Loss of Normal Profilaggrin and Filaggrin in Flaky Tail (*ft/ft*) Mice: an Animal Model for the Filaggrin-Deficient Skin Disease Ichthyosis Vulgaris

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Flaky tail (gene symbol *ft*) is an autosomal recessive mutation in mice that results in a dry, flaky skin, and annular tail and paw constrictions in the neonatal period. Previous studies demonstrated that the *ft* mutation maps to the central region of mouse chromosome 3, in the vicinity of the epidermal differentiation complex, a gene locus that includes many nonkeratin genes expressed in epidermis. In this study we report a detailed characterization of the flaky tail mouse. Affected homozygous *ft/ft* mice exhibit large, disorganized scales on tail and paw skin, marked attenuation of the epidermal granular layer, mild acanthosis, and orthokeratotic hyperkeratosis. Biochemical analysis demonstrated that *ft/ft* mice lacked normal high molecular profilaggrin (≈ 500 kDa), and instead expressed a lower molecular weight form of profilaggrin (220 kDa) that is not proteolytically processed to profilaggrin intermediates or filaggrin. Mutant mice lacked the large, irregular F-type keratohyalin granules that contain profilaggrin, and filaggrin was absent from the cornified layers of *ft/ft* epidermis. The expression of epidermal keratins was unchanged, whereas the cornified envelope proteins involucrin and loricrin were increased in *ft/ft* epidermis. Cultured *ft/ft* keratinocytes also synthesized reduced amounts of profilaggrin mRNA and protein, demonstrating that the defect in profilaggrin expression is intrinsic to epidermal cells. These findings demonstrate that flaky tail mice express an abnormal profilaggrin polypeptide that does not form normal keratohyalin F-granules and is not proteolytically processed to filaggrin. We propose that the absence of filaggrin, and in particular the hygroscopic, filaggrin-derived amino acids that are thought to function in epidermal hydration, underlies the dry, scaly skin characteristic of *ft/ft* mice. This animal model provides a tool for understanding the role of filaggrin in normal epidermal function and may provide insight into the molecular basis of the filaggrin-deficient human skin disorder ichthyosis vulgaris. ***Key words: epidermis/flaky tail/ichthyosis/profilaggrin. J Invest Dermatol 115:1072–1081, 2000***

During epidermal differentiation, keratinocytes express a large number of structural proteins including the cytoskeleton-associated keratin proteins K1, K10, and K2e, and proteins such as loricrin, involucrin, and the small proline-rich proteins that are cross-linked by transglutaminases into the cornified cell envelope as cells undergo terminal differentiation (for reviews, see Fuchs and Byrne, 1994; Presland and Dale, 2000). Two of the most abundant proteins expressed in the granular layer of epidermis are loricrin, a glycine/serine-rich protein that constitutes 70–90% of total cornified envelope proteins (Hohl and Roop, 1993; Hohl *et al*, 1993; Steinert and Marekov, 1995) and profilaggrin, a highly phosphorylated calcium-binding protein that is proteolytically processed to filaggrin during terminal differentiation (for reviews, see Dale *et al*, 1994; Presland and Dale, 2000). Filaggrin functions to aggregate keratin proteins to form the tightly aligned macrofibril bundles characteristic of cornified cells (Dale *et al*, 1978; Steinert *et al*, 1981) and similarly affects the organization of keratin filaments in cultured epithelial cells (Dale *et al*, 1997). In the stratum corneum, filaggrin is degraded by proteases and the amino acids are chemically modified to produce a mixture of hygroscopic compounds that are believed to be important for normal epidermal hydration and flexibility (reviewed in Rawlings *et al*, 1994). Both profilaggrin and loricrin are prominent components of keratohyalin granules in granular cells in mouse skin, where they form morphologically distinct granules termed F-granules and L-granules, respectively (Steven *et al*, 1990; Manabe *et al*, 1991; Dale *et al*, 1994). In human epidermis, only profilaggrin has been localized unequivocally to keratohyalin in epidermis, whereas loricrin antibodies diffusely label the cytoplasm and nuclear aggregates in most skin types studied (Manabe *et al*, 1991; Ishida-Yamamoto *et al*, 1996).

Ichthyosis vulgaris is a heterogeneous autosomal skin disease characterized by dry, scaly skin, mild hyperkeratosis, and a decreased or absent granular layer that either lacks, or contains

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Abbreviations: EDC, epidermal differentiation complex; *ft*, flaky tail; ichthyosis vulgaris AGL, ichthyosis vulgaris with absent granular layer; *ma*, matted; MEK, mouse epidermal keratinocytes.

morphologically abnormal, keratohyalin granules (Anton-Lamprecht and Hofbauer, 1972; Sybert *et al*, 1985; Manabe *et al*, 1991). A morphologically distinct subset of individuals with the clinical findings of ichthyosis vulgaris, in whom the granular layer is consistently absent (termed ichthyosis vulgaris AGL), has been described recently.[1] Both the skin of ichthyosis vulgaris AGL patients and keratinocytes cultured from affected individuals exhibit reduced or absent profilaggrin mRNA and protein levels compared with normal controls, implicating a profilaggrin defect (Sybert *et al*, 1985; Fleckman *et al*, 1987; Nirunsuksiri *et al*, 1995). Other epidermal differentiation markers such as loricrin and keratin 1 are not affected. More recent studies of ichthyosis vulgaris AGL keratinocytes have shown that profilaggrin gene transcription is normal, but that the profilaggrin mRNA is unstable and hence is poorly translated in ichthyosis vulgaris keratinocytes (Nirunsuksiri *et al*, 1995, 1998). To date, the genetic defect has not been identified; however, in one family where the proband lacks an epidermal granular layer, the morphologic phenotype maps to human chromosome 1q21[2] where profilaggrin, loricrin, involucrin, trichohyalin, and many other genes expressed in epidermis are clustered in an epidermal differentiation complex (EDC) (Volz *et al*, 1993; Mischke *et al*, 1996). Linkage to chromosome 1q21 was demonstrated using both autosomal dominant and recessive models[2] suggesting that ichthyosis vulgaris AGL could be a dominant or recessive disorder.

In this study, we report the detailed characterization of a spontaneous mouse mutation termed flaky tail (gene symbol *ft*) that has morphologic and biochemical similarities to human ichthyosis vulgaris AGL. Neonatal *ft/ft* mice have a dry, flaky skin that presents histologically with mild orthokeratotic hyperkeratosis and an attenuated granular layer that lacks profilaggrin keratohyalin, or F-granules. Previously, the flaky tail phenotype was shown to be due to a single autosomal recessive mutation that maps to mouse chromosome 3 (Lane, 1972), close to the genes encoding mouse loricrin and profilaggrin (Rothnagel *et al*, 1994). Here we demonstrate that *ft/ft* mice lack filaggrin and the normal high molecular form of profilaggrin. This filaggrin-deficient mouse provides a useful model for understanding the function of filaggrin in epidermal differentiation and desquamation and may provide insight into the underlying basis of profilaggrin/filaggrin-deficient ichthyosis vulgaris AGL.

MATERIALS AND METHODS

Mice Flaky tail (STOCK *ft/ft*, *ma/ma*) and C57BL/6J+/+ mouse colonies were derived and maintained at the Jackson Laboratory (Bar Harbor, ME). Groups of two or more female and male mice, doubly homozygous for the flaky tail (*ft/ft*) and matted (*ma/ma*) mutations, and equal numbers of normal, wild-type C57BL/6J mice (+/+), serving as controls, were killed at 3 d intervals from 0 to 30 d, at sexual maturity (6 wk of age), and at the age of sexual quiescence (8 mo of age) for phenotypic characterization. Mice were maintained on a 12 h light/dark cycle in polycarbonate double pen boxes. They were fed Rodent NIH-31 diet with 6% fat (Agway, Syracuse, NY) and were given acidified water *ad libitum*.

Mice were killed following standards approved by the Jackson Laboratory Institutional Animal Care and Use Committee. Routine colony surveillance and diagnostic work-up verified that mice were free of known mouse pathogens (http://jaxmice.jax.org/html/health/quality_control.shtml#Animalhealth).

Tissue collection Complete sets of organs were collected at major time points in the mouse's life as described previously (Sundberg *et al*, 1997). Serial sections of paraffin-embedded tissues were stained with hematoxylin and eosin for histopathologic examination (Sundberg *et al*, 1998; Relyea *et al*, 1999).

Immunohistochemistry Serial sections of dorsal or tail skin were reacted with polyclonal antibodies directed against mouse profilaggrin (antibody 1398, Resing and Dale, 1991) and keratins K5, K6, K1, K10, K14, loricrin, and profilaggrin/filaggrin (Covance Research Products, Richmond, CA). Immunohistochemistry was performed on methyl Carnoy's fixed samples embedded in paraffin using the ABC peroxidase method as previously described (Hsu *et al*, 1981). Reaction was detected using diaminobenzidine. For immunofluorescence, newborn skin was frozen and fixed in methyl Carnoy's and sections stained with profilaggrin, loricrin, and K1 polyclonal antibodies followed by a horse anti-rabbit biotinylated secondary antibody and streptavidin–Texas Red for detection (Vector Laboratories, Burlingame, CA). Samples were coverslipped using Prolong mounting media (Molecular Probes, Eugene, OR) and photographed using a Nikon Microphot-SA microscope equipped with a CCD camera (Photometrics, Tucson, AZ). Controls performed in the absence of primary antibody were routinely performed and showed no reaction with epidermal tissue.

Electron microscopy Transmission electron microscopy was performed on dorsal skin of six female *ft/ft* and six C57BL/6J+/+ mice at 14 d of age as described previously (Morita *et al*, 1995; Bechtold, 1999). Sections were embedded in Quetol 657 resin (EMS, Fort Washington, PA), stained with uranyl acetate and Reynold's lead citrate, and examined in a JEOL 100CXII transmission electron microscope.

For scanning EM, feet and tails were removed from mutant (one 3 d old female, one 6 d old female, one 6 d old male, and two 9 d old females) and control mice (one female and one male 5 d old C57BL/6J) at the time of necropsy and fixed in cacodylate-buffered glutaraldehyde. Samples were prepared for scanning electron microscopy using established procedures (Morita *et al*, 1995; Bechtold, 1999) and examined in a JEOL 35C scanning electron microscope operated at 10 kV. Immunoelectron microscopy was performed on epidermal tissue from newborn and 13 d old mice embedded in LR White resin as described previously (Presland *et al*, 1997). Detection of profilaggrin/filaggrin antibody was with goat anti-rabbit 15 nm gold-tagged antibody (Vector Laboratories). Tissue was examined using a JEOL 1200 transmission electron microscope operated in the transmission mode at 80 kV.

Mouse keratinocyte culture Newborn *ft/ft* and control C57/BL6 mice, 1–3 d old, were killed and skins shipped overnight to Seattle in transport media [modified Eagle's medium with Earle's balanced salt solution and 0.06 mM calcium (EMEM) containing 20% fetal bovine serum, 1% penicillin and streptomycin, 1% hydrocortisone, 0.1% gentamycin, 1% fungizone; Hager *et al*, 1999]. Mouse epidermal keratinocytes (MEK) were isolated and cultured from newborn mouse skin as described by Hager *et al* (1999). For all experiments, keratinocytes were maintained in medium that contained 0.06 mM $CaCl_2$ and differentiation induced by growing in keratinocyte growth medium containing 0.15 mM $CaCl_2$ for 48 h (Hager *et al*, 1999).

Western analysis for immunoblot studies, the entire dorsal skin and tail were removed from freshly killed *ft/ft* and control+/+ newborn (1–3 d old) mice. The epidermis was separated from dermis by immersion in PBS containing 5 mM EDTA at 50°C for 2 min, followed by rapid cooling in cold PBS. Separated dorsal epidermis and tail skin were extracted in urea/Tris buffer containing protease inhibitors by several strokes with a Dounce homogenizer (Presland *et al*, 1997). For immunoblotting, equal protein loadings were separated by discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis on either 7–12% or 5–12% gels (Laemmli, 1970) and electroblotted for 12 h at 100 mA on to nitrocellulose (0.45 μM, Schleicher & Schuell, Keene, NH). Blots were reacted with polyclonal antibodies to profilaggrin/filaggrin, loricrin, K1, involucrin, and K14 (Covance). Immunoreactive proteins were visualized with the avidin–biotin–peroxidase complex method using 4-chloro-1-napthol as substrate (Towbin *et al*, 1979; Haydock *et al*, 1993) or by enhanced chemiluminescence (Amersham, Arlington Heights, IL) as recommended by the manufacturer. Enhanced chemiluminescence blots were developed on BioMax ML X-ray film (Eastman Kodak, Rochester, NY). Immunoblots were calibrated with prestained protein standards (Life Technologies, Gaithersburg, MD).

For 2-D gels, urea/Tris extracts of newborn mouse epidermis were separated by charge in the first dimension using the nonequilibrium gradient system (NEpHGE, O'Farrell *et al*, 1977; Harding and Scott, 1983). Proteins were subsequently separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 4–15% gels (Jule Biotechnologies, New Haven, CT). Samples were blotted as described for 1-D gels and proteins detected with appropriate antibodies by the ABC method.

RNA isolation and analysis Total RNA was isolated from newborn mouse skin and cultured keratinocytes using Tri-Reagent (MRC, Cincinatti, OH) or RNAqeous (Ambion, Austin, TX). For reverse transcription–polymerase chain reaction, RNA was treated with RNAe-free DNAase I (Life Technologies) to remove genomic DNA as suggested

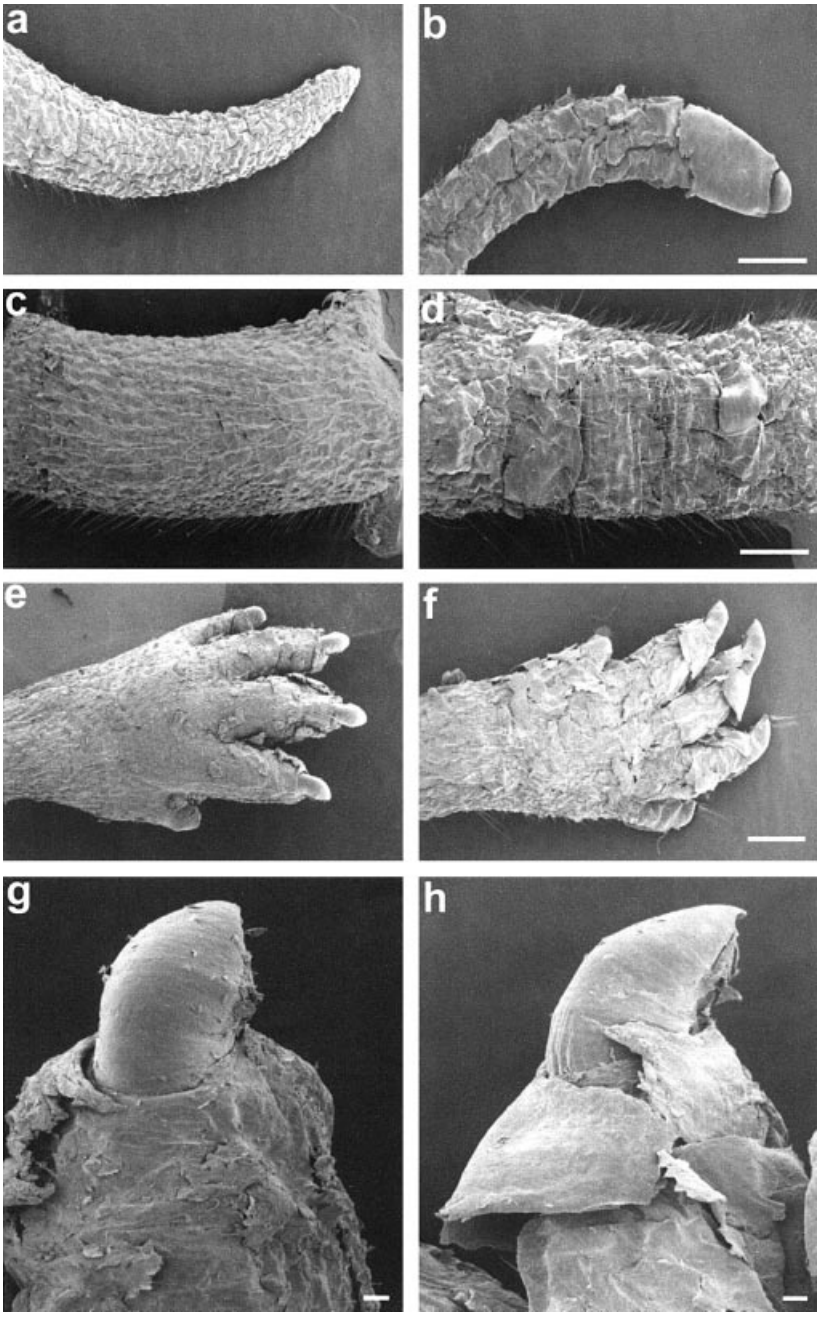

Figure 1. Tail and paw skin of *ft/ft* mice have disorganized scale. Shown are scanning electron micrographs from the tails of normal (*a*, *c*) and mutant (*b*, *d*) mice, normal (*e*) and mutant (*f*) paws, and higher magnification of the nail region of normal (*g*) and mutant (*h*) paws. Control animals have regular scales that are well organized, whereas mutant animals have disorganized, desquamating scales that vary in size, with some lifting off the epidermis. *Scale bars*: 1 mm (*a–f*), 0.1 mm (*g*, *h*).

by the manufacturer. Reverse transcription–PCR was performed using 0.5 μg of each RNA sample using the Titan One-Tube System (Boehringer Mannheim, Indianapolis, IN). Reverse transcription was performed for 30 min at 50°C followed by PCR using primer pairs for loricrin and K1 (Bickenbach *et al*, 1995), glyceraldehyde 3-phosphate dehydrogenase (GPDH) (Clontech Laboratories, Palo Alto, CA), and profilaggrin (5′ GAATCCATATTTACAGCAAAGCACCTTG 3′ (forward) and 5′ GGTATGTCCAATGTGATTGCACGATTG 3′ (reverse) (Rothnagel and Steinert, 1990). PCR was performed by heating at 94°C for 3 min followed by 25 cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C. Products were analyzed by 2% agarose gel electrophoresis. Product sizes were as follows: 417 bp for K1, 100 bp for loricrin, 452 bp for GAPDH, and 493 bp for profilaggrin. To check for genomic DNA contamination, DNase I-treated RNA samples (0.5 μg) were amplified with Taq polymerase (Boehringer Mannheim) and GPDH primers using the same cycling conditions. For northern analysis of keratinocyte RNA, 20 μg of total RNA was fractionated on 0.9% agarose/glyoxal gels, the products transferred to GeneScreen Plus (NEN, Boston, MA), and hybridized with a ^{32}P-labeled 750 bp probe containing a single mouse filaggrin unit as described previously (Nirunsuksiri *et al*, 1995, 1998). Filters were washed at high stringency (0.1 × 0.015 sodium citrate/0.0015 chloride buffer, 0.1% sodium dodecyl sulfate) and exposed to X-ray film. Blots were reprobed with a GAPDH probe to ascertain equal RNA loadings between samples and the data analyzed by scanning with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) as described (Nirunsuksiri *et al*, 1998).

DNA isolation and analysis Genomic DNA was isolated from the livers of newborn mice and from the spleens of older mice for southern blot and PCR studies as described (Sambrook *et al*, 1989). For southern analysis, genomic DNAs from two affected *ft/ft* mice and two +/+ control mice were digested with the restriction enzymes *Kpn*I, *Hin*dIII, and *Eco*RI as recommended by the manufacturer and fractionated on 1% agarose gels. DNA was transferred to Genescreen Plus and filters were hybridized with a profilaggrin probe (either a 750 bp filaggrin coding probe or a 3′ noncoding probe), washed, dried, and exposed to X-ray film.

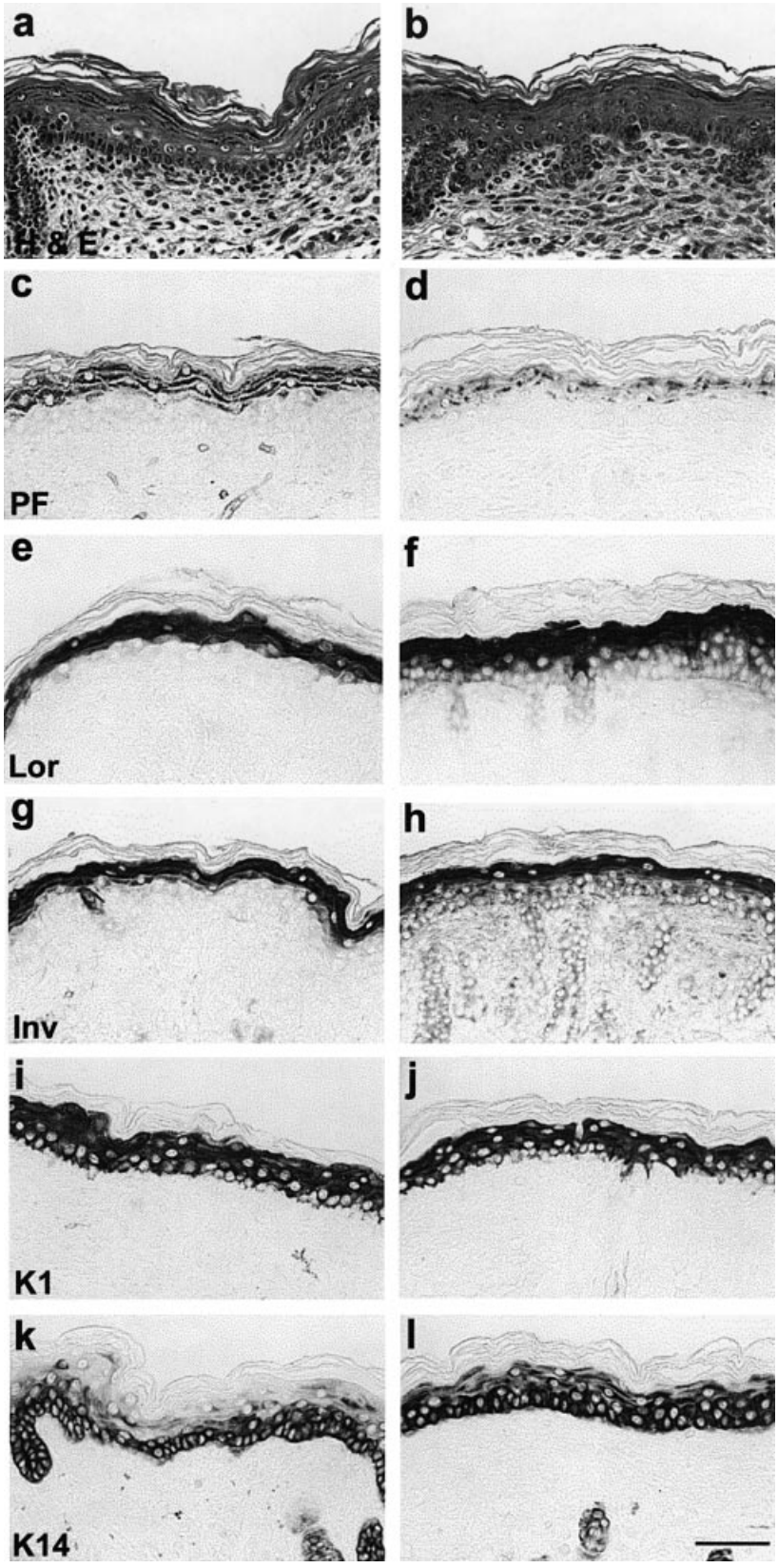

Figure 2. Histology and immunohistochemistry of *ft/ft* mouse epidermis. Hematoxylin–eosin staining (*a*, *b*) and immunohistochemistry (*c–i*) of normal (*a*, *c*, *e*, *g*, *i*, *k*) and *ft/ft* (*b*, *d*, *f*, *h*, *j*, *l*) dorsal skin from newborn mice. Note the near absence of keratohyalin granules in the granular layer of *ft/ft* epidermis, which is prominent in the control. Immunohistochemistry was performed with profilaggrin (PF; *c*, *d*), loricrin (Lor; *e*, *f*), involucrin (Inv; *g*, *h*), K1 (*i*,*j*), and K14 (*k*,*l*) antibodies. Note the marked reduction of profilaggrin expression in *ft/ft* epidermis (*d*). *Scale bar*: 50 μm.

RESULTS

Phenotypic and genetic description The flaky tail mutation arose spontaneously among the progeny of crosses between heterogeneous stocks at the Jackson Laboratory in 1958. Some of the offspring developed abnormally small ears, and, when backcrossed on to the C57BL/6J inbred strain, the offspring also developed tail constrictions and flaky tail skin. This latter phenotype provided the name for the mutation, flaky tail (Lane, 1972; Sundberg, 1994a; Sundberg *et al*, 1996). Homozygous affected mice appear normal at birth. The flaky tail phenotype appears at about 3 d of age with the skin on the dorsal and ventral surfaces having a flaky appearance. At 6 d of age the

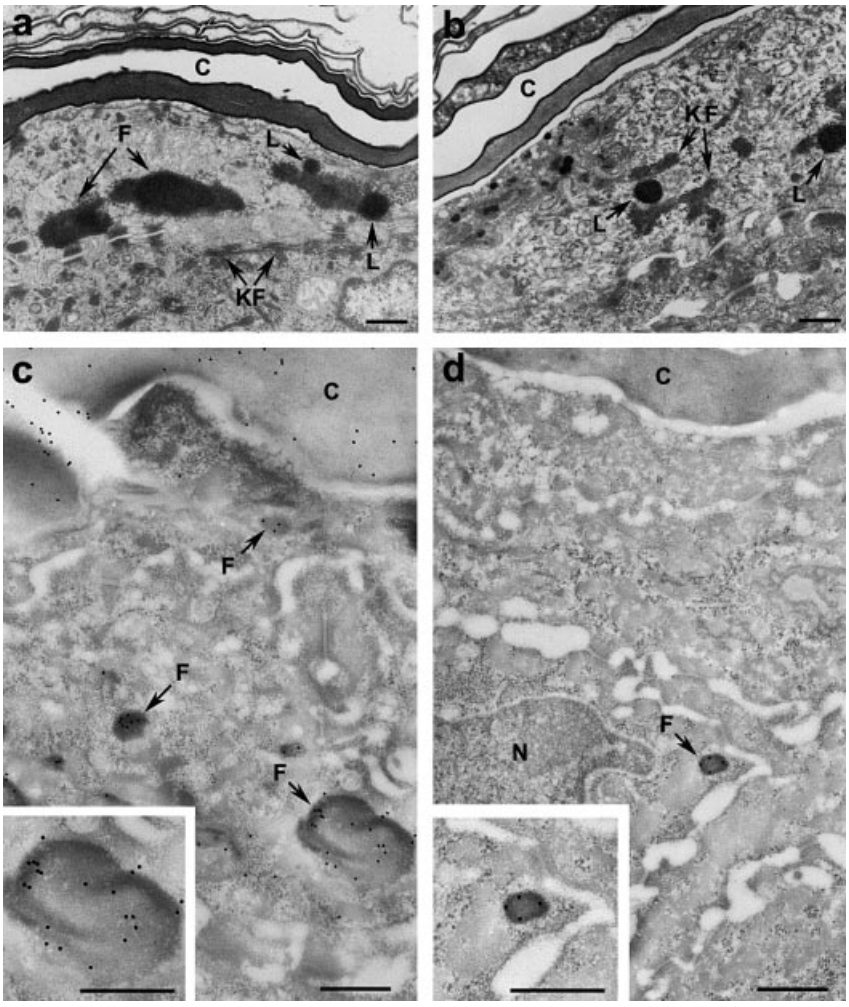

Figure 3. *ft/ft* mouse epidermis lacks normal profilaggrin F-granules. Shown are sections of control (*a*, *c*) and *ft/ft* (*b*, *d*) epidermis examined by electron microscopy for morphology and immunoelectron microscopy after immunolabeling with profilaggrin/filaggrin antibody. Note that while +/+ control mice contain both the large, irregular F-granules (F) and dense, round L-granules (L) that contain profilaggrin and loricrin, respectively (*a*), *ft/ft* epidermis lacks normal large F-granules (*b*). L-granules are often seen embedded in F-granules in normal mouse skin (*a*). In *ft/ft* mice, keratin filaments (KF) that are associated with polyribosomes appear to be clumped into aggregates (*b*) that are less prominent in the epidermis of control mice (*a*). Parts (*c*) and (*d*) show immunoelectron microscopy of control (*c*) and *ft/ft* (*d*) epidermis from newborn mice. The arrows indicate immunolabeled profilaggrin F-granules in control epidermis (*c*) and a smaller profilaggrin-containing granule in *ft/ft* epidermis (*d*). Insets show higher magnifications of profilaggrin-containing granules from control and *ft/ft* epidermis. Note the absence of immunolabeling of the cornified layers in *ft/ft* epidermis (*d*). C, cornified cell; F, F-granule; L, L-granule; KF, keratin filaments. *Scale bars*: 0.4 μm.

skin on both surfaces peels off in large sheets, similar to sunburned skin. The skin on the front and hind limbs and the tail also begins to show signs of peeling at this age and there are usually constrictions visible around the ankles of the hind limbs and on the tail. The constrictions on the tail can result in autoamputation of a portion of the tail (Sundberg *et al*, 1996). At 9 d of age, when the hair coat has covered most of the body, the flaking and peeling can be seen only on the tail and hind limbs that have sparse amounts of hair. Pups continue to show this phenotype, with gradual improvement until approximately 15 d of age. At 15–21 d the pups appear normal except for the shortened ears and loss of tail tips.

No pathologic lesions were seen in any tissues other than skin and associated appendages.

Scanning electron microscopy of *ft/ft* epithelia showed defects in tissue organization Tails, dorsal skin, and feet from five *ft/ft* homozygous mice, ranging in age from 3 to 9 d of age, and two normal C57BL/6J mice, 5 d of age, were examined by scanning electron microscopy. Regardless of age or sex, features were very similar between individuals within either group. The tail from a normal mouse had uniform scale formation along its entire length (**Fig 1*a***, ***c***). Tail skin from *ft/ft* mutant mice appeared to be crinkled up along its entire length. This was due to disorganized desquamating scales of various sizes (**Fig 1*b***, ***d***). Some of the *ft/ft* scales were large and had a smooth surface, particularly at the distal end of the tail (**Fig 1*b***). Sections of tail examined midway between the tail base and tip had small scales of various sizes lifting off the surface (**Fig 1*d***). The dorsal skin had a similar scale pattern as the tail of mutant mice except that the scales were much smaller and more uniform in size and *ft/ft* mice had greatly decreased numbers of hair fibers emerging from the follicles (data not shown). The feet of *ft/ft* mice exhibited prominent scale that lifted off the surface at articulation points and the entire dorsal skin surface of the foot had prominent desquamating scales (**Fig 1*f***) that were not observed in control animals (**Fig 1*e***). A similar disorganization was seen in scaling periungual skin adjacent to nails (**Fig 1*g***, ***h***). Both front and rear feet had similar features. The nails themselves were normal.

The epidermis of flaky tail mice exhibit an attenuated granular layer and reduced profilaggrin expression Histologically, newborn *ft/ft* mice exhibited a markedly attenuated granular layer (where loricrin and profilaggrin are expressed) compared with epidermis of normal controls that typically have two to three granular layers (**Fig 2*a***, ***b***). The basal and spinous layers appeared normal. Mutant mice consistently exhibited compact orthokeratotic hyperkeratosis with mild acanthosis.

To determine if any epidermal proteins were altered in distribution, *ft/ft* and +/+ mouse skin was immunostained with a panel of antibodies specific for mouse epidermal proteins. In *ft/ft* mutant skin, profilaggrin expression was markedly attenuated in the differentiated layers, whereas loricrin expression appeared to be slightly increased compared with controls (**Fig 2*c–f***). The residual profilaggrin remaining in *ft/ft* epidermis was localized in a punctate pattern in the cytoplasm of granular cells (**Fig 2*d***). Double label immunofluorescence analysis with profilaggrin and loricrin antibodies confirmed that the residual profilaggrin staining was in the granular layer where loricrin was expressed normally (data not shown). The expression patterns of the epidermal proteins involucrin, K1, and K14 were very similar between *ft/ft and* control mice (**Fig 2*g–l***), with the exception that K14 was consistently present in a greater number of epidermal layers in *ft/*

ft mice compared with controls (**Fig 2*k*, *l***). K6 expression was restricted to hair follicles of dorsal skin in affected mice, consistent with the observed lack of acanthosis in *ft/ft* epidermis (data not shown).

Granular cells of *ft/ft* mice lack normal profilaggrin F-granules and filaggrin in the stratum corneum Electron microscopy of *ft/ft* epidermis confirmed the light microscopic findings and unequivocally showed that the granular layer of *ft/ft* epidermis lacked the large, irregular shaped keratohyalin (referred to as F- or P–F-granules; Steven *et al*, 1990; Dale *et al*, 1994) that contain profilaggrin (**Fig 3*a*, *b***). On the other hand, the small round loricrin-containing keratohyalin (L-granules) were present in the cytoplasm (and sometimes nucleus) of epidermal granular cells in both control and *ft/ft* mice. In normal mouse skin, small L-granules were often seen embedded in the larger F-granules (**Fig 3*a***), as reported previously (Steven *et al*, 1990). Keratin filaments were present as clumped aggregates in *ft/ft* epidermis (**Fig 3*b***), a feature not generally seen in normal epidermis. These results show that *ft/ft* epidermis lacks typical profilaggrin F-granules, consistent with the greatly reduced level of profilaggrin expression (**Fig 2**).

To determine the location of the residual *ft/ft* profilaggrin protein, immunoelectron microscopy was performed on newborn epidermis of *ft/ft* and control mice using a profilaggrin/filaggrin-specific antibody. As expected, *ft/ft* epidermis displayed a greatly reduced immunolabeling compared with control tissue. In *ft/ft* mice, the profilaggrin antibody sparsely labeled the cytoplasm of granular cells in a diffuse pattern and, occasionally, small granular aggregates (**Fig 3*d***). These structures, which varied in size, resembled the small keratohyalin granules seen by electron microscopy in the granular layer of *ft/ft* mice (**Fig 3*b***). Control epidermis exhibited the large profilaggrin-reactive F-granules characteristic of granular cells as well as smaller granules similar to those seen in *ft/ft* mice (**Fig 3*c***; Steven *et al*, 1990). Similar results were seen with epidermal tissue from 14 d old mice (data not shown). Little if any labeling of the cornified layers was seen in *ft/ft* epidermis (**Fig 3*d***), consistent with the lack of processing of *ft/ft* profilaggrin to filaggrin (see below).

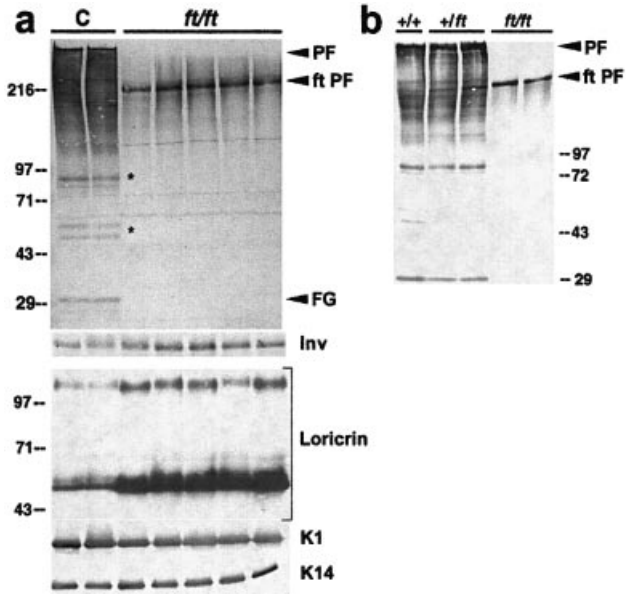

Figure 4. *ft/ft* mice express a smaller profilaggrin protein that is not processed to filaggrin. (*a*) Immunoblot analysis of equal protein loads of urea/Tris extracts from two control +/+ mice (C) and five mutant (*ft/ft*) mice with profilaggrin (top panel), involucrin, loricrin, K1, and K14 antibodies. Note that normal-sized profilaggrin (PF) and filaggrin (FG), as well as profilaggrin intermediates (*stars*) are absent from *ft/ft* mice. The ≈ 220 kDa form of profilaggrin present in *ft/ft* epidermis is labeled as ft PF. The loricrin antibody detected both the monomeric (≈ 60 kDa) and partially cross-linked forms (≈ 100 kDa) of loricrin protein. (*b*) Immunoblot analysis of control (+/+), heterozygous (+/*ft*), and homozygous *ft/ft* mice with profilaggrin/filaggrin antibody. Heterozygous mice contain both high molecular weight profilaggrin and the smaller profilaggrin molecule expressed by *ft/ft* mice ft PF. Molecular weight markers are in kilodaltons. PF, profilaggrin; ft PF, *ft/ft* profilaggrin; FG, filaggrin.

ft/ft* mice express reduced amounts of a lower molecular form of profilaggrin, and do not process it to filaggrin** The absence of normal F-granules (**Fig 3**) and markedly attenuated expression of profilaggrin (**Fig 2**) suggested that *ft/ft* mice express profilaggrin abnormally. To examine this further, immunoblot analysis was performed using epidermal extracts prepared from dorsal skins of newborn mice. Both the normal high molecular weight profilaggrin (≈ 500 kDa), and filaggrin (30 kDa), were absent from newborn *ft/ft* epidermis (n = 5) (**Fig 4*a). Profilaggrin intermediates, normally produced during the proteolytic conversion of profilaggrin to filaggrin, also were absent from *ft/ft* epidermis; instead a lower molecular weight profilaggrin of ≈ 220 kDa was detected (**Fig 4*a***). K1 and K14 were present at comparable levels in control and *ft/ft* epidermis, whereas the cornified envelope proteins involucrin and loricrin exhibited increased expression in *ft/ft* mice compared with controls. Heterozygous (+/*ft*) mice expressed both the normal high molecular weight and 220 kDa profilaggrin species, consistent with the notion that the inherited molecular defect lies in the profilaggrin gene (**Figs 4*b*** and **5**). In addition, heterozygous mice showed no gross or histologic abnormalities (data not shown), consistent with the recessive nature of the mutation (Lane, 1972; Sundberg, 1994a).

2-D immunoblot analysis with profilaggrin antibody confirmed the above findings, demonstrating that *ft/ft* mice express a smaller profilaggrin protein that is not processed to lower molecular weight intermediates or filaggrin (**Fig 5**). In addition, the profilaggrin molecules produced by *ft/ft* and control mice were similar in charge, suggesting that the *ft/ft* profilaggrin was normally phosphorylated *in vivo*. Heterozygous (+/*ft*) mice express both the normal and flaky tail forms of profilaggrin (**Fig 5**, *right panel*), confirming the 1-D immunoblot data (**Fig 4*a***).

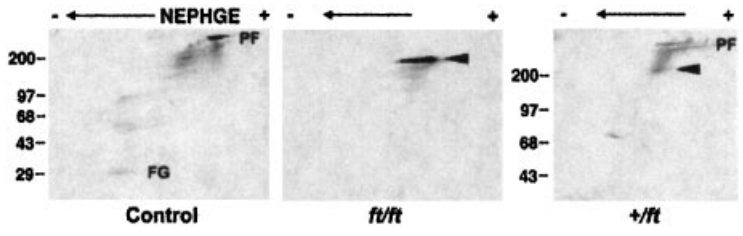

Figure 5. 2-D immunoblot analysis of *ft/ft* profilaggrin. Epidermal proteins were separated in the first dimension using the nonequilibrium gradient system (NEpHGE), and in the second dimension by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Arrowhead* indicates the lower molecular weight profilaggrin protein present in *ft/ft* epidermis. Note that heterozygous mice (+/*ft*) express both the high molecular weight profilaggrin (PF) and the smaller *ft* form of profilaggrin (*arrowhead*). The second dimension of the +/*ft* gel was run further in order to resolve the two profilaggrin polypeptides. Molecular weight markers are in kilodaltons. PF, profilaggrin; FG, filaggrin.

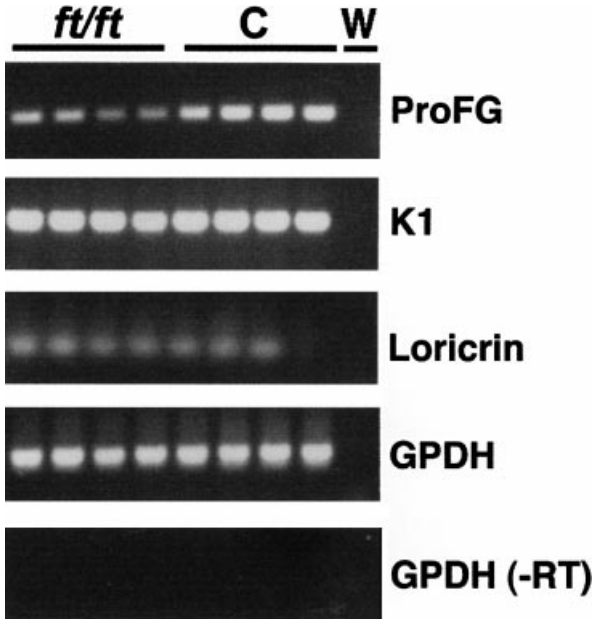

Figure 6. *ft/ft* epidermis have reduced levels of profilaggrin mRNA. Reverse transcription–PCR was performed on total RNA isolated from control (C) and *ft/ft* newborn mouse skins using primer pairs specific for profilaggrin (proFG), K1, loricrin, and GPDH. PCR was performed for 25 cycles, to allow a qualitative comparison of mRNA levels between samples. W, represents a –RNA control. GPDH (–RT) represents controls performed with RNA and GPDH primers, but in the absence of reverse transcriptase.

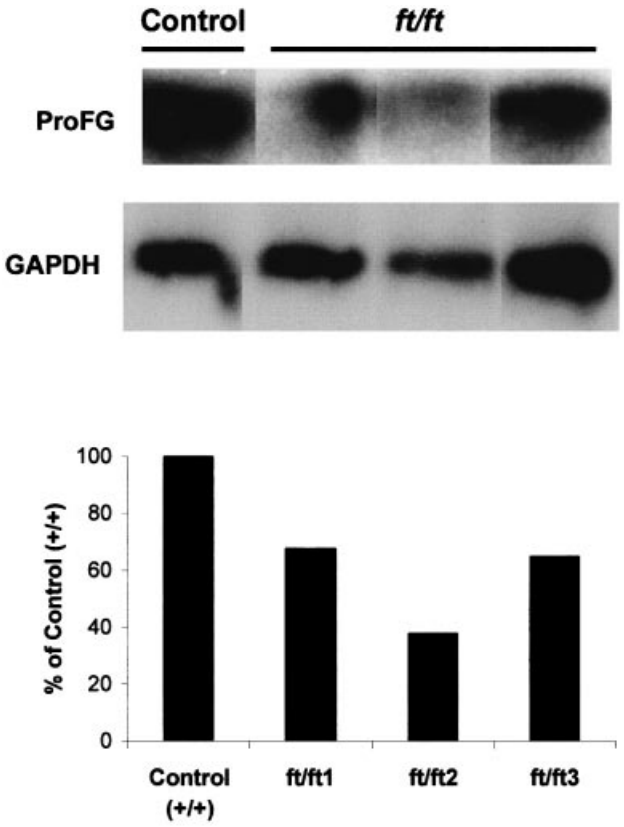

Figure 7. Cultured *ft/ft* keratinocytes express reduced profilaggrin mRNA. Equal amounts of total RNA from control and *ft/ft* MEK were fractionated on agarose gels, blotted, and probed sequentially with a single filaggrin repeat unit and GAPDH probes. Signal intensities were quantified by PhosphorImager analysis and the profilaggrin mRNA signal normalized relative to the GAPDH signal to correct for variation in RNA load. Results are plotted relative to the profilaggrin signal of control keratinocytes, which was set at 100%. The profilaggrin signals for the three *ft/ft* cultures (prepared from individual mice) were 67, 38, and 65% of the control +/+ value.

The profilaggrin gene in *ft/ft* and control mice is a similar size Our immunoblot data demonstrate *ft/ft* mice lack normal profilaggrin but express a lower molecular form of profilaggrin that is not processed to filaggrin. The altered molecular weight could result from: (i) *ft/ft* profilaggrin having fewer filaggrin units within the coding region, or (ii) a mutation in the center of the gene, in one of the filaggrin units, that creates a premature stop codon downstream. To examine the first possibility, we compared the size of the profilaggrin genes in *ft/ft* and control mice by performing southern analysis of *ft/ft* and control mice after digestion with restriction enzymes (*Kpn*I, *Hin*dIII, *Eco*RI) that cut in DNA sequences flanking the filaggrin repeats (Rothnagel *et al*, 1987; R. Presland, unpublished). The profilaggrin gene was similar in size between *ft/ft* and control mice, and therefore contained a similar number of filaggrin units (data not shown). Therefore, the molecular weight disparity is best explained by a mutation within one of the repeated filaggrin units, i.e., the polyfilaggrin region, that results in truncation of the profilaggrin gene product in *ft/ft* mice.

ft/ft mice express reduced levels of profilaggrin mRNA We further examined the profilaggrin deficiency in *ft/ft* mice to determine if mutant mice had reduced levels of mRNA, which would be indicative of an unstable or poorly translated mRNA as a consequence of mutation. Reverse transcription–PCR performed on total RNA isolated from age-matched *ft/ft* and +/+ mice showed that the level of profilaggrin mRNA was reduced significantly compared with control mice, whereas K1, loricrin, and GPDH mRNA exhibited no measurable differences (**Fig 6**). These results provide further evidence that a defect in profilaggrin, or in a gene that regulates its expression, is responsible for the epidermal abnormalities in *ft/ft* mice.

Cultured *ft/ft* keratinocytes synthesize reduced amounts of profilaggrin mRNA and protein To examine the growth of *ft/ft* keratinocytes *in vitro* and allow comparisons with keratinocytes from human ichthyosis vulgaris AGL patients that exhibit reduced profilaggrin expression (Fleckman *et al*, 1987; Nirunsuksiri *et al*, 1995), MEK were cultured from *ft/ft* and +/+ mice. Northern blot analysis demonstrated that low passage *ft/ft* keratinocytes expressed less profilaggrin mRNA than control MEK prepared and cultured in parallel from newborn mice (**Fig 7**). Quantitation of the profilaggrin mRNA signal relative to the GAPDH loading control demonstrated that the *ft/ft* profilaggrin mRNA was present at $57 \pm 16\%$ of the amount present in normal +/+ keratinocytes ($n = 3$) (**Fig 7**). Immunoblot analysis of *ft/ft* keratinocyte extracts also showed attenuated levels of profilaggrin protein, consistent with *in vivo* results (**Fig 8**). Normal MEK express profilaggrin as a high molecular weight immunoreactive smear, which is not processed to low molecular weight filaggrin due to the incomplete differentiation obtained under these submerged culture conditions (**Fig 8**; Hager *et al*, 1999). In addition to the expected lack of processing, the profilaggrin protein expressed by cultured *ft/ft* keratinocytes was smaller than that seen in control cells, as is also the case in epidermis. Other epidermal markers examined (i.e., K1, loricrin, involucrin, and K14) were expressed at similar levels between control and *ft/ft* keratinocytes (**Fig 8**, and data not shown).

Indirect immunofluorescence of cultured *ft/ft* keratinocytes showed that the distribution of K1- and K14-containing filaments within *ft/ft* keratinocytes was indistinguishable from control cells (data not shown). These findings demonstrate that *ft/ft* keratinocytes have no discernible alteration in the keratin cytoskeleton.

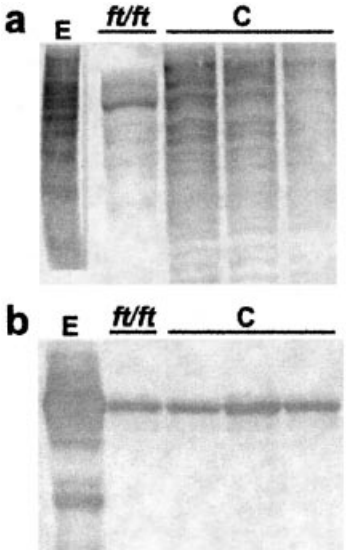

Figure 8. Cultured *ft/ft* keratinocytes express reduced levels of profilaggrin *in vitro*. Shown are immunoblots of epidermal extracts from MEK cultured from control and *ft/ft* mice reacted with profilaggrin (*a*) and K1 (*b*) antibodies. Note the reduced expression of *ft/ft* profilaggrin relative to controls and the smaller size of the *ft/ft* protein (*a*). These results are representative of keratinocytes cultured from several newborn control and *ft/ft* mice. *E* represents an epidermal extract from a control mouse.

DISCUSSION

The spontaneous flaky tail mouse mutation results in dry, flaky skin in neonatal mice, shortened ears, constriction and sometimes autoamputation of tail tips (Lane, 1972; Sundberg, 1994a; Sundberg *et al*, 1996). Previously it was shown to be an autosomal recessive mutation that maps to mouse chromosome 3 near profilaggrin, loricrin, and other genes that comprise the mouse EDC (Rothnagel *et al*, 1994; Krieg *et al*, 1997). In this study we describe a detailed characterization of this mutant mouse and demonstrate a specific biochemical defect that involves a deficiency of normal, high molecular weight profilaggrin, and the expression of a novel, low molecular weight form of profilaggrin that is not converted to normal profilaggrin intermediates or filaggrin.

Ichthyosis vulgaris is a relatively common inherited scaling disease that is characterized by absence of the granular layer in a subset of patients (referred to as ichthyosis vulgaris AGL[1,2]), coinciding with reduced or absent profilaggrin expression that correlates with a markedly reduced stability of profilaggrin mRNA in ichthyosis vulgaris AGL keratinocytes (Fleckman *et al*, 1987; Nirunsuksiri *et al*, 1995, 1998). In this study we have demonstrated that the flaky tail mutant mouse and human ichthyosis vulgaris AGL share a number of morphologic and biochemical features, including the appearance of dry, scaly skin that is not present at birth; the presence of an attenuated or absent granular layer that lacks profilaggrin-containing F-granules, as well as orthohyperkeratosis; and by attenuated or absent profilaggrin/filaggrin expression *in vivo* (**Table I**). Like *ft/ft* mice, human ichthyosis vulgaris also improves with age (Wells and Kerr, 1966; Merrett *et al*, 1967), perhaps reflecting a decreased need for filaggrin and its hygroscopic amino acids that coincides with a decline in filaggrin expression with age (our unpublished data). In addition, like keratinocytes from ichthyosis vulgaris AGL patients (Nirunsuksiri *et al*, 1995, 1998), cultured mouse *ft/ft* keratinocytes exhibit reduced or absent profilaggrin expression, whereas other markers of epidermal differentiation, including K1, K14, involucrin, and loricrin are expressed relatively normally. Thus, the flaky tail mutant mouse may be a useful model for the human subtype of ichthyosis vulgaris, which has an absent granular layer (**Table I**). It should be emphasized that some features of these disorders are unique to either the human or mouse condition, reflecting the fact that mice and humans do not necessarily show identical phenotypic characteristics, even when the mutation is in the same gene (Sundberg *et al*, 1996). For example, *ft/ft* mice show constricting bands around the ankles or tails, which is not a feature of ichthyosis vulgaris AGL, although it is seen in the fingers of patients with the skin disorder loricrin keratoderma (Maestrini *et al*, 1996). A second difference between the mouse and human disorders is that the *ft/ft* mutation is autosomal recessive, whereas ichthyosis vulgaris has classically been considered a dominant disorder (Wells and Kerr, 1966); however, recent studies have suggested that ichthyosis vulgaris AGL could be either a dominant or recessive disorder.[2] Pseudoxanthoma elasticum, a disease that affects elastic fibers in the skin and other tissues, is another example of a disease that can be either dominantly or recessively inherited and appears to involve a single gene on chromosome 16p (Struk *et al*, 1997; Bergen *et al*, 2000; Le Saux *et al*, 2000; Ringpfeil *et al*, 2000).

Filaggrin is believed to perform at least two functions in the stratum corneum of epidermis. In the lower stratum corneum, immediately after its formation from profilaggrin, filaggrin aggregates keratin filaments into macrofibrils. This aggregation facilitates disulfide bond formation between keratin polypeptide chains, resulting in the "keratin pattern" characteristic of cornified cells (Dale *et al*, 1978; Manabe *et al*, 1991). Filaggrin is subsequently modified by deamination and degraded to free amino acids by several proteases, including one or more cathepsins present in epidermis (Scott and Harding, 1986; Kawada *et al*, 1995a,b). These filaggrin-derived amino acids, some of which are chemically or enzymatically modified, are highly hygroscopic and have long been proposed to be important in maintaining the osmolarity and flexibility of epidermis (Scott *et al*, 1982; Scott and Harding, 1986; reviewed in Rawlings *et al*, 1994). Dry, flaky skin, a major characteristic of *ft/ft* mice, could readily be explained by the reduced or absent expression of filaggrin as has previously been suggested to cause the dry, flaky skin in human ichthyosis vulgaris (Manabe *et al*, 1991; Fleckman and Dale, 1993). This lack of epidermal flexibility (as a result of filaggrin deficiency) could also explain the shortened ears and susceptibility to tail amputation observed in these mice.

In both *ft/ft* mice and ichthyosis vulgaris AGL patients, the causative mutation has not been identified, although the profilaggrin gene remains a strong candidate in both cases. This is based on genetic mapping data that place the mutation close to profilaggrin in *ft/ft* mice (Lane, 1972; Rothnagel *et al*, 1994) and within the human EDC on chromosome 1,[2] and biochemical data demonstrating attenuated (reduced or absent) profilaggrin and filaggrin expression in both the human and mouse diseases (**Table I**) (Dale *et al*, 1985; Fleckman *et al*, 1987; Nirunsuksiri *et al*, 1995, 1998; this study). An alternative possibility is that a gene that regulates profilaggrin expression, located nearby, may be defective. Profilaggrin mRNA present in ichthyosis vulgaris AGL keratinocytes is unstable, consistent with either a mutation in profilaggrin itself or in a gene that regulates profilaggrin mRNA stability (Nirunsuksiri *et al*, 1998). Reduced levels of profilaggrin mRNA are also present in *ft/ft* mice, suggesting that the mRNA is susceptible to degradation via non-sense-mediated decay or other cellular mechanisms that facilitate the degradation of mutant mRNA molecules (Hentze and Kulozik, 1999). Both the *ft/ft* profilaggrin gene and mRNA are similar in size in *ft/ft* and control mice, suggesting that *ft/ft* mice contain a mutation within the polyfilaggrin portion of the gene that leads to premature termination of translation. Such a truncated profilaggrin protein would lack the unique C-terminal domain that is moderately conserved among mammalian profilaggrins (Presland *et al*, 1992). The small form of profilaggrin expressed by *ft/ft* mice does not form normal epidermal keratohyalin F-granules, is not processed to

[1]Fleckman P, Brumbaugh S: Submitted for publication.

[2]Compton JG, DiGiovanna JJ, Johnston KA, Fleckman P, Bale SJ: Submitted for publication.

Table I. Comparison of features of *ft/ft* mice and human ichthyosis vulgaris AGL

Characteristic	*ft/ft* mice	Human ichthyosis vulgaris AGL
Dry, scaly skin	Yes[a]	Yes
Maps to EDC	Yes (mouse chromosome 3)	Yes (human chromosome 1)[b]
Mode of inheritance	Autosomal recessive	Autosomal dominant or recessive[b]
Absent granular layer	Attenuated[c]	Yes
Loss of keratohyalin granules	Profilaggrin F-granules absent	Yes
Attenuated profilaggrin and loss of filaggrin expression *in vivo*	Yes	Yes
Attenuated profilaggrin expression *in vitro*	Yes	Yes
Expression of other epidermal differentiation markers normal	Yes	Yes

[a]Epidermal tissues such as tail and paws exhibit scaling from 2 to 14 d with recovery by 21 d after birth.
[b]Compton JG, DiGiovanna JJ, Johnston KA, Fleckman P, Bale SJ, manuscript submitted.
[c]Histologically, the granular layer is reduced but not absent. Remaining granules in *ft/ft* mice contain predominantly loricrin (**Figs 2** and **3**).

filaggrin, and is present at low levels in *ft/ft* epidermis. Our results suggest that the C-terminal domain of profilaggrin plays an important part in keratohyalin formation and the subsequent normal proteolytic processing to filaggrin. Hence, keratohyalin granule formation may function to stabilize the profilaggrin protein and prevent its destruction prior to epidermal cornification.

In flaky tail mutant mice, the scaling disorder is transient, i.e., it is only readily apparent for the first 2–3 wk of life, even on external epithelia (tail, feet) that do not grow a significant amount of hair. This could be explained by an adaptive (compensatory) response by the mice, involving altered expression of other genes encoding cornified envelope proteins or of other components that regulate barrier function (e.g., lipid biosynthesis; Chujor *et al*, 1998), as has been reported in loricrin-deficient mice (Koch *et al*, 2000). A second possible explanation for the transient nature of the *ft/ft* phenotype is that the mice no longer require filaggrin for normal epidermal desquamation as they become older. Normal adult mice express markedly reduced amounts of profilaggrin and filaggrin, consistent with the later possibility. Compensatory mechanisms may also be involved, however, as the cornified envelope proteins loricrin and involucrin showed elevated expression in *ft/ft* mice (**Fig 4**). The absence of scaling in adult *ft/ft* mice does not result from ''repair'' of the profilaggrin to filaggrin processing defect, as adult *ft/ft* mice, like their neonatal counterparts, are unable to process profilaggrin (data not shown).

The mutant mouse characterized in this study is actually a double homozygous of flaky tail and another mutation, matted (*ma*). The matted phenotype begins to be visible between 18 and 21 d during the second hair cycle as the hair coat reaches its full growth. The hairs appear to stick together in clumps, hence the name matted (Jarrett and Spearman, 1957; Searle and Spearman, 1957). The *ma* mutation allows homozygous adult *ft/ft* mice to be readily identified and is largely a tool for breeding purposes. The two mutations are currently placed ≈1.5 cM apart and were derived independently at different times in different laboratories, implying that they affect different genes (Sundberg, 1994a,b). A number of potential candidate genes for the matted mutation reside in the EDC, including involucrin (de Viragh *et al*, 1994) and trichohyalin (Rothnagel and Rogers, 1986; O'Guin *et al*, 1992; Manabe and O'Guin, 1994), that are both expressed in hair follicles as well as epidermis. Matted does not appear to result from the loss of function of involucrin or trichohyalin, as these proteins are expressed normally in homozygous mutant mice (**Figs 3** and **4**, and data not shown). The phenotypes responsible for the two mutations should be readily distinguishable by creation of a transgenic mouse from *ft/ft* embryos expressing mouse filaggrin (or profilaggrin) to correct the proposed filaggrin defect and determine if the dry, flaky skin and tail abnormalities are directly due to the loss of filaggrin protein.

In summary, we have shown that flaky tail mice, which exhibit a scaling skin disease in the neonatal period and a susceptibility to tail autoamputation, lack normal profilaggrin protein and its processed form, filaggrin, in stratified epithelia. The mutant mice show many similarities to human ichthyosis vulgaris AGL, including dry, scaly skin that has been proposed to result from filaggrin deficiency. These studies provide a firm basis for determining the nature of the genetic lesion in *ft/ft* mice and in the related human disease ichthyosis vulgaris AGL, and for further understanding the relationship between profilaggrin/filaggrin deficiency and dry, scaly skin disease.

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REFERENCES

Anton-Lamprecht I, Hofbauer M: Ultrastructural distinction of autosomal dominant ichthyosis vulgaris and X-linked recessive ichthyosis. *Humangenetik* 15:261–264, 1972

Bechtold LS. Ultrastructural evaluation of mouse mutations. In: Sundberg JP, Boggess D (eds). *Systematic Approach to Evaluation of Mouse Mutations*. Boca Raton: CRC Press, 1999, pp 121–129

Bergen AA, Plomp AS, Schuurman EJ, *et al*: Mutations in ABCC6 cause pseudoxanthoma elasticum. *Nat Genet* 25:228–231, 2000

Bickenbach JR, Greer JM, Bundman DS, Rothnagel JA, Roop DR: Loricrin expression is coordinated with other epidermal proteins and the appearance of lipid lamellar granules in development. *J Invest Dermatol* 104:405–410, 1995

Chujor CS, Feingold KR, Elias PM, Holleran WM: Glucosylceramide synthase activity in murine epidermis: quantitation, localization, regulation, and requirement for barrier homeostasis. *J Lipid Res* 39:277–285, 1998

Dale BA, Holbrook KA, Steinert PM: Assembly of stratum corneum basic protein and keratin filaments in macrofibrils. *Nature* 276:729–731, 1978

Dale BA, Resing KA, Lonsdale-Eccles JD: Filaggrin: a keratin filament-associated protein. *Ann NY Acad Sci* 455:330–342, 1985

Dale BA, Resing KA, Presland RB. Keratohyalin granule proteins. In: Leigh I, Lane B, Watt F (eds). *The Keratinocyte Handbook*. Cambridge: Cambridge University Press, 1994, pp 323–350

Dale BA, Presland RB, Lewis SP, Underwood RA, Fleckman P: Transient expression of epidermal filaggrin in cultured cells causes collapse of intermediate filament networks with alteration of cell shape and nuclear integrity. *J Invest Dermatol* 108:179–187, 1997

de Viragh PA, Huber M, Hohl D: Involucrin mRNA is more abundant in human hair follicles than in normal epidermis. *J Invest Dermatol* 103:815–819, 1994

Fleckman P, Dale BA. Structural protein expression in the ichthyoses. In: Bernard BA, Shroot B (eds). *Pharmacology and the Skin*. Basel: Karger, 1993, pp 1–15

Fleckman P, Holbrook KA, Dale BA, Sybert VP: Keratinocytes cultured from subjects with ichthyosis vulgaris are phenotypically abnormal. *J Invest Dermatol* 88:640–645, 1987

Fuchs E, Byrne C: The epidermis: rising to the surface. *Curr Opin Genet Dev* 4:725–736, 1994

Hager B, Bickenbach JR, Fleckman P: Long-term culture of murine epidermal keratinocytes. *J Invest Dermatol* 112:971–976, 1999

Harding CR, Scott IR: Histidine-rich proteins (filaggrins): structural and functional heterogeneity during epidermal differentiation. *J Mol Biol* 170:651–673, 1983

Haydock PV, Blomquist C, Brumbaugh S, Dale BA, Holbrook KA, Fleckman P: Antisense profilaggrin RNA delays and decreases profilaggrin expression and alters *in vitro* differentiation of rat epidermal keratinocytes. *J Invest Dermatol* 101:118–126, 1993

Hentze MW, Kulozik AE: a perfect message: RNA surveillance and nonsense-mediated decay. *Cell* 96:307–310, 1999

Hohl D, Roop DR: Loricrin. In: Darmon M, Blumenberg M (eds). *Molecular Biology of the Skin the Keratinocyte*. San Diego: Academic Press, 1993, pp 151–179

Hohl D, Ruf Olano B, de Viragh PA, Huber M, Detrisac CJ, Schnyder UW, Roop DR: Expression patterns of loricrin in various species and tissues. *Differentiation* 54:25–34, 1993

Hsu SM, Raine L, Fanger H: A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am Soc Clin Pathol* 75:734–738, 1981

Ishida-Yamamoto A, Eady RA, Watt FM, Roop DR, Hohl D, Iizuka H: Immunoelectron microscopic analysis of cornified cell envelope formation in normal and psoriatic epidermis. *J Histochem Cytochem* 44:167–175, 1996

Jarrett A, Spearman RI: The keratin defect and hair-cycle of a new mutant (matted) in the house mouse. *J Embryol Exp Morphol* 5:103–110, 1957

Kawada A, Hara K, Hiruma M, Noguchi H, Ishibashi A: Rat epidermal cathepsin L-like proteinase L. Purification and some hydrolytic properties toward profilaggrin and synthetic substrates. *J Biochem* 118:332–337, 1995a

Kawada A, Hara K, Morimoto K, Hiruma M, Ishibashi A: Rat epidermal cathepsin B. Purification and characterization of proteolytic properties toward filaggrin and synthetic substrates. *Int J Biochem Cell Biol* 27:175–183, 1995b

Koch PJ, de Viragh PA, Scharer E, *et al*: Lessons from loricrin-deficient mice. Compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. *J Cell Biol* 151:389–400, 2000

Krieg P, Schuppler M, Koesters R, Mincheva A, Lichter P, Marks F: Repetin (*Rptn*), a new member of the "fused gene" subgroup within the S100 gene family encoding a murine epidermal differentiation protein. *Genomics* 43:339–348, 1997

Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970

Lane PW: Two new mutations in linkage group XVI of the house mouse. Flaky tail and varitint-waddler-J. *J Hered* 63:135–140, 1972

Le Saux O, Urban Z, Tschuch C, *et al*: Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. *Nat Genet* 25:223–227, 2000

Maestrini E, Monaco AP, McGrath JA, *et al*: A molecular defect in loricrin, the major component of the cornified cell envelope, underlies Vohwinkel's syndrome. *Nat Genet* 13:70–77, 1996

Manabe M, O'Guin WM: Existence of trichohyalin-keratohyalin hybrid granules: co-localization of two major intermediate filament-associated proteins in non-follicular epithelia. *Differentiation* 58:65–75, 1994

Manabe M, Sanchez M, Sun TT, Dale BA: Interaction of filaggrin with keratin filaments during advanced stages of normal human epidermal differentiation and in ichthyosis vulgaris. *Differentiation* 48:43–50, 1991

Merrett JD, Wells RS, Kerr CB, Barr A: Discriminant function analysis of phenotype variates in ichthyosis. *Am J Hum Genet* 19:575–585, 1967

Mischke D, Korge BP, Marenholz I, Volz A, Ziegler A: Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ("epidermal differentiation complex") on human chromosome 1q21. *J Invest Dermatol* 106:989–992, 1996

Morita K, Hogan ME, Nanney LB, King LE, Manabe M, Sun TT, Sundberg JP: Cutaneous ultrastructural features of the flaky skin (*fsn*) mouse mutation. *J Dermatol* 22:385–395, 1995

Nirunsuksiri W, Presland RB, Dale BA, Fleckman P: Decreased profilaggrin expression in icthyosis vulgaris is a result of selectively impaired post-transcriptional control. *J Biol Chem* 270:871–876, 1995

Nirunsuksiri W, Zhang SH, Fleckman P: Reduced stability and bi-allelic, coequal expression of profilaggrin mRNA in keratinocytes cultured from subjects with ichthyosis vulgaris. *J Invest Dermatol* 110:854–861, 1998

O'Farrell PZ, Goodman HM, O'Farrell PH: High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* 12:1133–1142, 1977

O'Guin WM, Sun T-T, Manabe M: Interaction of trichohyalin with intermediate filaments: three immunologically defined stages of trichohyalin maturation. *J Invest Dermatol* 98:24–32, 1992

Presland RB, Dale BA: Epithelial structural proteins of skin and oral cavity: function in health and disease. *Crit Rev Oral Biol Med* in press, 2000

Presland RB, Fleckman P, Haydock PV, Nirunsuksiri W, Dale BA: Characterization of the human epidermal profilaggrin gene: Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus. *J Biol Chem* 267:23772–23781, 1992

Presland RB, Kimball JR, Kautsky MB, Lewis SP, Lo C, Dale BA: Evidence for specific proteolytic cleavage of the N-terminal domain of human profilaggrin during epidermal differentiation. *J Invest Dermatol* 108:170–178, 1997

Rawlings AV, Scott IR, Harding CR, Bowser PA: Stratum corneum moisturization at the molecular level. *J Invest Dermatol* 103:731–740, 1994

Relyea MJ, Miller J, Boggess D, Sundberg JP. Necropsy methods for laboratory mice. Biological characterization of a new mutation. In: Sundberg JP, Boggess D (eds). *Systematic Approach to Evaluation of Mouse Mutations*. Boca Raton: CRC Press, 1999, pp 57–89

Resing KA, Dale BA. Proteins of keratohyalin. In: Goldsmith LA (ed.) *Biochemistry, Physiology and Molecular Biology of the Skin*, 2nd edn. New York: Oxford University Press, 1991, pp 148–167

Ringpfeil F, Lebwohl MG, Christiano AM, Uitto J: Pseudoxanthoma elasticum: mutations in the MRP6 gene encoding a transmembrane ATP-binding cassette (ABC) transporter. *Proc Natl Acad Sci USA* 97:6001–6006, 2000

Rothnagel JA, Rogers GE: Trichohyalin, an intermediate filament-associated protein of the hair follicle. *J Cell Biol* 102:1419–1429, 1986

Rothnagel JA, Steinert PM: The structure of the gene for mouse filaggrin and a comparison of the repeating units. *J Biol Chem* 265:1862–1865, 1990

Rothnagel JA, Mehrel T, Idler WW, Roop DR, Steinert PM: the gene for mouse epidermal filaggrin precursor. its partial characterization, expression, and sequence of a repeating filaggrin unit. *J Biol Chem* 262:15643–15648, 1987

Rothnagel JA, Longley MA, Bundman DS, *et al*: Characterization of the mouse loricrin gene: linkage with profilaggrin and the flaky tail and soft coat mutant loci on chromosome 3. *Genomics* 23:450–456, 1994

Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning a Laboratory Manual*, 2nd edn. New York. Cold Spring Harbor Laboratory Press, 1989

Scott IR, Harding CR: Filaggrin breakdown to water binding compounds during development of the rat stratum corneum is controlled by the water environment. *Dev Biol* 115:84–92, 1986

Scott IR, Harding CR, Barrett JG: Histidine-rich protein of the keratohyalin granules. Source of the free amino acids, urocanic acid and pyrrolidone carboxylic acid in the stratum corneum. *Biochim Biophys Acta* 719:110–117, 1982

Searle AG, Spearman RI: "Matted", a new hair-mutant in the house-mouse: genetics and morphology. *J Embryol Exp Morphol* 5:93–102, 1957

Steinert PM, Marekov LN: The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. *J Biol Chem* 270:17702–17711, 1995

Steinert PM, Cantieri JS, Teller DC, Lonsdale-Eccles JD, Dale BA: Characterization of a class of cationic proteins that specifically interact with intermediate filaments. *Proc Natl Acad Sci USA* 78:4097–4101, 1981

Steven AC, Bisher ME, Roop DR, Steinert PM: Biosynthetic pathways of filaggrin and loricrin—two major proteins expressed by terminally differentiated epidermal keratinocytes. *J Struct Biol* 104:150–162, 1990

Struk B, Neldner KH, Rao VS, St Jean P, Lindpaintner K: Mapping of both autosomal recessive and dominant variants of pseudoxanthoma elasticum to chromosome 16p13.1. *Hum Mol Genet* 6:1823–1828, 1997

Sundberg JP: The flaky tail (*ft*) mutation, chromosome 3. In: Sundberg JP (ed.). *Handbook of Mouse Skin and Hair Mutations: Animal Models and Biomedical Tools*. Boca Raton: CRC Press, 1994a, pp 269–273

Sundberg JP: The matted (*ma*) mutation, chromosome 3. In: Sundberg JP (ed.) *Handbook of Mouse Skin and Hair Mutations: Animal Models and Biomedical Tools*. Boca Raton: CRC Press, 1994b, pp 345–349

Sundberg JP, HogenEsch H, King LE Jr: Mouse models for scaly skin diseases. In: Maibach H (ed.) *Dermatological Research Techniques*. Boca Raton: CRC Press, 1996, pp 61–89

Sundberg JP, Boggess D, Hogan ME, *et al*: Harlequin ichthyosis (*ichq*): a juvenile lethal mouse mutation with ichthyosiform dermatitis. *Am J Pathol* 151:293–310, 1997

Sundberg JP, Montagutelli X, Boggess D: Systematic approach to evaluation of mouse mutations with cutaneous appendage defects. In: Chuong M (ed.) *Cutaneous Appendages Molecular Biology Intelligence Unit I*. Austin: R.G. Landes Company, 1998, pp 421–435

Sybert VP, Dale BA, Holbrook KA: Ichthyosis vulgaris: identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. *J Invest Dermatol* 84:191–194, 1985

Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76:4350–4354, 1979

Volz A, Korge BB, Compton JG, Ziegler A, Steinert PM, Mischke D: Physical mapping of a functional cluster of epidermal differentiation genes on chromosome 1q21. *Genomics* 18:92–99, 1993

Wells RS, Kerr CB: Clinical features of autosomal dominant and sex-linked ichthyosis in an English population. *Br Med J* 1:947–950, 1966