The Epidermal Barrier: Biography of a Keratinocyte

Heide M. Newton, DVM, Diplomate ACVD
Dermatology for Animals
Tucson, Arizona

An Electron Microscope Study of Cornification in the Human Skin.

Arwyn Charles, 1959, *J Invest Dermatol* , 33:65-74.

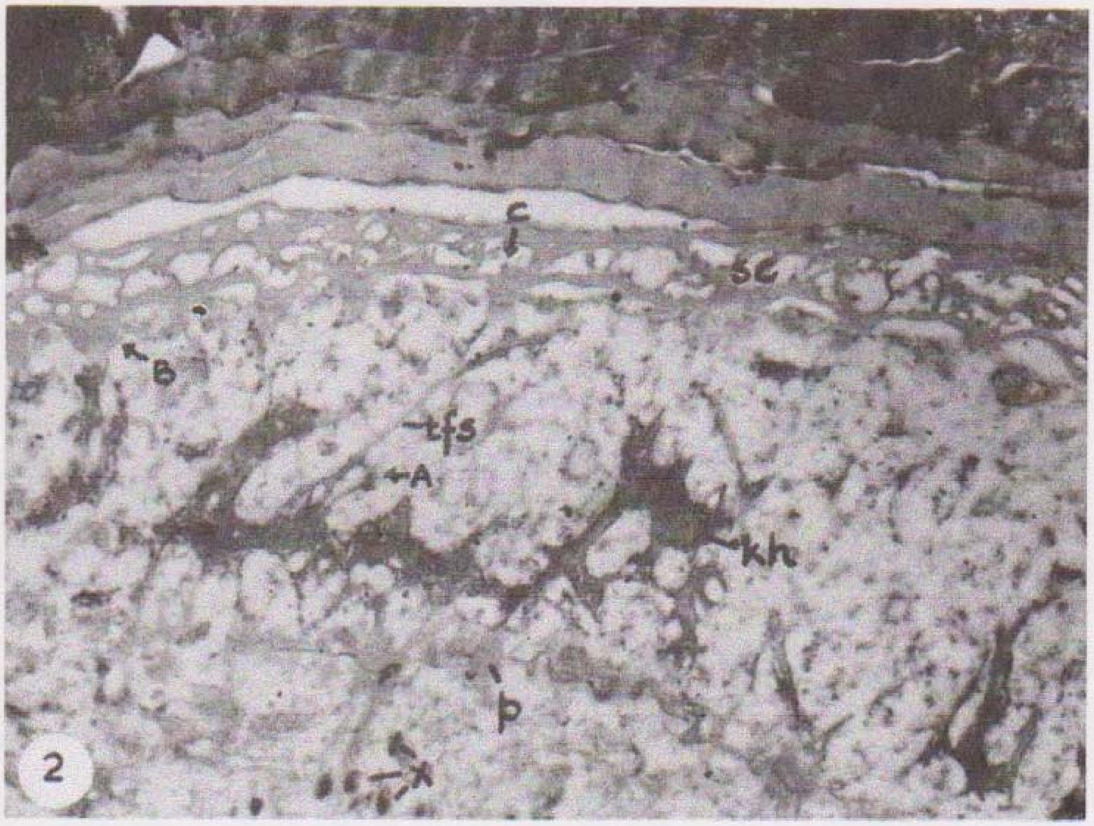
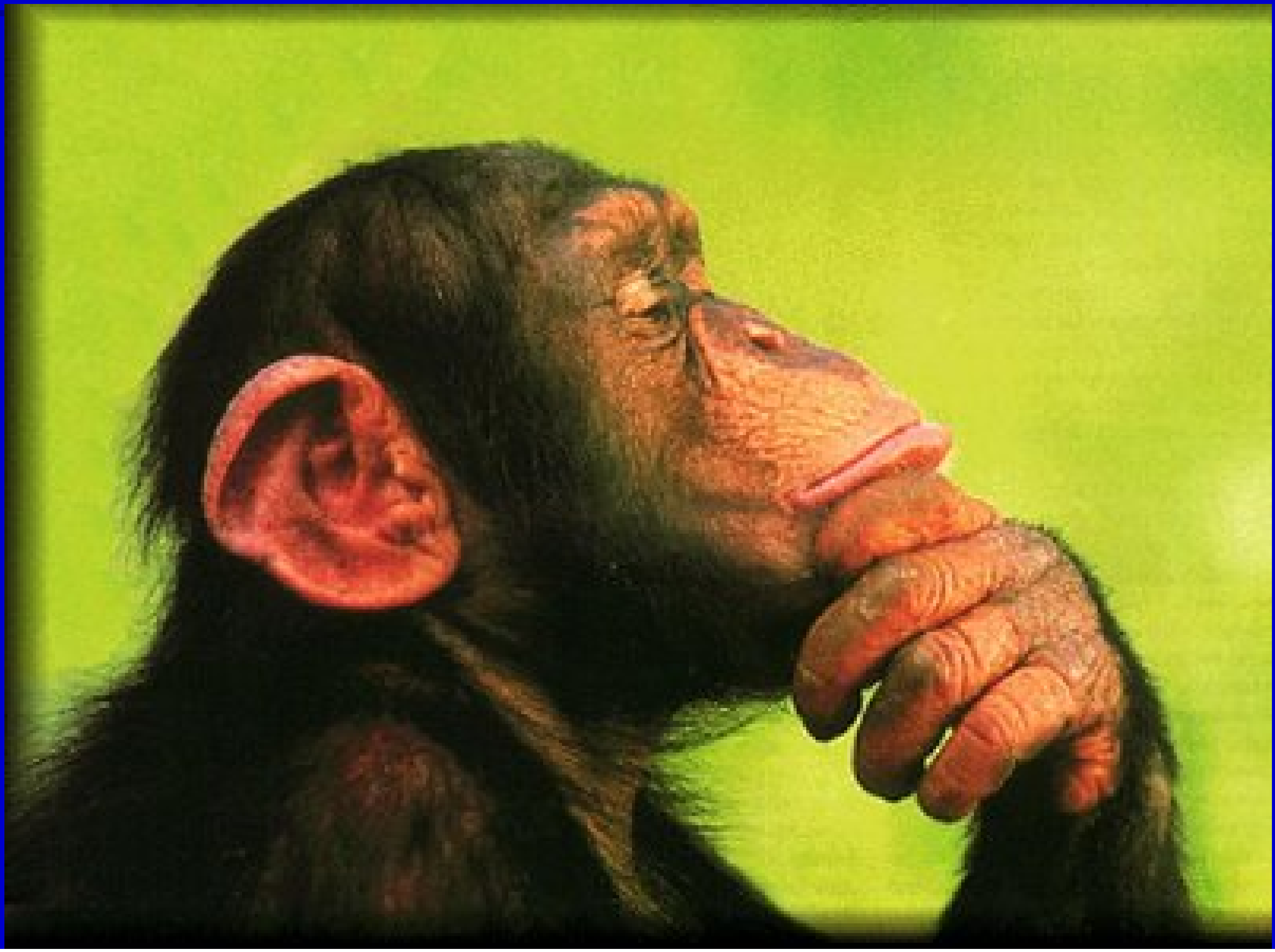


FIG. 2. Cells of the granular and horny layers. Keratohyalin forms a sheath around the tonofibrils at (tfs), the light lines (l) probably representing the fibrils; this region, indicated by arrow A, is enlarged as Fig. 3A. Arrow B indicates a prickle enlarged in Fig. 3B, in which the cell wall remnants 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.

Biography of a Keratinocyte??



Skin Functions

- Physical permeability barrier
- Protection from infectious agents
- Thermoregulation
- Sensation
- Physical appearance

Epidermal Functions

- Barrier between outside and inside
  - Mechanical protection
  - Compressive, tensile, bending strength
  - Prevents penetration of pathogenic microorganisms, allergens, toxins
  - Prevents water loss
  - Protects against damage from ultraviolet radiation

Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte adhesion
- Desquamation
- Epidermal barrier function

Layers of the Epidermis

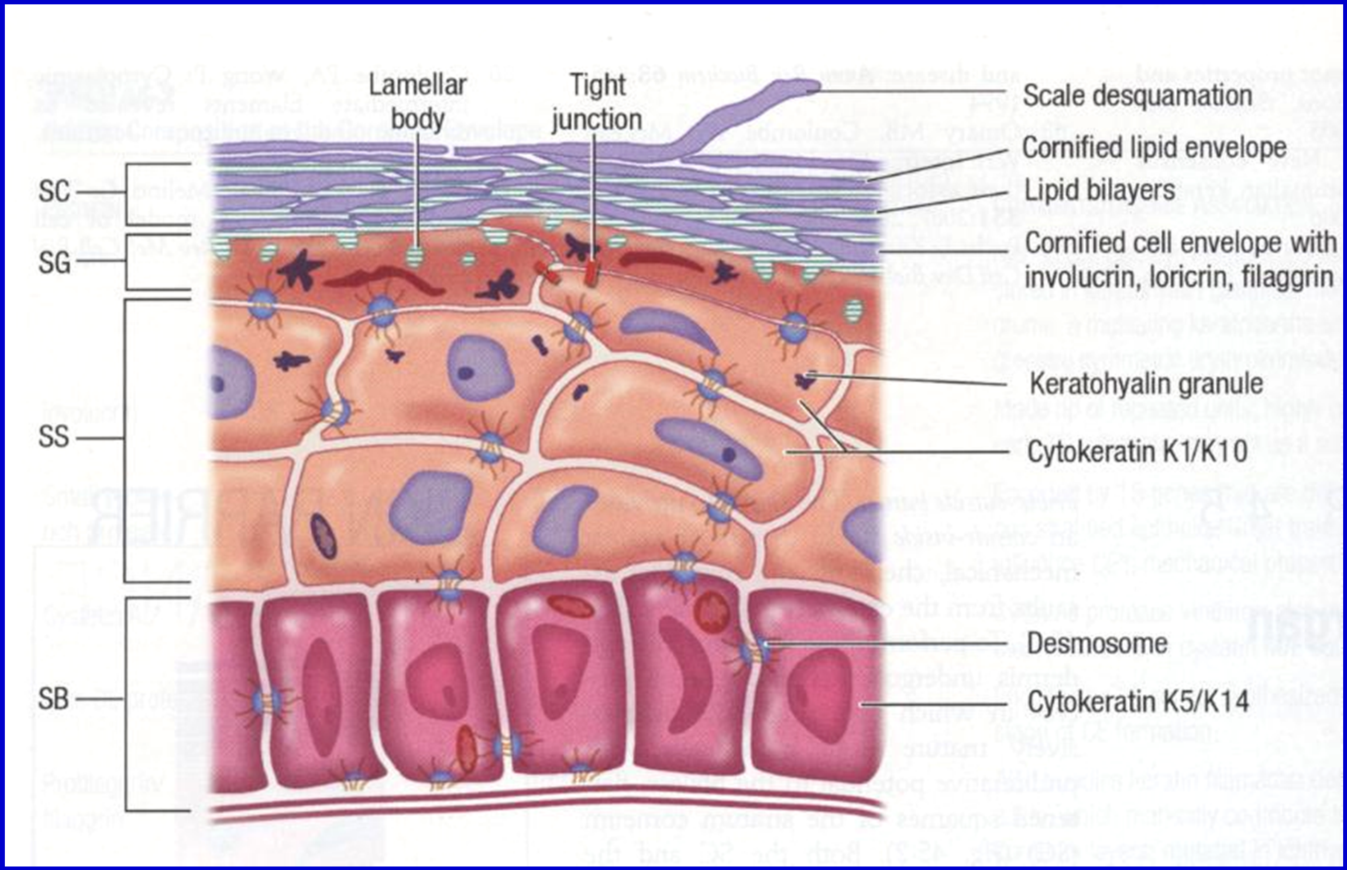


Figure 45-2 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. p. 384.

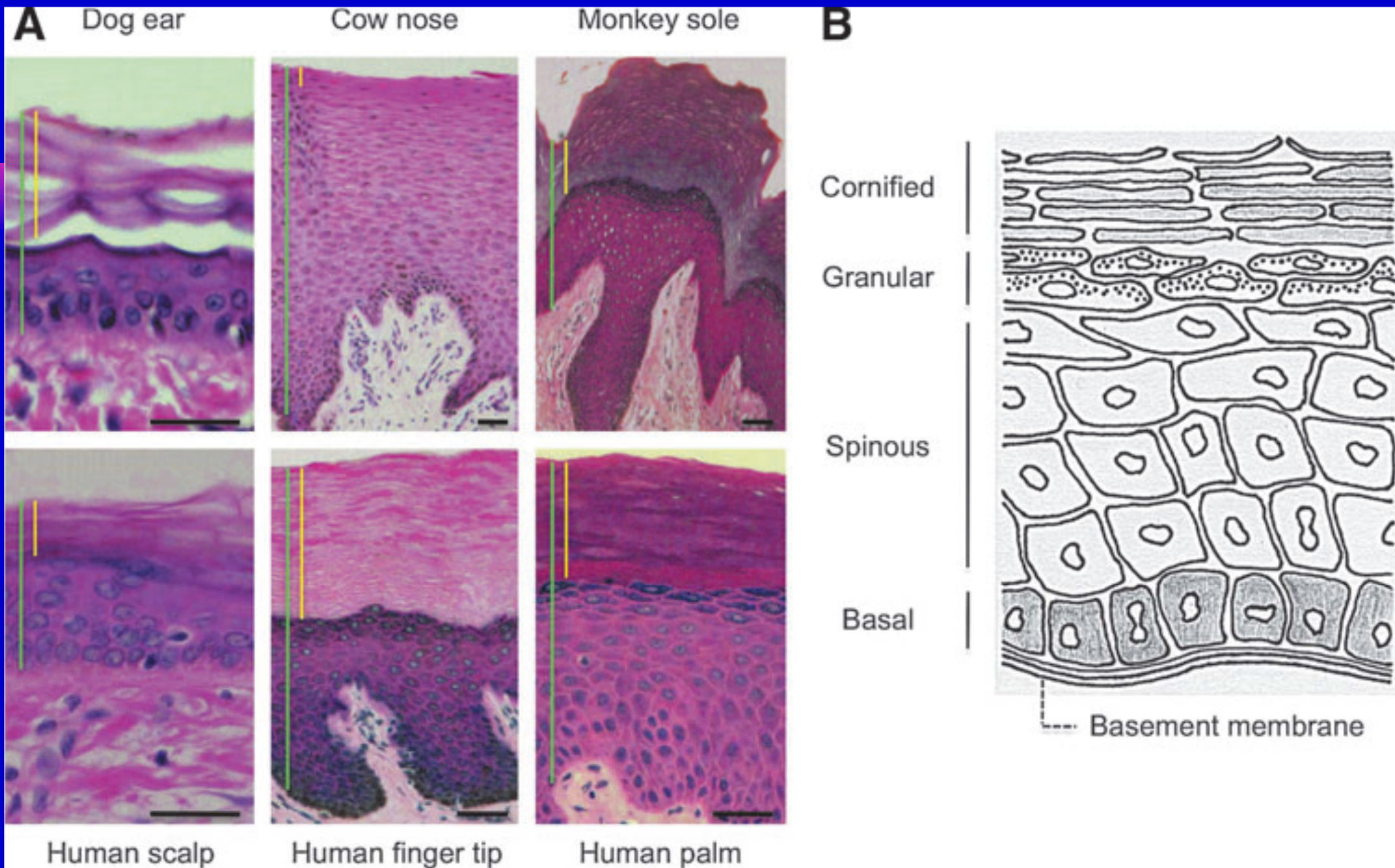
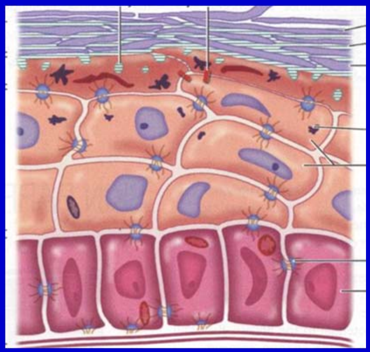


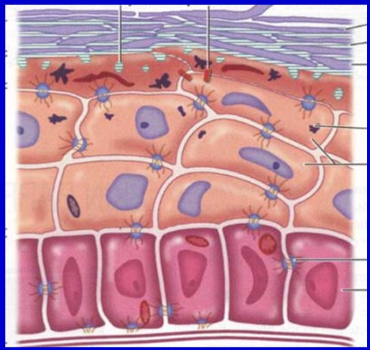
Figure 1 from Senoo M. Epidermal Stem Cells in Homeostasis and Wound Repair of the Skin. *Adv Wound Care (New Rochelle)*. 2013;2(6):274.

Stratum basale



- AKA basal cell layer
  - Basal keratinocytes attach to basement membrane at hemidesmosomes
  - Small polar undifferentiated cells
  - Express K5 and K14
    - Dogs also express K1 and K6

Stratum basale



- Stem cells
  - Slowly dividing, undifferentiated keratinocytes
- Transit amplifying (TA) cells
  - Rapidly proliferating

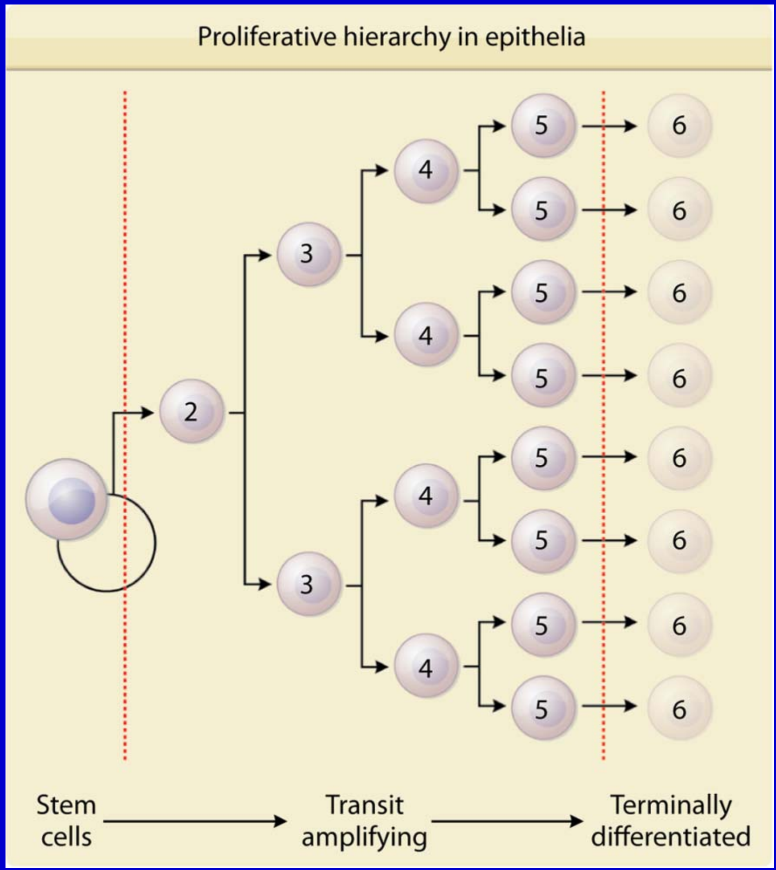
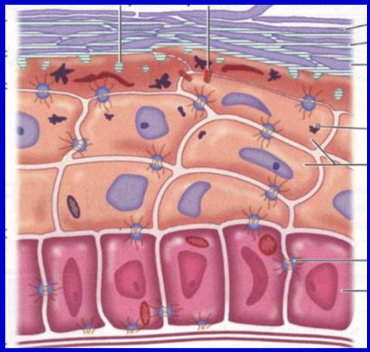


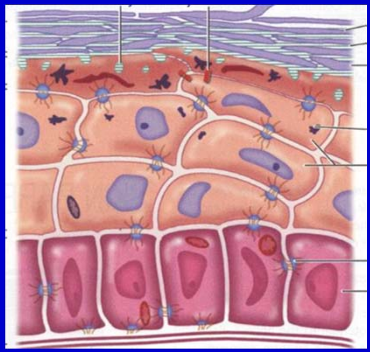
Figure 45-1 from Morris RJ. Epidermal stem cells. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 474.

Stratum basale

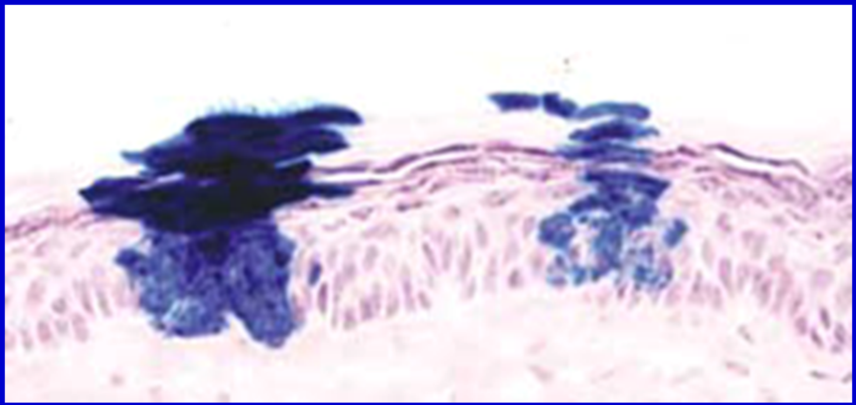


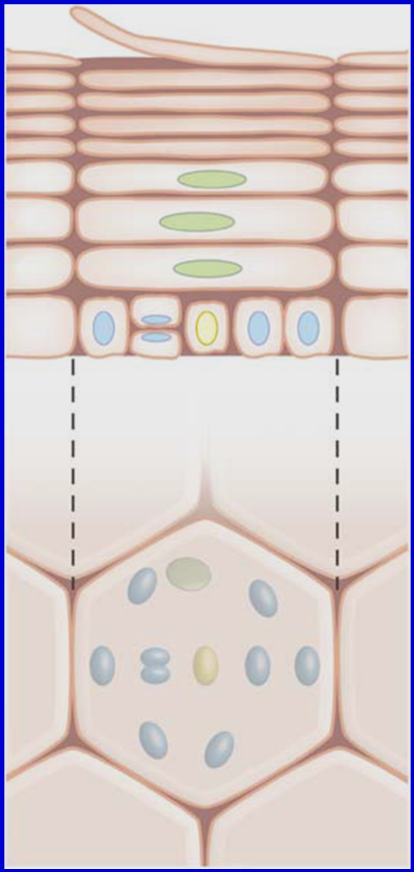
- Epidermal proliferative unit (EPU)
  - Hexagonal column of cells in all layers
  - Approximately 10 basal cells
    - Single stem cell
    - TA cells
  - Suprabasal differentiating progeny
- EPUs are functionally independent packets of clones of original TA cells

Stratum basale



- Clonal EPUs demonstrated
  - Mouse skin injected with retrovirus then stained to reveal cells expressing the transgene





Figures 1 and 3 from Kaur P. Interfollicular epidermal stem cells: Identification, challenges, potential. *J Invest Dermatol* 2006; 126: 1452, 1453.

Stratum basale

- Theories of epidermal homeostasis

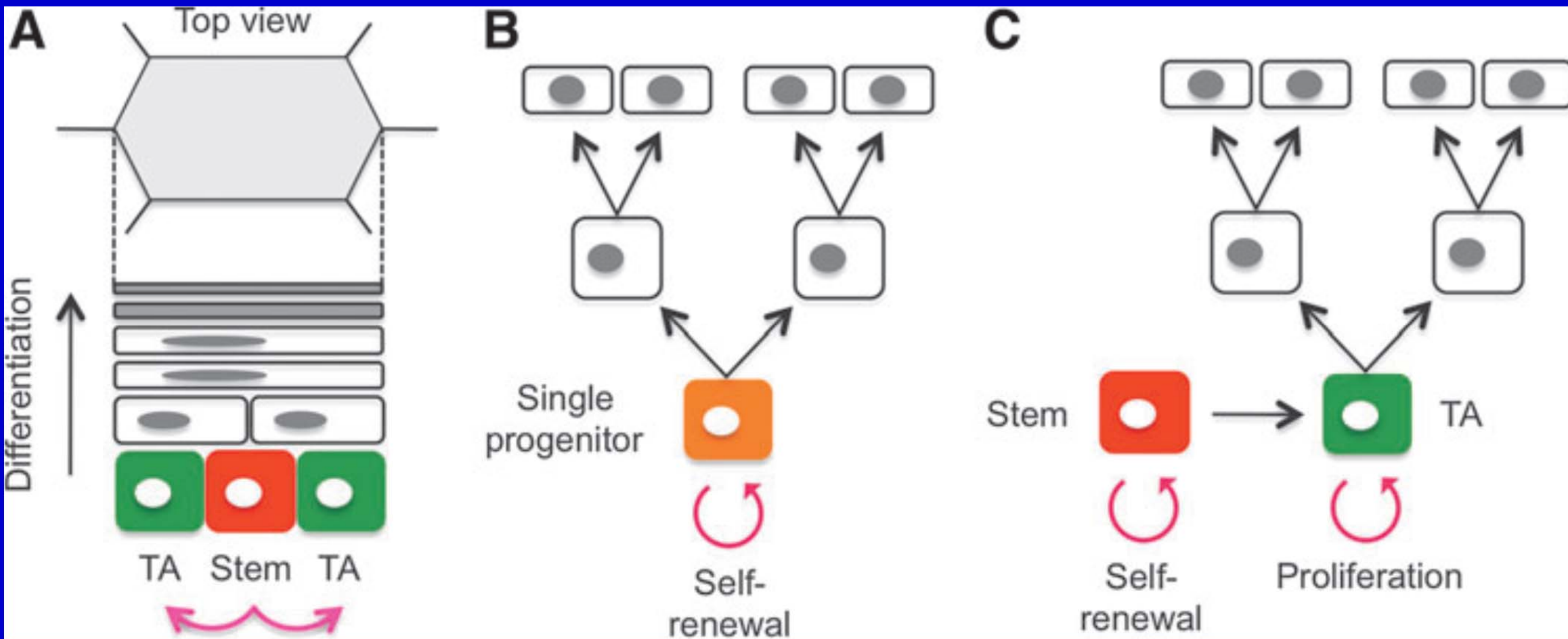
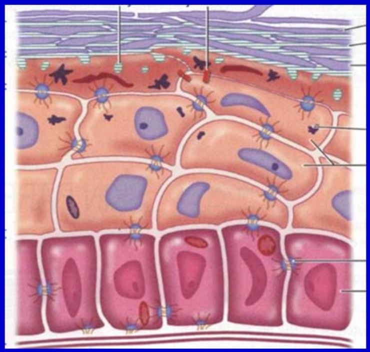


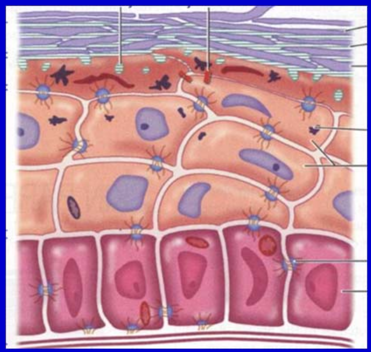
Figure 2 from Senoo M. Epidermal Stem Cells in Homeostasis and Wound Repair of the Skin. *Adv Wound Care (New Rochelle)*. 2013;2(6):276.

Stratum spinosum



- AKA spinous or prickle cell layer
  - "Spines" represent desmosomes
- Keratinocytes entering terminal differentiation pathway
  - Synthesizing K1 and K10
  - Dogs also express K4 and K15/16
- Start producing other components
  - Involucrin
  - Profilaggrin
  - Lamellar granules (LGs)

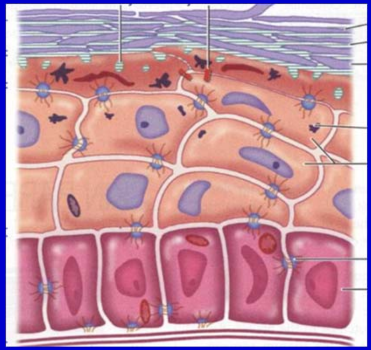
Stratum granulosum



- AKA granular layer
- Keratohyalin granules visible under light microscopy
- Granule components
  - Profilaggrin
  - Keratin filaments
  - Loricrin
- Keratin intermediate filament assembly
- Cornified cell envelope construction

Stratum granulosum

- At interface with stratum corneum
  - LGs fuse with plasma membrane at apical surface
  - Secrete contents into intercellular space



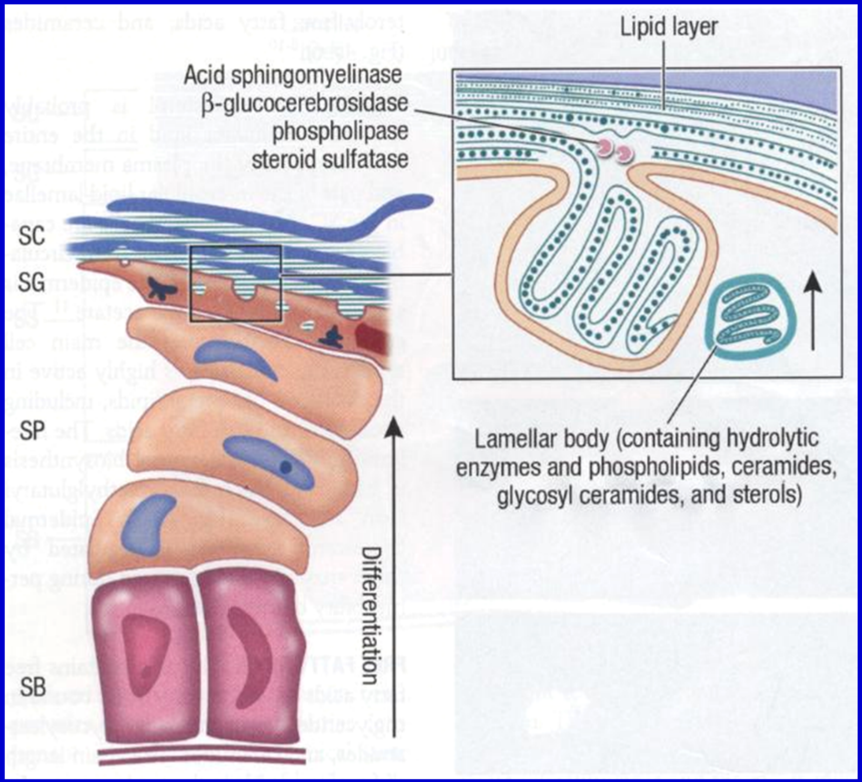


Figure 45-5 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. p. 386.

Stratum granulosum

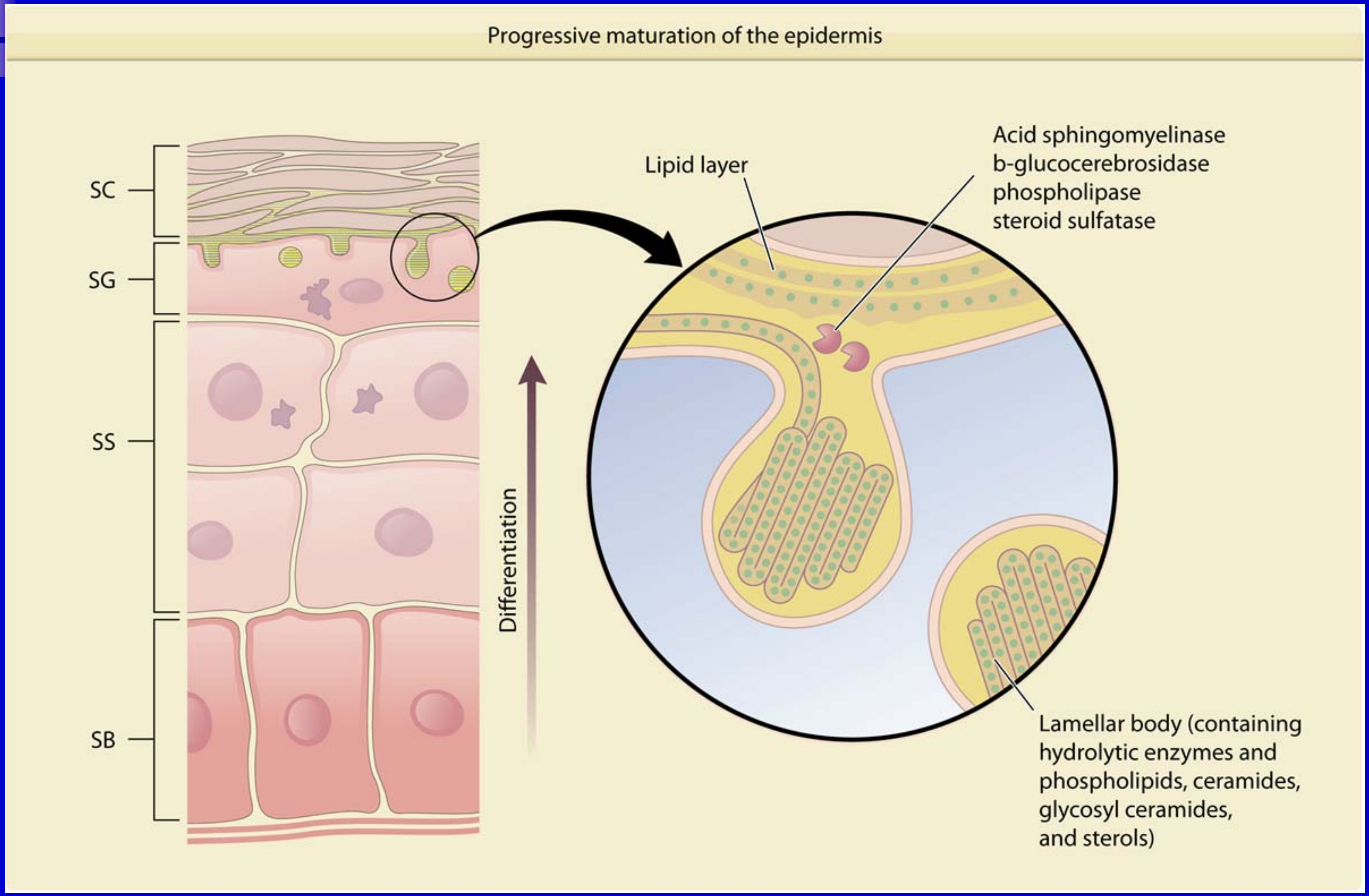
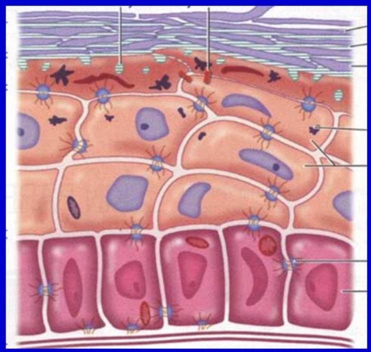


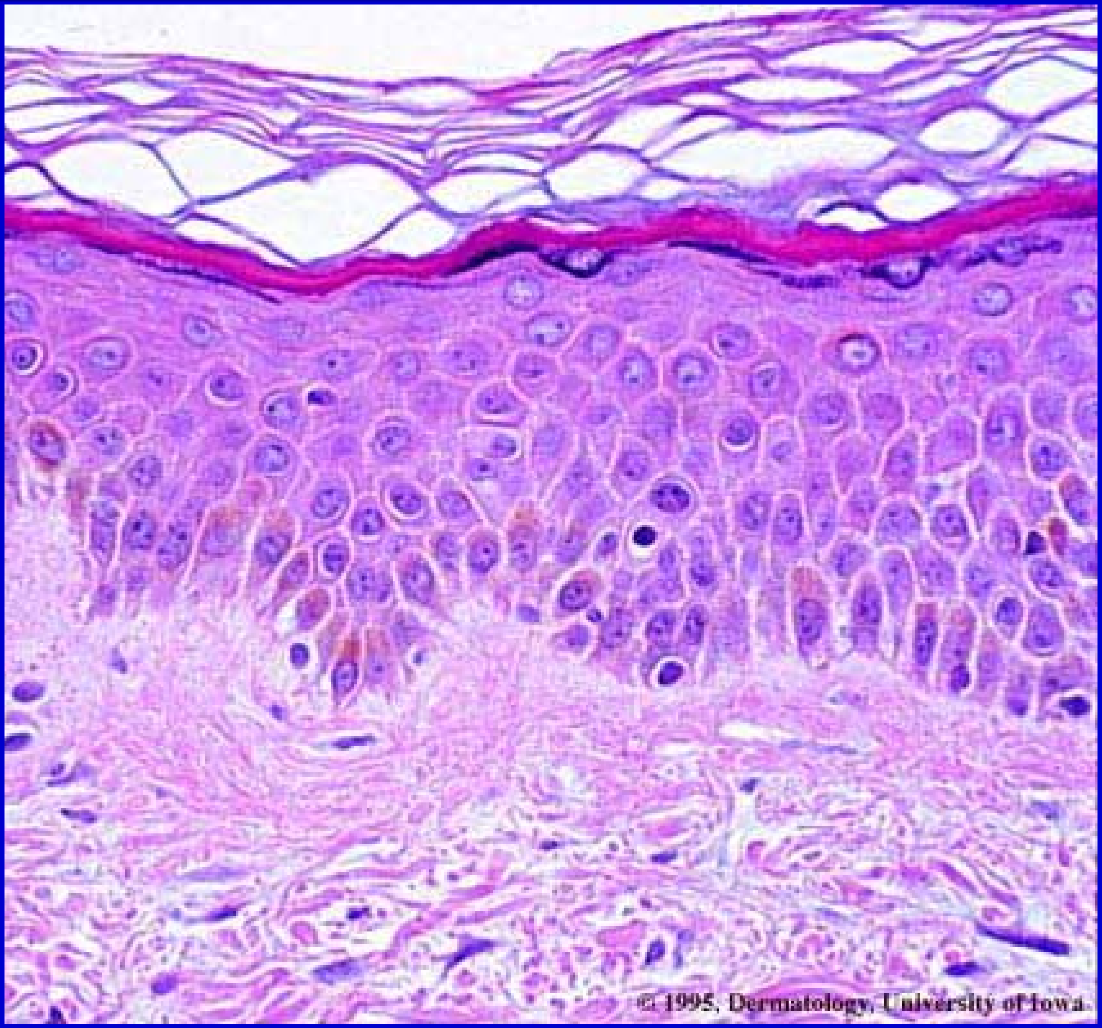
Figure 47-5 from Proksch E, Jensen JM. Skin as an Organ of Protection. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 489.

Stratum corneum



- AKA cornified layer, horny layer
- Corneocytes in extracellular lipid matrix
  - Corneocytes – dead, flattened terminally differentiated keratinocytes
    - Densely packed core of keratin and filaggrin
    - Coated in durable protein layer: cornified cell envelope
    - Loss of nuclei and organelles
  - Lipid matrix composed of ceramides, free fatty acids, and cholesterol

Layers of the Epidermis



Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte adhesion
- Desquamation
- Epidermal barrier function

Cytoskeleton of Epithelial Cells

- Intracellular transport
  - Microfilaments – actin, 7 nm diameter
  - Microtubules – α- and β-tubulin, 20 nm diameter
- Scaffold
  - Intermediate filaments – 7-12 nm diameter
    - Keratin

KIF Network

- Micrograph of human epidermal cells in culture
- Double–labeling by indirect immunofluorescence
  - Keratin = red
  - Desmoplakin = green
- KIFs span cytoplasm and attach at desmosomes
  - Arrowheads = desmosomes
  - n = nucleus, bar = ~50 µm

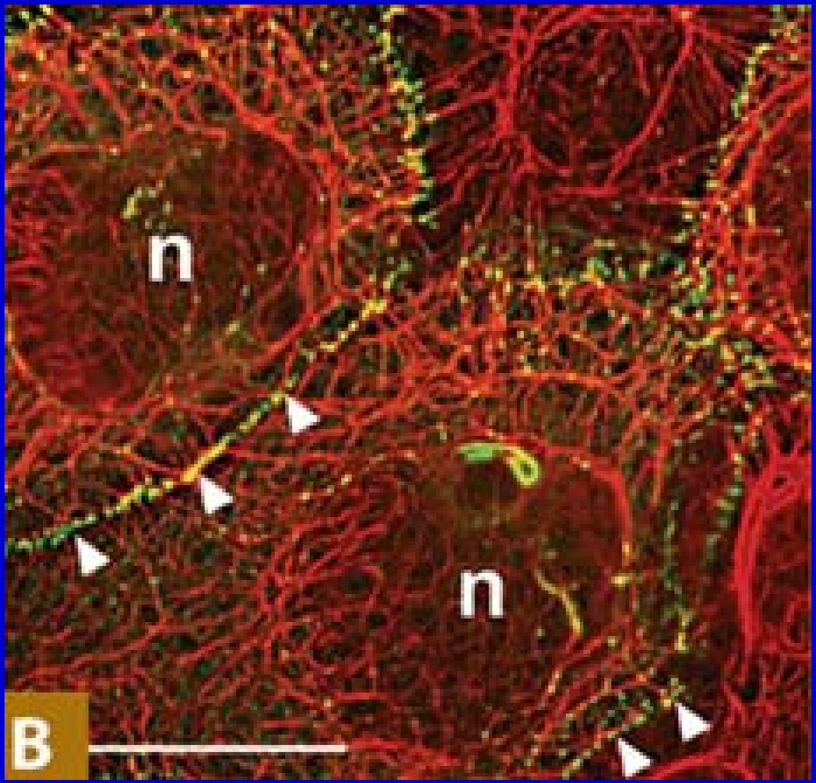


Figure 46-2 from Coulombe PA, Miller SJ, Sun T. Epidermal Growth and Differentiation. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 481.

Keratin Proteins

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

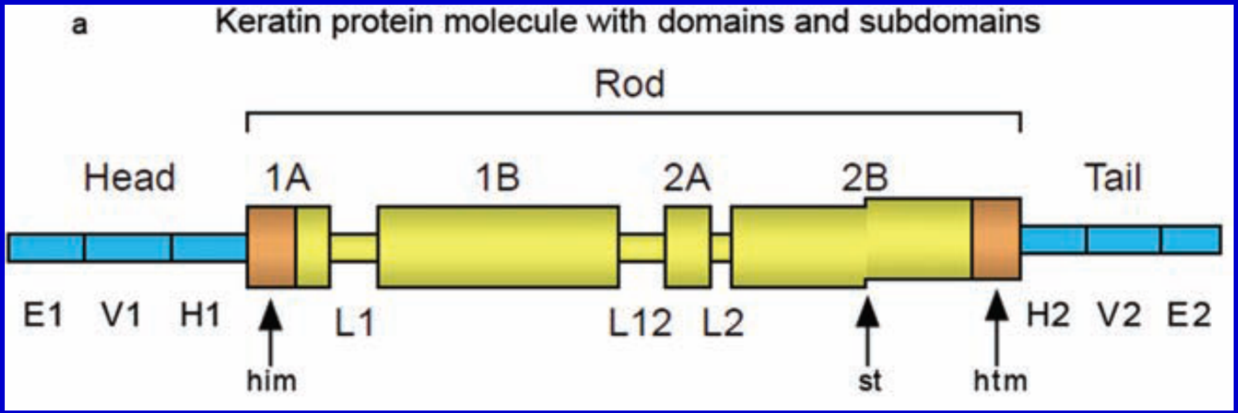


Figure 2a from Bragulla HH, Homberger DG. Structure and functions of keratin proteins in stratified, keratinized and cornified epithelia. *J Anat* 2009; 214:530.

Keratin Proteins

- Type I
  - Acidic
  - Smaller than type II
  - K9-19 in epidermis
- Type II
  - Basic to neutral
  - Larger then type I
  - K1-8 in epidermis

Acidic and basic proteins form pairs

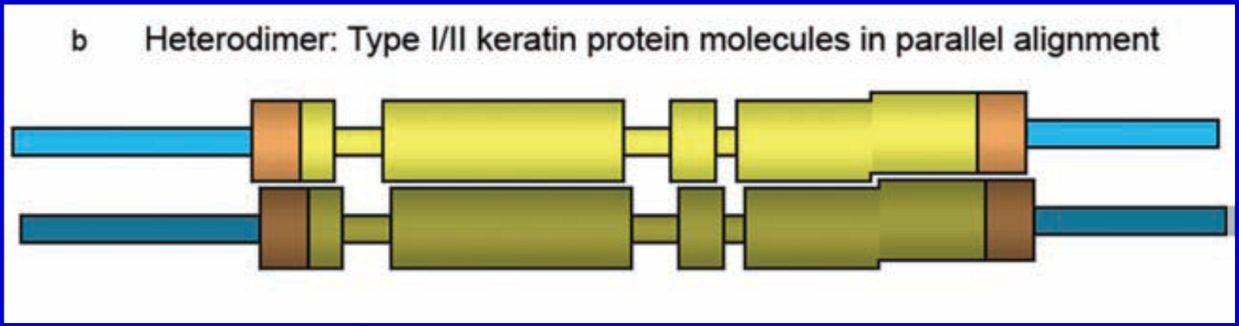


Figure 2b from Bragulla HH, Homberger DG. Structure and functions of keratin proteins in stratified, keratinized and cornified epithelia. *J Anat* 2009; 214:530.

Keratin Intermediate Filament (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

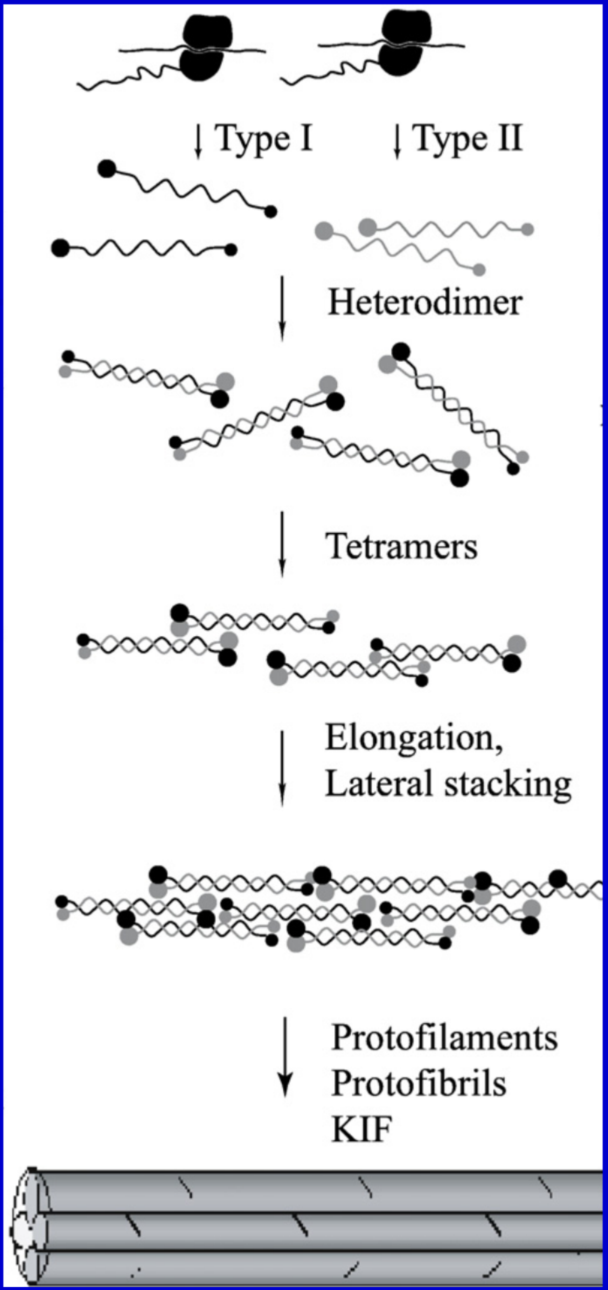


Figure 2 from Uitto J, Richard G, McGrath JA. Diseases of epidermal keratins and their linker proteins. *Exp Cell Res* 2007; 313: 1997.

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

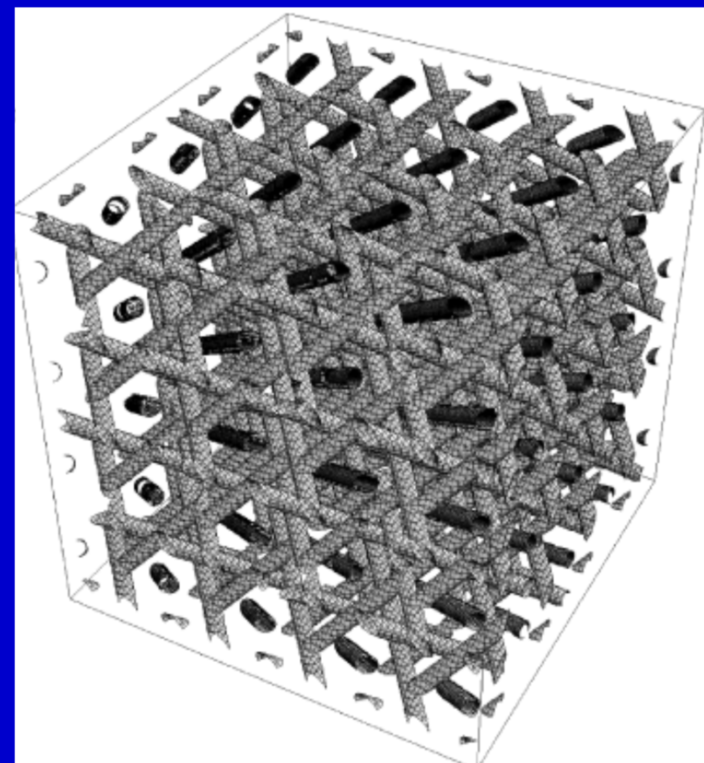


Figure 5B from Norlén L, Al-Amoudi A. Stratum corneum keratin structure, function, and formation: the cubic rod-packing and membrane templating model. *J Invest Dermatol* 2004; 123:721.

Keratin Intermediate Filaments

- Keratins cross-linked by disulfide bonds
- KIFs aggregated and bundled into densely packed parallel formation
- Filaggrin plays role in bundling KIFs

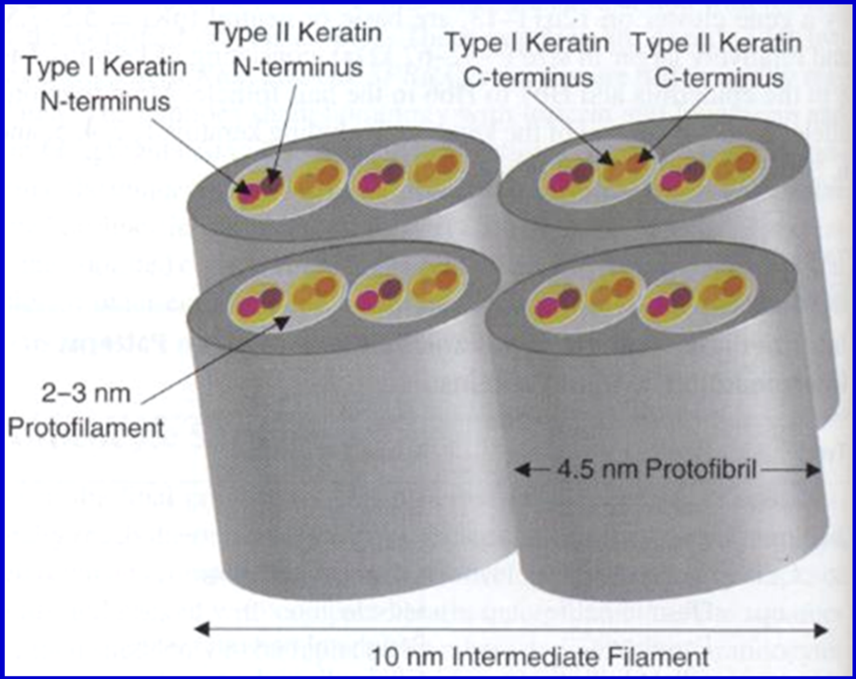
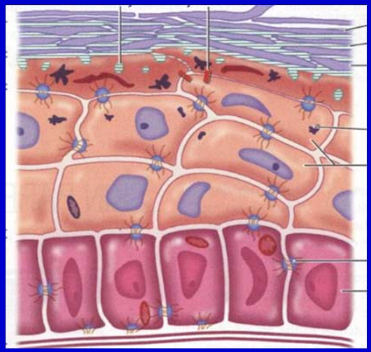


Figure 7-6 from Kimyai-Asadi A, Jih MH, Freedberg IM. Epidermal Cell Kinetics, Epidermal Differentiation, and Keratinization. In: Freedberg IM et al, editors, Fitzpatrick's Dermatology in General Medicine, 6th Ed.

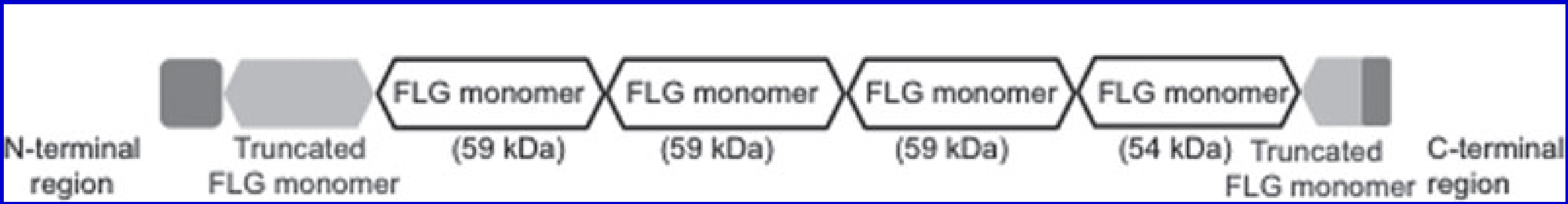
Filaggrin: Filament Aggregating Protein

- Histidine-rich, cationic protein
- Precursor: profilaggrin
  - Synthesis starts after K1 and K10
  - Consists of multiple filaggrin units between N- and C- terminal domains
    - N-terminal domain has calcium binding domain
      - Calcium may be involved in profilaggrin processing
  - Component of keratohyalin granules

Filaggrin

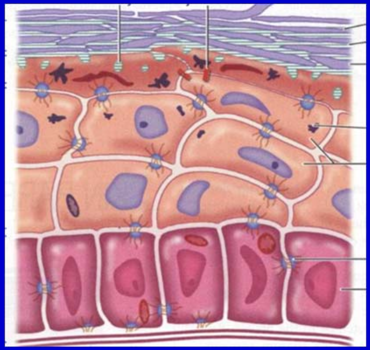


- Profilaggrin cleaved into filaggrin units in granular layer
  - 10-12 in humans, 12-20 in mice, 4 in dogs
- Structure of canine profilaggrin



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

Filaggrin



- Bundles KIFs into tight arrangement
- Degraded in stratum corneum
  - Pyrrolidone carboxylic acid and amino acids → maintain epidermal hydration and pH
  - Urocanic acid → photoprotection
- Caspase-14 involved in degradation
- Key to epidermal barrier function

Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte adhesion
- Desquamation
- Epidermal barrier function

Cornified Cell Envelope

- Construction begins in granular layer inside of cell membrane
- Transglutaminase enzymes
  - Catalyze formation of N(ε)-(γ-glutamyl)-lysine isopeptide bonds – highly resistant to proteolytic enzymes
  - Calcium dependent

Cornified Cell Envelope Components

- Involucrin
- Loricrin
- Envoplakin, periplakin
- Small proline-rich peptides (SPRs)
- Multiple other proteins

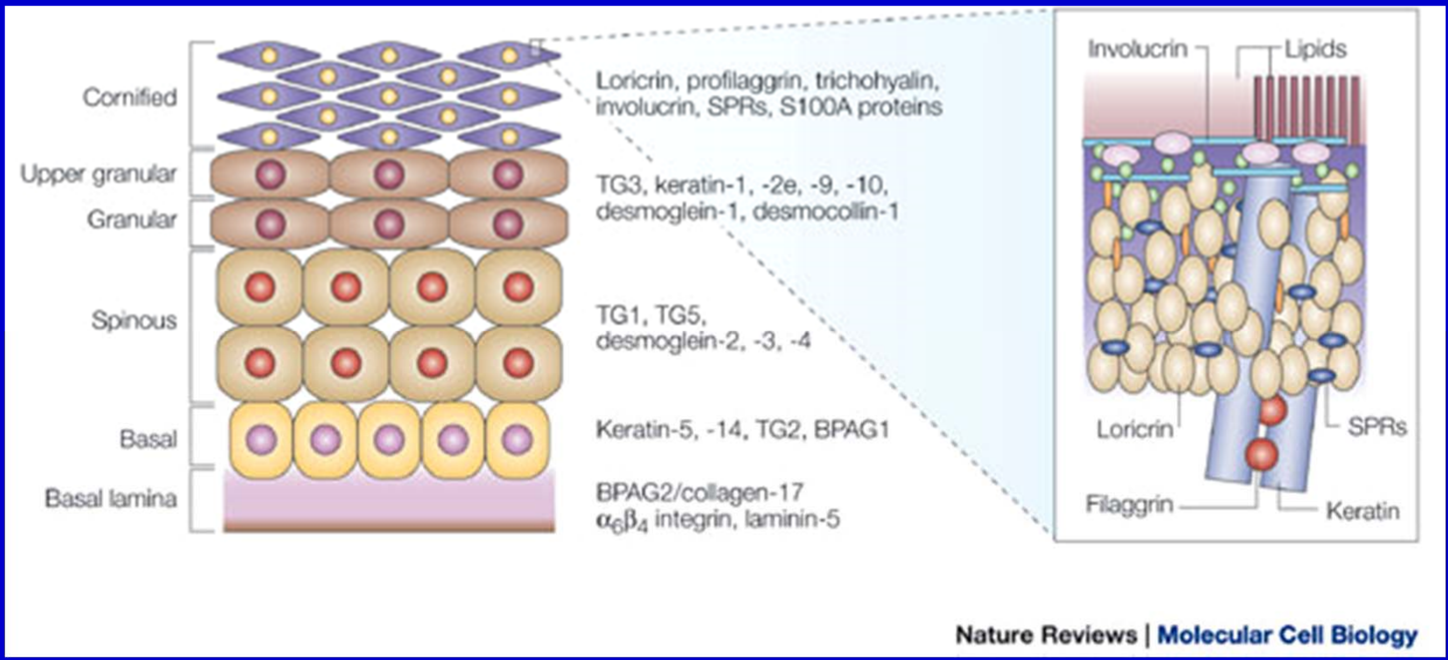


Figure 1 from Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol. 2005 Apr; 6(4):329.

Cornified Cell Envelope

- Involucrin
  - Glutamine-rich protein
  - Connects corneocytes to extracellular lipid layer
- Loricrin
  - Cysteine-rich highly flexible protein with glycine loops
  - Major structural component (>70%)

Cornified Cell Envelope (CE) Construction

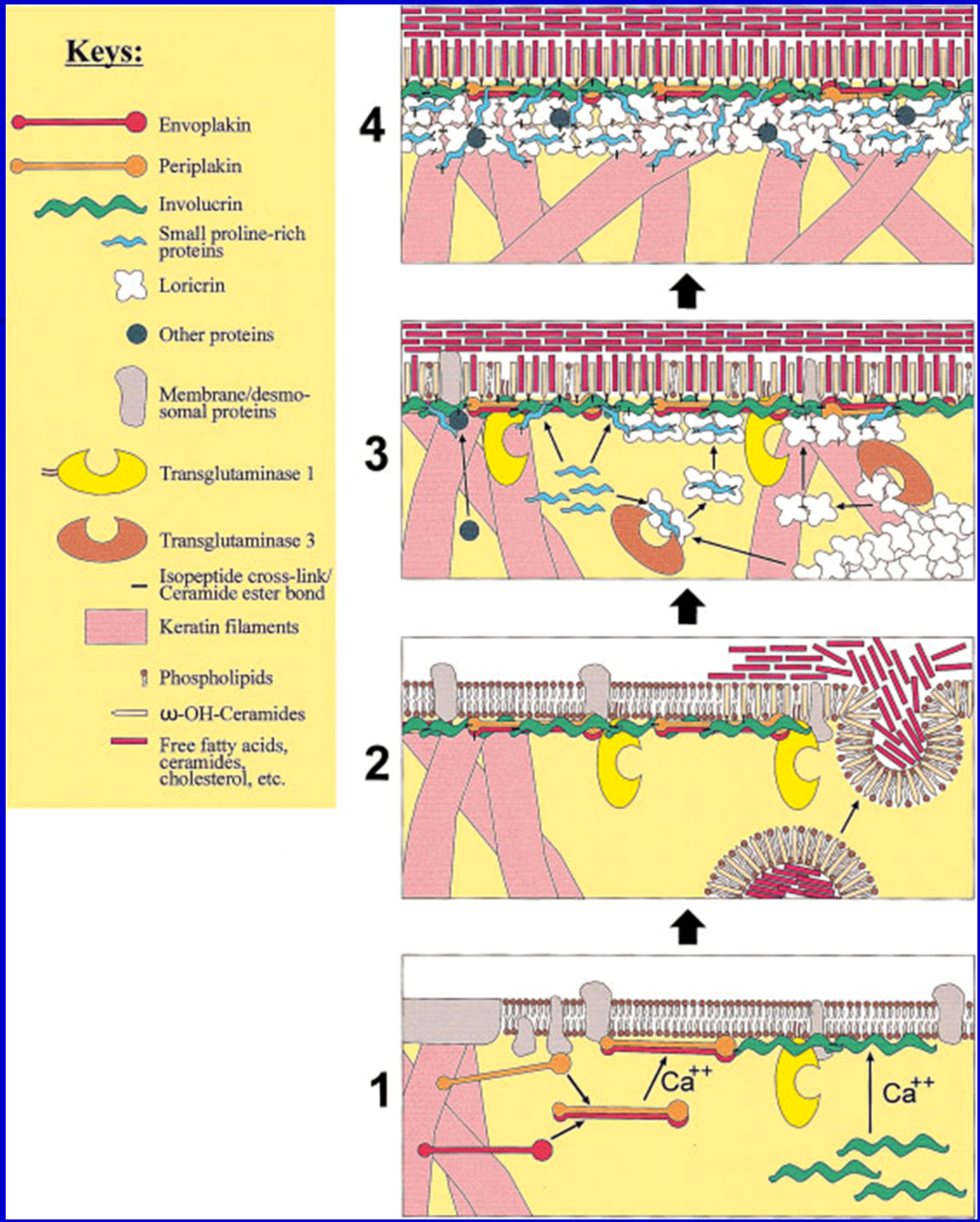
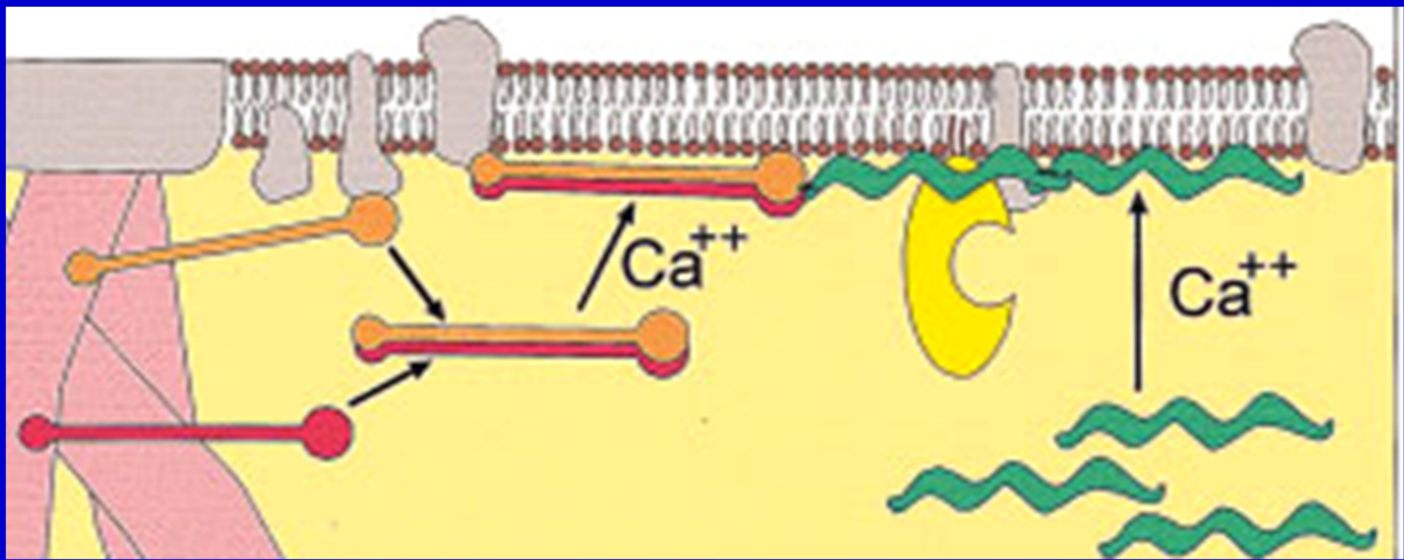


Figure 4 from Kalinin AE, Kajava AV, Steinert PM. Epithelial barrier function: assembly and structural features of the cornified cell envelope. *BioEssays* 2002; 24:796.

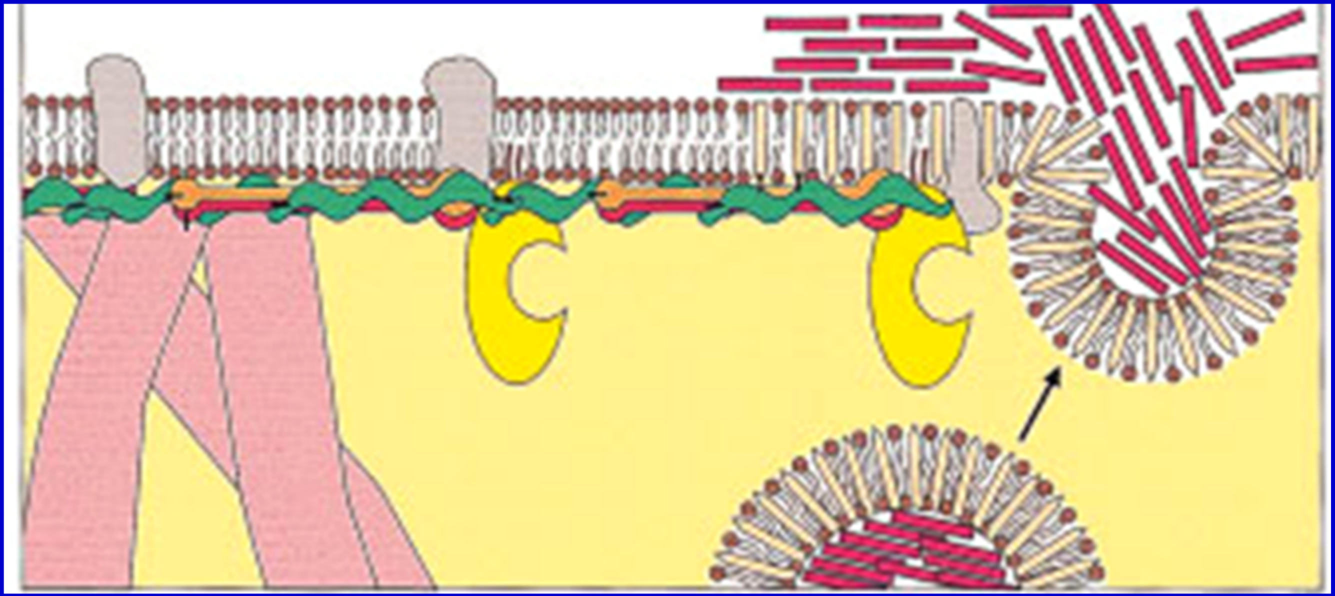
CE Construction

- Envoplakin, periplakin, and involucrin move to cell membrane
- Transglutaminases link involucrin to other proteins to form scaffold



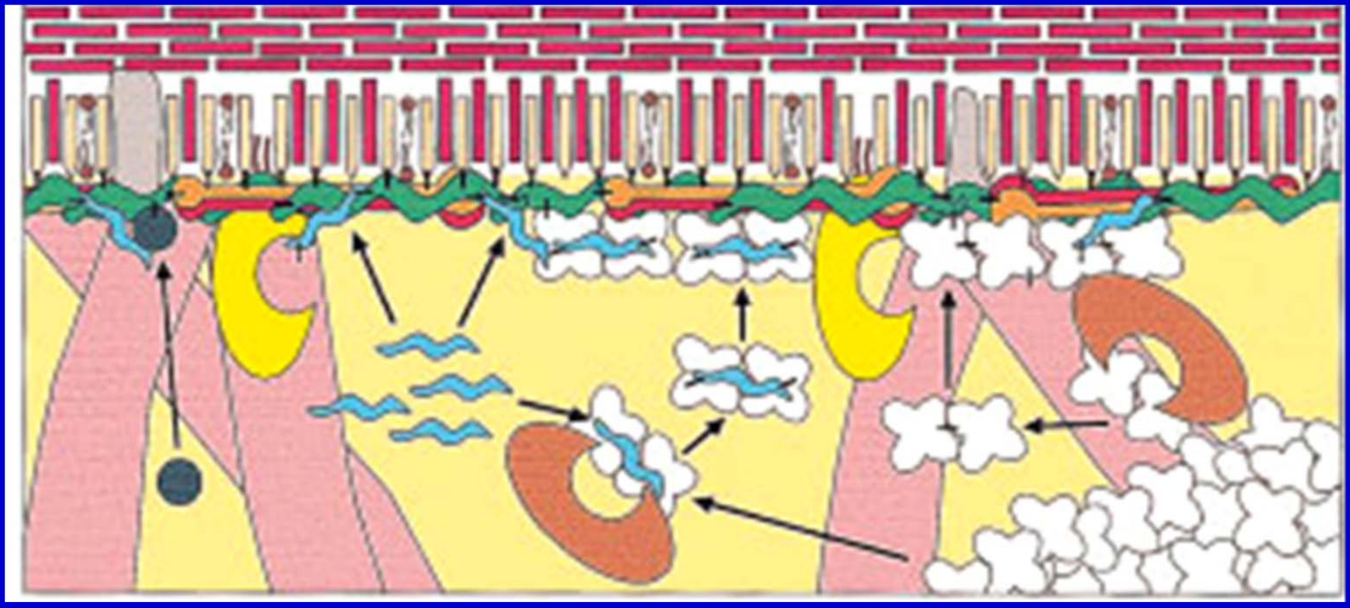
CE Construction

- Lamellar granules (LGs)
  - Move to apical surface of granular cells
  - Fuse with cell membrane
  - Secrete lipids and enzymes



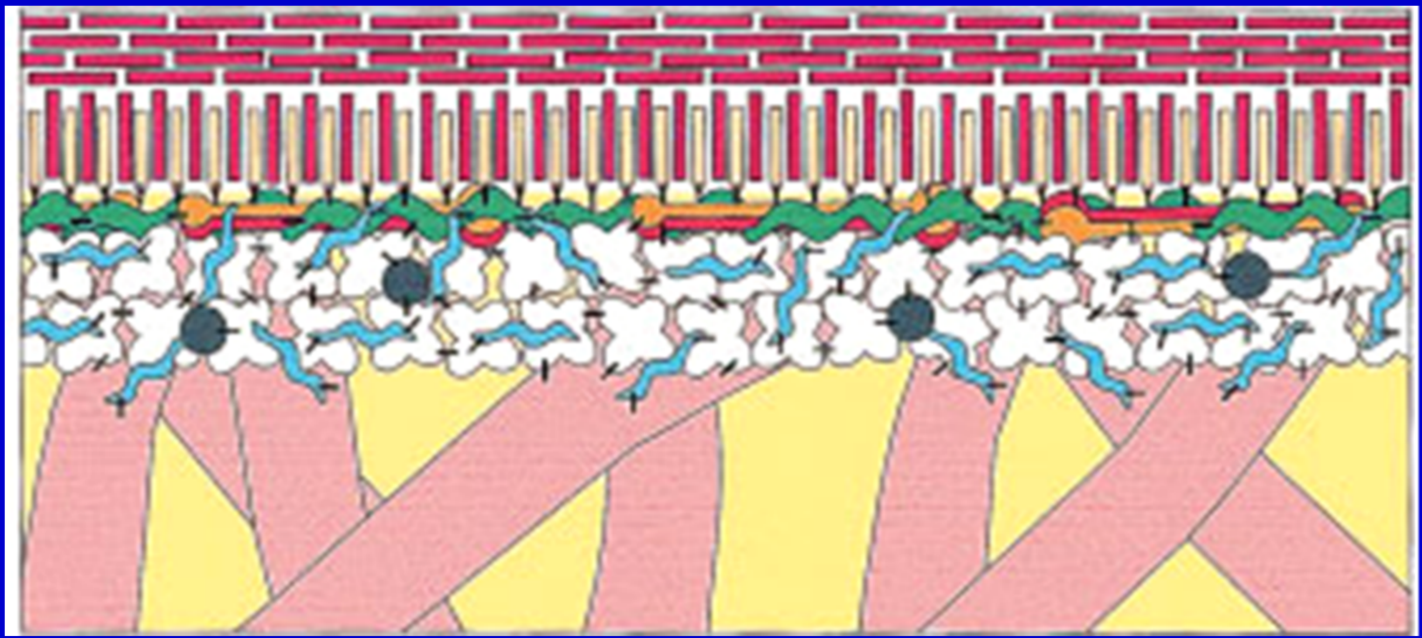
CE Construction

- Transglutaminase 3 links loricrin and SPRs
- Transglutaminase 1 links
  - Loricrin/SPRs and other proteins to involucrin scaffold
  - Involucrin to ω-hydroxyceramides in extracellular lipid layer



CE Construction

- CE replaces cell membrane
- KIFs linked to CE at type II head domains
- Loricrin inside, involucrin outside



CE Construction

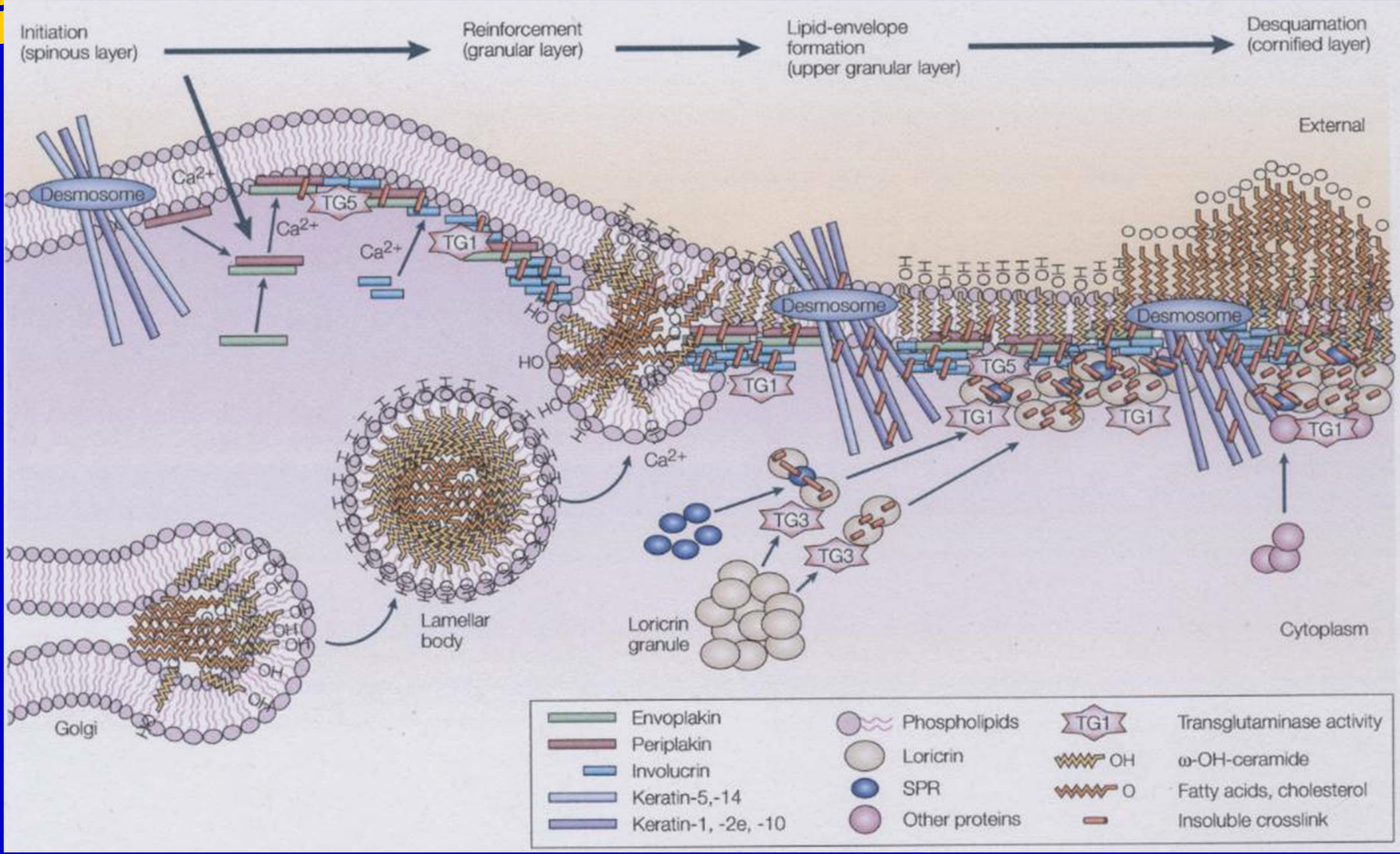


Figure 2 from Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol. 2005 Apr; 6(4):330.

Corneocyte

- Nucleus and organelles degraded
- KIF and filaggrin tightly bundled
- CE replaced plasma membrane
- Desmosomes modified into corneodesmosomes

Corneocyte

Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte adhesion
- Desquamation
- Epidermal barrier function

Stratum Corneum

- Corneocytes embedded in extracellular lipid matrix

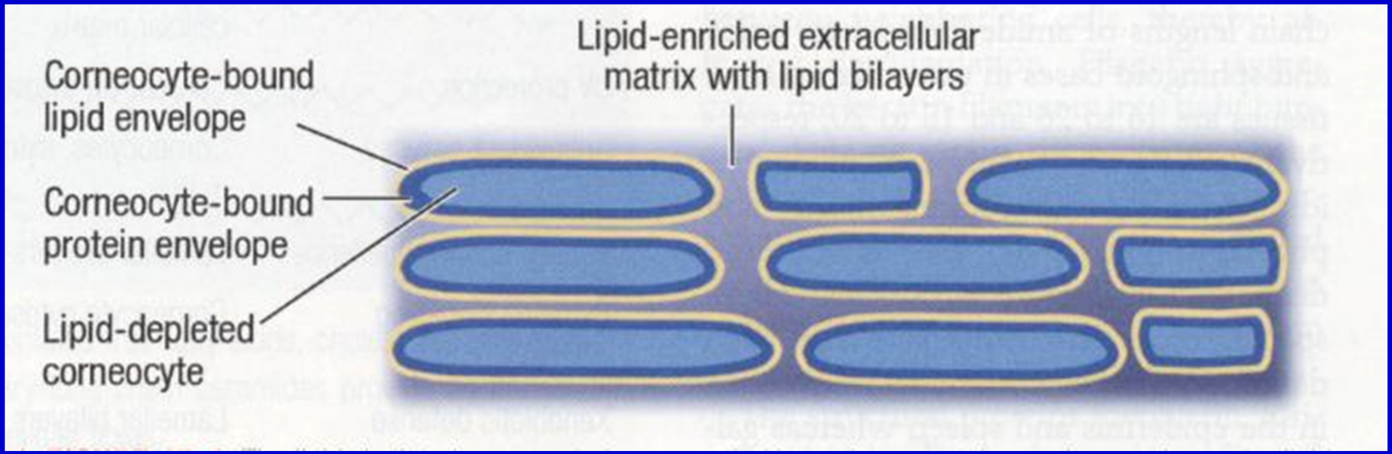


Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

Interface of Stratum Granulosum and Stratum Corneum

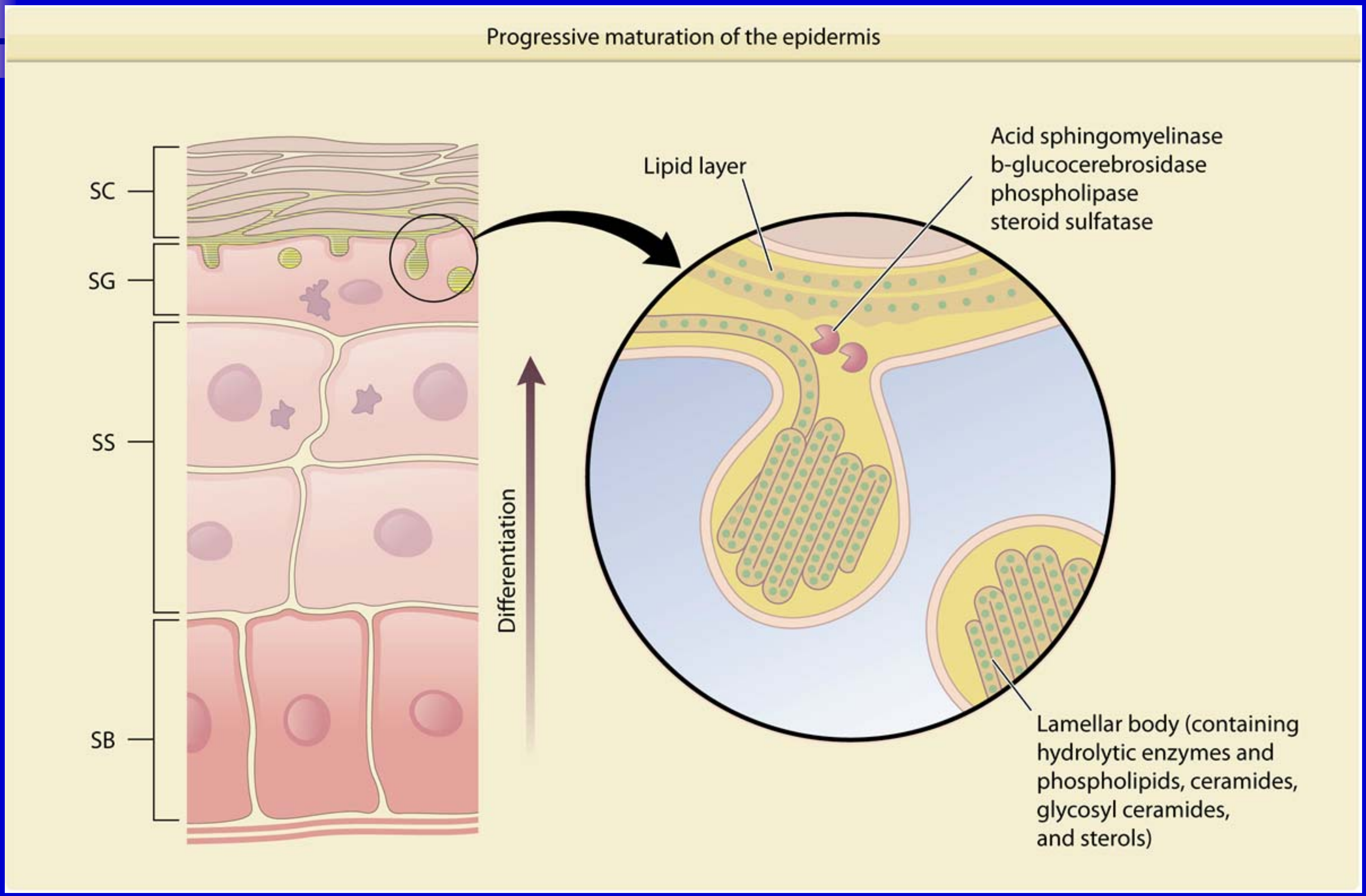


Figure 47-5 from Proksch E, Jensen JM. Skin as an Organ of Protection. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 489.

Extracellular Lipid Matrix

- Stratum corneum lipids
  - Ceramides
  - Free fatty acids
  - Cholesterol

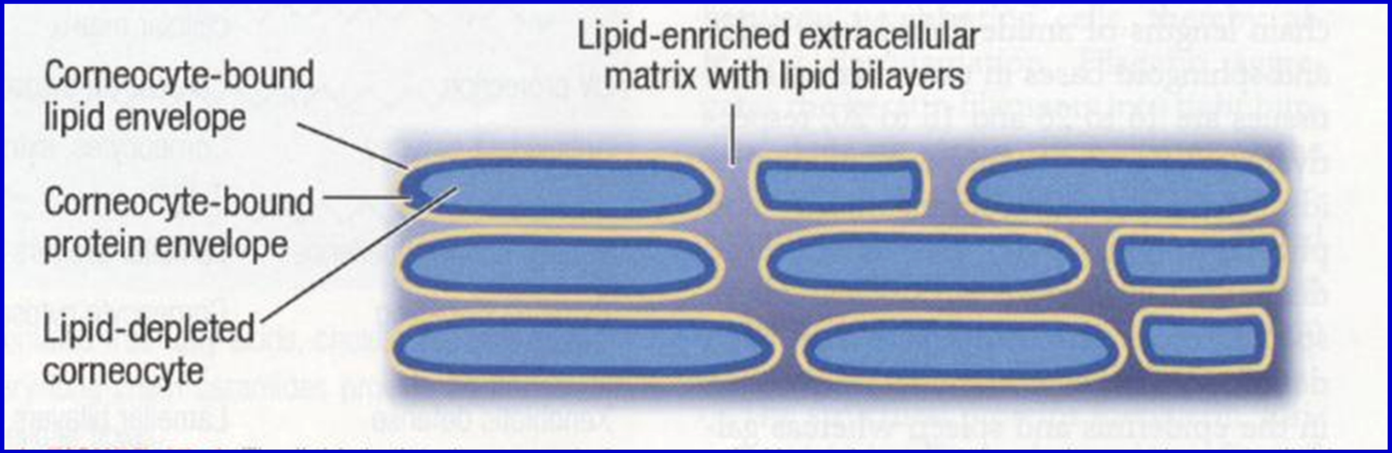
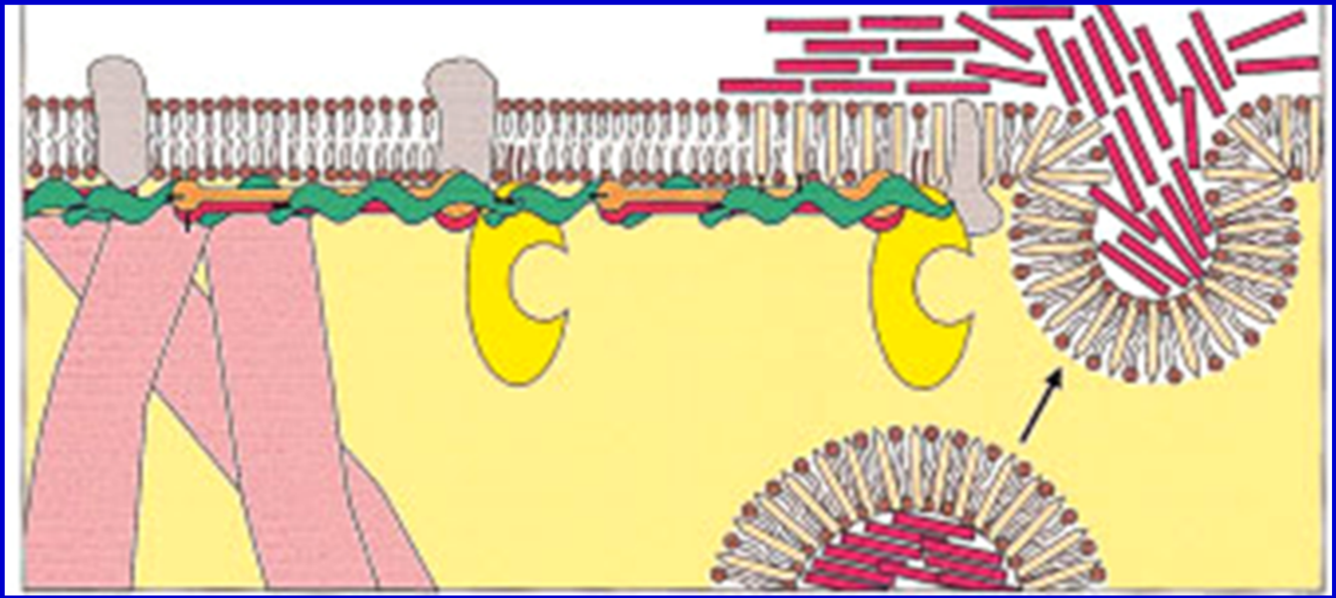


Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

CE and Extracellular Lipid Matrix Construction

- Lamellar granules (LGs)
  - Move to apical surface of granular cells
  - Fuse with cell membrane
  - Secrete lipids and enzymes



Extracellular Lipid Matrix Construction

- Increase in extracellular calcium concentration signals lamellar granule secretion
- Secreted lipids metabolized from polar to nonpolar products

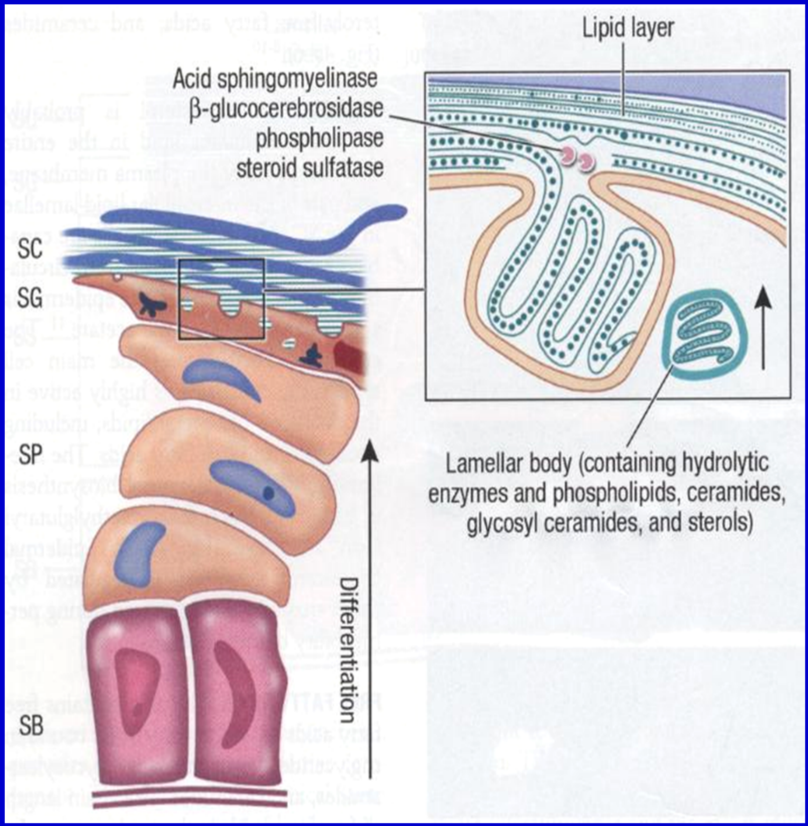
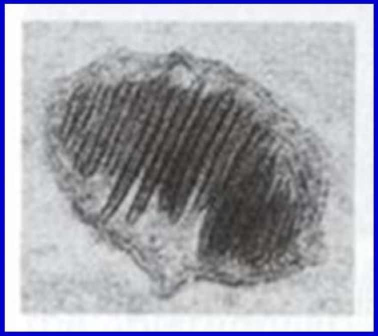


Figure 45-5 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. p. 386.

Lamellar Granules (LG)



- First described in detail in 1960s
- Unique to keratinizing epithelia
- Arise from Golgi apparatus
- Appear in spinous layer, accumulate in granular layer, and extrude contents at stratum granulosum/corneum interface
- Contain stacks of lipid vesicles

Figure 7-5 from Chu DH. Development and structure of skin. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 61.

Stratum Granulosum/Corneum

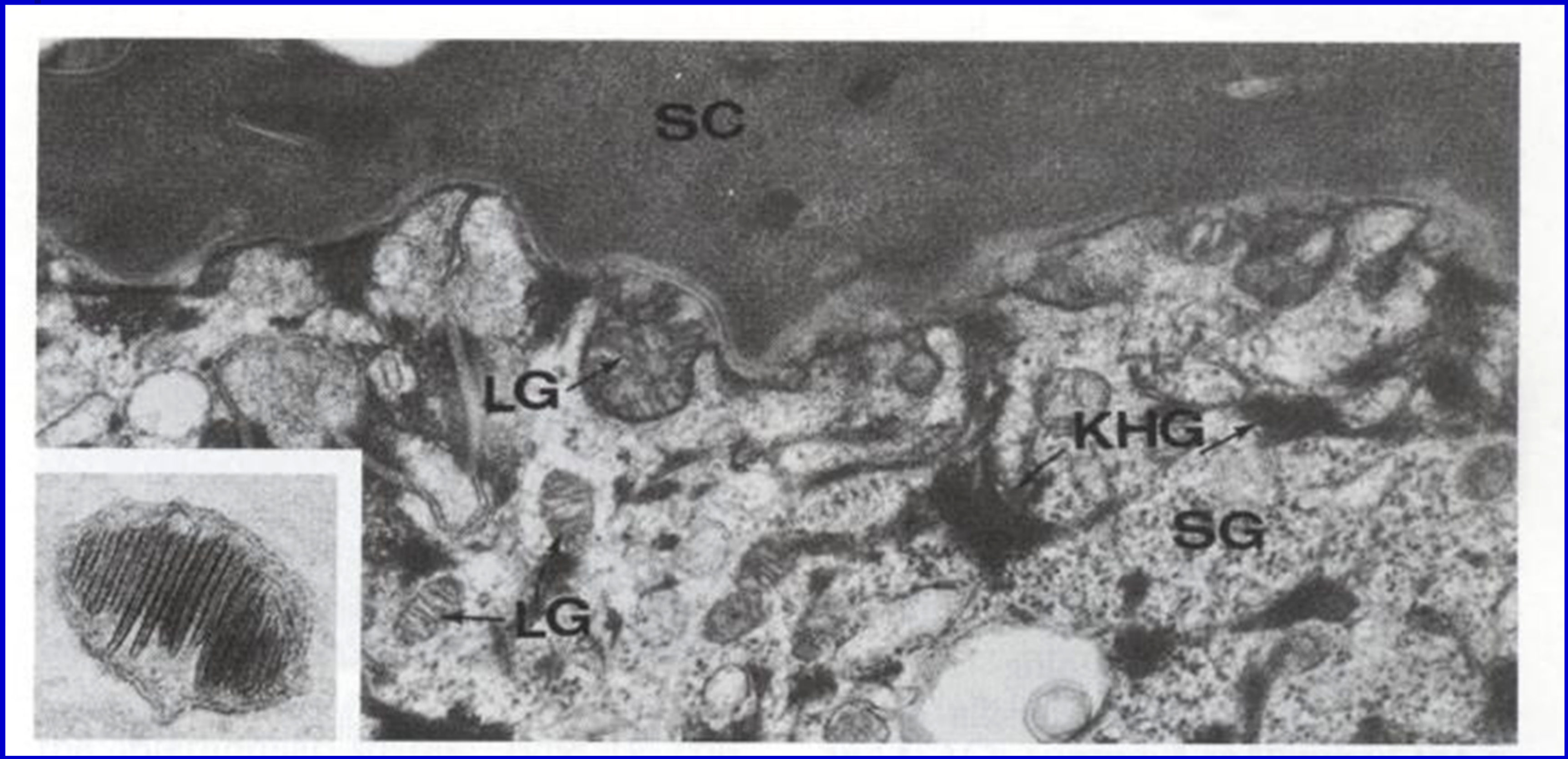


Figure 7-5 from Chu DH. Development and structure of skin. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 61.

LG Formation and Fusion

- Proteins involved in membrane trafficking
  - CHEVI tethering complex - C Homologues in Endosome-Vesicle Interaction
    - VPS33B and VIPAR
  - Rab11a – small GTPase
  - SNAREs - soluble N-ethylmaleimide-sensitive factor attachment protein receptor
    - v-SNAREs – on vesicular membrane
    - t-SNAREs – on target membrane

LG Trafficking via CHEVI complex

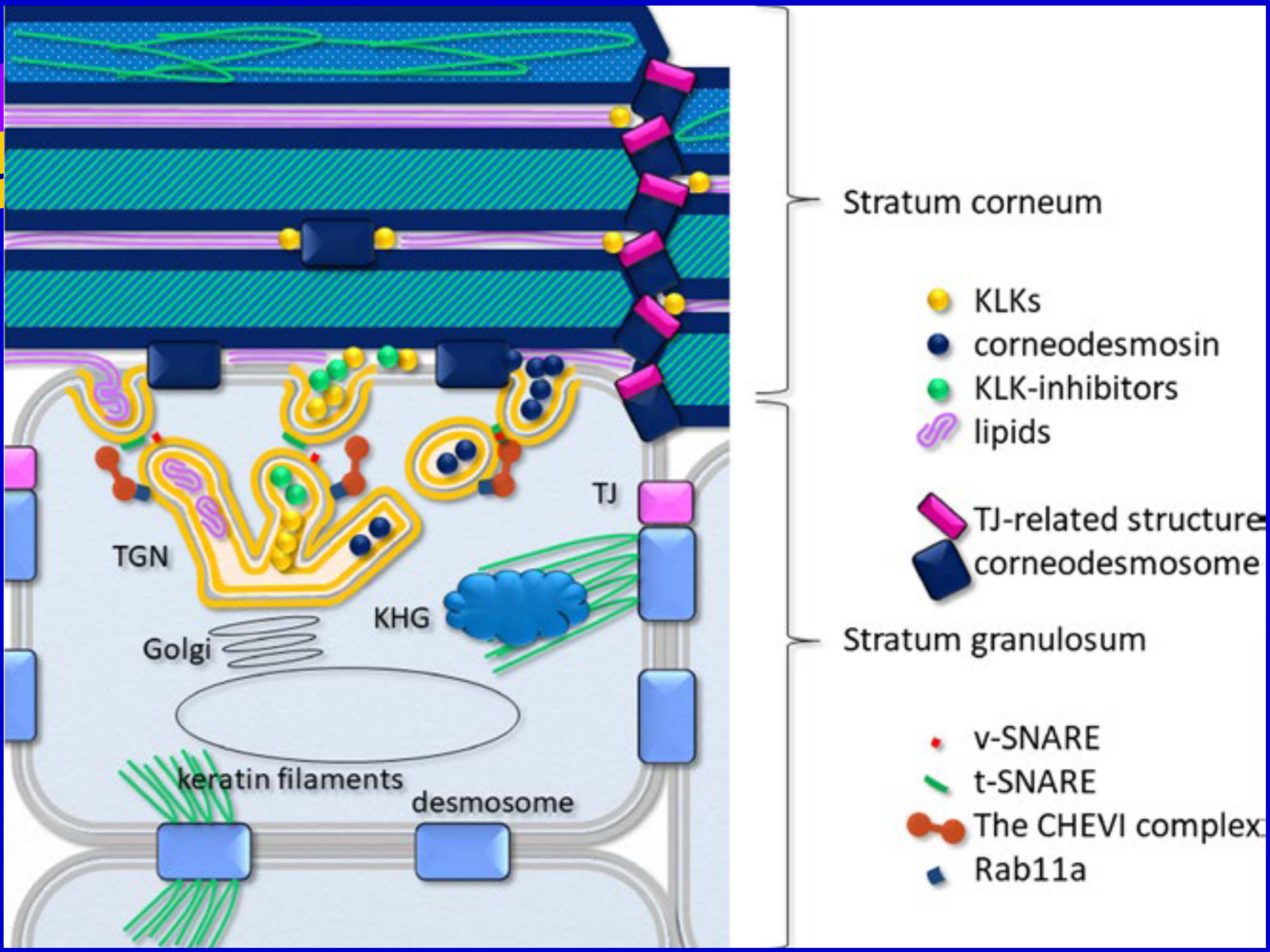
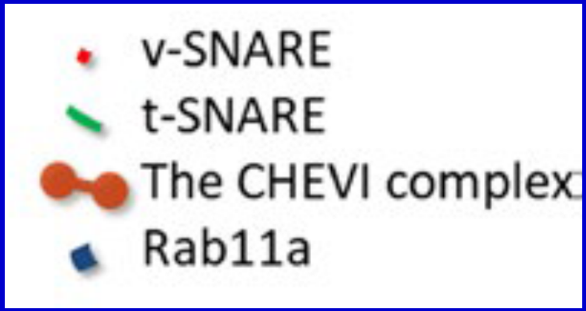
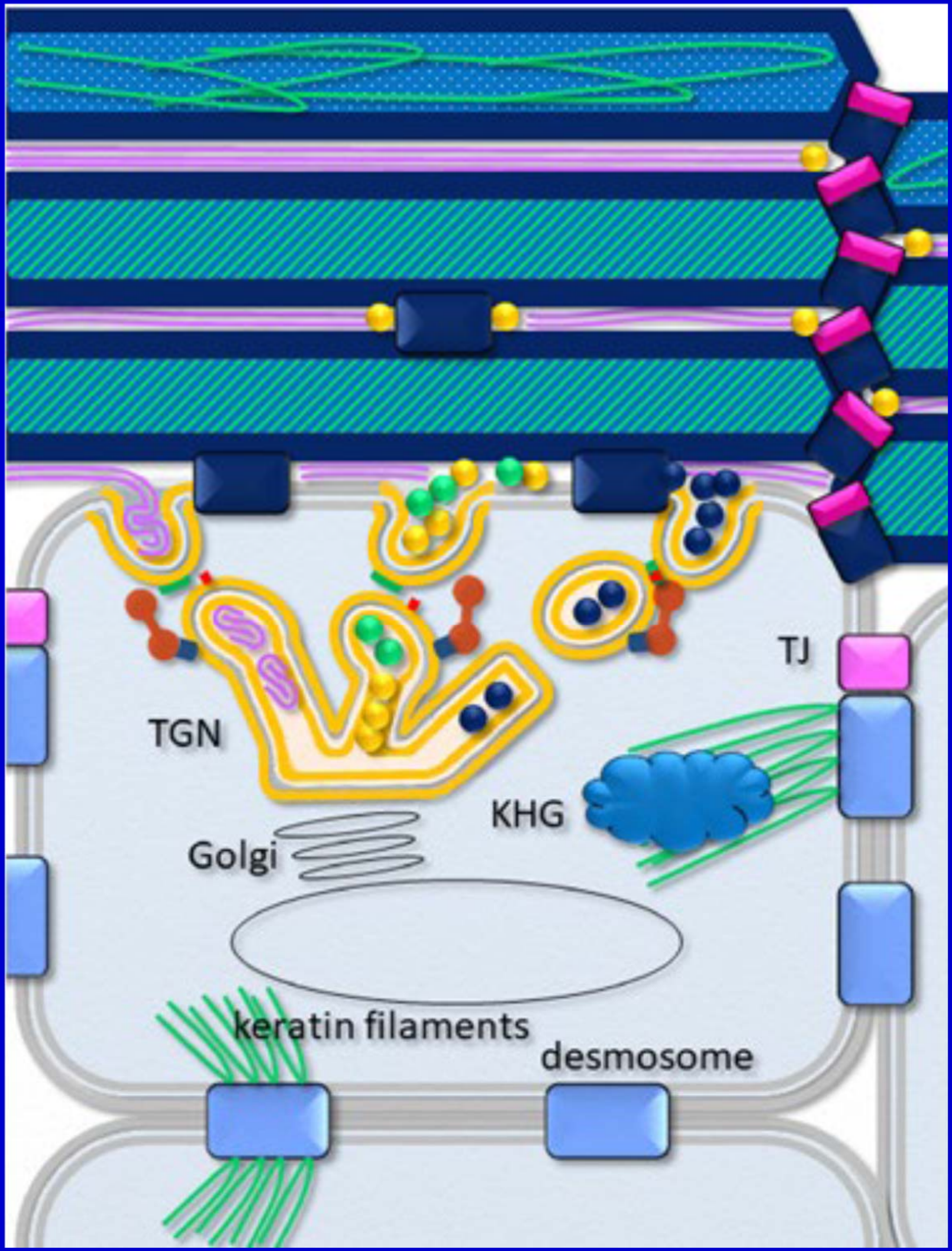


Figure 1 from Ishida-Yamamoto A, et al. Molecular basis of the skin barrier structures revealed by electron microscopy. *Exp Dermatol.* 2018;27:842.

LG Fusion



- Localization of cargo



LG Fusion

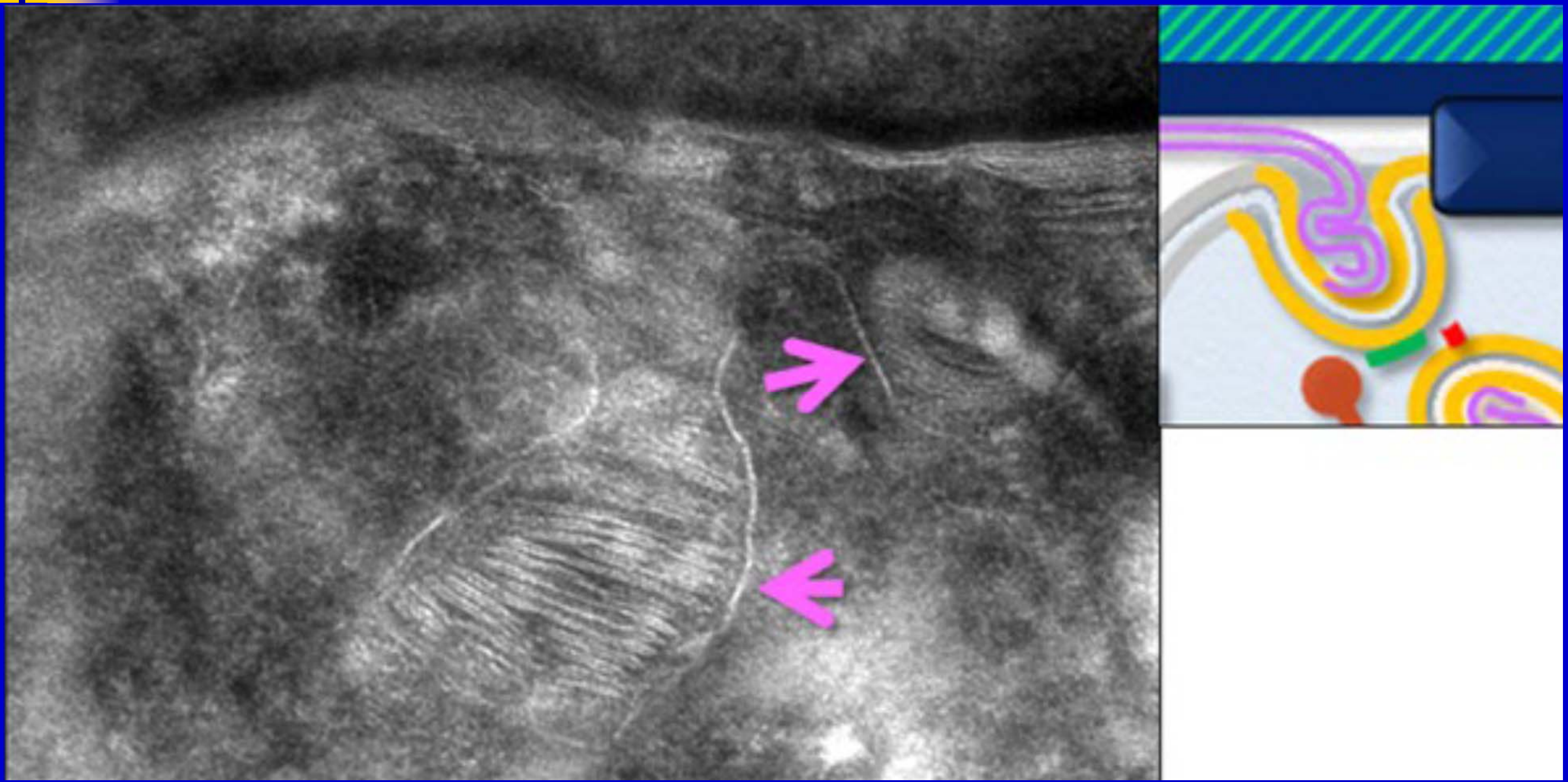


Figure 3A from Ishida-Yamamoto A, Igawa S, Kishibe M. Molecular basis of the skin barrier structures revealed by electron microscopy. *Exp Dermatol.* 2018;27:842.

Lamellar Granules

- Discrete organelle, or tubuloreticular network?
- Key precursor of permeability barrier

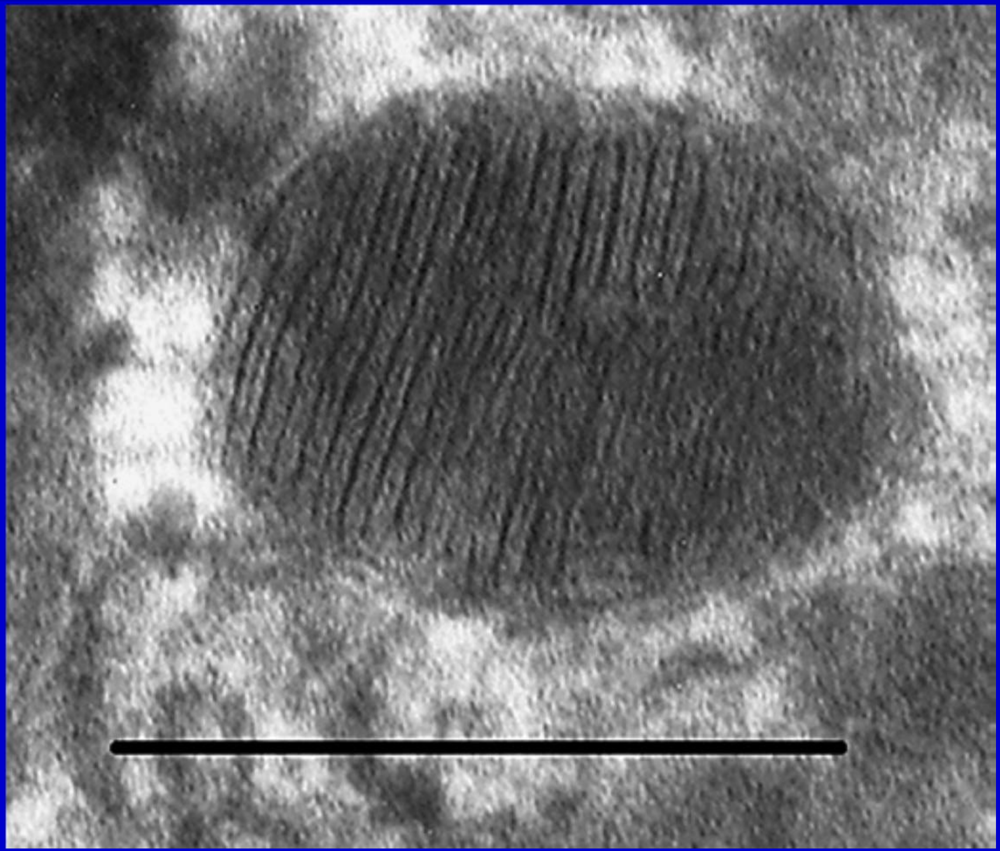


Figure 1 from Wertz P. Epidermal Lamellar Granules. *Skin Pharmacol Physiol* 2018;31:263. Scale bar 200 nm.

Contents of LGs

- Lipid precursors
  - Glucosylceramides
  - Sphingomyelin
  - Phospholipids
- Desquamation proteases
  - Kallikreins
  - Cathepsins
- Lipid hydrolases
  - β-glucocerebrosidase
  - Acid sphingomyelinase
  - Phospholipase A_2
- Corneodesmosin
- Steroid sulfatase
- Antimicrobial peptides

Extracellular Matrix Lipid Metabolism

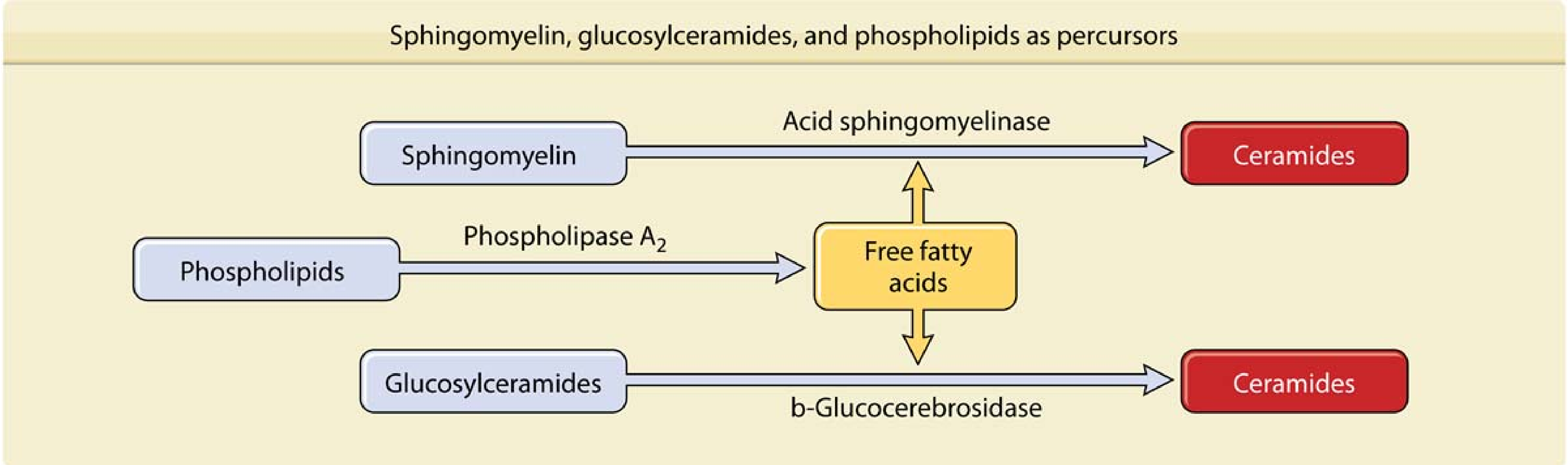


Figure 47-7 from Proksch E, Jensen JM. Skin as an Organ of Protection. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 491.

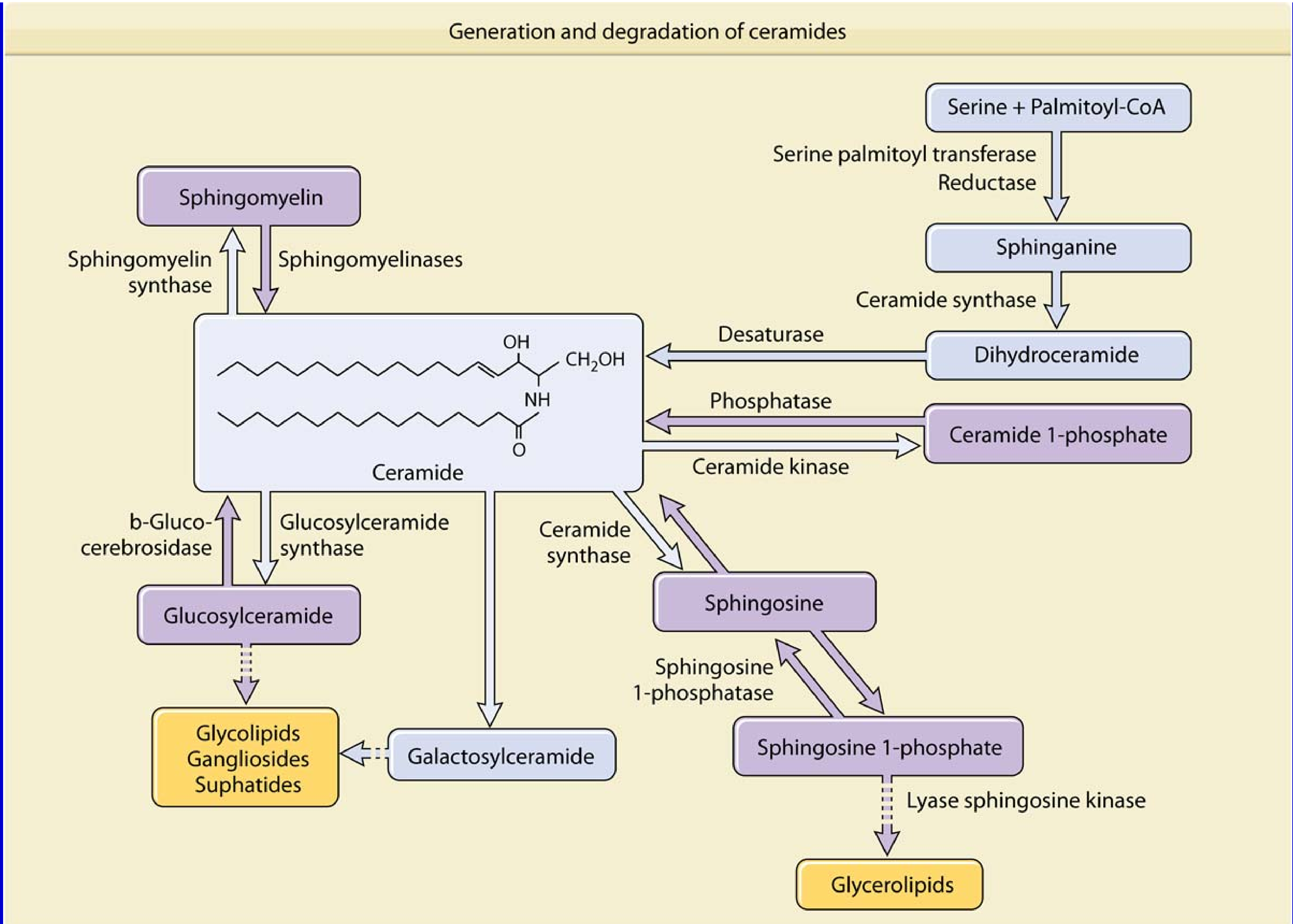


Figure 47-8 from Proksch E, Jensen J. Skin as an Organ of Protection. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 491.

Extracellular Lipid Matrix

- Ceramides in cornified layer
  - From precursors
    - Glucosylceramides
    - Sphingomyelin
  - Major lipid component of stratum corneum
    - Minor in other tissues

Extracellular Lipid Matrix

- Stratum corneum lipids
  - Ceramides
  - Free fatty acids
  - Cholesterol

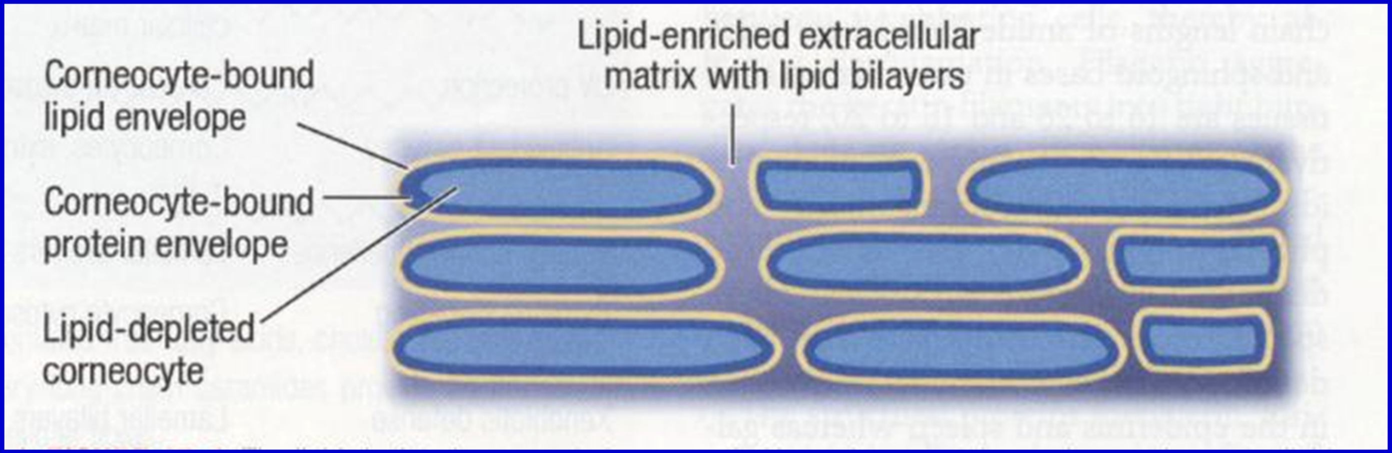


Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

Ceramide Structure

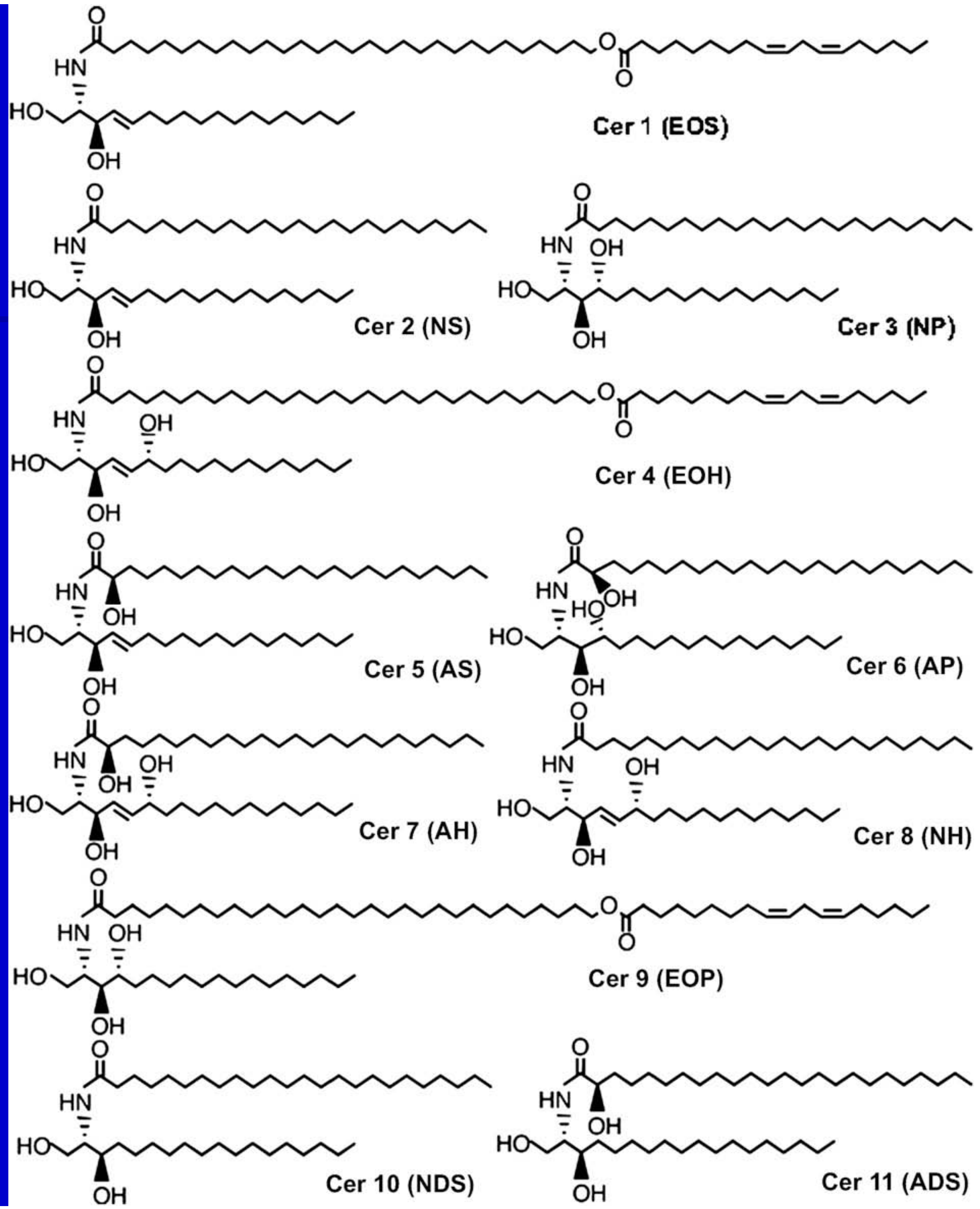
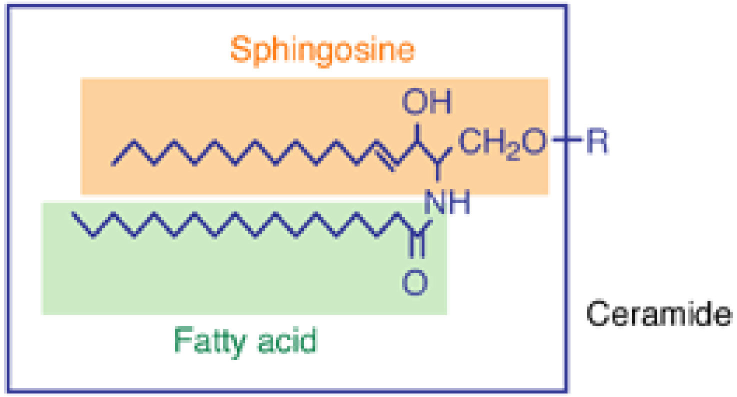


Figure 2 from Mizutani Y, Mitsutake S, Tsuji K, et al. Ceramide biosynthesis in keratinocyte and its role in skin function. *Biochimie* 2009; 91:786.

Ceramide Structure

- What is a ceramide?
  - Amide-linked fatty acid attached to sphingoid base
  - Type of sphingolipid
    - Sphingolipids ubiquitous in mammalian tissues with tissue-specific distribution



Substituent (R)	Sphingolipid
H	Ceramide
Phosphocholine	Sphingomyelin
Sugar(s)	Glycosphingolipid

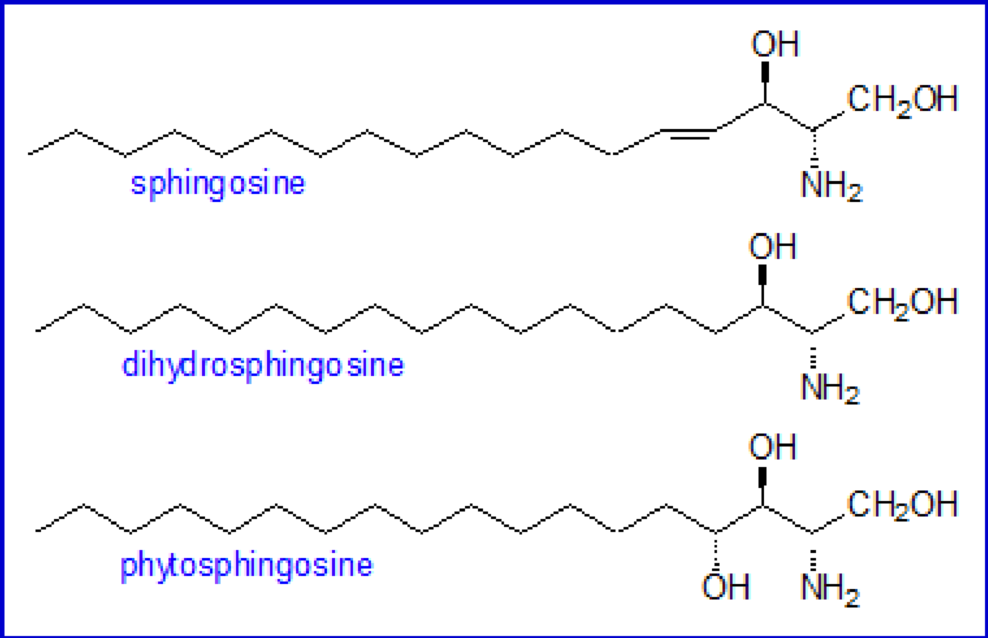
General sphingolipid structure

Expert Reviews in Molecular Medicine

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

Ceramide Structure

- Sphingoid base
  - Long-chain amino alcohol



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

Ceramide Structure

- 11 structural categories
  - Determined by combination of sphingoid base and fatty acids
- Numerical nomenclature for categories
  - Ceramide 1, 2, 3, …

Ceramide Structure

- Sphingoid bases
  - P=phytosphingosine
  - S=sphingosine
  - H=6-hydroxylsphingosine
  - D=dihydrosphingosine
- Fatty acids
  - EO=esterified ω-hydroxy fatty acids
  - A=α-hydroxy fatty acids
  - N=nonhydroxy fatty acids

Ceramide Structure

Exclusively in stratum corneum

Human hairs and stratum corneum

Sphingoid / Fatty acid	6-hydroxy sphingosine [H]	Phytosphingosine [P]	Sphingosine [S]	Dihydrosphingosine [D]
Esterified ω-hydroxy fatty acid [EO]	CER[EOH]	CER[EOP]	CER[EOS]	CER[EODS] (Not detected in SC)
α-hydroxy fatty acid [A]	CER[AH]	CER[AP]	CER[AS]	CER[ADS]
Nonhydroxy fatty acid [N]	CER[NH]	CER[NP]	CER[NS]	CER[NDS]

Figure 4 from: Nishifuji K, Yoon JS. The stratum corneum: the rampart of the mammalian body. *Vet Dermatol* 2013; 24:66.

Ceramide Structure

Exclusively in stratum corneum

Human hairs and stratum corneum

Sphingoid / Fatty acid	6-hydroxy sphingosine [H] 	Phytosphingosine [P] 	Sphingosine [S] 	Dihydrosphingosine [D] 
Esterified ω-hydroxy fatty acid [EO] 	CER[EOH] 4	CER[EOP] 9	CER[EOS] 1	CER[EODS] (Not detected in SC)
α-hydroxy fatty acid [A] 	CER[AH] 7	CER[AP] 6	CER[AS] 5	CER[ADS] 11
Nonhydroxy fatty acid [N] 	CER[NH] 8	CER[NP] 3	CER[NS] 2	CER[NDS] 10

Figure 4 from: Nishifuji K, Yoon JS. The stratum corneum: the rampart of the mammalian body. *Vet Dermatol* 2013; 24:66.

ω-hydroxyceramides

- EOS/Cer 1, EOH/Cer 4, EOP/Cer 9
  - Unique to stratum corneum
  - Very long chain fatty acids
  - Linoleic acid (ω-6 EFA) is component
  - Bonded to involucrin in CE and interdigitate with other lipids
  - Key to stratum corneum barrier function

Corneocyte Lipid Envelope

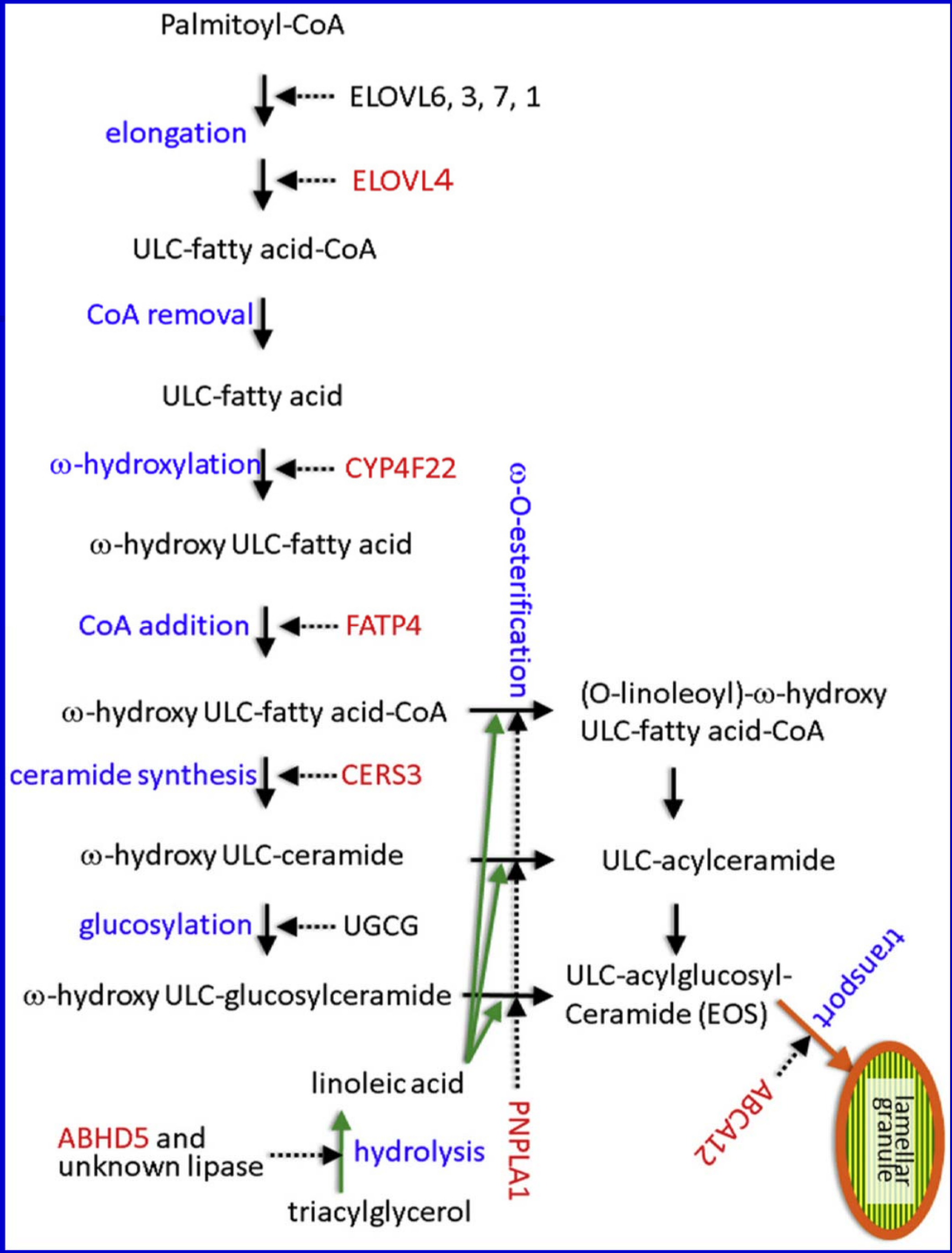
- Layer of ω-hydroxyceramides bonded to CE
- Forms scaffold for intercellular lipid layers of stratum corneum
- Key structure for skin barrier function and ichthyosis pathogenesis

  - Akiyama M. Corneocyte lipid envelope (CLE), the key structure for skin barrier function and ichthyosis pathogenesis. *Journal of Dermatological Science* 2017; 88:3-9.

EOS/Cer 1 Synthesis

- Dotted arrows: key enzymes
- Deficiencies that cause ichthyoses in red
- Green: linoleic acid supply

Akiyama M. Corneocyte lipid envelope (CLE), the key structure for skin barrier function and ichthyosis pathogenesis. *Journal of Dermatological Science* 2017; 88:6.



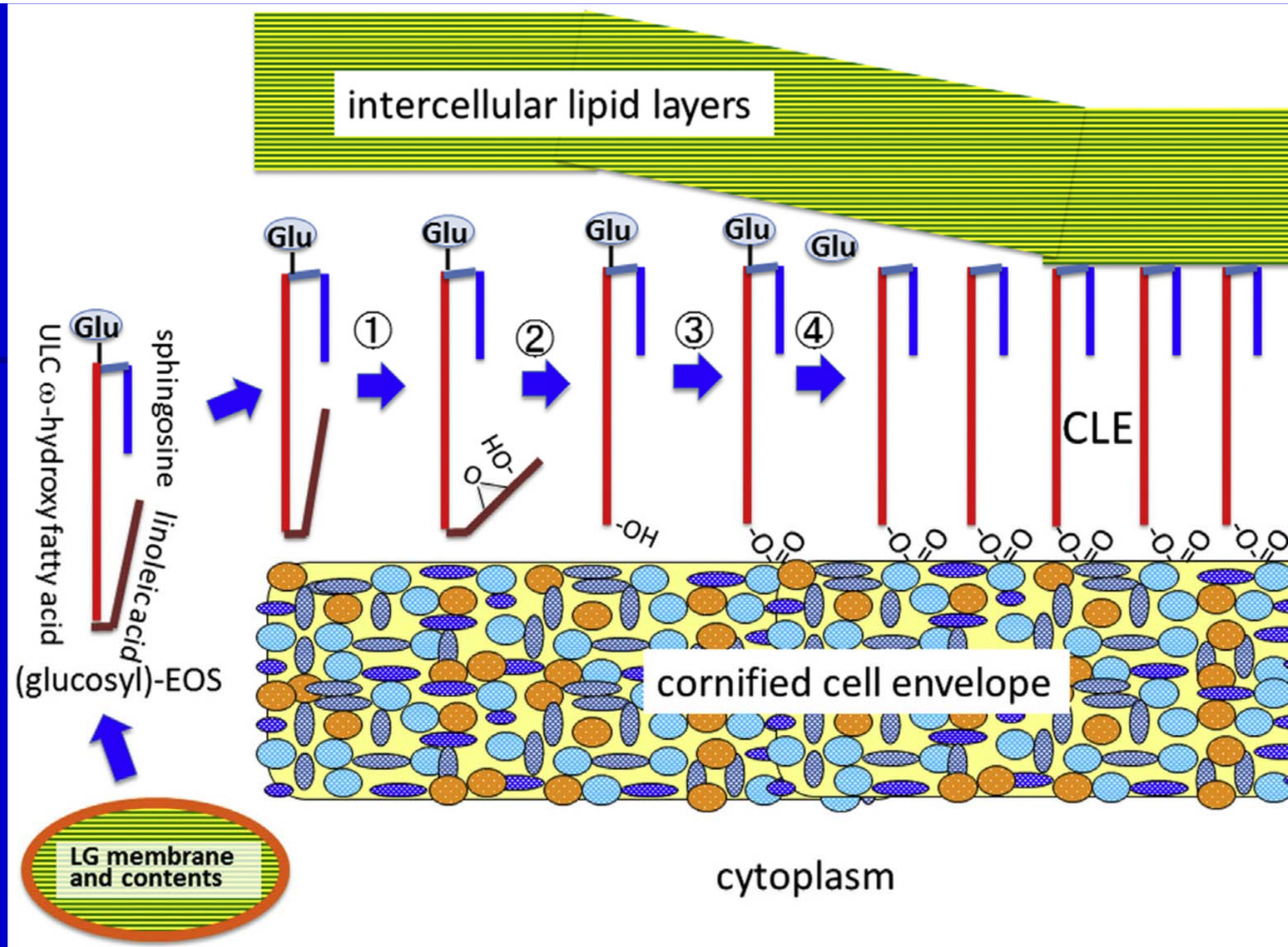


Fig. 3. The formation of the CLE during the late differentiation of keratinocytes: ① the oxidation of linoleic acid in ULC-acylglucosylceramide, ② the hydrolysis of oxidized linoleic acid, ③ the covalent linking of ULC-glucosylceramide to the outer surface of the CCE, ④ the deglucosylation of glucosylceramide

Akiyama M. Corneocyte lipid envelope (CLE), the key structure for skin barrier function and ichthyosis pathogenesis. *Journal of Dermatological Science* 2017; 88:6.

Extracellular Lipid Matrix

- Stratum corneum lipids
  - Ceramides
  - Free fatty acids
  - Cholesterol

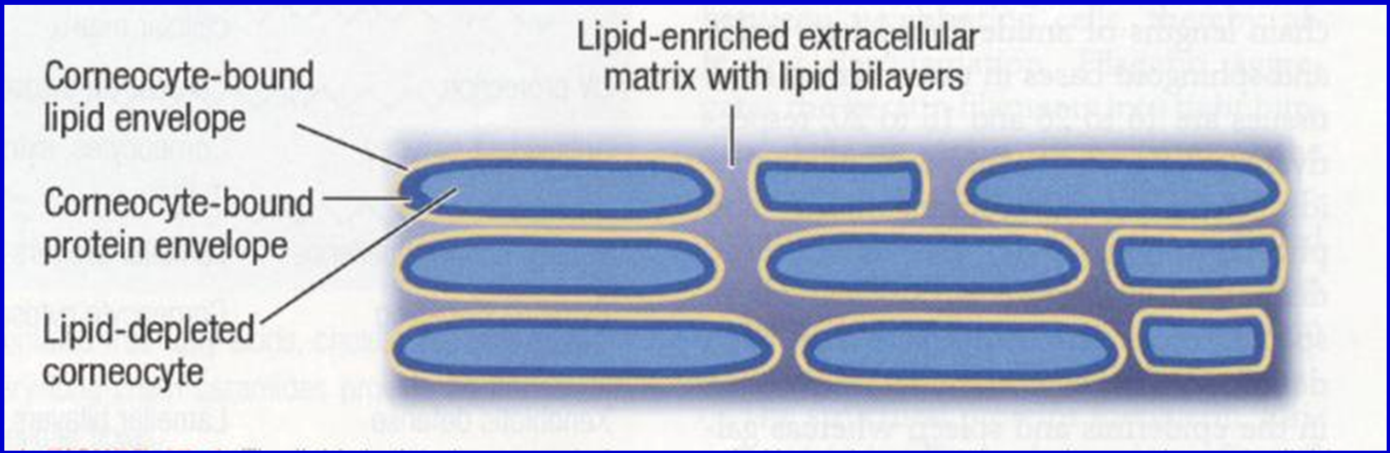


Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

Extracellular Matrix Lipid Metabolism

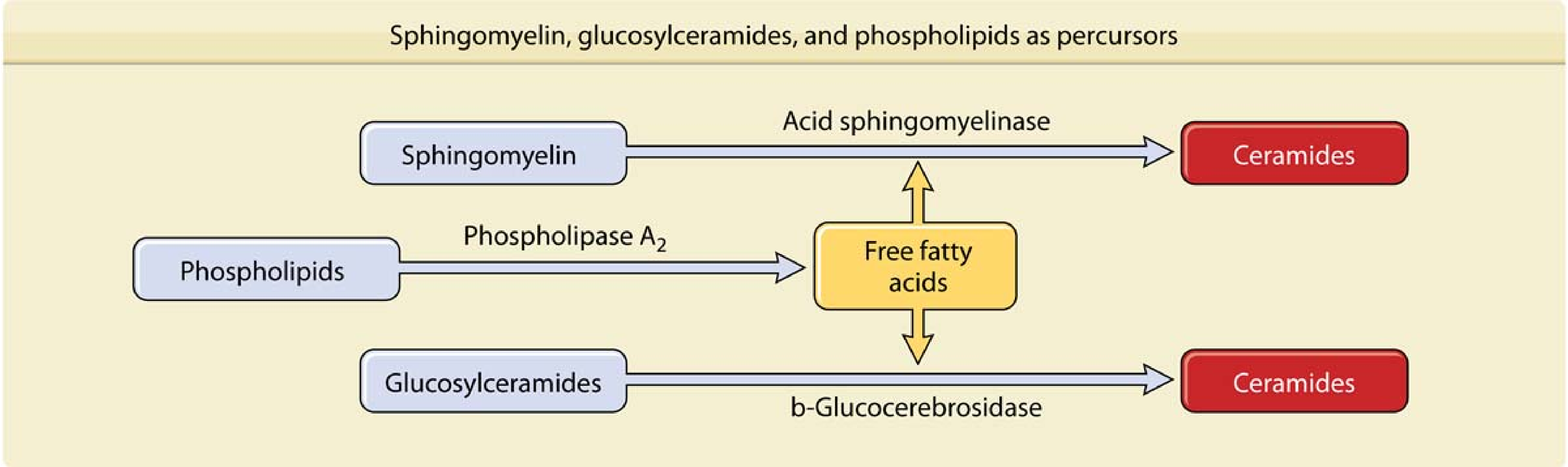


Figure 47-7 from Proksch E, Jensen JM. Skin as an Organ of Protection. In: Goldsmith, et al, Fitzpatrick's Dermatology in General Medicine, 8th edition. 2012, p 491.

Extracellular Lipid Matrix

- Phospholipids → free fatty acids and glycerol
- Free fatty acids
  - Acidify stratum corneum – assists enzymes
    - β-glucocerebrosidase
    - Acid sphingomyelinase
- Glycerol – helps with hydration

Extracellular Lipid Matrix

- Stratum corneum lipids
  - Ceramides
  - Free fatty acids
  - Cholesterol

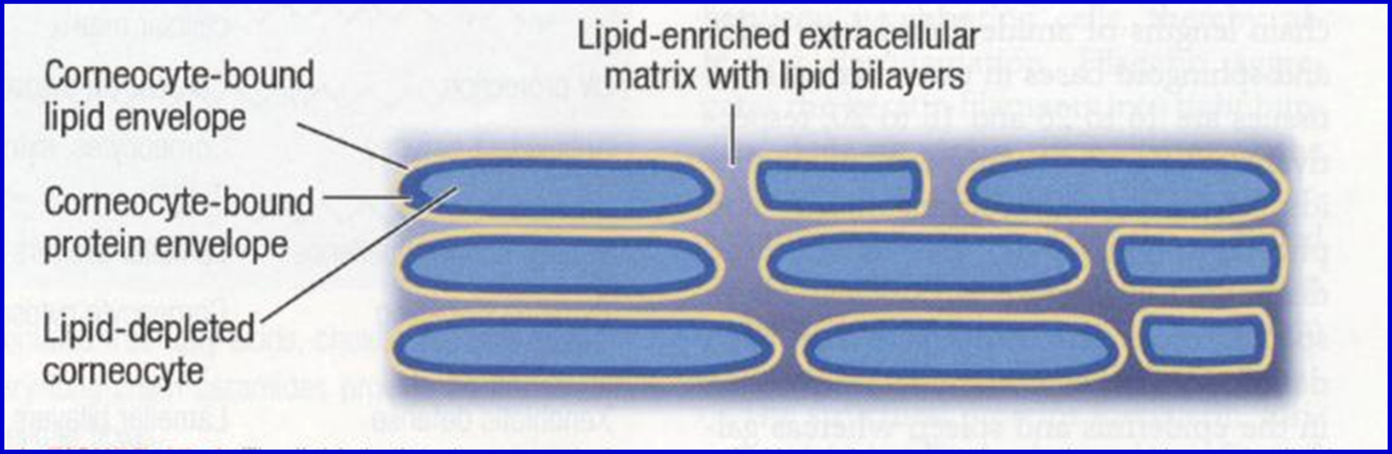


Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

Extracellular Lipid Matrix

- Cholesterol
  - Synthesized in lower epidermis
  - Some incorporated into LGs and secreted unchanged
  - Some converted to cholesterol sulfate
    - Cholesterol sulfate impairs desquamation
    - Metabolized to cholesterol by steroid sulfatase

Extracellular Lipid Matrix Construction

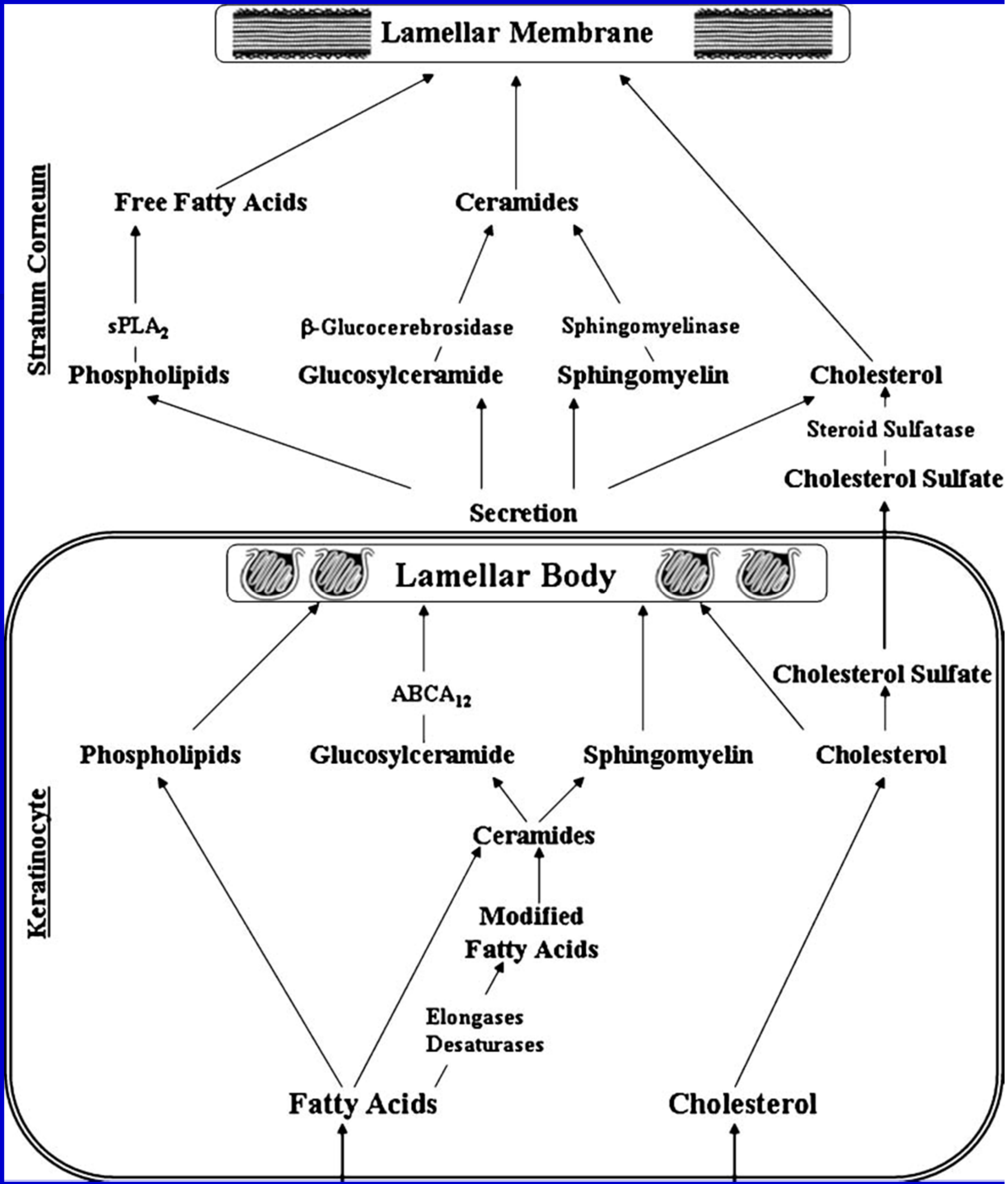


Figure 1 from Pathways for the formation of the extracellular lamellar lipid membranes that provide for the permeability barrier. From Feingold KR. The role of epidermal lipids in cutaneous permeability barrier homeostasis. *J Lipid Res* 2007; 48: 2533.

Stratum Corneum

- Corneocytes embedded in extracellular lipid matrix

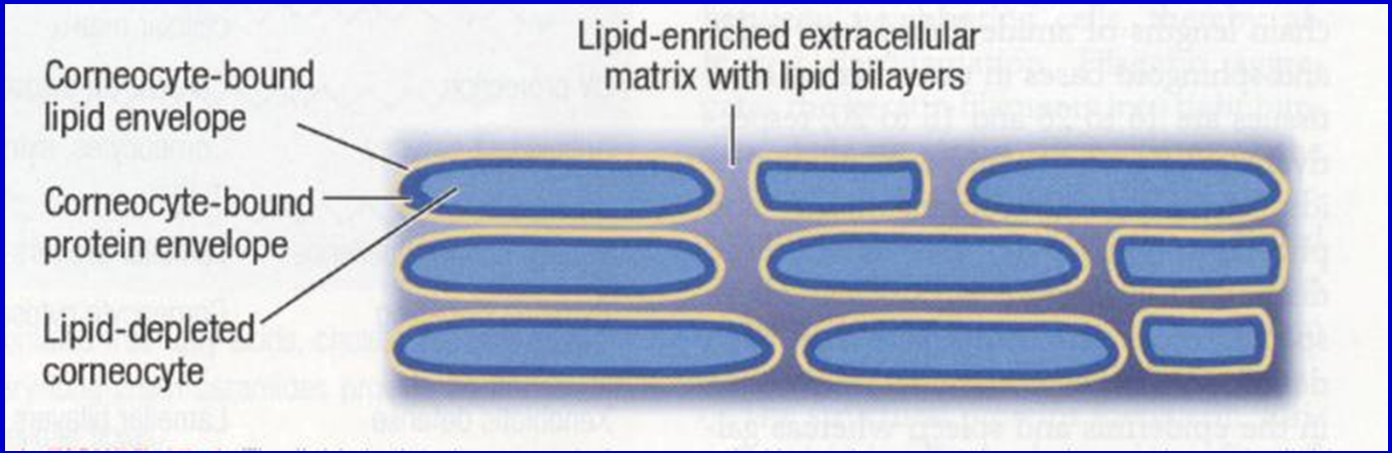
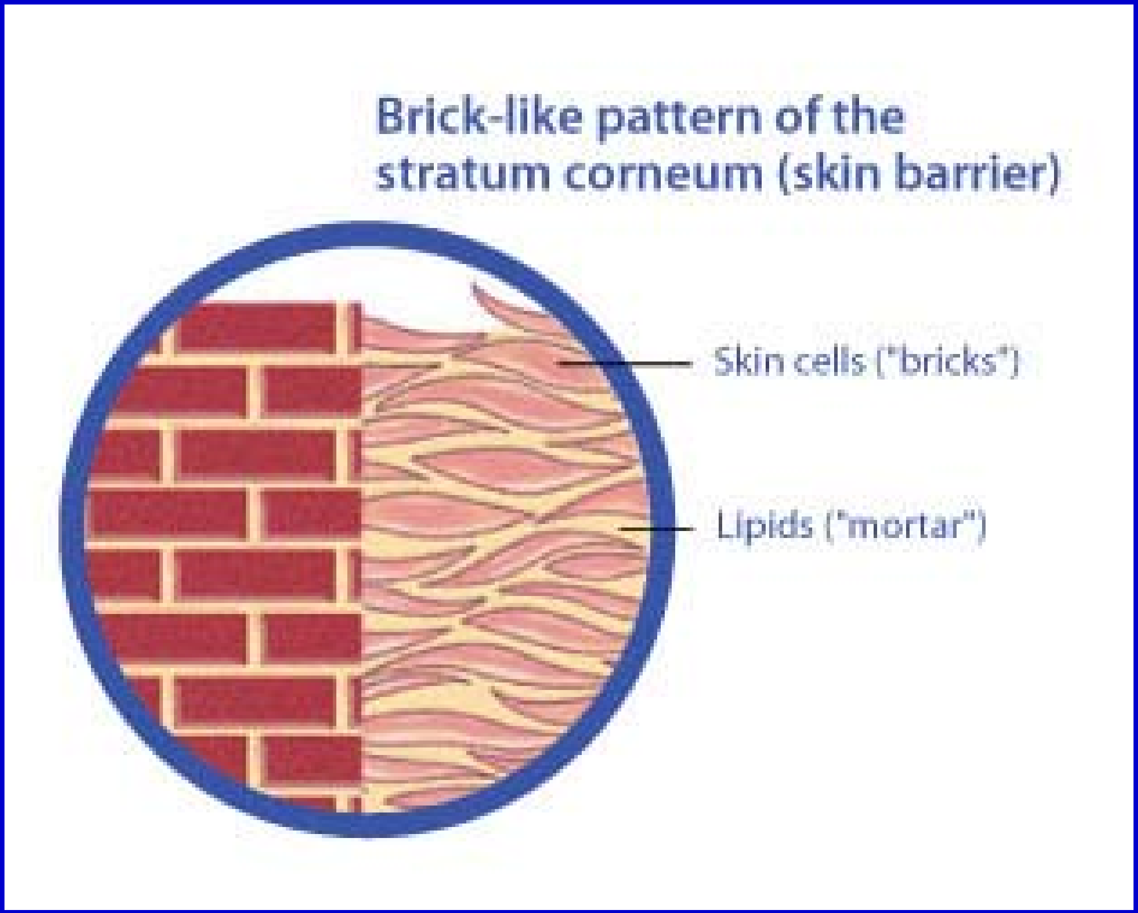


Figure 45-4 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. New York: McGraw-Hill Companies, Inc. 2008. p. 385.

Stratum Corneum: “Bricks and Mortar”



www.cerave.com/barrier/WHPSK507barrierV4_14.jpg

Stratum Corneum: “M&Ms and Caramel”


Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte adhesion
- Desquamation
- Epidermal barrier function

Layers of the Epidermis

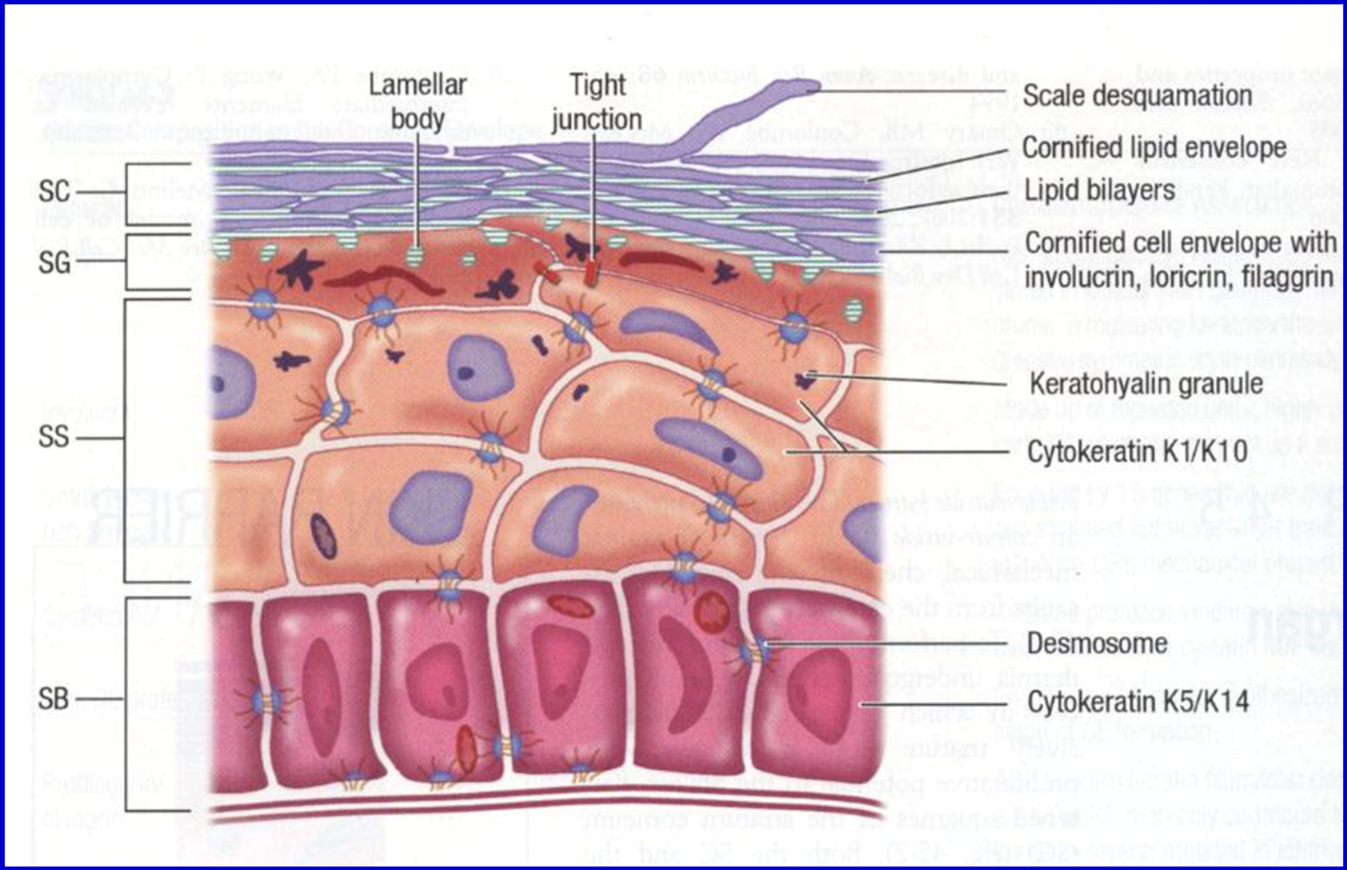


Figure 45-2 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. p. 384.

Keratinocyte Adhesion

- Desmosomes
- Corneodesmosomes
- Tight junctions
- Adherens junctions

Desmosomes

- Sites of intercellular adhesion
- Link neighboring keratinocytes and their KIFs
- Provide structural strength for epidermis
- Constantly rearranging as keratinocytes migrate suprabasally

Desmosome Structure

- Desmoglea – extracellular portion between adhered keratinocytes
- Within keratinocyte cell membrane
  - Outer dense plaque
  - Inner dense plaque – connects to KIFs

Desmosome Structure

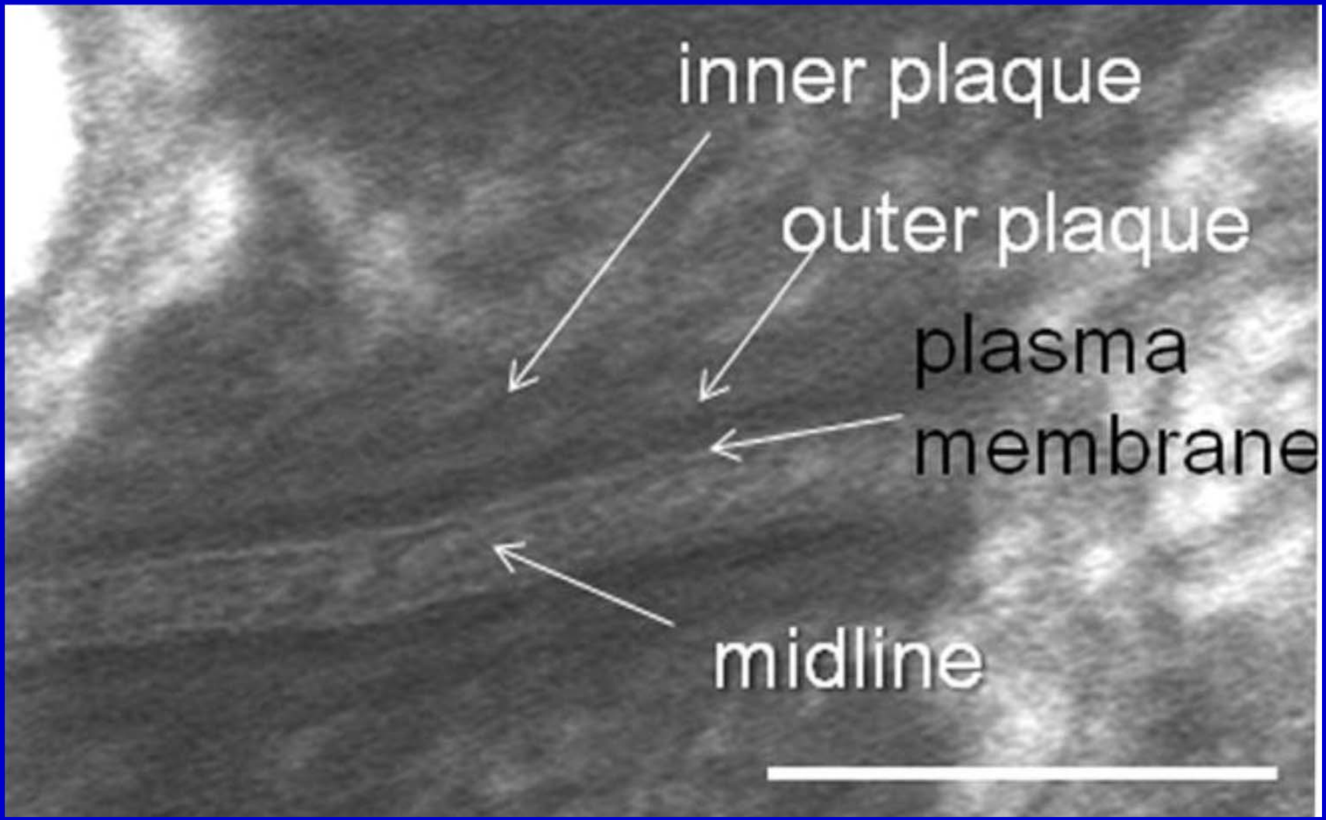


Figure 2 from Ishida-Yamamoto A, Igawa S. The biology and regulation of corneodesmosomes. *Cell Tissue Res* 2015: 360:479

Desmosome Components

- Cadherins (transmembrane, calcium dependent)
  - Desmogleins
  - Desmocollins
- Armadillo proteins (bind to cadherins)
  - Plakoglobin
  - Plakophilins
- Plakin proteins
  - Desmoplakin
  - Envoplakin, periplakin

Desmosomes

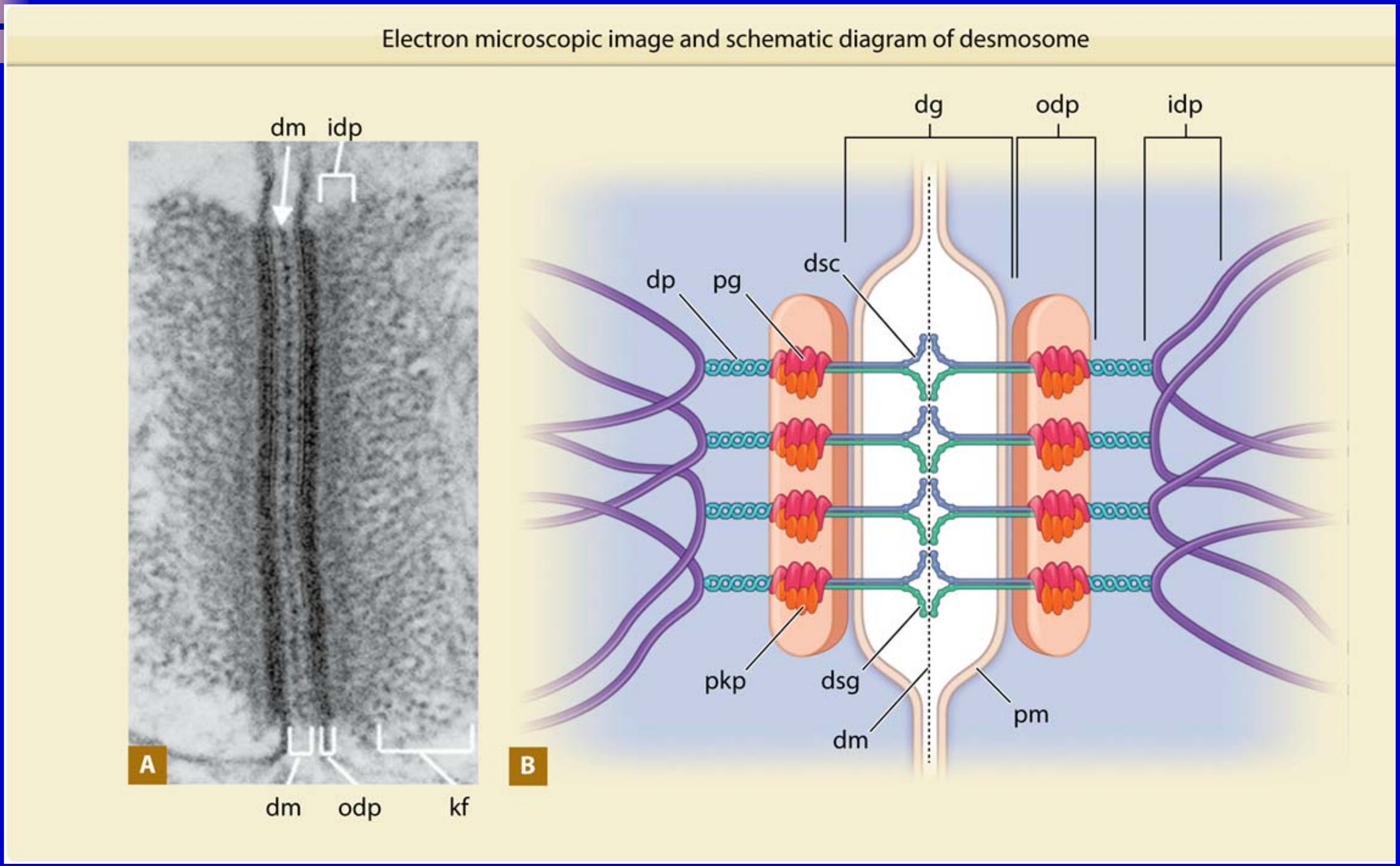


Figure 53-2 from Bruckner-Tuderman L, Payne AS. Epidermal and Epidermal-Dermal Adhesion. In: Goldsmith LA et al, editors, Fitzpatrick's Dermatology in General Medicine, 8th Ed. 2012, p. 570.

Desmosome/Corneodesmosome Structure

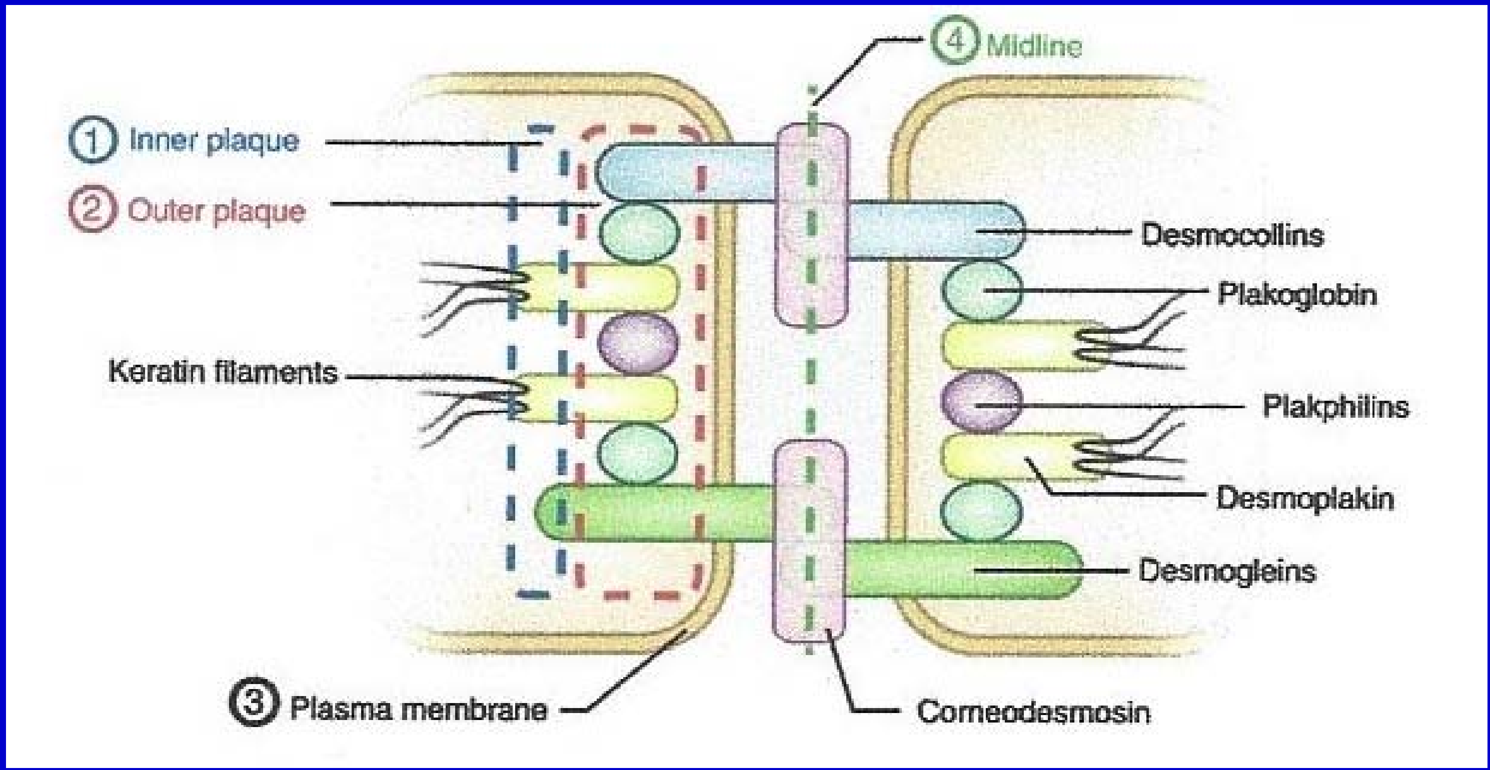


Figure 1 from Ishida-Yamamoto et al. Clinical and molecular implications of structural changes to desmosomes and corneodesmosomes. *Journal of Dermatology* 2018; 45: 386.

Corneodesmosomes

- Sites of corneocyte intercellular adhesion
- Components
  - Desmoglein 1
  - Desmocollin 1
  - Corneodesmosin

Corneodesmosin

- Serine and glycine rich protein
  - Forms “glycine loops” that act like velcro
    - Mediate reversible intermolecular adhesion
    - Glycine loops also in keratins and loricrin
- Secreted from lamellar granules
- Incorporated into desmoglea
- Plays a key role in stratum corneum cohesion

Corneodesmosomes

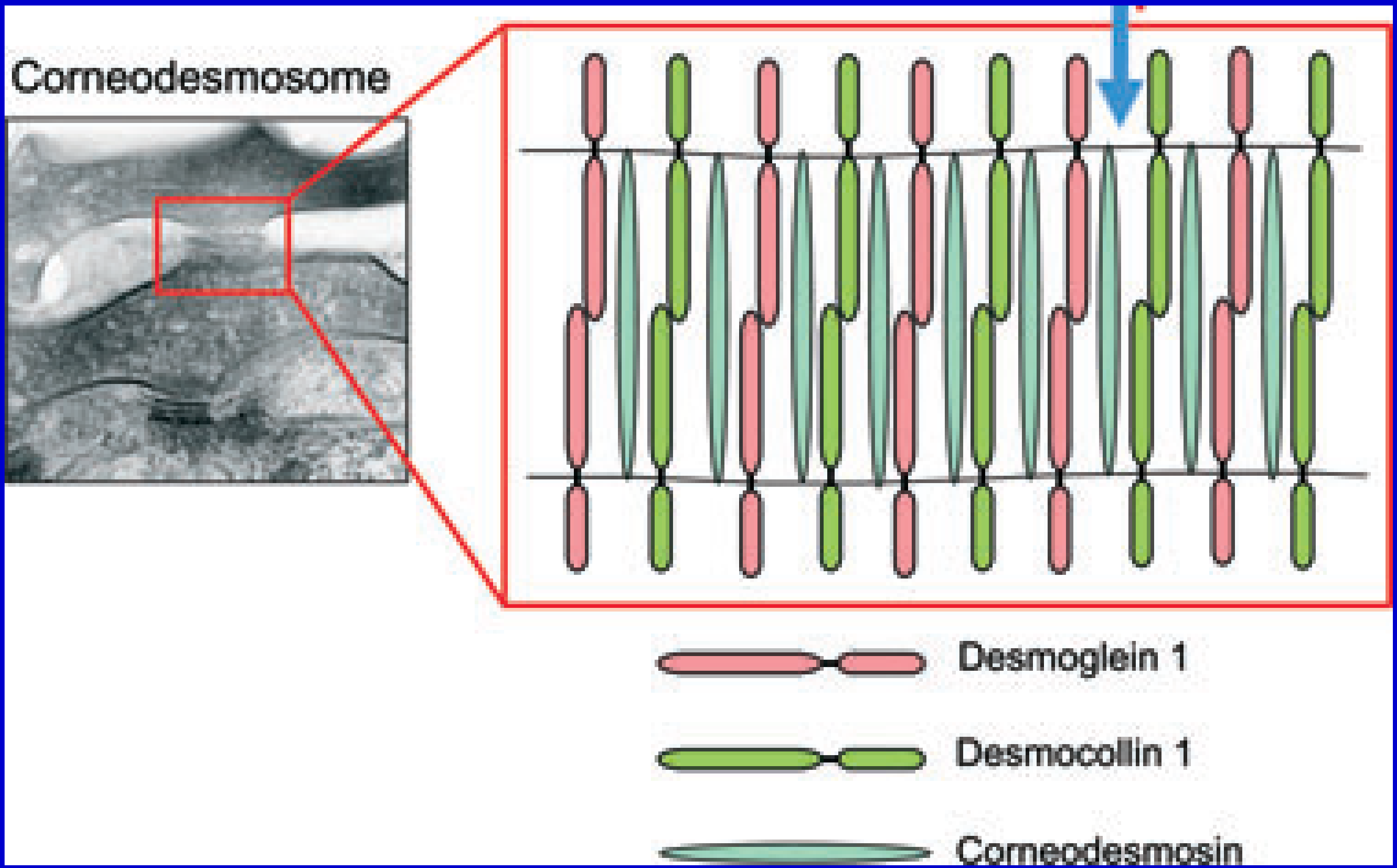


Figure 2 from Nishifuji K, Yoon JS. The stratum corneum: the rampart of the mammalian body. *Vet Dermatol* 2013; 24:64.

Transition from Desmosome to Corneodesmosome

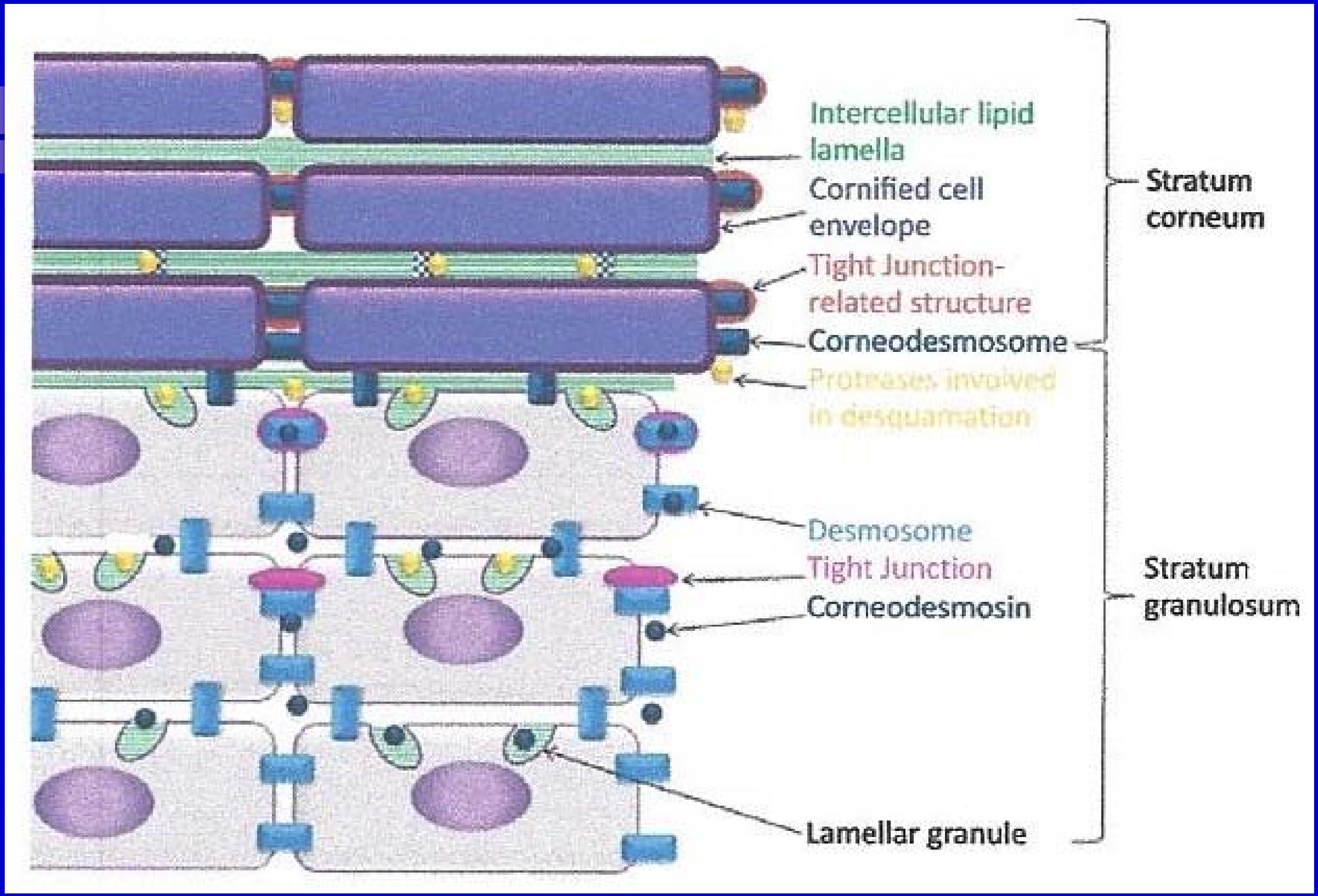


Figure 2 from Ishida-Yamamoto et al. Clinical and molecular implications of structural changes to desmosomes and corneodesmosomes. *Journal of Dermatology* 2018; 45: 386.

LGs Deliver Corneodesmosin

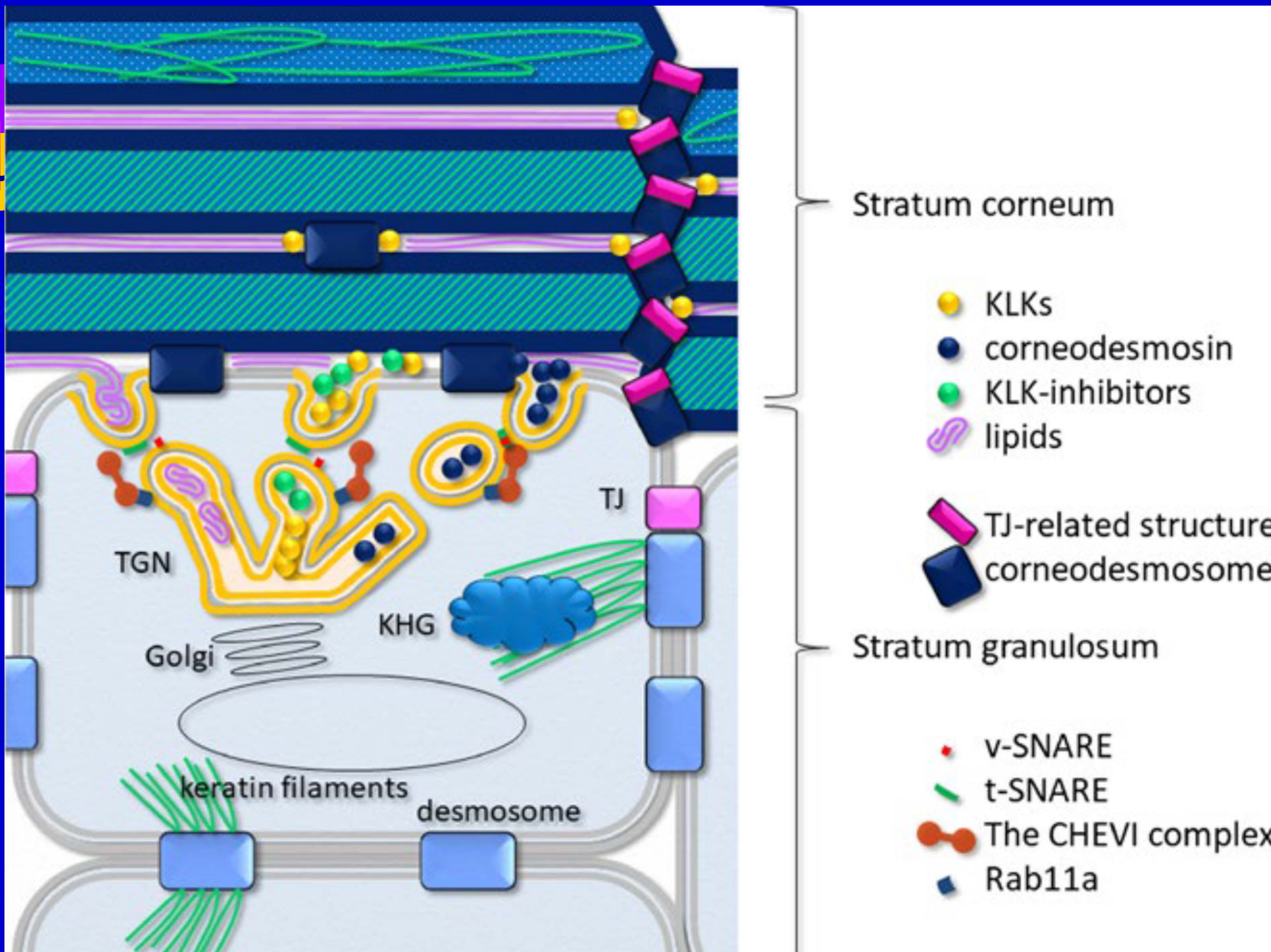
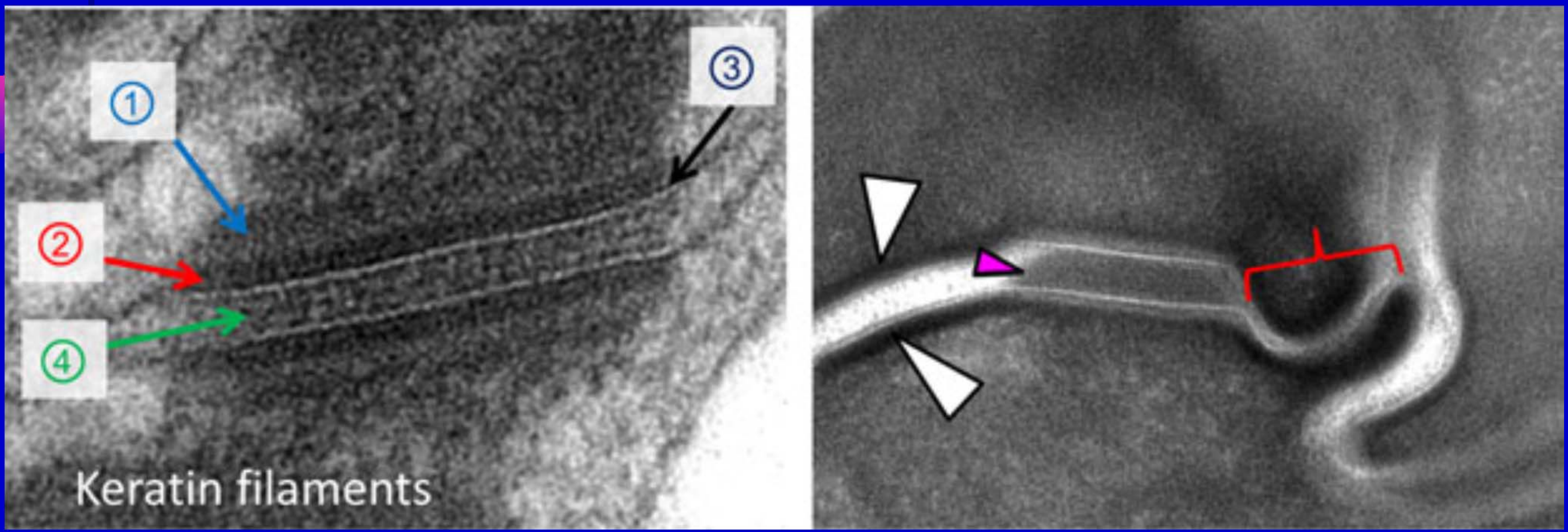


Figure 1 from Ishida-Yamamoto A, et al. Molecular basis of the skin barrier structures revealed by electron microscopy. *Exp Dermatol.* 2018;27:842.

Desmosome and Corneodesmosome



Desmosome and corneodesmosome ultrastructure in the epidermis.

- White arrowheads: cornified cell envelope.
- Magenta arrowhead: corneodesmosome electron-dense extracellular plaque.
- Red bracket: tight junction-related structure in the stratum corneum.

Figure 1 from Ishida-Yamamoto et al. Clinical and molecular implications of structural changes to desmosomes and corneodesmosomes. *Journal of Dermatology* 2018; 45: 386.

Keratinocyte Adhesion

- Desmosomes
- Corneodesmosomes
- Tight junctions
- Adherens junctions

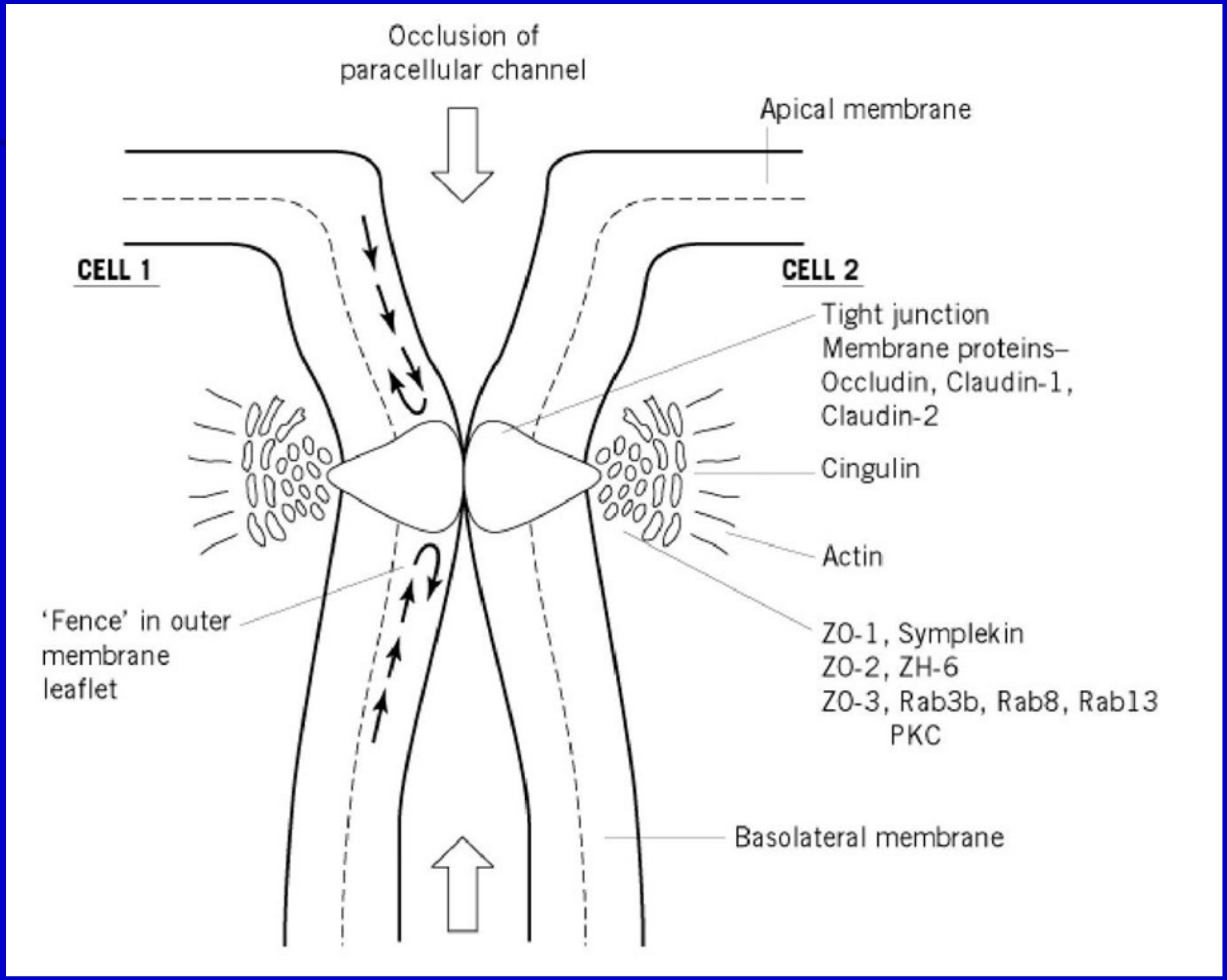
Tight Junctions in Epidermis

- Form key structural permeability barrier in granular layer
- Role still under investigation
  - Effects on keratinization and desquamation

Tight Junctions in Epithelia

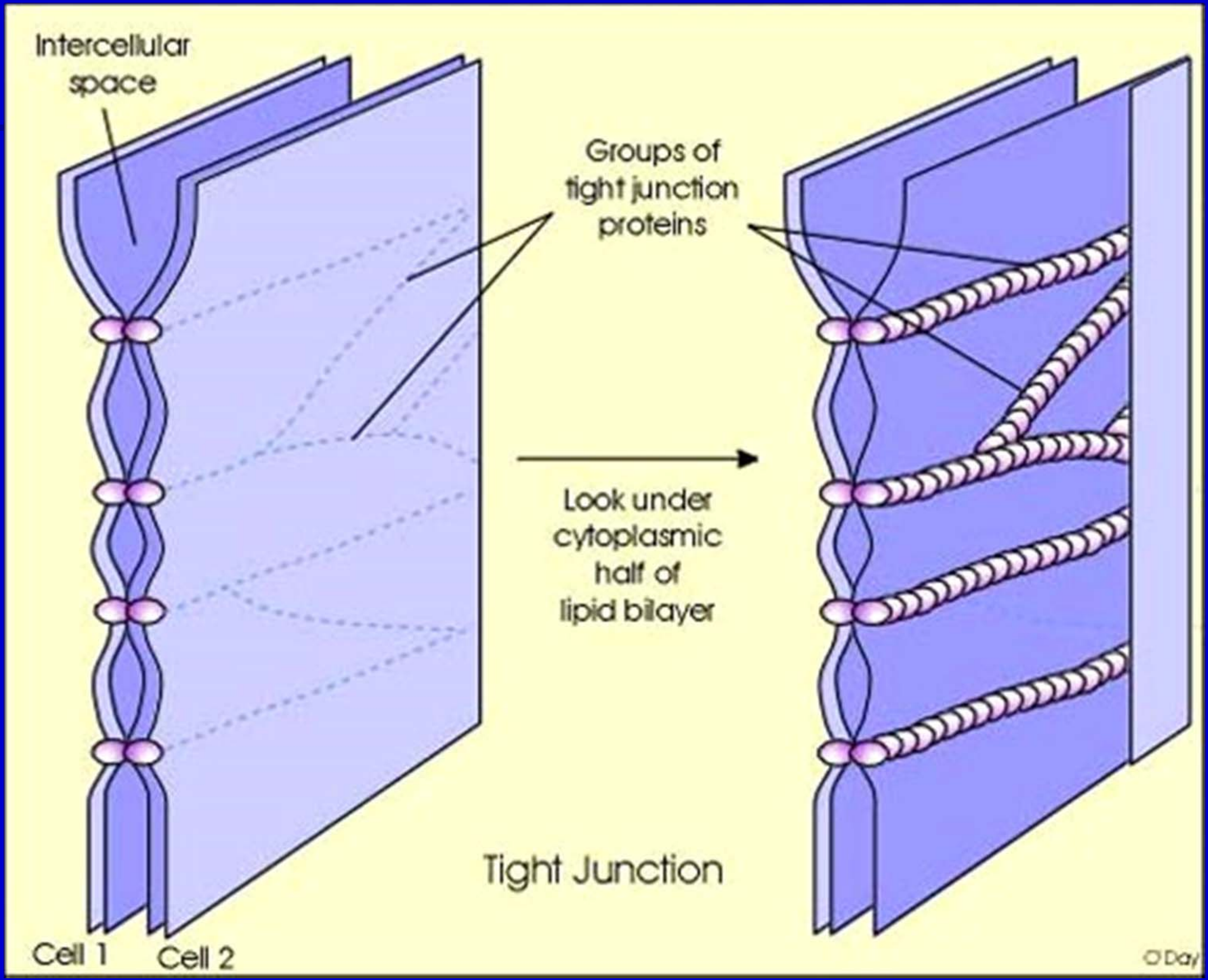
- Very close intercellular contacts
  - Seal intercellular space
- Control paracellular movement of molecules
  - Semipermeable size and ion-specific barrier
- Perform “fence function”
  - Restrict molecule diffusion within cell membrane
  - Demarcate apical and basolateral regions

Tight Junctions



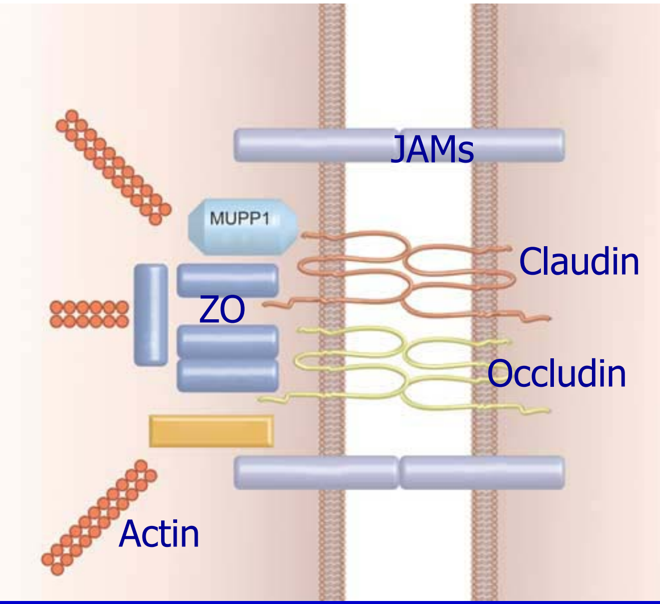
http://what-when-how.com/molecular-biology/tight-junction-molecular-biology/

Tight Junctions



www.erin.utoronto.ca/~w3bio315/lecture3.htm

Tight Junctions: Components



- Transmembrane molecules
  - Claudins – critical component
  - Occludin
  - Junctional adhesion molecules (JAMs) – IgG-like
- Intracellular proteins
  - Zonula occludens (ZO) proteins
    - Interact with claudins, occludin and actin

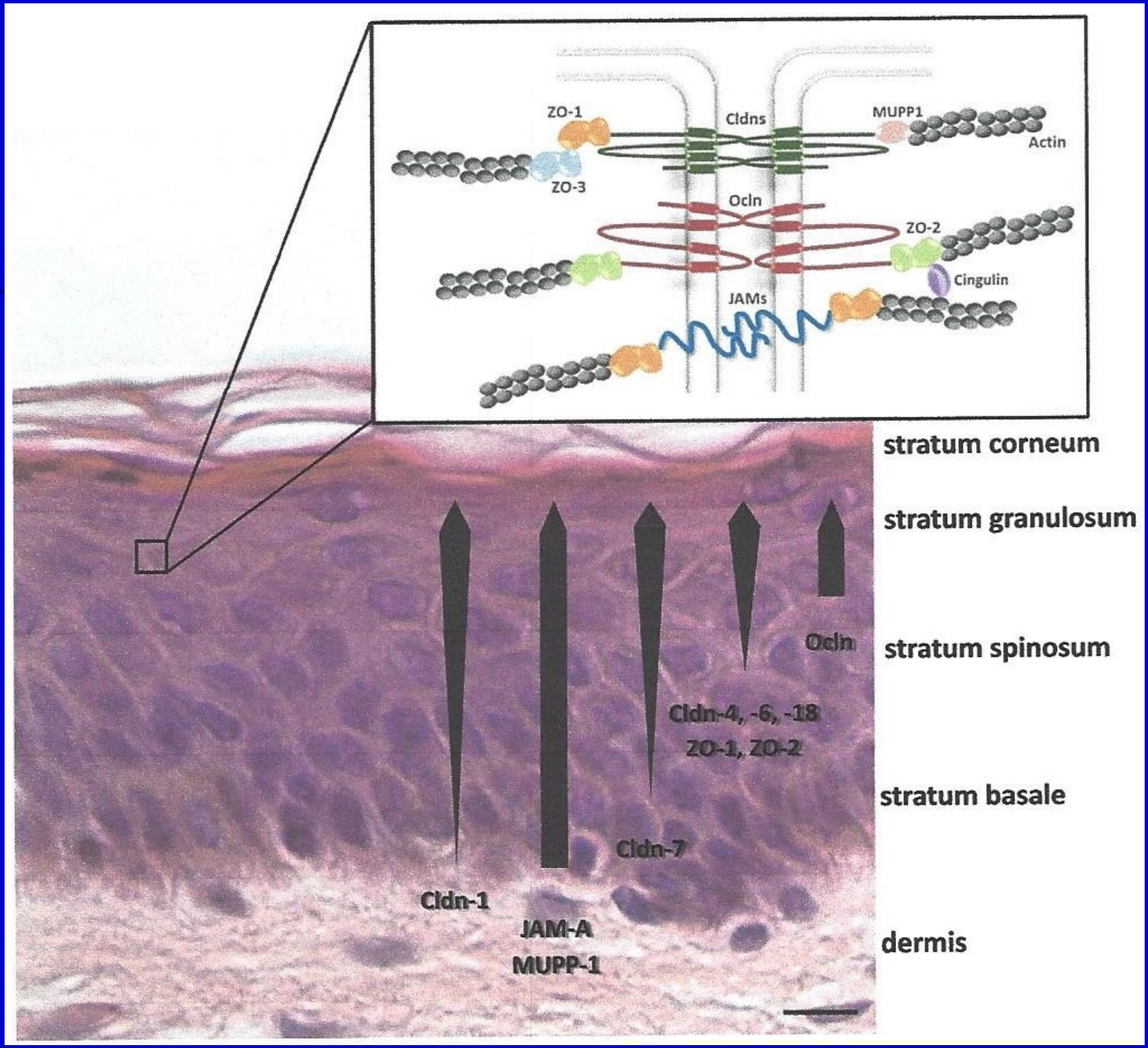


Figure 3 from Bäsler et al, The role of tight junctions in skin barrier function and dermal absorption. *Journal of Controlled Release* 2016: 242; 108.

Tight Junction-Related Structures

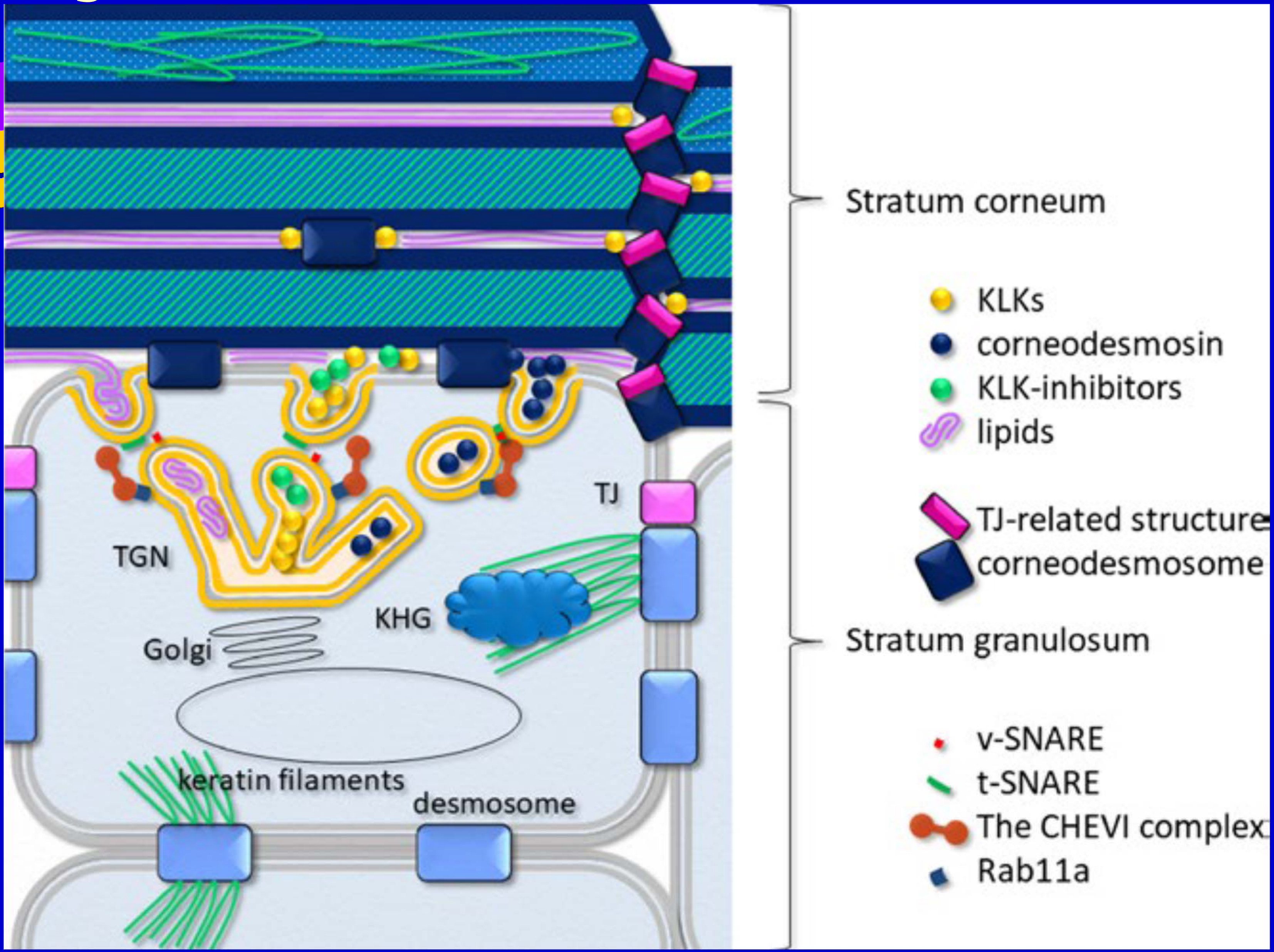
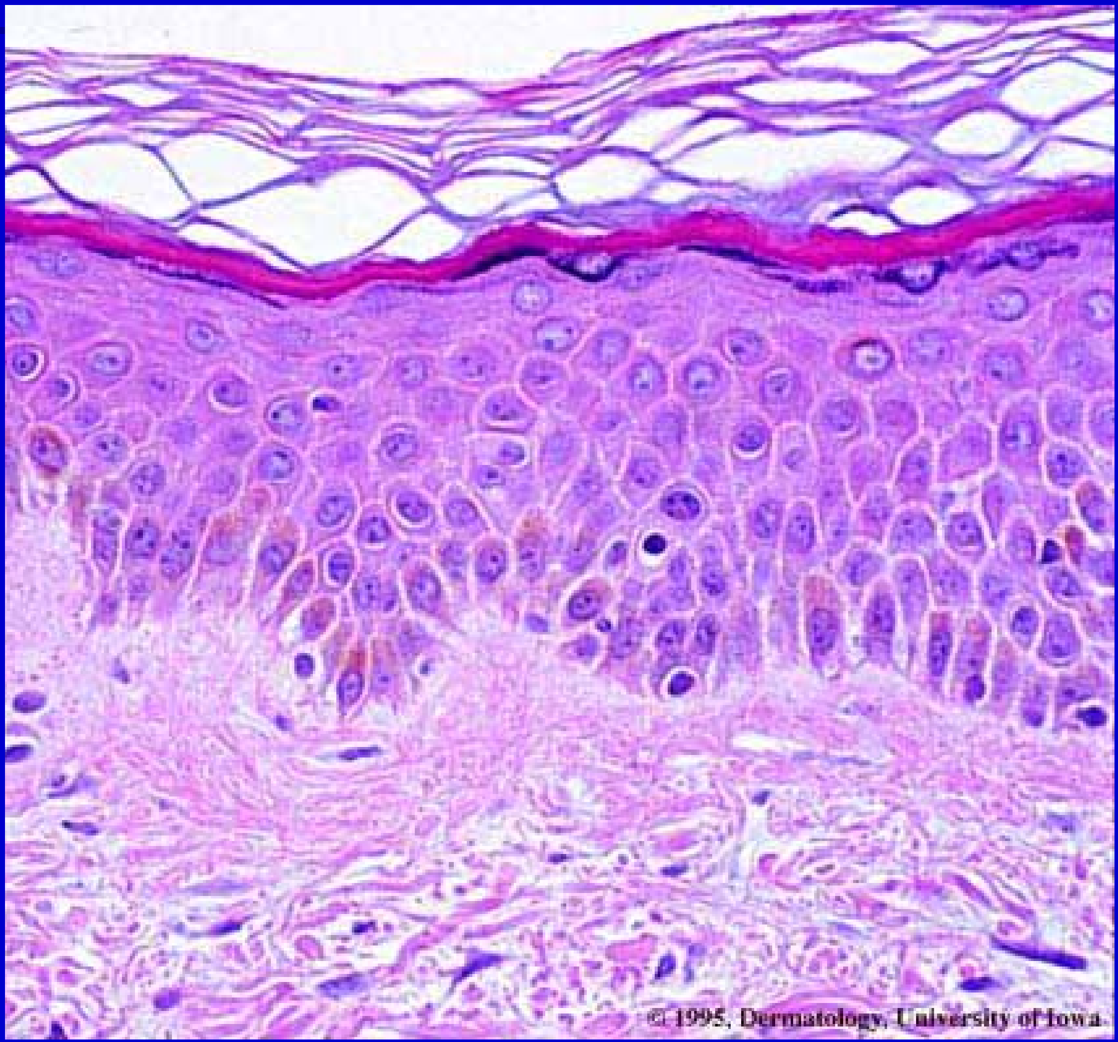


Figure 1 from Ishida-Yamamoto A, et al. Molecular basis of the skin barrier structures revealed by electron microscopy. *Exp Dermatol.* 2018;27:842.

Basket-weave Pattern

Corneodesmosome Distribution



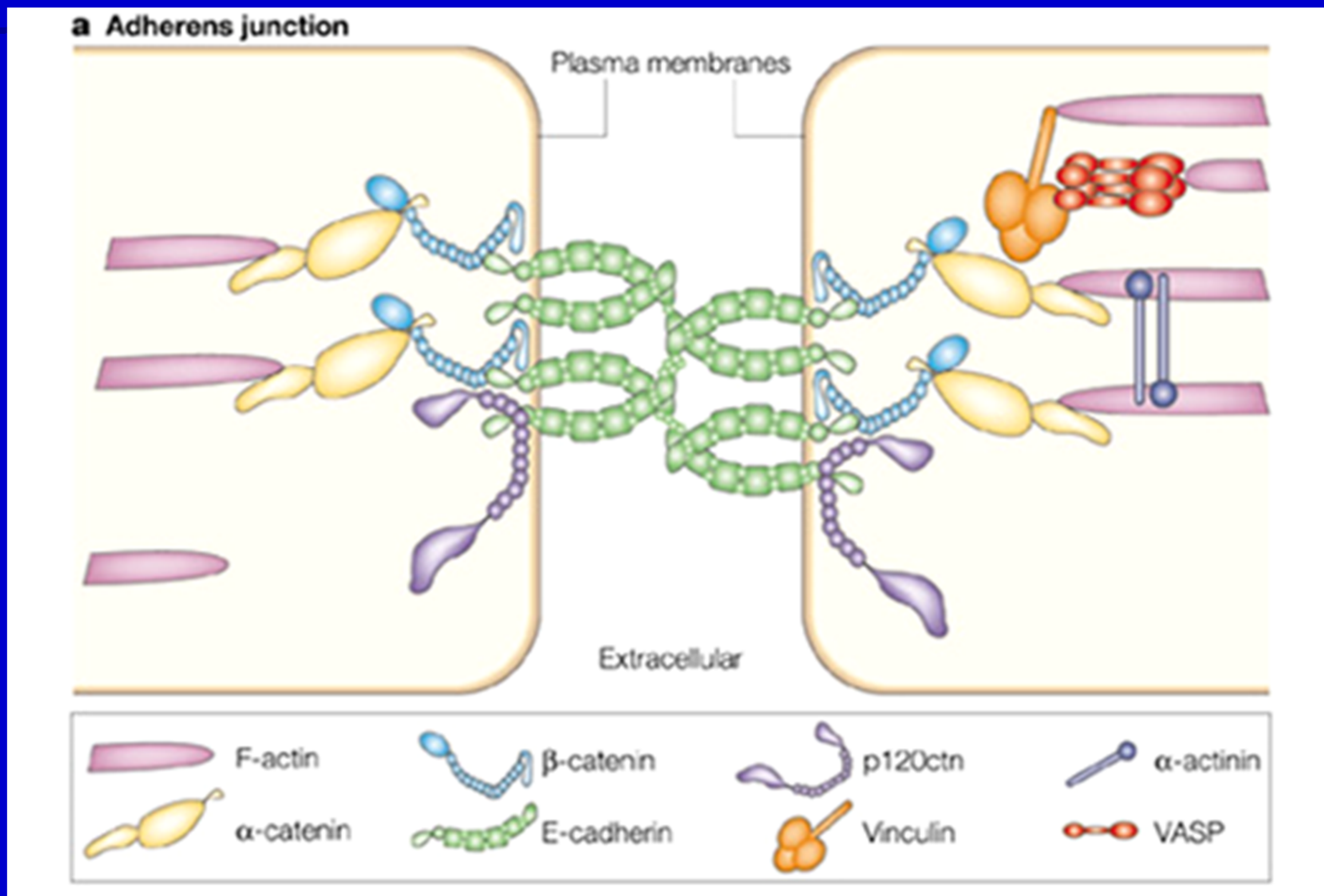
Keratinocyte Adhesion

- Desmosomes
- Corneodesmosomes
- Tight junctions
- Adherens junctions

Adherens Junctions

- Areas of intercellular contact
- Closely connected to actin microfilament cytoskeleton
- Cadherin-catenin complex binds actin
  - E-cadherin – transmembrane molecule
    - Calcium-dependent binding
  - Cytoplasmic catenins
    - β-catenin, p120 catenin, α-catenin

Adherens Junctions



From Fuchs E, Raghavan S. Getting under the skin of epidermal morphogenesis. *Nature Reviews Genetics* 2002; 3:199-209.

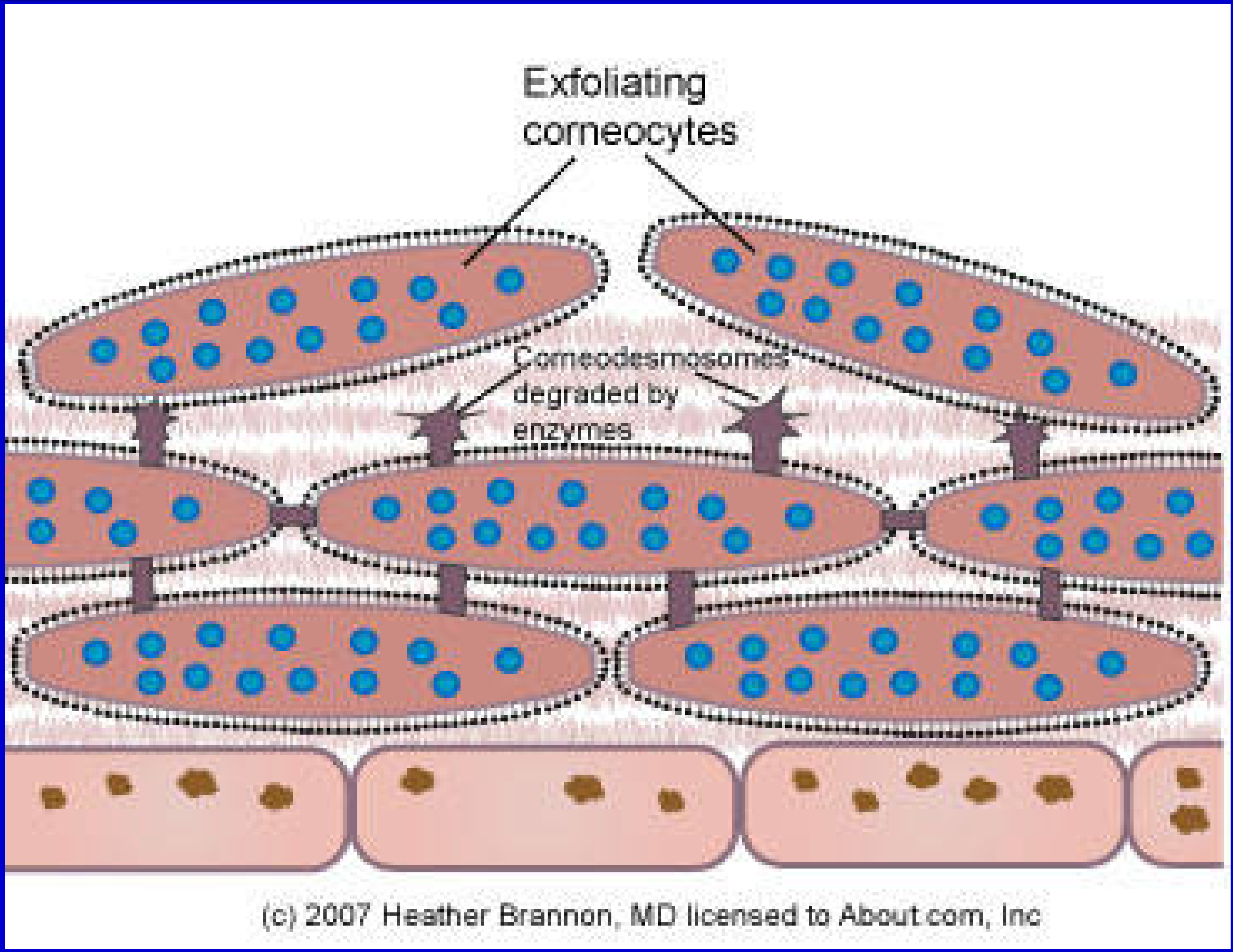
Adherens Junctions

- Initiate and maintain cell-cell adhesion
- Regulate organization of actin cytoskeleton
- Establish hub for cell signaling and regulation of gene transcription
- Involved in regulating keratinocyte proliferation and differentiation
- Play role in wound healing

Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte adhesion
- Desquamation
- Epidermal barrier function

Desquamation: Shedding of Corneocytes



http://dermatology.about.com/od/anatomy/ss/sc_anatomy_9.htm

LGs Deliver Desquamation Enzymes and Inhibitors

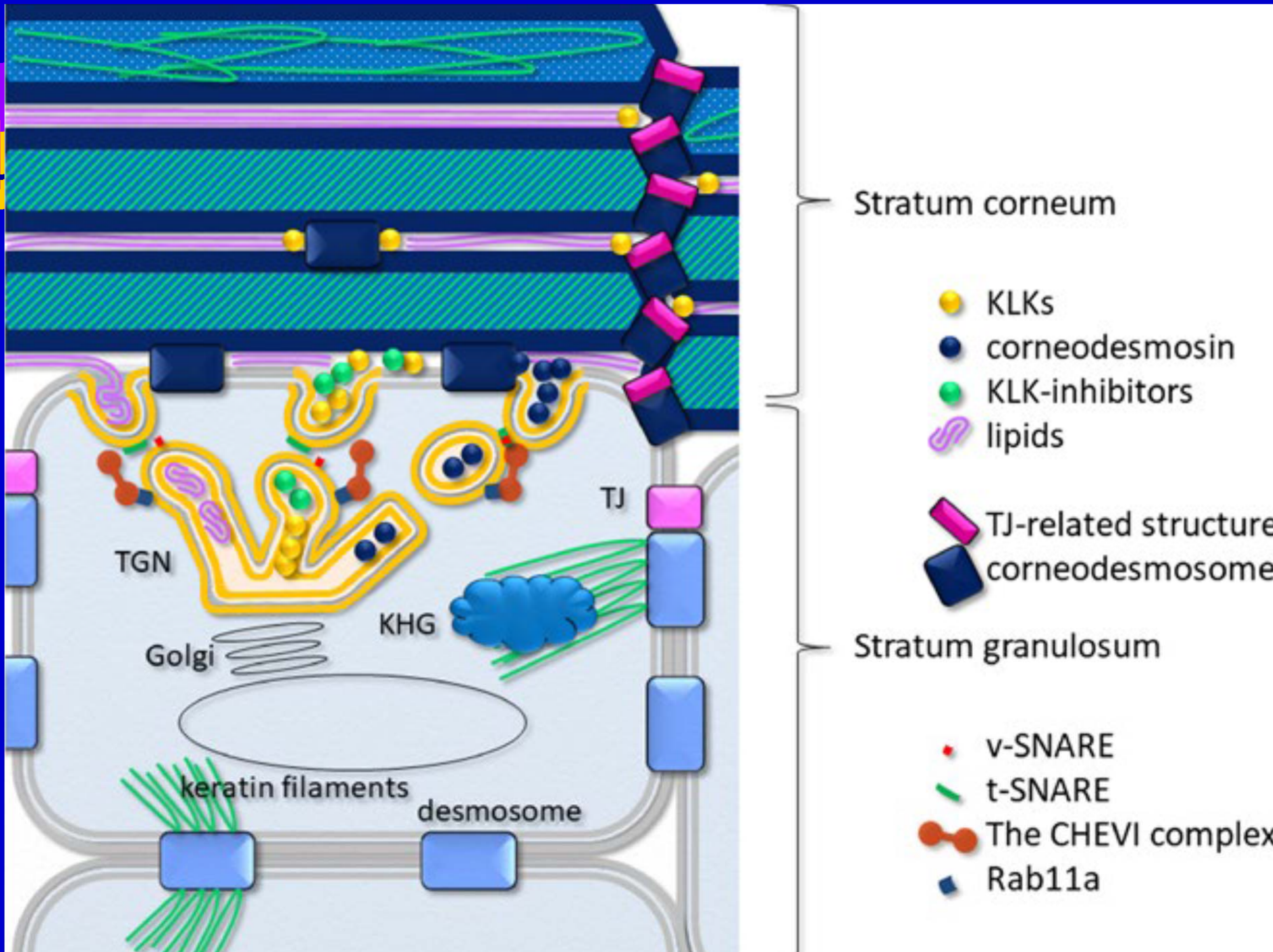
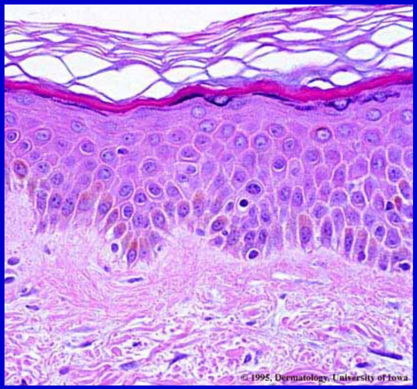


Figure 1 from Ishida-Yamamoto A, et al. Molecular basis of the skin barrier structures revealed by electron microscopy. *Exp Dermatol.* 2018;27:842.

Enzymatic Cleavage of Corneodesmosomes



- Kallikreins (KLKs) - serine proteases
  - KLK7 degrades corneodesmosin, desmocollin-1
  - KLK5 degrades corneodesmosin, desmocollin-1, & desmoglein-1
  - KLKs 1, 6, 14 degrade desmoglein-1
- Cathepsins – cysteine proteases
  - Degrade corneodesmosin

Corneodesmosome Cleavage

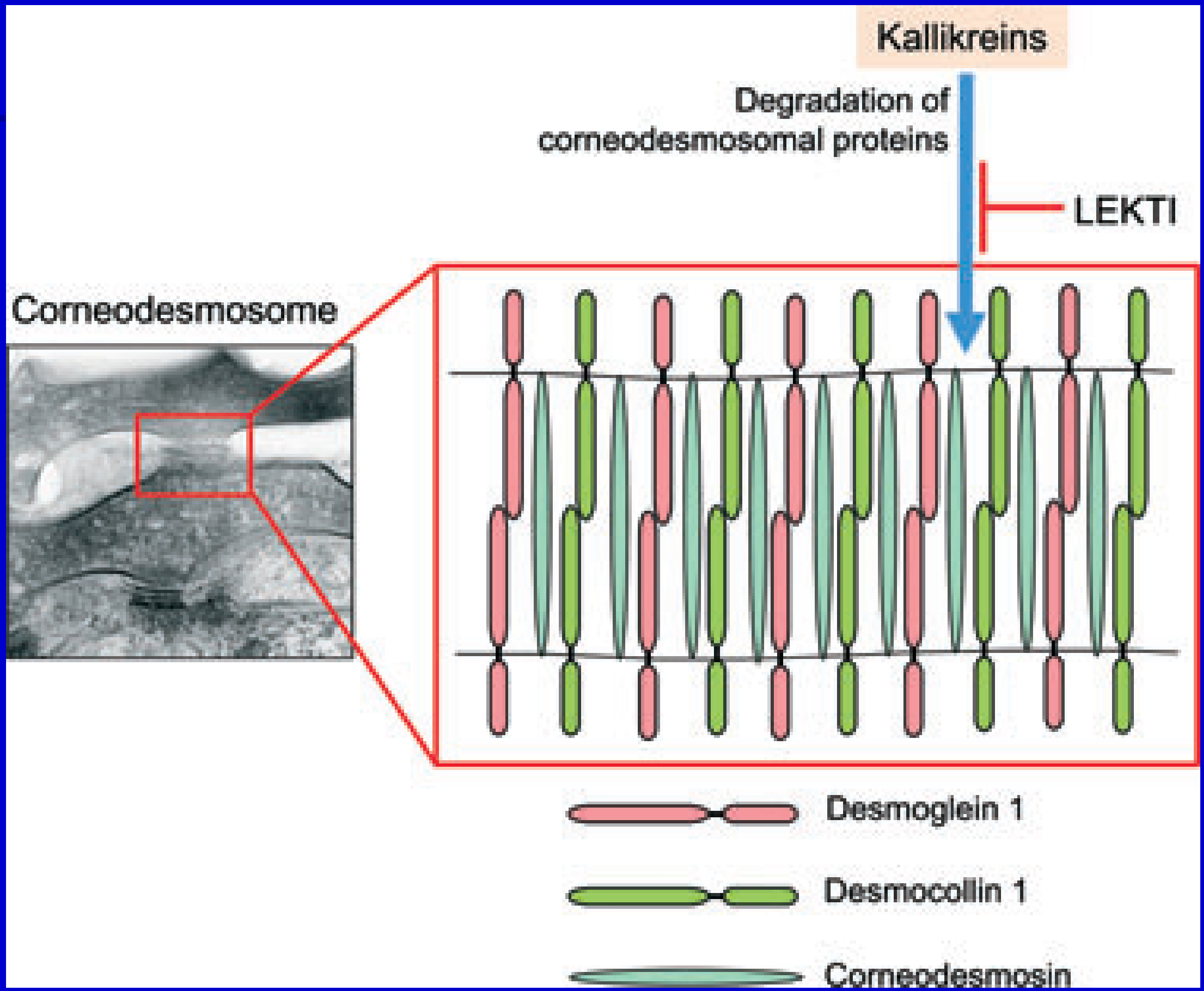


Figure 2 from Nishifuji K, Yoon JS. The stratum corneum: the rampart of the mammalian body. *Vet Dermatol* 2013; 24:64.

Regulation of Desquamation

- Complex pH dependent network of enzymes and their inhibitors
- LEKTI (Lympho-epithelial Kazal type inhibitor)
  - KLK inhibitor
  - Secreted from lamellar granules
  - Effective inhibition at neutral pH
- Balance between KLKs and LEKTI regulates normal desquamation

pH Regulates KLK and LEKTI Interaction

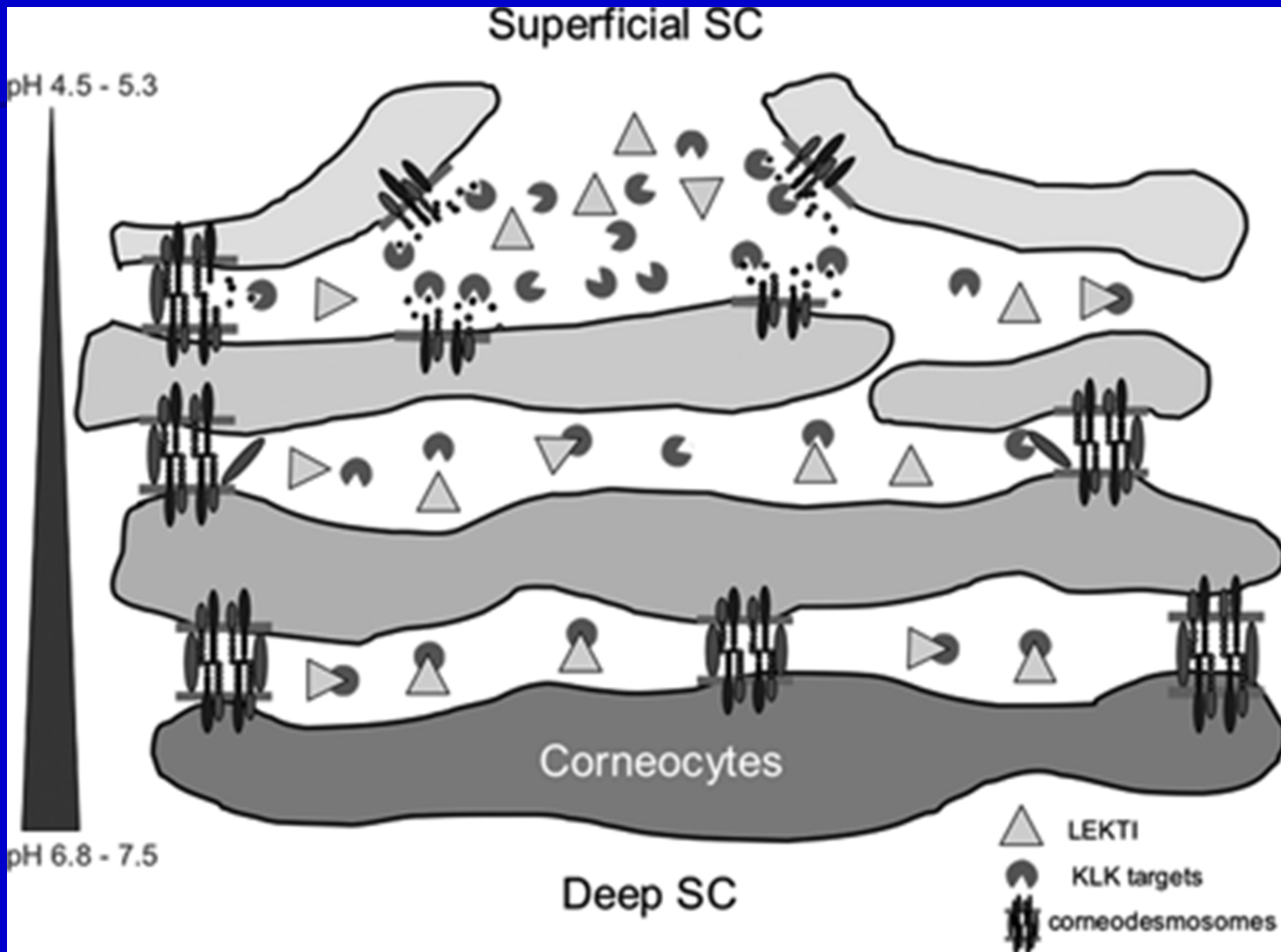


Figure 9 from Deraison C, et al. LEKTI Fragments Specifically Inhibit KLK5, KLK7, and KLK14 and Control Desquamation through a pH-dependent Interaction. *Mol. Biol. Cell September 1, 2007 vol. 18 no. 9 3607-3619 .*

Complexity of Desquamation

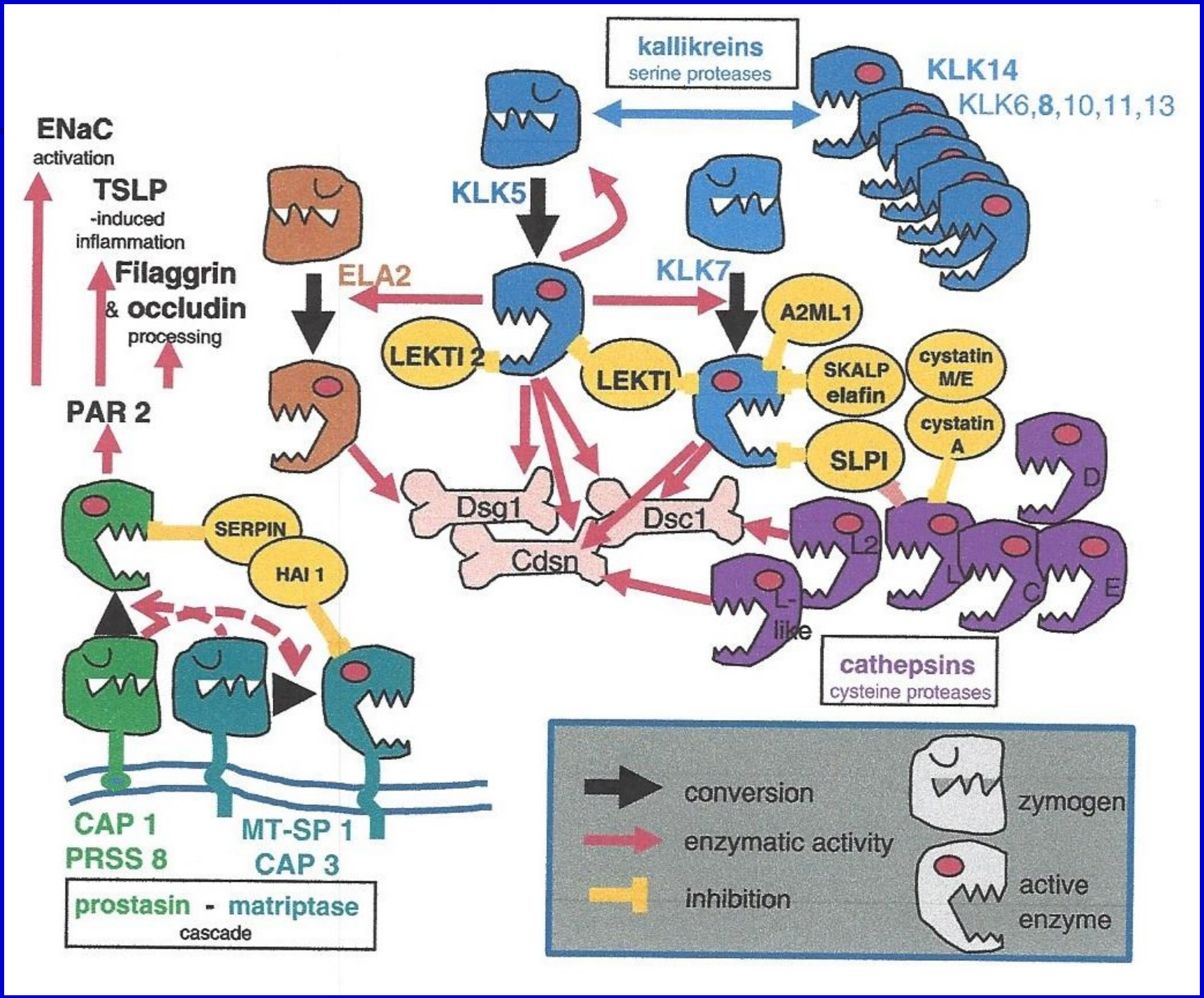


Figure 1 from Haftek M. Epidermal barrier disorders and corneodesmosome defects. *Cell Tissue Res* (2015) 360:484.

Overview

- Layers of the epidermis
- Keratinization
- Cornified cell envelope
- Extracellular lipid matrix
- Keratinocyte connections
- Desquamation
- Epidermal barrier function

Biography of a Keratinocyte

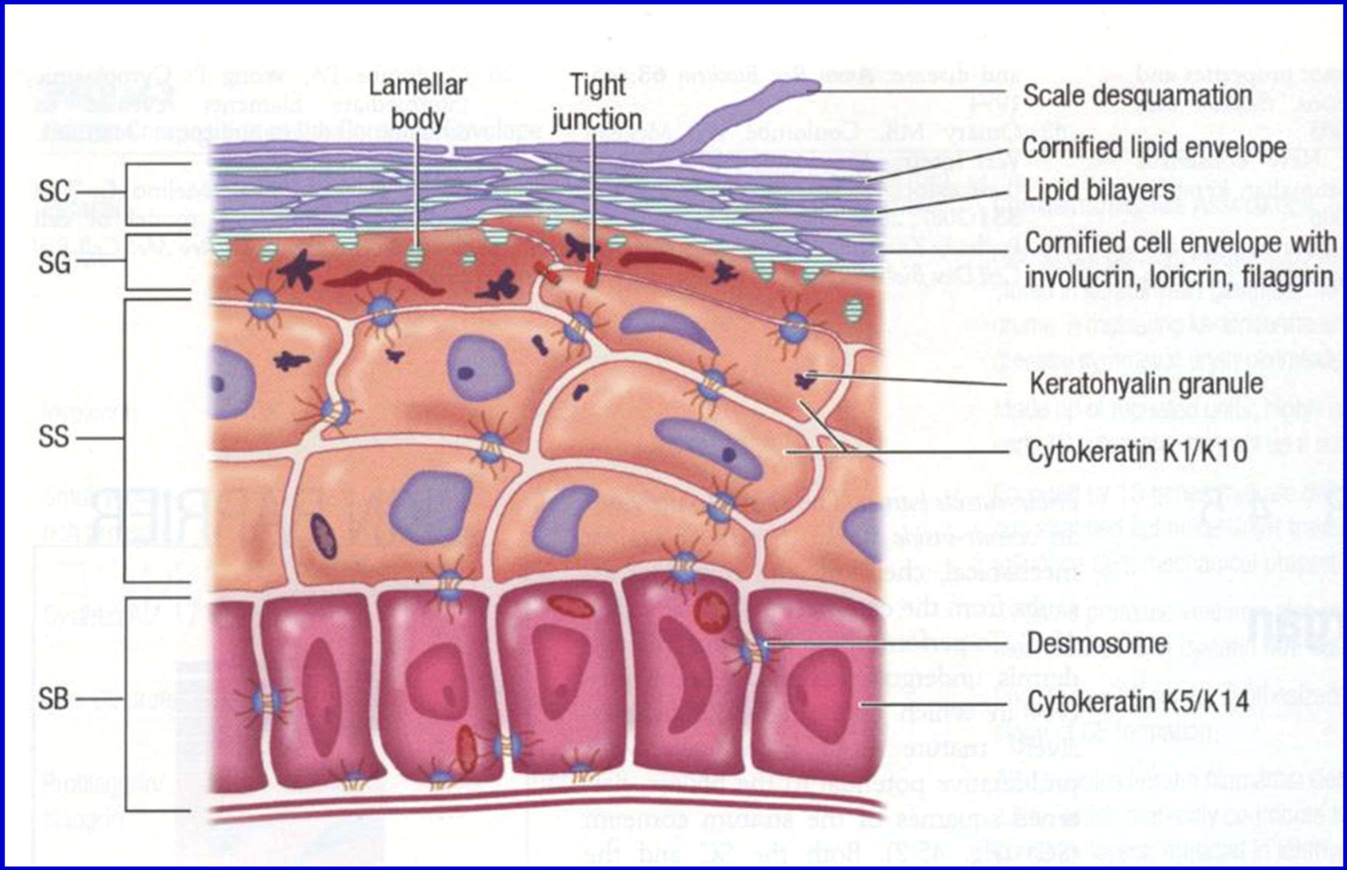


Figure 45-2 from Proksch E, Jensen JM. Skin as an organ of protection. In: Wolff K et al, editors, Fitzpatrick's Dermatology in General Medicine, 7th Ed. p. 384.

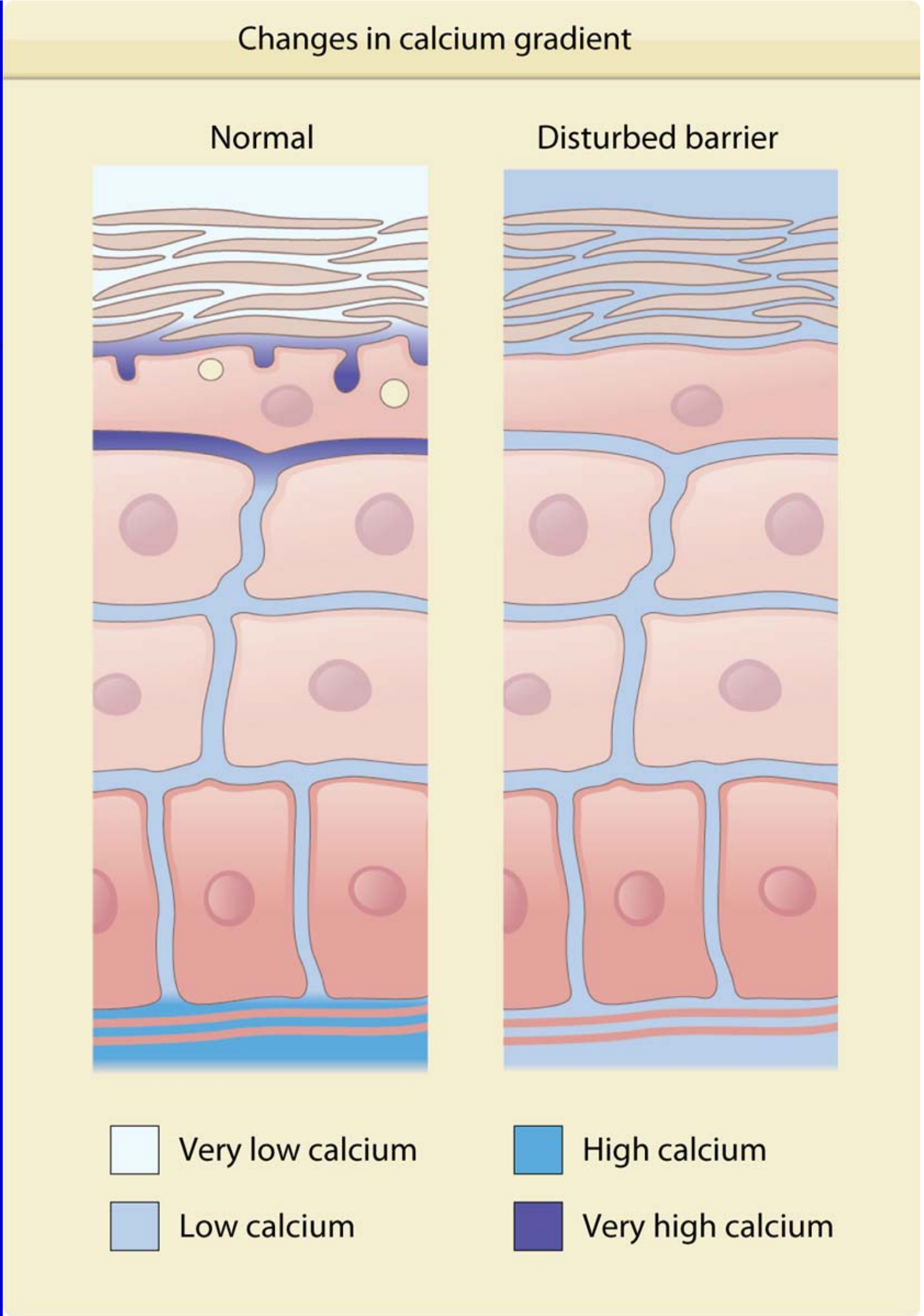
Epidermal Calcium Gradient and Barrier Function

- Ca^{2+} regulate formation of stratum corneum
  - Transcription of genes
  - Lamellar granule secretion
  - Transglutaminase activity
  - Cleavage of profilaggrin to filaggrin
- Gradient reflects relative Ca^{2+} concentration required for each layer's differentiation steps

Epidermal Barrier

- Dependent on calcium gradient
  - Basal – low
  - Spinous – low
  - Granular – high
  - Cornified – low

Figure 47-11 from Proksch E, Jensen JM. Skin as an Organ of Protection. In: Goldsmith LA, et al, editors, Fitzpatrick's Dermatology in General Medicine, 8th Ed. New York: McGraw-Hill Companies, Inc. 2012. p. 496.



Epidermal Calcium

- Intracellular Ca^{2+} in endoplasmic reticulum (ER)
  - Released in response to intracellular signals
- Tight junctions prevent extracellular Ca^{2+} diffusion to stratum corneum

Calcium in Barrier Homeostasis

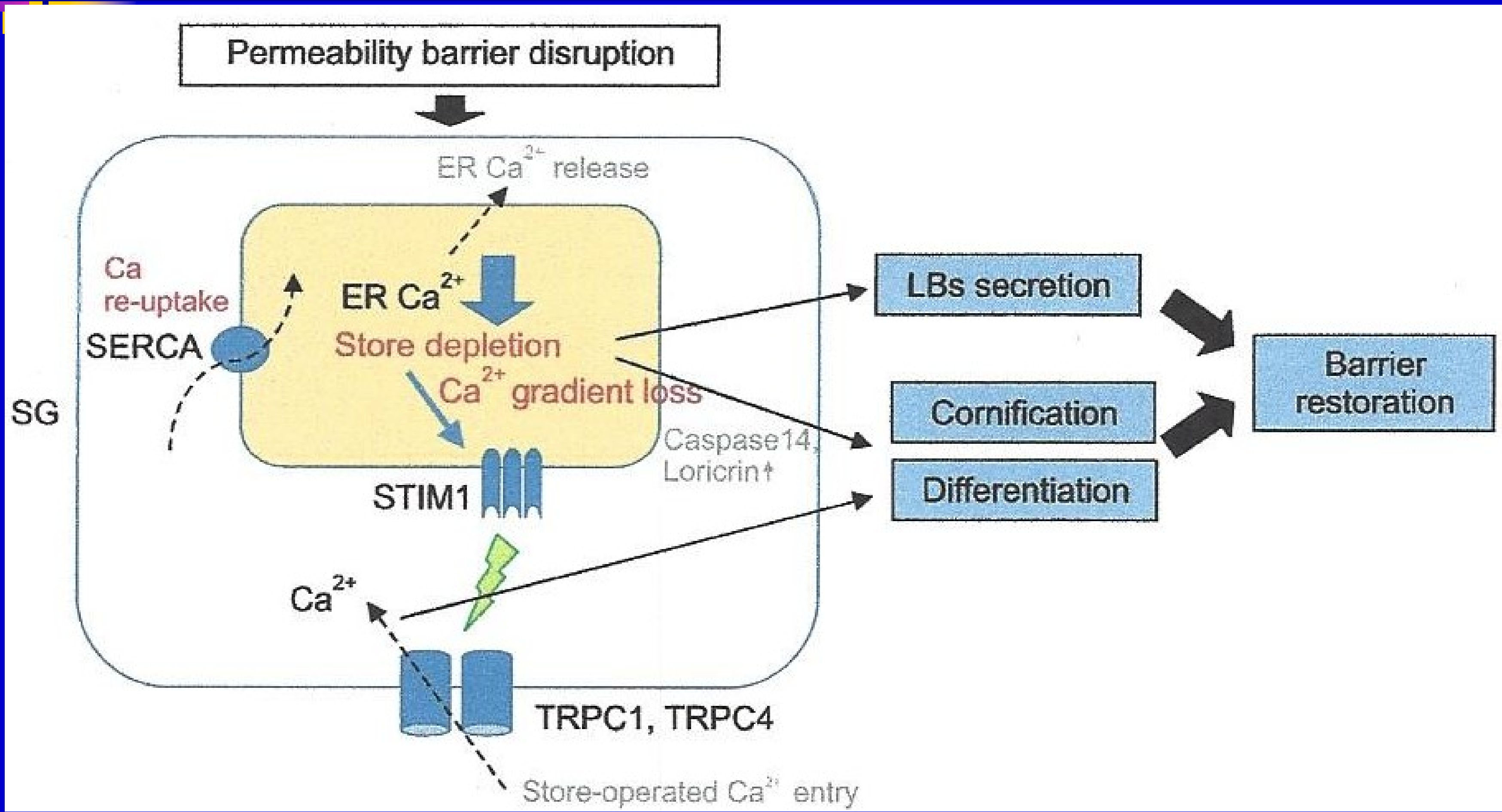


Figure 1 from Lee SE and Lee SH. Skin Barrier and Calcium. *Ann Dermatol* 2018; 30(3):268.

Epidermal Barrier Function

- "Biography of a keratinocyte"
  - Purpose of keratinocyte: to produce stratum corneum
  - Stratum corneum provides barrier

Questions?