

BIOLOGY OF MELANOCYTES

OBJECTIVES

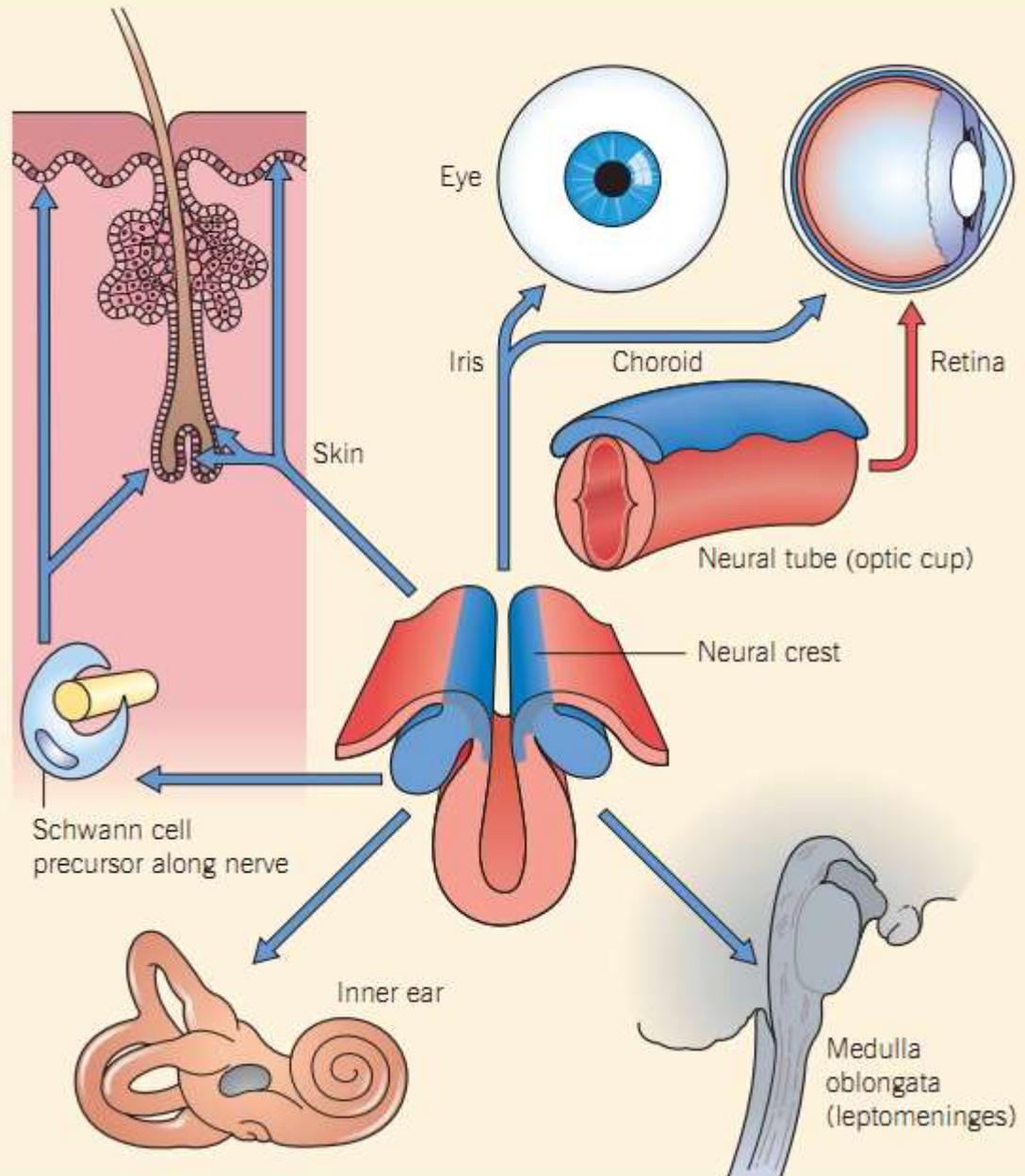
- Definition, development, function & Site-specific of **Melanocyte**
- Definition, formation & function of **Melanosomes**
- Definition & function of **Melanin**
- **Melanogenesis**

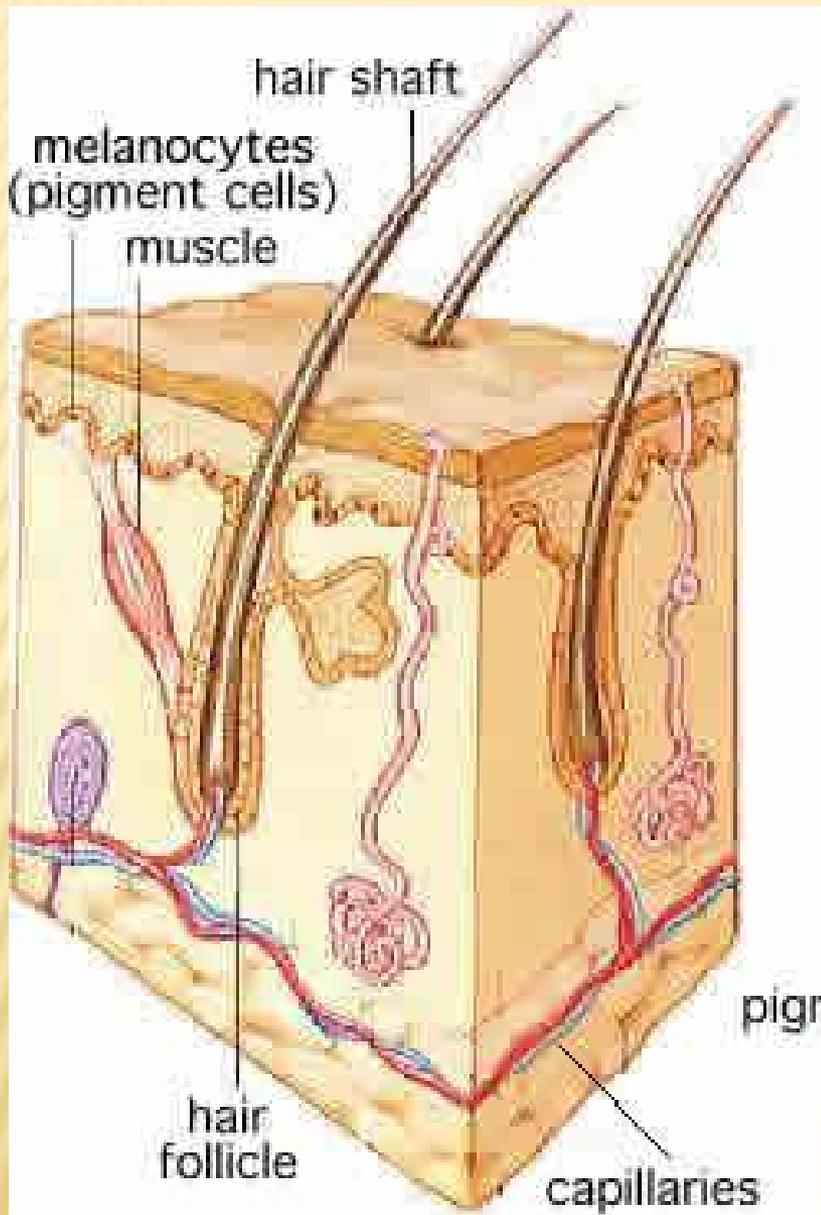
- **Definition of Melanocyte:**

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

MIGRATION OF MELANOCYTES FROM THE NEURAL CREST

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





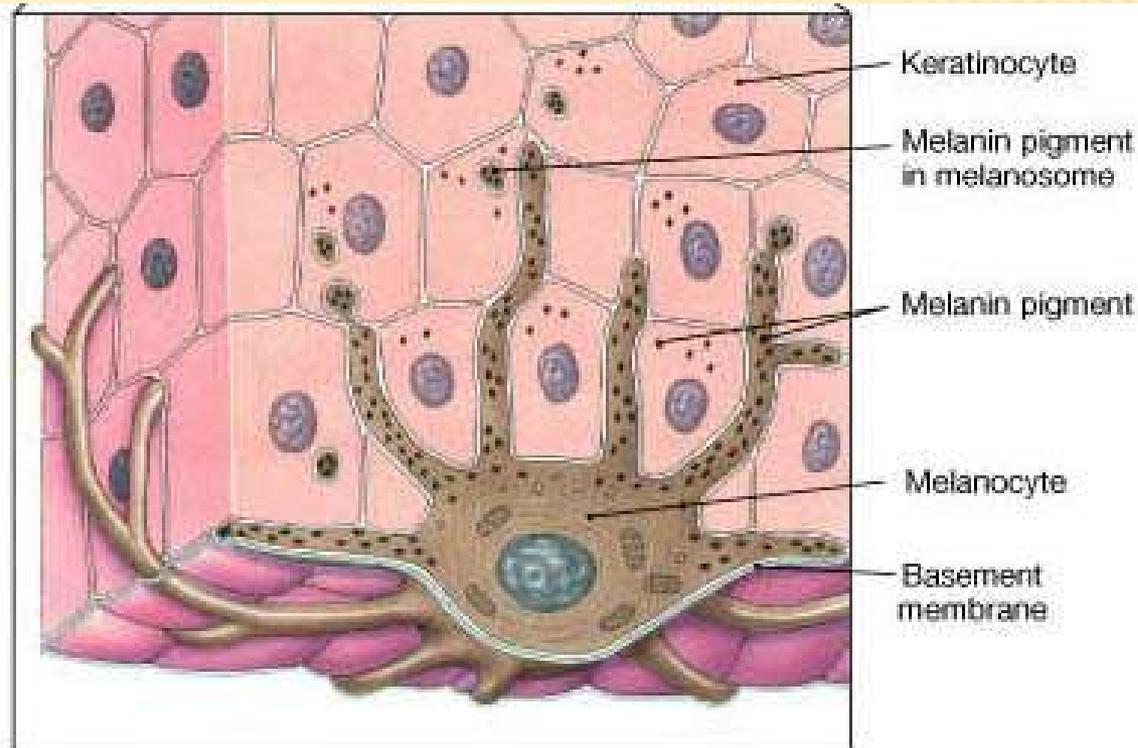
EMBRYOLOGY OF MELANOCYTE

- 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)**.



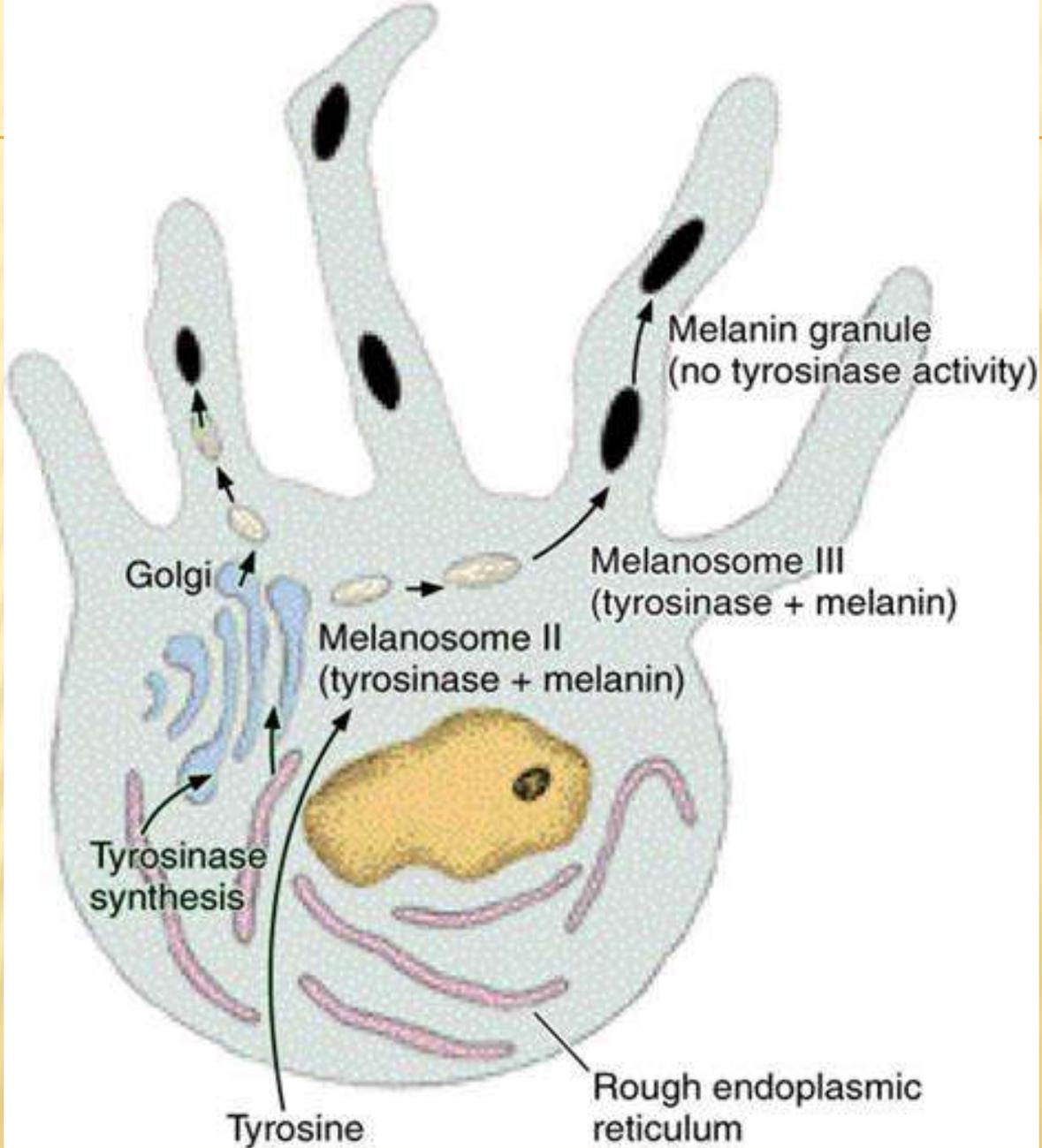
Association of a melanocyte with approximately **30–40** surrounding keratinocytes to which it transfers melanosomes has been called the

Epidermal melanin unit

1:36 ratio of melanocyte:keratinocyte

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.



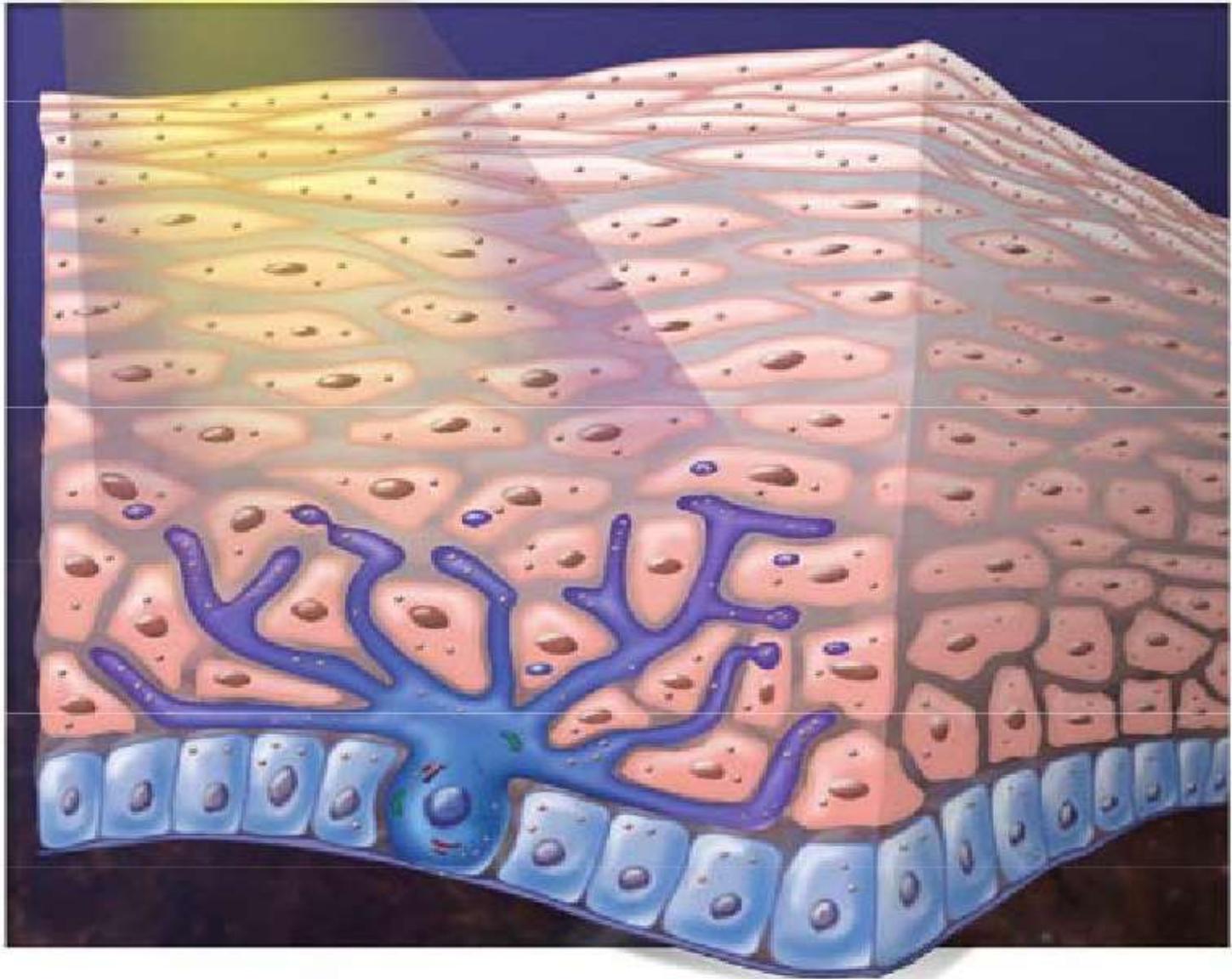
TYPES OF MELANOCYTES

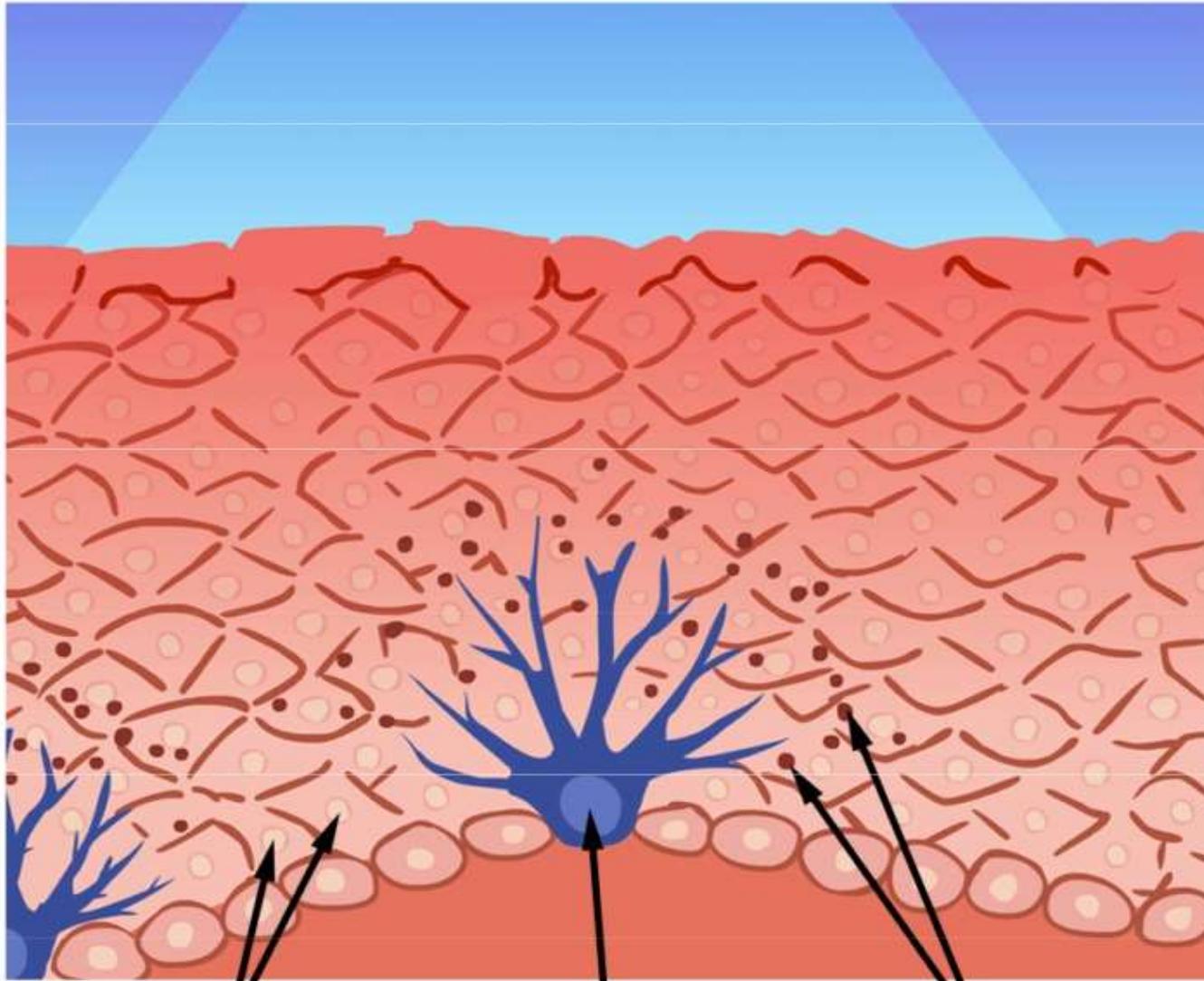
1- SECRETORY MELANOCYTES:

- ❑ 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 (**cytokrine** activity)

2- NON-SECRETORY MELANOCYTES:

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





Keratinocyte

Melanocyte

Melanosome

Epidermis

Basal Layer

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?

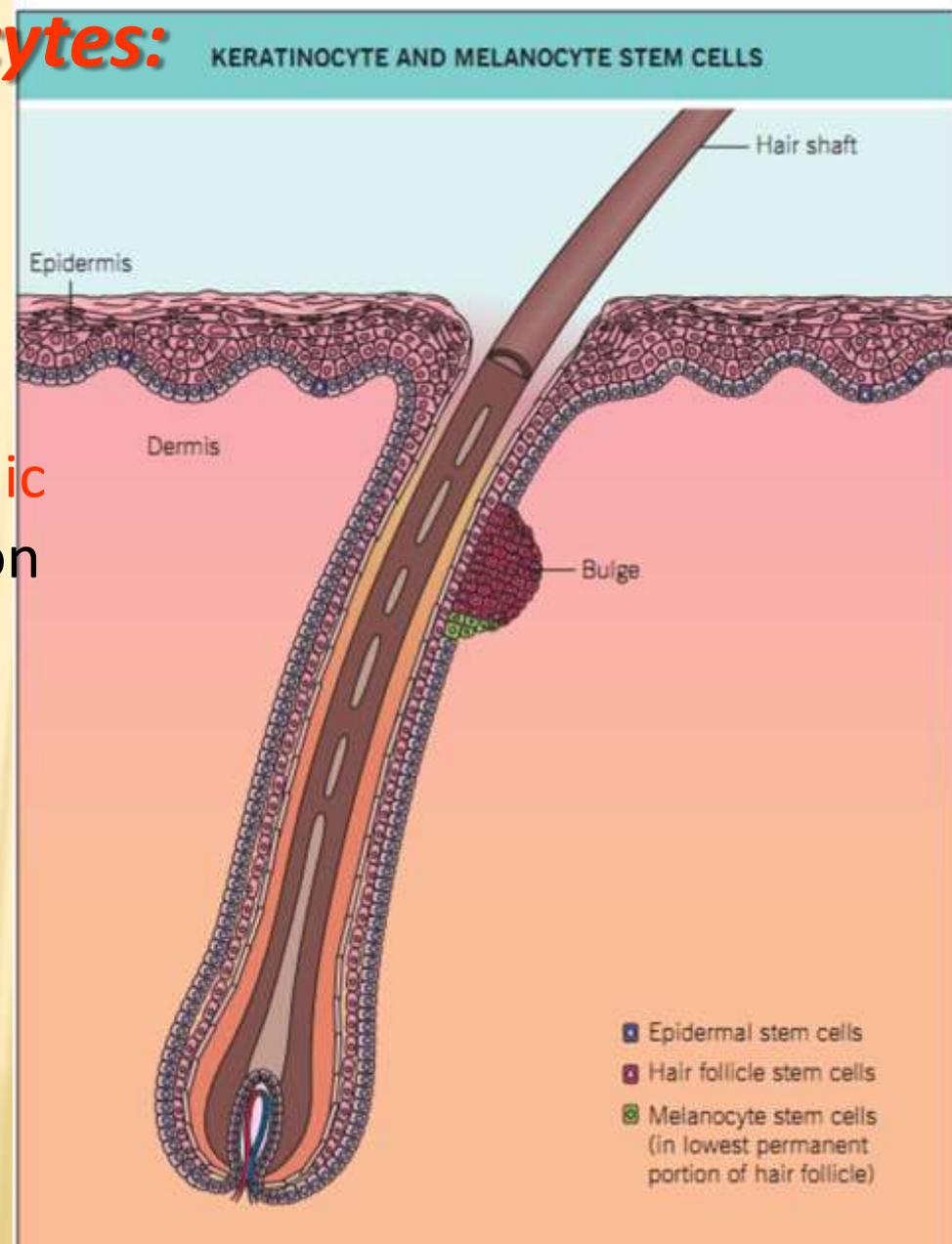
- Melanocyte **density** is almost the **same** in all individuals of different ethnic background & thus cutaneous pigmentation doesn't depend on melanocyte number.

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:

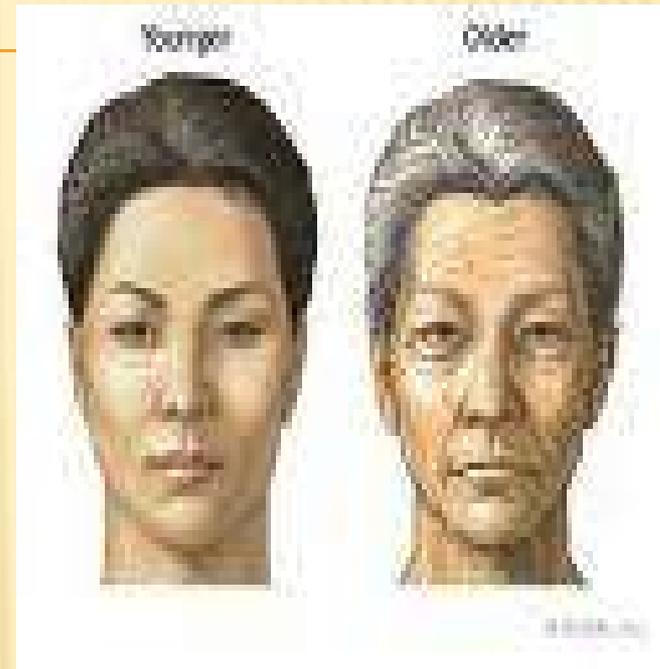
- Unlike cutaneous melanocytes, ocular melanocytes are in contact only with each other & don't transfer melanosomes.

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:

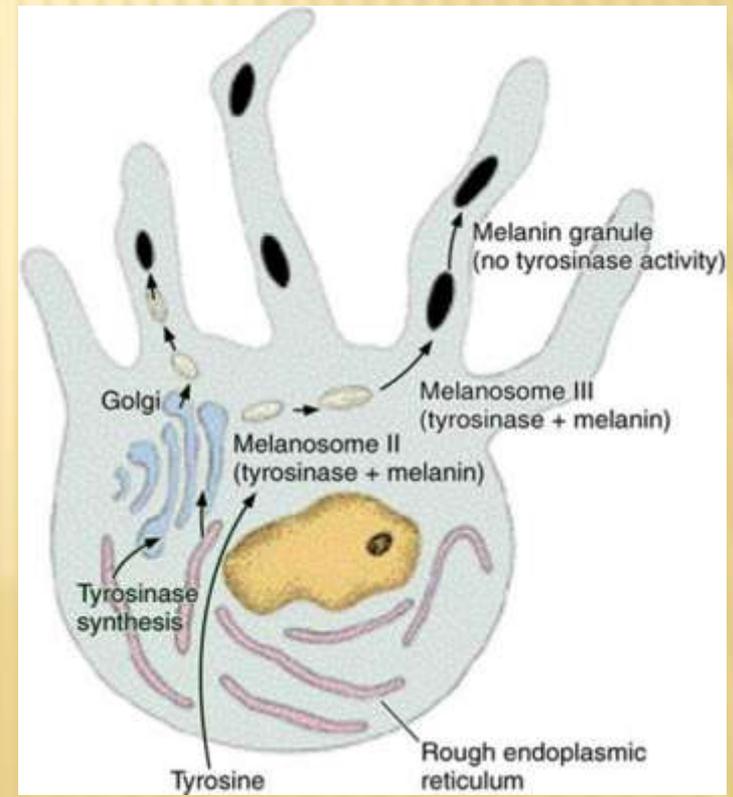
Is membrane-bound unique organelle Within the cytoplasm of melanocytes in which in which melanin pigments are synthesized, deposited and transported.

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

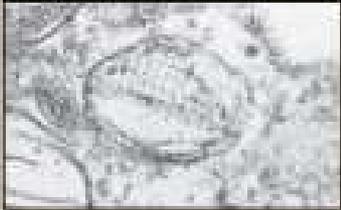
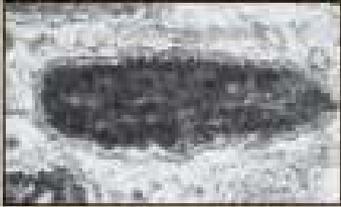
- **Eumelanosome**
- **Pheomelanosome**

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



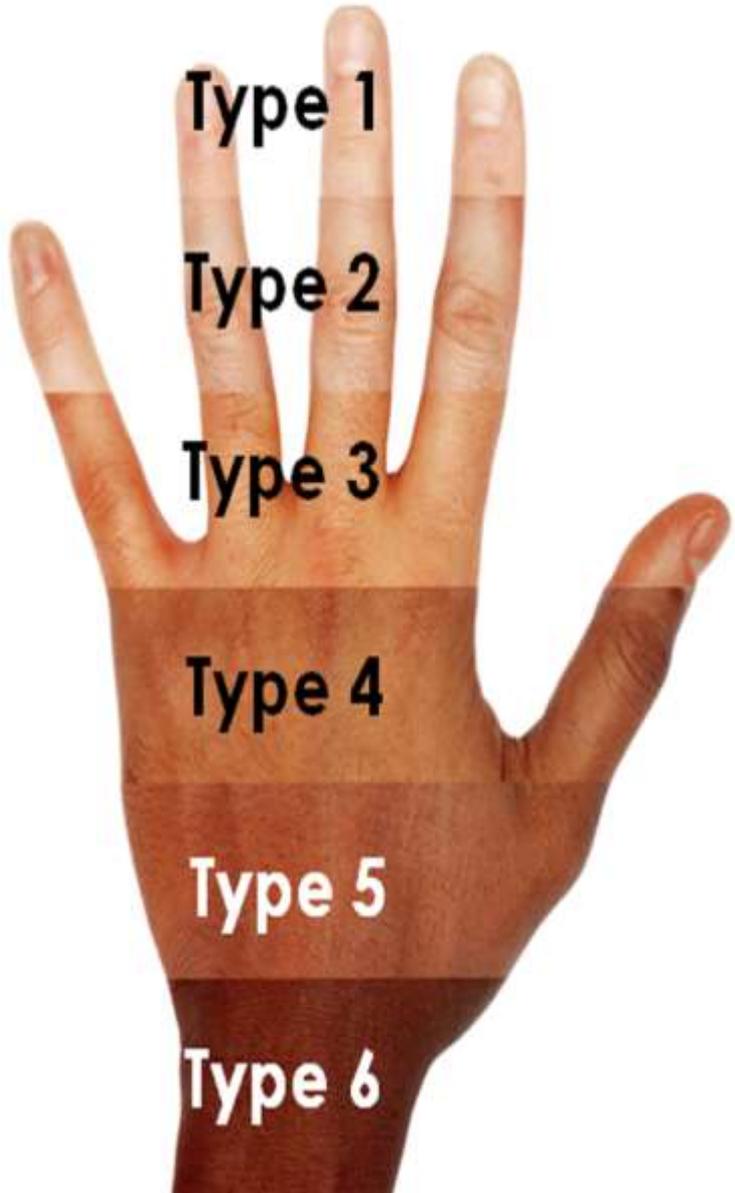
FOUR MAJOR STAGES OF EUMELANIN MELANOSOMES

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



Type 1

White: Always burns, never tans

Type 2

White: Usually burns, difficulty in tanning

Type 3

White: Sometimes burns, average tan

Type 4

Moderate Brown: Rarely burns, tans with ease

Type 5

Dark Brown: Very rarely burns, tans very easily

Type 6

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



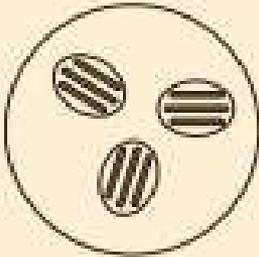
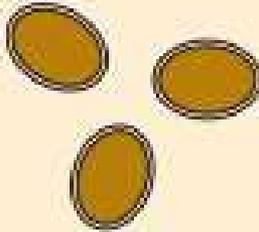
White people strive for tanning while brown and black people strive for a lighter skin

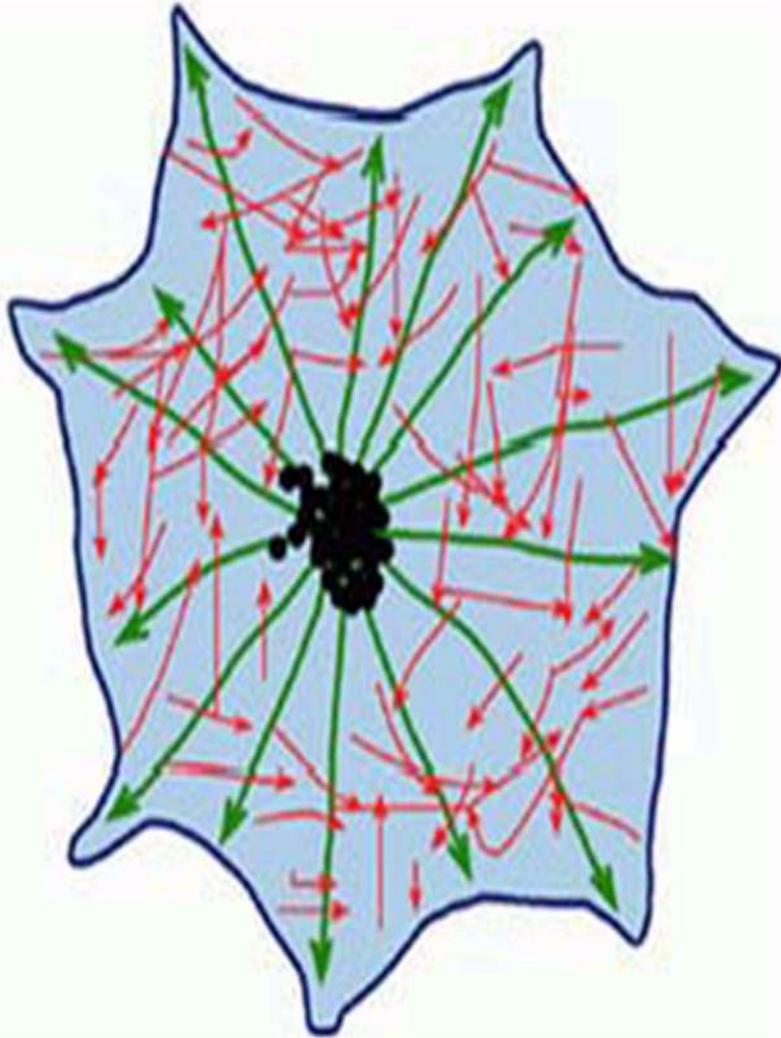
VARIATION OF PREDOMINANT MELANOSOMAL STAGES WITH LEVEL OF CUTANEOUS PIGMENTATION

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

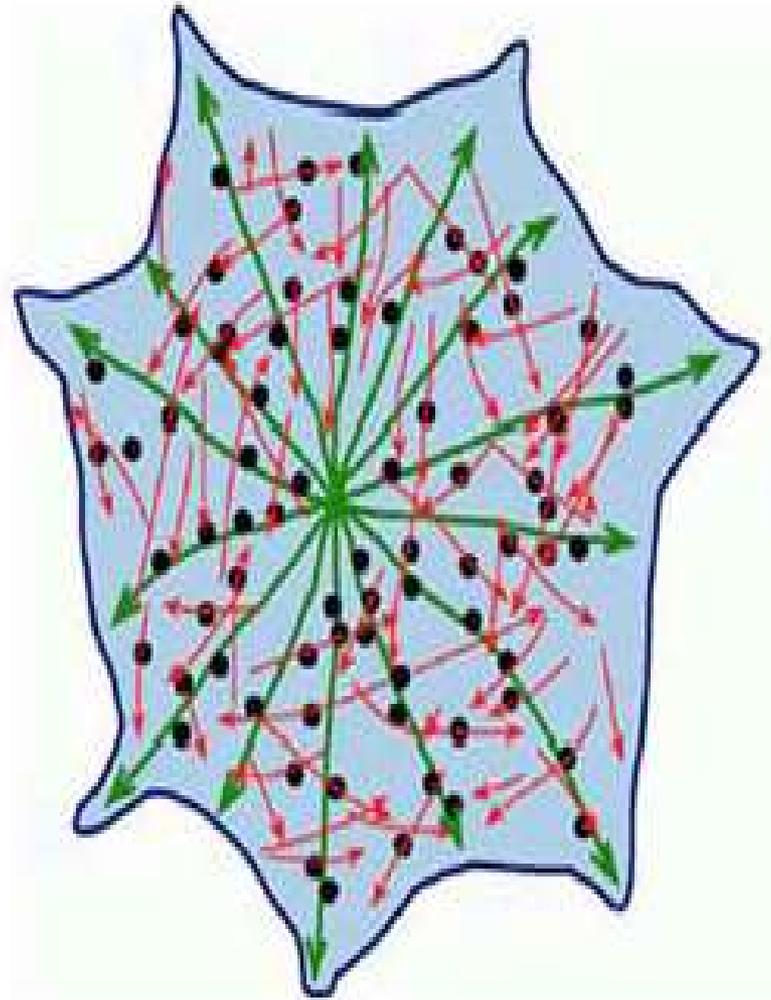


MELANOSOMES IN LIGHTLY PIGMENTED VERSUS DARKLY PIGMENTED SKIN

	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

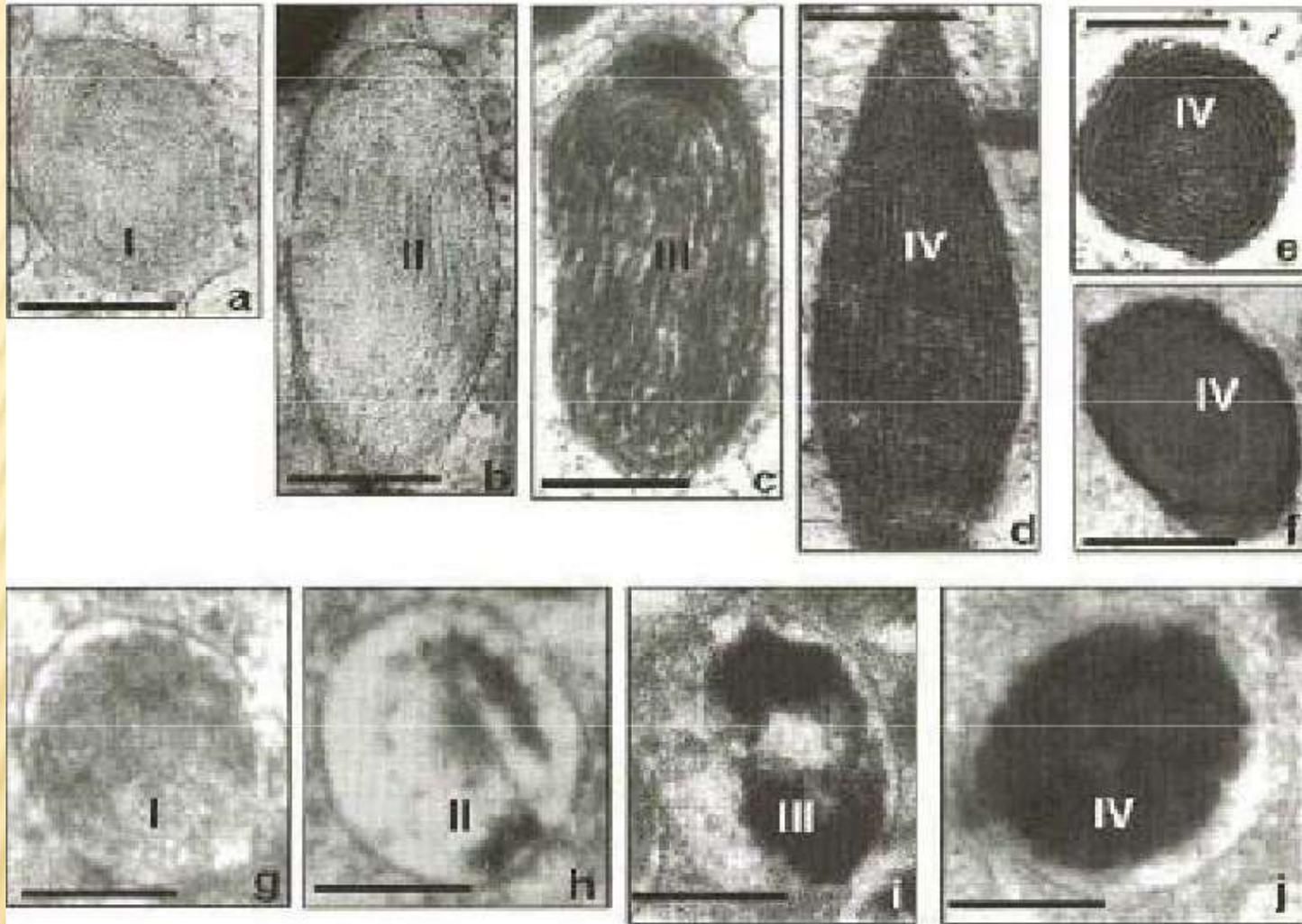
MELANOSOME BIOGENESIS

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

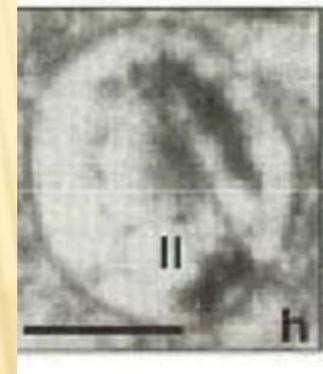
Stage 2

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

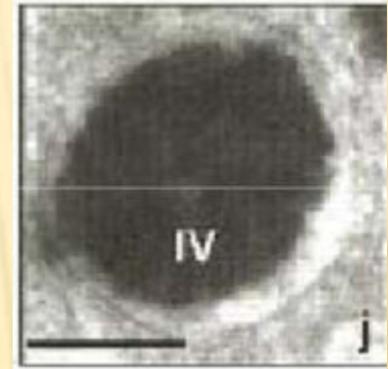
Internal structure of melanosomes is **partially** obscured by deposition of melanin
stage 3 pheomelanosomes: continuation of melanin synthesis & deposition



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

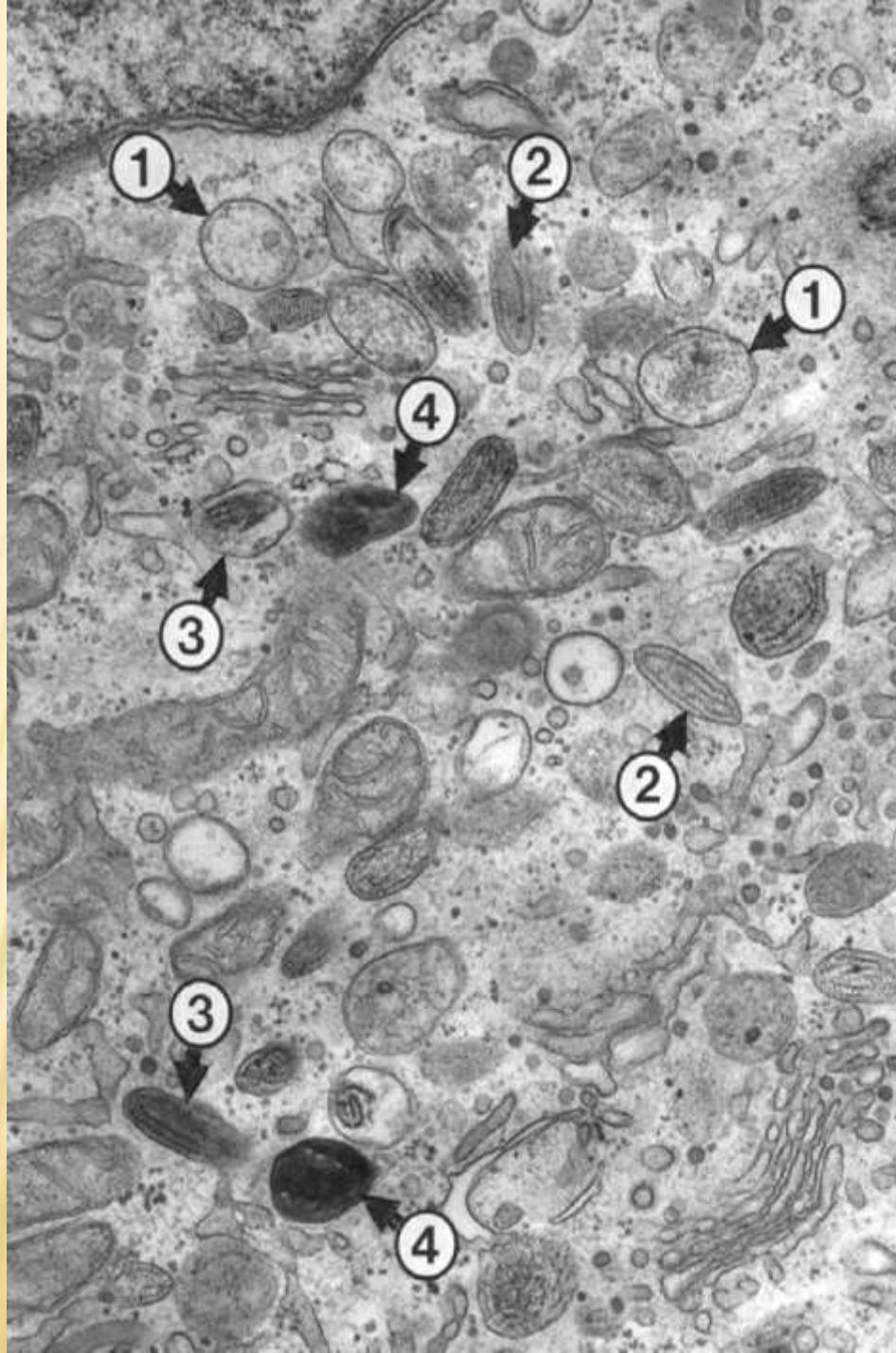


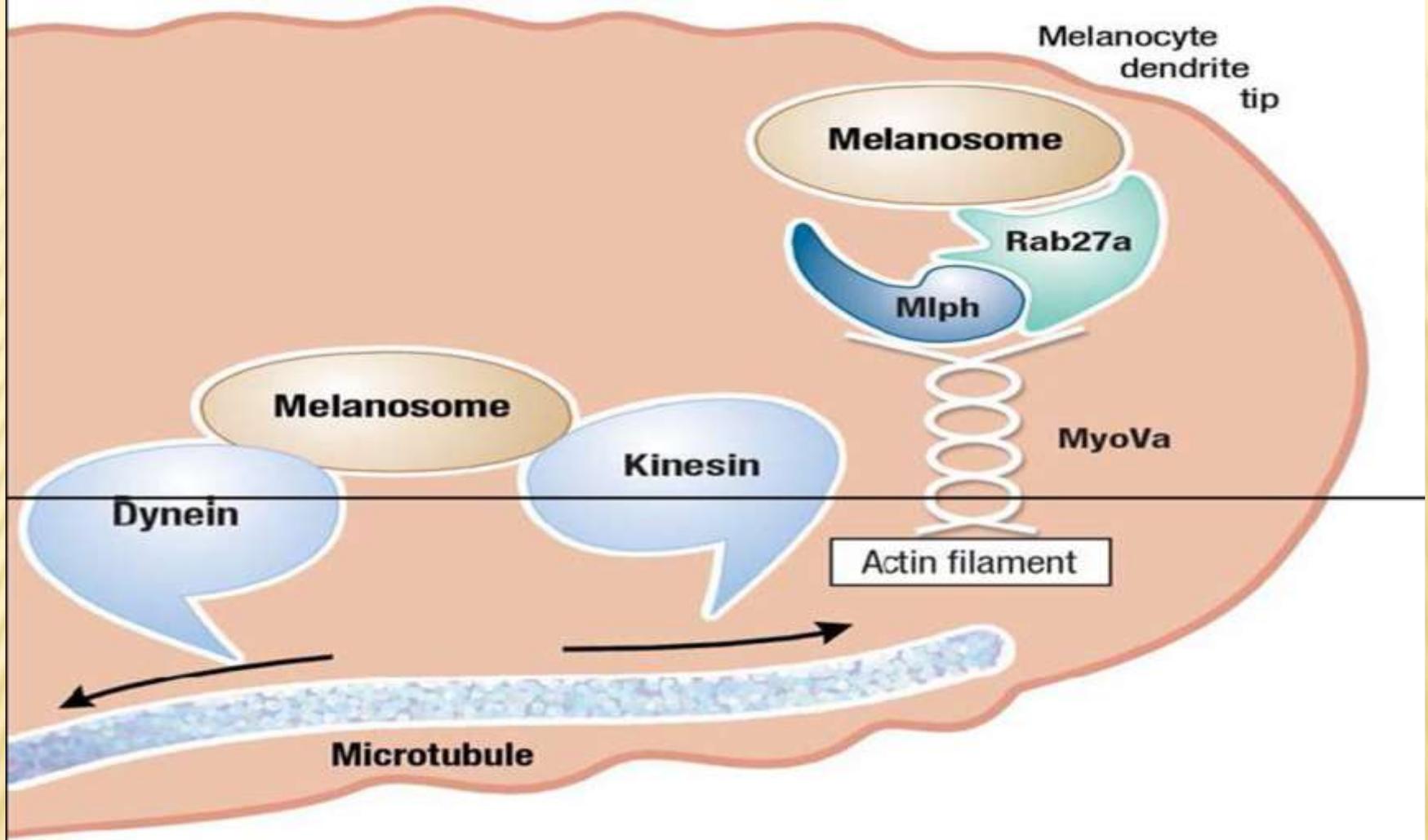
Stage 4



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



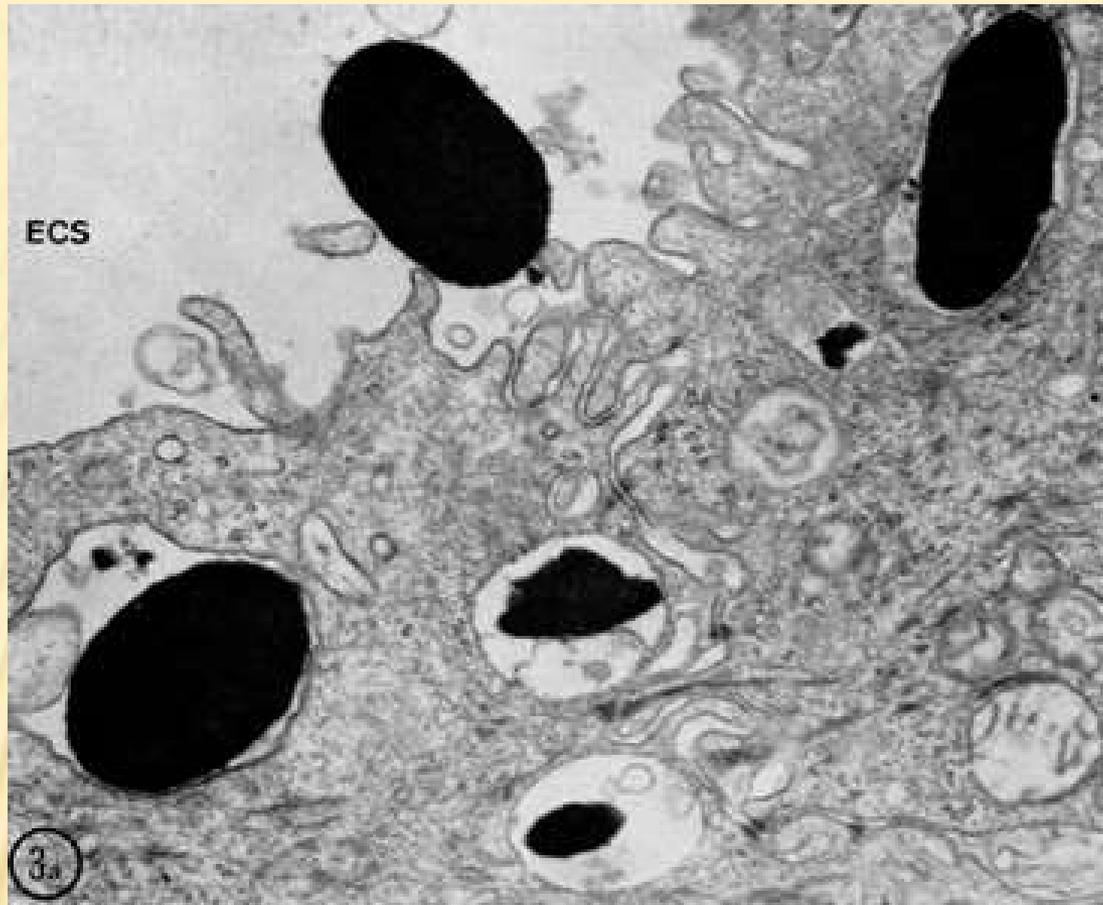




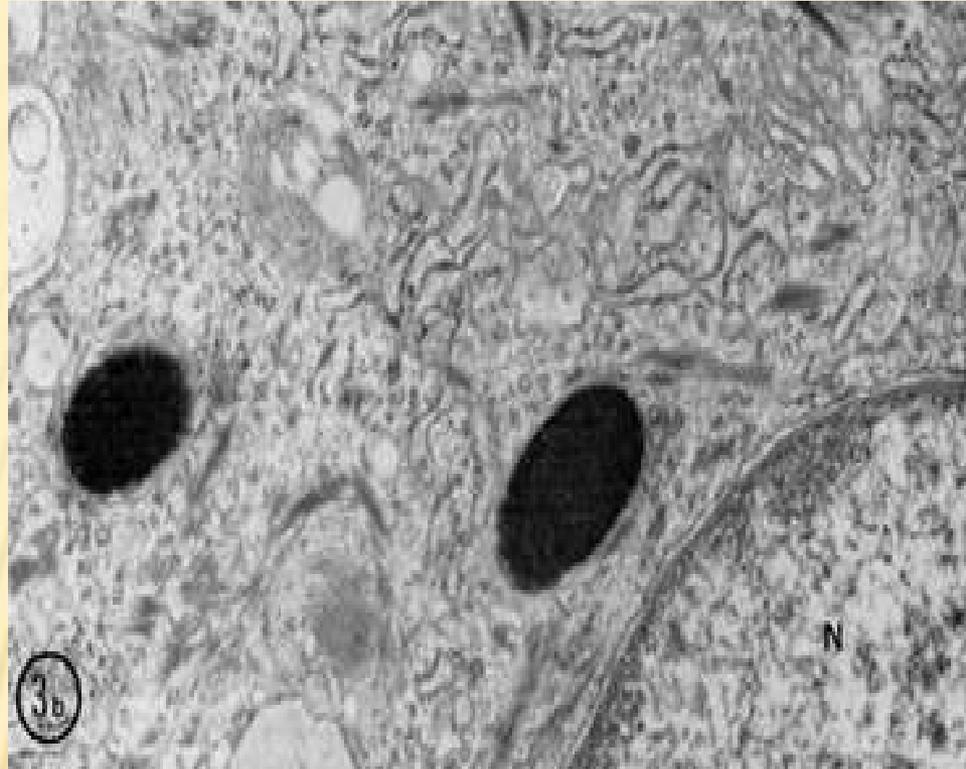
MELANOSOMES TRANSPORT TAKES PLACE ON MICROTUBULE THAT ARE ARRANGED PARALLEL TO THE LONG AXIS OF THE DENDRITE USING 2 MOTOR PROTEINS

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 cyto**phagocytose** 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

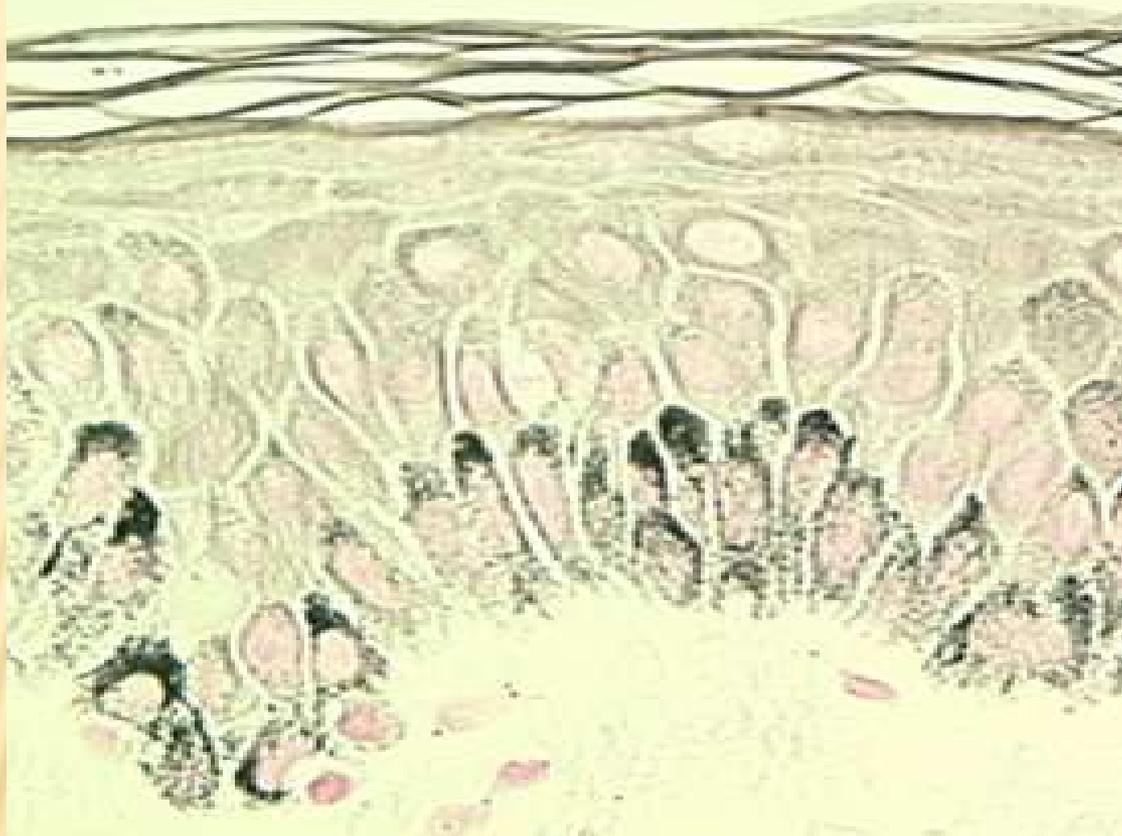


**MELANOSOME BEING PHAGOCYTOSED BY A
KERATINOCYTE**



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

A

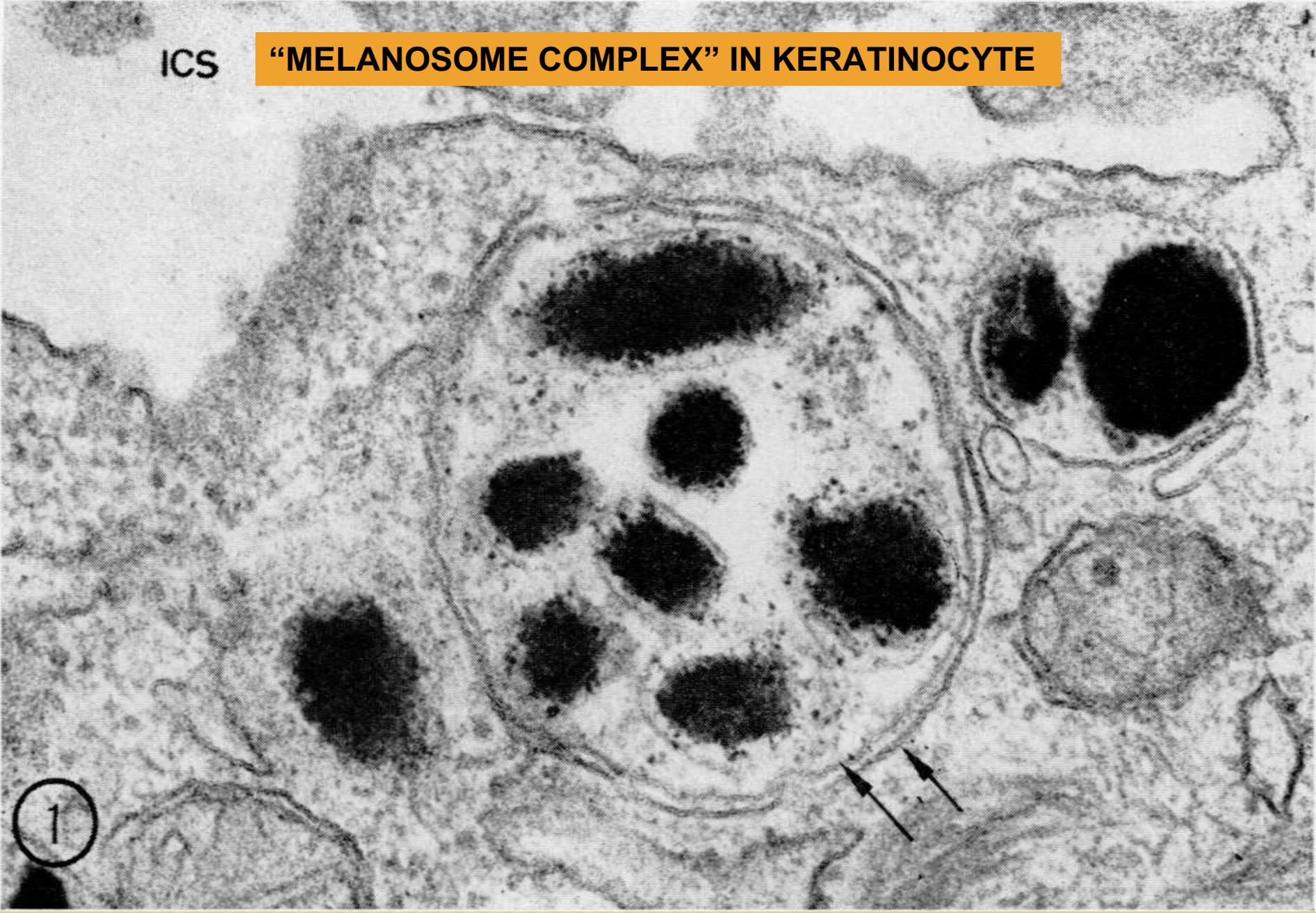


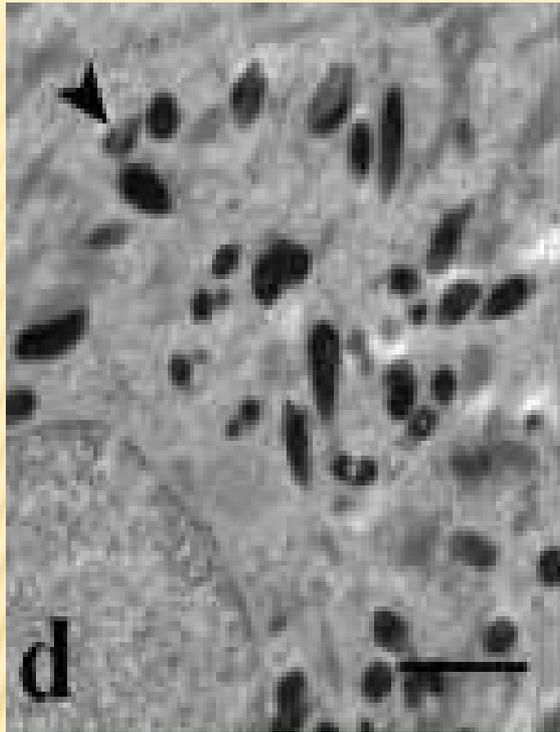
**MELANSOMES ARE TAKEN UP BY PHAGOCYTOSIS
INTO THE LOWER KERATINOCYTES**

- 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

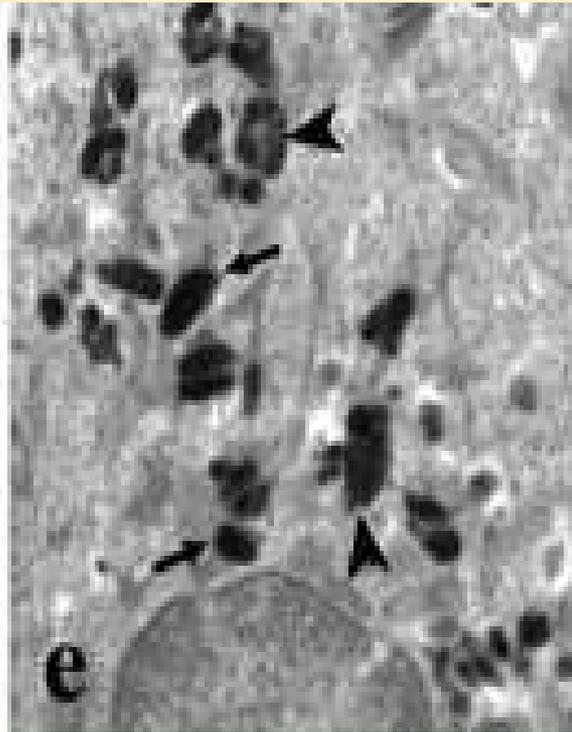
"MELANOSOME COMPLEX" IN KERATINOCYTE





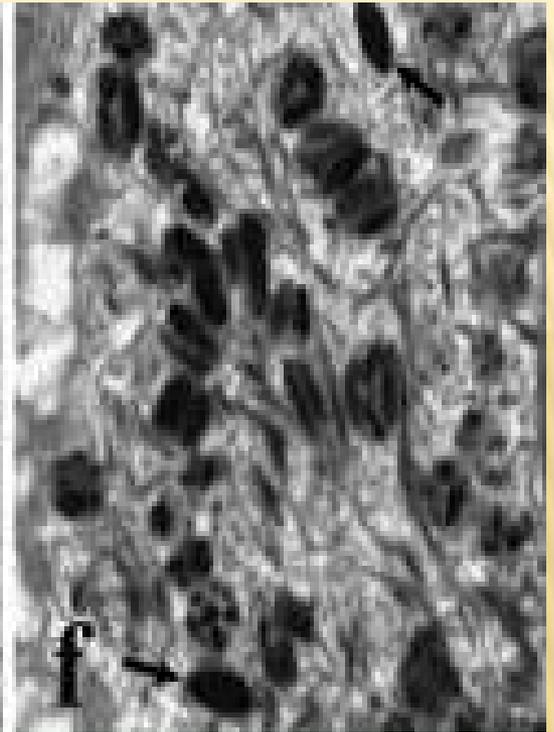
AFRICAN/AMERICAN

**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**)

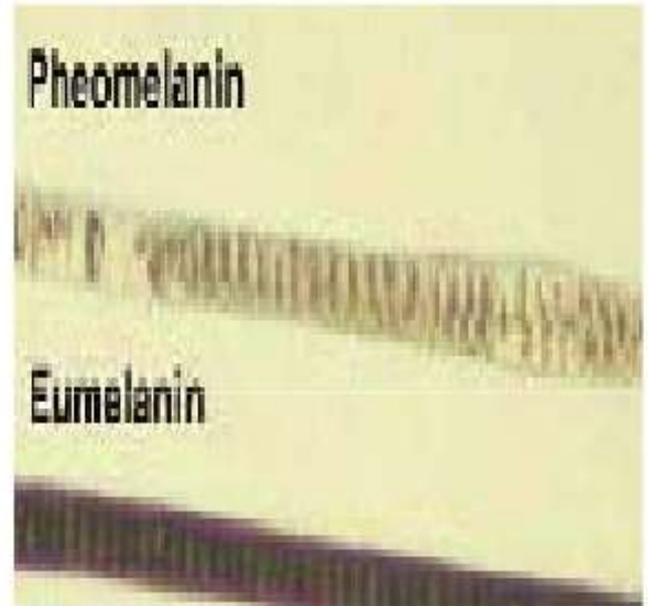
Eumelanin

Pheomelanin



Pheomelanin

Eumelanin



TYPES OF MELANIN PIGMENTATION

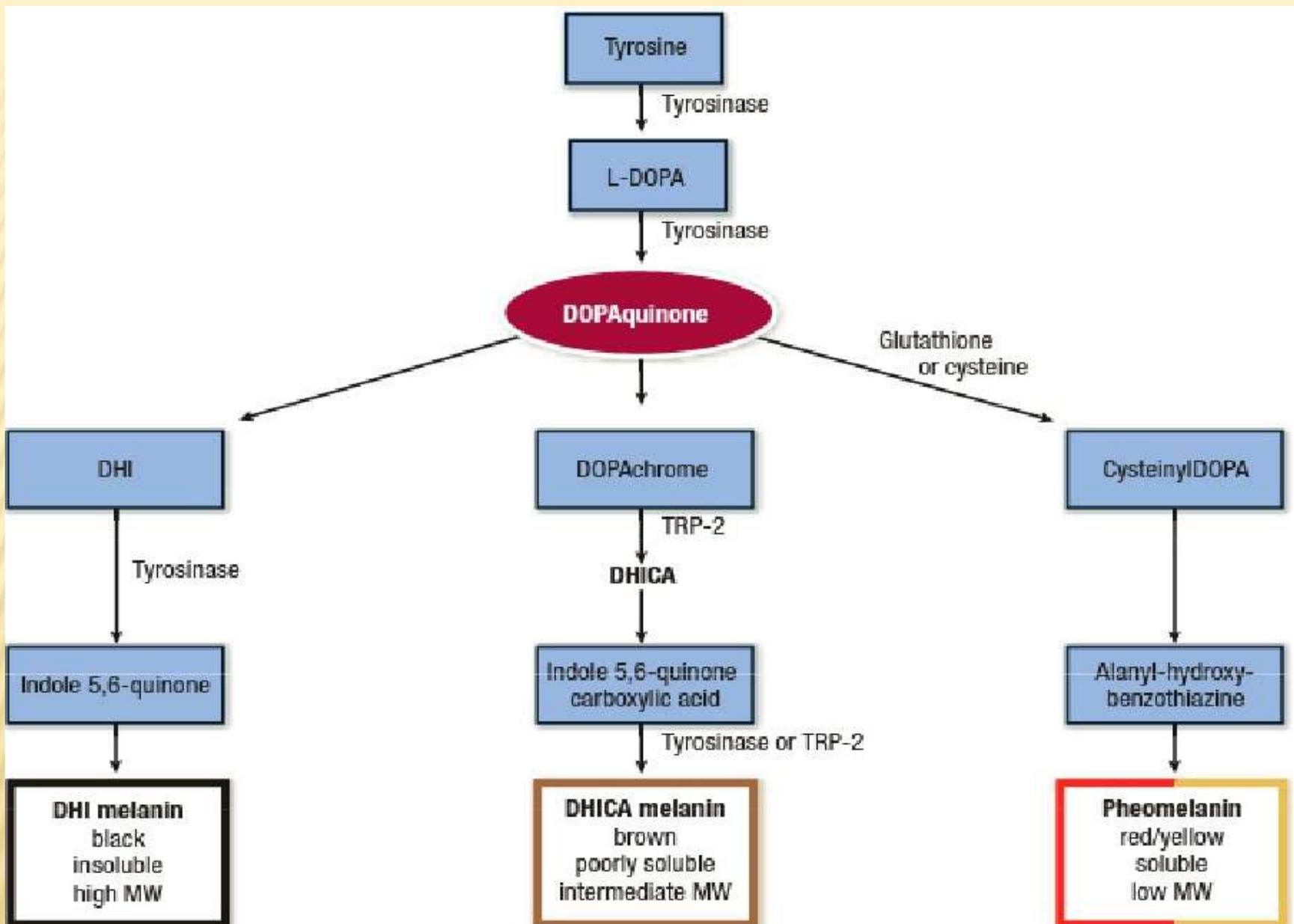
- **Constitutive:**

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

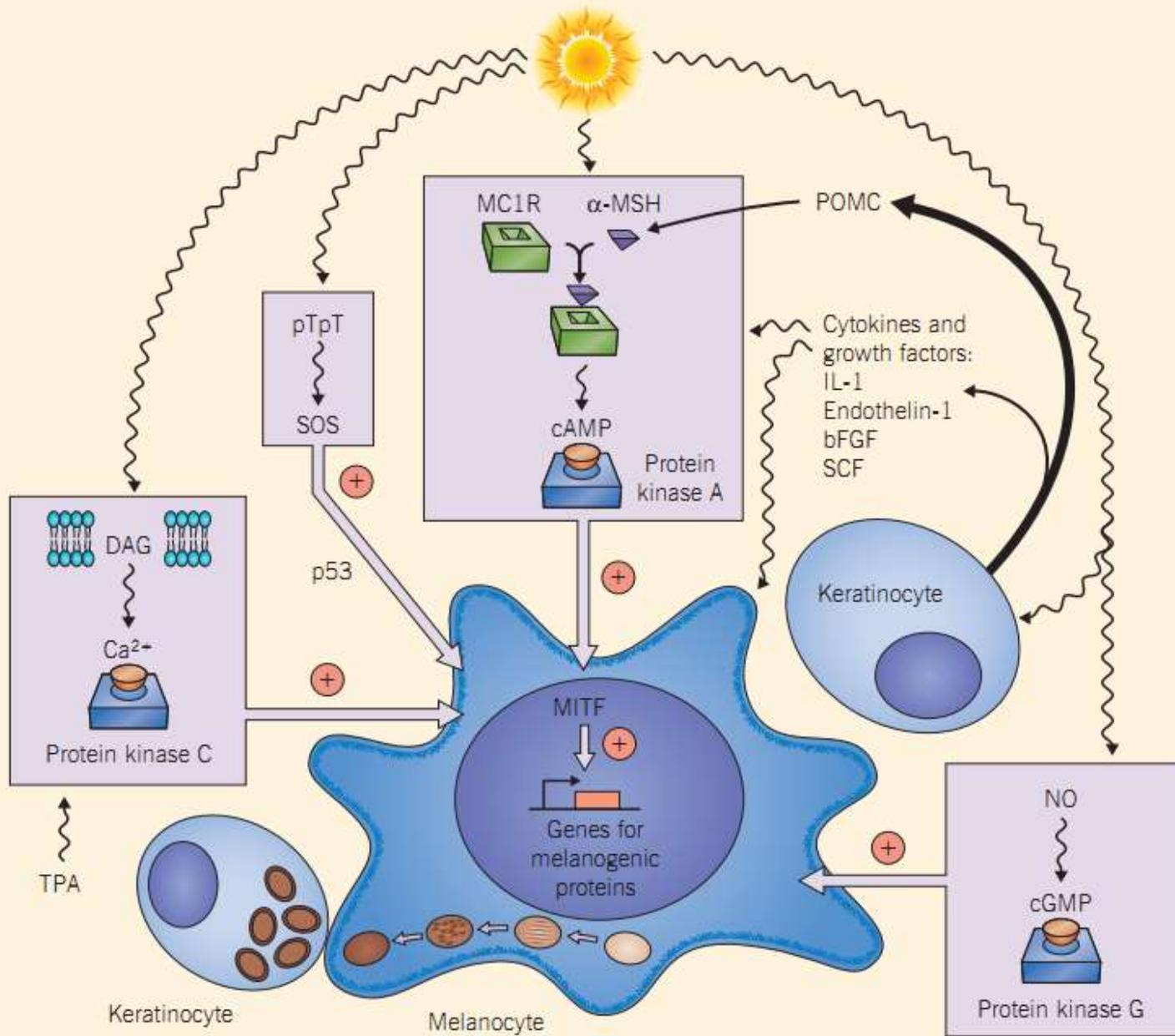
C. Biochemical factors:

- 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**

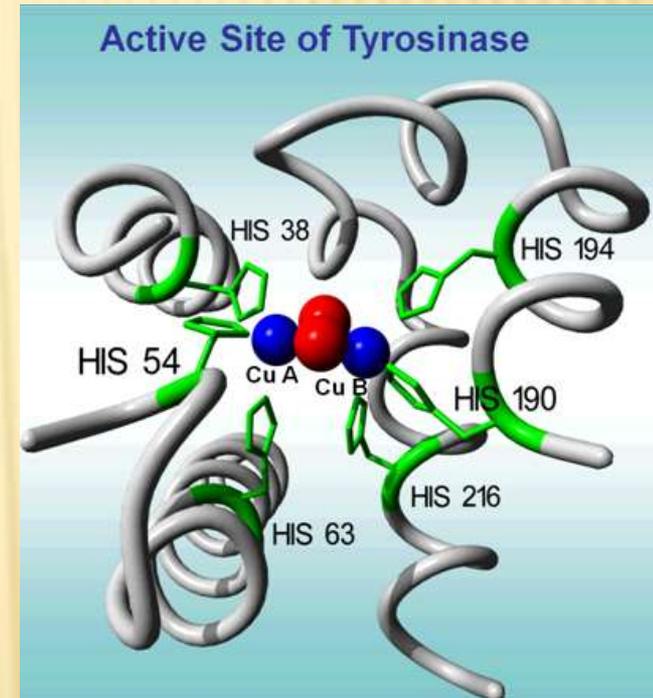
(P Protein, Heterotetrameric adaptor protein complexes “Aps”)

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

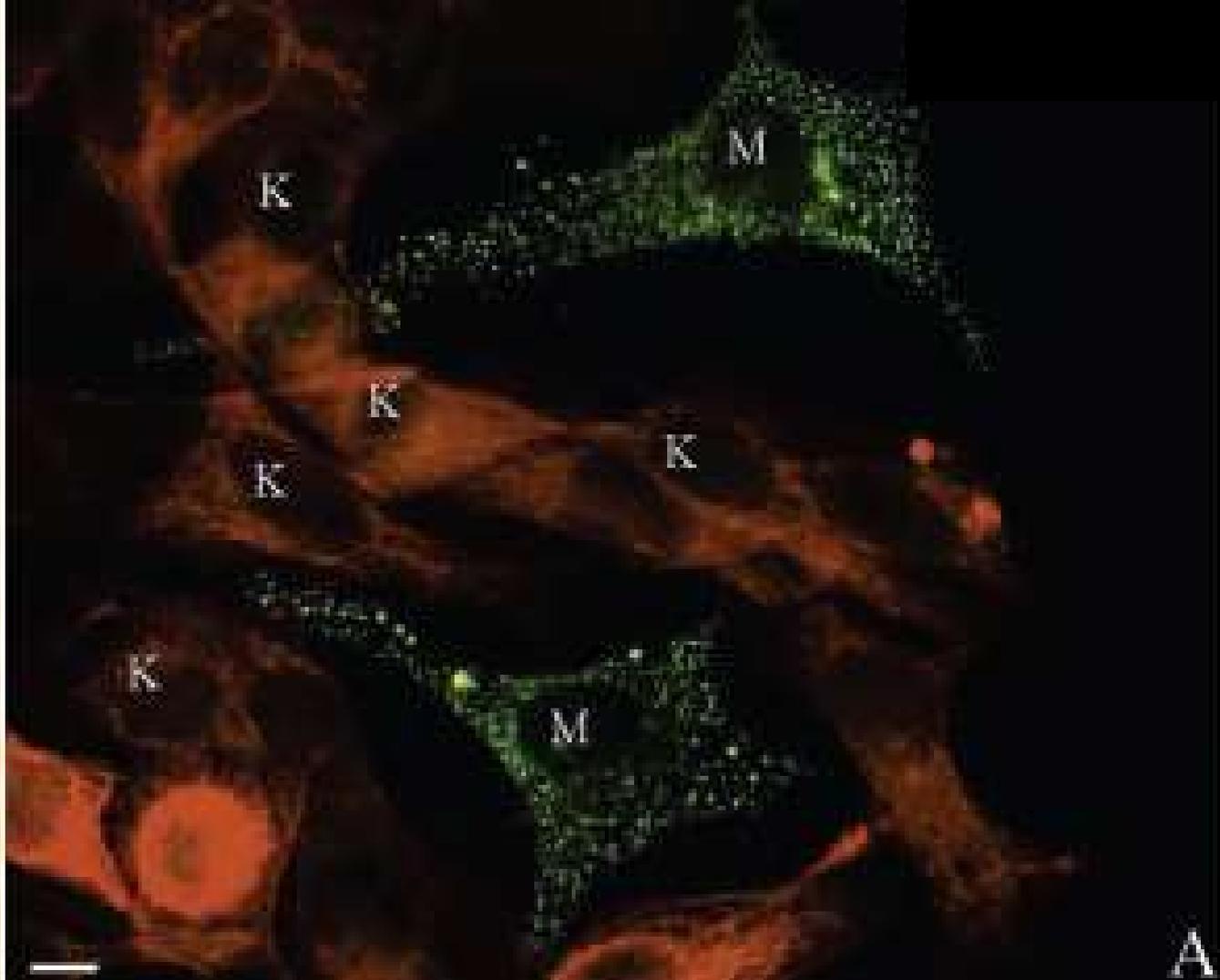
- **Histidine** residues present in the inner domain.
- Binds **two copper atoms** within its active site which are required for tyrosinase activity.
- Rare cases of **copper deficiency** can lead to *diffuse cutaneous pigmentary dilution*.
- In addition to that, two serine residues on the cytoplasmic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



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.



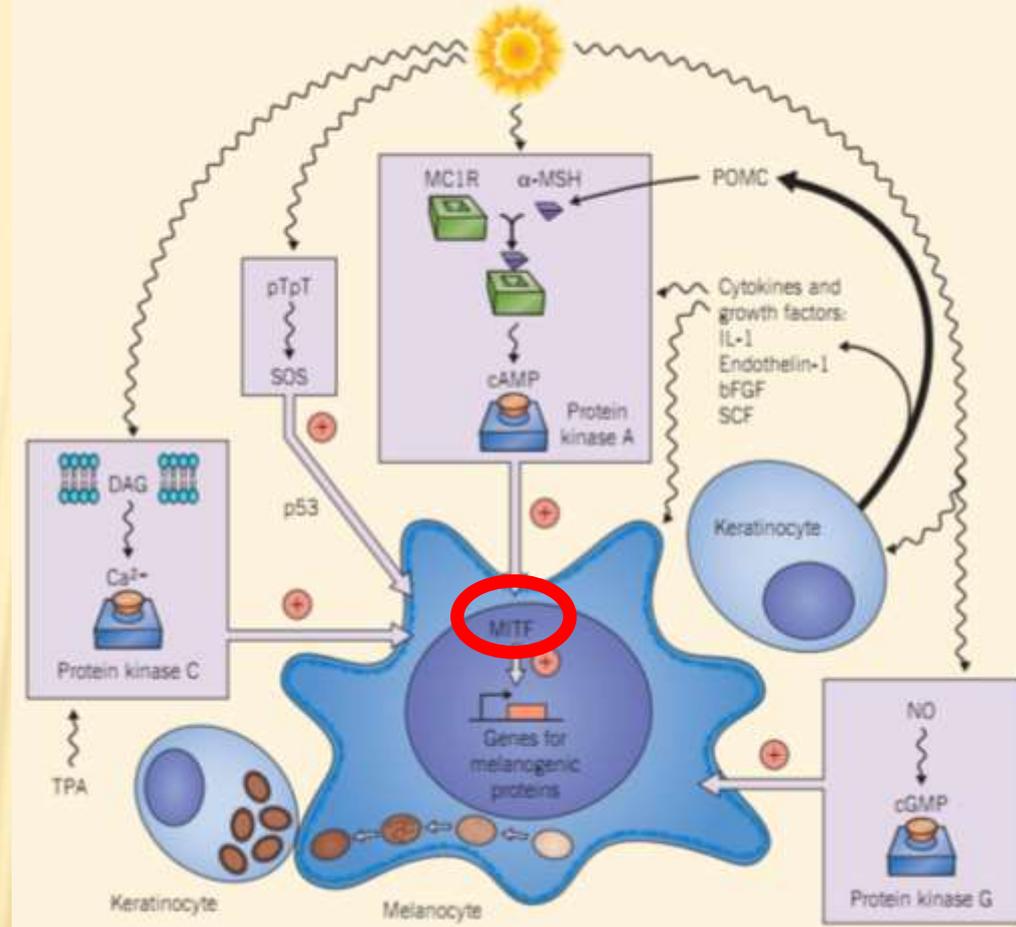


MELANOSMES ARE GREEN (**ANTI-TYROSINASE** IMMUNOFLUORESCENCE LABELLING)

KERATINOCYTES ARE RED (**ANTI-KERATIN** IMMUNOFLUORESCENCE LABELLING)

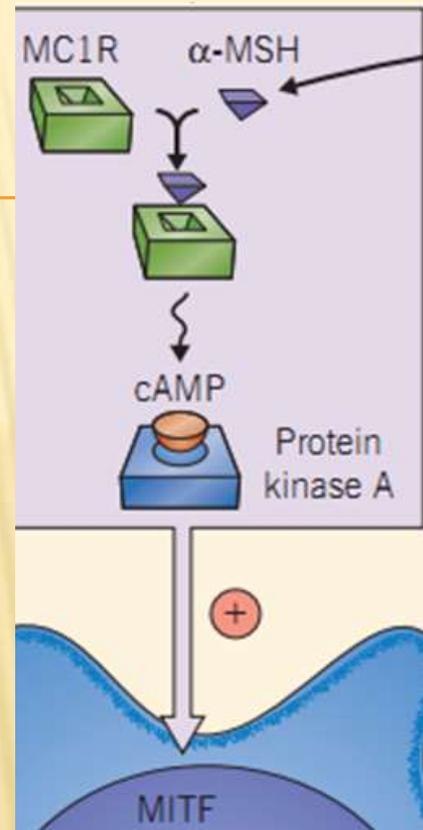
MITF

- 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**)



THANK