Extracellular Matrix of the Skin: 50 Years of Progress

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The extracellular connective tissue matrix of the skin is a complex aggregate of distinct collagenous and non-collagenous components. Optimal quantities and delicate interactions of these components are necessary to maintain normal physiologic properties of skin. This overview summarizes the progress made in understanding the normal biology and biochemistry of the extracellular matrix, and will highlight cutaneous diseases with underlying molecular defects in the structure and expression of extracellular matrix components.

Some 50 years ago, at the time of birth of *the Journal of Investigative Dermatology,* the extracellular connective tissue matrix was perceived as an inert, metabolically inactive substance, which was necessary to glue tissues together and provide support for cells. During the ensuing half a decade, this perception has been radically revised, and it is now appreciated that the extracellular matrix is a complex aggregate of distinct collagenous and non-collagenous protein components, which in physiologic situations are in a dynamic equilibrium. The optimal quantities of different matrix components and their delicate interactions are clearly necessary to maintain normal physiologic properties of tissues, such as skin. In fact, skin is a good example of an organ where the extracellular matrix plays an integral role in providing physiologic properties to the tissue. This point is well demonstrated by the fact that there are several examples where molecular aberrations in the structure or expression of components of the skin result in a phenotypically recognizable cutaneous disease.

It is now appreciated that the extracellular matrix of skin consists of a large number of distinct components, and the predominant ones, as they are recognized today, are listed in Table I. To illustrate the tremendous progress made in our understanding of the biology and biochemistry of the extracellular matrix, this overview will discuss some of the specific components of skin, *viz.* collagen, elastic fibers, and the basement membrane zone glycoprotein laminin, as examples of relatively well-characterized extracellular matrix components. For additional reviews on matrix research not covered by this overview, see Chapters in recent textbooks [1–3].

THE COLLAGENS

The predominant extracellular matrix component of the dermis and a variety of other human tissues is collagen [4], now known to be a superfamily of closely related, yet genetically distinct proteins [5,6]. As the name implies (Gr. *kolla,* glue; *gennan,* to produce; collagen, glue former), collagen was initially recognized as a tissue component which, when boiled, produced glue. In fact, this property of collagen was noted by the Romans as early as 50 A.D. when Pliny wrote ''glue is cooked from the hides of bulls'' (see Ref 7).

The initial scientific approaches employed to study collagen centered around the tanning industry, which attempted to enhance the tensile properties of leather products by introducing additional cross-links into collagen. The advent of modern technologies, such as electron microscopy, allowed the visualization of collagen fibers [8–10], and such fiber structures are essential for collagen to provide tensile strength to the tissues (Fig 1). Bit by bit, the biochemistry of collagen has been elucidated in a large number of studies by skilled researchers, including many individuals specialized in investigative dermatology. As a result of these studies it is now appreciated that there are as many as 25 different genes which code for the subunit polypeptides of at least 13 different collagen types [6,11]. Six different collagen types have been detected in human skin (Table I), and additional collagens have been shown to be synthesized by cultured human skin fibroblasts *in vitro* (see Ref 11).

Genetic Heterogeneity Each of the genetically distinct collagen types has an important functional role within its compartmentalized distribution in the skin. Collagen types I and III are considered to be the major interstitial, fiber-forming collagens in normal human dermis [12,13]. In addition, the dermis contains collagens types IV, V, VI, and VII [13–16]. Type IV collagen is also a major constituent of the basement membrane at the dermal-epidermal juncture [17–19].

Type I collagen is the classic collagen molecule consisting of three polypeptide chains in a characteristic triple-helical conformation. This conformation, which is the hallmark of collagenous proteins, results from the unique primary sequence of collagen α-chains. Specifically, the collagenous portion of these polypeptides consist of a repeating Gly-X-Y sequence, where the X and Y positions are often occupied by the imino acids, proline and hydroxyproline, respectively. The enzymatic synthesis of hydroxyproline, which requires ascorbic acid as a co-factor, stabilizes the triple-helical conformation under physiologic conditions. A similar enzymatically catalyzed pathway exists for the formation of hydroxylysine, an amino acid also characteristically found in collagens. In addition to prolyl and lysyl hydroxylations, the collagen polypeptides undergo other co- and post-translational modifications, including glycosylation, interchain disulfide bonding, and folding of the proα-chains into their triple-helical conformation [6,11]. Subsequent to the secretion of triple-helical procollagen molecules into the extracellular milieu, the precursor specific extensions both at the amino- and carboxy-terminal ends of the procα-chains, are proteolytically removed, and the processed collagen molecules spontaneously assemble into fiber structures [20,21]. Recent studies have suggested that the order in which the amino- and carboxy-terminal extensions are removed from the molecules determines, at least in part, the final diameter of the collagen fibers in tissues [22,23]. Additionally, several lines of evidence suggest that the presence of type III collagen serves as a factor limiting the fiber

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Table I. Structural Components of the Extracellular Matrix Relevant to Skin

Component[a]	Size of Native Molecule (daltons)	Molecular Organization	Tissue Distribution	Functional Role
Collagens				
Type I	285,000	$[\alpha1(I)]_2\alpha2(I)$	Ubiquitous in most connective tissues, including skin, bones, tendons, ligaments, etc.	Major component providing tensile strength
Type III	285,000	$[\alpha1(III)]_3$	Skin, blood vessels, predominant in fetal tissues	Contributes to tensile properties
Type IV	540,000	$[\alpha1(IV)]_2\alpha2(IV)$	Basement membranes, anchoring plaques	Major structural component of basement membranes
Type V	300,000	$[\alpha1(V)]_2\alpha2(V)$[b] $[\alpha1(V)]_3$	Ubiquitous	Pericellular location interfacing the cell surface and the surrounding matrx
Type VI	530,000	$\alpha1(VI)\alpha2(VI)\alpha3(VI)$	Extracellular microfibrils	Matrix assembly
Type VII[c]	510,000	$[\alpha1(V)]_3$	Skin, fetal membranes	Structural component of anchoring fibrils
Elastic fibers				
Elastin	70,000	Cross-linked polymer of fibers	Blood vessels, skin and lungs	Resilience and elasticity of the skin
Microfibrillar component	Unknown	Surrounding the elastic fibers	Same as elastin	Scaffolding in formation of the elastic fibers
Fibrillin	350,000	Part of the microfibrillar component	Same as microfibrillar component	Stabilization of the microfibril structure (?)
Basement membrane associated macromolecules				
Proteoglycans/GAGs	$>10^6$	Complex aggregates	Cartilage, skin	Maintenance of water and ion balance; regulation of growth, migration and attachment of cells
Heparan sulfate proteoglycan		Low and high density forms	Basement membranes	
Laminin	900,000	A, B1, B2	Basement membranes	Cell attachment and differentiation, neurite outgrowth
Nidogen/entactin	150,000	Stoichiometric binding to laminin	Co-localizes with laminin	Cell binding
Fibronectin	450,000	Disulfide-linked dimers	Cell surface, plasma	Attachment of cells to the extracellular matrix

[a]These are the major, relatively well-characterized matrix components in the skin. Several additional components, including bullous pemphingoid antigen, SPARC/BM-40/osteonectin, vitronectin, tenascin, and epinectin, are currently under biochemical characterization.
[b]Additional heterotrimeric forms may exist.
[c]The carboxy-terminal domain of type VII collagen is the acquired epidermolysis bullosa antigen.

growth during fetal development [24] and in pathologic conditions, such as scleroderma [25,26].

The entire primary sequence of the α1(I) and α2(I) chains of type I collagen, as well as several additional procollagen α-chains, has been elucidated through sequencing of the corresponding cDNAs (see, *e.g.*, Refs 27–29). Examination of the genes encoding type I collagen polypeptides has revealed that the genomic DNA consists of ~50 separate coding regions, exons, which are interspersed between noncoding, intervening sequences, introns (Fig 2). During initial stages of the expression of these genes, the nucleotide sequence in the collagen gene is faithfully transcribed into a messenger RNA precursor molecule, which contains sequences corresponding to both exons and introns (Fig 3). Post-transcriptionally, the sequences corresponding to the introns are removed by ''splicing,'' and after additional post-transcriptional modifications, such as ''capping'' of the 5′ end and polyadenylation of the 3′ end, the messenger RNA molecules serve as a template for translation of the preproα-chains (Fig 4). The newly synthesized polypeptides undergo several co- and post-translational modifications depicted in Fig 4. The triple-helical procollagen molecules are secreted out of the cells, and the non-helical extensions are removed by specific proteases. The collagen molecules then align to a fiber structure which is stabilized by the formation of intermolecular cross-links (Fig 4). The fibers have a characteristic banding pattern, as shown in Fig 1.

Examination of the regulatory sequences in the promoter regions of the type I collagen genes has revealed the presence of ''CAAT'' and ''TATA'' boxes, nucleotide sequences that serve as signals for expression of the corresponding genes by serving as binding sites for the transcriptional machinery [30,31]. In addition, recent studies have

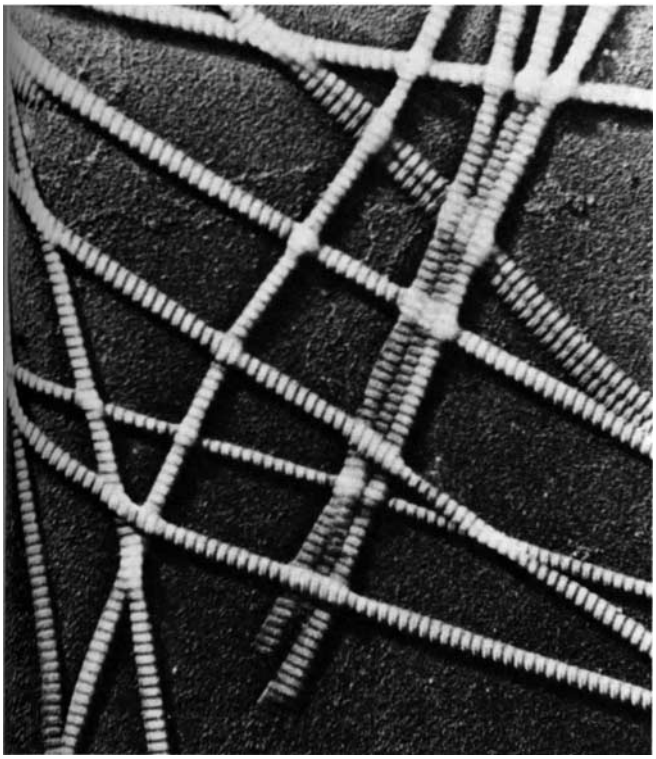

Figure 1. Electron micrograph of collagen fibers prepared from human dermis. Note the regular banding pattern at ~68-nm intervals (×45,000). (From Ref 206, with permission.)

identified other regulatory elements at the 5′ flanking region, upstream from the initiation site of transcription [32]. There are also distinct transcriptional enhancer elements located in the first intron of several collagen genes [33,34]. Many of these promoter and enhancer sequences serve as binding sites for specific regulatory proteins that control the expression of these genes.

Type III collagen was initially called fetal collagen because of its abundance in fetal tissues, including skin [35]. In fetal skin, type III collagen initially accounts for over half of the total collagen [35]. However, type I collagen synthesis exceeds the production of type III collagen in the postnatal period, and consequently, in adult human skin, the ratio of type I/III collagen is approximately 5–6:1 [36]. Initial immunofluorescence data suggested that type III collagen in human skin might be enriched in the papillary dermis as compared with the reticular dermis [37]. However, quantitative biochemical and biosynthetic measurements have not confirmed this initial observation [38,39].

Type IV collagen is a major component of the basement membrane in the dermal-epidermal junction [17–19]. Type IV collagen differs from the interstitial collagen types I and III in that the triple-helical conformation of the molecule is interrupted by several non-collagenous segments which do not demonstrate the repeating Gly-X-Y sequence. This feature provides the molecule with added flexibility, allowing these molecules to form a meshwork-like structure, as opposed to the ordered, staggered fibrils formed by interstitial collagen fibers. Type IV collagen molecules are thought to act as a scaffold which allows interactions with other noncollagenous basement membrane components, such as laminin, nidogen, and heparan sulfate proteoglycan [40,41]. It has also been shown that basal keratinocytes preferentially attach to type IV collagen [42]. This interaction, which may be mediated by laminin or nidogen, apparently contributes to the stability of the skin at the interface between the epidermis and the dermis at the dermal-epidermal basement membrane zone.

The type V collagen gene is expressed in a variety of tissues, but in normal human skin it is clearly a minor component [13,14,43,44]. It was initially suggested that type V collagen has a pericellular location, interfacing between the cells and their immediate environment [45]. The role of type V collagen in human skin is not clear, but it has been reported that migrating epidermal cells produce type V collagen and its continued synthesis is a prerequisite for migration [46].

Type VI collagen was also initially thought to be a minor collagen in tissues, such as the dermis [47]. However, recent improvements in the isolation techniques have demonstrated that type VI collagen may be a more abundant collagenous component in a variety of tissues, including skin [48]. In support of the latter suggestion is the recent demonstration that type VI collagen is a major gene product of cultured skin fibroblasts, as determined on the mRNA level [16]. In fact, molecular hybridizations with human sequence specific cDNAs have revealed that the ratio of type I/VI procollagen mRNAs in cultured skin fibroblasts is approximately 3:1, suggesting that type VI collagen may be even more abundant than type III collagen in the skin. Thus, type VI collagen, which is unusual in that it is highly disulfide bonded, may play an important role in the assembly of the collagenous matrix in the dermis.

Type VII collagen was initially termed long-chain collagen due to the fact that each α-chain is considerably larger (467 nm) than the α-chains of interstitial collagens, type I and III (297 nm) [49]. Type VII collagen is of particular interest from a dermatologic point of view because type VII collagen is known to be a predominant component of anchoring fibrils, structures which extend from the dermal-epidermal junction to the upper dermis [15,50]. It has been suggested that the structure of type VII collagen in tissues is an anti-parallel dimer linked through their amino-termini. The usually large carboxy-terminal, non-collagenous domains of type VII collagen (~150 kd) are thought to interact with type IV procollagen in the dermal-epidermal basement membrane and in the choring plaques [51]. The latter structures, which are embedded in the papillary dermis, were recently shown to contain type IV collagen. This type of structural organization of the anchoring fibrils is thought to stabilize the attachment of the dermal-epidermal basement membrane to the underlying dermis [40]. Thus, alterations in the expression, structure, or molecular interactions of type IV and/or type VII procollagens could result in fragility of the skin, as exemplified by epidermolysis bullosa, a group of heritable cutaneous disorders (see below).

Molecular Pathology Considering the complexity of collagen biology, it is clear that there are several features which predispose the collagenous proteins to faulty production leading to synthesis of abnormal collagen fibers. In cases where the abnormal fibers lead to altered functional properties of skin, such a situation could be manifested clinically as a disease. For example, as indicated above, collagens comprise a super-family of genetically distinct, yet closely related proteins that have a characteristic tissue distribution and specific molecular interactions (Table I). The collagens also have a complex gene structure and the expression of these genes in a precise and coordinate manner requires carefully regulated control mechanisms (Figs 2 and 3). There is also a need for the repetitive Gly-X-Y primary sequence in the collagenous portion of the molecule. The initial biosynthetic product undergoes multiple co- and post-translational modifications, which are necessary for deposition of functional collagen molecules (Fig 4). Finally, extracellular processing and fibrillogenesis, followed by formation of intermolecular cross-links are multi-step events, which are required for stabilization of the fiber structures (Fig 4). Thus, there are several distinct levels of collagen biology where processes can go awry, and such alterations may be manifested phenotypically as a connective tissue disease. In fact, several heritable collagen diseases are currently recognized and their clinical features, associated biochemical defects, and mode of inheritance are summarized in Table II.

Many dermatologists have been involved in studies on heritable and acquired collagen diseases, and this progress has been summarized in the *Journal of Investigative Dermatology* on several occasions. For example,

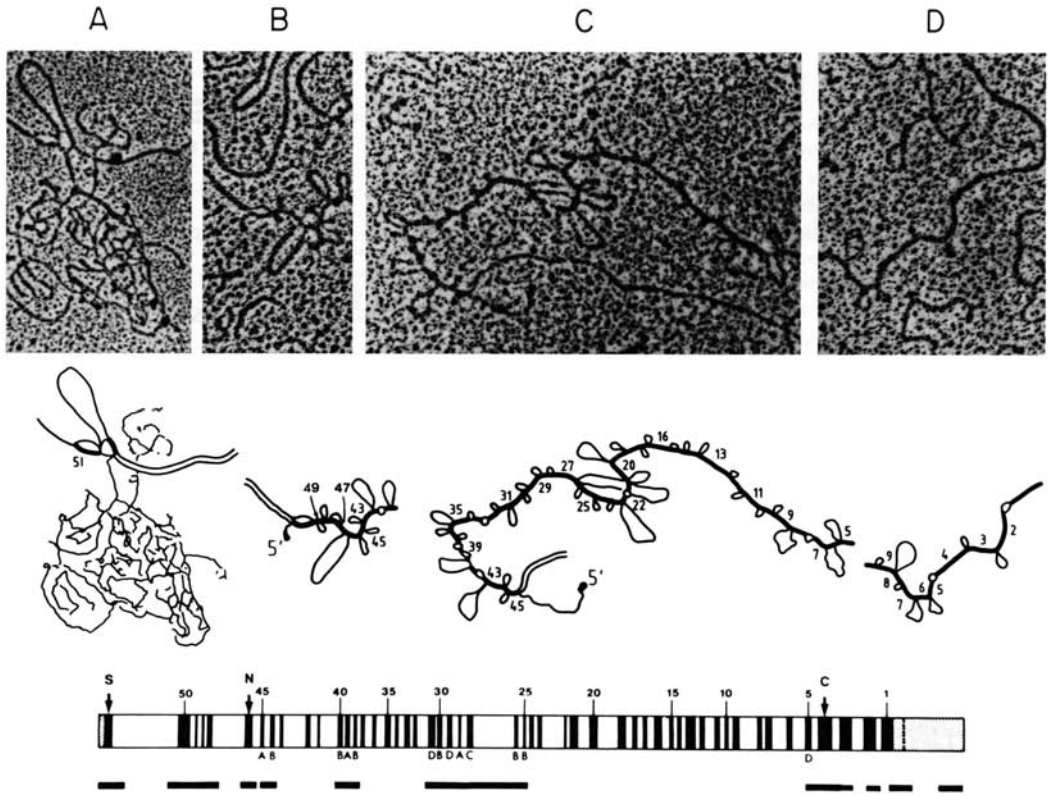

Figure 2. Structure of human pro-α1(I) collagen gene. The *upper panels* represent electron micrographs of DNA-RNA hybrids of different parts of the procollagen gene and the corresponding mRNA. This technique, called R-loop mapping, allows evaluation of the intron-exon structure of a gene; the introns, i.e., the intervening, noncoding sequences of DNA, loop out, while the exons, i.e., the coding sequences, bind through complementary base-pairing to the corresponding translatable sequences in the RNA. The *numbers* indicate the order of exons. The *diagram at the bottom* of the picture is a schematic presentation of the exon-intron arrangement of the gene. The *solid blocks* represent exons, *open areas* signify introns, and the *hatched areas* are regions corrcsponding to untranslated sequences in the mRNA. There are 51 exons in the pro-α1(I) procollagen gene separated by introns of variable sizes. The areas indicated by the *solid horizontal bars* at the bottom of the figure respond to sections for which the exact nucleotide sequences were determined. The positions of *S*, *N*, and *C*, indicated by the *arrows*, correspond to the sites of cleavage of the signal peptidase, procollagen N-protease and procollagen C-protease, respectively. (From Ref 207, with permission.)

in 1976, an updated review was presented [52]; this review ranks among the 100 most quoted articles in the Journal. In 1982, a special issue was devoted to the extracellular matrix [53]. This compilation was based on a William Montagna Symposium devoted to the extracellular matrix of the skin, which was held the prior year at the Salishan Lodge in Gleneden Beach, Oregon.

The Ehlers-Danlos Syndrome: The prototype of collagen diseases is the Ehlers-Danlos syndrome (EDS), a group of phenotypically related conditions manifested as hyperextensible skin, loose-jointedness, and fragility of tissues [11,54,59]. It is now appreciated that there are at least 11 different forms of EDS, and several of them result from specific molecular defects in collagen. In fact, the demonstration of hydroxylysine-deficient collagen in EDS VI by Pinnell and his co-workers in 1972 [55] was the first case of a heritable human connective tissue disorder in which a distinct abnormality in collagen was disclosed. Thus, EDS VI is a primary collagen disease in which the underlying molecular defect is directly related to collagen metabolism [55,56]. Subsequently, EDS IV was shown to result from deficient deposition of type III collagen in tissues [57,58], while EDS VII is a result of deficient conversion of type I procollagen to collagen [59,60]. Finally, the abnormality in EDS IX has been shown to involve deficient cross-linking of collagen, as a result of reduced lysyl oxidase activity [61]. The primary defect in EDS IX is, however, in the metabolism of copper, a cofactor for lysyl oxidase [62]. Strictly speaking then, EDS IX is a secondary collagen disease, in which collagen is clearly altered, but the primary defect is unrelated to collagen metabolism. It should be pointed out that the most common autosomal dominant forms of EDS, types I–III, may involve abnormalities in collagen, as suggested by aberrant organization of collagen fibers noted by ultra-structural analyses [63]; however, no biochemical defect has been disclosed as yet. It should also be emphasized that the initial reports of deficient lysyl oxidase activity in the X-linked EDS V were probably incorrect because further careful analyses [64] have not disclosed any abnormalities in collagen cross-linking in these patients.

Osteogenesis Imperfecta: Significant progress has recently been made in understanding the underlying molecular defects in osteogenesis imperfecta (OI), a disease characterized primarily by brittle bones, but also manifested by thin skin, blue sclerae, and aberrant scar formation. In a large number of cases with OI, specific defects in type I collagen have been demonstrated; these include insertions or deletions in the gene, splicing errors, or single point mutations [65,66]. Particularly instructive are the cases in which a single point mutation in one of the glycine codons in the repeating Gly-X-Y sequence has resulted in substitution of a glycine by a cysteine residue [67]. This amino acid substitution often leads to a lethal form of OI, and these observations clearly attest to the importance of maintaining glycine in every third position of the repetitive primary sequence of type I collagen [65].

Fibrotic Skin Diseases: A group of diseases of particular interest to dermatologists involves abnormalities in the regulation of collagen gene expression. It is clear that rigorous control at the transcriptional and translational levels has to be exerted in order to maintain physiologic

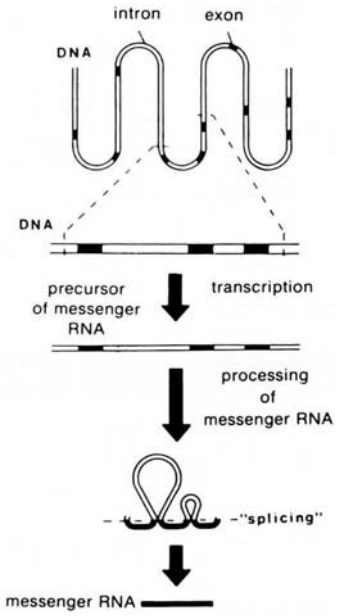

Figure 3. Schematic presentation of the expression of a gene in eukaryotic cells. DNA, containing the coding sequences (exons) and the intervening non-coding sequences (introns) of the gene, is transcribed to form a precursor of the mRNA molecule. The pre-mRNA molecule is processed during post-transcriptional events, including splicing, which removes the segments corresponding to the intron sequences. Following capping of the 5′ end and polyadenylation of the 3′ end, the functional mRNA molecule is ready to serve as template for translation of the polypeptides. (From Ref 208, with permission.)

concentrations of collagens in tissue [68]. The steady-state level of tissue collagen results from a balance between the biosynthetic pathway and the rate of degradation. Fibrotic conditions, demonstrating excessive accumulation of collagen, apparently result from an imbalance between synthesis and degradation of skin collagen. A classification of the fibrotic skin diseases has allowed recognition of at least five distinct categories, based on clinical, genetic, and biochemical considerations (Table III).

The prototype of fibrotic diseases is scleroderma, a condition in which fibrosis is evident not only in the skin but also in several internal organs, including the lungs, gastrointestinal tract, and the kidneys [69]. In addition to scleroderma, scleroderma-like skin changes are often encountered in other connective tissue disorders, including overlap syndromes and eosinophilic fasciitis. Particularly interesting is the observation that chronic graft-vs-host disease presents with a clinical picture similar to that of progressive systemic sclerosis [70]. The latter observation emphasizes the fact that progressive systemic sclerosis is often considered an autoimmune disease, with the underlying immune reaction perhaps triggering collagen production by skin fibroblasts [71]. Excessive collagen deposition is also noted by histopathologic examination of hamartomas of the collagen type, as well as in keloids and hypertrophic scars [72].

In attempts to elucidate the underlying molecular mechanisms leading to collagen deposition in fibrotic skin diseases, a series of studies has been published in *The Journal of Investigative Dermatology* [68,73–81]. These studies have primarily examined collagen biochemistry in fibroblast cultures established from patients with different forms of dermal fibrosis. The conclusions derived from several of these studies attest to the possibility that in many cases, collagen genes are overexpressed such that synthesis exceeds degradation. As a result of this imbalance, collagen accumulates, leading to a clinically recognizable fibrotic disease.

It is clear that fibrotic skin diseases comprise a heterogenous group of clinical conditions [72], and they also demonstrate distinct differences in

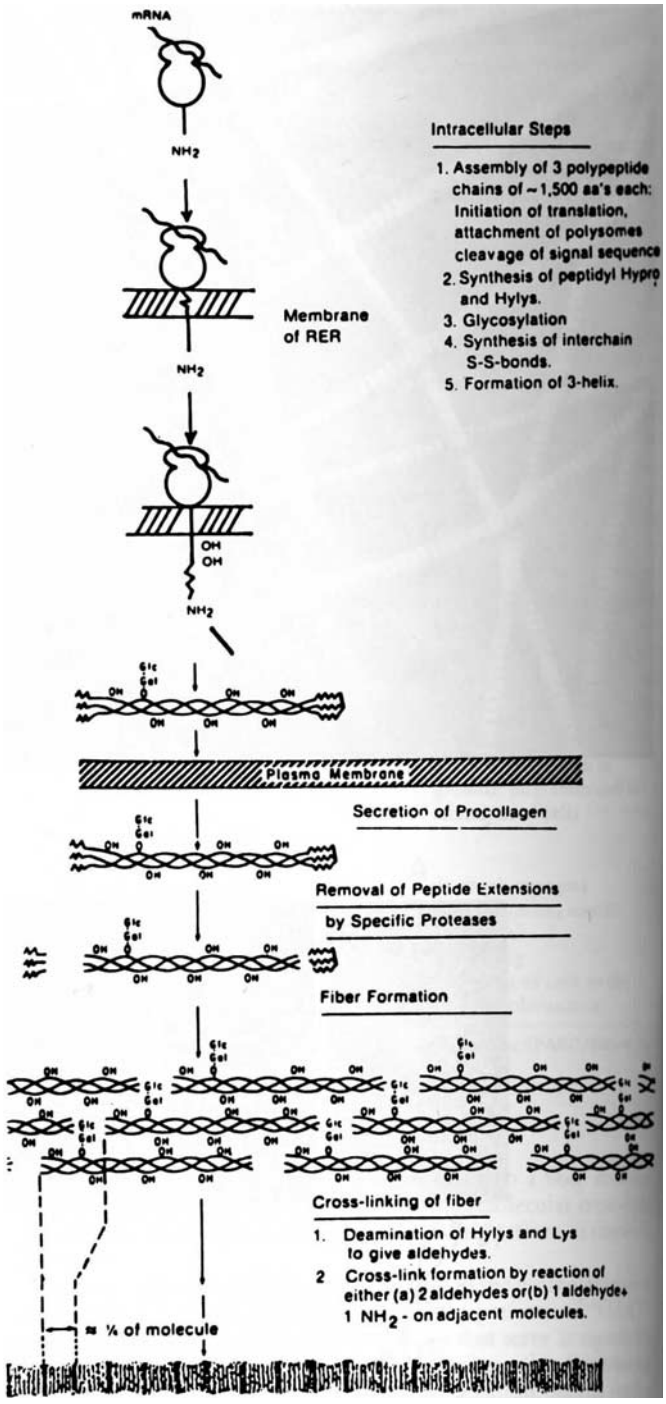

Figure 4. Biosynthesis of procollagen and the assembly of collagen molecules into extracellular fibers. Abbreviations: *mRNA,* messenger *RNA; aa's,* amino acids; *Hypro,* hydroxyproline; *Hylys,* hydroxyiysine; *Glc-Gal,* glucosylgalactose attached to a hydroxylysyl residue; S—S bonds, disulfide bonds; *Lys,* lysine; *NH_2-,* amino terminal end of the newly synthesized polypeptide chain or the ϵ-amino group of either a lysyl or hydroxylysyl residue. (From Ref 208, with permission.)

the mechanisms of connective tissue accumulation. For example, in the case of scleroderma, there is a coordinate activation of several matrix genes, so that the expression of type I and III collagen, as well as fibronectin genes is enhanced, resulting in generalized connective tissue accumulation [73,82]. In contrast, fibroblast cultures established from

Table II. Clinical Features, Biochemical Defects and Mode of Inheritance in Heritable Diseases with Collagen Abnormalities

Disease[a]	Major Clinical Features	Associated Biochemical Defect[b]	Mode of Inheritance[c]
Ehlers-Danlos syndrome	Hyperextensible skin, loose jointedness, fragility of tissues	Abnormalities in the structure or metabolism of collagen or procollagen	AR, AD, XR
Cutis laxa	Loose, sagging skin	Alterations in collagen or elastin	AD, AR, XR, NH
Osteogenesis imperfecta	Fragility of bones, thin skin	Structural abnormalities in type I collagen or procollagen	AD, AR
Marfan syndrome	Arachnodactyly and other skeletal abnormalities, aortic dilatation and dissection, heart valve abnormalities	Structural abnormalities in collagen, elastin or possibly other connective tissue components	AD
Homocystinuria	Skeletal abnormalities, thrombosis of the blood vessels, frequent mental retardation	Abnormal cross-linking of collagen	AR
Menkes' syndrome	Tortuosity of the blood vessels, brittle hair	Defective cross-linking of collagen and elastin	XR
Alkaptonuria	Degenerative changes of cartilage, osteoarthrosis	Deficient lysyl hydroxylation in collagen	AR
Focal dermal hypoplasia	Local areas of absence of dermis, skeletal abnormalities	Compromised growth potential of skin fibroblasts	XD
Familial cutaneous collagenoma	Multiple dermal nodules	Accumulation of collagen in the skin	AD
Epidermolysis bullosa	Fragility of the skin	Increased collagenase activity in the dystrophic recessive form, decreased glucosyl transferase in one family with EB simplex	AD, AR

[a]This list includes only those inherited diseases in which there is some biochemical evidence of collagen abnormalities. Most of these conditions represent a group of diseases with clinical, genetic, and biochemical heterogeneity.
[b]The biochemical abnormalities have been demonstrated only in a limited number of patients in each group, and it is not known whether the molecular aberrations are the same in each patient of any given type.
[c]AD: autosomal dominant; AR: autosomal recessive; NH: not hereditary; XD: X-linked dominant; XR: X-linked recessive.

keloid tissue demonstrate a selective activation of type I collagen genes, resulting in a significantly altered ratio of type I/III procollagens, as determined at the mRNA level [36,78]. In some fibrotic diseases, decreased collagen degradation, resulting from reduced collagenase activity, contributes to the accumulation of collagen in tissues [79,81]. In addition to collagens type I and III, a recent study [83] has demonstrated enhanced expression of type IV procollagen genes in fibroblast cultures established from a patient with lipoid proteinosis, a heritable disorder of basement membrane accumulation in the skin and vascular tissues (see below). All these studies suggest that altered expression of different matrix genes can lead to clinically recognizable diseases.

Diseases with Reduced Collagen Content of the Skin:–Lack of collagen deposition or increased degradation of collagen fibers by specific collagenases [84,85] can also lead to a clinical disease. Of particular interest to dermatologists has been epidermolysis bullosa (EB), a group of heritable diseases where the hallmark is fragile skin [86–88]. Several studies, have attested to the possibility that enhanced collagenase expression might lead to dissolution of collagenous structures, thus explaining the fragility of skin. In particular, collagenase activities in the skin and fibroblast cultures from pateints with recessive dystrophic EB have been shown to be elevated [89–93]. Thus, the degradation of collagens, such as type VII collagen in the anchoring fibrils, may explain the tissue fragility in some cases of EB [94]. It is clear, however, that EB is both a clinically and biochemically heterogeneous group of diseases, and it is expected that in several other cases a structural aberration in basement membrane zone genes may be the underlying defect.

Loss of collagen is also a clinical feature of focal dermal hypoplasia, an X-linked dominant disease with apparent lethality in hemizygous males [95]. Examination of fibroblast cultures from a patient with focal dermal hypoplasia has led to the suggestion that the primary abnormality resides in the fibroblasts, rather than in collagen metabolism per se [96]. Specifically, the fibroblasts from this case were shown to have a reduced proliferative capacity and lowered saturation density, phenomena which might explain reduced collagen deposition in the dermal microenvironment.

Regulation of Collagen Gene Expression The above examples are clear indications that the control of collagen deposition has to be closely regulated in order to maintain physiologic levels of collagen. This notion has been the impetus for a large number of studies which have addressed the control mechanisms regulating the expression of genes coding for collagen or collagenase. With respect to collagen gene expression, several variables have been tested, including tissue culture environment [97–103]. In particular, ascorbic acid has been shown to enhance collagen production in skin fibroblast cultures by at least two mechanisms. On the post-translational level, ascorbic acid is required for hydroxylation of prolyl residues [100,102]. The presence of hydroxyproline stabilizes the triple-helical conformation, a prerequisite for secretion of collagen at an optimal rate. The triple-helical conformation also prevents degradation of collagen polypeptides by non-specific proteases, thus leading to deposition of functional collagen fibers. The second mechanism by which ascorbic acid increases collagen synthesis involves enhanced transcription of at least type I and III collagen genes [103]. As a result of enhanced rate of transcription, steady-sate levels of the corresponding mRNAs are elevated and serve as a template for translation of preproα-chains. As a result, more collagen is being synthesized and deposited in the presence of ascorbic acid. Based on

Table III. Classification of Cutaneous Diseases with Dermal Fibrosis[a]

I. *SCLERODERMA*
A. Systemic Scleroderma
1. Progressive systemic sclerosis
2. CREST syndrome
B. Localized Scleroderma (Morphea)
1. Circumscribed morphea
2. Linear morphea
3. Guttate morphea
4. Generalized morphea
II. *SCLERODERMA-LIKE SKIN CHANGES IN SYSTEMIC DISEASES*
A. Inflammatory Connective Tissue Diseases
1. Mixed connective tissue disease
2. Features of scleroderma in lupus erythematosus and dermatomyositis
3. Eosinophilic fasciitis
B. Metabolic and Immunologic Disorders
1. Chronic graft *vs.* host disease
2. Porphyrias
3. Phenylketonuria
4. Carcinoid syndrome
5. Scleredema with paraproteinemia
6. Juvenile-onset diabetes mellitus
7. Acromegaly
C. Premature Aging Syndromes
1. Werner's syndrome
III. *CHEMICALLY INDUCED DERMAL FIBROSIS*
A. Drugs
1. Bleomycin
2. Pentazocine
B. Chemicals
1. Polyvinyl chloride
2. Silicates
3. Organic solvents
4. Contaminated rapeseed oil (toxic oil syndrome)
IV. *CONNECTIVE TISSUE HAMARTOMAS OF THE COLLAGEN TYPE*
A. Inherited
1. Familial cutaneous collagenoma
2. Shagreen patches in tuberous sclerosis
B. Acquired
1. Isolated collagenomas
2. Eruptive collagenomas
V. *KELOIDS AND HYPERTROPHIC SCARS*

[a]Modified from reference 78.

these observations, ascorbic acid has been proposed to enhance collagen synthesis in patients with EDS. In particular, in some cases of EDS VI, the K_m of lysyl hydroxylase with respect to ascorbic acid, a cofactor for the enzyme, is increased [104]. Thus, feeding of these patients with relatively large quantities of ascorbic acid (2–4 g/day) may partially overcome the genetic defect and lead to clinical improvement.

Pbarmacologic Inhibition of Collagen Deposition Based on the knowledge emerging from the studies on regulation of collagen gene expression in normal situations, attempts have also been made to develop pharmacologic approaches to control excessive collagen deposition in fibrotic skin diseases [105,106]. Although many of these compounds have been effective in tissue or cell culture environment, their efficacy in clinical situations has been compromised by toxicity. Thus, there has been a distinct need for further development of novel approaches to control collagen accumulation in patients with connective tissue abnormalities. The progress in this area of dermatopharmacology was reviewed in the Journal in 1982 [106], but several newer approaches have been developed since then.

Inhibitors of Triple-Helix Formation: Particularly interesting are recent attempts to interfere with collagen deposition by disrupting the formation of triple-helical conformation. One of these approaches centers around selective inhibition of prolyl hydroxylation by derivatives of 3,4-dihydroxybenzoic acid, a specific inhibitor of prolyl hydroxylase with respect to α-ketoglutarate, which is a cosubstrate in the hydroxylation reaction [107,108]. Thus, inhibition of triple-helix formation leads to enhanced degradation of collagen polypeptides reducing the deposition of collagen fibers. It is of interest that doxorubicin, an anthracycline compound used for cancer therapy, similarly inhibits collagen production by inhibiting prolyl hydroxylation, in addition to its direct effects on collagen synthesis on the transcriptional and translational levels [109]. The latter observations would explain the compromised wound healing in patients treated with doxorubicin.

Inhibition of triple-helix formation has also shown to be the mechanism of action of several proline analogues which inhibit collagen deposition in tissues [106]. The proline analogues, and *cis*-4-hydroxyl-L-proline and azetidine carboxylic acid in particular, unlike the naturally occurring *trans*-4-hydroxy-L-proline, are incorporated into newly synthesized preproα-chains in place of prolyl residues [110]. For steric reasons, the presence of the analogues prevents the polypeptides from folding into a stable triple helical conformation, and consequently synthesis of extracellular collagen fibers is impaired. In addition to preventing the triple-helix formation, *cis*-4-hydroxy-L-proline and azetidine carboxylic acid reduce the plating efficiency and proliferation of human skin fibroblasts, and another proline analogue, 3,4-dehydroproline, interferes with the hydroxylation of prolyl residues to *trans*-4-hydroxy-L-proline [106,110,111]. As a result of these effects, proline analogues inhibit collagen deposition in cell or tissue cultures. Thus, development of these compounds may provide a means to interfere with excessive collagen deposition in fibrotic skin diseases.

Steroids: As the topical steroids are the most commonly used therapeutic modality in dermatology, considerable interest has been set forth in elaborating their effects on collagen synthesis in the skin. This interest is partly due to the fact that prolonged topical cortico-steroid therapy leads towards side effects, such as dermal atrophy and striae distensae, which clearly involve connective tissue alterations [112]. Early studies in animal models demonstrated that potent corticosteroids were able to inhibit collagen production, thus explaining deficient accumulation of collagen in steroid-treated animals [113,114]. Subsequently, numerous studies have addressed the inhibition of collagen gene expression utilizing skin fibroblast cultures (see Ref 112). In general, the results indicate that fluorinated corticosteroids are more potent inhibitors of collagen biosynthesis than their non-fluorinated counterparts, and that the therapeutic efficacy of these compounds roughly parallels the inhibition of collagen synthesis [115,116]. The inhibition of collagen gene expression by steroids apparently occurs both on the transcriptional, translational and post-translational levels.

Retinoids: Recently, the effects of retinoids on connective tissue biochemistry have also been examined using cell and tissue culture

systems. These studies are based on the observations that connective tissue fragility is associated with prolonged treatment with retinoids [117]. Also, preliminary clinical observations have suggested that retinoids might be helpful in the treatment of keloids [118] and scleroderma [119], two conditions characterized by excessive collagen deposition in tissues (see above).

Several studies utilizing *in vitro* incubation systems have attested to the possibility that all-trans-retinoic acid and 13-*cis*-retinoic acid are potent inhibitors of connective tissue formation [120–124]. Specifically, these two retinoids have been shown to suppress collagen synthesis by cultured skin fibroblasts and this inhibition appears to occur on the pre-translational level [120,124]. These studies would also suggest that retinoids might be helpful for treatment of connective tissue disorders. It should be noted, however, that initial reports suggesting beneficial retinoid effects in patients with scleroderma utilized an aromatic retinoid, RO-10-9359 [119]. This particular retinoid was found to be ineffective *in vitro* in inhibiting collagen production [124], and therefore, any clinically beneficial effect would probably be unrelated to inhibition of collagen production, as noted *in vitro*. It should be noted that topical application of all-trans-retinoic acid (Retin A) has been suggested to increase collagen production in animal models of solar elastosis, as judged by histology (see below). The discrepancy between *in vitro* and *in vivo* observations has not been resolved as yet but may relate to differential metabolization of retinoids in isolated tissues and cells vs whole animals.

ELASTIC FIBERS

Investigative dermatologists have shown considerable interest in elastic fibers during the 50 years of publication of the Journal. This curiosity is well justified on the basis of our current understanding that elastic fibers play an important role in the structure and function of the skin, providing elasticity and resilience. Although the elastin content of normal adult skin is only about 2–4% of its dry weight [4,125,126], alterations in the mechanical properties of the skin accompanying aging or selected heritable disorders are clearly associated with abnormalities in the elastic fibers [127,128].

Fiber Structure Elastic fibers consist of two biochemically and ultrastructurally distinct components: (a) Elastin, a well-characterized connective tissue protein, and (b) the elastic-fiber-associated microfibrillar component, a less well-characterized complex of glycoproteins [129], Visualization of elastic fibers by scanning electron microscopy, after removal of other extracellular matrix components, reveals a randomly oriented, interconnected fiber network (Fig 5). It is conceivable that this network structure provides the elastic properties to the skin through its ability to rapidly recoil after being mechanically stretched.

The soluble elastin precursor polypeptide, tropoelastin, consists of approximately 700 amino acids, and the primary sequence of human, bovine, rat, and chick elastin has been elucidated through sequencing of the corresponding cDNAs (for human sequences, see Refs 130–132). Examination of elastin gene structure reveals that tropoelastin sequences are encoded by a large number of separate exons which correspond to alternating hydrophobic and cross-link domains (Fig 6). These exons are separated by unusually large in-trons, and consequently, only about 7% of the elastin gene contains coding information [130,133,134]. An interesting, and potentially important, phenomenon observed in the post-transcriptional processing of elastin precursor mRNA molecules involves alternative splicing, a phenomenon that results in the synthesis of mRNAs of slightly differing nucleotide sequences [130–132]. These mRNAs can serve as templates for the synthesis of polypeptides which differ in their primary sequences. As a result of alternative splicing, different isoforms of elastin are synthesized, and the assembly of these polypeptides may lead to formation of fibers that have different elastic properties.

Following translation of the tropoelastin mRNAs, the individual polypeptides are secreted into the extracellular space, and together with the microfibrillar component, they assemble to form an elastic fiber network [135]. The fiber structure is stabilized by formation of covalent cross-links, desmosines (desmosine and isodesmosine), in a reaction catalyzed by lysyl oxidase [129]. These two amino acid derivatives are not present in any other mammalian protein besides elastic fibers, and consequently, desmosine can serve as a specific marker for the quantity of crosslinked elastic fibers in tissues, such as skin [126,136].

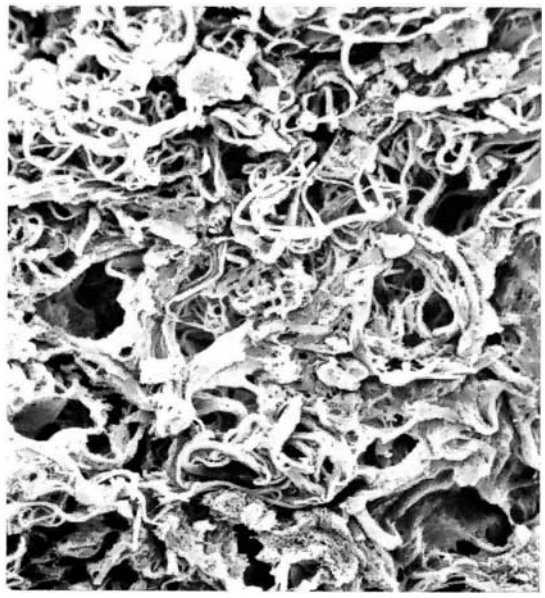

Figure 5. Scanning electron microscopy of the elastic fibers in human skin after removal of other extracellular matrix components. Note the randomly oriented meshwork structure consisting of elastic fibers of varying widths.

As indicated above, the microfibrillar component is poorly characterized, but it has been suggested to consist of aggregates of acidic, cysteine-rich glycoproteins [137]. One such protein may be fibrillin, a partially characterized glycoprotein that co-localizes with the elastic fibers [138]. Although the role that fibrillin may play in the assembly of elastic fibers is not currently understood, preliminary observations have suggested a fibrillin deficiency in some patients with the Marfan syndrome [139].

Aberrations in Diseases *The Journal of Investigative Dermatology* has served as a forum for several publications elucidating elastin abnormalities in both heritable and acquired cutaneous diseases. The progress in understanding the basis of such diseases has been documented on several occasions, including comprehensive review articles in 1979 and 1982 [127,129]. The progress since 1982 has been greatly accelerated by the improvement in techniques available for biochemical and molecular analysis of elastic fibers. For example, highly specific and sensitive radioimmunoassays for desmosines have been utilized to quantitate the amount of crosslinked elastic fibers in tissues [136]. Using computerized morphometric analyses, a correlation can be made between the histopathologic findings on elastic fibers and the concentration of desmosine in human skin [126]. These techniques have been applied to several cutaneous diseases. For example, the skin lesions of the Buschke-Ollendorff syndrome, known as dermatofibrosis lenticularis disseminata, which by histopathologic examination demonstrate an increase in elastic fiber content, also contain elevated levels of desmosine [140]. In addition to quantitation of elastic fibers by desmosine assays, the availability of specific cDNAs encoding human elastin sequences has allowed quantitation of elastin gene expression in tissues and cell cultures [131,133,141]. An example of the utilization of the techniques of molecular biology to study elastin is provided by cutis laxa, a systemic disease which affects skin, lung, and the blood vessels. In a recent study [142], skin fibroblast cultures established from patients with autosomal recessive cutis laxa exhibited significant reductions in

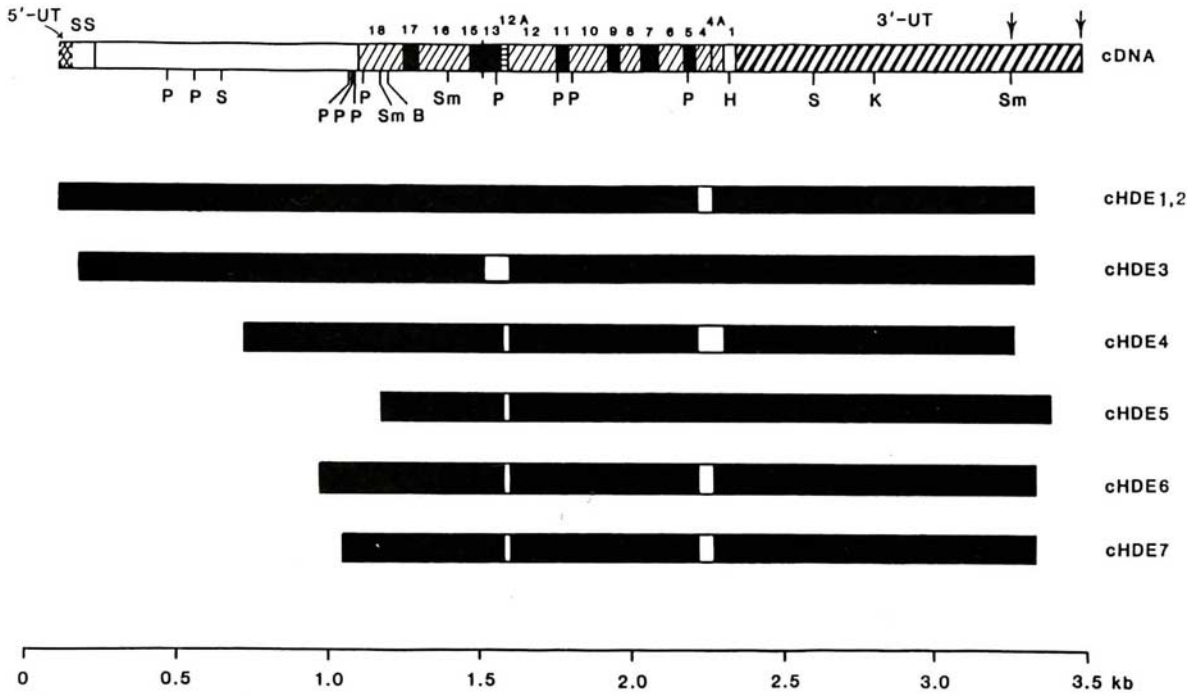

Figure 6. Schematic presentation of seven human skin fibroblast elastin cDNAs. Clones cHDEl through 7 are depicted below the composite cDNA. *Open boxes* represent exons which are differentially spliced from the respective cDNAs. The *bottom line* represents a 3.5 kb scale corresponding to the full-length human elastin mRNA. The composite cDNA encompasses: (1) ~1 kb of 3′ untranslated sequence (3′-UT) (▨) containing two polyadenylation signals (↓); (2) ~2.2 kb of translated sequence which includes two cysteinyl residues in exon 1, hydrophobic exons (▨), putative cross-link exons (■), and the 78 bp signal sequence (SS): and (3) a 49 bp 5′ untranslated sequence (5′-UT) (▩). Restriction endonucleases used: BamHI *(B),* HindIII *(H),* KpnI *(K),* PstI *(P),* Sad *(S),* SmaI *(Sm).* (From Ref 132, with permission.)

elastin gene expression, demonstrated at the mRNA steady-state levels by molecular hybridizations (Fig 7). Assuming an equal translational efficiency of the individual mRNA species from the control and cutis laxa cells, the reduced steady-state abundance of elastin mRNAs would lead to deficient production of tropoelastin polypeptides, and consequently, to reduced deposition of elastic fibers. This proposed scenario would explain the paucity of elastic fibers in the affected tissues in cutis laxa, and would correlate with the phenotypic characteristics of these patients [126]. It should be noted that in some cases with cutis laxa, the reduction in elastic fibers results from enhanced degradation by elastases [143,144]. For example, in a case with severe cutis laxa and pulmonary emphysema, significantly elevated levels of an elastase-like metalloprotease were noted in serum [144]. Although the origin of the serum elastase-like metalloprotease has not been established, fibroblasts are known to contain similar enzymes that are capable of degrading the human skin elastic fiber system [145]. Furthermore, in patients with acquired cutis laxa, with preceding inflammation, the release of neutrophil elastases may play a role in elastolysis. Thus, either reduced deposition or enhanced degradation of elastic fibers can lead to cutis laxa with similar clinical phenotype.

Cutaneous Aging Investigative dermatologists have devoted considerable efforts toward disclosing the molecular mechanisms underlying cutaneous aging. The early studies were primarily morphologic, demonstrating accumulation of elastotic material in actinically damaged skin and loss of elastic fibers during chronologic aging [146–148]. Recent biochemical studies have indicated that cultured human skin fibroblasts actively express the elastin gene, and these cells are the likely source of dermal elastic fibers [131,141,142]. The synthesis of elastin is initiated relatively late during fetal development and remains at a high level over several decades of post-natal life [131,151]. However, around the sixth decade, elastin synthesis appears to precipitously decline (Fig 8) [131]. This observation would account for loss of elastic fibers as part of innate aging affecting skin. Mechanistically, innate aging may be somewhat analogous to cutis laxa where elastic fibers can be deficient either as a result of reduced synthesis or increased degradation (see above).

The mechanisms leading to accumulation of elastotic material in actinically damaged skin, or even the exact composition of this material, are not entirely clear at this point, although it has been suggested that it is primarily composed of elastin and microfibrillar proteins with co-distributing fibronectin [152]. It has been postulated that UV irradiation, and UV-A irradiation in particular, might trigger fibroblasts to make excessive amounts of elastin. However, because of aberrant, poorly understood control mechanisms, these molecules do not assemble into functional elastic fibers, but demonstrate a pleomorphic appearance instead. The latter situation may be analogous to elastoderma, a disease of aberrant elastin accumulation within the skin [153].

Several studies have also attempted to reproduce the actinic elastosis in animal models using ultraviolet light irradiation. Many of these studies have been successful in inducing accumulation of elastotic material in the skin of the experimental animals. The accumulation of elastotic material has been demonstrated both by morphologic assessment, immunostaining, and biochemical analyses [154–162]. These animal models have then served as a test system to study the prevention and repair of actinic damage by compounds such as sunscreens and the retinoids [163–165]. In particular, sunscreens and retinoids have been shown to promote repair of actinically damaged skin by enhancing synthesis of dermal collagen, which replaces the elastotic material in the upper dermis. The biochemical mechanisms of this repair process have not been elucidated as yet, but it is conceivable that similar repair mechanisms may be operative in the skin treated with topical all-*trans*-retinoic acid (Retin-A) for reversal of cutaneous aging. It is of interest that the Journal served as forum for early publications on the development of paraaminobenzoic acid as a sunburn-protecting agent [167,168]. These

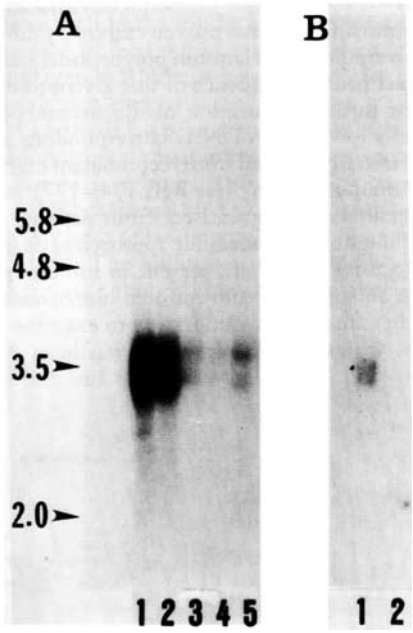

Figure 7. Northern transfer analysis of RNA isolated from cultured skin fibroblasts and hybridized with human elastin cDNA. *A:* total RNA (15 μg per lane) from control cells (*lanes 1* and *2*) and from three patients with autosomal recessive cutis laxa (*lanes 3–5,* respectively) was electrophoresed on a 1% agarose gel, transferred to nitrocellulose, and hybridized with a 2.5 kb human elastin cDNA labeled with $\alpha[^{32}P]$dCTP. *B:* 1 μg of poly(A)$^+$RNA *(lane 1)* or 10 μg of poly(A)$^-$-RNA *(lane 2)* from the same control fibroblast strain as shown in *panel A, lane 1,* was electrophoresed as in *panel A,* and hybridized with an exon 1-specific 35-base oligomer 5′-end-labeled with $\gamma[^{32}P]$ATP. Both the elastin cDNA and exon 1-specific oligomer hybridize with mRNA transcripts in the range of 3.5 kb, as estimated by parallel hybridizations with a human pro-α1(I) (5.8 and 4.8 kb polymorphic transcripts) and β-actin (2.0 kb mRNA) cDNAs. (From Ref 142, with permission.)

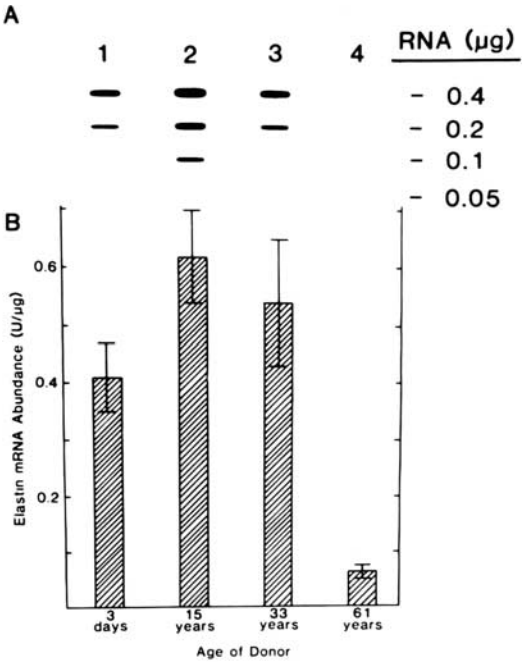

Figure 8. Elastin mRNA steady-state abundance in skin fibroblast cultures established from individuals of varying ages. *A:* Slot-blot hybridizations. Poly(A)$^+$RNA was isolated, dotted on the nitrocellulose filters, in varying amounts as indicated at the *right* (0.4–0.05 μg) and hybridized with a human elastin cDNA (cHE-1) labeled with $[^{32}P]$dCTP. The figure represents the autoradiogram of the $[^{32}P]$cDNA/mRNA hybrids. *Lanes 1–4* represent RNA isolated from fibroblast cultures established from individuals with ages of 3 d, 15 years, 33 years, and 61 years, respectively. *B:* Quantitation of elastin mRNA levels. The abundance of elastin mRNA was quantitated from the slot-blot hybridizations shown in *A* by scanning densitometry. Values are expressed as densitometric units (U)/μg of poly(A)$^+$RNA dotted, and corrected for the abundance of β-actin mRNA levels in the same preparations, as determined by parallel hybridizations with ^{32}P-labeled human β-actin cDNA. The values represent mean ± SEM of three to five determinations in the linear range of the mRNA hybridization curve from triplicate cultures of the same cell strain. (From Ref 131, with permission.)

studies were authored by Dr. Stephen Rothman, considered by many to be the father of modern investigative dermatology in the United States [167,168].

THE BASEMENT MEMBRANE ZONE

The progress made in understanding the complexity of specialized matrix structures within compartmentalized areas of the skin is best exemplified by the characterization of the basement membrane zone defining the dermal-epidermal junction. The basement membrane zone was early recognized histologically as an amorphous, poorly defined structure separating the epidermis and dermis, which matures during the seventh fetal month [169]. The complexity of this specialized compartment was initially suggested by immunologic observations demonstrating that sera from patients with certain autoimmune diseases specifically stain the basement membrane zone (Fig 9) [170–171]. It was clear, however, that several different epitopes were involved, suggesting complexity and molecular heterogeneity of the basement membrane zone [172], At the present time, it is recognized that basement membrane is a highly complex structure, containing at least eight distinct components, including type IV and VII collagens, laminin, nidogen, heparan sulfate proteoglycan, bullous pemphigoid antigen, fibronectin, and SPARC/BM-40/osteonectin (Table I).

Basement Membrane Components Individual components of the basement membrane zone demonstrate specific interactions which result in the formation of an organized, functional meshwork structure (Fig 10). Specifically, the major component of the basement membrane zone, type IV collagen, serves as an attachment site for basal keratinocytes [42]. At the same time, type IV collagen interacts with type VII collagen present in the anchoring fibrils (see above). These interactions apparently play a major role in securing a stable association between the epidermis and dermis in normal human skin [40].

Characterization of some basement membrane zone components utilizing recombinant DNA technology, has demonstrated the power of these state-of-the-art techniques. A good example of the utility of these techniques is offered by the studies that have elucidated the primary sequence and molecular structure of laminin [173–177]. This basement membrane zone protein is present in tissues in minute quantities, and it is insoluble and highly susceptible to proteolytic degradation. Consequently, isolation of laminin from normal human tissues has proven extremely difficult. Cloning of cDNAs corresponding to laminin polypeptides has allowed us to define the exact primary sequence of this glycoprotein, thus forming a basis for further delineation of the structure-function relationships (see below). The cDNAs corresponding to lamin sequences were initially isolated from recombinant cDNA expression libraries by immunoscreening (see Refs 174–177), suggesting that antibody detection utilizing antisera from patients with autoimmune diseases might also be a feasible approach to characterize other cutaneous structures which are present in minute quantities, and therefore, not amenable to conventional biochemical procedures. Such

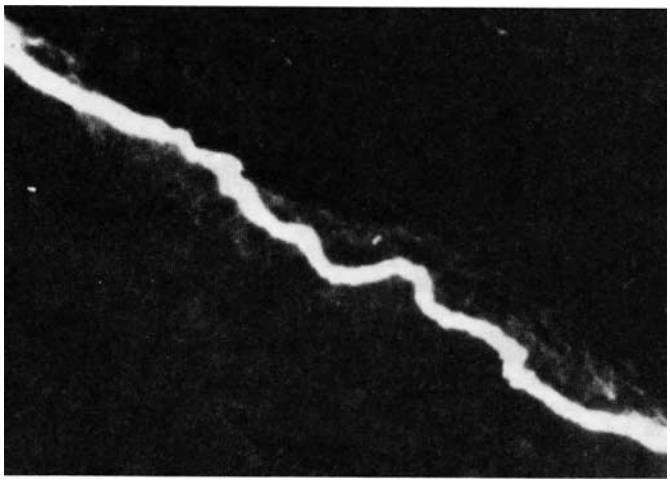

Figure 9. Indirect immunofluorescence of the dermal-epidermal basement membrane zone with a monoclonal antibody to an unidentified glycoprotein of ~150 kD. The staining is restricted to the dermal-epidermal basement membrane zone and does not reveal any staining of the adnexal structures, suggesting molecular heterogeneity of basement membranes. (From Ref 209, with permission.)

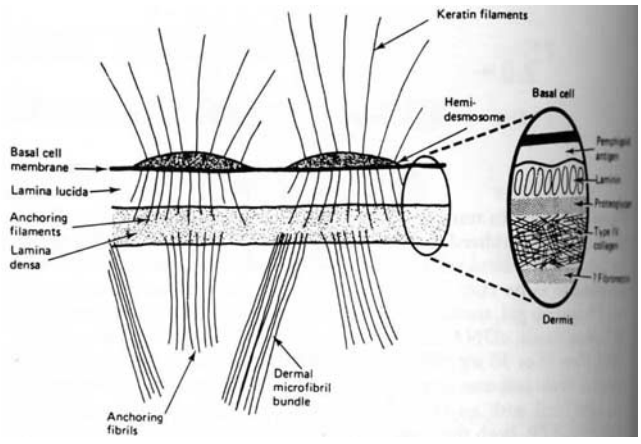

Figure 10. Schematic presentation of the dermal-epidermal basement membrane zone. An area on the *right* has been expanded to show the approximate spatial distribution of the components of the basement membrane. (From Ref 210, with permission.)

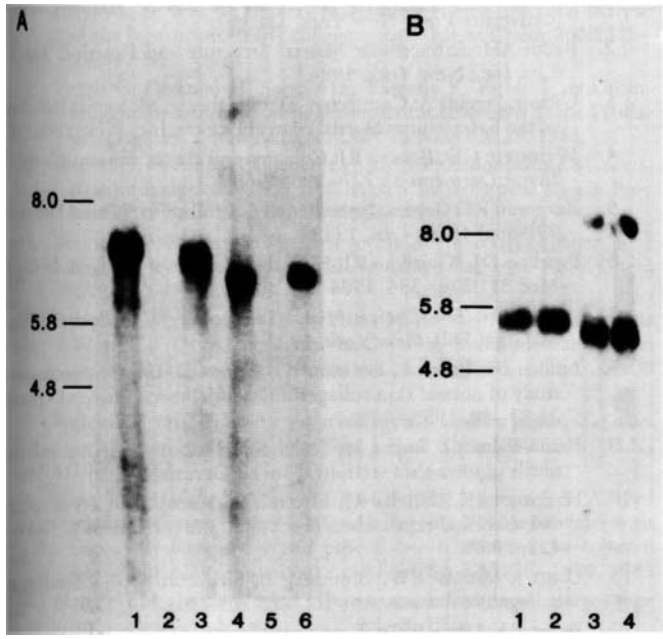

Figure 11. Northern transfer analysis of RNA isolated from human skin fibroblast cultures. Poly(A)$^+$RNA was isolated and electrophoresed on a 1% agarose gel. The RNAs, 1 μg per lane, were transferred to nitrocellulose filters and hybridized with type IV procollagen cDNAs *(panel A)* or laminim B1 and B2 chain cDNAs *(panel B)*. *Panel A:* Hybridizations with α1(IV) *(lanes 1–3)* α2(IV) *(lanes 4–6)* chain cDNAs. *Lanes 2* and *5* contain RNA from a 33-year old male, while *lanes 3* and *6* contain RNA from cultures isolated from a 14-week old fetus. For comparison, in *lanes 1* and *4*, RNA from fibroblast cultures established from a newborn male with perinatally lethal osteogenesis imperfecta is shown; this cell line has been previously shown to synthesize large quantities of type IV procollagen. *Panel B:* Hybridizations with human laminin B1 *(lanes 1* and *2)* or B2 *(lanes 3* and *4)* chain cDNAs. *Lanes 1* and *3* contain RNA from adult skin fibroblast cultures and *lanes 2* and *4* contain RNA from fetal cell cultures, as indicated in *Panel A*. The 5.8 and 4.8 kb markers indicate the migration positions of polymorphic transcripts of human pro-α1(I) collagen chain mRNA, while the 8.0 kb marker indicates the position of fibronectin mRNA, as determined by parallel hybridizations of RNA blots from the same electrophoretic runs. The mRNAs in *lanes 2* and *5 of Panel A* are not readily visible with this exposure; however, upon extended exposure to x-ray films, the bands are evident. These observations are consistent with differential regulation of basement membrane components, type IV collagen and laminin, during chronologic aging. (From Ref 186, with permission.)

approaches are currently underway to examine other components of skin, where the disease entity has been defined by the presence of specific autoantibodies, as in the case of pemphigus vulgaris, bullous pemphigoid, and acquired epidermolysis bullosa [178–181].

As indicated above, cloning and sequencing of cDNAs corresponding to laminin has allowed elucidation of the primary sequence of its subunit polypeptides, A, Bl, and B2 chains [177]. These sequence data provide a basis for the prediction of the tertiary structure of these molecules. These structural data can then be related to known biologic activities of laminin, and in this way, attempts to localize active domains within the molecule can be made. For example, sequencing of human laminin A chain cDNAs [177] has revealed the presence of a peptide sequence, arginine-glycine-aspartic acid (RGD), a sequence that has been shown to be crucial for the interaction with specific cell surface receptors for a variety of proteins, including fibronectin [182]. The RGD sequence in human laminin was found in the C-terminal portion of the A chain [177]. A proteolytic fragment of mouse laminin A chain has been shown to interact with a cell surface receptor [183]. Further biologic functions attributed to the globular domains within the laminin subunits are binding to basement membrane collagen type IV, nidogen, as well as to anionic heparin and heparan sulfate proteoglycans [173]. These interactions play a major role in the supramolecular assembly of the basement membrane zone components.

Cellular Origin of Extracellular Matrix Components Presently the cellular source of several extracellular matrix components has been identified using isolated dermal and epidermal cell cultures. Fibronectin, one of the predominant proteins in skin is produced by both fibroblasts and keratinocytes, as well as numerous other cell types in the skin and other tissues [184]. Type IV procollagen, the major structural component of the basement membrane, was initially thought to be a specific product of the basal keratinocytes [185]. However, more recent studies utilizing cDNA probes specific for the subunit polypeptides of type IV procollagen have demonstrated expression of the corresponding genes by normal dermal fibroblasts as well [83, 186]. In fact, fibroblast cultures established from biopsies of fetal skin contain abundant levels of

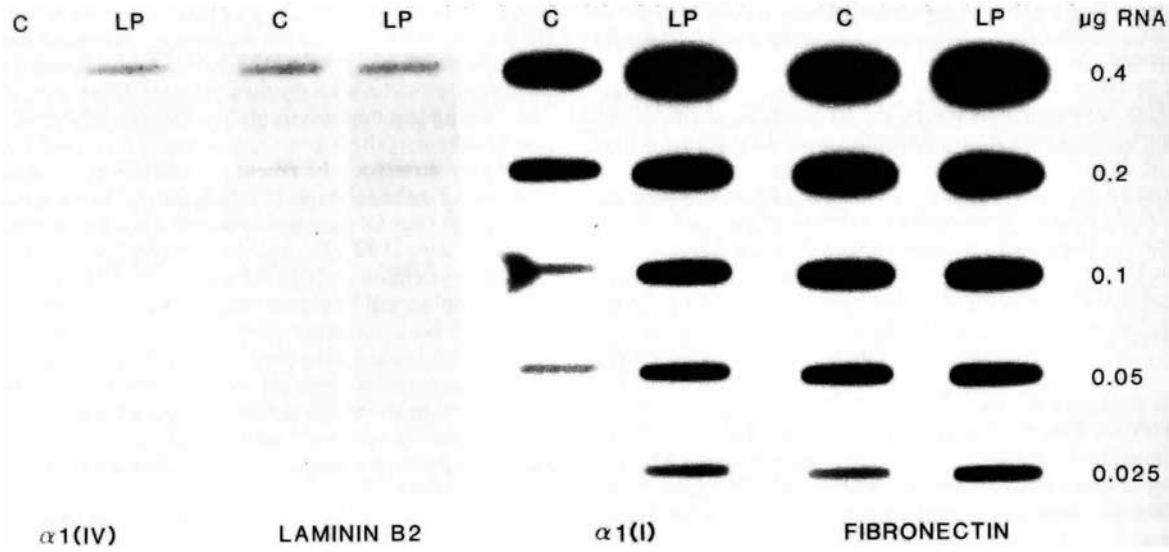

Figure 12. Slot-blot hybridizations of RNA isolated from control (C) or lipoid proteinosis *(LP)* fibroblast cultures. Poly(A)$^+$RNA was isolated, and different amounts, 0.4, 0.2, 0.1, 0.05, and 0.025 μg, were dotted onto nitrocellulose filters. The filters were hybridized with pro-α1(IV) and pro-α1(I) collagen, laminin B2 chain, or fibronectin cDNAs, and subjected to autoradiography for 7 d. Note the clearly detectable signal with a pro-α1(IV) cDNA in LP fibroblasts, reflecting elevated levels of the corresponding mRNA in comparison to control cells. Also note that the signal obtained with α1(IV) collagen and laminin B2 chain cDNAs is much lower than that obtained with α1(I) and fibronectin cDNAs, indicating that the mRNAs for basement membrane zone components are significantly less abundant than those for type I collagen and fibronectin in human skin fibroblasts (from Ref 83, with permission).

α1(1 V) ind α2(IV) collagen mRNAs, whereas fibroblast cultures established from adult skin express these two genes at very low levels [186]. Laminin synthesis has been demonstrated by metabolic labeling and immunoprecipitation or immunofluorescence both in keratinocyte and fibroblast cultures [185,187]. The expression of laminin A, B1, and B2 chain mRNAs by molecular hybridizations has confirmed the synthesis of laminin by normal human dermal fibroblasts [83,177,186]. The bullous pemphigoid antigen, a protein which is unique to the skin, is known to be a gene product of the basal keratinocyte [188,189], but the recent cloning of cDNAs for this gene will allow further analysis of potential expression of this protein by other skin cells [178,179]. Dermal fibroblasts and epidermal keratinocytes both synthesize type VII collagen, also known as the acquired epidermolysis bullosa antigen [190,191]. It is of interest to note that the human fibrosarcoma cell line HT 1080, which produces numerous basement membrane zone marcomolecules, does not synthesize type VII collagen, whereas epidermoid carcinoma and transformed amniotic cell lines do express the type VII collagen gene [192]. The cells responsible for the synthesis of nidogen and osteonectin (also referred to as BM-40, SPARC) in the dermal-epidermal basement membrane have not yet been defined. Although both antigens can be detected by immunofluorescence in mouse and human skin [193,194], neither dermal fibroblasts nor keratinocytes in culture have been examined directly for the synthesis of these proteins. Epithelial cells from other mouse tissues have been shown to express these antigens, however [195,196]. Several different proteoglycans are also synthesized by both fibroblasts and keratinocytes [197,198].

The implications of these *in vitro* and *in vivo* findings are that not only the basal keratinocytes, the cells which are juxtaposed to the basement membrane, but also dermal fibroblasts have the capacity to synthesize components of the basement membrane zone, and therefore contribute to the synthesis of the dermal-epidermal basal lamina during skin development.

Basement Membrane Zone Gene Expression Recently, we have demonstrated that cultured human skin fibroblasts express the genes encoding the basement membrane zone components, type IV collagen, and laminin [83,186,187] (Fig 11). As discussed above, the expression of these genes has been detected both at mRNA and protein levels. Demonstration of basement membrane zone gene expression by cultured fibroblasts allows the utilization of these gene probes and cell culture systems to examine heritable disorders affecting the basement membrane zone [83]. An example of such studies is the characterization of the molecular defect accompanying lipoid proteinosis (LP), a rare autosomal recessive condition of which one feature is reduplication of the basal laminae of the skin and vascular structures [199,200]. In a study by us, fibroblast cultures established from the lesional skin of a patient with LP contained over 4.5-fold higher steady-state levels of α1(IV) procollagen mRNAs than age-matched control cell cultures (Fig 12) [83]. The overexpression of the α1(IV) procollagen gene appeared to be selective in that the mRNA levels for the laminin B2 chain, fibronectin, type I procollagen, and β-actin were unaltered in the lesional fibroblasts. However, the mechanisms which result in the accumulation of type IV procollagen mRNA in LP are not known. This alteration could reflect an enhanced rate of transcription of type IV collagen gene or could result from greater stability of the corresponding mRNA. It is of interest to note that the laminin B2 chain mRNA levels remained unaltered in LP, suggesting that the reduplicated basement membranes seen in this disease may be deficient in laminin, and therefore, may not be entirely functional. These observations suggest that alterations in the expression of basement membrane zone genes can lead to clinically recognizable disease, analogous to accumulation of interstitial collagen in fibrotic skin diseases (see above).

Biologic Activities of Matrix Proteins Several extracellular matrix macromolecules appear to play key roles in biologic activities [201,202]. For example, during the early stages of the wound healing processes, exposed collagen matrices stimulate aggregation of platelets. Additionally, peptides derived from collagen, fibronectin, and elastin have all been shown to possess chemotactic activity for mononuclear cells. These cells in turn elaborate a variety of soluble factors capable of amplifying the wound repair processes [203–205]. Fibronectin also promotes several other aspects of cutaneous wound healing. During the formation of granulation tissue, fibronectin is thought to provide a

provisional matrix, along with type I and III collagens, allowing the reepithelializaton of the wound by keratinocytes at the margin of the injury [201,202]. Fibronectin also acts as a non-specific opsonin of cellular and bacterial debris during the initial inflammatory phase of the wound healing process, thus aiding in the removal of the debris by phagocytic cells [201].

The role of other noncollagenous glycoproteins, such as laminin, nidogen, osteonectin, fibrillin and elastin, in the wound healing processes has not yet been fully elucidated. As more is learned about the primary structures of these proteins through cloning and sequencing of their complementary DNAs and genes, specific biologic activities may be assigned to certain domains within the molecules. This information will undoubtedly provide valuable knowledge concerning their roles in normal cutaneous biology and how aberrations in their structures may result in cutaneous disorders.

FUTURE PERSPECTIVE

In the past five decades or so, much information has been gathered from work by many investigators who have examined the extracellular connective tissue matrix of the skin. The work has not been finished, however, and much remains to be elucidated on the structure and function of normal extracellular matrices. Even more strikingly, the extrapolations of our knowldge on features of normal cutaneous extracellular matrix to disease processes have been sparse, and our understanding of the molecular defects in many heritable and acquired diseases affecting the cutaneous structures is incomplete. It is clear that utilization of state-of-the-art recombinant DNA technologies will allow molecular dermatologists to pinpoint the underlying defects in the pathologic processes. Such information will clearly be helpful in further development of approaches for the diagnosis and treatment of skin diseases.

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