Transglutaminase Function in Epidermis

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Surface epithelial cells, such as the epidermal keratinocyte, undergo a process of terminal cell differentiation that results in the construction of a multilayered epithelium. This epithelium functions to protect the organism from the environment. Transglutaminases, enzymes that catalyze the formation of isopeptide protein-protein cross-links, are key enzymes involved in the construction of this structure. This brief review will focus on the role of these enzymes in constructing the epidermal surface.

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A major function of the epidermis is protection of the organism from the environment. Construction of the epidermal surface begins with regulated cell proliferation in the innermost epidermal cell layer—the basal layer (Cotsarelis *et al*, 1989; Fuchs and Byrne, 1994). The daughter cells are then displaced outward towards the cell surface. As the cells move outward, they lose the ability to proliferate and initiate the differentiation program (Fuchs and Byrne, 1994; Eckert *et al*, 1997) (Fig 1). The second epidermal layer, the spinous layer, is characterized by cells containing a large many of desmosomal connections. The desmosomes contribute strength. Cells in the third layer, the granular layer, contain granule-enclosed proteins and lipids (Matoltsy, 1966; Lavker and Matoltsy, 1971; Holbrook and Odland, 1975; Lavker, 1976; Ishida Yamamoto *et al*, 1993). Some of these granules, for example, contain cornified envelope precursors (Steven *et al*, 1990) that are ultimately deposited onto the inner surface of the developing cornified envelope. The transition zone is a region of extensive remodeling that marks the transition between living and dead epidermis. In this zone, the intracellular organelles are destroyed by proteases and nucleases. Concomitant with this destruction is assembly of the cornified envelope and the stabilization of the keratin intermediate filament bundles. The result of this process is the construction of the outermost epidermal layer, which is comprised of terminally differentiated keratinocytes—called corneocytes. Corneocytes consist of two major structures—a network of disulfide bond-stabilized keratin filament bundles surrounded by an envelope of covalently cross-linked protein (Nemes and Steinert, 1999) and associated lipid (Segre, 2003). Millions of corneocytes are required to cover the body surface.

The Cornified Envelope

The cornified envelope is a covalently cross-linked structure that forms beneath the plasma membrane in differentiating keratinocytes (Matoltsy and Odland, 1955; Matoltsy, 1976; Steven and Steinert, 1994; Nemes and Steinert, 1999). The envelope includes two functional parts—an insoluble 15 nm thick structure consisting of covalently cross-linked protein (10 nm thick) that comprises the backbone of the envelope and covalently linked lipids (5 nm thick) that coat the exterior (Matoltsy and Matoltsy, 1966; Elias and Friend, 1975; Grayson and Elias, 1982; Wertz *et al*, 1989b; Robinson *et al*, 1997; Nemes and Steinert, 1999; Nemes *et al*, 1999a; Steinert and Marekov, 1999). Various proteins function as substrates and are incorporated into this structure (Table I). These include soluble (e.g., involucrin and small proline-rich proteins (SPR)) and insoluble (e.g., loricrin, periplakin, and envoplakin) proteins. Several soluble precursors are initially deposited to form a scaffold (Eckert *et al*, 1993; Nemes and Steinert, 1999; Kalinin *et al*, 2002). The insoluble precursors are subsequently deposited on the inner surface of the scaffolding. For example, loricrin is a major protein of the envelope that is cross-linked to the inner surface of the scaffolding (Mehrel *et al*, 1990; Hohl *et al*, 1991b; Nemes and Steinert, 1999).

The interprotein cross-links that hold together this multiprotein assembly (Hennings *et al*, 1981; Yaffe *et al*, 1993; Steven and Steinert, 1994; Steinert and Marekov, 1995; Robinson *et al*, 1996; Robinson *et al*, 1997; Steinert and Marekov, 1997; Steinert *et al*, 1998) are formed by transglutaminases (TGase). These enzymes catalyze interprotein bond formation by forming a thiolester acyl-enzyme intermediate and subsequently transferring the acyl residue to a primary amine (Folk and Finlayson, 1977; Folk, 1980). The amine acceptor is generally provided by the ε-amino group of a protein-bound lysine and the ultimate link is an N^6-(γ-glutamyl)lysine isopeptide bond. Three TGase are known to be active in surface epithelia and are thought to participate to varying degrees in cross-link formation. These include TGase 1, TGase 3, and TGase 5 (Buxman and Wuepper, 1976; Goldsmith, 1983; Peterson and Wuepper, 1984; Rothnagel and Rogers, 1984; Thacher and Rice, 1985; Michel and Demarchez, 1988; Kim *et al*, 1991, 1993, 1995b; Polakowska *et al*, 1991; Hitomi *et al*, 1999, 2003). In

Abbreviations: SPR, small proline-rich proteins; TGase, transglutaminase

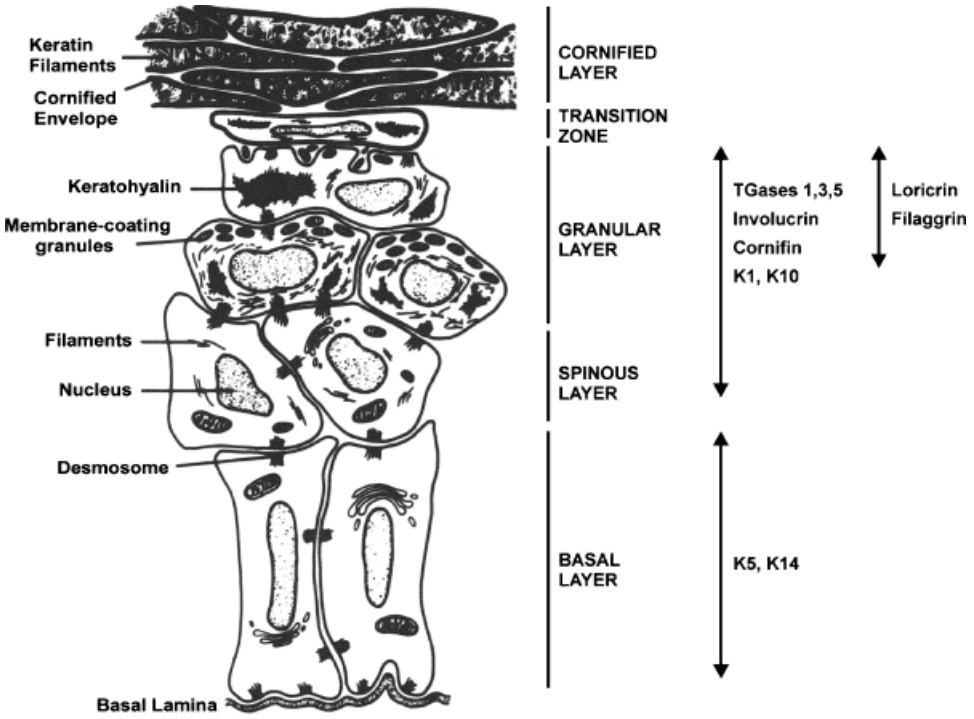

Figure 1
Keratinocyte differentiation. Keratinocytes begin in the basal layer as undifferentiated cells that have proliferative potential and express keratins 5 and 14. As they exit the basal layer, the daughter cells lose proliferative ability and give rise to differentiated cells comprising the spinous, granular, and cornified layers. Cornified envelope assembly begins in the late spinous layer and continues as the cells progress into the granular layer. The approximate range of expression of each protein is shown by the arrows. Transglutaminase (TGase) 1, TGase 3, and TGase 5 are expressed in the spinous and granular layers along with the indicated envelope precursors (e.g., involucrin, cornifin), and keratins K1 and K10. The cornified envelope precursors, loricrin and filaggrin, are expressed later in differentiation. The rate of incorporation of envelope precursors and the deposition of covalent cross-links accelerates in the transition zone as the available level of free calcium increases and activates TGase. Cross-linking in the transition zone finishes production of the cornified layer that comprises the dead protective epidermal surface.

this brief review, we will describe some of the structural precursors involved in keratinocyte cornified envelope assembly, and the role of the various TGase in assembling the cornified structure.

Structural Components of the Cornified Envelope

Involucrin As mentioned above, the cornified envelope consists of a host of proteins (Table I). Involucrin was the first precursor discovered and cloned (Rice and Green, 1979; Eckert and Green, 1986). It is expressed throughout the suprabasal layers (Rice and Green, 1979; Banks-Schlegel and Green, 1981; Crish *et al*, 1993, 1998) in stratified epithelia, including the cervix, epidermis, esophagus, cornea, trachea, and conjunctiva (Banks-Schlegel and Green, 1981; Walts *et al*, 1985; Crish *et al*, 1993; Crish *et al*, 2002). Involucrin expression is controlled by AP1, Sp1, and C/EBP regulatory factors (Welter *et al*, 1995; Banks *et al*, 1998; Crish *et al*, 1998, 2002; Banks *et al*, 1999; Eckert *et al*,

Table I. Cornified envelope precursors

Protein	Size (kDa)	Chromosome location	Cornified envelope (%)[b]	Other names
Involucrin[a]	65	1q21	5	
Loricrin[a]	26	1q21	80	
SPR[a]	5–26	1q21(Gibbs *et al*, 1993)	3–5	Cornifins, pancornulins
Cystatin A	12	3cen–q21	2–5	Keratolinin
Proelafin	10	20q12–q13	<1	Elastase-specific inhibitor, SKALP
Profilaggrin[a]	>400	1q21	<1	Filaggrin
Type II keratins	56–60	12q13	<1	
Desmoplakin	330/250	6p21–ter	<1	
Envoplakin	210	17q25	<1	
Periplakin	195	16p13.3	<1	
S100A10 and S100A11[a]	12	1q21	<1	Calpactin light chain and S100C/calgizzarin
Annexin I	36	9q12–q21.2	<1	Lipocortin I
Cornifelin	12.5	19q13		

[a]Localized in the epidermal differentiation complex (Zhao and Elder, 1997; South *et al*, 1999).
[b]Nemes and Steinert (1999).
SPR, small proline-rich proteins.

2004), and the function of these factors is controlled by an nPKC, Ras, MEKK1, MEK3, p38 MAPK signaling cascade (Efimova *et al*, 1998; Efimova *et al*, 2003; Eckert *et al*, 2004). Involucrin contains thirty-seven 10 amino acid repeats, with each repeat containing three glutamine and two glutamate residues (Rice and Green, 1979; Simon and Green, 1985; Etoh *et al*, 1986; Simon and Green, 1988; Lambert *et al*, 2000). The entire protein is 15% glutamine and 20% glutamate (Eckert and Green, 1986). The predominant structure is α-helical (Yaffe *et al*, 1992) but additional secondary structural features have been proposed (Lazo and Downing, 1999; Kajava, 2000). Although multiple glutamines on involucrin are TGase substrates (Etoh *et al*, 1986; LaCelle *et al*, 1998), TGase preferentially labels a single residue, glutamine496 (Simon and Green, 1988; Nemes *et al*, 1999b). Involucrin is one of the first precursors cross-linked during envelope assembly and is thought to form a scaffold for subsequent cross-linking of additional precursors (Nemes and Steinert, 1999).

Loricrin Loricrin is the major component of the cornified envelope in the epidermis (Steven and Steinert, 1994). Loricrin expression is induced by a variety of differentiating agents, including calcium (Hohl *et al*, 1991a; DiSepio *et al*, 1995), and is detected in granules in the granular layer (Steven *et al*, 1990; Ishida Yamamoto *et al*, 1993, 1996). It is likely that loricrin is sequestered in granules due to its low solubility. During terminal differentiation, in transition zone cells, loricrin is released from the granules and cross-linked to the inner surface of the nascent cornified envelope (Ishida-Yamamoto *et al*, 1999). Loricrin is also important in disease. Mutation of the glycine-rich domain of loricrin has been reported to result in the formation of arginine-rich nuclear localization sequences. The accumulation of mutated involucrin in the nucleus is thought to be the underlying cause of loricrin keratoderma (Ishida-Yamamoto, 2003).

SPR The SPR comprise a 14-member multigene family that contains three distinct subgroups–SPR1 (two members), SPR2 (11 members), and SPR3 (one member) (Tesfaigzi and Carlson, 1999). Expression of these genes is induced by calcium and other differentiating agents via activation of AP1, Sp1, and ets transcription factors (Gibbs *et al*, 1990; Fischer *et al*, 1996). The SPR structure includes eight or nine amino acid proline-rich repeats flanked by glutamine-, lysine-, and proline-rich ends (Gibbs *et al*, 1993). Glutamine and lysine residues, located at the amino and carboxy terminal ends of SPR proteins, participate in cross-link formation. These proteins have been proposed to function as bridges that link together other precursors (Steinert *et al*, 1998).

Cystatin and elafin Cystatin A, a cysteine protease inhibitor, is a minor component of the cornified envelope (Takahashi *et al*, 1994). Cystatin A expression is increased by treatment of keratinocytes with differentiating agents via an MAPK kinase cascade that includes Ras, MEKK1, MEK7, and JNK (Takahashi *et al*, 2001). A mutation in another cystatin family member, cystatin M/E, is thought to be an underlying cause of the disturbed epidermal cornification observed in harlequin ichthyosis (Zeeuwen *et al*, 2002, 2004). Elafin, a potent inhibitor of elastase and proteinase 3, is translated as preproelafin, which is cleaved to release proelafin by removal of the 25 amino acid signal peptide. The proelafin protein is then cleaved to release elafin and cementoin. Cementoin is encoded by the pro-domain of elafin, and like elafin, can serve as a TGase substrate (Molhuizen *et al*, 1993; Nara *et al*, 1994; Steinert and Marekov, 1995). Members of the cystatin family and elafin may function to regulate protease activity that is required for envelope maturation. For example, cystatin M/E deficiency is associated with disturbed cornification, impaired barrier function, and dehydration (Zeeuwen *et al*, 2004). The function of cystatin M/E is to inhibit legumain, an asparaginyl endopeptidase. Thus, the increase in legumain activity resulting from cystatin M/E deficiency may be an underlying cause of harlequin ichthyosis (Zeeuwen *et al*, 2004).

Filaggrin Filaggrin is synthesized as profilaggrin, which consists of numerous filaggrin units flanked by amino- and carboxy-terminal domains (Presland *et al*, 1992, 1997; Pearton *et al*, 2002). Like several other envelope precursors (i.e., S100 proteins), profilaggrin contains two calcium-binding EF hand motifs (Presland *et al*, 1992; Markova *et al*, 1993). Filaggrin is a product of proteolytic cleavage of profilaggrin (Presland *et al*, 1997). In addition to its role in the cornified envelope, filaggrin also functions to bundle intermediate filaments (Dale *et al*, 1978; Mack *et al*, 1993). Filaggrin may also play a role in facilitating apoptotic responses due to its ability to alter keratin filament distribution (Presland *et al*, 2001).

Desmoplakin, envoplakin, periplakin, and type II keratins As mentioned above, the corneocyte consists of keratin intermediate filament bundles contained within an envelope of covalently cross-linked protein. Keratins are the most abundant proteins in the corneocyte and the assembled keratin intermediate filament bundles connect to the cell periphery at desmosomes (Green and Gaudry, 2000). Considering that cornified envelope assembly takes place at the plasma membrane, it is perhaps not surprising that both desmosomal components and keratin filaments are incorporated into the cornified envelope. The connection of the keratin filaments to the desmosome occurs through several proteins, including desmoplakin and envoplakin (Virata *et al*, 1992; Green and Gaudry, 2000). During terminal differentiation, the keratin filaments become covalently linked to the envelope via a specific lysine residue located at the amino terminus of type II keratins (Candi *et al*, 1998). Several other desmosomal proteins, including desmoplakin, desmoglein 3, desmocollins 3A/3B, plakoglobin, and plakophilin, are also incorporated into the cornified envelope (Robinson *et al*, 1997).

S100 proteins and annexin I S100 proteins are calcium-regulated EF hand-containing proteins (Donato, 1999). The genes encoding many S100 proteins are clustered in the epidermal differentiation complex on chromosome 1q21 (Celis and Olsen, 1994). S100A10 and S100A11 are incorporated into the cornified envelope in normal human keratinocytes (Robinson *et al*, 1997). In addition, the cross-

linking sites have been identified on several S100 proteins at the amino- and carboxy-termini (Robinson and Eckert, 1998; Ruse *et al*, 2001). In response to increased calcium, S100A11 moves to the cell periphery via a tubulin filament-dependent mechanism indicating that tubulin filaments are required to position this envelope precursor in the vicinity of the plasma membrane (Broome and Eckert, 2004).

S100A11 forms a complex with annexin I The S100A11/annexin I complex is a heterotetramer consisting of two S100A11 and two annexin I proteins. Rety *et al* (2000) solved the structure of this complex, confirming a novel mode of interaction of S100A11 with annexin I, and suggesting a model whereby the calcium-regulated (annexin I/S100A11)$_2$ heterotetramer functions to organize membrane fusion events. In addition, almost all annexins, including annexin I and annexin II, display calcium channel activity in *in vitro* systems (Chen *et al*, 1993; Cohen *et al*, 1995; Benz *et al*, 1996; Burger *et al*, 1996; Gerke and Moss, 2002). This activity has not been demonstrated in cells under normal intracellular conditions, but such activity may be possible under oxidizing and reduced pH conditions (Gerke and Moss, 2002); thus, annexin-dependent channel activity may be possible in keratinocytes in the oxidizing, acidic environment observed during terminal keratinocyte differentiation and may contribute to calcium influx.

Cornifelin Cornifelin is a recently described protein that is encoded on chromosome 19q13 (Michibata *et al*, 2004). Cornifelin is expressed in the epidermal suprabasal layers (Michibata *et al*, 2004). Like several other cornified envelope precursor genes, including involucrin (Eckert and Green, 1986), the first exon of the cornifelin gene is non-coding. Robinson *et al* (1997) reported an amino acid sequence, ARELKIRE, obtained by CNBr digestion of highly purified cornified envelopes, that matches a segment of cornifelin sequence (Michibata *et al*, 2004), suggesting that cornifelin is a bona fide envelope precursor.

TGase in Keratinocytes

TGase comprise a family of calcium-dependent enzymes that catalyze the formation of isopeptide bonds. Several members of this family have been characterized (Phillips *et al*, 1990; Kim *et al*, 1991; Aeschlimann *et al*, 1998; Grenard *et al*, 2001) (Table II). These include Factor XIIIa, which is involved in stabilization of fibrin clots and in wound healing (Siefring Jr *et al*, 1978; Lorand and Graham, 2003), TGase 4, which is involved in the cross-linking of seminal fluid (Dubbink *et al*, 1998), Band 4.2, an inactive TGase, which is a structural protein in erythroblasts and erythrocytes (Lorand *et al*, 1987), TGase 2, a ubiquitously expressed TGase with multiple functions (Fesus and Piacentini, 2002; Griffin *et al*, 2002), and the epidermal-specific TGase, TGase 1 (Phillips *et al*, 1990; Polakowska *et al*, 1991), TGase 3 (Kim *et al*, 1994a), and TGase 5 (Candi *et al*, 2001, 2002, 2004; Grenard *et al*, 2001). Four TGase family members are expressed in keratinocytes and/or in epidermal tissues—TGase 1, TGase 2, TGase 3, and TGase 5 (Table III). These enzymes are discussed below.

TGase type 1 The TGase 1 protein is encoded by the TGM1 gene located on chromosome 14q11.2 (Yamanishi *et al*, 1992). As measured by *in situ* hybridization, TGase 1 expression is differentiation-dependent and initiates in the spinous layer (Michel *et al*, 1992). TGase 1 promoter activity in cultured keratinocytes appears to require two Sp1 sites and an AP1 site within the distal region of the gene (Floyd and Jetten, 1989; Liew and Yamanishi, 1992; Yamada *et al*, 1994; Mariniello *et al*, 1995; Medvedev *et al*, 1999; Jessen *et al*, 2000a, b, 2001; Phillips *et al*, 2004).

Most TGase 1 is anchored to the keratinocyte plasma membrane via fatty acyl linkages (myristate, palmitate) that are present at the TGase 1 amino terminus (Rice *et al*, 1990; Phillips *et al*, 1993; Steinert *et al*, 1996b). Treatment with the protein synthesis inhibitors suggests that the myristylation occurs co-translationally and that palmitate labeling occurs

Table II. The transglutaminase (TGase) family

TGase[a]	Synonyms	Chromosome location	Gene	Function	Size aa (kDa)	Location
Factor XIII	Fibrin stabilizing factor	6p24–25	F13A1	Blood clotting and wound healing	732 (83)	Cytosol, extracellular
Band 4.2	Erythrocyte membrane protein	15q15.2	EPB42	Structural protein in erythrocytes—no activity	690 (72)	Membrane
TGase 1	Keratinocyte TGase	14q11.2	TGM1	Cornified envelope assembly in surface epithelia	814 (90)	Cytosol, membrane
TGase 2	Tissue TGase	20q11–12	TGM2	Cell death/differentiation, adhesion, matrix assembly	686 (80)	Cytosol, nucleus, membrane, cell surface, extracellular
TGase 3	Epidermal TGase	20q11–12	TGM3	Cornified envelope assembly in surface epithelia	692 (77)	Cytosol
TGase 4	Prostate TGase	3q21–22	TGM4	Semen coagulation in rodents	683 (77)	Unknown
TGase 5	TGase X	15q15.2	TGM5	Epidermal differentiation	719 (81)	Nuclear matrix, cytoskeleton
TGase 6	TGase Y	20q11	TGM6	Unknown	Unknown	Unknown
TGase 7	TGase Z	15q15.2	TGM7	Unknown	710 (80)	Unknown

[a]Transglutaminase subtypes indicated in bold are expressed in epidermis.

Table III. Transglutaminases (TGase) in surface epithelia

TGase	Epidermal expression	Cleavage modification	Subcellular distribution	Activity regulators
TGase 1	Expressed in spinous and granular layers	Phosphorylation, myristylation, palmitylation, cleavage to release 10, 33, and 68 kDa products	Plasma membrane	Calcium
TGase 2	Absent or minimal levels, expressed under specialized conditions		Cytosol	Calcium, GTP, GTPase cycle
TGase 3	Expressed in spinous and granular layers	Zymogen to active form transition involves protease cleavage at a site in the flexible loop surrounding Ser^{469} (Ahvazi *et al*, 2002)	Cytosol	Calcium, GTP, GTPase cycle
TGase 5	Expressed in spinous and granular layers of epidermis, low level in basal layer (Candi *et al*, 2002)		Nuclear matrix and cytoskeleton (Candi *et al*, 2001)	Calcium, GTP

post-translationally (Steinert *et al*, 1996b). The target sites for myristate and palmitate addition are a cluster of five cysteine residues ($Cys_{47, 48, 50, 51,}$ and $_{53}$) within the sequence $C_{47}CGCCSC_{53}$ at the TGase 1 amino terminus (Phillips *et al*, 1993). Deletion or mutation of the cysteines in this cluster reduces TGase 1 association with the keratinocytes plasma membrane. Although only a single cysteine need be present in this cluster for palmitate addition, more than one cysteine residue must be present to direct TG1 to the membrane. Expression of TGase 1 in 3T3 fibroblasts also results in a similar modification of TGase 1, indicating that this processing is not specific for keratinocytes (Phillips *et al*, 1993). Moreover, the membrane targeting domain can function in other contexts—a fusion protein containing the amino terminus of TGase 1 fused to involucrin protein directs involucrin to the plasma membrane (Phillips *et al*, 1993). The half-lives of the myristoyl and the palmitoyl adducts are less than that of the TGase 1 protein, suggesting that the rate of modification may influence TGase 1 membrane association.

A fraction of anchored TGase 1 undergoes proteolysis to yield fragments of 10, 33, and 68 kilodaltons (Steinert *et al*, 1996a). The level of these cleavage products increases with keratinocyte differentiation, suggesting that the process is regulated (Kim *et al*, 1995a, b; Steinert *et al*, 1996a, b). The 10 kDa subunit of processed TGase 1 contains the amino terminal region of TGase 1. *In vitro* studies suggest that this cleavage increases TGase 1 activity (Kim *et al*, 1994b, 1995a).

In addition to lipid acylation and proteolytic cleavage, TGase 1 is phosphorylated at amino-terminal serine residues—predominantly at Ser_{82} (Chakravarty *et al*, 1990; Rice *et al*, 1996). Baseline phosphorylation is present in proliferating keratinocytes, and phorbol ester treatment produces a significant protein kinase c-dependent increase in TGase 1 phosphorylation at Ser_{82} (Chakravarty *et al*, 1990; Rice *et al*, 1996). These phosphorylated serine residues reside in close proximity to the acylation/membrane anchoring sites at the TGase 1 amino terminus, suggesting that phosphorylation may influence membrane anchorage.

TGase 1 in disease Lamellar ichthyosis is a disorder of cornification that affects both the epidermis and hair. The phenotype includes large plate-like scales with underlying erythroderma, orthokeratotic hyperkeratosis, and mild-to-moderate acanthosis (Traupe *et al*, 1984). Mutations of the TGase 1 enzyme that reduces TGase 1 activity have been reported in lamellar ichthyosis patients (Huber *et al*, 1995a; Russell *et al*, 1995). These findings suggest that the TGase 1 mutation may be an underlying causative factor in the pathogenesis of this disease. The finding that mice lacking TGase 1 have an ichthyosis-like phenotype (Matsuki *et al*, 1998; Kuramoto *et al*, 2002) supports the idea that reduced TGase 1 activity can cause the disease. However, additional evidence suggests that a substantial percentage of ichthyosis patients have normal TGase 1 activity, indicating that the disease is genetically heterogeneous (Huber *et al*, 1995a, b).

Control of TGase 1-dependent cross-linking The activity of TGase 1, which is known to require calcium as a cofactor, increases when cells are treated with calcium (Ogawa and Goldsmith, 1976; Hennings *et al*, 1981; Goldsmith, 1983). The increase in extracellular calcium is associated with an increase in intracellular calcium (Hennings *et al*, 1989). The observation that TGase 1 activity is calcium-dependent is physiologically important, as increased calcium levels have been reported in the suprabasal epidermal layers (Menon *et al*, 1985). Moreover, for cultured epidermal keratinocytes, a standard method of triggering increased TGase activity is shifting the cells from a low-calcium medium (0.09 mM calcium) to a culture medium containing >0.3 mM calcium (Green, 1980; Li *et al*, 1995a, b). This shift results in an increase in cornified envelope precursor protein expression, increased TGase level and activity (Eckert *et al*, 1997), and increased cornified envelope formation (Green, 1980; Hennings *et al*, 1989). Thus, calcium treatment increases TGase 1 protein level, TGase 1 activity, and the level of TGase 1 substrates (Hennings *et al*, 1981). Other agents, for example phorbol ester and retinoic acid, also influence TGase 1 level (Saunders *et al*, 1993; Medvedev *et al*, 1999).

Recently, a protein has been described that triggers TGase-associated cell death in the absence of increased extracellular calcium (Sturniolo *et al*, 2003). TIG3 is a member of a family of type II tumor suppressors (DiSepio *et al*, 1998; Deucher *et al*, 2000). TIG3 protein is expressed at very low levels, undetectable via immunoblot in cultured keratinocytes, but TIG3 mRNA is detected. TIG3 protein is detected in the suprabasal epidermal layers, in human epidermis (Duvic *et al*, 2000). Vector-mediated delivery of the TIG3 protein to keratinocytes results in substantial growth suppression and dramatic morphological changes to produce cells that resemble cornified envelopes (Sturniolo *et al*, 2003). TGase mRNA and protein levels are not changed by TIG3 expression, but TGase activity is increased. Detailed studies reveal that TIG3 co-localizes with TGase 1 and that TGase 1 activity is specifically increased at locations of co-localization (Sturniolo *et al*, 2003). Moreover, TIG3 activates TGase 1 in the absence of a level of extracellular calcium normally required to activate TGase 1. Recent studies indicate that TIG3 and TGase 1 co-precipitate, suggesting that these proteins interact as part of a complex (Sturniolo and Eckert, unpublished).

Lipids and TGase 1 As noted above, TGase 1 is unique among TGase in that it is anchored to the plasma membrane via a specific linkage. This location suggests that TGase 1 activity and specificity could be influenced by the membrane environment. Simon and Green showed that involucrin is a TGase substrate when incubated with keratinocyte membranes, and that glutamine$_{496}$ is the preferred site of cross-link formation (Simon and Green, 1988). Parallel studies revealed that additional glutamine residues function as TGase substrate sites when the involucrin protein is fragmented. This finding suggests that the overall structure of the involucrin protein has a role in defining which glutamine residues are selected for cross-link formation (Simon and Green, 1988). Steinert and coworkers combined recombinant TGase 1 and involucrin in a lipid vesicle system (Nemes *et al*, 1999b). Under these conditions, both involucrin and TGase 1 bind to the vesicles. Vesicle association of TGase 1 sensitizes the enzyme to calcium—enzyme activity is optimal at 10 μM calcium instead of at the 100 μM calcium concentration that is required in a lipid-free system. When incubated with TGase 1 in solution, involucrin is labeled at multiple glutamine residues; however, in the presence of the vesicles, glutamine$_{496}$ of involucrin is the primary TGase-reactive residue (Nemes *et al*, 1999b). Thus, both involucrin structure and TGase 1/involucrin association with membranes may influence which substrate residues are selected for cross-linking.

A role for lipids as TGase 1 substrates has also been identified. The cornified envelope is composed of a layer of covalently cross-linked proteins and a layer of associated lipids (Wertz *et al*, 1989b; Elias and Feingold, 2001). It had been proposed that ceramide lipids may be covalently linked to the protein scaffolding (Wertz *et al*, 1989a). Ultimately, Steinert and coworkers demonstrated that TGase 1, when reconstituted with involucrin and a ω-hydroxyceramide analog on phosphatidylserine vesicles, promotes lipid ester formation at Gln$_{107, 118, 122, 133,}$ and $_{496}$ of involucrin (Nemes *et al*, 1999a). Three of these residues are sites of ceramide modification of involucrin *in vivo* (Marekov and Steinert, 1998). Thus, TGase 1 can also function to cross-link lipids to the envelope.

TGase type 2 The TGM2 gene, which encodes TGase 2, is located on chromosome 20q11–12 (Wang *et al*, 1994). TGase 2 is only expressed in keratinocytes and other surface epithelial cells under specific conditions. Vitamin A treatment of cultured keratinocytes increases TGase activity (Yuspa *et al*, 1982). This is surprising since vitamin A treatment suppresses cornified envelope formation. It turns out, however, that the induced TGase activity in these experiments is TGase 2 (Lichti *et al*, 1985). It is not clear why TGase 2 is increased under these conditions, as vitamin A treatment of cultured ectocervical epithelial cells results in a suppression of TGase activity and a reduction in TGase 2 mRNA and protein level (Sizemore *et al*, 1993). TGase 2 can function as a calcium-activated cross-linking protein or as a guanine nucleotide-dependent signaling protein. Activation of the cross-linking function is associated with a reduction in its signaling activity and vice versa. Thus, TGase 2 has multiple roles (Nakaoka *et al*, 1994).

TGase 2 plays an important role in wound healing. In fact, wound healing in surface epithelia requires the concerted action of several TGase. For example, Factor XIIIa is involved in control of blood loss (i.e., clotting) after traumatic injury, TGase 1, TGase 3, and TGase 5 are involved in stabilization of resident keratinocytes, and TGase 2 is involved in matrix repair and remodeling (Haroon *et al*, 1999; Griffin *et al*, 2002; Zhang *et al*, 2004). For example, in rat corneal epithelial wounds, TGase 2 mRNA and protein are detected in migrating epithelial cells and the activity co-localizes with high levels of extracellular matrix proteins, suggesting a role in the maintenance of corneal integrity and cell migration (Zhang *et al*, 2004). In addition, TGase 2 profoundly influences fibroblast function—both by regulating intracellular signaling pathways (Stephens *et al*, 2004) and by regulating extracellular matrix remodeling (Griffin *et al*, 2002). TGase 2 is released into and interacts with the matrix in specific ways (Gaudry *et al*, 1999; Griffin *et al*, 2002). Thus, TGase 2 appears to have intracellular functions in surface epithelial cells and in the underlying fibroblasts, and to also have a role in stabilizing the extracellular matrix (Griffin *et al*, 2002).

TGase type 3 The TGM3 gene, encoding TGase 3, is located on chromosome 20q11–12 (Wang *et al*, 1994). TGase 3 expression is increased in calcium-treated keratinocytes by a mechanism that may involve interaction of ets and Sp1 transcription factors with binding sites in the TGM3 gene promoter (Lee *et al*, 1996; Kim *et al*, 2004). TGase 3 is expressed in the upper epidermal layers and is localized in the cytoplasm (Hitomi *et al*, 2003).

The TGase 3 zymogen consists of four folded domains that share a common structure with other TGase. An N-terminal β-sandwich domain, encompassing amino acids 1-134, contains nine β-sheets intermingled with three α-helices. The catalytic core encompasses amino acids from

Asn_{135} to Gly_{472} and includes 15 β-sheets interspersed with 15 α-helices. The longest of these helices encodes the active site Cys_{272} residue (Ahvazi *et al*, 2002, 2004). Adjacent β-sheets encode the remaining two residues, His_{330} and Asp_{353}, which comprise the active site triad. The barrel 1 and 2 domains span amino acids 473–592 and 593–692, respectively. The active site is buried in a narrow cleft formed by two β-sheets of the catalytic core and the barrel 1 c-terminus (Ahvazi *et al*, 2002). Residues 462–471 comprise a flexible loop that joins the last α-helix of the catalytic domain to the first β-strand of barrel 1. Cleavage at Ser_{469}, within this loop, converts the zymogen form to a form that can be activated by calcium. The zymogen form of TGase 3 contains one calcium ion per protein monomer. This calcium ion is retained in the inactive and activated form and is thought to be required for stability. For activation, however, binding of two additional calcium ions is required. Binding of the second calcium ion produces a minimal change in protein-active site conformation. Binding of the third calcium ion, in contrast, produces a dramatic change in structure that results in the opening of a channel. (Ahvazi *et al*, 2003). The active channel forms a cone that extends inward toward the catalytic triad (Ahvazi *et al*, 2002, 2003). Once the zymogen is proteolytically cleaved in the 462–471 loop, magnesium (intracellular concentration = 2 mM) fills the third calcium binding site and the enzyme remains inactive. Only when intracellular calcium levels rise does calcium displace Mg^{2+} at site three and activate the enzyme. Thus, TGase 3 activity requires the proteolytic cleavage of the zymogen and the binding of calcium at three locations. Once these requirements are met, cross-linking ensues (Ahvazi *et al*, 2003; Ahvazi and Steinert, 2003). In addition, TGase 3 activity is regulated by guanine nucleotides (Ahvazi *et al*, 2004). Binding of GTP is associated with substitution of Ca^{2+} with Mg^{2+} at the binding site three. This results in a conformation change that closes the active site channel. The channel remains closed as long as GTP is present; however, when this GTP is hydrolyzed to GDP the enzyme reverts to the active channel state. Both GTP and GDP bind to a pocket formed between the core domain and the β-barrel 1 domain (Ahvazi *et al*, 2004). The importance of the GTP-associated regulation is not well understood, although it may function in a manner similar to the GTPase function of TGase 2 (Iismaa *et al*, 1997, 2000).

TGase 3 is also an auto-antigen in skin disease. Gluten sensitivity typically presents as celiac disease, a common chronic small intestinal disorder (Reif and Lerner, 2004). In certain individuals, however, celiac disease is associated with dermatitis herpetiformis, a blistering skin disease characterized by granular IgA deposits in the papillary dermis. Antibodies produced by dermatitis herpetiformis patients show a high avidity for TGase 3 (Sardy *et al*, 2002). Moreover, the IgA precipitates that appear in the papillary dermis of patients with dermatitis herpetiformis contain TGase 3. These findings suggest that TGase 3 may serve as the auto-antigen involved in producing the skin phenotype.

TGase type 5 The gene encoding TGase 5 is localized on chromosome 15q15.2 (Grenard *et al*, 2001). TGase 5 is expressed in the spinous and granular layers of human epidermis (Candi *et al*, 2002), and TGase 5 expression in cultured keratinocytes is increased by treatment with the keratinocyte differentiating agent, calcium. Treatment of keratinocytes with elevated calcium resulted in a transient increase in TGase 5 mRNA, after 24 h of treatment, followed by a return to the level observed in untreated cells by 72 h. In contrast, the level of TGase 1 mRNA increases after 24 h and remains elevated. Thus, the kinetics of accumulation of the two TGase mRNA differs. RNA isolated from human keratinocytes reveals the presence of full-length TGase 5 mRNA and three alternate splice products—delta 3, delta 11, and delta 3/11—in which exons 3, 11, or 3 and 11 are absent (Candi *et al*, 2001). The spice products that lack exon 11 have a shift in frame that results in the translation of 25 new amino acids followed by a premature stop codon. Cell-free studies reveal that recombinant full-length and delta 11 spiced forms of TGase 5 are active, while spiced forms lacking the third exon (delta 3 and delta 3/11) are not active (Candi *et al*, 2001). The N-terminus of TGase 5 is also acetylated (Rufini *et al*, 2004).

Recombinant TGase 5 can utilize classical keratinocyte TGase substrates, including involucrin, loricrin, SPR1, and SPR2 (Candi *et al*, 1995). Moreover, the various TGase 5 forms have the expected reactivity - full-length and the delta 11 splice variant are fully active for all substrates, whereas the delta 3 and delta 3/11 splice variants are not active. TGase 5-dependent cross-linking of loricrin results in the formation of loricrin multimers, indicating that TGase 5 can utilize both glutamine and lysine residues from loricrin—both inter- and intra-protein cross-links are formed.

TGase 5 is half-maximally activated at 45 μM calcium (Candi *et al*, 2004). Like TGase 2 (Nakaoka *et al*, 1994; Iismaa *et al*, 2000), TGase 5 is regulated by nucleotides (Candi *et al*, 2004). GTP and ATP inhibit the calcium-dependent cross-linking activity of recombinant TGase 5. In the presence of high calcium more GTP or ATP is required to inhibit calcium-dependent cross-linking. This response reflects the inverse relationship between TGase cross-linking activity and the nucleotide-dependent signaling activity, as previously reported for TGase 2 and TGase 3 (Iismaa *et al*, 2000; Ahvazi *et al*, 2004). TGase 5 is a relatively uncharacterized enzyme; thus, it is not known how its GTPase activity is involved in regulating cell function.

TGase 5 also displays an interesting *in situ* resistance to extraction with detergent, salt and reducing agent. Due to the low level of expression, TGase 5 has been studied in keratinocytes by overexpressing the protein. Expressed TGase 5 is not extracted from keratinocytes by non-ionic detergents, reducing agent, varied ionic strength, or pH. It is only extracted by treatment with SDS or urea—conditions required to extract insoluble proteins. Cell fractionation studies reveal that TGase 5 is associated with nuclear matrix and the cytoskeleton. Confocal analysis indicates that transfected TGase 5 is co-localized in a perinuclear location with vimentin, but not with keratin intermediate filaments (Candi *et al*, 2001). Thus, TGase 5 associates with insoluble filamentous structures. TGase 5 level is also increased in several epidermal diseases—it may contribute to the hyperkeratotic phenotype observed in ichthyosis (Candi *et al*, 2002).

TGase—Progressive and Selective Roles in Cornified Envelope Assembly

The experiments summarized in this review support several conclusions. First, the conservation of protein cross-linking sites, the morphological conservation of epidermal structure, and the ordered deposition of envelope precursors suggest that assembly of the envelope is a directed process (Eckert *et al*, 1993; Steinert, 1995). For example, membrane-associated proteins, including desmoplakin, envoplakin, and periplakin, are always present at the plasma membrane. Thus, it is not surprising that these proteins are envelope components. An involucrin-enriched scaffolding is constructed against this background of membrane-associated proteins (Eckert *et al*, 1993; Murthy *et al*, 1993; Steinert and Marekov, 1997; Jarnik *et al*, 1998). The involucrin scaffolding then provides deposition sites for the cross-linking of other envelope proteins, including cystatin A, proelafin, SPR, and loricrin (Nemes and Steinert, 1999). Second, in spite of the fact that the assembly is ordered, the system is also resilient, since altering the expression of specific precursors does not produce a visible phenotype (Yoneda and Steinert, 1993; Djian *et al*, 2000). This is in part due to the fact that when one precursor is eliminated, for example by gene ablation, the level of others is increased. Third, discovery of three major TGase types in the epidermis, each with a different localization, suggests different roles in assembly. Several studies suggest that TGase may differentially participate to produce the cornified envelope (Candi *et al*, 1995, 1999; Tarcsa *et al*, 1997, 1998). For example, TGase 3 utilizes SPR1 head domain A sequences as cross-linking substrates, whereas TGase 1 utilizes SPR1 head domain B sequences (Candi *et al*, 1999). Experiments with SPR1 in the presence of both TGase 1 and TGase 3 indicate that SPR1 will not polymerize in the absence of TGase 1. This information suggests that each TGase has a specific role in cross-link formation *in vivo*. TGase type-specific patterns of cross-link formation are also observed for cross-linking of loricrin (Candi *et al*, 1995). TGase 3 promotes the formation of intrachain cross-links at favored lysine and glutamine residues in loricrin, whereas TGase 1 catalyzes the formation of loricrin multimers using many glutamine and lysine residues. The loricrin residues selected for *in vitro* cross-link formation by the combined action of TGase 1 and TGase 3 mirror the residues utilized for cross-link formation *in vivo* (Candi *et al*, 1995). These findings, and the observation that TGase 1, TGase 3, and TGase 5 are differentially distributed at the subcellular level, suggest that each TGase has distinct and complementary roles in envelope assembly.

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References

Aeschlimann D, Koeller MK, Allen-Hoffmann BL, Mosher DF: Isolation of a cDNA encoding a novel member of the transglutaminase gene family from human keratinocytes. Detection and identification of transglutaminase gene products based on reverse transcription-polymerase chain reaction with degenerate primers. J Biol Chem 273:3452–3460, 1998

Ahvazi B, Boeshans KM, Idler W, *et al*: Roles of calcium ions in the activation and activity of the transglutaminase 3 enzyme. J Biol Chem 278:23834–23841, 2003

Ahvazi B, Boeshans KM, Idler W, *et al*: Structural basis for the coordinated regulation of transglutaminase 3 by guanine nucleotides and calcium/magnesium. J Biol Chem 279:7180–7192, 2004

Ahvazi B, Kim HC, Kee SH, *et al*: Three-dimensional structure of the human transglutaminase 3 enzyme: Binding of calcium ions changes structure for activation. EMBO J 21:2055–2067, 2002

Ahvazi B, Steinert PM: A model for the reaction mechanism of the transglutaminase 3 enzyme. Exp Mol Med 35:228–242, 2003

Banks EB, Crish JF, Eckert RL: Transcription factor Sp1 activates involucrin promoter activity in non-epithelial cell types. Biochem J 337 (Pt 3):507–512, 1999

Banks EB, Crish JF, Welter JF, Eckert RL: Characterization of human involucrin promoter distal regulatory region transcriptional activator elements-a role for Sp1 and AP1 binding sites. Biochem J 331 (Pt 3):61–68, 1998

Banks-Schlegel S, Green H: Involucrin synthesis and tissue assembly by keratinocytes in natural and cultured human epithelia. J Cell Biol 90:732–737, 1981

Benz J, Bergner A, Hofmann A, *et al*: The structure of recombinant human annexin VI in crystals and membrane-bound. J Mol Biol 260:638–643, 1996

Broome AM, Eckert RL: Microtubule-dependent redistribution of a cytoplasmic cornified envelope precursor. J Invest Dermatol 122:29–38, 2004

Burger A, Berendes R, Liemann S, *et al*: The crystal structure and ion channel activity of human annexin II, a peripheral membrane protein. J Mol Biol 257:839–847, 1996

Buxman MM, Wuepper KD: Isolation, purification and characterization of bovine epidermal transglutaminase. Biochim Biophys Acta 452:356–369, 1976

Candi E, Melino G, Mei G, *et al*: Biochemical, structural, and transglutaminase substrate properties of human loricrin, the major epidermal cornified cell envelope protein. J Biol Chem 270:26382–26390, 1995

Candi E, Oddi S, Paradisi A, *et al*: Expression of transglutaminase 5 in normal and pathologic human epidermis. J Invest Dermatol 119:670–677, 2002

Candi E, Oddi S, Terrinoni A, *et al*: Transglutaminase 5 cross-links loricrin, involucrin, and small proline-rich proteins *in vitro*. J Biol Chem 276:35014–35023, 2001

Candi E, Paradisi A, Terrinoni A, *et al*: Transglutaminase 5 is regulated by guanine/adenine nucleotides. Biochem J 381:313–319, 2004

Candi E, Tarcsa E, DiGiovanna JJ, *et al*: A highly conserved lysine residue on the head domain of type II keratins is essential for the attachment of keratin intermediate filaments to the cornified cell envelope through isopeptide crosslinking by transglutaminases. Proc Natl Acad Sci USA 95:2067–2072, 1998

Candi E, Tarcsa E, Idler WW, *et al*: Transglutaminase cross-linking properties of the small proline-rich 1 family of cornified cell envelope proteins. Integration with loricrin. J Biol Chem 274:7226–7237, 1999

Celis JE, Olsen E: A qualitative and quantitative protein database approach identifies individual and groups of functionally related proteins that are differentially regulated in simian virus 40 (SV40) transformed human keratinocytes: An overview of the functional changes associated with the transformed phenotype. Electrophoresis 15:309–344, 1994

Chakravarty R, Rong XH, Rice RH: Phorbol ester-stimulated phosphorylation of keratinocyte transglutaminase in the membrane anchorage region. Biochem J 271:25–30, 1990

Chen JM, Sheldon A, Pincus MR: Structure-function correlations of calcium binding and calcium channel activities based on 3-dimensional models of human annexins I, II, III, V and VII. J Biomol Struct Dyn 10:1067–1089, 1993

Cohen BE, Lee G, Arispe N, Pollard HB: Cyclic 3′–5′-adenosine monophosphate binds to annexin I and regulates calcium-dependent membrane aggregation and ion channel activity. FEBS Lett 377:444–450, 1995

Cotsarelis G, Cheng SZ, Dong G, *et al*: Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: Implications on epithelial stem cells. Cell 57:201–209, 1989

Crish JF, Bone F, Banks EB, Eckert RL: The human involucrin gene contains spatially distinct regulatory elements that regulate expression during early versus late epidermal differentiation. Oncogene 21:738–747, 2002

Crish JF, Howard JM, Zaim TM, *et al*: Tissue-specific and differentiation-appropriate expression of the human involucrin gene in transgenic mice: An abnormal epidermal phenotype. Differentiation 53:191–200, 1993

Crish JF, Zaim T, Eckert RL: The distal regulatory region (DRR) of the human involucrin promoter is required for expression in epidermis. J Biol Chem 273:30460–30465, 1998

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

Deucher A, Nagpal S, Chandraratna RA, *et al*: The carboxy-terminal hydrophobic domain of TIG3, a class II tumor suppressor protein, is required for appropriate cellular localization and optimal biological activity. Int J Oncol 17:1195–1203, 2000

DiSepio D, Ghosn C, Eckert RL, *et al*: Identification and characterization of a retinoid-induced class II tumor suppressor/growth regulatory gene. Proc Natl Acad Sci USA 95:14811–14815, 1998

DiSepio D, Jones A, Longley MA, *et al*: The proximal promoter of the mouse loricrin gene contains a functional AP-1 element and directs keratinocyte-specific but not differentiation-specific expression. J Biol Chem 270: 10792–10799, 1995

Djian P, Easley K, Green H: Targeted ablation of the murine involucrin gene. J Cell Biol 151:381–388, 2000

Donato R: Functional roles of S100 proteins, calcium-binding proteins of the EF- hand type. Biochim Biophys Acta 1450:191–231, 1999

Dubbink HJ, de Waal L, van Haperen R, *et al*: The human prostate-specific transglutaminase gene (TGM4): Genomic organization, tissue-specific expression, and promoter characterization. Genomics 51:434–444, 1998

Duvic M, Helekar B, Schulz C, *et al*: Expression of a retinoid-inducible tumor suppressor, Tazarotene-inducible gene-3, is decreased in psoriasis and skin cancer. Clin Cancer Res 6:3249–3259, 2000

Eckert RL, Crish JF, Efimova T, *et al*: Regulation of involucrin gene expression. J Invest Dermatol 123:13–22, 2004

Eckert RL, Crish JF, Robinson NA: The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. Physiol Rev 77:397–424, 1997

Eckert RL, Green H: Structure and evolution of the human involucrin gene. Cell 46:583–589, 1986

Eckert RL, Yaffe MB, Crish JF, *et al*: Involucrin—structure and role in envelope assembly. J Invest Dermatol 100:613–617, 1993

Efimova T, Broome AM, Eckert RL: A regulatory role for p38 delta MAPK in keratinocyte differentiation. Evidence for p38 delta-ERK1/2 complex formation. J Biol Chem 278:34277–34285, 2003

Efimova T, LaCelle P, Welter JF, Eckert RL: Regulation of human involucrin promoter activity by a protein kinase C, Ras, MEKK1, MEK3, p38/RK, AP1 signal transduction pathway. J Biol Chem 273:24387–24395, 1998

Elias PM, Feingold KR: Coordinate regulation of epidermal differentiation and barrier homeostasis. Skin Pharmacol Appl Skin Physiol 1 14 Suppl:28–34, 2001

Elias PM, Friend DS: The permeability barrier in mammalian epidermis. J Cell Biol 65:180–191, 1975

Etoh Y, Simon M, Green H: Involucrin acts as a transglutaminase substrate at multiple sites. Biochem Biophys Res Commun 136:51–56, 1986

Fesus L, Piacentini M: Transglutaminase 2: An enigmatic enzyme with diverse functions. Trends Biochem Sci 27:534–539, 2002

Fischer DF, Gibbs S, van De Putte P, Backendorf C: Interdependent transcription control elements regulate the expression of the SPRR2A gene during keratinocyte terminal differentiation. Mol Cell Biol 16:5365–5374, 1996

Floyd EE, Jetten AM: Regulation of type I (epidermal) transglutaminase mRNA levels during squamous differentiation: Down regulation by retinoids. Mol Cell Biol 9:4846–4851, 1989

Folk JE: Transglutaminases. Annu Rev Biochem 49:517–531, 1980

Folk JE, Finlayson JS: The epsilon-(gamma-glutamyl)lysine crosslink and the catalytic role of transglutaminases. Adv Protein Chem 31:1–133, 1977

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

Gaudry CA, Verderio E, Aeschlimann D, *et al*: Cell surface localization of tissue transglutaminase is dependent on a fibronectin-binding site in its N-terminal beta-sandwich domain. J Biol Chem 274:30707–30714, 1999

Gerke V, Moss SE: Annexins: From structure to function. Physiol Rev 82:331–371, 2002

Gibbs S, Fijneman R, Wiegant J, *et al*: Molecular characterization and evolution of the SPRR family of keratinocyte differentiation markers encoding small proline-rich proteins. Genomics 16:630–637, 1993

Gibbs S, Lohman F, Teubel W, *et al*: Characterization of the human spr2 promoter: Induction after UV irradiation or TPA treatment and regulation during differentiation of cultured primary keratinocytes. Nucleic Acids Res 18:4401–4407, 1990

Goldsmith LA: Human epidermal transglutaminase. J Invest Dermatol 80 (Suppl.):39s–41s, 1983

Grayson S, Elias PM: Isolation and lipid biochemical characterization of stratum corneum membrane complexes: Implications for the cutaneous permeability barrier. J Invest Dermatol 78:128–135, 1982

Green H: The keratinocyte as differentiated cell type. Harvey Lect 74:101–139, 1980

Green KJ, Gaudry CA: Are desmosomes more than tethers for intermediate filaments? Nat Rev Mol Cell Biol 1:208–216, 2000

Grenard P, Bates MK, Aeschlimann D: Evolution of transglutaminase genes: Identification of a transglutaminase gene cluster on human chromosome 15q15. Structure of the gene encoding transglutaminase X and a novel gene family member, transglutaminase Z. J Biol Chem 276:33066–33078, 2001

Griffin M, Casadio R, Bergamini CM: Transglutaminases: Nature's biological glues. Biochem J 368:377–396, 2002

Haroon ZA, Hettasch JM, Lai TS, *et al*: Tissue transglutaminase is expressed, active, and directly involved in rat dermal wound healing and angiogenesis. FASEB J 13:1787–1795, 1999

Hennings H, Kruszewski FH, Yuspa SH, Tucker RW: Intracellular calcium alterations in response to increased external calcium in normal and neoplastic keratinocytes. Carcinogenesis 10:777–780, 1989

Hennings H, Steinert P, Buxman MM: Calcium induction of transglutaminase and the formation of epsilon(gamma-glutamyl) lysine cross-links in cultured mouse epidermal cells. Biochem Biophys Res Commun 102:739–745, 1981

Hitomi K, Kanehiro S, Ikura K, Maki M: Characterization of recombinant mouse epidermal-type transglutaminase (TGase 3): Regulation of its activity by proteolysis and guanine nucleotides. J Biochem (Tokyo) 125:1048–1054, 1999

Hitomi K, Presland RB, Nakayama T, *et al*: Analysis of epidermal-type transglutaminase (transglutaminase 3) in human stratified epithelia and cultured keratinocytes using monoclonal antibodies. J Dermatol Sci 32: 95–103, 2003

Hohl D, Lichti U, Breitkreutz D, *et al*: Transcription of the human loricrin gene *in vitro* is induced by calcium and cell density and suppressed by retinoic acid. J Invest Dermatol 96:414–418, 1991a

Hohl D, Mehrel T, Lichti U, *et al*: Characterization of human loricrin. Structure and function of a new class of epidermal cell envelope proteins. J Biol Chem 266:6626–6636, 1991b

Holbrook KA, Odland GF: The fine structure of developing human epidermis: Light, scanning, and transmission electron microscopy of the periderm. J Invest Dermatol 65:16–38, 1975

Huber M, Rettler I, Bernasconi K, *et al*: Mutations of keratinocyte transglutaminase in lamellar ichthyosis. Science 267:525–528, 1995a

Huber M, Rettler I, Bernasconi K, *et al*: Lamellar ichthyosis is genetically heterogeneous—cases with normal keratinocyte transglutaminase. J Invest Dermatol 105:653–654, 1995b

Iismaa SE, Chung L, Wu MJ, *et al*: The core domain of the tissue transglutaminase Gh hydrolyzes GTP and ATP. Biochemistry 36:11655–11664, 1997

Iismaa SE, Wu MJ, Nanda N, *et al*: GTP binding and signaling by Gh/transglutaminase II involves distinct residues in a unique GTP-binding pocket. J Biol Chem 275:18259–18265, 2000

Ishida-Yamamoto A: Loricrin keratoderma: A novel disease entity characterized by nuclear accumulation of mutant loricrin. J Dermatol Sci 31:3–8, 2003

Ishida Yamamoto A, Eady RA, Watt FM, *et al*: Immunoelectron microscopic analysis of cornified cell envelope formation in normal and psoriatic epidermis. J Histochem Cytochem 44:167–175, 1996

Ishida Yamamoto A, Hohl D, Roop DR, *et al*: Loricrin immunoreactivity in human skin: Localization to specific granules (L-granules) in acrosyringia. Arch Dermatol Res 285:491–498, 1993

Ishida-Yamamoto A, Tanaka H, Nakane H, *et al*: Antigen retrieval of loricrin epitopes at desmosomal areas of cornified cell envelopes: An immunoelectron microscopic analysis. Exp Dermatol 8:402–406, 1999

Jarnik M, Simon MN, Steven AC: Cornified cell envelope assembly: A model based on electron microscopic determinations of thickness and projected density. J Cell Sci 111 (Pt 8):1051–1060, 1998

Jessen BA, Phillips MA, Hovnanian A, Rice RH: Role of Sp1 response element in transcription of the human transglutaminase 1 gene. J Invest Dermatol 115:113–117, 2000a

Jessen BA, Qin Q, Phillips MA, *et al*: Keratinocyte differentiation marker suppression by arsenic: Mediation by AP1 response elements and antagonism by tetradecanoylphorbol acetate. Toxicol Appl Pharmacol 174: 302–311, 2001

Jessen BA, Qin Q, Rice RH: Functional AP1 and CRE response elements in the human keratinocyte transglutaminase promoter mediating Whn suppression. Gene 254:77–85, 2000b

Kajava AV: alpha-Helical solenoid model for the human involucrin. FEBS Lett 473:127–131, 2000

Kalinin AE, Kajava AV, Steinert PM: Epithelial barrier function: Assembly and structural features of the cornified cell envelope. Bioessays 24:789–800, 2002

Kim CD, Seo EY, Sung YH, *et al*: Characterization of Ets-binding sequence of human transglutaminase 3 gene promoter. Exp Dermatol 13:529–534, 2004

Kim HC, Idler WW, Kim IG, *et al*: The complete amino acid sequence of the human transglutaminase K enzyme deduced from the nucleic acid sequences of cDNA clones. J Biol Chem 266:536–539, 1991

Kim IG, Gorman JJ, Park SC, *et al*: The deduced sequence of the novel protransglutaminase E (TGase3) of human and mouse. J Biol Chem 268: 12682–12690, 1993

Kim IG, Lee SC, Lee JH, *et al*: Structure and organization of the human transglutaminase 3 gene: Evolutionary relationship to the transglutaminase family. J Invest Dermatol 103:137–142, 1994a

Kim SY, Chung SI, Steinert PM: Highly active soluble processed forms of the transglutaminase 1 enzyme in epidermal keratinocytes. J Biol Chem 270:18026–18035, 1995a

Kim SY, Chung SI, Yoneda K, Steinert PM: Expression of transglutaminase 1 in human epidermis. J Invest Dermatol 104:211–217, 1995b

Kim SY, Kim IG, Chung SI, Steinert PM: The structure of the transglutaminase 1 enzyme. Deletion cloning reveals domains that regulate its specific activity and substrate specificity. J Biol Chem 269:27979–27986, 1994b

Kuramoto N, Takizawa T, Takizawa T, *et al*: Development of ichthyosiform skin compensates for defective permeability barrier function in mice lacking transglutaminase 1. J Clin Invest 109:243–250, 2002

LaCelle PT, Lambert A, Ekambaram MC, *et al*: *In vitro* cross-linking of recombinant human involucrin. Skin Pharmacol Appl Skin Physiol 11:214–226, 1998

Lambert A, Ekambaram M, Robinson N, Eckert RL: Transglutaminase reactivity of human involucrin. Skin Pharmacol Appl Skin Physiol 13:17–30, 2000

Lavker RM: Membrane coating granules: The fate of the discharged lamellae. J Ultrastruct Res 55:79–86, 1976

Lavker RM, Matoltsy AG: Substructure of keratohyalin granules of the epidermis as revealed by high resolution electron microscopy. J Ultrastruct Res 35:575–581, 1971

Lazo ND, Downing DT: A mixture of alpha-helical and 3(10)-helical conformations for involucrin in the human epidermal corneocyte envelope provides a scaffold for the attachment of both lipids and proteins. J Biol Chem 274:37340–37344, 1999

Lee JH, Jang SI, Yang JM, *et al*: The proximal promoter of the human transglutaminase 3 gene. Stratified squamous epithelial-specific expression in cultured cells is mediated by binding of Sp1 and ets transcription factors to a proximal promoter element. J Biol Chem 271:4561–4568, 1996

Li L, Tucker RW, Hennings H, Yuspa SH: Chelation of intracellular Ca2+ inhibits murine keratinocyte differentiation *in vitro*. J Cell Physiol 163:105–114, 1995a

Li L, Tucker RW, Hennings H, Yuspa SH: Inhibitors of the intracellular Ca(2+)-ATPase in cultured mouse keratinocytes reveal components of terminal differentiation that are regulated by distinct intracellular Ca2+ compartments. Cell Growth Differ 6:1171–1184, 1995b

Lichti U, Ben T, Yuspa SH: Retinoic acid-induced transglutaminase in mouse epidermal cells is distinct from epidermal transglutaminase. J Biol Chem 260:1422–1426, 1985

Liew FM, Yamanishi K: Regulation of transglutaminase 1 gene expression by 12-O-tetradecanoylphorbol-13-acetate, dexamethasone, and retinoic acid in cultured human keratinocytes. Exp Cell Res 202:310–315, 1992

Lorand L, Barnes N, Bruner-Lorand JA, *et al*: Inhibition of protein cross-linking in Ca^{2+}-enriched human erythrocytes and activated platelets. Biochemistry 26:308–313, 1987

Lorand L, Graham RM: Transglutaminases: Crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol 4:140–156, 2003

Mack JW, Steven AC, Steinert PM: The mechanism of interaction of filaggrin with intermediate filaments. The ionic zipper hypothesis. J Mol Biol 232:50–66, 1993

Marekov LN, Steinert PM: Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope. J Biol Chem 273: 17763–17770, 1998

Mariniello L, Qin Q, Jessen BA, Rice RH: Keratinocyte transglutaminase promoter analysis. Identification of a functional response element. J Biol Chem 270:31358–31363, 1995

Markova NG, Marekov LN, Chipev CC, *et al*: Profilaggrin is a major epidermal calcium-binding protein. Mol Cell Biol 13:613–625, 1993

Matoltsy AG: Membrane-coating granules of the epidermis. J Ultrastruct Res 15:510–515, 1966

Matoltsy AG: Keratinization. J Invest Dermatol 67:20–25, 1976

Matoltsy AG, Matoltsy MN: The membrane protein of horny cells. J Invest Dermatol 46:127–129, 1966

Matoltsy AG, Odland GF: Investigation of the structure of the cornified epithelium of the human skin. J Biophys Biochem Cytol 1:191–196, 1955

Matsuki M, Yamashita F, Ishida Yamamoto A, *et al*: Defective stratum corneum and early neonatal death in mice lacking the gene for transglutaminase 1 (keratinocyte transglutaminase). Prod Natl Acad Sci USA 95:1044–1049, 1998

Medvedev A, Saunders NA, Matsuura H, *et al*: Regulation of the transglutaminase I gene. Identification of DNA elements involved in its transcriptional control in tracheobronchial epithelial cells. J Biol Chem 274:3887–3896, 1999

Mehrel T, Hohl D, Rothnagel JA, *et al*: Identification of a major keratinocyte cell envelope protein, loricrin. J Cell Biochem 61:1103–1112, 1990

Menon GK, Grayson S, Elias PM: Ionic calcium reservoirs in mammalian epidermis: Ultrastructural localization by ion-capture cytochemistry. J Invest Dermatol 84:508–512, 1985

Michel S, Bernerd F, Jetten AM, *et al*: Expression of keratinocyte transglutamine mRNA revealed by *in situ* hybridization. J Invest Dermatol 98:364–368, 1992

Michel S, Demarchez M: Localization and *in vivo* activity of epidermal transglutaminase. J Invest Dermatol 90:472–474, 1988

Michibata H, Chiba H, Wakimoto K, *et al*: Identification and characterization of a novel component of the cornified envelope, cornifelin. Biochem Biophys Res Commun 318:803–813, 2004

Molhuizen HO, Alkemade HA, Zeeuwen PL, *et al*: SKALP/elafin: An elastase inhibitor from cultured human keratinocytes. Purification, cDNA sequence, and evidence for transglutaminase cross-linking. J Biol Chem 268:12028–12032, 1993

Murthy S, Crish JF, Zaim TM, Eckert RL: A dual role for involucrin in the epidermis—ultrastructural localization in epidermis and hair follicle in humans and transgenic mice. J Struct Biol 111:68–76, 1993

Nakaoka H, Perez DM, Baek KJ, *et al*: Gh: A GTP-binding protein with transglutaminase activity and receptor signaling function. Science 264:1593–1596, 1994

Nara K, Ito S, Ito T, *et al*: Elastase inhibitor elafin is a new type of proteinase inhibitor which has a transglutaminase-mediated anchoring sequence termed "cementoin". J Biochem (Tokyo) 115:441–448, 1994

Nemes Z, Marekov LN, Fesus L, Steinert PM: A novel function for transglutaminase 1: Attachment of long-chain omega-hydroxyceramides to involucrin by ester bond formation. Proc Natl Acad Sci USA 96:8402–8407, 1999a

Nemes Z, Marekov LN, Steinert PM: Involucrin cross-linking by transglutaminase 1. Binding to membranes directs residue specificity. J Biol Chem 274: 11013–11021, 1999b

Nemes Z, Steinert PM: Bricks and mortar of the epidermal barrier. Exp Mol Med 31:5–19, 1999

Ogawa H, Goldsmith LA: Human epidermal transglutaminase: Preparation and properties. J Biol Chem 251:7281–7288, 1976

Pearton DJ, Dale BA, Presland RB: Functional analysis of the profilaggrin N-terminal peptide: Identification of domains that regulate nuclear and cytoplasmic distribution. J Invest Dermatol 119:661–669, 2002

Peterson LL, Wuepper KD: Epidermal and hair follicle transglutaminases and crosslinking in skin. Mol Cell Biochem 58:99–111, 1984

Phillips MA, Jessen BA, Lu Y, *et al*: A distal region of the human TGM1 promoter is required for expression in transgenic mice and cultured keratinocytes. BMC Dermatol 4:2, 2004

Phillips MA, Qin Q, Mehrpouyan M, Rice RH: Keratinocyte transglutaminase membrane anchorage: Analysis of site-directed mutants. Biochemistry 32:11057–11063, 1993

Phillips MA, Stewart BE, Qin Q, *et al*: Primary structure of keratinocyte transglutaminase. Proc Natl Acad Sci USA 87:9333–9337, 1990

Polakowska R, Herting E, Goldsmith LA: Isolation of cDNA for human epidermal type I transglutaminase. J Invest Dermatol 96:285–288, 1991

Presland RB, Haydock PV, Fleckman P, *et al*: 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, *et al*: Evidence for specific proteolytic cleavage of the N-terminal domain of human profilaggrin during epidermal differentiation. J Invest Dermatol 108:170–178, 1997

Presland RB, Kuechle MK, Lewis SP, *et al*: Regulated expression of human filaggrin in keratinocytes results in cytoskeletal disruption, loss of cell–cell adhesion, and cell cycle arrest. Exp Cell Res 270:199–213, 2001

Reif S, Lerner A: Tissue transglutaminase—the key player in celiac disease: A review. Autoimmun Rev 3:40–45, 2004

Rety S, Osterloh D, Arie JP, *et al*: Structural basis of the Ca(2+)-dependent association between S100C (S100A11) and its target, the N-terminal part of annexin I. Structure Fold Des 8:175–184, 2000

Rice RH, Green H: Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: Activation of the cross-linking by calcium ions. Cell 18:681–694, 1979

Rice RH, Mehrpouyan M, Quin Q, *et al*: Identification of phosphorylation sites in keratinocyte transglutaminase. Biochem J 320 (Pt 2):547–550, 1996

Rice RH, Rong XH, Chakravarty R: Proteolytic release of keratinocyte transglutaminase. Biochem J 265:351–357, 1990

Robinson NA, Eckert RL: Identification of transglutaminase-reactive residues in S100A11. J Biol Chem 273:2721–2728, 1998

Robinson NA, LaCelle PT, Eckert RL: Involucrin is a covalently crosslinked constituent of highly purified epidermal corneocytes: Evidence for a common pattern of involucrin crosslinking *in vivo* and *in vitro*. J Invest Dermatol 107:101–107, 1996

Robinson NA, Lapic S, Welter JF, Eckert RL: S100A11, S100A10, annexin I, desmosomal proteins, small proline-rich proteins, plasminogen activator inhibitor-2, and involucrin are components of the cornified envelope of cultured human epidermal keratinocytes. J Biol Chem 272:12035–12046, 1997

Rothnagel JA, Rogers GE: Transglutaminase-mediated cross-linking in mammalian epidermis. Mol Cell Biochem 58:113–119, 1984

Rufini A, Vilbois F, Paradisi A, *et al*: Transglutaminase 5 is acetylated at the N-terminal end. Amino Acids 26:425–430, 2004

Ruse M, Lambert A, Robinson N, *et al*: S100A7, S100A10, and S100A11 are transglutaminase substrates. Biochemistry 40:3167–3173, 2001

Russell LJ, DiGiovanna JJ, Rogers GR, *et al*: Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. Nat Genet 9:279–283, 1995

Sardy M, Karpati S, Merkl B, *et al*: Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. J Exp Med 195:747–757, 2002

Saunders NA, Bernacki SH, Vollberg TM, Jetten AM: Regulation of transglutaminase type I expression in squamous differentiating rabbit tracheal epithelial cells and human epidermal keratinocytes: Effects of retinoic acid and phorbol esters. Mol Endocrinol 7:387–398, 1993

Segre J: Complex redundancy to build a simple epidermal permeability barrier. Curr Opin Cell Biol 15:776–782, 2003

Siefring GE Jr., Apostol AB, Velasco PT, Lorand L: Enzymatic basis for the Ca^{2+}-induced cross-linking of membrane proteins in intact human erythrocytes. Biochemistry 17:2598–2604, 1978

Simon M, Green H: Enzymatic cross-linking of involucrin and other proteins by keratinocyte particulates *in vitro*. Cell 40:677–683, 1985

Simon M, Green H: The glutamine residues reactive in transglutaminase-catalyzed cross-linking of involucrin. J Biol Chem 263:18093–18098, 1988

Sizemore N, Kasturi L, Gorodeski G, *et al*: Retinoid regulation of human ectocervical epithelial cell transglutaminase activity and keratin gene expression. Differentiation 54:219–225, 1993

South AP, Cabral A, Ives JH, *et al*: Human epidermal differentiation complex in a single 2.5 Mbp long continuum of overlapping DNA cloned in bacteria integrating physical and transcript maps. J Invest Dermatol 112:910–918, 1999

Steinert PM: A model for the hierarchical structure of the human epidermal cornified cell envelope. Cell Death Differ 2:33–40, 1995

Steinert PM, Candi E, Kartasova T, Marekov L: Small proline-rich proteins are cross-bridging proteins in the cornified cell envelopes of stratified squamous epithelia. J Struct Biol 122:76–85, 1998

Steinert PM, Chung SI, Kim SY: Inactive zymogen and highly active proteolytically processed membrane-bound forms of the transglutaminase 1 enzyme in human epidermal keratinocytes. Biochem Biophys Res Commun 221:101–106, 1996a

Steinert PM, Kim SY, Chung SI, Marekov LN: The transglutaminase 1 enzyme is variably acylated by myristate and palmitate during differentiation in epidermal keratinocytes. J Biol Chem 271:26242–26250, 1996b

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, Marekov LN: Direct evidence that involucrin is a major early isopeptide crosslinked component of the keratinocyte cornified cell envelope. J Biol Chem 272:2021–2030, 1997

Steinert PM, Marekov LN: Initiation of assembly of the cell envelope barrier structure of stratified squamous epithelia. Mol Biol Cell 10:4247–4261, 1999

Stephens P, Grenard P, Aeschlimann P, *et al*: Crosslinking and G-protein functions of transglutaminase 2 contribute differentially to fibroblast wound healing responses. J Cell Sci 117:3389–3403, 2004

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

Steven AC, Steinert PM: Protein composition of cornified cell envelopes of epidermal keratinocytes. J Cell Sci 107:693–700, 1994

Sturniolo MT, Dashti SR, Deucher A, *et al*: A novel tumor suppressor protein promotes keratinocyte terminal differentiation via activation of type I transglutaminase. J Biol Chem 278:48066–48073, 2003

Takahashi H, Honma M, Ishida-Yamamoto A, *et al*: Expression of Human Cystatin A by Keratinocytes Is Positively Regulated via the Ras/MEKK1/MKK7/JNK Signal Transduction Pathway but Negatively Regulated via the Ras/Raf-1/MEK1/ERK Pathway. J Biol Chem 276:36632–36638, 2001

Takahashi M, Tezuka T, Kakegawa H, Katunuma N: Linkage between phosphorylated cystatin alpha and filaggrin by epidermal transglutaminase as a model of cornified envelope and inhibition of cathepsin L activity by cornified envelope and the conjugated cystatin alpha. FEBS Lett 340:173–176, 1994

Tarcsa E, Candi E, Kartasova T, *et al*: Structural and transglutaminase substrate properties of the small proline-rich 2 family of cornified cell envelope proteins. J Biol Chem 273:23297–23303, 1998

Tarcsa E, Marekov LN, Andreoli J, *et al*: The fate of trichohyalin. Sequential post-translational modifications by peptidyl-arginine deiminase and transglutaminases. J Biol Chem 272:27893–27901, 1997

Tesfaigzi J, Carlson DM: Expression, regulation, and function of the SPR family of proteins. A review. Cell Biochem Biophys 30:243–265, 1999

Thacher SM, Rice RH: Keratinocyte-specific transglutaminase of cultured human epidermal cells: Relation to cross-linked envelope formation and terminal differentiation. Cell 40:685–695, 1985

Traupe H, Kolde G, Happle R: Autosomal dominant lamellar ichthyosis: A new skin disorder. Clin Genet 26:457–461, 1984

Virata ML, Wagner RM, Parry DA, Green KJ: Molecular structure of the human desmoplakin I and II amino terminus. Proc Natl Acad Sci USA 89:544–548, 1992

Walts AE, Said JW, Siegel MB, Banks-Schlegel S: Involucrin, a marker of squamous and urothelial differentiation. An immunohistochemical study on its distribution in normal and neoplastic tissues. J Pathol 145:329–340, 1985

Wang M, Kim IG, Steinert PM, McBride OW: Assignment of the human transglutaminase 2 (TGM2) and transglutaminase 3 (TGM3) genes to chromosome 20q11.2. Genomics 23:721–722, 1994

Welter JF, Crish JF, Agarwal C, Eckert RL: Fos-related antigen (Fra-1), junB, and junD activate human involucrin promoter transcription by binding to proximal and distal AP1 sites to mediate phorbol ester effects on promoter activity. J Biol Chem 270:12614–12622, 1995

Wertz PW, Madison KC, Downing DT: Covalently bound lipids of human stratum corneum. J Invest Dermatol 92:109–111, 1989a

Wertz PW, Swartzendruber DC, Kitko DJ, *et al*: The role of the corneocyte lipid envelopes in cohesion of the stratum corneum. J Invest Dermatol 93: 169–172, 1989b

Yaffe MB, Beegen H, Eckert RL: Biophysical characterization of involucrin reveals a molecule ideally suited to function as an intermolecular cross-bridge of the keratinocyte cornified envelope. J Biol Chem 267: 12233–12238, 1992

Yaffe MB, Murthy S, Eckert RL: Evidence that involucrin is a covalently linked constituent of highly purified cultured keratinocyte cornified envelopes. J Invest Dermatol 100:3–9, 1993

Yamada K, Yamanishi K, Kakizuka A, *et al*: Transcriptional regulation of human transglutaminase1 gene by signaling systems of protein kinase C, RAR/RXR and Jun/Fos in keratinocytes. Biochem Mol Biol Int 34:827–836, 1994

Yamanishi K, Inazawa J, Liew FM, *et al*: Structure of the gene for human transglutaminase 1. J Biol Chem 267:17858–17863, 1992

Yoneda K, Steinert PM: Overexpression of human loricrin in transgenic mice produces a normal phenotype. Proc Natl Acad Sci USA 90:10754–10758, 1993

Yuspa SH, Ben T, Steinert P: Retinoic acid induces transglutaminase activity but inhibits cornification of cultured epidermal cells. J Biol Chem 257: 9906–9908, 1982

Zeeuwen PL, Vlijmen-Willems IM, Hendriks W, *et al*: A null mutation in the cystatin M/E gene of ichq mice causes juvenile lethality and defects in epidermal cornification. Hum Mol Genet 11:2867–2875, 2002

Zeeuwen PL, Vlijmen-Willems IM, Olthuis D, *et al*: Evidence that unrestricted legumain activity is involved in disturbed epidermal cornification in cystatin M/E deficient mice. Hum Mol Genet 13:1069–1079, 2004

Zhang W, Shiraishi A, Suzuki A, *et al*: Expression and distribution of tissue transglutaminase in normal and injured rat cornea. Curr Eye Res 28:37–45, 2004

Zhao XP, Elder JT: Positional cloning of novel skin-specific genes from the human epidermal differentiation complex. Genomics 45:250–258, 1997