Tropoelastin Interacts with Cell-surface Glycosaminoglycans via Its COOH-terminal Domain

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Using a biochemical and cell biological approach, we have identified a cell interaction site at the carboxyl terminus of tropoelastin. Cell interactions with the COOH-terminal sequence are not through the elastin-binding protein (EBP67) because neither VGVAPG-like peptides nor galactoside sugars altered adhesion. Our results also show that cell adhesion to tropoelastin is not promoted by integrins. Through the use of mutant Chinese hamster ovary cell lines defective in glycosaminoglycan biosynthesis, as well as competition studies and enzymatic removal of specific cell-surface glycosaminoglycans, the tropoelastin-binding mieties on the cell surface were identified as heparan and chondroitin sulfate-containing glycosaminoglycans, with heparan sulfate being greatly preferred. Heparin affinity chromatography combined with cell adhesion assays identified the last 17 amino acids as the sequence element at the carboxyl terminus of tropoelastin responsible for the adhesive activity.

The ability to interact with receptors or binding proteins on the cell surface is a property of all proteins of the extracellular matrix. This interaction provides important temporal and spatial signals to cells and influences such cellular processes as adhesion, migration, gene expression, proliferation, and apoptosis. In addition, several receptors function in ECM assembly to directly or indirectly assemble monomers of ECM proteins into their functional polymeric form.

Elastin is the major extracellular matrix protein capable of elastic recoil in tissues repeatedly subjected to cycles of reversible extension (1–3). It functions as an insoluble polymer made up of cross-linked tropoelastin molecules emmeshed in a network of filamentous microfibrils (4–6). The secreted form of elastin, tropoelastin, is a highly cationic protein because of an enrichment of lysine residues that serve as precursors to covalent cross-links that form between and within tropoelastin monomers. Lysine side chains are modified through oxidative deamination of ε-aminogroups that then condense with unmodified lysines or with other modified side chains to form a covalent linkage. It is important to note that almost all of the lysine residues in tropoelastin are modified to form cross-links in the mature protein. This changes the physical character of the protein from cationic to slightly anionic and hydrophobic in nature. In this respect, mature elastin has very different chemical and physical properties than does the precursor molecule tropoelastin.

Like other ECM proteins, elastin has been shown to specifically interact with binding proteins on the cell surface (7–10). Binding occurs with high affinity and to multiple cell types, including bacteria (11). In responsive cells, elastin has been shown to alter gene expression, influence mobilization of intracellular ions, modulate cell adhesion and movement, effect cell proliferation, and to induce actin polymerization (7, 12–18). There is evidence from multiple studies that implicate changes in calcium and inositol phosphate levels as one consequence of elastin-receptor interaction (19–21). In Lewis lung carcinoma cell lines, the affinity of receptors for elastin and chemotactic responsiveness are coordinately modulated by treatment of cells with agents that affect protein kinase C activity (22). Karnik et al. (13) found that an elastin-derived peptide activates a pertussis toxin-sensitive G-protein-coupled pathway that stimulates Goi, inhibits adenylate cyclase, reduces cAMP levels, and stimulates Rho-induced actin polymerization. Other studies have found that elastin peptides signal through a Ras-independent mechanism requiring both p110g/Raf-1 and protein kinase A/B-Raf (23).

The sequence in elastin assumed responsible for these activities is the hexapeptide VGVAPG and similar sequences (24, 25) located in the middle of the molecule. These peptides are thought to bind to a 67-kDa elastin-binding protein (EBP67) that is also a galactoside-binding lectin (8, 26–28). Other documented elastin-binding proteins include a 120-kDa protein (elastonecin) up-regulated in the presence of fragments of insoluble elastin (9), and a 59-kDa VGVAPG-binding protein identified on tumor cells (7). Elastin does not contain an RGD sequence and does not interact with most RGD-binding integrins, although αvβ3 integrin has been shown to bind to tropoelastin in solid phase binding assays (29). Whereas numerous biological activities have been demonstrated for VGVAPG and similar hydrophobic peptides from insoluble elastin, one thing these hydrophobic sequences do not do well is promote cell adhesion. Tropoelastin, in contrast, actively promotes cell attachment and spreading, suggesting that it interacts with a receptor that is different from that which recognizes the VGVAPG sequence.

This study localizes a specific cell interaction activity to a highly conserved domain at the COOH terminus of elastin. Results with inhibitors of integrin receptors and of elastin-binding protein ruled out either receptor class as mediating binding to this domain. Through the use of CHO wild-type and mutant cell lines defective in glycosaminoglycan (GAG) production, the identity of the COOH-terminal binding entity was established as heparan sulfate (HS) and chondroitin sulfate (CS) cell-surface proteoglycans, with a preference for HS chains.
Tropoelastin and Cell-surface GAGs

MATERIALS AND METHODS

Cells—Auricular chondrocytes (FBC) (30) obtained from a 170-day gestation fetal calf were maintained in DMEM + 10% cosin calf serum (Hyclone Inc.) and subcultured 1:3 weekly. The cells were used before passage 6 and were split 1:3 the night before use in adhesion assays. Wild-type Chinese hamster ovarian cells (CHO-K1 ATCC CRL-9618) and their mutants pgsA-745 (ATCC CRL-2242), deficient in cellular GAGs (CHO-745) (31), and CHO-M1, which lack surface HS,3 were maintained in F-12 media + 10% fetal calf serum and split 1:3 the night before use in adhesion assays. Cells treated with chondroitinase ABC (Seikagaku Corporation, 100330) were washed with DMEM + 10 mg/ml bovine serum albumin (BSA) then incubated at 37 °C for 1 h with 250 milliunits/ml of chondroitin ABC before use in adhesion studies.

Expression and Purification of Tropoelastin and Fragments—Tropoelastin and tropoelastin fragments were expressed as His6 fusion proteins using the pQE expression system (Qiagen, Inc.) in the M-15 strain of Escherichia coli. Expressed proteins were initially purified using nickel chromatography under denaturing conditions and the batch purification method according to the manufacturer. Eluted tropoelastin or tropoelastin fragments were dialyzed against 50 mM acetic acid and purified method according to the manufacturer. Eluted tropoelastin or tropoelastin fragments were dialyzed against 50 mM acetic acid and lyophilized. They were further purified using reverse phase high performance liquid chromatography on a Vydac 214TP510 C-4 column using a 0–50% linear acetonitrile gradient. Peak fractions were dried by rotary evaporation, dissolved in MilliQ water, then lyophilized. LC-mass spectrometry performed by the Protein and Nucleic Acid Chemistry Laboratory at Washington University Medical School verified the composition of each purified peptide and confirmed the presence of intra-chain disulfide bonds. The numbering of the peptide residues is based on the translated bovine A splice variant beginning with the initial methionine (33). The bovine C variant has exons 13 and 14 spliced out.

Peptides—All peptides were synthesized on an ABI-431A synthesizer using FastMoc chemistry on Wang-capped resins. After cleavage, peptides were dissolved in MilliQ water with 0.05% trifluoroacetic acid and purified with reverse phase high performance liquid chromatography on a Vydac 218TP1022 C-18 column using a linear 0–50% acetonitrile gradient. Peak fractions were dried by rotary evaporation, dissolved in MilliQ water, then lyophilized. Peptides containing cysteine were dissolved in water and disulfide bonds were allowed to form before use (34). We confirmed that the folded synthetic peptides were monomeric using gel filtration chromatography with a 1.5 × 70-cm Superdex-30 column. LC–mass spectrometry performed by the Protein and Nucleic Acid Chemistry Laboratory at Washington University Medical School verified the composition of each purified peptide and confirmed the presence of intra-chain disulfide bonds. The numbering of the peptide residues is based on the translated bovine A splice variant beginning with the initial methionine (33). The wild-type COOH-terminal peptide (CT-25) begins immediately after the cross-linking sequence in exon 35 (residue 723) and goes to the end of the molecule (residue 747): FGGAL-

3 L. Zhang, unpublished results.

RESULTS

A Cell Adhesive Site Is Located at the Carboxyl Terminus of Elastin—Bovine tropoelastin and a series of tropoelastin fragments (Fig. 1A) were expressed as His6 fusion proteins and purified to homogeneity (Fig. 1B).
The full-length protein was found to promote adhesion of multiple cell lines and strains, including fibroblasts from skin and the bovine ligamentum nuchae (FCL cells), smooth muscle cells from fetal bovine aorta, A2058 melanoma cells, and fetal bovine chondrocytes (FBC cells) from auricular elastic cartilage (data not shown). We selected FBC cells for further studies because of their in vitro elastogenic potential and their well characterized phenotype. Fig. 2A shows that FBC cells adhered to substrates coated with full-length tropoelastin (exons 1–36) and fragments containing the carboxyl-terminal region of the protein (exons 15–36 and exons 29–36). However, the cells failed to adhere to the amino-terminal portion of the molecule (exons 1–15) or to the back half-fragment truncated at exon 29 (exon 15–29). These results localize the binding activity to within the exon 29-36 fragment.

Synthetic peptides (see TABLE ONE for peptide sequences) were then used to localize the adhesive activity to a sequence contained in the last two exons of the molecule. Fig. 2B shows dose-dependent adhesion of FBC cells to CT-25, which contains a sequence that begins immediately after the cross-linking domain in exon 35 and includes all of exon 36 of bovine elastin, and Hu-29, which includes exons 33 and 36 of human elastin. A scrambled version of the bovine sequence (p35–36s) or peptides encoding the exon 35 or exon 36 portions of CT-25 alone (p35/H11032 and CT-14, respectively) did not support adhesion (Fig. 2B and C). Furthermore, adhesion was diminished when the basic charge of CT-25 was reduced by deleting the conserved arginine at residue 744 (ΔR744). Interestingly, substituting an aspartic acid residue for glycine at position 734 (G734D) completely ablated cellular binding in the concentration range tested. Altering the secondary structure of CT-25 by changing proline 733 to leucine (P733L) had no effect (Fig. 2C).

To determine which amino acids upstream of exon 36 are required to convert CT-14 to an active peptide, elastin sequences from different vertebrate elastins were compared. Our rationale was that amino acids critical for the biological activity of the peptide would be conserved across multiple species. TABLE TWO shows an alignment of the carboxy-terminal region of elastins from six species ranging from zebrafish to humans. The most prominent conservation was an aromatic residue (either Phe or Tyr) two amino acids from the COOH-terminal end of the penultimate exon. This residue was preceded by a hydrophobic amino acid (Gly, Ile, or Tyr) in all species. Fig. 2B shows that adding an aromatic residue (Phe) from the bovine sequence plus the amino acid before (Gly) and after it (Pro) to the amino terminus of CT-14 (producing CT-17) restored adhesive activity.

Cells in Contact with Tropoelastin or the COOH-terminal Elastin Peptide Show Fewer Stress Fibers and Reduced Vinculin-containing Focal Adhesions—FBC cells adherent in the presence of CT-25 spread to nearly the same size as cells plated with serum (Fig. 3A). On the peptide, however, the cells showed few if any stress fibers and lacked the


**Table One**

| Fragment/peptide | Residue/sequence | Peak | Peak width |
|------------------|------------------|------|------------|
| Tropoelastin      | 1–747            | 350  | 330–390    |
| Exons 1–15       | 1–274            | 360  | 330–420    |
| Exons 15–29      | 275–622          | 250  | 225–280    |
| Exons 15–36      | 275–747          | 355  | 335–380    |
| Exons 29–36      | 623–747          | 450  | 420–480    |
| CT-25            | FGALGALGFPGACLGKSCKGRKRK | 340 | 325–365    |
| CT-17            | GFGACLGKSCKGRKRK  | 420  | 400–450    |
| CT-14            | GGACLGKSCKGRKRK  | 415  | 400–440    |
| p35             | FGALGALGFPGACLGKSCKGRKRK | 0        | 300–330    |
| p35–36S         | GPKAFAKGAKACSPARGGLCGTPGRLGPGK | 0  | 300–330    |
| G734D           | FGALGALGFPGACLGKSCKGRKRK | 315 | 300–350 |
| ΔR747           | FGALGALGFPGACLGKSCKGRKRK | 230 | 210–250 |
| P733L           | FGALGALGFPGACLGKSCKGRKRK | 335 | 310–360 |
| Hu-29           | GVAARPFGGLSPIFGACLGKSCKGRKRK | 315 | 300–330 |

**Table Two**

| Species       | Exons  | Penultimate exon          | Final exon  |
|---------------|--------|---------------------------|-------------|
| Human         | 33/36  | GVAARPFGGLSPIFPGACLGKSCKGRKRK | GGA–C-LGKACGRKRK |
| Bovine        | 35/36  | GGAGGLGVGKKPPPFPGAGALGALGFP | GGA–C-LGKACGRKRK |
| Mouse         | 35/36  | GPPKPYGGALGALGYQ          | GG–GC–FGKSCGRKRK |
| Chicken       | 35/36  | GKPPKYGGALGALGFR          | GGVCAGKGCGRKRK |
| Xenopus       | 48/49  | AKQPSKSYR                 | GGGLCGQQKGCGRKRK |
| Zebrafish 1   | 57/58  | SKAAKYALQGGFLAGGGGYR      | GGAGC–QKGCGRKRK |
| Zebrafish 2   | 55/56  | AKAAYKGGgLTGFLGGGYYR      | GGAGC–QKGCGRKRK |

distinct vinculin-containing focal contacts that were observed for cells cultured on serum-coated substrates. A similar morphology was observed for FBC cells spread on surfaces coated with full-length tropoelastin (Fig. 3B), suggesting that the adhesion properties were similar for both substrates.

**Integrin Inhibitors Have No Effect on COOH-terminal-mediated FBC Adhesion and Spreading**—Integrins represent the major family of adhesion receptors for extracellular matrix molecules. The tripeptide RGD inhibits ligand binding to many of the integrin receptors (39). Fig. 4A compares the effect of the fibronectin peptide GRGDSP or a control peptide GRGESP on FBC adhesion to serum or CT-25. GRGDSP fully and specifically inhibited serum-mediated adhesion but had no effect on adhesion mediated by CT-25. Similarly, the inclusion of 10 mM EDTA to inhibit integrin function through the chelation of divalent cations had minimal effects on adhesion to CT-25 but completely blocked serum-mediated adhesion (Fig. 4B). Finally, addition of the antibody LM609, a specific inhibitor of αvβ3 integrin (40), partially inhibited serum-mediated adhesion but had no effect on adhesion mediated by CT-25 (Fig. 4C). There was also no effect of GRGDSP and EDTA on the ability of FBC cells to spread on CT-25 (Fig. 4D). Together, these data strongly suggest that FBC adhesion to CT-25 is not mediated by integrins.

**Elastin Receptor Inhibitors Have No Effect on COOH-terminal-mediated Adhesion and Spreading**—Elastin interacts with the elastin receptor (EBP67) through the sequence VGVAPG. The related sequences PGAIPG and LGTPG have also been shown to be active. Synthetic peptides encoding these sequences had no effect on FBC adhesion to CT-25 at concentrations up to 0.5 mM (Fig. 5A). Similarly, adhesion was not disrupted by the inhibitory galactosugars α or β lactose, D(+)-galactose, or by the control sugar β-D-(+)-glucose (Fig. 5B). There was also no effect of the VGVAPG peptide on cell spreading on CT-25 (data not shown).

**COOH-terminal Tropoelastin Peptides Specifically Bind Heparin**—The clustering of basic residues at the COOH terminus of tropoelastin is characteristic of heparin-binding motifs in peptides. To evaluate whether heparin binding is a property of the COOH-terminal sequence, the fragments and synthetic peptides described above were applied to a HiTrap heparin affinity column and eluted with an increasing salt gradient. TABLE ONE shows the concentration of NaCl required to elute each of the fragments and peptides from the column. Full-length tropoelastin and front half (exons 1–15) constructs bind tightly and require NaCl concentrations of 450 mM NaCl to elute from the heparin column. The central region of the molecule (exons 15–29) is also bound but is eluted at lower NaCl concentrations (250 mM). The inclusion of the COOH-terminal exons (exons 29–36) to the middle portion (producing exons 15–36) restores heparin binding comparable with full-length tropoelastin. Interestingly, the small COOH-terminal fragment (exons 29–36) requires the highest salt concentration for elution (440 mM NaCl) and likely contains the major heparin-binding domain in tropoelastin.

CT-25 requires ~345 mM NaCl to elute from the column, which is comparable with the elution requirements for full-length tropoelastin. The NH2-terminal 8 residues of CT-25 appear to attenuate the affinity...
of the peptide for heparin, as the truncations CT-17 and CT-14 require significantly higher NaCl (≈420 mM) concentrations for elution. As expected, the basic sequence (RKKR) at the COOH terminus plays a critical role in heparin binding as deletion of arginine 744 (ΔR744) lowers the required NaCl concentration for peptide elution by one-third, to 230 mM. The overall charge of the peptide can be altered with the addition of an aspartic acid residue upstream of the RKKR sequence (G734D) with little loss of heparin binding. The binding of CT-25 to heparin is specific, as the scrambled peptide p35–36s and the exon 35 peptide (p35+) failed to bind.

**Cell-surface Proteoglycans Are Required for CHO Adhesion to Tropoelastin and CT-25**—To test whether the binding moiety on cells is a GAG side chain of a proteoglycan, CHO wild-type K1 and two CHO K1-derived cell lines, CHO-745 and CHO-M1, were analyzed for their ability to adhere to CT-25. CHO-745 cells are deficient in xylosyltransferase, the enzyme involved in the first step of GAG biosynthesis. As a result, GAG expression on their cell surface is greatly reduced. CHO-M1 cells are deficient in HS polymerase, i.e. N-acetylglucosaminyl transferase/glucuronyl transferase, and therefore lack all HS GAG chains. Fig. 6 shows that CHO-745 cells were unable to adhere to either full-length tropoelastin (Fig. 6A) or CT-25 (Fig. 6B) in the cell attachment assay, confirming that sulfated GAGs are required for cell attachment to tropoelastin. Positive attachment was observed with the HS-deficient CHO-M1 cells, however, indicating that attachment is not restricted to HS GAGs. As expected, all 3 cell lines attached normally in the presence of serum (Fig. 6C).

To confirm that CS is a potential CT-25 binding partner, cellular GAG side chains were removed by treatment with chondroitinase ABC. Fig. 7 shows that enzyme treatment completely ablated adhesion of CHO-M1 cells to the peptide (Fig. 7A) but had no effect on CHO-K1 cell adherence (Fig. 7B). These results imply that CS GAG is capable of promoting adhesion to CT-25 but that adhesion can be completely supported by a HS GAG.

Soluble glycosaminoglycans can act as inhibitors of adhesion (Fig. 8). FBC cells together with differing concentrations of HS, dermatan sulfate, or CS were added to plates coated with the exon 29–36 fragment at 250 μM. Adhesion was completely inhibited by HS concentrations at ≈1 μg/ml. CS-A and dermatan sulfate, in contrast, required concentrations...
100 times higher to completely inhibit adhesion. Interestingly, CS-C at the concentrations used had minimal effects on adhesion (Fig. 8A). The soluble glycosaminoglycans had no effect on serum-mediated FBC adhesion (Fig. 8B).

**DISCUSSION**

The tropoelastin molecule has a unique domain structure characterized by repeating hydrophobic stretches in tandem with lysine-containing sequences. The lysine residues participate in cross-linking reactions that join tropoelastin monomers into the functional polymer. The hydrophobic domains, in contrast, are thought to contribute to the elastic properties of the protein. The carboxyl-terminal region of the protein encoded by the last exon is different from the rest of the molecule. It contains the only two cysteine residues of the protein as well as a cluster of basic amino acids (KXXXKRKRK). Indirect evidence suggests that this region of the protein is important in elastic fiber assembly, although its exact role is unknown. Tropoelastin molecules that lack this sequence do not properly assemble into fibers and antibodies directed against the sequence encoded by exon 36 can inhibit interactions between tropoelastin and microfibrillar proteins (41, 42). Here, we use cell adhesion assays to show that the tropoelastin COOH-terminal region also contains a cell interaction site.

By screening recombinant fragments of tropoelastin in cell adhesion assays, we identified adhesive activity for FBC and CHO-K1 cells associated with a tropoelastin fragment encoded by exons 29–36. No adhesion of either cell type was observed with the central portion of the molecule (exons 15–29), even though this fragment contains the VGVAPG sequence known to react with the elastin-binding protein. Weak adhesion to the front half of the molecule was observed for both CHO and FBC cells, but at peptide concentrations 20–30 times higher than what was observed for the back half-fragment (data not shown).

Mapping studies using synthetic peptides encoding sequences within the COOH-terminal region localized the cell adhesive activity to the last 17 amino acids of the protein. These peptides also helped identify several structural requirements that define cell adhesion to this domain. The first is a requirement for clustering of the lysine and arginine residues at the COOH terminus of the peptide. Cell adhesive activity was lost when the charged residues were distributed randomly throughout the sequence (p35–36s). A second requirement is a stretch of hydrophobic amino acids before the basic residue cluster, with the last three amino acids of the penultimate exon contributing to the active sequence. In human elastin these amino acids are contributed by exon 33 (exons 34 and 35 are missing from the human gene), indicating that the amino acid sequence of exon 33 behaves similarly to exon 35 in other species (TABLE TWO). This is also shown in Fig. 2B where the peptide Hu-29, encoding human exon 33 in-frame with 36, was fully active in the adhesion assays.

Cell attachment to the COOH-terminal sequence is not through the elastin-binding protein (EBP67) because neither VGVAPG-like sequences nor galactoside sugars altered adhesion to the peptide. Furthermore, we did not observe cell adhesion to fragments of tropoelastin that contain the VGVAPG sequence. Peptide fragments from insoluble elastin that contain VGVAPG are also poorly adhesive but do have potent chemotactic and signaling properties. This implies a functional signaling receptor for these peptides that is distinct from the binding protein that supports adhesion to the tropoelastin COOH terminus.

Our results also show that adhesion to the COOH-terminal peptide is
not promoted by integrins. EDTA, an inhibitor of integrin binding activity, had a minimal effect on FBC adhesion to CT-25. The inclusion of the inhibitory peptide GRGDS also did not change the adhesion profile, although, as expected, both GRGDS and EDTA greatly reduced cell binding to serum. Furthermore, the αvβ3 function-blocking antibody LM609 had no effect on FBC cell adhesion to CT-25 but did characteristically reduce adhesion of these cells to serum (this later result confirms the presence of active αvβ3 on FBC cells). Moreover, whereas FBC cells plated on tropoelastin or on the CT-25 peptide were able to spread on the coated substrate, they did not activate actin filaments and induce concomitant stress fiber formation, as did cells cultured on serum.

Adhesion studies with CHO cells also support a negligible role for integrins in adhesion to the COOH terminus of elastin. CHO cells deficient in GAGs, but expressing β1 and other integrins important for interacting with ECM proteins, did not adhere to tropoelastin, confirming that integrins cannot substitute for GAGs in recognizing the COOH-terminal sequence. Because CHO-K1 cells (and the mutants derived from CHO-K1s) lack αvβ3 integrins, their ability to bind the elastin COOH-terminal sequence when GAGs are present provides further evidence that αvβ3 is not required for binding to this region.

Recently, Rodgers et al. (29) showed that purified αvβ3 integrin binds to the COOH terminus of tropoelastin in a solid-phase assay. This is in contrast to our results suggesting that αvβ3, and integrins in general, do not promote adhesion to the COOH-terminal sequence. One explanation for the discrepancy in the two studies may reflect differences in how the receptor functions when purified compared with the context of live cells. Further studies will be required to evaluate this possibility.

Based on the following observations, the molecular identity of the tropoelastin-binding moiety on the cell surface was characterized as HS, with a lesser contribution by CS and CS able to substitute in the absence of HS. First, the CHO-745 cell mutant that is deficient in GAG production did not attach to either tropoelastin or to CT-25, indicating that GAGs are required for the interaction. Second, HS was 100 times more efficient on a weight basis at inhibiting adhesion to CT-25 than was CS. Adhesion to CT-25 was observed for CHO-M1 cells that produce CS but not HS, confirming that non-heparan-containing GAGs are capable of binding to the CT-25 sequence. However, five times higher peptide concentrations were required to obtain 50% maximal adhesion with the CHO-M1 cells when compared with wild-type CHO-K1 cells, suggesting that the reduction in adhesive properties was because of the missing HS chains. Enzymatic removal of CS with chondroitinase ABC from CHO-M1 cells completely inhibited adhesion, consistent with previous findings that cells lacking GAGs (i.e. CHO-745 cells) do not adhere to the CT-25 peptide. Interestingly, treatment of CHO-K1 cells with chondroitinase ABC did not diminish the ability of these cells to adhere to CT-25, consistent with HS being the preferred GAG when both HS and CS are present.

Our finding that GAGs are potential tropoelastin binding partners is consistent with numerous studies showing in vitro interactions between the molecules (38, 43–45). An important question germane to understanding the biological relevance of the interaction is whether the association is simply a nonspecific ionic interaction between the positively charged tropoelastin (pl > 12) and the negatively charged sulfated GAGs, or if there is specificity to where GAGs bind within the elastin molecule. Our studies indicate that binding to the COOH-terminal domain occurs with sequence requirements that support a high degree of specificity. Binding to heparin was disrupted, for example, when the basic residues normally clustered at the end of CT-25 were randomly distributed in a scrambled peptide. Furthermore, deleting one arginine residue from the KRRK cluster resulted in weaker binding to the heparin affinity column. There also appears to be structural requirements in terms of GAG recognition. Inhibition studies with purified GAGs showed that CS-A and -B (dermatan sulfate) inhibited cell binding to
the COOH-terminal sequence, whereas CS-C, with the same charge density as the other chondroitin sulfates, had little effect.

Whereas all of the recombinant fragments and synthetic peptides used in this study bound heparin (except for p35 and the scrambled peptide p35–36), heparin binding by itself is not sufficient to promote adhesion. CT-14, for example, bound tightly to the heparin column yet was inactive in promoting adhesion of FBC cells. In another peptide, the substitution of a negatively charged aspartic acid for a glycine near the amino terminus of CT-17 (G734D) had a minimal effect on binding to heparin, but significantly inhibited cell adhesion. Interestingly, this amino acid change is a recognized polymorphism in human elastin (G773D using the human transcript numbering system) that has recently been shown to have functional consequences relevant to the pathogenesis of chronic obstructive pulmonary disease (46). Another example is the front half of tropoelastin (exon 1–15), which bound to heparin with the same affinity as the full-length and back-half-portions but was not adhesive for FBC cells except at very high coating concentrations.

The nature of the CS- or HS-containing PG on the cell surface that mediates tropoelastin binding is not yet known, although syndecans and/or glypicans are probable candidates. Syndecans are hybrid PGs that carry both HS and CS chains (47, 48), whereas glypicans are thought to carry only HS GAGs. It is interesting to note that the morphology of cells that attach to tropoelastin or to CT-25 show a predominance of filopodial extensions, resembling the effects of syndecan-2 or syndecan-3 overexpression (49, 50). Similar effects are not observed with overexpression of glypicans, a glycosphingadinositol-anchored protein. Syndecans have recently been shown to modulate multiple cell-cell and cell-matrix signaling pathways and to provide direct signals through their cytoplasmic domain (51). Whether binding to cell-surface HS moieties defines a new signaling pathway for tropoelastin and can account for some of the previous signaling studies is not yet known.

It is unlikely that cell attachment is the only biological role for the GAG-tropoelastin interaction. Tropoelastin is rapidly modified upon secretion to form the cross-linked polymer, which is a poorly adhesive form of the protein. There is, however, accumulating evidence for a role of cell-surface GAGs in elastic fiber assembly. For example, inhibiting sulfation by adding chlorate to cultured cells will prevent elastin fiber formation (52, 53), implicating a sulfated proteoglycan in the cytoplasmic domain (51). Similar effects are not observed with overexpression of glypicans, a glycosphingadinositol-anchored protein. Syndecans have recently been shown to modulate multiple cell-cell and cell-matrix signaling pathways and to provide direct signals through their cytoplasmic domain (51). Whether binding to cell-surface HS moieties defines a new signaling pathway for tropoelastin and can account for some of the previous signaling studies is not yet known.

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