Issue n° 24 - February 1996

ESPCR BULLETIN

PUBLISHED BY THE EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH

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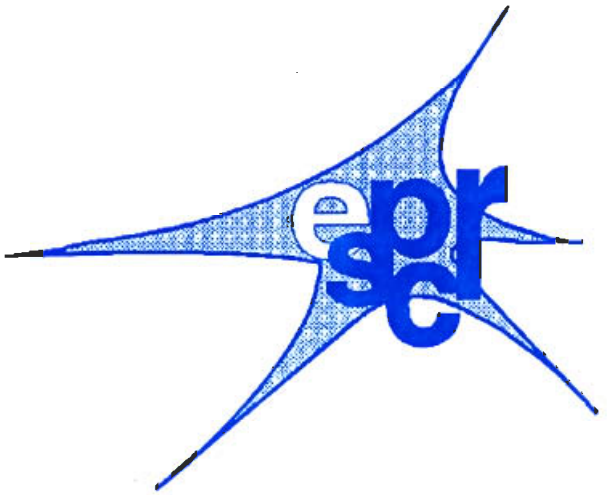

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH BULLETIN

N° 24 - February 1996

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The Golden Melanocyte Award
6th Meeting of the ESPCR (Lausanne, Switzerland) Oct. 1995

Melanin pigmentation plays an essential role in protecting the skin from the damaging effects of ultraviolet radiation (UVR)

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In mammals, there are at least two types of melanin, the red/yellow phaeomelanin and the brown/black eumelanin, both of which are present in human skin (1). Of these two types of melanin, eumelanin has the more significant role in protection against UVR whereas phaeomelanin, because of its greater potential to produce free radicals in response to UVR (2), which are capable of inflicting cell injury, may actually contribute to UV-induced skin damage. Thus, the susceptibility to photocarcinogenesis and possibly the tanning ability may depend not simply on the amount of melanin in the skin but also upon the type of melanin produced in the melanocytes.

In mice and other mammals the relative proportions of phaeomelanin and eumelanin are regulated by melanocyte-stimulating hormone (MSH), which acts via its receptor (MC1R), on melanocytes, to increase the synthesis of eumelanin (3,4) and the product of the agouti locus which antagonises this action (5). In mice, mutations at either the MC1R or agouti genes affect the pattern of melanin synthesis resulting in coat colour changes (6,7).

The purpose of the present study was to determine whether MC1R variants occur in humans as in other mammals and whether they are related to their pigmentation phenotype.

Because of the polygenic nature of human pigmentation, 60 British or Irish Caucasians were selected from individuals showing extremes of hair colour, that is either red or black. Genomic DNA was extracted from blood or mouthwash samples and the coding region of the MC1R gene analysed by nested PCR followed by direct cycle sequencing. This methodology enabled us to determine that the 30 dark-haired individuals that we examined had no variations respect to the sequence previously published for the coding region of the MC1R gene (8). In contrast, 70 % of the red-haired population had at least one substitution in this gene. In total, 9 different heterozygous variations were identified (A64S, F76Y, D84E, V92M, T95M, V97I, A103V, L106Q, D294H). 8 of them were located in a region of 42 aminoacids between the first cytoplasmic loop and the first extracellular loop spanning the second transmembrane domain. This location was very similar to that previously found for the dominant mutations of the MC1R gene in mice (9). On the other hand, the substitution at codon 294 was located in the seventh transmembrane domain. The D294H change was the most common (53 %)

followed by the variation at codon 92 (27 %). However, from the functional point of view, the variations at codons 84, 106 and 294 may be the most important, since these positions are highly conserved in the melanocortin receptor family (8,10,11).

Since we only found variations in the MC1R gene sequence from the red-haired individuals as opposed to those from the dark-haired ones, we examined whether these substitutions were related to a specific shade of red hair. To do this, we subclassified the red-haired population according to their shades of red hair as light-red, deep-red and auburn or brownish-red. The variation at codon 294 was found in all three groups, the substitution at codon 92 occurred in light-red and deep-red haired individuals, while all the other changes were seen either in light-red or deep-red haired ones. Moreover, 8 of these individuals with light-red or deep-red hair had a combination of two, three or even four substitutions in their MC1R gene (D294H & T95M; D294H & V97I; V92M & L106Q; two with D294H & V92M; D294H, V92M & A64S; D294H, V92M & D84E; D294H, V92M, A64S & D84E). The cloning of their PCR products and the subsequent sequencing of several clones showed that only one had the variations at the same allele of the gene (D294H & V97I) while the remaining 7 were compound heterozygous. These results pointed to a complex relationship between the red hair phenotype and the MC1R genotype. On the other hand, these data did not discount the possibility that the MC1R variants were present in individuals with intermediate hair phenotype between the extremes red and black. We therefore analysed a new group of 75 volunteers with different hair colours ranging from one extreme to the other. The results obtained from these 75 individuals were pooled whith those the 60 previously examined. 82 % of light-red/deep-red haired persons had changes in one or both alleles, compared with 22 % auburns, 33 % of fair or blondes and less than 20 % of the brown or blacks. The ocurrence of some subtitutions (only at codons 92, 103 or 294) in some individuals with intermediate hair phenotype and even with black hair indicated that the MC1R variants were not exclusively associated with red hair. It was possible however that they were related to the poor tanning ability which characterises the red-haired population. To examine this possibility, we classified the individuals' skin type by using the Fitzpatrick classification and found the highest frequency of any MC1R variation in individuals with skin type I (76.5 %), followed by those with skin type II (46.5 %). No individuals with skin type IV and only 5 % with skin type III had changes. In addition, only persons with skin type I or II, who also had light-red or deep-red hair had more than one substitution or substitutions at both alleles of the gene.

Our findings suggest that in humans as in other mammals, MC1R may be a control point in the regulation of pigmentation phenotype, and more importantly, that variations in this protein are associated with a poor tanning in humans. Functional and mapping studies are now in progress to determine whether MC1R may be used in the future as a marker for the study of human population genetics and to clarify the wide variations in pigmentation and the susceptibility to skin cancer in humans.

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The Silver Melanocyte Award
6th Meeting of the ESPCR (Lausanne, Switzerland) Oct. 1995

Expression of apoptosis related antigens in cultured melanocytes.

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During the last six years the investigators at AMC-UvA concentrated their studies on the role of melanocytes (MC) within the Skin Immune System (SIS), particularly in relation to the pathology of vitiligo. From *in vitro* investigations it has become clear that MC can have both an immune effector function as well as can become a target of the immune system (1). *In situ* immunohistochemical analysis of lesional, peri-lesional and non-lesional vitiligo skin showed the presence of interacting cellular infiltrates composed of T-cells and macrophages. Particularly in peri-lesional skin CD68 positive macrophages seem to constitute the major part of these infiltrates. These *in situ* data indicate that the interaction between MC, T-cells and macrophages is important in destruction of MC as seen in vitiligo. We hypothesize that immune mediated destruction of MC plays an important role at least in some of the vitiligo patients (2).

Numerous studies on the biology of MC indicate that toxic intermediates are formed during the process of melanin synthesis (3). Necrotic spill over of these substances by dying MC might damage the surrounding cells. Since tissue damage is scarcely observed in hypopigmentation we hypothesize that MC in vitiligo are induced to die by apoptosis. In this form of cell death the cell itself actively participates by activating a suicide program. This process finally leads to the formation of morphologically distinct apoptotic bodies that can be cleared by phagocytic cells like the infiltrating macrophages found in the peri-lesional vitiligo skin (4).

In order to substantiate such hypothesis an essential pre-requisite is to demonstrate that MC are able to undergo apoptosis. With the use of UVB we confirmed that MC *in vitro* are vulnerable to an apoptotic process. After UVB irradiation cells were incubated with the DNA binding dyes "Propidium Iodide" and "Hoechst 33342" and examined by epifluorescence microscopy, that allows discrimination of healthy, apoptotic and necrotic cells by morphological criteria. The results indicate that at 48 hours after UVB irradiation (40 J/m^2) apoptosis was induced in 30% of MC whereas necrosis occurred in less than 2% of the cells. Free radical species are considered to be one of the major intracellular mediators of apoptosis. Interestingly, antioxidant treatment of vitiligo patients has been reported a suitable therapeutic strategy by several groups (6,7). This suggests that vitiligenous MC either have an intrinsic defect in antioxidant defences or cannot overcome the extracellular oxidative stress as may be executed by the observed immune infiltrates. A possible intrinsic defect and the subsequent excess of intracellular Reactive Oxigen Species (ROS) may lead directly to ROS-mediated damage and apoptosis. Alternatively damage caused by ROS may lead to altered antigenicity of MC, this may render MC a target of the immune system.

The investigations were extended to study possible differences in baseline expression levels of Bcl-2, BAX, p53, p21 and FAS on MC. The Bcl-2 molecule has been suggested to function in an antioxidant pathway and plays an important role in cell resistance to apoptosis. BAX can form heterodimers with Bcl-2 and herewith inhibit its function; high levels of BAX as compared to Bcl-2 will therefore render cells more vulnerable to certain apoptotic stimuli. On the other hand the tumor suppressor p53 directly regulates gene expression of both Bcl-2 and BAX (8). In addition p53 can regulate

cell cycle arrest via p21 (9) and recent publications suggest that the cell membrane expressed FAS molecule can be upregulated by p53. FAS/FAS-ligand interaction is an essential for one of the two pathways by which cytotoxic T-cells can induce apoptosis in target cells (10). To inventory baseline expression levels of the above mentioned molecules melanocytic cell cultures of differentiation stages were grouped as follows: fetal (n=4), neonatal (n=5), normal adult (n=5) and adult naevus (n=5) as well as non-lesional adult vitiligo MC (n=5) and were investigated by FACS.

Donor to donor differences exist for all markers, also within the different groups of cells. Differences in average expression levels between groups are discussed below.

Examining Bcl-2:BAX ratios a trend can be seen in which the ratio is low in both fetal and neonatal cells and equally high in normal adult and non-lesional vitiligo MC. The Bcl-2:BAX ratio in adult naevus cells however resembles that of fetal and neonatal cells. Focussing on the BCL-2 defence system alone these results suggest that adult naevus cells as well as fetal and neonatal cells are more prone to be affected by certain apoptotic stimuly. One has to bear in mind though that although relative BCL-2:BAX expression levels are similar in normal adult and non-lesional vitiligo cells this does not exclude possible functional differences.

p53 expression levels are somewhat lower in fetal and naevus cells as compared to neonatal, non-lesional vitiligo and normal adult MC. This does not reflect the differences found in Bcl-2:BAX ratios as it has been reported that p53 upregulates BAX and downregulates Bcl-2 expression. At present it is not kown wether Bcl-2 and BAX levels can be regulated via other mechanisms.

The range of p21 expression levels is similar in all cultures except for naevus cells which have an increased p21 expression. In view of the relatively low p53 expression by naevus cells this result may be explained by a pathway for p53 independent induction of p21 as has been reported by others (9).

Average FAS expression is slightly upregulated in fetal cells whereas the naevus cells express almost three times as much FAS than cells in other differentiation stages. Therefore these cells are possibly more susceptible to FAS mediated induction of apoptosis by cytotoxic T-cells.

From the above mentioned investigation it can be speculated that fetal and naevus cells and not non-lesional vitiligo MC will be more prone to actually enter apoptosis after a stimulus has been applied. To unravel the possible role of MC apoptosis in hypopigmentation, further studies are in progress.

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The Bronze Melanocyte Award
6th Meeting of the ESPCR (Lausanne, Switzerland) Oct. 1995

Comparison of attachment of human ocular melanocytes and melanoma cells to extracellular matrix (ECM) proteins

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The aim of our study was to investigate whether the intracellular signalling systems used by the uveal melanoma cell differed significantly from those used by the uveal melanocyte in their attachment to ECM proteins. Any differences identified could be used to select a pharmacological approach to affect neoplastic but not normal cell attachment.

First of all we compared the substrate preference of the normal versus the neoplastic cell and found that the melanocyte expressed a clear preference for fibronectin over the other substrates studied (collagen I, collagen III, collagen IV, laminin and plastic). In contrast to this, the melanoma cells showed an equal preference for collagens I,III,IV and fibronectin. We then compared the timecourse of attachment of both cell types to fibronectin, but found that both normal and transformed cells attached similarly showing rapid attachment within 20 - 30 minutes.

In this study, we considered the post receptor events and examined which intracellular signalling systems which were important in mediating the early stages of cell attachment to matrix proteins. All initial studies were conducted using melanocytes in their mitogen rich medium that has one-tenth the normal physiological calcium concentration. Under these conditions we found that by manipulating the cyclic AMP system using dideoxyadenosine and, the protein kinase C system, using forskolin and phorbol 12-myristate 13-acetate and staurosporine, had little or no effects on both cell types.

Similarly, we found that inhibition of the calmodulin system using either the experimental calmodulin antagonist drug J8 or tamoxifen produced a profound inhibition of attachment. Both melanocyte and melanoma cells did not differ in their sensitivity to either drug.
We were surprised to discover that by manipulating the the intracellular calcium system using ionomycin and TMB8 was initially without effects on the attachment of the ocular melanocyte, while they inhibited the ocular melanoma cell attachment. As it may have offered an approach for selective pharmacological intervention we examined this result further.

In these further studies the melanocytes were transferred to medium containing physiological concentrations of calcium for two days prior to experimentation. These experiments revealed that the lack of response of the melanocytes to ionomycin was dependent upon the calcium concentration of the medium as all other additions to the medium were kept constant.

We also confirmed that melanocyte and melanoma cells responded equally well to an acute addition of ionomycin with a rapid increase in intracellular calcium. However, the action of ionomycin is entirely dependent on the concentration of extracellular calcium and therefore our results lead us to conclude that if uveal melanocytes are grown under concentrations of low extracellular calcium then

ionomycin is probably unable to elevate intracellular to a level which will inhibit cell attachment.

To conclude, we found that both normal uveal melanocytes and transformed uveal melanoma cells use the same intracellular signalling systems in their initial stages of attachment to ECM proteins This signalling involves calcium and calmodulin to a large extent, accordingly any drugs which affect calcium or calmodulin activity could reduce the likelihood of the attachment of either cell.

We can also state that the ocular melanocyte and melanoma cells show a requirement for a calcium and calmodulin sensitive intracellular signal within the first few minutes of adhesion suggesting that this is unlikely to be specific to a particular cell or even to any particular adhesion molecule/ECM substrate interaction.

In summary, while we are unable to identify a pharmacological target for intracellular signalling which would allow one to focus on the transformed rather than the normal cell, it is still possible that drugs affecting calcium or inhibiting calmodulin could be used in preventing adhesion of any unattached cells and this could be of value in preventing metastatic spread.

1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)

Oxidation of mono- and diphenolic compounds: The current period of literature surveyed seems to be marked by an ever increasing number of papers on the formation of dopamine-cysteine conjugates (CDA´s) in connection with oxidative stress and drug metabolism in brain. Palumbo et al. (Palumbo *et al.*, 1995) suggest that in the presence of Fe2+-EDTA, hydrogen peroxide and lipoperoxides may play an important role in generation of 5-S-Cys-DA and the minor isomer 2-S-Cys-DA. Fe2+-induced autoxidation is completely suppressed by ascorbic acid. Oxidation of dopamine by prostaglandin H synthase with arachidonic acid and hydrogen peroxide was investgated by (Hastings, 1995). Indomethacin blocks the reaction. Oxidized dopamine covalently binds to proteins as indicated by cysteinyl-dopamine residues. It is concluded that prostaglandin H synthase may have implications for the development of Parkinson's disease. Zang and Dryhurst (Zhang & Dryhurst, 1995) identified novel conjugates of D-penicillamine methyl ester (PME) as 2-S-, 5-S-, and 6-S-adducts with DA. These are further oxidized by unreacted DA-o-quinone to give, among others, 7-(2-aminoethyl)-5-hydroxy-2,2-dimethyl-1,4-benzothiazine-3-carboxylic acid methyl ester. At physiological pH 5-S-CyS-DA is oxidized chemically by a bicyclic DA *o*-quinone imine intermediate to 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2H-1,4-benzothiazine-3-carboxylic acid. This putative dihydrobenzothiazine endotoxin - which is lethal when administered into the brains of mice - is even more easily oxidized than 5-S-CyS-DA in a rather complex reaction that ultimately forms 7-(2-aminoethyl)-5-hydroxy-1,4-benzothiazine-3-carboxylic acid which might serve as a better analytical marker molecule than 5-S-Cys-DA for either elevated rates of DA autoxidation and/or for roles of GSH and CySH in the neurodegenerative mechanisms in the SN that contribute to PD. Though not related to melaniun chemistry directly, a study of Miller (Miller, Lau & Monks, 1995) should be mentioned who propose that some of the acute effects of 3,4-(+/-)-(methylenedioxy)-amphetamine and 3,4-(+/-)-(methylenedioxy)methamphetamine may be a consequence of the initial high concentrations of 5-S-Cys-α-methyldopamine, followed by the accumulation and persistence of 5-S-(N-acetyl-Cys)-α-MeDA, which contributes to the long-term neurotoxicity.

The toxicity dihydroxyphenylacetaldehyde (DOPAL) to dopaminergic neurons of was investigated with resepect to selective inhibition of dopamine uptake and membrane damage using rat neostriatal synaptosomes (Mattammal *et al.*, 1995).
Much progess has been achieved in the chemistry of serotonin oxidation which is possibly relevant in Alzheimer´s disease, especially by some very informative papers from the Dryhurst school. The Fenton system trensforms 5-HT rapidly to a mixture of 2,5- (major product), 4,5-, and 5,6-dihydroxytryptamine (DHT) (Wrona *et al.*, 1995). Many other secondary reaction products of 5-HT oxidation were identified. 5,6-DHT is formed in the brains of rats following a large dose of methamphetamine (MA) suggests that this drug might evoke HO radical formation and damage to serotoninergic neurons.Electrochemical and peroxidase-mediated oxidations of the dimer of 5-hydroxytryptophan, 5-[(3-(2-amino-2-carboxyethyl)-5-hydroxy-1H-indol-4-yl)oxy)-(3-(2-amino-2-carboxyethyl))-1H-indole leads under weakly oxidizing conditions to an equimolar mixture of 5-HTPP and tryptophan-4,5-dione (Wu, Shen & Dryhurst, 1995). Under more strongly oxidizing conditions oxidation of 5-HTPP reacts with the free hydroxyl residue of the dimer to form oligomers. Phamocological results concerning effects of the dimer on various neurotransmitters are also reported in that paper. 4-S-Cysteinyl-5-hydroxy-tryptamine (4-S-Cys-5-HT) is more easily electro-oxidized than 5-HT and, in the presence of free Cys-SH, undergoes a complex series of reactions leading to 8-(2-aminoethyl)-1,2,3,5,6,9-hexahydro-5,9-dioxo-pyrrolo[3, 2-g] [1, 4] benzothiazine-2-carboxylic acid and N-[7-[(2-amino-2-carboxyethyl) thio]-3-(2-aminoethyl)-1,4-dihydro-4-oxo-5H-indol-5-ylidene]-L-cysteine (Wrona, Singh & Dryhurst, 1994). Cys-SH also reacts with tryptamine-4,5-dione to give the same products. 5-HT is oxidized electrochemically or by tyrosinase/O_2 to give 5,5'-dihydroxy-4,4'-bitryptamine along with a number of other oligomers, or with water to give tryptamine-4,5-dione (Wrona, Singh & Dryhurst, 1995). In the presence of GSH 4-S-glutathionyl-5-hydroxytryptamine is formed. Glutathionyl conjugates of an indolic trimer and of two tetramers. Compound 1 might be a useful analytical marker molecule for unusual oxidation reactions of 5-HT in the Alzheimer brain.
Products of catechol (L-DOPA, dopamine, or DOPAC) oxidation can covalently crosslink neurofilaments (Montine, Farris & Graham, 1995). The crosslinking mechanism can involve lysine, and copper, iron, and manganese ions accelerate catechol-mediated protein crosslinking.
Papers on Drug Binding: Radwanska et al. determined differences in drug-melanin interactions for 13 phenothiazine neuroleptics and 2 dibenzazepine thymoleptics by HPLC using a stationary phase of immobilized synthetic L-dopa melanin on silica gel or by ultrafiltration (Radwanska *et al.*, 1995). The QSRR equation derived allows for the estimation of melanin binding based on the structure of a compound candidate, and thus rationalizes predictions of potential toxicity of drugs or drug candidates.
Biosynthesis of melanins: Koch and Kaufmann (Koch & Kaufmann, 1995) studied the biosynthesis of melanin related pigments from radiolabeled tyrosine, DOPA, and β-alanine in relation with DOPA decarboxylase activity in wings spots of the butterfly *Precis coenia.* Additional inhibitors of tyrosinase and melanin biosynthesis have been discovered. Among them are hydroxystilbenes and their galloylglycosides (Iida *et al.*, 1995) and extracts of the plant *Myrica rubra* containing, *inter alia,* flavanoids and their glycosides (Matsuda *et al.*, 1995). DHICA and DHI both inhibit tyrosinase (Wilczek &

Mishima, 1995), the latter beeing more effective. Thus, DOPAchrome tautomerase plays a role in positive control of the tyrosinase-catalyzed early phase of melanogenesis.

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2. Biology of pigment cells and pigmentary disorders

(Comments by Dr M. Picardo)

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  Commentary: Dendriticy of melanocytes probably plays a crucial role in pigmentation since a single melanocytes supply melanin to several keratinocytes and UV radiation promotes melanocyte dendriticy. The control of melanocyte dendriticy in skin is poorly known. Factors produced by basement membrane, such as laminin, collagen type IV are able to increase melanocyte dendriticy. Keratinocytes appear to be a major source of exogenous signals for inducing melanocytes dendriticy inflammatory cytokines, α-MSH, NGF and PGE2 have been reported to increase melanocyte dendriticy at least in vitro. Endothelin 1, another factor secreted also by keratinocytes, induces melanocyte proliferation and melanin synthesis. Cultured melanocytes, which express high affinity receptor for ET-1, exposed to ET-1 increase in their dendriticy. Moreover, medium from UV-irradiated keratinocytes which contains 25-fold more ET-1 than medium form non-irradiated cells, enhances melanocytes dendrites and the phenomenon was inhibited by anti ET-1 antibodies. The results of this paper provide supports for the concept of melanocyte-keratinocyte unit, demonstrating that melanocyte function in the skin is regulated, at least in part, by keratinocytes factors and functions.

- Hirobe T.
  Structure and function of melanocytes: microscopic morphology and cell biology of mouse melanocytes in the epidermis and hair follicle. Histol Histopathol. 10(1):223-37, 1995.

- Jimbow M, Marusyk H, Jimbow K.
  The in vivo melanocytotoxicity and depigmenting potency of N-2,4-acetoxyphenyl thioethyl acetamide in the skin and hair. Br J Dermatol. 133:526-536, 1995.
  Commentary: The possibility to use alternative substrate of tyrosinase to reach a selective melanocytoxicity and a specific depigmenting effect or a is one of the goals of the studies on pigmentation. Different papers have been published on the activity of alternative substrates of tyrosinase in melanocyte and melanoma cultures and in particularly on hydroquinone. The specificity of the action of these compounds has been discussed by some groups and a possible mechanism of toxicity throw the generation of reactive oxygen species during spontaneous autoxidation rather than the tyrosinase-mediated oxidation has been reported by different groups. Now Bologna et al. report that toxicity of hydroquinone is increased by the contemporaneous administration of buthionine sulfoxime, an agent which reduces the intracellular concentration of glutathione and suggest that the combination of the drugs could be used in the treatment of hyperpigmentary disorders.
  Jimbow and co-workers report the depigmentary effect and melanocytotoxicity of the acetyl derivative of N-acetyl-4-S-cysteaminylphenol, a substance previously described as specifically melanocytotoxic. The esterfication of the substance may increase the skin penetration and cellular uptake of the substance. The presence in the skin and body fluid of significant amounts of deacylating enzymes can account for the O-deacytilation of NAP-TEA in N-Ac-4-S-CAP which exerts the cytostatic effect. The experimental data reported support the view that the toxic effect is mediated by tyrosinase oxidation of the deacytilated compound.

- Kairiyama C, Slavutsky I, Larripa I, Morvillo V, Bravo AL, Bover L, Podhajcer OL, Mordoh J.
  Biologic, immunocytochemical, and cytogenetic characterization of two new human melanoma cell lines -IIB-MEL-LES and IIB-MEL-IAN. Pigment Cell Research. 8(3):121-131, 1995.

- Krasagakis K, Garbe C, Schrier PI, Orfanos CE.
  Paracrine and autocrine regulation of human melanocyte and melanoma cell growth by transforming growth factor beta in vitro. Anticancer Res. 14(6B):2565-71, 1994.

- Mortarini R, Gismondi A, Maggioni A, Santoni A, Herlyn M, Anichini A.
  Mitogenic activity of laminin on human melanoma and melanocytes - Different signal requirements and role of beta-1 integrins. Cancer Research. 55(20):4702-4710, 1995.

- Okura M, Maeda H, Nishikawa S, Mizoguchi M.
  Effects of monoclonal anti-c-kit antibody (ACK2) on melanocytes in newborn mice. J Invest Dermatol. 105(3):322-8, 1995.

- Thody AJ.
Epidermal melanocytes - their regulation and role in skin pigmentation. European Journal of Dermatology. 5(7):558-565, 1995.

- Yamanishi DT, Meyskens FL.
Alterations in gene expression and signal transductions in human melanocytes and melanoma cells. Critical Reviews in Oncogenesis. 5(5):429-450, 1994.

Melanocyte cultures
(Comments by Dr N. Smit)

In the paper of *Barker et al* in Cancer Res 55 melanocytes of extreme skin types were used to measure the effects and possible differences in sensitivities of the heavily pigmented melanocytes from dark skin and the lightly pigmented cells from lower skin type for UV-B irradiation. In the accompanying paper by *Medrano et al* the effect of UV-B irradiation on cell cycle control of the same melanocyte cultures are described.

- Barker D, Dixon K, Medrano EE, Smalara D, Im S, Mitchell D, Babcock G, Abdelmalek ZA.
Comparison of the responses of human melanocytes with different melanin contents to ultraviolet B irradiation. Cancer Research 55 (18): 4041-4046, 1995.

- Medrano EE, Im S, Yang F, Abdelmalek ZA.
Ultraviolet B light induces G(1) arrest in human melanocytes by prolonged inhibition of retinoblastoma protein phosphorylation associated with long-term expression of the p21(Waf-1/SDI-1/Cip-1) protein. Cancer Research 55 (18): 4047-4052, 1995.

- Piepkorn M, Hoovingh P, Dillberger A, Linker A.
Divergent regulation of proteoglycan and glycosaminoglycan free chain expression in human keratinocytes and melanocytes. In Vitro Cellular & Developmental Biology - Animal 31 (7): 536-541, 1995.

- Scott G, Liang H.
pp125(FAK) in human melanocytes and melanoma: Expression and phosphorylation. Experimental Cell Research 219 (1): 197-203, 1995.

3. MSH, MCH, other hormones, differentiation

- Airaghi L, Lettino M, Manfredi MG, Lipton JM, Catania A.
Endogenous cytokine antagonists during myocardial ischemia and thrombolytic therapy. Am Heart J. 130(2):204-11, 1995.

- Balm PH, Hovens ML, Wendelaar Bonga SE.
Endorphin and MSH in concert form the corticotropic principle released by tilapia (Oreochromis mossambicus; Teleostei) melanotropes. Peptides. 16(3):463-9, 1995.
Abstract: HPLC characterization of tilapia pituitary endorphins using an antibody specific for N-terminally acetylated endorphins yielded three major peaks in the neurointermediate lobe, but none in the pars distalis. The melanotropes secreted two of the immunoreactive products in vitro, one of which coeluted with Xenopus laevis N-ac-beta-END(1-8). This immunoreactive fraction also coeluted with diacetyl-alpha-MSH. Evidence is presented that the noteworthy corticotropic potency of this HPLC fraction, previously attributed to diacetyl-alpha-MSH, results from END and MSH acting in a coordinated fashion. Confinement stress had no effect on plasma N-ac-beta-END immunoreactivity, but led to a decrease in plasma alpha-MSH levels. Therefore, it seems unlikely that the corticotropic action of the peptides regulates the elevation of cortisol production that takes place during confinement, but it may play a role during other forms of stress that are known to activate the melanotropes.

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Agouti antagonism of melanocortin binding and action in the B16F10 murine melanoma cell line. Biochemistry. 34(33):10406-11, 1995.

- Cammas FM, Kapas S, Barker S, Clark AJ.
Cloning, characterization and expression of a functional mouse ACTH receptor. Biochem Biophys Res Commun. 212(3):912-8, 1995.

- Durando P, Celis ME.
Possible mechanism by which alpha-melanotropin advances the time of vaginal opening. Acta Physiol Pharmacol Ther Latinoam. 44(3):85-93, 1994.

- Dyer JK, Philipsen HL, Tonnaer JA, Hermkens PH, Haynes LW.
Melanocortin analogue Org2766 binds to rat Schwann cells, upregulates NGF low-affinity receptor p75, and releases neurotrophic activity. Peptides. 16(3):515-22, 1995.

- Hruby VJ, Lu D, Sharma SD, Castrucci AL, Kesterson RA, al Obeidi FA, Hadley ME, Cone RD.
Cyclic lactam alpha-melanotropin analogues of Ac-Nle4-cyclo[Asp5, D-Phe7,Lys10] alpha-melanocyte-stimulating hormone-(4-10)-NH2 with bulky aromatic amino acids at position 7 show high antagonist potency and selectivity at specific melanocortin receptors. J Med Chem. 38(18):3454-61, 1995.

- Nishii M, Moverus B, Bukovskaya OS, Takahashi A, Kawauchi H.
Isolation and characterization of [Pro2]somatostatin-14 and melanotropins from Russian sturgeon, Acipenser gueldenstaedti Brandt. Gen Comp Endocrinol. 99(1):6-12, 1995.
Abstract: A new form of somatostatin (SRIH), along with melanotropins (MSHs), was isolated from pituitaries of the Russian sturgeon Acipenser gueldenstaedti Brandt by gel filtration, ion exchange, and reversed-phase HPLC following acid-acetone extraction. The sturgeon SRIH consists of 14 amino acid residues and differs from mammalian SRIH-14 by the substitution Pro for Gly at position 2. Synthetic [Pro2]SRIH-14 was as potent as mammalian SRIH-14 in inhibiting release of growth hormone into medium from the organ-cultured pituitary of rainbow trout. Sturgeon alpha-MSH has the same amino acid sequence as those found in mammals. Sturgeon beta-MSH is composed of 17 amino acid residues, and its amino acid sequence is identical to the N-terminal 15 residues of salmon beta-MSH I and to the C-terminal 2 residues of mammalian beta-MSH.

- Star RA, Rajora N, Huang J, Stock RC, Catania A, Lipton JM.
Evidence of autocrine modulation of macrophage nitric oxide synthase by alpha-melanocyte-stimulating hormone. Proc Natl Acad Sci U-S-A. 92(17):8016-20, 1995.
Abstract: alpha-Melanocyte-stimulating hormone (alpha-MSH) is a potent inhibitory agent in all major forms of inflammation. To identify a potential mechanism of antiinflammatory action of alpha-MSH, we tested its effects on production of nitric oxide (NO), believed to be a mediator common to all forms of inflammation. We measured NO and alpha-MSH production in RAW 264.7 cultured murine macrophages stimulated with bacterial lipopolysaccharide and interferon gamma. alpha-MSH inhibited production of NO, as estimated from nitrite production and nitration of endogenous macrophage proteins. This occurred through inhibition of production of NO synthase II protein; steady-state NO synthase II mRNA abundance was also reduced. alpha-MSH increased cAMP accumulation in RAW cells, characteristic of alpha-MSH receptors in other cell types. RAW cells also expressed mRNA for the primary alpha-MSH receptor (melanocortin 1). mRNA for proopiomelanocortin, the precursor molecular of alpha-MSH, was expressed in RAW cells, and tumor necrosis factor alpha increased production and release of alpha-MSH. These results suggest that the proinflammatory cytokine tumor necrosis factor alpha can induce macrophages to increase production of alpha-MSH, which then becomes available to act upon melanocortin receptors on the same cells. Such stimulation of melanocortin receptors could modulate inflammation by inhibiting the production of NO. The results suggest that alpha-MSH is an autocrine factor in macrophages which modulates inflammation by counteracting the effects of proinflammatory cytokines.

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Melatonin antagonizes alpha-melanocyte-stimulating hormone enhancement of melanogenesis in mouse melanoma cells by blocking the hormone-induced accumulation of the c locus tyrosinase. Eur J Biochem. 232(1):257-63, 1995.

- van Strien FJ, Galas L, Jenks BG, Roubos EW.
Differential acetylation of pro-opiomelanocortin-derived peptides in the pituitary gland of Xenopus laevis in relation to background adaptation. J Endocrinol. 146(1):159-67, 1995.

- Willard DH, Bodnar W, Harris C, Kiefer L, Nichols JS, Blanchard S, Hoffman C, Moyer M, Burkhart W, Weiel J, et al.
Agouti structure and function: characterization of a potent alpha-melanocyte stimulating hormone receptor antagonist. Biochemistry. 34(38):12341-6, 1995.
Abstract: The murine agouti gene encodes for a novel 131 amino acid protein. The sequence includes a 22 residue putative secretion signal, an internal basic region, and a C-terminal domain containing 10 cysteines. Agouti has been found to antagonize the binding of certain pro-opiomelanocortin peptides, such as alpha-melanocyte stimulating hormone (alpha-MSH), to the murine melanocortin-1 receptor (MC1-R). We report the purification of a secreted murine agouti to homogeneity by a two-step procedure from baculovirus-infected Trichoplusia ni (T. ni). The protein is glycosylated and exhibits competitive, high-affinity antagonism (Ki = 0.8 nM) versus alpha-MSH in cell-based assays employing B16F10 cells. Association state analysis by analytical ultracentrifugation reveals that agouti exists in a monomer--dimer plus aggregate equilibrium at low micromolar concentrations. Data from secondary structure studies indicate that the protein is highly stable to thermal denaturation. Enzymatic digestion to probe disulfide bond arrangement yielded a discrete C-terminal (Val 83-Cys 131) domain. The isolated highly cysteine-rich C-terminal domain retains alpha-MSH antagonism equipotent with mature agouti. This bioactive domain contains all 10 cysteines which exhibit sequence homology when aligned with several conotoxins.

4. Photobiology and photochemistry

(Comments by Dr M. d'Ischia)

The biochemical mechanisms underlying the response of melanocytes to UVB irradiation, the role of melanin pigments and related metabolites in skin photoprotection, the validity of sunscreens as a preventive measure against melanoma and other skin cancers, and the sensitizing properties of psoralen derivatives are very active issues in current studies on skin photobiology and show prominently in the present survey of the literature. In a study on cultured human melanocytes from different skin phenotypes, **Barker et al.** provide evidence for a direct correlation between melanin content and resistance to UVB-induced damage, as determined by the capacity to resume proliferation, expression of tumor suppressor p53 protein and extent of formation of pyrimidine dimers in DNA. From these data, the authors propose that a prolonged induction of p53 in poorly pigmented melanocytes arrests these cells in G1, via inhibition of retinoblastoma protein phosphorylation (see **Medrano et al.**) which may account for the increased susceptibility of light skinned individuals to actinic damage. It would be of interest to assess this attractive hypothesis in also in relation to the quality, i.e. the eumelanin/pheomelanin ratio, of the pigments within melanocytes.

The results of an EORTC case control study in some European countries by **Autier et al.** would provide support to the view that sunscreens are unable to prevent development of melanoma. This is a point of great scientific and public concern that needs to be further addressed at multidisciplinary level, especially with regard to the safety of psoralencontaining sunscreen formulations.

Hill et al. describe the effects of a diffusible multitherapy factor (MTRF) produced by Cloudman S91 melanoma cells in vitro on the relative sensitivity of S91/amel cells to five different genotoxic factors, in comparison to near UV irradiation. The results would indicate a significant effect of MTRF after exposure of the target cells to all genotoxic agents, with the sole exception of UV irradiation, suggesting that different mechanisms are operative in the latter case. The definition of such mechanisms at the biochemical level would be an important focus for further studies, as it would open new perspectives for the treatment of tumor cells.

- Autier P, Dore JF, Schifflers E, Cesarini JP, Bollaerts A, Koelmel KF, Gefeller O, Liabeuf A, Lejeune F, Lienard D, et al.
  Melanoma and use of sunscreens: an Eortc case-control study in Germany, Belgium and France. The EORTC Melanoma Cooperative Group. Int J Cancer. 61(6):749-55, 1995.

- Barker D, Dixon K, Medrano EE, Smalara D, Im S, Mitchell D, Babcock G, Abdel-Malek ZA.
  Comparison of the responses of human melanocytes with different melanin contents to ultraviolet B irradiation. Cancer Res. 55(18):4041-6, 1995.

- Gonzalez VH, Hu LK, Theodossiadis PG, Flotte TJ, Gragoudas ES, Young LH.
  Photodynamic therapy of pigmented choroidal melanomas. Invest Ophthalmol Vis Sci. 36(5):871-8, 1995.

- Haylett AK, Ross S, Truscott TG, Moore JV.
  Pharmacokinetic and therapeutic outcome in melanoma cells, of the administration of symmetric and asymmetric cationic photosensitizers. Cancer Lett. 88(2):191-9, 1995.

- Hill HZ, Hill GJ, Cieszka K, Azure M, Chowdhary I, Sayre RM.
  A multitherapy resistance factor from melanoma reveals that killing by near UV is different from genotoxic agents. Photochem Photobiol. 61(5):479-83, 1995.

- Jimbow K.
  Current update and trends in melanin pigmentation and melanin biology. Keio J Med. 44(1):9-18, 1995.

- Joshi PC, Pathak MA.
  Photophysical and photobiological properties of 3-carbethoxypsoralen. Indian J Biochem Biophys. 32(2):63-73, 1995.

- Medrano EE, Im S, Yang F, Abdel-Malek ZA.
  Ultraviolet B light induces G1 arrest in human melanocytes by prolonged inhibition of retinoblastoma protein phosphorylation associated with long-term expression of the p21Waf-1/SDI-1/Cip-1 protein. Cancer Res. 55(18):4047-52, 1995.

- Pollock PM, Yu F, Qiu L, Parsons PG, Hayward NK.
  Evidence for u.v. induction of CDKN2 mutations in melanoma cell lines. Oncogene. 11(4):663-8, 1995.

- Duval C, Poelman MC.
  Scavenger effect of vitamin E and derivatives on free radicals generated by photoirradiated pheomelanin. J Pharm Sci. 84(1):107-10, 1995.

(Comments by Dr M. Picardo)

- Allan AE, Archambault M, Messana E, Gilchrest BA.
  Topically applied diacylglicerols increase pigmentation in guinea pig skin. J Invest Dermatol. 105(5):687-692, 1995.
  Commentary: In this paper the group of Barbara Gilchrest reports that topical application of diacylglycerol on the skin of guinea pigs induces iperpigmentation possibly throw the activation of protein kinase C. Prof. Gilchrest has presented some of these results during the ESPCR meeting in Vienna. The histological analyses have shown that the skin modification induced by diacylglycerol were comparable to those induced by direct UVB irradiation. The results are interesting for the contributes in understanding the mechanisms which control iperpigmentaion and could suggest a clinical use of these non toxic compounds for inducing tanning. By the experiments performed is not completely defined if the iperpigmentaion induced by diacylglycerol could be related to the direct activation of protein kinase C of melanocytes or if the phenomenon could be mediated by the secretion of paracrine factors form keratinocytes stimulated by DAG.

- Bessou S, Surlève-Bazeille J, Sorbier E, Taïeb A.
  Ex vivo reconstruction of the epidermis with melanocytes and the influence of UVB. Pigment Cell Res. 8:241-249, 1995.
  Commentary: The authors present an interesting model for reconstituted epidermis using keratinocytes and melanocytes culture seeded on de-epidermized dermis. Histological, immunohistochemical and electronmicroscopic examinations have shown an excellent level of differentiation in the reconstructed epidermis. UVB irradiations have induced iper-pigmentation and increased melanocyte number indicating that the system may be an useful moel for studies on skin pigmentation ex vivo.

- Bestak R, Barneston R, Nearm MR, Halliday GM.
  Sunscreen protection of contact hypersensitivity response from chronic solar stimulated ultraviolet irradiation correlated with the absorption spectrum of the sunscreen. J. Invest. Dermatol. 105(3):345-351, 1995.
  Commentary: Local or systemic immunosuppression is one of the effects of UV irradiation of the skin and the protective effect of commercial sunscreens has been questioned. Differences in the experimental protocols used are probably the cause of the conflicting results obtained. In their paper, Roberts and Beasley demonstrated that the sunscreens prevent UV-induced immune suppression after an acute dose of UV that simulates sunlight, and Bestak et al. have shown that some chemical (ethylhexyl p-methoxycinnamate) and physical (titanium dioxide) sunscreens are able to reduce immunesuppression induced by chronic UV exposure. The application of other sunscreens (Padimate O) was associated with more immunesuppression. The studies indicate that a mixture of UVA and UVB sunscreens may provide better protection than a single agent. The definition of the mechanisms of UV-mediated damage and the activity of sunscreens are certainly important steps for practical application of the photobiology studies.

- de Leeuw SM, Simons JW, Vermeer BJ, Schothorst AA.
  Comparison of melanocytes and keratinocytes in ultraviolet-induced DNA damage per minimum erythema dose sunlight: applicability of ultraviolet action spectra for risk estimates. J Invest Dermatol. 105(2):259-63, 1995.

- Roberts LK, Beasley DG.
  Commercial sunscreen lotions prevent ultraviolet-radiation-induced immune suppression of contact hypersensitivity. J. Invest. Dermatol. 105(3):339-344, 1995.

- Schallreuter KU, Wood JM, Lemke KR, Levening C.
  Treatment of vitiligo with a topical application of pseudocatalase and calcium in combination with short term UVB exposure: a case study on 33 patients. Dermatology. 190:223-229, 1995.
  Commentary: The group of Schallreuter and Wood have proposed in several papers a new pathogenetic hypothesis of vitiligo demonstrating that an alteration of biopterin metabolism with a subsequent intracellular hydrogen peroxide accumulation and decrease of catalase activity exists in the skin of vitiligo patients. Their results support the view that an oxidative stress may be the cause of melanocyte damage in vitiligo. Consequently, the authors performed a therapy in 33 patients affected from active vitiligo with topical applications of a vehicle containing a low molecular weight pseudo-catalase and calcium twice daily on the total body from 4 to 27 months. Suberythemagenic UVB exposures twice weekly were also performed. The authors report the arrest of depigmentation in all patients and in 90% the appearance of repigmentaion. This therapeutic approach is very interesting both for the effectiveness and the absence of side effects. Long term results after the suspension of the treatment are waiting.

5. Neuromelanins

(Comments by Dr M. d'Ischia)

Current studies of Substantia nigra neuromelanin place considerable emphasis to the ironchelating properties of the pigment and the possible biological consequences under conditions of oxidative stress. By means of 57Fe Mossbauer spectroscopy Gerlach et al. provide evidence suggesting that the iron binding sites in purified human neuromelanin are similar to those of human hemosiderin (ferritin). These and other data that are reported make a serious point about the risk of overlooking

the role of the attached protein matrix in studies of the biological properties of neuromelanin.
In another study, **Mareba et al.** use a combination of ESR spin trapping techniques and the salicylate hydroxylation assay to monitor the effect of synthetic dopamine melanin, as a model of neuromelanin, on the formation of hydroxyl radicals by the Fenton system.
The results are consistent with the ability of the pigment to act both as an iron sequestering agent, thereby exerting a protective effect, and as an enhancer of hydroxyl radical production by redox activation of the ions. However, in considering the possible implications of these data for the etiology of Parkinson's disease, attention should be paid to the actual relevance of the model pigment chosen, synthetic dopamine melanin, to human neuromelanin. This is a most critical issue in the light of the possible differences between natural and synthetic melanins in terms of basic structural units, redox properties and stability to autoxidation.

- Gerlach M, Trautwein AX, Zecca L, Youdim MB, Riederer P.
  Mossbauer spectroscopic studies of purified human neuromelanin isolated from the substantia nigra. J Neurochem. 65(2):923-6, 1995.

- Zareba M, Bober A, Korytowski W, Zecca L, Sarna T.
  The effect of a synthetic neuromelanin on yield of free hydroxyl radicals generated in model systems. Biochim Biophys Acta. 1271(2-3):343-8, 1995.

6. Genetics, molecular biology

(Comments by Dr F. Beerman)

Brn-2 (brain-2):
Two recent reports suggest the possible importance of the transcription factor Brn-2 in melanocyte biology and melanoma (Eisen et al., 1995; Angus et al., 1995, see below).
What is Brn-2? Brn-2 belongs to a class of transcription factors, which bind DNA by interacting with the octamer control sequence. Brn-2 also contains the so-called POU domain, an essential conserved protein region. POU domain transcription factors are interesting, because they are expressed in specific cell lineages, and Brn-2 is expressed specifically in the nervous system and the developing cortex, thereby corresponding to the defects in hypothalamus and brain, when Brn-2 is deleted following homologous recombination (Nakai et al., 1995, Genes & Development 9: 3109-3121; Schonemann et al., 1995, Genes & Development 9: 3122-3135).
Brn-2 in melanocytes? Earlier studies had reported on a melanoma-specific octamer-binding protein (Cox et al., 1988, Nucleic Acids Research 16: 11047-11056; Sturm et al., 1994, Pigment Cell Research 7: 235-240). It has now been convincingly shown, that this octamer-binding factor is indeed Brn-2, also named N-Oct3. Eisen et al. (1995) now report that Brn-2 binds to the promoter of the tyrosinase gene thereby preventing activation of the promoter by the Microphthalmia transcription factor. Brn-2 thus represses activity of the tyrosinase (and possibly TRP-1 and -2) promoter. Angus et al. (1995) used antisense RNA of brn-2 to reduce Brn-2 expression in melanoma cells. In their study, reduction of brn-2 led to morphological changes of the cells, including loss of pigmentation and downregulatotion of the Microphtalmia transcription factor. Even though these reported results appear to be somewhat contrasting, they both demonstrate that Brn-2 might be a serious candidate for a transcription factor directly involved in expression of pigmentation genes and melanocyte differentiation.

- Angus J, Thomson F, Murphy K, Baker E, Sutherland GR, Parsons PG, Sturm RA
  The Brn-2 gene regulates the melanocytic phenotype and tumorigenic potential of human melanoma cells. Oncogene. 11(4):691-700, 1995.

- Artuc M, Nurnberg W, Czarnetzki BM, Schadendorf D.
  Characterization of gene regulatory elements for selective gene expression in human melanoma cells. Biochemical Biophys Res Commun. 213(2):699-705, 1995.

- Beermann F, Orlow SJ, Boissy RE, Schmidt A, Boissy YL, Lamoreux ML.
  Misrouting of tyrosinase with a truncated cytoplasmic tail as a result of the murine platinum (c(p)) mutation. Experimental Eye Research. 61(5):599-607, 1995.
  Summary: The c-locus mutation platinum is characterized by severe oculocutaneous albinism despite the presence of substantial amounts of tyrosinase activity. This is explained by a point mutation changing a lysine residue to a termination codon, and leading to truncation of the cytoplamic tail. Tyrosinase therefore bypasses melanosomes and is found at the cell surface.

- Breimer LH, Winder AF, Panayiotidis P, Jay M, Moore A, Jay B.
  A trinucleotide deletion together with a base duplication event at codon 439 in the human tyrosinase gene identifies a mutational hotspot. Clinica Chimica Acta. 243 (1): 35-42, 1995.

- Dunaief JL, Kwun RC, Bhardwaj N, Lopez R, Gouras P, Goff SP.
  Retroviral gene transfer into retinal pigment epithelial cells followed by transplantation into rat retina. Human

Gene Therapy. 6(9):1225-1229, 1995.

- Eisen T, Easty DJ, Bennett DC, Goding CR.
The POU domain transcription factor Brn-2 - elevated expression in malignant melanoma and regulation of melanocyte-specific gene expression. Oncogene. 11(10):2157-2164, 1995.

- Englaro W, Rezzonico R, Durandclement M, Lallemand D, Ortonne JP, Ballotti R.
Mitogen-activated protein kinase pathway and AP-1 are activated during cAMP-induced melanogenesis in B-16 melanoma cells. Journal of Biological Chemistry. 270(41):24315-24320, 1995.

- Fukai K, Oh J, Frenk E, Almodovar C, Spritz RA.
Linkage disequilibrium mapping of the gene for Hermansky-Pudlak syndrome to chromosome 10q23.1-q23.3. Human Molecular Genetics. 4(9):1665-1669, 1995.

- Hoon DSB, Wang Y, Dale PS, Conrad AJ, Schmid P, Garrison D, Kuo C, Foshag LJ, Nizze AJ, Morton DL.
Detection of occult melanoma cells in blood with a multiple-marker polymerase chain reaction assay. Journal of Clinical Oncology. 13(8):2109-2116, 1995.

- Imokawa G, Miyagishi M, Yada Y.
Endothelin-1 as a new melanogen - coordinated expression of its gene and the tyrosinase gene in UVB-exposed human epidermis. Journal of Investigative Dermatology. 105(1):32-37, 1995.

- Kagore F, Lund PM.
Oculocutaneous albinism among schoolchildren in Harare, Zimbabwe. Journal of Medical Genetics. 32(11):859-861, 1995.

- Klungland H, Vage DI, Gomezraya L, Adalsteinsson S, Lien S. **The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination.** Mammalian Genome. 6(9):636-639, 1995.

- Koga A, Inagaki H, Bessho Y, Hori H.
Insertion of a novel transposable element in the tyrosinase gene is responsible for an albino mutation in the medaka fish, Oryzias latipes. Molecular & General Genetics. 249(4):400-405, 1995.

- Kucera GT, Bortner DM, Rosenberg MP.
Overexpression of an Agouti cDNA in the skin of transgenic mice recapitulates dominant coat phenotypes of spontaneous mutants. Developmental Biology. 173: 162-173, 1996.
Summary: Agouti cDNA was overexpressed in transgenic mice using keratin 14 promoter or the TRP-1 promoter. K14 promoter directed high constitutive expression of *Agouti* mRNA in skin, with coat colors as in *Agouti* alleles. Transgenic mice were not different from controls with regard to obesity and hypoglycemia, indicating that *Agouti* product does not act as endocrine factor. In addition, *Agouti* transgenes can be used as dominant phenotypic markers of coinjected transgenes, as has been shown for tyrosinase (see Methot et al., 1995, below).

- Lecoin L, Lahav R, Martin FH, Teillet MA, LeDouarin NM.
Steel and c-kit in the development of avian melanocytes - a study of normally pigmented birds and of the hyperpigmented mutant silky fowl. Developmental Dynamics. 203(1):106-118, 1995.

- Methot D, Reudelhuber TL, Silversides DW.
Evaluation of tyrosinase minigene co-injection as a marker for genetic manipulations in transgenic mice. Nucleic Acids Research. 23(22):4551-4556, 1995.
Commentary: This report evaluated the use of tyrosinase minigenes as visual marker when coinjected during production of transgenic mice on an albino (c) background, as had been reported earlier (Beermann et al., 1991, Nucleic Acids Research 19: 958; Overbeek et al., 1991, Transgenic Research 1: 31-37). Cosegregation of the tyrosinase minigene and the transgene of interest occurred in more than 90 % of double transgenic mice. The degree of pigmentation seems to be correlated to the degree of expression of the expressed transgene of interest, presumably by indicating integration into an "open" chromatin domain thus favouring expression.

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Transcriptional activation of the melanocyte-specific genes by the human homolog of the mouse microphthalmia protein. Journal of Biochemistry. 118(5):874-881, 1995.

7. Tyrosinase, TRP1, TRP2 and other enzymes

(Comments by Prof. J.C. Garcia-Borron)

Donatien and Orlow (Eur. J. Biochem, 1995, 232:159-164) present an interesting discussion concerning the interaction of melanosomal proteins with the melanized-matrix of the organelle. Their results can provide an explanation for previous observations on the differential solubilization of the TRPs. Moreover, they provide a convenient method to carry out a preliminary screening aiming to discriminate between melanosomal proteins interacting with the matrix. Their results, therefore, constitute an attractive and straight forward biochemical approach for the characterization of the melanosomal architecture.

The controversy on the catalytic potential of TRP-1 is still obviously unsettled. Although the most commonly accepted hypothesis is that TRP-1 acts as a DHICA-oxidase (see, for example, Kobayashi et al. J Cell Sci. 1995, 108:2301-2309), some experimental data suggest a dopachrome tautomerase function (Winder et al. Biochim Biophys Acta. 1995, 1268:300-310). Since this is a major issue in the biochemistry of melanogenesis, a vis-à-vis comparison of the catalytic activities of

the purified proteins is clearly to be considered. This is not an easy task, owing to the difficulties in purifying to homogeneity relatively large amounts of each enzyme, and may call for the collaboration between several laboratories.

Wilczek and Mishima (Pigment Cell Res. 1995, 8: 105-112) present some interesting data on the role of the DHICA/DHI ratio in the central of melanogenesis, as related to the different ability of these indoles to inhibit the rate-limiting enzyme of the pathway, i.e. tyrosinase. The ratio DHICA-DHI was previously reported by Solano and coworkers to be important for the incorporation of DHICA into melanin. Since this ratio reflects the balance between the catalytic activities of tyrosinase and the two TRPs, these data allow to predict that minor changes in the relative levels of the three enzymes could have comparatively larger effects on the kinetics of the pathway, and, hence, on the composition of the melanin polymer. This situation is further complicated by the possibility of an independent regulation of the enzymatic proteins (see, for example, Vijayasaradhi et al. J Invest Dermatol. 1995, 105:113-119).

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8. Melanoma and other pigmented tumours

Melanoma therapy I
(Comments by Dr N. Smit)

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Melanoma-experimental therapy
(Comments by Dr N. Smit)

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Melanoma cytotoxicity, experimental.
(Comments by Dr N. Smit)

Hudgins et al describe the effects of phenylacetate and analogues. The cytotoxic effects of these compounds on different tumour cell lines including melanoma was correlated with the calculated lipophilicity. In another paper of this group (*L. Liu et al*) the role of TGF-alpha in tumour differentiation induced by phenylacetate or phenylbutyrate is discussed. In our own paper (*Smit and Pavel*) we describe the possibility of using catechols for the induction of cytotoxicity in melanoma cells making use of melanin metabolism. In this respect it is interesting that certain flavonoids such as quercetin and myricetin effect melanin biosynthesis possibly via inhibition of tyrosinase according to *Matsuda et al* but possibly also because they can serve as substrates for catechol-O-methyltransferase. In the paper by *Menon et al* the effects of different flavonoids and related polyphenolic compounds have also been studied in order to find out if these compounds could prevent melanoma metastasis. In another study by *Piantelli et al* it is shown that quercetin and tamoxifen inhibit melanoma cell growth by interacting with the type II estrogen binding sites expressed by three melanoma cell lines. The tyrosinase dependent cytotoxicity of p-alkoxyphenols such as 4-hydroxyanisole towards melanoma cells has been thorougly studied in previous studies. *Potsch et al* now describe that the alkoxyphenols can act via reduction of ribonucleotide reductase (RR) and they show that reduction of the tyrosyl radical of RR is correlated with the length of the alkyl side chain of the alkoxyphenol.

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Effect of trypan blue on the activity of lysosomal enzymes, tumor growth and cell ultrastructure in B16 melanotic melanoma in mice. Neoplasma 42 (4): 173-178, 1995.

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Transcriptional upregulation of TGF-alpha by phenylacetate and phenylbutyrate is associated with differentiation of human melanoma cells. Cytokine 7 (5): 449-456, 1995.
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Studies of cuticle drugs from natural sources. III. Inhibitory effect of *Myrica rubra* on melanin biosynthesis. Biol Pharm Bull 18 (8): 1148-1150, 1995.

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Inhibition of lung metastasis in mice induced by B16F10 melanoma cells by polyphenolic compounds. Cancer Letters 95 (1-2): 221-225, 1995.

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Induction of cytotoxicity in melanoma cells through inhibition of catechol-O-methyltransferase.
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Recognition of multiple epitopes in the human melanoma antigen gp100 by peripheral blood lymphocytes stimulated in vitro with synthetic peptides. Cancer Res 55 (21): 4972-4979, 1995.

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Cytotoxicity effects of transition-metal chelators of the 5-substituted 2-hydroxyacetophenones and their oximes. European Journal of Medicinal Chemistry 30 (7-8): 553-560, 1995.

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Apoptosis and c-jun induction by cisplatin in a human melanoma cell line and a drug-resistant daughter cell line. Anti-Cancer Drug 6 (5): 657-668, 1995.

Melanoma Radiotherapy
(Comments by Dr R. Peter and Dr G. Krähn)

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Ultraviolet B irradiation-induced G2 cell cycle arrest in human keratinocytes by inhibitory phosphorylation of the cdc2 cell cycle kinase. Oncogene. 11:2151-56.
UVB induced cell cycle arrest was investigated. UVB induced G2 arrest in mammalian cells is mediated by inhibitory tyrosine phosphorylation of cdc2 and acts as a defense mechanism against DNA damage irrespective of the cells p53 status. This is an important explanation for cell cycle control after UV-irradiation and also shows that the tumor suppressor status is of no influence in keratinocytes.

- Soulieres D, Rousseau A, Tardif M, Larochelle M, Tremblay M, Vaillancourt L, Pelletier G.
The radiosensitivity of uveal melanoma cells and the cell survival curve. Graefes Arch Clin Exp Ophthalmol. 233(2): 85-9, 1995.
Commentary: Uveal melanoma cells were irradiated with Cobalt-60 and radiosensitivity was measured by soft-agar bilayer assay, tritiated tymidine incorporation and BrdU incorporation. This cell line is according to these results radioresistant.
This is a well established way to determine radiosensitivity and should be applied more often, especially when primary cell cultures of high risk melanoma patients are available.

- Umebayashi Y, Uyeno K, Tsujii H, Otsuka F.
Proton radiotherapy for malignant melanoma of the skin. Dermatology. 190(3):210-3, 1995.
Primary melanomas and lymph node metastases of melanoma were irradiated with proton beam. Macular lesion regressed either completely or showed a regression of up to 80%.
It is certainly of great interest to further develop therapeutic regimens for melanoma especially with proton beams. However, surgical treatment would have resulted in a close to 100% rate of "regression" in primary tumors.
Radiation therapy with proton beam could be a new way of treating melanomas which are not accessible by surgery.

ANNOUNCEMENTS & RELATED ACTIVITIES

1996 **5th Melanoma Workshop**
Hamburg, Germany, 15 - 16 June
Contact: Holger Voigt, M.D.
Melanoma Research Project
31 B Dammtorstrasse
Hamburg D-20354, Germany
FAX: 49 40/3480 525

1996 **EORTC BTDG Meeting**
King's College School of Medicine and Dentistry
London, 16 - 17 October

1996 **XVIth International Pigment Cell Conference**
Anaheim, California, 29 October - 3 November
Contact: MMC/UCI Center for Health Education
PO Box 1428, Long Beach
CA 90801-1428
FAX: 310/933 2012 http://lenti.med.umn.edu/paspcr/ipcc.htm

Note from Prof. Martin G. Peter:
A limited number of travel stipends is available in form of a contribution to the cost of the rail or air ticket for students who wish to attend the meeting but cannot obtain support from institutional or personal funds or other sources. Applications should be sent together with a statement of the supervisor about non-availability of funds within six weeks after publication of this notice to the treasurer of ESPCR, indicating the reason for application and the approximate amount of money requested. The stipend will be paid after the meeting upon submission of the original spent travel documents. Applicants must be members of ESPCR.

1997 **4th World Conference on Melanoma**
Sydney, Australia, 10 - 14 June
Contact: The Melanoma Foundation
PO Box M123
Camperdown
NSW 2050 Australia
FAX: 61 2/550 6316

1997 **VIIth PASPCR Annual Meeting RI**
Providence, 15 - 18 June
Contact: Dr. Walter C Quevedo, Jr.
Brown University, Division of Biology and Medicine
Providence, RI 02912
FAX: 401/863 1971

1997 **International Meeting "Pigmentary Disorders from a Global Perspective"**
Bali, Indonesia, 22 - 24 June
Contact: Bureau PAOG
Tafelbergweg 25
1105 BC Amstersdam
The Netherlands
FAX: 31 20/696 3228

1997 **ESPCR Meeting: Krakow - Dr T. Sarna**
1998 **ESPCR Meeting: Bordeaux - Dr A. Taieb**

Note in the memory of Professor F. Serri

By Prof. Giannetti
(transmitted by Dr M. Picardo)

Professor Ferdinando Serri's lecture on skin embryology was among the most fascinating for students and Dermatology residents, as well as his "pièce de résistance". 'The slide series that he commented upon with more enthusiasm, and which derived from his own research and observations, showed the close connection between the early melanocytes, recognizable among the epithelial cells, and the nervous fibers, "as if the nerves led the melanocytes path from the neural crest to the dermis and the epidermis", in his words.

He used to remind us that his interest in melanocytes and melanin (at the time, the melanins were unknown) went back to his own Dermatology residency, when he heard arguing about these matters his old professor, Giorgio Falchi. The latter was an open minded scholar, very receptive to the new fields that were hardly making their way in Dermatology in the early 1930, often on intuitive knowledge only.

At the end of the second world war, Serri was Assistant Professor in Dermatology at the University of Pavia. Straight after the war, he went to the most prestigious Institutions of the time, where he was to develop his studies on skin embryology: the St. Louis' Hospital, The Istituto Fournier. NY University Skin and Cancer, and the Boston University. From then on, Serri kept up strict relations with the international Dermatological community. We was the scientific secretary at the International Meeting in Venice in 1972, Board Member in the International Ligue from 1968 to 1982, His career went on as appointed Chairman in Dermatology at the University of Sassari from 1962 to 1965, in Pavia from 1965 to 1977 and in Rome from 1977 to 1987.

In Europe, he was among the pioneers in photochemiotherapy of vitiligo. Around that time, he had a chance to meet and develop a long lasting friendship with Professor Giuseppe Prota. Serri knew well the European Dermatological community, and remained always an advocate of the free exchange and frequent meetings between researchers from different countries, in order to further develop the scientific Research in Dermatology. He was a founding father of the ESDR and its first President in 1970.

For the same reasons, he founded and directed the "Fondazione pro Ricerca Dermatologica", through which he supported generously young researchers and scientific and cultural events. Serri was also an advocate of scientific multidisciplinary, and he knew and befriended many scientists, not only physicians and dermatologists, who were interested in cutaneous biology. Ferdinando Serri had a full and happy family life and leaves his wife Marisa and two daughters, Riccarda and Isabella.

His "joie de vivre" and his vigour were well known among his pupils and his many friends, who were astonished at the news of his death, on ....

Addendum

Among these a particular attention was devoted to ESPCR. Professor Serri was a member of the Society and actively participated at all the meetings and supported for many years the Bulletin of the ESPCR providing an useful service to our Society. He was also the Editor of the Bulletin from the foundation up to last year. During these years he has improved the quality of the bulletin.

ESPCR Web Pages

Dear ESPCR member, Dear Colleague,

The President and Council members of the ESPCR decided to diffuse informations about the society throught the growing electronic networks worldwide. The utility of such a service is evident especially in providing freshly updated informations to you, in addition to the possibility of downloading usefull pages including coloured figures and photos. However, some of the informations will only be the privilige of ESPCR members and available through Keywords. The full text bulletin will be one of these.
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Should you have any suggestions of any kind to help improving this service, please feel free to forward it to me on my Fax / E-Mail Address below.

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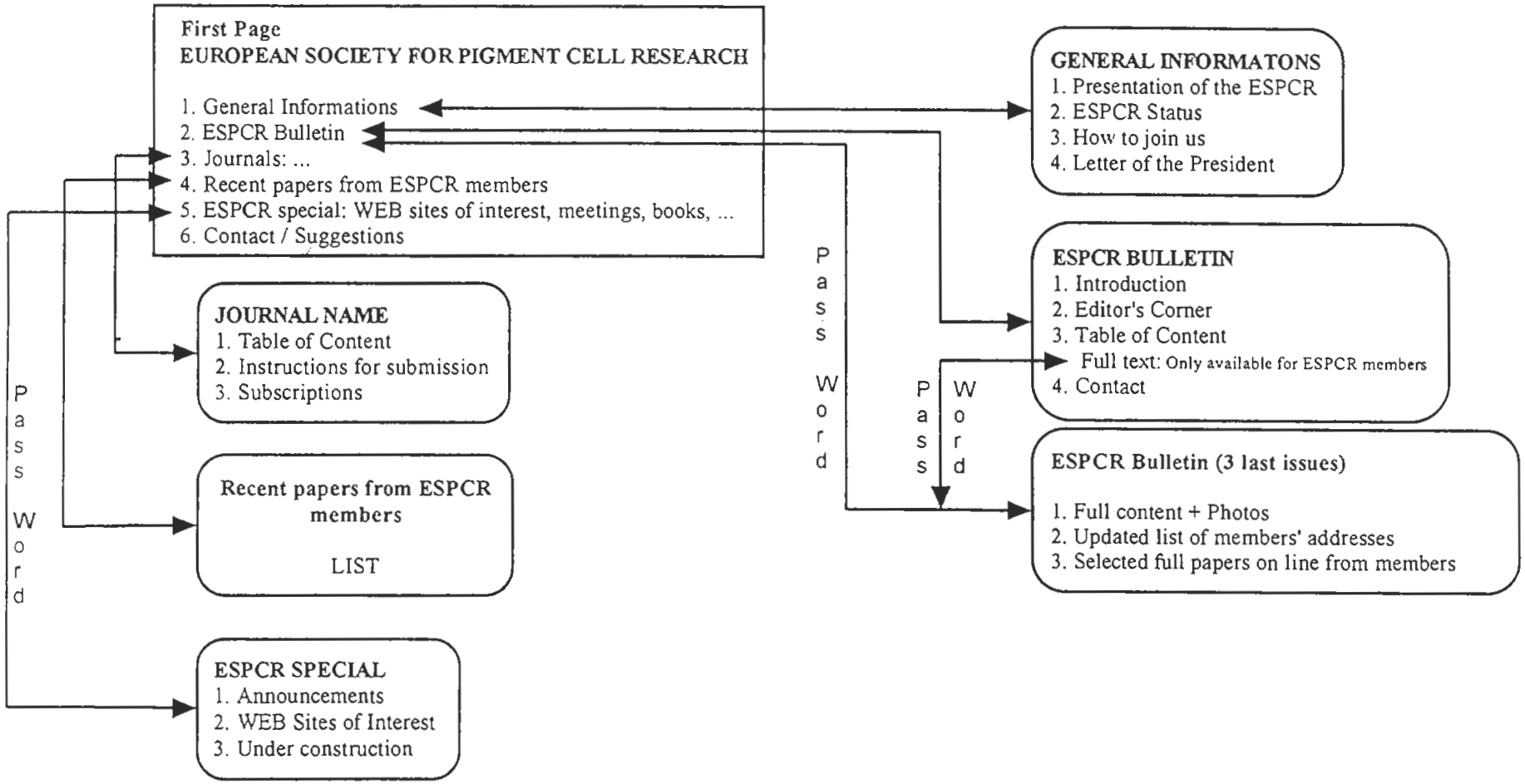
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Meeting Report by Koichiro Kameyama.

10th Annual Meeting of the JSPCR, Osaka, Japan December 9-10, 1995.

This meeting was organized by Osaka University School of Medicine with Prof. Morita as Chairman; it was composed of six special lectures by council members of IFPCS and 25 papers. Special lecture "Modulation of expression and activity of tyrosinase, tyrosinase-related proteins and other melanosomal proteins in murine melanocytes treated with MSH or agouti signal protein" Dr. Vincent J. Hearing discussed the interaction of tyrosinase gene family products and MSH or agouti signal protein (ASP). Recently ASP has been reported to antagonize the ability of MSH using a cloned MSH receptor, and ASP and MSH are key substances of the switching between eumelanogenesis and pheomelanogenesis. To further characterize the switch between eumelanogenesis and pheomelanogenesis, Dr. Hearing studied the responses of cultured melan-a black melanocytes exposed to MSH and/or recombinant ASP. After exposure to ASP, the color of the cell pellets changed from black to light brown, the number of melanosomes decreased, they became pheomelanosome-like at the ultrastructural level, and the amount of total melanin or eumelanin was much decreased, while the amount of pheomelanin was slightly but significantly increased. At the same time the amount of tyrosinase, TRP1 or TRP2 were decreased at mRNA level or protein synthesis level. When MSH was added to the culture, the amount of tyrosinase was increased at transcriptional level or translational level, and total melanin was increased due to increasing eumelanin, while the amount of pheomelanin was not changed significantly. Interestingly, in cells treated with MSH and ASP, the level of tyrosinase, TRP1, TRP2 and melanin production was increased. These data suggest that ASP suppress tyrosinase, TRP1 and TRP2 expression primary at the level of transcription and that the effects of ASP are not mediated solely by inhibition of MSH binding. After his lecture, there was one interesting question which asked the mechanism of the interaction between ASP and MSH. He answered that agouti is a functional antagonist of MSH binding and the possible mechanism is that; 1) via competitive inhibition of MSH binding, 2) via distinct agouti receptor, 3) via distinct effect on MSH receptor, and to resolve the question completely, further study such as binding study is necessary.
Special lecture " The genetic complexities of human oculocutaneous albinism: a model of melanin regulation" Dr. Richard King reported the model system for the study of normal and abnormal human pigmentation in oculocutaneous albinism (OCA); he identified four major genes responsible. Mutations of the tyrosinase gene produce OCA1 or tyrosinase related OCA, and at least 100 different mutations of this gene have been identified. Mutations of the P gene are responsible for OCA2 or tyrosinase positive OCA, the most common type of OCA in humans. He analyzed P gene mutation on individuals with a phenotype compatible with OCA2. One common 2.7 kb deletion mutation has been found in African-American and African individuals. A mutation in the TRP1 gene has been found in one individual with OCA3 or brown OCA. Cultured melanocytes from this individual have no TRP1 mRNA and demonstrate a significant reduction but not a total loss of DHICA oxidase function. After his presentation there were several interesting questions. One question asked if there is a relationship between tyrosinase gene mutation and P gene mutation or not. His answer was yes and he mentioned two possible mechanisms; one possibility is that the two genes exist on very close site, and another possibility is that P gene plays an important role on the management of promoter of tyrosinase gene.

One another question asked the presence of the patient with TRP2 mutation. His answer was no. Analysis of melanin - Dr. Shosuke Ito developed microanalytical methods to qualify eumelanin (EM) and pheomelanin (PM) based on the HPLC analysis of their specific degradation products pyrrole-2,3,5-tricarboxylic acid (PTCA) and aminohydroxyphenylalanine. He showed that 1) total melanin (EM+PM) can be dissolved in hot Soluene-350, 2) EM can be dissolved in NaOH-H2O2 after removal of PM by heating in hydriodic acid, and 3) PM can be preferentially dissolved in NaOH. He also mentioned that to see the total melanin, spectrophotometric method using Soluene-350 is the best, and this method is available for cultured cells. He also commented that an important point is that Soluene-350 is the trade name, to check the total melanin, he uses 500 nm not 350 nm. Dr. Ozeki Hiroyuki in Dr. Ito s group presented a comparison of spectrophotometric methods (Sp. EM)/spectrophotometric method using Soluene-350 (total M). He found that the comparison of Sp. EM/total M ratio of brown eumelanin in mice hair was about a half that of black eumelanin, although the PTCA/total M ratio indicates that the proportions of DHICA-derived units were similar. He also showed that 1) Sp. EM/total M ratio correlates to the percentage content of DHICA-derived units, 2) the Sp. EM/total M ratio of DHICA melanin increases as the polymerization proceeds, and 3) the Sp. EM/total M ratio is a parameter as useful as the PTCA/total M ratio in distinguishing chemical properties of eumelanins. Adult T cell leukemia-derived factor (ADF) / thioredoxin - Dr. Yoko Funasaka in Kobe University studied the effect of ADF/ human homologue of thioredoxin in cultured human keratinocytes. She has already reported that UVB irradiation induces the strong expression of ADF in cultured human keratinocytes. To study the effect of ADF on UVB-induced melanogenesis, she analyzed the MSH receptor binding activity using 125I labeled MSH, the expression of MSH receptor mRNA using the melanocortin 1 receptor (MC1-R) cDNA and 3H-thymidine uptake in normal human melanocytes. She showed that ADF increased MSH receptor binding activity of MSH and MC1-R mRNA expression. ADF has not stimulated DNA synthesis neither alone nor with endothelin-1, stem cell factor, hepatocyte growth factor, and basic fibroblast growth factor, but ADF increased MSH-induced DNA synthesis. These results indicate that ADF is one of the stimulatory factors for UVB induced melanogenesis via upregulating MSH receptor binding activity with increasing MC1-R mRNA expression and increasing the DNA synthesis by MSH stimulation. After her presentation, there was a question which asked the mechanism of increasing of MSH binding activity, so that, increasing the number of MSH receptor or increasing the affinity of them. Her answer was the mechanism is still not clear.

Tyrosinase related proteins - The enzymatic functions of tyrosinase, TRP1 and TRP2 are already becoming clear. TRP1 can oxidize DHICA produced by TRP2. However the effect of those enzymes on each other is still not clear. Dr. Kobayashi studied the stabilizing function of the b-locus protein on tyrosinase. He investigated the effect of mutations at the brown locus on stability of tyrosinase in cells. Pulse labeling and chase experiments showed that tyrosinase degraded more quickly in melan-b melanocytes, homozygous for the brown mutation than in melan-a melanocytes, the wild type. Less stability of tyrosinase in melan-b cells was partly rescued by transfection of the wild-type TRP1 gene along with phenotypic rescues. His results clearly showed that TRP1 could stabilize tyrosinase in melanocytes and might also contribute to the polymerization of DHI into melanin. Melanogenic inhibitor - Dr. Yokota and Dr. Kameyama reported the new melanogenic inhibitor, ascorbic acid 2-phosphate sodium salt (APS). They reported that APS is an ascorbic acid derivative that is stable in aqueous solution unlike ascorbic acid. 8X10-3 M APS decreased the total amount of melanin approximately 30% , and suppressed 14C-thiouracil uptake approximately 50% without suppression of 3H-thymidine uptake in cultured B16 melanoma cells. Relatively high concentration of APS (2.7X10-2M) suppressed the activities of tyrosinase, TRP1 and TRP2 significantly on cultured melan-a cells. Then 3% APS cream was applied to the patients with hyperpigmented disorders such as melasma. The results showed that APS cream was effective on 9 out of 20 patients. These findings clearly indicated that APS application can be useful for some patients with hyperpigmented diseases. After their presentation there was a question which ask the absorption of APS. Dr. Kameyama

answered although they don t have the experimental data of APS, similar ascorbic acid derivative magnesium L-ascorbyl-2-phosphate showed that approximately 2% was absorbed into epidermis or dermis on diffusion cell chamber assay.

Obituary Notice
Dr. Takuji Takeuchi
(1930-1996)

Dr. Takuji Takeuchi, Editor-in-Chief for Pigment Cell Research, died of liver failure in January 2, 1996. He was in the position of the President of Nihon Gene Research Laboratories in Sendai and Professor of Ishinomaki Senshu University in Ishinomaki, Japan.

He was born in Kagawa Prefecture, Japan in 1930, graduated from Tohoku University in Sendai in 1955 and, via Graduate School of Science there, appointed as Assistant Professor of Fukushima Prefectural Medical College in Fukushima in 1958. He stayed in Department of Zoology in Rochester University in 1960-61 and Department of Genetics (Professor M. Foster's laboratory) at University of Michigan in 1961-63.

After the return to Japan, he became Associate Professor of Miyagi University of Education in Sendai in 1965 and moved to Tohoku University in 1970. He was promoted to Professor in 1979 and became Professor of Animal Embryology there in 1985. In 1993 he retired at age of 63 according to the age-limit system in Tohoku University.

Dr. Takeuchi dedicated himself to the organization of the XIth IPCC held in Sendai in 1980, together with the late Professor Makoto Seiji. He also organized the Symposium on Molecular Biology of Pigment Cells which was held in Sendai in 1990. He received Seiji Award in 1984, the Zoological Society prize from the Zoological Society of Japan in 1992, and Myron Gordon Award from the IFPCS in 1993.

Dr. Takeuchi was the member of the Board of Associate Editors after the foundation of PCR in 1987, and nominated as the successor of Professor Joseph T. Bagnara, the founding Editor-in-Chief, under recommendation of IFPCS, The Japanese Government conferred the Order of Secret Treasure (Gold) with Neck Ribbon on him after the death.

Jiro Matsumoto
Secretary/Treasurer of JSPCR

Shosuke Ito
Secretary/Treasurer of IFPCS

INTERPIG DataBase by Vincent Hearing

The INTERPIG database is on the InterNet! You can now access the InterPig DataBase at the following address: **http://lenti.med.umn.edu/paspcr/interpig.html.**
Please note that as of this time, I estimate that less than 5% of the various IFPCS members have contributed entries. Think of how useful and complete this list would be if everyone took the time to supply their own information. Please take a moment to fill out the database data entry form and send it back to Dr. Hearing.

XVIth IPCC (International Pigment Cell Conference) by Roger Bowers, Frank Meyskens
The XVIth International Pigment Cell Conference will be held from October 29th to November 3rd, 1996 at the Disneyland Hotel in Anaheim, California. Frank Meyskens is the Organizer of this meeting with Roger Bowers and Alistair Cochran serving as co-chairs of the Organizing Committee. The PASPCR has established a Web page that contains relevant information for this meeting; take a look at:
"http://lenti.med.umn.edu/paspcr/ipcc.htm". Following is the tentative Program of the IPCC:

XVIth International Pigment Cell Conference
Tentative Conference Agenda

Tuesday, **October 29, 1996**
3:00 - 7:00 pm Pre-registration/View Exhibits
7:00 - 10:00 pm Welcome Reception: Fashion Show: "Safe and Sexy in the Sun"

Wednesday, October 30, 1996 Conference Attendees
7:00 - 8:00 am Registration/Continental Breakfast/View Exhibits
8:00 - 8:05 am Welcome: Chairman, Frank L. Meyskens, Jr.
Introduction: Laurel Wilkening, Chancellor, Univ of California, Irvine
8:05 - 8:35 am Special Lecture, R. Sherwood Rowland, Nobel Laureate, 1995, Chemistry
"Ozone Depletion, Ultraviolet Light, and the Pigment Cell"
Symposium I: Economic and Societal Implications of Melanin and Melanogenesis
8:35 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm Workshop A: "Extracutaneous Melanin"
Posters and Discussion #1 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 2:00 pm Lunch on your own
Symposium II: Molecular Biology of Pigment Cells
2:00 - 2:30 pm Keynote Speaker
2:30 - 4:00 pm Invited and Competitive Abstract Speakers

4:00 - 4:05 pm IFPCS InterPig Database on the WorldWideWeb: Vincent Hearing
4:05 - 4:15 pm Break
4:15 - 6:15 pm Workshop B: "Regulating Mechanisms of Melanocyte Proliferation"
4:15 - 6:00 pm Posters and Discussion #2 TBN* (4:15 - 5:30 Viewing; 5:30 - 6:00 Discussion)
5:30 - 7:00 pm Workshop C: "Biophysics of Melanin"
6:15 pm Adjourn Free evening

Accompanying Guests
(8:00 - 11:00 am) Welcome/Introduction: Buffet Breakfast;
(12:00 - 5:00 pm) Group Activity

Thursday, October 31, 1996
7:00 - 8:00 am Continental Breakfast/View Exhibits
8:00 - 8:30 am Seiji Lectureship: Introduction: Giuseppe Prota, President, IFPCS
Symposium III: Melanoma Research: Basic and Applied
8:30 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm Workshop D: "Control of Melanogenesis"
11:00 - 12:30 pm Simultaneous Business Meetings of Regional Societies
12:30 - 2:00 pm Lunch on your own
Symposium IV: Photobiology of Melanocytes: Etiology and Prevention
2:00 - 2:30 pm Keynote Speaker
2:30 - 4:00 pm Invited and Competitive Abstract Speakers
4:00 - 7:00 pm Workshop E: The "Blues" Symposium
4:00 - 7:00 pm Poster Viewing
Adjourn - Free evening

Friday, November 1, 1996
7:00 - 8:00 am Continental Breakfast/View Exhibits
8:00 - 8:30 am Introduction: Sally Frost-Mason, President, PASPCR
Gelb Lectureship: Seth Orlow
Symposium V: Melanogenesis and Pigmentary Disorders
8:30 - 9:00 am Keynote Speaker
9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm Workshop F: "Biology and Biochemistry of Melanosomes"
11:00 - 12:30 pm Posters and Discussion #3 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 1:15 pm Controversy Session: "Semiquinone Radicals are not Important during Melanin Synthesis"
Pro: Patrick Riley Con: Stan Pavel
1:15 pm Adjourn Scientific Session
1:15 - 6:30 pm Break
6:30 - 7:30 pm Reception
7:30 - midnight Banquet, Awards and Dancing

Saturday, November 2, 1996
7:00 - 8:00 am Continental Breakfast/View Exhibits
8:00 - 8:30 am Presidential Address: Giuseppe Prota, President IFPCS
Symposium VI: Comparative Developmental Biology of Pigment Cells
8:30 - 9:00 am Keynote Speaker

9:00 - 10:30 am Invited and Competitive Abstract Speakers
10:30 - 11:00 am Break
11:00 - 12:30 pm Workshop G: "Genetic Aspects of Albinism"
11:00 - 12:30 pm Posters and Discussion #4 TBN* (11:00 - 12:00 Viewing; 12:00 - 12:30 Discussion)
12:30 - 2:00 pm Lunch on your own
2:00 - 4:00 pm Educational Forum: "Living with the Sun"
4:00 - 6:00 pm Family Farewell Reception and Wine Tasting

Sunday, November 3, 1996
8:00 - 5:00 pm
1. Satellite Meeting (all day): Classification of Cutaneous Melanoma: Alistair Cochran
2. Satellite Meeting (3 hours): Safety of Sunscreens and Tanning Parlors: J.P. Cesarini, et al.
3. Satellite Meeting (3 hours): Ocular Melanin: Giuseppe Prota

Workshops and poster and poster discussion sessions will be simultaneous.
The poster sessions and discussions will feature areas that do not overlap with the workshop. The chairs of these sessions will be selected from submitted competitive abstracts and the Chairman in turn will organize this session with help from the Organizing Committee.