Complementary Distributions of Vinculin and Dystrophin Define Two Distinct Sarcolemma Domains in Smooth Muscle

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Abstract. The sarcolemma of the smooth muscle cell displays two alternating structural domains in the electron microscope: densely-staining plaques that correspond to the adherens junctions and intervening uncoated regions which are rich in membrane invaginations, or caveolae. The adherens junctions serve as membrane anchorage sites for the actin cytoskeleton and are typically marked by antibodies to vinculin.

We show here by immunofluorescence and immunoelectron microscopy that dystrophin is specifically localized in the caveolae-rich domains of the smooth muscle sarcolemma, together with the caveolae-associated molecule caveolin. Additional labeling experiments revealed that β₁ integrin and fibronectin are confined to the adherens junctions, as indicated by their codistribution with vinculin and tensin. Laminin, on the other hand, is distributed around the entire cell perimeter.

The sarcolemma of the smooth muscle cell is thus divided into two distinct domains, featuring different and mutually exclusive components. This simple bipartite domain organization contrasts with the more complex organization of the skeletal muscle sarcolemma: smooth muscle thus offers itself as a useful system for localizing, among other components, potential interacting partners of dystrophin.

The sarcolemma of smooth muscle cells exhibits two structurally distinct regions: those bearing submembranous dense plaques ~0.2 μm thick and 0.5 μm wide, and intervening uncoated zones which bear many vesicular invaginations or caveolae (see Bagby, 1983). The submembranous plaques are junctions of the adherens type, involved in actin anchorage and characterized by the presence of a specific set of junctional proteins (Geiger and Ginsborg, 1991) including vinculin (Geiger et al., 1981) and talin (Volberg et al., 1986; Drenckhahn et al., 1988; Draeger et al., 1989). Antibodies against the latter proteins reveal a longitudinal rib-like organization of adherens junctions at the smooth muscle cell surface (Small, 1985; Drenckhahn et al., 1988), with only rare exceptions (avian gizzard) in which the ribs are discontinuous (Draeger et al., 1989). In surface views of the smooth muscle cell membrane, obtained by freeze-fracture EM (Gabella and Blundell, 1978), longitudinal channels containing membrane vesicles or caveolae are seen to alternate with smooth-surfaced channels, taken to be the adherens junction domains.

In comparison with smooth muscle, the sarcolemma of skeletal muscle appears to be more complex, as regards the complement of peripheral cytoskeletal proteins (see review by Small et al., 1992). Adherens junction proteins have been localized in submembranous specializations flanking the Z-disc (costameres; Pardo et al., 1983), whereas other cytoskeletal components, including certain spectrin isoforms (Appleyard et al., 1984; Vybiral et al., 1992) and dystrophin, have been reported to be more uniformly distributed over the sarcolemma. Particular interest has focused on dystrophin since this protein is absent from, or altered in sufferers of Duchenne muscular dystrophy (DMD) (Hoffman et al., 1987; Hoffman et al., 1988). The widely demonstrated localization of dystrophin along the skeletal muscle plasma membrane (Arahata et al., 1988; Bonilla et al., 1988; Zubrzycka-Gaarn et al., 1988), together with the identification of a tightly linked oligomeric complex of membrane glycoproteins, has been taken to suggest a structural role for dystrophin in linking the subsarcolemmal cytoskeleton to the plasma membrane (Campbell and Kahl, 1989; Ervasti and Campbell, 1991) and, via laminin-binding complexes, to the extracellular matrix (Tbraghimov-Beskrovnya et al., 1992). However, taking the apparently homogeneous distribution of dystrophin along the skeletal muscle sarcolemma seen by immunogold labeling (Watkins et al., 1988; Cullen et al., 1990; Byers et al., 1991) and the complexity of organization of other membrane skeleton components, skeletal muscle appears a less favorable model for testing the colocalization of putative dystrophin-associated proteins in situ.

Preliminary data of Byers et al. (1991) suggested that dystrophin may not be distributed uniformly over the smooth muscle cell membrane. In the present work we confirm and extend these latter studies by showing that dystrophin and...
vinyl alcohol; RT, room temperature.

**Materials and Methods**

**Antibodies**

The primary antibodies used were: monoclonal antibodies directed against human vinculin (generously supplied by Prof. V. Koteliansky (CNRS, Paris, France); clone no. 15-1-33 from Sigma Immunochemicals, St. Louis, MO), chicken vinculin (a kind gift from Prof. B. Geiger, Weizman Institute, Rehovot, Israel) and tensin (Glenney and Zokas, 1989; Bockholt et al., 1992); human fibronectin (clone no. FN-382, Sigma Immunochemicals); and cavolin (Rothberg et al., 1992); and polyclonal antibodies against dystrophin, affinity-purified antibody 6-10 (produced in a rabbit immunized with a dystrophin polypeptide expressed in bacteria from dystrophin cDNA residues 6,181-9,544; Lidov et al., 1990; Byers et al., 1991); mouse laminin (affinity-isolated antibody L 9393 from Sigma Immunochemicals); and rat β1 integrin (Bottger et al., 1989; generously supplied by Dr. S. Johannson, University of Uppsala, Sweden). For double labeling in combination with fibronectin, tensin, and cavolin, we used a polyclonal antiseraum against pig stomach vinculin raised in collaboration with Dr. M. Moeremans (Janssen Pharmaceuticals, Beerse, Belgium) and purified on a pig vinculin affinity column by Dr. M. Gimona (Institute of Molecular Biology, Salzburg, Austria). Polyclonal antibodies against various mammalian erythrocyte and brain spectrin subunits were generously donated by Dr. B. Riederer (University of Lausanne, Switzerland), Prof. J. Morrow (Yale University, New Haven, CT), and Dr. K. Burridge (University of North Carolina).

**Gel Electrophoresis and Immunoblotting**

SDS-gel electrophoresis was carried out using 2.5-12.5% linear gradient mini-gels (0.5% bis-acrylamide; Fürst et al., 1988) and Laemmli buffer system (Laemmli, 1970). Proteins were transferred electrophoretically (Towbin et al., 1979) onto nitrocellulose sheets (Schleicher & Schuell, Germany) and the blots processed for immunogold silver staining as described previously (Moeremans et al., 1984).

**Preparation of Semi-Thin Cryosections**

Thin longitudinal strips of guinea pig *Taenia coli* muscle or chicken gizzard were dissected into a Ca$^{2+}$-free balanced salt solution (solution 1, Small et al., 1986), tied to plastic plates and fixed for 30 min in 2% paraformaldehyde (PFA) in the same solution, at room temperature (RT). After several washes in ice-cold solution 1, the strips were cryoprotected by infusion for at least 2 h with a polyvinyl pyrrolidone/sucrose/muscure solution (Tokuyasu, 1990), mounted on aluminium cryopins (Reichert, Vienna) and plunge frozen in a drop of liquid nitrogen. Semi-thin cryosections, ~0.25 μm thick, were prepared according to the general method of Tokuyasu (1980) using a Reichert-Jung FC4E cryo-ultramicrotome in combination with tungsten-coated glass knives (Roberts, 1975). Sections were retrieved on drops of 2 M sucrose plus 0.75% gelatin (Tokuyasu and Singer, 1976), transferred to 4-mm glass coverslips coated with 1 mg/ml poly-L-lysine and stored on gold buffer (GB, 155 mM NaCl, 2 mM MgCl$_2$, 2 mM EGTA, 20 mM Tris-base, pH 7.6) at 4°C before immunolabeling.

**Tissue Whole Mounts**

Short strips (~8 mm long) of guinea pig *T. coli* and chicken gizzard were tied to plastic plates and incubated in ice-cold solution 1 for 4–5 h. They were then digested in 1 mg/ml collagenase (Sigma type V) for 1.5 h at 37°C in 137 mM NaCl, 5 mM KCl, 4 mM NaHCO$_3$, 5.5 mM glucose, 2 mM MgCl$_2$, 2.5 mM CaCl$_2$, and 10 mM Pipes, pH 6.5. After rinsing in cold solution 1, the samples were fixed for 10 min in 2% PFA, 0.2% Triton X-100 at RT and then immunolabeled. After labeling, thin strips were teased from the exposed surface, using a fine needle under a dissecting microscope, retrieved on coverslips and flattened onto a slide coated with a drop of Gelvatol (20–30) containing 2.5 mg/ml n-propyl gallate (Giboh and Sedat, 1982) or 1 mg/ml phenylene-diamine (Johnson et al., 1982) as anti-bleach agent.

**Preparation of Sections for EM**

For immunoelectron microscopy the method of embedding in polyvinyl alcohol (PVA; Small et al., 1986) was specifically adapted for use with fixation-sensitive antibodies. Guinea pig *T. coli* strips were prepared and fixed as for cryoultramicrotomy, and then infiltrated sequentially with 5 and 10% PVA (10 kD; Air Products and Chemicals Inc. Allentown, PA) in solution 1 followed by 20% PVA plus 8% trehalose in water, over a total period of 36 h. After final embedding in fresh 20% PVA and 8% trehalose, the sections were allowed to dry at RT to a jelly-like consistency, and refract in this state in the vapor from a solution of 2% PFA for 1 h at RT. Sections were cut at low specimen temperatures (~60–80°C) with a diamond knife onto 50% DMSO at ~40°C. Spreading of sections was effected by transfer onto 87% glycerol at room temperature by the use of a wire loop, before retrieval on formvar-coated nickel grids.

**Immunocytochemistry**

Immunolabeling was performed on sections by transferring the coverslips or grids between droplets of antibodies and washing solutions arranged on a sheet of paraffin. For tissue whole mounts, the plates carrying the muscle strips were inverted on paraffin and 40 μl of antibody pipetted underneath. Antibodies were dissolved in GB containing 1% BSA, and washes were carried out in multwell dishes using the same buffer containing 0.1% BSA. Before the first antibody incubation, sections were treated for 10 min with 0.02 M glycine, followed by 10 min with 2% normal goat serum (NGS) plus 1% BSA (whole mounts), or with 5% NGS, 1% BSA, 2% PVA, and 2% gelatin (sections), and then rinsed on a drop of 0.1% BSA.

Fluorescent labeling was performed using the biotin-streptavidin-FITC system supplied by Amersham International (Amersham, UK), a FITC-conjugated anti-lgM antibody (Sigma Immunochemicals) and rhodamine-conjugated secondary antibodies prepared according to Brandzaeg (1973). Final mounting was in Gelvatol (20–30) containing an antibleach agent as described above. Microscopy was carried out using a Zeiss Axioskop epifluorescence microscope (Carl Zeiss, Inc., Oberkochen, Germany).

Double immunogold labeling on PVA-embedded sections of smooth muscle was performed using a 5-nm gold-conjugated anti-mouse IgG (Bio-Cell Research Laboratories, Cardiff, UK) to detect the monoclonal vinculin antibody, and a biotinylated secondary antibody (Amersham) followed by an anti-biotin antibody conjugated to 10 nm colloidal gold (BioCell Research Laboratories) to detect bound anti-dystrophin antibodies. After thorough washing in GB, sections were fixed for 5 min in 2.5% glutaraldehyde in solution 1, rinsed in water, and contrasted by negative staining using 2% ammonium molybdate or a mixture of equal parts of 2% ammonium molybdate and 2% sodium ortho-oxanade, the latter being added to the negative stain to reduce electron density without reducing the thickness of the final embedding layer.

Sections were examined using a Zeiss EM 10A electron microscope (Carl Zeiss, Inc.).

**Results**

The reactivity of the different antibodies on immunoblots (Fig. 1) was monospecific and essentially in accord with previously published data, giving the following labeling patterns: dystrophin, a major band at ~400 kD (Byers et al., 1991), a second, weaker band of slightly lower molecular weight, possibly a degradation product, was also labeled; vinculin/metavinculin, two bands at an apparent molecular mass of 130 and 150 kD (Gimona et al., 1987); tensin, two bands of ~150 and 200 kD (Wilkins et al., 1986); fibronectin, a single band at 240 kD; and laminin, a band at ~220 kD (Hedin et al., 1988). In our hands, the β1 integrin antibody (Bottger et al., 1989) gave a diffuse reaction at ~180 kD, and the monoclonal caveolin antibody labeled three bands corresponding to the monomer (22 kD) and higher molecular weight complexes of around 220 and 350 kD, which may arise from aggregation (Glenney, unpublished data).
In transverse semi-thin cryosections of guinea pig T. coli, antibodies against vinculin and dystrophin both labeled discontinuous streaks of fluorescence at the cell periphery (Fig. 2, a and b), which appeared to occupy complementary positions. In general the vinculin label was confined to a discrete narrow band corresponding to the adherens junctions (Geiger et al., 1981), whereas the dystrophin label tended to be somewhat more diffuse.

In tissue whole mounts (Fig. 2, c and d) vinculin and dystrophin were seen to occupy alternating sets of complementary and nonoverlapping surface ribs arranged parallel to the long axis of the cell. The ribs of dystrophin immunolabel were not always continuous, a feature that appeared to be an artefact of Triton extraction, in that longer Triton treatments resulted in an almost spotty appearance (not shown).

The complementary distributions of vinculin and dystrophin were most strikingly seen in superimposed images of double immunofluorescence-labeled cross sections (Fig. 2 g). The alternating positions of vinculin (green) and dystrophin (red) label in such double exposures contrasted with the superimposed pattern (yellow) obtained with vinculin and tensin, a further component of adherens junctions (Wilkins et al., 1986; Fig. 2 h). One feature of the labeling of adherens junction regions, particularly noticeable in the case of tensin, was the intense staining of cells of apparently smaller profile (Fig. 2, e and f). It is probable that these cells had been sectioned across their terminal portions, where the adherens junctions have been shown to occupy almost 100% of the plasmalemmal area (Gabella, 1984).

The relative localizations of dystrophin and vinculin were also investigated in chicken gizzard, which has previously been shown to possess an unusual pattern of vinculin distribution (Draeger et al., 1989). The transverse, banded or chevron-like arrays of vinculin-positive streaks characteristic of gizzard cells is seen in Fig. 3 a. Unfortunately, an even greater susceptibility of dystrophin in chicken gizzard towards Triton extraction rendered the use of such whole mount preparations unsuitable for its localization. However, the exclusion of dystrophin from vinculin-rich domains could be clearly demonstrated in semi-thin, longitudinal cryosections (Fig. 3, b and c).

Immunoelectron microscopy confirmed the localization of dystrophin and vinculin in different and complementary sarclemma domains (Fig. 4). Since the human vinculin antibody did not react with glutaraldehyde-fixed tissue, the embedding and sectioning protocol had to be modified to allow covisualization of sarclemma structure and immunolabel. As indicated in Materials and Methods this was achieved by using a variation of the PVA embedding technique (Small et al., 1986) involving curing of soft blocks with formaldehyde vapor and low temperature sectioning onto DMSO. As shown in Fig. 4 a, dystrophin label was confined to the infolded, caveolae-rich regions of the sarclemma found between the adhesion plaques marked by vinculin antibodies. The visibility of the 5-nm gold particles could be enhanced by the use of a composite mixture of ammonium molybdate and sodium vanadate as negative stain (Fig. 4 b). The restricted localization of dystrophin label in smooth muscle contrasted markedly with the homogeneous distribution of immunogold label reported for skeletal muscle by several laboratories (see Discussion).

Does the differential distribution of dystrophin and vinculin reflect the segregation of other sarclemma-associated proteins in smooth muscle? This was tested using other antibody probes on semi-thin cryosections of guinea pig T. coli muscle in double label combinations with vinculin antibodies (Fig. 5). Caveolin, a protein component of caveolae membrane coats (Rothberg et al., 1992), was confined, like dystrophin, to the vesicular domains (Fig. 5 b). β integrin was colocalized with vinculin (Fig. 5 d), as was fibronectin (Fig. 5 f). In contrast, laminin showed an almost continuous distribution around each muscle cell (Fig. 5 h), implying an association with components of both domains. In some areas the laminin label assumed a zig-zag appearance between neighboring cells, which appeared to arise from excessive spreading of the sections or cell shrinkage, leading to an artefactual enlargement of the extracellular space.

A range of anti-spectrin antibodies, directed against different chains of mammalian erythrocyte and brain spectrin, were also tested on semi-thin cryosections of guinea pig T. coli. No antibody was found to react with smooth muscle cells, although some of them, including one originally reported to react weakly with smooth muscle (Burridge et al., 1982), labeled the membranes of cells localized in the nerve plexus (Fig. 5 f).

Discussion

The results described above show that the two structurally distinct domains of the smooth muscle sarclemma are associated with different complements of cytoskeletal, integral membrane, and extracellular matrix proteins. The clear-cut division of these domains contrasts with the more complex organization of the skeletal muscle sarclemma, and permits us to make suggestions concerning possible molecular inter-
actions. Before discussing these we draw attention to two technical points.

First, we found that the use of semi-thin cryosections was imperative to obtain optimal resolution in the light microscope. In sections thicker than ~0.25 μm, the punctate labeling of dystrophin and vinculin was more difficult to discern. This finding explains the apparently continuous and erroneous pattern of dystrophin immunolabel previously obtained using 8-μm cryostat sections of chicken gizzard (Pons et al., 1990; Hurricane et al., 1991). Second, although ultrathin sections of PVA-embedded tissue have previously been used in this laboratory for immunofluorescence microscopy, cell profiles were smaller than in cryosections and the intensity of immunolabeling sometimes varied across the section. Thus, for light microscopy, the standard cryosectioning method gave slightly superior results. However, the situation was reversed for the electron microscope preparations in which the structural integrity in transverse ultrathin sections was significantly better in PVA-embedded tissue than in standard cryosections. In particular, the cells were held together in a more coherent manner and the preservation of filaments within the cell was improved. This technique is thus well suited for ultrastructural immunocytochemistry of mildly fixed muscle tissue.

The total exclusion of dystrophin from the adherens junctions of smooth muscle is at variance with the suggested interaction of this protein with vinculin (Porter et al., 1992). It is also inconsistent with a possible interaction between dystrophin and integrins (Otey et al., 1990), although the presence of a different integrin isoform in the caveolar regions or of a transient interaction during development, as suggested for skeletal muscle (Lakonishok et al., 1992), cannot be ruled out. This finding of dystrophin outside adherens junctions would also appear to contradict a suggestion that dystrophin may be a focal adhesion protein (Kramarcy and Sealock, 1990), based on its colocalization with talin in cultured Xenopus muscle. Our results are consistent with the reported indirect association of dystrophin with laminin (Ibragimov-Beskrovnaya et al., 1992), and also prompt the suggestion that caveolin is a potential interacting partner of dystrophin (see also below).

Vinculin (Geiger et al., 1981), talin (Volberg et al., 1986; Drenckhahn et al., 1988; Draeger et al., 1989), metavinculin (Gimona et al., 1988), filamin (Small et al., 1986; Draeger et al., 1990), paxillin (Turner et al., 1991), and plectin (Wiche et al., 1983) have previously been localized to the adherens junctions of smooth muscle. We now formally add to this list the protein tensin, as well as β integrin, both of which have been earlier localized to fibroblast focal adhesions (Wilkins et al., 1986; Hynes, 1987) and are considered to be adherens junction components (Geiger and Ginsborg, 1991). The β integrins constitute the largest subfamily of integrins and are represented in receptors to fibronectin and laminin (for reviews, see Hynes, 1987; Damsky and Werb, 1992); they have been identified in smooth muscle extracts (Kelly et al., 1987) and localized in sections of smooth muscle tissue (De Strooper et al., 1989) and in cultured smooth muscle cells (Clyman et al., 1990). In this context, the different distributions of fibronectin and laminin in the pericellular matrix of smooth muscle cells is noteworthy. As we show, laminin occurs around the entire cell periphery, consistent with the presence of a continuous basal lamina, whereas fibronectin shows a restricted distribution over the adherens junctions. Kurisu et al. (1987) noted a higher concentration of fibronectin label within apposed adhesion plaques, but failed to note the specific association described below.

**Figure 2.** Relative localizations of vinculin and dystrophin (a−d and g) and of vinculin and tensin (e, f, and h) in guinea pig T. coli muscle. Double immunofluorescent labeling of vinculin and dystrophin on a transverse semi-thin cryosection (a and b) shows the two proteins to occupy complementary positions at the cellular periphery (arrows). In a tissue whole mount (c and d) the two antibodies label complementary ribs at the cell surface. The labeling of dystrophin and vinculin contrasts with the coincident labeling of vinculin and tensin obtained on transverse semi-thin cryosections (e and f; see arrows). The relative distributions of dystrophin, vinculin and tensin are most clearly seen in double exposure color micrographs of transverse semi-thin cryosections immunolabeled in (g) for vinculin (green) and dystrophin (red) and in (h) for vinculin (red) and tensin (green). Overlap of the two colors in (h) gives rise to a yellow product. Bars, 5 μm.

**Figure 3.** Relative localizations of vinculin and dystrophin in chicken gizzard. (a) Vinculin immunolabeling of a tissue whole mount demonstrates the complex, chevron-like pattern of vinculin distribution at the surface of gizzard cells. In longitudinal semi-thin cryosections, double immunolabeling of vinculin (b) and dystrophin (c) reveals the mutual exclusion of vinculin- and dystrophin-associated domains also in gizzard muscle. Bar, 5 μm.

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Figure 4. Localization of vinculin and dystrophin at the periphery of smooth muscle cells as demonstrated by immunogold labeling. Vinculin (5 nm gold) is localized at the adherens junctions (AJ) and dystrophin (10 nm gold) in the intervening regions containing caveolae and membrane invaginations (CV; caveolar regions; arrows indicate caveolar invaginations). Transverse ultrathin sections of PVA-embedded guinea pig T. coli were negatively stained with 2% ammonium molybdate (a) and 1% ammonium molybdate plus 1% sodium ortho-vanadate (b). The vesicular regions are most clearly seen in a but the 5 nm gold is more easily visualized using the weaker stain combination (b). Bars, 0.1 μm.

The presence of fibronectin over the adhesion plaques containing β1 integrin points to the involvement of these receptors in restricting the deposition of fibronectin in the basal lamina in vivo. Exactly how this final arrangement is achieved is unclear, but it probably involves an interplay between intrinsic (cytoskeletal) and extrinsic factors (Burridge, 1986) including interactions between the matrix molecules themselves (Ruoslahti, 1988; Damsky and Werb, 1992). The circumferential distribution of laminin suggests that receptors for this matrix molecule are present over the whole cell surface. However, we suppose that different classes of receptors are involved and that those resident in the caveolar domains include the dystrophin-associated glycoprotein complex that is thought to link dystrophin to laminin in skeletal muscle (Ibraghimov-Beskrovnaya et al., 1992). This proposal remains to be tested in future work. In any case, dystrophin distribution is clearly determined not by the accessibility of laminin, but by intracellular factors.

In view of the widespread occurrence of spectrin in the membrane skeleton of diverse cell types (Coleman et al., 1989), including striated muscle cells, we considered it necessary to probe for the presence of spectrin in the smooth...
Figure S. Immunofluorescent localization of other sarcolemma-associated proteins relative to vinculin (a, c, e, g, and i) on transverse semi-thin cryosections of guinea pig T. coli. Caveolin (b) is localized, like dystrophin, in the regions between those labeled with vinculin, while $\beta_1$ integrin (d) and fibronectin (f) are colocalized with vinculin at the adherens junctions. Arrows indicate regions where the relative localizations are most clearly seen. Laminin (h) is localized more or less continuously around the cell periphery. Various anti-spectrin antibodies failed to label smooth muscle cells, although some of them labeled the membranes of cells in the nerve plexus (j) (anti-mammalian brain spectrin from Dr. B. Riederer). Bars, 5 $\mu$m.
muscle sarcolemma. Mammalian skeletal muscle is characterized by multiple spectrin isofoms that are variously distributed in a restricted (Bloch and Morrow, 1989; Vybral et al., 1992; Porter et al., 1992) or uniform (Appleyard et al., 1984) manner along the cytoplasmic surface of the plasma membrane. However, we observed no cross-reactivity of any of the donated anti-spectrin antibodies with smooth muscle cells. The strong labeling of nerve cells by some of the antibodies could explain the weak cross-reactivity with smooth muscle tissue reported by certain laboratories (Levine and Willard, 1981; Burridge et al., 1982; Glenney and Glenney, 1983). We conclude that either spectrin is absent from smooth muscle cells or they possess a unique isoform.

A final question concerns the functional significance of the restricted distribution of dystrophin. The absence of dystrophin from dystrophic skeletal muscle has been proposed to result in mechanical damage to the plasma membrane (Weller et al., 1990; Menke and Jockusch, 1991), possibly by altering the threshold for work-induced injury (Stedman et al., 1991), or in altered calcium regulation across the membrane (Franco and Lansman, 1990; Fong et al., 1990). During smooth muscle contraction, the major burden of longitudinal force transmission is believed to fall on the sarcolemma, the vesicle-rich regions of the membrane being forced outwards relative to the dense bands (Gabella, 1984), analogous to the festooning of the membrane which occurs in skeletal muscle (Pierobon-Bormioi, 1981). This behavior of the dystrophin-rich membrane would be consistent with the frequently proposed role for dystrophin in providing mechanical support to the membrane, possibly via anchorage of the membrane skeleton to the extracellular matrix (Ervasi et al., 1990; Ervasi and Campbell, 1991). However, it is also possible that the location of dystrophin in the caveolea-rich domains may reflect a more direct functional interaction. A recent immunofluorescence study suggested that dystrophin is highly concentrated adjacent to the I-band regions of skeletal muscle, a result taken to suggest an involvement of dystrophin in the subsarcolemmal lattice which is believed to mediate attachment of the contractile apparatus to the plasma membrane (Porter et al., 1992). However, since further results suggest that vinculin and dystrophin are not exactly colocalized in skeletal muscle (Masuda et al., 1992), alternative interpretations should be considered. Thus the characteristic distribution of caveolea over the I bands of fast skeletal muscle fibers, together with the apparent loss of this banding pattern in Duchenne muscle (Shotton, 1982), would be consistent with an interaction of dystrophin with caveolea. Although the role of caveolea in muscle cells remains elusive (Severs, 1988), proposed functions include their involvement in the transport of calcium across the membrane (Popescu, 1974) and in stretch reception (Prescott and Brightman, 1976). Hence, a putative interaction between dystrophin and caveolea would be consistent with the suggestion that dystrophin acts as a tension-sensing molecule, conveying information about sarcolemmal stress to mechano-sensitive calcium channels (Lansman and Franco, 1991). The localization in skeletal muscle of caveolin or caveolin-like molecules, relative to dystrophin, remains to be established.

It is clear that much information is lacking about the organization of the smooth muscle sarcolemma. Nevertheless, we propose that smooth muscle provides a more simple system than skeletal muscle for identifying proteins which might interact with dystrophin, on the initial basis of their colocalization in the vesicle-rich domain. Of primary interest will be to determine whether all of the components of the dystrophin-associated glycoprotein complex are present at, and restricted to, the dystrophin-associated membrane areas in this tissue.

We acknowledge Drs. K. Burridge, B. Geiger, V. Kotelyansky, S. Johansson, J. Morrow, and B. Riederer for generously donating antibodies and for interesting discussion. We are grateful to Drs. J. Slot and Dr. G. Griffiths for helpful advice concerning immunogold labeling. We also thank Dr. M. Gimona for antibody purification and Mr. A. Weber for photographic assistance.

A. J. North acknowledges the generous support of the Wellcome Trust in the form of a Traveling Research Fellowship. This work was supported in part by grants from the Austrian Science Research Council and the Austrian National Bank (to J. V. Small).

Received for publication 18 August 1992 and in revised form 27 November 1992.

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