The horny cell layer acts like a film of plastic wrap, allowing the body to retain moisture and protecting it from invasion by foreign substances. If the horny cell layer is lost or defective, a human being can survive for no more than 24 hours due to loss of liquid components leading to dehydration. The layer comprises various substances such as keratins, produced by the epidermal keratinocytes, and lipids. The epidermal keratinocytes divide in the basal layer, produce keratins and differentiate, and migrate to the upper layers as they mature. This process is called keratinization. Recent studies have proved that epidermal keratinocytes secrete various cytokines.

**c. Keratinization**

Keratin forms tonofilaments that act as a cytoskeleton to maintain the structure of the keratinocyte. Keratins are classified as type I (acidic) or type II (neutral to basic). Type I and type II keratins bind to each other in pairs to form intermediate filaments. Pairs of keratins with characteristic molecular characteristics form, depending on the keratin pairs and state of differentiation of the keratinocytes. For example, K5/K14 pairs in basal cells and K1/K10 pairs in the suprabasal cell layers to form the cell cytoskeleton (Table 1.1, Fig. 1.14).

When keratinized, keratin fibers in the granular cell layer aggregate with help from the protein called filaggrin to form the characteristic condensed keratin pattern. Profilaggrins, abundantly found in keratohyalin granules, decompose into filaggrins by the action of the protease peptidylarginine deiminase during keratinization (Fig. 1.15). Released filaggrins aggregate keratin fibers in the horny cell cytoplasm and keratins decompose into amino acids, for example, in the horny cell upper layer. The

**Table 1.1 Expression regions of main keratin pairs, and congenital disorders from mutations of keratins.**

<table>
<thead>
<tr>
<th>Keratins</th>
<th>Main area of expression</th>
<th>Genetic disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1, K10</td>
<td>Keratinocytes in the suprabasal cell layer and granular cell layer</td>
<td>Bullous congenital ichthyosiform erythroderma</td>
</tr>
<tr>
<td>K2e</td>
<td>Superficial epidermis</td>
<td>Ichthyosis bullosa of Siemens</td>
</tr>
<tr>
<td>K3, K12</td>
<td>Anterior epithelium of the cornea</td>
<td>Juvenile epithelial corneal dystrophy (Meesman)</td>
</tr>
<tr>
<td>K4, K13</td>
<td>Mucosa</td>
<td>White spongy nevus (on tongue)</td>
</tr>
<tr>
<td>K5, K14</td>
<td>Keratinocytes in the basal cell layer</td>
<td>Epidermolysis bullosa simplex</td>
</tr>
<tr>
<td>K6, K16</td>
<td>Nails</td>
<td>Pachyonychia congenita</td>
</tr>
<tr>
<td>K9</td>
<td>Keratinocytes in the suprabasal cell layer and granular cell layer on the palmoplatant region</td>
<td>Palmoplantar keratodsis (Vörner)</td>
</tr>
</tbody>
</table>
decomposed filaggrins, which function in moisture retention and ultraviolet absorption are called natural moisturizing factors (NMF).

2. Cornified cell envelope

The cornified cell envelope (marginal band) is an extremely large and strong, insoluble structure lining the horny cell membrane. It appears under the electron microscope as an electron-dense structure at the periphery of the horny cells (Fig. 1.16). The main structural components of the cornified cell envelope are involucrins, produced in the lower keratinocyte suprabasal cells, and loricrins, produced in the granular layer keratinocyte cells. The cornified cell envelope forms when these proteins are successively cross-linked by enzymes such as transglutaminases during keratinization. Transglutaminases are calcium dependent and are activated by the influx of calcium ions into the cells accompanying cell death after keratinization.

3. Horny layer (stratum corneum) intercellular lipids

More than 50% of the lipids in the horny cells are ceramides, followed in decreasing order of abundance by cholesterols, free fatty acids and cholesterol sulfates. Lamellar granules are abundantly found in the cytoplasm of the granular cell layers. These granules are released from cells when the cells become apoptotic, forming horny layer intercellular fat. The enzyme ABCA12 plays a significant role in the release of lipid from lamellar granules from granular cells. Ceramides are released from lamellar granules, and free fatty acids are secreted from granular cell membranes. Cholesterol sulfates connect and stabilize the layered

**Ceramide and its moisturizing function**

The ceramide content in the horny cell layer is reduced in patients with atopic dermatitis. Ceramide is thought to relate to dry skin and to disorders of skin barrier function.

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**Fig. 1.14 Expression of keratin types in the epidermis.**

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**Fig. 1.15 Keratohyalin granules.**

Profilaggrin is composed of linearly arranged filaggrin domains that connect with each other by linker proteins. These filaggrin domains have many serine-threonine residues, which are kept phosphorylated. During keratinization these domains are cut by proteases and are dephosphorylated to form filaggrin, which aggregates (hence: “fil” for filament, and “aggrin” for aggregate) keratin filaments. The filaggrin degrades into small peptides, which act to moisturize and to absorb UV rays (quoted from; Iizuka H. Hyperkeratosis. In: Arata J, editor. General Dermatology. 7th ed. Igaku Shoin; 2004).

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**Fig. 1.16 Cornified cell envelope.**
lipid structures between horny cells. The horny layer intercellular fat is important in preventing excessive transepidermal water loss.

4. Exfoliation of horny cells

As horny layer intercellular lipids move upward with the horny cell layers, they gradually get more decomposed by lipases, a group of catabolic lipid enzymes, and steroid sulfatases. Subsequently, adhesion between keratinocytes is disrupted by proteases from the upper skin surface, which causes gradual exfoliation of horny layer cell.

**d. Melanocytes and melanin synthesis**

1. Form and distribution of melanocytes

Melanocytes (pigment cells) are neural crest (ectoderm)-derived dendritic cells found in the basal cell layer and hair matrix (Fig. 1.17). Since their cytoplasm contracts during the processes of dehydration and fixation in light microscopy, such as in HE staining, melanocytes, as well as Langerhans cells, are called clear cells. Melanocytes stain a characteristic brownish black in DOPA (Fig. 1.17). Approximately 1,000 to 1,500 melanocytes are seen per square millimeter of skin. Dense melanocytes are found in sun-exposed sites of the body, such as the face, and in physiologically pigmented sites, such as external genitalia.

The Golgi apparatus develops in a cell and contains various melanosomes in various formative stages (stages I, II, III and IV). Melanin is produced from the amino acid tyrosine in the melanosome. Mature melanosomes are packaged and transported to the neighboring basal cells and suprabasal cells. Basal cells that are provided with melanosomes aggregate them in the upper part of the cytoplasm over the nucleus, forming a melanin cap to protect their DNA from UV rays.

Racial differences in skin color are determined by the number and size of melanosomes. There is no difference in the distribution or density of melanocytes between races.

2. Biosynthesis of melanin

Melanin is a generic term for a group of polymer pigmented molecular phenolic substances. The melanins in human skin are various indole compounds synthesized from tyrosines through polymer formation (Fig. 1.18).

Melanins in humans are roughly classified as eumelanin, which is black (intrinsic melanin), or pheomelanin (yellow melanin). Melanins in human skin and hair are complexes of these two types, and their ratio determines hair color.

Tyrosines, supplied by the blood, are oxidized by tyrosinase, which contains copper, and are metabolized into dopas and then
Tyrosinase is the enzyme that catalyzes these two reactions. This metabolism is the rate-limiting reaction in the synthesis of melanins (Fig. 1.18).

Dopaquinones are automatically oxidized to become indole compounds that are connected to each other to synthesize eumelans. If cysteins are involved at this stage, dopaquinons connect with cysteins and change into 5-S-cysteinyl dopa (5-S-CD), which polymerizes to synthesize pheomelans.

The melanosome is a subcellular organelle, enclosed by a lipid double membrane, in which melanins are exclusively produced. When tyrosinases, which are synthesized by the Golgi apparatus, are carried to premelanosomes, which are isolated from the agranular endoplasmic reticula, melanin synthesis begins. As the amount of synthesis increases, melanosomes enlarge. The formation of melanosomes is divided into stages I to IV by the degree of melanin deposition (Fig. 1.19). A melanosome in stage IV is 500 nm to 700 nm along its major axis, football shaped, and supplied from the dendrites to the neighboring epidermal keratinocytes.

The most important role of melanin is protecting the skin from UV rays and preventing the occurrence of malignant tumors and sunlight injury to the skin. The darker the skin of a particular race, the lower is the incidence of skin cancer caused by UV light.

Exposure to sunlight darkens the skin. This darkening may occur immediately after exposure and may be temporary, when melanins are oxidized temporarily, or it may occur after several days of exposure, when there is an increase in melanin synthesis and mature melanosome formation.

Melanins can also act to absorb harmful active enzymes, metals and drugs.

The Langerhans cell is a bone marrow-derived dendritic cell specific to stratified squamous epithelia such as the skin. Langerhans cells are frequently seen isolated in the middle and upper suprabasal cell layers (Fig. 1.20). The cells are distributed at a density of 400/mm² to 1,000/mm². They lack tonofilaments and cell attachment structures, such as desmosomes, and they migrate. By electron microscopy, a few fibrillar components and Birbeck granules, whose cross-section is a characteristic tennis racquet shape, are observed in the cell cytoplasm (Fig. 1.21a). Birbeck granules are known to be Golgi-apparatus-derived or membrane-derived, and carry antigens in the cells.