Spatial and temporal relationships between vinculin and talin in the developing chicken gizzard smooth muscle

Tova Volberg, Helena Sabanay, and Benjamin Geiger

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

Abstract. The spatiotemporal relationships between vinculin and talin in developing chicken gizzard smooth muscle were investigated. Immunofluorescence and immunoelectron-microscopic labeling revealed that both proteins are associated with membrane-bound dense plaques in muscle cells; however, the most intense labeling for vinculin was located rather closer to the membrane than that for talin. The localization of vinculin and talin in embryonic chicken gizzards indicated that both are primarily cytoplasmic during the first 2 embryonic weeks. Only around days 16-18 does talin apparently become associated with the plasma membrane, this being concomitant with the appearance of distinct myofilament-bound dense plaques. Vinculin, on the other hand, remains primarily cytoplasmic and appears in the plaques only 1-3 days after hatching. It is thus proposed that the interactions of the dense plaque with myofilaments or with the membrane do not depend on the presence of vinculin in the plaque. Electrophoretic analyses indicated that, during development, there is no major change in the differential expression of specific vinculin isoforms. Quantitative immunoblotting analysis indicated that the vinculin content (relative to total extracted protein) is virtually constant during the last week of embryonic life. However, within 3 days of hatching, the vinculin concentration increases remarkably to over twice the embryonic level, and then slowly increases until it reaches the adult levels, which are three to four times higher than the embryonic level. The concentration of metavinculin (a 160-Kd vinculin-related protein) showed only a limited increase after hatching. We discuss the possible roles of vinculin and talin in the assembly of membrane-bound dense plaques during the different phases of smooth-muscle development.

Introduction

The major sites of actin-membrane interaction in smooth-muscle cells are the membrane-bound dense plaques [9, 10, 30, 32, 33]. These structures appear as electron-dense layers beneath the plasmalemma and have some structural similarity to cytoplasmic dense bodies. In previous studies, we have shown that, although both dense plaques and dense bodies contain actin filaments and α-actinin, only the former contain the adherens-junction proteins, vinculin and talin [18, 20]. Vinculin has been shown to be present in the junctional plaques of all adherens junctions studied so far, including cell-cell contacts (i.e., zonula adherens, fascia adherens) and cell-matrix adhesions (i.e., focal contacts, dense plaque; see [13, 14, 16, 17, 18, 21]). In several cell types, notably in smooth-muscle cells, a vinculin-related molecule called metavinculin with a molecular mass of about 160 kilodaltons (Kd) has been detected [31]. The other plaque protein, namely talin, is a 215-Kd protein isolated from chicken smooth muscle. Talin has been shown to coincide with vinculin in cell-substrate focal contacts of cultured cells [4, 5], and to be present in platelets [28] and in the dense plaques of smooth muscle. This protein is apparently absent from vinculin-rich cell-cell adherens junctions [20]. The specific and almost exclusive localization of vinculin and talin in junctional plaques has suggested that these proteins are involved in the attachment of actin filaments to the membrane at these sites [8, 14]. To substantiate this idea, attempts have been made to characterize directly the interactions of these two proteins with each other and with actin. The results obtained in several laboratories have shown that purified talin can bind vinculin as well as a few additional proteins, but that it has no apparent affinity for actin (i.e. [7, 29, 40]). The results concerning actin-vinculin interaction have been more controversial. While studies from several groups have indicated that vinculin binds to F-actin and reduces its apparent viscosity [6, 24, 38], recent experiments have suggested that this effect is largely due to the presence of a minor contaminant in the vinculin preparations used [11, 39].

Another aspect of vinculin-talin relationships has recently been noted in studies dealing with the molecular heterogeneity of adherens junctions. As already pointed out, we have shown that, while vinculin is present in essentially all adherens junctions, talin is only associated with cell-matrix contacts and is absent from the corresponding intercellular junctions. In cultured lens cells, for example, talin is associated with the vinculin-rich focal contact but not with the junctional plaque of cell-cell contacts formed by the same cells [20]. To study the molecular interrelationships between vinculin and talin in membrane-bound junctional plaques, we examined in the present study the spatial and temporal relationships between the two proteins in developing chicken gizzard smooth muscle. To approach the former aspect, we used high-resolution immunoelectron-microscopic labeling of ultrathin frozen sections of smooth-muscle cells, while for the latter, we immunocytochemically localized the two proteins in smooth muscle at various
stages of embryonic and post-hatching development. We showed that vinculin and talin are both associated with the dense plaques of adult smooth-muscle cells (as previously shown for vinculin alone [18]), there being only limited differences with respect to their fine topology. However, examination of embryonic gizzard indicated that talin, which is initially located mainly in the cytoplasm, becomes associated with the membrane at embryonic days 16–18, while vinculin becomes associated with the membrane considerably later, i.e. around the first day post-hatching. The significance of these results and their relevance to the functional properties and molecular interactions of vinculin and talin are discussed.

Methods

Tissues

Fertilized chicken eggs at various stages of development were purchased from a local supplier. Embryonic development was largely comparable to that described by Hamburger and Hamilton [22] for embryos of similar ages. Gizzard tissues of embryos, and newly hatched and adult chickens were removed and immediately processed for biochemical or immunocytochemical analyses.

Immunocytochemical reagents

Vinculin was prepared from chicken gizzard according to the method of Feramisco and Burridge [12]. Antibodies to vinculin were prepared in rabbits (polyclonal) or in mice (monoclonal; Vin-11.5). Rabbit antibodies to talin were kindly provided by K. Burridge (University of North Carolina, Chapel Hill). As secondary-antibody reagents, we used affinity-purified goat anti-rabbit Ig. These antibodies were coupled to rhodamine-tissamine sulfonyl chloride [27]. As secondary antibodies for immunoblotting, we used iodinated (125I) affinity-purified goat anti-mouse Ig antibodies. Goat anti-rabbit IgG coupled to 15-nm gold particles was purchased from Janssen Pharmaceutica (Beerse, Belgium).

Electron microscopy

Small blocks of chicken gizzard smooth muscle (1–2 mm) were dissected and fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 5 mM CaCl2. The tissues were rinsed, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon (Polybed 812, Polysciences, USA). Sections were cut using a diamond knife (Diatome, Switzerland), stained with uranyl acetate and lead citrate, and examined using a Philips 410 electron microscope at an accelerating voltage of 80 kV.

Immunocytochemical labeling

Light microscopy. Gizzard tissues were fixed with 5% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, containing 5 mM CaCl2 for 3 h, washed, and incubated for >1 h with 0.9 M sucrose. Sections (thickness, 0.5 mm) were cut at about -60°C in a Sorvall MT2B ultramicrotome equipped with a cryoattachment (Sorvall Ultromicrrotome LTC2) according to the method of Tokuyasu ([34]; see also [18]). Indirect-immunofluorescence labeling was carried out as previously described [13], and the sections were examined using a Zeiss photomicroscope III equipped with filter sets for rhodamine and fluorescein fluorescence.

Electron microscopy. Tissue blocks were fixed according to a three-stage method [35] including primary fixation with 8% paraformaldehyde (1 h on a shaker), fixation with 20 mM ethylacetimidate (Aldrich Chemical, USA) in 8% paraformaldehyde (5 min on a shaker), and incubation with a mixture of 4% glutaraldehyde and 4% paraformaldehyde (1 h on a shaker). The fixed gizzard tissues were infiltrated with 2.3 M sucrose or with 5 M fructose, sectioned, and indirectly labeled as described elsewhere [18, 34, 35]. As the secondary reagent, used goat anti-rabbit IgG coupled to 15-nm gold particles. The stained sections were postfixed with glutaraldehyde and 0.05% osmium tetroxide, stained with uranyl acetate, and embedded in methyl cellulose. Quantitative evaluation of the immunoelectron-microscopic labeling was carried out by direct measurement of the dis-
Fig. 2A–F. Distribution of talin (A, C, E) and vinculin (B, D, F) in adult chicken gizzard smooth muscle. A, B Immunofluorescence labeling of cross-sections through smooth-muscle fibers showing dotted labeling along the membrane. C–F Immunogold labeling; note the association of label for both antigens with the membrane-bound dense plaques but not with the cytoplasmic dense bodies. The intercellular space is of variable width, and connective-tissue elements (notably collagen) can be detected in it (col). Arrowheads show the profile of the membrane bilayer. Bar in B, 20 μm (also for A); bars in C–F, 0.2 μm.
Compared to the labeling for talin (Fig. 3), was carried out by immunoblotting, using purified vinculin as a standard run on the same gels. The bands on the autoradiograms (both pure vinculin standards and tested samples) were scanned using a spectrophotometer DU-8 (Bek- man USA), and the relative content of vinculin in the samples was determined.

Results

Immunoelectron-microscopic localization of vinculin and talin in adult chicken gizzard myocytes

In the present study, we used immunofluorescence and immunoelectron-microscopic approaches to localize the constituents of dense plaque in adult and developing smooth muscle. Dense plaques are abundant structures characteristic of mature smooth-muscle cells, as shown in Fig. 1. The dense plaques of chicken gizzard smooth muscle are elongated structures with a mean thickness of 500-700 Å, measuring 0.5-2 μm in width, and of variable length. They often appear in a rather nonsymmetric fashion on the membranes of neighboring cells, and elements of connective tissue are often detected in the intercellular cleft (Fig. 2) associated with the cell surface. At their cytoplasmic aspects, dense plaques are often flanked by intermediate filaments (not shown; see [6]), and the submembrane areas between adjacent plaques often contain arrays of caveolae. The rest of the cytoplasm of smooth-muscle cells contains tightly packed arrays of myofilaments and dense bodies, as well as some small, peri-caveolar areas.

The localization of vinculin and talin in adult smooth muscle was carried out by immunolabeling at both the light- and electron-microscope levels. Indirect-fluorescence labeling of thin (0.5 μm) frozen sections of chicken gizzard for talin and vinculin (Fig. 2A, B) revealed dense arrays of peripheral spots. Corresponding labeling of ultrathin frozen sections using the immunogold technique verified that the staining for talin and vinculin was specifically associated with membrane-bound dense plaques (Fig. 2C, F). Cytoplasmic dense bodies were negative for both antigens. High-power electron photomicrographs, in which the section angle was nearly perpendicular to the plane of the membrane, were used to quantitate the labeling density as a function of distance from the membrane. The results, which are summarized in Fig. 3, indicate that talin is widely distributed throughout the dense plaque, exhibiting a broad peak at distances of 150-500 Å from the plasma membrane, with some enrichment being seen in the 200-250 Å zone. Vinculin had a generally similar distribution, although most of the label was confined to a narrower, partially overlapping area 100-400 Å away from the membrane, there being a significant peak in the 100-150 Å zone (see Discussion).

The assembly of dense plaques and their molecular constituents during the development of chicken gizzard smooth muscle

Gizzard tissues were cut into small blocks and extracted using Laemmli sample buffer [25]. Protein concentration was determined using a modification of the Lowry method [26], and fixed amounts of protein were subjected to 8% polyacrylamide gel electrophoresis. Two-dimensional gel separation was carried out according to the method of O’Farrell [27] using LKB (Sweden) ampholines (pH 3.5-10.0) boosted with ampholines of pH 4.0-7.0. Immunoblotting analysis was performed according to the procedure of Towbin et al. [36]. Electrotransfer was carried out for 5 h. The nitrocellulose sheets were blocked with 3% bovine serum albumin (BSA) and were then labeled with the monoclonal antibody to vinculin (clone Vin 11.5) followed by 125I-labeled goat anti-mouse immunoglobulin.

Quantitative analysis of the vinculin in crude extracts was carried out by immunoblotting, using purified vinculin as a standard run on the same gels. The bands on the autoradiograms (both pure vinculin standards and tested samples) were scanned using a spectrophotometer DU-8 (Bekman USA), and the relative content of vinculin in the samples was determined.
Fig. 4A–F. Transmission electron micrographs of developing embryonic gizzards (A, B 14 day; C, D 18 day; E 21 day) and the gizzard of a 3-day-old chick (F). Note the presence of cytoplasmic dense bodies at all stages, and the absence of defined myofilament-bound dense plaques in the 14-day embryo. Defined dense plaques with filaments attached to their cytoplasmic aspects are visible in the 18-day gizzards (arrow in C). Examination of the 21-day embryonic gizzard and the gizzard at 3 days post hatching shows an increase in the abundance of dense plaques and cytoplasmic myofilaments. Bars, 0.2 µm.
Fig. 5. Immunofluorescence labeling of embryonic and post hatching chicken gizzards with antibodies to talin (TAL) and vinculin (VIN). The numbers in the upper-left corners represent the embryonic age or post hatching age (+1 and +3) in days. Note that vinculin is mostly cytoplasmic until day +1 and is clearly associated with the membrane in the gizzard at 3 days post-hatching. Talin, on the other hand, is apparently associated with the membrane on the 18th embryonic day. Unlabeled areas occupied by connective tissue are marked with asterisks. Bar, 10 μm
Fig. 6A–H. Immunelectron-microscopic labeling of ultrathin frozen sections for talin (A, C, E, G) and vinculin (B, D, F, H). The tissues were obtained from 14-day (A, B), 18-day (C, D), and 21-day (E, F) embryos as well as from 3-day-old chicks (G, H). Note the presence of talin (C, E) and the absence of vinculin (D, F) along the membrane of 18- and 21-day embryonic smooth muscle. Significant vinculin labeling of dense plaques was only noted in 3-day-old chick gizzards (H). Bars, 0.2 μm
Table 1. Relative concentrations of vinculin and metavinculin in developing chicken gizzards as determined by quantitative immunoblotting analysis

<table>
<thead>
<tr>
<th>Age</th>
<th>Relative concentrations</th>
<th>Vinculin/metavinculin ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vinculin</td>
<td>Metavinculin</td>
</tr>
<tr>
<td>Embryo day 14</td>
<td>0.25</td>
<td>0.15</td>
</tr>
<tr>
<td>Embryo day 18</td>
<td>0.31</td>
<td>0.18</td>
</tr>
<tr>
<td>Embryo day 21</td>
<td>0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>Chick day 3</td>
<td>0.67</td>
<td>0.30</td>
</tr>
<tr>
<td>Adult chicken</td>
<td>1</td>
<td>0.31</td>
</tr>
</tbody>
</table>

a Gizzard extracts containing 20 μg total protein were subjected to immunoblotting along with pure vinculin standards. The relative concentrations were determined by densitometric scanning of the autoradiograms.

b For comparison, the amount of vinculin in adult gizzard was taken to be 1.

surrounded by intermediate filaments. Examination of a large number of specimens indicated that, at this stage of development, distinct membrane-bound dense plaques are rarely recognizable; when submembrane density was observed, attachment to myofilaments was rarely detected. Only later, i.e., around the 16th-18th day of embryonic life, were distinct dense plaques noticed along the membrane (Fig. 4C, D). These plaques, when examined at high magnifications, exhibited many of the characteristics of ‘mature’ dense plaques, including association with cytoplasmic myofilaments. In 1-day-old chicks, both the abundance and extent of the structural development of myofilaments increased considerably, this being concomitant with the appearance of more dense plaques and dense bodies.

Immunofluorescence labeling of thin frozen sections of embryonic chicken gizzards for talin and vinculin revealed distinct differences between the organization of the two proteins (Fig. 5). In gizzards of 14- to 15-day embryos, the staining for both talin and vinculin was mostly cytoplasmic, with only slight enrichment along the cell membrane. About 3 days later, concomitant with the appearance of dense plaques, most of the cellular talin had apparently become confined to the cell periphery, and its relative abundance in the cytoplasm was markedly reduced. Patterns of talin distribution largely resembling those seen in adult chickens were detected in older embryos as well as in 1- and 3-day-old chicks (Fig. 5). Comparison of the organization of vinculin and talin indicated that the former associates with the membrane much later: the vinculin found in gizzard smooth muscle was mostly cytoplasmic, and only after hatching did significant submembrane labeling become predominant (Fig. 5). These results were further corroborated by immunoelectron-microscopic labeling of corresponding samples. Ultrathin frozen sections of chick gizzards from embryos and early post-hatching stages were labeled for both talin and vinculin using the immunogold technique. Figure 6 shows that, at embryonic day 15 (or earlier), talin and vinculin were detectable throughout the cytoplasm, both exhibiting a relatively low spatial density (Fig. 6A, B). It should be pointed out that the total amount of vinculin in cells at this stage was relatively low (see Table 1).

At more advanced embryonic stages, defined membrane-bound dense plaques were labeled with antibodies against talin (Fig. 6C), while the vinculin labeling was still mostly sparse and not strictly confined to the subplasmalemmal area. Only after hatching was significant labeling for vinculin first noted along the dense plaques (Fig. 6H).

In conclusion, both the light- and electron-microscopic data indicate that talin becomes organized along the membrane concomitant with the assembly of distinct myofilament-associated dense plaques, while vinculin is largely absent from these sites until the first day after hatching.

Quantitative and qualitative analysis of vinculin isoforms and metavinculin in developing chick gizzard smooth muscle

Chicken gizzards at the different stages of development were analyzed biochemically in order to determine whether the amount of vinculin or the expression of its various variant forms change during smooth-muscle development. Chicken gizzard smooth muscle was extracted with SDS sample buffer or with O'Farrell's lysis buffer, and the solubilized proteins were separated by one- or two-dimensional gel electrophoresis, respectively. The relative content of vinculin and metavinculin per fixed amount of total protein was determined by quantitative immunoblotting analysis. The relative concentrations of immunoreactive vinculin (130-Kd band) and metavinculin (~160-Kd band) present during different developmental stages are summarized in Table 1. The vinculin concentration as well as the ratio of vinculin to metavinculin remained almost constant throughout the last embryonic week. In the first few days after hatching (concomitant with vinculin incorporation into dense plaques), there was a marked increase in the total vinculin level accompanied by only a moderate increase in the metavinculin level; thus, the ratio between immunoreactive metavinculin and vinculin dropped from about 0.6 in 14-day embryonic gizzard to about 0.3 in adults.

Vinculin isoform analysis was carried out by applying two-dimensional gel analyses to total gizzard proteins. As shown previously [15], five distinct isoforms are present in adult gizzard. Examination of the smooth muscle of day-14, -16, -18, and -21 embryos and that of 3-day-old and adult chicks showed the presence of all isoforms in roughly similar proportions in all specimens tested regardless of the developmental stage (results not shown).

Discussion

Adherens junctions are complex cellular structures composed of several molecular subdomains [1, 14, 19, 21]. The outermost elements consist of specific ‘contact receptors’ whose nature is still poorly defined, while within the cytoplasm, there are bundles of actin-rich microfilaments that are associated with the membrane at these sites via a membrane-bound plaque structure. It has been proposed that, upon contact formation, components of the membrane domain become clustered and immobilized, leading to local assembly of the plaque which nucleates the assembly of the actin-filament bundles.

Attempts to characterize the molecules involved in this process have focused on two junctional-plaque proteins, vinculin and talin. It has been shown that these two proteins largely coincide in focal contacts and may bind to each other in vitro [7, 29, 40]. However, despite their affinity to each other in vitro, the co-localization of vinculin and talin in cells is not complete: whereas vinculin is present in all adherens junctions tested for far, talin is only asso-
ciated with contacts formed with noncellular matrices, but not with cell-cell contacts [20]. This selective association suggests that additional factors, beside the presence of vinculin, selectively determine the incorporation of talin into the junctional plaque.

In order to gain some insight into the organization and specific roles of vinculin and talin, we examined the differential assembly and spatial relationships of the two proteins during smooth-muscle development. Our study of vinculin and talin in embryonic gizzard provided some insight into the embryonic development of gizzard smooth muscle in general, as well as into the formation of the membrane-bound, myofilament-associated dense plaques.

The major morphological features of gizzard myocytes noted during various stages of development were generally in good agreement with those reported in the extensive study of Bennett and Cobb [2] and will therefore not be discussed in detail. It is, however, noteworthy that the medullary contractile system, which is composed of myofilament bundles with distinct cytoplasmic dense bodies, could be detected in the gizzard at embryonic day 9 [2] and had become quite prominent by embryonic day 14 (Fig. 4). Throughout these early stages of muscle development, most of the myofilaments were confined to the core of the muscle fibers, i.e., at a considerable distance from the plasma membrane. During the early stages (before day 14), most of the cell cortex contained ribosomes and some intermediate filaments which often extended to the cell periphery. Only later (at days 16–18) did myofilaments apparently approach the cell periphery and form visible associations with the membrane-bound dense plaques.

Membrane-bound dense plaques have been reported to be present in the gizzards of day-10 embryos [12]. In our experience, however, these early submembrane dense areas are relatively rare, have a granular appearance and do not exhibit a clear association with the myofilament system. The absence of both vinculin and talin from these plaques suggests that the two proteins are not obligatory for initial dense-plaque formation. The results of immunocytochemical labeling for vinculin and talin, combined with ultrastructural data suggest that there are several molecular and structural stages of dense-plaque development. The initial phase is apparently induced by local contacts between the plasma membrane and extracellular-matrix filaments [39].

A similar process has been proposed for the initiation of cell-substrate focal contacts [14, 19]. It is noteworthy that the myofilament bundles present in early (less than 16 days) embryos develop in the cell medulla independently of the newly formed plaques. Only later (embryonic age of about 16–18 days) does extensive association between the dense plaques and the myofibrillar network occur, this being concomitant with the appearance of talin in the plaque. The basis for the developmentally regulated changes in the composition and organization of dense plaques, particularly the reason for the late incorporation of vinculin, is not yet clear.

Immunoelectron-microscopic labeling indicated that the distribution of talin and vinculin in dense plaques is largely overlapping yet exhibits small but significant differences with regard to their fine localization within the plaque itself. This was shown by the apparent enrichment of vinculin immediately next to the plasmalemma, while the area with the most intense talin labeling was slightly further from the inner surface of the membrane. This suggests that the plaque does not contain a homogeneous mixture of the two proteins but rather has a distinct molecular substructure. Given the intrinsic resolution of double immunogold labeling (200–250 Å) and assuming free accessibility to the epitope at the surface of the section, we propose that talin is in fact absent from the immediate vicinity of the membrane or is only present in relatively low concentrations.

This finding should be considered vis-a-vis several recent studies concerning vinculin-talin-actin relationships. We have recently shown that the association of both vinculin and talin with focal contacts is largely actin independent; thus, extensive severing of actin filaments by fragmin has almost no effect on the association of the two proteins with focal contacts [1, 19]. These observations have been interpreted as suggesting that the two proteins interact with some membrane-bound junctional component other than actin. This suggestion did not indicate, however, which of the two proteins is primarily involved in this interaction. Our attempts to selectively extract vinculin and talin from focal-contact membranes did not yield meaningful results, since both proteins are comparable sensitive to the treatments we employed, including high- and low-ionic-strength buffers, deoxycholate, and urea [19]. A different approach reported by Herman and Pledger has suggested that talin may interact with the membrane in a vinculin-independent manner [23]. These investigators have shown that vinculin transiently dissociates from the focal contacts of 3T3 cells treated with platelet-derived growth factor, while talin remains associated with these structures. Our studies of the molecular heterogeneity of adherens junctions on the other hand, have suggested that vinculin may interact with the junctional membrane in a talin-independent manner in cell-cell contacts which are devoid of talin [20]. This mutual independence is further corroborated by the present results, which demonstrate, on the one hand, closer association of vinculin to the membrane and, on the other hand, the exclusive association of talin with the embryonic plaque. This is especially interesting in view of the already mentioned finding that vinculin and talin can interact with each other in vitro [7, 29, 40]. In an attempt to explain the differential distribution and assembly of vinculin and talin, we considered two possibilities: first, that the interactions of the two proteins with the plaque is independently regulated by additional, as yet undefined, proteins (see above), and second, that post-translational modification or selective isoform expression of the two may play a role in their selective incorporation into the plaque. In the absence of information about other plaque proteins, we examined the second possibility, which implies that the late incorporation of vinculin into pre-existing plaques is triggered by quantitative or qualitative changes in vinculin itself. Analysis of the total vinculin content at various developmental stages indicated that, within 1 day after hatching, there is a significant increase in the overall vinculin concentration. There seemed, however, to be no apparent change in the specific vinculin isoforms expressed at this stage. Moreover, the relative content of metavin with bound inhibitory, which has been implicated in the process of vinculin-membrane interaction [31], actually decreased. While we still do not know the molecular basis for the apparent post-hatching increase in the relative vinculin content, it may be relevant to point out that vinculin synthesis in cells may be regulated by cell-contact formation and affected by cell configuration. We have recently shown that changes in culture density
or substrate adhesiveness may significantly affect vinculin synthesis: cells seeded sparsely or on a poorly adhesive substrate may synthesize considerably less vinculin (relative to total protein) than densely plated cells which form extensive cell-substrate and cell-cell adhesions [37].

In conclusion, it appears that further progress in the elucidation of adherens-junction formation in general, and the attachment of the contractile apparatus of smooth muscle to the membrane in particular, will be made by pursuing two major lines of study. The first involves extending our knowledge of additional constituents of these junctions, the exact timing of their expression during junction formation and ontogenesis, and their relationship to vinculin and talin. The other direction is related to the controlled differential expression of vinculin and talin in culture or in intact tissue. Investigations in both directions, currently in progress, will hopefully shed light on the molecular mechanism involved in the regulation of adherens-junction formation during smooth-muscle development.

References


Received April 1986 / Accepted in revised form June 7, 1986