**Actin Filaments Are Required for Fibripositor-mediated Collagen Fibril Alignment in Tendon***

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Cells in tendon deposit parallel arrays of collagen fibrils to form a functional tissue, but how this is achieved is unknown. The cellular mechanism is thought to involve the formation of intracellular collagen fibrils within Golgi to plasma membrane carriers. This is facilitated by the intracellular processing of procollagen to collagen by members of the tolloid and ADAMTS families of enzymes. The carriers subsequently connect to the extracellular matrix via finger-like projections of the plasma membrane, known as fibripositors. In this study we have shown, using three-dimensional electron microscopy, the alignment of fibripositors with intracellular fibrils as well as an orientated cable of actin filaments lining the cytosolic face of a fibripositor. To demonstrate a specific role for the cytoskeleton in coordinating extracellular matrix assembly, cytochalasin was used to disassemble actin filaments and nocodazole or colchicine were used to disrupt microtubules. Microtubule disruption delayed procollagen transport through the secretory pathway, but fibripositor numbers were unaffected. Actin filament disassembly resulted in rapid loss of fibripositors and a subsequent disappearance of intracellular fibrils. Procollagen secretion or processing was not affected by cytochalasin treatment, but the parallelism of extracellular collagen fibrils was altered. In this case a significant proportion of collagen fibrils were found to no longer be orientated with the long axis of the tendon. The results suggest an important role for the actin cytoskeleton in the alignment and organization of the collagenous extracellular matrix in embryonic tendon.

Collagen fibrils are indeterminate in length, range in diameter from 10–500 nm (depending on the tissue and stage of development), and are some of the largest protein polymers in animals. The fibrils are comprised of individual triple helical collagen molecules arranged in a pseudo quarter-staggered array to produce fibrils with a 67-nm periodic axial repeat (the D-periodicity) (1). The three-dimensional structure of the fibrils is beginning to be understood, but the mechanism of how cells orchestrate the three-dimensional arrangement of fibrils in different tissues remains elusive. Tendon contains the simplest fibrillar architecture in that virtually all the fibrils are aligned in the same direction, along the long axis of the tendon. The parallel arrangement of the fibrils is determined early in tendon development and appears to be coordinated by tendon fibroblast cells that actively synthesize large amounts of collagen (2). Collagen is synthesized as a proform, known as procollagen (3, 4), which contains N- and C-propeptides that are removed to facilitate fibril formation (5). Processing of procollagen to collagen occurs partly within the extracellular matrix and partly inside tendon fibroblasts (6). Tendon fibroblasts in situ have a highly convoluted three-dimensional shape, in contrast to fibroblasts in cell culture which are flattened, and are associated with intracellular and extracellular collagen fibrils (7). Specifically, collagen fibrils can be found completely enclosed within tendon cells in vivo and also within plasma membrane structures that protrude from the surface of the cell, known as fibripositors (6). The fibripositors are co-aligned with the collagen fibrils in the extracellular matrix and with the long axis of the tendon. These observations led to the hypothesis that collagen fibrils are formed inside the late secretory pathway of tendon fibroblasts by the processing of procollagen to collagen and are secreted via fibripositors by a mechanism that produces a parallel arrangement of collagen fibrils in the extracellular matrix (8). It has been suggested that the cytoskeleton of the cell could therefore be involved in the parallelism of extracellular collagen fibrils (9).

The role of microtubules and actin filaments in cells actively producing procollagen has been investigated. Using colchicine to disassemble microtubules, procollagen secretion was inhibited in bone (10), periodontal ligament (11, 12), isolated tendon cells (13), and cultured fibroblasts (14). Furthermore, in bone (10, 15, 16), dermis (17, 18), odontoblasts (19), and periodontal ligament (11, 12, 20) colchicine administration was shown to result in Golgi complex disassembly and an accumulation of compartments containing condensed collagen aggregates. Using cytochalasin B to disassemble actin filaments in cranial bone explants, heterogeneous effects on procollagen secretion were observed (10, 21).

To test the hypothesis that the cytoskeleton controls the formation and secretion of collagen fibrils through fibripositor-like structures in developing tendon, the effect of the disruption...
of microtubules (using nocodazole and colchicine) and actin filaments (using cytochalasin B) was investigated. The secretion of procollagen was investigated using a previously established pulse-chase protocol for embryonic tendon explants, the relative numbers of cell-associated collagen fibrils were determined by serial section transmission electron microscopy, and direct visualization of actin filaments and microtubules was achieved by electron tomography.

**EXPERIMENTAL PROCEDURES**

*Pulse-Chase Analysis of Procollagen Processing and Secretion*—Reagents were obtained from Invitrogen or Sigma unless otherwise stated. Fertile hens eggs were supplied by Joice and Hill Poultry Ltd. (Peterborough, UK) and metatarsal tendons obtained from day 13 chick embryos. Pulse-chase experiments were performed at 37 °C in Dulbecco’s minimal essential medium/nutrient mix F12 containing Pen-Strep (1% v/v), L-glutamine (2 mM), L-ascorbic acid 2-phosphate (200 μM), and β-aminopropionitrile (400 μM) and supplemented with Me2SO (0.1% v/v), nocodazole (10 μM, 0.1% v/v Me2SO), cytochalasin (10 μM, 0.1% v/v Me2SO), or colchicine (100 μM) as required. Pulse-chase experiments were performed at 37 °C; tendons were pre-equilibrated for 0.5–3 h, labeled for 10 min with 2.5 μCi/ml of [14C]proline (Amersham Biosciences), and then transferred to unlabeled medium for 15 min to 3 h. Pulse-chase was stopped by transferring the tendons to EDTA (25 mM), Tris-HCl (50 mM, pH 7.5) at 4 °C. Tendons subjected to pulse-chase analysis in 1-ml aliquots of supplemented medium were then extracted in 100-μl aliquots of salt extraction buffer (NaCl (1 M), EDTA (25 mM), Tris-HCl (50 mM, pH 7.5)) containing protease inhibitors and supplemented as required with Nonidet P-40 detergent (1% v/v). A concentrated stock solution of protease inhibitors was made using EDTA-free Protease Inhibitor Mixture tablets (Roche Applied Science). Tendons were extracted in four changes of salt extraction buffer: overnight (S1), 6 h (S2), overnight (S3), 6 h (S4), and overnight in salt extraction buffer containing Nonidet P-40 (N). Extracts were analyzed on 4% precast SDS-polyacrylamide gels under reducing conditions. The gels were fixed for 2 × 20 min in methanol (10% v/v) with acetic acid (10% v/v) and dried under vacuum. The gels were exposed to a BAS-MS phosphorimaging plate (Fujifilm) that was processed using an FLA3000 phosphorimager (Fujifilm). The relative amounts of procollagen, pCcollagen, pNcollagen, and fully processed collagen (collectively denoted *collagen) in the salt (S1) and detergent (N) extracts were determined by densitometry using AIDA 2.0 software.

*Transmission Electron Microscopy*—Metatarsal tendons were obtained from day 13 chick embryos. Following incubation in nocodazole (10 μM, 0.1% v/v Me2SO), cytochalasin (10 μM, 0.1% v/v Me2SO), or Me2SO (0.1% v/v) for 1 h, tissues were fixed for 2 h at room temperature in glutaraldehyde (2.5% v/v) in sodium cacodylate buffer (0.1 M, pH 7.3). After washing three times in sodium cacodylate buffer (0.1 M, pH 7.3) for 10 min they were post-fixed in osmium tetroxide (1% w/v) in sodium cacodylate buffer (0.1 M, pH 7.3) for 2 h. After washing three times in distilled water for 10 min they were en bloc stained with aqueous uranyl acetate (0.5% w/v) overnight. The samples were then dehydrated in a graded acetone series and embedded in Spurr’s resin. Embryonic mouse tails (15.5 dpc) were incubated in nocodazole (10 μM, 0.1% v/v Me2SO), cytochalasin (10 μM, 0.1% v/v Me2SO), or Me2SO (0.1% v/v) for 30 min or 3 h and fixed in glutaraldehyde (2%) in phosphate buffer (100 mM, pH 7.0) for 30 min at room temperature and then for 2 h at 4 °C in fresh fixative. After washing in phosphate buffer (200 mM) they were fixed in glutaraldehyde (2% v/v) with osmium tetroxide (1% w/v) in phosphate buffer (50 mM, pH 6.2) for 40 min at 4 °C. After extensive washing in distilled water they were en bloc stained with aqueous uranyl acetate (1% w/v) at 4 °C overnight. The samples were then dehydrated in a graded acetone series, treated with propylene oxide for 10 min at room temperature, and embedded in Spurr’s resin. Ultrathin (60–70 nm) sections were collected on uncoated copper 200 grids and stained with lead citrate (0.3% in 0.1 M sodium hydroxide) for 1 min. Sections were examined on a FEI Tecnai 12 Biowitn transmission electron microscope and images recorded on 4498 film (Kodak) and digitized using an Imacon Flexight 848 scanner (Precision Camera and Video).

*Image Analysis*—The numbers of transverse sections of lumen containing collagen fibrils within the main body of the cell and within cellular projections (see Fig. 3A) were quantified from transmission electron microscopy (TEM) images of embryonic tendons incubated in nocodazole (10 μM, 0.1% v/v Me2SO), cytochalasin (10 μM, 0.1% v/v Me2SO), or Me2SO (0.1% v/v). Using a magnification of ×4800, 6 non-overlapping images per treatment (1–h incubation) for the embryonic chick metatarsal tendon samples and 15–20 separate tendon fascicles per treatment (30 min- or 3-h incubation) from the embryonic mouse tail samples were analyzed. Cellular projections containing collagen fibrils were determined to have a roughly elliptical profile with a diameter of ≤320 nm across the minor axis and to contain no other cellular organelles. Counts were normalized for the number of nuclei profiles observed to obtain relative counts of the numbers of cell-associated fibrils and expressed as the mean ± S.D. The data were analyzed by one-way analysis of variance or using a t test as appropriate.

To determine the percentage of extracellular fibrils that were sectioned at an oblique angle in transverse sections of embryonic tendons incubated in cytochalasin (10 μM, 0.1% v/v Me2SO) or Me2SO (0.1% v/v) for 3 h, the extracellular collagen fibrils or bundles of fibrils were traced using IMOD (22, 23). Oblique fibrils were classified as those in which the length of the fibrillar profile was observed to be more than three times the width of a collagen fibril. The relative areas of the oblique and transverse profiles were then used to calculate the percentage of extracellular fibrils that were oblique rather than transverse in 15–17 separate fascicles per treatment. The results were expressed as the mean ± S.D. and analyzed using a t test.

*Electron Tomography and Three-dimensional Reconstruction*—Embryonic mouse tails (15.5 dpc) were prepared for electron microscopy and serial section reconstruction performed as described (6). Semi-thick (300 nm) serial sections were also

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2 The abbreviations used are: TEM, transmission electron microscopy; dpc, days post coitum; LPC, large pliomorphic carrier; pCcollagen, procollagen lacking the N-propeptides but retaining the C-propeptides; pNcollagen, procollagen lacking the C-propeptides but retaining the N-propeptides.
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FIGURE 1. Effects of nocodazole and cytochalasin on collagen secretion and processing in embryonic chick metatarsal tendon. A, pulse-chase analysis was carried out in the presence of nocodazole (10 μM) or cytochalasin (10 μM) for the chase times indicated as described under “Experimental Procedures.” Bands corresponding to each of the type I procollagen processing intermediates are indicated (H, procollagen; *, pCcollagen; ▲, pNcollagen; ▼, collagen, where the upper band in each lane corresponds to the α1 chain and the lower band to the α2 chain for each intermediate). The proportion of *collagen in the intracellular detergent extract (N) at each time point (x-axis) was then expressed as a percentage (y-axis) to assess the rate of secretion for each treatment. N denotes the use of Nonidet P-40 detergent to generate the extract of intracellular proteins.

RESULST

Disruption of Microtubules Retards Procollagen Secretion and Interaction with Processing Enzymes—To investigate the relative contributions of microtubules and actin filaments to the intracellular trafficking and secretion of procollagen, pulse-chase analysis was carried out in freshly dissected chick embryonic metatarsal tendons in the presence of nocodazole or cytochalasin B. Nocodazole prevents the addition of tubulin to microtubules resulting in microtubule depolymerization, and cytochalasin B is a fungal toxin that inhibits actin polymerization and results in actin filament disassembly. This type of pulse-chase experiment has been optimized with a pulse time of 10 min in [14C]proline to specifically label type I procollagen and chase times of up to 3 h, at which point most of the procol-

lagon has been secreted and processed at both the N- and C-propeptides to collagen (6). Subsequent extractions first remove extracellular proteins using a buffer containing 1 M NaCl and then intracellular proteins in the same buffer supplemented with the detergent Nonidet P-40. Supplementing the cultures with nocodazole resulted in a delay in the processing of procollagen to collagen and in the transfer of labeled type I collagen- and procollagen-processing intermediates from the intracellular detergent extract to the extracellular salt extract (Fig. 1). Cytochalasin B had no effect on the procollagen processing and secretion profile in this system. These results suggest that microtubules are involved in the transport of procollagen through the secretory pathway. To confirm this result the pulse-chase analysis was repeated in the presence of colchicine. Colchicine acts in a similar manner to nocodazole but is soluble in water. Secretion and processing of procollagen were also found to be retarded in the presence of colchicine (Fig. 2).

Disruption of Actin Filaments Results in Rapid Loss of Fibri-

![Image](https://example.com/image.png)

FIGURE 2. Effect of colchicine on collagen secretion and processing in embryonic chick metatarsal tendon. A, pulse-chase analysis was carried out with or without the addition of colchicine (100 μM) for the chase times indicated as described under “Experimental Procedures.” Bands corresponding to each of the type I procollagen processing intermediates are indicated (H, procollagen; *, pCcollagen; ▲, pNcollagen; ▼, collagen, where the upper band in each lane corresponds to the α1 chain and the lower band to the α2 chain for each intermediate). B, the relative amounts of labeled procollagen, pCcollagen, pNcollagen, and fully processed collagen (collectively denoted “collagen”) in each lane were quantified as described under “Experimental Procedures.” The proportion of “collagen in the intracellular (N) extract at each time point (x-axis) was then expressed as a percentage (y-axis) to assess the rate of collagen secretion.
Actin and Collagen Fibril Alignment

Continued Inhibition of Actin Polymerization Disturbs the Parallelism of Extracellular Fibrils and Coincides with a Loss of Intracellular Fibril Profiles but Does Not Affect Procollagen Processing or Secretion—Longer (3 h) incubations with cytochalasin B caused gross morphological changes in embryonic mouse tail tendon, including speckling on the surface of the fibrils (Fig. 4A), apparent death of cells surrounding the developing tendons (not shown), and disturbances to the orientation of the extracellular fibril bundles (Fig. 4B). Furthermore, the number of cellular projections containing collagen fibrils and the number of collagen fibrils observed in the main body of the cell were reduced in both embryonic mouse tail tendon (Fig. 4C) and chick embryonic tendon (not shown). To investigate whether procollagen processing and secretion would be affected by longer incubations with cytochalasin B, the pulse-chase experiment was repeated using preincubation times of 1, 2, and 3 h. Surprisingly, incubation times of up to 3 h had no effect on the pulse-chase profile (Fig. 5).

Intracellular Fibrils as Well as Fibripositor Structures Are Aligned with the Tendon Axis—To show the coalignment of intracellular collagen fibrils and fibripositors, membrane-associated collagen fibrils were tracked through a 250-section series (corresponding to a tissue depth of 21.4 μm). These were then classified as fibripositor-associated (blue) or intracellular (green) fibrils. Those located within the main body of the cell but connected to a fibripositor were also identified (green). The results showed that the intracellular fibrils and those in fibripositors were aligned with the long axis of the tendon (Fig. 6).

Collagen Fibrils inside a Fibripositor Are Parallel to Cytoskeletal Actin Filaments—Automated electron tomography of serial semi-thick (300 nm) sections of mouse tail tendon (15.5 dpc) was used to obtain a detailed three-dimensional image of the cytoskeletal elements surrounding the base of a fibripositor. This is not possible with lower resolution techniques such as light microscopy or by electron microscopy of thin sections, which shows superimposed details in projection. Electron tomography permitted examination of the three-dimensional arrangement of the cytoskeleton in the vicinity of the fibripositor. Furthermore, using serial semi-thick sections in combination with electron tomography it was possible to obtain structural information from a suitable volume of tissue. Three longitudinal serial sections through a tendon fibroblast from developing mouse tail tendon, which included the base of a fibripositor and surrounding features, were analyzed. Features of interest within the three-dimensional volume were reconstructed by tracing individual components in serial 1-nm virtual slices to produce a model (Fig. 7A). Collagen fibrils present within the fibripositor were recognized by their distinctive 67-nm D-periodicity (Fig. 7B). Actin filaments were readily identified (Fig. 7C), but because of numerous branch points the filaments appeared as short segments (Fig. 7D) coursing alongside the fibripositor membrane. The reconstructed region of the cell also contained a microtubule that pointed from the centriole to the plasma membrane (bottom left to top right of Fig. 7A, centriole and plasma membrane not shown). Notably, a large pleiomorphic carrier (LPC) was associated with the microtubule via two attachment points (Fig. 7F). Tracking through serial virtual slices (not shown) indicated that the

shown), consistent with cell survival during the course of the experiment. To investigate the relationship between collagen fibrils and cellular membranes, the numbers of collagen fibrils observed either within fibripositor-like cellular projections or within the main body of the cell in transverse section were quantified. In samples treated with cytochalasin B the number of cellular projections containing collagen fibrils was significantly reduced (Fig. 3).

FIGURE 3. Cytochalasin treatment reduces the number of collagen fibrils present in fibripositor-like cellular projections. A, electron micrograph to show the appearance of transverse sections through cell-associated collagen fibrils within the main body of the cell (green striped arrows) and within cellular projections (blue arrows). The image shown is from embryonic mouse tail tendon at 15.5 dpc. Scale bar, 1 μm. B, the relative numbers of cell-associated collagen fibrils (abbreviated to relative counts on the y-axis) in embryonic chick metatarsal tendon (E13) after treatment with nocodazole (10 μM) or cytochalasin (10 μM) for 1 h were quantified and compared with the control treatment as described under “Experimental Procedures.” *, p < 0.05 by one-way analysis of variance. The color coding is the same as in panel A. C, the relative numbers of cell-associated collagen fibrils (abbreviated to relative counts on the y-axis) in embryonic mouse tail tendon (15.5 dpc) after treatment with nocodazole (10 μM) or cytochalasin (10 μM) for 30 min were quantified and compared with the control treatment as described under “Experimental Procedures.” **, p < 0.01 by one-way analysis of variance. The color coding is the same as in panel A.
material inside the LPC was fibrous in nature, and the size of the largest compartment (~500 nm in length) indicates that it could readily accommodate procollagen or processed collagen molecules.

**DISCUSSION**

In this study we have shown that microtubules and actin filaments play distinct and assignable roles in tendon fibripositor cells; microtubule disruption retards secretion, whereas actin filaments stabilize the structure of the fibripositor. Microtubules have been implicated in endoplasmic reticulum to Golgi (24) and Golgi to plasma membrane (25, 26) trafficking as well as in the organization of the Golgi complex (27). Furthermore, they are important for the spatial distribution of the endoplasmic reticulum and Golgi complex within the cell (28). The result that procollagen secretion is slowed by microtubule disruption could therefore be attributed to protein traffic disruption at any point in the secretory pathway. Interestingly, secretion was not prevented by microtubule disruption, and the percentage of labeled collagen secreted by a 3-h chase was just below that of the control sample. This is consistent with previ-
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FIGURE 7. Automated electron tomography and three-dimensional reconstruction to identify cytoskeletal components surrounding the base of a fibripositor. Scale bars, 100 nm. A, three-dimensional reconstruction showing mitochondria (blue) and the base of a fibripositor (semi-transparent light blue) containing collagen fibrils (purple). An actin cable (red) runs alongside the cytosolic face of the fibripositor. One microtubule (yellow) attached to an LPC (gold) is also shown. B, virtual slice (7 nm thick) showing one of the cross-banded collagen fibrils (black arrow) present within the fibripositor. C, virtual slice (7 nm thick) showing the actin filaments (boxed) that run alongside the fibripositor. The actin cable is found in a different tomographic plane to that of the fibripositor and collagen fibrils. D, three-dimensional reconstruction showing the relative orientations of the fibripositor membrane (semi-transparent light blue), collagen fibrils (purple), and the actin filaments (red). E, virtual slice (7 nm thick) showing part of the microtubule and associated LPC (outlined) that contains fibrous material. F, three-dimensional reconstruction shows a number of points in the model (gray arrows) where the LPC (gold) appears to be associated with the microtubule (yellow).

ous observations that drug-induced disruption of microtubules only moderately disturbs protein traffic (27). It seems likely that disruption of microtubules alters the organization of the secretory pathway, creating a logistical hurdle to protein secretion. This can also affect the microtubule-dependent targeting of post-Golgi carriers to the plasma membrane (26). It may be the case that procollagen or processing intermediates secreted into the salt-extractable extracellular matrix in the absence of microtubules do not follow the default pathway for secretion and would not result in the formation of a functional tissue due to mistargeting. The observation of a microtubule-associated LPC containing fibrous material is reminiscent of early electron microscopy studies in which ∼500-nm-long secretory vacuoles, often found associated with microtubules, were implicated in the post-Golgi trafficking and maturation of procollagen (20, 29–34). The results indicate that the transport and processing of procollagen does not depend on an intact actin microfilament system. However, the observed decrease in the number of fibri-

positors within 30 min of incubation with cytochalasin B is consistent with rapid disruption of the actin cytoskeleton. These data indicate that the structure of the fibripositor depends on actin filaments; the decrease in fibripositor counts could correspond to a decrease in the total numbers of fibri-positors/cell or a decrease in the length of all fibripositors. Actin filaments are structural components of a number of other cellular protrusions, including microvilli (35), filopodia (36), invadopodia (37, 38), and Drosophila bristles (39, 40). Electron tomography indicated that fibripositor formation or stabilization may be accomplished by the formation of a bundle of fila-

ments at one side of the membrane encasing the collagen fibrils. Longer incubations in cytochalasin B also resulted in a decrease in the relative numbers of membrane-bounded fibril cross-sections within the main body of the cell. Again this may be due to a decrease in the number or length of internal collagen fibrils or because these fibrils are no longer aligned with the tendon long axis.

The loss of parallelism of extracellular collagen fibrils and fibril bundles after cytochalasin treatment implicates the actin cytoskeleton and cell shape in the maintenance of the ordered arrangement of extracellular collagen fibrils via fibripositors. How tendon fibroblasts orchestrate the localized and directional assembly of actin filament bundles is as yet unknown. Conceptually, this could occur through any number of cell signaling pathways, potentially mediated by mechanotransduction (41, 42) or diffusion of growth factors from surrounding tissues (43). However, the best molecular candidates for this function are probably members of the Rho family of small GTPases. Both Cdc42 (44) and Rif (Rho in filopodia) (45, 46) have been implicated in the formation of filopodia in cultured fibroblasts, and it may be the case that they are also involved in fibripositor dynamics. Identification of the molecular mechan-

ism of fibripositor formation, whether involving signaling pathways, cytoskeletal proteins, or cell matrix-interacting pro-

teins, will require the development of a robust cell culture-based system in which the levels of candidate molecules can be knocked down or up-regulated. Unfortunately, such a system is presently unavailable because embryonic tendon cells released from tissue and placed in culture rapidly lose fibripositors, presumably because of flattening of the cytoskeleton as the cells adhere to culture plates (data not shown).

It is possible that fibripositors have further functions in addition to the secretion of preassembled collagen fibrils. They could be involved in the alignment of extracellular collagen fibrils not just through fibril deposition but potentially by reor-
iering and pulling on extracellular fibrils to prevent them turning out of a parallel arrangement. This conclusion is consistent with the observation that depolymerization of actin filaments led to a loss of parallelism of some of the extracellular collagen fibril bundles. The disappearance of the fibripositors and intra-

cellular fibrils and the concomitant rearrangement of existing extracellular fibrils after cytochalasin treatment suggests that fibripositors, or the fibrils contained within a fibripositor, stab-

bilize the parallelism of the extracellular matrix. Alternatively, the observed disorganization of the collagen fibril bundles after cytochalasin treatment could be related to additional effects on neighboring tissues or changes in cell shape.
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Furthermore, it may be the case that fibripositors are involved in collagen fibril remodeling; an alternative hypothesis for the presence of intracellular collagen fibrils in different tissues, particularly in periodontal ligament and periosteum, is that they are taken up by phagocytosis and digested, a process that involves matrix metalloproteinases and cathepsin family enzymes (47). In peristomal fibroblasts no intracellular collagen fibrils were observed after treatment of bone explants with cytochalasin B for 24 h (48). In support of the view that some or all the intracellular collagen fibrils observed in embryonic tendon are being turned over rather than deposited, no adverse effect on procollagen processing or secretion was observed in the presence of cytochalasin B in our experiments. However, these data could be interpreted to mean that secretion and processing of procollagen is able to proceed as normal even without using the default fibripositor pathway for secretion. If collagen fibril remodeling occurs in developing tendons it would be of interest to determine what proportion of extracellular collagen fibrils are degraded after synthesis. Perhaps the entire population of thin collagen fibrils observed in the extracellular bundles of embryonic tendons are sequentially removed and subsequently replaced by a population of longer and wider collagen fibrils. In this model the embryonic fibrils would act as a template for the formation of mature tendons with mechanical properties suitable for the demands placed on the tissue after birth/hatching. Interestingly, immunoreactivity for type XIV collagen, which is located on the surface of collagen fibrils (49), disappears from developing chick tendons at hatching (50). This corresponds to the time when fibril diameters switch from a unimodal (narrow diameter) to a multimodal distribution (51).

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