The Development of Skin Whitening Agents

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Cosmeceuticals

- Biologically active materials
  - Efficacy
  - Safety
- Formulations enhancing efficacy
  - Stability
  - Bioavailability

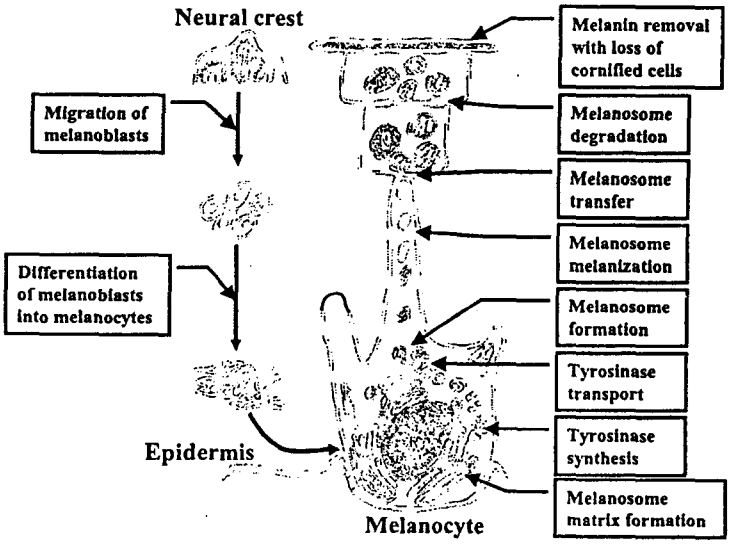
Development of biologically active materials

- Research areas
  - **Biological Target Mechanisms**
  - **Bioassay**
    - **Efficacy**
    - **Bioavailability**
    - **Safety**
  - **Materials development**
    - **Natural compounds**
    - **Synthesis**
    - **bioengineering, ...**

Human skin pigmentation

Biological Target Mechanisms

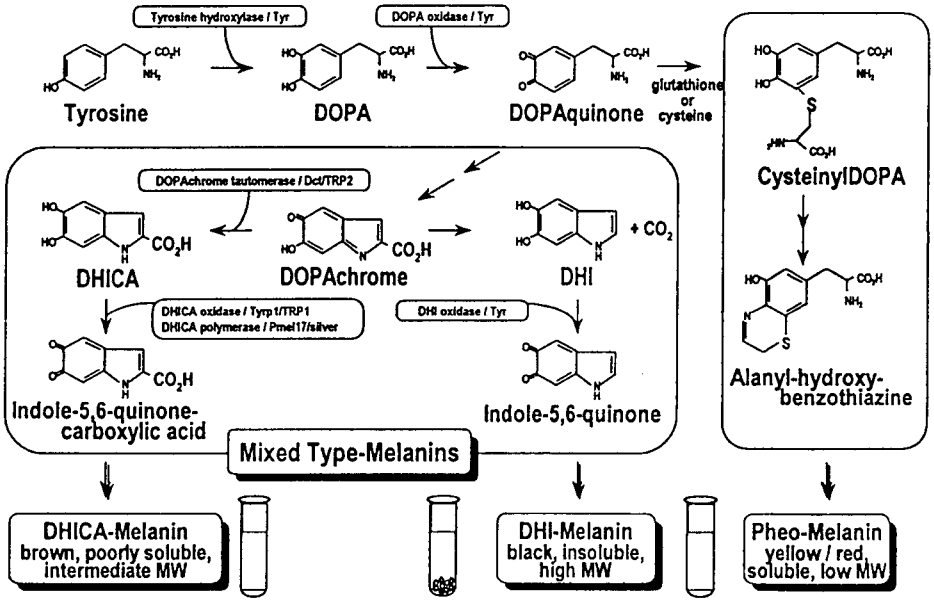
- Tyrosinase
- Enzymens and Proteins in Melanogenesis
- Signal transduction in Melanocyte
- Communication between cells in the skin
- UV, oxidative stress
- Bleaching

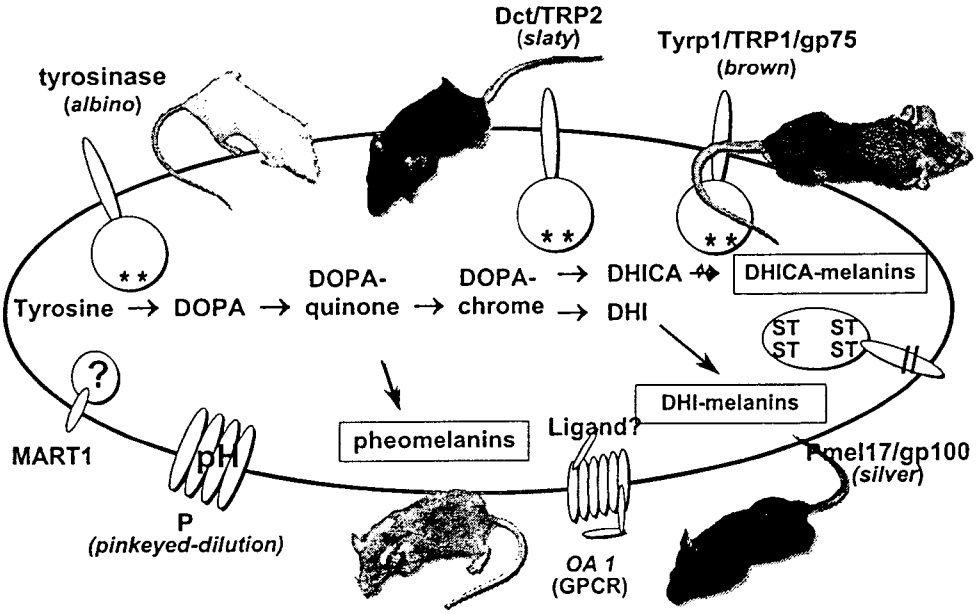


UV-induced skin darkening

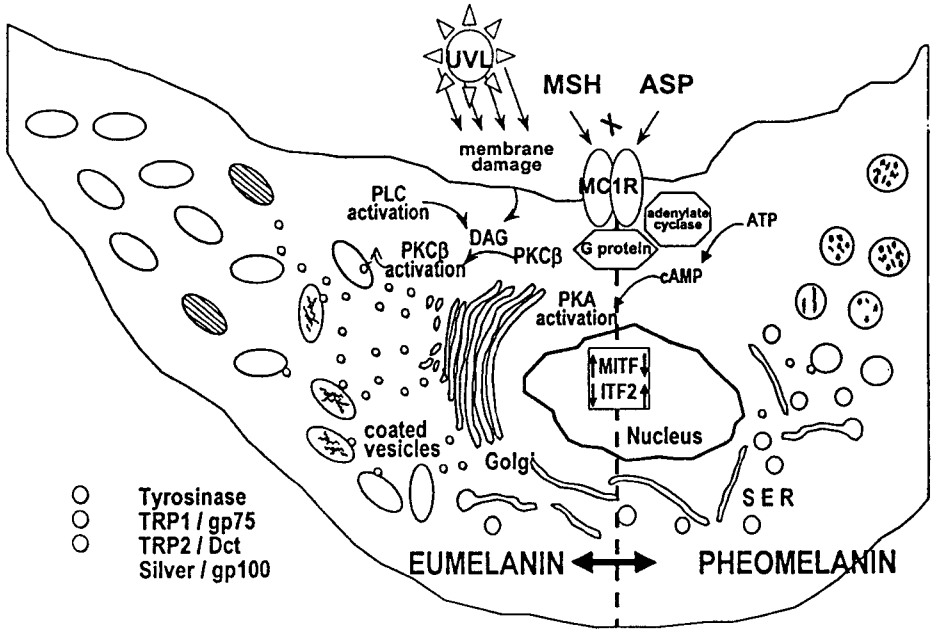
- Increase in the melanocyte number
- Stimulation of melanin neosynthesis
- Stimulation of melanocyte dendricity

Melanin pigment synthesis

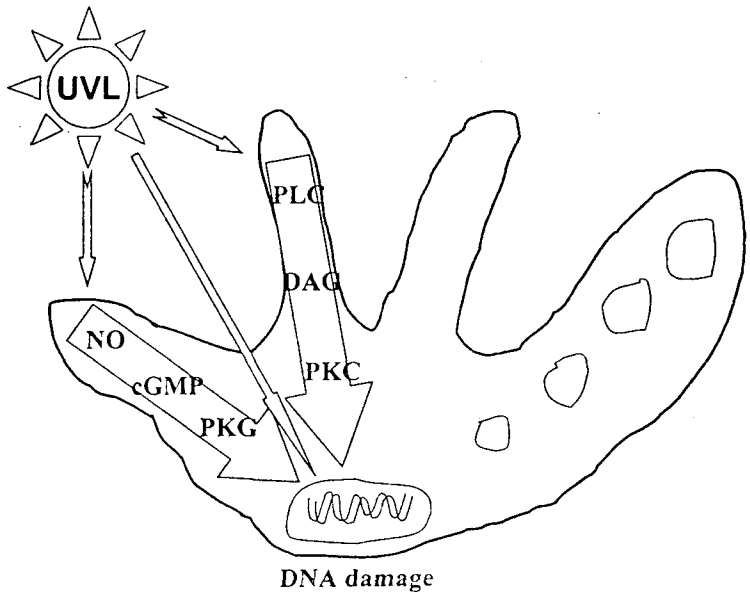




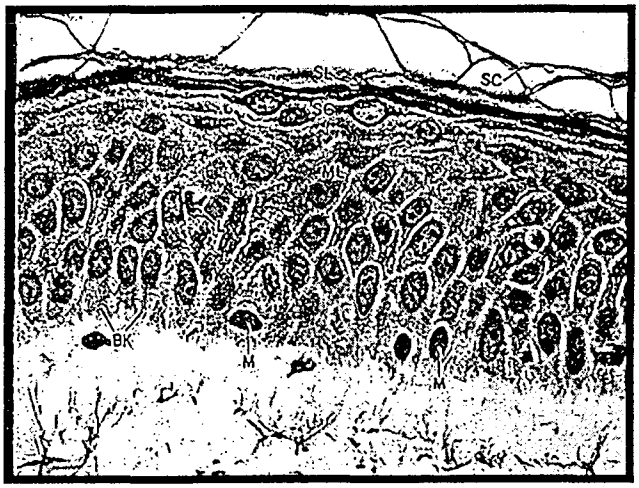
• Proteins in melanosome transport and transfer



Direct effects of UV on melanogenesis



Indirect effects of UV



Epidermal Cells
Keratinocytes
Melanocytes
Langerhans cells

Dermal Cells
Fibroblasts
Inflammatory cells
Endothelial cells
Mast cells

UV light
MSH
agouti protein

interleukins
leukotrienes
endothelins

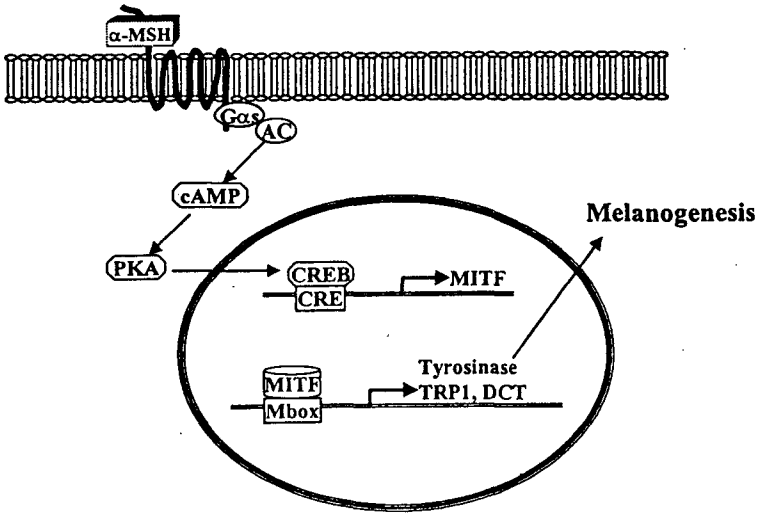
vitamins A, D
interferons
prostaglandins

retinoids, neurotropins
growth factors {bFGF,
PDGF, MSGF & SLF}

α-MSH and the MC1R receptor

- Pro-opiomelanocortin (POMC) peptides;
  α-MSH, ACTH
- UV increases
  - POMC production by keratinocytes
  - the expression of MC1R receptor

cAMP-dependent pathway



Regulation of MITF expression

- MITF expression is restricted to specific cell types and tissues.
- PAX3 and SOX10 synergistically transactivates the MITF promoter activity

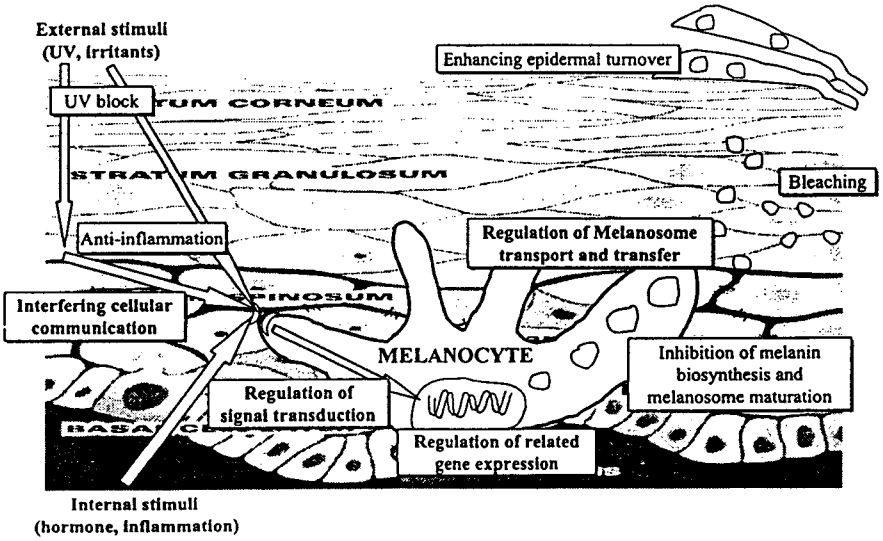
Agouti signal protein

- In vitro, ASP inhibits;
  - eumelanin synthesis, tyrosinase activity and reducesthe level of TRP1 and tyrosinase expression
  - MSH-stimulated expression of tyrosinase, TRP1, and DCT
  - MSH-induced expression of the MITF gene
- In vivo, ASP decreases eumelanin synthesis due to;
  - a slight inhibition of tyrosinase
  - an almost complete loss of TRP1 and DCT expression

Agouti signal protein

- ASP: An antagonist of α-MSH signaling mediated by the MC1R
- ASP up-regulates ITF2, which, in turn, decreases melanogenic enzymes as well as MITF expression

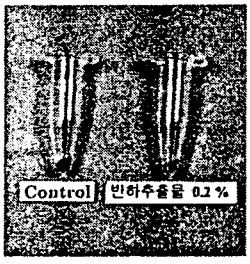
Biological Target Mechanisms



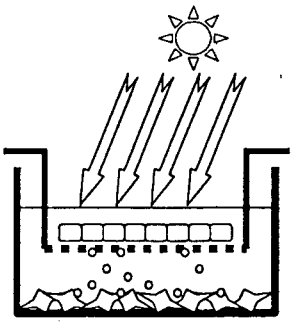
Bioassay

- In vitro screening
  - Physicochemical evaluations: free radical scavenging, UV absorption/blocking
  - Enzymes: tyrosinase
  - Cell culturess: melanoma cells, melanocytes, melanocyte/keratinocyte co-culture
  - 3D skin cultures: reconstituted epidermis, Dermal and Skin equivalents
- In vivo efficacy evaluations
  - Brown guinea pig, mouse
  - Human

In vitro bioassay

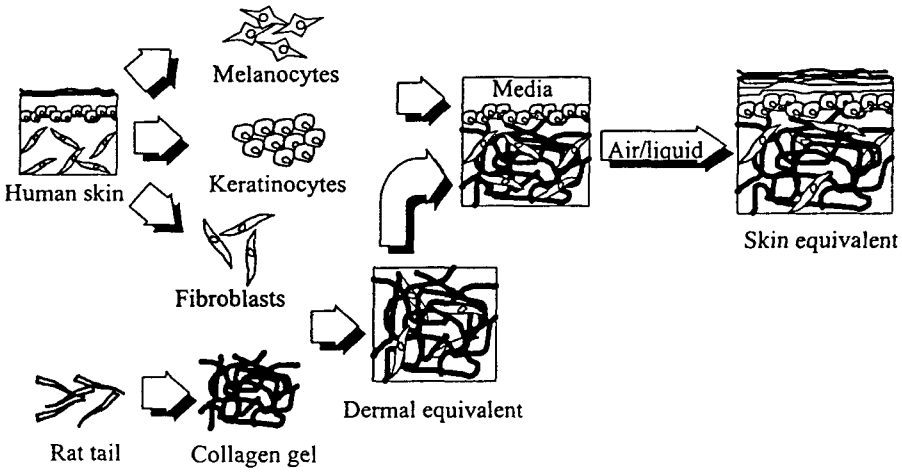


B16 melanoma cells



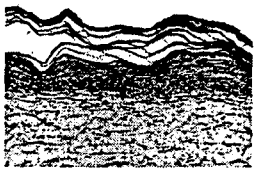
MC/KC co-culture

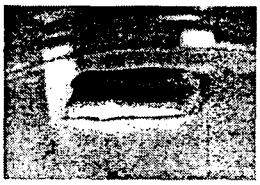
Skin equivalent



Skin equivalents

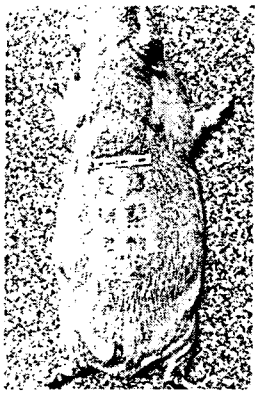
On dermal equivalent



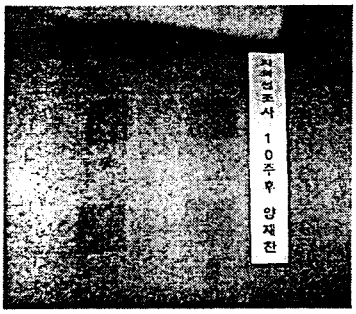


On de-epidermized dermis

In vivo bioassay



Brown guinea pig



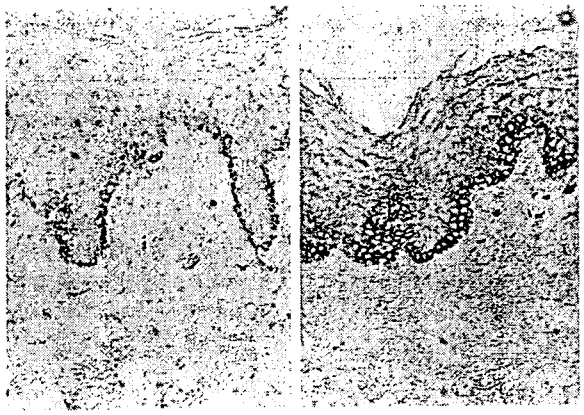
Human in vivo test

In vivo bioassay

SKH2 hairless mouse

In vivo bioassay

Fontana-Masson stain of human skin

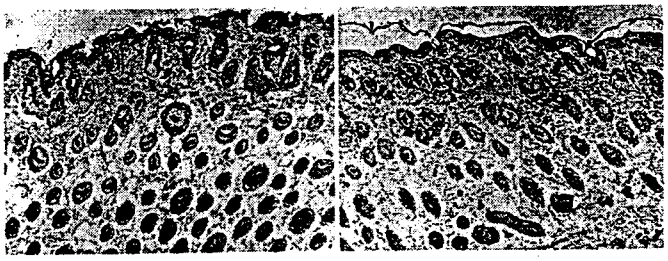


normal skin tanned skin

In vivo bioassay

Effect of Kligman formula on mouse hair color

Fontana-Masson stain

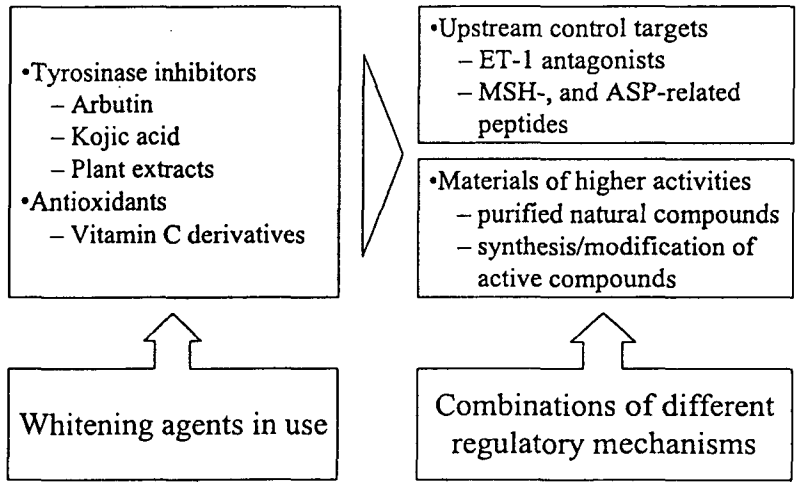


pigmented area depigmented area

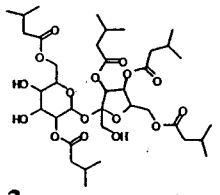
Whitening agents

	Past	Present	Future
Target mechanisms	Limited understanding about melanogenesis - tyrosinase - melanocytes	Multiple regulatory points of melanogenesis - irritation - hormone	Understanding network of regulations
Bioassays	Objective efficacy test Simple assay methods - tyrosinase inhibition - B16 melanona test	Subjective efficacy test Various screening method - enzyme, cells, animals	Novel methods for other target mechanisms
Materials	Animal/plant extracts - vitamin C derivatives - placenta - plant extracts	Active compounds of natural origin - Kojic acid Organic synthesis - Arbutin	Purified actives of various natural sources Modification/synthesis of active compounds
Efficacy	Doubtful	Weak	Obvious Various concepts

Whitening agents



Phytoclear-EL1 and -EL3



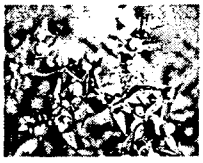
EL1 (5,15-diacetyl-3-benzoyllathyrol)

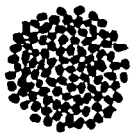
EL3 (sucrose-pentaisovalerate)

	IC_{50} (B16)
Phytoclear-EL1	5 μg/ml
Phytoclear-EL3	5 μg/ml
Arbutin	300 μg/ml
Kojic acid	280 μg/ml

- Non-tyrosinase inhibitor
- Safe and stable

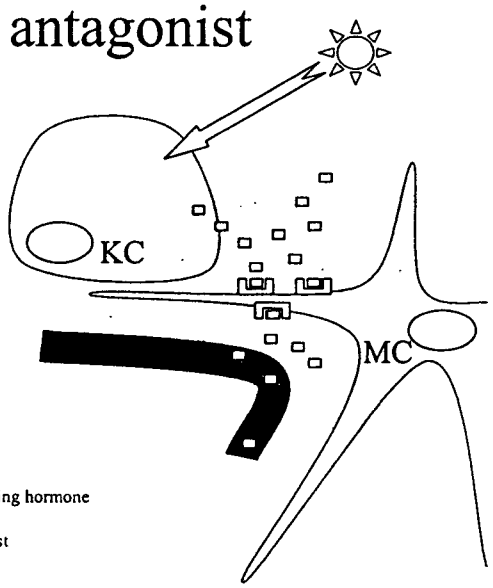
Mole plant (*Euphorbia lathyris L.*)





- 續隨子 莖中白汁 (中藥大辭典)

MC1R antagonist



KC: keratinocytes
MC: melanocytes
MC1R: Melanocortin receptor 1
MSH (melanocortin): Melanocyte stimulating hormone
ACTH: Adrenocorticotropic hormone
MC1Ra: Melanocortin receptor 1 antagonist

Percutaneous absorption

- Target cells, melanocytes reside in the basal layer of epidermis

- Physicochemical barrier of S. corneum
- Biological barrier of epidermis (metabolism)

Safety

- Unlike pharmeceuticals, cosmetics are used for long-term bases
- Most active materials act on living cells
  - more side effects than conventional cosmetics
  - skin problems induced by secondary metabolites