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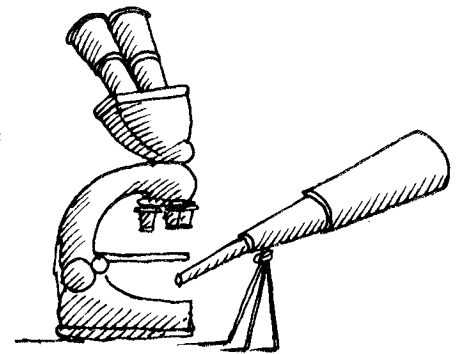
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PERSPECTIVES IN DERMATOPATHOLOGY

Kurt Stenn, *Editor*



Changing concepts of keratin

Leonard M. Milstone, M.D.

Recent biochemical studies have dramatically advanced our understanding of keratin and the process of keratinization. Unexpected and fundamental questions have arisen concerning the family of proteins most closely identified with keratinization, the tonofilament proteins, and terms such as "keratin" and "keratinization" have acquired new meanings. In this "perspective," keratinization will be used to describe the process of terminal differentiation in which the cells of certain epithelia are transformed into keratin.

The word "keratin," derived from the Greek word *keras* meaning horn, is well over 800 years old. It was originally intended to identify the organic substance or substances which gave horn-like cutaneous structures, such as hair, nail, and epidermal scale, their peculiar physical and textural properties. The predominantly protein nature of hair and nail was established in the 19th century and it was then concluded that keratin was a protein. Chemists in the early 20th century placed intact pieces of hair in x-ray beams and discovered that a characteristic diffraction pattern was produced. This pattern, called the α -keratin pattern, was consistent with the protein nature of keratin and was the first objective way of identifying keratin. In the 1950s, a controversy arose over the exact molecular interpretation of the α -keratin diffraction pattern.^(1,2) The pro-

tagonists were Francis Crick and Linus Pauling. The interest of these eminent scientists in the x-ray analysis of keratin conferred immeasurable importance on the diffraction pattern as the crucial identifying feature of keratin.

At about the same time, people began to notice bundles of 8- to 10-nm filaments (tonofilaments) in electron micrographs of keratinizing mammalian epithelia. Since the keratin-type diffraction pattern implied a highly ordered rope-like arrangement of the keratin proteins, it was only a short step conceptually to imagine that the tonofilaments were the filamentous form of keratins. During the ensuing 20 years a variety of methods was applied to the extraction and characterization of filament-forming polypeptides from keratinizing epithelia.⁽³⁻⁶⁾ These studies all indicated that a group of relatively insoluble, abundant proteins could be extracted from keratinizing epithelia and could form filaments *in vitro* with the dimensions of tonofilaments *in vivo* and the diffraction pattern of intact keratin. It seemed that the major keratin proteins had been identified. The only unanswered question was how (or whether) the filaments were able to confer the essential physical and textural properties implied by the word "keratin."

We now know that virtually all mammalian cells contain 8- to 10-nm intracellular filaments, generically referred to as intermediate filaments^(8,12); a single tonofilament is morphologically indistinguishable from any other intermediate filament. In addition to their morphologic similarity, these fila-

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ments also share certain biochemical and biophysical parameters such as amino acid composition, amount of α -helix, and x-ray diffraction pattern.⁽⁷⁾

There are four classes of 8- to 10-nm filaments, distinguishable by immunochemical reactivity, solubility, intracellular distribution, or polypeptide composition.⁽⁸⁾ These four classes are: 1) filaments found in most epithelial cells, composed of two to seven filament-forming polypeptides, called keratins or cytokeratins; 2) filaments found in fibroblasts and endothelial cells, composed of one filament-forming polypeptide, called vimentin; 3) filaments found in striated and smooth muscle cells, composed of one filament-forming polypeptides, called desmin or skeletin; 4) filaments found in nerve axons composed of three unnamed filament-forming polypeptides. Each class is formed by a set of unique polypeptides. Within the class of epithelial filaments some filament-forming polypeptides are common to several epithelia, while others appear to be tissue-specific.⁽⁹⁾ Within a given epithelial tissue, such as epidermis or hoof, each of the filament-forming polypeptides is distinct and none is a product or precursor of any of the others.^(10,11)

The heterogeneity of the filament-forming polypeptides has raised unanticipated questions. Why are there so many filament-forming polypeptides? Did they arise independently or from a common ancestral gene? While solutions to these questions require more information about the primary sequence of the filament-forming polypeptides, currently available indirect evidence—such as immunochemical cross-reactivity and one-dimensional peptide mapping—has provided some clues as to which filament-forming polypeptides are likely to have segments of similar amino acid sequence. The data indicate that, within a given tissue, epidermis or hoof, for example, the individual filament-forming polypeptides probably contain significant regions of sequence homology.^(10,11) In morphologically related tissues, such as hoof and esophagus, the filament-forming polypeptides are related as judged by immunological cross-reactivity,⁽¹³⁾ but have very different peptide maps.⁽¹¹⁾ Finally, there is no evidence, at present, for homologous sequences in filament-forming polypeptides from the four different classes of filaments.

The single most important void in our understanding of the various classes of intermediate filaments relates to their function and to whether the occurrence of different filament-forming polypeptides implies different functions for the filaments they form. We must be especially cautious, therefore, in interpreting changes in the number or character of filament-forming polypeptides in dis-

orders of keratinization. Three observations underscore this point. The lower portion of the epidermis synthesizes different proportions of the various filament-forming polypeptides than the upper portion.⁽¹³⁾ Any disease which accelerates the vertical transit of basal cells to shed squames may cause the stratum corneum to exhibit the same pattern of filament-forming polypeptides as the basal cells. Second, normal epidermal cells in culture synthesize a family of filament-forming polypeptides whose molecular weights are quite different from those of normal, viable epidermis.⁽¹⁰⁾ Third, many of the filament-forming polypeptides of hoof are identical to some of those in snout, but there are filament-forming polypeptides in each tissue which do not occur in the other.⁽¹⁴⁾ Do these different filament-forming polypeptides confer different functions on the filaments they form and do they account for differences in the final keratin produced by these tissues?

Other aspects of keratinization may have more to do with the textural and physical properties of keratin than do the filaments. Much of the toughness and resistance of a squame to physical and chemical damage is undoubtedly attributable to the intermolecular glutamyl-lysyl bonds of the cornified envelope or marginal band.⁽¹⁵⁾ A specific epidermal enzyme, epidermal transglutaminase, catalyzes the formation of these cross-links in terminally differentiating epidermal cells.⁽¹⁶⁾

A protein, called the stratum corneum basic protein, has been isolated from rat epidermis and is probably related to the histidine-rich protein of keratohyalin granules. This protein has the ability to cause lateral aggregation and clumping of epidermal intermediate filaments.⁽¹⁷⁾ It will be important to determine whether stratum corneum basic protein occurs only in epidermis and whether it can cause aggregation of filaments other than epidermal filaments. Intermediate filaments in different tissues form characteristic associations with each other. For example, filaments in epidermis clump in discrete tonofilament bundles, generally parallel to the surface but randomly arranged with respect to a vertical axis. Filaments in hair and nail are closely packed in parallel arrays with the long axes of the filaments parallel to the long axis of the hair or nail. Filaments in noncutaneous stratified squamous epithelia, such as those in calf esophagus, form bundles in which the packing appears to be looser than that of epidermal tonofilaments. Thus, the interaction of filaments with matrix or filament-associated proteins may be far more important to the overall properties of an epithelium than are any intrinsic differences in the filaments themselves.

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2. Crick, *Nature* 191: 1046 (1962).
3. Rudall, *In Advances in Protein Chemistry*, Vol. 17, Academic Press, New York, 1962, p. 1.

The intercellular diffusion barrier established by the cells of the stratum granulosum⁽¹⁸⁾ is another aspect of keratinization of enormous physiological importance. While little is known about how this barrier is established or what macromolecules are involved, it may protect the keratinized cell from external physical and chemical damage as well as prevent outward diffusion.

In summary, there are multiple events in the keratinization process, namely, synthesis of the filament-forming polypeptides, production of a cornified envelope, accumulation of stratum corneum basic protein, and elaboration of an intercellular permeability barrier. It is the sum of these individual events that accounts for the unique physical, textural, and biological properties of a given epithelium.

Our current understanding of the process of keratinization has made the term "keratin" ambiguous. Some dermatopathologists refer to the stratum corneum as "keratin" and some cell biologists call the filament-forming polypeptides of various epithelia "keratin." There is ample historical precedent for calling filament-forming polypeptides "keratins," but the occurrence of filament-forming polypeptides in all epithelia suggests that the filament-forming polypeptides are necessary but not sufficient for determining whether terminal differentiation will result in the production of a horn-like material. Accurate communication is best assured if "keratin" is used to signify the complex of organic substances produced during terminal differentiation of certain epithelia, and other names are used for individual components of that material. In 1952 Rudall proposed the name "epidermin" for the epidermal tonofilament polypeptides.⁽³⁾ In 1980 "epitheliin," rather than keratin, would be an appropriate generic name for the polypeptides which form the epithelial intermediate filaments. □

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