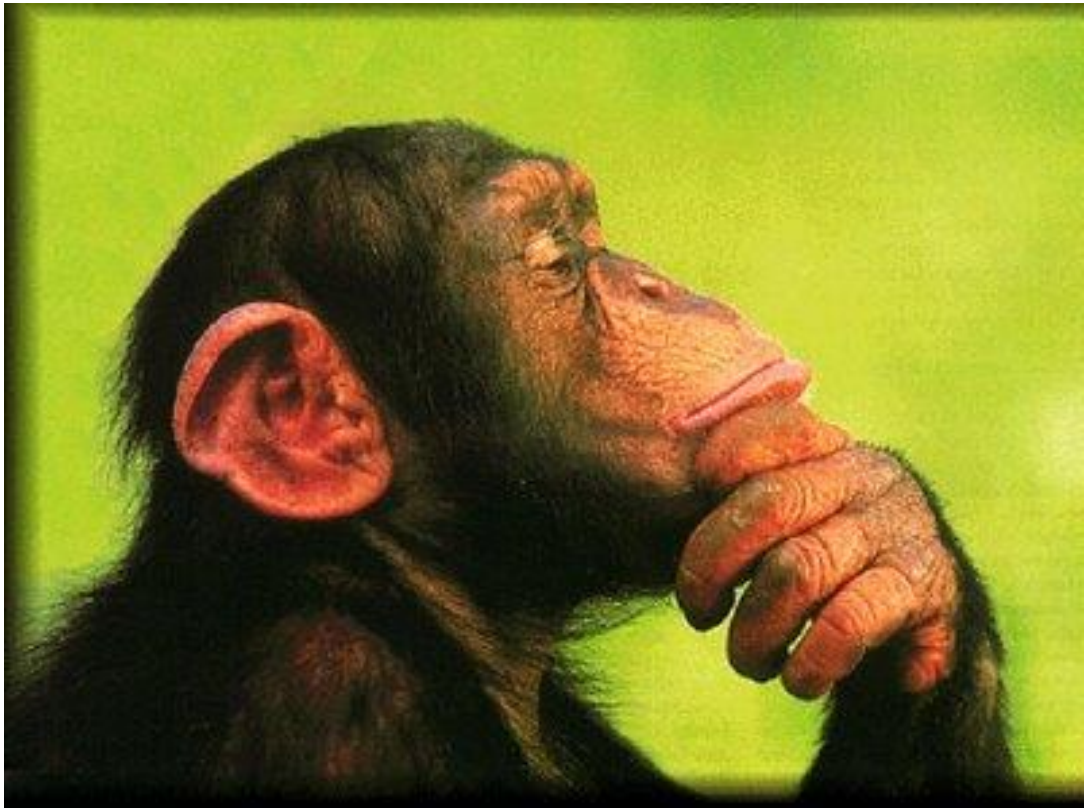
The Epidermal Barrier: Biography of a Keratinocyte

HEIDE M. NEWTON, DVM, DACVD

Biography of a Keratinocyte??



An Electron Microscope Study of Cornification in the Human Skin. Arwyn Charles, 1959, *J Invest Dermatol* , 33:65-74.

Abbreviations Used

cw, cell wall; hl, horny layer; kh, keratohyalin; mit, mitochondria; n, nucleus; p, prickle; pn, prickle nodule; sc, spongy cell.

Fig. 1. Cells of the upper epidermis. The cells at lower left are ordinary Malpighian-layer cells, above which occur flattened Malpighian cells, then cells of the granular layer containing keratohyalin, and finally, at the top right-hand corner, a cell of the horny layer. The unknown bodies can be seen at (x) × 9,000

Fig. 2. Cells of the granular and horny layers. Keratohyalin forms a sheath around the tonofibrils at (tfs), the light lines (l) probably representing the fibrils; this region, indicated by arrow A, is enlarged as Fig. 3A. Arrow B indicates a prickle enlarged in Fig. 3B, in which the cell wall remnants appear as less-electron-dense lines (pl) with a darker region (pd), the point of intercellular adhesion, between. Note the increased electron-density of the two upper horny layers. × 17,500.

Skin Functions

EPIDERMIS-DERMIS-SUBCUTIS

Physical permeability barrier

Protection from infectious agents

Thermoregulation

Sensation

Physical appearance

EPIDERMIS – BARRIER BETWEEN INSIDE AND OUTSIDE

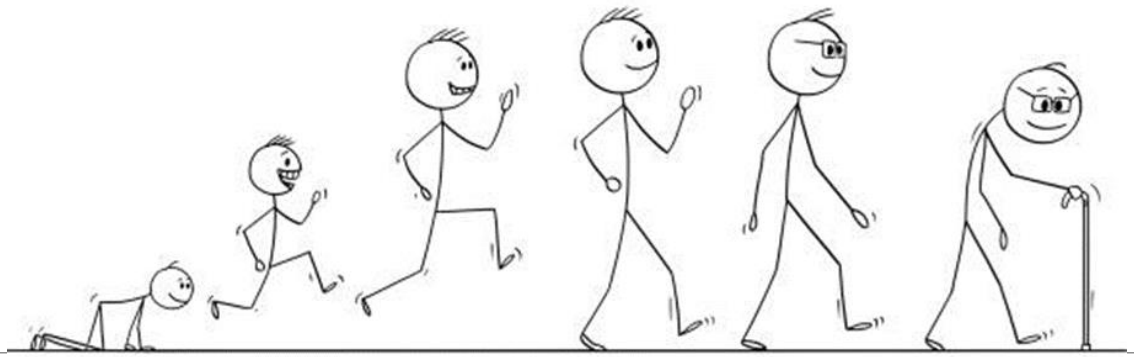
Compressive, tensile, bending strength

Mechanical protection

Prevents penetration of pathogenic microorganisms, allergens, toxins

Protects against damage from ultraviolet radiation

Prevents water loss



BIOGRAPHY OF A KERATINOCYTE

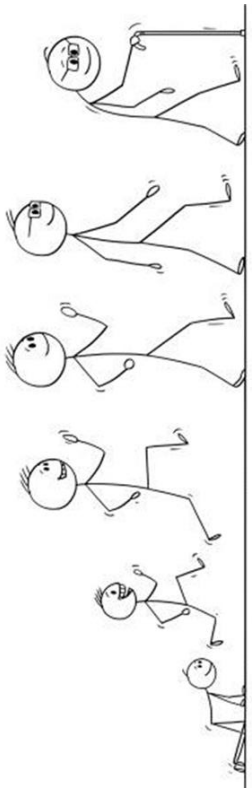
Overview of epidermal layers

Cornification

Keratinocyte adhesion

Desquamation

Epidermal barrier



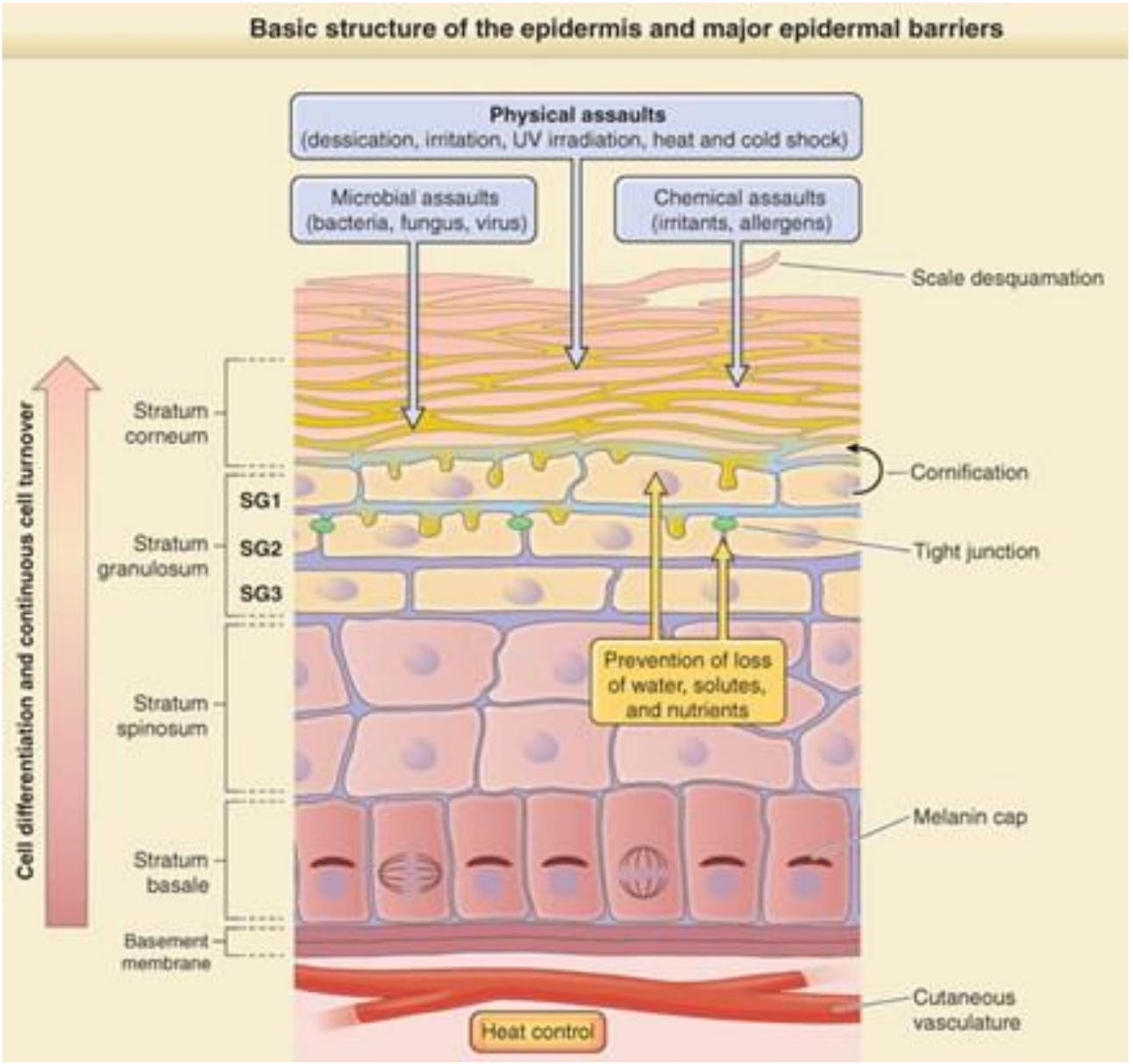


Figure 14-2 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Stratum basale

Basal keratinocytes

- Small polar undifferentiated cells
- Express K5 and K14
  - Dogs also express K1 and K6
- Attach to basement membrane at hemidesmosomes

Stratum basale

Theories of epidermal homeostasis

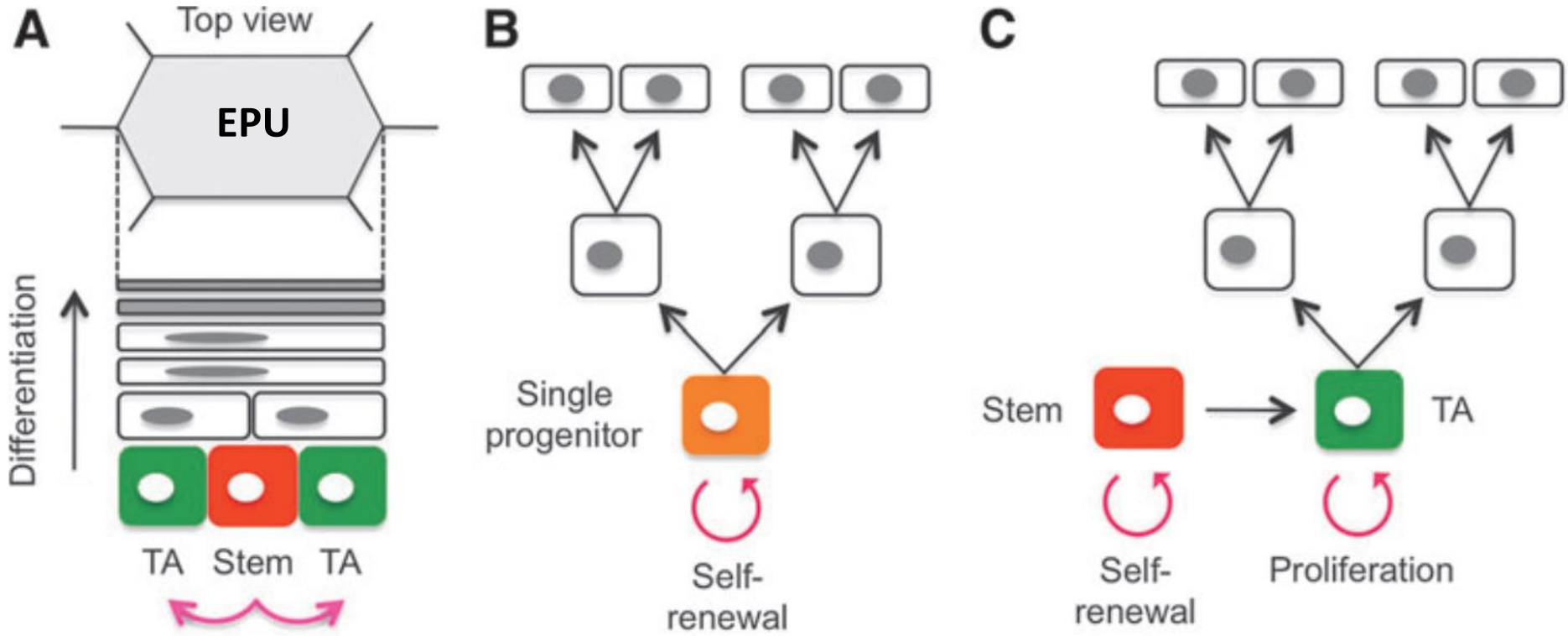


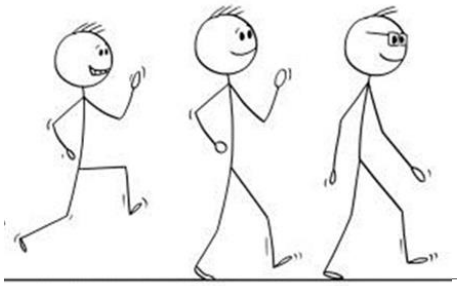
Figure 2 from Senoo M. Epidermal Stem Cells in Homeostasis and Wound Repair of the Skin. *Adv Wound Care (New Rochelle)*. 2013;2(6):276.

Stratum spinosum

AKA spinous or prickle cell layer
- “Spines” represent desmosomes

Keratinocytes enter terminal differentiation pathway
- Synthesizing K1 and K10
- Dogs also express K4 and K15/16

Start producing other components
- Involucrin
- Profilaggrin
- Lamellar bodies



Stratum granulosum

Keratohyalin granules visible under light microscopy

Granule components

- Profilaggrin
- Keratin filaments
- Loricrin

3 layers: SG3 (deepest) → SG2 → SG1

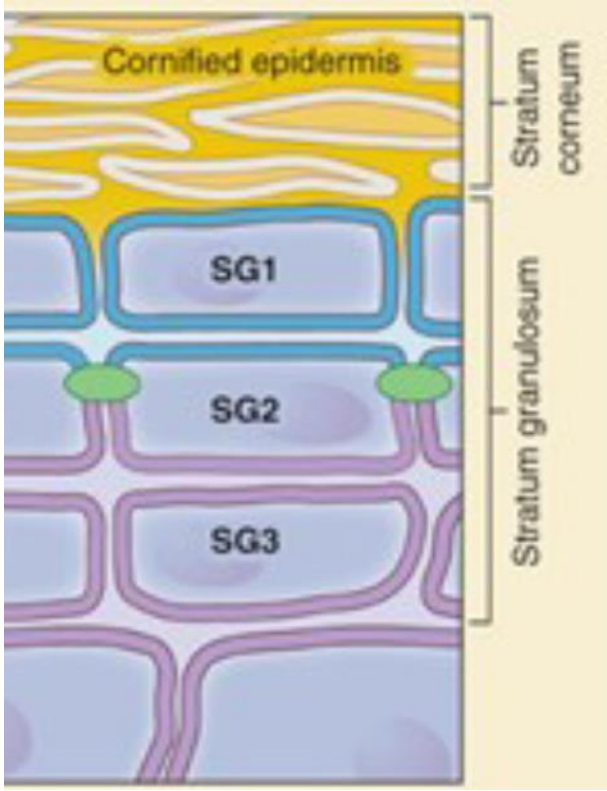


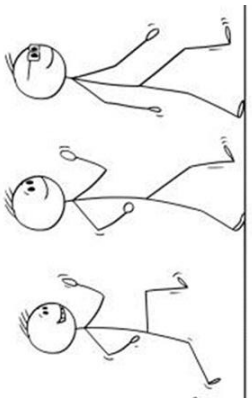
Figure 14-9 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

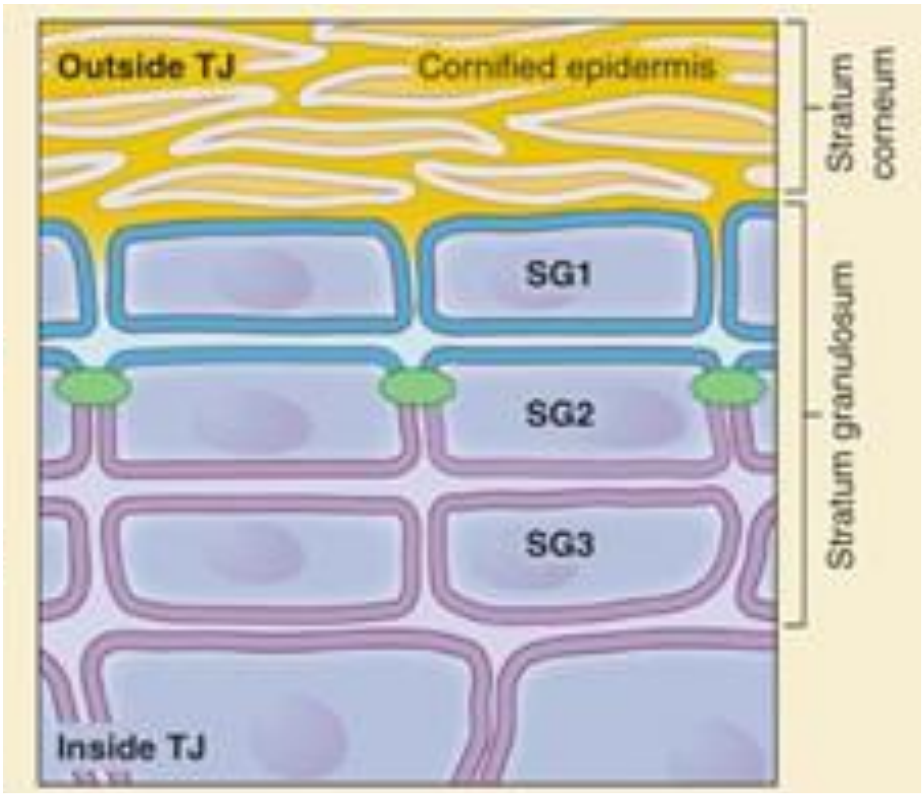
SG2

SG2 creates permeability barrier

Tight junctions seal intercellular spaces

- Holds H_2O inside
- Prevents entry of antigens from outside





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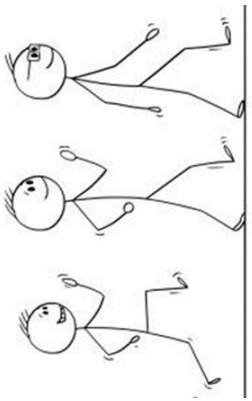
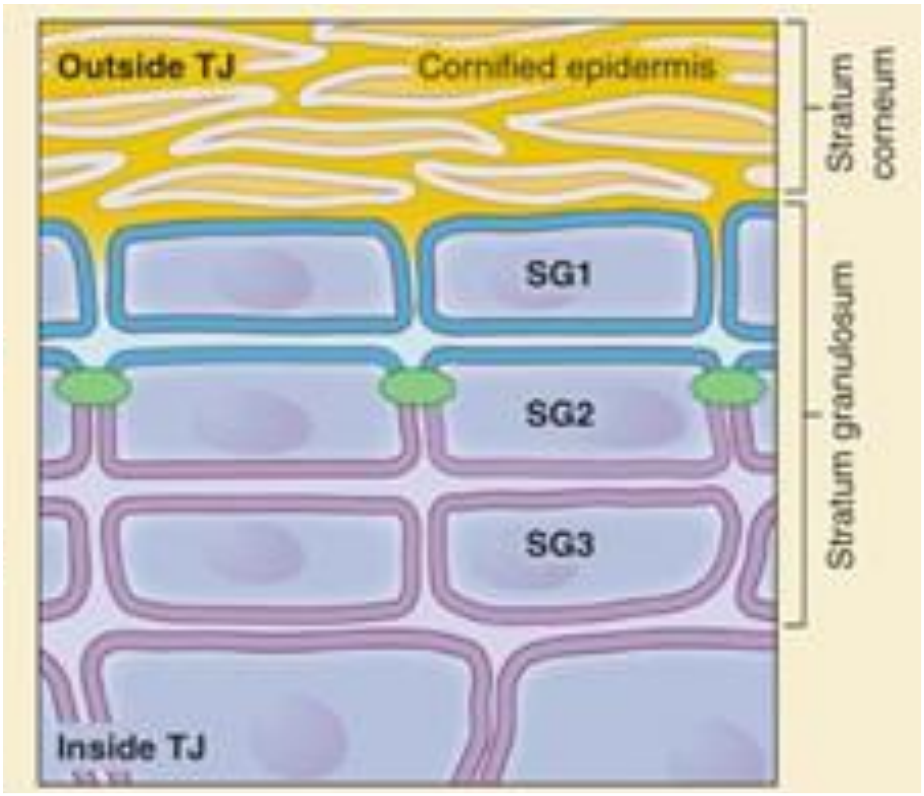
SG1

Keratin intermediate filament assembly

Cornified cell envelope construction

Lamellar body exocytosis and creation of corneocyte lipid envelope and intercellular lipid lamellae





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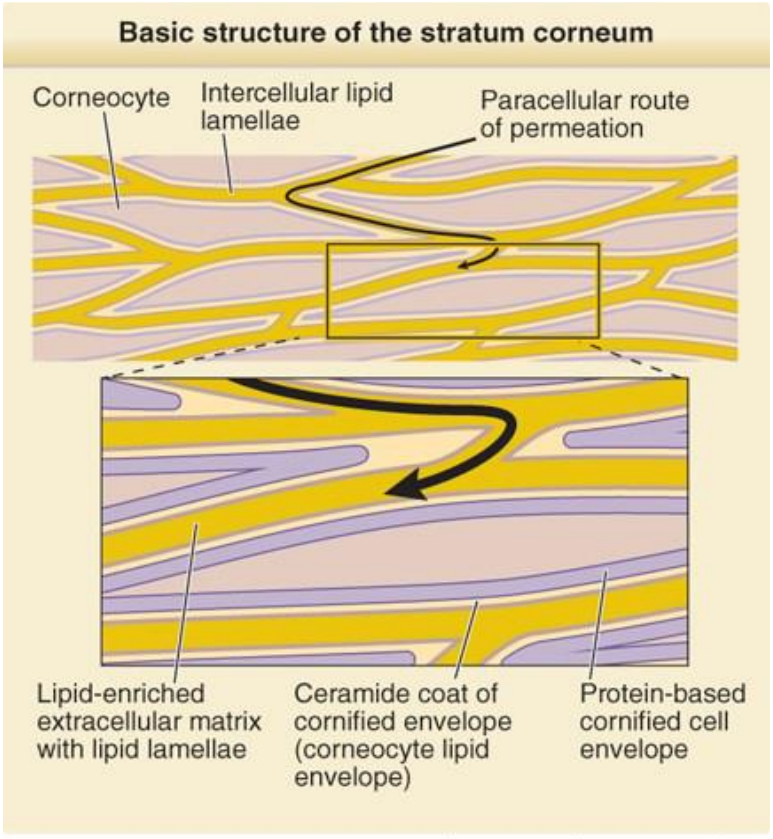
Stratum corneum

Corneocytes in extracellular lipid matrix



Source: S. Kang, M. Amagai, A.L. Bruckner, A.H. Enk, D.J. Margolis, A.J. Mcmichael, J.S. Orringer: Fitzpatrick's Dermatology, Ninth Edition

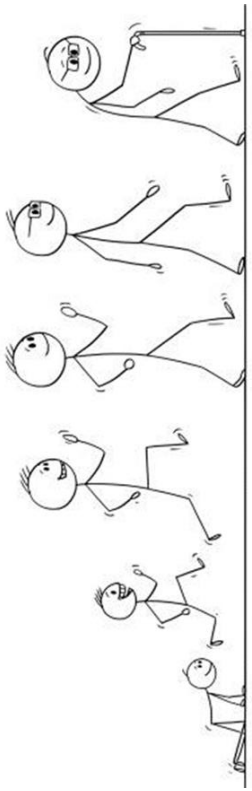
Corneocytes – dead, flattened terminally differentiated keratinocytes

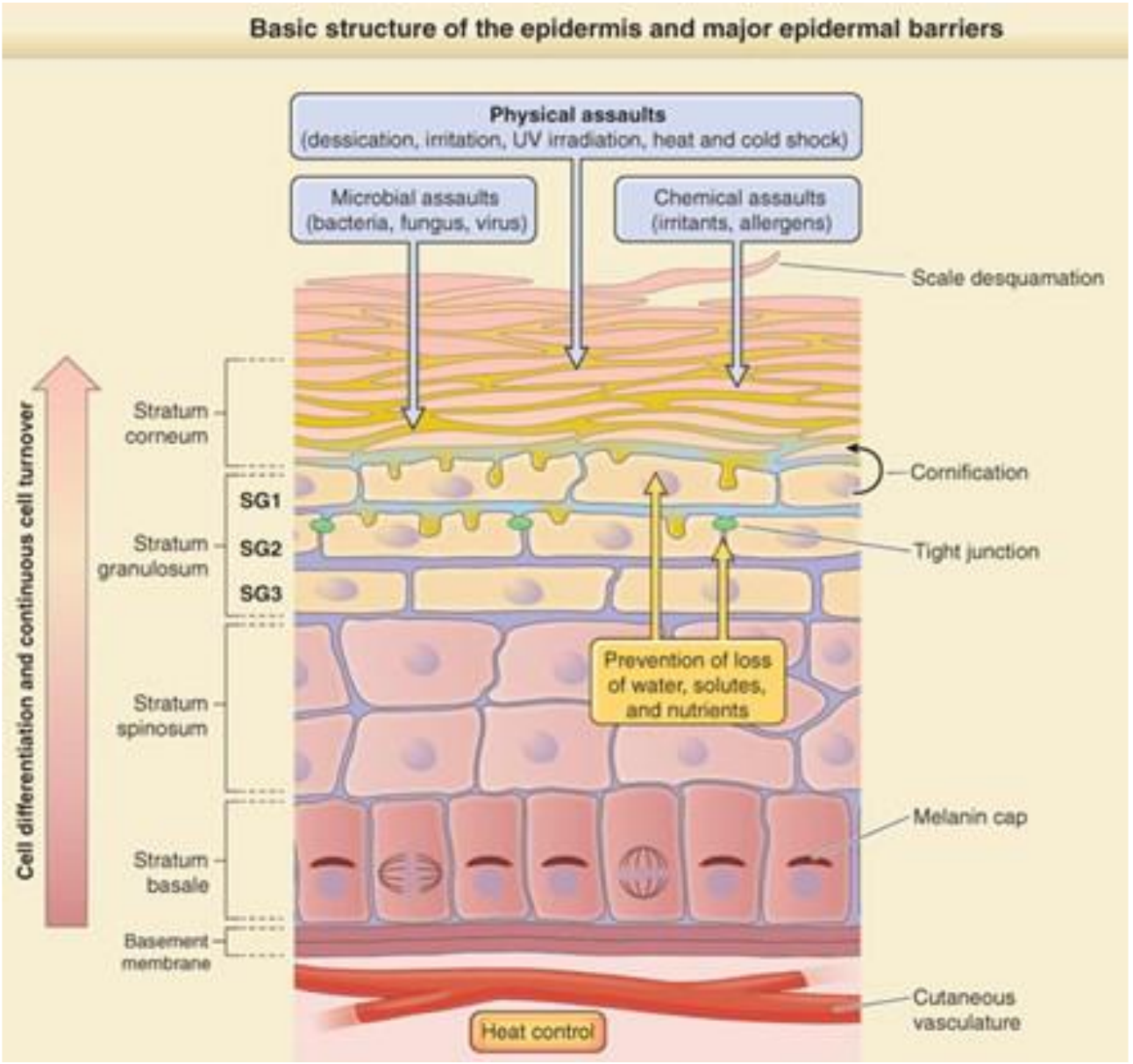
- Densely packed core of keratin and filaggrin
- Coated in durable protein layer: cornified cell envelope (CE)
- Loss of nuclei and organelles
- Corneodesmosomes attach corneocytes

Corneocyte lipid envelope (CLE) attaches corneocytes to lipid lamellae composed of ceramides, free fatty acids, and cholesterol

Figure 14-3 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Corneocyte analogy





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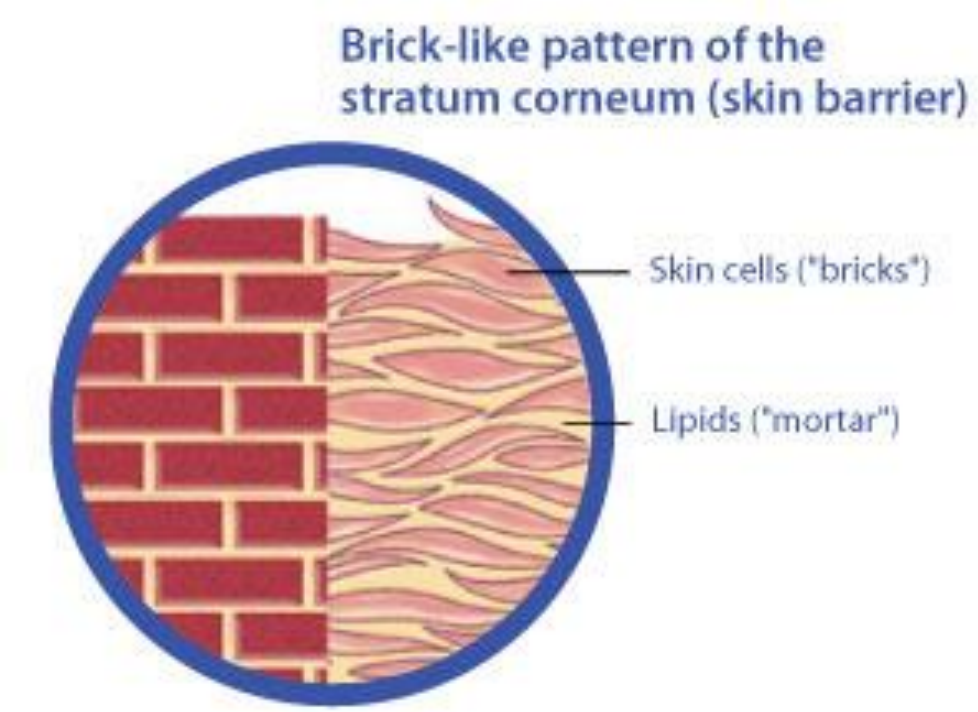
“Bricks and mortar”



www.cerave.com/barrier/WHPSK507barrierV4_14.jpg

Stratum corneum analogy - “m&m’s and caramel”

Cornification - the “bricks”

Keratin intermediate filament (KIF) assembly

Cornified cell envelope (CE) construction

Epithelial cytoskeleton

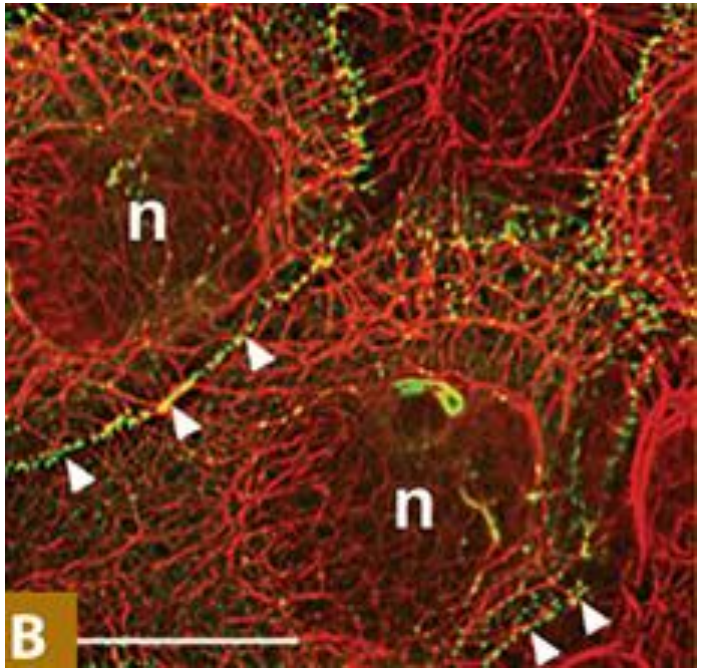
Intracellular transport

- Microfilaments – actin, 7 nm diameter
- Microtubules – α- and β-tubulin, 20 nm diameter

Scaffold - keratin

- Intermediate filaments – 7-12 nm diameter

KIFs span cytoplasm and attach at desmosomes



Cultured human epidermal cells
Keratin = red
Arrowheads = desmosomes

Figure 46-2 from Coulombe PA, Miller SJ, Sun T. Epidermal Growth and Differentiation. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 481.

Keratin proteins

Structure

- Central alpha-helical rod domain
- Amino (N)-terminal head and carboxy (C)-terminal tail that exhibit “glycine loops”

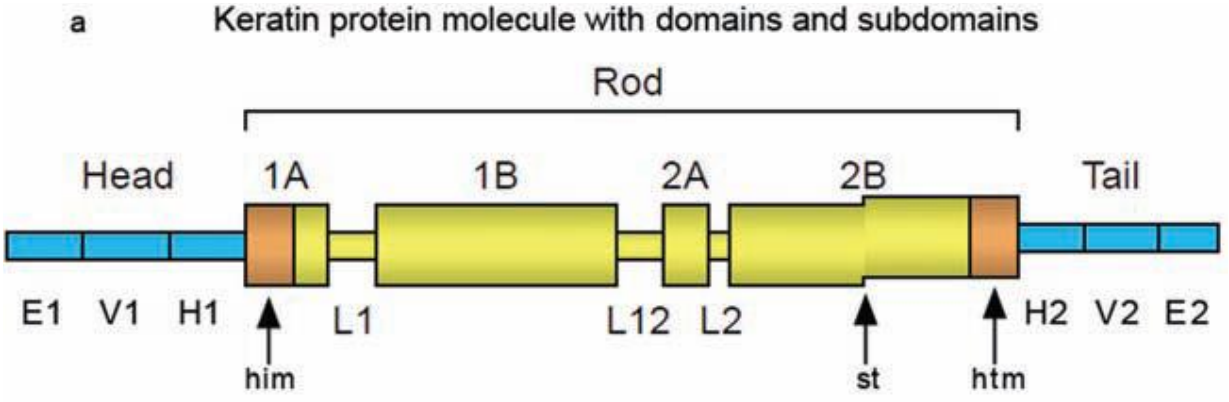


Figure 2a from Bragulla HH, Homberger DG. Structure and functions of keratin proteins in stratified, keratinized and cornified epithelia. *J Anat* 2009; 214:530.

Keratin proteins

Type I
- Acidic
- Smaller than type II
- K9-19 in epidermis

Type II
- Basic to neutral
- Larger than type I
- K1-8 in epidermis

Acidic and basic proteins form pairs

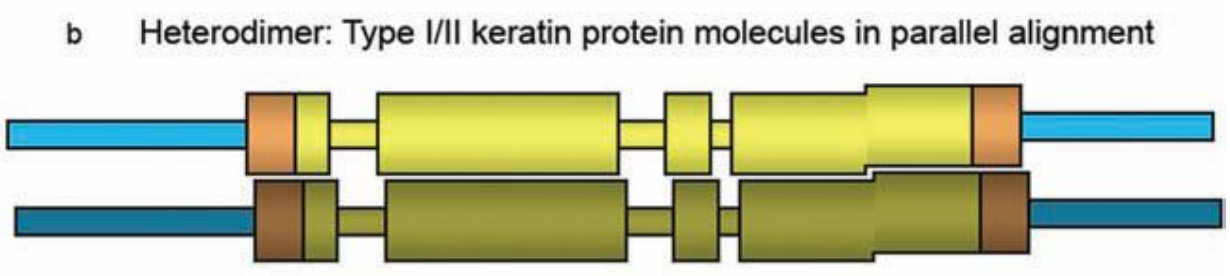


Figure 2b from Bragulla HH, Homberger DG. Structure and functions of keratin proteins in stratified, keratinized and cornified epithelia. *J Anat* 2009; 214:530.

KIF Assembly: Classic Model

Type I and II keratins→

Heterodimers x 2→

- Staggered, antiparallel

Tetramers end to end→

Protofilaments x 2→

Protofibrils x 4→

KIF

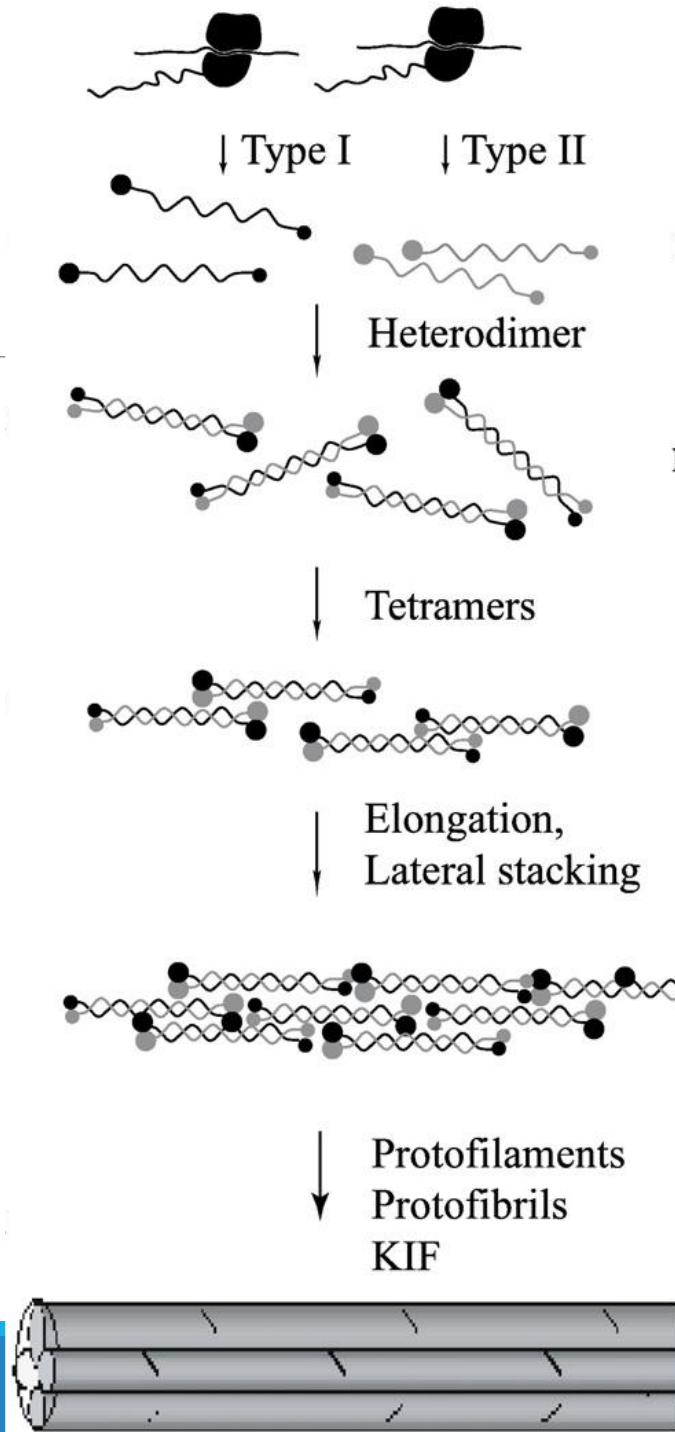


Figure 2 from Uitto J, Richard G, McGrath JA. Diseases of epidermal keratins and their linker proteins. *Exp Cell Res* 2007; 313: 1997.

Keratin Intermediate Filaments

Keratins cross-linked by disulfide bonds

KIFs aggregated and bundled into densely packed parallel formation

Filaggrin plays role in bundling KIFs

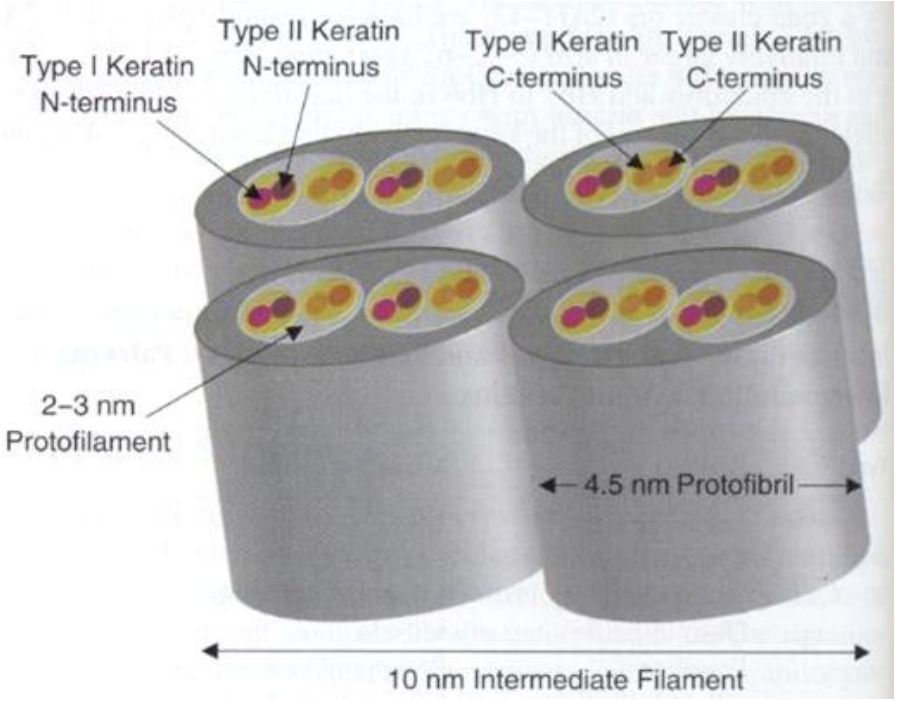


Figure 7-6 from Kimyai-Asadi A, Jih MH, Freedberg IM. Epidermal Cell Kinetics, Epidermal Differentiation, and Keratinization. In: Freedberg IM et al, editors, Fitzpatrick's Dermatology in General Medicine, 6th Ed.

KIF Assembly: Cubic Rod-Packing and Membrane Templating Model

Disputes keratin self-assembly

Proposes membrane template organizes keratin assembly

Explains strength and water-holding capacity of epidermis

Explains findings with cryo-transmission electron microscopy on fully-hydrated epidermis

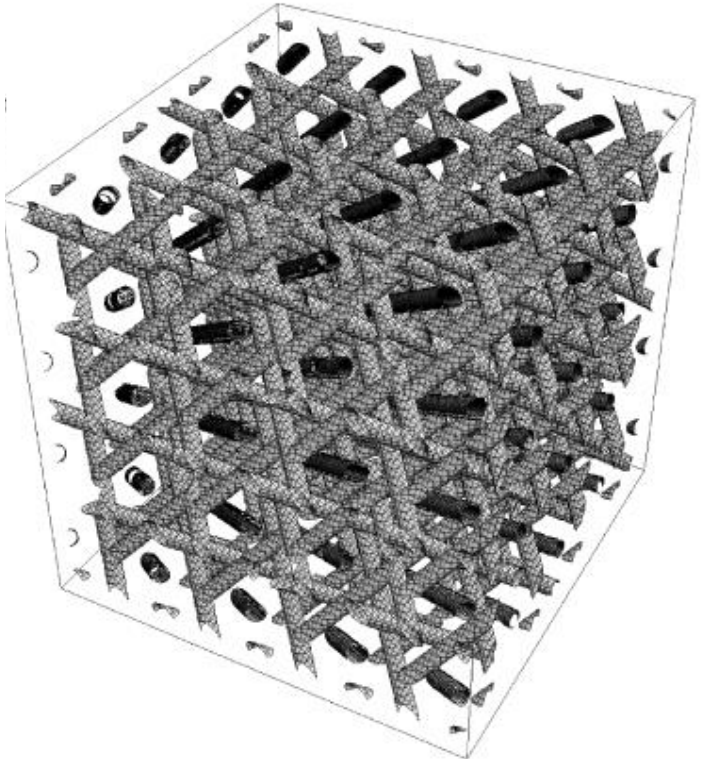


Figure 5B from Norlén L, Al-Amoudi A. Stratum corneum keratin structure, function, and formation: the cubic rod-packing and membrane templating model. *J Invest Dermatol* 2004; 123:721.

Filaggrin (Filament Aggregating Protein)

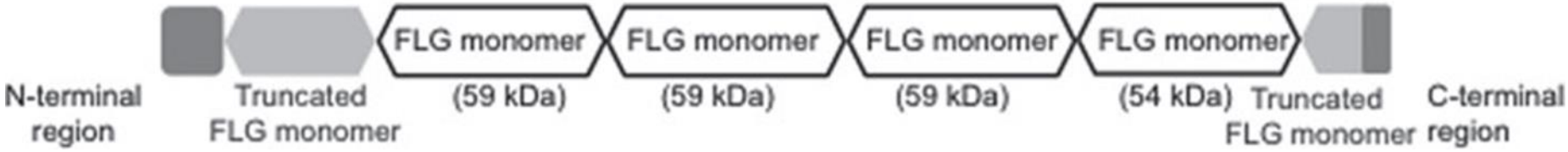
Key to epidermal barrier function

- Role in KIF assembly and flattening of corneocytes
- Becomes natural moisturizing factor (NMF)

In SS - synthesis of profilaggrin starts after K1/K10

- Multiple filaggrin units between N- and C- terminal domains
  - 10-12 in humans, 12-20 in mice, 4 in dogs

Structure of canine profilaggrin:



Kanda S, et al. Characterization of canine filaggrin: gene structure and protein expression in dog skin. *Vet Dermatol* 2013; 24: 27.

Filaggrin in SG

Filaggrin bundles KIFs into tight arrangement

Profilaggrin cleaved into filaggrin units

Component of keratohyalin granules

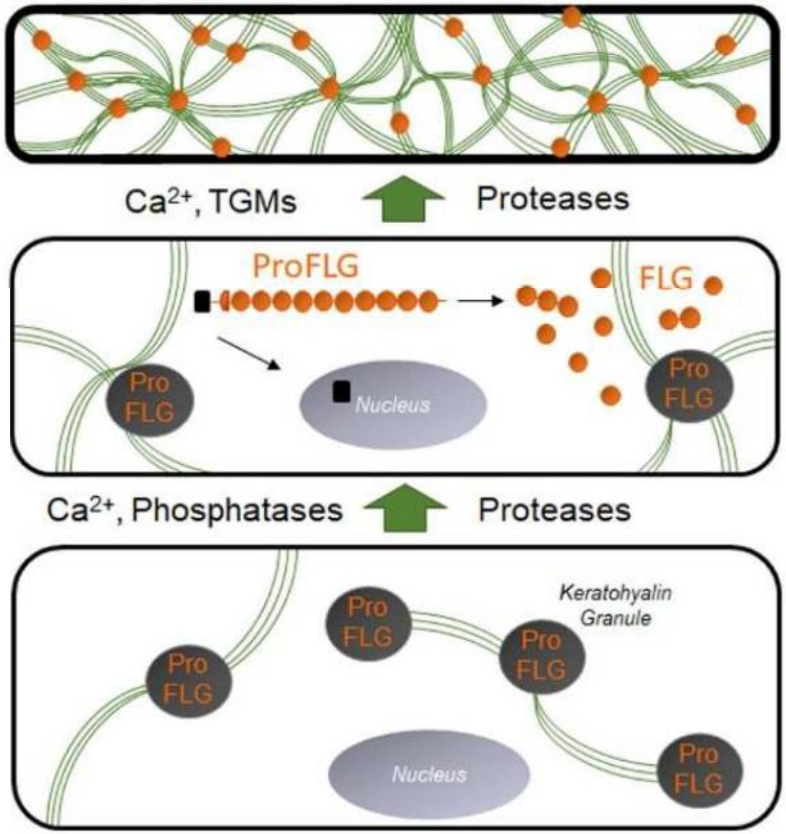


Figure 1 from Moosbrugger-Martinz V, Leprince C, Méchin MC, Simon M, Blunder S, Gruber R, Dubrac S. Revisiting the Roles of Filaggrin in Atopic Dermatitis. Int J Mol Sci. 2022 May 10;23(10):5318

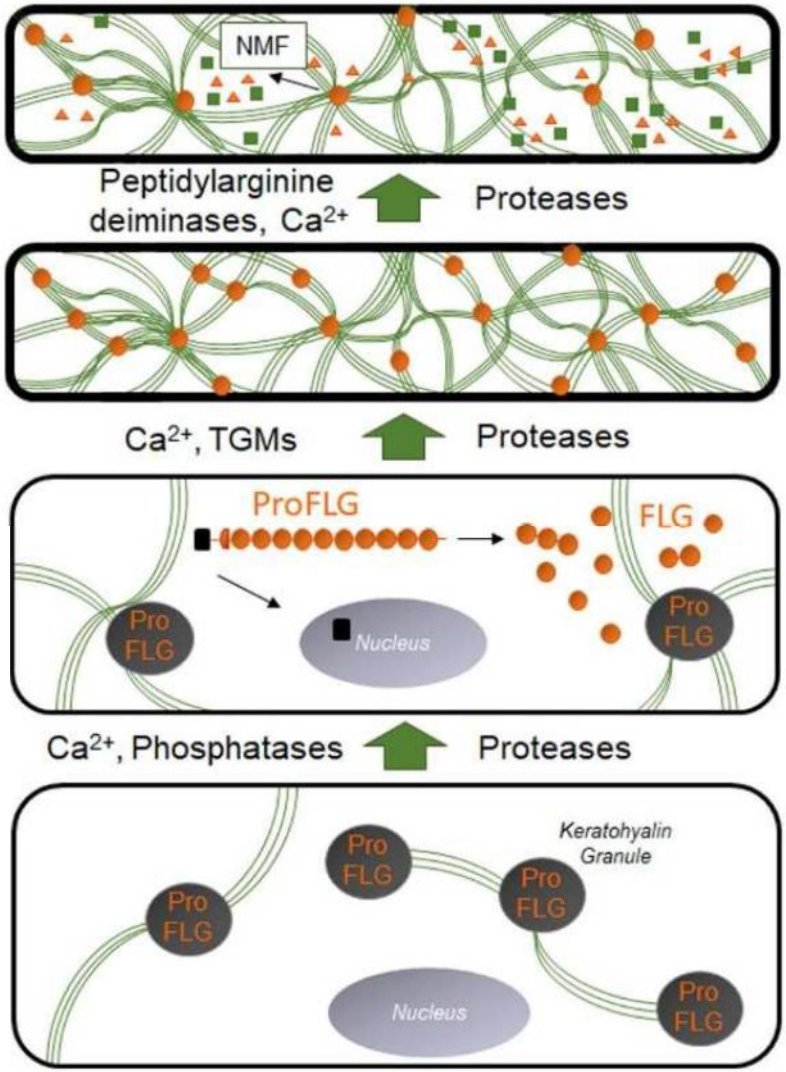
Filaggrin in SC

Enzymatically degraded into NMF

- Pyrrolidone carboxylic acid (PCA)
- Urocanic acid (UCA)
- Amino acids, lactic acid, citrate, urea, sugars

NMF contributes to epidermal hydration and pH

UCA plays role in photoprotection



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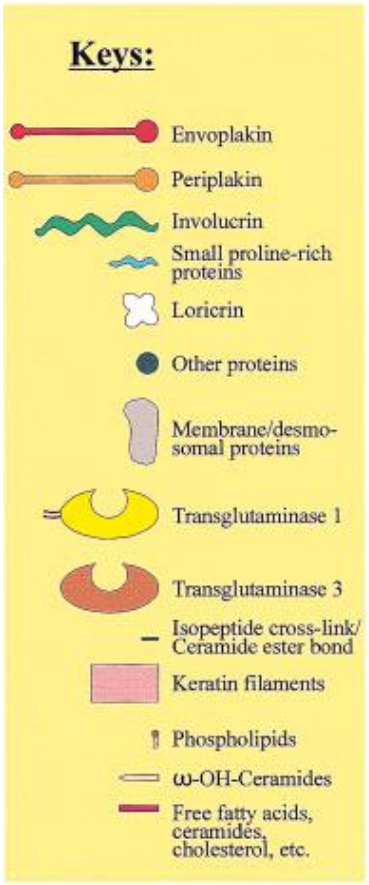
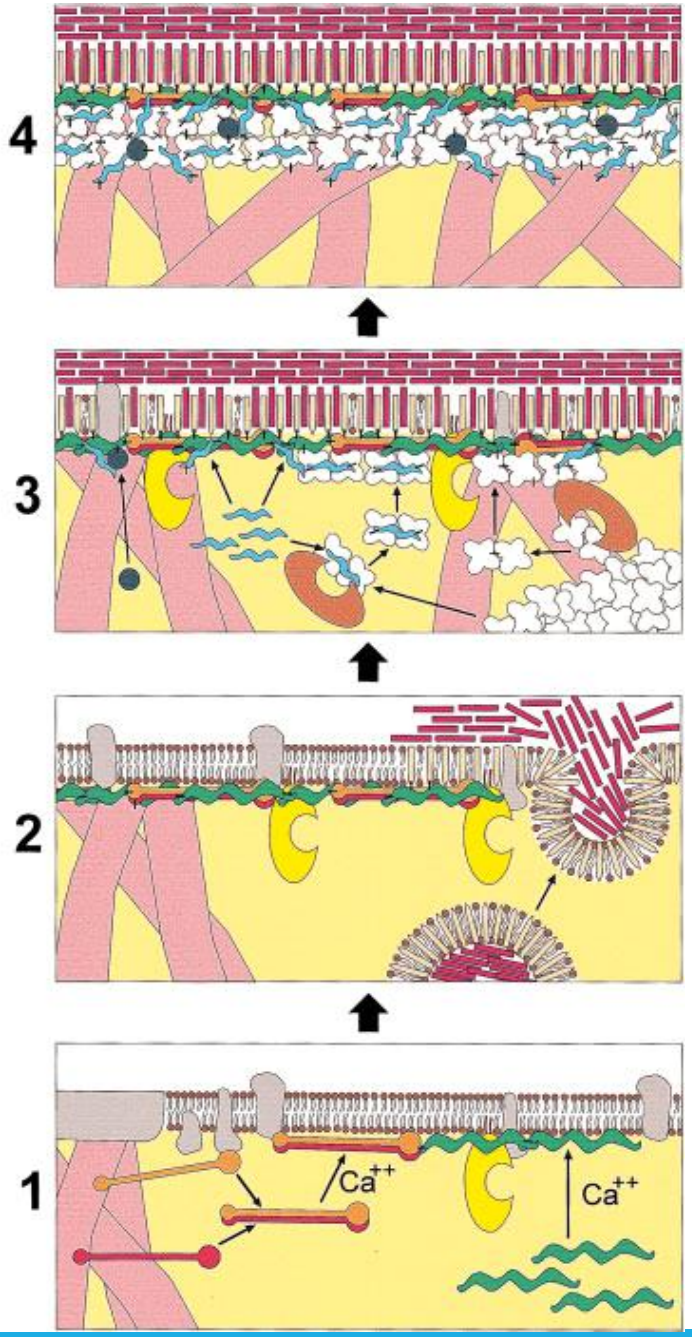
Cornification - the “bricks”

Keratin intermediate filament (KIF) assembly

Cornified cell envelope (CE) construction

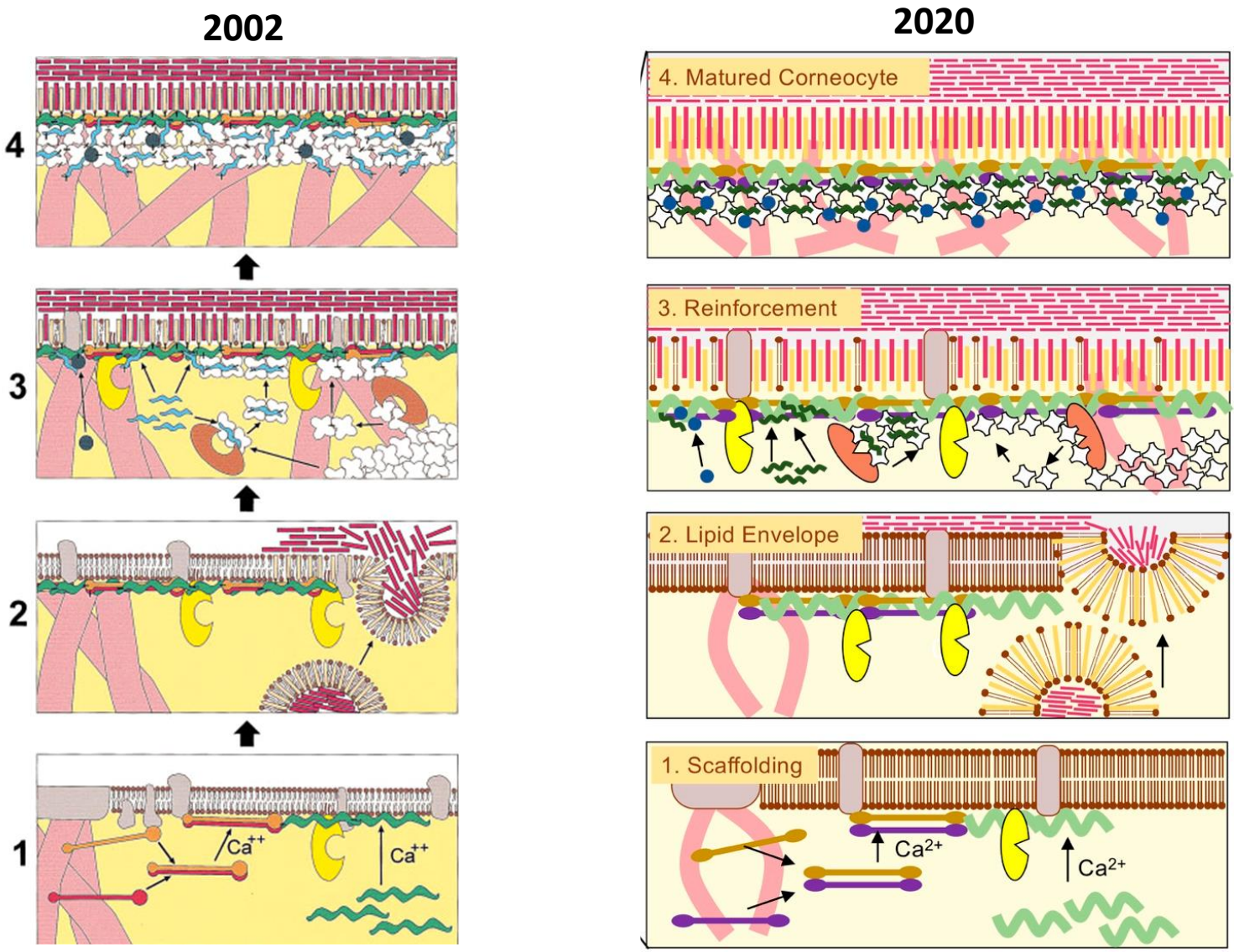




Cornified cell envelope (CE) construction

Figure 4 from Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. *BioEssays* 2002; 24:796.

Cornified cell envelope (CE) construction



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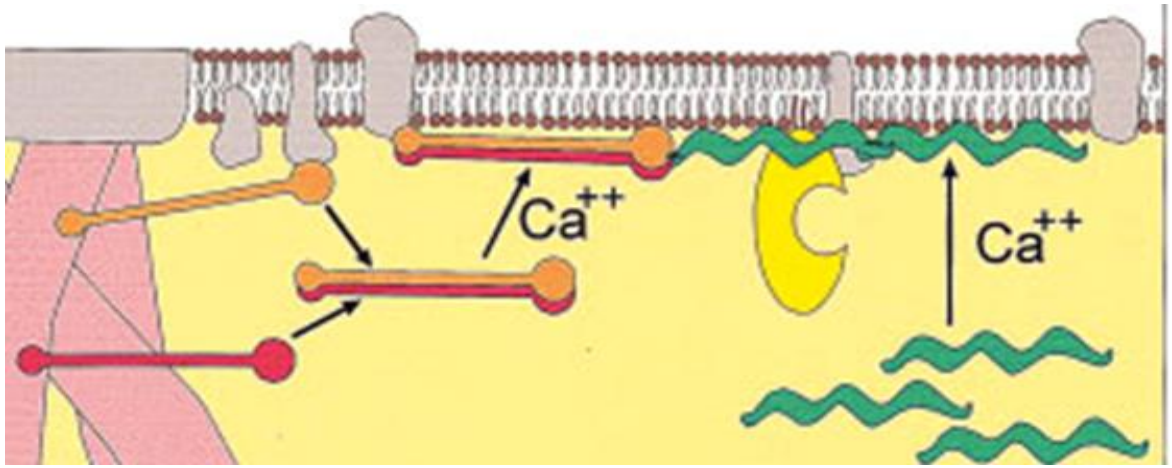
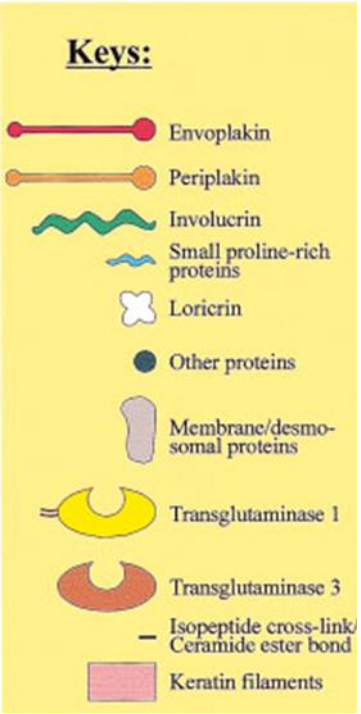
CE construction

Envoplakin, periplakin, and involucrin move to cell membrane

Transglutaminases link involucrin to other proteins to form scaffold





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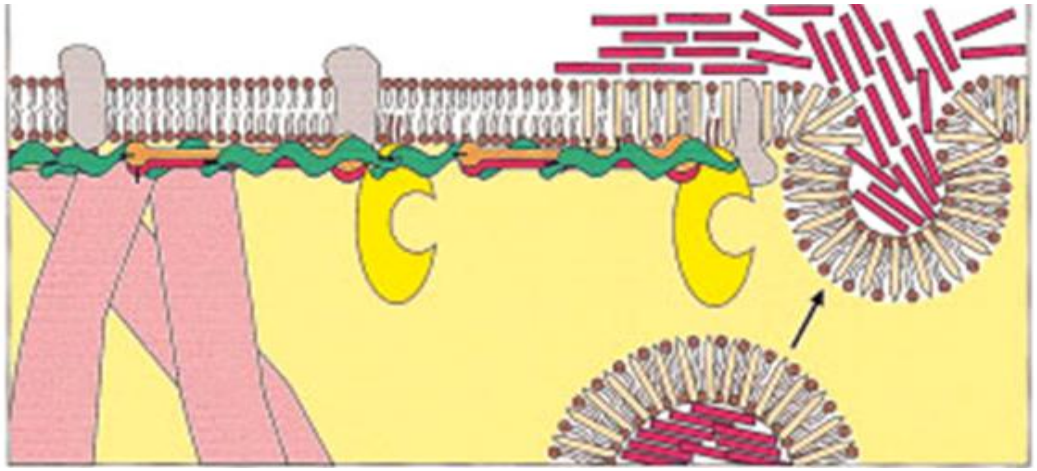
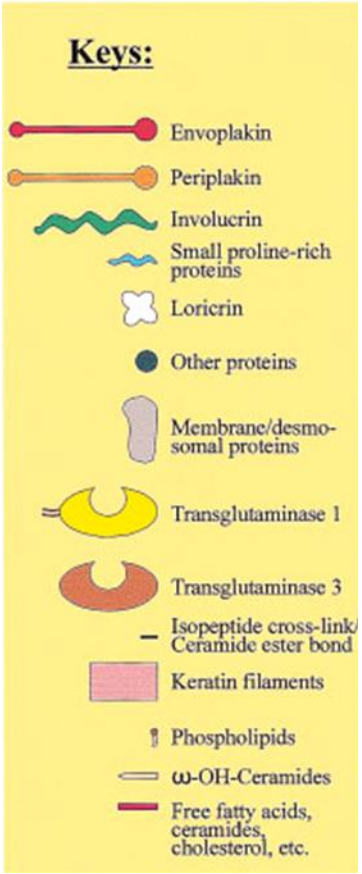
CE and CLE construction

Lamellar bodies secrete lipids and enzymes





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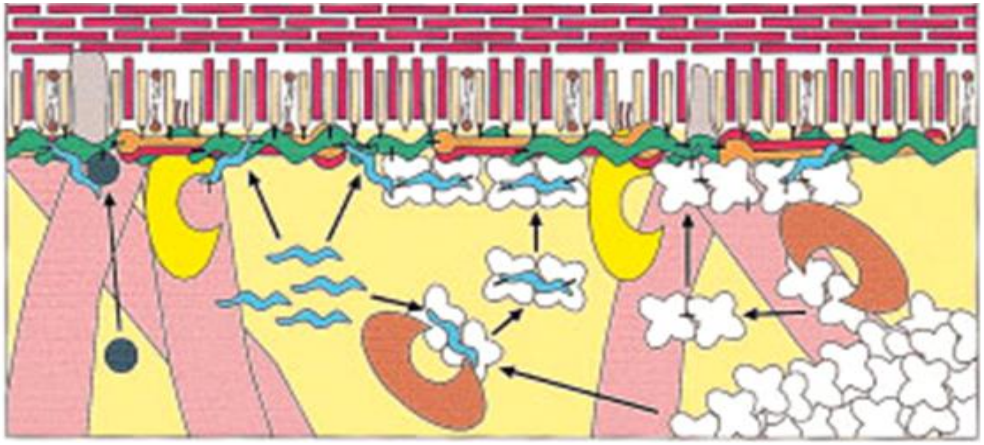
CE construction

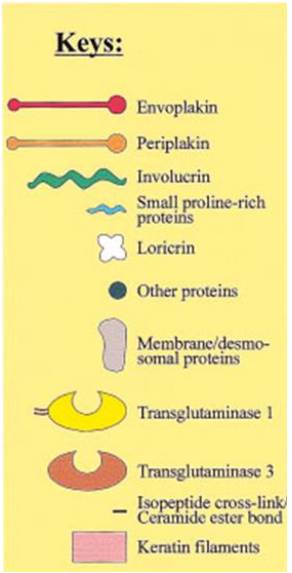


Loricrin

- Cysteine-rich flexible protein with glycine loops
- Major structural component (>70%)

Transglutaminase (TG) enzymes

- Catalyze formation of N(ε)-(γ-glutamyl)-lysine isopeptide bonds
  - Highly resistant to proteolytic enzymes
- Calcium dependent
- TG1 links
  - Loricrin to involucrin scaffold
  - Involucrin to ω-hydroxyceramides to form CLE
- TG3 links loricrin to other cytoplasmic proteins



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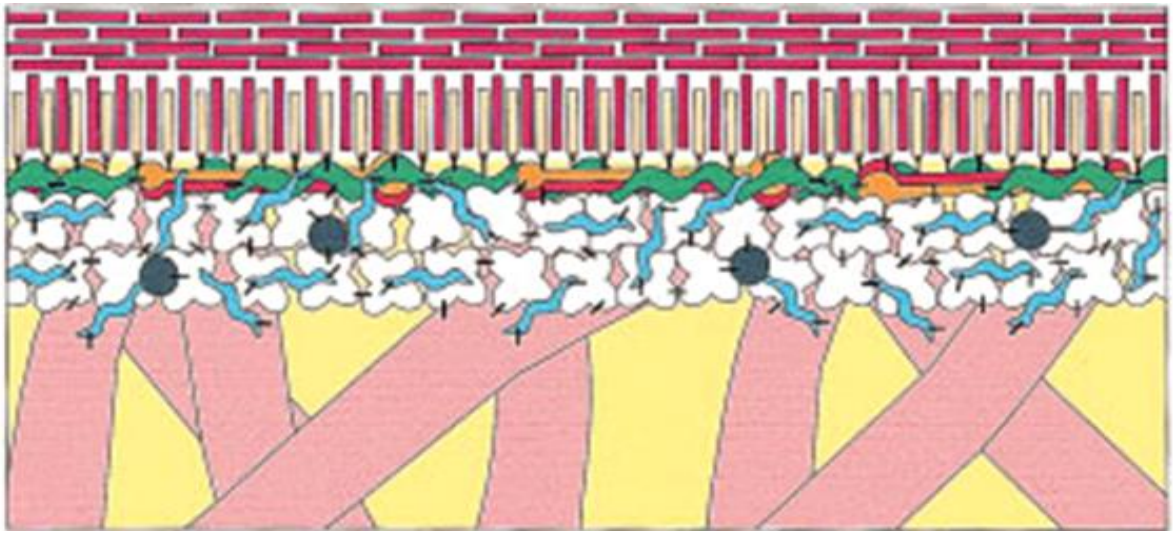
CE construction

CE replaces cell membrane

- Loricrin inside, involucrin outside

KIFs linked to CE



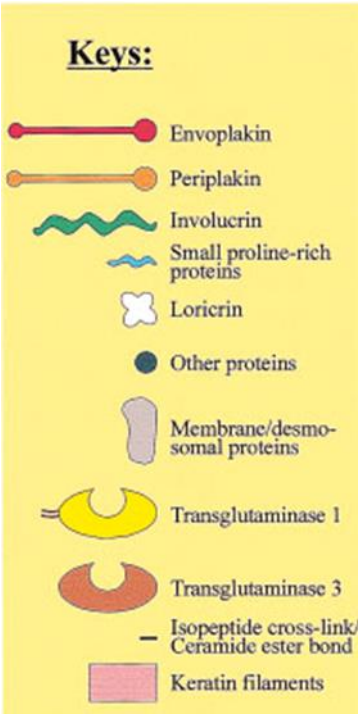


Figure 4 from Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. *BioEssays* 2002; 24:796.

Cornification - the “bricks”

Inside to out: keratin→ loricrin→ involucrin

Cornification - the “mortar”

Composed of ceramides, free fatty acids, and cholesterol

Extracellular lipid matrix

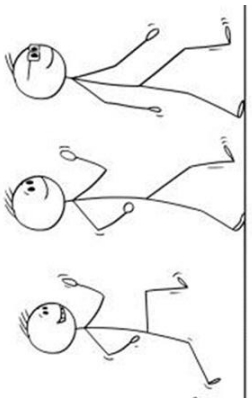
- Corneocyte lipid envelope (CLE)
- Intercellular lipid lamellae

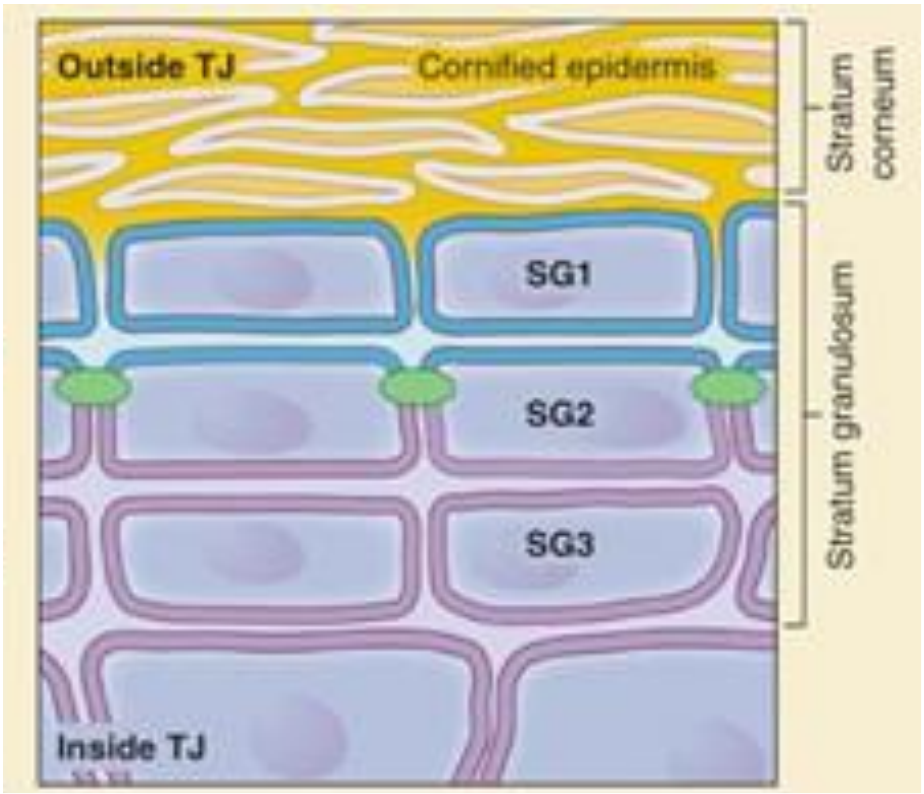
SG1

Keratin intermediate filament assembly

Cornified cell envelope construction

Lamellar body exocytosis and creation of corneocyte lipid envelope and intercellular lipid lamellae





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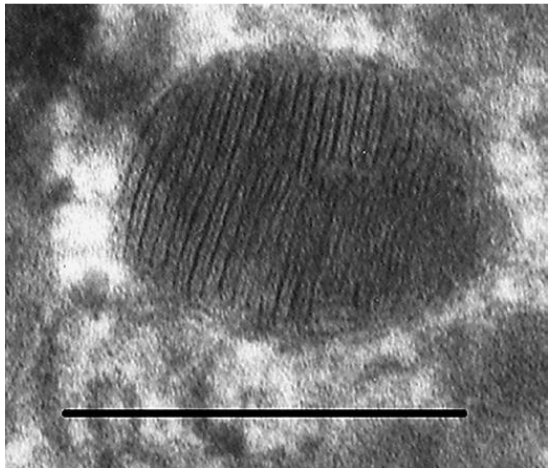
Lipid synthesis is complex

Keratinocytes synthesize lipid precursors and enzymes

Lamellar bodies deliver lipid precursors and enzymes to SG1 and stratum corneum interface



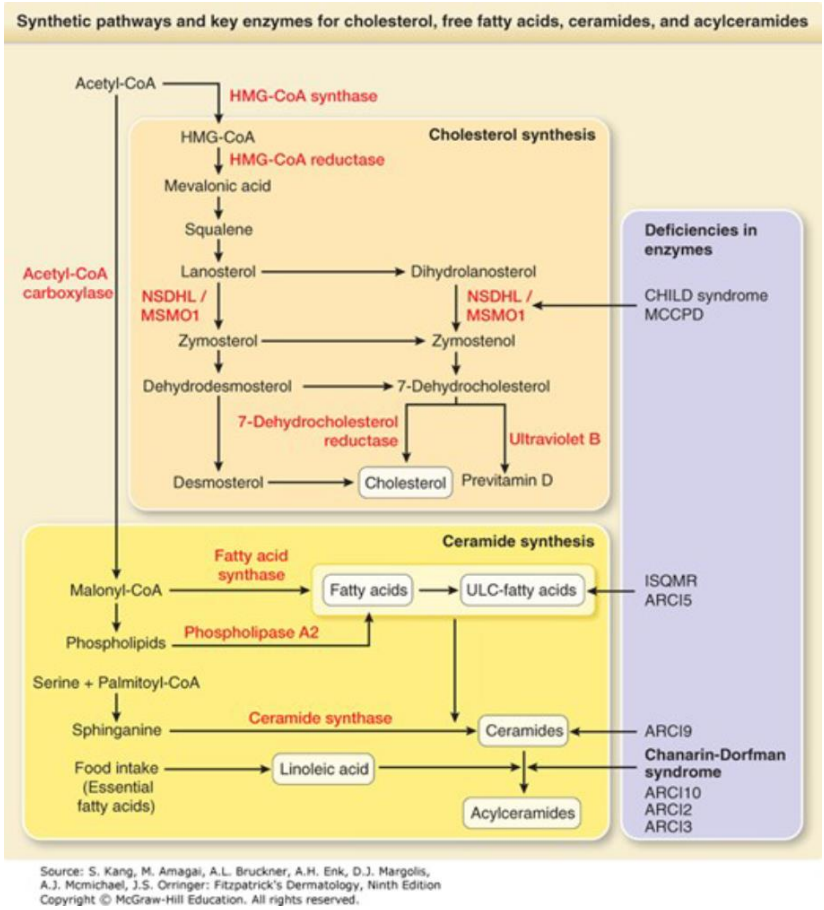
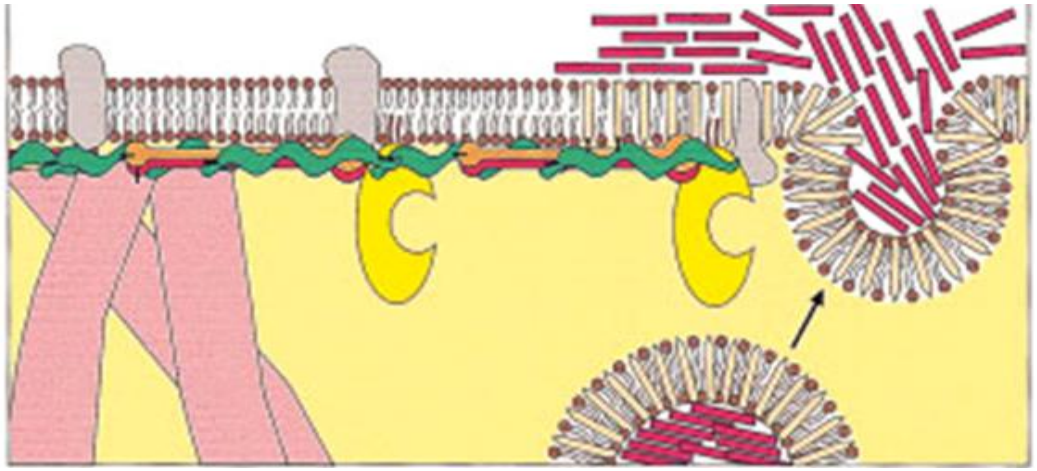


Fig 1 from Wertz P. Epidermal Lamellar Granules. *Skin Pharmacol Physiol* 2018;31:263. and Fig 14-7 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

CE and CLE construction

Lamellar bodies secrete lipids and enzymes



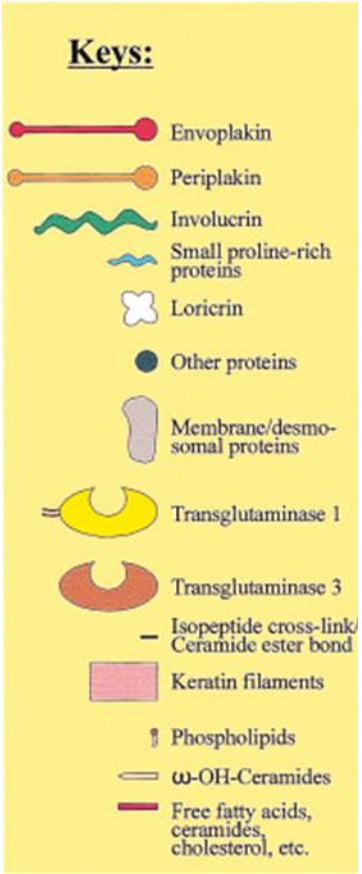
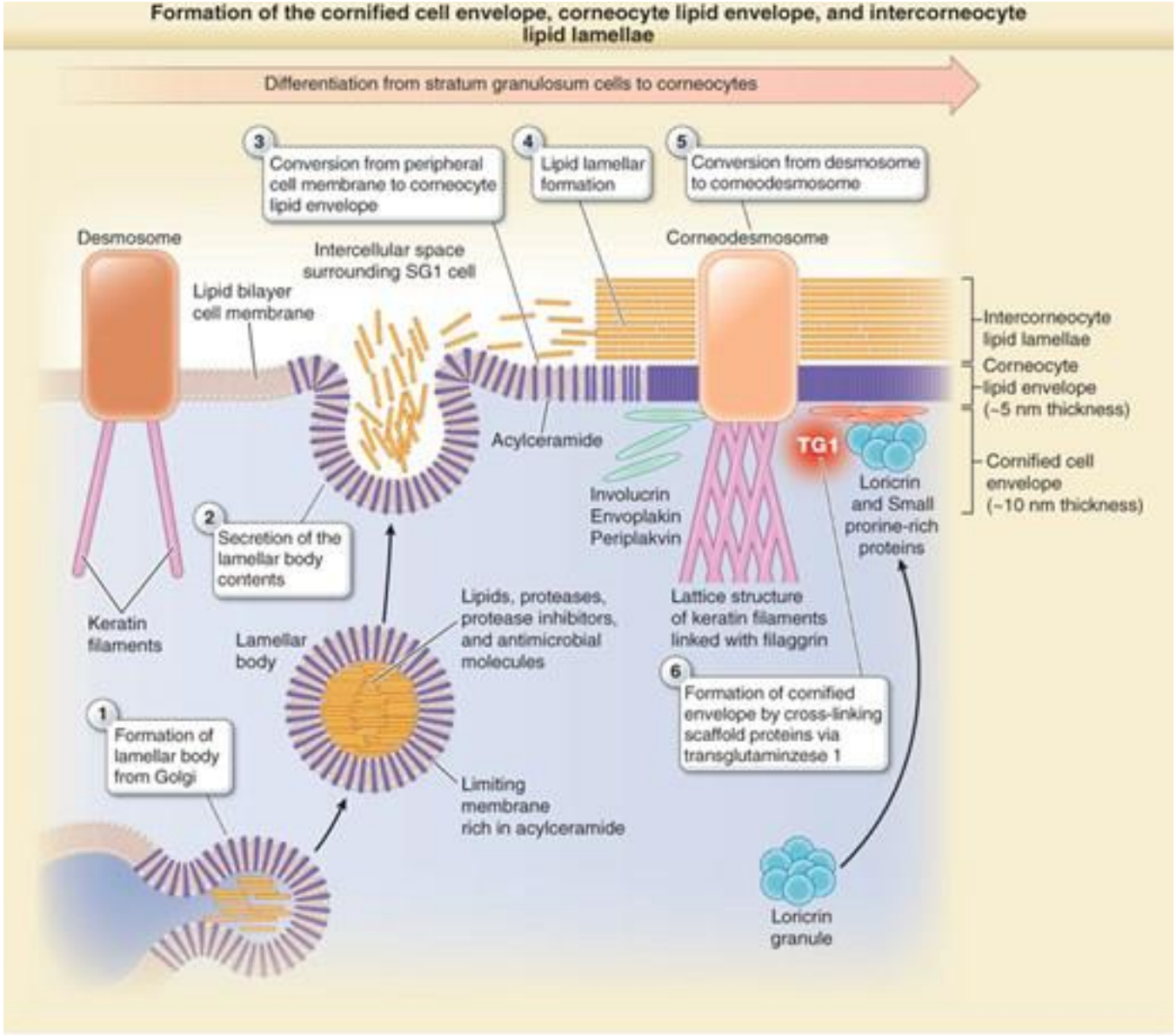


Figure 4 from Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. *BioEssays* 2002; 24:796.



Lamellar body contents

Lipid precursors

Enzymes

- Lipid hydrolases
- Steroid sulfatase
- Kallikreins
- Cathepsins

Corneodesmosin

Antimicrobial peptides

Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Extracellular lipid matrix

Corneocyte lipid envelope (CLE)

- ω-hydroxyceramides

Intercellular lipid lamellae

- Ceramides, free fatty acids, and cholesterol

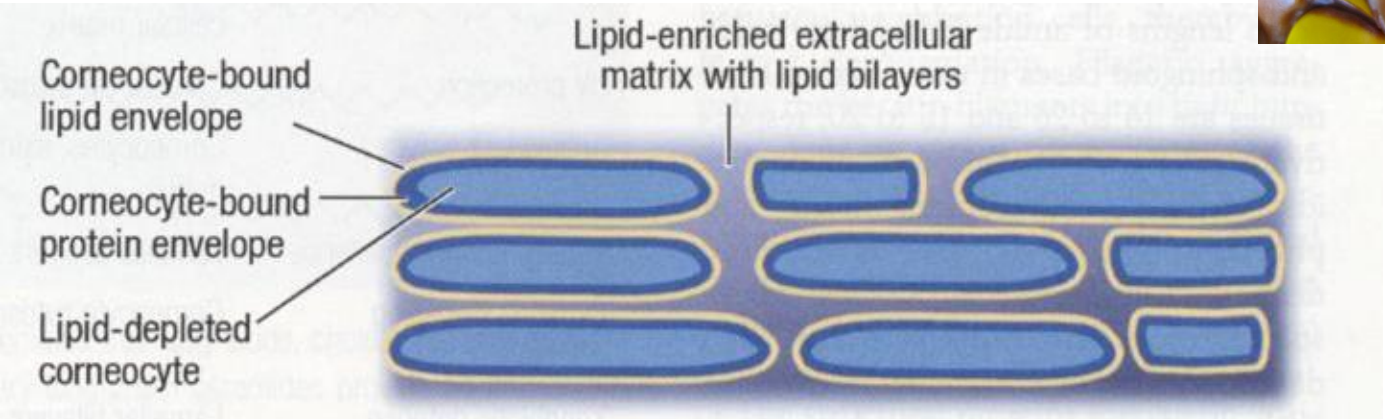
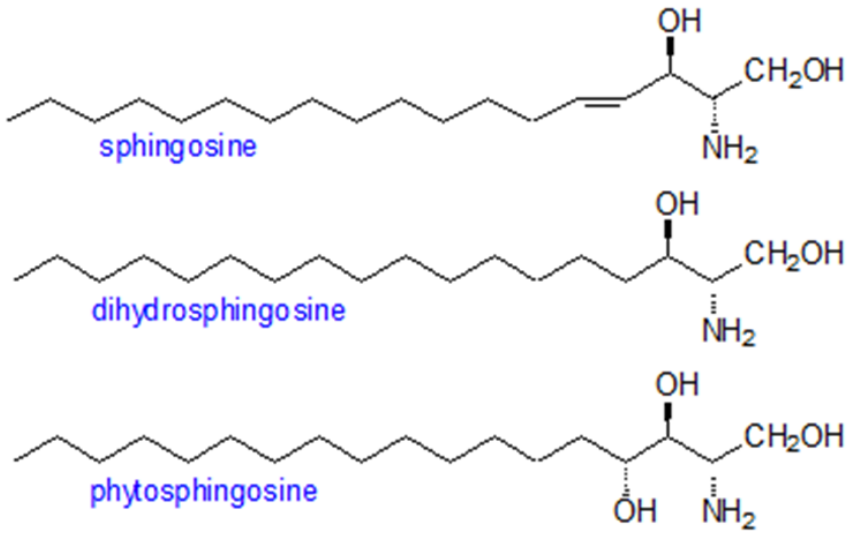


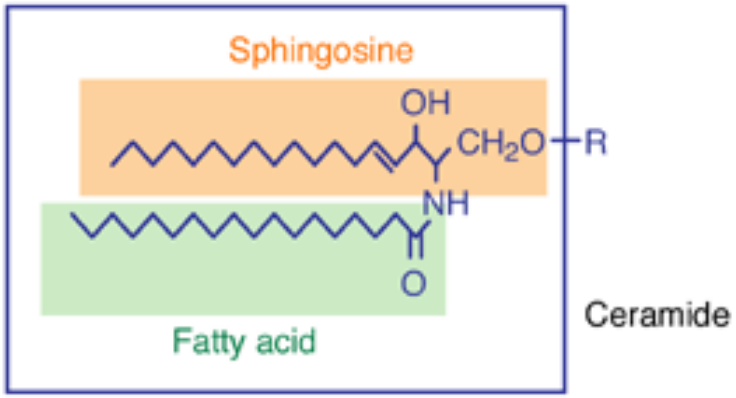
Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

What is a ceramide?

Type of sphingolipid: amide-linked fatty acid attached to sphingoid base

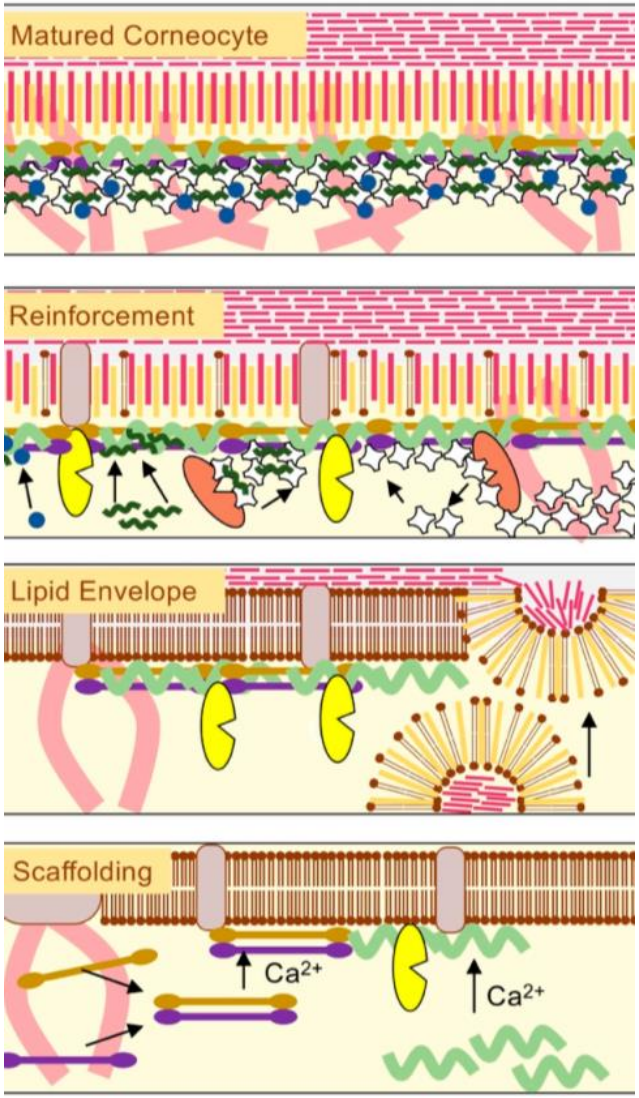
Sphingoid base: long-chain amino alcohol





http://lipidlibrary.aocs.org/lipids/lcb/index.htm

http://www.meritnation.com/ask-answer/question/what-is-sphingolipids/biomolecules/2879804



Corneocyte lipid envelope (CLE)

Layer of ω-hydroxyceramides bonded to CE

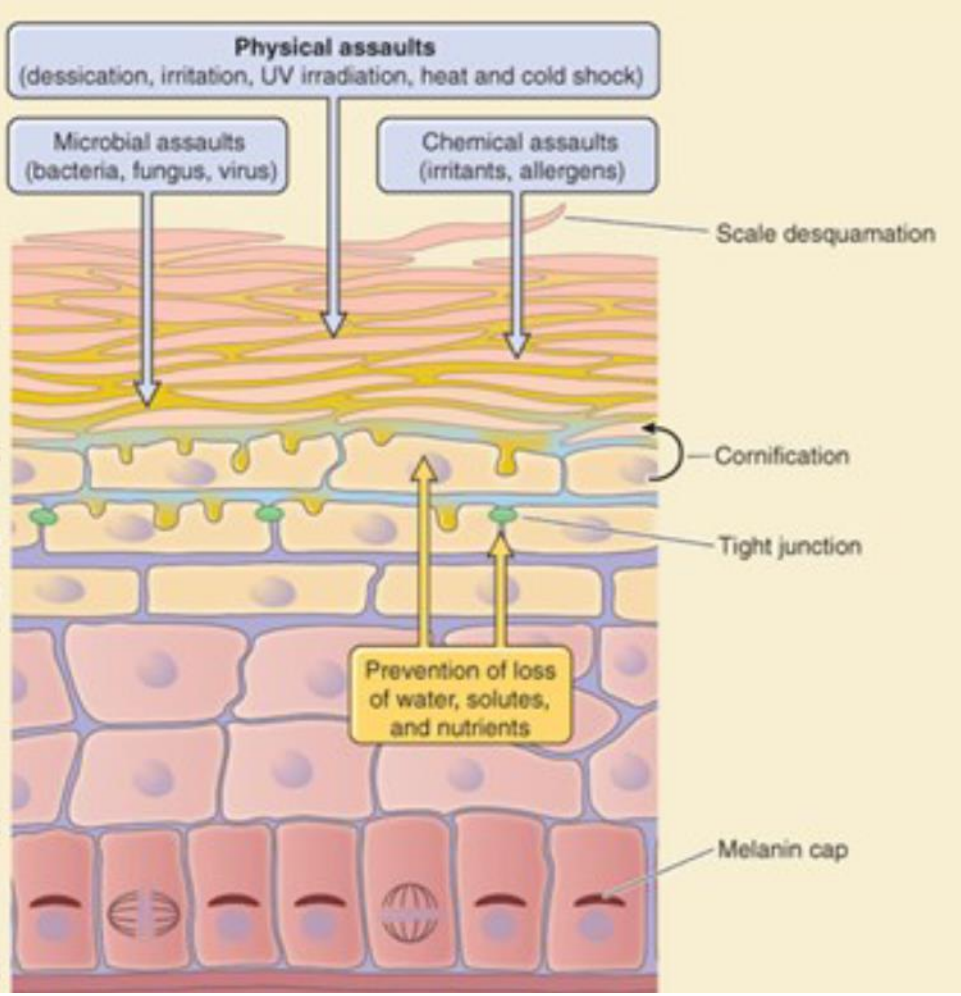
- Unique to stratum corneum
- Ultra long chain (ULC) fatty acids
- Linoleic acid (ω-6 EFA) is component
- Bonded to involucrin in CE

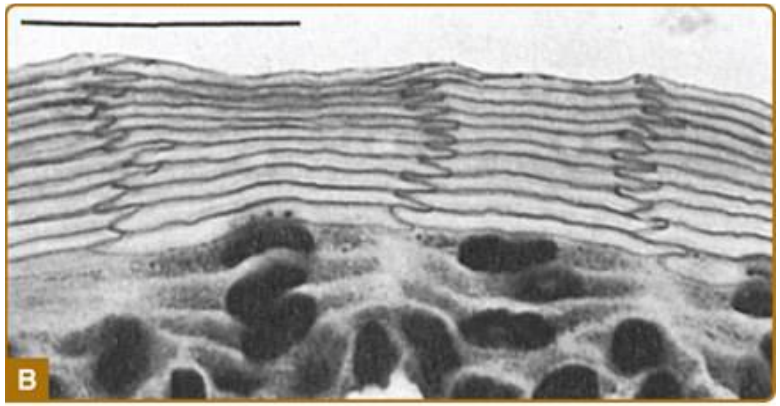
Forms scaffold for intercellular lipid layers of stratum corneum

Key structure for skin barrier function and ichthyosis pathogenesis

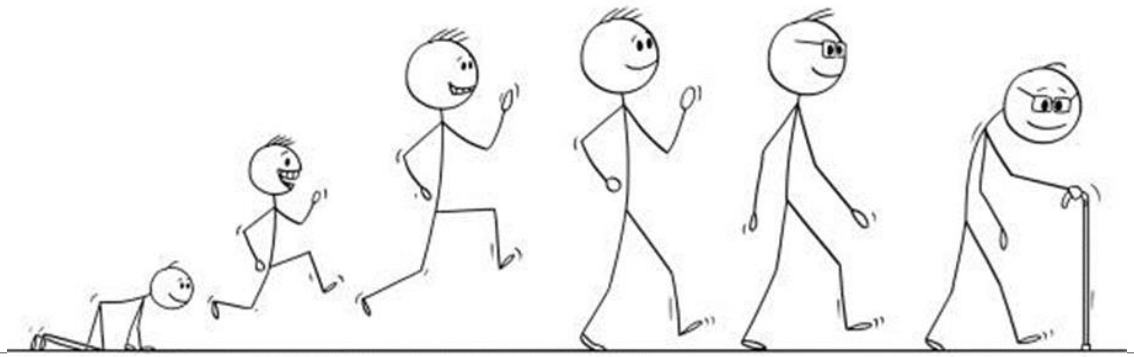
Ishitsuka Y, Roop DR. Loricrin: Past, Present, and Future. Int J Mol Sci. 2020 Mar 25;21(7):2271.

Stratum corneum





Figures 14-2 and 14-5 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.



BIOGRAPHY OF A KERATINOCYTE

Overview of epidermal layers

Cornification

Keratinocyte adhesion

Desquamation

Epidermal barrier

Keratinocyte adhesion

Desmosomes

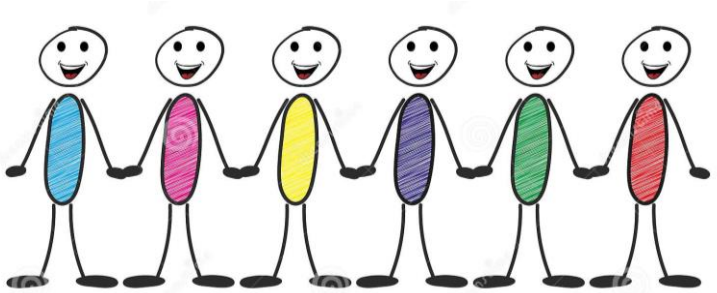
- Link neighboring keratinocytes

Corneodesmosomes

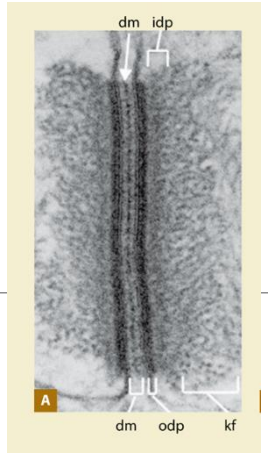
- Link neighboring corneocytes

Tight junctions

- Permeability barrier
- Found only in SG2



Desmosome structure



Desmoglea – extracellular portion between adhered keratinocytes

- Cadherins: desmogleins, desmocollins

Within keratinocyte cell membrane

- Outer dense plaque (plakoglobin)
- Inner dense plaque (desmoplakin) connects to KIFs

Provides structural strength

Constantly rearranging

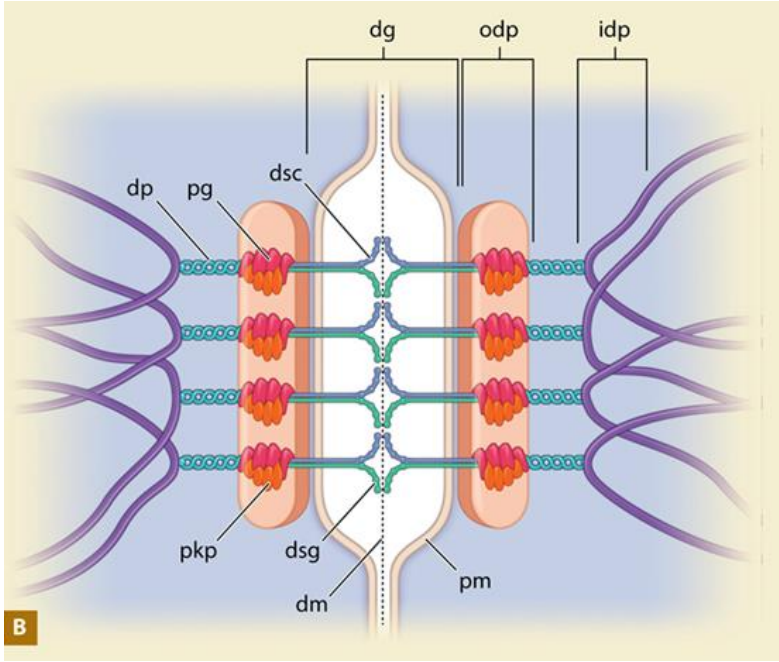


Figure 53-2 from Bruckner-Tuderman L, Payne AS. Epidermal and Epidermal-Dermal Adhesion. In: Goldsmith LA et al, editors, Fitzpatrick's Dermatology in General Medicine, 8th Ed. 2012, p. 570.

Corneodesmosome structure

Corneodesmosin

- Serine and glycine rich protein
  - Forms glycine loops
- Secreted from lamellar bodies
- Attaches to desmoglea
- Key to stratum corneum cohesion

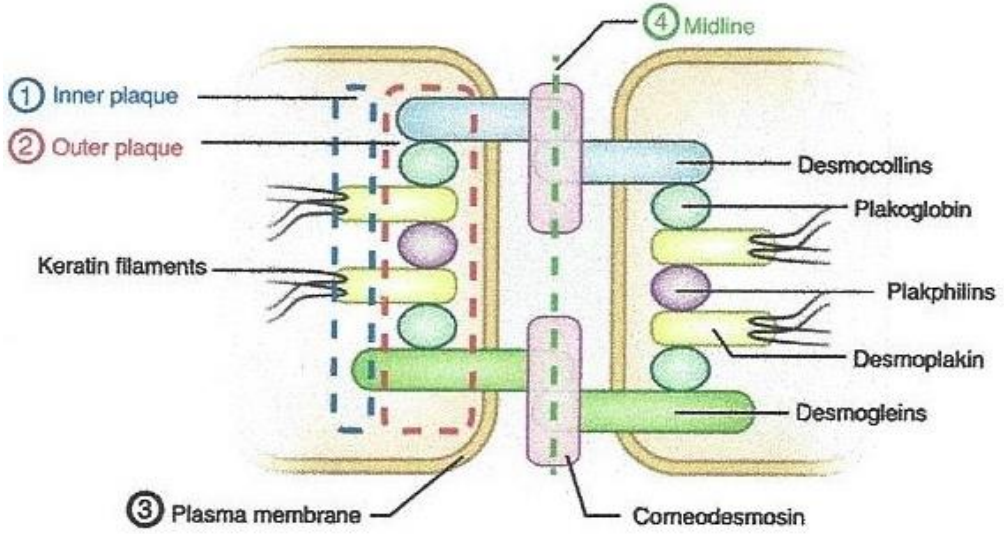


Figure 1 from Ishida-Yamamoto A, Igawa S, Kishibe M, Honma M. Clinical and molecular implications of structural changes to desmosomes and corneodesmosomes. Journal of Dermatology 2018; 45: 385-389.

Keratinocyte adhesion

Desmosomes

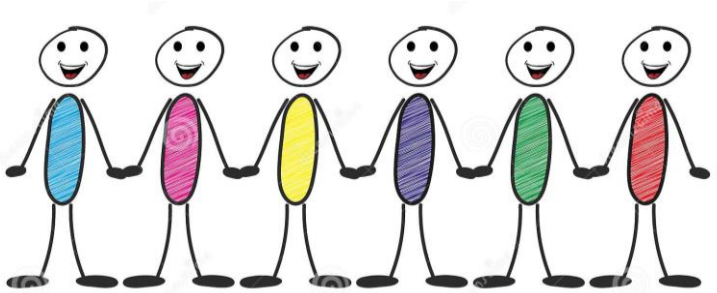
- Link neighboring keratinocytes

Corneodesmosomes

- Link neighboring corneocytes

Tight junctions

- Permeability barrier
- Found only in SG2



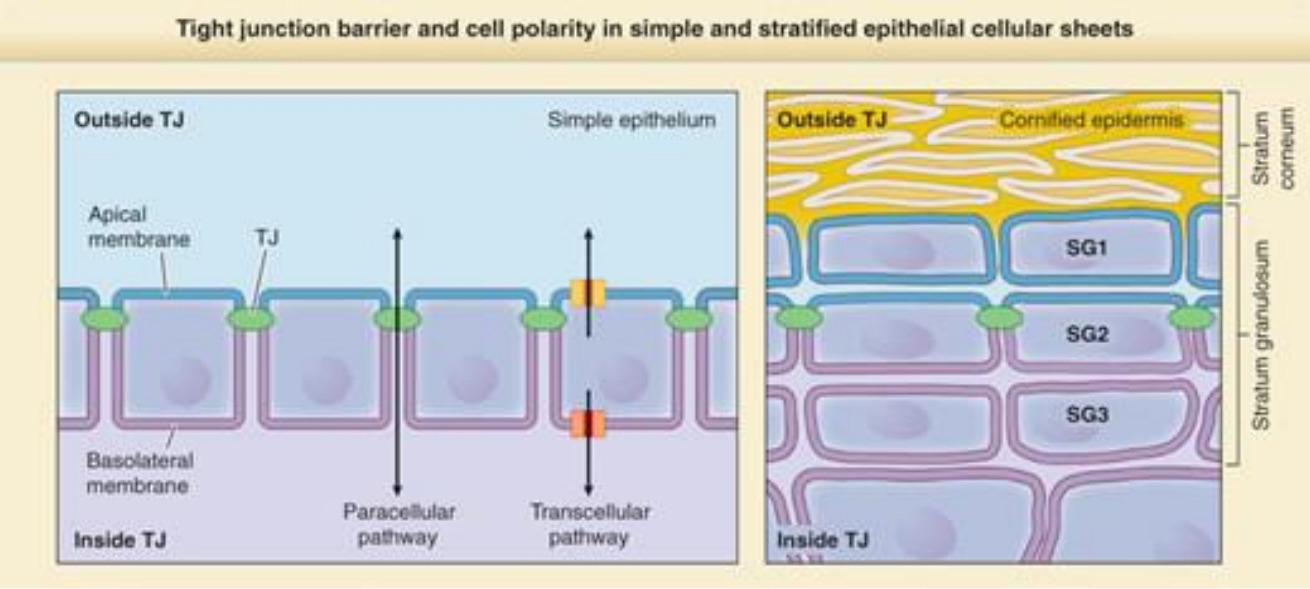
Function of tight junctions

Very close intercellular contacts

- Seal intercellular space

Control paracellular movement of molecules

- Semipermeable size and ion-specific barrier

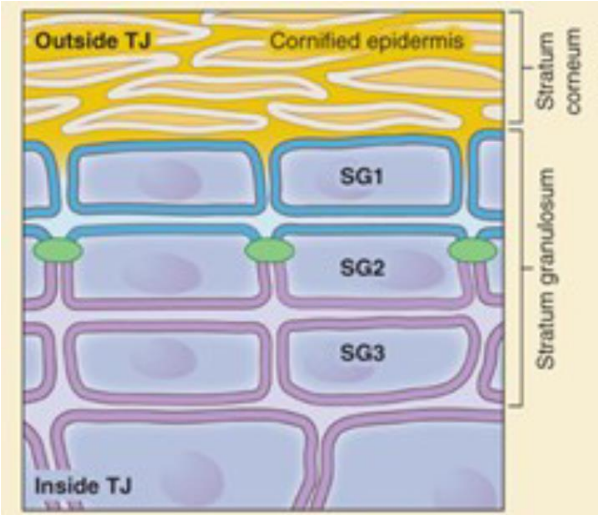


Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Function of tight junctions

Perform “fence function”

- Restrict molecule diffusion within cell membrane
- Demarcate apical and basolateral regions



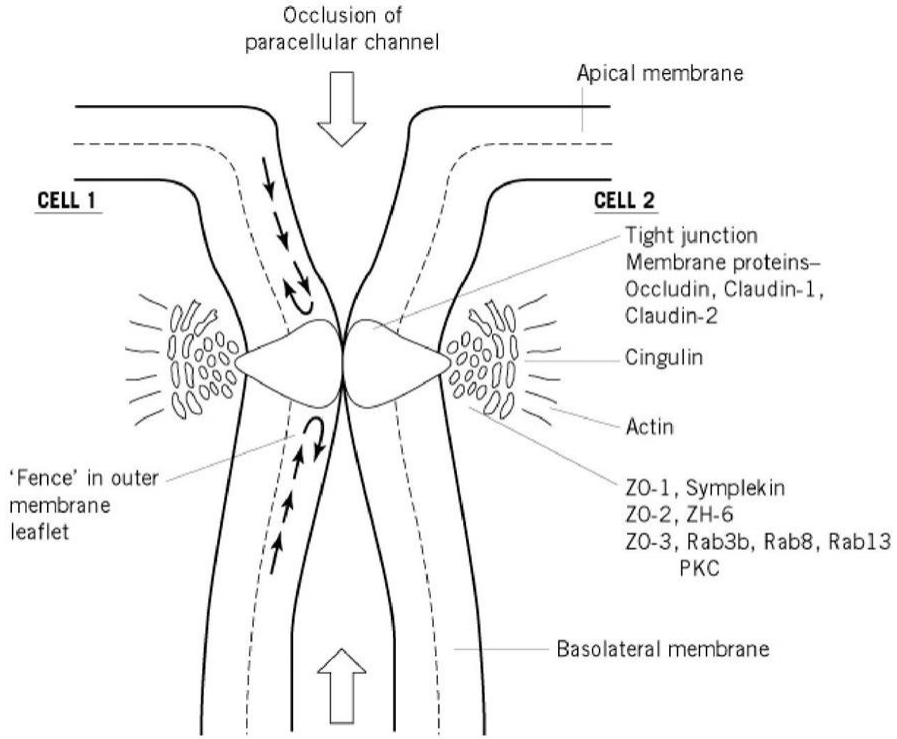


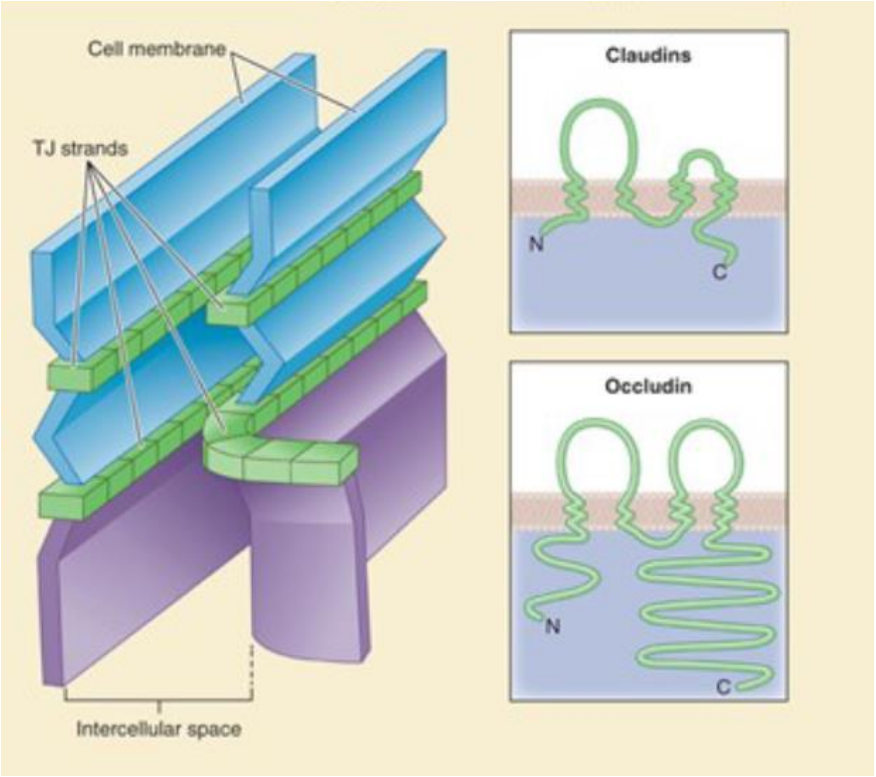
Figure 14-9 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019 and http://what-when-how.com/molecular-biology/tight-junction-molecular-biology/

Tight junction structure

Transmembrane molecules
- Claudins – critical component
- Occludin
- Junctional adhesion molecules (JAMs)

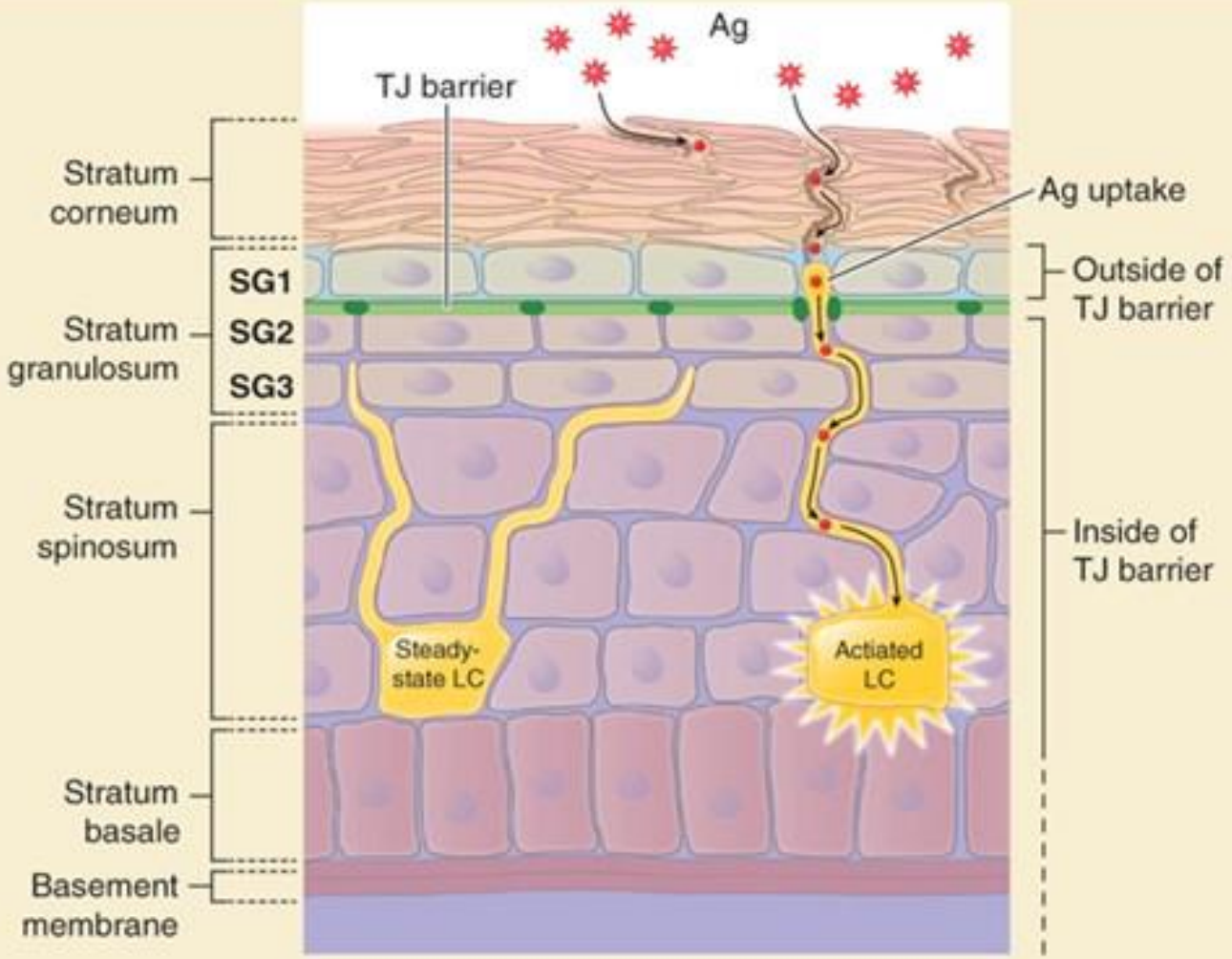
Intracellular proteins
- Zonula occludens (ZO) proteins
  - Interact with claudins, occludin, actin

Create “zip lock” seal
- Holds H_2O in, antigens out

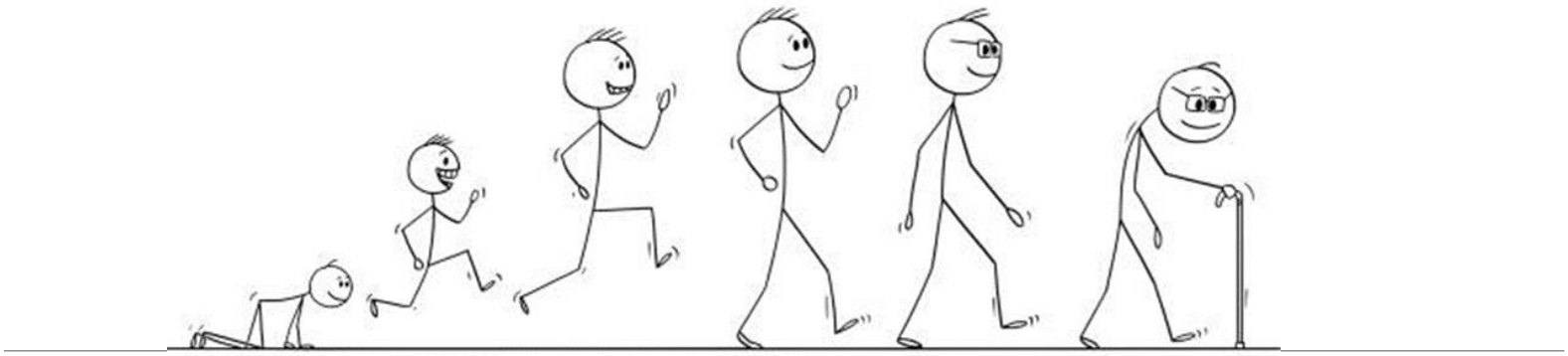


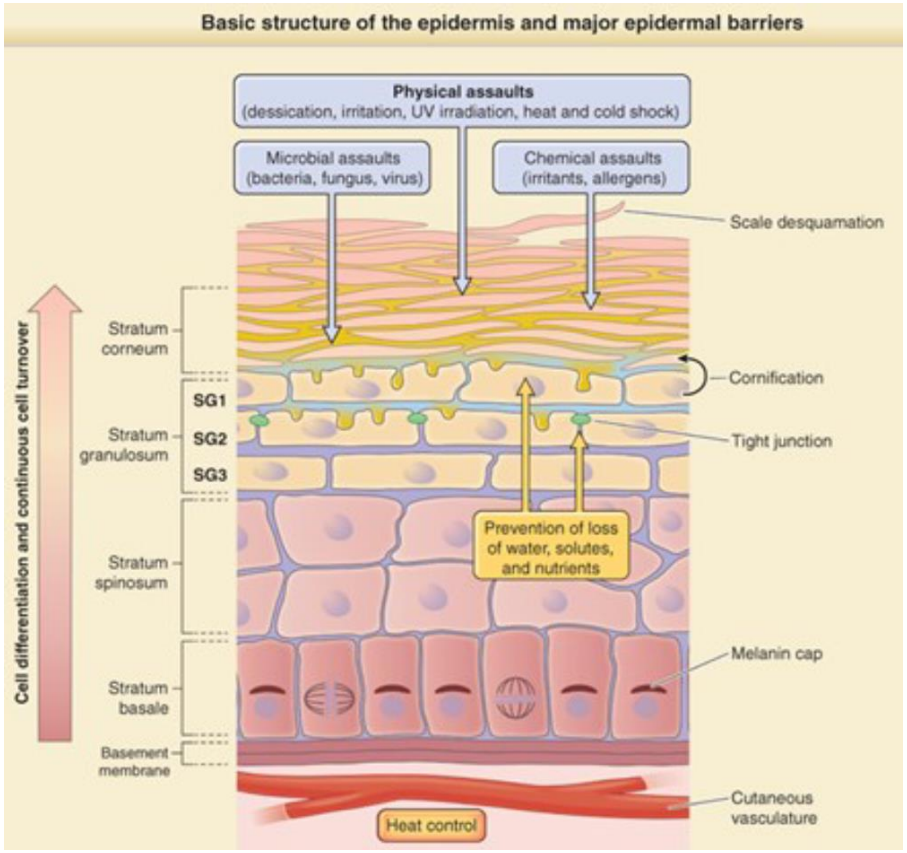
Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Spatial location of the stratum corneum, tight junctions, and Langerhans cells



Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.





BIOGRAPHY OF A KERATINOCYTE

Overview of epidermal layers

Cornification

Keratinocyte adhesion

Desquamation

Epidermal barrier

Figure 14-2 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Desquamation

Shedding of corneocytes

Regulated enzymatic cleavage of corneodesmosomes

Maintains epidermal homeostasis

Removes microorganisms preventing colonization

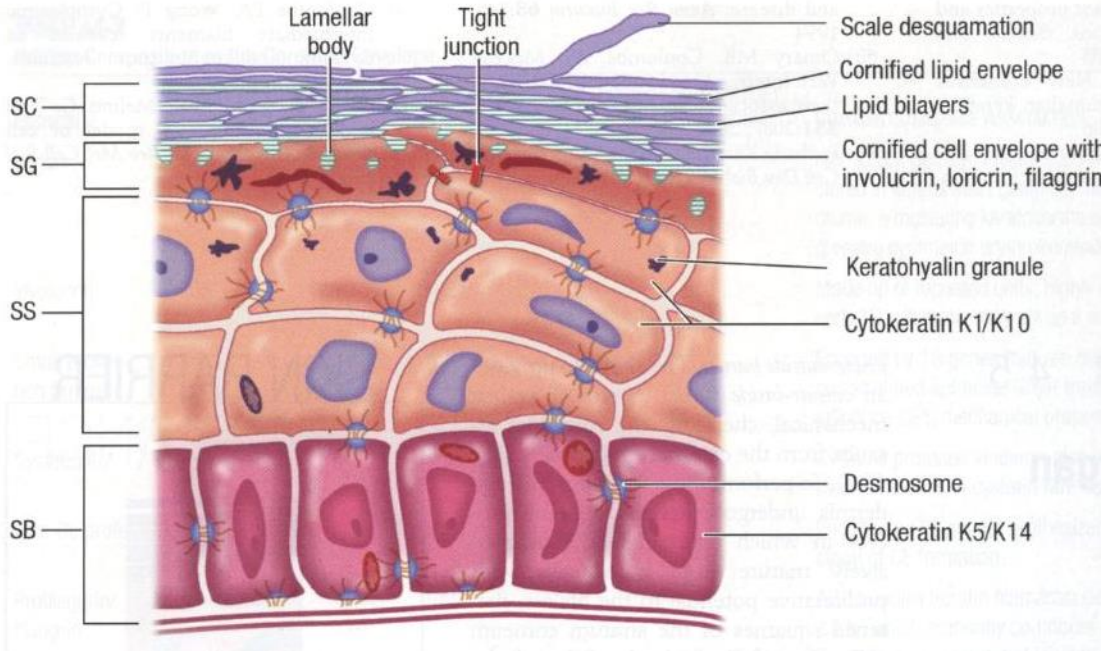


Figure 45-2 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. p. 384.

Enzymatic Cleavage of Corneodesmosomes

Enzymes and inhibitors released from lamellar bodies

- Proteases – kallikreins (KLK) and cathepsins
- Inhibitors – LEKTI (lymphoepithelial Kazal-type-related inhibitor)

Complex pH dependent network of enzymes and their inhibitors

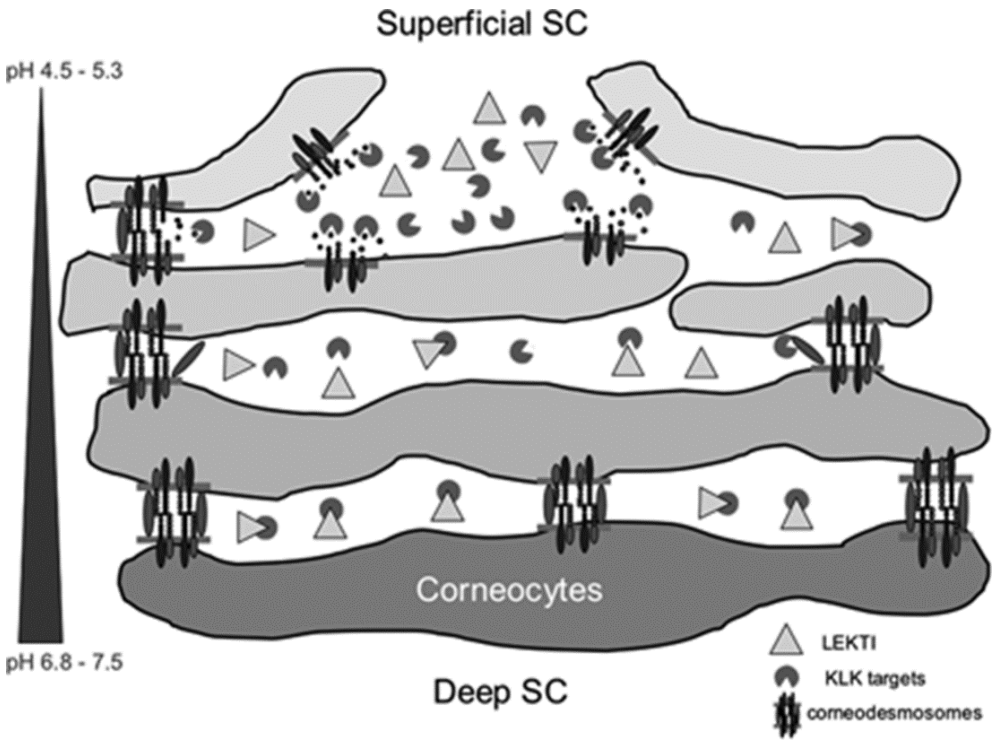


Figure 9 from Deraison C, et al. LEKTI Fragments Specifically Inhibit KLK5, KLK7, and KLK14 and Control Desquamation through a pH-dependent Interaction. *Mol. Biol. Cell* September 1, 2007 vol. 18 no. 9 3607-3619.

Selective degradation of corneodesmosomes

Figure 14-5 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

Complexity of Desquamation

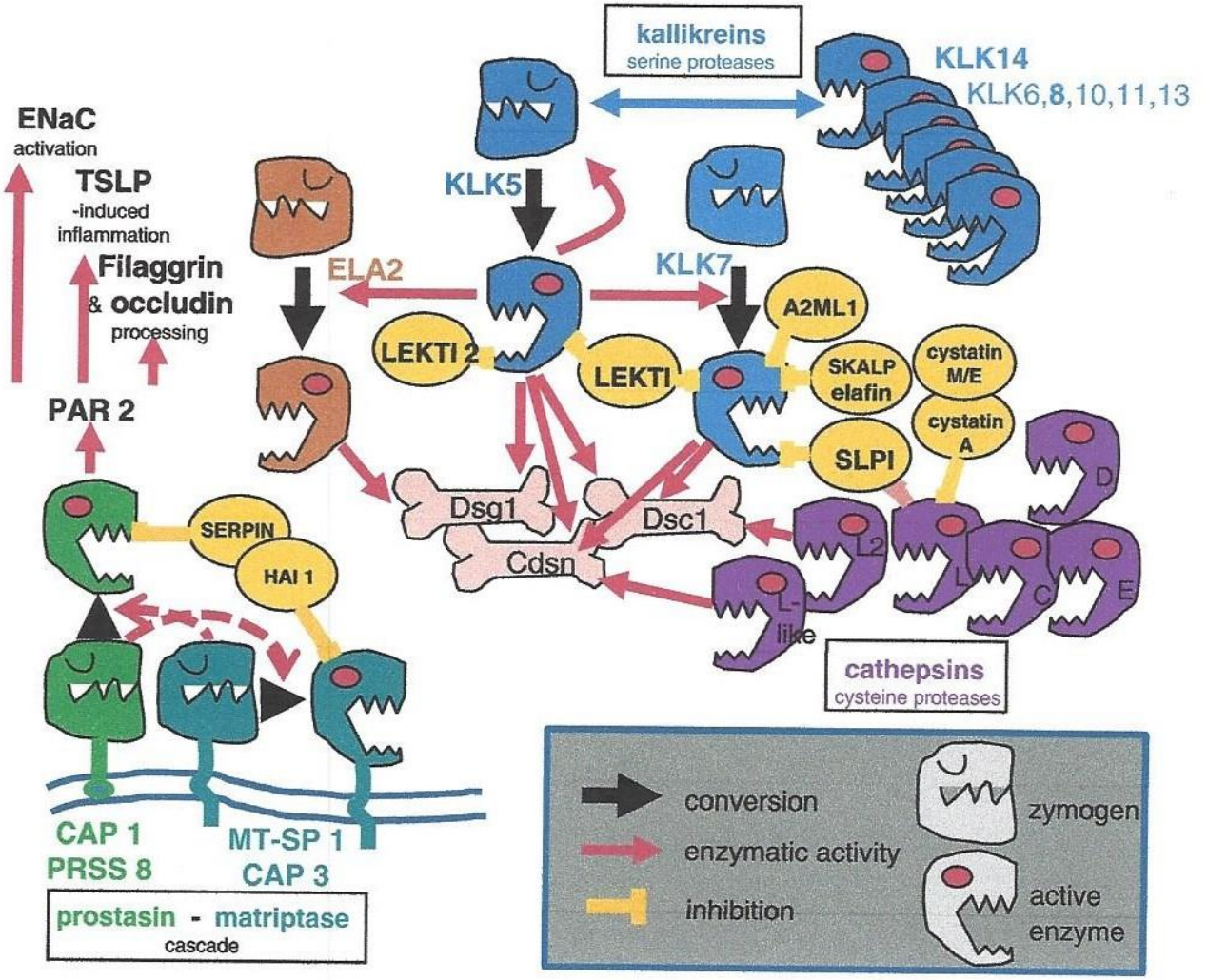
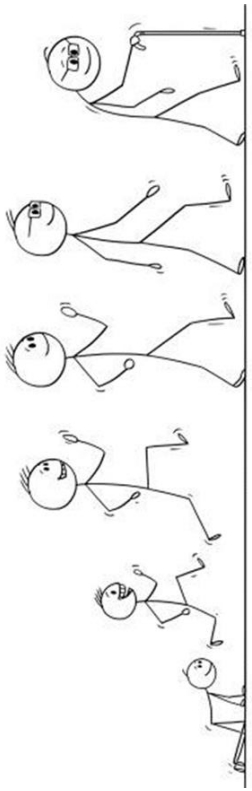


Figure 1 from Haftek M. Epidermal barrier disorders and corneodesmosome defects. *Cell Tissue Res* (2015) 360:484.



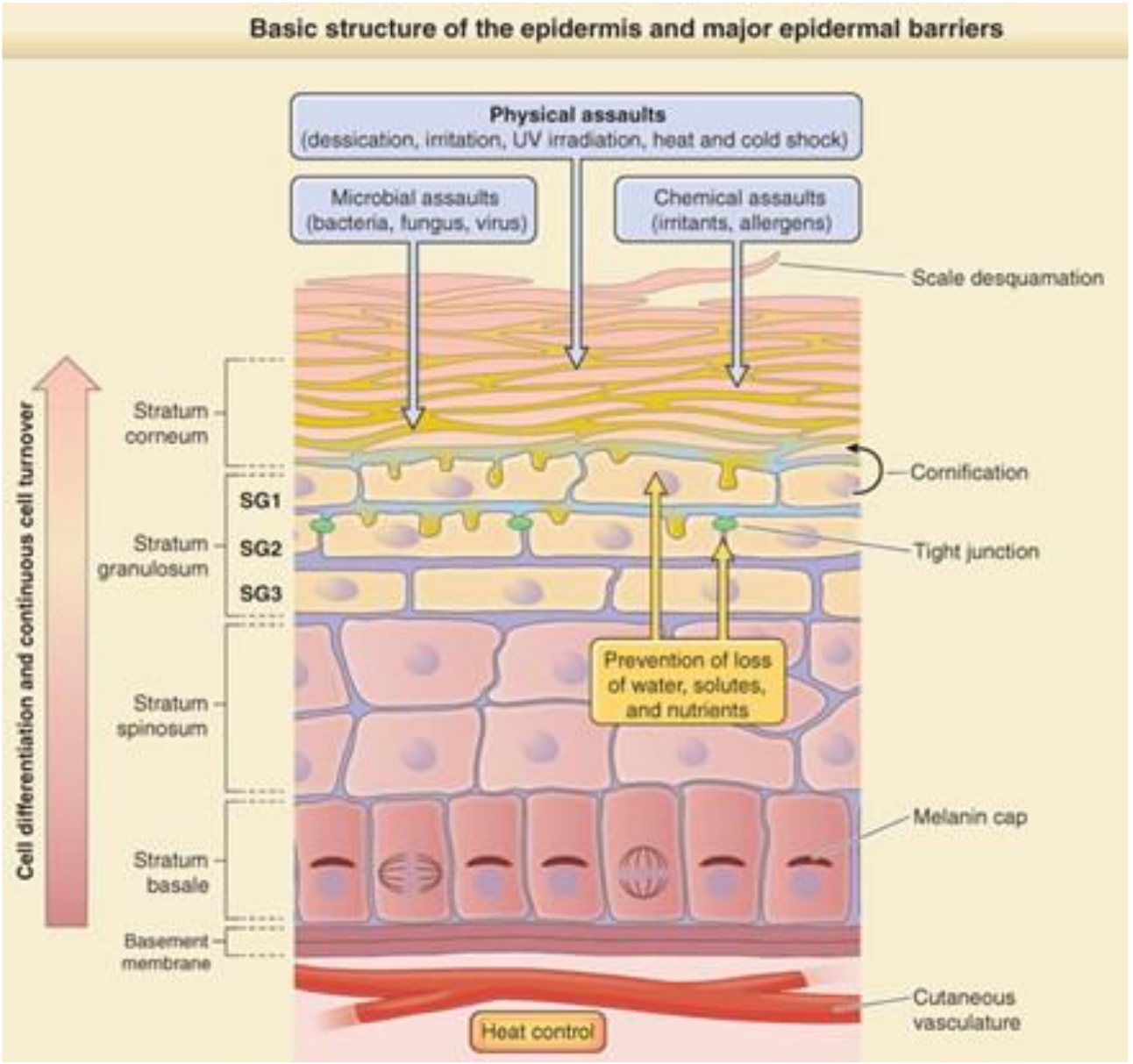
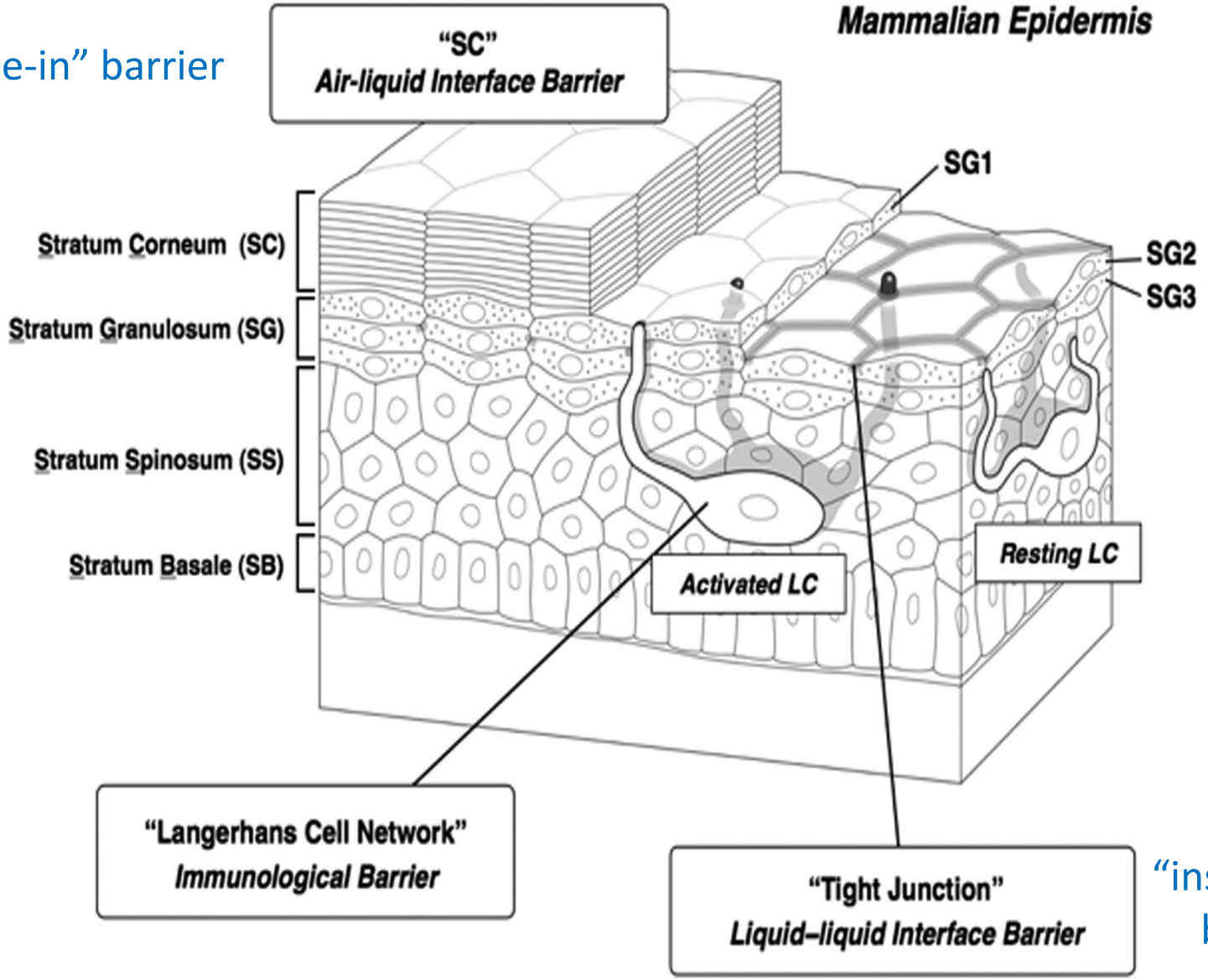


Figure 14-2 from Kubo A, Amagai M. Skin Barrier. In: Kang S, Amagai M, Bruckner AL, Enk AH, Margolis DJ, McMichael AJ, Orringer JS. eds. Fitzpatrick's Dermatology, 9e. McGraw Hill; 2019.

“outside-in” barrier



“inside-out” barrier

Figure 1 from Matsui T, Amagai M. Dissecting the formation, structure and barrier function of the stratum corneum. Int Immunol. 2015 Jun;27(6):269-80.

Thank you! Any questions?