June 2007
Volume 6

CENTER FOR CANCER RESEARCH
frontiers
IN SCIENCE

MOLECULAR BIOLOGY

# Regulation of Skin Pigmentation via Modification of Tyrosinase Function

Ando H, Kondoh H, Ichihashi M, and Hearing VJ. Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. *J Invest Dermatol* 127:751–61, 2007.

Melanin synthesis in the skin, hair, and eyes is ultimately regulated by tyrosinase, the critical rate-limiting enzyme produced by melanocytes within those tissues (Figure 1). Following the translation and subsequent processing of tyrosinase in the endoplasmic reticulum (ER) and Golgi, it is trafficked to specialized organelles, termed melanosomes, wherein melanin is synthesized and deposited. In the skin and hair, melanosomes are transferred from melanocytes to neighboring keratinocytes and are distributed in those tissues to produce visible color. Melanin in the skin is not only important for cosmetic appearance, but has other critical functions, such as photoprotection from UV radiation. Excess melanin production or its abnormal distribution can cause irregular hyperpigmentation of the skin. In order to develop therapies or prophylactics that improve or prevent hyperpigmentary disorders, such as melasma and age spots, disruption of tyrosinase activity has usually been targeted.

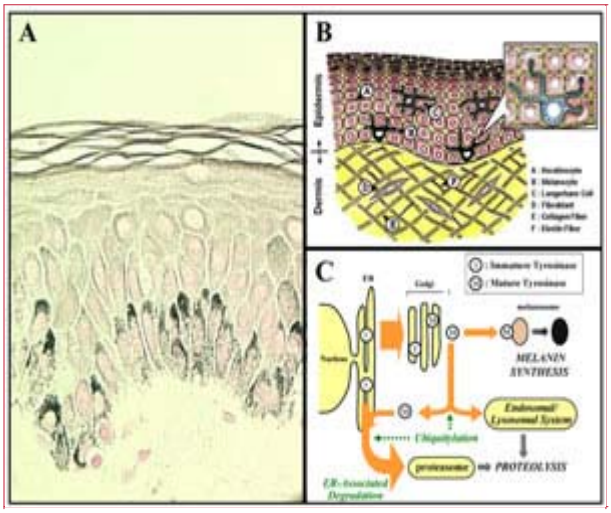


**Figure 1.** Regulation of skin pigmentation. *A)* Histology of human skin; black melanin pigment is seen just above the dermal-epidermal border. *B)* Schematic showing types of cells present in the epidermis and dermis. *C)* Schematic of tyrosinase processing and degradation within melanocytes. After

maturation in the Golgi, tyrosinase is trafficked either to melanosomes for melanin synthesis or to the degradation machinery. ER, endoplasmic reticulum.

Levels of intracellular proteins are regulated by a balance between their synthesis and degradation, which is also true for tyrosinase. However, in contrast to effects on other proteins, reduced stability and function of tyrosinase has dramatic results on ensuing pigmentation. Tyrosinase is degraded endogenously, at least in part, by proteasomes, and several types of inherited hypopigmentary diseases (e.g., oculocutaneous albinism [OCA] and Hermansky Pudlak syndrome) involve the aberrant processing/trafficking of tyrosinase and its degradation or secretion to the extracellular milieu. In this study, we consider the quality control of tyrosinase and its stability and implications for the regulation of skin pigmentation.

Many targets exist for controlling melanin synthesis via the regulation of tyrosinase function. These include the following:

- **Inhibiting tyrosinase transcription.** Decreases of tyrosinase mRNA levels can be elicited by 5-BRDUR (5-bromodeoxyuridine), TPA (12-*O*-tetradecanoylphorbol-13-acetate, TGF-β1 (transforming growth factor-β1), and TNF-α (tumor necrosis factor-α). The transcription factor MITF (microphthalmia-associated transcription factor), termed the master regulator of melanocyte function, directly controls tyrosinase gene expression, and a number of factors that decrease levels of mRNAs encoding tyrosinase and/or MITF have been identified, including agouti signal protein, ceramide, dihydrolipoic acid, sphingosine-1-phosphate, lysophosphatidic acid, and sphingosylphosphorylcholine.
- **Affecting tyrosinase maturation.** Tyrosinase is a glycoprotein with 6 conserved N-linked glycosylation sites. Mutations in the tyrosinase gene are responsible for OCA type 1 and mutations of critical glycosylation sites reduce its catalytic function. Abnormal glycosylation of tyrosinase in the ER/Golgi inhibits its folding and maturation and results in hypopigmentation. Thus glycosylation inhibitors, such as glucosamine and tunicamycin, inhibit melanin synthesis.
- **Inhibiting catalytic function.** Inhibitors of tyrosinase activity include competitive and non-competitive inhibitors. Hydroquinone and azelaic acid, both used as potent therapies for hyperpigmentary disorders, are competitive inhibitors but are cytotoxic to melanocytes. Arbutin, a derivative of hydroquinone, also inhibits tyrosinase activity, as does 4-tertiary butylphenol, aloesin, 4,4′-dihydroxybiphenyl, and 4-n-butylresorcinol. Tyrosinase activity depends on 2 copper atoms bound at its active site, and metal chelators inhibit its activity (e.g., phenylthiourea, kojic acid, and ellagic acid).
- **Accelerating tyrosinase degradation.** Many physiological factors in the skin can regulate tyrosinase degradation (e.g., TGF-β1, TNF-α, and 25-hydroxycholesterol). Linoleic acid, an unsaturated fatty acid, is a major component of cell membranes and is of special interest in this regard. Topical application of linoleic acid decreases UV-induced hyperpigmentation of the skin. Fatty acids can regulate tyrosinase degradation in contrasting manners via their effects on proteasomes—for example, linoleic acid accelerates whereas palmitic acid decelerates its degradation. We have shown that linoleic acid increases levels of ubiquitylated tyrosinase, which leads to its accelerated degradation by proteasomes.

We have shown that tyrosinase destined for degradation in the ER is degraded by proteasomes, via ER-associated protein degradation (ERAD). ERAD is a mechanism for quality control of proteins, which involves their retention in the ER or retro-translocation to the cytosol if misfolded or unassembled. This is followed by their deglycosylation, ubiquitylation, and subsequent proteolysis by proteasomes. Tyrosinase degradation can occur following its maturation in the Golgi, which suggests that it is also subject to post-Golgi–associated protein degradation.

Thus, skin pigmentation is regulated physiologically at many levels that affect the function of tyrosinase. Such regulation has dramatic effects on visible pigmentation and the function of the skin and thus provides an ideal model for the study of such processes. Each of those levels of regulation is a tempting target for affecting pigmentation and thus optimizing skin morphology and function.

**Hideya Ando, PhD**
Research Fellow
Laboratory of Cell Biology

**Vincent J. Hearing, PhD**
Senior Investigator
Laboratory of Cell Biology
NCI-Bethesda, Bldg. 37/Rm. 2132
Tel: 301-496-1564
Fax: 301-402-8787
hearingv@nih.gov

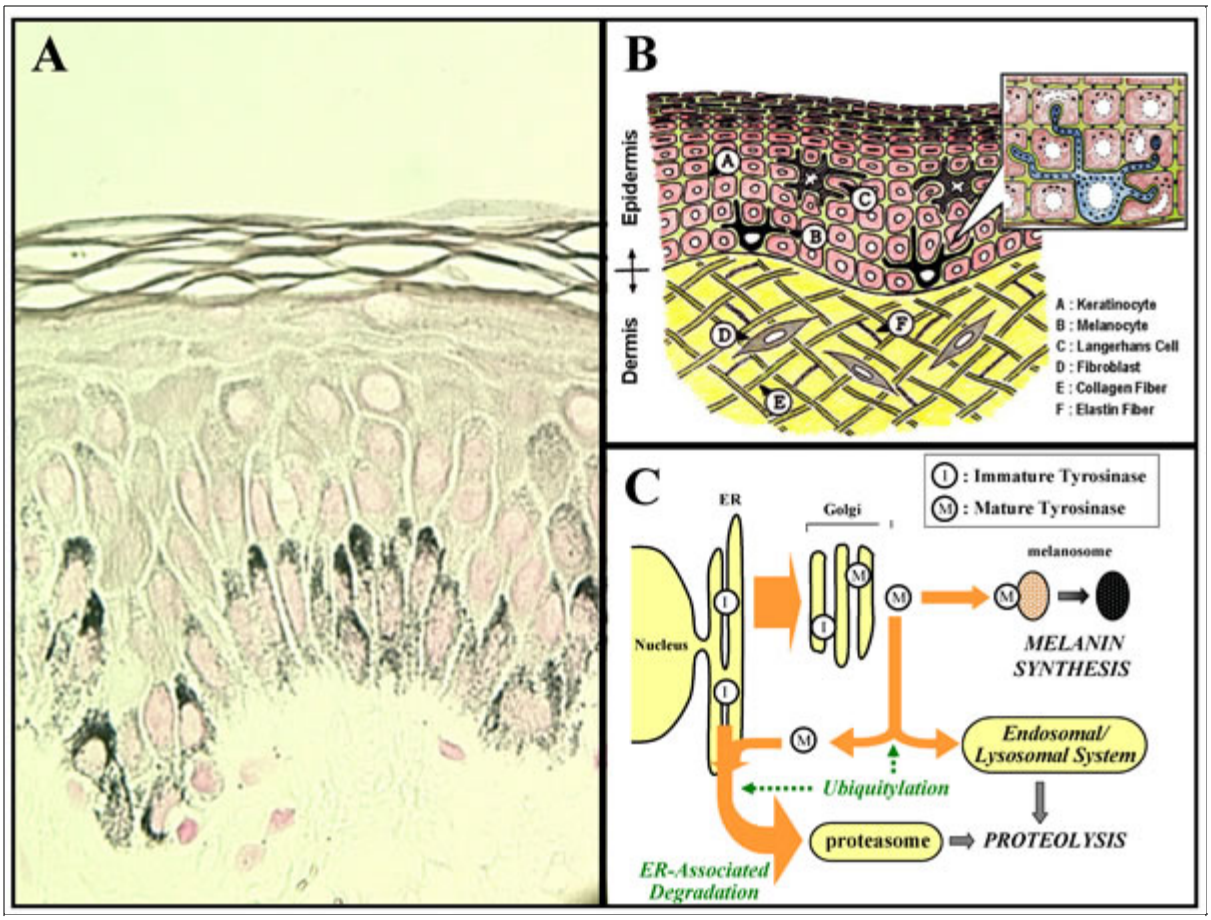


**Figure 1.** Regulation of skin pigmentation. *A)* Histology of human skin; black melanin pigment is seen just above the dermal-epidermal border. *B)* Schematic showing types of cells present in the epidermis and dermis. *C)* Schematic of tyrosinase processing and degradation within melanocytes. After maturation in the Golgi, tyrosinase is trafficked either to melanosomes for melanin synthesis or to the degradation machinery. ER, endoplasmic reticulum.