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CHEMICAL DYNAMICS IN EPIDERMAL DIFFERENTIATION*

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The sequence of morphological changes associated with the migration of the epidermal cell from the germinative layer to the cornified layer —cessation of mitosis, formation of tonofilaments, appearance of keratohyalin granules, loss of the nucleus and development of keratin fibers —must coincide with a series of molecular changes resulting from alterations in the cellular profile of catalytic activities. Undoubtedly, this chemical differentiation is genetically programmed in time and in substance, although environmental influences may modulate the activities of specific metabolic systems. It is not yet possible to sketch a dynamic picture of enzymatic differentiation in the epidermis but some aspects of the story are becoming evident.

NUCLEIC ACID SYNTHESIS

Mitosis and the synthesis of nuclear DNA in the epidermis, as observed autoradiographically after the administration of thymidine-H^3, appear to be normally restricted to cells in the basal layer in the rat and the mouse (1–4) but to include cells just above the basal layer in the human (5–6). The results of cytophotometric analysis of nuclei in the various cellular layers of the human epidermis have confirmed the expectation that only a very small fraction of the spinous cells have more than the diploid amount of DNA (7). It appears possible, therefore, that the decision to cease mitosis is implemented by inhibiting or degrading one or more of the requisite enzymes or by making unavailable necessary precursor molecules.

Preliminary results of experiments carried out by Vaughan‡ in this laboratory appear to rule out the absence of the enzymes or precursor molecules as the mechanism for inhibition of DNA synthesis in the differentiated cells. Free nuclei, isolated in 0.25 M sucrose from populations of separated differentiated cells (8), incorporated tritiated thymidine into DNA although no nuclear labeling was observed when whole cells were exposed to this precursor. Tritium became acid-insoluble and a large portion of the nuclei showed the characteristic autoradiographic picture of the formation of tritiated DNA from thymidine-H^3. In contrast, populations of intact basal cells, under comparable conditions, incorporated the tracer into nuclear DNA very well. Although these data are only qualitative, and it must be demonstrated that they represent the synthesis of new DNA rather than non-specific chain elongation or repair of damaged DNA, it would appear that all the enzymes necessary to convert thymidine-H^3 into DNA are present in the nuclei of differentiated cells. Furthermore it appears likely that all the precursors for DNA synthesis are present. It is not likely that the failure of intact differentiated cells to synthesize DNA is a function of membrane impermeability to tritiated thymidine since some cytoplasmic labeling has been seen in differentiated cells exposed to this tracer, *in vivo*.

Synthesis of RNA appears to occur normally in nucleated cells at all levels in the rat (9) and the normal human (10–11) epidermis as measured by the autoradiographic localization of uridine-H^3. If differentiative changes in the synthesis of RNA, with respect to amount and species, do occur, they have gone undetected to date.

Interestingly, the "parakeratotic" nuclei in psoriasis do not appear to be associated with the synthesis of RNA (12) although, in the epidermis of the newborn rat, kinetic autoradiography indicates that normal nuclei are an early site of the incorporation of tritiated uridine (9). In the psoriatic tissue, either the "parakeratotic" nuclei are functionally deficient in regard to the synthesis of RNA or the tracer does not reach these nuclei in sufficient amount to demonstrate synthesis of RNA.

Coincident with the normal loss of the nucleus in the transition from the granular to the cornified state, the cell loses its nucleic acids. By the usual autoradiographic techniques, no label from tritiated uridine or thymidine are found in the cornified cells (1, 9). This observation should provide an experimental "handle" for investigating the phenomenon of nuclear degeneration as a function of cornification.

PROTEIN SYNTHESIS

From ultrastructural observation of cells in the various layers of the normal mammalian epidermis, one could expect major loci of protein synthesis to coincide with the formation of tonofilaments in the basal and spinous cells and with the biogenesis keratohyalin in the granular layer. Presumably, keratin fibers develop from a combination of tonofilaments and keratohyalin (13). Autoradiographic observations, using tritiated amino acids (14–15), have indeed demonstrated the existence of two such loci of amino acid incorporation and have shown that certain amino acids preferentially incorporate *initially* in one or the other locus. Leu-

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cine, phenylalanine, valine, lysine and methionine are first seen to accumulate in the lower cells of the newborn rat epidermis while glycine, histidine, arginine and serine initially make their appearance in the granular layer. This differential localization of labeled amino acids appears to be a general phenomenon, since glycine has been shown to concentrate initially in the granular cells of the keratinizing epithelium in the esophageal and forestomach of the mouse (16); histidine-H^3 and arginine-H^3 are first seen in the granular cells of the human epidermis (10, 17) and arginine-H^3 probably localizes similarly in white pig skin (18). Strikingly, the localization of histidine-H^3 *does not occur* in the involved psoriatic epidermis (12, 19, 20).

Proline and tyrosine do not seem to preferentially localize and are seen in the cells of all layers at the same time. Labeled cystine has been reported to be incorporated first in the granular cells of the newborn rat epidermis (21), the horse's hoof (22) and the estrogen-stimulated vaginal epithelium of the mouse (23), but in the oral mucosa in the mouse, the lower cells are said to be the initial site of labeling (4).

Although it seems likely that the two loci of amino acid incorporation are associated with the biogenesis of tonofilaments and keratohyalin, respectively, data are available only in regard to the latter. Presumably, both cases involve the ribosomal mechanism of protein synthesis and differ in the input of genetic information *via* messenger RNA.

The presence of a high concentration of histidine in keratohyalin has been shown histochemically (24, 25). Ultrastructural autoradiography indicated that keratohyalin in the granular cell becomes labeled upon injection of histidine-H^3 (26, 27)—possibly by way of synthesis of a labeled protein in the cytoplasm outside the granule and transfer of this protein into the granule (27). The isolation from cattle hoof keratohyalin of ribonucleoprotein containing a high level (7.0 residues/100 residues) of histidine has recently been reported (28). Earlier reports have described the presence of a histidine-rich protein in the granular cells of the newborn rat (29–31) and human epidermis (20). This protein accounted for a *minimum* of 23 percent of the total histidine-H^3 found in the epidermis one hour after the intraperitoneal injection of the tracer into newborn rats (32) and contained histidine at a concentration of 11 residues per 100 residues (33). The histidine-rich protein also contains glycine, arginine and serine but no significant amount of leucine, phenylalanine, valine or methionine. Cysteine residues are absent. In addition to glycine, arginine, serine and histidine, the protein contains aspartic acid, glutamic acid, threonine and alanine. These eight amino acids constitute about 97% of the protein (33).

The involvement of the histidine-rich protein in the biogenesis of keratohyalin was first surmised from the observation that the protein could not be isolated from the involved psoriatic epidermis which contains no keratohyalin (20). The fact that urocanic acid is low in the psoriatic epidermis (34) coupled with the finding (35) that the histidine-rich protein appears to be linked *in situ* to a large protein through a urocanic acid-like moiety—possibly through a peptide linkage (35, 36)—further supports this relationship.

Preliminary data from experiments by Sibrack indicate that the histidine-rich protein is present in keratohyalin. Using the method described for isolation of keratohyalin from cattle hoof epi-

TABLE I

Isolation of histidine-rich protein from keratohyalin granules of newborn rat epidermis

SKIN

↓ 0.24 M NH_4Cl, pH 9.5, 0°, 5 Min

EPIDERMIS

↓ Minced, 1.0 M phosphate, pH 7.0, 37°, Centrifuged

PHOSPHATE EXTRACT	RESIDUAL TISSUE
↓ Dialyzed H_2O, 0°, Centrifuged	→ Homogenized, 8 M urea, Dialyzed, etc.
MACROAGGREGATES	
↓ Homogenized, 8 M urea-0.2 M Tris, pH 8.5, Dialyzed 0.01 M NH_4OH Lyophilized	
DRIED RESIDUE	
↓ 0.12 N $HClO_4$, 24°, 30 Min	
0.01 N $HClO_4$-SOLUBLE FRACTION	
↓ pH 4.5, Centrifuged	
pH 4.5 PRECIPITATE	
↓ Extracted 0.02 M Na_2CO_3, Chromatographed Sephadex G-100 or Sepharose 6B	
HISTIDINE-RICH PROTEIN	

dermis (28), material was extracted from newborn rat epidermis with 1.0 M phosphate, pH 7.0 and precipitated as macroaggregates by dialysis against water. These keratohyalin aggregates and the residual tissue (Table I) were exposed to the usual technique for isolation of the histidine-rich protein. From the keratohyalin, protein was obtained, which on Sepharose 6B behaved as a single entity with molecular weight of about 390,000. When isolated from animals which had received histidine-H^3, the protein was labeled. The tritium chromatographed coincidently and symmetrically with the protein. From the residual tissue, however, two protein fractions were obtained on Sepharose 6B. One, highly labeled with histidine^3, showed a symmetric peak at the position of molecular weight about 190,000. The other was symmetrically distributed at the position of about 45,000 but was essentially unlabeled. The labeled histidine-rich protein isolated from keratohyalin accounted for about 54% of the protein and 45% of the tritium in the macroaggregated keratohyalin indicating the presence in keratohyalin of histine-labeled protein which does not appear to be histidine-rich protein. The keratohyalin macroaggregates never accounted for more than about 33% of the histidine-H^3 in the epidermis between 30 min and 6 hr after the intraperitoneal injection of the tracer. About 18% of the histidine-H^3 was isolated as histidine-rich protein from the residual tissue indicating the existence of an extragranular concentration of this protein. The kinetics of incorporation of histidine-H^3 into these two "compartments" of histidine-rich protein allow consideration of the hypothesis that the extragranular compartment constitutes the precursor of the histidine-rich protein in the keratohyalin. Table II presents data on the fraction of the labeled histidine-rich protein which is in the keratohyalin at various times after the intraperitoneal injection of the tracer. At 30 min after administration of histidine-H^3, 30% of the labeled histidine-rich protein is in the keratohyalin. By 3 hours, this figure has risen to over 50%. Preliminary data indicate that the specific activity of the purified protein in the residual tissue is higher than it is in the keratohyalin.

Data obtained, *in vitro*, on the mechanism of synthesis of histidine-rich protein indicates that a precursor-peptide of low molecular weight, (possibly 5000) synthesized by the usual ribosomal mechanism, is polymerized to a protein of larger molecular weight (37). In this *in vitro* study, the polymerization went to units of about 30,000 and 60,000 molecular weight. From the recent data of Sibrack§ cited above, it would appear that the polymerization *can* continue to a molecular weight of 190,000 outside the granule. Whether the polymerization is enzymatic or not is as yet uncertain. Also uncertain is the relationship of the oligomeric peptides from cattle hoof epidermal keratohyalin (28) to the histidine-rich protein.

§ L. A. Sibrack, Ph.D. Thesis.

TABLE II

Distribution of labeled histidine-rich protein in extragranular (residual tissue) and keratohyalin (KHG) locations after intraperitoneal injection of histidine-H^3 to newborn rats

Hr Post-Administration	Total cpm × 10^{-3}		% of total in KHG $\frac{a \times 100}{a + b}$
	KHG (a)	Residual Tissue (b)	
½	60	144	30
1	141	160	47
3	310	229	57
6	520	474	52

Since synthesis of histidine-rich protein begins with a step sensitive to puromycin—an indication of a ribosomal mechanism—it should be possible to use synthesis of this protein as a means of investigating control mechanisms for epidermal differentiation. For example, since populations of cells from the basal and granular layers, respectively, can be obtained (8), it will be possible to attempt the isolation of messenger RNA separately from the two. If synthesis of histidine-rich protein is limited to the granular cells by a transcriptional control mechanism, messenger RNA for this protein should only be present in the granular cell population. It is possible that in psoriasis, synthesis of the precursor peptide for histidine-rich protein is never "turned on" because the proper gene is repressed and the appropriate messenger RNA is not synthesized. Alternatively, the polymerizing enzyme may not be synthesized because the gene for that enzyme remains repressed in this disease.

In the absence of a certain etiology for the hyperplasia in the psoriatic epidermis, it seems useful to attempt the elucidation of molecular mechanisms for each abnormality in epidermal differentiation seen in this condition. An understanding of the chemistry involved may indicate where the basic difficulty lies.

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