A Unique Talin Antigenic Determinant and Anomalous Megakaryocyte Talin Distribution Associated With Abnormal Platelet Formation in the Wistar Furth Rat

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Rats of the Wistar Furth (WF) strain have hereditary macrothrombocytopenia with decreased platelet α-granule proteins. The autosomal recessive pattern of inheritance of the large mean platelet volume (MPV) phenotype and platelet α-granule protein deficiencies suggest that a component common to both formation of platelet α-granules and subdivision of megakaryocyte cytoplasm into platelets is quantitatively or qualitatively abnormal in WF megakaryocytes and platelets. We examined WF platelets for such an abnormality using electrophoretic and immunologic analyses. Rabbit antisera prepared against WF rat platelets and absorbed with Wistar rat platelets recognized a major 235-Kd band, and minor bands of WF rat platelets ranging from 200 to 130 Kd, not present in immunoblots of Wistar, Sprague-Dawley, or Long-Evans rat platelets. The minor bands were labeled with affinity-isolated antibody to the 235-Kd band, indicating that all bands contained the same unique antigenic site. The 235-Kd antigen had the same mobility as rat platelet talin identified with a platelet antitalin antibody. Activation of calcium-dependent proteases during Triton X-100 extraction caused conversion of the 235-Kd antigen into a major fragment of 200 Kd and minor fragments ranging to 115 Kd, identical in mobility to fragments of rat platelet talin produced in the same samples. The absorbed anti-WF platelet antiserum also detected a 235-Kd antigen in WF lung, kidney, and small intestine by immunoblotting. Finally, the 235-Kd antigen unique to WF rats was immunoprecipitated from Triton X-100 supernatants of WF platelets with an antitalin monoclonal antibody (MoAb). These data indicate that the unique antigenic site is on WF talin. Examination of talin distribution in Wistar megakaryocytes showed localization beneath the plasma membrane, on the cytosolic face of demarcation membranes, associated with α-granule membranes, and diffusely throughout the cytoplasm. Although WF megakaryocytes showed the same general distribution pattern, some differences were apparent. In contrast to membrane systems of the Wistar rat, the large membrane complexes in WF megakaryocytes contained little or no talin. In addition, approximately half of WF megakaryocytes showed an increased peripheral localization of talin, often associated with membrane blebs, with decreased talin in the cytoplasmic interior. The association of the unique talin antigenic determinant and anomalous megakaryocyte talin distribution with abnormal platelet formation in WF rats suggests that talin is abnormal in this rat strain and that talin plays an important role in subdivision of megakaryocyte cytoplasm into platelets.

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Materials and Methods

Wistar Furth, Wistar, Wistar Kyoto, Wistar Munich, Sprague-Dawley, Lewis, and Long-Evans hooded rats were purchased from Harlan Industries (Indianapolis, IN). Rabbit antisera prepared against purified human platelet P235 (talin) were provided by Dr Joan Fox of the Gladstone Foundation Laboratories for Cardiovascular Disease, University of California, San Francisco and by Dr Mary Beckerle, University of Utah, Salt Lake City. A mouse monoclonal antibody (MoAb 8d4) to chicken gizzard talin was supplied by Dr Carol Otey of the Department of Cell Biology and Anatomy, University of North Carolina at Chapel Hill. Alkaline phosphatase-conjugated goat anti-rabbit IgG and goat anti-mouse IgG and alkaline phosphatase substrate kits were purchased from BioRad Laboratories, Richmond, CA. Alkaline phosphatase-
conjugated protein A was purchased from Boehringer Mannheim, Indianapolis, IN. 125I-Labeled protein A was obtained from ICN Biomedicals, Costa Mesa, CA. Platelet counts were determined by phase microscopy.

Collection and solubilization of platelets and other tissues. Blood was collected from the dorsal aorta or through cardiac puncture of methofane-anesthetized rats into syringes containing Na,EDTA or acid-citrate-dextrose. Platelets were separated by differential centrifugation. Platelets were pelleted and routinely washed three times in EHS buffer (0.001 mol/L Na,EDTA, 0.01 mol/L HEPES buffer, and 0.15 mol/L NaCl). Platelets suspended in EHS buffer were solubilized for electrophoresis by boiling for 5 minutes in one-third volume of gel sample buffer containing 0.125 mol/L Tris-HCl, pH 6.8, 4% glycerol, 8% sodium dodecyl sulfate (SDS), 160 mmol/L dithiothreitol (DTT), and 0.01% bromphenol blue. RBC were separated from EDTA-anticoagulated blood by differential centrifugation and washed free of platelets. Whole RBC were solubilized by boiling in one-third volume of the SDS gel sample buffer described above.

Other rat tissues also were solubilized for electrophoresis. To eliminate platelet contamination, these other tissues (lung, kidney, and small intestine) were collected 7 days after rats were myelosuppressed by lethal Cs-137 irradiation. Platelet counts at the time of tissue collection were 1 x 10¹⁰ and 7 x 10¹⁰/mL, respectively, in WF and Wistar rats. Tissues were homogenized in a tissue grinder in EHS with protease inhibitors, including 1 mg/mL leupeptin, 8 mg/mL benzamidine-HCl, and 2 mmol/L phenylmethylsulfonylfluoride (PMSF). The homogenates were solubilized for electrophoresis by boiling 5 minutes in one-third volume of SDS gel sample buffer.

Preparation of platelet antiserum. Rabbit antiserum against WF platelets was prepared by intravenous (IV) injection of isolated, washed platelets twice weekly for 3 weeks followed by periodic booster immunizations. Blood was collected from the central ear artery, and serum was prepared, heat-inactivated, and absorbed with intact Wistar platelets and 15,850- and 100,000-g pellets of Wistar platelets after extraction with 1% Triton X-100. One-tenth milliliter of antiserum was absorbed with 1 mL of packed Wistar platelets and subsequently with the low-speed (15,850 g) and high-speed (100,000g) Triton X-100 pellets of those platelets. In some cases, the antiserum also was absorbed with Triton X-100 supernatants from Wistar rat platelets.

Triton X-100 extraction of platelets. For Triton X-100 extraction experiments, washed platelets suspended in EHS buffer were extracted with an equal volume of 2% Triton X-100 (Sigma Chemical Co, St Louis, MO) in 0.1 mol/L Tris-HCl buffer (pH 7.4) containing 10 mmol/L EGTA, 2 mmol/L PMSF, 1 mg/mL leupeptin, and 8 mg/mL benzamidine-HCl for 30 minutes on ice. Lysates were centrifuged at 4°C for 4 minutes at 15,850g and, in some cases, the Triton X-100 supernatants were centrifuged for 2½ hours more at 100,000g. The low-speed and high-speed pellets and the high-speed supernatants were then solubilized for electrophoresis by boiling for 5 minutes in one-third volume of SDS gel sample buffer.

For Triton X-100 extraction experiments in which calcium-dependent proteases were to be activated, platelets were collected in acid-citrate-dextrose, washed, and resuspended in EHS. EGTA was omitted from the Triton X-100 extraction buffer, and calcium-dependent proteases were activated by addition of calcium at a final concentration of 3 mmol/L. The extraction time was 5 minutes instead of 30 minutes to distinguish the differential sensitivities of different proteins to hydrolysis by calcium-dependent proteases.

Immunoprecipitation. Blood was collected into EDTA, and platelets were separated by differential centrifugation. Platelets were washed, suspended in EHS at a concentration of 2 x 10¹²/mL, and extracted with an equal volume of 2% Triton X-100 containing 10 mmol/L EGTA, 1 mg/mL leupeptin, 8 mg/mL benzamidine-HCl, and 2 mmol/L PMSF. Insoluble material was pelleted at 15,850g for 4 minutes, and 2 mL of supernatant was preabsorbed by incubation with protein A-sepharose for 30 minutes at 4°C. The protein A-sepharose was pelleted, and the supernatant was divided into two equal samples. One sample was incubated for 2 hours at 4°C with 300 μL of 8d4 antitalin monoclonal antibody (MoAb). The other sample was incubated in parallel with an irrelevant MoAb of the same IgG isotype as that of the 8d4 MoAb (IgG). Next, the Triton X-100 supernatants were incubated for 2 hours at 4°C with 150 μL of a 50% suspension of protein A-sepharose preincubated for 2 hours with 400 μg of affinity-isolated rabbit anti-mouse IgG. Then the protein A-sepharose-bound antigen-antibody complexes were pelleted, and the pellets were washed six times with EHS and Triton X-100 extraction buffer in a ratio of 1:1. Finally, the precipitates were solubilized by boiling directly in the SDS gel sample buffer described above.
The nitrocellulose strips were incubated overnight with the specific antiserum, washed, and incubated with $^{125}$I-labeled protein A or with alkaline phosphatase-conjugated second antibody or alkaline phosphatase-conjugated protein A. Two percent casein was included in all incubations and washes to reduce nonspecific binding. The strips were dried, and autoradiograms were prepared with Kodak XAR-5 film when $^{125}$I-protein A was used as the antibody label. Alkaline phosphatase substrate kits were used to detect binding of alkaline phosphatase conjugates. In some cases, after the initial blots were photographed, the blots were developed with Kodak XAR-5 film.

20% methanol. The nitrocellulose strips were incubated overnight with the specific antiserum, washed, and incubated with $^{125}$I-labeled protein A or with alkaline phosphatase-conjugated second antibody or alkaline phosphatase-conjugated protein A. Two percent casein was included in all incubations and washes to reduce nonspecific binding. The strips were dried, and autoradiograms were prepared with Kodak XAR-5 film when $^{125}$I-protein A was used as the antibody label. Alkaline phosphatase substrate kits were used to detect binding of alkaline phosphatase conjugates. In some cases, after the initial blots were photographed, the blots were developed with Kodak XAR-5 film.

**Fig 2. Immunoblot of Wistar, WF, Sprague-Dawley, and Long-Evans platelets indicating high-mol-wt antigenic determinants unique to WF platelets as detected with a Wistar platelet-absorbed rabbit anti-WF platelet antiserum: the most prominent protein recognized by the antiserum has a mol wt of 235 kDa. Antibody binding was detected with $^{125}$I-protein A. MW, molecular weight; LE, Long-Evans rat; SD, Sprague-Dawley rat.**

**Gel electrophoresis.** One-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) was performed in linear gradients of acrylamide using the discontinuous buffer system of Laemmli. Usually, about 65 μg of protein contained in 40 μL of buffer was loaded per lane. Gels were stained with Coomassie blue for visualization of proteins.

**Immunoblotting of antibodies to electrophoretically separated proteins.** Proteins separated in polyacrylamide gels were electrophoretically transferred to nitrocellulose sheets in a transfer buffer consisting of 25 mmol/L Trizma base, 192 mmol/L glycine, and 20% methanol. The nitrocellulose strips were incubated overnight with the specific antiserum, washed, and incubated with $^{125}$I-labeled protein A or with alkaline phosphatase-conjugated second antibody or alkaline phosphatase-conjugated protein A. Two percent casein was included in all incubations and washes to reduce nonspecific binding. The strips were dried, and autoradiograms were prepared with Kodak XAR-5 film when $^{125}$I-protein A was used as the antibody label. Alkaline phosphatase substrate kits were used to detect binding of alkaline phosphatase conjugates. In some cases, after the initial blots were photographed, the blots were developed with Kodak XAR-5 film.

**Fig 3. Immunoblot of Wistar and WF platelet fractions after extraction with Triton X-100 showing that the unique antigenic determinants detected in WF platelets by a Wistar platelet-absorbed rabbit anti-WF platelet antiserum were present in the high-speed (100,000 g) pellet and supernatant but not in the low-speed (15,850 g) pellet. Antibody was detected with $^{125}$I-protein A. MW, molecular weight; W, Wistar rat.**
were probed with another antibody (without removing the initial antibody) so that mobility of the detected antigens could be determined precisely. Alkaline phosphatase conjugates were used for detection of antibody binding in such cases. In some instances, antibody to the unique 235-Kd WF antigen and antibody to human platelet talin were affinity purified from nitrocellulose transfers of WF or Wistar platelets, respectively, that had been incubated with the appropriate antiserum, the antibody detected with alkaline phosphatase-conjugated second antibody, the 235-Kd band excised, and the antibody eluted with glycine-HCl.

Preparation of marrow for immunoelectron microscopy. To increase marrow megakaryocyte concentration, Wistar and WF rats received 150 mg/kg 5-fluorouracil IV through a tail vein 12 days before they were killed. Rats were exsanguinated under metofane general anesthesia. Femurs were removed and cracked open with bone clippers. Femoral marrow was removed, placed in a few drops of 8% paraformaldehyde (in 0.1 mol/L PIPES buffer, pH 7.4) in a plastic Petri dish, and cut into small pieces with a razor blade. The marrow pieces were then fixed for 90 minutes at room temperature in a larger volume of the 8% paraformaldehyde solution. The fixed tissue was washed three times in 0.1 mol/L PIPES buffer, pH 7.4, in 10% sucrose, dehydrated in a graded ethanol series, and then infiltrated and embedded in LR White (Ted Pella, Redding, CA). Thin sections were cut and placed on collodion-coated grids.

Immunogold labeling. Tissue sections were incubated for 30 minutes in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) and 0.01 mol/L glycine and then incubated overnight at 4°C with antitalin antibodies diluted in this same buffer. They were then washed and incubated for 1 hour at room temperature with protein A-gold (9-nm particles; Upstate Biotechnology, Lake Placid, NY), prepared as a 1:20 dilution in this same buffer. Infiltrated and embedded in LR White (Ted Pella, Redding, CA). Antiserum labeled a major band with a molecular weight (mol wt) of 235 Kd, and minor ones with mol wt ranging from 130 to 200 Kd, which were unique to WF platelets. However, after absorption with intact Wistar platelets and with 15,850-g and 100,000-g Triton X-100 Wistar platelet pellets, the antiserum labeled a major band with a molecular weight (mol wt) of 235 Kd, and minor ones with mol wt ranging from 130 to 200 Kd, which were unique to WF platelets (Fig 2). These unique antigenic determinants were not detected in Long-Evans, Sprague-Dawley, Wistar (Fig 2), Wistar-Kyoto, Wistar-Munich, or Lewis rats (data not shown). The WF platelet bands uniquely detected by the antiserum had the same mol wt when platelets were prepared without reducing agent (not shown). In addition to the unique antigenic sites detected on WF platelets, the absorbed antiserum labeled three platelet bands with mol wts of approximately 80, 70, and 45 Kd in all rat strains, indicating that not all rat strain cross-reacting platelet antibodies were removed by absorption of the antiserum with whole Wistar

RESULTS

A rabbit antiserum raised against WF platelets recognized a number of proteins in immunoblots of reduced samples of both Wistar and WF platelets (Fig 1); antibody binding to a 235-Kd antigen was somewhat more intense in WF than in Wistar platelets, whereas binding to some other bands was more intense in Wistar platelets. However, after absorption with intact Wistar platelets and with 15,850-g and 100,000-g Triton X-100 Wistar platelet pellets, the antiserum labeled a major band with a molecular weight (mol wt) of 235 Kd, and minor ones with mol wt ranging from 130 to 200 Kd, which were unique to WF platelets (Fig 2). These unique antigenic determinants were not detected in Long-Evans, Sprague-Dawley, Wistar (Fig 2), Wistar-Kyoto, Wistar-Munich, or Lewis rats (data not shown). The WF platelet bands uniquely detected by the antiserum had the same mol wt when platelets were prepared without reducing agent (not shown). In addition to the unique antigenic sites detected on WF platelets, the absorbed antiserum labeled three platelet bands with mol wts of approximately 80, 70, and 45 Kd in all rat strains, indicating that not all rat strain cross-reacting platelet antibodies were removed by absorption of the antiserum with whole Wistar platelets before and after calcium activation of calcium-dependent proteases showing that the unique antigenic determinants detected in WF platelets by a Wistar platelet-absorbed rabbit anti-WF platelet antiserum have the same mol wt on SDS-PAGE analysis as platelet P235 (talin) and its proteolytic fragments. (A) Coomassie blue-stained proteins of Wistar and WF platelets separated by SDS-PAGE in 7.5% to 20% linear polyacrylamide gradients before or after 5-minute incubation with 3 mmol/L calcium to activate calcium-dependent proteases. (B) Immunoblot of a duplicate gel as in A with affinity-isolated Wistar platelet-absorbed rabbit anti-WF platelet antibody. (C) Reblot of B blot with affinity-isolated rabbit antihuman platelet talin antibody. MW, molecular weight; ABP, actin-binding protein. Antibody to the unique antigenic sites on WF platelets was affinity isolated by binding, detection, and glycine-HCl elution from blots of WF platelets. Antiplatelet P235 antibody was affinity isolated by binding, detection, and glycine-HCl elution from blots of Wistar platelets. Antibody binding was detected with alkaline phosphatase-conjugated goat anti-rabbit IgG.
platelets and their low- and high-speed Triton X-100 pellets. The antigenic sites unique to WF platelets were not released by thrombin activation (not shown).

The unique WF antigenic sites were observed in the 100,000-g pellet and supernatant but not in the 15,850-g pellet after immunoblotting of Triton X-100 extracts of WF platelets with Wistar platelet-absorbed anti-WF platelet serum (Fig 3).

The similar mol wt of platelet talin and the unique 235-Kd WF antigenic site detected by the anti-WF platelet serum prompted an examination of whether the unique 235-Kd antigenic site was indeed on talin. Two questions were addressed. First, was the mol wt of the unique 235-Kd platelet antigenic site of WF rat platelets identical to that of platelet talin? Second, was the unique 235-Kd WF antigenic site cleaved by calcium-dependent proteases as is talin? Affinity-isolated anti-WF platelet antibody to the 235-Kd unique WF platelet antigenic site was prepared by immunoblotting electrophoresed proteins of WF platelets with Wistar platelet-absorbed anti-WF platelet serum, excising the antibody-reactive band, and acid-eluting the antibody. Affinity-isolated antibody to platelet talin was prepared in the same way from immunoblots of Wistar platelets using the rabbit anti-human platelet talin antibody provided by Dr Joan Fox. These affinity-isolated antibodies were used in the following experiment. Triton X-100 extracts of Wistar and WF platelets were prepared with and without activation of calcium-dependent proteases. Electrophoresed samples of the Triton X-100 extracts were first immunoblotted with the affinity-isolated anti-WF platelet antibody, photographed, and then rebotted with affinity-isolated antitalin antibody. Examination of these gels and immunoblots (Fig 4) showed the following: (a) the mol wt of the 235-Kd protein detected by the affinity-isolated anti-WF platelet antibody (Fig 4B) was identical to that of talin (Fig 4C); (b) the other unique bands detected in WF platelets by the Wistar platelet-absorbed anti-WF rat platelet serum also are detected by the affinity-isolated anti-WF antibody (Fig 4B), indicating that the same antigenic site is being recognized in all the unique WF bands; (c) these other unique WF bands (Fig 4B) have the same mol wt as minor bands recognized by the affinity-isolated antitalin antibody (Fig 4C); and (d) activation of calcium-dependent proteases by addition of millimolar calcium during Triton X-100 extraction cleaved the 235-Kd antigen into a major fragment of approximately 200 Kd and minor ones of lower mol wt (Fig 4B), identical in size to fragments of rat platelet talin produced in the same samples (Fig 4C).

Next, we examined whether the 235-Kd WF platelet protein with the unique antigenic site could be immunoprecipitated with MoAb to talin. Triton X-100 extracts of WF platelets were prepared; one half were incubated with a MoAb (8d4) to talin, and the other half were incubated with an irrelevant MoAb of the same IgG isotype. After electrophoresis and transfer to nitrocellulose, the immunoprecipitated proteins were immunoblotted with Wistar platelet-absorbed anti-WF platelet antibody. The antitalin MoAb immunoprecipitated the 235-Kd WF platelet protein with the unique antigenic site, but the irrelevant MoAb did not (Fig 5).

Because talin exists in many cell types, we examined other WF tissues, including lung, kidney, small intestine,
showing that the unique antigenic site detected on a 235-Kd protein of WF platelets also exists in WF rat lung, small intestine, and kidney. Nitrocellulose transfers of platelet, lung, small intestine, and kidney proteins were blotted with the Wistar platelet-absorbed anti-WF platelet antibody (A), photographed, and reblotted with polyclonal antibody to human platelet talin (B). Antibody binding was detected with alkaline phosphatase-conjugated protein A or second antibodies. P, platelets; L, lung; S, small intestine; K, kidney.

Next we examined the talin distribution in Wistar and WF megakaryocytes by immunogold electron microscopy to determine whether any differences were associated with the unique antigenic determinant in WF talin. In Wistar megakaryocytes, talin was localized beneath plasma membranes, on the cytosolic face of demarcation membranes, and diffusely throughout the cytoplasm (Fig 7A). In addition, label was apparent on a substantial proportion of granules. The granule-associated label (usually one or two gold particles) was localized on or immediately adjacent to granule membranes, never in the granule matrix. Mitochondria and cisternae of the demarcation membrane system were devoid of label. Very low background staining was observed when sections were incubated with protein A-gold alone (Fig 7C). Although WF megakaryocytes showed the same general talin localization, some important differences were observed (Fig 7B). In contrast to membrane systems of the Wistar rat, the large membrane complexes present in WF megakaryocytes contained little or no talin. In addition, approximately half of the WF megakaryocytes showed an increased peripheral localization of talin, often associated with membrane blebs, with decreased talin in the cytoplasmic interior.

DISCUSSION

WF platelets have abnormally large mean platelet volume with increased platelet size heterogeneity, reduced platelet counts, and decreased levels of α-granule proteins, inherited as autosomal recessive traits. Both megakaryocytes and platelets of this rat strain contain large membrane complexes and tubular structures. Megakaryocytes show haphazard arrangement of the demarcation membrane system, which is believed to be involved in platelet membrane formation. The autosomal recessive inheritance pattern of this WF platelet phenotype suggests that megakaryocytes have qualitative or quantitative abnormalities in a macromolecule involved in the process of platelet formation.

The unique 235-Kd antigenic site detected in WF platelets with antiserum raised against WF platelets and absorbed with Wistar platelets has the same electrophoretic mobility as rat platelet P235 (talin). Platelet talin also is solubilized by Triton X-100 extraction as was the unique 235-Kd antigen. Human platelet talin is cleaved by calcium-dependent proteases into a major fragment with a mol wt 200 Kd. The identical electrophoretic mobilities of rat platelet talin and the unique 235-Kd antigen of WF platelets coupled with the cleavage of the unique 235-Kd antigen by calcium-dependent proteases to a major 200-Kd fragment and minor ones ranging to 115 Kd, identical in size to rat platelet talin fragments formed in the same samples, suggested that the unique antigenic site is on platelet talin. Immunoprecipitation of the unique 235-Kd antigen by an antitalin MoAb provided further evidence that the unique antigenic site detected by the absorbed anti-WF platelet antisera is on talin. Recognition of the 200-Kd but not the 46-Kd calpain fragment of WF platelet talin by the anti-WF rat platelet antibody indicates that the...
Fig 7.
antibody recognizes a unique epitope in the C-terminal portion of talin, because Rees et al showed that the 200-Kd calpain fragment is C-terminal. The tissue distribution of the unique 235-Kd WF antigen (absent in RBC but present in lung, kidney, and small intestine) is also consistent with the tissue distribution of talin.

In resting platelets, talin is homogeneously distributed; however, in cells that show attachment, such as smooth muscle cells and fibroblasts, talin is localized to sites where bundles of actin filaments interact with plasma membranes. The talin distribution in Wistar megakaryocytes showed localization beneath plasma membranes, on the cytosolic face of demarcation membranes, on or adjacent to granule membranes, and diffusely throughout the cytoplasm. WF megakaryocytes showed a similar pattern of talin distribution, but with some interesting differences. In contrast to membrane systems of Wistar megakaryocytes, the large membrane complexes of megakaryocytes showed little or no talin and peripheral concentration of talin (often in membrane blebs) was increased, whereas that in the interior cytoplasmic region was decreased, in approximately half of the WF megakaryocytes. Several conclusions can be made from these megakaryocyte talin distribution results. First, association of talin with demarcation and α-granule membranes as well as plasma membranes suggests that talin may play a role in platelet and α-granule membranes. In resting platelets, talin is homogeneously distributed. Second, the anomalous talin distribution in WF megakaryocytes is consistent with our hypothesis that talin is abnormal in WF and that a talin defect is responsible for the abnormal α-granule and platelet formation in this animal. Third, the membranous association of talin in megakaryocytes (reported here), but homogeneous distribution in resting platelets, suggests that talin undergoes a posttranslational modification that causes its dissociation from megakaryocyte membranes as talin in megakaryocytes at or near the time of platelet formation. Phosphorylation is a likely candidate. Evidence for this possibility is provided by a report that phosphorylation of talin increased in a kidney cell line as the distribution of this protein changed from focal adhesion localization to a homogeneous cytoplasmic staining pattern after treatment with the phorbol ester TPA, an activator of protein kinase C.

What is the function of talin? Based on its localization pattern in attached cells, talin is postulated to be a link between membrane proteins such as the integrin family and filamentous actin. Mouse talin DNA has been cloned and sequenced and encodes a protein of 2,541 amino acids. A 200 to 220 amino-acid segment in the N-terminal portion of talin bears significant homology to the N-terminal 200 residues of an RBC membrane-skeletal protein, protein 4.1, which links the spectrin-actin cytoskeleton to the RBC membrane suggesting that the homologous region of talin may bind to a plasma membrane protein. A membrane attachment site for talin may be β integrins, to which weak talin binding has been reported in vitro. The exact nature of the linkage of talin to actin is unclear. Purified talin has been reported to bind directly to both purified G- and F-actin and to promote actin filament nucleation. Purified human platelet talin also binds tightly to vinculin and metavinculin, which colocalize with talin to areas where actin filaments terminate at plasma membranes.

If the unique talin antigenic determinant of WF rats is associated with a megakaryocyte talin functional abnormality, do other talin-containing tissues also show abnormal function? This question has not been addressed directly; however, WF rats are known to have a lower reproductive rate with smaller litter size than other rat strains. Other subtle changes in organ function may have been overlooked previously because the major interest in these rats has been their high spontaneous tumor incidence.

Association of the unique antigenic site detected on a 235-Kd protein in WF platelets, identified as talin, and the anomalous distribution of talin in WF megakaryocytes, with haphazard arrangement of the demarcation membrane system and abnormal development of α-granules in megakaryocytes of these rats, lead us to speculate that talin is involved in α-granule formation and subdivision of megakaryocyte cytoplasm into platelets and that an abnormality in this protein in WF megakaryocytes results in disorganized platelet formation.

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