Intracellular Trafficking of Tropoelastin

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Abstract

Elastin is secreted as soluble tropoelastin monomers which are then cross-linked in the presence of extracellular microfibrils to form insoluble elastic fibers. Although the secretion of tropoelastin is thought to be mediated and targeted by an intracellular chaperone complex, the intracellular route taken by this protein and the role of such a chaperone complex remain undefined. In the present study, the specific pathway of tropoelastin secretion was investigated in fetal bovine chondrocytes and ligamentum nuchae fibroblasts by immunofluorescence staining and immunoprecipitation of tropoelastin following treatment with secretion-disrupting agents. In untreated cells, tropoelastin is secreted in approximately 30 min. In both cell types, brefeldin A and monensin inhibited secretion of tropoelastin and caused an intracellular accumulation of the protein in the fused ER/Golgi compartment or in the Golgi stacks, respectively. Incubations of longer than 1 h in the presence of brefeldin A result in eventual degradation of tropoelastin in the ER/Golgi compartment (Davis and Mecham, 1996). In contrast, the tropoelastin trapped in the Golgi as a result of monensin treatment steadily accumulated. Agents that elevate intracellular pH, such as ammonium chloride and chloroquine, also caused an intracellular accumulation of tropoelastin which appeared by immunofluorescence staining to be localized in secretory vesicles and/or endosomes. Since weak bases and ionophores alter the morphology of vacuolar compartments, the effect of bafilomycin A1 on tropoelastin secretion was also investigated. This vacuolar H⁺-ATPase inhibitor prevents acidification of the trans-Golgi network and endosomal compartments without disrupting intracellular organelle formation. When the elastogenic cells were treated with bafilomycin A1, tropoelastin secretion was diminished and an intracellular accumulation of tropoelastin was detected in the trans-Golgi network and small secretory vesicles. These results suggest that tropoelastin may be diverted from the constitutive pathway after exiting the Golgi and instead targeted to an acidic compartment prior to transport to the cell surface. The identity and role of such a compartment in the sorting and/or trafficking of tropoelastin has yet to be determined.

Key words: endosome, Golgi, secretion, tropoelastin.

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Abbreviations used: ALLN, N-acetyl-leu-leu-norleucinal; BFA, brefeldin A; FBC, fetal bovine chondrocyte; FCL, fetal calf ligamentum nuchae; TGN, trans-Golgi network.
Introduction

Elastic fibers are an abundant and integral part of many extracellular matrices and are critical for providing the property of elastic recoil to tissues such as the lung, skin and blood vessels. Morphologically, these fibers consist of two distinct components, elastin and 10 nm fibrillin-containing microfibrils (Parks et al., 1993; Rosenbloom et al., 1993; Mecham and Davis, 1994). Evidence to date suggests that these two components interact for the first time at the cell surface, where tropoelastin monomers are nucleated and guided by a scaffold of microfibrils to assemble into an insoluble elastin fiber.

Over the past several years, considerable information has been generated concerning the protein constituents of elastic fibers. Remarkably little is known, however, about the intracellular pathways and events required for delivery of these proteins to the cell surface. Early autoradiographic studies demonstrated the ability of aortic smooth muscle cells to synthesize and secrete tropoelastin by showing the uptake of [3H]-proline into the cells and the subsequent incorporation of the radiolabel into elastic laminae (Ross and Klebanoff, 1971; Gerrity et al., 1975). Although these studies also showed specific radioautographic labeling of ER, Golgi apparatus and secretory vesicles, the label corresponding to tropoelastin as opposed to that of collagen could not be distinguished. In further attempts to explore the synthesis and secretion of tropoelastin, morphological and cytochemical techniques showed the presence of small vesicles with electron dense cores in the vicinity of the Golgi apparatus and cell periphery in endothelial cells, smooth muscle cells and fibroblasts in the developing aortic wall. Since the amorphous material of the dense core had staining properties identical to that of elastin, and similar material was observed within the cisternae of ER and Golgi saccules, it was suggested that tropoelastin probably follows the classical pathway of protein secretion (Thyberg et al., 1979). The presence of tropoelastin in the ER, Golgi apparatus and vesicles containing electron dense material was later supported by immunolocalization studies of tropoelastin in cells of the embryonic chick aorta (Damiano et al., 1984; Daga-Gordini et al., 1987). However, an abundance of electron-dense vesicles and vesicles that immunolabel positively for the presence of tropoelastin is not observed in elastogenic cells. Since tropoelastin undergoes little, if any, post-translational modifications, with no glycosylation or proteolytic cleavage, the necessity of newly synthesized tropoelastin to follow a Golgi-dependent pathway has remained under question. Indeed, several studies have suggested that tropoelastin may follow a non-classical pathway of secretion, possibly even directly from the ER to the plasma membrane via secretory vesicles (Thyberg et al., 1979; Saunders and Grant, 1985; Hinek et al., 1995).

In the present study, the intracellular pathway of tropoelastin trafficking was investigated using five secretion-disrupting drugs. These drugs were chosen based on their ability to block transport of secretory proteins at various levels en route to the cell surface. Brefeldin A (BFA) has been used extensively to block protein transport from the ER to the Golgi (Pelham, 1991). In the presence of this agent, the Golgi apparatus disassembles and the ER becomes extensively dilated due to retrograde fusion of cis-, medial- and trans-Golgi cisternae into the ER (Misumi et al., 1986; Fujiwara et al., 1988; Doms et al., 1989; Lippincott-Schwartz et al., 1989; 1990). Since the effect of BFA on protein synthesis is minimal, secretory proteins tend to accumulate within the mixed ER/Golgi compartment (Misumi et al., 1986; Magner and Papagiannes, 1988). In contrast, the monovalent ionophore monensin causes dilation and retention of proteins within the Golgi apparatus, leaving the function and structure of the ER unaltered (Tartakoff, 1983). Since the newly synthesized proteins that accumulate in monensin-treated cells fail to acquire terminal sugars, the block appears to be in the passage of proteins from cis- to trans-Golgi (Ledger et al., 1983; Tartakoff, 1983). Three other drugs, chloroquine, ammonium chloride and bafilomycin A1, all disrupt the intracellular pH gradient that exists along the secretory pathway. Chloroquine and ammonium chloride are weak bases that accumulate in any organelle or vesicle with an acidic pH, thus leading to dilation of the trans-Golgi network (TGN) and the formation of large vacuolar structures in the cell periphery (Wibo and Poole, 1974). Because of the elevated intravesicular pH that results, integral membrane proteins accumulate in endosomes, receptor-ligand interactions are disrupted and acid proteases in the endosome/lysosome degradative pathway are inhibited (Mellman et al., 1986). In contrast to these weak bases, bafilomycin A1 is a specific inhibitor of vacuolar H+-ATPase (Bowman et al., 1988), and although it has effects similar to those of the weak bases on protein trafficking and receptor recycling (Henomatsu et al., 1993; van Weert et al., 1995), it prevents acidification of the TGN and endosomal compartment without disrupting the morphology of intracellular organelles (Johnson et al., 1993).

With these five secretion-disrupting drugs, we have investigated the production and distribution of tropo-
lastin by immunoprecipitation and immunofluorescence microscopy. Results from this work clearly demonstrate the utilization of the Golgi apparatus by tropoelastin as it traverses from the ER to the cell surface and provide evidence for the role of an acidic compartment in the trafficking and/or sorting of tropoelastin to assembly sites on the cell membrane.

Materials and Methods

Cells and reagents

Fetal calf ligamentum nuchae (FCL) fibroblasts were obtained from explants of 240-260 day gestation nuchal ligament tissue. Fetal bovine chondroblasts (FBCs) were obtained from 160-180 day fetal bovine auricular cartilage by collagenase digestion as previously described (Mecham, 1987). Cells were grown to confluency in DMEM supplemented with L-glutamine, nonessential amino acids, antibiotics and 10% fortified fetal calf serum (Hyclone Laboratories, Inc., Logan, UT). All experiments in this report were conducted with first passage cells.

For metabolic labeling, [4,5-3H]-L-leucine (1 mCi/ml) was purchased from ICN Pharmaceuticals, Inc. (Irvine, CA). Dialyzed fetal bovine serum (FBS) was purchased from Hyclone Laboratories, Inc. (Logan, UT). Protease inhibitors, e-amino-n-caproic acid, phenylmethylsulfonyl fluoride and N-ethylmaleimide were purchased from Sigma Chemical Company (St. Louis, MO) and used in the lysis buffer at final concentrations of 10 mM, 5 mM and 5 mM, respectively. For immunoprecipitation and immunofluorescence experiments, a monoclonal tropoelastin antibody BA-4, raised to bovine a-elastin (Wrenn et al., 1986), was used. As a marker of the Golgi apparatus, a monoclonal antibody to β-COP (a gift from Dr. Philip Stahl, Washington University, St. Louis) was used. Immune complexes were precipitated using heat-killed Staphylococcus aureus (Pansorbin cells, Calbiochem-Novabiochem Corporation, San Diego, CA).

Reagents used during metabolic labeling included ammonium chloride, bafilomycin A1, brefeldin A (BFA), chloroquine and monensin (Sigma Chemical Company, St. Louis, MO). Ammonium chloride and chloroquine were prepared fresh as 2 M and 10 mM stocks, respectively, in distilled water. Monensin was prepared fresh as a 10 mM stock in ethanol. Bafilomycin A1 and BFA were stored at -20 °C as 100 μM and 10 mg/ml stocks, respectively, in DMSO. Final concentrations for all secretion-disrupting agents are as indicated in the text and figures.

Metabolic labeling and immunoprecipitation

Metabolic labeling experiments were carried out with confluent monolayers of either FBCs or FCL fibroblasts grown in 6-well tissue culture plates. For metabolic labeling studies, cells were incubated in leucine-free medium containing 5% dialyzed FBS for 1 h prior to metabolic labeling with 50 μCi/ml of [3H]-leucine. Secretion-disrupting drugs were added to the medium at the beginning of the pulse. After metabolic labeling, the medium was collected and the cell layer washed three times with cold PBS. To each well, 1 ml of cold lysis buffer (25 mM Tris HCl (pH 7.5), 5 mM EDTA (pH 7.5), 250 mM NaCl, 0.1% Triton X-100) with protease inhibitors was added. The culture dishes were placed on a platform shaker at 4 °C, and cell lysates together with the detached cells were collected after 30 min. Cellular debris was pelleted by centrifugation and the cell lysates transferred to clean microfuge tubes.

For immunoprecipitation, medium and lysates were incubated with primary antibody over night at 4 °C with gentle agitation; the following day, 40 μl of Pansorbin suspension was added to each tube and further incubated for 1 h at 4 °C with gentle agitation. The immune complexes were pelleted and the pellets washed two times with lysis buffer and once with non-detergent buffer (10 mM Tris HCl (pH 7.5), 5 mM EDTA (pH 7.5)). After the final wash, each pellet was resuspended in 35 μl of Laemmli sample buffer containing 100 mM DTT and incubated at 100 °C for 6 min. The samples were electrophoresed on SDS-polyacrylamide gels, fixed, treated with ENHANCE (NEN Research Products, Boston, MA) for 1 h, then dried and exposed to Kodak XAR-5 x-ray film.

Immunofluorescence

For immunolocalization of tropoelastin, sub-confluent cultures of FBCs in 4-well LabTek Chamber Slides (Nunc no. 177437, Thomas Scientific, Swedesboro, NJ) were left untreated or treated with 10 μg/ml BFA for 1 h, or 10 μM monensin, 1 μM bafilomycin A1, 200 μM chloroquine or 50 mM ammonium chloride for 3 h. Cell layers were then washed with PBS and fixed with 2% paraformaldehyde in PBS for 30 min. Following three rinses in PBS containing 1% BSA and 0.1% saponin, cells were further permeabilized for 15 min two times in the same buffer. For β-COP immunolocalization, cells were either left untreated or incubated with 10 μg/ml BFA for 4 h before being fixed and permeabilized with -20 °C methanol. The permeabilized cells were then in-
cubated with primary antibody for 1 h at room temperature. All antibody dilutions and washes were with the permeabilization buffer for tropoelastin immunostaining, and PBS containing 1% BSA for β-COP. After washing for 5 min four times, the cells were incubated with goat anti-mouse fluorescence-conjugated IgG (Cappel, West Chester, PA) diluted 1:200 for 1 h. Cell layers were then washed for 5 min four times, rinsed once with PBS, and then mounted in 50% glycerol in PBS containing 1 mg/ml p-phenylenediamine and visualized with a Zeiss axophot microscope.

**Results**

*Effect of secretion-disrupting drugs on the secretion of tropoelastin*

FCL cells were metabolically labeled for 4 h with [3H]-leucine in the absence or presence of secretion-disrupting agents. Cell lysates and medium were collected and immunoprecipitated for tropoelastin (Fig. 1). In the absence of any drug, radiolabeled tropoelastin was observed in both the cell lysate and medium. Since tropoelastin takes approximately 30 min to be secreted (Davis and Mecham, 1996), more radiolabeled tropoelastin was present in the medium then cell lysate by the end of the 4 h continuous pulse. Treatment of the cells with bafilomycin A1, chloroquine, monensin or ammonium chloride during the pulse all resulted in diminished secretion of tropoelastin and an intracellular accumulation of the protein being observed. In the case of bafilomycin A1, the drug was effective at inhibiting secretion of tropoelastin even at doses as low as 0.5 μM. Ammonium chloride was equally effective, with a large pool of intracellular tropoelastin being detected. When monensin was tested, a clear dose-dependent response was observed. In a previous study, tropoelastin synthesis was shown to be repressed in rat smooth muscle cells when secretion of tropoelastin was inhibited by monensin (Frisch et al., 1985). The concentration and incubation time used in the present work, however, should have little or no effect on mRNA levels for tropoelastin. A dose-dependent response was also seen with chloroquine, al-

![Figure 1](image-url)

Figure 1. Monensin (MON), bafilomycin A1 (BAF), chloroquine (CLQ) and ammonium chloride (NH₄Cl) all inhibit the secretion of tropoelastin in FCL cells. In two separate experiments, FCL cells were pulsed with [3H]-leucine for 4 h in the absence (CON) or presence of secretion-disrupting agents. Tropoelastin was immunoprecipitated from the cell lysates and media using *Staphylococcus aureus* to collect the immune complexes. Samples were run on an 8.75% SDS-polyacrylamide gel, fixed, treated with EN3HANCE, and exposed to x-ray film. Note that in Experiment 2, both isoforms of tropoelastin are resolved on the gel.
though the drug appears to have a negative effect on tropoelastin production at doses higher than 200 μM.

Immunoprecipitation experiments to study tropoelastin secretion were also conducted with BFA, with results equivalent to those previously published (Davis and Mecham, 1996). It was found that treatment of FCL cells with BFA completely inhibited the secretion of tropoelastin. In contrast to the results obtained with the four drugs described above, however, an intracellular accumulation of tropoelastin was not observed following a 4 h continuous pulse. After additional experimentation, it was determined that, following an initial accumulation of tropoelastin for approximately 1 h, the protein was rapidly and selectively degraded by a cysteine protease in the fused ER/Golgi compartment.

Despite high levels of tropoelastin synthesis and secretion, FCL fibroblasts do not assemble soluble tropoelastin monomers into an insoluble elastic fiber matrix under tissue culture conditions (Mecham et al., 1981). Since this may reflect some cell-specific alteration of how tropoelastin is folded and/or transported through the FCL cell, the effect of the secretion-disrupting drugs was also tested on a different cell type. In contrast to FCL cells, FBCs maintain their ability to cross-link tropoelastin monomers in culture and form insoluble elastic fibers that can be detected at both the light and electron microscope level (Lee et al., 1994). Consistent with the results obtained with FCL cells, all four drugs caused a dose-dependent inhibition of tropoelastin secretion in FBCs (dose-dependent results are not shown for bafilomycin A1), with a resulting intracellular accumulation of the protein (Fig. 2). In addition, results similar to those described above for FCL cells were also obtained with FBCs following treatment with BFA (not shown).

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Localization of intracellular tropoelastin following treatment with secretion-disrupting drugs

Although the various secretion-disrupting drugs used in this study block the transport of secretory proteins at specific locations along the pathway from ER to cell surface, all of the drugs have been known to have uncharacteristic effects on the secretion of certain proteins. Thus, to verify the predicted site of intracellular retention of tropoelastin and more directly determine the organelles involved in trafficking the protein to the cell surface, indirect immunofluorescence microscopy was used to localize tropoelastin in FBCs treated with each of the five secretion-disrupting agents. In untreated cells, fixed and permeabilized with saponin, immunostaining for tropoelastin was seen in a perinuclear Golgi cap and in small vesicles distributed throughout the cell (Fig. 3A). In contrast, when cells were treated with BFA for 1 h, a strong immunofluorescent signal was observed throughout the cell (Fig. 3B). This result is consistent with an accumulation of tropoelastin in the fused ER/Golgi compartment, since previous electron microscopic studies have shown that this compartment occupies much of the cell volume (Davis and Mecham, 1996). To confirm the location and structure of the Golgi apparatus in FBCs and to verify the disruption of this organelle by BFA treatment, untreated and BFA-treated FBCs were immunostained for β-COP (Fig. 3C, 3D). β-COP, one of the major coat proteins (COPs) of Golgi vesicles (Allan and Kreis, 1986; Duden et al., 1991), rapidly redistributes from a Golgi-like staining pattern into a diffuse cytosolic pattern on BFA treatment (Klausner, 1992). The immunostaining pattern observed with β-COP demonstrates the presence of a perinuclear Golgi apparatus in FBCs and the disruption of this organelle by BFA.

Figure 2. Monensin (MON), bafilomycin A1 (BAF), chloroquine (CLQ) and ammonium chloride (NH4Cl) all inhibit the secretion of tropoelastin in FBCs. FBCs were pulsed with [3H]-leucine for 4 h in the absence (CON) or presence of secretion-disrupting agents. Tropoelastin was immunoprecipitated from the cell lysates and media as described in Figure 1.
Figure 3. Immunolocalization of tropoelastin in BFA-treated cells. FBCs were either left untreated (A, C) or incubated with 10 μg/ml of BFA for 1 h (B) or 4 h (D). Cells were fixed, permeabilized, and tropoelastin (A, B) or β-COP (C, D) were localized by indirect immunofluorescence. In control cells (A), faint tropoelastin staining is seen in the Golgi apparatus (arrows) and small vesicles. In contrast, those cells treated with BFA show staining throughout the cell, representing tropoelastin trapped in the fused ER/Golgi compartment (B). In untreated and BFA-treated cells, immunostaining for β-COP verifies the location of the Golgi apparatus (A, arrows) and demonstrates the disruption of this organelle by BFA treatment (B). Original magnification: (A, B) 490×, (C, D) 590×. Bars = 20 μm.

Immunofluorescence staining for tropoelastin in cells treated for 3 h with either monensin or bafilomycin A1 showed strong staining of the perinuclear Golgi apparatus (Fig. 4A, 4B). Although the immunofluorescence pattern appears basically the same, a clear distinction between the two drugs can be seen at higher magnification (Fig. 5). In monensin-treated cells, the fluorescence staining is located only in a perinuclear Golgi cap, whereas in cells treated with bafilomycin A1, immunostaining for tropoelastin was observed not only in the

Figure 5. Localization of tropoelastin in the Golgi apparatus of monensin and bafilomycin A1 treated cells. FBCs were treated for 3 h with either 10 μM monensin (A) or 1 μM bafilomycin A1 (B) and stained for tropoelastin as described in Figure 3. In monensin treated cells, tropoelastin appears localized in a perinuclear cap of distended Golgi cisternae. In contrast, a looser, less disrupted Golgi staining is present when tropoelastin is accumulated in the TGN in cells treated with bafilomycin A1. Note the presence of an array of small vesicles that stain strongly for tropoelastin in the bafilomycin A1 treated cells. Original magnification 985×. Bar = 10 μm.
Figure 4. Tropoelastin accumulates in different cellular compartments with various secretion-disrupting agents. FBCs were incubated for 3 h with either 10 μM monensin (A), 1 μM bafilomycin A1 (B), 200 μM chloroquine (C) or 50 mM ammonium chloride (D). Cells were fixed and permeabilized with saponin, and intracellular tropoelastin was localized by indirect immunofluorescence. Monensin and bafilomycin A1 treated cells show tropoelastin accumulated in the perinuclear Golgi region. Both chloroquine and ammonium chloride show an accumulation of tropoelastin in vesicles around the cell periphery. Original magnification 490×. Bar = 20 μm.
Golgi cisternae, but also in numerous small vesicles that radiate out from the Golgi region. This result is consistent with a cis-to trans-Golgi block for monensin and a disruption of the pH gradient at the level of the TGN and secretory vesicles following bafilomycin A1 treatment. In FBCs treated for 3 h with either ammonium chloride or chloroquine, tropoelastin was localized in vesicular structures (Fig. 4C, 4D). In contrast to ammonium chloride, however, where the vesicles were small in size and distributed throughout the cell, chloroquine treatment resulted in the appearance of large, centrally located tropoelastin-containing vacuoles that clearly demonstrated the disruption of organelle structure.

**Discussion**

In the present study, immunoprecipitation and immunofluorescence experiments following treatment of elastogenic cells with secretion-disrupting drugs demonstrate that tropoelastin follows the classical pathway of protein secretion. More specifically, immunofluorescence localization of tropoelastin in the perinuclear Golgi region following treatment of cells with monensin clearly establishes that tropoelastin traverses the Golgi apparatus en route to the cell surface. These results are consistent with kinetic studies on the intracellular trafficking of other matrix proteins where monensin treatment of human fibroblasts has been shown to inhibit the secretion of both procollagen and fibronectin and lead to their accumulation within the Golgi apparatus (Uchida et al., 1980).

Retention of tropoelastin in the Golgi apparatus was also demonstrated in the present study by bafilomycin A1 treatment. In contrast to monensin, however, the block appeared to be located more at the trans-face of the Golgi, with an abundance of small vesicles staining for tropoelastin radiating from the Golgi apparatus. Chloroquine and ammonium chloride, which also raise intravesicular pH but in a more general manner, also blocked the secretion of tropoelastin in vesicular compartments. The inhibition of tropoelastin secretion following treatment of cells with agents that prevent acidification of endosomes demonstrates a requirement for an acidic compartment along the intracellular pathway utilized by tropoelastin. Apart from the TGN, however, the identity of the intracellular compartments that contain the accumulated tropoelastin is difficult to determine. This is due primarily to the fact that little is known concerning the specific pathways taken by newly synthesized proteins as they travel from the TGN to the cell surface in non-polarized cells. Increasing evidence suggests that there are distinct biosynthetic routes in these cells similar to the apical and basolateral routes that have been well described in polarized cells (Yoshimori et al., 1996). The difficulty of delineating these transport routes, however, is compounded by the complex and diverse nature of the endocytic compartments through which some secreted proteins traverse. It is not clear to what extent these tubulo-vesicular structures, which include sorting and recycling endosomes, represent distinct permanent compartments, subcompartments, or compartments that progressively change and mature. In certain cell types, furthermore, subpopulations of specialized endosomes have been identified, such as the MHC class II compartment in antigen-presenting cells (Amigorena et al., 1994; Tulp et al., 1994), compartments for the storage and regulation of surface expression of GLUT4 and other transporters in adipocytes, and compartments for the retrieval and regeneration of synaptic vesicles in neurons (Kelley, 1993).

The inhibition of tropoelastin secretion by acidotropic agents also raises the intriguing possibility that tropoelastin may use a pH-sensitive receptor for trafficking to the cell surface. Receptor-ligand interactions usually involve high affinity binding at neutral pH and only weak binding at low pH. Internalized receptor-ligand complexes, therefore, dissociate upon reaching the acidic environment of the endosome, leaving the receptor free to return to the cell surface (Mellman et al., 1986). Obviously, if a receptor mediates the transport of tropoelastin to the cell surface, the affinity of this receptor for its ligand would need to be reversed in such a way that the receptor would bind tropoelastin at low pH and release the monomer after being exposed to the neutral pH of the extracellular matrix. Although there is very little evidence for "receptor-mediated exocytosis", there are examples to support the possibility of such a process. For instance, while transferrin has a high affinity for its receptor at neutral pH, apotransferrin (iron-free transferrin) has high affinity at acidic pH (Dautry-Varsat et al., 1983). Thus, apotransferrin binds tightly to its receptor in the acidic endosome and, upon recycling of the receptor to the cell surface, dissociates as a result of exposure of the complex to the neutral pH of the matrix.

Recently, we have shown that tropoelastin forms an association with at least two molecular chaperones, BiP and the peptidyl-prolyl cis-trans isomerase FKBP65 (Davis et al., 1998). Based on their known functions, these chaperones undoubtedly facilitate tropoelastin folding. A role for a proline isomerase in the folding of tropoelastin is attractive, since approximately 12% of
the residues in tropoelastin are proline. It is also likely
that tropoelastin associates with protein disulphide-isomerase (PDI), since the only two cysteine residues in the
protein form an intramolecular disulphide bond in the
C-terminus (Brown et al., 1992). The implication of these
results is that tropoelastin requires the help of
molecular chaperones in the ER to fold correctly and
thus be ready for further transport through the cell and
assembly at the cell surface. Such a hypothesis is sup-
ported by the fact that an accumulation of tropoelastin
in the ER by BFA treatment leads to rapid degradation
of the protein, probably as a result of being recognized
as mis-folded or aggregated (Davis and Mecham, 1996).

Another candidate for assisting in the secretion of
tropoelastin is the 67-kD elastin binding protein
(EBP67) first characterized by Wrenn and colleagues
(1988). The identity of this protein remains uncertain,
however, with candidate proteins including a 67-kD
high affinity lamin receptor, galactosyltransferase,
5'nucleotidase, an alternatively spliced form of β-galac-
otosidase and several others (Mecham and Hinek, 1996).
Until the identity of any elastin-binding protein is clari-
fied, the role of such a protein in events leading to sort-
ing and/or trafficking of tropoelastin to membrane sites
for the assembly of elastic fibers cannot be determined.

A consistent observation made during the course of
this study was that not all the cells showed intracellular
staining for tropoelastin when analyzed by immunofluo-
rescence. Although this could result from inefficient cell
permeabilization, several lines of evidence suggest that
this is not the case. First, all of the cells labeled for β-
COP thus showing complete accessibility to intracellular
antigens. Second, it has been shown that elastin expres-
sion correlates closely with cell cycle, so that proliferat-
ing cells have relatively low levels of elastin expression
as compared to quiescent cells (Mecham et al., 1981;
Wachi et al., 1995). Since the cultures used for immuno-
fluorescence were subconfluent, one would expect that
many of the cells would be actively dividing and thus
not synthesizing elastin.

In summary, although tropoelastin undergoes no post-
translational modifications, including no glycosylation,
results from the present study confirm that tropoelastin
traverses the Golgi apparatus en route to the cell surface.
At the level of the TGN, tropoelastin appears to require
a sorting event to allow for transport to an acidic com-
partment prior to trafficking of the monomer to cell sur-
face assembly sites. As discussed by Mellman and col-
leagues (1986), it is conceivable that within the exocytic
pathway, elevation of intravesicular pH by acidotropic
agents affects the interaction of certain secreted proteins
with a possible class of receptors that are responsible for
transporting these proteins to the plasma membrane.
The existence of such a system of “receptor-mediated
exocytosis” for tropoelastin, however, remains to be
demonstrated.

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