

## 7.2

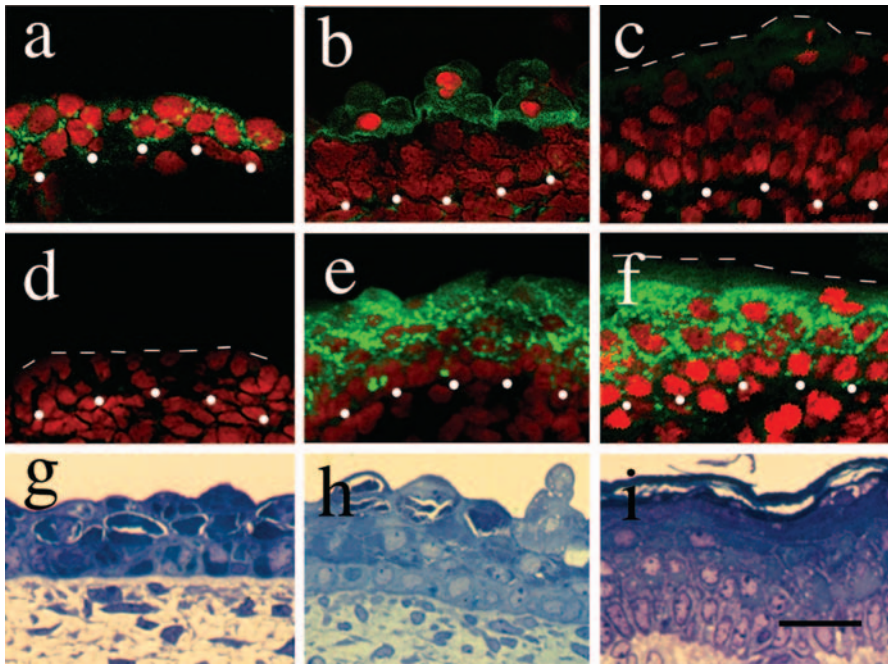
# The Gap Junction System of Human Skin

### 7.2.1

## Changing Patterns of Connexin Expression During Human Skin Development

In the human embryo, skin development starts at around 4 weeks of estimated gestational age (EGA) with the proliferation of the single-layered ectoderm into a two-layered epidermis. The outer layer is the periderm and the inner layer is formed by proliferating basal cells adhering to an immature basement membrane (Holbrook and Odland 1975; Holbrook 1979). Around the 11th week of EGA an intermediate layer is established. By 21 weeks, the epidermis has further stratified into spinous, granular, and cornified layers and the periderm is gradually shed into the amniotic fluid (Holbrook 1979). At birth, a dramatic change in environmental conditions takes place, which requires the epidermis to mature and adapt to a terrestrial milieu by forming a competent skin barrier (Williams et al. 1998).

The different stages of morphogenesis of the epidermis are accompanied by changes in gap junction distribution and connexin expression. In fetal mouse skin, Cx26 and Cx43 were found to be uniformly expressed in all epidermal layers during the early phase. In the later stages of development, Cx43 expression is limited to the intermediate layer and Cx26 expression is restricted to the outermost differentiated epidermal layer (Choudhry et al. 1997). In human fetal skin, Cx26 appears to be expressed during the early stages of fetal epidermal development and Cx43 during the stratification and differentiation (Figs. 7.1, 7.2). As early as 7 weeks EAG, Cx26 can be detected in the basal layer and the periderm. As the epidermis further stratifies, Cx26 expression becomes limited to the periderm while basal expression declines and ceases. After 16 weeks EAG, no Cx26 immunostaining has been detected in the four-layered epidermis (Arita et al. 2002). In neonatal and adult interfollicular epidermis, Cx26 is usually found only in a patchy distribution between basal keratinocytes in the skin of palms and soles. However, there is a high expression of Cx26 in hair follicles and eccrine sweat glands, including acroinfundibulum and sweat duct epithelium (Salomon et al. 1994; Lucke et al. 1999). Cx43 expression has been detected at the surface of basal cells after 8 weeks EAG (Hentula et al. 2001), and later also in the intermediate layer around 12–13 weeks EAG (Arita et al. 2002). Nevertheless, the gap-junction plaques formed before 16 weeks EAG appear immature on electron microscopic examination. Contrary to Cx26, Cx43 expression increases throughout the differentia-



**Fig. 7.1a–i.** Connexin 26 is expressed in the early stages of human fetal epidermal development and Cx43 in the later stages. **a** Expression of Cx26 within the periderm and basal cells in the two-layered stage of fetal epidermis (49 days EGA). **b** Cx26 is restricted to the periderm at the four- or more layered stage (96 days EGA). **c** Lack of epidermal Cx26 during the stage of interfollicular keratinization (163 days EGA). **d** Conversely, there is no expression of Cx43 in the epidermis at 49 days EGA. **e** Cx43 expression is detectable within the periderm and the intermediate cells of the four or more layered epidermis (88 days EGA). **f** Cx43 is visible between all intermediate cells, but there is only weak expression at basal cell borders during the stage of interfollicular keratinization (163 days EGA). **a–f** Fluorescent micrographs. Green represents FITC staining of connexins; red is nuclear PI staining. **g–i** Light micrographs depict representative morphological features of developing human epidermis (toluidine blue). **g** The two-layered stage. **h** The four- or more layered stage. **i** The stage of interfollicular keratinization. White dots represent the basement membrane zone, dashed line the top of the epidermis (top of the periderm or cornified layer). Bar 100  $\mu$ m. (Reproduced with permission from Arita et al. 2002)

tion process of fetal epidermis. In neonatal epidermis, Cx43 shows a typical punctate plasma membrane staining, which is strongest in the granular and spinous cell layers and less intense and focal in the basal layer (Hentula et al. 2001; Arita et al. 2002). After 17–21 weeks EAG, ultrastructural analyses revealed morphologically mature and intact gap junction plaques with typical pentalaminar structures, predominantly between cells of the periderm and less frequently between cells of the intermediate layers. Basal

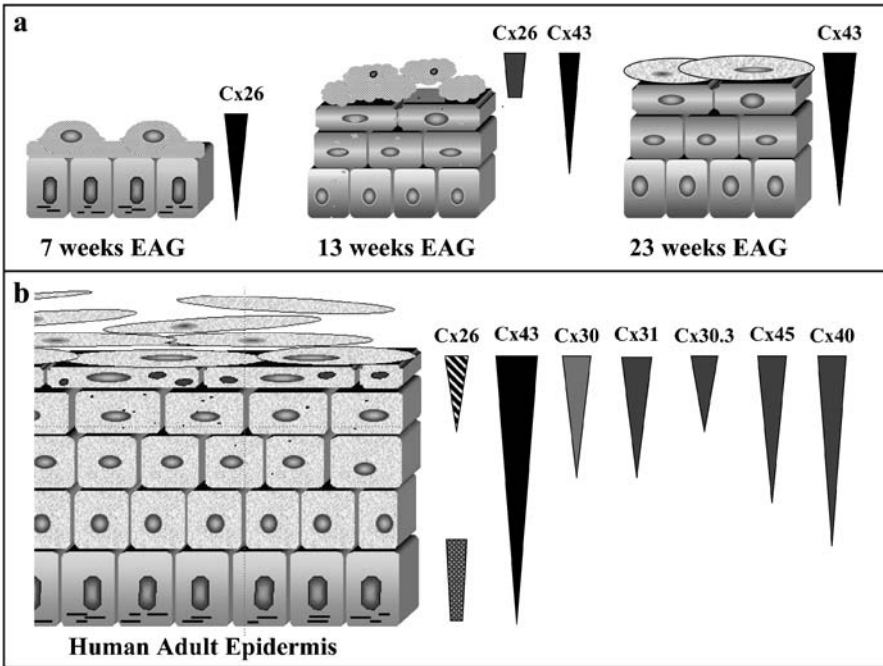


Fig.7.2. Schematic of the connexin expression patterns in fetal and mature epidermis

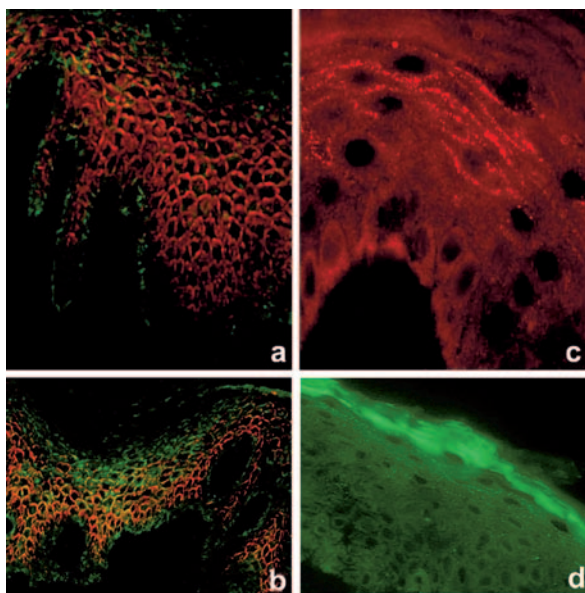
cells showed no obvious gap junctions at this stage, but developed a small number of gap junctions later on. Overall, the number of gap junction plaques increases significantly with gestational age, especially in the upper differentiated layers (Arita et al. 2002). It still remains to be determined, if and which other connexin proteins are required during fetal skin development.

It is also difficult to determine the actual level and pattern of intercellular communication that is established by this intricate network of different gap junctions in vivo. In developing mouse epidermis, the cytoplasmic microinjection of a fluorescent tracer into single cells revealed a rapid and extensive vertical dye spread between the basal and all differentiating layers. Surprisingly, the obvious switch in connexin expression during maturation and differentiation of the epidermis did not result in a change in the pattern of dye transfer, perhaps suggesting that more sensitive methods have to be developed to detect all biological changes in intercellular communication (Choudhry et al. 1997).

### 7.2.2

#### Connexin Expression in the Mature Human Epidermis

Similar to other epithelia of ectodermal origin, the epidermis and its appendages are exceptionally well coupled by gap junctions. During terminal differentiation, keratinocytes utilize at least nine different connexin proteins, thus producing a very complex and redundant system (Di et al. 2001). Keratinocytes of the basal layer encompass predominantly proliferating, transiently amplifying cells and a small number of slow-cycling stem cells (Watt 2002). The only connexin consistently expressed within this layer in interfollicular epidermis is Cx43, although spotty Cx26 immunostaining has been observed in palmoplantar basal epidermis. With initiation and progress of keratinocyte differentiation, cells gradually expand their gap junction network and express different connexins, including Cx30, Cx30.3, Cx31, Cx40, and Cx45 (Fig. 7.2). The expression patterns of Cx43 and Cx26 in adult epidermis do not significantly deviate from those of neonatal skin. Cx43 is expressed throughout the interfollicular epidermis preferentially in differentiated keratinocytes (Fig. 7.3). It is also abundant in the sebaceous glands and hair follicles, especially in the cortex, inner and outer root sheets



**Fig. 7.3a–d.** Expression pattern of selected epidermal connexin isoforms. Immunolocalization of Cx26 (green) and Cx43 (red). **a** In normal plantar epidermis and **b** in a patient with PPK and SNHL due to mutation E42del in Cx26. **c** Immunolocalization of Cx30 (red) and **d** Cx31 (green) in normal truncal epidermis. (Reproduced with permission from Richard 2003)

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