Bone morphogenetic protein-1 (BMP-1) plays key roles in regulating the deposition of vertebrate extracellular matrix; it is the procollagen C-proteinase that processes the major fibrillar collagen types I–III, and it may process prolylsyl oxidase to the mature enzyme necessary to the formation of covalent cross-links in collagen and elastic fibers. Type V collagen is a fibrillar collagen of low abundance that is incorporated into and helps regulate the shape and diameter of type I collagen fibrils. Here we show that, in contrast to its action on procollagens I–III, BMP-1 does not cleave the C-propeptide of pro-α1(V) homotrimers. Instead, the single BMP-1-specific cleavage site within pro-α1(V) chains, lies within the large globular N-propeptide. This cleavage site is immediately upstream of a glutamine, thus redefining the specificity of cleavage for BMP-1-like enzymes. It also produces an NH₂-terminus that corresponds to an equivalent NH₂-terminus on the processed matrix form of the similar α1(XI) chain, thus suggesting physiological significance. Cleavage of the C-propeptide occurs efficiently in recombinant pro-α1(V) homotrimers produced in 293-EBNA human embryonic kidney cells, and this cleavage is shown to occur immediately downstream of the sequence RTRR. This is similar to sites cleaved by subtilisin-like proprotein/prohormone convertases and is shown to be specifically cleaved by the recombinant subtilisin-like proprotein/prohormone convertase furin.

Collagen types I–III, the major fibrous components of vertebrate matrix, are synthesized as procollagens, precursors with N- and C-propeptides¹ that are cleaved to yield mature monomers capable of forming fibrils (1–3). In particular, failure to remove the C-propeptide seems incompatible with fibrillogenesis (4). The C-propeptides of procollagens I–III are cleaved by procollagen C-proteinase (5–7), an activity of bone morphogenetic protein-1 (BMP-1) and mammalian tolloid (mTld), two proteins encoded by alternatively spliced mRNAs of the BMP1 gene (8–10). BMP-1 is the prototype of a subfamily of astacin-like proteases involved in embryogenetic patterning in diverse organisms (11), in some cases by liberating transforming growth-factor-like morphogens from latent complexes (12–14). Thus, identification of BMP-1 as procollagen C-proteinase provided a link between enzymes involved in matrix deposition and genes involved in pattern formation and suggested that such enzymes may be involved in coordinating various molecular events underlying morphogenesis.

Monomers of the low abundance or minor fibrillar collagen types V and XI are incorporated into the fibrils of the much more abundant collagen types I and II, respectively, and act as regulators of the sizes and shapes of the resultant heterotypic fibrils (15–21). Type V collagen is most widely distributed in tissues as a heterotrimer of the chain composition α1(V)α2(V) (22) but is also found, almost exclusively in placenta, as the heterotrimer α1(V)α2(V)α3(V) (23), and in certain cell types and tissues as an α1(V)₃ homotrimer (22, 24–26). Type XI collagen, in the form of an α1(XI)α2(XI)α3(XI) heterotrimer (27), was first characterized as a minor collagen of cartilage. However, findings of type XI chains in noncartilaginous tissues (28), of type V chains in cartilage (29), and of cross-type heterotrimers composed of both type V and XI chains (30, 31) now suggest that type V and XI chains constitute a single collagen type in which different combinations of chains associate in a tissue-specific manner. Unlike the major fibrillar collagens I–III, collagens V and XI retain N-propeptide sequences (22, 24, 26, 32–36). These appear to be of functional importance, since, as shown for type V collagen, they protrude beyond the surface of heterotypic fibrils and may directly control fibrillogenesis by sterically hindering the further addition of collagen monomers to the fibril surface (34).

In the present study, we have produced recombinant pro-α1(V) homotrimers and subjected them to cleavage with BMP-1. Surprisingly, BMP-1 did not cleave the pro-α1(V) C-propeptide but, instead, cleaved at a single specific site within the pro-α1(V) N-propeptide. Cleavage at this site produces an NH₂-terminus that corresponds to the NH₂-terminus of the processed matrix form of the similar α1(XI) chain deposited by chick chondrocytes (35), thus suggesting physiological significances by BMP-1; α1(V) processed pro-α1(V) chain in which the C-propeptide has been removed and part of the N-propeptide has been removed by BMP-1; P1, P2, P1', P2', etc., cleavage site residues amino-terminal to (nonprimed) and carboxyl-terminal to (primed) the cleaved bond.
cance. NH₂-terminal sequencing of the pro-α(I) C-propeptide showed it to have been cleaved, in 293-EBNA embryonic kidney cell cultures, by a subtilisin-like proprotein convertase (SPC) (37, 38). Implications of the data for collagen deposition and morphogenesis are discussed.

**EXPERIMENTAL PROCEDURES**

**pro-α(I) Expression Construct—**A 3310-base pair Ncol–EcoRI fragment from cDNA clone CW334 (39), corresponding to the 5’ portion of human pro-α(I) sequences, was subcloned between the EcoRV and EcoRI sites of pBluescript II KS+ (Stratagene) and re-excised with the AgeI restriction enzyme to create a 10-bp palindrome of the poly(A) addition site. The samples had EcoRI–AgeI sites added to the 5’ of the pro-α(I) fragment. A 926-base pair EcoRI–PstI fragment of cDNA CW197 (39), corresponding to the 3’ portion of pro-α(I) sequences, was separately subcloned between the EcoRI and PstI sites of pBluescript II KS+, and the EcoRI–SoI fragment derived from CW334 was added to this construct between the EcoRI site and the SalI site of the pBluescript polylinker. A 1811-base pair EcoRI fragment of CDNA CW32 (39), corresponding to the middle portion of pro-α(I), was inserted into the EcoRI site of the preceding construct to reconstitute a full-length pro-α(I) coding sequence. The full-length pro-α(I) cDNA, extending from nucleotide 224 to nucleotide 6276 of the original pro-α(I) sequence (39), was then excised with HindIII and NotI, which cut within the pBluescript polylinker, and inserted between the HindIII and NotI sites of expression vector pCEP-Pu (40).

**Production of pro-α(I) Procollagen—**293-EBNA human embryonic kidney cells (Invitrogen) were maintained in growth medium consisting of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (HyClone). Cells of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (HyClone). Cells were switched to fresh serum-free DMEM containing 40 g/ml puromycin (Sigma), and surviving cells were applied to a 1.5 ml DEAE-cellulose column (DE-52 Whatman) and made 15% in glycerol for storage at −70 °C.

**Preparation of Type I Procollagen—**Confluent human neonatal foreskin fibroblasts, grown to confluence in DMEM containing 10% fetal bovine serum and 1% l-glutamine, were washed three times with phosphate-buffered saline and then incubated for 24 h in serum-free DMEM containing 50 μg/ml ascorbic acid. Conditioned medium was made 1% in phenylmethylsulfonyl fluoride, p-aminobenzoic acid, and 10 mM EDTA. Samples were centrifuged to remove cell debris, and the supernatants were stored at −70 °C.

**Enzyme Assays—**Recombinant BMP-1 produced in a baculovirus system and purified chromatographically, as described (8), was kindly provided by Dr. David Solow-Cordero (FibroGen Inc.). BMP-1 (30 ng) was incubated with 2 μg of type I procollagen or −1.5 μg of pro-α(I) + pNa1(V) collagen in a total reaction volume of 40 μl of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM CaCl₂ at 37 °C for 17 h. A recombinant truncated/secreted form of furin produced in a baculovirus system and a wild type baculovirus control were the kind gifts of Dr. Claire M. Dubois (Université de Sherbrooke, Sherbrooke, Quebec, Canada). High levels of furin activity, with 1 unit defined as the amount of enzyme that digests 1 pmol of the bovine RVRR-aminomethylcoumarin substrate (42, 43). Incubations were performed with 3 μl of furin (−1 unit) or control preparation and either −2 μg of type I procollagen or −1.5 μg of pro-α(I) collagen in a total reaction volume of 40 μl of 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM CaCl₂, 1 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin at 30 °C for 5 h. For collagenase treatment, −2 μg of recombinant pro-α(I) + pNa1(V) collagen in buffer A (see above) was made 2 mM in CaCl₂, 1 mM N-ethylmaleimide and incubated for 3 h at 37 °C in a total reaction volume of 50 μl with 1 unit of bacterial collagenase (Advanced Biofactures).

Peptidase type V collagen (used as a size marker in Fig. 1B) was prepared from human placenta as described by Miller and Rhodes (44). Samples were centrifuged to remove cell debris, and the supernatants were stored at −70 °C.

**RESULTS**

To examine the ability of BMP-1 to process pro-α(I) homotrimers, a full-length pro-α(I) cDNA was inserted into the episomal expression vector pCEP-Pu (40) for production of recombinant pro-α(I) in 293-EBNA human embryonic kidney cells. Mass cultures of 293-EBNA cells, transfected with the pro-α(I)/pCEP-Pu construct produced −1 μg/ml of a ~230-kDa recombinant protein and lesser amounts of a ~40-kDa recombinant protein, not produced by 293-EBNA cells transfected with an empty pCEP-Pu vector (Fig. 1A, lanes 2 and 3) or by parental 293-EBNA cells (not shown). The appearance of a single high molecular weight recombinant protein band and a second ~40-kDa band, similar to the expected size of cleaved pro-α(I) C-propeptide (22), suggested that the large band represented a pN form, lacking the C-propeptide but retaining the N-propeptide. Recently, others using a system similar to that described here, also found that the C-propeptide of recombinant pro-α(I) is rapidly cleaved in 293-EBNA cultures (41). The previous report (41) also described rapid processing of the pN form to a form similar in size to pepsin-treated α(I) chains, although such a form was not apparent as a significant band in our system. Pepsin treatment of the recombinant material from our system produced a pepsin-resistant band identical in size to the α(I) C-propeptide of type V collagen from human placenta (Fig. 1B), showing the presumptive pNa1(V) form to have a native triple-helical configuration.

Arginine has previously been found to inhibit the normal proteolytic removal of the C-propeptides of procollagens I, II, and III in cell-free systems and in tissues (26). We therefore cultured transfected 293-EBNA cells in the presence of 100 μM arginine, resulting in the appearance in the collagen cultures of a ~260-kDa form with a concomitant reduction in amounts of 230- and 40-kDa forms detected (Fig. 1A, lane 4). The appear-
The 260-kDa form also disappeared under nonreducing conditions, consistent with identity of the 40-kDa band as the C-propeptide. The various electrophoretic results described above suggested that BMP-1 cleaved at a single specific site within the pro-α1(V) N-propeptide. Moreover, since the 40-kDa band did not increase in intensity upon cleavage of substrate with BMP-1 (Fig. 2A, lanes 1 and 2), these results also suggested that BMP-1 does not cleave pro-α1(V) C-propeptides. Samples previously run on 4–15% acrylamide gradient gels (Fig. 2A) were electrophoresed on 5% acrylamide gels (Fig. 2B) for better resolution of high molecular weight forms, and the predicted identities of the various forms, based on the electrophoretic data described above, are shown in Fig. 2C.

To ascertain whether the identities of the various SDS-PAGE bands were as inferred above, 260-, 230-, and 40-kDa bands derived from substrate that had not been incubated with BMP-1 were isolated from a reducing gel; 40- and 35-kDa conditions, consistent with its identity as full-length, C-propeptide-containing, pro-α1(V) chains. Interestingly, the ~200- and ~35-kDa bands did not disappear under nonreducing conditions, but the ~35-kDa form did show an increase in mobility to ~25 kDa. The increase in mobility of the 35/25-kDa form was consistent with its identity as a proteolytic cleavage product of the N-propeptide, containing cysteine residues capable of affecting electrophoretic mobility through formation of intramolecular disulfide bonds. The fact that the 200-kDa form was smaller than the presumptive pNa1(V) band and the fact that it did not disappear under nonreducing conditions suggested that this form lacked both a C-propeptide and some portion of the N-propeptide, presumably that portion corresponding to the 35/25-kDa fragment. The 230-kDa band, when derived from substrate not incubated with BMP-1, was not diminished in intensity on gels under nonreducing conditions. In contrast, when derived from substrate incubated with BMP-1, the 230-kDa band was reduced in intensity by about one-half under nonreducing conditions (Fig. 2, A and B). This was interpreted to mean that the 230-kDa band from BMP-1-digested material was a doublet containing pNa1(V) forms, incapable of forming intermolecular disulfide bonds, and a pCa1(V) form of similar size that retained the C-propeptide but from which a portion of the N-propeptide had been removed by BMP-1.
bands derived from substrate that had been incubated with BMP-1 were isolated from a reducing gel; and 230- and 200-kDa bands derived from substrate that had been incubated with BMP-1 were isolated from a nonreducing gel (see Fig. 3) and subjected to automated Edman degradation for determination of NH₂-terminal amino acid sequences. As expected, NH₂-terminal sequences of both the 260-kDa form and the 230-kDa form (from either reducing or nonreducing gels) corresponded to the NH₂ terminus of the pro-α1(V) N-propeptide (Fig. 3), beginning with the first amino acid after the predicted cleavage site (39) of the prepro-α1(V) signal peptide. This confirmed the identities of the 260- and 230-kDa forms as pro-α1(V) and pNα1(V) chains, respectively. In addition, the 35-kDa product resulting from cleavage with BMP-1, was found to have the same NH₂-terminal sequences as pro-α1(V) and pNα1(V) chains, thus confirming it as a cleavage product of the pro-α1(V) N-propeptide.

The NH₂-terminal sequence of the 200-kDa form revealed

FIG. 2. BMP-1 cleavage and predicted identities of recombinant pro-α1(V)- and pro-α1(V)-derived forms produced by transfected 293-EBNA cultures. A and B, electrophoretic patterns are compared for proteins derived from 293-EBNA cultures transfected with a pro-α1(V) expression vector and treated with 100 mM arginine (lanes 1 and 3) and the same samples after incubation with BMP-1 (lanes 2 and 4). Samples were run either under reducing (lanes 1 and 2) or nonreducing (lanes 3 and 4) conditions, on 4–15% acrylamide (A) or 5% acrylamide (B) SDS-PAGE gels, and stained with Coomassie Blue. Positions and molecular masses (in kDa) are indicated for protein standards (A). Predicted identities of the various bands are indicated (A and B) and are represented diagrammatically, with molecular masses in kDa (C). C, C-propeptide; N, N-propeptide. Vertical and diagonal lines between cleaved propeptides and other forms represent disulfide bonds. The arrowheads (B) represent possible pro-α1(V) trimers.
that BMP-1 had cleaved the pro-α1(V) N-propeptide between residues 254 and 255 of the published pro-prepro-α1(V) amino acid sequence (39, 47) (Fig. 3). This site occurs immediately downstream of a pair of cysteines that divide the pro-α1(V) N-propeptide into a somewhat basic upstream domain and a downstream domain rich in acidic residues and tyrosines (39). The upstream domain is fairly conserved between pro-α1(V), pro-α1(XI), and pro-α2(XI) chains and has been designated the PARP domain, whereas the downstream domain, which is not conserved among the same three chains, has been designated the variable region (48) (see Fig. 3). The NH2 terminus remaining on the 200-kDa form corresponds to an equivalent NH2-terminus of the processed matrix form of the very similar α1(XI) collagen chain (35). Pro(1) V, pro-α1(XI), and pro-α2(XI) chains form a subgroup among fibrillar procollagen chains on the basis of sequence similarities, structures of cognate genes, and size and configuration of N-propeptides (39, 47–51). Thus, an alignment of pro-α1(V), pro-α1(XI), and pro-α2(XI) sequences, from various species, is shown for the region in which BMP-1 cleaves human pro-α1(V) and in which chicken pro-α1(XI) is cleaved to produce the α1(XI) chain of chondrocyte matrix (Fig. 4). Glutamines at the P1 and P2 positions, and a proline at the P3’ position of the pro-α1(V) cleavage site are conserved in the pro-α1(XI) and pro-α2(XI) chains. Comparison of these residues with residues flanking previously described BMP-1/mTld cleavage sites (8, 52, 53) (data not shown) found prolines also located at the P3’ position of the pro-α1(III), pro-α2(I), and prolyl oxidase sites. Prolines two or three amino acids from cleavage sites can strongly affect the activity of astacin-like proteases (11). However, prolines are not found at similar positions at other previously identified BMP-1/mTld cleavage sites. Other features common to the pro-α1(V) BMP-1 cleavage site and previously described sites were not immediately apparent.

The NH2-terminal amino acid sequence of the 40-kDa form shows it to be cleaved C-propeptide (Fig. 3). However, the

![Diagram](image)

**Fig. 3. Comparison of peptide sequences of recombinant pro-α1(V)- and pro-α1(V)-derived forms with the human pro-α1(V) sequence deduced from cDNA.** To determine NH2-terminal amino acid sequences, 260- (V7), 230- (V5 and V6), and 200-kDa (V4) bands were isolated from a 5% acrylamide SDS-PAGE gel, and 40- (V2 and V3) and 35-kDa (V1) bands were isolated from a 12% acrylamide SDS-PAGE gel for automated Edman degradation. Bands were derived from substrate either incubated or not incubated with BMP-1 and were from samples either reduced or not reduced with β-mercaptoethanol (β-ME), as indicated. Residues derived from NH2-terminal sequencing are aligned with the published human pro-α1(V) sequence deduced from cDNA (39, 47), and positions of residues in the published sequence (39, 47) corresponding to the first residues of the various peptides are given. Residues corresponding to a consensus sequence for cleavage by furin-like SPases in are in **boldface type**. Positions of BMP-1 and furin cleavage sites are shown in relation to a schematic of the pro-α1(V) chain. C-pro, C-propeptide; proline/arginine-rich protein (PARP) and Var, PARP and variable subdomains of the N-propeptide, respectively.

![Diagram](image)

**Fig. 4. Alignment of pro-α1(V), pro-α1(XI), and pro-α2(XI) sequences in regions corresponding to pro-α1(V) N- and C-propeptide cleavage sites.** Available sequences from pro-α1(V) chains of humans (39, 47), hamsters (39), and chickens (34); from pro-α1(XI) chains of humans (49, 57), mice (58), rats (57, 59), and chickens (60); or from pro-α2(XI) chain of humans (48, 61) and mice (62), were manually aligned in the two regions of interest. The arrows denote sites of pro-α1(V) cleavage by BMP-1 and by a furin-like SPC. Residues found in peptide sequences from the NH2 termini of BMP-1-cleaved human pro-α1(V) and the matrix form of chicken α1(XI) (35) are _underlined_. Residues conserved in all three chains for all species in the area of BMP-1 cleavage of pro-α1(V) are in _boldface type_.
Furin cleaves pro-α1(V) but not type I procollagen. Proteins derived from 293-EBNA cultures transfected with a pro-α1(V) expression vector and then treated with 100 mM arginine were incubated with a control preparation from medium of insect cells infected with wild type baculovirus (lane 1) or with recombinant furin (lane 2). Human type I procollagen is shown before (lane 3) and after incubation with BMP-1 (lane 4) or furin (lane 5). Samples were analyzed by SDS-PAGE on 4–15% acrylamide gels stained with Coomassie Blue. C, pro-α1(V) C-propeptide; C1 and C2, C-propeptide subunits of the pro-α1 and pro-α2 chains, respectively, of type I procollagen. SBTI, soybean trypsin inhibitor.

stream of the sequence RTRR, thus predicting that the pro-α1(V) C-propeptide is cleaved in 293-EBNA cultures by a mammalian SPC of which furin is the prototype (37, 38). Interestingly, alignment shows similar (R/K)X(X/R) sequences, suitable for cleavage by furin-like SPCs, in the C-telopeptide regions of the pro-α1(V), pro-α1(XI), and pro-α2(XI) chains of various species (Fig. 4). As noted above, intensity of the 40-kDa band did not increase upon incubation of substrate with BMP-1, thus suggesting that BMP-1 cleavage of the pro-α1(V) C-propeptide did not occur. Similarly, NH2-terminal sequences were the same whether the 40-kDa band had been derived from substrate incubated, or not incubated, with BMP-1, (Fig. 3, bands V2 and V3, respectively), with no evidence of any additional NH2-sequences in the 40-kDa form from sample incubated with BMP-1. Thus, cleavages by BMP-1 did not occur at additional sites within the C-telopeptide region.

To further explore the apparent cleavage of the pro-α1(V) C-propeptide by furin-like SPCs, substrate containing pro-α1(V) and pNα1(V) chains was incubated with a recombinant truncated/secerted form of furin (42) produced in a baculovirus system. As can be seen (Fig. 5, lane 2), furin cleaves pro-α1(V) chains to produce additional pNα1(V) chains and C-propeptides, with the absence of any nonspecific cleavages elsewhere in the pro-α1(V) molecule. Interestingly, just as 100 mM arginine inhibited cleavage of about 50% of pro-α1(V) C-propeptides by endogenous SPC in our culture system, 100 mM arginine also inhibited cleavage of about 50% of pro-α1(V) C-propeptides by furin in this in vitro assay (data not shown).

As a control for the specificity of the furin and BMP-1 preparations used in this study, both preparations were incubated with type I procollagen substrate. As expected, BMP-1 cleaved type I procollagen only at C-propeptide cleavage sites, producing the C1(1) and C2(1) C-propeptide subunits of the pro-α1(I) and pro-α2(1) chains, respectively (Fig. 5, lane 4). In contrast, there was an absence of observable cleavages of type I procollagen upon incubation with furin (Fig. 5, lane 5). In addition, we have found an absence of cleavage of type II procollagen by furin.2 These results strengthen the conclusion that furin and BMP-1 cleavages at particular sites within the pro-α1(V) C- and N-propeptides, respectively, are highly specific, similar to the degree of specificity with which BMP-1 cleaves type I procollagen C-propeptides.

**DISCUSSION**

BMP-1 and mTld play multiple roles in regulating matrix deposition including provision of procollagen C-proteinase activity for procollagens I-III (8, 9), activation of lysyl oxidase (52), and biosynthetic processing of laminin 5 (53), a component of skin basement membranes. Here, we describe a new BMP-1 activity, processing of the pro-α1(V) N-propeptide, suggesting an additional role in matrix deposition. Biological significance for this cleavage is suggested, since it creates an NH2 terminus corresponding to the NH2 terminus of the chondrocyte matrix form of the highly similar α1(XI) collagen chain (35). In fact, previous rotary shadowing analysis of α1(V) chains in chick cornea (34) estimated an NH2 terminus only 2 amino acids upstream of the cleavage site demonstrated here, while a number of other studies (22) show retention of α1(V) N-propeptide sequences consistent in size with cleavage at the BMP-1-cleaved site. Some of the latter studies (22) show retained sequences to contain most or all sulfated tyrosines found in full-length pro-α1(V) N-propeptides, also consistent with cleavage at the site described here. In contrast, some studies (24, 32, 36) show α1(V) chains extracted from tissues to contain N-propeptide sequences shorter than would be produced by cleavage at the BMP-1 site. These latter findings may suggest either additional processing of the α1(V) N-propeptide in some tissues or artifactual proteolysis of α1(V) chains during tissue extraction.

The pro-α1(V) BMP-1 cleavage site identified here differs from previously described sites (8, 52, 53),2 most notably in lacking an otherwise invariant aspartate at the P1' position. It should be stressed, however, that even the limited conservation found in residues flanking previously described BMP-1/mTld cleavage sites is somewhat misleading, since (i) conserved residues flanking procollagen I-III chain sites may reflect the similar evolutionary origin of these chains rather than an absolute functional requirement for such residues and (ii) prolyl oxidase and laminin 5 were first identified as possible BMP-1/mTld substrates based on similarities of in vivo cleavage sites, to those of procollagens I-III (52, 53). The relative lack of similarity between residues immediately flanking the pro-α1(V) site and previous BMP-1/mTld cleavage sites indicates that BMP-1 and mTld, like other astacin-like proteases (11), are not highly specific for such residues and suggests a reappraisal of features that influence cleavage of substrates by BMP-1 and mTld. Certainly, future candidates for BMP-1/mTld substrates should not be limited to proteins with cleavage sites resembling those of procollagen I-III C-propeptides.

Original predictions of potential pro-α1(V) C-propeptide cleavage sites (39, 47) assumed cleavage by an activity similar to that which cleaves procollagens I–III and were based on the positions of aspartates in the C-telopeptide region. Nevertheless, sequence differences in this region between pro-α1(V) and procollagen I-III chains and differential processing of pro-α1(V) and pro-α1(D) C-propeptides in a given cell type had suggested that different enzymes might cleave the C-propeptides of these different procollagen chain types (39). Data presented here indicate that the pro-α1(V) C-propeptide is not cleaved by BMP-1 but that it is cleaved by cells at a site consistent with cleavage by a furin-like SPC and by furin itself. Moreover, alignment shows conservation of the sequence (R/K)X(X/R), suitable for cleavage by furin-like SPCs (37, 38), in the C-telopep-

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2 Y. Inamura, B. M. Steiglitz, and D. S. Greenspan, unpublished observations.
tide regions of pro-α1(V), pro-α1(XI), and pro-α2(XI) chains (Fig. 4), suggesting a common mechanism of cleavage. In support of this possibility, preliminary data indicate that endogeo-

pro-α(XI) C-propeptide is cleaved at the predicted (R/K)XRR site in the A204 rhadomyosarcoma cell line.1,2 SPC processing of some procollagen V/XI C-propeptides adds another level of regulation to the fibrillogenesis of type IV and type II/XI heterotypic fibrils and may link fibrillogenesis with other processes, since the same types of furin-like SPCs process growth factors, receptors, matrix metalloproteinases (37, 38), and probably BMP-1 and Tld-like proteases (11).

Of the known mammalian SPCs, only furin (SPC1), PACE 4 (SPC4), PC5/PC6 (SPC6), and PC7/PC8 (SPC7) have the broad growth factors, receptors, matrix metalloproteinases (37, 38), and probably BMP-1/mtid-like proteases (11).

By some criteria pro-α2(V) is more similar to procollagen I-III chains than to pro-α1(V), pro-α1(XI), and pro-α2(XI) chains (50, 55–57), whereas the pro-α2(XI) chain is a modified product of the type II collagen pro-α(1/II) gene (29). Interest-

pro-α(C) C-propeptide lacks an (R/K)XRR or similar sequence, and the pro-α3(XI) C-propeptide presumably resembles that of the pro-αII chain in lacking such a site. We have found that furin does not cleave type I procollagen (or type II collagen) at the predicted cleavage site (29). Interest-

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