Dystrophin-related Protein in the Platelet Membrane Skeleton

INTEGRIN-INDUCED CHANGE IN DETERGENT-INSOLUBILITY AND CLEAVAGE BY CALPAIN IN AGGREGATING PLATELETS*

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The platelet membrane is lined with a membrane skeleton that associates with transmembrane adhesion receptors and is thought to play a role in regulating the stability of the membrane, distribution and function of adhesive receptors, and adhesive receptor-induced transmembrane signaling. When platelets are lysed with Triton X-100, cytoplasmic actin filaments can be sedimented by centrifugation at low g-forces (15,600 × g) but the membrane skeleton requires 100,000 × g. The present study shows that DRP (dystrophin-related protein) sediments from lysed platelets along with membrane skeleton proteins. Sedimentation results from association with the membrane skeleton because DRP was released into the detergent-soluble fraction when actin filaments were depolymerized. Interaction of fibrinogen with the integrin αIIbβ3 induces platelet aggregation, transmembrane signaling, and the formation of integrin-rich cytoskeletal complexes that can be sedimented from detergent lysates at low g-forces. Like other membrane skeleton proteins, DRP redistributed from the high-speed pellet to the integrin-rich low-speed pellet of aggregating platelets. One of the signaling enzymes that is activated following αIIbβ3-ligand interactions in a platelet aggregate is calpain; DRP was cleaved by calpain to generate a ~140-kDa fragment that remained associated with the low-speed detergent-insoluble fraction. These studies show that DRP is part of the platelet membrane skeleton and indicate that DRP participates in the cytoskeletal reorganizations resulting from signal transmission between extracellular adhesive ligand and the interior of the cell.

Duchenne muscular dystrophy is one of the most common inherited human diseases. It is caused by a defective gene that codes for a 427-kDa protein, dystrophin (1–5). The deduced amino acid sequence of dystrophin shows that it consists of four domains and suggests that it is a cytoskeletal protein (6). The major rod-shaped domain contains 24 spectrin-like repeats. This domain is flanked on the amino terminus by a domain that has a high degree of homology to the actin-binding domains of spectrin and α-actinin, and on the carboxy terminus by a cysteine-rich domain that shows some homology to a Ca2+-binding region in α-actinin. The most carboxyl-terminal end of dystrophin consists of a short domain that has no homology to any known protein and appears to play a role in linking the molecule to the plasma membrane (7, 8). Recent studies using purified protein or recombinant fragments containing the putative actin-binding domain (9–11) have shown that the protein can bind to actin filaments in vitro, supporting the idea that this molecule functions as a cytoskeletal protein. The finding that dystrophin exists in a submembranous location (8, 12, 13) and that the carboxy-terminal end of the molecule associates tightly with a complex of membrane glycoproteins (termed dystroglycan) (14–17) suggests that dystrophin is a component of a submembranous cytoskeleton.

Although there is now considerable information concerning the structure and interactions of dystrophin, little is known about the way in which the absence of dystrophin leads to muscle cell necrosis. It is well established that in the absence of dystrophin, there are increased concentrations of cytoplasmic Ca2+ and activation of the Ca2+-dependent protease, calpain (18–21), suggesting that dystrophin may play a role in stabilizing the sarcolemma or in regulating the activity of Ca2+ channels (22–25). One of the problems associated with studying the function of dystrophin is that it is found primarily in muscle and brain tissue. In contrast, a related protein, dystrophin-related protein (DRP)1 (26, 27) is present in many different cell types (28). DRP is an autosomal gene product that is 80% homologous to dystrophin (26, 29). Recent studies have shown that DRP is present in a submembranous location (30, 31) and associates with the same complex of transmembrane glycoproteins as does dystrophin (32). Because the extracellular domain of dystroglycan can bind laminin (11, 33) and agrin (34, 35), and DRP co-localizes with agrin-induced acetylcholine receptor clusters, it has been suggested that DRP may play a role in the transmission of signals between the extracellular matrix and intracellular cytoskeleton (35). Given the similarities between dystrophin and DRP, it appears that DRP may serve a similar function to dystrophin. However, as with dystrophin, direct evidence that DRP is part of a membrane skeleton in intact cells or that it is involved in transmembrane signaling is lacking.

One cell-type that has a membrane skeleton that can be readily isolated and analyzed is the blood platelet (36). This skeleton coats the plasma membrane and associates with membrane glycoproteins. It has been visualized morphologically (37, 38), isolated from detergent-solubilized platelets by centrifugation (37), and shown to be composed of short actin filaments, vinculin, spectrin, actin-binding protein and other unidentified proteins.

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‡ The abbreviations used are: DRP, dystrophin-related protein; PCR, polymerase chain reaction; RGDS, Arg-Gly-Asp-Ser.
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Preparation of Platelet Suspensions—Venous blood was drawn from healthy donors, from donors with Duchenne muscular dystrophy, and from patients with Galmann's thorabastenia (whose platelets lack α₁β₂ (43)). Platelets were isolated by centrifugation as described previously (37). Prostacyclin (Sigma) was included in all wash steps at a concentration of 50 ng/ml. Washed platelets were resuspended at a concentration of 1 × 10⁹ platelets/ml at 37°C in a Tyrode's buffer containing 138 mM sodium chloride, 2.9 mM potassium chloride, 12 mM sodium bicarbonate, 5 mM calcium chloride, and 0.4 mM magnesium chloride, pH 7.4. Platelet suspensions were activated with 1.0 NIH unit of thrombin/ml (the thrombin was a generous gift of Dr. J. W. Fenton II of the New York Department of Health, Albany, NY). In some experiments, platelets were preincubated with the calpain inhibitors MLD (44) and EST (45). MLD was the generous gift of Dr. S. Mihdi of Merrill Dow (Cincinnati, OH); EST was from Dr. Tamai of Taisho Pharmaceutical (Saitama, Japan). In other experiments, as described in the figure legends, platelets were preincubated with a synthetic peptide consisting of the sequence Arg-Gly-Asp-Ser (RGDS) (Tetio Pharmaceuticals, Inc., San Diego, CA). Incubations were terminated by addition of one-third volume of ice-cold lysis buffer containing 2% Triton X-100, 10 mM EGTA, 100 mM Tris-Cl, 2 mM MgCl₂, 100 mM benzamidine, and 2 mM phenylmethylsulfonyl fluoride, pH 7.4 (37). Lysates were immediately centrifuged at 15,000 g at 4°C for 4 min to sediment the cytoplasmic actin filaments. The 15,000 g supernatant was further centrifuged at 2× 10⁵ g for 2.5 h at 4°C to 4°C to sediment components of the membrane skeleton (37). In some experiments, as indicated in the text, actin filament depolymerization was induced by omitting EGTA from the lysis buffer, including DNase I (2 mg/ml, Boehringer Mannheim) (39), and incubating lysates at 4°C for 1 h prior to centrifugation. Sedimented material was solubilized by addition of an SDS-containing buffer in the presence of reducing agent (37) and either 1 mg/ml leupeptin or 4 mM EGTA. Samples were analyzed on SDS-polyacrylamide gels according to the method of Laemmli (46). When DRP was under study, samples were electrophoresed through gels containing 6% acrylamide and a ratio of acrylamidel bisacrylamide of 37.5:1. For all other proteins, 7.5% gels and a 29:1 ratio of acrylamide: bisacrylamide were used.

Preparation of Subcellular Fractions—Platelets were lysed by addition of an equal volume of ice-cold lysis buffer containing 2% Triton X-100, 10 mM EGTA, 100 mM Tris-Cl, 2 mM MgCl₂, 100 mM benzamidine, and 2 mM phenylmethylsulfonyl fluoride, pH 7.4 (37). Lysates were immediately centrifuged at 15,000 × g at 4°C for 4 min to sediment the cytoplasmic actin filaments. The 15,000 g supernatant was further centrifuged at 2× 10⁵ g for 2.5 h at 4°C to sediment components of the membrane skeleton (37). In some experiments, as indicated in the text, actin filament depolymerization was induced by omitting EGTA from the lysis buffer, including DNase I (2 mg/ml, Boehringer Mannheim) (39), and incubating lysates at 4°C for 1 h prior to centrifugation. Sedimented material was solubilized by addition of an SDS-containing buffer in the presence of reducing agent (37) and either 1 mg/ml leupeptin or 4 mM EGTA. The 100,000 g supernatant was solubilized by addition of one-third volume of a four times concentrated SDS buffer (37). All samples were heated to 95°C and electrophoresed through SDS-polyacrylamide gels. In any given experiment, detergent-insoluble fractions originating from the same number of blood units were typically 3 × 10⁹ cells and analyzed.

Immunoblotting—Proteins were transferred from SDS gels to nitrocellulose membranes (Bio-Rad) by standard techniques (47). The membranes were stained with Ponceau S solution, blocked overnight in a buffer (TBS) containing 0.1% Tween 20, 137 mM NaCl, 25 mM Tris-HCl, pH 8.0, to which 5% nonfat milk (Carnation) was added. Membranes were incubated overnight at room temperature in the TBS buffer containing the primary antibodies and 1% bovine serum albumin. Membranes were either incubated in alkaline phosphatase-conjugated secondary antibodies (Bio-Rad) and developed with Bio-Rad's 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium color development solutions or were incubated with horseradish peroxidase-conjugated antibody and developed using the enhanced chemiluminescence Western blot detection system (Amersham Corp.).

Polymerase Chain Reaction (PCR) Amplification of Platelet cDNA—Fresh human platelets were isolated and platelet total RNA prepared (48). Approximately 600 ng of RNA were used to synthesize cDNA, using random hexamers and the GeneAmp RNA PCR kit (Perkin-Elmer). The resulting cDNA was used in the PCR-amplification reaction. PCR conditions were 0.15 μM of each primer, 2.5 units of Taq polymerase, denaturation at 94°C for 1 min, primer annealing at 57°C for 2 min, and extension at 72°C for 4 min for 40 cycles. Primer reaction products were separated on an agarose gel and stained with ethidium bromide. Primer pairs from the amino-terminal end of DRP corresponded to nucleotides 28–47 (5'-AGTCTGGACATGCGCAGAA-3') and nucleotides 823–842 (5'-GCCCTTTCTTCACATTTC-3') (29). Primer pairs from the carboxyl-terminal end corresponded to nucleotides 9089–9111 (5'-AACGACGGATAATGTGAAGGAC-3') and 10272–10294 (5'-CTGTGGCTGGGAGAATTT-3') (29). Primers were chosen in separate exons to eliminate possible amplification of any contaminating DNA and were obtained from Operon Technologies Inc. (Alameda, CA). Leukocytes were isolated from human blood on Ficol (Accurate Chemical and Scientific Corp., Westbury, NY) and leukocyte RNA obtained using the same method as was used to isolate platelet RNA. Primers for the light and heavy chains of IgG were obtained from Operon Technologies Inc.

Preparation of Mouse Muscle Microsomes—Skeletal muscle from freshly killed mice (C57BL/10SNJ), The Jackson Laboratory, Bar Harbor, ME was homogenized essentially as described by Ervasti et al. (15), with some modifications. Briefly, tissue from one mouse was homogenized on ice in a buffer containing 50 mM Tris-HCl, 120 mM iodoacetamide, 20 mM Tris-HCl, 120 μM leupeptin, 6 μM benzamidine, and 120 μM phenylmethylsulfonyl fluoride, pH 7.4, using a microscrew attachment to the VirtuShear (Virtis Co., Gardiner, NY) high-speed homogenizer. The tissue suspension was centrifuged in microcentrifuge tubes at 14,000 × g for 10 min. Supernatants were centrifuged at 125,000 × g for 30 min. The pellets were resuspended and washed twice in a buffer containing 5 mM EDTA, 50 mM Tris-HCl, 120 μM leupeptin, 6 μM benzamidine, and 120 μM phenylmethylsulfonyl fluoride, pH 7.4. The washed pellets were resuspended in washing buffer that contained higher concentrations of protease inhibitors (1 mg/ml leupeptin, 50 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride) and solubilized for analysis on SDS-polyacrylamide gels as described below for platelets.

Antibodies—The polyclonal antibody against DRP was generously provided by Dr. Louis Kunkel (Harvard Medical School, Boston, MA). This antibody was generated against a bacterial fusion protein produced using a PCR-amplified 646-base pair sequence located near the carboxyl terminus of the DRP protein product (27). Dystrophin was detected using a polyclonal rabbit antisera, 6-10, also from Dr. Kunkel, which was raised against a fusion protein generated from dystrophin cDNA encoding a large region of the distal portion of the dystrophin rod domain (49). Monoclonal antibodies, NCL-DYS1, against the mid-rod domain of human dystrophin, and NCL-DYS2, against a synthetic polypeptide consisting of the last 17 amino acids of the carboxyl-terminal domain of human dystrophin, were obtained from Novoceastra, Inc. (Newcastle upon Tyne, United Kingdom). Monoclonal antibody 327, against pp60⁶¹⁵, was a generous gift of Dr. J. own Brugge (ARIAID Pharmaceuticals, Inc., Cambridge MA). Monoclonal antibodies against α₁β₂(43) were a gift of Dr. David Phillips of COR Therapeutics (South San Francisco, CA). Polyvalent antibodies against actin-binding protein, talin, and glycoprotein Ib, were raised, affinity-purified, and characterized as described previously (50, 51).

RESULTS

Presence of DRP in Platelets—To determine whether dystrophin or dystrophin-related protein were present in platelets, platelet proteins were electrophoresed through SDS-polyacrylamide gels and analyzed on Western blots. As shown in Fig. 1 (panel A), an antibody (6-10) against the rod domain of dystrophin (49) reacted strongly with dystrophin in mouse muscle but showed negligible reactivity against human platelets. Two other antibodies (NCL-DYS1 and NCL-DYS2) against the mid-rod domain and carboxyl-terminal amino acids of dystrophin, respectively, also reacted with normal mouse muscle but did not react with a protein in the molecular weight range of dystrophin in platelets (data not shown). In contrast, an antibody against DRP (27) showed weak reactivity against mouse muscle but reacted strongly with a protein in human platelets (Fig. 1, panel B). The reactivity was comparable in platelets from normal controls and from patients with Duchenne mus-
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A. Dystrophin  

B. DRP  

C. ABP

**Fig. 1.** Western blots showing the presence of dystrophin-related protein in human platelets. Samples of mouse skeletal muscle or suspensions of human platelets (plts) from a normal control or from a patient with Duchenne’s muscular dystrophy (dys) were electrophoresed through SDS gels and transferred to nitrocellulose paper. Blots were incubated with antibodies raised against dystrophin (panel A), dystrophin-related protein (panel B), or actin-binding protein (ABP) (panel C). Antibody-antigen complexes were detected with alkaline phosphatase-conjugated second antibodies.

**Fig. 2.** Agarose gel electrophoresis showing products from PCR analysis of platelet RNA using primers specific for dystrophin-related protein. Platelet RNA was subjected to reverse transcriptase PCR with primer pairs from the carboxyl-terminal (lane 1) or amino-terminal (lane 2) end of dystrophin-related protein. PCR reaction products were separated on an agarose gel and stained with ethidium bromide. The left-hand lane shows a 1-kilobase pair DNA ladder.

**Fig. 3.** Western blots showing the sedimentation of dystrophin-related protein with cytoskeletal fractions from platelet lysates. Suspensions of platelets (1 × 10⁹ platelets/ml) were solubilized in an SDS-containing buffer (lane 1) or were lysed by addition of a Triton X-100 lysis buffer (lanes 2–4). Lysates were centrifuged for 4 min at 15,600 × g. The resulting pellet was solubilized in SDS-containing buffer (lane 2) and the Triton X-100 supernatant was centrifuged for a further 2.5 h at 100,000 × g. The resulting high-speed pellet (lane 3) and the high-speed supernatant (lane 4) were solubilized in SDS-containing buffer. All samples were electrophoresed through SDS-polyacrylamide gels and transferred to nitrocellulose paper. Blots were incubated with antibody against DRP. Antibody-antigen complexes were detected by enhanced chemiluminescence.

...inherently insoluble in Triton X-100. To distinguish between these possibilities, a lysis buffer that induces depolymerization of actin filaments (39, 51) was used (the buffer contained DNase I and free Ca²⁺) and the effect of this on the solubility of DRP determined. Analysis of the high-speed detergent-insoluble pellets confirmed that the amount of filamentous actin was decreased in lysates containing free Ca²⁺ and DNase I (Fig. 4, compare the first and second lanes of panel A). Depolymerization of actin filaments in the detergent lysates was accompanied by decreased sedimentation of several proteins known to be associated with the membrane skeleton in un-stimulated platelets (42, 51): glycoprotein Ibα (Fig. 4, panel B), αIIbβ3 (Fig. 4, panel C), and pp60⁺⁻SRC (Fig. 4, panel D). Depolymerization of actin was also accompanied by decreased sedimentation of DRP (Fig. 4, panel E).

Incorporation of DRP into Integrin-rich Cytoskeletal Complexes—When platelets are activated, actin polymerization occurs and the cytoskeleton reorganizes such that increased ac-tin, myosin, and other cytoskeletal proteins sediment at low g-forces from detergent-lysed platelets (36, 52, 53). In addition, fibrinogen is secreted from intracellular granules and binds to αIIbβ3. Because it is a bivalent molecule, fibrinogen binds to αIIbβ3 on adjacent platelets and induces platelet aggregation. This cross-linking of αIIbβ3 on adjacent platelets results in...
signaling across the ligand-occupied integrin which in turn results in a second set of cytoskeletal reorganizations (36, 40, 52). The result of this second reorganization is that the redistribution of integrin and membrane skeletal proteins to the low-speed detergent-insoluble fraction occurs slowly in un-stirred platelet suspensions (in which cell-cell contact and, therefore, aggregation is minimized) (Ref. 42 and left-hand panels of Fig. 5) and much more rapidly in suspensions that are stirred (middle panels in Fig. 5); redistribution of the integrin and membrane skeletal proteins occurs more slowly in stirred suspensions if aggregation is inhibited by inclusion of the tetrapeptide RGDS (that binds to the ligand-binding site of \( \alpha_{IIb}\beta_3 \), preventing binding of dimeric adhesive ligand) (Ref. 42 and Fig. 5, right-hand panels). As shown in Fig. 5, the amount of DRP that sedimented at low g-forces increased as platelets were activated with thrombin. The redistribution to the low-speed pellet occurred slowly in un-stirred suspensions (left-hand panel) and more rapidly in stirred suspensions (middle panel); inclusion of RGDS in stirred suspensions decreased the rate of redistribution (right-hand panel). There was a close correlation between the rate at which DRP was incorporated into the membrane skeleton and that at which \( \alpha_{IIb}\beta_3 \) and the other components of the membrane skeleton were incorporated.

Platelets from patients with Glanzmann’s thrombasthenia are deficient in \( \alpha_{IIb}\beta_3 \) (43). Thus, although they undergo other activation-induced events (42), they do not undergo \( \alpha_{IIb}\beta_3 \)-induced transmembrane signaling. Therefore, the integrin-dependent redistribution of membrane skeleton proteins to the low-speed detergent-insoluble fraction does not occur when these platelets are activated (42). In the present study, we observed that although DRP redistributed to the low-speed detergent-insoluble pellet of normal platelets (Fig. 6A), it did not redistribute to the low-speed detergent-insoluble fraction of thrombasthenic platelets that were activated in the same way (Fig. 6B). This finding is consistent with the idea that DRP is part of a submembraneous skeleton that is incorporated into integrin-rich cytoskeletal complexes as a result of integrin-induced transmembrane signaling in platelets.

Integrin-induced Cytoskeletal Reorganizations Lead to the Cleavage of DRP by Calpain—One consequence of the formation of integrin-rich cytoskeletal complexes in aggregating platelets is that calpain is activated (54). Several of the proteins that are cleaved by the protease (spectrin, talin, and actin-binding protein) are components of the integrin-rich cytoskeletal complexes (39, 42, 50). Since the findings described...
above suggest that DRP is a component of these cytoskeletal complexes, we determined whether DRP was also cleaved by calpain. As shown in Fig. 7, the amount of DRP-reactive protein in platelets decreased when platelets were stirred with thrombin (compare lane 2 with lane 1). The decrease in intact DRP was accompanied by the appearance of a fragment of 140 kDa (lane 2 of Fig. 7). The cleavage of DRP did not occur in thrombasthenic platelets (see Fig. 6). Furthermore, a concentration of the tetrapeptide RGDS that partially inhibited fibrinogen binding to \( \alpha_{IIb}\beta_3 \) (as shown by a partial inhibition of aggregation (data not shown)) partially inhibited the cleavage of DRP (Fig. 7, lane 3; see also Fig. 5).

The finding that cleavage of DRP could be inhibited with RGDS and did not occur in thrombasthenic platelets is consistent with cleavage being induced by calpain. To directly test this, platelets were incubated with agonist in the presence of the membrane permeable inhibitors of calpain, MDL (44) and EST (45). The concentration of MDL used was such that it partially inhibited the activity of calpain, as shown by the partial inhibition of the appearance of the degradation products of actin-binding protein (Fig. 8, panel A) (actin-binding protein is cleaved to generate fragments of \(~200\) and \(~100\) kDa; the \(~100\)-kDa fragment is then cleaved further to generate a fragment of \(~91\) kDa (50)). The concentrations of EST used were such that they almost completely prevented degradation of actin-binding protein in thrombin-activated platelets (Fig. 8, panel A). Similarly, MDL partially inhibited degradation of DRP while EST completely prevented any detectable degradation (Fig. 8, panel B). The amount of calpain activation in thrombin-activated platelets can be quite variable. The ex-
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Although the molecular cause of muscular dystrophy is now known, little information is available about the way in which the absence of functional dystrophin leads to muscle necrosis. One of the problems in elucidating the function of dystrophin is that it is present predominantly in muscle and brain. In contrast, a related protein, DRP, is present in many non-muscle cells. In the present study, we show that DRP exists in platelets. In this cell, the membrane is lined by a skeleton that is readily isolated from detergent-lysed platelets by centrifugation (37) and has been visualized by electron microscopy (37, 38). Several lines of evidence show that DRP is a component of the platelet membrane skeleton. First, it was recovered along with the membrane skeleton in the high-speed fraction from detergent-lysed platelets. Second, like other membrane skeleton proteins, it was released from the detergent-insoluble material when actin filaments were depolymerized. Third, DRP redistributed, along with other membrane-skeleton proteins, to the low-speed detergent-insoluble fraction from aggregating platelets. Finally, like other membrane-skeleton proteins (40, 42), the redistribution from the high- to the low-speed pellet in aggregating platelets was dependent on binding of adhesive ligand to $\alpha_{IIb}{\beta}_3$ and did not occur in platelets that lacked this integrin. The observations that dystrophin binds to actin in vitro (9–11), that dystrophin and DRP associate with membrane glycoproteins (14–17, 32), and that dystrophin and DRP exist in a submembranous location (8, 12, 13, 30, 31), have provided circumstantial evidence that these proteins are part of a submembranous cytoskeletal structure. The present study provides direct evidence that DRP is indeed a component of a membrane skeleton in an intact cell.

The finding that DRP is a component of the platelet membrane skeleton suggests a number of potential functions for this protein. For example, the skeleton coats the entire plasma membrane and it is thought that it may regulate the function and distribution of membrane glycoproteins (37, 52), and also stabilize the membrane, preventing microvesicles from being shed (55). It also binds signaling molecules and appears to be involved in transmembrane signaling following integrin-ligand interactions (42). In other cells, binding of extracellular ligand to dystroglycan has been implicated in inducing changes in the organization of a membrane skeleton (34, 35). It is not known whether dystroglycan is present in platelets; the reorganizations of the DRP-containing membrane skeleton detected in the present study were initiated by interaction of extracellular ligand with the integrin $\alpha_{IIb}{\beta}_3$. In platelets, at least two adhesive receptors are associated with the membrane skeleton (glycoprotein Ib-IX and $\alpha_{IIb}{\beta}_3$) (42, 51) and transmembrane signaling is induced as a consequence of ligand binding to both of them (42, 56–59). Thus, it appears possible that DRP is a component of a skeletal structure that reorganizes in response to interaction of associated glycoproteins with their extracellular adhesive ligands. Additional work will be needed to investigate this possibility and to identify the membrane glycoprotein with which DRP associates in platelets.

Based on the fact that the integrin-rich cytoskeletal complexes in platelets associate with cytoplasmic actin (42) and that they contain a number of the proteins present in focal contacts of cultured cells (e.g. talin and vinculin (42)), we have suggested that they may be analogous to focal contacts in adherent cells (36, 42). Interestingly, there have been previous indications that dystrophin may be present in integrin-rich domains in other cells (60). As in focal contacts, a number of signaling molecules appear to associate with the integrin-cytoskeletal complexes in platelets (e.g. pp60$^{c-src}$, pp125$^{FAK}$, phosphoinositide 3-kinase, and calpain (41, 42, 57, 58, 61–64)). At least in some cases, the recruitment of signaling molecules to the integrin-rich cytoskeletal complexes appears to be involved in activation of the enzymes (41, 64). The specific protein-protein interactions that mediate the recruitment of the signaling enzymes to the integrin-cytoskeletal complexes are not known. A number of the proteins present in these cytoskeletal complexes contain the SH2 and SH3 domains that have been implicated in protein-protein interactions. Interestingly, an additional motif present in a number of signaling molecules has recently been identified and shown to be present in DRP (65, 66). It will be of interest to determine whether DRP plays a simple structural role in the integrin-cytoskeletal complexes or whether it is also involved in binding and regulating signaling molecules.

One enzyme that is recruited to focal contacts in cultured cells (67) and is incorporated into the detergent-insoluble integrin-rich cytoskeletal fraction in aggregating platelets (64) is calpain. This protease is selectively activated at sites where the integrin clusters with cytoskeletal proteins (64). Thus, we have suggested that recruitment of the protease to the “focal-contact-like” structures in platelets is the first step in activation of this protease (64). The finding in the present study that DRP is part of the integrin-rich detergent-insoluble cytoskeletal fraction is of interest because one of the characteristics of muscle from patients with Duchenne's muscular dystrophy is activation of calpain and subsequent degradation of muscle proteins. In muscle, it is thought that the absence of dystrophin may result in decreased membrane stability and thus, increased Ca$^{2+}$ concentrations and calpain activation. An alternative idea is that dystrophin normally serves to directly regulate Ca$^{2+}$ fluxes (21, 24, 25). In platelets, integrins have been implicated in the regulation of Ca$^{2+}$ fluxes (68, 69) and calpain activation (54). An increased understanding of the role of the integrin-cytoskeletal complexes and of DRP in regulating calpain activation in platelets may shed light on the way in which the absence of dystrophin leads to increased calpain activation in muscle.

The fact that DRP is cleaved by calpain suggests that it plays an active role in inducing the cytoskeletal remodeling that is induced by integrin-ligand interactions. Previous studies have shown that dystrophin is a substrate for calpain in vitro (70, 71) but it has not been known whether it is cleaved by this protease in an intact cell. The major DRP fragment detected in the present study was one of $\sim 140$ kDa that reacted with an antibody against the carboxyl-terminal end of the molecule. While this end of the molecule contains the binding site for dystroglycan, it does not contain the binding site for actin; despite this, the fragment remained associated with the cytoskeleton in aggregating cells. Future work will be needed to determine whether additional proteolytic fragments remain present in platelets.
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associated with the cytoskeleton and to identify cytoskeletal and membrane proteins that mediate the interaction of DRP and its calpain-induced fragment with the integrin-rich cytoskeletal fraction.

In summary, it is becoming increasingly apparent that the membrane skeleton in platelets binds signaling molecules and is involved in transmitting signals from extracellular adhesive proteins to the interior of the cell. The present study shows that DRP is part of this structure. The finding that integrin-induced transmission of signals from extracellular ligand to the interior results in cleavage of DRP by calpain, suggests that DRP may play an important role in mediating integrin-induced cytoskeletal remodeling and transmembrane signaling. Because the platelet membrane skeleton can be readily obtained from detergent-solubilized platelets, signaling events can be rapidly induced, and DRP-containing integrin-rich cytoskeletal complexes can be isolated, the platelet may provide a useful model in which to characterize the interactions of DRP and to identify the function of this protein. The high degree of homology between dystrophin and DRP suggests that these proteins may serve the same function. Thus, studies on the platelet could lead to an increased understanding of the way in which the absence of dystrophin in patients with Duchenne's muscular dystrophy leads to cell necrosis.

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