Type VII Collagen, Anchoring Fibrils, and Epidermolysis Bullosa

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The anchoring fibrils at the dermal-epidermal junction have been well characterized as ultrastructural entities. From their appearance, it was proposed that they fortified the attachment of the epidermis to the dermis. This hypothesized function was strengthened by observations indicating that the anchoring fibrils were abnormal, diminished, or absent from individuals with dystrophic epidermolysis bullosa. Therefore, characterization of the molecular constituents of the anchoring fibrils and their interactions with other basement membrane and dermal components might lead to identification of the gene defects underlying at least some forms of epidermolysis bullosa. Type VII collagen was identified as the protein component of anchoring fibrils in 1986. Since then, the major characteristics of the molecule have been described. These are consistent with a model wherein secreted type VII collagen molecules form disulfide-bond stabilized antiparallel dimers. The dimers then condense laterally into unstaggered arrays that are the anchoring fibrils. This arrangement allows for the protrusion of large globular domains (NC-1) from both ends of the fibrils. The aggregated triple-helical domains extend into the papillary dermis and entrap fibrous dermal components. The NC-1 domains are believed to interact with components of the basement membrane and thus to mediate the attachment of the basement membrane to the dermis. This model predicts that mutations in the type VII collagen gene that prevent the secretion of the molecule will be the most devastating, whereas mutations in the regions encoding the globular domains may show more variable phenotype. Ultimately, understanding the function of type VII collagen at the molecular level will be the key to devising strategies to moderate the pathophysiology of dystrophic epidermolysis bullosa.

The purpose of this brief review is to summarize the current status of studies of type VII collagen and its function as the anchoring fibril protein. I will try to indicate those aspects of past studies that have withstood the test of time during the ten years since type VII was first reported, and try to make corrections where more recent studies contradict past findings. I will also try to point out certain aspects of type VII collagen function that are based upon correlations or modeling, which may or may not be correct in detail, as well as those that are based upon solid structural information. It will be very interesting to observe the modifications of these concepts that will inevitably occur as characterization of the molecular defects underlying dystrophic epidermolysis bullosa (DEB) become increasingly abundant.

At this point, as a result of the initial biochemical analyses and subsequent primary structure prediction, the structure of the type VII molecule is quite well understood. The amino terminus contains the large NC-1 domain [1–6]. Rotary shadowed images (Fig 1) of this domain examined by transmission electron microscopy appear as three apparently identical extended structures joined by disulfide bonds at their C-terminus within a large globule. In some micrographs, the N-termini appear to end in smaller globules, but most often the individual arms appear as elongated structures increasing in thickness from the C- to the N-terminus [2]. Biochemical analysis of the NC-1 domain subunit chains described three polypeptides of about 150 kDa that could not be separated by any applied technology, suggesting that all three subunits were derived from the same gene product. This estimate compares well with the predicted amino acid sequence of the a1(VII) chain, which indicates that the domain is a composite of a cartilage matrix protein (CMP) homology, followed by nine fibronectin type III (FNIII) repeats and a von Willebrand factor (vWF) repeat [5–8] (Fig 2). The sequence of the complete amino terminus is not yet published. The data suggest that the C-terminal large globule of NC-1 joining the individual arms is contributed by the second vWF repeat, while the extension of the arms results from the series of FNIII repeats. The CMP repeat may coincide with the N-terminal globule sometimes observed by rotary shadowing.

We originally assigned the NC-1 domain to the C-terminus of the a1(VII) chain [3]. This was based upon a comparison of the amino acid sequence determined for the intact triple-helical domain with the sequence of the P1 pepsin fragment, which was identified by electron microscopy to be involved in dimer formation. These peptides both produced the same sequence, indicating that the P1 fragment was at the amino terminus of the triple-helical domain and therefore P2, which was adjacent to the NC-1 domain, must be at the C-terminus. Predictions of amino acid sequence from analysis of the cDNA of a1(VII) [7] has proved this to be incorrect. Presumably, the sequence of P1 we reported actually derived from contaminating P2 peptides that produced the dominant sequence signal because the N-terminus of P1 was probably blocked by spontaneous cyclization. The occurrence of NC-1 at the amino terminus is far more consistent with the structures of other collagen types. The large non–triple-helical domains of types IV, XII, and XIV collagens occur at the amino terminus. It is also consistent with the generally accepted concept that the triple-helical domain of colla-
caps fold intracellularly from the C-terminus. It has always been difficult to reconcile the folding of type VII collagen with this mechanism when it was believed that the independently folded arms of the NC-1 domain somehow nucleated triple-helix assembly.

The triple-helical domain of α1(VII) is unusually long (424 nm, 170 kDa) and flexible [1,9]. The flexibility of the domain is due to the presence of a large discontinuity in the triple-helical structure at the junction of the major pepsin fragments P1 and P2. Shorter disruption of the Gly-X-Y motif specifying helical structure occur throughout the length of the domain.

The C-terminal NC-2 domain forms a small globule visualized by rotary shadowing [10] (Fig 1). Biochemical estimates of the molecular mass of this domain predicted masses of 32 and 34 kDa. This is in considerable excess of the cDNA prediction. The reason for this discrepancy may eventually be explained by post-translational glycosylation (which has yet to be characterized), or by secondary structures that are resistant to sodium dodecysulfate denaturation and hold the polypeptide in an extended conformation, slowing its migration through electrophoretic gels.

It is assumed that the type VII collagen molecule is composed of a single gene product, α1(VII). This prediction derives from the failure of biochemical methods to fractionate the whole type VII chains or the P1 and P2 peptides into more than one chromatographic or electrophoretic fraction. This is supported by the finding of single mRNA species encoding type VII-like sequences [7]. Further support derives from the mapping of all DEB mutations to the same genomic region [11–15]. However, there are several observations that urge caution in fully accepting this conclusion. Attempts to characterize the NC-2 domain always showed two species: one with an electrophoretic migration consistent with a mass of 32 kDa, another with a predicted mass of 34 kDa. This heterogeneity has never been understood. Secondly, protein sequencing of CNBr fragments of the triple-helical domain by Dr. Ivan Rocos, and of NC-1 by Dr. Louise Rosenbaum when the work was done in Portland, identified a number of peptide sequences that have not been found in the reported cDNA predictions. The percentage of unidentified sequences to sequences placed within α1(VII) is significant, and consistent with the possibility that another type VII chain may exist. The DEB linkage studies do not rule out this possibility if a second type VII gene is closely linked to COL7A1. Alternatively, these observations could have trivial explanations. In any case, those directly working with type VII collagen might bear this possibility in mind.

α1(VII) is synthesized by keratinocytes [2,4,16–22] and to a lesser extent by dermal mesenchymal cells [4,20]. In vitro, the mesenchymal contribution is incorporated into the basement membrane made by dermal equivalents (Marinkovich, Nishiyama, and Burgeson, unpublished observations). After secretion, the type VII molecules dimerize [3] (Fig 3). Subsequently, the dimers become disulfide bonded, but this crosslink is not made in cell culture under standard conditions, even though non-covalently stabilized dimers are observed in vitro. We have assumed that dimerization is catalyzed by binding of the NC-2 domain to a specific region of the triple-helix. This assumption is based upon the observation that the dimers formed in vitro retain the NC-2 domains on the C-termini of the molecules; therefore it is not necessary that NC-2 be removed to obtain dimer formation. Also, we have never been able to form dimers in vitro from isolated triple-helical domains or from P1
anchoring fibrils originate within the lamina densa and extend perpendicularly into the upper regions of the papillary dermis where they condense with the ends of other anchoring fibrils and with ubiquitous basement membrane components to form anchoring plaques. Additional anchoring fibrils bridges these plaques with plaques deeper in the papillary dermis. The extended network thus formed is capable of entrapping large numbers of dermal fibrous elements, thereby securing the lamina densa to the dermis.

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2. Lunstrum GP, Sakai LY, Keene DR, Morris NP, Burgeson RE: Large collagen defects to the dystrophic phenotype. Further support for this close association derives for studies of the acquired form of DEB. The autoantibodies characteristic of this disease specifically recognize the NC-1 domain of the type VII molecule [40–44]. The autoimmunity appears to be associated with the class II HLA allele [45].
3. Very recently, the first mutation in COL7A1 has been identified as the cause of recessive DEB within one kindred [46]. The single base transmutation encodes a methionine to lysine substitution, near the NC-2–triple-helix junction. The mutation lies within a highly conserved region containing cysteine residues that are likely to be involved in the process of type VII dimerization.

The anchoring fibril network (Fig 4) has been described as primary fibrils originating in the lamina densa of the basement membrane, looping into the upper regions of the papillary dermis and reinserting into the lamina densa. The loops surround and entrap the fibrous dermal elements, securing the basement membrane to the dermis. We described a secondary network [23,24] in which anchoring fibrils originating in the lamina densa extend perpendicularly into the dermis and insert into amorphous elements containing type IV collagen and probably laminin, and perhaps other ubiquitous basement membrane components. We called these structures anchoring plaques. The acceptance of these structures has been controversial; however, none of our subsequent experience with ultrastructural visualization of the upper papillary dermis contradicts this original conclusion. A similar arrangement of these molecules has been reported in cornea [25].

The formation of the anchoring fibril network requires the NC-1 domain to interact with constituents of the lamina densa and the anchoring plaques. Solid-phase binding studies indicated that isolated NC-1 could bind type IV collagen and laminin [26] (and kalinin/K-laminin; Burgeson, unpublished), suggesting that the assembly and stability of the anchoring fibril network is dependent upon the interactions of basement membrane components with NC-1. The molecular mechanisms of these potential interactions have not been further characterized. Potential interactions of the NC-1 domains with keratinocyte basal surface transmembrane proteins has never been documented. With the identification of the cDNA encoding NC-1 the possibility of further dissecting the binding functions of the CMP, vWF, and FNIII subdomains using recombinant fragments exists. Although difficult to perform, and even more difficult to interpret unambiguously, such studies would greatly facilitate the interpretation of molecular defects that are certain to be found in the region of the gene encoding NC-1. From the lessons learned [27] from the studies of defects underlying osteogenesis imperfecta, the Ehlers-Danlos syndromes, and the chondrodystrophies, it is likely that the most severe forms of dystrophic epidermolysis bullosa will result from gene defects that prevent accumulation of type VII molecules in the basement membrane zone, predominantly by predisposing the molecule to intracellular degradation. However, mutations that interrupt the binding capacities of the NC-1 subdomains are also likely, and these might result in some of the more mild forms of the disease. Hybrid mutant/wild-type molecules could exist, exhibiting partial ligand binding due to the predicted trivalency of NC-1.

The predicted relationship of type VII collagen to DEB is now well documented. The absence of anchoring fibrils from the basement membrane zone of patients with severe generalized recessive epidermolysis bullosa has been a characteristic of the disease. Lack of type VII collagen correlates with the same condition [17,21,28–34]. Type VII has also been identified intracellularly within keratinocytes in some patients [16,21], suggesting that a mutation may prevent secretion. COL7A1 has now been localized to the human gene locus 3p21.3 [7,35,36]. Use of observed polymorphisms [35] has allowed the demonstration of linkage of dominant and recessive epidermolysis bullosa to the type VII gene [11–15]. The consistency of the linkage studies of all forms of DEB to type COL7A1 is surprising. It has been generally predicted that some of the defects resulting in anchoring fibril defects would be secondary to other gene defects. The possibility that anchoring fibrils were degraded due to aberrant synthesis or activation of collagenase or another protease is a particularly attractive possibility [37]. The type VII molecule has been shown to be a potential substrate for a variety of degradative enzymes [38]. The linkage studies also suggest that type VII is the only component of the anchoring fibril. This is very unexpected. Although the banding pattern of the segment long spacing fibers made from type VII collagen triple-helical domain do account for most, if not all, of the centrosymmetric banding seen in anchoring fibrils, and type VII is the major structural component of the fibrils, there are indications that other molecules may be present in the structures. The antigens recognized by the monoclonal antibodies AF-1 and AF-2 [39] are prime candidates. The epitopes recognized by these antibodies have been localized to the anchoring fibrils, but have not been found within the type VII molecule. This discrepancy remains a mystery, and the AF-1 and AF-2 antigens have not been further characterized. However, the possibility that additional components contribute to anchoring fibril structure and function remains.

The linkage studies firmly tie type VII collagen defects to the dystrophic phenotype. Further support for this close association derives for studies of the acquired form of DEB. The autoantibodies characteristic of this disease specifically recognize the NC-1 domain of the type VII molecule [40–44]. The autoimmunity appears to be associated with the class II HLA allele [45].

The anchoring fibril network is comprised of a complex organization of molecules, which include collagen type IV, laminin, fibronectin, and various proteins specific to the anchoring fibrils. The network is dynamic and constantly undergoes turnover, with new fibrils being added and old ones being degraded. The anchoring fibrils are responsible for attaching the epidermis to the dermis, and any defect in their formation or function can lead to the symptoms of epidermolysis bullosa. The study of these fibrils has been facilitated by the identification of the genes encoding the different components, allowing for the identification of mutations that are responsible for the disease.

**Figure 4.** Drawing of the proposed anchoring fibril network. As shown, anchoring fibrils originate within the lamina densa and extend perpendicularly into the upper regions of the papillary dermis where they condense with the ends of other anchoring fibrils and with ubiquitous basement membrane components to form anchoring plaques. Additional anchoring fibrils bridges these plaques with plaques deeper in the papillary dermis. The extended network thus formed is capable of entrapping large numbers of dermal fibrous elements, thereby securing the lamina densa to the dermis.
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