Aberrant Signal Peptide Cleavage of Collagen X in Schmid Metaphyseal Chondrodysplasia

IMPLICATIONS FOR THE MOLECULAR BASIS OF THE DISEASE*

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Schmid metaphyseal chondrodysplasia results from mutations in the collagen X (COL10A1) gene. With the exception of two cases, the known mutations are clustered in the C-terminal nonhelical (NC1) domain of the collagen X. In vitro and cell culture studies have shown that the NC1 mutations result in impaired collagen X trimer assembly and secretion. In the two other cases, missense mutations that alter Gly18 at the −1 position of the putative signal peptide cleavage site were identified (Ikegawa, S., Nakamura, K., Nagano, A., Haga, N., and Nakamura, Y. (1997) Hum. Mutat. 9, 131–135). To study their impact on collagen X biosynthesis using in vitro cell-free translation in the presence of microsomes, and cell transfection assays, these two mutations were created in COL10A1 by site-directed mutagenesis. The data suggest that translocation of the mutant pre-α1(X) chains into the microsomes is not affected, but cleavage of the signal peptide is inhibited, and the mutant chains remain anchored to the membrane of microsomes. Cell-free translation and transfection studies in cells showed that the mutant chains associate into trimers but cannot form a triple helix. The combined effect of both the lack of signal peptide cleavage and helical configuration is impaired secretion. Thus, despite the different nature of the NC1 and signal peptide mutations in collagen X, both result in impaired collagen X secretion, probably followed by intracellular retention and degradation of mutant chains, and causing the Schmid metaphyseal chondrodysplasia phenotype.

Collagen X is the most abundant extracellular matrix component synthesized by hypertrophic chondrocytes during the transition from cartilage to bone in endochondral ossification. It is classified as a short-chain nonfibrillar collagen and consists of three distinct protein domains; a central, short triple helical COL1 domain (463 amino acids) flanked by a small N-terminal nonhelical NC2 domain (38 amino acids) and a larger, more conserved, nonhelical C-terminal NC1 domain (161 amino acids). The chains are synthesized with an N-terminal signal peptide, which is proteolytically removed from the pre-α1(X) chains during biosynthesis (1, 2). In collagen biosynthesis, the polypeptide chains are translocated into the lumen of the ER, the chains of the trimer associate via their C-terminal globular domains. This allows nucleation and folding of the triple helix to occur sequentially from this end of the molecule.

Mutations in the collagen X gene (COL10A1) result in Schmid metaphyseal chondrodysplasia (SMCD), an autosomal dominant skeletal disorder characterized by short to normal stature, bowed legs, coxa vara, and flaring of the metaphyses of long bones. To date, all reported SMCD mutations in COL10A1, except two, are localized to the C-terminal NC1 domain of the protein (see review by Chan and Jacenko, Ref. 3). These NC1 domain mutations (COL10-NC1m) were proposed to affect the initial stages of the folding and chain assembly of collagen X (4, 5), hindering nucleation of the triple helix and, therefore, impairing secretion of collagen X trimers. The disruption of collagen X assembly as a mechanism underlying SMCD is supported by studies using in vitro transcription and translation of mutant and normal COL10A1 cDNAs in a cell-free system (1). In this system, the inability of COL10-NC1m chains to trimerize was clearly demonstrated. Expression of COL10-NC1m cDNAs in cells resulted in poor expression levels, with little or no secretion of the mutant chains (6). Together, these results indicate that the inability of mutant collagen α1(X) chains to assemble can lead to their intracellular retention and subsequent degradation. As a consequence, in SMCD, collagen X in the matrix could be reduced, potentially to 50% of normal levels, because of haploinsufficiency. The absence of detectable mutant COL10A1 mRNA in extracts of growth plate cartilage from a SMCD patient with a nonsense mutation in the NC1 domain, probably caused by nonsense-mediated mRNA degradation, is consistent with haploinsufficiency as a mechanism in SMCD (7).

However, there is increasing evidence suggesting that other mechanisms could also underlie SMCD. For example, trace amounts of heterotrimers can be detected during cell-free coexpression of normal and mutant chains with amino acid substitutions in the NC1 domain (6). In addition, the clustering of COL10A1 mutations in the NC1 domain, the absence of null mutations and the autosomal dominant inheritance of SMCD in patients are more consistent with a dominant-negative mechanism. That is, the expression of mutant collagen X chains could impact on the assembly and secretion of normal chains as well. We (8) and others (9, 10) have recently demonstrated through in vitro approaches that a dominant-negative mechanism could also underlie some SMCD mutations. Molecular modeling of the NC1 domain based on the crystal structure

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‡ The abbreviations used are: SMCD, Schmid metaphyseal chondrodysplasia; COL10-NC1m, a collective term for mutations within the collagen X NC1 domain; PAGE, polyacrylamide gel electrophoresis; SPm, signal peptide mutant; wt, wild type; PCR, polymerase chain reaction; ER, endoplasmic reticulum.
of ACRP30 (9) showed that NC1 amino acid substitutions in SMCD are localized to two regions of the folded domain and that these mutations may not totally abolish the ability of the mutant chains to form trimers.

Recently, two missense mutations in SMCD patients were identified in the putative signal peptide of the molecule at nucleotide positions 148 and 149 (G148A and G149A) (11), altering Gly18 at the position of the signal peptide cleavage site (2). The molecular consequences of these mutations on collagen X biosynthesis are unknown but the predicted consequence is that secretion of the molecules will be impaired as the signal sequence plays a key role in this process (12). We have obtained a better understanding of the molecular mechanisms underlying SMCD in these patients by studying the impact of these two signal peptide mutations (SPm) on collagen X biosynthesis and assembly. Our data show that the G148A and G149A mutations do result in impaired secretion of collagen X.

EXPERIMENTAL PROCEDURES

Construction of a 1(X) Signal Peptide Mutations—Overlap extension PCR (13) was used to reproduce the two human missense mutations in collagen X identified by Ikegawa et al. (11). Both mutations altered Gly18 at the position of the putative signal peptide cleavage site. These were the G148A and G149A nucleotide substitutions which change the codon for Gly (Gly18) to codons for Arg (G18R) and Asp (G18D), respectively. To create these changes, 5 ng of a plasmid pTM1−h10wt (6) containing a full-length human collagen X cDNA was used as a template for the primary rounds of PCR with primer pairs: sense, pTM1−1; antisense, G18R−2 or G18D−2; and sense, G18R−1 or G18D−1; antisense, 1(X) reporter construct, helix a, lanes 1–3, representing transla-

| Primers | Primer sequence (5′ to 3′) | Primer location |
|---------|-----------------------------|------------------|
| G18R−1  | TGAAGCTTGGTCATAGAGTGTTCACCTGCT | 134−162          |
| G18R−2  | AGCTTACAAACTCTACTAGACCAAAGTCTCA | 162−194          |
| G18D−1  | GAAGCTTGGTCATAGAGTGTTCACCTGCT | 134−162          |
| G18D−2  | CAGCTTACAAACTCTACTAGACCAAAGTCTCA | 162−194          |
| HX10    | TTATGCTTCACTCATCACGACCAACGACCC | 660−691          |
| pTM1−1  | TATAAGATACACCTGCAAAG | 1171−1190          |

Primers for site-directed mutagenesis using strand overlap extension PCR

The introduced nucleotide substitution in each of the primers for overlapping PCR is highlighted in bold and underlined. Sequences and numbers for collagen X primers are taken from Reichenberger et al. (24) and for pTM1 from Fuerst et al. (25).

### Table I

| Primers | Primer location |
|---------|------------------|
| G18R−1  | 134−162          |
| G18R−2  | 162−194          |
| G18D−1  | 134−162          |
| G18D−2  | 162−194          |
| HX10    | 660−691          |
| pTM1−1  | 1171−1190          |

In Vitro Cell-free Transcription and Translation—Cell-free transcription/translation was performed as described previously (1, 6), in a coupled transcription and translation system (Promega). The reactions were carried out in a total volume of 12.5 μl and labeled with 10 μCi of translation grade l-[35S]methionine (1000 Ci/mmol, PerkinElmer Life Sciences). To determine whether the mutant chains can form heterotrimers with normal chains, cotranslation experiments using an assembly-competent helix deletion a1(X) reporter construct, helix a (6), were performed with either the G18R or G18D mutant constructs.

Coupled transcription and translation experiments were carried out at 30 °C for 90 min using a total of 100 ng of purified plasmids. When required, the microsomes were separated from the reticulocyte lysate by centrifugation and analyzed separately. To assay for the location of the translation products, proteins external to the isolated microsome vesicles were digested with trypsin and chromatography on a 15% SDS-polyacrylamide gel. Electrophoretic conditions and fluorography of radioactive gels have been described previously (16). Radioactive bands were imaged and quantified using a Phosphorimage (Molecular Dynamics).

### Coupled transcription and translation

Collagen X Signal Peptide Mutations

#### Expression of Normal and Mutant Collagen X in Mammalian Cells—COL10A1 cDNA constructs were expressed in a rat osteogenic sarcoma cell line, UMR 106−01 (American Type Culture Collection, ATCC-CRL 1661), using the vaccinia-driven T7 bacteriophage expression system (7). The vaccinia-T7 bacteriophage expression system, previously described (6), was used with minor modifications, to determine whether the mutant chains remained as an integral component of the lipid bilayer. Following cell-free synthesis, microsome vesicles were separated from the reticulocyte lysate by centrifugation at 14,000 rpm for 15 min, and washed with 0.5 ml of KHBM buffer (110 mM KOAc, 20 mM HEPES, pH 7.2, 2 mM Mg(OAc)2). Unbound proteins were extracted from the microsome vesicles with 0.1 M Na2CO3, pH 11.5, on ice for 30 min and then centrifuged as described above. The lipid bilayer was washed with 0.5 ml of KHBM buffer. The supernatants were neutralized to pH 7.5 with 1 M HC1 and the extracted proteins precipitated with 75% (v/v) ethanol. Proteins in both the Na2CO3-soluble, and the membrane fractions were dissolved in 40 μl of sample loading buffer, as described above, prior to analysis on a 7.5% SDS-polyacrylamide gel.

### Mutations at Gly18 Prevent Cleavage of the Signal Peptide—

Sequence analysis was used to study collagen X secretion and degradation. Transfected cells in 6-well plates were preincubated in 1 ml of Dulbecco’s modified Eagle’s medium without t-methionine (Life Technologies, Inc.) for 1 h, then pulse-labeled for 2 h with 15 μCi of l-[35S]methionine (1110 Ci/mmol, PerkinElmer Life Sciences). For secretion studies, labeled cells were chased with fresh medium containing excess unlabeled methionine over a period of 2 h. Degradation of A1(X) chains from the cell and medium fractions was recovered by immunoprecipitation using a specific antibody (gift from Dr. Olena Ja- 

### Results

#### RESULTS

Mutations at Gly18 Prevent Cleavage of the Signal Peptide—Sequencing confirmed that the G148A and G149A mutations had been introduced in the cDNA constructs (Fig. 1). In vitro transcription and cell-free translation demonstrated that the mutant cDNAs were translated into pre-a1(X) chains with the same molecular size as the wild type (Fig. 2a, lanes 1–3). The lower molecular weight band in lanes 1–3 represents translation products initiated from the second methionine residue. In the presence of microsomes (Fig. 2a, lanes 4–6), the initiation of translation was more accurate, with only a single translation product for each of the constructs. A reduction in the molecular size can be clearly demonstrated when wt pre-a1(X) chains (Fig. 2a, lane 1) were processed to a1(X) chains (Fig. 2a, lane 4). In contrast, the mutant translation products remained as pre-
Procedures” for details). The resultant [35S]methionine-labeled prod-
and the deduced amino acid sequence resulting from the mutations are
sequences (G148A and G149A) created by overlapping extension PCR (see
mutagenesis.

Sequencing gel showing the wild-type and mutated se-
corresponding sequencing gel. The underlined sequence represents the region of sequence listed on the
nal peptide cleavage site.

bly. The percentages of mutant pre-
percentage of the total translation product (trimers
association for wt as well as G18R and G18D translation prod-
the reticulocyte lysate prior to analysis, both the mutant and
jected signal peptide. When microsomes were separated from
chains migrated with a slightly increased molecular size, sug-

Mutant Chains Can Associate in Vitro via the NC1 Domain to
Form Heterotrimers with Signal Peptide-cleaved Chains—Because SMCD is an autosomal dominant disorder, an important
consideration is whether the mutant pre-a1(X) chains are able to
heterotrimers with normal a1(X) chains. To
address this issue, reporter (helixΔ) and SPm plasmids were
tcotranscribed and translated in the presence of microsomes. The helixΔ plasmid is an
is less efficient compared with cotranslations of wt with
D 1(X) or wt chain and two helix
bands comprised of one mole-
chains can assemble with the helix
b chains, the stoichiometry
of these complexes can readily be determined by assessing the
photograph migration of the multimers.

All translation products were localized to the microsomal
membrane fraction (Fig. 3). Cotranslation of the SPm or wt
transcripts with the helixΔ transcripts showed that in addition
to the trimeric components of each product, two additional
intermediate multimeric bands, labeled as a and b, were also
observed (Fig. 3, lanes 8–10). This is consistent with band a
containing heterotrimers of two pre-a1(X) or wt chains and one
helixΔ a1(X) chain, and band b containing heterotrimers of one
pre-a1(X) or wt chain and two helixΔ a1(X) chains.

To assess trimer assembly efficiency, the trimer bands,
a1(X)/pre-a1(X), band a, band b, and helixΔa1(X), in lanes 8–10,
were quantified using phosphor imaging to estimate trimer assembly preference (Table II). The data showed that
relative to the formation of helixΔa1(X), the formation of trimer,
containing one or more SPm chains in cotranslations with
helixΔ is less efficient compared with cotranslations of wt with
Integral Membrane Proteins—Treatment of membrane vesicles of microsomes, the vesicles were treated with 0.1M Na2CO3, pH 11.5 and centrifuged to determine whether the mutant chains remain integrated with the lipid bilayer. This approach revealed that the majority of the monomers and trimers containing uncleaved SPm chains associate primarily with the membrane pellet (Fig. 4a). Thus it appears that, in the absence of cleavage by signal peptidase, mutant pre-a1(X) chains remain anchored to the translocons and behave as integral membrane components. We observed a similar pattern for cotranslation products of trimers containing SPm chains with an uncleaved signal peptide, which were preferentially retained in the membrane-bound fraction following treatment with Na2CO3 (Fig. 4b). These included mutant pre-a1(X) homotrimers and the heterotrimers labeled a and b described above (Fig. 4b, lanes 2 and 3). In contrast, the heterotrimers of the reporter (helixΔ), wt chains, and heterotrimers of helixΔ and wt chains, were found predominantly in the soluble fraction (Fig. 4b, lanes 4–6).

Mutations in the Signal Peptide Cleavage Site Impair Stable Triple Helix Formation and Secretion from Cells—The in vitro cell-free system does not promote stable triple helix formation because of the lack of appropriate post-translational modifying enzymes (6). To address the issue of helix formation, secretion, and the fate of the mutant chains, normal and mutant plasmids were transiently transfected into cells. Expression studies in UMR-106-01 cells, a collagen-producing osteoblast-like cell line, indicated that the wt chains assembled as trimers (Fig. 5), forming stable triple helical molecules (Fig. 6), which were secreted efficiently by the cells (Fig. 5). In contrast, whereas the SPm chains (G18R and G18D) associated into homotrimers intracellularly (Fig. 5), they were sensitive to pepsin digestion (Fig. 6), demonstrating a lack of helical configuration. In Fig. 6, the pepsin-resistant a1(I) and a2(I) chains are endogenous collagen I expressed by the UMR106-01 cells and were present in control untransfected cells that were only infected with the vTF7-3 virus. Secretion of the mutant chains was also severely impaired as no immunoprecipitable products could be detected in the medium fractions over the 2-h chase period (Fig. 5).

Unsecreted Mutants Chains Are Degraded Intracellularly in UMR106-01 Cells—Even though SPm trimers or chains were not secreted, the intracellular concentration decreases over the chase period, suggesting that the mutant chains are being degraded intracellularly (Fig. 5). To determine the likely degradative pathway involved, cells were treated from the preincubation period with either a proteasome inhibitor (clasto-lactacystin β-lactone) or a vesicular transport inhibitor for the endosome/lysosome pathway (brefeldin A). The concentration of mutant collagen X was compared after 1 h of the chase period. Cells transfected with the G18R or G18D constructs showed a similar response (Fig. 7). Incubation with brefeldin A resulted in a 4-fold increase in intracellular mutant chains compared with untreated cells and an approximately 8-fold increase was observed in the presence of clasto-lactacystin β-lactone.

**DISCUSSION**

Signal peptide sequences of secreted proteins share common features that include a net positive charge at the N terminus, a central hydrophobic region, and a C-terminal region with small nonpolar amino acids at positions −1 and −3 from the cleavage site (12). The −1 position of the putative signal peptide in collagen X is Gly13 (2). In view of the common requirement for signal peptide sequences, the G18R and G18D mutations are significant, changing the nonpolar glycine residue at the −1 position to the highly charged arginine or aspartate residues. Based on reports of signal sequence mutations in other secretory proteins (15, 19), the most likely consequence of these mutations is impaired cleavage of the signal peptide and inhibition of collagen X secretion.

Cell-free translations in the presence of microsomal membranes will allow the interaction of the signal recognition particle with the newly synthesized polypeptides, followed by docking and translocation into the microsome vesicles, where cleavage of the signal peptide will occur (20). This system was utilized to test whether the G18R and G18D mutations in COL10A1 alter these events. When microsomes were separated from the reticulocyte lysate following translation, the translation products of wt, G18R, and G18D were found associated with the microsome vesicles. Only small amounts of translation product remained in the reticulocyte lysate thus showing no indication of a preferential retention of mutant chains in the reticulocyte lysate when compared with wt translation. This suggests that interaction with the signal recognition particle and targeting to the translocons are not affected, as the mutant chains are delivered to the microsome vesicles during cell-free synthesis. Translocation into the lumen of the microsome vesicles was also not affected because we were able to demonstrate that the translation products are inside the microsomes with a protease protection assay using exogenous trypsin and chymotrypsin (data not shown). As the exogenous proteases cannot enter the vesicle, the presence of intact pre-a1(X) chains implies that they are inside the microsomes (15).

Our in vitro data suggest that signal peptidase failed to recognize or cannot cleave the mutant sites. With no cleavage, the mutant pre-a1(X) chains would not be released from the translocons and remain anchored to the microsomal membranes. The molecular consequence of mutations involving the signal peptide has not been characterized in the collagen family of proteins or other matrix components. However, there are a number of reports characterizing signal peptide mutations in secreted proteins that are associated with several diseases. For example, in human coagulation factor X deficiency (15) and diabetes insipidus (19, 21), signal peptide mutations have been identified in factor X and prepro-vasopressin, respectively. For these nonmatrix-secreted proteins, failure to cleave the signal peptide resulted in intracellular retention of the mutant products, with a severe reduction in the amount of circulating protein and protein activity.

As shown by pulse-chase analysis in transiently transfected cells, impaired secretion of the mutant collagen X chains is clearly evident in the two cases reported here. That the nonsecreted SPm chains are targeted for intracellular degradation is

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**Table II**

| pre-a1(X)/a1(X) | Heterotrimer band a | Heterotrimer band b | helixΔ a1(X) |
|----------------|---------------------|---------------------|-------------|
| Wt + helixΔ    | 0.8                 | 1.2                 | 1           |
| G18R + helixΔ  | 0.1                 | 0.6                 | 0.8         |
| G18D + helixΔ  | 0.1                 | 0.5                 | 0.7         |

helixΔ. In cotranslational experiments of SPm and helixΔ chains, the assembly of heterotrimers containing one or two SPm chains appears to be more favored then the assembly of SPm homotrimers.
supported by the continued decline of intracellular levels of SPm chains over the chase period in the absence of secretion. The precise mechanism(s) for this intracellular degradation is

**Fig. 4.** Extraction of microsomes with sodium carbonate. The wt, helixΔ, and mutant cDNA constructs were transcribed and translated in a coupled cell-free translation system supplemented with microsomal membranes (see “Experimental Procedures” for details). Panel a represents individual translation products, and panel b shows products from cotranslation experiments. Following the completion of translation, the microsomes were isolated and treated with 0.1 M Na2CO3 (18). The [35S]methionine labeled translation products in the membrane-bound fractions (MB) and the soluble fractions (S) from single or cotranslation experiments were analyzed on a 7.5% SDS-polyacrylamide gel. The identity of the lanes, the migration positions of pre-α1(X) chains containing the signal sequence, α1(X) chains after cleavage of this sequence, and trimeric α1(X)3 components are indicated. The identities of bands labeled α and β are as described in Fig. 3.

**Fig. 5.** Retention of SPm collagen X in transfected UMR106-01 cells. Transfections and biosynthetic labeling were performed as described under “Experimental Procedures.” Cells were pulse-labeled with L-[35S]methionine for 2 h and chased for 0–2 h as indicated. Collagen X from cell and medium fractions was recovered by immunoprecipitation and analyzed by SDS-PAGE. The migration position of α1(X) trimers (α1(X)3) and monomers (α1(X)) are indicated. Pepsin treated collagens from human skin fibroblasts are included as standard (std) molecular size references. The positions of the α1(I) and α2(I) chains of collagen I, and the trimer of unreduced collagen III are shown.

**Fig. 6.** SPm collagen X homotrimers failed to form stable triple helical molecules in transfected UMR106-01 cells. Transfections and biosynthetic pulse labeling with L-[35S]methionine were performed as described under “Experimental Procedures.” Cell fractions after 1 h of chase were harvested. Protein fractions were analyzed by SDS-PAGE with (+ pepsin) or without (− pepsin) digestion by pepsin. The position of collagen X trimers (α1(X)3), and the pepsin-resistant α1(I) and α2(I) chains of collagen X of collagen X are indicated. The bands labeled as procollagen I chains represent procollagen I α-chains and various processed intermediates containing N- or C-propeptides.

**Fig. 7.** Degradation of SPm collagen X chains in UMR106-01 cells is prevented by proteasome and vesicular transport inhibitors. Transfections and biosynthetic pulse labeling with L-[35S]methionine were performed as described under “Experimental Procedures.” Cells were treated throughout the preincubation, pulse, and chase periods with (+) or without (−) inhibitors for intracellular protein degradation; 5 μM clasto-lactacycin β-lactone (Lac) for the proteasome, or 1 μg/ml brefeldin A (BFA) for the endosome/lysosomal pathways. Cells were harvested after 1 h of chase and collagen X recovered by immunoprecipitation and analyzed on a 7.5% SDS-polyacrylamide gel. A standard (std) of pepsin-treated collagens from human skin fibroblasts was included as a molecular size reference. The position of the α1(I) and α2(I) chains of collagen I and the trimer of the unreduced collagen III are shown. Radioactive bands were imaged and quantified using a phosphor imager. The quantified value for the bands indicated as collagen X trimers and monomers were combined as a measure of total collagen X present in the cell fractions. The degree of protection against intracellular degradation by the addition of inhibitors was estimated relative to the corresponding untreated cells.
not clear at this stage. Our preliminary study using inhibitors of lysosomal and proteasome pathways suggests that both degradative pathways may be involved. However, the more significant level of protection from degradation shown by the proteasome inhibitor, clasto-lactacystin β-lactone, suggests this could be the major degradative pathway.

The ability of SPm chains to associate into homo- and heterotrimers contrasts with our previous reports on SMCD mutations in the NC1 domain, where in vitro association of NC1 mutant collagen X chains was impaired (1, 6). Although SPm chains do associate into homo- and heterotrimers, there appears to be some constraint on the nature of the trimers that are formed. In cell-free translation experiments expressing SPm or wt chains, we noted that the assembly of SPm pre-α1(X) chains into homotrimers was less efficient relative to the assembly of wt homotrimers. When cotranslated with helixΔ α1(X) chains, SPm chains showed a preference for assembly into heterotrimers over SPm homotrimers, with heterotrimers containing one SPm chain most favored.

Collagen X triple helix formation is initiated via nucleation and extension following the assembly of the C-terminal NC1 domains, so a possible reason why heterotrimers are preferred is the physical constraint imposed by the anchoring of SPm chains to the membrane. For the formation of homotrimers, only chains that are close enough will be able to associate; however, this constraint will have a lesser effect on the formation of heterotrimers containing two SPm chains, and be smallest for heterotrimers containing one SPm chain. We reason that whereas SPm chains can associate into homo- and heterotrimers via the NC1 domain in vitro, folding of SPm chain-containing molecules into a triple helix is likely to be inefficient because the N termini will be anchored to the ER membrane. A lack of stable triple helical structure was demonstrated for SPm homotrimers by the absence of intracellular pepsinresistance SPm collagen X molecules in transfected cells.

The precise impact on the level of available normal molecules in vivo is not clear. In transient transfected cells, it is difficult to make reliable assessments of the yield of trimers because of the variable transfection efficiency and the added complication of intracellular degradation. However, we noted a reduction of ~60% in the amount of collagen X molecules secreted in a cotransfection experiment (data not shown), supporting a dominant-negative effect of SPm chains that could affect the level of normal collagen X molecules. We propose that SPm chains anchored to the translocons impair triple helix formation, and the unfolded chains are removed from the ER by retrograde translocation and targeted for degradation via the proteasome pathway (22, 23).

It would appear that although the signal peptide mutations and the NC1 SMCD mutations reported previously (6) are at opposite ends of the molecule and have different molecular effects on collagen X biosynthesis, the net phenotype is similar, with intracellular retention and degradation of mutant collagen X. The fact that both types of mutations should give rise to SMCD is consistent with a similar molecular consequence for the mutations. Whereas an apparent 50% reduction in collagen X level has been shown in one patient to be a mechanism for SMCD (7), this may not be the only mechanism. The current finding together with other in vitro studies (8–10) suggest that a dominant-negative effect may be a more common mechanism. Intracellular retention and active degradative processes of collagen molecules containing mutant chains could lead to deregulated cellular metabolism, altering cell differentiation, proliferation, and apoptosis. These are the critical cellular events in endochondral ossification that induce precise alterations in the ECM. An imbalanced ECM not only compromises matrix integrity but also alters cell-matrix interactions, eliciting aberrant cellular responses with unknown consequences. Further understanding of the impact of SPm and COL10-NC1 SMCD mutations on the metabolism and differentiation of chondrocytes in the growth plate will need to await investigation of these mutations in animal models.

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