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QUARTERLY

Smooth muscle actomyosin promotes Ca^{2+}-dependent interactions between annexin VI and detergent-insoluble glycosphingolipid-enriched membrane domains★✪

Victoria S. Babiychuk[1], Annette Draeger[2] and Eduard B. Babiychuk[1, 2] ✉

[1]*Institute of Physiology, Kiev University, 252031 Kiev, Ukraine;*
[2]*Institute of Anatomy, University of Bern, 3012 Bern, Switzerland*

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The mechanical link coupling cytoskeletal and contractile proteins to the sarcolemma of smooth muscle cells is essential for transmitting tension from the cell's interior to exterior. In addition to the well-characterized actin-integrin associations present in *adhaerens* junctions, our recent work has postulated the existence of a reversible annexin-dependent membrane-cytoskeleton complex, forged in response to a rise in intracellular Ca^{2+} concentration following smooth muscle cell stimulation (Babiychuk *et al., J. Biol Chem.* 1999, 274, 35191–35195). Detailed biochemical characterization of the interactions responsible for the formation of this complex revealed that annexins II and VI interact with actomyosin, or detergent-insoluble glycosphingolipid-enriched membrane domains (rafts) purified from smooth muscle, in a concentration- and Ca^{2+}-dependent manner. Annexin II interacted with lipid rafts with high Ca^{2+}-sensitivity, while for annexin VI this interaction required non-physiologically high concentrations of free Ca^{2+}. However, the Ca^{2+}-sensitivity of the latter interaction strongly increased in the presence of purified smooth muscle actomyosin. The detailed biochemical analysis of the interactions occurring between annexin II, annexin VI, actomyosin and rafts suggests that annexins regulate sarcolemmal organization during smooth muscle cell contraction.

The actomyosin-based contractile apparatus of smooth muscle cells exerts a force, which is transmitted to the surrounding extracellular matrix *via* actin attachment sites residing

★75th Anniversary of Membrane Lipid Bilayer Concept.

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✉Corresponding author: Eduard B. Babiychuk, Department of Cell Biology, Institute of Anatomy, University of Bern, Bühlstr. 26, CH-3012 Bern, Switzerland; tel: (41 31) 631 4625; Fax: (41 31) 631 3807; e-mail: edik@ana.unibe.ch

Abbreviation: DIGs, detergent-insoluble glycosphingolipid-enriched membrane domains.

within the sarcolemma. In order to protect the sarcolemma from mechanical damage, the membrane-cytoskeleton constituents have to adapt instantly to alterations in cell length. It is therefore conceivable that changes in membrane organization and interactions with the cytoskeleton and contractile apparatus are regulated analogously to cellular contractions, by Ca^{2+}-dependent pathway(s).

Members of the annexin protein family have been implicated in Ca^{2+}-dependent interactions between membranes and cytoskeletal elements (reviewed in Raynal & Pollard, 1994; Gerke & Moss, 1997). Recently, we have demonstrated the existence of a reversible annexin-dependent membrane-cytoskeleton complex connecting actin-based cytoskeletal elements with detergent-insoluble glycosphingolipid-enriched membrane domains (DIGs). This complex is forged in smooth muscle cells in response to a rise in intracellular Ca^{2+} concentration (Babiychuk *et al.*, 1999). The purpose of the present study was to pinpoint the interactions governing the formation of this complex and to elucidate the mode of its biochemical regulation.

MATERIALS AND METHODS

Tissue preparation and immunohistochemistry. For antibody production, annexin II and annexin VI were purified as described in "Results". Subcutaneous immunization of rabbits was performed in compliance with the protocol established by Harlow & Lane (1988). The antibodies thereby generated were purified by anion-exchange chromatography (Q-Sepharose FF; Pharmacia Biotech, Uppsala, Sweden).

Processing for contraction/relaxation experiments, thin cryo-sectioning and immunolabeling were performed as described previously (Babiychuk *et al.*, 1999). Fluorescent labeling was performed using Cy3- (Jackson, Baltimore, U.S.A.) or Alexa-conjugated (Molecular Probes, Eugene, U.S.A.) secondary antibodies. Negative controls were generated by absorbing the antibody with purified antigen. Tissue sections were examined in a Zeiss Axiophot fluorescent microscope and images collected using a digital CCD camera (Ultrapix, Astrocam, U.K.).

Purification of a smooth muscle annexin-dependent membrane-cytoskeleton complex and isolation of its individual components. Unless otherwise stated, all procedures were performed at 4°C or on ice. Minced porcine stomach muscle (100 g) was extracted in 300 ml of buffer A (60 mM KCl; 2 mM $MgCl_2$ and 20 mM imidazole, pH 7.0) containing 0.5% Triton X-100 and 1 mM EGTA. After centrifugation at 6 000 × ***g*** for 30 min, the supernatant was filtered through glass wool and clarified at 50 000 × ***g*** for 90 min. The resulting supernatant (concentration of free Ca^{2+} adjusted to 200 μM) was subjected to high-speed centrifugation at 50 000 × ***g*** for 90 min. The pellet thereby obtained was washed 3 times (6000 × ***g*** for 30 min) in 10 volumes of buffer B (120 mM KCl and 20 mM imidazole, pH 7.0) containing 0.2 mM $CaCl_2$. This preparation, designated as "Ca^{2+} high-speed pellet", represented the annexin-dependent membrane-cytoskeleton complex.

For purification of individual components, preparations of the annexin-dependent membrane-cytoskeleton complex were extracted with buffer B containing 1 mM EGTA and centrifuged at 10 000 × ***g*** for 30 min. The supernatant thereby obtained was further centrifuged at 20 000 × ***g*** for 60 min. The resulting pellet was washed (20 000 × ***g*** for 60 min) and finally resuspended in buffer B. This represented the purified DIG fraction. The resulting supernatant was clarified at 20 000 × ***g*** for 60 min, representing the purified annexin fraction.

Smooth muscle actomyosin was purified according to the method described by Sobieszek & Bremel (1975). Minced porcine stomach muscle (100 g) was extracted in 300 ml of buffer A containing 0.5% Triton X-100 and 1 mM EGTA. The homogenate was centri-

fuged at 6 000 × ***g*** for 30 min and the pellet washed 3 times in the same buffer (6 000 × ***g*** for 30 min). The pellet thereby obtained was extracted by 3 volumes of buffer A containing 2 mM EDTA, 2 mM EGTA and 5 mM ATP. The supernatant obtained after centrifugation at 15 000 × ***g*** for 30 min was filtered through glass wool and its $MgCl_2$ concentration raised to 25 mM. This fraction was incubated overnight at 4°C and then centrifuged at 6 000 × ***g*** for 30 min. The resulting pellet was washed extensively in buffer A containing 2 mM EGTA; it consisted of purified actomyosin.

Purification of annexins II and VI. For antibody production, annexin II and VI were separated by anion-exchange chromatography. The annexin fraction was dialyzed against buffer A and applied on Q-Sepharose FF column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with the same buffer. Annexin II was recovered in the flow-through fraction, while annexin VI was eluted by a linear salt gradient (60–300 mM NaCl). Annexin II was dialyzed against buffer A (pH = 5.8) and further purified by cation-exchange chromatography (CM-Sepharose; Pharmacia Biotech, Uppsala, Sweden). The column was equilibrated in the same buffer and eluted with 300 mM NaCl. A final purification step for both annexins consisted of gel filtration chromatography (Sephacryl S-200 HR; Pharmacia Biotech, Uppsala, Sweden).

Miscellaneous. Annexin VI null mutant mice (Hawkins *et al.*, 1999) were kindly provided by Prof. S.E. Moss (London). Thin-layer chromatography (TLC) was performed using a modified version (Babiychuk *et al.*, 1999) of the protocol published by Macala *et al.* (1983). SDS/PAGE was carried out using the procedure described by Laemmli (1970). Polypeptides were visualized by staining with Coomassie Brilliant Blue. Blotting of gels on Immobilon-P membranes (Millipore Corporation, Bedford, MA, U.S.A.) was performed as detailed by Towbin *et al.* (1979). Immunoreactivity was detected using a secondary antibody conjugated to horseradish peroxidase (Amersham, Switzerland) and visualized with metal- diaminobenzidine. Protein concentrations were determined according to the method described by Bradford (1976) using bovine serum albumin as a standard. Free Ca^{2+} concentrations ($[Ca^{2+}]_{free}$) were calculated using MAX CHELATOR software (Chris Patton, Stanford University, Hopkins Marine Station, U.S.A.).

RESULTS

Characterization of polyclonal anti-annexin II and anti-annexin VI antibodies

In tissue homogenates (Fig. 1*a*, lane 1; Fig. 1*b*, lanes 1, 2), in the preparations of the annexin-dependent membrane-cytoskeleton complex (Fig. 1*a*, lane 3; Fig. 1*b*, lanes 3, 4) and in cultured cells (Fig. 1*a* and Fig. 1*b*, lanes 5, 6), the anti-annexin VI- and the anti-

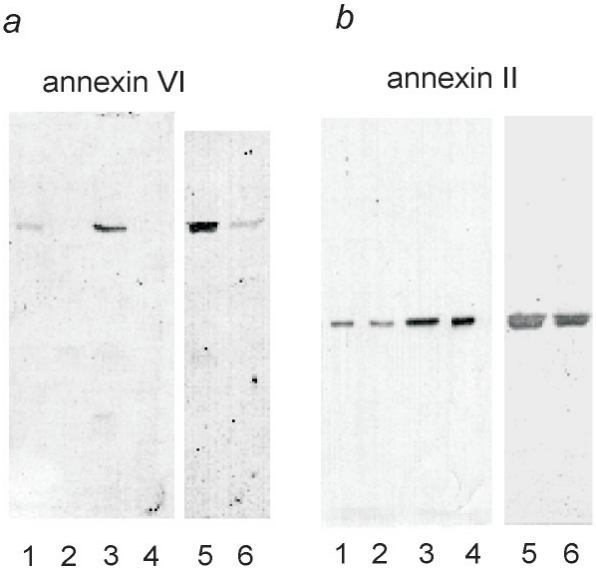

Figure 1. Characterization of polyclonal anti-annexin II and VI antibodies.

Western blotting of pig stomach smooth muscle homogenates (1, 2), preparations of the annexin-dependent membrane-cytoskeleton complex (3, 4) obtained from pig- (1, 3) or annexin VI knock-out mouse source (2, 4), cultured pig aorta- (5) and human myometrium (6) cells. The blots were treated either with anti-annexin VI- (*a*) or anti-annexin II- (*b*) antibodies.

annexin II antibodies recognized 68 kDa and 34 kDa polypeptides, respectively. Anti-annexin VI antibodies showed no reaction with tissue homogenates or preparations of annexin-dependent membrane-cytoskeleton complex obtained from annexin VI- -/- mouse samples (Fig.1*a*, lanes 2, 4).

Ca^{2+}-sensitive translocation of annexin II and annexin VI to smooth muscle sarcolemma and formation of an annexin-dependent membrane-cytoskeleton complex

As reported previously, the distribution of annexin VI in smooth muscle cells depended upon their state of contraction (Babiychuk *et al.*, 1999). When fixed in rigor, this protein was located exclusively within the sarcolemma (Fig. 2a). After relaxation of the tissue strips in a Ca^{2+} free solution, annexin VI became diffusely distributed within the cytosol (Fig. 2c). The Ca^{2+}-dependent redistribution of annexin II followed the pattern observed for annexin VI (Fig. 2b, d).

The Ca^{2+}-dependent translocation of annexins II and VI to the smooth muscle sarcolemma led to their accumulation in the Ca^{2+} high-speed pellet obtained in fractionation experiments (Fig. 3*a, b, c*). Biochemical analysis of this pellet revealed a Ca^{2+}-dependent coprecipitation of cytoskeletal elements such as actin, myosin and tropomyosin (Fig. 3*c*, lanes 5, 6) and sarcolemmal cholesterol- and sphingomyelin-enriched DIGs (Fig. 3*d*, lanes 4, 5) together with the annexins as a result of annexin-dependent membrane-cytoskeleton complex formation (Babiychuk *et al.*, 1999).

Isolation of individual components of annexin-dependent membrane-cytoskeleton complex

To study the interactions governing the formation of the annexin-dependent membrane-cytoskeleton complex, we developed a protocol for isolation of its individual components (Fig. 3*a*). The supernatant obtained after an initial low speed centrifugation of

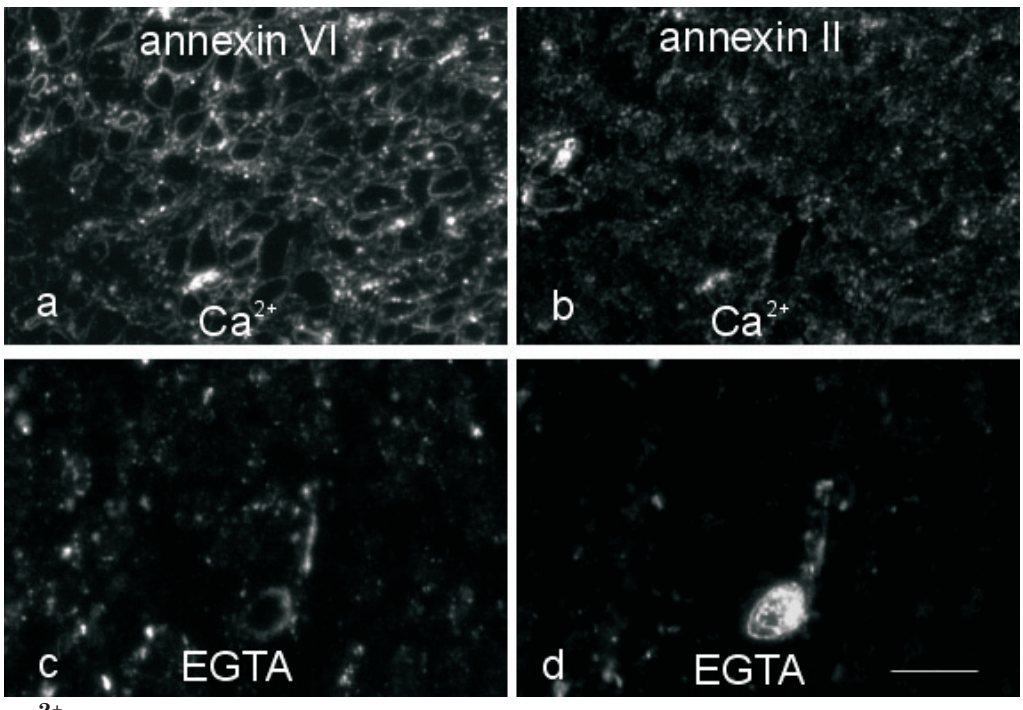

Figure 2. Ca^{2+}-dependent translocation of annexin VI and annexin II in smooth muscle cells.

Transverse sections of human *taenia coli* labeled with antibodies against annexin VI (**a, c**) or annexin II (**b, d**). Within smooth muscle cells fixed in *rigor* (**a, b**), annexin VI and annexin II are localized to the plasmalemma. After relaxation of cells for 2 h in an EGTA-containing solution, these proteins become diffusely distributed throughout the cytoplasm (**c, d**). Bar = 20 μm.

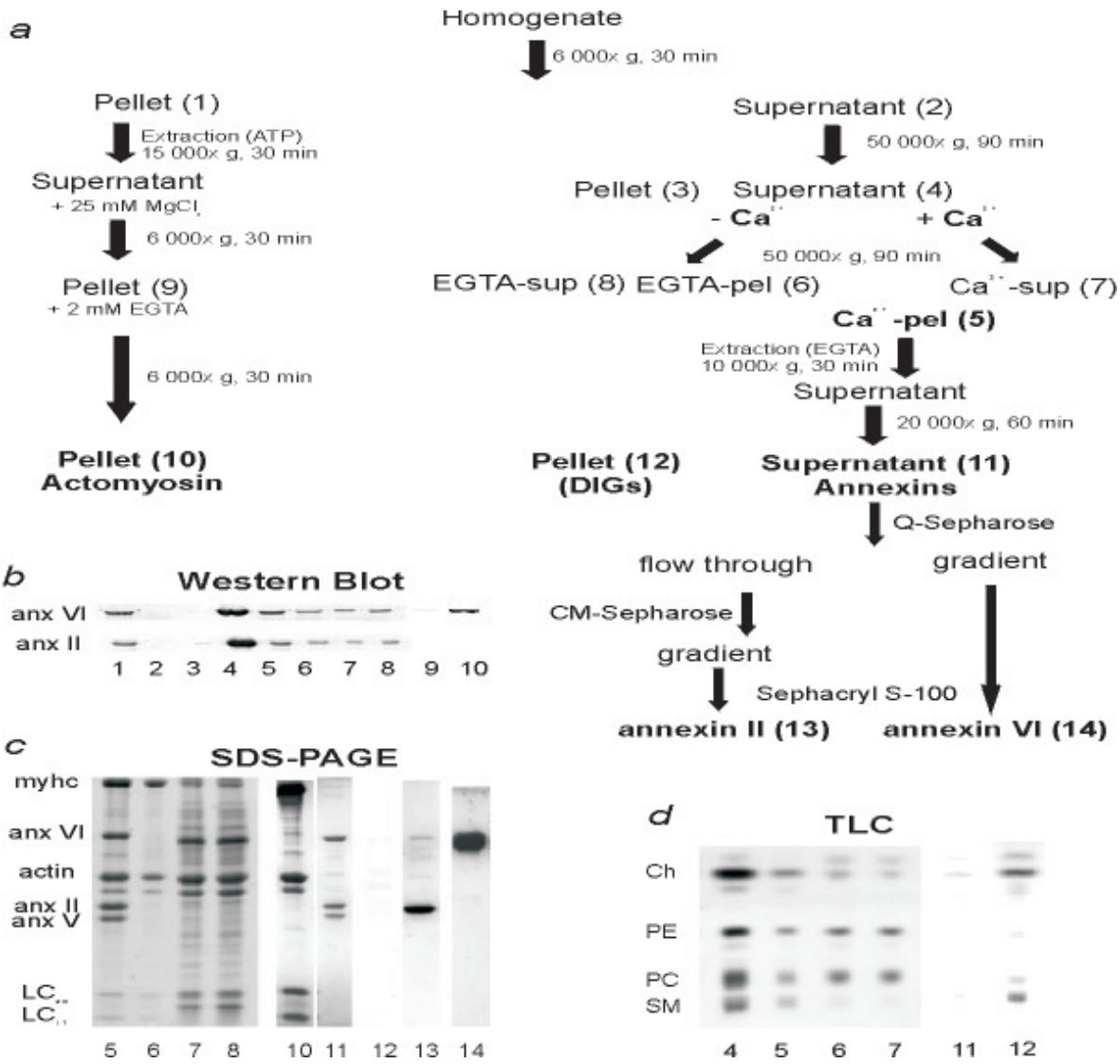

Figure 3. Purification and characterization of individual components of the annexin-dependent membrane-cytoskeleton complex.

a*, Purification protocol.** Low-speed centrifugation of smooth muscle homogenates obtained in the presence of 0.5% Triton X-100 and 1 mM EGTA resulted in a pellet (1), which was used for actomyosin preparation and a supernatant (2) used for purification of annexin- and DIG-fractions. For actomyosin preparation, the low-speed centrifugation pellet was extracted in the presence of ATP. Extracted actomyosin was precipitated by $MgCl_2$ (9) and extensively washed in the presence of EGTA (10). For isolation of annexin- and DIG-fractions, the low-speed centrifugation supernatant (2) was clarified at 50 000 × ***g for 90 min. The pellet obtained (3) was discarded, while the supernatant (4) was divided into two parts; one supplemented with $CaCl_2$ to obtain $[Ca^{2+}]_{free}$ = 0.2 mM. After high-speed centrifugation, the Ca^{2+}-pellet (5), the EGTA-pellet (6), Ca^{2+}-supernatant (7) and the EGTA-supernatant (8) were analyzed. The Ca^{2+}-pellet (5) represented the annexin-dependent membrane-cytoskeleton complex and was further used for isolation of its annexin- and DIG-fractions. It was extracted in the presence of EGTA. After low-speed centrifugation, the resulting supernatant was subjected to centrifugation at 20 000 × ***g*** for 60 min giving rise to a supernatant (11) representing purified annexin-fraction and a pellet (12) representing the purified DIG-fraction. Annexin II (13) and annexin VI (14) were further purified by liquid chromatography. ***b*, Western blot** showing the distribution of annexin II and annexin VI through different purification steps (1–10, see legend to Fig. 1*a*). ***c*, SDS/PAGE** depicting the protein distribution through different purification steps (5–8, 10–14, see legend to Fig. 1*a*). Positions of myosin heavy- (myhc) and light- (LC_{20} and LC_{17}) chains, annexin VI (anx VI), annexin II (anx II), annexin V (anx V) and actin are indicated. ***d*, Thin-layer chromatography** showing lipid distribution through different purification steps (4–7, 11–12, see legend to Fig. 1*a*). Positions of cholesterol (Ch), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyelin (SM) are indicated.

smooth muscle homogenates (0.5% Triton X-100 and 1 mM EGTA) was used for the purification of the annexins and DIGs, while the pellet was used as a source for actomyosin.

For isolation of the annexin- and DIG-fractions, the clarified low-speed centrifugation supernatant was supplemented with 1.2 mM $CaCl_2$ and immediately subjected to high-speed centrifugation. The resulting Ca^{2+} high-speed pellet represents the annexin-dependent membrane-cytoskeleton complex. EGTA treatment of this pellet led to the dissociation of the membrane-cytoskeleton complex allowing separation of its cytoskeletal fraction (actomyosin) from the annexin- and DIG-fractions. Under these conditions, actomyosin remained in the pellet, while the annexins and DIGs were recovered in the EGTA-supernatant. The annexin- and DIG-fractions were further separated by centrifugation (Fig. 3*c*, lanes 11, 12; Fig. 3*d*, lanes 11, 12) and used for reconstitution experiments.

For antibody production, annexin II and annexin VI were further purified by liquid chromatography. The first step involved anion-exchange chromatography. Annexin II was recovered in the flow-through fraction while annexin VI was separated from annexin V by eluting the column with a linear gradient of NaCl. Annexin II was further purified by cation-exchange chromatography. A final purification step for both annexin II (Fig. 3*c*, lane 13) and annexin VI (Fig. 3*c*, lane 14) included gel-filtration chromatography.

Since, after separation, the actomyosin fraction of the annexin-dependent membrane-cytoskeleton complex contained significant amounts of DIGs, smooth muscle actomyosin for recombination experiments was purified according to the method described by Sobieszek & Bremel (1975). The first low-speed centrifugation pellet (Fig. 3*a*) was extracted in the presence of ATP. The supernatant obtained after centrifugation at 15 000 × ***g*** for 30 min was filtered through glass wool, and its $MgCl_2$ concentration raised to 25 mM. Precipitated actomyosin was harvested by low-speed centrifugation and extensively washed in the presence of 1 mM EGTA. This step was necessary for the removal of residual annexins VI (Fig. 3*b*, lanes 9, 10) and II (not shown) from the actomyosin preparations.

Reconstruction of the annexin-dependent membrane-cytoskeleton complex

As shown in Fig. 4*b*, both annexin VI and annexin II interacted with purified actomyosin with high Ca^{2+}-sensitivity. The interaction required a half-maximal $[Ca^{2+}]_{free}$ of 1–3 μM, consistent with the Ca^{2+}-sensitivity of annexin-dependent membrane-cytoskeleton complex formation (Babiychuk *et al.*, 1999). However, the stoichiometry of the annexin-actomyosin interactions was very low (Fig. 4*a*). An approximately 100 times weight-to-weight excess of actomyosin was required for total binding of the annexins, whereas the actomyosin-to-annexin weight-to-weight ratio in the annexin-dependent membrane-cytoskeleton complex was estimated at 2:1 (see Fig. 3*c*, lane 5).

Both annexins II and VI interacted with purified DIGs preparations in a concentration-dependent manner (Fig. 5*a*). Annexin II, in particular, exhibited a highly Ca^{2+}-sensitive interaction with DIGs (Fig. 5*b*), which could account for its interaction within the annexin-dependent membrane-cytoskeleton complex (Babiychuk *et al.*, 1999). In contrast, the interaction of annexin VI with purified DIGs required much higher concentrations of $[Ca^{2+}]_{free}$ (Fig. 5*b*). Therefore, the interactions of annexin VI within the annexin-dependent membrane-cytoskeleton complex or its translocation to the smooth muscle sarcolemma observed previously (Babiychuk *et al.*, 1999) cannot be explained by an interaction with either DIGs (low Ca^{2+}-sensitivity, see Fig. 5*b*) or actomyosin (low stoichiometry, see Fig. 4*b*). However, as shown in Fig. 5*b*, recombination of all three components of the annexin-dependent membrane-cytoskeleton

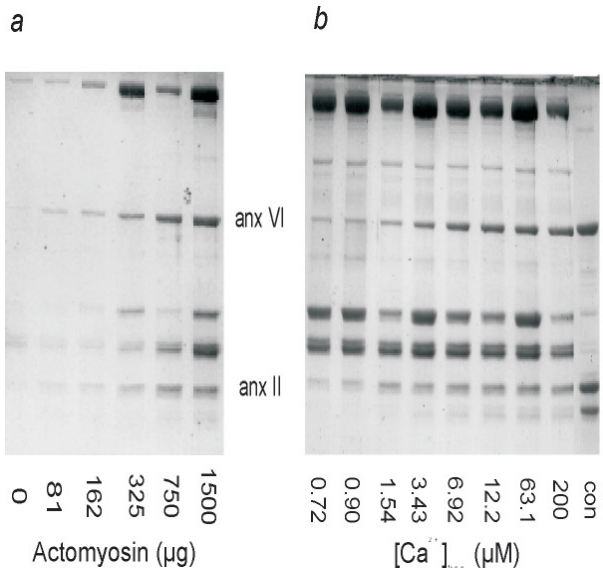

Figure 4. Annexin II and annexin VI interact with purified actomyosin with high Ca^{2+}-sensitivity but low stoichiometry.

The purified annexin-fraction (10 μg of protein) was recombined (buffer B, final volume: 400 μl) with the indicated amounts of purified actomyosin at 200 μM of $[Ca^{2+}]_{free}$ (*a*) or with 1 500 μg of purified actomyosin at the indicated $[Ca^{2+}]_{free}$ (*b*). The samples were incubated for 15 min at ambient temperature. Pellets obtained after centrifugation at 6000 × ***g*** for 30 min were made up to a final volume of 30 μl with buffer B. The annexins present in the pellet were extracted by adding 10 μl of 20 mM EGTA. After centrifugation at 6 000 × ***g*** for 30 min, equal aliquots of the resulting supernatants were analyzed by SDS/PAGE. In a control experiment (con), 10 μg of annexin-fraction were applied to the gel.

complex was clearly accompanied by an increased Ca^{2+}-sensitivity of annexin VI interactions, even at relatively low actomyosin-to-annexin ratios.

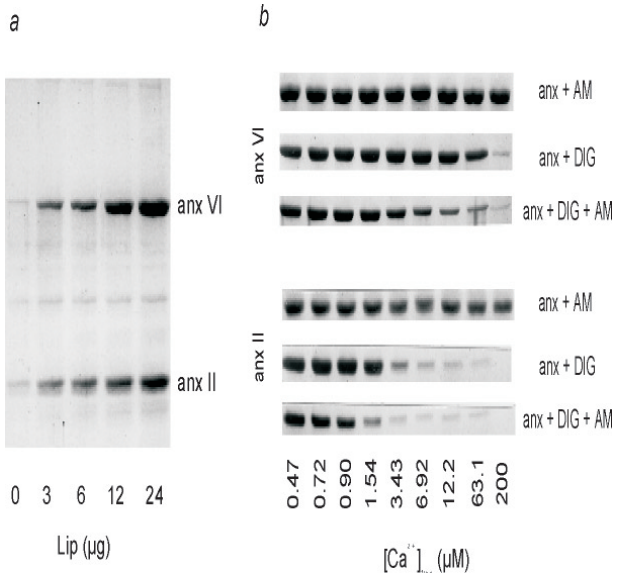

Figure 5. Actomyosin increases the Ca^{2+}-sensitivity of the annexin VI-lipid interaction.

The purified annexin-fraction (12.5 μg of protein) was recombined (buffer B, final volume: 25 μl) with: *a*, indicated amounts of DIGs at 200 μM of $[Ca^{2+}]_{free}$; *b*, constant amounts of DIGs (25 μg; **anx + DIG**) or actomyosin (50 μg; **anx + AM**) or both DIGs (25 μg) and actomyosin (50 μg; **anx + DIG + AM**) at the indicated $[Ca^{2+}]_{free}$. After centrifugation at 6 000 × ***g*** for 30 min, equal aliquots of each pellet (***a***) or supernatant (***b***) were analyzed by SDS/PAGE. The bands corresponding to annexin VI (anx VI) and annexin II (anx II) are marked.

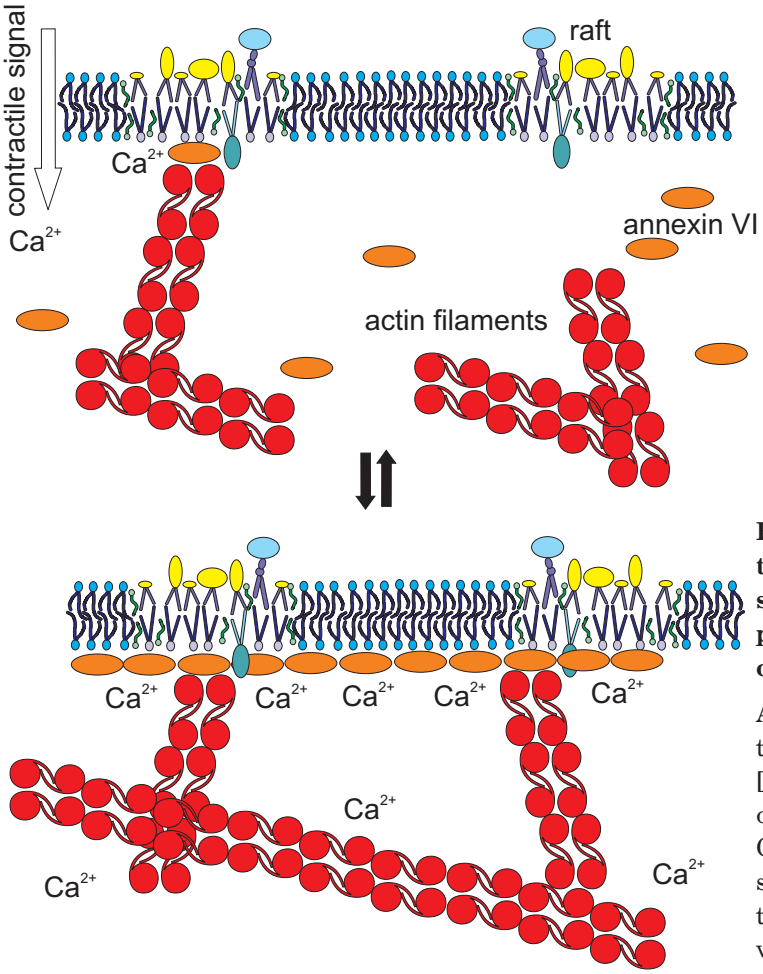

Figure 6. Ca^{2+}-dependent formation of the annexin-dependent membrane-cytoskeleton complex occurs due to actin-dependent oligomerization of annexin VI on the intracellular membrane surface.

An initial contractile signal leads to a raise in the intracellular $[Ca^{2+}]_{free}$. At elevated $[Ca^{2+}]_{free}$, the oligomerization of annexin VI occurs on lipid surfaces around initial, highly Ca^{2+}-sensitive sites; these sites being represented by single annexin VI molecules positioned and affixed to the membrane by individual cytoskeletal filaments.

DISCUSSION

Stimulation of smooth muscle cells leads to a rise in intracellular Ca^{2+} concentration and ensuing contraction. The need for communication between the Ca^{2+}-signal-generating structures within the plasma membrane and their executing agents in the contractile apparatus is obvious. It is all the more surprising that we still know so little about the role of the annexins, proteins that combine lipid- and actin-binding properties, in regulation of smooth muscle contraction.

The redistribution of annexins in response to stimulation of smooth muscle cells

The localization of different members of the annexin protein family and their translocation to distinct intracellular compartments remain a subject of controversy (for reviews, see Raynal & Pollard, 1994; Gerke & Moss, 1997). In addition to the Ca^{2+}-dependent translocation of annexin VI to the sarcolemma (Babiychuk *et al.*, 1999), here we show that annexin II, also translocates to sarcolemmal sites at elevated intracellular $[Ca^{2+}]$. Our data are in accord with the previously reported relocation of annexin II from cytoplasmic to membrane sites after stimulation of a number of cell types (Barwise & Walker, 1996; Blanchard *et al.*, 1996; Chasserot-Golaz *et al.*, 1996; Kang *et al.*, 1996; Sagot *et al.*, 1997).

The interactions of annexin II and annexin VI with cytoskeletal- and sarcolemmal elements

Recently, we have described the Ca^{2+}-dependent formation of annexin-dependent membrane-cytoskeleton complex as a result of

interactions occurring between annexins II and VI, actomyosin and membrane DIGs (Babiychuk *et al.*, 1999). In the present paper we have undertaken a detailed characterization of these interactions using purified components of the annexin-dependent membrane-cytoskeleton complex.

Both annexin II and annexin VI interacted with purified actomyosin in a Ca^{2+}-dependent manner. Indeed, the actin binding properties of the annexins are well known (Gerke & Weber, 1984; Glenney, 1986; Glenney *et al.*, 1987; Ikebuchi & Waisman, 1990; Hosoya *et al.*, 1992) and their co-localization with F-actin has been reported (Hosoya *et al.*, 1992; Diakonova *et al.*, 1997). Whilst there is little doubt that the actin-based cytoskeleton participates in annexin-dependent membrane-cytoskeleton complex formation, the precise cytoskeletal binding partner has yet to be identified. Although both α-smooth muscle and β-cytoplasmic actin were found to be present within the complex, Western blot analyses showed no evidence of cytoskeletal proteins, such as α-actinin, vinculin, filamin or calponin.

Stimulation of smooth muscle cells evokes a plasma membrane-based redistribution of annexin VI and annexin II leading to their Ca^{2+}-dependent interaction with DIGs. These sarcolemmal sites are obvious loci for the formation of reversible links with the underlying cytoskeleton since DIGs are closely apposed to cytoskeletal elements. Actin and myosin subfragment 1 have been identified in caveolae- and DIG-preparations (Izumi *et al.*, 1988; Chang *et al.*, 1994; Lisanti *et al.*, 1994; Smart *et al.*, 1995; Schnitzer *et al.*, 1995). In addition, the caveolae-specific plasmalemmal inositol 1,4,5-triphosphate receptor-like protein is aligned along actin filaments in bovine aortic endothelial cells (Fujimoto *et al.*, 1992). More recently, annexin II has been identified as a component of an EGTA-insoluble complex containing membrane-associated actin, α-actinin, ezrin and moesin (Harder *et al.*, 1997).

Our detailed biochemical analyses established the degree of Ca^{2+}-dependency characterizing the interaction between annexins II or VI and actomyosin or DIGs. These experiments revealed a highly Ca^{2+}-sensitive interaction for annexin II with DIGs. In contrast, the interaction of annexin VI with purified DIGs was characterized by low Ca^{2+}-sensitivity that increased in the presence of actomyosin, which itself was able to bind both annexins with high Ca^{2+}-sensitivity but low stoichiometry. The low stoichiometry of annexin-actomyosin interactions suggests that the primary target for the annexins is most likely not actin or myosin, but rather actin- or myosin-associated proteins. It is conceivable that these consist of actin filament capping proteins, which would link the very end of the filaments to sites on the plasmalemma. The low Ca^{2+}-sensitivity of annexin VI interaction with purified DIGs, which is greatly promoted in the presence of actomyosin, suggests that its binding to the sarcolemma is a result of its co-operative oligomerization on the intracellular membrane surface (Zaks & Creutz, 1991). Such oligomerization would occur around initial, highly Ca^{2+}-sensitive sites; these sites being represented by single annexin VI molecules positioned and affixed to the membrane by individual actin filaments (Fig. 6).

The Ca^{2+}-sensitivity of annexin-dependent membrane-cytoskeleton complex formation corresponds to that of annexin VI- but not annexin II interactions within the complex (Babiychuk *et al.*, 1999). Our data suggest that annexin VI is the determinant factor in the complex formation. The role of annexin II in this process remains enigmatic. Annexin II is able to aggregate individual DIGs, thus promoting their association (Babiychuk & Draeger, unpublished). At present it is unclear if the annexin II-dependent DIG-association, which occurs with higher Ca^{2+}-sensitivity than the annexin-dependent membrane-cytoskeleton complex formation, precedes the annexin VI-dependent DIG-acto-

myosin interactions or if these two processes are unrelated. The observation that annexin II interacts exclusively with DIGs in purified smooth muscle microsomal preparations, while annexin VI is also able to interact with glycerophospholipid membrane compartments (Babiychuk & Draeger, unpublished), favor the latter assumption.

Whereas the main force of contraction is undoubtedly transmitted *via* the firm actin-attachment sites, or focal adhesions, it is our understanding that the subtle fine-tuning of force transduction might be mediated by the reversible, annexin-dependent linkage of the cytoskeleton to the lipid bilayer.

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