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# Molecular Genetics of Epidermolysis Bullosa

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**Blisters following minor trauma characterize epidermolysis bullosa, a group of hereditary diseases of the skin. In the simplex type, epidermal basal cells are fragile, and mutations of genes encoding keratin intermediate filament proteins underlie that fragility. In the dystrophic types, the causative mutation appears to be in the gene encoding type VII collagen, which is the major component of anchoring fibrils. These recent findings afford solid evidence that at least one function of the cytoskeletal intermediate filament network is the provision of mechanical stability and that anchoring fibrils indeed do anchor the epidermis to the underlying dermis.**

Job 7:5—*My skin closeth up and breaketh out afresh.*

Skin diseases present a particular fascination and, by their visibility, cause particular embarrassment for the afflicted. They are so common that all of us have had some personal experience with them on our own skin or at least on the skin of others we see about us. Hence, it is not surprising that skin disease was so prominent among Job's trials. Because the eye can make such fine discriminations, dermatologists can appreciate an enormous number of skin diseases, and many of these are inherited. Among the latter, a group of diseases marked by blistering, epidermolysis bullosa, is quite prominent, less for their frequency and more for their distinctive appearance. Quite recently, our understanding of the defects underlying this group has advanced markedly through the application of the techniques of molecular genetics. As is typical for such studies, new understanding of normal functioning of skin molecules is inherent in the identification of the defects. Less typical, however, is that some of the tissue-specific technology that might be necessary for gene therapy of epidermal disease already exists: removal of skin, in vitro culture of keratinocytes, and reattachment of epidermal sheets at their normal site are techniques already used routinely for treatment of patients with burns. Hence, skin disease would seem an especially promising substrate for DNA-based therapies. In this review, I will focus on epidermolysis bullosa (EB), on the defective cytoskeletal keratins underlying EB simplex and the defective dermal collagen underlying dystrophic EB, and will mention some of the array of hereditary skin diseases, the underlying defects of which are likely to be discovered soon by further applications of molecular genetics.

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## Hereditary Blisters of the Skin

The term "epidermolysis bullosa" was attached a century ago to a clinically heterogeneous group of disorders characterized by blistering of the skin following minimal to inapparent trauma. Onset is early in life, often neonatally or during infancy, and, except for the rather uncommon EB aquisita, the disease is heritable. The inheritance may be dominant or recessive, and the disease may be severe or mild and may be generalized or localized to a portion of the skin surface. Most importantly, blisters may heal with mutilating scarring or with no scarring at all (*1*).

Although some workers had noted histologic heterogeneity previously, subclassification of EB was rationalized three decades ago when electron microscopy permitted accurate assessment of the blister site in the skin (Fig. 1) (*2*). Pearson initially and subsequently Anton-Lambrecht and others established that the split in all variants of EB simplex (termed "simplex" because healing generally occurs without scarring) is within the basal cells of the epidermis, such that the base of the blister contains remnants of the basal plasma membrane and the roof contains the nucleus and supranuclear portion of the basal cells. This site is quite different from that of ordinary friction blisters, in which the split is considerably higher, in the upper spinous cells of the epidermis (*3*). In junctional EB (one variety of which had been termed EB letalis because of its dismal prognosis) the split is within the basement membrane zone in the lamina lucida, and blisters heal with varying degree of scarring. In dystrophic EB, so called because chronic cycles of blistering invariably lead to scarring and, in more severe cases, to disability, inanition, and death, the split is in the upper dermis.

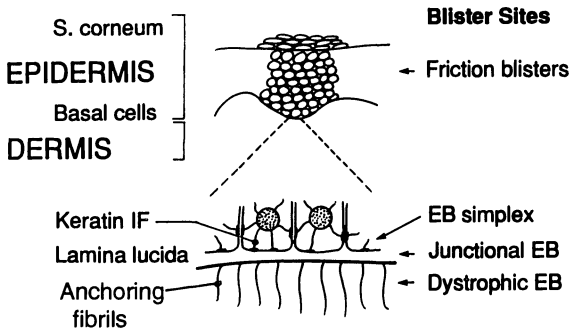


**Fig. 1.** Schematic drawing of histologic section of skin indicating sites of cleavage in friction blisters and in the three major types of epidermolysis bullosa.

At least part of the recent great increase in information derived from the clinic and from the laboratory is attributable to a concerted effort by the Dystrophic Epidermolysis Bullosa Research Association (DEBRA) of America, a patient advocacy group that has been remarkably effective in bringing EB to the attention of both the legislative and scientific communities. One result was the establishment of the National EB Registry, funded by the National Institutes of Health, that now has data on approximately 1800 EB patients in the United States. Such a large clinical sampling will allow real gains in knowledge of clinical course and responses to therapy as well as the identification of kindreds for investigators interested in studying pathophysiology. Although exact cause and effect are uncertain, at the very least there has been some temporal correlation between legislative "targeting" of research in these diseases and the new insights now emerging.

## EB Simplex and the Cytoskeleton

EB simplex (EBS) is characterized by mechanical fragility of the basal cells of the epidermis, and this fragility has been linked with mutations of genes encoding intermediate filament components of the epidermal cytoskeleton.

The cytoskeleton of nucleated cells extends throughout the cytoplasm and is composed of a series of linear structures that were postulated, on the basis of microscopic appearance, to provide stability to the cytoplasm. The structures appear in three size fractions: 25-nm tubulin-containing microtubules, 5-nm actin-containing microfilaments, and 8- to 10-nm intermediate filaments (IF).

IF generally are insoluble in physiologic buffers or salt concentrations but can be denatured by 8 M urea and will repolymerize when the denaturant is removed by dialysis. These protein components form a family whose members share a quite similar overall structure comprised of a central rod-shaped portion of approximately 310 amino acids flanked by globular heads and tails of varying size (*4*). The central rod consists of four longer stretches of α-helix interrupted by three shorter nonhelical linker regions. The basic building blocks of IF are parallel dimers wrapped about each other in a coiled coil configuration that apposes the hydrophobic residues found at positions one and four of repeating heptads.

Although essentially all eukaryotic cells contain IF, these IF are assembled from components that differ according to cell lineage and that can be grouped into six different subfamilies (Table 1). In general, the sequences of members of one subfamily share at least 50% identity, and those of different subfamilies share approximately 25% identity. All IF polypeptides except the keratins can form homopolymers in cell-free reassembly studies and, apparently, in vivo as well. By contrast, the IF of epithelial cells always are heterodipolymers, containing a relatively smaller type I (generally acidic) and a relatively larger type II (generally neutral to basic) component. This obligate pairing of a type I and a type II keratin molecule occurs at the first step of polymerization, when the parallel dimer is formed that then serves as the building block of the keratin IF (*5*). There are at least 20 epithelial keratins as well as perhaps another dozen keratins in epithelial derivatives (hair and nails). For convenience, these have been numbered according to their size and charge as detected by two-dimensional gel electrophoresis: keratins 1 through 8 are type II, and keratins 9 through 19 are type I. Within each type, the keratins are numbered according to size, for example K1 is the largest and K8 is the smallest of the type II keratins.

Several laboratories have identified and sequenced genes encoding individual keratins, and the chromosomal location of nearly a dozen of these has been reported. The genes encoding the type I keratins are clustered on chromosome 17q, and genes encoding type II on chromosome 12q.

Simple epithelia generally contain K8 and K18, and the basal layers (that is, the cells closest to the underlying connective tissue substrate) of stratified epithelia contain K5 and K14. Suprabasal cells of stratified epithelia express different keratin pairs that are characteristic of a particular epithelium. K1 and K10 are expressed in normal suprabasal epidermis, K4 and K13 in normal suprabasal esophagus, and K3 and K12 in normal suprabasal cornea. The predominant cells of the epidermis are so rich in keratin IF that they are termed "keratinocytes." Final differentiation of keratinocytes results in the stratum corneum, which consists of disk-shaped anucleate bags containing little more than keratin IF. These cellular remnants are somewhat homologous to the hemoglobin-stuffed anucleate mature erythrocyte.

## Functions of Intermediate Filaments

Judging from their near universal presence, one might expect that IF would serve some function highly essential to survival of individual cells; judging by their cell-specific expression, one might expect that IF would serve some function highly essential to the functioning of individual tissues. Yet, until recently, it has been difficult to prove any function for these near ubiquitous structures.

Efforts to demonstrate function experimentally have concentrated on trying to identify consequences of disruption of the IF network. Intracellular injection of an antibody to IF (anti-IF) into fibroblasts specifically caused vimentin IF to collapse into perinuclear aggregates without affecting microtubule or microfilament arrays, but these cells retained their normal size and shape, were capable of normal locomotion, and were mitotically active (*6*). Although drug-induced disruption of microfilaments and microtubules or, in some instances, of microfilaments alone, causes reorganization of intermediate filaments (*7*), no drug with disrupting effects limited clearly to IF has been described. Further efforts to study the consequences of IF disruption therefore depended on the development of DNA-based techniques, including for example transfection with DNA that encoded mutant IF monomers. Although the details of the results of transfection studies differ a bit depending on which IF gene is transfected and which cell is used for testing the mutation, several common conclusions have emerged.

First, despite the insolubility of IF in physiologic salt concentrations and neutral pH following disruption of cells, subunits produced by transfected DNA [or introduced into cells by microinjection (*8*)] are incorporated rapidly into the endogenous IF network.

Second, deletion of much of the nonhelical tails does not prevent such incorporation (*9*, *10*). However, expression of mutant polypeptides lacking even a few amino acids from either end of the helical rod

domains causes collapse of the endogenous IF network. The IF proteins may then form small aggregates in the cytoplasm and around the nucleus similar to aggregates that form after intracellular injection of anti-IF antibodies. Such disruption can occur when the mutant protein represents as little as 1 to 2% of the total IF complement (*11*).

Third, structural requirements for de novo polymerization of components into IF may differ from structural requirements for interaction of soluble components with preexisting IF, and hence transfection of cells lacking endogenous IF of the type under study may be useful. Small deletions near the $NH_2$-terminus of the globular head of desmin produce marked effects on de novo polymerization of desmin and vimentin (with which desmin can co-polymerize) but produce little effect on polymerization when endogenous vimentin IF are present (*12*). Similarly, transfection of keratin genes into fibroblasts, which lack endogenous keratin expression, indicated that, as found in cell-free assembly studies (*13*), only one member of a keratin pair must have both $NH_2$- and COOH-terminal domains for keratin IF network formation. Although type I and type II keratins can pair promiscuously, pairing of those subunits normally expressed together in the same cells produces a more normal appearing keratin IF meshwork (*14*).

Fourth, mutations in IF-associated proteins, such as desmoplakin, also can disrupt IF networks (*15*). This is a reminder that IF interact, in many instances, with the nucleus and with the plasma membrane. The latter interaction often is at desmosomes, intercellular junctions that appear to help mediate adhesion between cells. Desmoplakin, which is predicted to have a central α-helical rod flanked by a globular head and tail, is located at the cytoplasmic portion of the desmosome, where the keratin IF appear to interact (*16*). Expression of mutant desmoplakins lacking the head region or lacking both head and rod regions not only can disrupt both keratin and vimentin IF in cells expressing desmosomes but, surprisingly, also can disrupt vimentin IF even in cells that do not express desmosomes.

Thus, transfection studies have enabled the identification of molecular features important in IF formation. However, as in earlier studies, few changes in cell function were observed. Therefore, cytoplasmic IFs seem not to be critical for cellular viability (*17*). Indeed, at least one cell line appears to express no IF proteins at all (*18*). Hence, more recently, investigators have turned from the study of isolated cells to the study of interactions between cells following IF disruption. Early results of these studies have been "mixed" but have produced at least some glimmers of hope that functions for IF will be identified.

In one such study, glial fibrillary acidic protein (GFAP) expression was suppressed by stable transfection of an astrocytoma cell line with an antisense oligonucleotide specific for GFAP (*19*). These cells contained vimentin IF but were deficient in GFAP IF and failed to extend GFAP-rich processes in response to the presence of neurons in cell culture. The processes are believed to direct and support neuron migration and thus may be crucial for normal brain development. The exact mechanism by which GFAP IF permit glial process outgrowth is uncertain but it is of note that other responses of GFAP-deficient astrocytoma cells to the presence of neurons (for example, cessation of proliferation) are unimpaired.

Other investigators have studied the effect of IF disruption on early embryonic cells. Disruption of vimentin IF by injection of DNA that encodes mutant vimentin into fertilized frog eggs (*20*) or disruption of keratin IF in mouse embryoid bodies by targeted inactivation of both K8 alleles (*21*) failed to produce detectable changes.

Also, complete disruption of IF in myoblasts transfected with a complementary DNA encoding a desmin truncated at the COOH terminus (*22*) did not inhibit the interaction of these cells and the formation of contractile striated myofibrils. However, disruption of the vimentin IF and keratin IF of differentiating F9 embryonal carcinoma cells did inhibit the formation of a normal visceral endoderm layer (*23*).

Several years ago, it occurred to us that the cellular fragility of EBS basal keratinocytes resembled the cellular fragility of erythrocytes in a group of hereditary anemias caused by mutations of genes encoding the cytoskeletal proteins of these cells. The erythrocytes of patients with these anemias, which include hereditary spherocytosis, elliptocytosis, and pyropoikilocytosis, often are misshapen and fragile. Hence we tested the hypothesis that EBS might be a disorder of the keratinocyte cytoskeleton, the predominant components of which are the keratin IFs. We determined by linkage analysis that the EBS disease gene mapped in one large kindred to the type II keratin gene cluster on chromosome 12q, and in another kindred to the type I keratin gene cluster on chromosome 17q. In the latter family, a single nucleotide change caused substitution of a proline for a leucine in the middle of the longest helical region, an exchange which would be expected to disrupt locally the α-helix (*24*). Others also have reported linkage of EBS in other families to keratin gene clusters on chromosome 12q and 17q (*25, 26*).

As is so common, in retrospect a selective, careful, insightful reading of previous reports could have made obvious a connection between keratin IF and EBS. Thus, clumping of keratin IF is the characteristic abnormality visible with the use of electron microscopy in the Dowling-Meara subtype of EBS (*27*). Sutherland and Hinton reported a family with EBS and a heritable fragile breakpoint on chromosome 12q a decade ago, even before the first assignment of type II keratin genes to this location (*28*). Kitajima and colleagues in 1989 (*29*) observed with the use of light microscopy of keratinocytes cultured from the skin of several patients with EBS changes in the IF that were quite similar to those induced in normal keratinocytes by transfection of genes encoding a truncated keratin 14 (*9*).

These latter studies were part of an extended targeted effort by Fuchs and colleagues to identify critical aspects of keratin gene structure, initially by studying the effects on the IF network of the introduction of mutant keratins through in vitro transfection studies of epithelial cells. Most recently, they have constructed transgenic mice expressing a mutant K14 lacking the tail and the COOH-terminal 30% of the α-helical rod, thus continuing the experimental journey that had begun with reassembly studies in cell-free conditions and progressed through study of the effects of IF disruption first on individual cells and then on interactions of individual cells in culture. Not only did these transgenic mice have keratinocytes with keratin IF abnormalities similar to those seen in cells transfected with the same DNA constructs, but also the epidermis was startlingly fragile. Basal keratinocytes readily underwent cytolysis, and the mice died soon after birth (*30*). Taken together, the identification of keratin gene mutations in patients with EBS and the production of EBS-like changes in transgenic mice expressing mutant keratins indicated with reasonable certainty that keratin gene mutations do underlie at least some cases of EBS.

**Table 1.** Intermediate filament proteins.

| Type | Examples | Location |
|---|---|---|
| I | Keratins (acidic) | Epithelia |
| II | Keratins (neutral to basic) | Epithelia |
| III | Vimentin | Mesenchymal cells |
| | Desmin | Muscle cells |
| | Glial fibrillary acidic protein | Glial cells, astrocytes |
| | Peripherin | Neurons |
| IV | Neurofilaments | Neurons |
| V | Nuclear lamins | All cells |
| VI | Nestin | Central nervous system stem cells |

Subsequently, mutations were identified in three patients with the more severe EBS–Dowling-Meara. Interestingly, all three of these mutations are located at the ends of the α-helical rods, sequences that are highly conserved among all IF proteins. Thus, Coulombe *et al.* found two patients with single point mutations in K14 at positions two and three of the same codon with resulting substitutions of cysteine and histidine for the normal arginine at the $NH_2$-terminus of the rod (*31*). Lane *et al.* found one kindred with a single point mutation in K5 with resulting substitution of glycine for glutamic acid three residues before the COOH-terminus of the rod (*26*). Experimentally produced point mutations in these highly conserved helix boundary regions also have marked effects on cell-free IF assembly and on the endogenous keratin IF network when expressed in transfected keratinocytes (*32, 33*). By contrast, the effects of experimental introduction of proline residues into the helix region are much more subtle when assessed by cell-free assembly or transfection studies (*33*). Such results are consistent with the production of the less severe EBS-Koebner phenotype by the mutation that introduced a proline into the helical rod (*24*). Keratinocytes cultured from patients in the latter family have a keratin IF network that appears essentially normal (*34*), emphasizing again the importance of assaying IF function in the context of the whole animal.

Taken together, these findings provide strong evidence that the keratin IF network contributes to mechanical stability of the epidermis. Thus far, it is tempting to conclude that the clinical severity due to mutations in the keratin genes is correlated not with which gene is affected but rather with the location of the mutation and how deleterious it is to normal IF functioning.

Several uncertainties remain. First, keratin gene mutations may not underlie all cases of EBS. There is seemingly very firm linkage of one subtype of EBS, that found in one large family the proband of which came from the Norwegian town of Ogna, to the glutamic-pyruvic transaminase (GPT) locus on chromosome 8q, a site far removed from any known keratin gene (*35*). In addition, the disruption of epithelial cell keratin IF by transfection of a mutant desmoplakin gene is a reminder that keratin IF interact with other proteins such as plectin at unknown sites as well as at their ends in conjunction with the nuclear and plasma membranes. Hence, mutations of genes encoding IF-associated proteins might disrupt the keratin IF network and produce the EBS phenotype.

Second, patients with EBS display marked heterogeneity. There is heterogeneity at a genetic level in that EBS, which nearly always is inherited as an autosomal dominant trait, recently has been described in several families as an autosomal recessive trait with severe blistering, associated abnormalities such as muscle disease, and early death. There is heterogeneity at the clinical level in that blisters in kindreds with the EBS–Weber-Cockayne (EBS-WC) subtype are limited to the hands and feet, but blisters in the EBS-Koebner subtype are more generalized with relatively less fragility of the skin of the hands and feet. The disease in some patients with EBS-WC is so mild that diagnosis is made only when the patient joins the army and has the opportunity to stroll in stiff boots for previously unattempted distances. Thus, the incidence of case reports of EBS-WC increased in the early 1940s, and there is one report of a family with this disease in which the proband's father was discharged from the army because of intractable blisters of the feet during the American Civil War (*36*). Fuchs and colleagues have developed three additional lines of transgenic mice and found a correlation between severity and localization of blistering of the murine skin and severity of disruption of cell-free assembly of filaments (*37*). Yet it still is far from obvious why some human mutations cause blisters mostly on the hands and feet and minimally elsewhere, whereas other mutations produce few blisters on the hands and feet yet more elsewhere. Similarly, the more severe histologic abnormalities of EBS–Dowling-Meara indeed may turn out to correlate well with mutations of conserved regions at the ends of the helices, but it is unclear why blisters in these patients typically appear in crops and in a grouped (herpetiform) array. At the least, it will be important to identify far more mutations and to try to correlate these with as precise a clinical description as is possible.

Third, why do these patients have so few extracutaneous manifestations? K5 and K14 are present in the basal cells of all stratified epithelia, but perhaps only one third of EBS patients have oral blisters and erosions, and corneal blistering has been reported only rarely (*38*). Explanations based on differences in degree of physical trauma are convenient but quite possibly inadequate. Similarly, why do most patients have far more blistering in the summer than the winter (one young woman even was able to prevent blisters by cooling her feet with ice water before running the 100-yard dash), but patients with EBS–Dowling-Meara may have a remission of their blisters following a febrile illness?

These more detailed questions aside, the finding that keratin gene mutations can cause cellular fragility provides, for perhaps the first time, evidence for physiologic function of the IF network. This work argues strongly that one real function of IF is to impart mechanical stability to the cell, that is, that the IF network really does act in vivo as a cytoskeleton.

What other epidermal functions might be disrupted by IF gene mutations? One that comes immediately to mind in the skin is the production of a normal stratum corneum, for keratins comprise the great bulk of the protein complement of this outermost layer. A generation ago, investigators assumed that skin diseases with abnormal stratum corneum ("disorders of keratinization") might be disorders of keratin molecules. However, work by Elias and others a decade ago turned the attention of most in this field away from study of keratins to study of the lipids that seal the interstices between the cells of the stratum corneum. These junctions are important in stratum corneum cell cohesion and shedding. One well-studied disease of the lipids of the stratum corneum is recessive X-linked ichthyosis, in which impaired shedding of the stratum corneum is caused by deficient desulfation of cholesterol sulfate due to a lack of the enzyme steroid sulfatase (*39*). However, at least some patients with EBS–Dowling-Meara have thickened stratum corneum of the palms and soles. Could keratin IF abnormalities also cause thickening of the stratum corneum? This question is of particular interest in epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma), another autosomal dominant epidermal disease. This disease is characterized clinically not only by blistering due to cytolysis of keratinocytes but also by marked thickening of the stratum corneum. The fragile suprabasal cells contain clumps of K1 and K10, just as the fragile basal cells of EBS–Dowling-Meara contain clumps of K5 and K14 (*40*). Although transgenic mice with IF-related abnormalities of the stratum corneum have not yet been reported, it is of note that overexpression of keratin IF in hair (another product of terminal differentiation of the epidermis) causes weakening and ready fracture of the hair shaft (*41*).

More generally, might mutations of genes encoding nonepidermal IF proteins cause other diseases? What phenotype would be expected to result from disruption of IF of other cells? One such phenotype theoretically might be mechanical fragility of extracutaneous cells. Few other cells are likely to be subjected to mechanical insults similar to those of the epidermis, and extracutaneous homologs of EBS do not spring to mind. However, such a concept of cellular fragility may be too narrow because, as discussed by Humphries (*42*), the type III IF protein peripherin appears to be crucial for maintenance of the highly specialized photoreceptors of the retina. Mutations of the

gene that encodes peripherin underlie one form of photoreceptor degeneration both in humans and in mice (*43*). Although peripherin is widely distributed in the nervous system (just as keratins 5 and 14 are widely distributed in the epithelia), clinical findings attributable to these mutations have been limited to the eye.

## Dystrophic EB

Whereas EBS generally annoys, discomforts, and occasionally can be occupationally disabling, dystrophic EB (DEB), especially in the autosomal recessive form, can be life-ruining. The blister split is deeper, and, as with deeper burns, scarring ensues. Unlike with burns, however, there is not only one but rather multiple cycles of blistering, denudation, and healing, which may never be complete before new blisters form. The scarring commonly takes the form of webbing of the fingers, encasing the hand in a mitten-like contracture. Oral blisters and scarring, loss of teeth, and strictures of the cervical esophagus may combine to prevent adequate nutrition just when loss of erythrocytes and protein through unhealed erosions makes such nutrition especially necessary (*44*), and death in the second to fourth decade is common.

The electron microscopic appearance of skin from DEB led directly to competing hypotheses about the primary defect in this disease. The resemblance of the upper papillary dermis to the dermis of the resorbing tadpole tail, in which collagenase activity is known to be high, led to the hypothesis that excess collagenase activity might underlie DEB. Some, albeit not all, fibroblasts cultured from DEB skin also show increased release of collagenase (*45*). More recently, a reduction in number and abnormal appearance of anchoring fibrils (AF) has been noted, even in apparently never-blistered skin of affected newborns (*46*). AF appear by electron microscopy to be thin threads that insert into the lamina densa at the basement membrane zone between dermis and epidermis and stream downward into the superficial dermis. This position called forth the anchoring fibril title when no functional data were available.

During the mid-1980s, Burgeson and colleagues identified type VII collagen as the major constituent of cutaneous AF, initially by identity of banding patterns between AF and collagen VII and subsequently by biochemical characterization. By morphologic studies, they demonstrated that AF are composed of two molecules of collagen VII tethered to each other at overlapping ends. The dermal ends of AF insert into type IV collagen- and laminin-containing islands termed anchoring plaques (*47*). Hence, the AF form a net in the upper dermis through which fibers containing types I and III collagens are threaded, a most satisfying configuration for a structure mediating adhesion of the overlying epithelium to the subjacent dermis. The gene encoding type VII collagen (COL7A1) remained recalcitrant to isolation until recently, when antibodies to collagen VII purified from the sera of several patients with acquired EB (EBA) were used to identify a fragment of collagen VII cDNA from an expression library (*48*). Patients with EBA develop antibodies to type VII collagen with consequent blistering upon minor mechanical trauma. The COL7A1 gene is located at chromosome band 3p21, and an intragenic Pvu II restriction fragment length polymorphism has been identified. In all families with dominant DEB thus far tested, inheritance of the disease is linked to this site (combined lod score 8.77 and no recombinants), thus providing very strong evidence that defects of collagen VII and consequently of AF underlie dominant DEB (*49*).

Evidence was presented last year that recessive DEB was not linked to the collagenase gene (*50*), and study of the same families very recently has indicated that mutations of the COL7A1 gene underlie the recessive form of the disease as well (*51*).

Thus, although specific mutations have not yet been reported, it appears highly likely that DEB is the third true genetic collagen disease, joining osteogenesis imperfecta and some forms of Ehlers-Danlos syndrome, in which collagen gene mutations underlie bone and dermal fragility, respectively.

These results of the application of molecular genetics to study of inherited disorders of the skin are likely to be but the initial ripples of a wave of new information. Careful clinical delineation and then light and electron microscopic studies were the first two waves. This third wave similarly should produce new classifications of cutaneous ailments with consequent improved ability to diagnose. More excitingly, it is reasonable to hope that new therapeutic approaches based on fundamental understandings may allow us to wash clear the skin of at least some of the modern-day descendents of Job.

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# On the Molecular Genetics of Retinitis Pigmentosa

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**The human retina carries specialized neurons, the rod and cone photoreceptors, which absorb and transduce light energy and transmit impulses through the optic nerve to the brain. The most prevalent group of inherited retinopathies, affecting approximately 1.5 million people, is collectively termed retinitis pigmentosa (RP). Mutations responsible for RP have now been found in two genes encoding transmembrane proteins of the rod photoreceptor outer segment disc, and a number of additional causative genes have been localized. It is likely that characterization of the majority of such genes over the next few years will lead to a substantial elucidation of the molecular pathology of this debilitating group of hereditary conditions.**

The term retinitis pigmentosa (RP) is used to describe a genetically and clinically heterogeneous group of human inherited retinopathies. These debilitating disorders collectively represent the most frequent inherited forms of human visual handicap, with an estimated prevalence of approximately 1 in 3000 (*1*, *2*). RP may be inherited autosomal dominantly (adRP), autosomal recessively (arRP), or in an X-linked fashion (xlRP). A number of studies have estimated the relative proportions of these various forms of RP (*3*). Typically, arRP together with single isolated cases seems to be the most frequent, representing at least half of the families affected by the disease. In contrast, on average both adRP and xlRP are present in between 10 to 15% of RP families. However, estimates vary between studies and are affected by the extent of consanguinity, by ascertainment biases, and so on.

In the past 5 years many of the technical developments in human molecular genetics have been applied successfully in the study of RP and have led to a greater understanding of the underlying mechanisms of pathogenesis in this group of disorders. The rapid emergence of a genetic map of the human genome has facilitated the localization of genes responsible for some forms of RP. The development of polymerase chain reaction (PCR) technology, together with the identification of many highly polymorphic genetic markers based on simple sequence repeats, has speeded the localization of disease-causing genes. Moreover, the development of methods for rapid identification of sequence variation, such as single strand conformational polymorphism electrophoresis (SSCPE) (*4*), heteroduplex analysis (*5*), denaturing gradient electrophoresis (*6*), and direct sequencing of amplified DNA products (*7*), has provided a means of investigating genes that are considered to be "candidates" for a particular disease. As a result of such technical developments two xlRP genes have been localized to the short arm of the X chromosome (*8*). Furthermore, three adRP genes have been mapped: to the long arm of chromosome 3 (*9*, *10*), to the short arm of chromosome 6 (*11*, *12*), and to the pericentric region of chromosome 8 (*13*). More recently, mutations within the rhodopsin gene, which maps to 3q (*14*), and the peripherin/*RDS* gene (*15*, *16*), which maps to 6p, have been implicated as causative in some forms of adRP. Both of these genes encode proteins that traverse the outer segment disc membranes of the rod photoreceptor cells. Thus, we have begun to elucidate the cause of some forms of this large group of retinopathies. However, although we have made great strides in this direction, as yet many of the genes involved in RP have not been localized. Moreover the means by which mutant rhodopsin and peripherin proteins cause photoreceptor degeneration remain unknown. In this review we will discuss the recent developments in the field and the predicted future directions that research will need to take to address these important questions.

RP is a degenerative disease characterized initially by the development of night blindness, or nyctalopia, owing to the progressive death of the rod photoreceptor cells. Long before the outward signs of RP develop however, electrophysiological changes may be detectable in the retina. A test to screen for such changes is called an electroretinogram, or ERG. This measures the electrical activity generated by the retina in response to flashes of light. Although first discovered by the Swedish physiologist Holmgren in 1870 (*17*), it was not until 1945 that Karpe (*18*) documented that the ERG response was abnormal in patients with RP. Such abnormalities are usually recordable even in presymptomatic cases. Rod responses are reduced in amplitude but may be normal or delayed in timing (Fig. 1). Cone responses may also be affected, wave forms being abnormal in amplitude or timing or both. In advanced cases of the condition all responses are extinguished. This may be the case even where central vision is relatively well preserved.

The death of the rod cells precipitates more extensive tissue degeneration (Fig. 2). Cone cells begin to die off and the vessels supplying blood to the retinal membranes become attenuated. As a result, daytime vision becomes noticeably affected. The retinal epithelium itself becomes thinner, and as the pathological changes develop, deposits of pigment often build up on the retinal surface, the latter phenomenon accounting for the name of the disease. The visual fields become constricted, and affected patients develop tunnel vision and often lose all effective sight. [For comprehensive clinical reviews, see (*3*) and (*19*).]

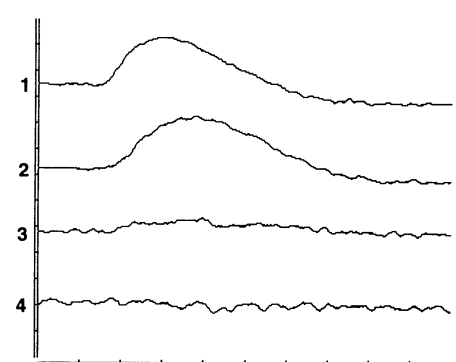


**Fig. 1.** Shown here are rod responses from a normal individual and from RP patients. Tracing 1 is from a normal individual. Tracings 2 and 3 demonstrate reduced amplitude and delayed responses from RP patients. Tracing 4 shows an extinguished response from a patient with advanced RP. The horizontal time base is 30 milliseconds per division. The vertical amplitude axis is 250 μV per division for tracing 1 and 100 μV per division for all other tracings.

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