Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.



COSMOFARMA 2006
Roma, 26 maggio 2006

# FOTOESPOSIZIONE E PREVENZIONE DERMOCOSMETICA PER I DANNI DA SOVRAESPOZIONE E PRINCIPALI CONSEGUENZE

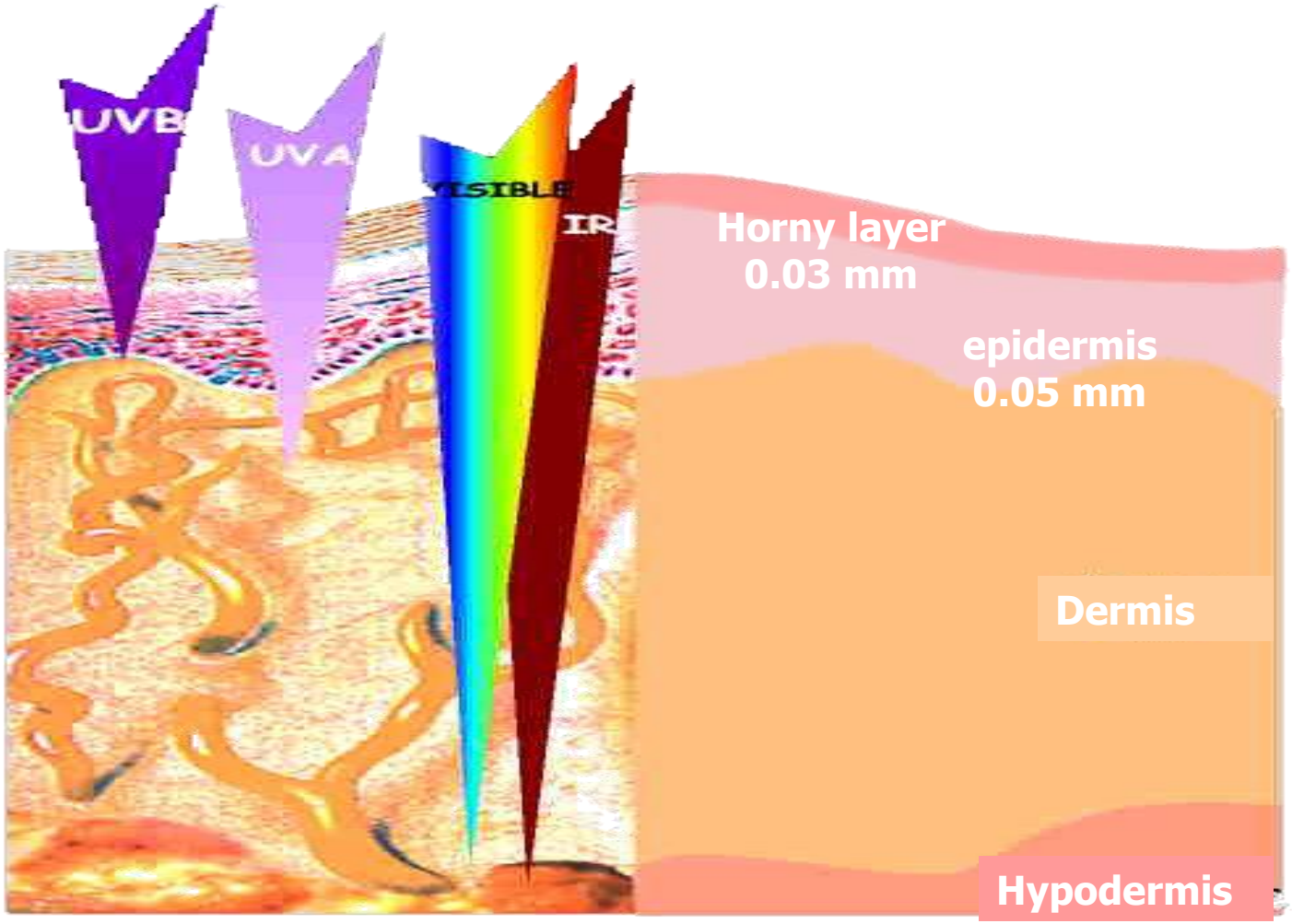
**Leonardo Celleno**
Direttore Centro Ricerche Cosmetologiche
Università Cattolica Sacro Cuore
Roma

# CUTANEOUS AGING

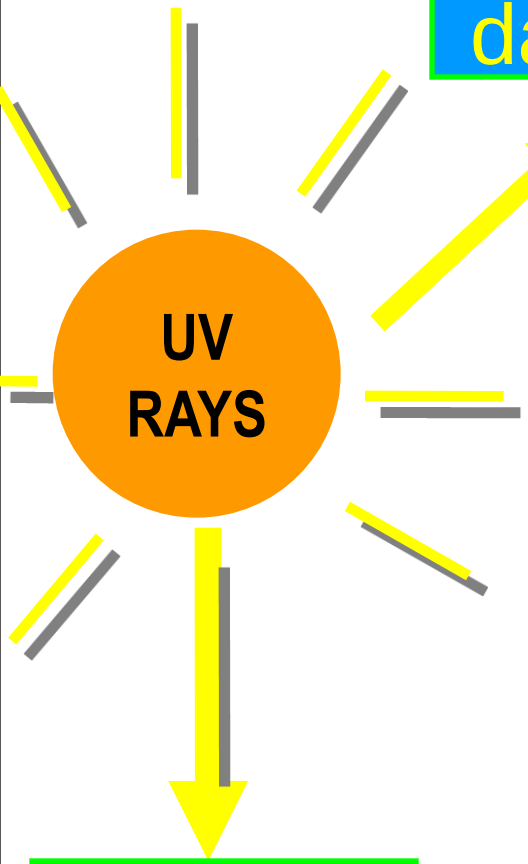
CHRONOAGING

PHOTOAGING

# PENETRATION



# Photobiological mecanisms of UV damaging




Direct damage → Molecular breakdown and molecular modifications

GENOMIC DAMAGE

Indirect damage → Free radicals → Molecular alterations

# DIRECT UV PHOTOBIOLOGICAL DAMAGE

**UVB**

**chromophore**

- **keratin**
- **haemoglobine**
- **porphirin**
- **nucleic acids**
- **melanin**
- **lipoprotein**
- **aromatic aminoacids (tyrosine-histidine)**

# DIRECT INDUCED CHANGES BY UVB

UVB (280-320 nm)

300 nm

PYRIMIDINE – PYRIMIDINE (DNA)

NUCLEOTIDE EXCISION REPAIR

THYMINE DIMERS

PYRIMIDINE – PYRIMIDINE

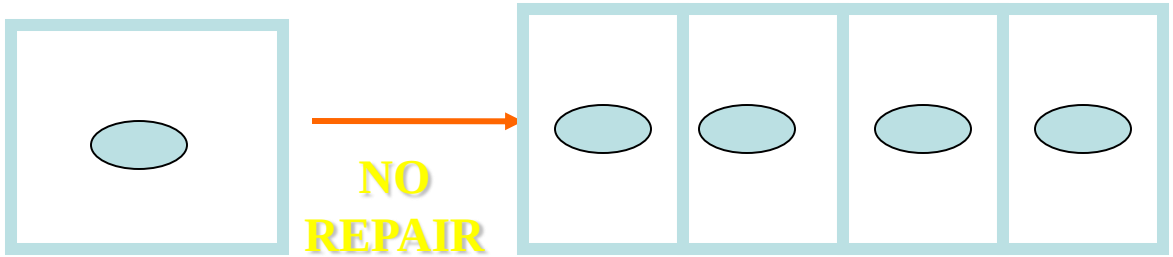
P53 PROTEIN UVB INDUCED

C → T  CC → TT

APOPTOSIS

ALSO UVA CAN INDUCE THYMIDINE DIMERS

UVB



NO REPAIR

NO APOPTOSIS

*MUTATION ON P 53 GENOME*

*CLONAL EXPANSION*

***CELLULAR ALTERATION LEADING TO PHOTOAGING AND SKIN CANCER***

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzata di nuovo la x rossa, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

# Clinical expression of *p53* genomic damage

Single allele damage ⟶ Actinic keratosis

Double allele damage ⟶ Sq cells carcinoma

Patch damage of genoma ⟶ Basal cells carcinoma

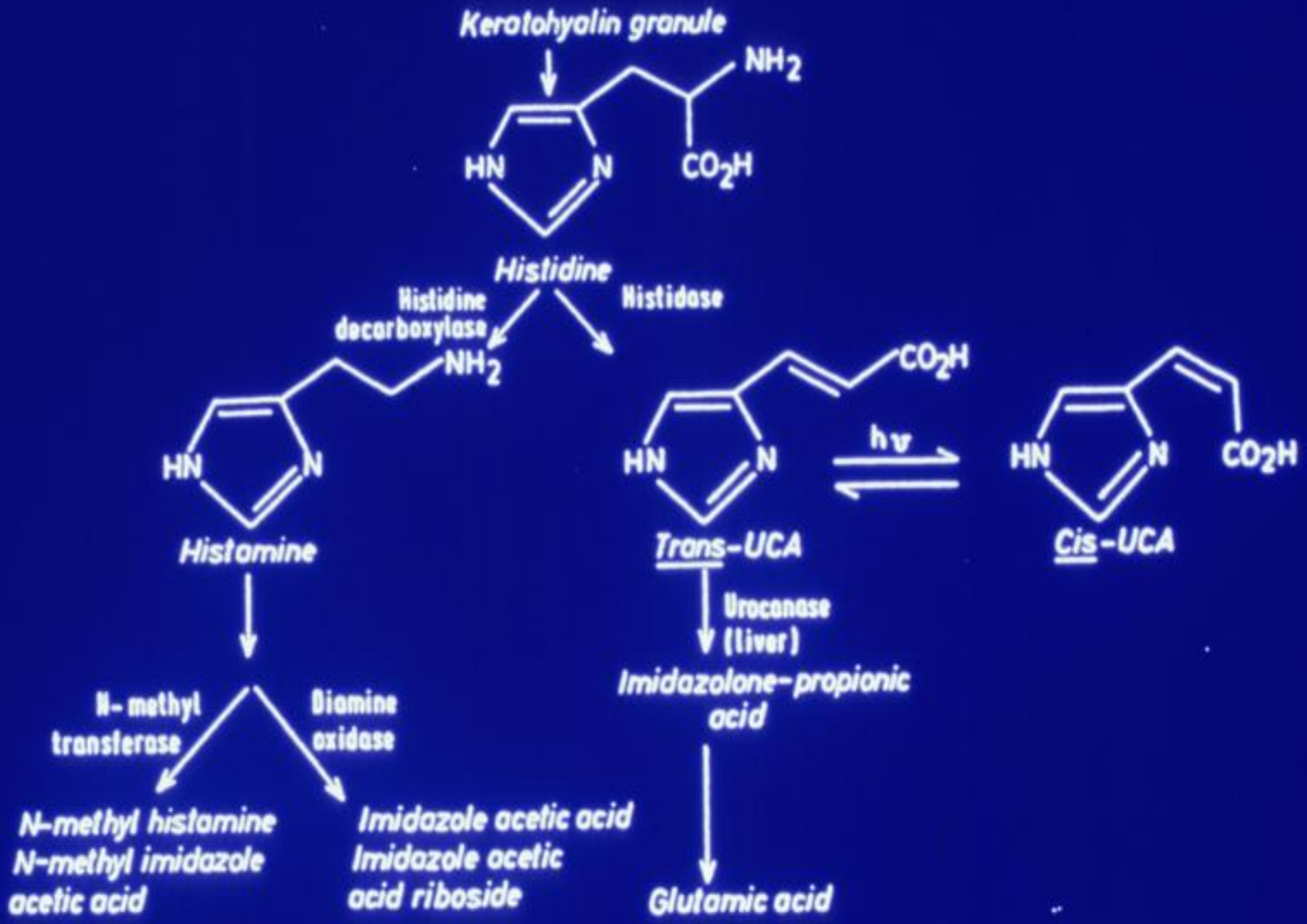



Figure 1. Metabolic pathways of histidine, histamine and urocanic acid.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, data, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

**UVA/UVB 295-350**

→

**Urocanic acid isomerization**

↓

**Immunosuppression**

**Immunomodulation**

↓

- **Numerical reduction, morphological and antigenic modifications of Langerhans cells**
- **T lynfocites lines cells activation**

# INDIRECT UV PHOTOBIOLOGICAL DAMAGE

**UVA**

**chromophore**

- keratin
- haemoglobine
- porphirin
- nucleic acids
- melanin
- lipoprotein
- aromatic aminoacids (tyrosine-histidine)

**photonic absorption**

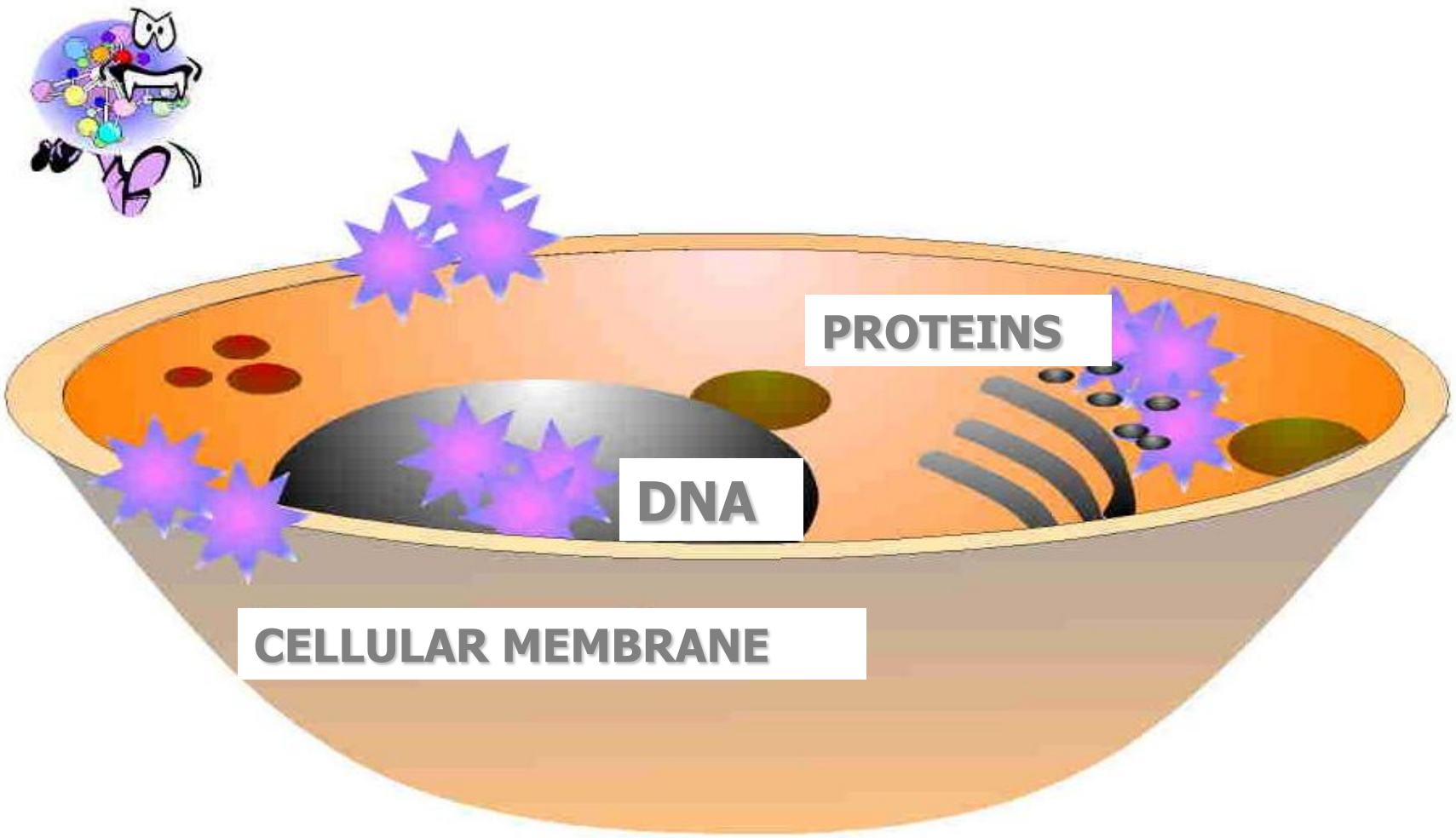
**excited molecules and free radicals formation**

**biochemical reaction**

**cellular damage**

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, data, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

# FREE RADICALS



Biological oxidations

Radiations and chemical pollutans

Tocopherol, β-Carotene, Ascorbic acid, Glutathione

$O_2^-$, $OH^-$, $^1O_2$, $H_2O$

Enzyme inactivation

DNA damage

Lipid peroxidation

Superoxide dismutase (SOD)

$2O_2^- + 2H^+ \longrightarrow H_2O + O_2$

$H_2O_2$

Catalase

$2H_2O \longrightarrow O_2$

Glutathione peroxidase (GSH-Px)

$2GSH + H_2O_2 \quad GSSG + 2H_2O$

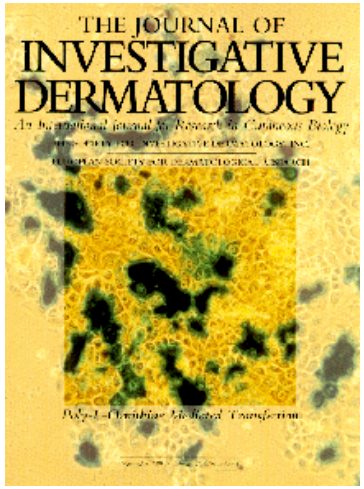
$H_2O$

# The Journal of Investigative Dermatology

Volume 124 Issue 2 Page 428 - February 2005


# B-Carotene Interferes with Ultraviolet Light A-Induced Gene Expression by Multiple Pathways

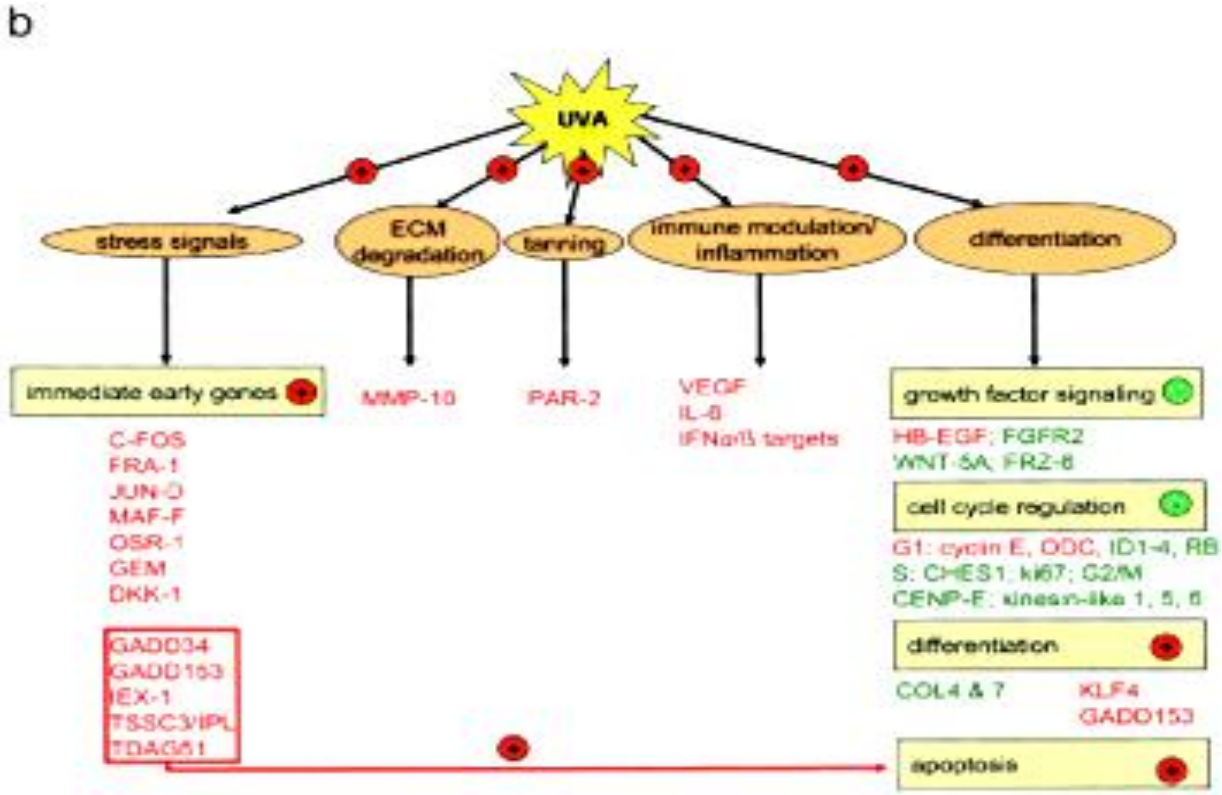

Karin Wertz*, Petra Buchwald Hunziker*, Nicole Seifert*, Georges Riss*, Martin Neeb , Guido Steiner , Willi Hunziker 1 and Regina Goralczyk*



Ultraviolet light A (UVA) exposure is thought to cause skin aging mainly by singlet oxygen ((1)O(2))-dependent pathways. Using microarrays, we assessed whether pre-treatment with the (1)O(2) quencher beta-carotene (betaC; 1.5 microM) prevents UVA-induced gene regulation in HaCaT human keratinocytes. Downregulation of growth factor signaling, moderate induction of proinflammatory genes, upregulation of immediate early genes including apoptotic regulators and suppression of cell cycle genes were hallmarks of the UVA effect. Of the 568 UVA-regulated genes, betaC reduced the UVA effect for 143, enhanced it for 180, and did not interact with UVA for 245 genes. The different interaction modes imply that betaC/UVA interaction involved multiple mechanisms. In unirradiated keratinocytes, gene regulations suggest that betaC reduced stress signals and extracellular matrix (ECM) degradation, and promoted keratinocyte differentiation. In irradiated cells, expression profiles indicate that betaC inhibited UVA-induced ECM degradation, and enhanced UVA induction of tanning-associated protease-activated receptor 2. Combination of betaC-promoted keratinocyte differentiation with the cellular "UV response" caused synergistic induction of cell cycle arrest and apoptosis. In conclusion, betaC at physiological concentrations interacted with UVA effects in keratinocytes by mechanisms that included, but were not restricted to (1)O(2) quenching. The retinoid effect of betaC was minor, indicating that the betaC effects reported here were predominantly mediated through vitamin A-independent pathways.

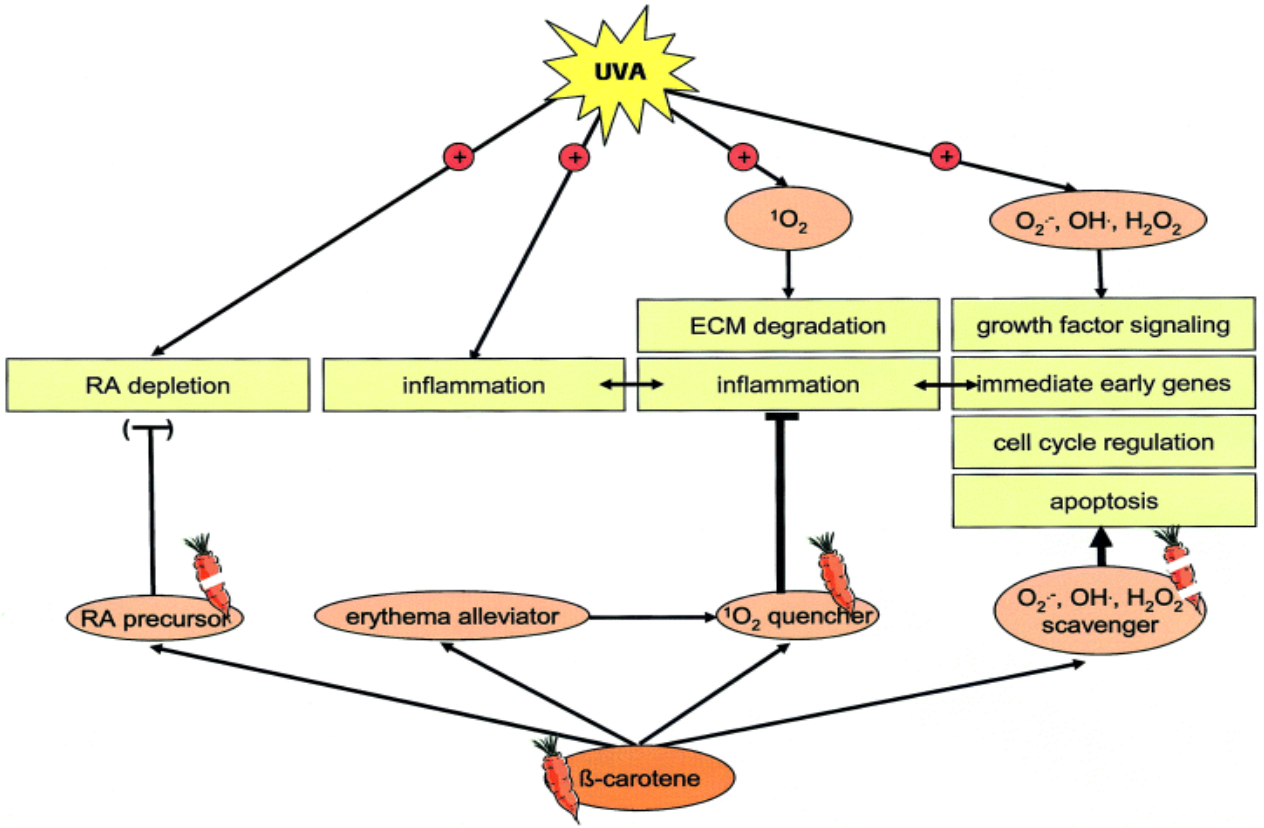
# Ultraviolet light A (UVA) effects in keratinocytes



# Proposed relationship of the modes of action of β-carotene to its influence on Ultraviolet light A-induced biological processes

- β-carotene inhibited gene regulations by UVA, which promote ECM degradation, arguing for a photoprotective effect

- β-carotene enhanced UVA-induced PAR-2 (protease- activated receptor 2) expression, suggesting that β-carotene enhances tanning after UVA exposure.




- the combination of β-carotene induced differentiation with the cellular "UV response" led to a synergistic induction of cell cycle arrest and apoptosis by UVA and β-carotene.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, tale potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

Our results explain and integrate many conflicting reports on the efficacy of

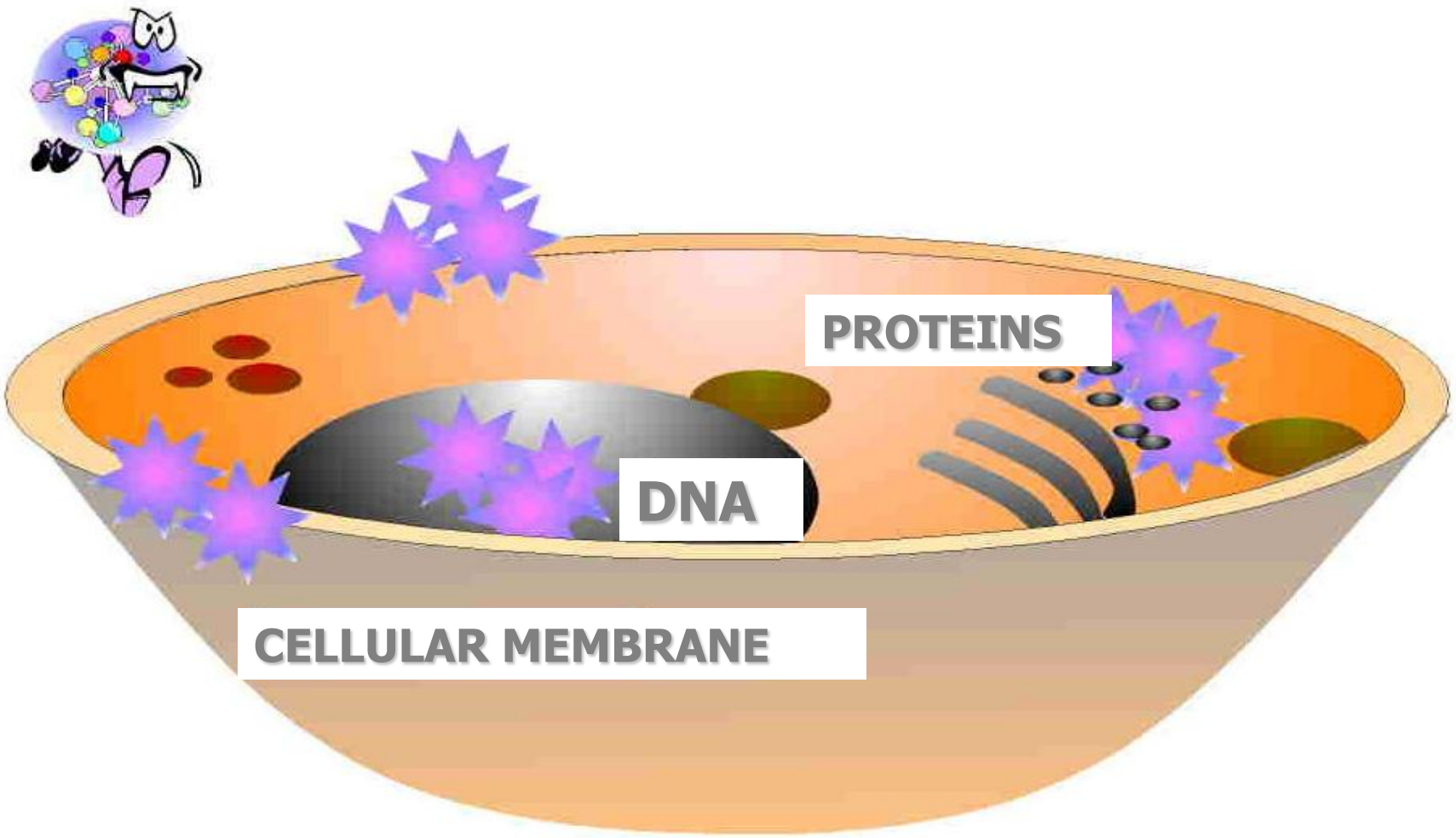
β-carotene as a $^1O_2$ quencher and as a general antioxidant in living cells.

The identified mechanisms, by which β-carotene acts on the skin have

implications on

skin photoaging, as well as on relevant skin diseases, such as skin cancer and psoriasis.

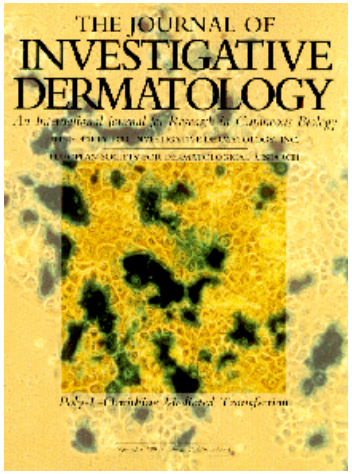
# FREE RADICALS



The Journal of Investigative Dermatology
Volume 124 Issue 2 Page 428 - February 2005

# The Creatine Kinase System in Human Skin: Protective Effects of Creatine Against Oxidative and UV Damage *In Vitro* and *In Vivo*

Lenz H, Schmidt M, Welge V, Schlattner U, Wallimann T, Elsasser HP, Wittern KP, Wenck H, Stab F, Blatt T.
Department of Cytobiology and Cytopathology, Philipps University Marburg, Marburg, Germany.




Cutaneous aging is characterized by a decline in cellular energy metabolism, which is mainly caused by detrimental changes in mitochondrial function. The processes involved seem to be predominantly mediated by free radicals known to be generated by exogenous noxes, e.g., solar ultraviolet (UV) radiation. Basically, skin cells try to compensate any loss of mitochondrial energetic capacity by extra-mitochondrial pathways such as glycolysis or the creatine kinase (CK) system. Recent studies reported the presence of cytosolic and mitochondrial isoenzymes of CK, as well as a creatine transporter in human skin. In this study, we analyzed the cutaneous CK system, focusing on those cellular stressors known to play an important role in the process of skin aging. According to our results, a stress-induced decline in mitochondrial energy supply in human epidermal cells correlated with a decrease in mitochondrial CK activity. In addition, we investigated the effects of creatine supplementation on human epidermal cells as a potential mechanism to reinforce the endogenous energy supply in skin. Exogenous creatine was taken up by keratinocytes and increased CK activity, mitochondrial function and protected against free oxygen radical stress. Finally, our new data clearly indicate that human skin cells that are energetically recharged with the naturally occurring energy precursor, creatine, are markedly protected against a variety of cellular stress conditions, like oxidative and UV damage in vitro and in vivo. This may have further implications in modulating processes, which are involved in premature skin aging and skin damage.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzata di nuovo la x rossa, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

Oxidative damage of cellular and extracellular components activates intrinsic repair mechanisms, which necessarily require ATP for full functionality.

The PCr (phosphocreatine)/CK (creatine kinase) system together with the recently discovered Epidermal Creatine Transporter (CRT) (schlattner et al, 2002) provide human skin with an important tool to cope efficiently with conditions of high-energy demand.

In skin  CK-activity may be caused by the generation of ROS during cutaneous aging (Harman, 1956; Dolder *et al*, 2001). Specifically Mi-CK is a primary target for ROS, especially peroxynitrite (Stachowiak *et al*, 1998).

The epidermal creatine system, which is very important for cellular energy metabolism, obviously declines under oxidative stress conditions, including skin aging processes.

This study shows the significance of the PCR/CK system for epidermal energy supply and the beneficial effects of creatine supplementation both *in vitro* and *in vivo*.

**The creatine supplementation results in less UV-induced mitochondrial DNA mutations in skin cells (krutmann, 2001).**

# The Journal of Investigative Dermatology

Apr 2005;124(4):825-32


# Ultraviolet A irradiation induces NF-E2-related factor 2 activation in dermal fibroblasts: protective role in UVA-induced apoptosis.


Hirota A, Kawachi Y, Itoh K, Nakamura Y, Xu X, Banno T, Takahashi T, Yamamoto M, Otsuka F.
Department of Dermatology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan.




Ultraviolet (UV) radiation is one of the most important environmental factors involved in the pathogenesis of skin aging and cancer. Many harmful effects of UV radiation are associated with the generation of reactive oxygen species, and cellular antioxidants act to prevent the occurrence and reduce the severity of UV-induced skin disorders. Transcription factor NF-E2-related Factor 2 (Nrf2) and its cytoplasmic anchor protein Kelch-like-ECH-associated protein 1 (Keap1) are central regulators of the cellular antioxidant response. In this study, we investigated the effects of UV irradiation on the activation of Nrf2 in dermal fibroblasts. We found that UVA irradiation, but not UVB, causes nuclear translocation and accumulation of Nrf2 by a factor of 6.5 as compared with unirradiated controls. The nuclear accumulation of Nrf2 induced by UVA was enhanced by the photosensitizer hematoporphyrin. To evaluate the protective role of Nrf2 against UVA radiation, we examined UVA-induced apoptosis using dermal fibroblasts derived from nrf2 or keap1 gene knockout mice. Whereas disruption of nrf2 increased the number of apoptotic cells following UVA irradiation by 1.7-fold, disruption of keap1 decreased the apoptotic cell number by half as compared with wild-type controls. These findings thus demonstrate that the Nrf2-Keap1 pathway plays an important role in the protection of the skin against UVA irradiation.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso e, tasto, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

Both UVA and UVB irradiations provoke apoptosis of the dermal cells. The mechanism of apoptosis induced by UVA has been suggested to be different from that induced by UVB:

- UVA induces apoptosis mainly through downregulation of Bcl-2 expression
- UVB-induced apoptosis accompanies the accumulation of p53 (wang *et al*, 1998).

In this regard, Godar *et al*. classified the mechanisms of UV-induced apoptosis as either immediate or delayed.

THE UVA IRRADIATION IS MUCH LESS MUTAGENIC THAN UVB, IT FULLY ACTIVATES THE **NRF2 KEAP1 PATHWAY** WITHIN 4 H AFTER EXPOSURE TO UVA.

Key proteins in the coordinate transcriptional induction of various antioxidant-matabolizing enzymes

SHOWED THAT THE **NRF2**-MEDIATED INDUCTION OF A SET OF CYTOPROTECTIVE GENES IS AN IMPORTANT PROCESS FOR THE PROTECTION OF THE DERMAL CELLS FROM UVA-INDUCED OXIDATIVE STRESS.

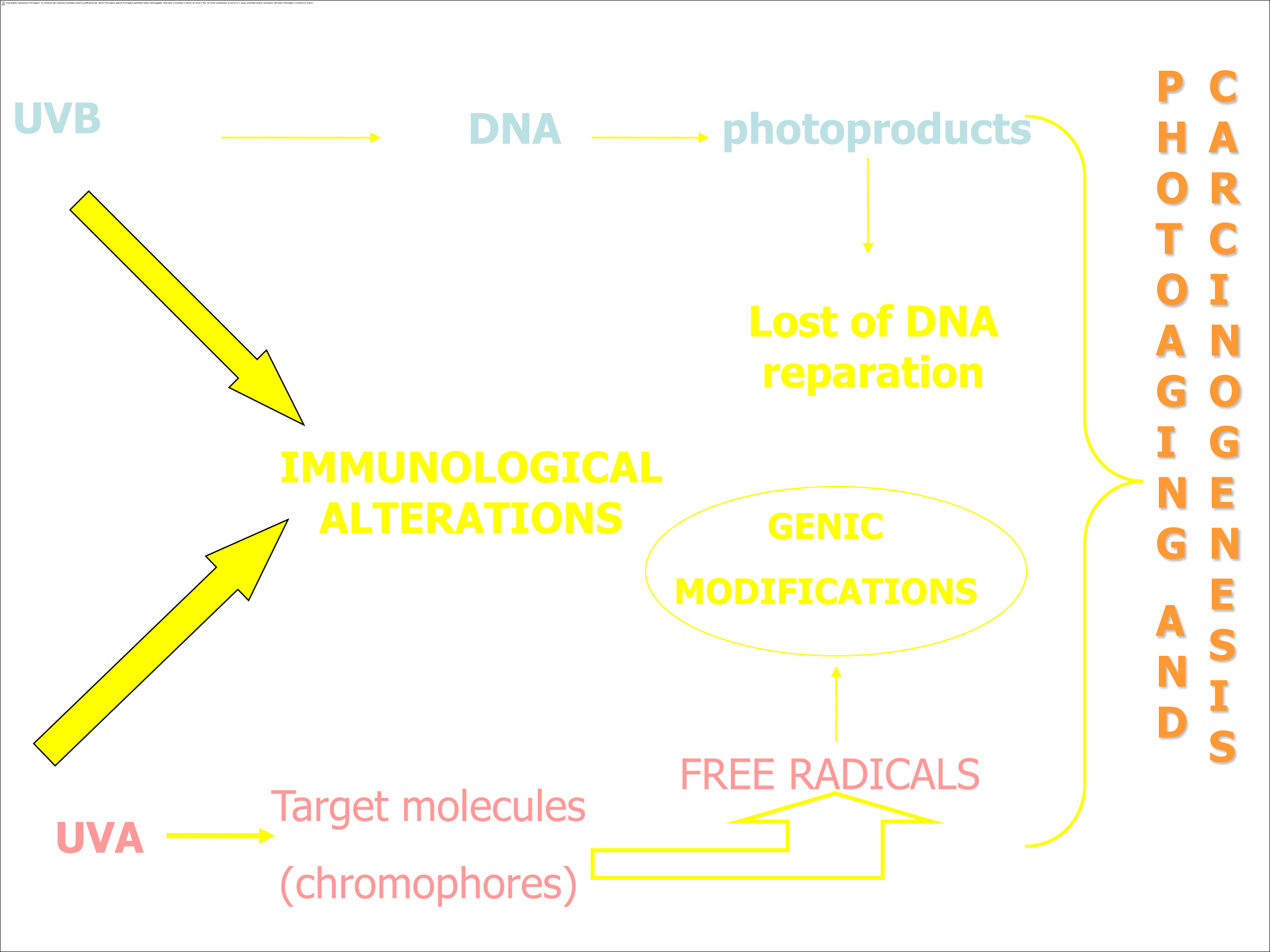
**THE PROTECTION OF CELLS FROM UVA-INDUCED APOPTOSIS WAS SIGNIFICANTLY DIMINISHED BY THE DISRUPTION OF *NRF2* .**

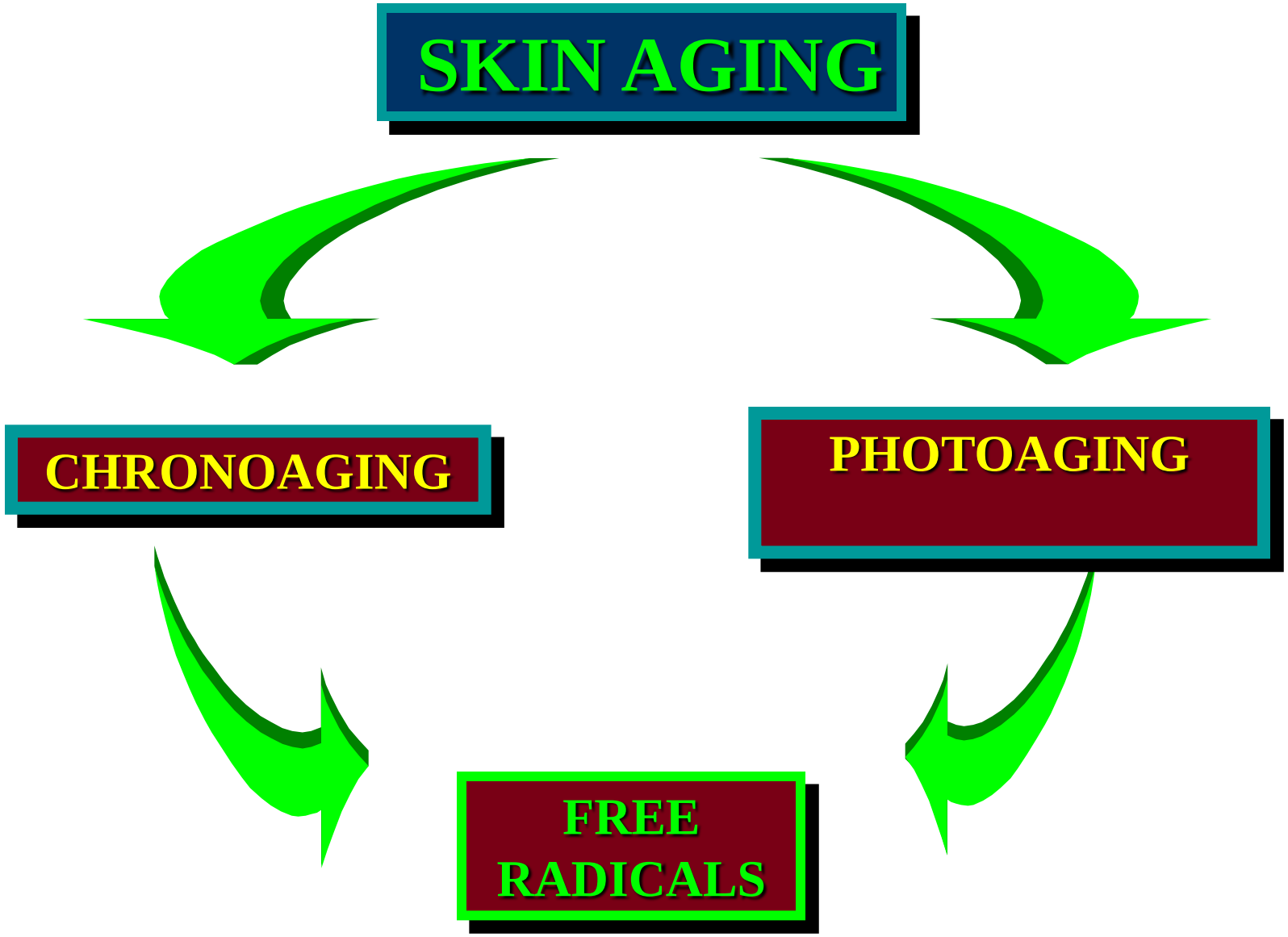
Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzata di nuovo la x rossa, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

CATALASE, SUPEROXIDE DISMUTASE, AND GLUTATHIONE PEROXIDASE (GPX), ALL OF WHICH ELIMINATE ROS, ARE ALSO DEPENDENT ON **NRF2** (LEE *ET AL*, 2003).

# UVA IRRADIATION INDUCES THE NUCLEAR ACCUMULATION OF **NRF2** IN DERMAL FIBROBLASTS.

**THE NRF2 KEAP1 SYSTEM PLAYS A CRITICAL ROLE IN THE PROTECTION OF DERMAL CELLS FROM THE DELETERIOUS EFFECTS PROVOKED BY THE EXPOSURE TO UVA.**


UVB
DNA
photoproducts
Lost of DNA reparation
IMMUNOLOGICAL ALTERATIONS
GENIC MODIFICATIONS
FREE RADICALS
UVA
Target molecules (chromophores)
PHOTOAGING AND CARCINOGENESIS


SKIN AGING
CHRONOAGING
PHOTOAGING
FREE
RADICALS

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, data, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

**“The effect of sunscreen on skin elastase activity induced by ultraviolet-A irradiation”**

Tsukahara K, Moriwaki S, Hotta M, Fujimura T, Sugiyama-Nakagiri Y, Sugawara S, Kitahara

It has been reported that application of sunscreens prevents the photoaging of skin in animal models and in humans. .....

In the UVA sunscreen group, both the UVA induced skin damage and the increase in skin elastase activity were significantly inhibited, as compared to the vehicle group

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, tale potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

Dermatol Clin. 2006 Jan;24(1):35-51

# Human safety and efficacy of ultraviolet filters and sunscreen products.

Nash JF.
Central Product Safety, The Procter and Gamble Company, Cincinnati, OH 45241, USA. nash.jf@pg.com

Ultraviolet (UV) filters are the active ingredients in sunscreens. The concentration and combination of UV filters determine the efficacy of sunscreens as measured by sun protection factor. The safety of individual UV filters, and, more generally, sunscreen products, is a matter of a few related components: objective toxicologic evaluation, phototoxicologic potential, and human health consequences of using products that may reduce some but not all of the solar UV. Of 16 UV filters approved by the US Food and Drug Administration, 9 are used in different combinations in the most currently marketed sunscreens. Most of these compounds are considered safe and effective alone or in combination with other UV filters based on extensive toxicologic/phototoxicologic evaluations and market history. The benefits from proper use of sunscreens outweigh real or perceived human health concerns, establishing a favorable benefit-to-risk ratio. Future UV filters will require complete human safety evaluations alone and in combination with select benchmark ingredients.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, tale potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

J Photochem Photobiol B. 1994 Jan;22(1):29-36.

# Relationship between the ability of sunscreens containing 2-ethylhexyl-4'-methoxycinnamate to protect against UVR-induced inflammation, depletion of epidermal Langerhans (Ia+) cells and suppression of alloactivating capacity of murine skin in vivo.

Walker SL, Morris J, Chu AC, Young AR.

Department of Photobiology, St. John's Institute of Dermatology, United Medical School, Guy's Hospital, University of London, UK.

The UVB sunscreen 2-ethylhexyl-4'-methoxycinnamate was evaluated in hairless albino mouse skin for its ability to inhibit UVR-induced (i) oedema, (ii) epidermal Langerhans cell (Ia+) depletion and (iii) suppression of the alloactivating capacity of epidermal cells (mixed epidermal cell-lymphocyte reaction, MECLR). The sunscreen, prepared at 9% in ethanol or a cosmetic lotion, was applied prior to UVB/UVA irradiation. In some experiments there was a second application halfway through the irradiation. Single applications in both vehicles gave varying degrees of protection from oedema and Langerhans cell depletion but afforded no protection from suppression of MECLR. **When the sunscreens were applied twice there was improved protection from oedema and Langerhans cell depletion and complete protection was afforded from suppression of MECLR. There was a clear linear relationship between Langerhans cell numbers and oedema with and without sunscreen application**. The relationship between Langerhans cell numbers and MECLR was more complex. These data confirm published discrepancies between protection from oedema (a model for human erythema) and endpoints with immunological significance, but show that 2-ethylhexyl-4'-methoxycinnamate can afford complete immunoprotection, although protection is dependent on the application rate and vehicle.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, tale potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

Skin Pharmacol Physiol. 2005 Jul-Aug;18(4):201-8. Epub 2005 May

# Efficacy of sunscreens containing pre-tocopheryl in a surviving human skin model submitted to UVA and B radiation.

Boisnic S, Branchet-Gumila MC, Merial-Kieny C, Nocera T

The aim of the present study was to evaluate by means of histological and biochemical tools the additive efficacy of pre-tocopheryl during photoprotection using a sunscreen containing mineral sunblock agents 50B-10A (TiO(2), ZnO) and pre-tocopheryl in comparison to a cream containing only mineral sunblock agents 50B-10A. For this purpose, an ex vivo technique and an acetone-impaired human skin model were used in order to approximate in vivo metabolic conditions. Creams were topically applied to the surface of the epidermis and submitted to UV radiations. Then, human skin explants were maintained alive in organ culture for 3 days. Free radical modulation was analysed by hydroperoxide assay. Epidermal (involucrin, cell proliferation, stratum corneum lipids) and dermal changes (elastic fibres and collagen) were studied. Analysis of ex vivo surviving skin samples impaired by UV irradiations and treated with the mineral sunscreen 50B-10A showed a significant decrease in hydroperoxide production and an improvement in the elastic fibre and collagen network in the dermis. Adding pre-tocopheryl to this formula induced an increase in involucrin and epidermal lipids such as squalenes and ceramides. Altogether, these results confirm the efficacy of the combination of a mineral sunscreen and pre-tocopheryl in photoprotection and free radical protection

# Topical Retinoic Acid for the Amelioration of Photoaging

**Albert M. Kligman, MD, PhD James J. Leyden, MD Lorraine H. Kligman, PhD**

Department of Dermatology University of Pennsylvania School of Medicine

Philadelphia, PA;

**Gary L. Grove, PHD**

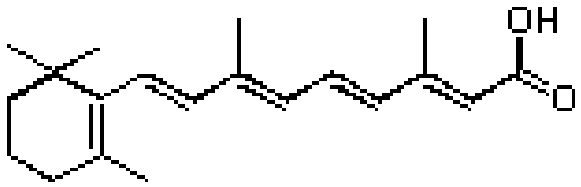
Skin Study Center

Philadelphia, PA

**1986**

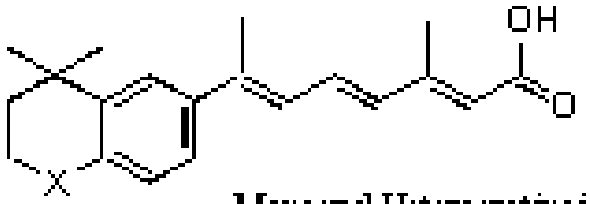
# Fig. 1: Retinoidi di prima generazione

**Parent Compounds**

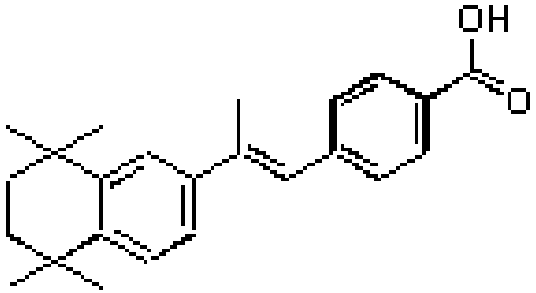



all-trans retinoic acid, MTD 10 mg/kg/day

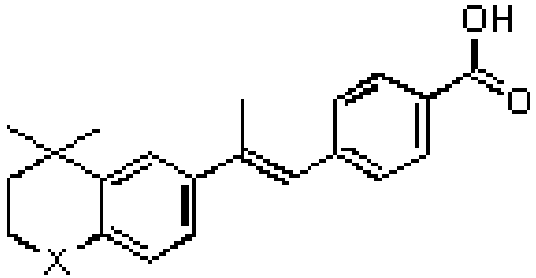
**First Generation Heteroarotinoids**




Monoaryl Heteroarotinoids
X = O, MTD = 32 mg/kg/day
X = S, MTD = 34 mg/kg/day

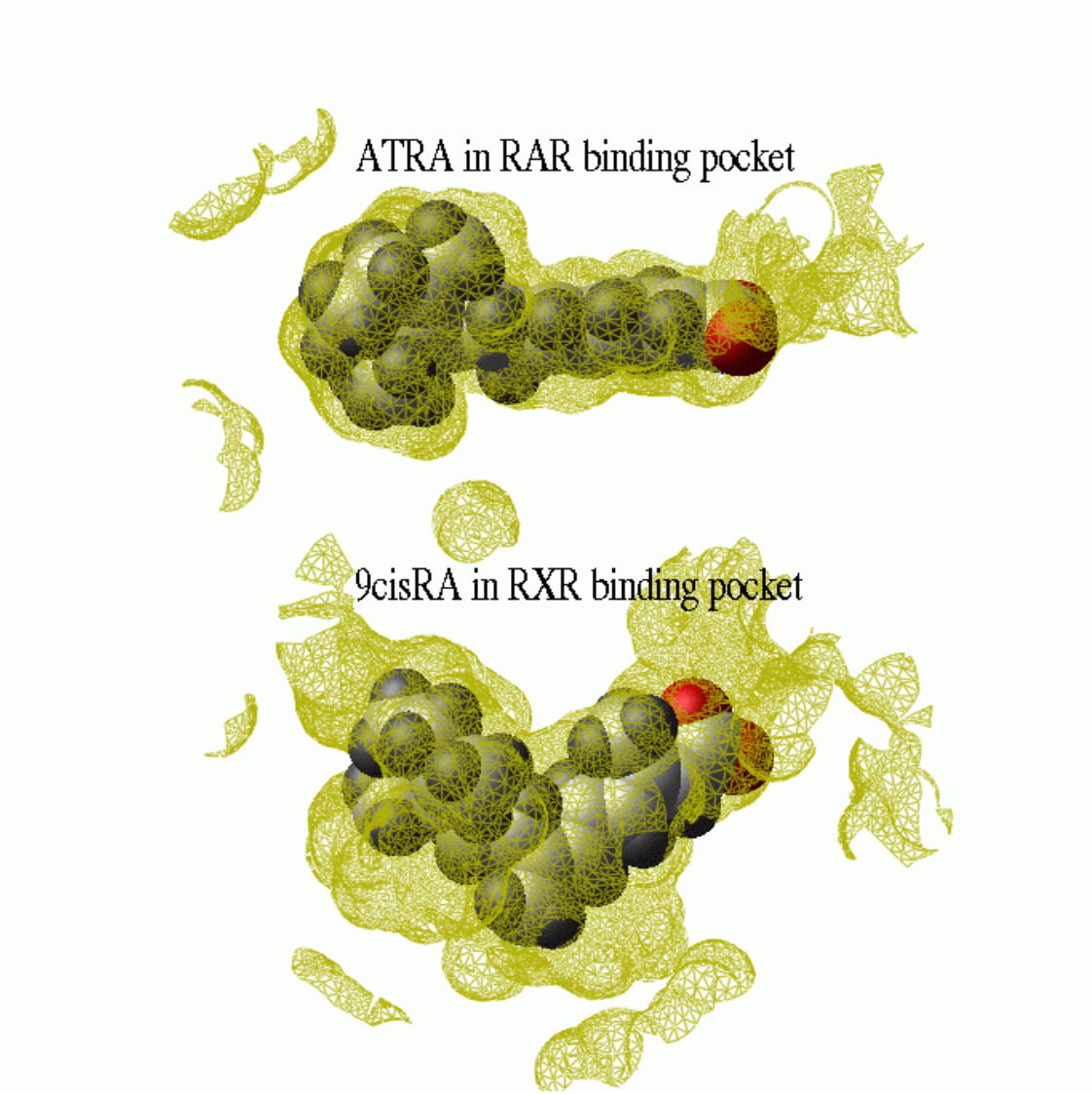



Arotiroid
TTNPB, MTD = 0.01 mg/kg/day

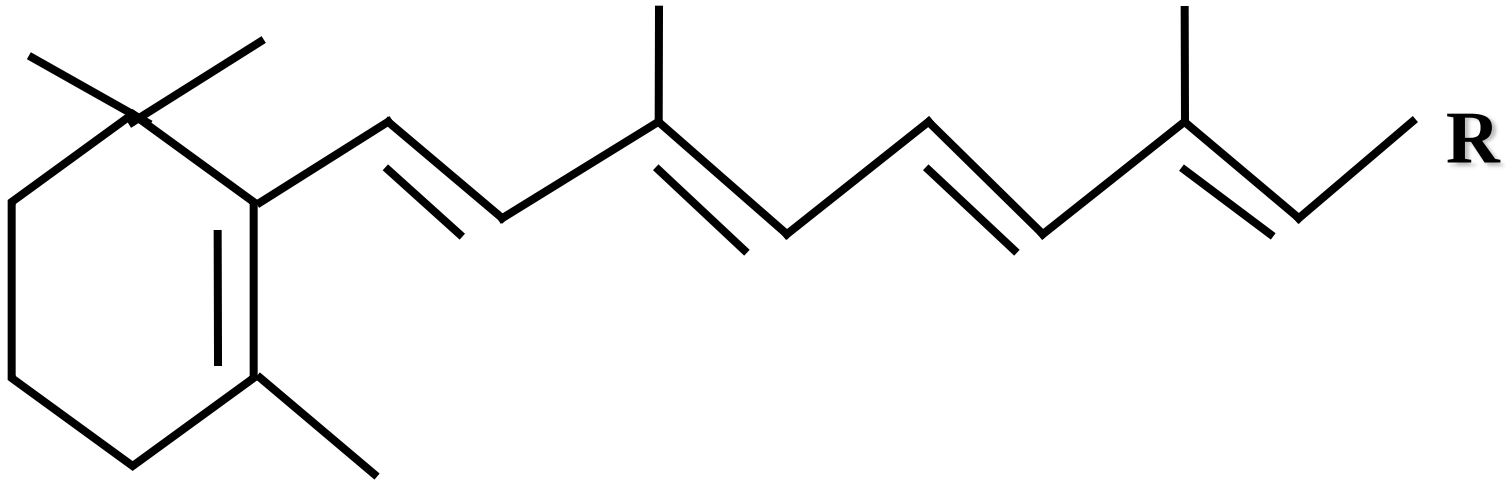



Diaryl Heteroarotinoid
X= O, MTD = 9.4 mg/kg/day

# Fig. 3: Legame ligando-recettore.



# Natural Vitamin A Compounds




**R= $CH_2$ OH : Retinol**

**R= CHO : Retinal**

**R= COOH : Retinoic Acid**

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, data potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

# ANTIOXIDANT SUBSTANCES

- alpha-tocopherol
- ascorbic acid
- beta-carotene
- Superoxide dismutase
- Q10
- resveratrol
- ginkgo biloba

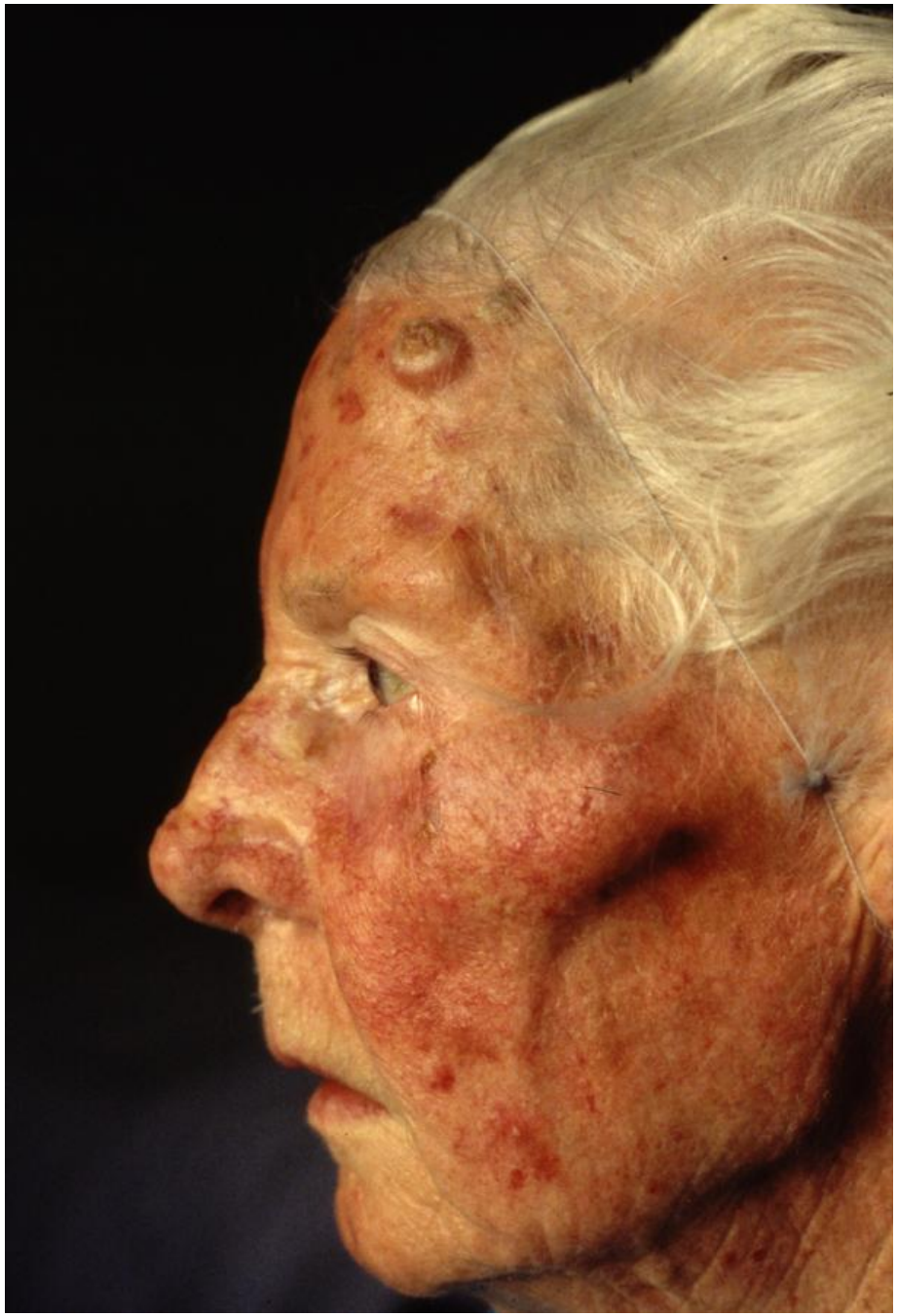
# Actual research lines

New molecules with anti free radicals activity

Improving of biodisponibility and stability of active principles all ready knewd.

Sinerging mixing of anti radical substances.

Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzato di nuovo il rosso x, tale potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.



Impossibile visualizzare l'immagine. La memoria del computer potrebbe essere insufficiente per aprire l'immagine oppure l'immagine potrebbe essere danneggiata. Riavviare il computer e aprire di nuovo il file. Se viene visualizzata di nuovo la x rossa, potrebbe essere necessario eliminare l'immagine e inserirla di nuovo.

# *THANK YOU !*

*Leonardo Celleno*