epidermal morphogenesis depends on α-catenin, which connects the cadherin complex to F-actin. We show that the adhesion modulation domain (AMD) of *Caenorhabditis elegans* HMP-1/α-catenin regulates its F-actin-binding activity and organization of junctional–proximal actin in vivo. Deleting the AMD increases F-actin binding in vitro and leads to excess actin recruitment to adherens junctions in vivo. Reducing actin binding through a compensatory mutation in the C-terminus leads to improved function. Based on the effects of phosphomimetic and nonphosphorylatable mutations, phosphorylation of SS09, within the AMD, may regulate F-actin binding. Taken together, these data establish a novel role for the AMD in regulating the actin-binding ability of an α-catenin and its proper function during epithelial morphogenesis.

**INTRODUCTION** Stable intercellular adhesions mediated by a highly conserved cadherin–catenin complex (CCC) play key roles in maintaining tissue integrity during metazoan embryonic development (Harris and Tepass, 2010; Takeichi, 2014). The intracellular tail of cadherin binds to β-catenin (Ozawa et al., 1989); α-catenin binds β-catenin and physically links the CCC at the membrane to the F-actin cytoskeleton (Pokutta and Weis, 2000; Pokutta et al., 2002; Buckley et al., 2014). α-Catenin is an actin-binding and -bundling protein with three vinculin homology domains (Herrenknecht et al., 1991). The N-termini of mammalian αE- and αN-catenin contain overlapping β-catenin-binding and homodimerization sites (Pokutta and Weis, 2000; Pokutta et al., 2014); the C-terminus contains the actin-binding domain (ABD; Imamura et al., 1999; Pokutta et al., 2002). αE-Catenin is regulated in several ways: 1) αE-catenin homodimers have a higher affinity for F-actin than do αE-catenin–β-catenin heterodimers (Drees et al., 2005; Rangarajan and Izard, 2013); 2) the affinity of αE-catenin for F-actin is regulated by whether the actin is under tension (Buckley et al., 2014); 3) the first α-helical region of the ABD may regulate the affinity of the ABD for F-actin (Ishiyama et al., 2018); and 4) the middle (M) domain, composed of three helical bundles designated M1, M2, and M3 (Ishiyama et al., 2013) contains a vinculin-binding site (Watabe-Uchida et al., 1998; Choi et al., 2012) buried by M2 and M3 when αE-catenin is not under tension (Yonemura et al., 2010; Choi et al., 2012; Rangarajan and Izard, 2012; Ishiyama et al., 2013; Thomas et al., 2013).

Further functional investigation of the M domain, especially in vivo, is warranted. The functional importance of vinculin binding remains unclear, because deletion of the relevant region within M1 does not lead to striking changes in cultured cells (Huveneers et al., 2012), and constructs lacking the VH2 domain in *Drosophila* E-catenin AMD (Izard, 2012; Ishiyama et al., 2013). We decided to focus on the adhesion modulation domain (AMD), comprising residues 509–643 of αE-catenin, which correspond to the M3 domain plus the linker region between M3 and the ABD (Figure 1A). The αE-catenin AMD supports lateral clustering of cadherins and nascent junction formation in cultured cells (Imamura et al., 1999) and it serves as a docking...
Caenorhabditis elegans provides a unique model to study the function of the AMD in vivo. C. elegans is amenable to genetic manipulation, visualization of morphogenetic movements, and it has single conserved homologues of core CCC components (Costa et al., 1998; Pettitt et al., 2003). HMP-1/α-catenin possesses a canonical M
domain (Kang et al., 2017), but is incapable of forming homodimers (Kwiatkowski et al., 2010; Callaci et al., 2015; Kang et al., 2017).

We show here that the AMD negatively regulates F-actin binding of HMP-1 and that S509 within the AMD, which is endogenously phosphorylated (Callaci et al., 2015), is important for proper function of HMP-1 during embryonic development. We also show that the N-terminus of the HMP-1 ABD is required for its full actin-binding ability. These results identify novel roles for the AMD and the adjacent C-terminal region in a biologically relevant context.

RESULTS AND DISCUSSION

The HMP-1 AMD negatively regulates F-actin binding

We sought an internal domain within HMP-1 that might be responsible for negative regulation of the full-length protein. To this end, we generated a series of internal deletions of recombinant SUMO::HMP-1 and used these in actin cosedimentation experiments (Figure 1B). Consistent with our previous findings (Kang et al., 2017), full-length recombinant HMP-1 (HMP-1FL) cosedimented with F-actin. Surprisingly, however, it did so to a markedly lesser extent than the C-terminus alone (Figure 1, C and D). Significantly, deletion of the AMD increased F-actin binding to nearly the same level as the isolated HMP-1 C-terminus, indicating that the AMD is necessary for negative regulation of full-length HMP-1 (Figure 1, C and D). We next tested the sufficiency of the AMD for negative regulation. HMP-1 constructs lacking the N-terminus and the N-terminal portion of the M domain but retaining the AMD (aa504–927) exhibited F-actin binding comparable to HMP-1FL (Figure 1E); thus, the AMD appears to be necessary and sufficient to confer intramolecular autoinhibition on the HMP-1 C-terminus.

The HMP-1 AMD is important for normal F-actin recruitment to adherens junctions

That the AMD regulated HMP-1 binding to F-actin in vitro led us to investigate the role of the AMD in vivo. Previous studies used a deletion allele, hmp-1(zu278), which encodes a protein that retains part of the N-terminus and M domain of HMP-1 (Costa et al., 1998; Kwiatkowski et al., 2010; Maiden et al., 2013). To avoid possible complexities in analysis, we generated a new null mutant allele, hmp-1(jc48), via CRISPR (Supplemental Figure S1), and assessed the ability of transgenes encoding various GFP-tagged HMP-1 deletions to rescue lethality in hmp-1(jc48) homozygotes. Expression levels of rescuing transgenes were found to be essentially identical (Supplemental Figure S2).

Full-length HMP-1::GFP fully rescues hmp-1(jc48) mutants (Figure 2A); rescued mutants develop indistinguishably from wild type. We next investigated the function of HMP-1::GFP lacking the AMD. HMP-1ΔAMD::GFP localized to junctions in a manner indistinguishable from full-length HMP-1::GFP (Figure 1, C and D). We next tested the sufficiency of the AMD for negative regulation. HMP-1 constructs lacking the N-terminus and the N-terminal portion of the M domain but retaining the AMD (aa504–927) exhibited F-actin binding comparable to HMP-1FL (Figure 1E); thus, the AMD appears to be necessary and sufficient to confer intramolecular autoinhibition on the HMP-1 C-terminus.

The HMP-1 AMD is important for normal F-actin recruitment to adherens junctions

That the AMD regulated HMP-1 binding to F-actin in vitro led us to investigate the role of the AMD in vivo. Previous studies used a deletion allele, hmp-1(zu278), which encodes a protein that retains part of the N-terminus and M domain of HMP-1 (Costa et al., 1998; Kwiatkowski et al., 2010; Maiden et al., 2013). To avoid possible complexities in analysis, we generated a new null mutant allele, hmp-1(jc48), via CRISPR (Supplemental Figure S1), and assessed the ability of transgenes encoding various GFP-tagged HMP-1 deletions to rescue lethality in hmp-1(jc48) homozygotes. Expression levels of rescuing transgenes were found to be essentially identical (Supplemental Figure S2).

Full-length HMP-1::GFP fully rescues hmp-1(jc48) mutants (Figure 2A); rescued mutants develop indistinguishably from wild type. We next investigated the function of HMP-1::GFP lacking the AMD. HMP-1ΔAMD::GFP localized to junctions in a manner indistinguishable from full-length HMP-1::GFP in a wild-type background,
indicating that an intact N-terminal HMP-2/β-catenin-binding domain (Shao et al., 2017) is sufficient to target HMP-1ΔAMD::GFP to junctions. HMP-1ΔAMD::GFP rescues hmp-1(jc48) mutants, although poorly relative to HMP-1FL::GFP. Embryonic lethality in hmp-1(jc48);Ex[hmp-1::gfp] is 52.5%, compared with 88.3% for hmp-1(jc48);Ex[hmp-1ΔAMD::gfp]; given that transmission of the extrachromosomal arrays in each case is 52.1 and 53%, respectively, this indicates virtually 100% efficiency of rescue by full-length HMP-1::GFP, but only 23% rescue efficiency in the case of HMP-1ΔAMD::GFP. These results suggest that the AMD is important for the normal function of HMP-1.

To assess how the AMD regulates F-actin recruitment in vivo, we performed phalloidin staining (Figure 2, A–D). The width of the junctional–proximal actin network was significantly greater in hmp-1(jc48);Ex[hmp-1ΔAMD::gfp] than in hmp-1(jc48);Ex[hmp-1::gfp] embryos (842.4 ± 64.27 nm, n = 6 vs. 441.8 ± 29.82 nm, n = 4 embryos; significantly different, p < 0.01, Student’s t test). Excessive recruitment of F-actin to adherens junctions by HMP-1ΔAMD::GFP in vivo is consistent with the increased ability of recombinant SUMO::HMP-1ΔAMD to cosediment with F-actin in vitro compared with HMP-1FL.

Unlike HMP-1FL::GFP, which presumably undergoes dynamic, regulated binding to F-actin, the HMP-1ΔAMD::GFP may bind to F-actin in a constitutively active manner, explaining its weak rescue ability. To test this possibility, we introduced an additional mutation (S823F; Maiden et al., 2013) into the HMP-1ΔAMD::GFP construct to slightly reduce its ability to bind F-actin. HMP-1ΔAMD(S823F)::GFP localized to junctions in a manner indistinguishable from HMP-1FL::GFP in a wild-type background (Supplemental Figure S3A). There was only a slight difference in the ability of HMP-1ΔAMD(S823F)::GFP to rescue hmp-1(jc48) than HMP-1ΔAMD::GFP (embryonic lethality of hmp-1(jc48);Ex[hmp-1ΔAMD::gfp] worms is 88.3%, compared with 78.4% for hmp-1(jc48);Ex[hmp-1ΔAMD(S823F)::gfp]; p < 0.01, Fisher’s exact test), despite ~50% array transmission in each case. However, we found a marked difference in the relative proportions of embryos that died early (Figure 3, A and B). Dead hmp-1(jc48);Ex[hmp-1::gfp] embryos tended to die at earlier stages of morphogenesis, with the majority arresting at the 1.5-fold stage or earlier with ruptures; only a small percentage survived beyond the twofold stage. In contrast, the majority of the dead hmp-1(jc48);Ex[hmp-1ΔAMD(S823F)::gfp] embryos were able to survive to a later stage, with most arresting at the three- to fourfold stage (Figure 3B). Moreover, less HMP-1ΔAMD(S823F) recombinant protein cosedimented with F-actin in vitro than with HMP-1ΔAMD (Figure 3C and Supplemental Figure S4B). Taken together, these results suggest that deleting the HMP-1 AMD leads to higher binding of F-actin and

FIGURE 3: The S823F mutation ameliorates the effects of deletion of the HMP-1 AMD. (A) Differential interference contrast (DIC) images of representative embryos used in scoring rescue of morphogenetic defects. Early rupture; the arrow shows extruded anterior cells. Expansion at the 1.5-fold stage; the arrow points to the ruptured anterior region. Head explosion at the twofold stage; the arrow indicates the site of rupture. Arrest at the three- to fourfold stage with body morphology defects; the arrow points to a rupture in the posterior part of the embryo. Scale bar = 10 µm. (B) Scoring of stage of arrest of dead hmp-1(jc48) mutants expressing HMP-1ΔAMD::GFP or HMP-1ΔAMD(S823F)::GFP. (C) Quantification of data from actin cosedimentation experiments for SUMO::HMP-1ΔAMD and SUMO::HMP-1ΔAMD(S823F). Error bars represent mean ± SEM (n = 3 for HMP-1ΔAMD(S823F) and HMP-1ΔAMD; **, p ≤ 0.05).
excessive F-actin recruitment at junctions, which can be partially offset by a reduction in the intrinsic binding affinity of the C-terminus for F-actin.

The N-terminus of the HMP-1 ABD is required for full function

Our previous studies (Kwiatkowski et al., 2010; Maiden et al., 2013; Kang et al., 2017) did not assess the importance of the N-terminal portion of the HMP-1 ABD for actin binding. We therefore made a SUMO::HMP-1(704–927) construct, which deletes the N-terminal region of the HMP-1 ABD (aa677–703; Figure 4A). Actin cosedimentation assays indicated that the N-terminally truncated HMP-1 C-terminus (704–927) does not bind F-actin as well as the entire HMP-1 ABD (aa677–927; Figure 4, B and C). The corresponding GFP construct (HMP-1Δ677–703::GFP) localized to junctions in a manner indistinguishable from HMP-1FL::GFP in a wild-type background (Figure 4D), and it rescued the embryonic lethality of hmp-1(jc48) mutants (introducing HMP-1Δ677–703::GFP into hmp-1(jc48) heterozygotes reduced embryonic lethality among their progeny from 25.3%, identical to the 25% expected for 100% lethality among homozygotes, to 15.1%). However, the rescued hmp-1(jc48) homozygotes displayed tail morphology defects (white arrow, Figure 4E) not seen in lines rescued with HMP-1FL::GFP. Moreover, although embryonic lethality was rescued, all hmp-1(jc48);Ex[hmp-1(Δ677–703)::gfp] embryos died as L1 or L2 larvae. This result suggests that the N-terminus of the HMP-1 ABD is essential for full function. Owing to lack of rescue, we could not unambiguously identify hmp-1(jc48);Ex[hmp-1(Δ677–703)::gfp]
Phosphorylation of HMP-1 at residue S509 is important for proper function

Phosphorylation and dephosphorylation of the P-linker region (the region between the M and ABD domains) is essential for proper function of Drosophila α-catenin (Escobar et al., 2015). However, whether phosphorylation of α-catenin affects its ability to bind F-actin is unclear. We previously found that HMP-1 residues S312, S509, S649, and S910 are subject to phosphorylation (Callaci et al., 2015). S509 and S649 lie within the AMD, so we examined them in more detail by introducing non-phosphorylatable and phosphomimetic mutations into our HMP-1FL::GFP construct. Both HMP-1(SS09A)::GFP and HMP-1(SS09E)::GFP localize normally to junctions in wild type and were indistinguishable from HMP-1FL::GFP (Supplemental Figure S3, C–F). Both HMP-1(SS09A)::GFP and HMP-1(SS09E)::GFP rescued the embryonic lethality of hmp-1(jc48) mutants; however, L1 offspring of hmp-1(jc48);Ex[hmp-1(S509A)::gfp] worms exhibited morphological defects, including Dumpy larvae, Sick L1s, and Lumpy tails (Supplemental Figure S3, G–K). In contrast, progeny of hmp-1(jc48);Ex[hmp-1(SS09E)::gfp] worms appeared indistinguishable from hmp-1(jc48) animals rescued by HMP-1FL::GFP (Figure 5A). These results suggest that phosphorylation of HMP-1 S509 is important for normal function.

We next investigated whether disrupting phosphorylation at S509 affects F-actin recruitment to junctions. Phalloidin staining of hmp-1(jc48);Ex[hmp-1(SS09E)::gfp]embryos revealed that circumferential actin filament bundles (CFBs) are indistinguishable from those in hmp-1(jc48);Ex[hmp-1(SS09E)::gfp] embryos (Figure 5E). In contrast, CFBs in hmp-1(jc48);Ex[hmp-1(SS09A)::gfp] embryos exhibited a range of CFB morphologies, from normal (Class I; Figure 5, B and B’) to partially irregular (Class II; Figure 5, C and C’) to merging of CFBs at seam-ventral junctions (Class III; Figure 5, D and D’). Quantification of junctional–proximal actin width in hmp-1(jc48);Ex[hmp-1(SS09A)::gfp] embryos showed that in embryos with normal CFBs the width of the junctional–proximal actin array did not differ significantly from hmp-1(jc48);Ex[hmp-1(SS09E)::gfp] or hmp-1(jc48);Ex[hmp-1::gfp] embryos (Figure 5F). However, in hmp-1(jc48);Ex[hmp-1(SS09A)::gfp] embryos exhibiting Class II and Class III defects, the width of junctional–proximal actin was significantly greater than in hmp-1(jc48);Ex[hmp-1(SS09E)::gfp] or hmp-1(jc48);Ex[hmp-1::gfp] embryos (Figure 5F), suggesting that the abnormal CFB organization...
in hmp-1(jc48);Ex[hmp-1(S509A)::gfp] embryos may be due to excessive actin recruited to junctions.

In contrast to the effects upon mutating S509, both HMP-1(S649E)::GFP and HMP-1(S649A)::GFP localized correctly to junctions, with HMP-1(S649E)::GFP showing more cytoplasmic signal (Supplemental Figure S4, A and B). Although both HMP-1(S649A)::GFP and HMP-1(S649E)::GFP rescued hmp-1(jc48) embryonic lethality, HMP-1(S649A)::GFP rescued more efficiently than HMP-1(S649E)::GFP (56.4% embryonic lethality for hmp-1(jc48);Ex[hmp-1(S649A)::gfp] worms vs. 69.3% embryonic lethality for hmp-1(jc48);Ex[hmp-1(S649E)::gfp] worms, respectively). Phalloidin staining indicated that CFBs and junctional–proximal actin were normal in both strains (Supplemental Figure S4, C and D), with no difference from mutants rescued with unmutated HMP-1FL::GFP (Supplemental Figure S4E). Thus the phosphorylation status of HMP-1 S649 has only subtle effects on proper HMP-1 function during embryogenesis compared with S509.

Conclusion: The HMP-1 AMD negatively regulates F-actin binding
Although the AMD of α-catenin has been suggested to be a regulator of α-catenin function (Imamura et al., 1999), the in vivo significance of the AMD has remained unclear for nearly two decades. Our results suggest that the HMP-1 AMD negatively regulates F-actin binding, because hmp-1(jc48);Ex[hmp-1::GFP] embryos possess excessive F-actin at junctions compared with wild type. Actin cosedimentation assays further suggest that recruitment of excess F-actin is due to excessive binding of HMP-1 AMD to F-actin. Excessive binding to F-actin may be deleterious during dynamic morphogenetic movements; at such times, the organization and assembly of the F-actin cytoskeleton must presumably be labile enough to accommodate dramatic changes in the shape and position of cells during epithelial morphogenesis. Adding a point mutation within the C-terminus of HMP-1 AMD::GFP known to weaken binding to F-actin (Maiden et al., 2013) supports the idea that the AMD normally maintains the affinity of HMP-1 for F-actin within an optimal functional range, because the mutated HMP-1 AMD::GFP functions better in a rescue assay.

Our results suggest a model for negative regulation of HMP-1 binding to F-actin via its AMD that involves multiple mechanisms (Supplemental Figure S5). One potential regulator is tension; either tension applied to HMP-1 or tension applied to the F-actin networks to which HMP-1 attaches. Recent research has shown that the affinity of the cadherin–β-catenin–αE-catenin ternary complex for F-actin increases dramatically when tension is applied to the actin (Buckley et al., 2014). Future experiments aimed at determining whether HMP-1 exhibits a tension-mediated increase in binding affinity for F-actin, and the role the AMD plays in this response, would prove extremely interesting.

Second, the AMD may be regulated through phosphorylation. That hmp-1(jc48) mutants rescued by a nonphosphorylatable mutant form of HMP-1::GFP (HMP-1(S509A)::GFP) exhibited more morphological defects compared with those rescued by an S509 phosphoryomimetic mutant suggests that HMP-1 phosphorylation at S509 is important for its proper function. Dephosphorylation of S509 may lead to excessive binding of HMP-1 to F-actin. That hmp-1(jc48);Ex[hmp-1(S509A)::gfp] embryos display abnormalities in F-actin organization in vivo is consistent with this possibility. Phosphoregulation of S641 in mammalian αE-catenin and the corresponding T645 in Drosophila α-catenin appears to be required for normal function (Escobar et al., 2015). Our results suggest that phosphoregulation of the corresponding residue in HMP-1, S649, plays a relatively minor role.

Finally, although not shown in Supplemental Figure S5, the HMP-1 AMD could regulate binding to other partners. The most heavily studied M domain binding partner of vertebrate αE-catenin is vinculin (Yonemura et al., 2010; Choi et al., 2012; Rangarajan and Izard, 2012; Ishiyama et al., 2013; Thomas et al., 2013). Even though the M1 domain of HMP-1 can bind vertebrate vinculin avidly, its binding to DEB-1, the C. elegans vinculin homologue, is very weak (Kang et al., 2017), and DEB-1 is not expressed in the epidermis in C. elegans (Barstead and Waterston, 1989). Although HMP-1 appears to have lost the ability to associate with C. elegans vinculin, other binding partners may associate with HMP-1 via unfurling of its M domain.

In addition to our analysis of the AMD, we identified aa677–704 of HMP-1 as necessary for F-actin binding. While this work was under review, a role for the α1 helix of the ABD (aa669–675 of αE-catenin, corresponding to aa676–681 of HMP-1) in negatively regulating the binding of α-catenin to F-actin was identified (Ishiyama et al., 2018). In that study deletion of α1 led to increased binding to F-actin, similar to our AMD deletions. Our larger N-terminal ABD deletion includes α1, α2, and part of the α3 domains of the ABD, which likely accounts for the difference in results from Ishiyama et al. Thus there may be several conformation-dependent regulatory domains within α-catenins that regulate their binding to F-actin, including the AMD and α1 within the ABD.

MATERIALS AND METHODS
Nematode strains and genetics
C. elegans strains were maintained using standard methods (Brenner, 1974). Bristol N2 was used as wild type. The following allele was utilized in this study: hmp-1(jc48). The following transgenic arrays were made for or used in this study: hmp-1::gfp, hmp-1::504–676::gfp, hmp-1::504–676::r5823F::gfp, hmp-1::677–703::gfp, each under its endogenous promoter.

Standard genetic crosses were used to create hmp-1(jc48);Ex[HMP-1::GFP] homozygotes, along with the introduction of other hmp-1 rescuing constructs.

Strains were made by DNA microinjection: 1 ng/μl of the transgene of interest, in addition to 20 ng/μl noncoding DNA (F35D3) and 79 ng/μl rol-6(su1006), was injected into the gonads of N2, as described previously (Mello and Fire, 1995). Comparable expression of all transgenes was confirmed using methods described previously (Shao et al., 2017).

CRISPR
The hmp-1(jc48) null mutant allele was generated via CRISPR following a protocol described previously (Arribere et al., 2014). Primer XS78(FOR): 5′ TGGCTCTGAGTCTTTTTTTTGCGATCTTCTACGTAGATTTTTAATTATTTTCGAAATGCTCGTTATAAATACATGCTCATGTTATTTACACACATCGCAACAGTGCGTCGTCGGAAATAG 3′ was synthesized by Integrated DNA Technologies (Coralville, IA).

Imaging
Embryos were isolated from gravid hermaphrodites, mounted on a 5% agarose pad, and aged at 20–25°C until the onset of morphogenesis. For four-dimensional differential interference contrast microscopy, embryos were imaged with 1-μm slice spacing at 3-min intervals using a Nikon Optiphot 2 microscope with a 60×/1.4 NA oil immersion objective at 20°C with a Macintosh computer running ImageJ (https://imagej.nih.gov/ij/; Schneider et al., 2012) using custom
macros/plug-ins (available at worms.zoology.wisc.edu/research/4d/4d.html). For fluorescent imaging, a Perkin-Elmer UltraView spinning disk confocal microscope, mounted on a Nikon Eclipse E600 microscope, equipped with a Hamamatsu ORCA-ER camera and controlled by Micro-Manager software (https://micromanager.org/; Edelstein et al., 2014), was used to collect images of GFP expressing embryos, using 0.5-μm slices at 3-min intervals with a 60×/1.4 NA oil objective at 20°C. Antibody staining (0.6-μm slices) and phalloidin staining (0.2-μm slices) images were collected with the same confocal microscope using a 100×/1.45 NA total internal reflection fluorescence objective.

Protein expression and purification
SUMO- His-tagged proteins were expressed in BL21-Gold(DE3) Escherichia coli cells and purified as described (Mayers et al., 2011; Maiden et al., 2013). Cells were induced with 0.1 mM isopropyl β-D-thiogalactopyranoside at 18°C for 6 h. Wash and elution buffers were as follows: His wash (50 mM Na-phosphate, pH 8.0, 300 mM NaCl, 0.1% Tween-20, 10 mM imidazole), and His elution (250 mM imidazole, 100 mM NaCl, 10% glycerol, 50 mM HEPES, pH 7.6).

Actin-pelleting assays
Actin-pelleting assays were performed as described previously (Maiden et al., 2013). Briefly, 5 μM purified proteins (quantified via a Bradford assay; Pierce/Thermo Scientific) was incubated at room temperature for 1 h with 0, 2, or 5 μM polymerized chicken F-actin (Cytoskeleton, Denver, CO). Samples were then centrifuged at 100,000 rpm for 20 min at 4°C in a TLA-100 rotor in a tabletop ultracentrifuge (Beckman Optima TL 100 Ultracentrifuge). Samples were run on 12% SDS–PAGE gels and stained with Coomassie Brilliant Blue, and bands were quantified using ImageJ software. To determine the percentage of protein bound to F-actin, background sedimentation of the protein (no actin control) was subtracted first and each band intensity was then normalized to the pelleted F-actin.

Antibody and phalloidin staining
Freeze-cracking was used for immunostaining embryos (Albertson, 1984). Staining was performed as described previously (Leung et al., 1999). Embryos were incubated with primary antibodies in phosphate-buffered saline + 0.1% Tween-20 (PBST) and 1% nonfat dry milk overnight at 4°C. Embryos were then incubated with secondary antibodies in PBST and 1% nonfat dry milk for 4 h at room temperature. The following primary antibodies were used: 1:1000 mouse-anti-GFP (Invitrogen), 1:4000 polyclonal rabbit-anti-HMP-1 (Zaidel-Bar et al., 2010), and 1:200 mouse monoclonal anti-AJM-1 (MH27; Francis and Waterston, 1991). The following secondary antibodies were used: 1:50 anti-rabbit immunoglobulin G Texas Red, 1:50 anti-rabbit Cy3, and 1:50 anti-mouse fluorescein isothiocyanate.

Phalloidin staining of mutant and wild-type embryos was used to visualize F-actin in fixed embryos (Costa et al., 1998). Embryos were fixed using the following: 4% paraformaldehyde, 0.1 mg/ml lyssolecithin, 48 mM PIPES, pH 6.8, 25 mM HEPES, pH 6.8, 2 mM MgCl2, and 10 mM EGTA (ethylene glycol-bis[β-aminoethyl ether] N,N′,N′,N′-tetraacetic acid) for 20 min at room temperature. Phalloidin-Texas Red (1:20, Thermo Fisher) was incubated with embryos overnight at 4°C. Quantification of junctional actin width was performed using a single-blind approach from unidentified stained specimens in ImageJ using a microscope stage micrometer as a reference for normalizing scale.

ACKNOWLEDGMENTS
We thank Tim Loveless for helpful discussions regarding experiments and manuscript preparation and Bill Weis for comments on the manuscript. This project was funded by Grant no. GM058038 from the National Institutes of Health and Grant no. 60698 from the John Templeton Foundation to J.H.

REFERENCES
Albertson DG (1984). Formation of the first cleavage spindle in nematode embryos. Dev Biol 101, 61–72.
Arrinere JA, Bell RT, Fu BX, Artiles KL, Hartman PS, Fire AZ (2014). Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in Caenorhabditis elegans. Genetics 198, 837–846.
Barstead RJ, Waterston RH (1989). The basal component of the nematode dense-body is vinculin. J Biol Chem 264, 10177–10185.
Brenner S (1974). The genetics of Caenorhabditis elegans. Genetics 77, 71–94.
Buckley CD, Tan J, Anderson KL, Hanein D, Volkman N, Weis WI, Nelson WJ, Dunn AR (2014). Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. Science 346, 1254211.
Callaci S, Morrison K, Shao X, Schuh AL, Wang Y, Yates JR 3rd, Hardin J, Audhya A (2015). Phosphoregulation of the C. elegans cadherin-catenin complex. Biochem J 472, 339–352.
Choi HJ, Pokutta S, Cadwell GW, Bobkov AA, Bankston LA, Liddington RC, Weis WI (2012). α-Catenin is an autoinhibited molecule that cooperates vinculin. Proc Natl Acad Sci USA 109, 8576–8581.
Costa M, Raich W, Agbunan C, Leung B, Hardin J, Pries JR (1998). A putative cadherin-catenin system mediates morphogenesis of the Caenorhabditis elegans embryo. J Cell Biol 141, 297–308.
Desai R, Sarpal R, Ishiyama N, Pellikka M, Ikura M, Tepass U (2013). Monomeric α-catenin links cadherin to the actin cytoskeleton. Nat Cell Biol 15, 261–273.
Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI (2005). α-Catenin is a molecular switch that binds E-cadherin-β-catenin and regulates actin filament assembly. Cell 123, 903–915.
Edelstein AD, Tsuchida MA, Amogadji N, Pinkard H, Vale RD, Stuurman N (2014). Advanced methods of microscope control using μManager software. J Biol Methods 1, e10.
Escobar DJ, Desai R, Ishiyama N, Folmsbee SS, Novak MN, Flozak AS, Daugherty RL, Mo R, Nanavati D, Sarpal R, et al. (2015). α-Catenin phosphorylation promotes intercellular adhesion through a dual-kinase mechanism. J Cell Sci 128, 1150–1165.
Francis R, Waterston RH (1993). Muscle cell attachment in Caenorhabditis elegans. J Cell Biol 114, 465–479.
Harris TJ, Tepass U (2010). Adherens junctions: from molecules to morphogenesis. Nat Rev Mol Cell Biol 11, 502–514.
Herrenknecht K, Ozawa M, Eckerskom C, Lotspeich F, Lenter M, Kemler R (1991). The uvomorulin-anchorin protein alpha catenin is a vinculin homologue. Proc Natl Acad Sci USA 88, 9156–9160.
Huveneers S, Oldenberg J, Spanjaard E, van Krogt G, Grigoriev I, Akhmanova A, Rehmhan A, de Rooij J (2012). Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. J Cell Biol 196, 641–652.
Imamura Y, Itoh M, Maeno Y, Tsukita S, Nagafuchi A (1999). Functional domains of α-catenin required for the strong state of cadherin-based cell adhesion. J Cell Biol 144, 1311–1322.
Ishiyama N, Sarpal R, Wood MN, Barnick SK, Nishikawa T, Hayashi H, Kobab AB, Flozak AS, Yemelyanov A, Fernandez-Gonzalez R, et al. (2018). Force-dependent allostery of the α-catenin actin-binding domain controls adherens junction dynamics and functions. Nat Commun 9, 5121.
Ishiyama N, Tanaka N, Abe K, Yang YJ, Abbass YM, Umitsu M, Nagar B, Bueler SA, Rubinstein JL, Takeichi M, Ikura M (2013). An autoinhibited structure of α-catenin and its implications for vinculin recruitment to adherens junctions. J Biol Chem 288, 15913–15925.
Kang H, Bang I, Jin KS, Lee B, Lee J, Shao X, Heier JA, Kwiatkowski AV, Nelson WJ, Hardin J, et al. (2017). Structural and functional characterization of Caenorhabditis elegans α-catenin reveals constitutive binding to β-catenin and F-actin. J Biol Chem 292, 7077–7086.
Kwiatkowski AV, Maiden SL, Pokutta S, Choi HJ, Benjamin JM, Lynch AM, Nelson WJ, Weis WI, Hardin J (2010). In vitro and in vivo reconstitution of the cadherin–catenin–actin complex from Caenorhabditis elegans. Proc Natl Acad Sci USA 107, 14591–14596.
Leung B, Hermann GJ, Priess JR (1999). Organogenesis of the Caenorhabditis elegans intestine. Dev Biol 216, 114–134.
Maiden SL, Harrison N, Keegan J, Cain B, Lynch AM, Pettitt J, Hardin J (2013). Specific conserved C-terminal amino acids of Caenorhabditis elegans HMP-1/α-catenin modulate F-actin binding independently of vinculin. J Biol Chem 288, 5694–5706.
Mandai K, Nakanishi H, Satoh A, Obashi H, Wada M, Nishioka H, Itoh M, Mizoguchi A, Aoki T, Fujimoto T, et al. (1997). Afadin: A novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. J Cell Biol 139, 517–528.
Mayers JR, Fyle I, Schuh AL, Chapman ER, Edwardson JM, Audhya A (2011). ESCRT-0 assembles as a heterotetrameric complex on membranes and binds multiple ubiquitylated cargoes simultaneously. J Biol Chem 286, 9636–9645.
Mello C, Fire A (1995). DNA transformation. In: Caenorhabditis elegans: Modern Biological Analysis of an Organism, Amsterdam: Elsevier BV, 451–482.
Ozawa M, Baribault H, Kemler R (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J 8, 1711–1717.
Pokutta S, Choi HJ, Ahlsen G, Hansen SD, Weis WI (2014). Structural and thermodynamic characterization of cadherin-β-catenin-α-catenin complex formation. J Biol Chem 289, 13589–13601.
Pokutta S, Drees F, Takai Y, Nelson WJ, Weis WI (2002). Biochemical and structural definition of the l-afadin- and actin-binding sites of α-catenin. J Biol Chem 277, 18868–18874.
Pokutta S, Weis WI (2000). Structure of the dimerization and β-catenin-binding region of α-catenin. Mol Cell 5, 533–543.
Rangarajan ES, Izard T (2012). The cytoskeletal protein α-catenin unfurls upon binding to vinculin. J Biol Chem 287, 18492–18499.
Rangarajan ES, Izard T (2013). Dimer asymmetry defines α-catenin interactions. Nat Struct Mol Biol 20, 188–193.
Schneider CA, Rasband WS, Eliceiri KW (2012). NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671–675.
Shao X, Kang H, Loveless T, Lee GR, Seok C, Weis WI, Choi HJ, Hardin J (2017). Cell-cell adhesion in metazoans relies on evolutionarily conserved features of the α-catenin-β-catenin-binding interface. J Biol Chem 292, 16477–16490.
Takeichi M (2014). Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. Nat Rev Mol Cell Biol 15, 397–410.
Watabe-Uchida M, Uchida N, Imamura Y, Nagafuchi A, Fujimoto K, Uemura T, Vermeulen S, van Roy F, Adamson ED, Takeichi M (1998). α-Catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. J Cell Biol 142, 847–857.
Yonemura S, Wada Y, Watanabe T, Nagafuchi A, Shibata M (2010). alpha-Catenin as a tension transducer that induces adherens junction development. Nat Cell Biol 12, 533–542.
Zaidel-Bar R, Joyce MJ, Lynch AM, Witte K, Audhya A, Hardin J (2010). The F-BAR domain of SRGP-1 facilitates cell-cell adhesion during C. elegans morphogenesis. J Cell Biol 191, 761–769.
**Supplemental Materials**

**Figure S1.** hmp-1(jc48) mutants show classic Hmp phenotypes. (A) A schematic representation of the hmp-1(jc48) lesion. hmp-1(jc48) mutates Serine 3 to a stop codon. (B) DIC and confocal images of a typical hmp-1(jc48) mutant embryo. Anterior is left, dorsal is up. Scale bar is 10 µm. hmp-1(jc48) homozygotes arrest during embryonic elongation with the characteristic dorsal epidermal folds previously described in hmp-1 mutants (Costa et al., 1998; Maiden et al., 2013). A dorsal fold has formed in the epidermis (arrow) at the elongation stage as the embryo reflexes dorsally, indicative of loss of function of HMP-1. (C-E) Double antibody staining of hmp-1(jc48) mutant embryos. Anterior is left, posterior is right. Anti-HMP-1 in red; anti-AJM-1 in green, marking cell-cell contacts. No HMP-1 protein was detectable via immunostaining in dead embryos (Fig. S1C-S1E), indicating that hmp-1(jc48) is a true protein null allele. (F-G) Staining with fluorophore-conjugated phalloidin in a wild-type (WT) (F) and hmp-1(jc48) mutant embryo (G) to visualize F-actin organization. In the wild-type embryo in (F), parallel CFBs are prominent; white arrowhead indicates the underlying muscle. In contrast, in the hmp-1(jc48) mutant embryo in (G), large gaps between CFBs are evident, as well as thicker bundles of CFBs (red arrow); this embryo also lacks junctional proximal actin in the epidermis, as evidence by gaps in phalloidin signal at the seam-dorsal boundary (green arrow). Scale bar = 10 µm.

**Figure S2: Quantification of in vivo expression level of hmp-1::gfp and related variants.** Quantification of hmp-1::gfp, hmp-1ΔAMD::gfp, hmp-1ΔAMD(S823F)::gfp, hmp-1Δ677-703::gfp, hmp-1S509A::gfp, hmp-1S509E::gfp, hmp-1S649A::gfp, hmp-
1S649E::gfp revealed no significant difference in expression level. (p=0.3139, one-way ANOVA). Z-projections of 8 focal planes were measured for each junction.

**Figure S3. Effects of point mutations on HMP-1 function.** (A) HMP-1ΔAMD(S823F)::GFP localizes correctly to junctions in an elongating embryo. Scale bar = 10 µm. (B) Coomassie-stained SDS-PAGE gels of actin cosedimentation experiments using SUMO::HMP-1ΔAMD protein constructs at the concentrations indicated. S = supernatant fraction, P = pellet fraction. (C-D) DIC and confocal images of a wild-type embryo expressing HMP-1(S509A)::GFP, which localizes to cell-cell junctions. Scale bar is 10 µm. (E-F) DIC and confocal images of a wild-type embryo expressing HMP-1(S509E)::GFP, which likewise localizes to cell-cell junctions. (G-K) DIC images illustrating representative body morphology defects in L1 offspring of hmp-1(jc48);Ex[hmp-1(S509A)::gfp] worms. (G) Wildtype; (H) Dpy/Dumpy larva; (I) larva with Lumpy tail; (J) Sick L1: larva with disorganized bulges (arrow) within different regions of the body. Scale bar = 20µm.

**Figure S4. Phosphorylation of HMP-1 at S649 does not have a significant role in regulating F-actin organization during embryonic development.** (A-B) Spinning disc confocal microscopy shows that both HMP-1(S649A)::GFP and HMP-1(S649E)::GFP localize to cell-cell junctions. Note that a higher percentage of HMP-1(S649E)::GFP is in the cytoplasm compared with HMP-1(S649A)::GFP. Scale bar =10 µm.(C-D) Staining with fluorophore-conjugated phalloidin in a hmp-1(jc48) mutant embryos rescued by HMP-1(S649A)::GFP (C) and HMP-1(S649E)::GFP (D) to visualize the F-actin organization. F-actin organization in both embryos appears indistinguishable from
wildtype, with parallel CFBs. In both figures, white arrowheads indicate the underlying muscle and white arrow points to junctional-proximal actin that concentrates along the junction. (E) Quantification of the width of junctional-proximal actin in the embryos of hmp-1(jc48) mutants rescued by HMP-1::GFP, HMP-1(S649A)::GFP and HMP-1(S649E)::GFP. (n.s., not significant; Student’s T-test).

**Figure S5. A model for AMD function in regulation of HMP-1 binding to F-actin.**

HMP-1 binds weakly to F-actin in its autoinhibited state, which is conferred in part through the conformation of the AMD. When autoinhibition is relieved, possibly through dephosphorylation of HMP-1 S509 or via tension, the activated C terminus of HMP-1 binds F-actin more readily (red star), leading to increased recruitment of F-actin to adherens junctions.
Figure S1
Figure S2
**Figure S3**

A. DIC and GFP images of different genotypes:

- **+/+; Ex[hmp-1ΔAMD(S823F)::gfp]**
- **HMP-1S509A::GFP**
- **HMP-1S509E::GFP**

B. Gel analysis showing SUMO::HMP-1ΔAMD(S823F) under different conditions:

|          | 3µM | 6µM |
|----------|-----|-----|
|          | S   | P   | S   | P   | S   | P   |
| no actin |     |     |     |     |     |     |
| + actin  |     |     |     |     |     |     |

- Actin expression under 3µM and 6µM conditions with and without actin addition.
Figure S4
Figure S5