BIOLOGY OF MELANOCYTES

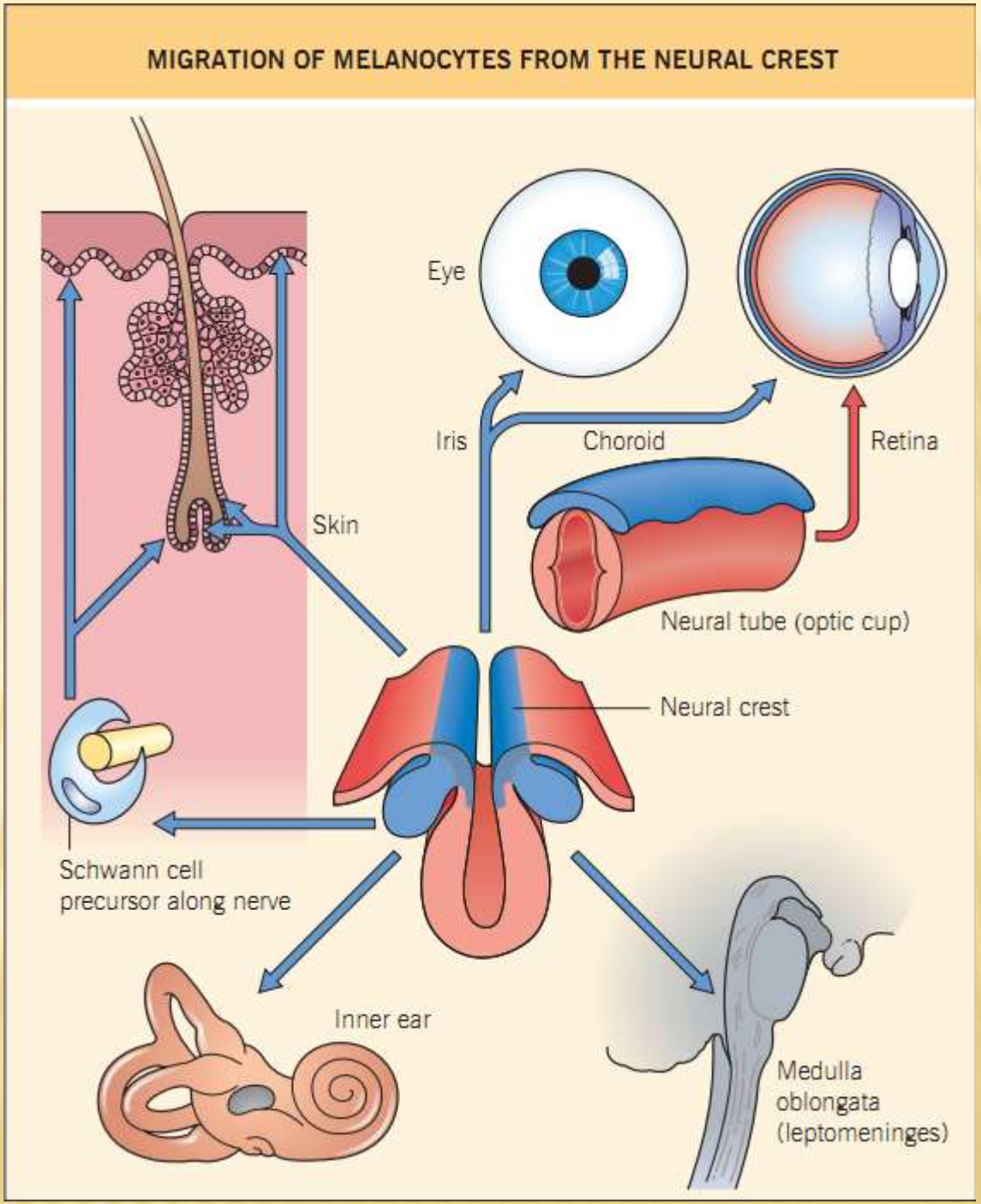
OBJECTIVES

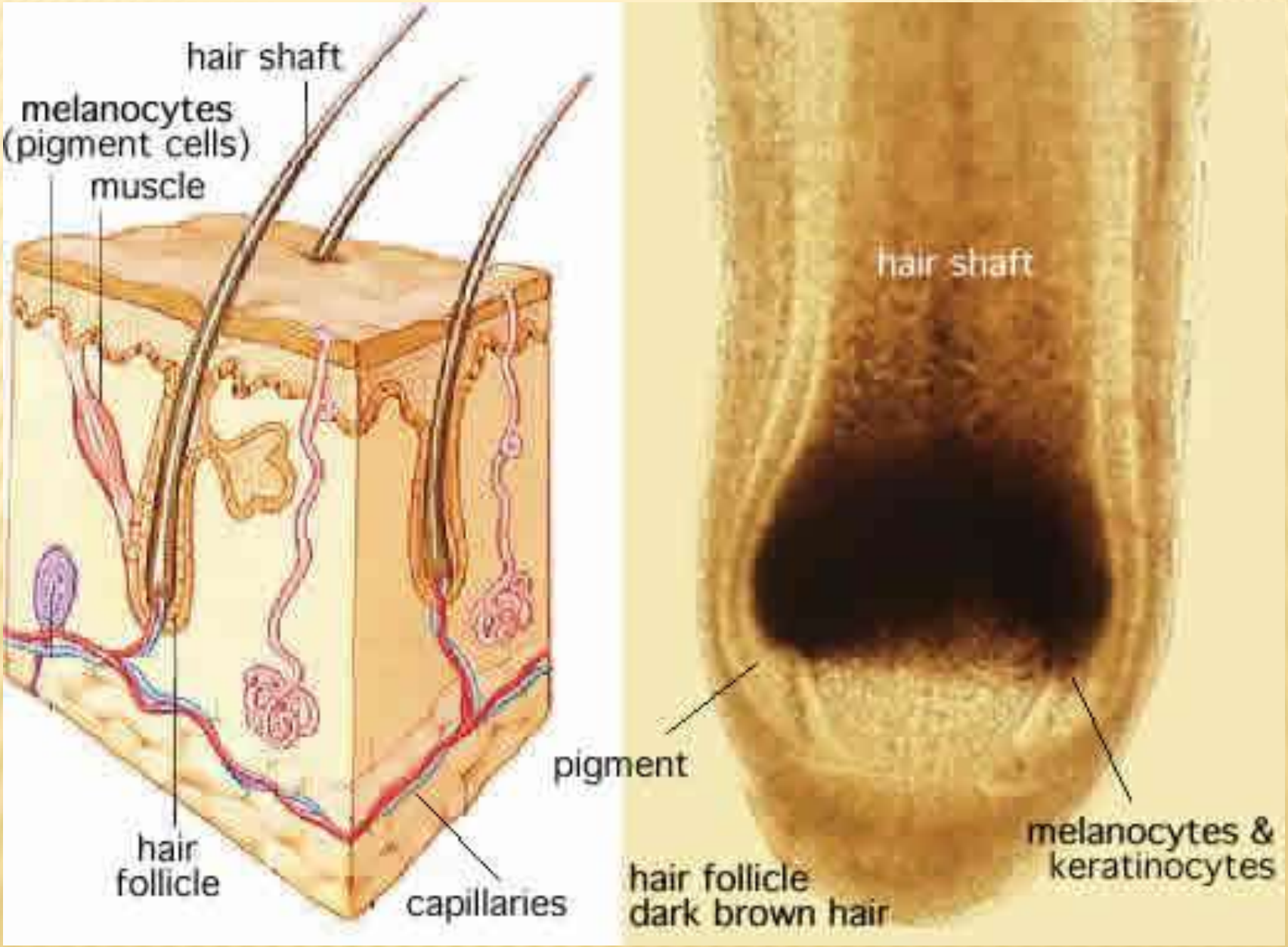
- Definition, development, function & Site-specific of Melanocyte
- Definition, formation & function of Melanosomes
- Definition & function of Melanin
- Melanogenesis

- Definition of Melanocyte:

Are neural crest-derived dendritic cells mainly located in the stratum basale of skin's epidermis & synthesize melanosomes.

Melanocytes, like other neural-derived tissues, have a low mitotic rate




hair shaft
melanocytes
(pigment cells)
muscle
hair
follicle
capillaries
hair shaft
pigment
melanocytes &
keratinocytes
hair follicle
dark brown hair

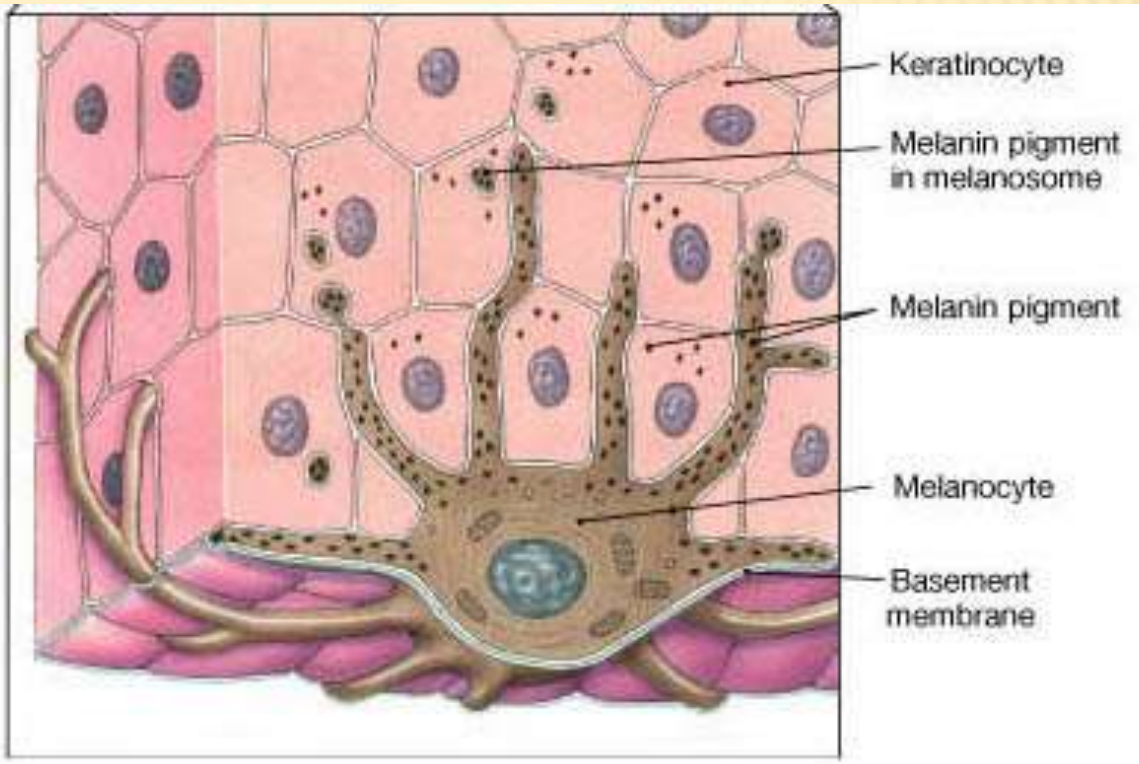
EMBRYOLOGY OF MELANOCYTE

- Primitive melanocytes: 8th wk. gestation
- Melanosome showing early melanization:10th wk. gestation
- Fetal skin contain melanocytes in the dermis basally, suprabasally
- Neonatal skin contain melanocytes only basally

EMBRYOGENESIS OF MELANOCYTES

- Melanoblasts (Melanocyte precursors) migration moving dorsolaterally and then ventrally around the trunk to the ventral midline & differentiation into melanocytes. During embryogenesis, melanin-producing melanocytes are found diffusely throughout the dermis.
- However, by the end of gestation, active dermal melanocytes have "disappeared", except in three anatomic locations
    1. the head and neck,
    2. the dorsal aspects of the distal extremities,
    3. the presacral area

that coincide with the most common sites for dermal melanocytosis and dermal melanocytomas (blue nevi).



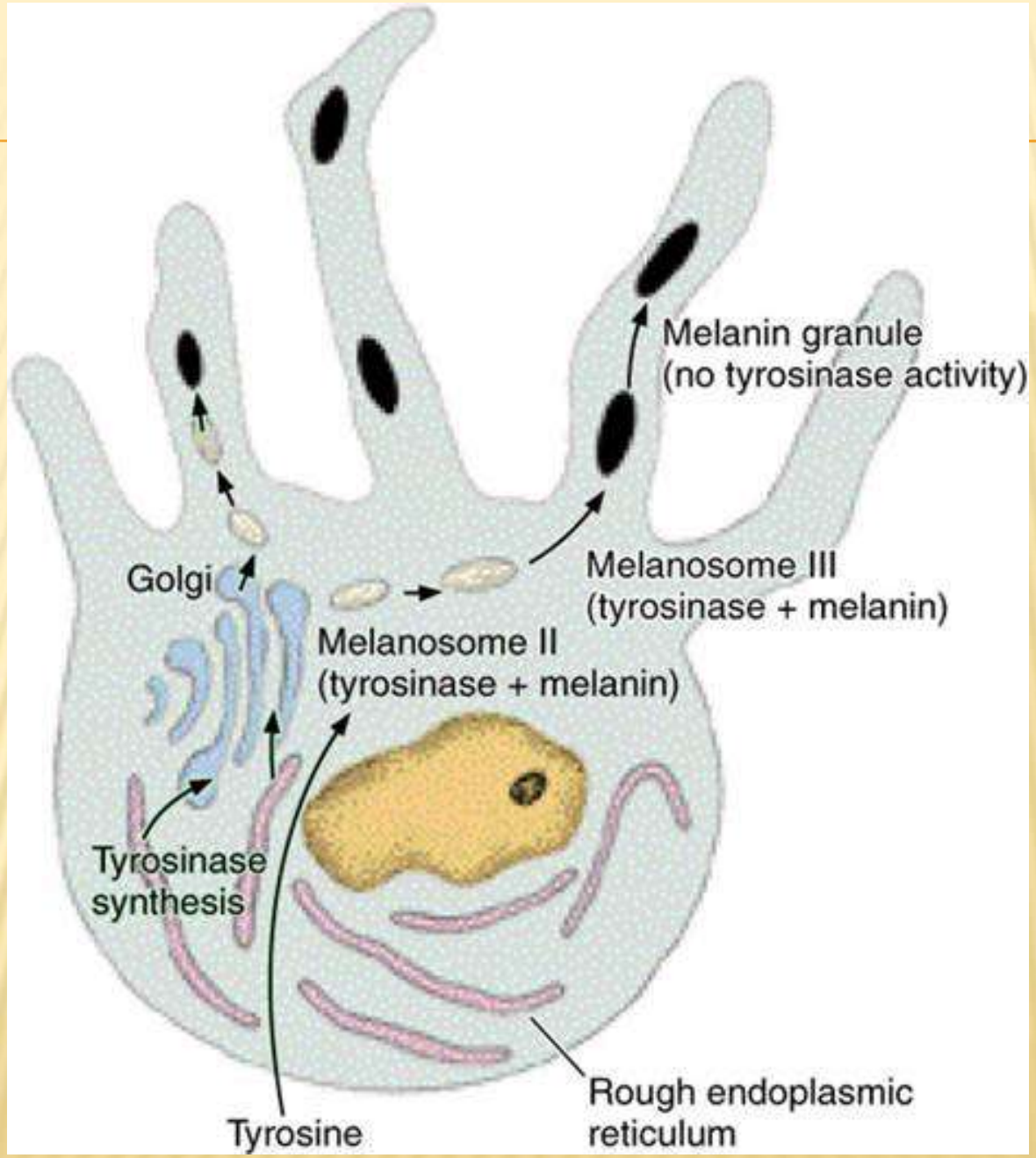
Association of a melanocyte with approximately 30–40 surrounding keratinocytes to which it transfers melanosomes has been called the

Epidermal melanin unit

1:36 ratio of melanocyte:keratynocyte

FUNCTION OF CUTANEOUS MELANOCYTES:

Is to synthesize melanin in membrane-bound organelles called melanosomes & to transfer melanosomes to neighbouring keratinocytes to provide protection from UV irradiation.


Melanin granule
(no tyrosinase activity)
Golgi
Melanosome III
(tyrosinase + melanin)
Melanosome II
(tyrosinase + melanin)
Tyrosinase
synthesis
Tyrosine
Rough endoplasmic
reticulum

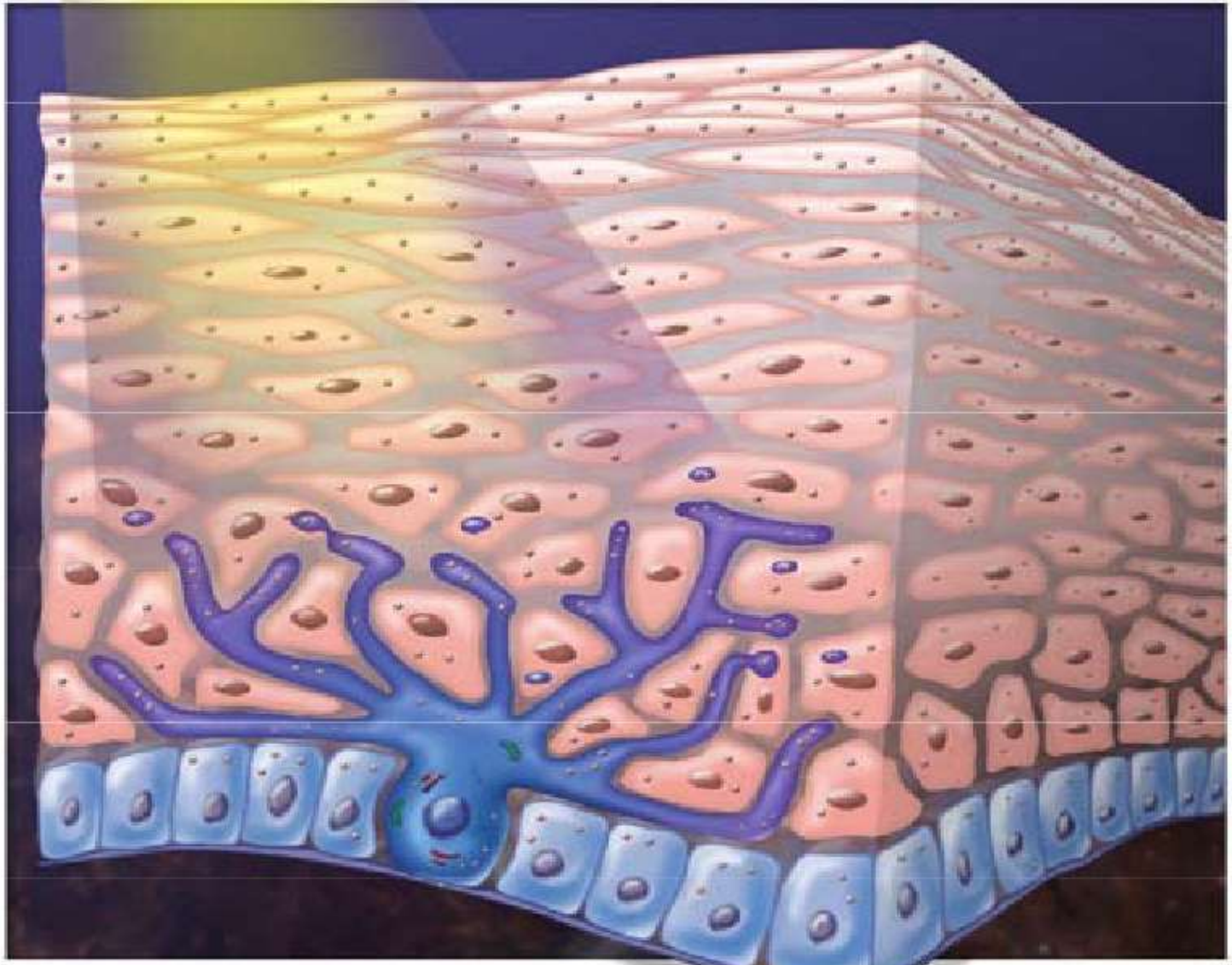
TYPES OF MELANOCYTES

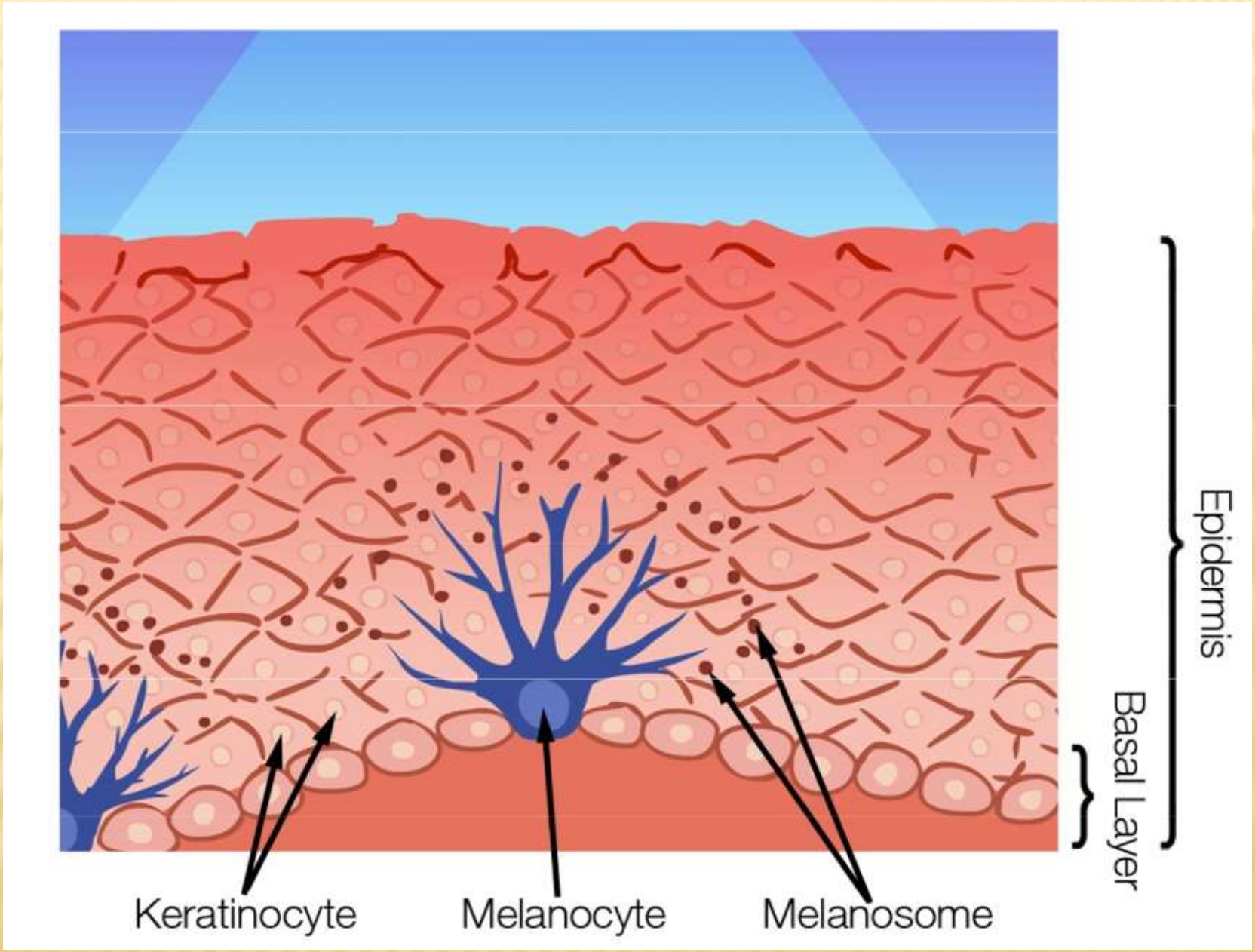
1- SECRETORY MELANOCYTES:

- Present in basal layer of epidermis form network of dendrites in the basal layer
- They act as unicellular glands producing melanosomes that are transferred to surrounding epidermal keratinocytes (cytocrine activity)

2- NON-SECRETORY MELANOCYTES:

- Called *MELANOPHORES* do not transfer melanosome but redistribute them from perinuclear zone into dendrites and back again




Epidermis
Basal Layer
Keratinocyte
Melanocyte
Melanosome

FACTORS CAUSING MELANOCYTES TO INCREASE MELANIN PRODUCTION

1) UV irradiation (direct effect)

2) Keratinocyte paracrine factors induced by UV irradiation (indirect effect)

• NORMAL SKIN COLOR IS PRODUCED BY FOUR SKIN PIGMENTS.

Epidermis

- by exogenously produced **carotenoids** (yellow).
- by endogenously produced **melanin** (**brown, black, Or yellow-red**) amount & location.

N.B. Normal skin pigmentation is influenced by the thickness of the horny layer.

Dermis

- by **oxygenated haemoglobin** (**red**) in capillaries
- by **reduced haemoglobin** (**blue**) in venules.

SITE SPECIFIC MELANOCYTES:

A. Cutaneous melanocytes:

- There is approximately one melanocyte per 5-10 keratinocytes
- Melanocytes synthesize & store melanin in cytosolic organelles called melanosomes that are transferred to keratinocytes.

- As keratinocytes are continuously being desquamated, there is a constant need for synthesis & transfer of melanosomes from melanocytes to keratinocytes to maintain cutaneous pigmentation.

- Melanocyte density/square mm ranges from 550 to 1500, with the highest concentration within face & genitalia.

- Melanocyte density is almost the same in all individuals of different ethnic background & thus cutaneous pigmentation doesn't depend on melanocyte number.

Q- it depends on what?

- Melanocyte density is almost the same in all individuals of different ethnic background & thus cutaneous pigmentation doesn't depend on melanocyte number.

Q- it depends on what?

1) Melanogenic activity within the melanocyte
2) The proportion of mature melanosomes
3) Size of melanosomes
4) Type of melanin (eumelanin, or pheomelanin)
5) Melanosomes transfer & distribution within the keratinocytes

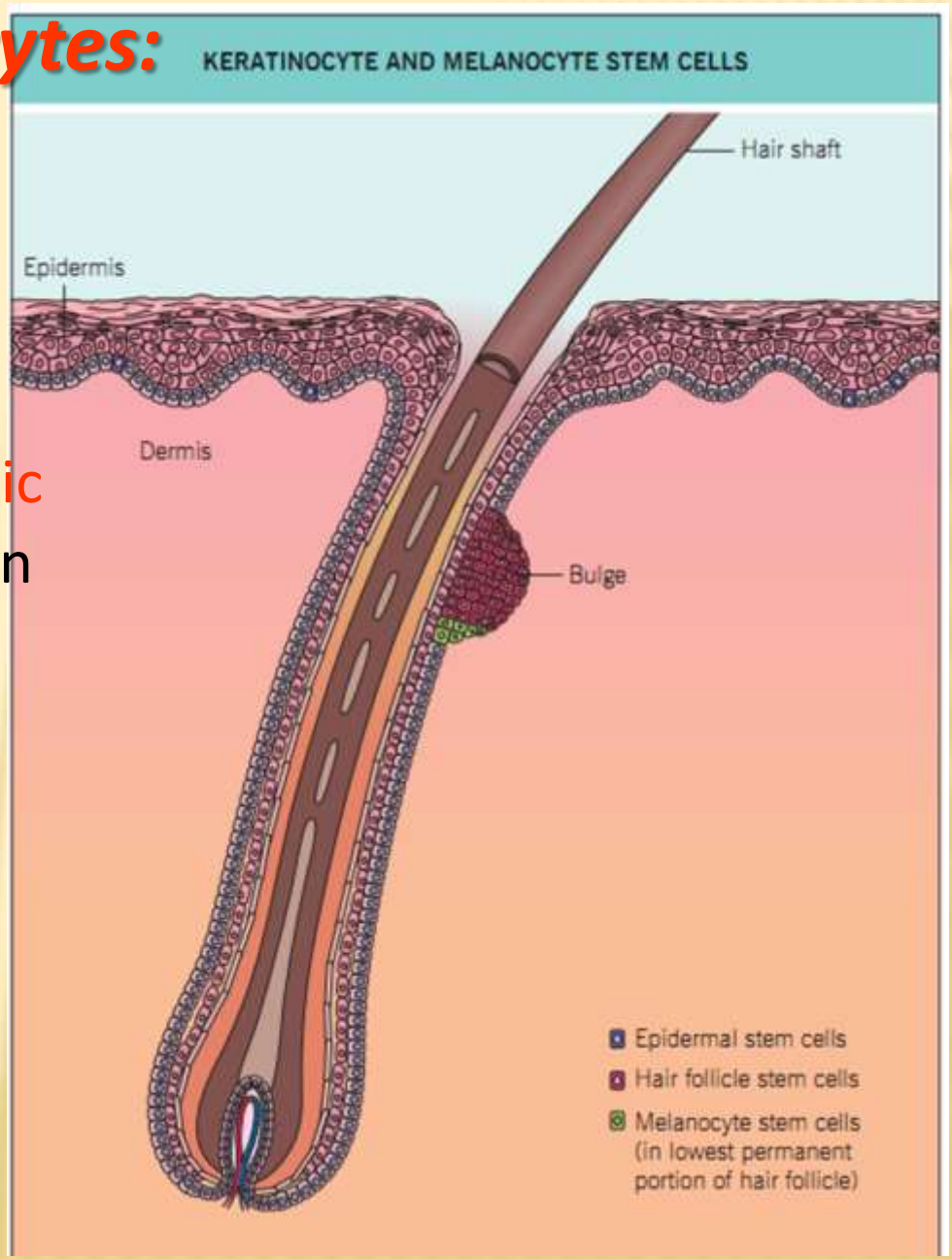
B. Hair follicle melanocytes:

- In contrast to interfollicular melanocytes, the follicular

melanin unit undergoes cyclic modifications in coordination with hair cycle.

- Hair color is determined by amount of melanin transferred to keratinocytes forming the hair shaft as well as by the ratio

of eumelanin to pheomelanin



C. Ocular melanocytes:

- Unlike cutaneous melanocytes, ocular melanocytes are in contact only with each other & don't transfer melanosomes.

Albinos may have visual abnormalities due to absence of melanin

D. Otic melanocytes:

- Reside in cochlea & are important for hearing, as loss of otic melanocytes may leads to **deafness** as in **Waardenburg syndrome TYPE II**.

- Reduction in number & activity of melanocytes occurs with ageing i.e. 6-8% per decade, more of **follicular melanocytes** & spared epidermal melanocytes from sun exposure are declined leading to graying of hair & lightening of skin color.

COMPARATIVE FEATURES OF PREMATURE, NEWBORN, AND ADULT SKIN MELANOCYTES

- **Adult**: Numbers decrease with age; melanin production dependent on skin type, body area
- **Newborn**: Similar number of cells to young adult; low melanin production
- **Premature**: High number of cells; few mature melanosomes

MELANOSOME

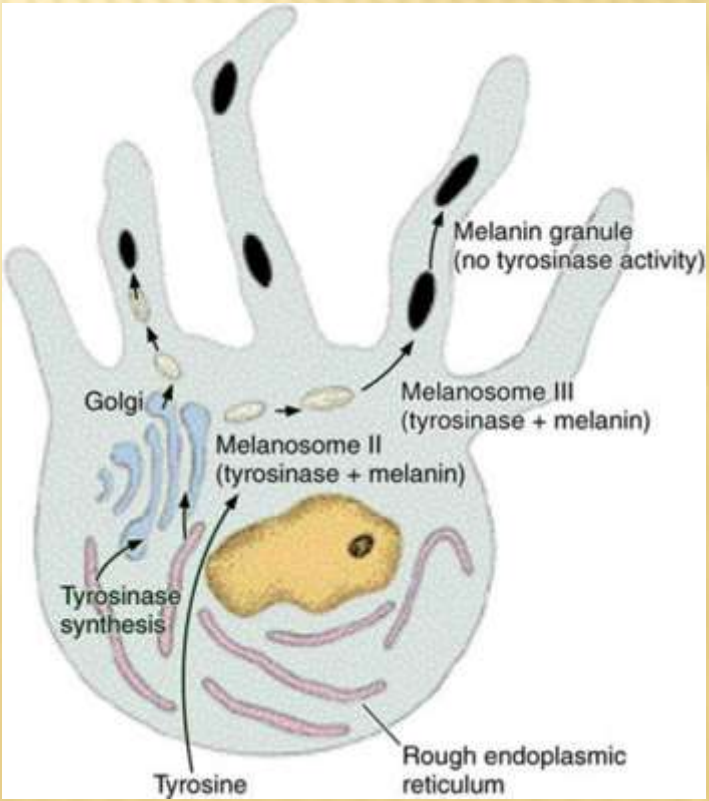
Definition:
Is membrane-bound unique organelle Within the cytoplasm of melanocytes in which in which melanin pigments are synthesized, deposited and transported.

And depending on the type of melanin (eumelanin or pheomelanin) synthesized, melanosomes can be divided into:

- **Eumelanosome**
- **Pheomelanosome**

SYNTHESIS & DISTRIBUTION OF MELANIN IN THE EPIDERMIS INVOLVES SEVERAL STEPS

1) Transcription of proteins required for melanin synthesis
2) Melanosome biogenesis
3) Sorting of melanogenic proteins into melanosomes to initiate melanin synthesis within the melanosome
4) Transport of the mature melanosomes to the tips of melanocyte dendrites migrates via microtubules
5) Transfer of melanosomes to keratinocytes

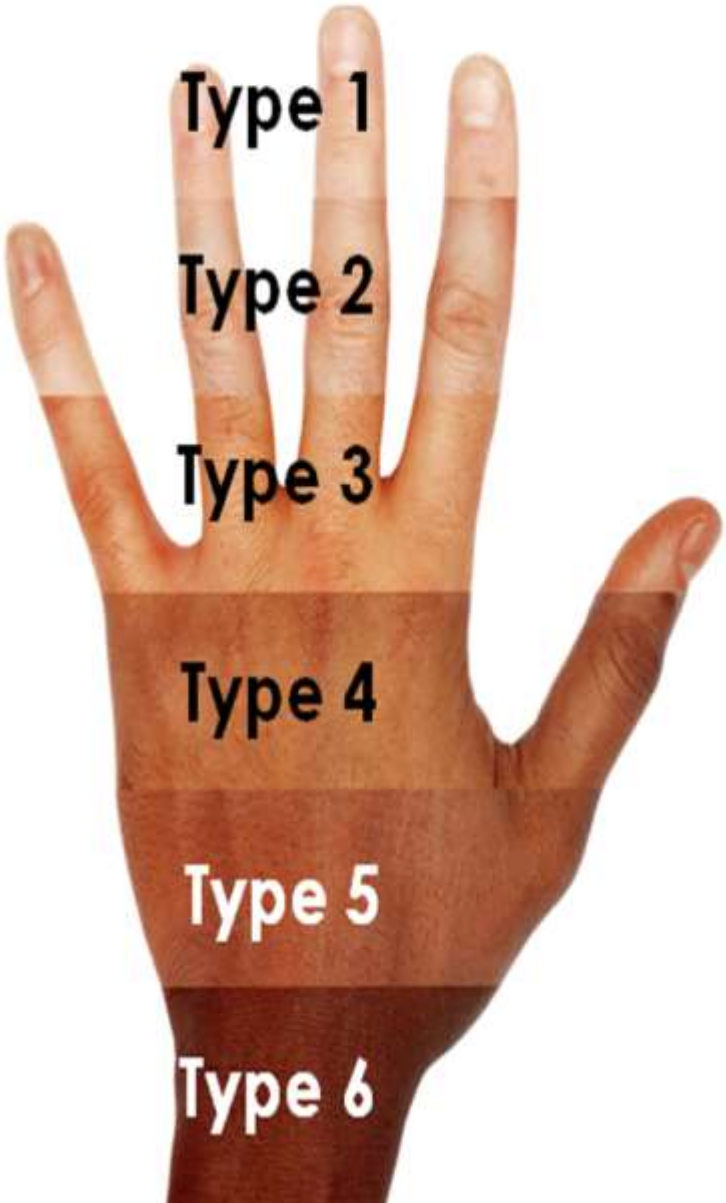


FOUR MAJOR STAGES OF EUMELANIN MELANOSOMES

Stage	Description	Electron micrographs
I	Spherical; no melanin deposition	
II	Oval; obvious matrix in the form of parallel longitudinal filaments; minimal deposition of melanin; high tyrosinase activity	
III	Oval; moderate deposition of melanin; high tyrosinase activity	
IV	Oval; heavy deposition of melanin; electron-opaque; minimum tyrosinase activity	

Human beings come in a glorious spectrum of different colors: light, dark, plain or freckly skin; black, brunette, blond, auburn, and white hair; and eyes that are blue, hazel, green, amber and brown, to name just a few. It’s amazing to realize that most of this color is attributed to a single class of pigments: the melanins.

Fitzpatrick Skin Types



White: Always burns, never tans

White: Usually burns, difficulty in tanning

White: Sometimes burns, average tan

Moderate Brown: Rarely burns, tans with ease

Dark Brown: Very rarely burns, tans very easily

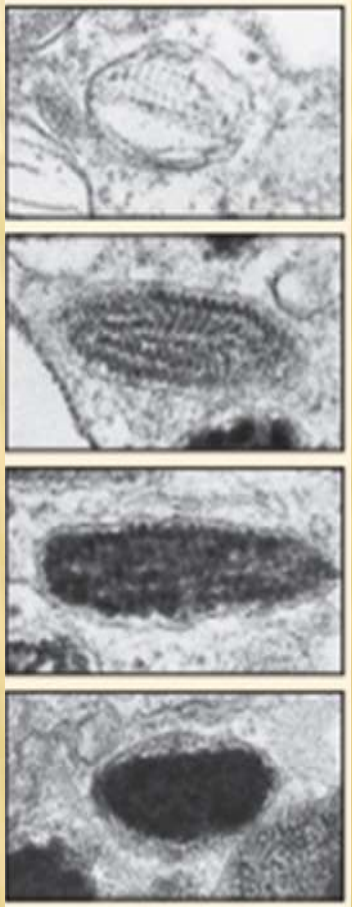
Black: Does not burn, tans very easily

FITZPATRICK SCALE OF SKIN PHOTOTYPES		
Skin phototype	**Skin color**	**Response to UV irradiation**
I	White	Always burns, does not tan
II	White	Burns easily, tans with difficulty
III	Beige	Mild burns, tans gradually
IV	Brown	Rarely burns, tans easily
V	Dark brown	Very rarely burns, tans very easily
VI	Black	Never burns, tans very easily

White people strive for tanning which while brown and black people strive for a lighter skin

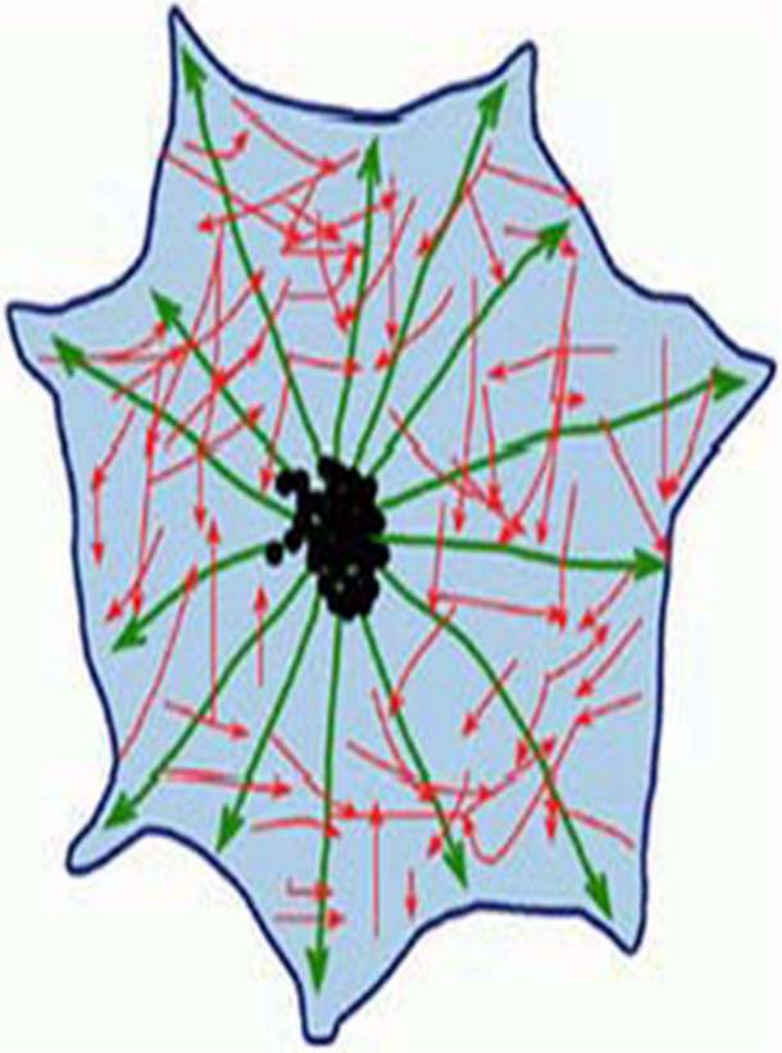
VARIATION OF PREDOMINANT MELANOSOMAL STAGES WITH LEVEL OF CUTANEOUS PIGMENTATION

	Predominant melanosomal stages	
Pigmentation of skin	Melanocytes	Keratinocytes
Fair	II, III	Occasional III
Medium	II, III, IV	III, IV
Dark	IV > III	IV

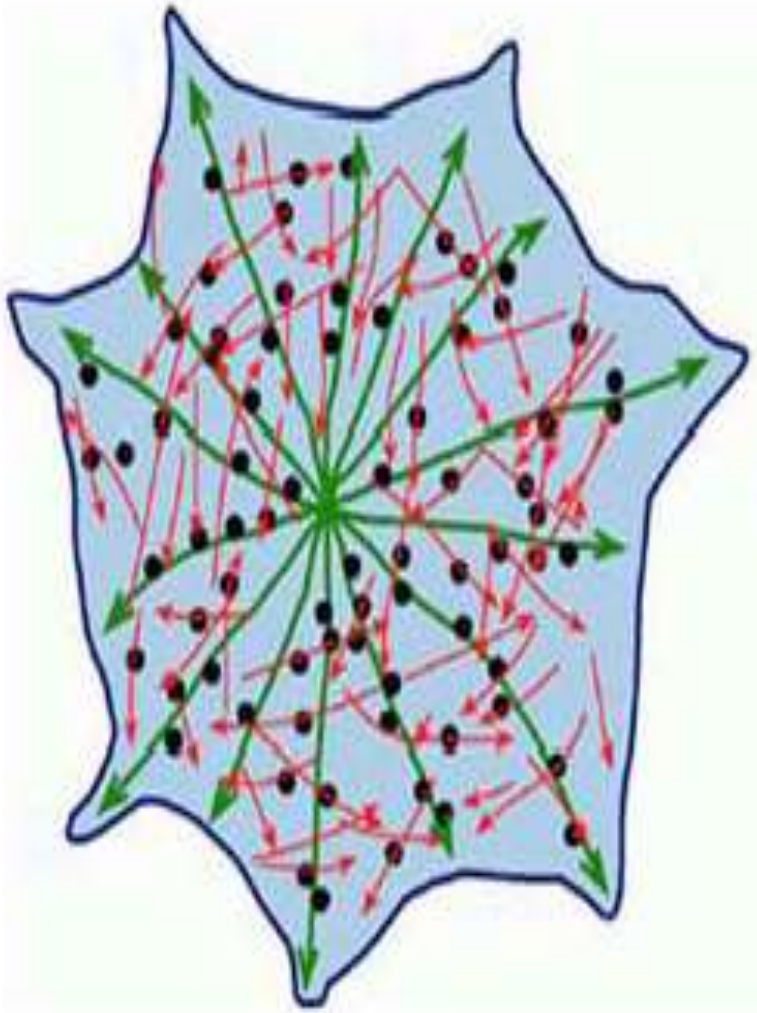


MELANOSOMES IN LIGHTLY PIGMENTED VERSUS DARKLY PIGMENTED SKIN

	Lightly pigmented skin	Darkly pigmented skin
Melanization	Stages II, III	Stage IV
Size (diameter)	0.3–0.5 microns	0.5–0.8 microns
Number per cell	<20	>200
Distribution of melanosomes within the lysosomes of keratinocytes	Groups of 2–10 	Single 
Degradation	Fast	Slow



Aggregated melanosomes



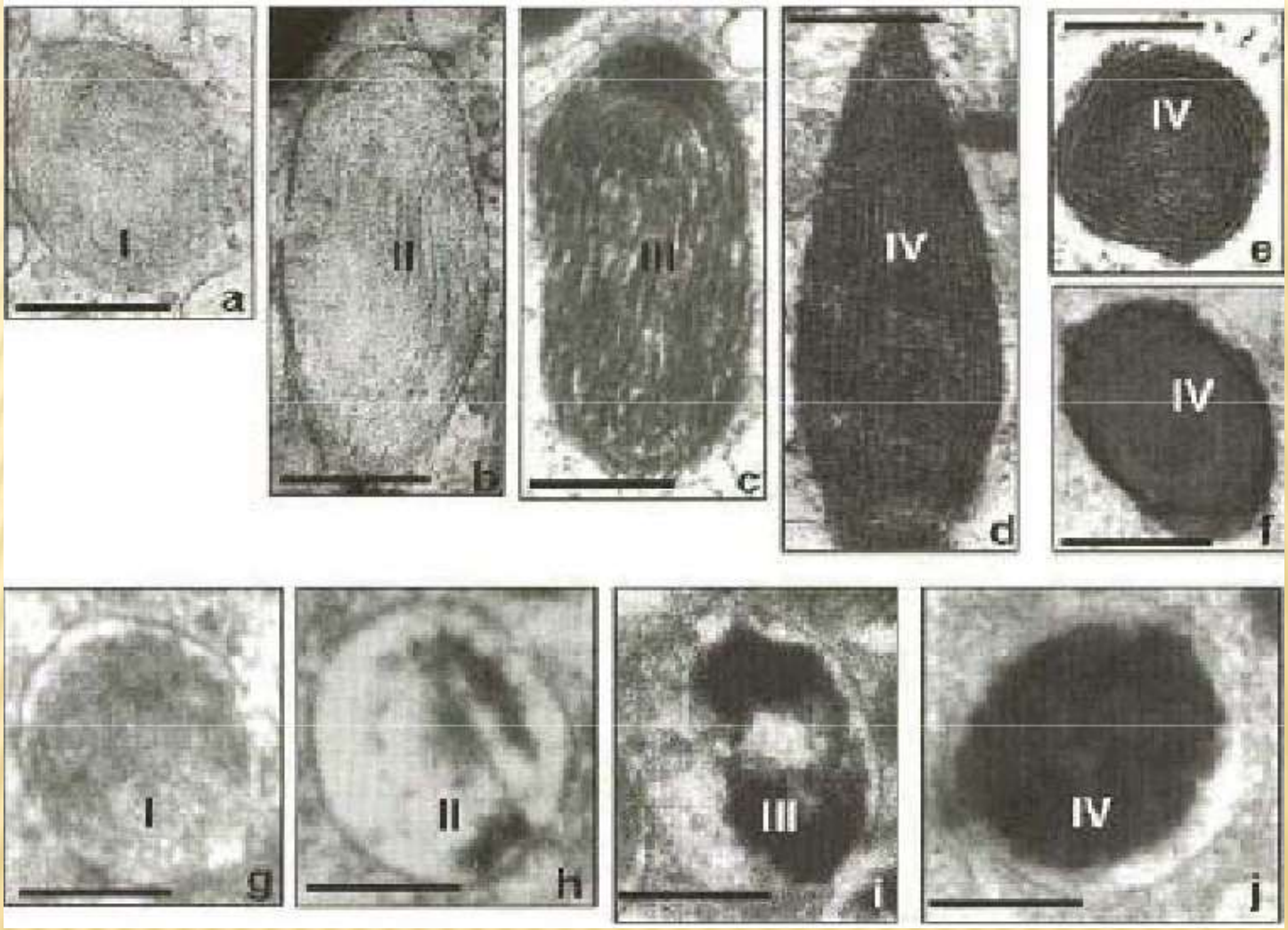
Dispersed melanosomes

MELANOSOME BIOGENESIS

Display 4 maturation stages:

Stage 1

- spherical, membrane bound vesicles develop from the endoplasmic reticulum & they have amorphous fibrillar matrix that acts as a scaffold upon which melanin is deposited
- Possess tyrosinase activity.



Pheomelanosomes is more spherical shape

Stage 2

Both (eumelanosomes & pheomelanosomes have
organized structured fibrillar (thread-like) matrix but;
stage 2 pheomelanosomes: ***melanin synthesis already takes place***, while

stage 2 eumelanosomes: oval minimal or ***no active melanin synthesis***

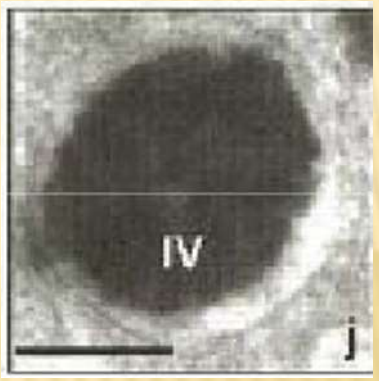
Stage 3

Internal structure of melanosomes is partially obscured by deposition of melanin

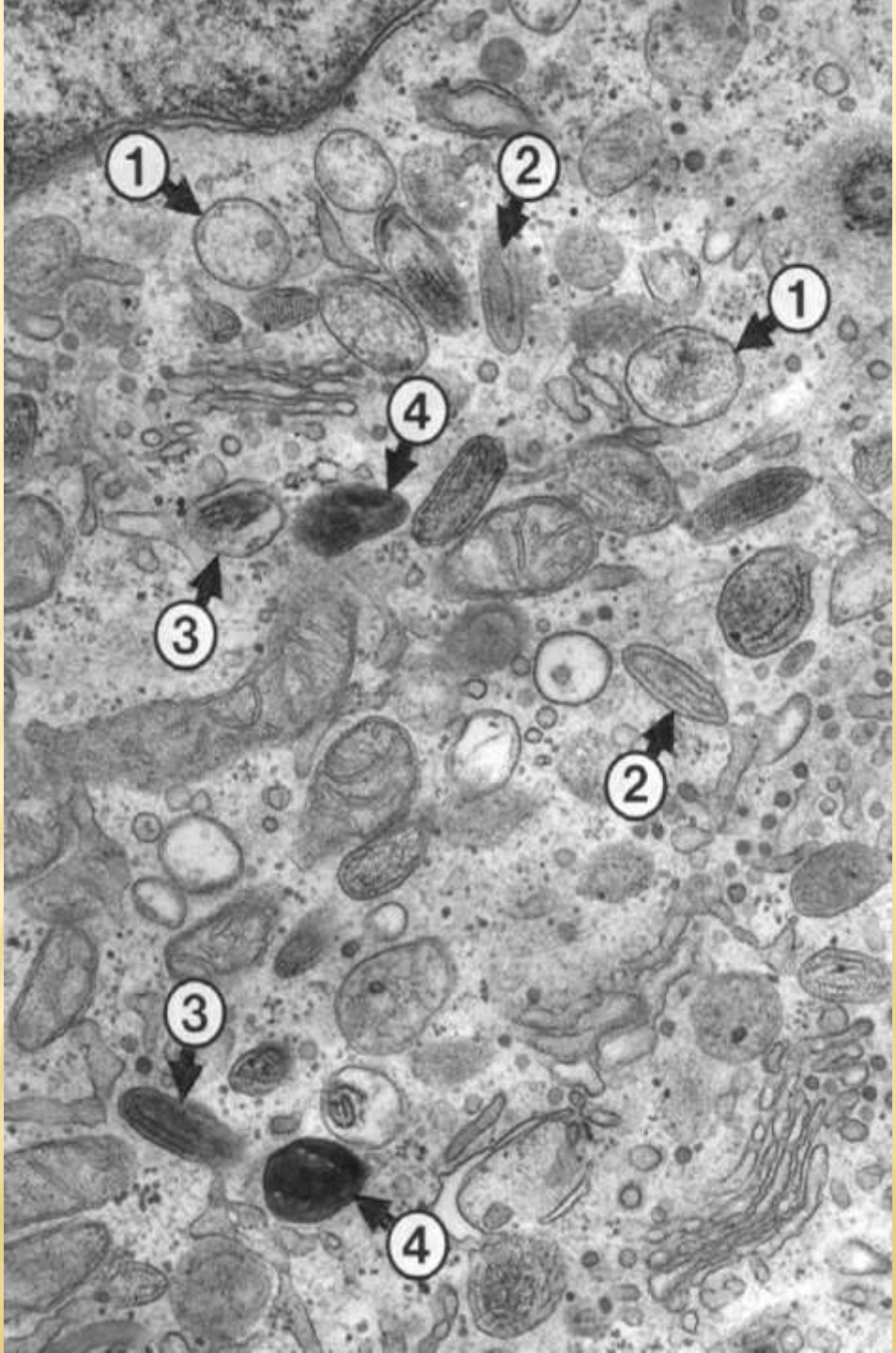
stage 3 pheomelanosomes: *continuation* of melanin synthesis & deposition

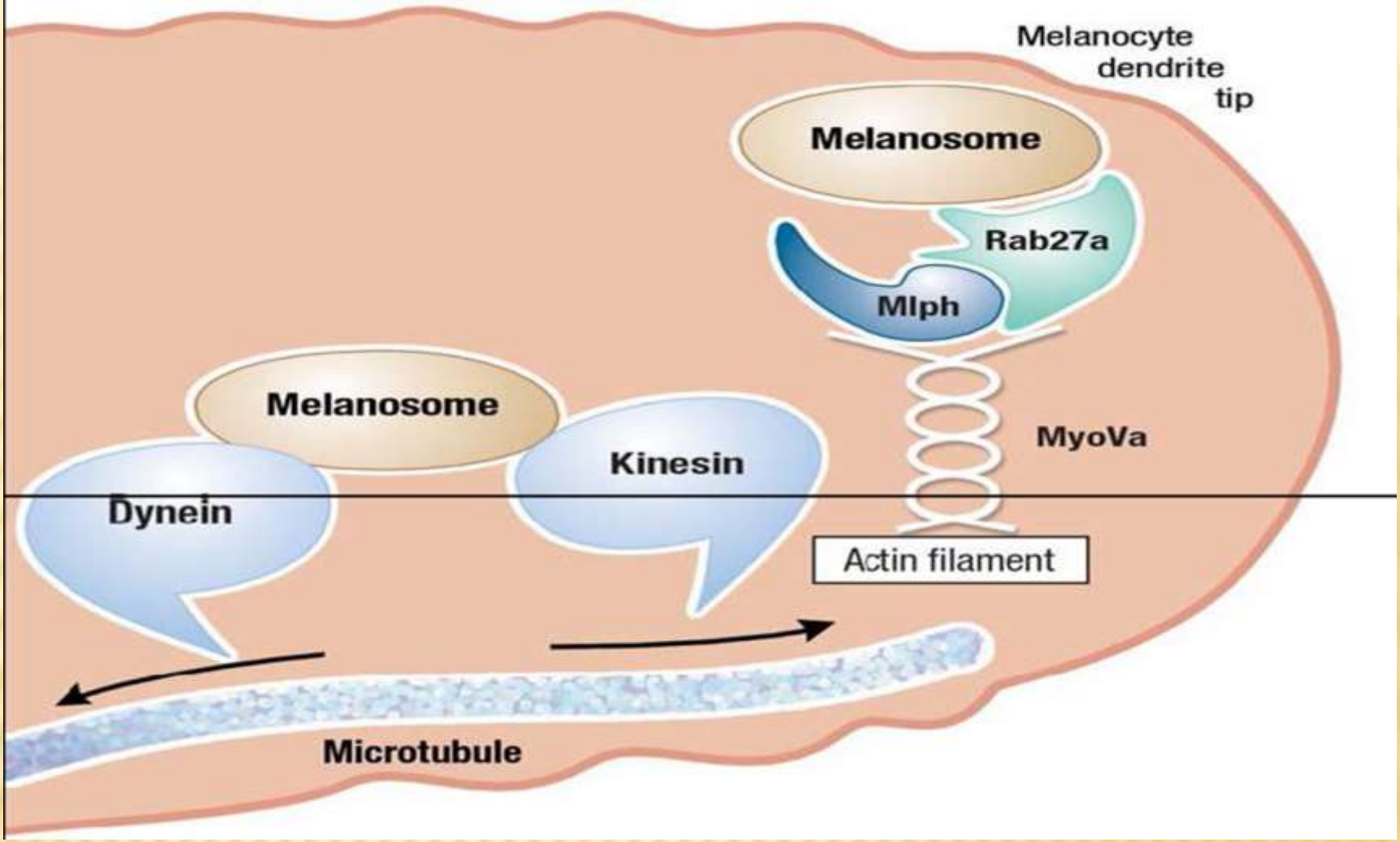
stage 3 eumelanosomes: *active melanin synthesis & deposition starts in this stage*

Stage 4



- mature melanosome appears electron dense.
- Both pheomelanosomes & eumelanosomes are fully melanised


1
2
1
4
3
2
3
4



MELANOSOMES TRANSPORT TAKES PLACE ON MICROTUBULE THAT ARE ARRANGED PARALLEL TO THE LONG AXIS OF THE DENDRITE USING 2 MOTOR PROTEINS

TRANSFER OF MELANOSOME TO KERATINOCYTE

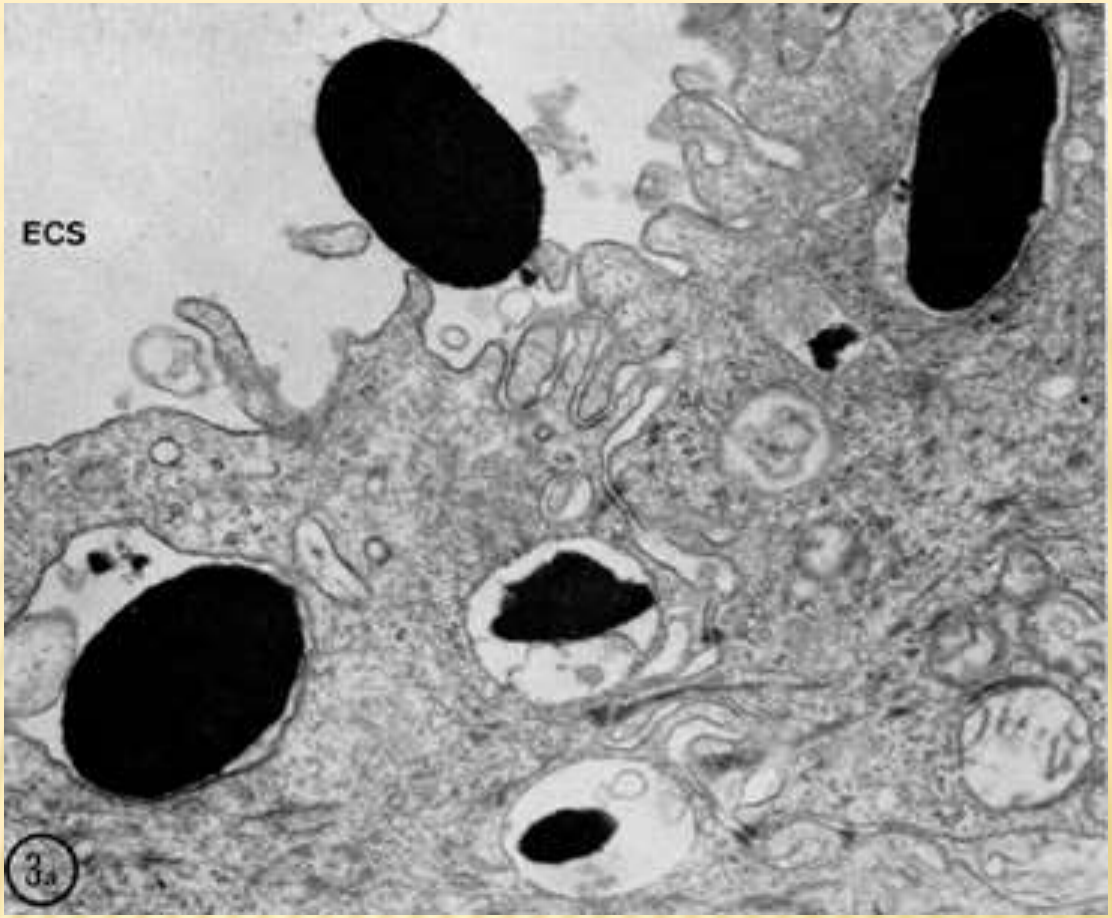
1) **Exocytosis**: fusion of the melanosomal membrane with the melanocyte plasma membrane, melanosome is released to the intercellular space and phagocytosis by surrounding keratinocytes occur.

2) **Cytophagocytosis**: projection of dendrites into keratinocyte cytoplasm then keratinocytes cytophagocytose the tip of a melanocyte dendrite.

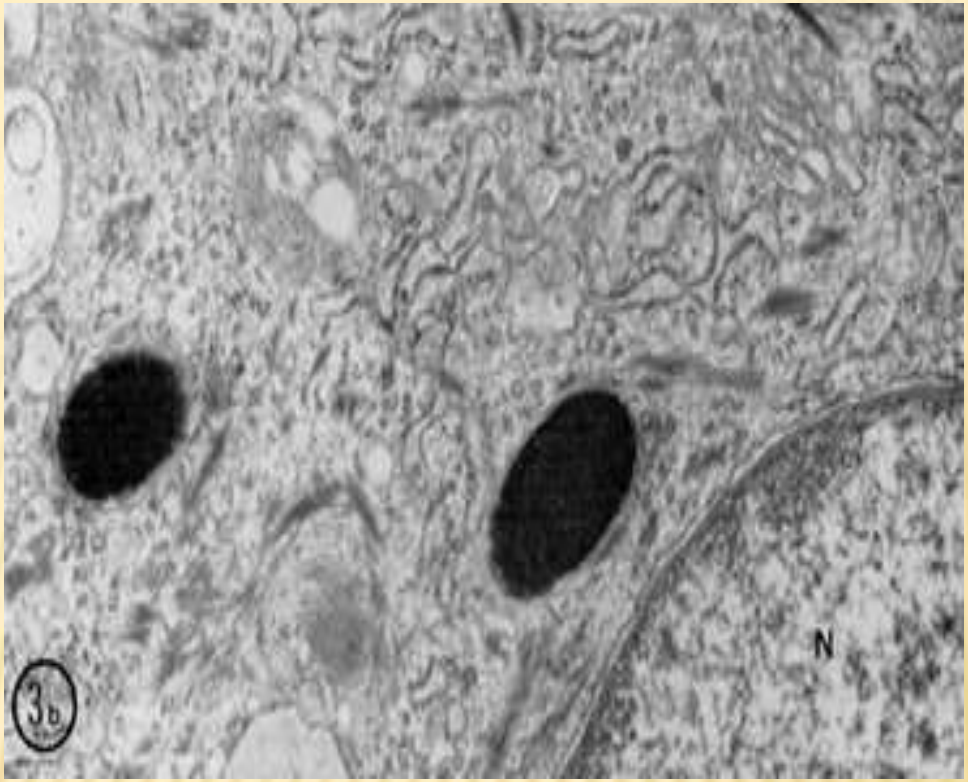
3) **Fusion of melanocyte & keratinocyte plasma membrane**

create a space through which melanosomes are transferred

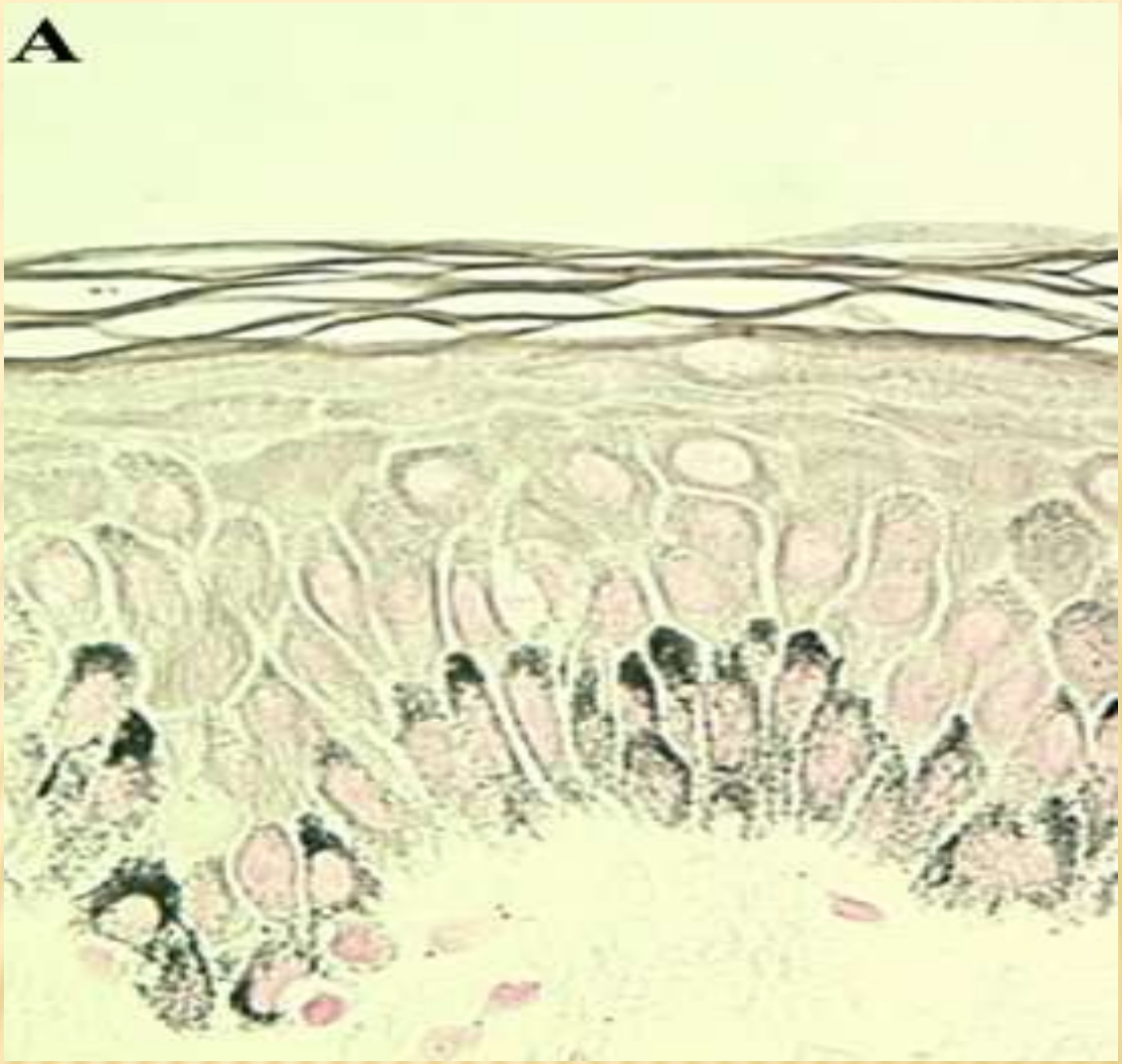
4) **Shedding of melanosome-filled vesicles** followed by phagocytosis of the vesicles by keratinocytes



MELANOSOME BEING PHAGOCYTOSED BY A KERATINOCYTE

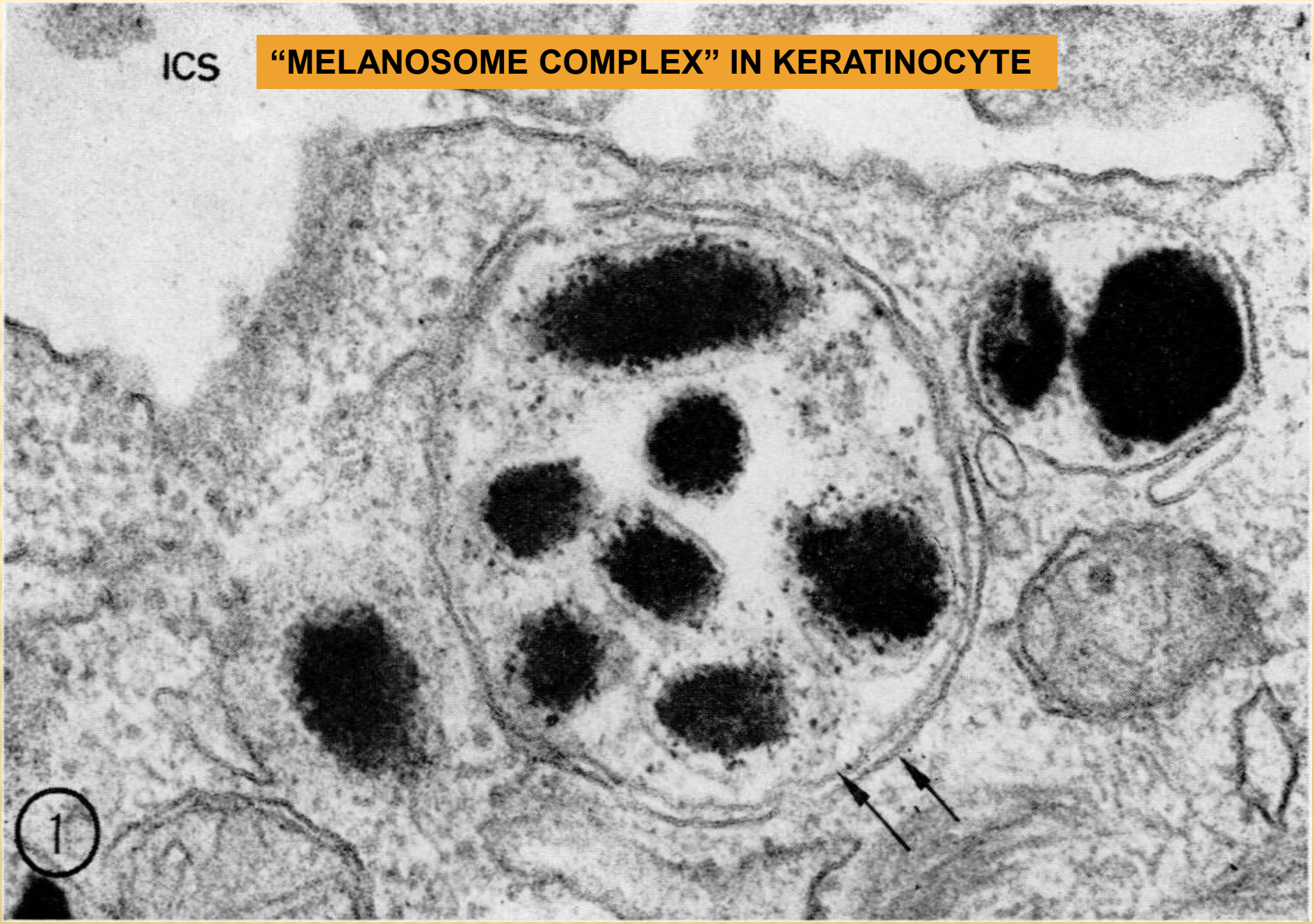


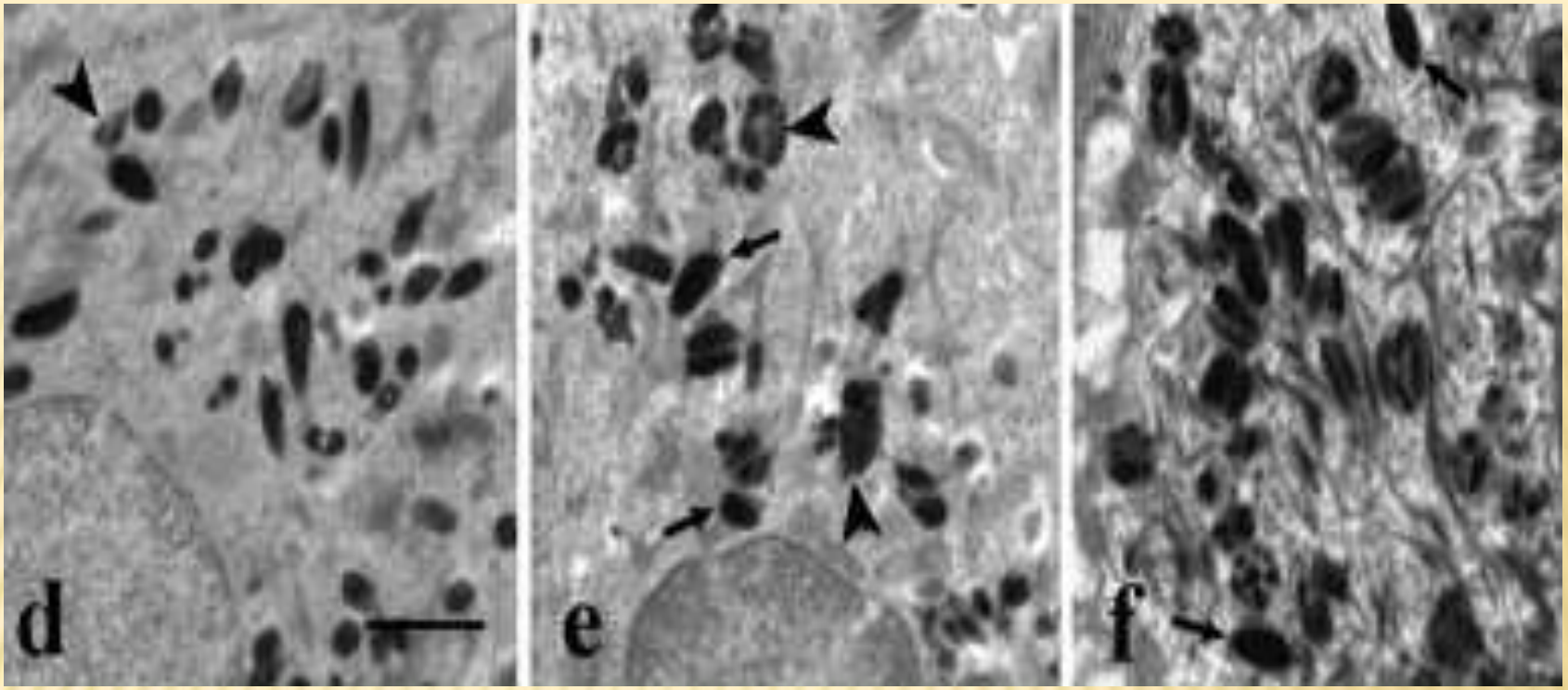
PHAGOCYTOSED MELANOSOMES ARE DISTRIBUTED AS A CAP AT TOP OF THE NUCLEUS



MELANSOMES ARE TAKEN UP BY PHAGOCYTOSIS INTO THE LOWER KERATINOCYTES

- Non-exposed skin of Caucasoids, especially those with light skin, such transferred melanosomes are found almost exclusively in the basal cell layer and, to a slight degree, in the layer of keratinocytes above the basal cell layer.
- However, in dark skin, in whom melanosomes are also principally seen in the basal cell layer, moderate quantities of melanosomes are found throughout the epidermis, including the stratum corneum


ICS
“MELANOSOME COMPLEX” IN KERATINOCYTE
1



AFRICAN/AMERICAN

11% MELANOSOMES IN MEMBRANE-BOUND PACKETS

ASIAN

37% MELANOSOMES IN MEMBRANE-BOUND PACKETS

CAUCASIAN

85% MELANOSOMES IN MEMBRANE-BOUND PACKETS

MELANINS

- Def: Melanins represent a group of complex polymers made from tyrosine that gives color to hair, skin, and the iris of the eye. Produced by melanocytes.

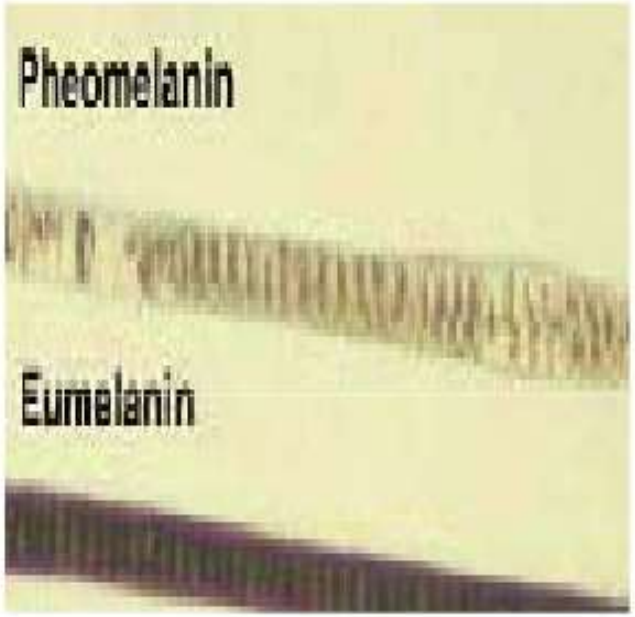
FUNCTIONS OF MELANIN

1) Provide protection against UV-induced DNA damage by absorbing & scattering UV radiation (280-400 nm)
2) Gives the skin, eyes, hair their color
3) Important for proper ocular function
4) May provide some protective effect against noise induced hearing loss
5) Neutralizer of toxic, free radical oxygen derivatives, by products of various inflammatory processes.

TYPES OF MELANIN

Eumelanin (**brown, black**)

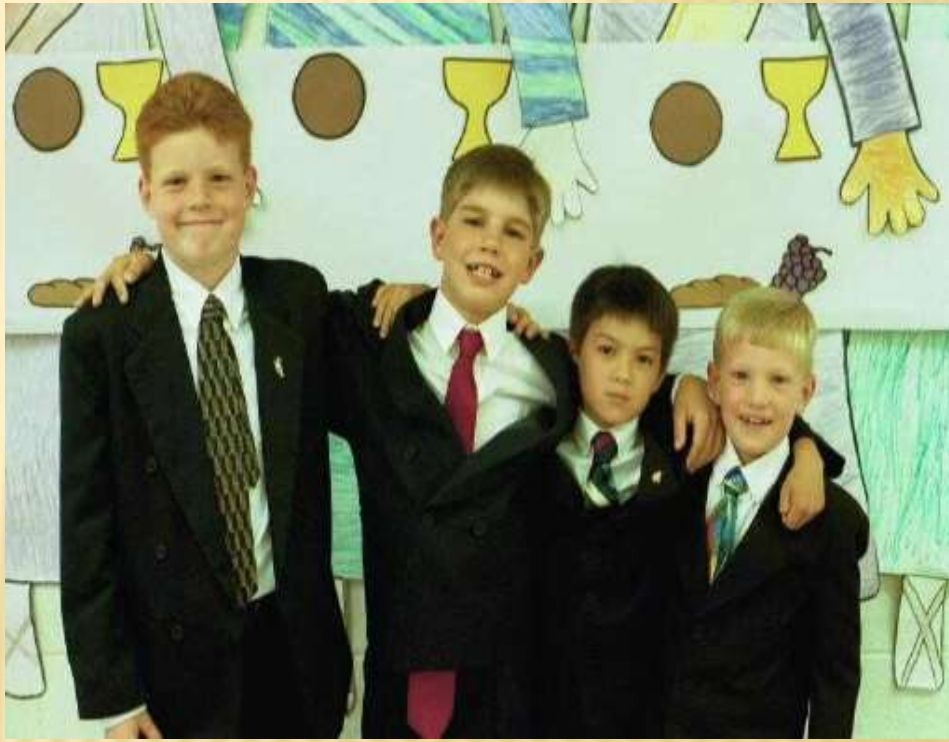
Pheomelanin (**red-yellow**)



TYPES OF MELANIN PIGMENTATION

- **Constitutive:**

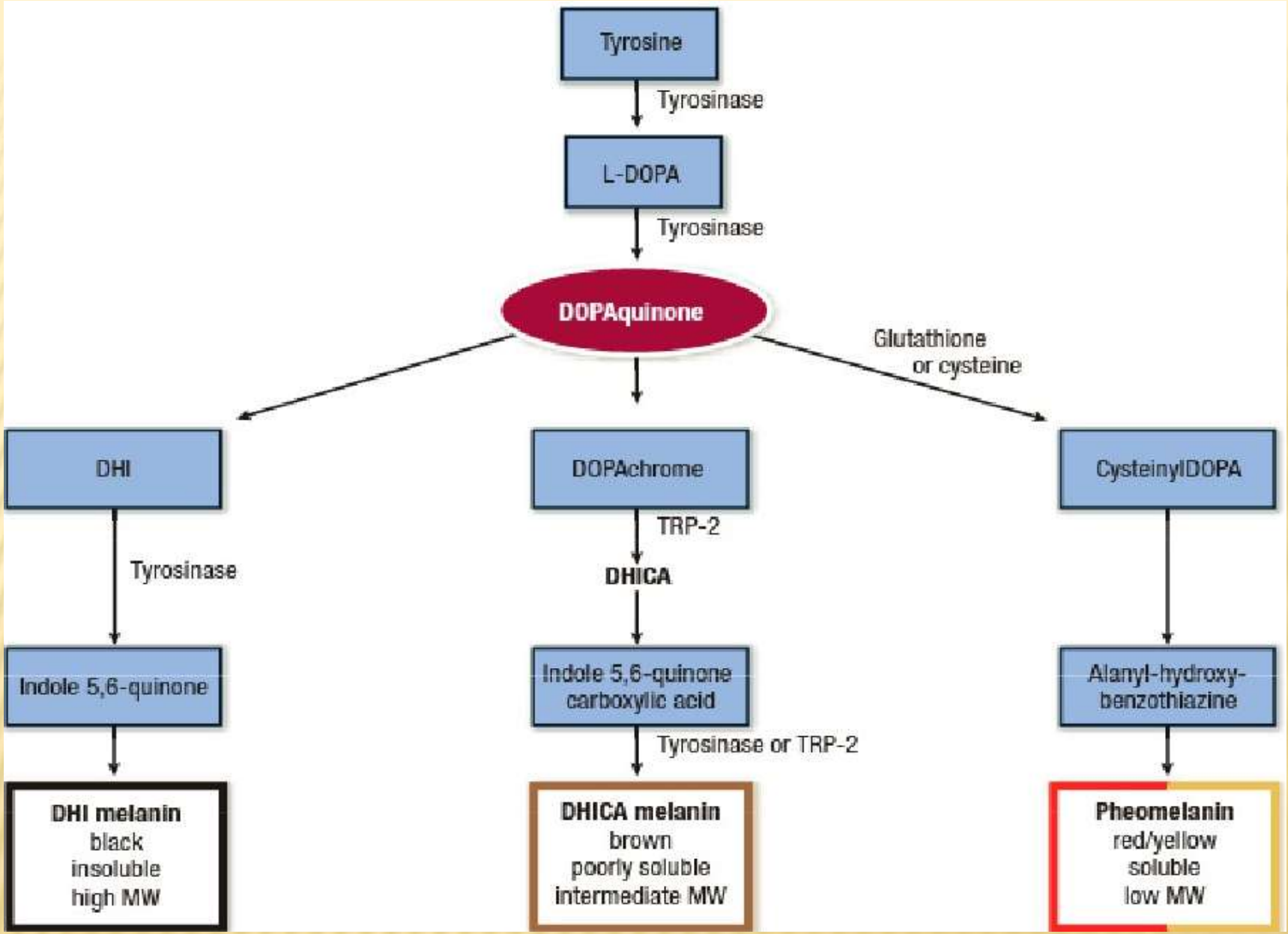
  Genetically determined melanin pigmentation in the absence of sun exposure and other influences.



- **Facultative (inducible):**

  stimulated Pigmentation result from sun exposure

- **Other**: Hormonal effect



THE MELANIN BIOSYNTHETIC PATHWAY

EUMELANINS

- BLACK OR BROWN
- INSOLUBLE IN ALL SOLVENTS
- NITROGENOUS PIGMENT
- OXIDATIVE POLYMERISATION OF

5-6DIHROXYINDOLE DERIVED FROM TYROSIN

- MANUFACTURED IN ELLIPSOIDAL MELANOSOMES

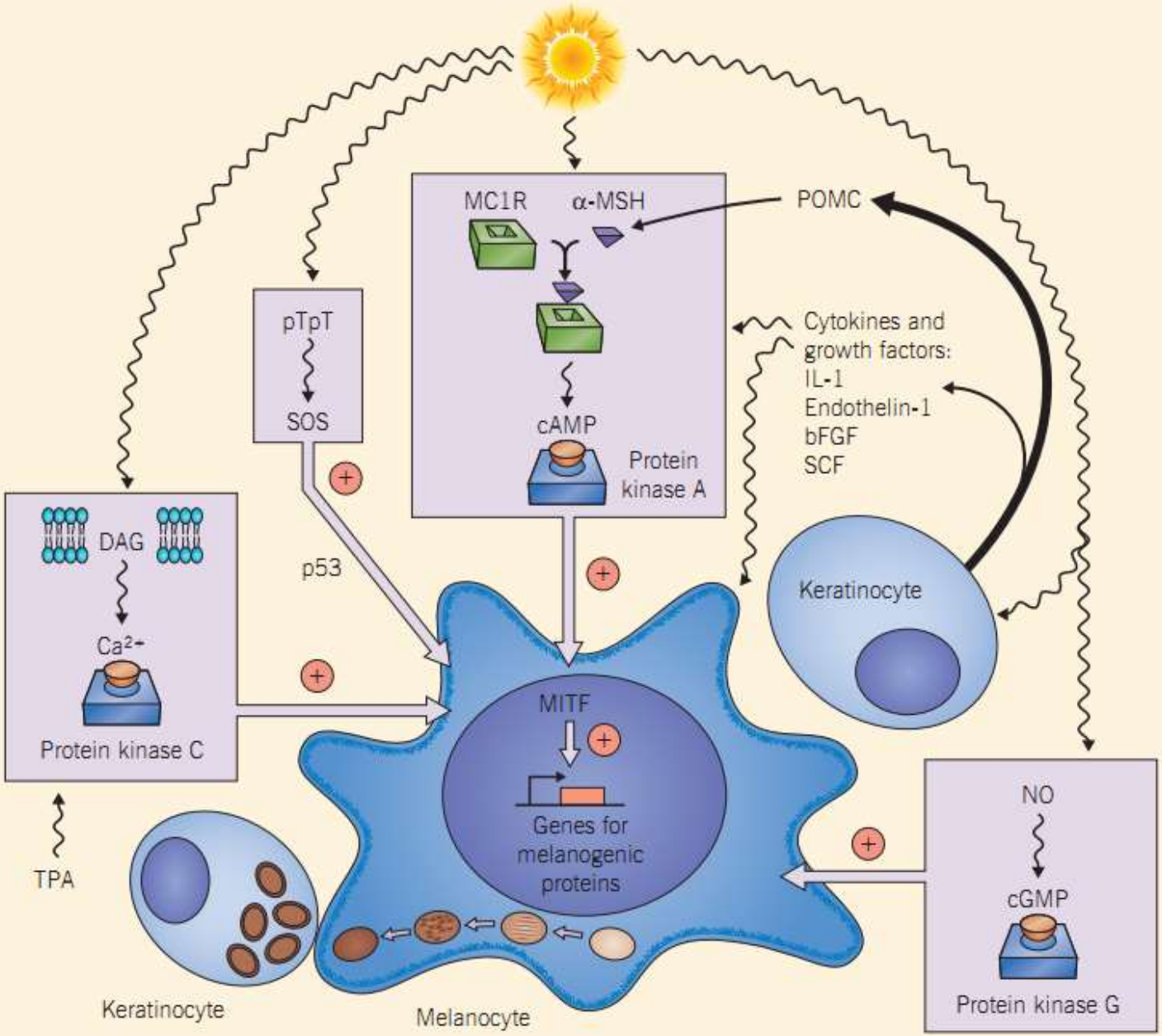
PHEOMELANINS

- YELLOWISH TO REDDISH BROWN
- ALKALI SOLUBLE
- CONTAIN SULPHUR IN ADDITION TO NITROGEN
- OXIDATIVE POLYMERISATION OF CYSTINE-S-YI-DOPA VIA 1-4BENZOTHIAZINE INTERMEDIATES
- MANUFACTURED IN SPHERICAL MELANOSOMES
- FEMALES >MALE
- lips, nipples, glans of the penis, and vagina
- CARCINOGENESIS

FACTORS INFLUENCE THE ACTIVITY OF KEY PROTEINS OF MELANOGENESIS

A. **Genetics.**
B. **Hormones**:
   1- MSH
   2- ACTH
   3- Estrogens
C. **Biochemical factors:**
   1- IL-1
   2- IL-6
   3- TNF-alpha
   4- basic fibroblast growth factor (bFGF)
   5- Endothelin-1
D. **External factors**:
   1- UV light (amount and wave-length)
   2- melanocyte stimulating chemicals like photosensitizers.

CAUSES OF INCREASED MELANIN PRODUCTION



MELANOGENIC PROTEINS

1) **Enzymes** regulate the biosynthesis of melanin.
   a) Tyrosinase,
   b) TRP-1 (Tyrosinase-Related Protein 1)
   c) TRP-2
   d) Protein kinase c-beta

2) **Structural proteins** matrix proteins which form a scaffolding upon which the melanin is deposited
   a) Pmel17 (Premelanosome protein)
   b) MART-1 (Melanoma Antigen Recognized by T cells)

4) **Regulatory proteins**
   a) MITF (Microphthalmia-Associated Transcription Factor)
   b) MC-1R (Melanocortin 1 receptor)

3) **Additional melanogenic proteins**

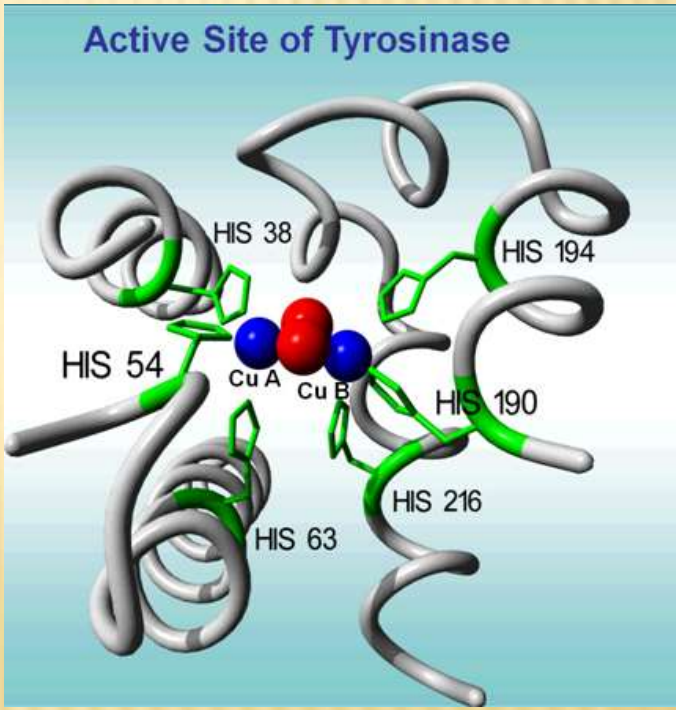
(P Protein, Heterotetrameric adaptor protein comlexes “Aps”)

TYROSINASE

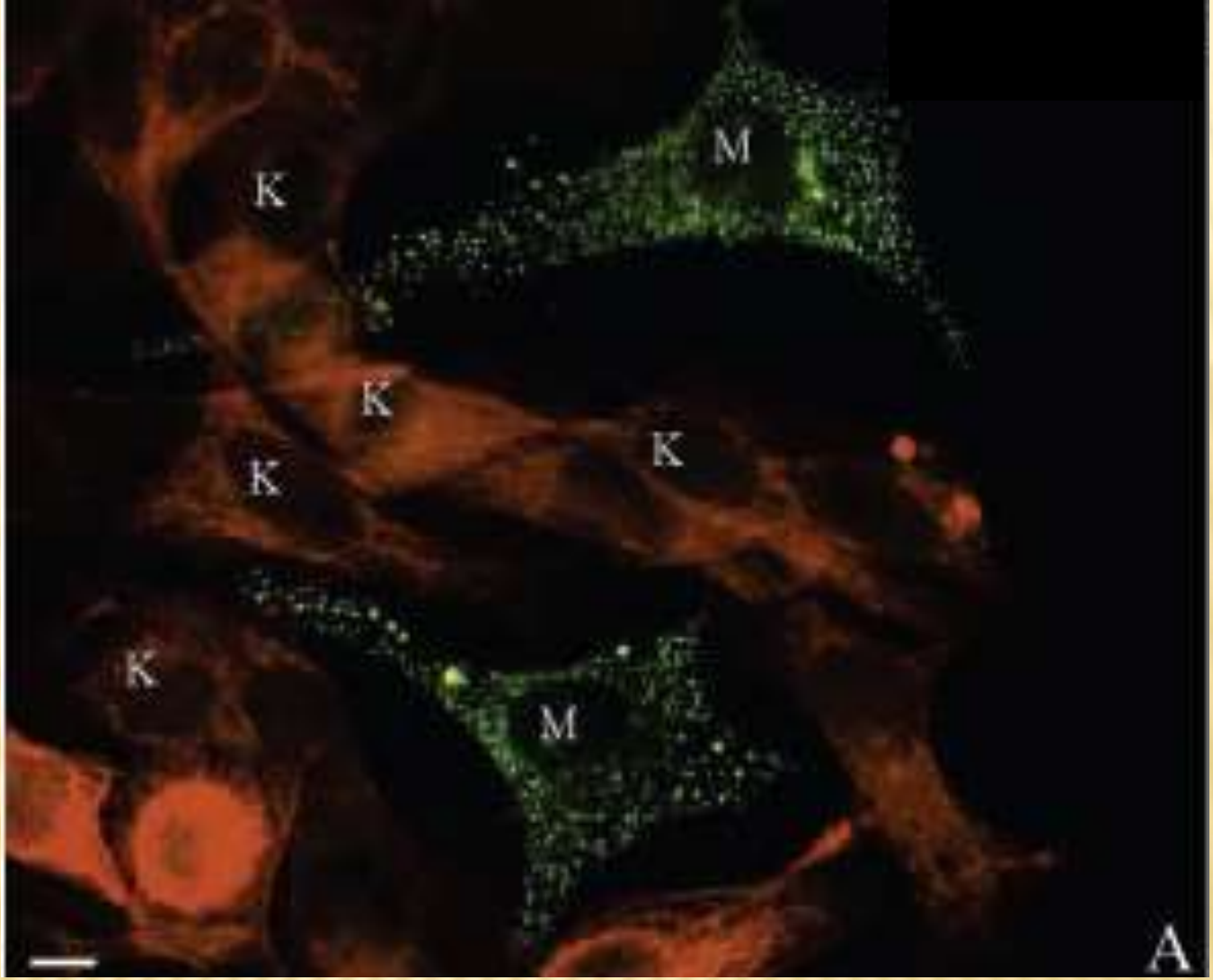
- The ***key enzyme*** for melanin synthesis within the melanosome.
- Catalyses the oxidation of phenols (such as tyrosine).
- Spans the *melanosomal outer membrane. It* has three domains:
  1. Inner melanosomal domain (form 90% of the protein).
  2. Melanosomal transmembrane domain.
  3. Cytoplasmic domain.

TYROSINASE

- Histidine residues present in the inner domain.
- Binds two copper atoms within its active site which are required for tyrosinase activity.
- Rare cases of copper deficiency can lead to *diffuse cutaneous pigmentary dilution.*
- In addition to that, two serine residues on the cytoplasmic domain must be phospholyrated by *protein kinase C-beta* to *activate tyrosinase*, in the absence of this phosphorylation, pigmentation does not occure.



Normal melanocyte.
A whole mount of epidermis has been stained for the presence of the enzyme *tyrosinase*, which typifies melanocytes. Note that the surrounding keratinocytes are virtually invisible. An adjacent *hair shaft* passes through the specimen. Melanocytes contain long dendritic processes that deliver protective melanosomes to nearby keratinocytes.

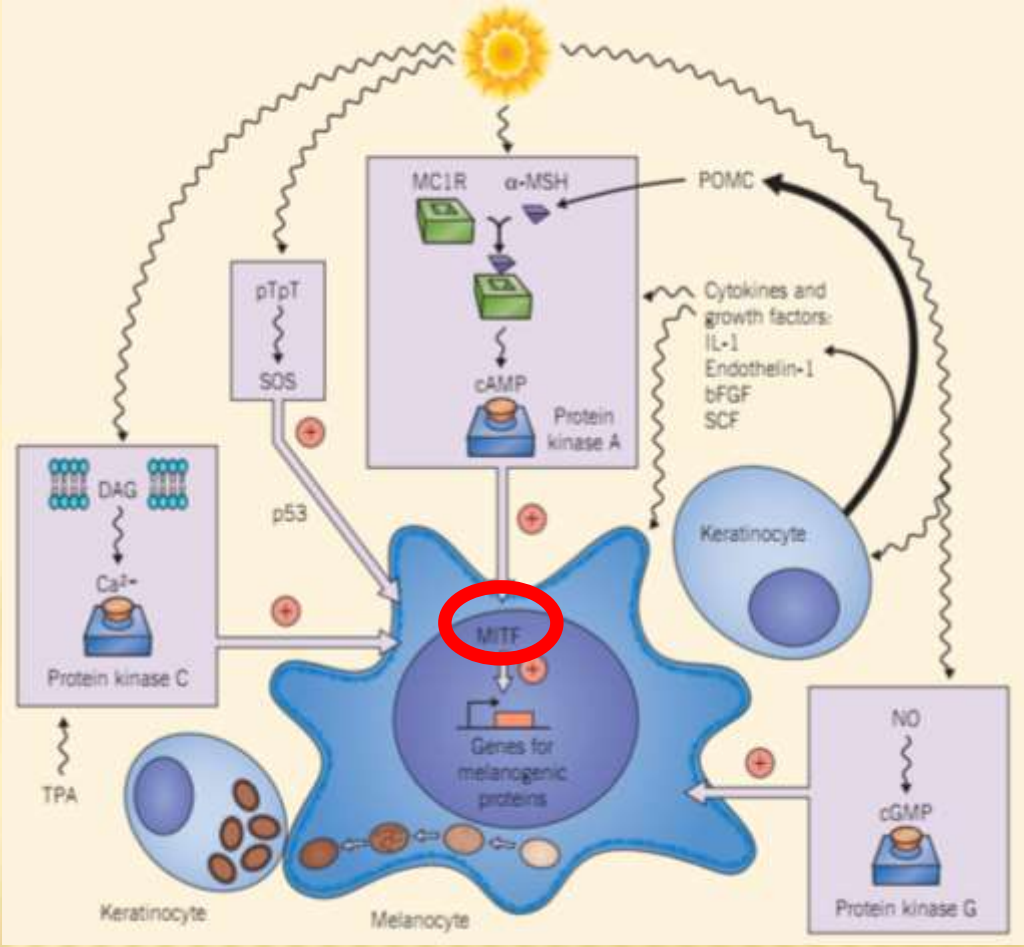


MELANOSMES ARE GREEN (ANTI-TYROSINASE IMMUNOFLUORESCENCE LABELLING)

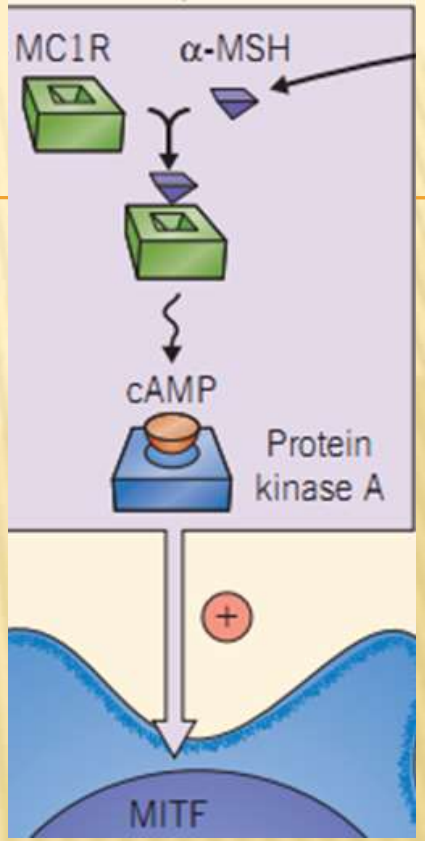
KERATINOCYTES ARE RED (ANTI-KERATIN IMMUNOFLUORESCENCE LABELLING)

MITF

- MITF gene is the master gene for melanocyte **survival** & is a key factor **regulating the transcription of the major melanogenic proteins** (tyrosinase, TRP-1 , TRP-2 & MART1)
- Mutations in MITF found in some pigmentary disorders:
  a) **Waardenburg syndrome type 2**
  b) **Tietz syndrome**



MC1-R



- ACTH & MSH activate MC1-R & stimulate melanogenesis (eumelanin over pheomelanin).
- That's why patients with **Addison disease** have generalized skin hyperpigmentation
- It's Major Distribution in Melanocytes
- □ intracellular concentration of cAMP → □ in tyrosinase activity → □ eumelanin production
- If the MC1-R is dysfunctional and fails to initiate a significant rise in the intracellular level of cAMP, then pheomelanogenesis is favored (majority of individuals with **red hair**)

THAN Q