Identification of a Myosin VII-Talin Complex*

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Myosin VII (M7) plays a role in adhesion in both Dictyostelium and mammalian cells where it is a component of a complex of proteins that serve to link membrane receptors to the underlying actin cytoskeleton. The nature of this complex is not fully known, prompting a search for M7-binding proteins. Co-immunoprecipitation experiments reveal that Dictyostelium M7 (DdM7) interacts with talinA, an actin-binding protein with a known role in cell-substrate adhesion. No additional proteins are observed in the immunoprecipitate, indicating that the interaction is direct. The N-terminal region of the DdM7 tail that lies between the region of predicted coil and the first MyTH4 domain is found to harbor the talinA binding site. Localization experiments reveal that talinA does not serve as a membrane receptor for DdM7 and vice versa. These findings reveal that talinA is a major DdM7 binding partner and suggest that their interaction induces a conformational change in each that, in combination with membrane receptor binding, promotes the assembly of a high avidity receptor complex essential for adhesion of the cell to substrata.

A diverse range of cellular movements is driven by members of the myosin superfamly (1). Myosins generate movement along actin filaments in an ATP-dependent manner and are characterized by the presence of a conserved motor domain, typically located at the N terminus, and a highly divergent C-terminal tail region. The class VII myosins (M7) are among the most widely expressed of the myosin family members, having been identified in organisms ranging from Dictyostelium to human. The M7 tail domain contains a tandem repeat of two MyTH4/FERM domains separated by an SH3 domain. In many cases, the tail begins with a region of predicted coiled-coil. In vertebrates, M7a is expressed almost exclusively in cells that possess specialized actin structures, most notably in the sensory hair cells of the ear in mice and zebrafish (2, 3). Loss of M7 function results in significant defects in all organisms where examined (3–6). Mouse and zebrafish M7a mutants have defects in hearing that are due to disorganization of the actin-filled stereocilia of critical sensory cells (3, 7). Consistent with these observations, M7a is localized to regions of the stereocilia where links between adjacent stereocilia are formed and current evidence suggests that M7a is critical for providing a connection between the actin cytoskeleton and these extracellular links via interaction with vezatin and harmonin, proteins that interact directly or indirectly with cadherins (8, 9).

The lower eukaryote Dictyostelium discoideum expresses a single M7, DdM7, an initially surprising finding given that this organism does not possess any highly specialized actin-based structures. DdM7 is localized at the plasma membrane where it is transiently found in actively extending actin-rich regions of the cell, such as the leading edge and filopodial tips. Dictyostelium lacking DdM7 adhere poorly to surfaces, lack calcium-dependent cell-cell adhesion, are defective in extending filopodia, and are smaller in size than control cells (6, 10). The defect in adhesion cannot be accounted for by the failure to transport adhesion proteins to the cell surface, because DdM7 does not appear to be localized to intracellular vesicles and the levels of candidate membrane-associated adhesion receptors on the plasma membrane are unchanged when they are compared between mutant and wild type cells (6). Taken together, the analysis of M7 function in two widely divergent systems suggests that this actin-based motor protein provides a link between the actin cytoskeleton and cell surface receptors (6, 8, 9).

The phenotype of the DdM7 null mutant is quite similar to that of a Dictyostelium mutant lacking talinA. Talin is a conserved cytoskeletal protein characterized by the presence of an N-terminal FERM domain that binds to the cytoplasmic domain of integrins and a C-terminal I/LWEQ actin binding domain (11). Mammalian talin is localized to sites of cell adhesion (11) and cells lacking talin exhibit adhesion defects (12). Dictyostelium expresses two distinct talins, talinA and talinB. TalinB, distinguished from talinA by the presence of a villin headpiece at its C terminus, is expressed at low levels in vegetative cells and is essential for development (13). Dictyostelium talinA has been shown to play a role in cell-substrate adhesion. It is localized to the leading edge of chemotactic cells, the cortex and filopodia (14, 15). The talinA null mutant is defective in adhesion to both particles and surfaces as well as in calcium-dependent cell-cell adhesion (16). In contrast to the DdM7 null mutant, however, the talinA null mutant still extends filopodia (6). The functional similarities between DdM7 and talinA suggest that the two proteins may interact with each other or with the same adhesion receptors. Consistent with this view, a recent report indicates that, in the absence of DdM7, talinA levels are significantly reduced (17). A search for DdM7-interacting proteins was undertaken to gain information about the potential functional interaction between these two adhesion proteins, as well as others, as a first step toward...
defining how an actin-based motor protein links cell surface receptors to the actin cytoskeleton.

**EXPERIMENTAL PROCEDURES**

**Strains, Cell Growth, and Maintenance—**All Dictyostelium strains were maintained using standard methods (18). Cells were grown on tissue culture plates in HL5 growth medium supplemented with 10,000 units/ml penicillin G (Fisher Scientific) and 10 μg/ml streptomycin sulfate (Sigma). The DdM7 null strain HTD17 (6) and talinA null strain HG1666 (16) were maintained in HL5 supplemented with 10 μg/ml blasticidin (ICN Biomedicals, Costa Mesa, CA); the DdM7 null strain HTD15 was maintained in HL5 only. Transformants expressing GFP-M7 (6), GFP-M7 tails (see below), GFP-talinA (15), or GFP alone were generated by electroporation as before (6) and maintained in HL5 supplemented with 10 μg/ml G418 (Geneticin, Invitrogen).

**Generation of Expression Plasmids—**All cloning was performed using enzymes and reagents from either New England Biolabs (Beverly, MA) or Roche Applied Science following the manufacturers’ protocols. An extrachromosomal plasmid expressing GFP-DdM7 was generated by a PCR-based approach in combination with TOPO cloning (Invitrogen) to add restriction sites at the 5′-and 3′-ends of the coding region using Ddel and XhoI as a template (19). The sequence of all generated clones was verified by sequencing. The entire myoI gene encoding DdM7 (19) was inserted into the XhoI site of pTX-GFP, a low copy number extrachromosomal plasmid carrying the gfp gene under the control of the actin15 promoter (20). The resulting expression plasmid was designated pdTI112. The GFP-tagged mutant DdM7 tail expression plasmids were generated using a similar approach except that the cDNA encoding the tail was used as a template for PCR. The sequence encompassing either the full-length tail (amino acids 809–2357), which includes the N-terminal predicted coiled-coil region or the tail lacking either the N-terminal or C-terminal FERM domains (amino acids 1314–1554 and 2057–2357, respectively), the tail lacking both FERM domains, the tail lacking most of the central SH3 and C-terminal proline-rich region alone (amino acids 1584–1790) were each cloned in-frame into pd60B, a vector expressing a gene encoding GFP driven by the actin15 promoter (21). The entire insert (i.e. the promotor, gfp gene, myoI tail sequence, and terminator) from each was then cloned into pSmall, a low copy number Dictyostelium extrachromosomal plasmid that carries a neomycin resistance cassette (22). The tail lacking both of the MyTH/FERM domains (MyTH4a/ferMI: amino acids 1157–1554; MyTH4b/ferM2: amino acids 1879–2357), the N-terminal region of predicted coiled-coil alone (809–901) and the N-terminal proline-rich region (amino acids 902–1156) was cloned directly into pTX-GFP. The GFP fusion expression plasmids, pdTI35 (full-length tail), pdTI35 (ΔFERM1 tail), pdTI76 (ΔFERM2 tail), pdTI114 (ΔFERMI/ΔFERM2 tail), pdTI155 (ΔMA/FERM), pdTI159 (ΔSh3/Pr2) (22), and pdTI183 (Coil), or the GFP-talinA plasmid (15), were transformed into either wild type, DdM7 null, or talinA null cells. Transformants were selected on 10 μg/ml G418 for fluorescence and then analyzed for expression of GFP-M7, GFP-M7 mutant, or wild type tails or tail fragments or GFP-talinA by Western blotting. A control strain expressing GFP alone was generated by transforming Ax2 cells with pTX-GFP.

**Immunoprecipitation—**A co-IP approach was used to initially identify DdM7-binding proteins from total cell extracts. A vector that drives GFP-DdM7 expression under the control of the actin15 promoter was transformed into DdM7 null cells to create a strain that stably expressed GFP-DdM7. Cells were either physically lysed by passage through 3-μm filters or chemically lysed by incubation with 1% Triton X-100 (Pierce Chemical Co.). The lysate was then centrifuged at 21,000 × g for 10 min, and the supernatant was collected. To remove proteins that nonspecifically bound the beads, 700 μl of supernatant was incubated sequentially with three separate solutions of Protein A Sepharose 4B Fast Flow beads (Amersham Biosciences). For each incubation, supernatant was added to 100 μl of protein A beads that had been washed twice with ILB, the sample was gently mixed at 4 °C for 1 h, and then the beads were pelleted by centrifugation at low speed for 3 min. The resulting supernatant was collected for the next incubation with beads. A rabbit polyclonal anti-GFP antibody (Molecular Probes Inc., Eugene, OR) was added to the pre-cleared supernatant, at a final concentration of 12 μg/ml, and the sample was incubated with gentle mixing at 4 °C for 1–2 h. The antibody-supernatant solution was then added to 100 μl of Protein A beads that had been washed with ILB, and the slurry was incubated with gentle mixing for an additional 3–4 h. The beads were collected by gentle centrifugation, and the supernatant was saved for analysis. The bead complex was then washed several times with ILB containing PI, and the final bead pellet was carefully drained of all excess liquid. A total of 30 μl of urea-containing SDS sample buffer (ULSB; 125 mM Tris, pH 6.8, 50% glycerol, 4% SDS, 6 μl urea, and 20 μl diethiothreitol) was added to the beads, and the samples were heated at 100 °C for 3 min and then applied either to a 6% or 12.5% SDS-PAGE gel. The samples were then either transferred to polyvinylidene difluoride membrane (Millipore, Bedford, MA) for immunoblotting or gels stained with either Coomassie R-250 or silver.

**Sucrose Gradients—**Log-phase cells were collected, washed twice in PB, resuspended to a density of 3 × 10⁷ cells/ml in sucrose gradient lysis buffer (phosphate buffered saline, pH 7.2, 10 mM Mg-ATP, and PI), and physically lysed by passage through 3-μm filters (as above). The lysate was centrifuged in a TL100.3 rotor in an Optima TLX Ultracentrifuge (Beckman Coulter, Fullerton, CA) at 126,000 × g at 4 °C for 30 min. The resulting high speed supernatant was collected, and 500 μl was loaded on to a 10-ml linear gradient of 5–20% sucrose in gradient buffer (1 mM EDTA, 1 mM Mg-ATP, 50 mM NaCl, 10 mM Mg-ATP, and PI), and the sample was centrifuged in an SW41 rotor (Beckman) at 200,000 × g at 4 °C for 16 h. Fractions of equal volume were collected, and the sucrose percentage was measured by a hand refractometer. The samples were concentrated by acetone precipitation, dissolved in 20 μl of ULSB, heated at 100 °C for 3 min, and then analyzed by SDS-PAGE or immunoblotting. Sucrose gradient standards consisted of cytochrome c (12 kDa), catalase (62.5 kDa monomer), and thyroglobulin (670-kDa tetramer (Sigma)) with sedimentation coefficients of 2.1 S, 11.3 S, and 19.4 S, respectively (23). A total of 75 μg of each standard protein was loaded onto a 10-ml sucrose gradient.

**Immunoblotting—**The IgG fraction from rabbit serum containing antibodies directed against a glutathione S-transferase fusion protein encompassing the DdM7 predicted coiled-coil region (amino acids 809–901; UMN87) was used at 1:1,500 for immunoblotting. A rabbit polyclonal antibody specific for the heavy chain of the class I myosin, myoB, was used at 1:1,500 (24). Two mouse monoclonal antibodies specific for Dictyostelium talinA (monoclonal antibody 477 and monoclonal antibody 341, mixed at 1:1 (14, 16)) and a monoclonal antibody specific for Dictyostelium actin (monoclonal antibody 244; used at 1:400) were generous gifts of Dr. Günther Gersich (Max-Planck Institut, Martinsried, Germany). A rat polyclonal antibody specific for the dynein intermediate chain (Rat 144; used at 1:1,500 (25)) was a generous gift of Dr. Rex L. Chisholm (Northwestern University School of Medicine). All of the primary antibodies, with the exception of the talinA antibodies were diluted in 5% nonfat dried milk in Tris-buffered saline. Washed blots were incubated with horseradish peroxidase goat anti-rabbit, goat anti-mouse (Bio-Rad Laboratories, Hercules, CA), or donkey anti-rat (Jackson Immunologicals, West Grove, PA) secondary antibodies and bands detected by chemiluminescence using SuperSignal (Pierce) followed by exposure to X-Omat film (Eastman Kodak).

**Other Methods—**Total cellular membranes and cytosol were prepared as described previously (26). Cells were prepared for confocal microscopy by placing them at subconfluent densities on tissue culture plates in HL5 the day before analysis. The cells were collected and washed twice with PB, allowed to adhere to coverslips, and then analyzed by confocal microscopy as described previously (6).

**RESULTS**

A co-IP approach was used to initially identify DdM7-binding proteins from total cell extracts. A vector that drives GFP-DdM7 expression under the control of the actin15 promoter was transformed into DdM7 null cells to create a strain that stably expressed GFP-DdM7. Cells were either physically lysed by passage through 3-μm filters or chemically lysed by incubation with 1% Triton X-100 and the GFP-DdM7 immunoprecipitate using a commercially available antibody directed against GFP. The GFP pellet was analyzed first by SDS-PAGE and found to contain two high molecular weight bands, one at the predicted ~300-kDa size of GFP-DdM7. The second band migrated slightly below, at ~260 kDa (Fig. 1A). The same result was obtained if the IP was performed with growth-phase cells that were actively extending filopodia and with aggregation-compe-
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The tail domains of myosins have been shown to interact with a range of binding proteins (1). Furthermore, a number of M7 tail-binding proteins have been identified through yeast two-hybrid and immunoprecipitation experiments and the majority of these have been shown to interact with the C-terminal FERM domain (i.e. harmonin, MAP2B, protein kinase A regulatory subunit (RIα), MyRIP, and vezatin (8, 9, 27–29)) and mutations in the C-terminal FERM domain of human M7 result in deafness (30). Thus, the possibility that talinA interacts with the DdM7 tail, and does so by binding to one or both of the FERM domains, was investigated. A GFP-tagged full-length DdM7 tail (from the predicted coiled-coil region through to the end of the C terminus) or tails lacking one or both FERM domains (Fig. 2A) were expressed in Ax2 cells. The expression level of the GFP-tagged tail fragments was analyzed by Western blotting. The blots were also probed with an antibody to myoB (24), a class I myosin, to provide an internal loading control. All tails were stably expressed at modest levels relative to the endogenous full-length DdM7 (Fig. 2B). The GFP-tagged fragments were immunoprecipitated as before, and the IP pellet was analyzed by both 6% and 12.5% SDS-PAGE. Inspection of the silver-stained gels revealed, in addition to the IgG bands and a band just below 100 kDa that is also routinely observed in control IP pellets with no antibody (see Fig. 1B), only two other bands were present in the GFP-tail IP pellet, one at 150–200 kDa and the other at ~260 kDa (Fig. 2C). The sizes of the two bands were consistent with those of the expressed GFP-tail and endogenous talinA. Each of the three GFP-tagged deletion tails, one that lacked the N-terminal FERM domain...
FIG. 2. The DdM7 tail binds to talin. A: Wild type Dictyostelium cells expressing either the full-length GFP-DdM7 tail (Tail) or the tail lacking either the N-terminal FERM domain (ΔFERM1 tail) or the C-terminal FERM domain (ΔFERM2 tail) or both FERM domains (ΔFERM1-ΔFERM2) were generated, and the binding of the different tails to talinA was assessed by IP. A, schematic illustration of the full-length DdM7 and the GFP-tagged DdM7 tail fragments expressed in wild type cells. The filled ovals indicate the light chain binding IQ motifs. The small gray shaded box represents the SH3 domain. The region of predicted coiled-coil is labeled “C,” and the MyTH4 domains are indicated by the black box labeled “M.” B, total cell lysates from each of the GFP-expressing lines were run on a 6% SDS-PAGE gel, and the level of the expressed tail fragments was analyzed by immunoblotting with antibodies directed against DdM7. The blot was also probed with an antibody directed against a class I myosin, myoB, to better assess the loading in each lane. C, the immunoprecipitated pellets were run on either a 6% or 12% SDS-PAGE gel and analyzed by staining with silver. The position of the IgG heavy and light chains are marked by asterisks.
(ΔFERM1), one that lacked the C-terminal FERM domain (ΔFERM2), or a tail lacking both (ΔFERM1-ΔFERM2), was found to co-IP with talinA (Fig. 2C). These results indicated that talinA interacts directly with the DdM7 tail domain and that the FERM domains of DdM7, either alone or in combination, are not required for this interaction.

A series of GFP-tagged DdM7 tail fragments were expressed in wild type Dictyostelium cells to identify the region that binds to talinA (Fig. 3A). These results indicated that talinA interacts directly with the DdM7 tail domain and that the FERM domains of DdM7, either alone or in combination, are not required for this interaction.

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levels in wild type cells (Fig. 3B) and found to co-IP with talinA (Fig. 3C).

M7 has been suggested to be a dimer based on the presence of a region of predicted coiled-coil at the N-terminal region of the tail domain. Gel filtration analysis of baculovirus-expressed mouse M7a confirmed that M7a is a dimer, although it was shown that the region of predicted coil could not promote dimerization of an “HMM” construct comprised of the motor domain, the IQ motifs, and the region of predicted coiled-coil (31). This finding suggested that the full-length DdM7 might be present in GFP-tail IP pellets. However, a high molecular weight band running at the expected position of DdM7 was not observed in either silver-stained gels or immunoblots of the IP pellet (Figs. 2C and 4). To rule out the possibility that the endogenous DdM7 does not co-IP with the GFP-tagged tail because the dimer is co-translationally assembled from full-length molecules, GFP-DdM7 was expressed in wild type cells and isolated by IP. The IP pellet was analyzed by electrophoresis on a 6% SDS gel that was run for several hours to separate the tagged from the untagged DdM7 heavy chain. Similarly to what was observed for the GFP-tail IP, the endogenous DdM7 was not found to co-precipitate with the GFP-tagged DdM7 (Fig. 4). These data suggest that DdM7 does not form a dimer in vivo.

The finding that DdM7 and talinA interact with each other in vivo is consistent with the recent suggestion that talinA is significantly reduced in cells lacking DdM7 (17). The levels of talinA in two different DdM7 null strains, a mutant line made in a KAx-3-derived thymidine auxotroph (19) and a blasticidin-resistant mutant made in the Ax2 line (6), were examined to confirm this observation. Potential differences in sample preparation were controlled for by performing the analysis as described by Gebbie et al. (17) in addition to the usual method that consists of collecting cells by centrifugation and dissolving them in urea-containing sample buffer immediately to minimize proteolysis. Regardless of the method of sample preparation or the cell line examined, talinA did appear to be present, albeit at a somewhat reduced level, in the two different DdM7 null cell lines (Fig. 5). The levels of DdM7 in the talinA null cells was also investigated and found to be unchanged when compared with wild type cells (Fig. 5).

The properties of the in vivo DdM7-talinA complex were investigated further by sucrose density centrifugation. A Dictyostelium high speed supernatant was applied to a linear 8–20% sucrose gradient, and fractions were analyzed for the presence of both DdM7 and talinA by immunoblotting. The two proteins were found to co-sediment in the lighter fractions of the gradient, with a peak of each located at ~12% sucrose (fraction 5) (Fig. 6A). The DdM7-talinA complex sedimented more slowly than the 20 S dynein complex (32) (Fig. 6A) and was estimated to sediment at ~14 S. The sedimentation of each protein in the absence of the other was examined to rule out the possibility that the individual proteins both happen to sediment at 14 S due to homodimerization or to a highly elongated structure. DdM7 from talinA null cells was found in a lighter fraction on the sucrose gradient (Fig. 6B) than observed for wild type samples (10% sucrose, fraction 3). Similarly, talinA from DdM7 null cells sedimented more slowly than observed in wild type samples with a peak observed at 9–10% sucrose (fractions 2 and 3) (Fig. 6C). These results are consistent with the findings from the IP experiments showing that DdM7 and talinA interact exclusively with each other.

The strikingly similar localization of DdM7 and talinA to the leading edge of migrating cells as well as to filopodia (6, 14, 15) and the finding that they interact exclusively with each other suggests that one may serve as a plasma membrane receptor for the other. The localization of GFP-DdM7 in the talinA null mutant and GFP-talinA in the DdM7 null cells was examined to test the possibility. The localization of GFP-DdM7 in the
DdM7 and talinA null mutants was examined first (Fig. 7, panels a–d). GFP-DdM7 was clearly localized to the leading edge of motile cells and in the tips of filopodia in both of the null mutants. Similarly, when the localization of GFP-talinA in either the talinA or DdM7 null mutant cells was examined, it was observed at the leading edge of both and in the filopodia of the talinA null cells (Fig. 7, panels e–h). The level of plasma membrane-associated DdM7 in the talinA null mutants was also examined. A total cell membrane fraction was prepared, and, as predicted, a similar level of DdM7 was found in samples from control and the talinA null mutant cells (Fig. 8). Together, these data reveal that talinA does not serve as a membrane receptor for DdM7 and vice versa.

DISCUSSION

A combination of immunoprecipitation and co-sedimentation experiments has revealed that DdM7 and talinA are associated with each other. They interact directly via the asterisks mark regions of the plasma membrane enriched for the GFP-tagged protein. The bar represents 10 μm.

**FIG. 7. Localization of M7 and talinA in null mutants.** Shown is a gallery of representative confocal images of GFP-M7 in the M7 null mutant (a and b), GFP-M7 in the talinA null mutant (c and d), GFP-talinA in the talinA null mutant (e and f), and GFP-talinA in the DdM7 null mutant (g and h). Arrows point to filopodial tips and filopodia; asterisks mark regions of the plasma membrane enriched for the GFP-tagged protein. The bar represents 10 μm.

DdM7 tail (Figs. 1–3) and, interestingly, no other proteins appear to be present in the complex. Analysis of a series of truncated or deleted DdM7 tails reveals that talinA binds to the N-terminal region of the DdM7 tail that lies between the region of predicted coil and the first MyTH4 domain (Fig. 3). Comparison of this region of DdM7 with other M7s reveals that it is unique to DdM7. However, given that both talin and M7 contribute to adhesion in a wide range of organisms, it is possible that they are both present in an adhesion complex, but their interaction is mediated by another protein. Alternatively, there may be poor sequence conservation in the M7 talin binding region, but both mammalian M7 and DdM7 share a common structural element. It will be interesting to explore the potential relationship between M7 and talin in other organisms to test these possibilities.

Contrary to what might have been expected based on recent work (17), talinA is still present in cells lacking DdM7, although the overall levels do appear to be reduced somewhat (Fig. 5). This indicates that, although the two proteins are associated with each other, they do not depend entirely on each other for stability in vivo. The talinA and DdM7 null mutants have similar phenotypes. They exhibit reduced binding to a range of surfaces, as measured by interference reflection contrast microscopy, bead binding, and particle uptake assays, and they both have lost calcium-dependent adhesion (mediated by gp24/DdCAD-1) (6, 16). The gross localization of DdM7 and talinA is similar. The two proteins are both found on the plasma membrane in areas of dynamic membrane movements as well as in filopodia (6, 14, 15). These findings indicate that DdM7 and talinA have closely related functions and localization, yet talinA is not required for the localization of DdM7 to the plasma membrane in *Dictyostelium* or vice versa (Figs. 7 and 8). This is not entirely unexpected, because talinA and DdM7 do have independent functions. DdM7 is essential for filopodia formation, whereas talinA is dispensable for this process (6). TalinA has a minor role in cytokinesis (15–17), whereas DdM7 does not appear to have any role (19). Thus, although the two proteins do make similar contributions to the ability of a *Dictyostelium* cell to adhere to a range of substrata, they also have clearly distinctive functions.

One of the most notable features of the M7 tail region is the presence of the tandem MyTH4/FERM domains. Several different M7a-binding proteins that link this motor to calcium-dependent adhesion proteins in the plasma membrane interact with the C-terminal MyTH4/FERM domain (8, 9, 27–29). These include vezatin, a protein that interacts with the cadherin-catenin complex and harmonin, a PDZ protein that interacts with cadherin 23 (9). Given that that C-terminal region of M7a is the binding site for these adhesion adaptor proteins, it was initially unexpected to find that talinA does not interact with

**FIG. 8. Normal levels of membrane-associated M7 in the talinA null mutant.** The gross levels of DdM7 on total cellular membranes were compared in wild type and talinA null mutant cells lines by immunoblotting of samples of the initial lyase (postnuclear supernatant (PNS)), the cytosolic fraction (Cytos), and total membranes (Mem). The blot was probed with antibodies against DdM7 and talinA. The same number of cells was used in each preparation. Note that the membrane sample is 10× concentrated relative to the cytosol sample. Total cell lysates from wild type cells (Ax2) and talinA null mutants (talA−) were also included on the blot.
the C-terminal FERM domain of DdM7. However, this is consistent with the finding that talinA is not required for DdM7 membrane association. Binding proteins that serve to link DdM7 to membrane receptors, perhaps via interaction with one or more of the FERM domains, still remain to be identified. Searches of the Dictyostelium genome for homologues of mammalian M7a-binding proteins (or even the regions of those proteins known to interact with M7a) have not uncovered any obvious homologues.2 Given the differences in the organization of the cytoskeleton in the highly specialized sensory cells of the ear and eye, compared with the dynamic cytoskeleton of amoebae, the lack of clear homologues is not surprising. The homology between the M7 tail binding sites of each interacting protein may only encompass a small region and not be readily identified using routine data base search methods. Alternative approaches, such as genetic screens and affinity chromatography, will be necessary to find those proteins responsible for the recruitment of DdM7 (and talinA) to the plasma membrane.

The major question prompted by this work is what the functional significance of the interaction between DdM7 and talinA might be. The interaction between DdM7 and the plasma membrane is dynamic. When a Dictyostelium binds to a particle, DdM7 is immediately present at the plasma membrane of the growing phagocytic cup and remains until the phagosome is formed, then disappears (6). This behavior indicates that the association of DdM7 with its membrane receptor(s) is exquisitely regulated, perhaps accounting for the lack of any additional proteins in the IP. The dynamics of talinA association with the membrane are not yet known, but given their specific interaction it would seem likely that the dynamics of talinA are similar to those of DdM7. One possibility is that there may be a "ready-to-go" pool of DdM7-talinA in the cytosol that is available for recruitment to the membrane where and when it is needed. Once on the membrane, the complex interacts with membrane receptors and/or associated proteins, organizing them into a high avidity complex that allows the cell to bind tightly to a variety of substrata. The dynamics of DdM7 suggest that these proteins are likely to be required early in the adhesion process, either to establish or reinforce initial binding to surfaces. Loss of either protein does not disturb interaction of the remaining partner with the membrane (Figs. 7 and 8), but perhaps the conformation of that remaining partner is such that it can no longer promote formation or stabilization of the adhesion complex. Continued searches for additional DdM7 and talinA binding partners will ultimately provide insight into the contribution of each to adhesion and should aid in better clarification of their functional relationship.

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2 M. A. Titus, unpublished observation.
Identification of a Myosin VII-Talin Complex
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