Actin isoform compartments in chicken gizzard smooth muscle cells

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SUMMARY

Differentiated smooth muscle cells typically contain a mixture of muscle (α and γ) and cytoplasmic (β and γ) actin isoforms. Of the cytoplasmic actins the β-isoform is the more dominant, making up from 10% to 30% of the total actin complement. Employing an antibody raised against the N-terminal peptide specific to β-actin, which labels only the β-isoform on two-dimensional gel immunoblots, we have shown that this isoform has a restricted localisation in smooth muscle. Using double-label immunofluorescence and immunoelectron microscopy of ultrathin sections of chicken gizzard, β-actin was localised in the dense bodies and in longitudinal channels linking consecutive dense bodies that were also occupied by desmin. It was additionally found in the membrane-associated dense plaques, but was excluded from the actomyosin-containing regions of the contractile apparatus. Taken together with earlier results these findings identify a cytoskeletal compartment containing intermediate filaments, cytoplasmic actin and the actin cross-linking protein filamin.

Using an antibody specific only for muscle actin, labelling was found generally around the myosin filaments of the contractile apparatus, but was absent from the core of the dense bodies that contained β-actin. Thus, if dense bodies act as dual-purpose anchorage sites, for the cytoskeletal actin and the contractile actin, the thin filaments of the contractile apparatus must be anchored at the periphery of the dense bodies. A model of the structural organisation of the cell is presented and the possible roles of the cytoskeleton are discussed.

Key words: actin isoform, smooth muscle, cytoskeleton

INTRODUCTION

In contrast to skeletal muscle, whose molecular organisation has been largely understood for many years, the arrangement of the molecular components of smooth muscle cells remains an enigma. Not only is the conformation of the contractile unit unclear, but the arrangement of the contractile apparatus with respect to the cytoskeleton and the mechanism by which they are coupled are still a matter of debate (see Bagby, 1990; Small and North, 1993). Previous studies from this laboratory identified a cytoskeleton domain in the smooth muscle cell, characterised by the presence of desmin intermediate filaments and the protein filamin (Small et al., 1986; Draeger et al., 1990). Since filamin is a potent actin-binding protein (Hartwig and Kwiatkowski, 1991) and actin appeared from immunlabelling studies to be distributed throughout the cytoplasm (Small et al., 1986) it was concluded that actin must also be contained in the cytoskeleton. Included in the cytoskeleton are also the α-actinin-rich dense bodies (Geiger et al., 1981), which show a common association with the intermediate filaments (see Bagby, 1990). The presence of actin and filamin in the cytoskeleton prompted the suggestion that their interaction may contribute to tension maintenance in smooth muscle. However, even if this were so, it was still unclear how the actin filaments of the cytoskeleton were organised with respect to those of the contractile apparatus; that is, whether they were common or segregated.

Vertebrates express at least six distinct actin isoforms in a tissue-specific manner (Vandekerckhove and Weber, 1978, 1981). These isoactins are highly homologous in their primary structure with the exception of the N-terminal region (Vandekerckhove and Weber, 1978, 1981) and can be resolved by isoelectric focusing into α-, β- and γ- forms (see Herman, 1993). In smooth muscle cells, α- and γ-smooth muscle type actins are expressed, together with the two cytoplasmic actins (β and γ), the relative proportions of each isoform being dependent upon the particular muscle type. In cultured cells derived from smooth muscle, specific antibodies have been used to demonstrate subcellular sorting of isoactins (Herman, 1993), but the localisation of actin isoforms in smooth muscle in vivo has yet to be established.

We demonstrate here that an antibody directed against β-cytoplasmic actin specifically labels the cytoskeletal compartment of smooth muscle cells. In contrast, an antibody that cross-reacts with the smooth muscle α- and γ-isoforms, but not with the β-isoform, labels the myosin-containing contractile regions. These results confirm the existence of at least two separate populations of thin filaments and permit us to propose a more complete model of the organisation of the smooth muscle cell.

MATERIALS AND METHODS

Antibodies

Monoclonal antibodies, actin 15 (AC-15, subclass IgG1) and actin 74 (AC-74, subclass IgG2a), were raised in mice against the N-terminal
synthetic peptide of β-actin (sequence: AcD-D-I-A-A-L-V-I-D-N-G-S-G-K) coupled to keyhole limpet hemocyanin. More complete details of antibody production are given elsewhere (Gimona et al., 1994). A monoclonal antibody that cross-reacts with all muscle isoactins (α-skeletal, α-cardiac, α-smooth and γ-smooth), but with neither of the cytoplasmic isoforms, was obtained from Enzo Diagnostics Inc. (clone no. C34931, subclass IgG1; Syosset, New York; Tsukada et al., 1987).

The affinity-purified antibodies against chicken gizzard myosin subfragment 1 (SF1) and chicken gizzard desmin were described previously (Langanger et al., 1986; Small et al., 1986). Antibodies against α-actin were a polyclonal antiserum generously donated by Dr T. Miron (Weizmann Institute of Sciences, Rehovot) and a mouse monoclonal antibody (clone no. BM-75.2 from BioMakor, Sigma Immunochemicals, Rehovot). The monoclonal antibody against filamin was a kind gift from Dr C. Otey and Dr K. Burridge (mAb 6E; Pavalko et al., 1989).

Electrophoresis

Analytical gel electrophoresis was carried out on either 8% to 22% gradient or 15% polyacrylamide minislab gels according to the procedure of Matsudaira and Burgess (1978) in the buffer system of Laemmli (1970). Proteins were processed for electrophoresis and stained as described (Gimona et al., 1992).

Two-dimensional electrophoresis

Isoelectric focussing of first-dimension tube gels was performed essentially according to Bravo et al. (1982) for 14 hours using the Bio-Rad Mini Protein 2D system. Gels contained 2% ampholytes (Serva, CH) pH 4-6 and 0.5% pH 3-10. Second-dimension gels were run as above.

Immunoblotting

Western blotting of polyacrylamide gels onto nitrocellulose sheets was carried out according to Towbin et al. (1979). Silver-enhanced immunogold staining was performed following the procedure described by Moeremans et al. (1984) using a secondary antibody with a gold tag (Amersham, UK). Samples of purified smooth and skeletal muscle actin, obtained from acetone-dried muscle powder, were kindly donated by Ms I. Majcher.

Preparation of tissue and sectioning

Gizzards from 6-week-old chickens were freshly excised and stored for 30–40 minutes on ice. Thin radial slices were then cut using a razor blade and the slices placed into a Ca²⁺-free balanced salt solution (Solution 1: Small et al., 1986). Using forceps, thin strips of muscle were teased from the slices, tied at an extended length to plastic plates and fixed in 2% paraformaldehyde in Solution 1 for 30 minutes at room temperature: 0.05% glutaraldehyde was additionally included in the fixative used for strips to be embedded in polyvinylalcohol (PVA), and these strips were treated after fixation with 3 changes (10 minutes each) of 1 mg/ml sodium borohydride (in Solution 1, on ice). Strips of guinea pig taenia coli were dissected into Solution 1, allowed to relax and then fixed in 2% paraformaldehyde in Solution 1 for 30 minutes at room temperature.

In all other respects, the preparation of tissue as well as cryosectioning was according to the general method of Tokuyasu (1980), using section thickness settings of 150-250 nm for semi-thin sections and 80 nm for ultrathin sections. The preparation of ultrathin sections by the low temperature PVA-embedding method was performed as described previously (North et al., 1993).

For immunofluorescence microscopy of longitudinal semi-thin cryosections, the sections were artificially expanded by inverting them on the surface of Tris-buffered saline prior to retrieval on 4 mm glass coverslips, as described in more detail in the accompanying paper (North et al., 1994).

Immunocytochemistry

Immunolabelling was performed on sections mounted on 4 mm × 4 mm glass coverslips or Formvar-coated nickel grids as described previously (North et al., 1993). For immunofluorescence, bound primary antibodies were detected using either an FITC-conjugated anti-mouse IgG antibody (Dako, Glostrup, Denmark), an FITC-conjugated anti-mouse IgM antibody (Sigma, Munich), Texas Red-conjugated anti-rabbit antibodies (Vector Laboratories Inc. Burlingame, CA) or rhodamine-conjugated secondary antibodies prepared according to Brandtzaeg (1973). Specimens were embedded in Gelvatol (Vinol 205, Air Products Inc., PA) containing 1 mg/ml phenylene diamine (Johnson et al., 1982) and microscopy was performed using a Zeiss Axioskop epifluorescence microscope. Immunogold labelling was carried out using conjugates of 5 nm and 10 nm colloidal gold with goat anti-mouse and goat anti-rabbit antibodies (BioCell Research Laboratories, Cardiff, Wales).

Contrasting for electron microscopy

After the final washing step, ultrathin sections for electron microscopy were post-fixed for 5 minutes in 2.5% glutaraldehyde (in Solution 1), and rinsed with water (4 times 1 minute). Longitudinal cryosections were stained for 5 minutes in 2% uranyl acetate oxalate, then briefly rinsed in water prior to staining and embedding in aqueous 0.2% uranyl acetate plus 2% PVA (Tokuyasu, 1989). Contrasting of sections of PVA embedded muscle was performed using a negative stain comprising 0.7% sodium silicotungstate plus 1.3% sodium orthovanadate. Microscopy was performed using a Zeiss EM 10A electron microscope, operating at 80 kV.

RESULTS

Antibody specificity

The specificity of the β-actin antibodies is described in some detail elsewhere (Gimona et al., 1994) and therefore only brief mention of their characteristics is necessary here. Since both
Localisation of \( \beta \)-actin in smooth muscle antibodies showed identical specificity only one is described. On one-dimensional gel immunoblots the \( \beta \)-actin peptide antibody (AC-15) reacted with actin purified from hog stomach and chicken gizzard smooth muscle (not shown) and with a single band of the same molecular mass in total extracts of smooth muscles (Gimona et al., 1994). It also labelled the band

**Fig. 2.** (a-f) Longitudinal, expanded, semi-thin cryosections of chicken gizzard muscle double-labelled with antibodies against the proteins indicated. Arrowheads mark matching positions in the double-label pairs (ab, cd, ef). (g-i) Transverse, semi-thin cryosections of chicken gizzard double-labelled with antibodies against the proteins indicated. Arrowheads in (i) and (j) point to the same cell. Bars, 10 \( \mu \)m.
Fig. 3. (a-c) Double-exposure micrographs of longitudinal, expanded (a,b) and transverse (c) semi-thin cryosections of chicken gizzard muscle double-labelled with antibodies against β-actin (green) and myosin (red). (d-f) Longitudinal, expanded cryosections of chicken gizzard muscle double-labelled with antibodies against α-actinin (green, d, e; red, f) in combination with myosin (d; red), desmin (e; red) and β-actin (f; green). Bars, 10 μm.
Fig. 4. Longitudinal ultrathin cryosections labelled with the following antibody combinations: (a and b) β-actin (5 nm gold) and α-actinin (10 nm gold); (c) β-actin (10 nm gold) and myosin (5 nm gold); (d) β-actin (5 nm gold) and desmin (10 nm gold). Black arrowheads point to dense bodies and white arrowheads to β-actin channels. my, myosin filaments. Bars, 0.25 μm.
of non-muscle actin in cultured fibroblasts and non-muscle tissues but showed a negative reaction with purified skeletal muscle actin and with total extracts from skeletal and cardiac muscle (Gimona et al., 1994). In two-dimensional electrophoresis gels of 11-day embryonic chicken gizzard (Fig. 1a) the amounts of β-cytoplasmic and γ-smooth actin are comparable (Hirai and Hirabayashi, 1983) in contrast to the dominance (about 80%) of γ-smooth actin in the adult (Vandekerckhove and Weber, 1978). Immunoblotting of such embryonic samples showed that the antibody was reactive with only the β-isofrom (Fig. 1b).

The muscle actin isoform antibody (HHF35) reacted with both smooth and striated muscle actins but showed no reaction with non-muscle actins (not shown), confirming the original characterisation of this antibody by Tsukada et al. (1987). The monoclonal antibody against α-actinin (Sigma, BM 75.2) and the polyclonal α-actinin antibody both labelled a single band of around 100 kDa in smooth muscle extracts as well as purified smooth muscle α-actinin that co-migrated with the same band (not shown).

**Restricted localisation of β-cytoplasmic actin in chicken gizzard**

Using indirect immunofluorescent labelling of expanded longitudinal semi-thin (150-250 nm) cryosections of chicken gizzard smooth muscle, β-actin was localised in narrow channels running parallel to the long axis of the cell (Fig. 2a,c). In transverse sections, a corresponding punctate label was observed (Fig. 2g,i). Double-labelling with antibodies directed against myosin showed the β-actin channels and the myosin-containing domains to be complementary and mutually exclusive (Fig. 2a,b; g,h); in contrast there was a striking, though incomplete, co-localisation of β-actin with desmin (Fig. 2c,d; i,j). The localisation of the β-actin isoform also showed strong similarity to that of filamin, which is likewise confined to the desmin-rich cytoskeletal domain (Fig. 2e,f) (Small et al., 1986). The complementary nature of the labelling for myosin and the β-actin isoform was most clearly seen in double-exposure colour micrographs of expanded sections (Fig. 3a-c), the fibrillar arrangement of both proteins being evident in the thinnest areas of the sections (Fig. 3a). (Due to slight inaccuracies in registration of the filter positions between channels some minor shift in the patterns was sometimes apparent.)

To gain more information about the relationship of the dense bodies to the cytoskeleton, we double-labelled longitudinal semi-thin cryosections for α-actinin in conjunction with myosin, desmin or β-actin (Fig. 3d-f). Given the relatively small size of dense bodies (around 0.1-0.2 mm in diameter), the results were most easily interpreted from double-exposure colour micrographs of highly expanded sections in which the cells showed greater separation of the different components. In these preparations it was clear that the dense bodies were not associated with myosin, but located in the myosin-free channels between the contractile domains (Fig. 3d). In confirmation of previous ultrastructural data (see Bagby, 1990), dense bodies were invariably associated with the desmin-containing intermediate filaments, which formed a branching, but largely longitudinal, network throughout the cell (Fig. 3e; see also Draeger et al., 1990). More striking, however, was the co-localisation of dense bodies with β-actin (Fig. 3f). Indeed, fibrils of β-actin appeared to encompass or link several consecutive dense bodies into longitudinal arrays or ‘strings’ of dense bodies (Fig. 3f, right half; arrowheads).

**Immunoelectron microscopy**

For immunoelectron microscopy we found that the attainment of a suitable balance between ultrastructure and antibody label required different techniques, according to whether longitudinal or transverse sections of the muscle tissue were being used. For longitudinal sections, the cryosectioning and contrasting method of Tokuyasu (1980, 1989) gave the best result, whereas for transverse sections the PVA method (Small et al., 1986) adapted for low temperature sectioning (North et al., 1993) proved most suitable.

In longitudinal ultrathin cryosections of chicken gizzard muscle, narrow, parallel, longitudinal channels of β-actin label corresponding to those seen in the fluorescence microscope were clearly visualised (Fig. 4). In sections double-labelled for β-actin and α-actinin it was additionally evident that β-actin label was highly concentrated in the α-actinin-rich dense bodies, that it flanked these structures, and that it could also be found to link consecutive dense bodies (Fig. 4a,b). Myosin antibodies labelled the regions containing recognisable thick filaments that were spatially separated from the β-actin channels (Fig. 4c). As expected, antibodies to desmin labelled the same channels as those occupied by β-actin, but desmin was excluded from the dense bodies (Fig. 4d). Sometimes a large co-axial region of desmin label was observed, probably corresponding to the central bundle of intermediate filaments occasionally seen in smooth muscle cells (Stromer and Bendayan, 1988), and this region was largely devoid of β-actin label (not shown).

In order to visualise 5 nm gold particles on ultrathin PVA-embedded transverse sections a negative staining protocol was developed that involved a composite stain comprising a mixture of 0.7% sodium silicotungstate and 1.3% sodium orthovanadate. Fig. 5 shows sections stained with this mixture that were double-labelled for β-actin (5 nm gold) in combination with α-actinin (Fig. 5a), myosin (Fig. 5b) and desmin (Fig. 5c).

The dense bodies (black arrowheads in Fig. 5) can be positively identified by the presence of α-actinin (Fig. 5a) and by the association at their periphery with desmin-containing intermediate filaments (as detected by labelling for desmin, Fig. 5c, inset), as well as by their homogeneous, structureless appearance after negative staining. As shown, the label for β-actin was concentrated in the dense bodies. In addition, small patches of β-actin label could also be identified in myosin filament-free regions of the cytoplasm (Fig. 5b, white arrowhead), which we conclude correspond to cross-sections of the β-actin channels identified in the longitudinal sections. The adhesion plaques on the membrane were also labelled heavily with the β-actin antibodies (Figs 4c, 5b and c).
Spatial segregation of cytoplasmic and muscle actin
The results described above suggest that the dense bodies couple β-actin filaments into linear arrays in the cytoskeleton. In order to assess whether they are, at the same time, an integral part of the contractile apparatus, and hence could act as a coupling site between the two filament systems, we employed an antibody that cross-reacts with both α- and γ-smooth muscle actin isoforms, but not with cytoplasmic isoactins. In gizzard,
the primary muscle isoactin is γ, which comprises about 80% of the total actin (Vandekerckhove and Weber, 1978). On both longitudinal and transverse cryosections, immunofluorescent labelling with this antibody resulted in staining of almost the entire cell (Fig. 6a). However, in the thinnest sections, small holes and longer narrow gaps were seen in the labelling pattern: after double-labelling against α-actinin some of these unlabelled holes were seen to correspond in position to dense bodies (not shown). The thick, central bundles of intermediate filaments were also devoid of label for γ-actin (not shown), but γ-actin-free channels, corresponding to the β-actin fibrils, could not be clearly distinguished.

Immunoelectron microscopy confirmed that γ-smooth muscle actin was largely absent from the interior of the dense bodies (Fig. 6b) and from the membrane-associated dense plaques (not shown), although labelling could be seen close to the periphery of these structures. The label for γ-actin generally coincided with the regions containing myosin filaments (Fig. 6b).

**DISCUSSION**

Since the realisation that multiple actin isoforms can be expressed within a single cell type (Vandekerckhove and Weber, 1978), considerable interest has focused on the question of whether such isoactins are differentially localised and perform different functions (see review by Herman, 1993). In differentiated skeletal muscle, minor amounts of non-muscle γ-cytoplasmic actin have been detected, but a consensus about the localisation of this actin, which has been described as around the mitochondria (Pardo et al., 1983), under the membrane (Craig and Pardo, 1983), at the neuromuscular junction (Hall et al., 1981) and in the sarcomere (Otey et al., 1988) has not been reached. In vertebrate smooth muscles up to four actin isoforms (two muscle and two non-muscle) can exist in significant amounts (Vandekerckhove and Weber, 1981). The complement of actin isoforms in chicken gizzard is, however, more simple: α-smooth muscle actin and β-cytoplasmic actin. These two actins show primary sequence differences that are essentially restricted to seven exchanges in the N-terminal 16 amino acids (Vandekerckhove and Weber, 1981). As we show here and elsewhere (Gimona et al., 1994) the antibodies raised against the N-terminal peptide of β-actin are specific for this isoform, whereas the muscle actin-specific antibody (Tsukada et al., 1987) was unreactive with both β- and γ-cytoplasmic actins. Both these antibodies reacted well with aldehyde-fixed tissue and thus proved suitable for immunocytochemistry in con-

![Fig. 6. Localisation of muscle actin isoforms. (a) Longitudinal semi-thin cryosection of chicken gizzard labelled for α,γ-smooth muscle actin. (b) Transverse PVA section of chicken gizzard cell double-labelled with antibodies against α,γ-smooth muscle actin (5 nm gold) together with α-actinin (10 nm gold). Arrowheads point to dense bodies. Note the presence of muscle actin in regions containing myosin filaments and the absence of label in the dense bodies, marked by α-actinin antibodies. Bars: (a) 10 μm; (b) 0.2 μm.](image-url)
Our current ideas about the structural organisation of the smooth muscle cell, based on the present and previous findings, are summarised in Fig. 7. In this scheme, the dense bodies are situated at the mid-point of bipolar arrays of non-muscle actin filaments that interdigitate and overlap with each other to form linear arrays. Intermediate filaments are situated peripherally to the β-actin arrays and are presumed to be bound, by undefined links, to the dense bodies. The results from antibody labelling suggest that β-actin is distributed only longitudinally and does not branch between adjacent channels of the cytoskeleton, as intermediate filaments appear to do (Bond and Somlyo, 1982; Draeger et al., 1990; and this study). The intermediate filaments are then presumed to link the cytoskeleton into a network and this network to the membrane skeleton, via association with the adherens junctions (Tsukita et al., 1983; Bagby, 1990). Such a linkage of dense bodies into linear arrays and their incorporation into a three-dimensional network is consistent with the earlier findings of Fay and co-workers (Fay et al., 1983; Kargacin et al., 1989). Since the β-actin network is largely longitudinal, it might be expected to anchor primarily at the terminal regions of the cell. We have already noted that β-actin is a component of the adherens junctions throughout the cell surface: at the ends of the cell the terminal bundles of β-actin presumably associate with the adherens junctions and in these terminal regions the junctional material may be accordingly more abundant. In support of this a notable increase in labelling for the adherens junction protein tensin has recently been described at the ends of smooth muscle cells (North et al., 1993). It has also been suggested that intermediate filaments terminate principally at the ends of the cells, based on their tendency to cluster along the central axis of the cell in stretched tissue (Cooke and Fay, 1972; Cooke, 1976; Bagby, 1983): in thin sections of muscle or in isolated cells stained with desmin antibodies (this study; and Draeger et al., 1990) we have, however, observed a more or less homogeneous distribution of filament bundles throughout the cell as well as a general association of desmin label at the periphery of dense plaques at the cell surface.

Whilst the elements of the cytoskeleton of smooth muscle appear to be oriented in a mainly longitudinal fashion, evidence derived largely from isolated smooth muscle cells

junction with ultrathin cryosections obtained from sucrose-infiltrated or PVA-embedded tissue.

The localisation in gizzard of β-actin, in the dense bodies, in the dense plaques, and in channels congruent with those occupied by the intermediate filaments, is indicative of a cytoskeletal role for this actin isotype in smooth muscle. The β-actin distribution closely matches that of the other cytoskeletal component filamin, with the exception that filamin is absent from the dense bodies (Small et al., 1986): in these structures actin cross-linking is presumably mediated by α-actinin. From our former studies on filamin distribution (Small et al., 1986) we concluded that actin must be co-distributed with filamin in the cytoskeleton domain, but we had no information then on the nature of this actin component. It turns out from the present work that the cytoskeleton and membrane skeleton harbour their own cytoskeletal actin isoform. In preliminary studies we have begun to test the generality of this actin isoform segregation among smooth muscles and have already found in guinea pig taenia coli, that β-actin is likewise segregated into fibrils in the cytoplasm that co-distribute with labelling for desmin.

Immunoprecipitation studies on chicken gizzard extracts with filamin and caldesmon antibodies (Lehman et al., 1987) identified two different thin filament fractions that were enriched, respectively, with one or other of these proteins. The filamin-rich thin filaments also tended to be associated with desmin and α-actinin, consistent with their being derived from the cytoskeletal domain; the isoform of actin present in the thin filaments was not, however, established in this work. Contrary to the present findings, Drew et al. (1991) obtained results with isolated smooth muscle thin filaments suggesting that there was actin isoform mixing within the same filament. However, the structure of isolated filaments is distorted during the many steps required for immunocytochemistry and it is then questionable whether single filaments can be distinguished from filament aggregates. We suggest that the images presented by these authors may depict lateral aggregates of muscle and non-muscle type actin filaments.

The present results place the dense bodies firmly in the cytoskeletal domain: intermediate filaments are distributed around them and β-actin fibrils clearly pass through them. So how do the dense bodies relate to the contractile apparatus? Fig. 7. Schematic illustration of the smooth muscle cytoskeleton. In cross-section only the components of the cytoskeleton are included: large dots represent intermediate filaments and small dots filaments of β-cytoplasmic actin (the numbers are not representative). Dense bodies are encircled by a continuous line and the cytoskeleton domain by a broken line. At the cell membrane adherens junctions (aj) containing cytoskeletal actin alternate with the caveolae-rich domains. In longitudinal section the intermediate filaments (thick lines, if) encase the channels occupied by dense bodies (db) and β-cytoplasmic actin (longitudinal thin lines, a). The β-cytoplasmic actin passes through the dense bodies. Actin filaments of the contractile apparatus (thin lines, a) are envisaged as having anchorage sites at the surface of the dense bodies and at the periphery of the adherens junctions. Other abbreviations: ca, region occupied by contractile elements; cs, cytoskeleton; my, myosin filaments.

apparatus (thin lines, a) are envisaged as having anchorage sites at the surface of the dense bodies and at the periphery of the adherens junctions. Other abbreviations: ca, region occupied by contractile elements; cs, cytoskeleton; my, myosin filaments.
indicates that the contractile elements are arranged obliquely with respect to the cell’s long axis (Fay and Delise, 1973; Small, 1974; Fisher and Bagby, 1977; Small et al., 1990). A more recent study has indicated that the contractile actin filaments are around three times longer than the myosin filaments (Small et al., 1990), and since the myosin label displayed no sign of periodicity we conclude that the myosin filaments are staggered along the thin filaments. Nevertheless, it must be admitted that the precise conformation of the contractile unit is still unsettled. Although γ-smooth muscle actin filaments were found to be absent from the core of the dense bodies, we suggest that the latter could still serve as coupling elements between the cytoskeleton and the contractile apparatus. In our scheme (Fig. 7) the contractile actin filaments are presumed to bind peripherally to the dense bodies and, together with myosin, to form obliquely arranged contractile units that link dense bodies with those in adjacent channels and with the adherens junctions at the cell surface. Bond and Somlyo (1982) as well as Tsukita et al. (1983) depicted actin filaments emanating from dense bodies in ultrathin sections, but in the light of our present results we cannot be sure that these belonged to the contractile apparatus. Indeed, although β-actin was clearly segregated from the contractile domains, we could not be sure whether the γ-smooth muscle actin label was conversely segregated from the cytoskeletal domain, since such a narrow channel free of immunofluorescent label might be obscured by the strong γ-actin labelling across the majority of the cell. The absence of γ-smooth actin from the dense bodies suggests that it is not a component of the cytoskeletal filaments themselves, but we cannot exclude the possibility that the more oblique contractile filaments could cross through the cytoskeletal domains. In short, the details of the arrangement of contractile actin around the dense body remain to be clarified.

In the light of the present and earlier data (Small et al., 1986) we suggest that the components of the cytoskeleton may contribute significantly to the mechanical properties of smooth muscle. As transcellular linkage elements the intermediate filaments would appear to serve to maintain three-dimensional integrity of the contractile and cytoskeletal apparatus and to prevent over-extension of the cell. The cytoskeletal actin may assume a more active role. The striking co-distribution of filamin and β-actin prompts us to reiterate the earlier suggestion (Small et al., 1986) that actin cross-linking could contribute to tension maintenance. Contraction may be accompanied by the disassembly of the cross-links, or even of the actin filaments themselves, to allow passive shortening of the cytoskeleton. In any case, we still have much to learn about the dynamics of interaction between the contractile apparatus and cytoskeleton of the smooth muscle cells. Further work is needed to address questions concerning the developmental establishment of the separate isoactin domains, the relative locations of muscle and non-muscle actins in different smooth muscle types, the identity and roles of other proteins in the cytoskeletal domain, and the mode and coupling of the different classes of actin filaments to the dense bodies and dense plaques.

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