Chapter 3

Anatomy and Organization of Human Skin

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Components of normal human skin

Human skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue [1–5]. The dermal–epidermal junction is undulating in section; ridges of the epidermis, known as rete ridges, project into the dermis. The junction provides mechanical support for the epidermis and acts as a partial barrier against exchange of cells and large molecules. Below the dermis is a fatty layer, the panniculus adiposus, usually designated as 'subcutaneous'. This is separated from the rest of the body by a vestigial layer of striated muscle, the panniculus carnosus.

There are two main kinds of human skin. Glabrous skin (non-hairy skin), found on the palms and soles, is grooved on its surface by continuously alternating ridges and sulci, in individually unique configurations known as dermatoglyphics. It is characterized by a thick epidermis divided into several well-marked layers, including a compact stratum corneum, by the presence of encapsulated sense organs within the dermis, and by a lack of hair follicles and sebaceous glands. Hair-bearing skin (Fig. 3.1), on the other hand, has both hair follicles and sebaceous glands but lacks encapsulated sense organs. There is also wide variation between different body sites. For example, the scalp with its large hair follicles may be contrasted with the forehead, which has only small vellus-producing follicles, albeit associated with large sebaceous glands. The axilla is notable because it has apocrine glands in addition to the eccrine sweat glands, which are found throughout the body. Regional variation is further considered below.

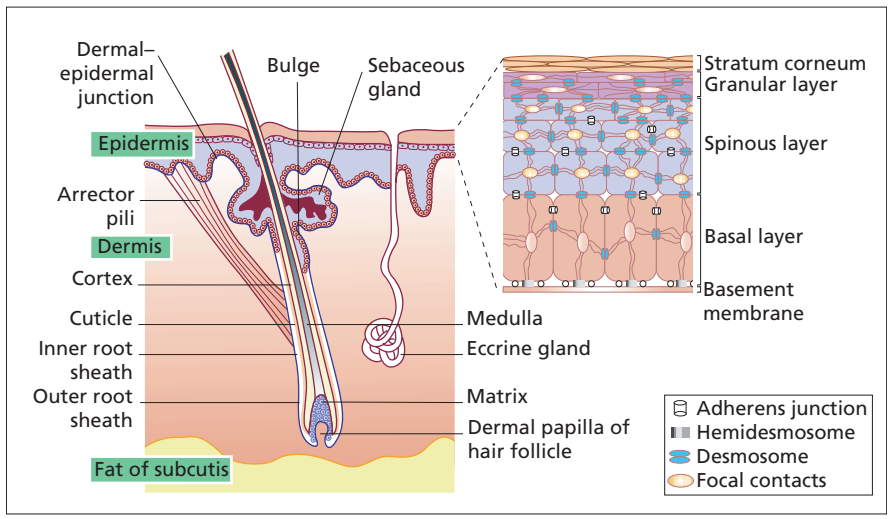

Fig. 3.1 The skin and its appendages.

The superficial epidermis is a stratified epithelium largely composed of keratinocytes that are formed by division of cells in the basal layer, and give rise to several distinguishable layers as they move outwards and progressively differentiate. Within the epidermis, there are several other cell populations, namely melanocytes, which donate pigment to the keratinocytes (Chapter 39), Langerhans' cells, which have immunological functions (Chapter 10) and Merkel cells.

The hair follicles comprise pockets of epithelium that are continuous with the superficial epidermis. They undergo intermittent activity throughout life. During the active phase, the follicle envelops at its base a small papilla of dermis. A bundle of smooth muscle, the arrector pili, extends at an angle between the surface of the dermis and a point in the follicle wall. Above the insertion, the holocrine sebaceous gland opens by a short neck into the pilary canal, and some follicles in certain areas of the body, notably the axilla, have, in addition, an apocrine gland. Also derived from the epidermis, and opening directly to the skin surface, are the eccrine sweat glands, present in every region of the body in densities of 100–600/cm^2.

The basis of the dermis is a supporting matrix or ground substance in which polysaccharides and protein are linked to produce macromolecules with a remarkable capacity for retaining water. Within and associated with this matrix are two kinds of protein fibre: collagen, which has great tensile strength and forms the major constituent of the dermis, and elastin, which makes up only a small proportion of the bulk. The cellular constituents of the dermis include fibroblasts, mast cells and histiocytes (monocyte/macrophages). The dermis has a very rich blood supply, although no vessels pass through the dermal–epidermal junction.

The motor innervation of the skin is autonomic, and includes a cholinergic component to the eccrine sweat glands and adrenergic components to both the eccrine and apocrine glands, to the smooth muscle and the arterioles and to the arrector pili muscle. The sensory nerve endings are of several kinds: some are free, some terminate in hair follicles and others have expanded tips. Only in glabrous skin are some nerve endings encapsulated. Sense organs are described later in this chapter.

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Embryology [1,2]

Origin of the skin

The skin arises by the juxtaposition of two major embryological elements: the prospective epidermis, which originates from a surface area of the early gastrula, and the prospective mesoderm, which is brought into contact with the inner surface of the epidermis during gastrulation [3,4]. The mesoderm not only provides the dermis but is essential for inducing differentiation of the epidermal structures, such as the hair follicle in mammals [5]. Indeed, an influence from the dermis is essential for the maintenance of adult epidermis [6], although organized dermis is not in this instance mandatory, the property also residing in powdered dermis or tendon [7].

The neural crest also makes an important contribution to the skin, namely the pigment cells, although their bulk is small.

The timing of the events during development is summarized in Table 3.1.

Epidermis

The development of the epidermis (and its appendages) relies on specific initiation signals. Although complex, critical events appear to be governed by opposing interplay between the Notch and Wnt (wingless-related) signalling pathways, with β-catenin, Lef1 and Notch peptide all having key roles [9]. Signals from the Sonic hedgehog pathway and bone morphogenetic proteins (BMPs) also are important in early embryogenesis, notably in determining whether cells have an ectodermal or neural fate. Specifically, BMP signalling promotes ectodermal development, while Sonic hedgehog promotes neural tube and

Table 3.1 Morphological events during fetal skin development. (Data from Holbrook and Hoff [8].)

	Month (gestation)					
	1	2	3	4	5	6
Hair peg				+		
Exposed hair					+	
Nail				+		
Sebaceous gland					+	
Apocrine gland						+
Eccrine gland						+
Follicular keratinization					+	
Interfollicular keratinization						+
Non-keratinocytes						
Melanocytes						
Non-functioning			+			
Active				+		
Langerhans' cells				+		
Merkel cells				+		

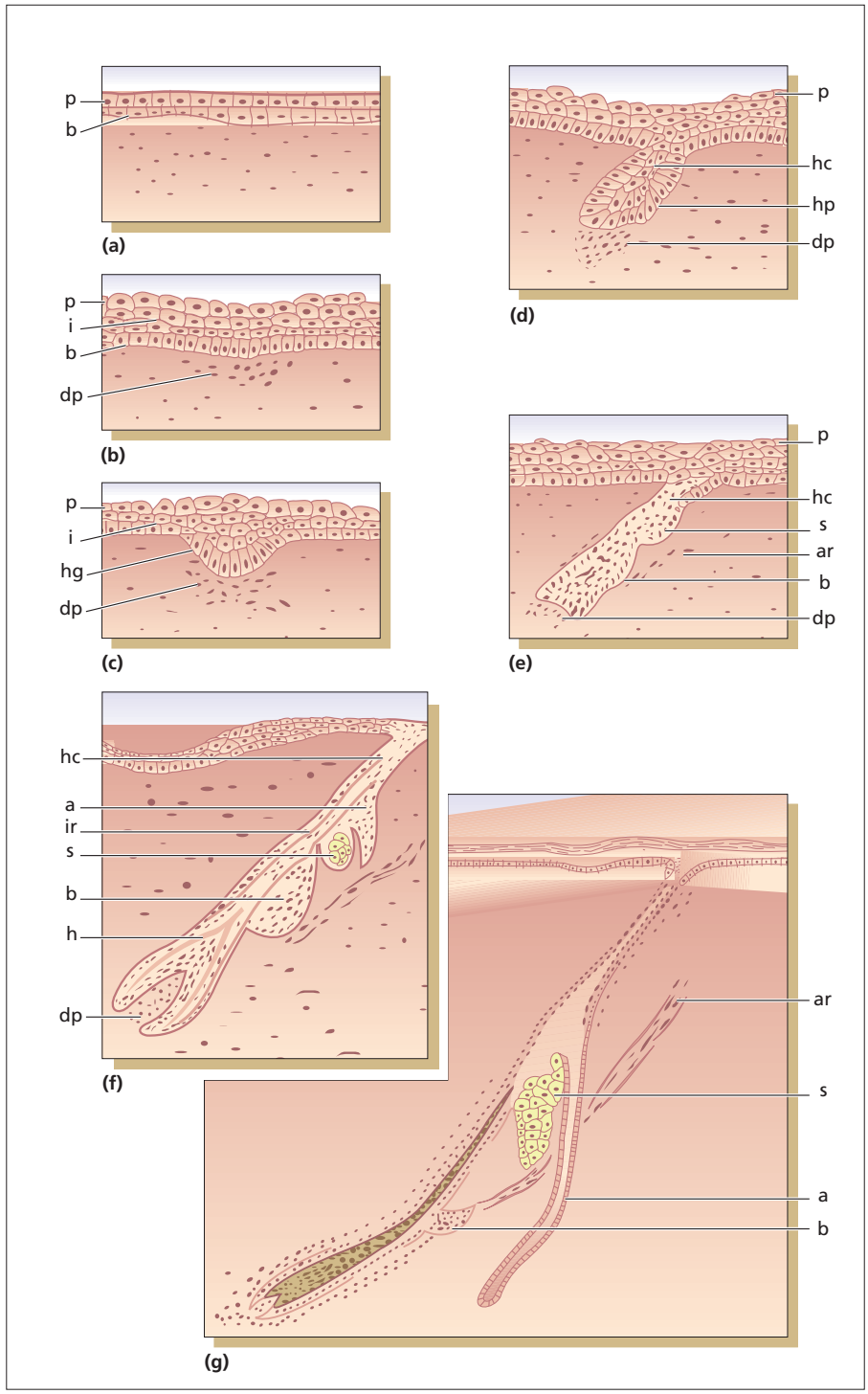

Fig. 3.2 Development of epidermis, hair follicle and associated structures. (a) Section of skin of embryo at about 4 weeks. The periderm (p) is clearly seen, and a basal or germinative layer (b) appears in some areas. (b) Skin at about 11 weeks. The epidermis is made up of basal cells, cuboidal in shape (b), and cells of the stratum intermedium (i) are beginning to appear above them. The periderm (p) consists of a single cell layer. Mesenchyme cells (dp) are beginning to aggregate below a presumptive hair follicle. (c) Hair germ (hg) stage. Basal cells are now columnar and starting to grow downwards. (d) Hair peg (hp) stage. Cells of the so-called 'hair canal' (hc) form a solid strand. (e) Bulbous hair peg. Note the solid 'hair canal' (hc), sebaceous gland rudiment (s), bulge (b) for attachment of developing arrector muscle (ar). (f) Later stage showing apocrine rudiment (a), sebaceous gland (s) now partly differentiated, and bulge (b). The dermal papilla (dp) has been enclosed and a hair (h) is starting to form, with an inner root sheath (ir). (g) Complete pilosebaceous unit of axillary skin from a 26-week-old fetus. The sebaceous gland (s) is well differentiated and the apocrine gland (a) is canalized.

central nervous system (CNS) development [10]. Thus, a complex interaction between these two components, as well as signals from fibroblast growth factors (FGFs) and additional regulatory control mechanisms from the Wnt pathway, underlies the preliminary stages of epidermal development.

In about the third week of fetal life, the epidermis consists of no more than a single layer of undifferentiated, glycogen-filled cells [8]. In a 4- to 6-week-old fetus [2], however, two layers of cells can be distinguished, the periderm or epitrichial layer and a stratum germinativum (Fig. 3.2). The periderm [8] is a purely embryonic structure (Fig. 3.3), which is unique to primates: it is ultimately lost *in utero* as the true epidermis keratinizes beneath it.

Between 8 and 11 weeks (crown to rump length 26–50 mm) a middle layer starts to form (Fig. 3.2). Glycogen is

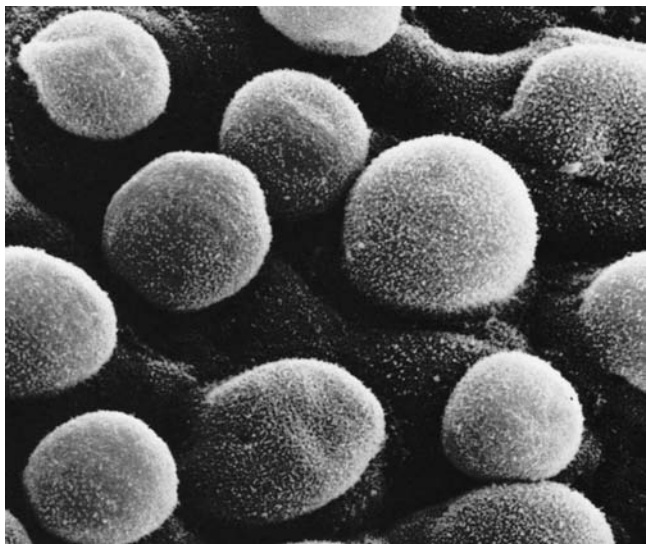

Fig. 3.3 Scanning electron micrograph of an 85–110 day (estimated gestation age) human embryo. Single globular blebs project from the periderm cells. (Courtesy of Professor K.A. Holbrook, University of Florida, Gainesville, FL, USA.)

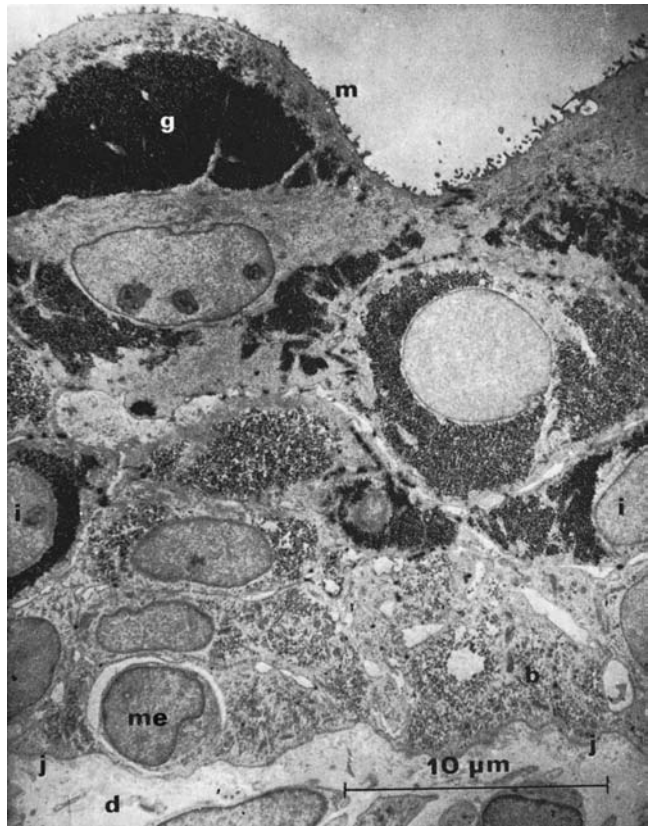

Fig. 3.4 Electron micrograph of the full-thickness epidermis from the back of a 14-week human fetus. Osmium fixation and lead staining. The periderm cells are full of glycogen (g) and have microvilli (m) at their amniotic border. Cells of the intermediate layer (i) also contain glycogen. Basal-layer cells (b) have lost glycogen by this stage. Just above the dermal–epidermal junction (j) is seen a melanocyte (me); the surrounding space indicates that it is a recent immigrant from the dermis (d). (Courtesy of Professor A.S. Breathnach, St John's Institute of Dermatology, London, UK.)

abundant in all layers, and a few microvillous projections occur at the surface of the periderm. The surface cells, as viewed by the scanning electron microscope, are flat and polygonal [11].

By 12–16 weeks (crown to rump length 69–102 mm), there are one or more intermediate layers. These cells contain mitochondria, Golgi complexes and a few tonofilaments, as well as abundant glycogen both within and between the cells (Fig. 3.4). Microvilli become much more numerous.

From this stage onwards, dome-shaped blebs start to project from the centres of the periderm cells (Fig. 3.3). At first the blebs are simple (Fig. 3.4), but later their surface becomes dimpled and infolded. Between 16 and 26 weeks, the intermediate layers increase in number, and by 21 weeks keratohyalin granules appear in the uppermost layer. The elevations of the periderm become cast off into the amniotic fluid, and by 24 weeks the periderm cells start to separate from the embryo. Together with shed lanugo, sebum and other materials, they form the vernix caseosa.

Hemidesmosomal and desmosomal proteins are already demonstrable in the basal keratinocytes at 10 weeks. By 14 weeks, basal keratins are expressed by the basal cells and skin-differentiation keratins are expressed by cells of the middle layer. Filaggrin, the protein of the granular layer, is first detectable at 15 weeks.

The periderm may be no more than a protective investment for the fetus before keratinization of the epidermis. On the other hand, features such as the abundant microvilli, raised blebs, coated- and smooth-membrane vesicles and increasing cell size, suggest it may have an important embryonic function. The microvilli with their 'fuzz' coat of mucopolysaccharide are similar to the modifications of the luminal border of the intestinal mucosa cell. All these considerations suggest that the periderm might be concerned with the uptake of carbohydrate from the amniotic fluid [11].

Hair follicles and apocrine glands

The earliest development of the hair rudiments [12–14] occurs at about 9 weeks in the regions of the eyebrow, upper lip and chin. This represents an initial response to the first of three discrete mesenchymal-epithelial exchanges that orchestrate hair follicle formation [15]. The first sign of a hair follicle is a crowding of nuclei in the basal layer of the epidermis, the so-called primitive hair germ or pregerm stage (Fig. 3.2b). This occurs in response to a primary message from the subjacent mesenchyme. The pregerm passes rapidly into the hair germ stage, the

basal cells become high; the nuclei become elongated and the structure starts to grow downward into the dermis. At the same time, mesenchymal cells and fibroblasts increase in number to form the rudiment of the hair papilla beneath the hair germ. These events are mediated by a second series of signals from the expanding epithelial cells. At this stage it is known as the hair peg (Fig. 3.2d). The outer cells of the hair peg are arranged radially to the long axis, and are columnar in shape, those at the advancing matrix end being conspicuously tall and narrow. As the germ develops, it grows obliquely downwards, and the advancing extremity becomes bulbous, gradually enveloping the mesodermal papilla. Proliferation and differentiation are then enhanced by a third series of signals emanating from the dermal papillae. At this bulbous hair-peg stage, two epithelial swellings appear on the posterior wall of the follicle. The lower one is the bulge to which the arrector muscle becomes attached, and the upper is the rudiment of the sebaceous gland. In many follicles, a third bud later appears above the sebaceous gland; this is the rudiment of the apocrine gland. Such rudiments develop in a large number of the follicles, including some on the scalp, face, chest, abdomen, back and legs, as well as in the axilla, mons pubis, external auditory meatus, eyelids, circumanal area, areola region of the breast, labia minora, prepuce and scrotum, where they survive in the adult.

As the bulbous hair peg grows downwards and differentiates, the first cells of the inner root sheath (IRS) begin to form above the region of the matrix. The matrix continues to burrow deeper, and above the root sheath the inner cells of the follicle grow upwards into the epidermis, to form the hair canal.

The different mesenchymal-epithelial cues involve several signalling pathways including Notch, Sonic hedgehog and Wnt, as well as contributions from FGFs and BMPs. There are also marked changes in certain cell adhesion proteins, notably E-cadherin and P-cadherin [15].

The hair follicles are arranged in patterns, usually in groups of three. It appears that the first follicles develop over the surface at fixed intervals of between 274 and 350 μm. As the skin grows, these first germs become separated, and new rudiments develop between them when a critical distance, dependent on the region of the body, has been reached. Commonly, follicles occur in groups of three, with the hairs arranged on a straight, short line, more or less transverse to the grain or slant of the hair. There is no large-scale destruction of follicles during postnatal development, only a decrease in actual density as the body surface increases; nor do any new follicles develop in adult skin.

Sebaceous glands [16,17]

These are, at first, solid, hemispherical protuberances on the posterior surfaces of the hair pegs. The cells contain moderate amounts of glycogen, but soon the cells in the centre lose this, and become larger and foamy as they accumulate droplets of lipid. The sebaceous glands become differentiated at 13–15 weeks, and are then large and functional. The sebum forms a part of the vernix caseosa. At the end of fetal life, sebaceous glands are well developed and generally large. After birth, the size is rapidly reduced, and they enlarge to become functional again only after puberty.

Eccrine glands [18,19]

These start to develop on the palms and soles at about 3 months, but not over the rest of the body until the fifth month. In embryos of 12 weeks, the rudiments of eccrine sweat glands are first identifiable as regularly spaced undulations of the stratum germinativum. Cells that form the anlagen are oblong, palisading and lie closely together, but otherwise they do not differ from the rest of the stratum germinativum. By 14–15 weeks, the tips of the eccrine sweat gland rudiments have penetrated deeply into the dermis, and have begun to form the coils. In the overlying epidermis, columns of cells that are destined to form the intraepidermal sweat ducts are recognizable. Each column is composed of two distinct cylindrical layers, comprising two inner cells that are elongated and curved so that they embrace the inner cylinder.

The intraepidermal duct appears to form by the coalescence of groups of intracytoplasmic cavities formed within two adjacent inner cells. In the intradermal segment, on the other hand, the lumen appears to form by dissolution of the desmosomal attachment plaques between the cells that compose the inner core of the eccrine duct germ.

Nails [20,21]

Nails begin to develop in the third month. In fetuses at 16–18 weeks (crown to rump length 120–150 mm) keratinizing cells from both dorsal and ventral matrices can be distinguished.

Melanocytes [22]

Melanocytes take their origin from the neural crest. This can be identified in early human embryos, but the elements arising from it soon lose themselves in the mesenchyme, and pigmented melanocytes cannot be identified, even in black skin fetuses, before 4–6 months of gestation. However, dopa-positive melanocytes can be demonstrated earlier.

Langerhans' cells [22,23]

These are derived from the monocyte–macrophage–histiocyte lineage and enter the epidermis at about 12 weeks.

Merkel cells [24]

These appear in the glabrous skin of the fingertips, lip, gingiva and nail bed, and in several other regions, around 16 weeks.

Dermis

It was at one time believed that the mesenchymal cells forming the dermis came from the ventrolateral part of the somite, which for that reason was named the dermatome. Although some cells may migrate from the dermatome and take part in the formation of the skin, most of the dermis is formed by mesenchymal cells that migrate from other mesodermal areas. These mesenchymal cells give rise to the whole range of blood and connective tissue cells, including the fibroblasts and mast cells of the dermis and the fat cells of the subcutis. Nevertheless, a new type of stem cell from the dermis, called skin-derived precursor (SKP) cells, has been identified [25]. Such cells are capable of being converted into several different cell types *in vitro* (e.g. neurones, smooth muscle cells or adipocytes) and might constitute a highly accessible source of pluripotential autologous stem cells.

The embryonic dermis is at first very cellular, and in the second month the dermis and subcutis are not distinguishable from each other. Fibrillar components shortly make their appearance, and regular bundles of collagen fibres are evident by the end of the third month. Later, the papillary and reticular layers become distinct and, at the fifth month, the connective tissue sheaths are formed around the hair follicles. Elastic fibres are first detectable at 22 weeks [26]. Beneath the dermis is a looser tissue characterized by fat islands that begin to form in definite places.

In embryos of 6–14 weeks, three types of cell have been described in the dermis: stellate cells, phagocytic macrophages and a granule-secretory cell, either a melanoblast or a mast cell [27]. From weeks 14–21, fibroblasts are numerous and active, and perineurial cells, pericytes, melanoblasts, Merkel cells and mast cells [28] can be individually identified. Another cell, of bone marrow origin, may be ancestral to the Langerhans' cell and the histiocyte [27].

At first, the undersurface of the epidermis is smooth, but during the fourth month, at the same time as the hair follicle starts to develop, it becomes irregular.

Touch pads become recognizable on the hands and fingers, and on the feet and toes, by the sixth week, and reach their greatest development at the 15th week. After this, they flatten and become indistinct. It is these areas, however, that determine the pattern of dermatoglyphs —the systems of papillary ridges—that take their place [29].

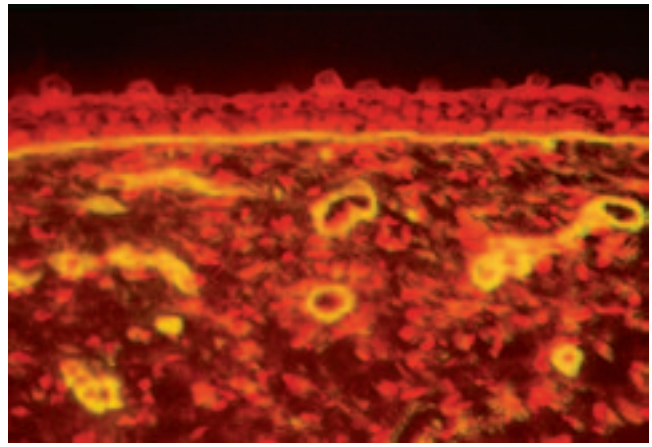

Fig. 3.5 Immunofluorescence photomicrograph showing staining with antitype IV collagen antibody of a section of the skin of a fetus of 15 weeks' gestational age. Note the surface periderm and the bright fluorescence at the dermal–epidermal junction and around the blood vessels. × 250.

Dermal–epidermal junction

A continuous lamina densa of the basement membrane becomes evident in the second month of gestation (Fig. 3.5), and hemidesmosomes appear in the third month [30].

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Epidermis

Structure and ultrastructure [1,2]

The normal epidermis is a terminally differentiated, stratified squamous epithelium (Fig. 3.6). The major cell, making up 95% of the total, is the *keratinocyte*, which moves progressively from attachment to the epidermal basement membrane towards the skin surface, forming several well-defined layers during its transit. Thus, on simple morphological grounds, the epidermis can be divided into four distinct layers: *stratum basale* or *stratum germinativum*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. The term *Malpighian* layer includes both the basal and spinous cells. Other cells resident within the epidermis include melanocytes, Langerhans' cells and Merkel cells.

The stratum basale (Fig. 3.7) is a continuous layer that is generally described as only one cell thick, but may be two to three cells thick in glabrous skin and hyperproliferative epidermis. The basal cells are small and cuboidal (10–14 nm) and have large, dark-staining nuclei, dense cytoplasm containing many ribosomes and dense tonofilament bundles. Immediately above the basal cell layer,

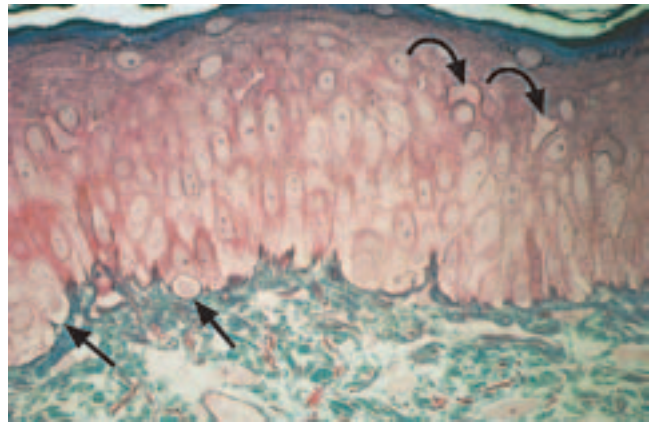

Fig. 3.6 Photomicrograph of a 1-µm-thick plastic section of normal human skin. The tissue was fixed with half-strength Karnovsky's medium and embedded in Epon. This technique allows the cellular components of the epidermis, including keratinocytes, melanocytes (straight arrows) and probable Langerhans' cells (curved arrows) to be clearly resolved. × 400. Basic fuchsin and methylene blue.

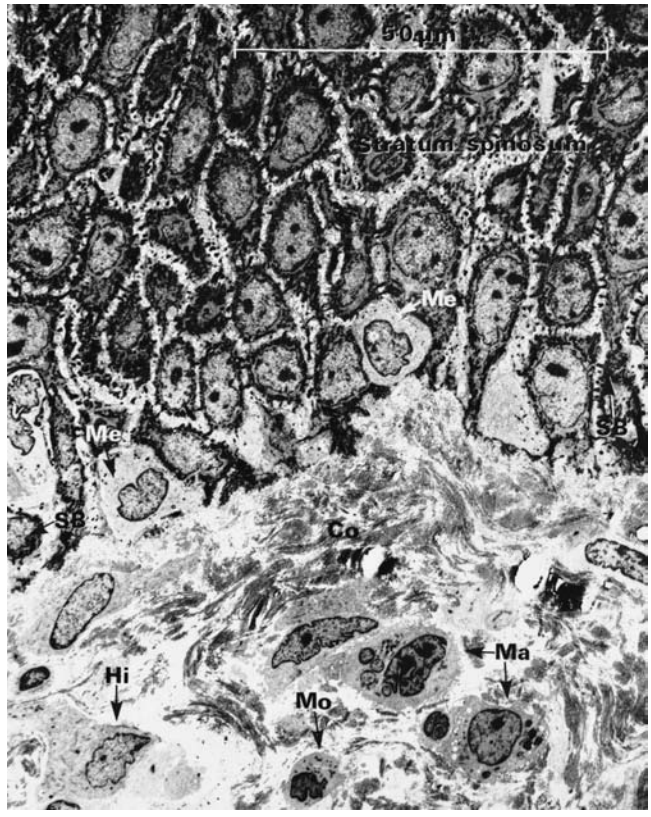

Fig. 3.7 The stratum basale (SB) and part of the stratum spinosum together with underlying dermis of skin from the forearm. Two melanocytes (Me) can be seen between the basal cells of the epidermis. In the dermis, collagen fibres (Co), histiocytes (Hi), monocytes (Mo) and mast cells (Ma) can be identified. ×1400. (Courtesy of Professor A.S. Breathnach, St John's Institute of Dermatology, London, UK.)

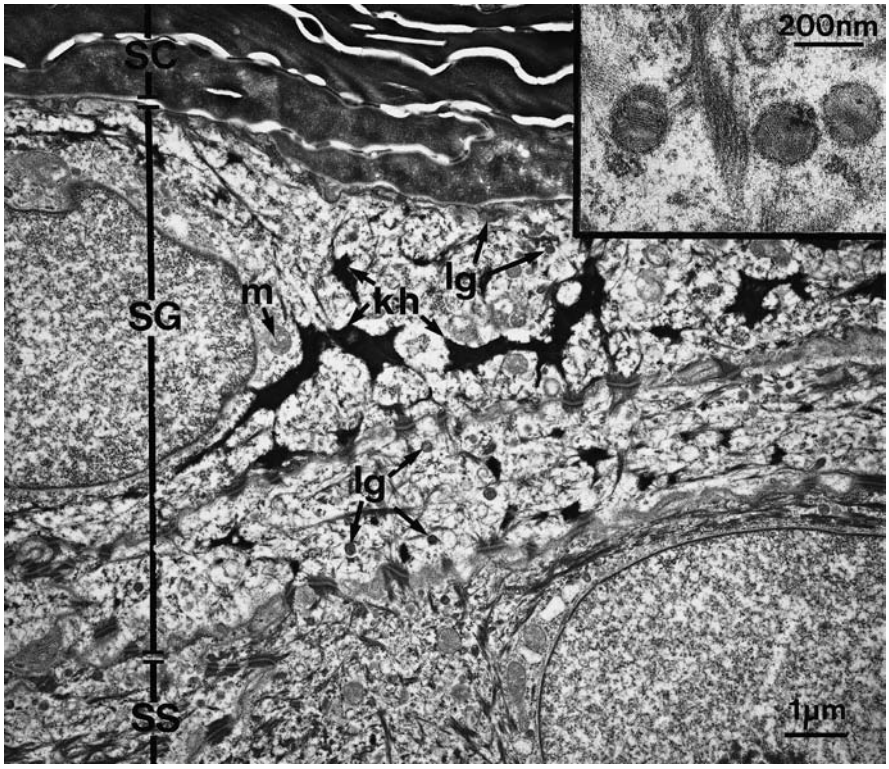

Fig. 3.8 Electron micrograph showing details of upper part of epidermis including stratum corneum (SC), stratum granulosum (SG) and the most superficial cell layer of stratum spinosum (SS). Note the irregularly shaped keratohyalin granules (kh) and the small, round lamellar granules (lg). The latter are present in both SS and SG and are smaller than mitochondria (m). Inset shows details of lamellar granules. See also Figs 3.19 and 3.20. Scale bar = 1 μm.

the epibasal keratinocytes enlarge to form the spinous/prickle-cell layer or *stratum spinosum* (Fig. 3.8).

The stratum spinosum is succeeded by the *stratum granulosum* or granular layer (see Fig. 3.8) because of the intracellular granules of keratohyalin. At high magnification, the dense mass of keratohyalin granules from human epidermis has a particulate substructure, with particles of irregular shape on average 2 nm length and occurring randomly in rows or lattices [3]. The cytoplasm of cells of the upper, spinous layer and granular cell layer also contains smaller lamellated granules averaging 100–300 nm in size, which are known as lamellar granules or bodies, membrane-coating granules or Odland bodies [2] (see Fig. 3.8). These are numerous within the uppermost cells of the spinous layer and migrate towards the periphery of the cells as they enter the granular cell layer. They discharge their lipid components into the intercellular space, playing important roles in barrier function and intercellular cohesion within the stratum corneum.

The outermost layer of epidermis is the stratum corneum (see Fig. 3.8) where cells (now corneocytes) have lost nuclei and cytoplasmic organelles. The cells become flattened and the keratin filaments align into disulphide cross-linked macrofibres, under the influence of *filaggrin*, the protein component of the keratohyalin granule, responsible for keratin filament aggregation [4]. The corneocyte has a highly insoluble cornified envelope within the plasma membrane, formed by cross-linking of the soluble protein precursor, *involucrin* [5], following the action of a specific epidermal transglutaminase also synthesized in the high stratum spinosum [6]. The process of desquamation involves degradation of the lamellated lipid in the intercellular spaces and loss of the residual intercellular desmosomal interconnections. In palmoplantar skin there is an additional zone, also electronlucent, the *stratum lucidum* between the granulosum and corneum. These cells are still nucleated, and may be referred to as 'transitional' cells.

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Intercellular junctions

Several types of cellular junction exist that link adjacent keratinocytes and which are responsible for mechanical,

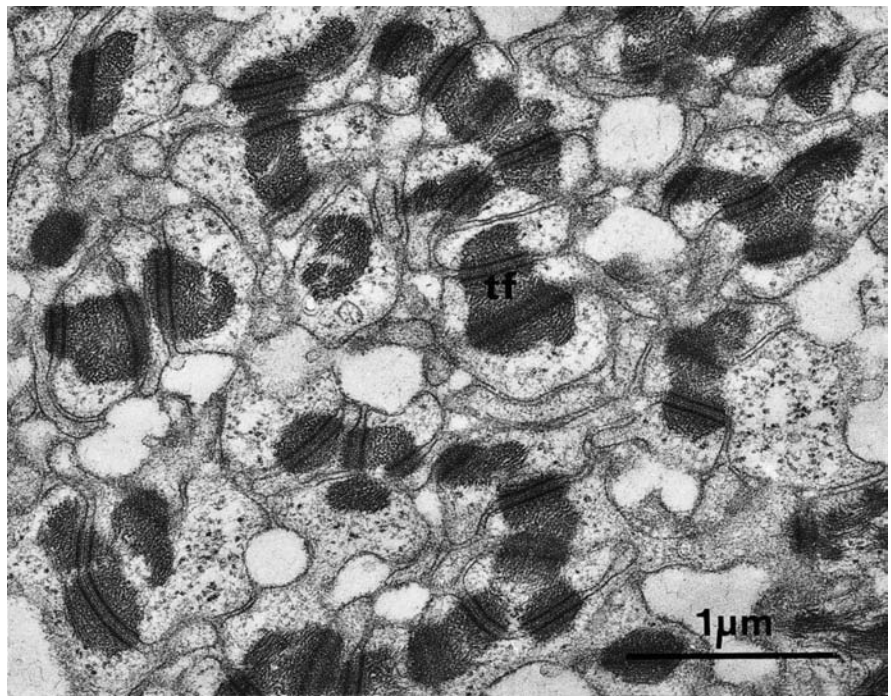

Fig. 3.9 Electron micrograph of desmosomes in spinous layer. These intercellular junctions are closely associated with tonofilaments (tf), many of which, in this view, are cross-sectioned. Scale bar = 1 μm.

biochemical and signalling interactions between cells. These include desmosomes, adherens junctions, gap junctions and tight junctions.

Desmosomes

Desmosomes are the major adhesion complex in epidermis, anchoring keratin intermediate filaments (IFs) to the cell membrane and bridging adjacent keratinocytes, and allowing cells to withstand trauma. The desmosome has a characteristic ultrastructure, in which the cell membrane of two adjacent cells forms a symmetrical junction with a central intercellular space of 30 nm containing a dense line (Fig. 3.9). Plaques of electron-dense material run along the cytoplasm parallel to the junctional region, in which three ultrastructural bands can be distinguished: an electron-dense band next to the plasma membrane, a less dense band, then a fibrillar area. Intermediate filaments loop through this region, and traversing filaments extending between the IFs and globular elements in the cell membrane may be unravelling IF protofilaments or associated proteins [1].

The main components of desmosomes consist of the products of three gene superfamilies: the desmosomal cadherins, the armadillo family of nuclear and junctional proteins, and the plakins [2]. The transmembranous cadherins comprise heterophilic associations of desmogleins and desmocollins. There are three main epidermis-specific desmogleins (Dsg1–3) and likewise for the desmocollins (Dsc1–3), all of which show differentiation-specific expression. For example, Dsg1 and Dsc1 are preferentially expressed in the superficial layers of the epidermis whereas Dsg3 and Dsc3 show greater expression in basal keratinocytes. The intracellular parts of these glycoproteins are attached to the keratin filament network via desmoplakin, plakoglobin and other macromolecules, the nature of which has been gleaned from a combination of yeast two hybrid, coimmunoprecipitation, recruitment assays in cultured cells and immunoelectron microscopy studies [2,3]. These have identified the armadillo protein, plakophilin 1, as an important stabilizer of keratinocyte adhesion in differentiated keratinocytes [4], as well as other site-specific plakin cell envelope proteins, such as envoplakin and periplakin [5,6]. The network of the major interactive desmosomal proteins is depicted in Fig. 3.10.

Further clues to the biological function and *in vivo* contribution to keratinocyte adhesion of these desmosomal components have arisen from various mouse models and human diseases, both inherited and acquired [2]. A summary of recent findings is represented in Table 3.2.

Adherens junctions

Adherens junctions are electron dense transmembrane structures that associate with the actin skeleton, part of the keratinocyte filament network concerned with cell motility, changes in cell shape and cell interactions. The transmembrane component of adherens junctions is E-cadherin, which forms calcium-dependent homophilic adhesive interactions with E-cadherin on opposing cells. The main linkage to the actin cytoskeleton is through α-catenin, although other adherens junction components

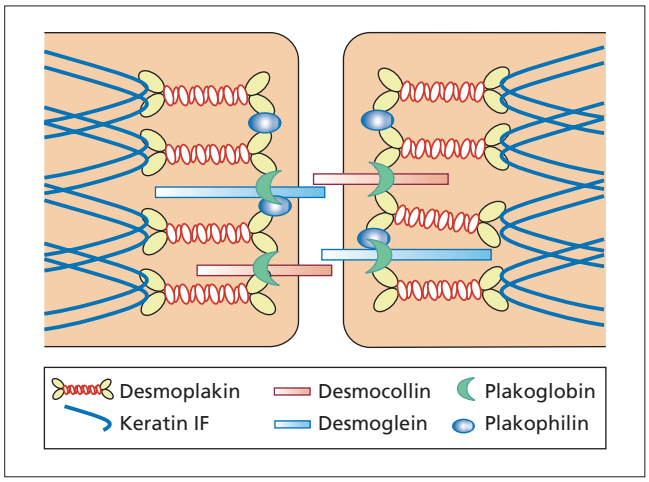

Fig. 3.10 Macromolecular composition of desmosomes linking adjacent keratinocytes. Cells are connected via transmembranous cadherin glycoproteins (desmogleins and desmocollins). Attachment of these molecules to the keratin filament cytoskeleton occurs via a network of desmosomal plaque proteins (desmoplakin, plakoglobin and plakophilin).

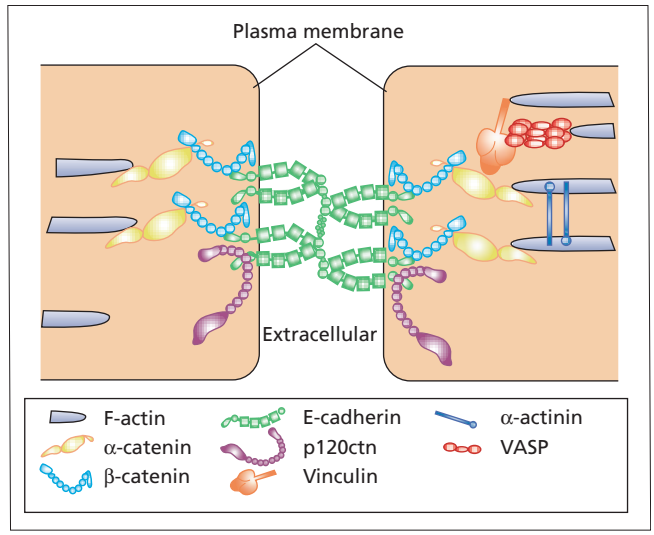

Fig. 3.11 Macromolecular composition of adherens junctions linking adjacent keratinocytes. Cells are connected via transmembranous E-cadherin and linked to the actin cytoskeleton via a network of adhesive proteins including β-catenin, α-catenin and p120ctn.

include p120ctn, β-catenin, plakoglobin (also present in desmosomes), α-actinin, vinculin, VASP (vasodilator-stimulated phosphoprotein), Mena and ZO1 (Fig. 3.11). Apart from forming E-cadherin–catenin complexes, α-catenin also appears to have a role in organizing the entire multiprotein complexity of adherens junctions and in determining the actin-binding and polymerization activities [7]. Clues to the precise function of individual components are gradually being realized through extensive conditional gene targeting and cultured cell

Table 3.2 Mouse models and human diseases related to desmosome genes/proteins.

Mutation/target antigen	Phenotype
Mouse models	
Plakoglobin knock-out	Lethal in early embryonic development (cardiac defects) Later survivors show epidermal fragility
Desmoglein-3 knock-out	Hair loss and epithelial fragility
Epidermally targeted truncated desmoglein-3 transgenic	Flakiness of back skin and paw swelling within 2 days of birth Desmosomes are reduced in number Hyperproliferation and inflammation in some areas
Desmoplakin knock-out	Lethal in early embryos
Desmocollin-1 knock-out	Flaky skin, defective epidermal barrier, hair loss
Inherited human diseases (autosomal recessive)	
Plakoglobin carboxy-terminal truncation	Naxos disease (arrhythmogenic right ventricular cardiomyopathy, keratoderma and woolly hair)
Desmoplakin carboxy-terminal truncation	Cardiomyopathy, keratoderma and woolly hair
Desmoplakin nonsense/missense combination of mutations	Skin fragility, keratoderma, woolly hair
Plakophilin-1 ablation	Skin fragility—ectodermal dysplasia syndrome
Desmoglein-4 ablation	Congenital hypotrichosis
Inherited human diseases (autosomal dominant)	
Desmoplakin haploinsufficiency	Striate palmoplantar keratoderma
Desmoglein-1 haploinsufficiency or dominant-negative mutations	Striate palmoplantar keratoderma
Autoimmune human diseases	
Desmoglein-3	Pemphigus vulgaris
Desmoglein-1*	Pemphigus foliaceus
Desmocollin-1	IgA pemphigus (subcorneal pustular dermatosis subtype)

* Desmoglein-1 is also the target/cleavage site of bacterial toxins in staphylococcal scalded skin syndrome and bullous impetigo.

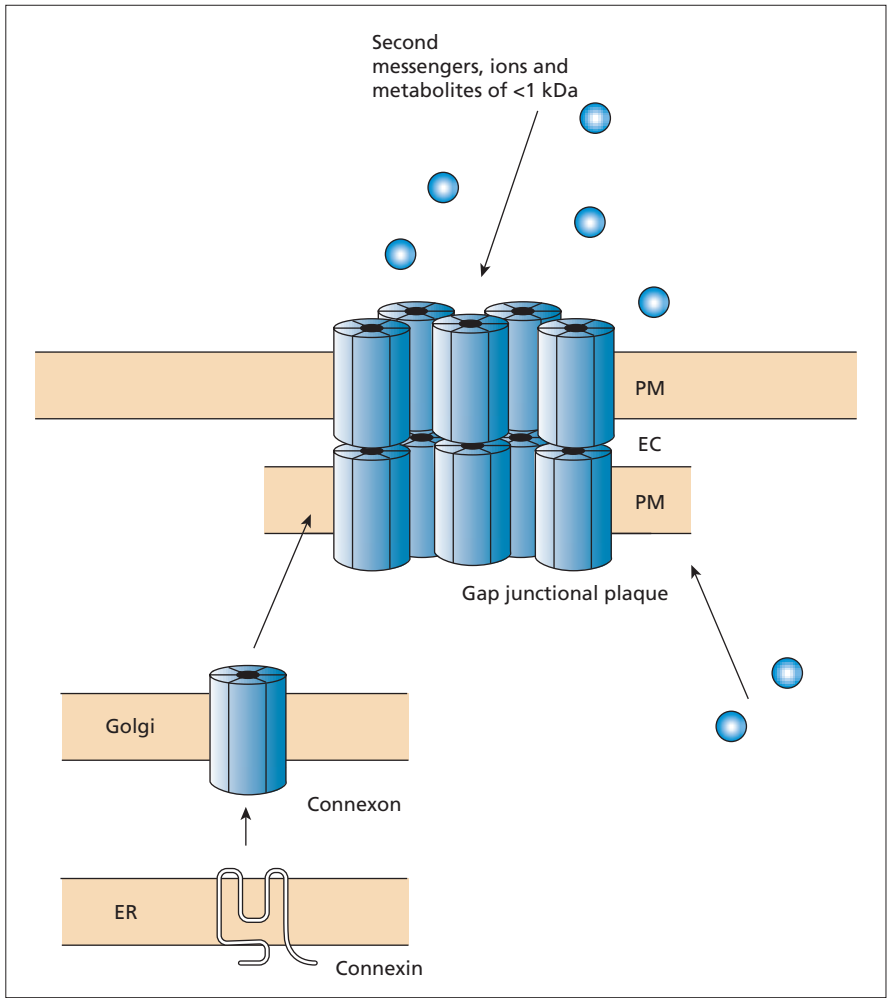

Fig. 3.12 Formation of gap junctions in human skin. In the Golgi network six connexin subunits assemble to form a connexon. The connexon is then transported to the plasma membrane. Other connexons then coaggregate and, in combination with aggregates of connexons on adjacent keratinocytes, a gap junction is formed that allows for the transfer of low-molecular-weight molecules (< 1 kDa) between cells. EC, extracellular; ER, endoplasmic reticulum; PM, plasma membrane.

reconstitution experiments, although the mechanisms regulating the dynamics of adherens junction formation are not yet clear. Nevertheless, the small GTPases of the Rho family appear to be important [8]. Likewise, VASP/Mena proteins have been implicated in the reorganization and polymerization of actin filaments [9]. Aside from actin polymerization, the myosin family of actin motor proteins may also contribute to generating the cellular movement necessary for intercellular adhesion by inducing contraction of actin filaments akin to pulling on a purse string [10]. The dynamics of the actin filament network allow for the extension, protrusion, embedding and anchorage of filipodia into neighbouring keratinocytes. This then encourages formation of adherens junctions and, in turn, permits other cell–cell junctions such as desmosomes to form, thus sealing adhesion between adjacent keratinocytes.

No human skin disorders have been linked to primary abnormalities in the structural components of adherens junctions, although plakoglobin—which may be mutated in Naxos disease (see Table 3.2)—is a component of both desmosomes and adherens junctions.

Gap junctions

Gap junctions comprise clusters of intercellular channels, known as connexons, that directly form connections between the cytoplasm of adjacent keratinocytes (and other cells) [11,12]. Thirteen different human connexins have been described [11]. Connexons originate following assembly of six connexin subunits within the Golgi network that are then transported to the plasma membrane. Here, connexons associate with other connexons to form a gap junction (Fig. 3.12). Homotypic or heterotypic connexins (formed from one or more than one type of connexin, respectively) are possible and the formation and stability of gap junctions can be regulated by protein kinase C, Src kinase, calcium concentration, calmodulin, adenosine 3′,5′-cyclic monophosphate (cAMP) and local pH. The function of gap junctions is to permit sharing of low-molecular-mass metabolites (< 1000 Da) and ion exchange between neighbouring cells, thus allowing intercellular coordination and uniformity [13].

Inherited abnormalities in genes encoding four different connexins (Cx26, 31, 30.3 and 30) have been detected in

several forms of keratoderma and/or hearing loss [12]. Specific connexin-associated genodermatoses include Vohwinkel's syndrome, autosomal dominant and recessive forms of erythrokeratoderma, Clouston's syndrome and *k*eratitis–*i*chthyosis–*d*eafness (KID) syndrome [14].

Tight junctions

Intercellular (tight) junctions are the major regulators of permeability in simple epithelia, but they are also present in skin, with a key role in skin barrier integrity [15]. Tight junctions, including those linking keratinocytes, are composed of transmembrane and intracellular molecules that include occludin, junction adhesion molecule and claudins [16]. As well as controlling permeability, tight junctions also have a role in maintaining cell polarity. Claudins may regulate epidermal permeability either through formation of tight junctions or via direct binding to certain transcription factors. However, a direct link to other transcription factors (e.g. Kruppel-like factor 4, Klf4) or enzymes (e.g. transglutaminase 1), that are known to be involved in regulating epidermal permeability through cross-linking of cornified cell envelope proteins, has yet to be established. Nevertheless, genetic ablation of claudin-1, or Klf4, or transglutaminase 1, in mice has been shown to cause similar patterns of epidermal barrier disruption [15,17,18]. This suggests that both the correct organization of tight junctions in the stratum granulosum and of cornified cell envelopes in the stratum corneum are required for full control of skin permeability.

No human skin disease has been associated with primary abnormalities in tight junction proteins, although abnormal expression of tight junction components, such as occludin, has been noted in a variety of inflammatory dermatoses, including psoriasis [19].

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Organization and kinetics

In adult life, cell division maintains differentiated tissues and replaces lost cells. There are three broad categories of tissues according to proliferative potential. In nerve and skeletal muscle there is no cell division. In other tissues, such as liver, cell division can occur in response to injury—'conditional renewal', which occurs little in normal states. In many tissues including skin and mucosa (stratified squamous epithelia) and gastrointestinal tract (simple epithelia), permanently renewing populations are produced by rapid and continuous cell turnover from a small population of 'stem' cells into differentiated cells having short lifespan. The epidermis has classically been viewed as a stratified squamous epithelium maintained by cell division within the basal layer, which is attached to the epidermal basement membrane. Differentiating cells are gradually displaced outwards through the stratum spinosum to the stratum corneum. The anucleate corneal cells (squames), corneocytes or cornified cells, which protect the viable cell layers, are continually shed from the skin surface, and the rate of production of cells in the basal layer must match the rate of loss from the surface to produce the normal skin thickness, although increased rates of loss and cell division occur in pathological states.

Dynamics of epidermis

Stem cells

Stem cells can be defined as cells that have an unlimited capacity for self-renewal and the ability to generate daughter cells that undergo terminal differentiation [1,2]. However, not all dividing basal keratinocytes are stem cells [2]. It is evident that a stem cell daughter cell that is

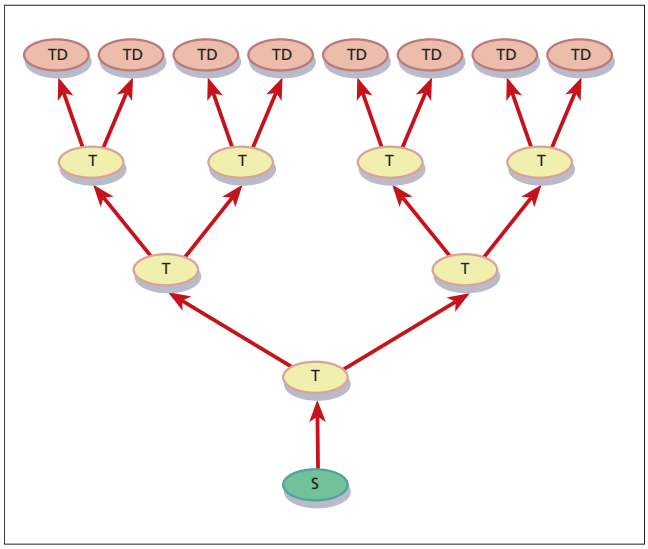

Fig. 3.13 Keratinocyte stem cell and transient amplifying cell division in human skin. Transient amplifying cells (T) are capable of increasing the number of keratinocytes that undergo terminal differentiation (TD) following a single stem cell (S) division. In this example, one stem cell division has resulted in eight terminally differentiated cells.

destined to undergo terminal differentiation can first proliferate and divide a small number of times (perhaps 5–6 mitoses): such cells are known as transient amplifying cells (Fig. 3.13). This expansion of proliferation therefore increases the number of terminally differentiating keratinocytes generated from each original stem cell division. Thus stem cells in the epidermis have a large capacity for proliferation but actually divide infrequently.

Stem cells are located in small clusters in the basal interfollicular epidermis and, in particular, in the bulge region of follicles. Although morphologically similar to other keratinocytes, stem cells are, to some extent, associated with a profile of particular chemical, molecular and biological characteristics. For example, stem cells retain labelling with injected ^{3}H-thymidine or Brdu after repeated cell division. In culture, actively growing clones present after serial passaging are considered to indicate origins from stem cells. Stem cells have also been shown to display increased β1 integrin expression as well as high levels of Notch ligand Delta 1. Other markers with altered expression in epidermal stem cells include the transferrin receptor, the nuclear-export protein 14-3-3σ, and the cytoskeletal keratins, K19 and K15.

Some putative stem cell markers, such as the c-Myc-regulated protein or the psoriasis-associated fatty acid binding protein (PA-FABP), are also expressed in the transient amplifying compartment. The same is true for the transcription factor p63.

Stem cells in the bulge region have the capacity to migrate (e.g. to the base of the hair follicle in follicular regeneration), as well as to differentiate into diverse lineages (e.g. outer root sheath [ORS], IRS, hair shaft, sebocytes and interfollicular epidermis). The precise lineage of terminal differentiation is governed by several local environmental cues. For example, Sonic hedgehog, Wnt and BMP are important in both embryonic and postnatal hair follicle development. Indeed, overexpression of the Wnt signalling component, β-catenin, leads to *de novo* follicle formation in skin. Thus, Wnt signalling appears to be important in making a follicle and Sonic hedgehog in maintaining it. Bone morphogenetic protein signalling influences differentiation of the hair shaft but not the IRS. However, little is known about the control of other adnexal differentiation, although Myc activation may be relevant for sebocyte differentiation.

The mechanisms that control exit from the stem cell compartment are incompletely understood, but clearly several molecular networks and signalling pathways are important in balancing epidermal growth and differentiation. Key components include NF-κB, Wnt/β-catenin, Sonic hedgehog/Patched, p63, 14-3-3σ, α-catenin and β1 integrin.

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Epidermal kinetics

Cell kinetics are complicated in the epidermis by the balance between growth with differentiation and cell death. A differentiated cell may have no proliferative capacity but may be extensively metabolically active, and can increase tissue volume or mass without an increase in cell number. There are a number of kinetic concepts that underly skin biology.

A major concept is that of *turnover time*, which is the amount of time for the whole cell population to replace itself (regeneration time or replacement time). This depends both on the time taken for individual cells to divide, *cell cycle*, and the proportion of basal cells dividing the *growth fraction*.

The *cell cycle* or intermitotic time (Tc) represents the interval between two successive mitoses (M). On histopathology of skin, dividing cells can be recognized by mitotic figures, but a longer period of time is spent between mitoses in interphase. Radiolabelled thymidine is incorporated into DNA, because of the salvage pathway for DNA, only during a specific period of DNA synthesis, the 'S' phase. All proliferating cells go through a cycle (Fig. 3.14), in which mitosis (M) is followed by the interphase or post-mitotic growth phase (G1), a period of active DNA synthesis (S) and a short resting or premitotic

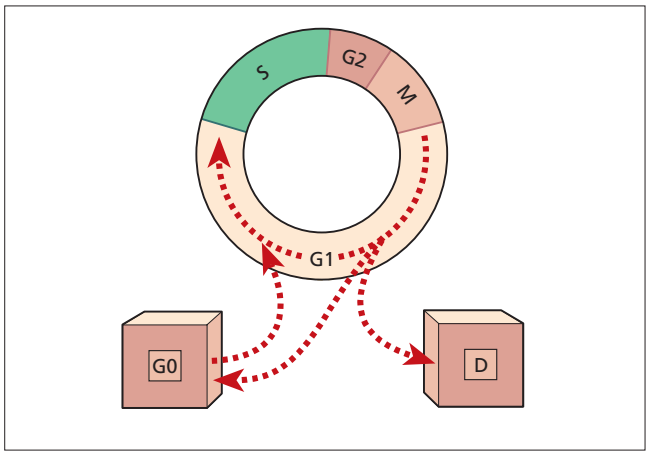

Fig. 3.14 Compartments of a cell proliferation system: M, mitosis, G1, interphase or post-mitotic growth phase; S, DNA synthesis; G2, resting or premitotic phase; G0, stem cells not proliferating; D, cells differentiating.

growth phase (G2). Some basal cells may remain quiescent in the so-called G0 phase, which permits them to re-enter the cell cycle and continue proliferation when required to do so by various stimuli. The balance of cell loss, from death, desquamation and apoptosis, and cell birth, decides the rate of increase or decrease of a cell population.

The *growth fraction* is the proportion of basal cells that are proliferative at any one time: in normal mouse skin this is estimated to be 60% of cells [1]. High proliferative rates can be achieved by a shorter cell cycle, or a higher proportion of proliferating cells, or both.

The proliferative index is familiar as the mitotic index and the flash-labelling index. The *mitotic index* is the fraction of basal or viable cells that is in mitosis at any point, and the *labelling index* is the fraction of basal cells in DNA synthesis. The labelling index is measured by exposing the skin to tritiated thymidine (^{3}HTdR), by intradermal or systemic injection, which is selectively taken up in DNA synthesis or rapidly broken down. This 'flash' labels the cells in S phase, which can then be detected by high-resolution autoradiography. Flash labelling the normal human epidermis labels about 30% of suprabasal cells, so this has to be included in some calculations [2].

These indices are referred to as state parameters, as they reflect the state of a particular component. However, rate parameters reflect the rate at which cells enter any phase. The rate of entry into mitosis is referred to as the *birth rate* in cells per 1000 cells per hour and can be measured by the accumulation of arrested metaphases after application of a stathmokinetic agent such as vincristine or colcemid [3]. The rate of entry into S phase is measured by double labelling, and is equivalent to the birth rate if all cells entering the S phase eventually divide, which is often not the case for keratinocytes in hyperproliferation. It is not known when cells leave the cycle to enter differentiation, as cells expressing involucrin as late markers of proliferation can still undergo scheduled DNA synthesis.

The *epidermal turnover time*, or transit time, has been used to represent the time taken for a cell to pass from basal layer to the surface of the skin, comprising passage through the living compartment to the upper stratum Malpighi and on through the non-viable compartment to the surface. Epidermal transit through stratum corneum can be estimated by injecting radioactive label or fluorescent dye, and measuring their appearance or disappearance at the surface of the skin. In normal skin, the total time is 52–75 days, but this is greatly reduced for psoriatic epidermis.

The best way of measuring the epidermal cell cycle and its component phases is the fraction of labelled mitoses technique, which involves determining the proportion of labelled mitoses after flash labelling with tritiated thymidine. From plotting the curve of the percentage of labelled mitoses with time, the duration of different phases of the cell cycle can be determined. Most cell populations show a distribution of cell-cycle times. *In vitro* studies, including explant cultures and cultures of disaggregated keratinocytes, have now been extensively used to analyse parameters of proliferation. Time-lapse photography can be used to directly measure intermitotic time *in vitro*. Initial colony formation is not dependent on multiplication, as keratinocytes appear to reassociate to colonies of four to six cells before replication, which occurs after 24–48 h. The kinetic parameters also illustrate a diversity of cell-cycle time *in vitro*. Scintillation counting is often used to measure incorporation of tritiated thymidine into DNA, but errors can be introduced by the size of the endogenous thymidine pool, and activity of thymidine-incorporating enzymes.

There are few direct measures of the cell-cycle time in normal skin, but they vary from 50 h (flow cytometry) to 457 h (turnover time). In psoriatic skin, the labelling index is greatly increased; the cell-cycle time is consistently reported to be reduced to around 50 h, and the growth fraction increased to 100% [4]. Keratinocytes *in vitro* have a mean intermitotic time of 22–24 h.

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The regulation of epidermal differentiation

Recent years have seen considerable progress in our

understanding of the patterns of expression of different structural genes in the epidermis and its appendages. However, just how the specific programmes of terminal differentiation are orchestrated at the transcriptional level remains poorly understood.

In skin development, several signalling pathways such as Hedgehog, Wnt and transforming growth factor-β (TGF-β) have been implicated. Indeed, many of these pathways show evolutionary conservation in aspects of epithelial differentiation, proliferation and tumourigenesis. These pathways may be variably activated, both spatially and temporally, leading to a diverse series of transcribed genes. In keratinocyte differentiation, three main classes of transcription factors, AP1, AP2 and Sp1, appear to be relevant, although gene-targeting experiments have shown evidence for functional redundancy. Nevertheless, key elements include the Fos/Jun family of *AP1* genes. *In vitro* studies have also identified several other relevant transcription factors such as basonuclin, C/EBP, Oct6 and Oct11 (POU domain transcription factors), ESE2 (ELF5), Klf4 and retinoic acid receptors (RARs).

Regulation of the nuclear transcription factor NF-κB also appears to be significant in the process of terminal differentiation in epidermis. I kappa kinase alpha (IKKα) is a kinase that, along with IKKγ, phosphorylates and destroys IκB, a cytoplasmic inhibitor of NF-κB. Targeted inactivation of the gene encoding IKKα results in impaired terminal differentiation, although actual levels of NF-κB are normal. It is thought that the defects result from loss of an unknown secreted factor normally dependent on IKKα activity. However, NF-κB activity is abnormal if IKKβ is compromised. IKKβ usually increases NF-κB activity but *IKKβ* gene-targeted heterozygous mice (X-linked gene) display signs of keratinocyte proliferation, skin inflammation and increased apoptosis. The *IKKβ* gene is clearly important in human epidermal physiology too since mutations underlie most cases of incontinentia pigmenti. Overall these *in vitro* data, mouse models and human gene mutations demonstrate that compromising NF-κB perturbs the balance between growth and differentiation in the epidermis (and perhaps also in certain immune response cells).

Terminal differentiation is also influenced by retinoids that act at the mRNA level to inhibit aspects of differentiation and to promote proliferation. These changes are mediated by nuclear RARs and their heterodimeric binding partners, retinoid X receptors (RXRs). Transgenic mice with disrupted RARs have impaired epidermal barrier function and suppressed epidermal differentiation. In addition, targeted disruption of RXRα leads to further changes in epidermal hyperplasia and aberrant terminal differentiation. These mice also have alopecia, indicating a further role for these receptors in hair follicle morphogenesis and cycling.

Growth stimulatory signals [1,2]

Epidermal growth factor (EGF) family

Human EGF is a 6-kDa polypeptide with 53 amino acids that stimulates cell proliferation and differentiation in a wide range of tissues. Transcripts are not found in the epidermis but in the salivary glands and intestinal tract. EGF has been shown to increase the growth and persistence of epidermal keratinocytes *in vitro* via binding to specific cell-surface receptors (170-kDa EGFr1), that have been detected in the basal layer of human epidermis and throughout the epidermis in psoriasis [3]. The EGFr has a large cytoplasmic domain with tyrosine kinase activity stimulating protein phosphorylation, and the cerB1 oncogene encoding truncated receptors acts by mimicking receptor activation [4].

In contrast to EGF, TGF-α, a single-chain 26-kDa polypeptide, is synthesized by epidermal keratinocytes and stimulates keratinocyte growth in an autocrine fashion following binding to the EGFr [5], although keratinocyte lifespan *in vitro* is mainly enhanced by stimulation of lateral migration of dividing colonies [6]. TGF-α can up-regulate its own production, as well as that of EGFr1. Overexpression of TGF-α has been found in suprabasal psoriatic epidermis [7].

Amphiregulin is a unique member of the EGF family of growth factors, with binding affinity for heparin-like glycosaminoglycans (GAGs) [8]. The amphiregulin gene maps to chromosome 4q, and encodes a transmembrane precursor protein, cleaved proteolytically to the active form, which binds to the EGFr. Amphiregulin appears to be a major autocrine keratinocyte growth factor, regulated by cellular GAGs and up-regulated by exogenous EGF and TGF-α, being overexpressed in hyperproliferative skin disease and squamous carcinomas [8,9].

More than 90% of autocrine growth of keratinocytes is mediated through the EGFr [8], but other cytokines synthesized by keratinocytes including interleukin-1 (IL-1), IL-6 and granulocyte–macrophage colony-stimulating factor (GM-CSF) can also stimulate growth [10–12]. Paracrine factors may be produced by dermal fibroblasts and microvascular endothelial cells. Some members of the FGF family stimulate keratinocyte growth, including acidic and basic FGF (bFGF). *Keratinocyte growth factor* (KGF: FGF7), in particular, has a high specificity for keratinocytes and is induced in dermal fibroblasts within 24 h of wounding [13]. Factors produced by leukocytes and macrophages—platelet-derived growth factor (PDGF) [14], TGF-α, IL-1β and tumour necrosis factor (TNF)—induce the *KGF* gene and may induce this rapid response.

REFERENCES

1 McKay IA, Leigh IM, eds. *Growth Factors: a Practical Approach*. Practical Approach Series. Oxford: Oxford University Press, 1993.