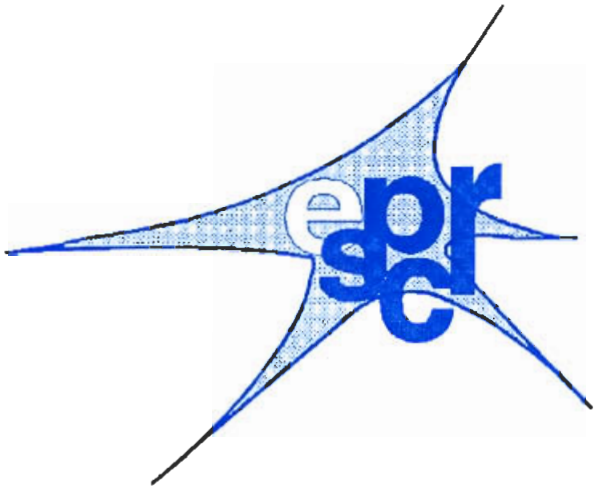
Issue n° 30 - April 1998

ESPCR BULLETIN

PUBLISHED BY THE EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH



EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH BULLETIN

N° 30 - April 1998

Editorial Office: G. Ghanem (Editor), C. Meunier, R. Morandini (Production Team), Laboratory of Oncology and Experimental Surgery (L.O.C.E.), Université Libre de Bruxelles, Institut J. Bordet, Rue Héger-Bordet 1, B - 1000 Brussels, Belgium.
Phone: 32-2-535.35.46 Fax: 32-2-534.95.50 E-Mail: gghanem@ulb.ac.be

ESPCR and ESPCR Bulletin WEB site
http://www.ulb.ac.be/medecine/loce/espcr.htm

CONTENTS

MEETING REPORT

12th JSPCR Meeting
Chiba, Japan 6 - 7 December 1997
by Dr. Jiro Matsumoto

This meeting was organized by Toho University with Professor Noriko Oshima (Department of Biomolecular Science, Faculty of Science) as chairperson.

The science programs consisted of a presidential address, an invited lecture, 2 special lectures, a Symposium, 4 research seminars and 28 oral presentations, with a special emphasis on the symposium.

The symposium entitled with "Phylogenic aspects of tyrosinase genes" was organized with an expectation to provide up-to-date, unified concepts on the evolution of genes playing a central role in pigmentation.
Four speakers reported their own recent works on tyrosinase or its associated genes deriving from different phyletic levels of organisms and their biological significance.

Melanogenesis and prokaryotes: Dr. Y. Nishimura reviewed recent findings on the wide distribution of homologues of eukaryotic tyrosinase in prokaryotes and pointed out that melanin-like pigments in bacteria are formed mostly by the tyrosinase catabolic pathway forming homogentisic acid as observed in human alkaptonuria. It was emphasized that bacteria with melanins or alike indicate a high survival rate against UV-irradiation, and that melanogenic Escherichia coli carrying plasmid tyrosinase genes from actinomycete (S. antibioticus) is available for screening of inhibitors for melanogenesis.

Insect pro-phenol oxidase and its activation mechanism: Dr. M. Ashida reported the homology of pro-phenol oxidase and hemocyanin present in the hemolymph of arthropods based on the similarity in the base sequence of their cloned cDNA, and presumed possible evolution of these two molecules from the common ancestral binuclear copper protein. He mentioned that dimeric phenol oxidase in insect hemolymph is activated by breakage of the peptide-bond at Arg (50)-Phe (51) under the presence of zymogen proteinase and then is released into tissues, and that quinones thus produced by activated phenol oxidase cause hardening of exoskeleton, attributing the protection against bacterial infections.

Expression of the ascidian tyrosinase gene in the developing brain: Dr. H. Yamamoto and his associate cloned tyrosinase gene from ascidian species, Halocynthia roretzi, and found that its open reading frame has 36 - 39% homology with its counterpart of vertebrate origin in terms of amino acid sequence. He also reported that this gene is expressed in the neural plate of early neurulae, even though the neural crest of this species shows no further differentiation, yielding only two sensory cells having different functions in the brain. Based on the presence of melanin granules, these two sensory cells were considered to be an ancestry type of pigment cells. Albinism in fish: insertion of an

Ac-like transportable element in the tyrosinase gene: Dr. H. Hori reported that albinism in the i4-mutant of Medaka fish is caused by insertion of a 4.6 kb sequence into the exon 5 of the tyrosinase gene, and that the inserts, Tol-2 in their designation, are homologous in its basic structure and mobility inside the genome to a maize transposable element, Ac. It was also shown that the gene for tyrosinase, TRP1 and TRP2 in this fish are similar in their basic structure to those of mammalian origin, but are different with regard to the size of their introns which are about 10 kb smaller than those of mammals.

MITF isoform multiplicity and a new aspect in melanin research: Dr. S. Shibahara reported that microphthalmia-associated transcription factor (MITF) plays an important role in cell type-specific transcription of the tyrosinase and the TRP-1 gene, and that MITF fails to transactivate the TRP-2 promoter, despite the presence of an MITF-binding site in the promoter of this gene. He pointed out that transgenic insertional mutation and/or mutation at the mi locus are closely associated with various dysfunctional phenotypes such as small non-pigmented eyes, a lack of melanocytes in the inner ear associated with deafness, a deficiency in mast cell and osteopetrosis, suggesting an intimate correlation of such disorders with the multiplicity of MITF isoforms.

Melanin monomer genesis investigations leading to therapeutic control of malignant melanoma and melanin pigmentation -molecular to clinical level : Dr. Y. Mishima reviewed a recent successful development of the selective boron neutron capture therapy by application of 10B-p-boronphenylalanine (10B-BPA) which had accentuated polymer forming ability within melanoma cells. He emphasized that 1) the accumulation of 10B-BPA becomes possible by formation of complexes with DHICA and DHI which are abundant in these cells, 2) this therapy becomes applicable even to amelanotic melanoma upon transfection of the genes for tyrosinase or its related proteins, and 3) complex forming ability of BPA with DHICA and DHI in vitro led to the idea of using this compound as an inhibitor for melanogenesis in hyperpigmented human skin. At the end of this presentation, he emphasized the necessity of conceptual designs in the research.

Structural and enzymatic components of mammalian melanosomes: Dr. V. J. Hearing, an invited speaker, addressed current understanding of mammalian melanosomes with a particular emphasis on the role of two intercellular signaling molecules, melanocyte stimulating hormone (MSH) and agouti signal protein (ASP), which switch between eumelanin and pheomelanin synthesis. He indicated based on his recent findings that ASP elicits down-regulation in transcription of several pigment specific loci such as albino, brown, slaty, silver and pink eyed-dilution, all of which encode proteins associated with catalytic and structural components of melanosomes. He set forth a view that melanosomes emerge as organelles common to lysosomes, which then differentiate into two distinguishable forms with spherical or elongated fibrillar phenotypes known as pheomelanosomes and eumelanosomes, and that selection of such differential courses of maturation can be defined by combination of specific proteins that are synthesized and deposited under the guidance of signaling molecules.

Signals and molecules regulating melanosome biosynthesis and transport of tyrosine-related protein: Dr. K. Jimbow reported his group's recent findings regarding the fate of tyrosinase-related protein (TRP-1) in the course of melanosome formation, with particular interest in biosynthesis and structural integration of this protein in the ER and Golgi and its transport via the trans Golgi network to the melanosomes under guidance of signal molecules. He pointed out that (1) calnexin plays an important role in folding of tyrosinase and TRP-1 in the ER, suggesting its correlation with melanosomal proteins, (2) newly synthesized TRP-1 is fully glycosylated in the trans Golgi network and then transported to late endosomes installed with the mannose-6-phosphate receptors, possibly by binding with its tyrosine-dileucine residues, (3) several small GTP-binding proteins present in melanosomes, particularly rab 7, are implicated in trapping of TRP-1, together with PI-3-kinase.

Pigment cells and gene transfer in Medaka: Dr. K. Ozato reviewed recent progress in gene-transfer studies using Medaka fish and reported his own success in production of transgenic Medaka carrying the gene for salmon melanin concentrating hormone (MCH) with CMV promoter. He addressed that the transgenes of salmon origin are expressed in a variety of tissues of homozygous progenies, indicating the presence of a detectable level of MCH in their serum and thereupon causing a distinguishable paler body coloration over 10 generations. He also reported his current success in establishing embryonic stem cell lines from Medaka embryos, suggesting the usefulness of the nuclear transplantation technique in production of chimeric animals.

At the research seminars, four topics were dealt with: the chemistry of melanins (Dr. K. Wakamatsu), the effects of ACF (stem cell factor) on the development of cultured neural crest cells into melanocytes (Dr. H. Ono), the role of c-KIT expression in melanoma (Dr. Y. Funasaka), and the expression of

SCF and c-KIT in cutaneous mastocytosis (mastocytoma) (Dr. Y. Kubota). In this seminar, Ono reported clear evidence indicating the necessity of stem cell factor for melanocyte differentiation which is considered to be inducible solely by endothelin-3, based on in vitro neural crest culture using homozygous sl mutant mice. Dr. Funasaka reported a marked decrease of c-kit expression in terms of mRNA levels in dysplastic nevus and malignant melanoma cells. She predicted, based on the inhibitory effects of SCF on the growth of melanoma cells, that apoptosis would be inducible by application of phosphorylation to c-kit proteins.

In the regular presentation, Dr. T. Hirobe reported that the differentiation-inducing potential of ACTH for murine melanocytes exists in the site corresponding to 1 to 13 amino acid residues, showing a higher activity with shortening of accessory residues and reaching to its maximal with the sequence similar to MSH.

1. Melanins and other pigments chemistry

(Prof. M. Peter)

General Aspects: The occurence and functional aspects of melanins (Riley) and potential applications of biopolymers from marine prokaryotes, including melanins, were reviewed (Weiner).

Structure and Analysis: The origin, structure, and functional significance of neuromelanin was reviewed in relation to Parkinson's disease (d'Ischia and Prota).
Hoof horn material from equids was analyzed by EPR spectroscopy and the presence of stable sulfur-centred radicals suggests occurrence of melanin (Cope et al.).
Fungal melanins contain amino sugars, ranging from 192 to 635 mg kg-1. They are composed of glucosamine (80-99%), galactosamine, and mannosamine (up to 10%) (Coelho et al.). EPR spectra of dark-coloured micromycetes isolated from marble monuments in the Mediterranean Basin revealed the presence of melanins (Panina et al.)
An improved sensitive method for the analysis of pheomelanin in biological samples, based on hydriodic degradation and HPLC determination with electrochemical detection of 4-amino-3-hydroxyphenylalanine and 3-amino-L-tyrosine was developed (Kolb et al.).
A spectrophotometric method developed for assaying the total amount of eu- and pheomelanins dissolved in Soluene-350. This was applied to characterize natural eumelanins with various DHI/ DHICA ratios and to estimate the DHICA content and the degree of polymerization (Ozeki, Wakamatsu, et al.).

Oxidation Chemistry: Melanins mediate electron transfer between hydroxybenzene donors (tyrosine, dopa, chemical depigmenters) and model acceptors (ferricyanide, tyrosinase) accelerate markedly the tyrosinase-catalyzed oxygenation of p-hydroxyanisole. Sepia melanin appears to protect against UV-induced damage to acid-soluble collagen (Menter and Willis).
The lag phase observed in the oxidation of monophenols by tyrosinase and catechols was investigated with various substrates. Oxygen consumption is retarded in N-acetyldopamine due to a diminished rate of cyclization. N,N-Dipropyldopamine was oxidized with normal kinetics and formation of a stable indoliumolate. N,N-dimethyltyramine shows an indefinite lag in the absence of a dihydric phenol cofactor (Cooksey et al.).
The Michaelis constant for the oxidation of 4-hydroxyanisole catalyzed by mushroom tyrosinase increased with decreasing pH. However the maximum steady-state rate did not change with the pH. The apparent catalytic constant was around twenty three times higher than that previously described for L-tyrosine (Espin et al.). The cytotoxicity of 4-hydroxyanisole is positively correlated with dopa oxidase activity as shown with three mouse and human melanoma cell lines (Rodriguez-Vicente et al.). Tyrosinase- or auto-oxidation of 4-S-Cysteaminylphenol (4-S-CAP) and 4-S-cysteaminylcatechol (4-S-CAC) produced a violet pigment, dihydro-1,4-benzothiazine-6,1-dione(BQ) which is the ultimate toxic metabolite in this reaction. BQ deprives melanoma cells of GSH and may inactivate SH enzymes. The cytotoxicity of 4-S-CAC was mostly prevented by catalase and superoxide dismutase (Hasegawa et al.).
In the presence of thiols, tyrosine hydroxylase (TH) oxidizes L-DOPA. The reaction products have been identified tentatively as thioether derivatives of DOPA. The DOPA oxidase activity of TH can lead to errors in the estimation of in vivo or in vitro TH activity (Haavik). Cinnamic acids enhanced the apoplastic peroxidase-dependent oxidation of dopa in bean leaves, probably by a radical mechanism. In plants, fhe formation of melanins is thought to be part of the defense system (Takahama). Maltol (3-hydroxy-2-methyl-4H-pyran-4-one) prevented the conversion of DL-DOPA, dopamine, and norepinephrine to their corresponding melanins via tyrosinase (Kahn and Ben-Shalom). Kojic acid [5-hydroxy-2-hydroxymethyl)-4H-pyran-4-one] and maltol inhibited the rate of N-acetyl tyrosine (NAT) hydroxylation by mushroom tyrosinase in the absence or presence of H2O2. Kojic acid is oxidized by the quinone of NAT to give a yellow product, while maltol apparently forms a conjugate (structure not reported) (Kahn and Ben-Shalom).
Dopamine and related catecholamines are oxidized in vitro in the presence of H2O2 by xanthine oxidase to give corresponding melanin pigments (Foppoli et al.). Dityrosine can be formed by the action of an enzymatic system alternative to peroxidase as was shown by oxidation of tyrosine and enkephalins with cytochrome c in the presence of H2O2 to give an o,o'-biphenyl link (Foppoli et al.). Peroxidase promotes oligomerization of 5-hydroxytryptamine with the formation of dimers, trimers and tetramers. The presence of H2O2 favours further oligomerization, leading to decamers. Tyrosinase promotes a higher degree of 5-hydroxytryptamine oligomerization, even in the absence of H2O2 (Favretto et al.) Certain dihydroxytetrahydroisoquinolines which are represented by some alkaloids and may also occur as endogenous metabolites arising from the condensation of catecholamines with aldehydes, are easily oxidized in the presence of hydrogen peroxide by peroxidase, lipoxygenase, and xanthine oxidase into the corresponding melanins (Mosca et al.). Another review on the formation of so called opiomelanins from opioid peptides has appeared (Rosei et al.).
The cytotoxicity of quinone compounds derived from the melanogenic pathway was demonstrated in rabbit erythrocytes and human K562 tumor cells (Cammarata et al.). Involvement of the o-quinone/quinone methide pathway in catechol toxicity depends on a combination between the rate of enzymatic formation of the o-quinone, the rate of isomerization to the more electrophilic quinone methide, and the chemical reactivity of the quinoids (Bolton et al.). The role of

neuromelanin bound Fe3+ in the overproduction of hydroxyl radicals was discussed in the context of the pathogenesis of Parkinson's disease (Bertrand et al.). Free radicals and MPTP-induced selective destruction of substantia nigra compacta neurons was reviewed (Chiueh and Rauhala).

Drug and Metal Binding: Drug-melanin binding of a set of 16 compounds has been analyzed with correlation analysis and a set of theoretical molecular parameters in order to better understand and characterize drug-melanin interactions. The resulting correlation equation supports a charge transfer model for drug-melanin complex formation and can also be used to estimate binding constants for related compounds (Lowrey et al.). Dependence of drug content on hair pigmentation was discussed (Potsch et al.). Guinea pigs receiving codeine with their drinking water showed righer contents in black than in light hair (Potsch et al.). The Saponins, elliptosides A-J, isolated from the tropical plant Archidendron ellipticum (Leguminosae) were particularly cytotoxic to certain renal and melanoma cancer cell lines (Beutler et al.). Stereoisomers of gossypol and gossypolone were investigated in two human melanoma cell lines. Racemic gossypol showed equal activity in both cell lines. (-)-Gossypol was significantly more active as compared to the (+)-isomer (Blackstaffe et al.).
Melanin-like pigments of Piedraia hortae may sequester ionic compounds of P, S, and Ca as determined by electron microscopy X-ray microanalysis. Environmental contaminants such as Al, Si, and Fe were detected exclusively on the surface of the nodule (Figueras and Guarro).

Biosynthesis and Inhibition: The formation of melanins from DOPA and tyrosine in the presence of cysteine was studied. Analysis of AHP / total melanin, changes in the tyrosine and cysteinyldopa concentrations, and changes absorbance ratio, A650/A500, led to the conclusion that lower tyrosinase concentration favors pheomelanogenesis even when the availability of cysteine is limited. Tyrosinase activity is the major factor controlling the course of melanogenesis with respect to eumelanogenesis or pheomelanogenesis (Ozeki, Ito, et al.). A review on biologically active fungal metabolites mentions also melanin biosynthesis inhibitors (Pearce). A new peptide, γ-(ß-histidinoalanino)homoalanine, named amphistin, was isolated from the fermentation broth of an actinomycete strain KP-3052. Amphistin inhibited the melanogenesis of B16 melanoma cells at concentration of 6.8 μM (Arai et al.).
2,3-dihydro-2,5-dihydroxy-4H-benzopyran-4-one is oxidized by trihydroxynaphthalene reductase to 4,5-dihydroxy-2H-benzopyran-2-one and dehydrated by scytalone dehydratase, enzymes that are involved in melanin biosynthesis from trihydroxynaphthalene in fungi (Thompson et al.).

Other topics: Several synthesis of gangliosides that are potentially useful in developing vaccines against melanoma, were described (Castropalomino et al.; Wilstermann and Magnusson).

-Arai N, Shiomi K, Takamatsu S, Komiyama K, Shinose M, Takahashi Y, Tanaka Y, Iwai Y, Liu JR, Omura S.
Amphistin, a new melanogenesis inhibitor, produced by an actinomycete. J. Antibiotics. 50: 808-814, 1997.

-Bertrand E, Lechowicz W, Szpak GM, Dymecki J.
Current views on the mechanisms of dopaminergic neuron death of the nigrostriatal system in Parkinson's disease. Neurol. Neurochir. Pol. 31: 295-302, 1997.

-Beutler JA, Kashman Y, Pannell LK, Cardellina JH, Alexander MRA, Balaschak MS, Prather TR, Shoemaker RH, Boyd MR.
Isolation and characterization of novel cytotoxic saponins from Archidendron ellipticum. Bioorg. Med. Chem. 5: 1509-1517, 1997.

-Blackstaffe L, Shelley MD, Fish RG.
Cytotoxicity of gossypol enantiomers and its quinone metabolite gossypolone in melanoma cell lines. Melanoma Res. 7: 364-372, 1997.

-Bolton JL, Pisha E, Shen L, Krol ES, Iverson SL, Huang ZW, Vanbreemen RB, Pezzuto JM.
The reactivity of o-quinones which do not isomerize to quinone methides correlates with alkylcatechol-induced toxicity in human melanoma cells. Chem. Biol. Interact. 106: 133-148, 1997.

-Cammarata M, Arizza V, Parrinello N, Candore G, Caruso C.
Phenoloxidase-dependent cytotoxic mechanism in ascidian (Styela plicata) hemocytes active against erythrocytes and K562 tumor cells. Eur. J. Cell Biol. 74: 302-307, 1997.

-Castropalomino JC, Ritter G, Fortunato SR, Reinhardt S, Old LJ, Schmidt RR.
Efficient synthesis of ganglioside GM2 for use in cancer vaccines. Ang. Chem. Int. Edn. Engl. 36: 1998-2001, 1997.

-Chiueh CC, Rauhala P.
Free radicals and MPTP-induced selective destruction of substantia nigra compacta neurons. Adv. Pharmacol. 42: 796-800, 1998.

-Coelho RRR, Sacramento DR, Linhares LF.
Amino sugars in fungal melanins and soil humic acids. Eur. J. Soil Sci. 48: 425-429, 1997.

-Cooksey CJ, Garratt PJ, Land EJ, Pavel S, Ramsden CA, Riley PA, Smit NPM.
Evidence of the indirect formation of the catecholic intermediate substrate responsible for the autoactivation kinetics of tyrosinase. J. Biol. Chem. 272: 26226-26235, 1997.

-Cope BC, Hopegood L, Latham RJ, Linford RG, Reilly JD, Symons MCR, Taiwo FA.
Studies of equid horn material by EPR spectroscopy. J. Mat. Chem. 8: 43-45, 1998.

-d'Ischia M, Prota G.
Biosynthesis, structure, and function of neuromelanin and its relation to Parkinson's disease: A critical update. Pigm. Cell Res. 10: 370-376, 1997.

-Espin JC, Varon R, Tudela J, Garciaca-Novas F.
Kinetic study of the oxidation of 4-hydroxyanisole catalyzed by tyrosinase. Biochem. Mol. Biol. Int. 41: 1265-1276, 1997.

-Favretto D, Bertazzo A, Costa CVL, Allegri G, Donato N, Traldi P.
The role of peroxidase in the oligomerization of 5-hydroxytryptamine investigated by matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun. Mass Spectrom. 12: 193-197, 1998.

-Figueras MJ, Guarro J.
X-ray microanalysis of black piedra. A. Van Leeuwenhoek Int. J. Gen. Mol. Microbiol. 72: 275-281, 1997.

-Foppoli C, Coccia R, Cini C, Rosei MA.
Catecholamines oxidation by xanthine oxidase. Biochim Biophys Acta. 1334: 200-6, 1997.

-Foppoli C, De Marco C, Blarzino C, Coccia R, Mosca L, Rosei MA.
Dimers formation by cytochrome c-catalyzed oxidation of tyrosine and enkephalins. Amino Acids 13: 273-280, 1997.

-Haavik J.
L-DOPA is a substrate for tyrosine hydroxylase. J. Neurochem. 69: 1720-1728, 1997.

-Hasegawa K, Ito S, Inoue S, Wakamatsu K, Ozeki H, Ishiguro I.
Dihydro-1,4-benzothiazine-6,7-dione, the ultimate toxic metabolite of 4-S-cysteaminylphenol and 4-S-cysteaminylcatechol. Biochem. Pharmacol. 53: 1435-1444, 1997.

-Kahn V, Ben-Shalom N.
Effect of maltol on the oxidation of DL-DOPA, dopamine, N-acetyldopamine (NADA), and norepinephrine by mushroom tyrosinase. Pigm. Cell Res. 10: 139-149, 1997.

-Kahn V, Ben-Shalom N.
N-acetyl-L-tyrosine (NAT) as a substrate for mushroom tyrosinase. Pigm. Cell Res. 11: 24-33, 1998.

-Kolb AM, Lentjes E, Smit NPM, Schothorst A, Vermeer BJ, Pavel S.
Determination of pheomelanin by measurement of aminohydroxyphenylalanine isomers with high- performance liquid chromatography. Anal. Biochem. 252: 293-298, 1997.

-Lowrey AH, Famini GR, Loumbev V, Wilson LY, Tosk JM.
Modeling drug-melanin interaction with theoretical linear solvation energy relationships. Pigm. Cell Res. 10: 251-256, 1997.

-Menter JM, Willis I.
Electron transfer and photoprotective properties of melanins in solution. Pigm. Cell Res. 10: 214-7, 1997.

-Mosca L, Blarzino C, Coccia R, Foppoli C, Rosei MA.
Melanins from tetrahydroisoquinolines: Spectroscopic characteristics, scavenging activity and redox transfer properties. Free Radical Biol. Med. 24: 161-167, 1998.

Ozeki H, Ito S, Wakamatsu K, Ishiguro I.
Chemical characterization of pheomelanogenesis starting from dihydroxyphenylalanine or tyrosine and cysteine. Effects of tyrosinase and cysteine concentrations and reaction time. Biochim. Biophys. Acta. 1336: 539-48, 1997.

-Ozeki H, Wakamatsu K, Ito S, Ishiguro I.
Chemical characterization of eumelanins with special emphasis on 5,6-dihydroxyindole-2-carboxylic acid content and molecular size. Anal. Biochem. 248: 149-57, 1997.

-Panina LK, Badalian AG, Bogomolova EV, Vollentsien U, Gorbushina AA, Krumbian VE, Sukharzhevskyi SM.

EPR-spectra of dark-coloured micromycetes isolated from the marble. Mikologiya I Fitopatologiya. 31: 46-51, 1997.

-Pearce C.
Biologically active fungal metabolites. Adv. Appl. Microbiol. 44: 1-80, 1997.

-Potsch L, Skopp G, Moeller MR.
Influence of pigmentation on the codeine content of hair fibers in guinea pigs. J. Forensic Sci. 42: 1095-1098, 1997.

-Potsch L, Skopp G, Moeller MR.
Biochemical approach on the conservation of drug molecules during hair fiber formation. Forensic Sci. Int. 84: 25-35, 1997.

-Riley PA.
Melanin. Int. J. Biochem. Cell Biol. 29: 1235-1239, 1997.

-Rodriguez-Vicente J, Vicente-Ortega V, Canteras-Jordana M, Calderon-Rubiales F.
Relationship between 4-hydroxyanisole toxicity and dopa oxidase activity for three melanoma cell limes. Melanoma Res. 7: 373-381, 1997.

-Rosei MA, Mosca L, De Marco C.
New possible pathways for melanogenesis: opiomelanins. Recenti Prog Med. 88: 134-9, 1997.

-Takahama U.
Enhancement of the peroxidase-dependent oxidation of dopa by components of Vicia leaves. Phytochemistry 46: 427-432, 1997.

-Thompson JE, Basarab GS, Pierce J, Hodge CN, Jordan DB.
2,3-dihydro-2,5-dihydroxy-4H-benzopyran-4-one: A nonphysiological substrate for fungal melanin biosynthetic enzymes. Anal. Biochem. 256: 1-6, 1998.

-Weiner RM.
Biopolymers from marine prokaryotes. Trends Biotech. 15: 390-394, 1997.

-Wilstermann M, Magnusson G.
Synthesis of Xyl beta Cer, Gal beta 1-4Xyl beta Cer, NeuAc alpha 2-3Gal beta 1-4Xyl beta Cer and the corresponding lactone and lactam trisaccharides. J. Org. Chem. 62: 7961-7971, 1997.

2. Biology of pigment cells and pigmentary disorders

(Dr M. Picardo)

Several papers have been published in the last few months on the possible role of MSH and its receptor. The group of **Abdel-Malek** presents data showing that alpha MSH and endothelin 1, that act synergistically to modulate melanocyte proliferation, have opposite effects on the melanocyte-matrix interaction. In fact MSH-analog treatment induces reorganization of cytoskeleton associated with an increase adhesion to fibronectin, ET-1 produces a distinct form of actin reorganization and decreased melanocyte adhesion to fibronectin. Moreover ET-1, but not MSH, activates the focal adhesion kinase pp125FAK, a non receptor kinase associated with focal contact formation. **Im and co-workers,** have shown that stimulation of cAMP formation is obligatory for the melanogenic response of cultured human melanocytes to UVB and that alpha MSH allows the cells to enter the S-phase acting as a mitogen rather than as a survival factor. **Wakamatsu et al** identified the presence of ACTH in the epidermis and the products of ACTH cleavage to MSH. Moreover the authors report that ACTH1-17 increases the dendriticity and melanin content of cultured melanocytes and conclude that ACTH peptides may have a role in the regulation of human melanogeneis acting through the MC1 receptor. **Nakazawa and co-workers** have established a new technique for reconstituting a pigmented human skin equivalent (PSE) and have evaluated its functional responses to environmental stimulus UVB. The PSE is reconstituted by grafting an epithelial sheet consisting of keratinocytes and melanocytes onto a porous non contractile dermal equivalent populated with mitotically and metabolically active fibroblasts . Morphologically this system presents the characteristics of a well differentiated epidermis and it is potentially useful as a model for studying cellular interactions.
The group of **Alain Taieb** is continuing their interesting work on the model of reconstructed epidermis. Now they have used this technique to study keratinocytes and melanocytes in vitiligo performing autologous and allogenic reconstructs. Macroscopical, microscopical and immuno histochemical examinations did not reveal any significant alteration in either melanocytes or keratinocytes from vitiligo subjects nor did they discriminate between keratinocytes and melanocytes as inducers of the disease. The authors conclude that the basic abnormality in vitiligo probably needs extrinsic factors to be revealed.
Sermadiras et al. have investigated the expression of Bcl-2 and Bax, two oncogenes which regulate the apoptosis process, in cultivated keratinocytes and melanocytes from the same donors, respectively induced to differentiate and produce

melanin. The authors report that in keratinocytes Bax level remains constant in differentiating medium while Bcl-2 expression decreases. In melanocytes they found that together with Bcl-2 also Bax is constantly expressed and this suggests that the high Bcl-2/Bax ratio could be the basis of the high resistance of melanocytes to UV-induced apoptosis. In conclusion the authors demonstrate that the modification of the ratio between the two oncogenes is associated with the differentiation of keratinocyte and melanocyte pigmentation, two important processes of skin function.

Yaar and Gilchrest present an interesting review which suggests that melanocytes could be a valuable in vitro model to study Alzheimer's disease. The authors report that human melanocytes appear to respond to environmental signals in the same manner as neurons in the central nervous system and that they can undergo apoptosis following exposure to beta amiloid, the mechanism proposed for neuron-loss in Alzheimer's. The authors suggest that melanocytes can be useful in evaluating possible therapies.

Abdel-Naser et al. report that a circulating factor that can stimulate melanocyte and fibroblast growth can be detected in patients following PUVA therapy and suggest that this could be one of the mechanisms by which PUVA can induce repigmentation in vitiligo. Anthony and co-workers report that the adducts 8-methoxy psoralen fatty acids prepared in vitro could be substituted for diacylglicerol to activate PKc in a cell free system and proposed this as a possible mechanism of action in PUVA exposure.

Tronnier et al. have evaluated by histology and by immunohistochemistry using different monoclonal antibodies, the modification of melanocytes from naevi following a single erythemagenic dose of UV or fractionally applied high doses. The authors have found that a single erythemagenic dose is more effective in inducing morphological changes and an enhanced proliferative riparative activity of melanocytes

Weiss et al analyzed Rap1-GAP expression, a genomic region associated with familial melanoma, in benign and malignant melanocytic tumors. Positivity was observed in 45% of ?nevi? and in 57% of melanomas and in the latter group, the frequency of RAP1-GAP expression increased with the thickness of primary tumors and was highest in metastatic lesions: Expression of this protein was detected in 79% of subsequently recurring primary melanomas but only in 47% of patients who remained free of disease for at least 6 years. Although not being an indicator for malignant transformation of melanocytic lesions the authors suggest that Rap1-GAP over-expression may represent a useful marker for identifying thin high risk melanomas.

Wenczl et al. modified a immunochemical technique, the sandwich enzyme-linked immunosorbent assay to detect UV-induced ssb (Single-stranded DNA) directly on monolayer-grown human melanocytes. The method is based on the binding of a monoclonal antibody to ssb. This assay is of great interest for further investigations regarding the photo-protecting and for the photo-sensitizing effects of melanins in human melanocytes derived from different skin types.

Rallis et al. analysed mRNA transcription of protoncogenes c-Jun, c-fos and Jun-B in nevus cells, melanocytes and melanoma cells, to determine if biological differences among these cells was due to their level of proto-oncogene expression . When the cells were serum starved end reefed, the level of c-FOS mRNA was elevated in cultures of nevus cells and only in one melanoma cell line. The authors conclude that the regulation of the proto-oncogene c-fos is different in nevus cells than in normal melanocytes and that this difference may contribute to different growth characteristics seen with nevus cells.

Zeff et al. briefly overview the evidence for the participation of the immune response in the genesis of the halo nevus. Although no direct demonstration of melanocyte killing has been observed by the immune effector cells found within the halo , the abundance of antigen-presenting cells in the regressing nevus and the presence of T lymphocytes at the site of depigmentation suggests that the immune system participates in the halo phenomenon. In particular CD8 T cells are considered to be potential effectors in the destruction of nevo-melanocytes. The break in tolerance that triggers migration and of these and other lymphocytes in the nevus, in the apparent absence of the disuse , remains unclear.

Bar-Eli reviews the molecular mechanisms of melanoma metastasis underlying the possible role of c-Kit. The expression of this tyrosine kinase receptor decreases progressively during local tumour growth and invasion and the author suggest that the loss of c-Kit may allow melanoma cells to escape stem cell factor induced apoptosis. Since the expression of c-Kit is regulated by the transcription factor AP-2, the loss of AP-2 expression might be a crucial event in the progression of melanoma.

Smit at al. investigated the effect of varying concentration of l-tyrosine and l-cysteine in culture medium on melanin production by human skin melanocytes (skin phototype II/III). They analysed dopa oxidase activity and total melanin, pheomelanin production in the cells . With varying concentrations of both amminoacids, profound changes in the pigmentation patterns of the melanocytes were observed. They concluded that melanogenesis in cultured melanocytes can be substantially influenced by L-Tyrosine and L-Cysteine and they suggest that variations in the concentrations of both amminoacids in culture medium can be used to regulate the melanogenetic phenotype under in vitro conditions.

Benathan presented new data on the metabolism of thiols in melanoma cells. The author evaluated the capability of melanoma cells to metabolise GSH, studying the activity of the membrane associated enzyme γ-glutamyl transpeptidase. The results showed that melanoma cells have a low capability to metabolise the GSH and that could be the reason for the secretion of GSH in the culture medium.

Finally Harada and co-workers have studied the clonality of nevocellular naevus and melanoma using an expression based clonality analysis at the X linked genes by PCR. The results showed that all nevocellular naevi analysed were policlonal in the origin and that all the melanoma were monoclonal independently on the histological type or if the neavi were congenital or acquired. The authors concluded that naevi can be considered amartomas and that the analysis of clonality could be used to differentiate benign from malignant melanocytic lesions.

-Abdel-Malek Z.A.

Epidermal reconstructs in vitiligo: an extrinsic factor is needed to trigger the disease. Br. J. Dermatol.137: 890-897, 1997.

-Abdel-Naser M.B., Hann S.K. , Bystryn J.C.
Oral Psoralen with UV-A therapy releases circulating growth factors that stimulate cell proliferation. Arch.Dermatol.133 (12): 1530-1533, 1997.

-Anthony F.A., Laboda H.M., Costlow M.E.
Psoralen-fatty acid adducts activate melanocyte protein kinase C: a proposed mechanism for melanogenesis induced by 8-methoxypsoralen and ultraviolet A light. Photodermatol. Photoimmunol. Photomed. 13: 9-16, 1997.

-Bar-Eli M.
Molecular mechanisms of melanoma metastasis. J. Cell Physiol. 173(2): 275-8, 1997.

-Benathan M.
Glutathione efflux associated with a low (-glutamyl transpeptidase activity in human melanoma cells. Br. J.Dermatol. 137: 721-727, 1997.

-Bessou S, Gauthier Y, Serleve-Bazeille, Pain C, Taieb A.
Epidermal reconstructs in vitiligo: an extrinsic factor is needed to trigger the disease. Br. J. Dermatol.137: 890-897, 1997.

-Castanet J., Ortonne J.P.
Pigmentary changes in aged and photoaged skin. Arch. Dermatol. 133 (10): 1296-1299. 1997.

-Chen W., Zouboulis C.C., Fritsch M., Blume-Peytavi U., Kodelja V., Goerdt S., Luu-The V., Orfanos C.E.
Evidence of heterogeneity and quantitative differences of the type 1 5 alpha -reductase expression in cultured human skin cells - evidence of its presence in melanocytes. J. Invest. Dermatol. 110:84-89, 1998.

-Graham A., Wakamatzu K., Hunt G., Ito S., Thody A.J.
Agouti protein inhibit the production of eumelanin and pheomelanin in the presence and absence of alpha-melanocyte stimulating hormone. Pigment Cell Res.10: 298-303, 1997.

-Im S., Moro O., Peng F., Medrano E.E., Cornelius G.,Babcock G., Nordlund JJ., Abdel-Malek Z.A.
Activation of the cyclic AMP patway by alpha-melanotropin mediates the response of human melanocytes to ultraviolet B radiation. Cancer Res. 58: 47-54, 1998.

-Imokawa G., Higuchi K., Yada Y.
Purification and characterization of an allergy-induced melanogenic stimulating factor in brownish pig skin. J Biol Chem 273 (3): 1605-1612, 1998.

-Luan J., Shattuck-Brandt R., Haghnegahdar H., Owen J.D., Strieter R., Burdick M., Nirodi C., Beuchamp D., Johnson K.N., Richmond A.
Mechanism and biological significance of constitutive expression of MGSA/GRO chemokines in malignant melanoma tumor progression. J. Leukoc. Biol. 62(5): 588-597, 1997.

-Nakajima M, Shinoda I, Mikogami T, Iwamoto H, Hashimoto S, Miyauchi H, Fukuwatari Y, Hayasawa H.
Beta -lactoglobulin suppresses melanogenesis in cultured human melanocytes. Pigment Cell Res.10: 410-413, 1997.

-Nakazawa K., Nakazawa H., Sahuc F., Lepavec A., Collombel C., Damour O.
Pigmented human skin equivalent: new method of reconstitution by grafting an epithelial sheet onto a non-contractile dermal equivalent. Pigment Cell Res. 10 (6): 382-390, 1997.

-Owen J.D., Strieter R., Burdick M., Haghnegahdar H., Nanney L., Shattuck-Brant R., Richmond A.
Enhanced tumor-forming capacity for immortalized melanocytes expressing melanoma growth stimulatory activity/growth-regulated cytokine beta and gamma proteins. Int. J. Cancer 73:94-103, 1997.

-Postaire E., Jungmann H., Bejot M., Heinrich U., Tronnier H.
Evidence for antioxidant nutrients-induced pigmentation in skin: results of a clinical trial. Biochem. Mol.Biol. Int. 42(5),:1023-1033, 1997.

-Rallis T.M., Larkin M.T., Schimidt L.A., Meyer L.J.
Expression of c-fos in cultured human nevus cells: an increase over melanocytes and melanoma cells. Exp. Dermatol. 6 (6): 308-313, 1997.

-Riley P.A.
Melanin. Int. J. Biochem. Cell. Biol. 29 (11): 1235-1239, 1997.

-Scott G., Cassidy L., Abdel-Malek Z.

Alpha-melanocyte-stimulating hormone and endothelin-1 have opposing effects on melanocyte adhesion migration and pp125FAK phosphorylation. Exp. Cell Res. 237: 19-28, 1997.

-Sermadiras S., Dumas M., Joly-Berville R., Bontje F., Meybeck A., Ratinaud M.H.
Expression of Bcl-2 and Bax in cultured normal Human keratinocytes and melanocytes: relationship to differentiation and melanogenesis. Br. J. Dermatol. 137: 883-889, 1997.

-Smith N.P., Van der Meulen H., Koerten H.K., Kolb R.M., Mommaas A.M., Lentjes E.G., Pavel S.
Melanogenesis in cultured melanocytes can be substantially influenced by L-tyrosine and L-cysteine. J. Invest. Dermatol.109:796-800, 1997.

-Tronnier M., Rudolph P., Koser T., Raasch B., Brinckmann J.
One single erythemagenic UV irradiation is more effective in increasing the proliferative activity of melanocytes in melanocytic naevi compared with fractionally applied high doses. Br. J. Dermatol. 137: 534-539. 1997.

-Wakamtsu K, Graham A., Cook D., Thody A.J.
Characterization of ACTH peptides in Human skin and their activation of the melanocortin-1 receptor. Pigment Cell Res.10 (5),: 288-297, 1997.

-Weiss J., Biwer B., Schliz M., Jung E.G.
Clinico-pathological and prognostic relevance of Rap-1 GAP expression in melanocytic tumors. Arch. Dermatol. Res. 289 (10): 573-577, 1997.

-Wenczl E., Pool S., Timmerman A.J., van der Schans G.P., Roza L, Schotohorst A.A.
Physiological doses of ultraviolet irradiation induce DNA strand breaks in cultured human melanocytes as detected by means of an immunochemical assay. Photochem, Photobiol 66 (6): 826-830, 1997.

-Wong K.Y., Rajora N., Boccoli G., Catania A., Lipton J.M.
A potential mechanism of local anti-inflammatory action of alpha-melanocyte-stimulating hormone within the brain: modulation of tumor necrosis factor alpha production by human astrocytic cells. Neuroimmunomodulation 4(1): 37-41, 1997.

-Yaar M., Gilchrest B.
Human Melanocytes as a model system for studies of Alzheimer's disease. Arch Dermatol. 133: 1287-1291, 1997.

-Zeff R.A., Freitag A., Grin C.M., Grant-Kels J.M.
The immune response in halo nevi. J. Am. Acad. Dermatol. 37: 620-624, 1997.

Melanocyte cultures
(Dr N. Smit)

Although the paper by Ozeki et al (BBA-Gen 1336) does not deal with melanocyte cultures at all, this study examining the process of pheomelanin formation may be of great importance for understanding what happens inside cultured human melanocytes. From the results of this study; "It was found that (1) mixed melanogenesis is a heterogeneous process in which pheomelanogenesis proceeds first, followed by eumelanogenesis and (2) lower tyrosinase concentration favors pheomelanogenesis even when the availability of cysteine is limited" the authors propose that tyrosinase activity is the major factor controlling the course of melanogenesis. In the melanocyte culture system the process of pigment production and deposition inside the melanosomal matrix is much more complex but still resembles the situation where tyrosine (dopa) and cysteine are combined in the presence of tyrosinase in a reaction vessel as in the study by Ozeki et al. The results described in our paper (Smit et al JID 109) show that also in the melanocyte culture system pheomelanin production is considerable and increases when the tyrosine concentrations in the culture medium are elevated. When the cysteine concentrations are reduced this results in an increase in the tyrosinase activity in the cell. Under these conditions and with sufficient levels of tyrosine present the pheomelanin to total melanin content in the melanocyte cultures was reduced. This indicates that the choice of culture medium strongly influences pigment content and composition of cultured melanocytes. Inducing differences in the pigmentation of cultured melanocytes may be of great use for studying the photo-protecting/sensitizing effects of melanins in the same melanocyte cultures. The method described by Wencl et al, (P&P 66) can be used to detect single strand breaks in melanocyte cultures resulting from irradiations using UV-A, UV-B and solar simulated light. Imokawa (JBC 273) describes the isolation of an allergy induced melanogenic stimulating factor (PIMSF) and its action on the intracellular transduction system in guinea pig melanocytes. As described before by the same group for endothelin-1 also the PIMSF influenced Ca2+-levels in the cells. The mechanism of action of endothelin-1 on the induction of [Ca2+](i) in cultured melanocytes is the topic of the paper by Kang et al. Using the fura-2/AM system for detection of intracellular Ca2+-levels influences of ET-1 were studied. It was found that prior depletion of intracellular Ca2+ stores with thapsigargin, an inhibitor of Ca2+-ATPase of the endoplasmic reticulum, abolished the ET-1-induced Ca2+ transient, whereas removal of extracellular Ca2+ with EGTA eliminated the sustained rise.
In the paper of Wagner et al the effects of modulating calmodulin and intracellular free calcium on attachment of uveal

normal and transformed melanocytes to extracellular matrix is described.

-Abdelnaser MB, Hann SK, Bystryn JC.
Oral psoralen with uv-a therapy releases circulating growth factor(s) that stimulates cell proliferation. Arch Dermatol 133(12):1530-1533, 1997.
Design: We examined the effect of serum samples obtained from patients with vitiligo before and following 2 and 4 months of PUVA therapy, and from non-PUVA-treated patients with vitiligo and normal individuals on the growth of melanocytes in vitro. Results: Proliferation of melanocytes in serum collected after 4 months of PUVA therapy was on the average 3-fold greater than that in serum samples collected from the same patients prior to therapy with PUVA. This circulating growth factor was absent in serum samples of non-PUVA-treated patients with vitiligo and normal individuals.

-Borovansky J, Blasko M, Siracky J, Schothorst AA, Smit NPM, Pavel S.
Cytotoxic interactions of zn2+ in vitro: melanoma cells are more susceptible than melanocytes. Melanoma Res 7(6):449-453, 1997.

-Chen WC, Zouboulis CC, Fritsch M, Blumepeytavi U, Kodelja V, Goerdt S, Luuthe V, Orfanos CE.
Evidence of heterogeneity and quantitative differences of the type 1 5 alpha-reductase expression in cultured human skin cells -evidence of its presence in melanocytes. J Invest Dermatol 110(1):84-89, 1998.

-Hedley SJ, Gawkrodger DJ, Weetman AP, Macneil S.
Alpha-msh and melanogenesis in normal human adult melanocytes. Pigm Cell Res 11(1):45-56, 1998.
Commentary: The aim of this study was to establish media conditions in which to obtain a reproducible melanogenic response to alpha-MSH in these cells. Twenty-five media of varying mitogen composition were examined. In only one medium condition, where basic fibroblast growth factor (bFGF) was the sole mitogen present, alpha-MSH induced both an increase in dopa oxidase activity (at 48%) and in melanin content (of 283%).

-Henion PD, Weston JA.
Timing and pattern of cell fate restrictions in the neural crest lineage. Development 124(21):4351-4359, 1997.
Commentary: Distinct neurogenic and melanogenic sublineages were also present in the outgrowth population almost immediately, but melanogenic precursors dispersed from the neural tube only after many neurogenic precursors had already done so, A discrete fate-restricted neuronal precursor population was distinguished before entirely separate fate- restricted melanocyte and glial precursor populations were present, and well before initial neuronal differentiation.

-Im SB, Moro O, Peng FP, Medrano EE, Cornelius J, Babcock G, Nordlund JJ, Abdel-Malek ZA.
Activation of the cyclic amp pathway by alpha-melanotropin mediates the response of human melanocytes to ultraviolet b radiation. Cancer Res 58(1):47-54, 1998.

-Imokawa G, Higuchi K, Yada Y.
Purification and characterization of an allergy-induced melanogenic stimulating factor in brownish guinea pig skin. J Biol Chem. 273(3):1605-1612, 1998.
Commentary: We have demonstrated recently that phenylazonaphthol (PAN) allergy-induced hyperpigmentation in brownish guinea pig skin is associated with the concomitant appearance of a melanogenic soluble factor(s) that activates the intracellular signal transduction system, including phosphatidylinositol turnover subsequent to ligand-receptor binding in cultured guinea pig melanocytes. In cultured guinea pig melanocytes, this purified PIMSF (the PAN-induced melanogenic stimulating factor) had the potential of activating an intracellular signal transduction system such as inositol 1,4,5-trisphosphate formation and intracellular calcium levels through a pertussis toxin- sensitive G protein-coupled receptor.

-Ivanova K, Lepoole IC, Gerzer R, Westerhof W, Das PK.
Effects of nitric oxide on the adhesion of human melanocytes to extracellular matrix components. J Pathol 183(4):469-476, 1997.

-Kang HY, Kang WH, Lee CO.
Endothelin-b receptor-mediated ca2+ signaling in human melanocytes. Pflugers Arch-Eur J Physiol 435(3):350-356, 1998.

-Sviderskaya EV, Novak EK, Swank RT, Bennett DC.
The murine misty mutation: phenotypic effects on melanocytes, platelets and brown fat. Genetics 148(1):381-390, 1998.
Commentary: These results suggest that in cultured human melanocytes the binding of ET-1 to ET-B receptors and the subsequent activation of PLC mediate ET-1- induced [Ca2+](i) increase, The transient [Ca2+](i) increase is attributed to mobilization of Ca2+ from inositol 1,4,5-trisphosphate-sensitive intracellular Ca2+ stores, and the sustained [Ca2+](i) level may be related to the influx of extracellular Ca2+.

-Kippenberger, S., Bernd, A., Bereiterhahn, J., Ramirezbosca, A., and Kaufmann, R.
The mechanism of melanocyte dendrite formation: the impact of differentiating keratinocytes. Pigm Cell Res 11(1):34-37, 1998.

Commentary: Time lapse studies revealed that only differentiated keratinocytes enhance melanocyte dendricity. Differentiated keratinocytes form connected cell sheets, which attach to part of the melanocyte plasma membrane. By contraction and retraction of keratinocyte units, new dendrites were drawn out from the melanocytes.

-Korchev YE, Milovanovic M, Bashford CL, Bennett DC, Sviderskaya EV, Vodyanoy I, Lab MJ.
Specialized scanning ion-conductance microscope for imaging of living cells. J Microsc-Oxford 188(:Part 1):Part 1):17-23, 1997.

-Lakshmi MS, Parker C, Sherbet GV.
Expression of the transmembrane glycoprotein cd44 and metastasis associated 18a2/mts1 gene in b16 murine melanoma cells. Anticancer Res 17(5A):3451-3455, 1997.
Commentary: The 18A2/mtsl gene, which codes for a Ca2+- binding protein of the S-100 family, is a metastasis associated gene and its expression has been shown to be related to cell proliferation, cancer metastasis and invasion. The association of 18A2/mts1 expression with invasion has been attributed to its ability to promote depolymerisation of cytoskeletal elements and it appears to also participate in the remodelling of the extracellular matrix. We found that metastatic potential was not related to the overall CD44 expression. In Fl cells treated with a- MSH where 18A2/mtsl expression was up-regulated, CD44v6 showed redistribution into a patchy focal pattern. It is postulated that this induction of patching could provide discrete and strong adhesive foci promoting cell adhesion and invasive behaviour.

-Nakajima M, Shinoda, I., Mikogami, T., Iwamoto, H., Hashimoto, S.I., Miyauchi, H., Fukuwatari, Y., and Hayasawa, H. Beta-lactoglobulin suppresses melanogenesis in cultured human melanocytes. Pigm Cell Res 10(6):410-413, 1997.

-Nakajima M, Shinoda I, Fukuwatari Y, Hayasawa H.
Arbutin increases the pigmentation of cultured human melanocytes through mechanisms other than the induction of tyrosinase activity. Pigm Cell Res. 11(1):12-17, 1998.

-Nakazawa K, Nakazawa H, Sahuc F, Lepavec A, Collombel C, Damour O.
Pigmented human skin equivalent: new method of reconstitution by grafting an epithelial sheet onto a non-contractile dermal equivalent. Pigm Cell Res. 10(6):382-390, 1997.

-Ozeki H, Ito S., Wakamatsu, K., and Ishiguro, I. Chemical characterization of pheomelanogenesis starting from dihydroxyphenylalanine or tyrosine and cysteine. effects of tyrosinase and cysteine concentrations and reaction time. Bba-Gen Subjects 1336(3):539-548, 1997.

-Roguet R, Schaefer H.
Overview of in vitro cell culture technologies and pharmaco-toxicological applications. Toxicol In Vitro. 11(5):591-599, 1997.

-Scott G, Cassidy L, Abdel-Malek, Z.
Alpha-melanocyte-stimulating hormone and endothelin-1 have opposing effects on melanocyte adhesion, migration, and pp125(fak) phosphorylation. Exp Cell Res. 237(1):19-28, 1997.
Commentary: These data indicate that while alpha-MSH and ET-I act synergistically to modulate melanocyte proliferation, they have opposite effects on melanocyte-matrix interactions.

-Shoji T, Park HY, Jalbert N, Bhawan J, Byers HR.
In situ and in vitro expression of protein kinase c alpha in human melanocytes. Pigm Cell Res. 11(1):18-23, 1998.

-Sieberblum M, Zhang J.M.
Growth factor action in neural crest cell diversification. J Anat. 191 (Part 4):493-499, 1997.
Commentary: Stem cell factor (SCF) is trophic for pluripotent neural crest cells. Contrary to expectation, SCF plus a neurotrophin, rather than SCF alone, is trophic for committed melanogenic cells. Basic fibroblast growth factor (bFGF) is mitogenic both for pluripotent cells and committed melanogenic cells. However, the cells become dependent on another factor for survival. Whereas any neurotrophin tested can rescue bFGF-activated pluripotent neural crest cells, the factor that rescues melanogenic cells remains to be determined.

-Smit NPM, Vandermeulen H, Koerten HK, Kolb RM, Mommaas AM, Lentjes EGWM, Pavel S.
Melanogenesis in cultured melanocytes can be substantially influenced by l-tyrosine and l-cysteine. J Invest Dermatol 109(6):796-800, 1997.

-Wagner M, Bielby S, Rennie IG, Macneil S.
Attachment of human uveal melanocytes and melanoma cells to extracellular matrix proteins involves intracellular calcium and calmodulin. Melanoma Res. 7(6):439-448, 1997.
Commentary: We noted that the intracellular free calcium in melanoma cells was less than half that seen in melanocytes. Fibronectin, laminin and Arg-Gly-Asp (RGD) were all capable of acutely increasing the intracellular free calcium in both cells.

-Wenczl E, Pool S, Timmerman AJ, Vanderschans GP, Roza L, Schothorst AA.
Physiological doses of ultraviolet irradiation induce dna strand breaks in cultured human melanocytes, as detected by means of an immunochemical assay. Photochem Photobiol. 66(6):826-830, 1997.

-Yaar M, Zhai S, Pilch PF, Doyle SM, Eisenhauer PB, Fine RE, Gilchrest BA.
Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis - a possible mechanism for alzheimer's disease. J Clin Invest. 100(9):2333-2340, 1997.

3. MSH, MCH, other hormones, differentiation

-Graham A, Wakamatsu K, Hunt G, Ito S, Thody AJ.
Agouti protein inhibits the production of eumelanin and phaeomelanin in the presence and absence of alpha-melanocyte stimulating hormone. Pigment Cell Res. 10(5):298-303, 1997.

-Knigge KM, Wagner JE.
Melanin-concentrating hormone (MCH) involvement in pentylenetetrazole (PTZ)-induced seizure in rat and guinea pig. Peptides. 18(7):1095-7, 1997.

-Lakshmi MS, Parker C, Sherbet GV.
Expression of the transmembrane glycoprotein CD44 and metastasis associated 18A2/MTS1 gene in B16 murine melanoma cells. Anticancer Res. 17(5A):3451-5, 1997.

-Masini MA, Sturla M, Pestarino M, Gallinelli A, Facchinetti F, Uva BM.
Pro-opiomelanocortin (POMC) expression and immunolocalization of POMC-related peptides in the ovary of Protopterus annectens, an African lungfish. Peptides. 18(9):1411-4, 1997.

-Nascimento AA, Amaral RG, Bizario JC, Larson RE, Espreafico EM.
Subcellular localization of myosin-V in the B16 melanoma cells, a wild-type cell line for the dilute gene. Mol Biol Cell. 8(10):1971-88, 1997.

-Scott G, Cassidy L, Abdel-Malek Z.
Alpha-melanocyte-stimulating hormone and endothelin-1 have opposing effects on melanocyte adhesion, migration, and pp125FAK phosphorylation. Exp Cell Res. 237(1):19-28, 1997.

-Sungbin Im, Osamu Moro, Fuping Peng, Estela E. Medrano, J. Cornelius, G. Babcock, J.J. Nordlund, Z.A. Abdel-Malek.
Activation of the Cyclic AMP pathway by α-melanotropin mediates the response of human melanocytes to ultraviolet B radiation. Cancer Research, 58:47-54, 1998.

-Wakamatsu K, Graham A, Cook D, Thody AJ.
Characterisation of ACTH peptides in human skin and their activation of the melanocortin-1 receptor. Pigment Cell Res. 10(5):288-97, 1997.

-Wong KY, Rajora N, Boccoli G, Catania A, Lipton JM.
A potential mechanism of local anti-inflammatory action of alpha-melanocyte-stimulating hormone within the brain: modulation of tumor necrosis factor-alpha production by human astrocytic cells. Neuroimmunomodulation. 4(1):37-41, 1997.

-Yan L, Zhu X, Tseng JL, Desiderio DM.
Beta-endorphin-containing proteins in the human pituitary. Peptides. 18(9):1399-409, 1997.

4. Photobiology and photochemistry

(Dr M. d'Ischia)

The role of melanin in skin photoprotection and the mechanisms of UV-induced melanogenesis are currently very actively investigated in various laboratories. A modified immunochemical assay, i.e. sandwich enzyme-linked immunosorbent assay, has been studied by Wenczl et al. to detect UV-induced damage in cellular DNA of monolayer-grown human melanocytes. The results from melanocytes expressed in ssb/Da DNA are shown to be comparable and to have the same sensitivity toward UVA as well as toward UVB as nonpigmented skin cells, and support the potential of the assay for further investigations about the photoprotecting and/or photosensitizing effects of melanins in human melanocytes from different skin types. The central role of alpha-melanotropin-induced cAMP in mediating UV-stimulated melanogenesis is demonstrated by Im et al., who report that stimulation of cAMP formation is obligatory for the melanogenic response of cultured normal human melanocytes to UVB radiation, since in the absence of cAMP inducers, UVB radiation inhibits

melanogenesis. The possible role of lipids in skin photoprotection was addressed by Lasch et al., who found that sterols, mainly free cholesterol, with their high concentration in the lipid barrier of the stratum corneum can effectively compete with the peroxidation of other human skin lipids (ceramides and free fatty acids), thus contributing to the skin defense mechanisms in addition to melanin.
The relationship between the UV sensitivity and constitutive skin pigmentation and thickness of the stratum corneum was investigated by Lock-Andersen J, et al. in 34 normal people and in 39 skin cancer patients (20) patients with cutaneous malignant melanoma and 19 patients with basal cell carcinoma of the skin. Epidermal thickness was independent of skin type and was not correlated to constitutive skin pigmentation. The most interesting finding was that stratum corneum thickness is of minor importance for the constitutive UV sensitivity, which was mainly determined by skin pigmentation.
Finally, the ability of synthetic and biological melanins to mediate electron transfer between hydroxybenzene donors (tyrosine, dopa, chemical depigmenters) and model acceptors (ferricyanide, tyrosinase) was investigated by Menter et al., while Wroebel et al. made an elegant study of the interaction of synthetic dopa melanin with porphyrins with a variety of spectroscopic techniques.

-Im S, Moro O, Peng F, Medrano EE, Cornelius J, Babcock G, Nordlund JJ, Abdel-Malek ZA.
Activation of the cyclic AMP pathway by alpha-melanotropin mediates the response of human melanocytes to ultraviolet B radiation. Cancer Res. 58(1): 47-54, 1998.

-Lasch J, Schonfelder U, Walke M, Zellmer S, Beckert D.
Oxidative damage of human skin lipids. Dependence of lipid peroxidation on sterol concentration. Biochim Biophys Acta. 15;1349(2): 171-181, 1997.

-Lock-Andersen J, et al.
Epidermal thickness, skin pigmentation and constitutive photosensitivity.
Photodermatol Photoimmunol Photomed. 13(4): 153-158, 1997.

-Menter JM, Willis I.
Electron transfer and photoprotective properties of melanins in solution. Pigment Cell Res. 10(4): 214-217, 1997.

-Wenczl E, et al.
Physiological doses of ultraviolet irradiation induce DNA strand breaks in cultured human melanocytes, as detected by means of an immunochemical assay. Photochem Photobiol. 66(6): 826-830, 1997.

-Wrobel D, Planner A, Hanyz I, Wielgus A, Sarna T.
Melanin-porphyrin interaction monitored by delayed luminescence and photoacoustics. J Photochem Photobiol B. 41(1-2): 45-52, 1997.

5. Neuromelanins

(Dr. M. d'Ischia)

Of the many research papers relating to neuromelanin that appeared in the last few months, two seem especially worthy of note. One by Tief et al. deals with the identification of tyrosinase promoter activity in cortex, olfactory system, hippocampus, epithalamus and substantia nigra in adult brain. The Swiss authors also found expression in several locations of developing forebrain and midbrain during embryogenesis. If confirmed in further studies, these results would definitively settle the longlasting and controversial issue of brain tyrosinase and would open interesting perspectives in the understanding of the mechanisms of neuromelanin formation in the substantia nigra also in relation to neurodegenerative disorders like Parkinson's disease.
The other paper, by Offen et al. is a continuation of previous studies by the same group on the function of neuromelanin within the dopamine-producing cells in the human and primate substantia nigra. Following exposure of PC12 cells to synthetic dopamine melanin, the authors found 50% cell death with the typical apoptotic DNA ladder, indicating inter-nucleosomal DNA degradation. The contribution of oxidative stress in this model of dopamine-melanin-induced cell death was also examined and the results indicated enhanced toxicity in the presence of iron and limited inhibition by antioxidants like reduced glutathione (GSH), N-acetyl cysteine, catalase and Zn/Cu superoxide dismutase (SOD). Although the authors conclude that neuromelanin may play a role in the selective vulnerability of dopaminergic neurons in Parkinson's disease, it is clear that the validity of this conclusion depends to a considerable extent on the suitability of dopamine melanin as a model of intraneuronal neuromelanin.
Other papers include a study on the role of dopa as a substrate of tyrosine hydroxylase (by Haavik) and two reviewing articles dealing with the role of peroxidase in neuromelanin synthesis, by Okun, and a survey of current views on the origin, structure and function of neuromelanin and its role in relation to neuronal degeneration, by d'Ischia and Prota.

-d'Ischia M, Prota G.
Biosynthesis, structure, and function of neuromelanin and its relation to Parkinson's disease: a critical update.
Pigment Cell Res. 10(6): 370-376, 1997. Review.

-Haavik J.
L-DOPA is a substrate for tyrosine hydroxylase. J Neurochem. 69(4): 1720-1728, 1997.

-Offen D, Ziv I, Barzilai A, Gorodin S, Glater E, Hochman A, Melamed E.
Dopamine-melanin induces apoptosis in PC12 cells; possible implications for the etiology of Parkinson's disease. Neurochem Int. 31(2): 207-216, 1997.

-Okun MR.
The role of peroxidase in neuromelanin synthesis: a review. Physiol Chem Phys Med NMR. 29(1): 15-22, 1997. Review.

-Smythies JR.
Oxidative reactions and schizophrenia: a review-discussion. Schizophr Res. 24(3): 357-364, 1997. Review.

-Tief K, Schmidt A, Beerman F.
New evidence for presence of tyrosinase in substantia nigra, forebrain and midbrain. Brain Res Mol Brain Res. 53(1-2): 308-311, 1998.

6. Genetics, molecular biology

(Dr. F. Beermann)

-April CS, Jackson IJ, Kidson SH.
The cloning and sequencing of a cDNA coding for chick tyrosinase-related protein-1. Biochimica et Biophysica Acta 1395(1):7-12, 1998.

-Bertolotto C, Busca R, Abbe P, Bille K, Aberdam E, Ortonne JP, Ballotti R.
Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP: pivotal role of M boxes (GTCATGTGCT) and of Microphthalmia. Molecular & Cellular Biology 18(2):694-702, 1998.

-Box NF, Wyeth JR, Mayne CJ, Ogorman LE, Martin NG, Sturm RA.
Complete sequence and polymorphism study of the human Tyrp1 gene encoding tyrosinase-related protein 1. Mammalian Genome 9(1):50-53, 1998.

-Box NF, Wyeth JR, Ogorman LE, Martin NG, Sturm RA.
Characterization of melanocyte stimulating hormone receptor variant alleles in twins with red hair. Human Molecular Genetics 6(11):1891-1897, 1997.

-Brouwenstijn N, Slager EH, Bakker A, Schreurs M, Vanderspek CW, Adema GJ, Schrier PI, Figdor CG.
Transcription of the gene encoding melanoma-associated antigen gp100 in tissues and cell lines other than those of the melanocytic lineage. British Journal of Cancer 76(12):1562-1566, 1997.

-Caracciolo A, Gesualdo I, Branno M, Aniello F, Dilauro R, Palumbo A.
Specific cellular localization of tyrosinase mRNA during Ciona intestinalis larval development. Development Growth & Differentiation 39(4):437-444, 1997.

-Chin L, Pomerantz J, Polsky D, Jacobson M, Cohen C, Cordoncardo C, Horner JW, Depinho RA.
Cooperative effects of INK4a and Ras in melanoma susceptibility in vivo. Genes & Development 11(21):2822-2834, 1997.
Summary: The authors generated transgenic mice which express an activated Ha-ras transgene regulated by the tyrosinase enhancer-promoter. These transgenic mice were crossed to mice deficient for the tumor suppressor gene INK4a (MTS1/ CDKN2/ p16). On a INK4a -/- deficient background, tumors arise after short latency and with high penetrance. In melanomas occurring on a INK4a +/- heterozygous background, the wildtype allele was consistently lost, as observed in human melanomas. The results show that loss of INK4a and activation of Ras can cooperate in melanoma development. They provide experimental evidence for a causal relationship between INK4a deficiency and melanoma.

-Diaz RM, Eisen T, Hart IR, Vile RG.
Exchange of viral promoter/enhancer elements with heterologous regulatory sequences generates targeted hybrid long terminal repeat vectors for gene therapy of melanoma. Journal of Virology 72(1):789-795, 1998.

-Fukuzawa T, Okumoto H, Nishioka M.
The site and time of expression of MIF in frog development. Pigment Cell Research 10(6):401-409, 1997.

-Hemesath TJ, Price ER, Takemoto C, Badalian T, Fisher DE.
MAP kinase links the transcription factor Microphthalmia to c-kit signalling in melanocytes. Nature

391(6664):298-301, 1998.
Abstract: Germline mutations at loci encoding the transcription factor Microphthalmia (Mi), the cytokine receptor c-Kit, or its ligand Steel factor (Sl) result in strikingly similar defects in mast cell and melanocyte development(1-3). Here we describe a biochemical link between Kit signalling and the activity of Mi. Stimulation of melanoma cells with Sl results in activation of MAP kinase, which in turn phosphorylates Mi at a consensus target serine. This phosphorylation upregulates Mi transactivation of the tyrosinase pigmentation gene promoter. In addition to modulating pigment production, such signalling may regulate the expression of genes essential for melanocyte survival and development. The pathway represents a new application of the general MAP kinase machinery in transducing a signal between a tissue-specific receptor at the cell surface and a tissue-specific transcription factor in the nucleus.

-Hyodo-Taguchi Y, Winkler C, Kurihara Y, Schartl A, Schartl M.
Phenotypic rescue of the albino mutation in the medakafish (Oryzias latipes) by a mouse tyrosinase transgene. Mechanisms of Development 68(1-2):27-35, 1997.
Summary: A mouse tyrosinase minigene (as used to complement murine albinism; see EMBO J, 9, 2819, 1990) was used to rescue the albino mutation in Medakafish. The rescue was not possible in b mutant fish. The authors place the i-3 locus upstream and the b locus downstream of the tyrosinase locus i-1 in the genetic hierarchy leading to wildtype pigmentation.

-Koga A, Hori H.
Albinism due to transposable element insertion in fish. Pigment Cell Research 10(6):377-381, 1997.

-Lee ST, Park SK, Lee H, Lee JS, Park YW.
DNA-based prenatal diagnosis of a Korean family with tyrosinase-related oculocutaneous albinism (OCA1). Japanese Journal of Human Genetics 42(4):499-505, 1997.

-Liu ZZ, Zhu LQ, Eide FF.
Critical role of trkb and brain-derived neurotrophic factor in the differentiation and survival of retinal pigment epithelium. Journal of Neuroscience 17(22):8749-8755, 1997.

-Mackenzie M, Jordan SA, Budd PS, Jackson IJ.
Activation of the receptor tyrosine kinase kit is required for the proliferation of melanoblasts in the mouse embryo. Developmental Biology 192(1):99-107, 1997.
Summary: The authors have generated transgenic mice carrying Dct/lacZ (TRP-2/lacZ) fusion genes. During development, they observe lacZ expression in the pigment epithelium of the retina, in melanoblasts/melanocytes and in the telencephalon, as reported earlier for Dct protein and mRNA. Using Dct/lacZ as a marker for the melanocyte lineage, they crossed the transgene into kit mutant mice (kit-W-v) to identify stages where melanoblasts rely on kit signalling. They conclude that survival of melanoblasts depends upon kit signalling up until E11.

-Mahalingam H, Watanabe A, Tachibana M, Niles RM.
Characterization of density-dependent regulation of the tyrosinase gene promoter - role of protein kinase C. Experimental Cell Research 237(1):83-92, 1997.

-Mochii M, Mazaki Y, Mizuno N, Hayashi H, Eguchi G.
Role of Mitf in differentiation and transdifferentiation of chicken pigmented epithelial cell. Developmental Biology 193(1):47-62, 1998.
Summary: The authors report on isolation of the chicken homologue of Mitf, which is predominantly expressed in the embryonic retinal pigment epithelium (RPE). Overexpression of Mitf within the RPE inhibits dedifferentiation and transdifferentiation as induced for example by bFGF. Mitf overexpression led to hyperpigmentation and induced expression of tyrosinase (and mmp115) and inhibited expression of Pax6.

-Nakayama A, Nguyen M, Chen CC, Opdecamp K, Hodgkinson CA, Amheiter H.
Mutations in Microphthalmia, the mouse homolog of the human deafness gene Mitf, affect neuroepithelial and neural crest-derived melanocytes differently. Mechanisms of Development 70(1-2):155-166, 1998.
Shortened abstract: Mitf expression in neuropithelium and neural crest precedes that of the melanoblast marker Dct, is then co-expressed with Dct, and gradually fades away except in cells in hair follicles. In embryos with severe Mitf mutations, neural crest-derived Mitf-expressing cells are rare, lack Dct expression, and soon become undetectable. In contrast, the neuroepithelial-derived Mitf-expressing cells of the retinal pigment layer are retained, express Dct, but not the melanogenic enzyme genes tyrosinase and Tyr1, and remain unpigmented. The results show that melanocyte development critically depends on functional Mitf and that Mitf mutations affect the neural crest and the neuroepithelium in different ways.

-Nicoletti A, Kawase K, Thompson DA.
Promoter analysis of RPE65, the gene encoding a 61-kDa retinal pigment epithelium-specific protein. Investigative Ophthalmology & Visual Science 39(3):637-644, 1998.

-Ollmann MM, Lamoreux ML, Wilson BD, Barsh GS.
Interaction of agouti protein with the melanocortin 1 receptor in vitro and in vivo. Genes & Development 12(3):316-330, 1998.

-Schreurs M, Deboer AJ, Schmidt A, Figdor CG, Adema GJ.
Cloning, expression and tissue distribution of the murine homologue of the melanocyte lineage-specific antigen gp100. Melanoma Research 7(6):463-470, 1997.

-Shin MK, Russell LB, Tilghman SM.
Molecular characterization of four induced alleles at the EDNRB locus. Proceedings of the National Academy of Sciences of the United States of America 94(24):13105-13110, 1997.

-Shioda T, Fenner MH, Isselbacher KJ.
MSG1 and its related protein MRG1 share a transcription activating domain. Gene 204(1-2):235-241, 1997.

-Shoji T, Park HY, Jalbert N, Bhawan J, Byers HR.
In situ and in vitro expression of protein kinase C alpha in human melanocytes. Pigment Cell Research 11(1):18-23, 1998.

-Southardsmith EM, Kos L, Pavan WJ.
Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. Nature Genetics 18(1):60-64, 1998.

-Steingrimsson E, Favor J, Ferredamare AF, Copeland NG, Jenkins NA.
Mitf(mi-enu122) is a missense mutation in the HLH dimerization domain. Mammalian Genome 9(3):250-252, 1998.

-Sviderskaya EV, Novak EK, Swank RT, Bennett DC.
The murine misty mutation - phenotypic effects on melanocytes, platelets and brown fat. Genetics 148(1):381-390, 1998.

-Tief K, Schmidt A, Beermann F.
New evidence for presence of tyrosinase in substantia nigra, forebrain and midbrain. Molecular Brain Research 53(1-2):307-310, 1998.

-Watanabe A, Takeda K, Ploplis B, Tachibana M.
Epistatic relationship between Waardenburg syndrome genes Mitf and Pax3. Nature Genetics 18(3):283-286, 1998.

8. Melanoma and other pigmented tumours

Experimental melanoma therapy (Dr. N. Smit)

9-acetoxy-2,7,12,17-tetrakis-(beta-methoxyethyl)- porphycene (ATMPn) and Si(IV)-methoxyethylene-glycol-naphthalocyanine (SiNc) were used in photodynamic therapy (PDT) in the studies by Abels et al and Mantareva et al. The use of the synthetic porphycene was reasonably successful for the PDT of melanoma tumors. PDT using SiNc only showed minor effects on tumour growth of B16 melanoma in C57Bl/6 mice whereas an efficient tumour response was obtained for Lewis lung carcinoma. In the study by Palumbo et al the selective incorporation of thiourea into melanotic melanoma was investigated. Thiourea inhibited the formation of dopachrome from dopa in the presence of tyrosinase and a 1:1 dopa-thiourea adduct was identified. The effect of thiourea on the oxidative polymerization of indolic precursors of melanin was much less and the incorporation of thiourea into dopa melanins was found to be the most significant. Rodriguez-Vicente et al report on the toxicity of antineoplastic agents and 4-hydroxyanisole (4HA) in three melanoma cell lines in two different papers.
As compared to melphalan, lomustine and fotemustine, 4HA was the least effective drug. Nevertheless its effect could be increased by BSO and was related to the dopa-oxidase activities in the cells.

-Abels C, Szeimies RM, Steinbach P, Richert C, Goetz AE.
Targeting of the tumor microcirculation by photodynamic therapy with a synthetic porphycene. J Photochem Photobiol B-Biol. 40(3):305-312, 1997.

-Blackstaffe L, Shelley MD, Fish RG.
Cytotoxicity of gossypol enantiomers and its quinone metabolite gossypolone in melanoma cell lines. Melanoma Res. 7(5):364-372, 1997.

-Braslavsky SE, Muller M, Martire DO, Porting S, Bertolotti SG, Chakravorti S, Kocweier G, Knipp B, Schaffner K.
Photophysical properties of porphycene derivatives (18 pi porphyrinoids). J Photochem Photobiol B-Biol. 40(3):191-198, 1997.

-Citro G, Dagnano I, Leonetti C, Perini R, Bucci B, Zon G, Calabretta B, Zupi G.

C-myc antisense oligodeoxynucleotides enhance the efficacy of cisplatin in melanoma chemotherapy in vitro and in nude mice. Cancer Res. 58(2):283-289, 1998.

-Greene JF, Morgan CD, Rao A, Amoss MS, Arguello F.
Regression by differentiation in the sinclair swine model of cutaneous melanoma. Melanoma Res. 7(6):471-477, 1997.

-Hagiwara A, Sawai K, Sakakura C, Shirasu M, Ohgaki M, Imanishi T, Yamasaki J, Togawa T, Takahashi T.
Prevention of peritoneal metastasis of cancer with dextran sulfate - an experimental study in mice. Anti-Cancer Drug. 8(9):894-897, 1997.

-Hersh EM, Stopeck AT.
Recent advances in the treatment of malignant melanoma with gene therapy. Mol Med. 3(10):636-651, 1997.

-Igarashi Y.
Functional roles of sphingosine, sphingosine 1-phosphate, and methylsphingosines: in regard to membrane sphingolipid signaling pathways. J Biochem Tokyo. 122(6):1080-1087, 1997.
Commentary: Recent findings on the functional roles of sphingolipids are described focusing on (i) functional roles of sphingosine 1-phosphate in cell motility regulation and platelet activation (ii) involvement of sphingosine in cell signaling (iii) effects of methylsphingosines in cancer cell apoptosis induction and in the regulation of inflammatory processes. Based upon these findings from our studies and others, the perspective of future sphingosine research (sphingology or sphingophysiology) is briefly discussed.

-Larsen RH, Akabani G, Welsh P, Zalutsky MR.
The cytotoxicity and microdosimetry of astatine-211- labeled chimeric monoclonal antibodies in human glioma and melanoma cells in vitro. Radiat Res. 149(2):155-162, 1998.

-Lartigau E, Randrianarivelo H, Avril MF, Margulis A, Spatz A, Eschwege F, Guichard M.
Intratumoral oxygen tension in metastatic melanoma. Melanoma Res. 7(5):400-406, 1997.
Commentary: Thus a decrease in PO2 values, probably corresponding to tumour hypoxia, was found in most of the metastatic tumours when compared with normal tissues. The prognostic value of these PO2 measurements in melanoma remains to be demonstrated in the tumour response to radiotherapy or alkylating agents. However, tumour hypoxia can already be investigated as a target for new treatment modalities in metastatic melanoma.

-Mantareva V, Shopova M, Spassova G, Wohrle D, Muller S, Jori G, Ricchelli F.
Si(iv)-methoxyethylene-glycol-naphthalocyanine: synthesis and pharmacokinetic and photosensitizing properties in different tumour models. J Photochem Photobiol B-Biol. 40(3):258-262, 1997.

-Palumbo A, Mars U, Demartino L, D'Ischia M, Napolitano A, Larsson BS, Prota G.
Selective incorporation of the prototype melanoma seeker thiourea into nascent melanin: a chemical insight. Melanoma Res. 7(6):478-485, 1997.

-Perng YP, Lin CC, Perng IM, Shen YC, Chuang CK, Liao SK.
Culture medium induced morphological changes of melanoma cells associated with change in sensitivity to lysis by lymphokine-activated killer cells. Cancer Biother Radiopharm. 12(5):317-331, 1997.
Commentary: Taken together the results demonstrate the presence of heterogeneous subpopulations within the CaCL 73-36 melanoma cell line regarding their pigmentary status, antigenic profile, growth pattern and responsiveness to NK/LAK cytolysis. The results also call attention to the importance of utilizing a same medium in short-and long-term cultures of melanomas for biological studies and response evaluations of therapeutic agents such as LAK cells, when multiple cell targets from different patients or multi-metastatic cell lines from individual patients are to be compared Finally, these melanoma sublines may be valuable for further elucidation of the relationship between MHC expression, and increased sensitivity to LAK cytolysis, and the role of the components of DMEM in the mechanism for the observed induction of cell differentiation and enhanced LAK cytolysis.

-Porgador A, Mandelboim O, Restifo NP, Strominger JL.
Natural killer cell lines kill autologous beta(2)-microglobulin-deficient melanoma cells: implications for cancer immunotherapy. Proc Natl Acad Sci Usa. 94(24):13140-13145, 1997.
Commentary: We show that beta(2)-microglobulin-deficient class I-negative melanoma variants derived from patients undergoing specific T cell therapy are lysed by heterologous as well as autologous natural killer (NK) lines and clones, but not by specific T cells. Adoptive autologous NK therapy may be an important supplement to consider in the design of new cancer immunotherapies.

-Reisfeld RA, Becker JC, Gillies SD.
Immunocytokines: a new approach to immunotherapy of melanoma. Melanoma Res. 7(:Suppl 1994;2):Suppl 1994;2):S99-S106, 1997.
Commentary: Targeted interleukin-2 (IL-2) therapy with immunocytokines (i.e. antibody-cytokine fusion proteins) is effective in eradicating established hepatic and pulmonary metastases of melanoma in animal model systems. The results

demonstrate the ability of immunocytokines to induce a T-cell-dependent host immune response capable of eradicating established melanoma metastases in clinically relevant organs and offers an effective, new tool for immunotherapy of malignant melanoma.

-Rodriguezvicente J, Vicenteortega V, Canterasjordana M, Calderonrubiales F.
Relationship between 4-hydroxyanisole toxicity and dopa oxidase activity for three melanoma cell limes. Melanoma Res 7(5):373-381, 1997.

-Rodriguezvicente J, Vicenteortega V, Canterasjordana M.
The effects of different antineoplastic agents and of pretreatment by modulators on three melanoma lines. Cancer 82(3):495-502, 1998.

-Rols MP, Delteil C, Golzio M, Dumond P, Cros S, Teissie J.
In vivo electrically mediated protein and gene transfer in murine melanoma. Nat Biotechnol. 16(2):168-171, 1998.
Commentary: It is shown that efficient permeabilization of murine melanoma can be obtained in vivo by applying electric pulses. More than 80% of the cell population is affected as shown by the penetration of propidium iodide.

-Uno T, Chen PW, Murray TG, Podack ER, Ksander BR.
Gene transfer of the cd80 costimulatory molecule into ocular melanoma cells using a novel episomal vector. Invest Ophthalmol Visual Sci 38(12):2531-2539, 1997.
Commentary: The B45-Neo episomal vector induces stable expression of the CD80 costimulatory molecule on ocular melanoma cells. Our results indicate that this vector is suitable for experiments designed to genetically engineer ocular melanoma cells to stimulate CD8(+) T cells.

-Wang RF.
Tumor antigens discovery: perspectives for cancer therapy. Mol Med 3(11):716-731, 1997.
Commentary: To understand the molecular basis of T cell-mediated antitumor immunity, several groups started to search for such tumor antigens in melanoma as well as in other types of cancers. This led to the subject reviewed in this article. The current status and progress toward identifying human tumor antigens and their potential applications to cancer treatment are summarized.

ANNOUNCEMENTS & RELATED ACTIVITIES

The ESPCR is delighted to welcome the following colleagues to membership and hope that they will play a full and active part in the Society.

Dr. Enikö WENCZL
Dept of Dermatology
University Hospital Leiden
P.O. Box: 9600
NL- 2300 RC LEIDEN

Dr. Patrick VERRANDO
Laboratoire d'investigation des
Maladies de la peau
46, Bd. de la Gaye
F- 13009 MARSEILLE

Calendar of events:

Also available in more details from address: http://www.ulb.ac.be/medecine/loce/espcr.htm

1998 **1st International Symposium on Melanoma, Rome, Italy, June 18 - 20**

Contact: B. Serra CARACCIOLO, G. ALEO, E. Del COCO, A. Di BONA
Ufficio Congressi IDI - IDI Congress Office
IDI - Via dei Monti di Creta, 104
I- 00167 Rome
Phone: 39.6.6646.4488
Fax: 39.6.6646.4483
E-Mail: congressi@idi.it

1998 **8th ESPCR Meeting: Prague, 23 - 26 September**

Contact: KAHLEN spol. s r.o.
Vlkova 24
Czech republic- 130 00 Praha 3
Phone: 00420-2-671 953 02
Fax: 00420-2-671 953 04
E-mail: kahlen@kahlen.cz

1998 **Frontiers in Melanoma: Vienna, Austria, October 1 - 4**

Scientific and Administrative Secretariat
Vienna Academy of Postgraduate Medical
Education and Research
Alserstrasse 4
A- 1090 Vienna, Austria
Phone: 43/1-405-13-83-13
Fax: 43/1-405-13-83-23
E-mail: medacad@via.at
Internet:http://www.via.at/medacad

1998 **VIII Meeting of European Immunodermatology Society: Rome, Italy, November 19 - 21**

Contact: Triumph P.R. S.r.l.
Via Proba Petronia, 3
I- 00136 Roma
Phone: 06.39727707
Fax: 06.39735195
E-mail: triumph@tin.it

1999 XVIIth International Pigment Cell Conference: Nagoya Congress Center, Japan, October 30 - November 3

Contact: Kazumasa WAKAMATSU, Ph.D.
Secretary-General, IPCC - Nagoya
Fujita Health University School of Health Sciences
J- Toyoake, Aichi 470-1192
Phone: 81-562-93-2518
Fax: 81-562-93-4595
E-mail: kwaka@fujita-hu.ac.jp

2000 9th ESPCR Meeting: Krakow

Contact: Dr T. SARNA
Jagellonian University
Al. Mickiewicza 3
Poland- 31-120 Krakow
Phone: 48-12-342008 (direct) or 48-12-341305 (switchboard)
Fax: 48-12-336907
E-mail: tsarna@mol.uj.edu.pl

New Web page

"For ESPCR members only"

in: http://www.ulb.ac.be/medecine/loce/espcr.htm

Dear Colleague,

A new web page dedicated to ESPCR members has been created to update you with specific information. For now: - download an Electronic Version of the Bulletin; - Photos taken at the last meeting in Bordeaux
However, this facility remains the privilege of ESPCR members and is consequently locked by a password.
Your password will be regularly sent to you to your personal E-Mail.
Please also note that you may obtain your passsword anytime by writing to: **gghanem@ulb.ac.be**

I hope you'll find this new facility both easy and helpful,
G. Ghanem, ESPCR Bulletin Editor

Call for E-Mail addresses

Dear Colleague,

In order to improve our service to you, your E-Mail address is a valuable tool to diffuse useful information very quickly.
PLEASE SEND a "Hello" to my E-Mail Address below and that's it. Thank you.

G. Ghanem, ESPCR Bulletin Editor
gghanem@ulb.ac.be

Press Release: 2nd February 1998
STIEFEL Research in Dermatology

Pseudocatalase

For the past 8 years, Stiefel has devoted a major portion of its research activities to a fundamental investigation of the basis of pigment formation and destruction with especial reference to Vitiligo. To this end we involved a number of leading investigators in this field notably in Canada, Germany, Netherlands and the U.K.
Over the last 18 months we have undertaken a multicentre double-blind placebo controlled clinical trial of Pseudocatalase, a formulation devised by Professors Schallreuter and Wood, now at Bradford University, for the treatment of Vitiligo. This has generated considerable interest amongst vitiligo sufferers around the world who are anxiously waiting to know the results.
We regret to say that the results of the trial were not as good as we had hoped and certainly not as good as those obtained by Professor Schallreuter in her original uncontrolled studies. We believe that in the main this was due to an ingredient which we felt necessary to add to the original formulation for good manufacturing practise reasons and which may have had an adverse effect on the Pseudocatalase moiety making it less active.
Our faith in Professor Schallreuter's original findings in such that we have now devised a new formulation without the additional ingredient and in much more patient friendly form. New clinical trials are about to start in a number of countries in Europe. North and South America and the Far East.
We trust that vitiligo sufferers will have still more patience and await the outcome of these new trials.
Even then, if they go successfully, there will be still a lot more work needed before a marketed product will be available.
We would like to take this opportunity to thank all those long suffering patients who volunteered for the trials and persevered with the treatment throughout the 12 months of the study.

THE PIGMENTARY SYSTEM

Physiology and Pathophysiology

Edited by **James J. Nordlund**, Professor and Chairman of the Department of Dermatology, University of Cincinnati College of Medicine, **Raymond Boissy**, Associate Professor of Dermatology, University of Cincinnati, **Vincent Hearing** , Senior Investigator, Laboratory of Cell Biology, National Cancer Institute, **Richard King**, Professor of Genetics, University of Minnesota, and **Jean-Paul Ortonne**, Doctor of Dermatology, Central Hospital of Nice, France.

- Contributions from 106 world-renowned experts for an international perspective
- Over 570 photographs, including three sections of 4 colour plates - the most comprehensible collection of pigment disorders photos available for clinicians
- Carefully links concepts of basic science to clinical diagnosis
- Covers normal as well as abnormal pigmentation (excluding melanoma)

Our knowledge about the function of the melanocyte has expanded beyond boundaries not previously imagined. The melanocyte is no longer considered merely a factory for the production of the pigment melanin. Old data not contradict the new but must be reinterpreted in light of modern concepts of molecular and cellular biology, enzymology, biochemistry, chemistry, and physics. Diseases of the pigmentary system must be understood in terms of modern science. The editors, each with special knowledge of the pigmentary system, have combined their expertise and talents to produce a book that will serve as the ultimate resource for the study of all aspects of pigment cell biology. There is no comprehensive, scholarly reference available which compiles both old and new data into a single source. This book fills that void. There are monographs to assist dermatologists caring for individuals with disorders of pigmentation, and textbooks with an introductory chapter on the physiology of pigmentation and a clinical chapter on the disorders manifested by common abnormalities of the pigmentary system. These resources continue to be invaluable, however they are written for a specific type and level of audience. This volume is encyclopaedic in scope, so that the biologist, chemist, cosmetic scientist, and clinician, whether novice or sophisticated expert, can peruse any section of the book with confidence that it contains most of the worlds knowledge on pigmentation, including historical work. The bibliographies are also prepared to be as comprehensive and all-inclusive as possible. The first part of the book brings together the molecular and cellular biology, biochemistry, chemistry , physics, and physiology of the normal melanocyte as known in the 1990s. The second part continues this theme, presenting a comprehensive discussion of most disorders of pigmentation described to date. Information about pathophysiology, treatment and other clinical data is included. The goal of the editors is to provide the ultimate reference for practising physicians who care for patients with the rarest or most common disorders of pigmentation, the laboratory scientist studying disease in order to help the study of basic processes which affect the pigmentary system, and the cosmetic scientist who seeks comprehensive information on the pharmacopoeia available for treating pigmentary disorders. All specialists interested in some aspect of the pigmentary system can seek current answers to questions related to their work.

Oxford Medical Publications
June 1998, 1168 pages, 574 halftones, 138 line figures

JOURNAL OF TUMOR MARKER ONCOLOGY
Volume 12, Number 4, Winter 1997
The International Academy of Tumor Marker Oncology Inc. Publishers

People in the News

Fritz Anders, the Awardee of the 1997 Prince Hitachi Prize for Comparative Oncology

Fritz Anders is one of those members of the International Academy of Tumor Marker Oncology who, together with John Klavins, Henry Lynch, Harris Busch, Peter Karlson, Andrew Schally, Robert Gallo and others, followed the call of the late Enrique Pimentel for a meeting on Human Oncology and Tumor Markers to Caracas, Venezuela, about 25 years ago. As a biologist searching for genes that code for neoplasia in plants and lower animals, especially in fish, he is a consequent participant of the annual IATMO meetings and is continuously contributing to ideas on the usefulness of oncodeterminants as tumor markers.

On May 27 this year he was awarded the prestigious Prince Hitachi Price for Comparative Oncology by the hands of Their Emperial Highnesses Prince and Princess Hitachi in Tokyo. The impressive ceremony was opened by the Emperial Message read by the Prince, and continued by messages from the members of the Japanese Foundation for Cancer Research, from the President of the International Union of Biological Sciences, Dr. Tokindo Okada, and from the Minister for Education, Science and Culture of Japan. The ceremony was extended by the Acceptance Address of the Awardee and his Awarding Lecture entitled „Contributions of Comparative Oncology to the Present Concept of Neoplasia". The following days were filled with special lectures and seminars given by Fritz Anders and his wife, Annerose Anders, in the Japanese Cancer Institute.

In 1948, at the age of 30, Fritz Anders came back to Germany from captivity of war. In Potsdam and Mayence he started his studies in Biology to earn his doctorate in Genetics and Zoology. Subsequently he was employed as a research assistant in a Grape Breeding Institute at the banks of the Rhine River. Here he concentrated on susceptibility, resistance and tolerance of grapevine leaves to plantgalls and tumors, and looked out for studies on tumorous outgrowth in lower animals. As a lecturer of General Genetics and as Professor and Director of the Genetics Institute of the Justus-Liebig-University in Giessen he extended his research to the development

of models for studies on neoplasia in Drosophila and teleost fish. Since 1988 he is Professor Emeritus in Giessen and concentrates more than ever on teaching and research in comparative oncology in fish and humans.

His career in experimental cancer research began in 1958 on the occasion of the Xth International Congress of Genetics in Montreal, when he viewed the exhibition material on melanoma inheritance in hybrids in *Xiphophorus* fish (platyfish × swordtails) from Central America presented by Myron Gordon from the Museum of Natural History in New York. He subsequently accessed the field of cancer genetics by means of this fish model. With fish, provided by Gordon and collected by himself in Mexico, he developed breeding systems to study the genetic pathways leading to hereditary melanoma. Together with his wife and his doctoral students, he hybridized fish from different wild populations and selected stable genotyoes that, besides melanoma, develop hereditary thyroid adenocarcinoma, reticulosarcoma and neuroblastoma. He showed step by step that neoplastic transformation can be traced to „tumor genes", known today as *oncogenes*, which are deregulated by consecutive loss or impairment of „controllers", known today as *suppressor genes*. His paper „Tumour Formation in Platyfish-Swordtail Hybrids as a Problem of Gene Regulation" (Experientia 23:1-10, 1967) was the first to specify Mendelian genetic elements of the present Oncogene-Suppressorgene Concept of neoplasia. In retrospect, according to the Imperial Message, this paper marks the beginning of the modern focus on genetics causation of neoplasia which is enormously expanding in the search for tumor markers.

Other breeds were genetically composed that, following the exposure to known carcinogens, yield hematopoietic mesenchymal and neurogenic neoplasms, or epithelial tumors of thyroid, pancreas, kidney, and liver, comparable to those human neoplasms that are not inherited through the germ line. Genes for tumor susceptibility and sensitivity to carcinogens were identified by means of these breeds (Proceedings of the 11th International Princess Takamatsu Cancer Research Symposium pp. 289-309, 1981). Over hundred carcinogen-suspected agents were tested for carcinogenicity and many of them have been identified either as mutagenic *tumor initiators* or as differentiation-provoking *tumor promoters*, or as both (Ann. Res. Vet. 22:273-294, 1991).

Based in the observations of melanoma suppression in fish by a particular chromosome, the *Xiphophorus* team proposed the appearance of human melanoma, retinoblastoma, Wilm´s tumor, phaeochromocytoma, and tumors of breast, ovaries, stomach and colon many years before the molecular identification of the genes by other laboratories, as loss or impairment of oncogene-specific suppressorgenes (in R.C.Gallo [ed.], Recent Advances in Cancer Research, CRC Press, pp. 103-117, 1977). Today, the formal similarity of the phenomenon of suppression or permission of tumorigenicity by a particular chromosome observable in the animals themselves to that observable in human cell lines, is self-evident and reveals a problem of general cancerology in animals and humans.

The first molecularly specified oncogene in *Xiphophorus* fish was the *x-scr* oncogene which is a homologue of the Rous Sarcoma Virus *v-src* oncogene from chicken (Cancer Research 42:2429-2433; 4222-4227, 1982). *x-src* and the 20 additional xiphophorine oncogenes studied by the Anders´ group evolved from sponge, sea anemone, cuttle fish, Limulus, Amphioxus, lamprey, shark, *Xiphophorus*, frog, snake, chicken, mouse, pig, up to humans. They express and overexpress in neoplasia of the fish in concert. One oncogene alone may not make a tumor in *Xiphophorus*, and Anders and his colleagues suggest that this will also be

true in humans (Aids Research and Human Retroviruses, Vol. 8:834-851, 1993). Moreover, they also state that one particular xiphophorine oncogene, *x-erbB*, that codes for the epidermal growth factor receptor, behaves like an ignition spark for the other oncogenes in a large variety of different tumor types (Oncogene 3:605-617, 1988). According to recent citations in the literature, of which many come from papers published in the Journal of Tumor Marker Oncology, it appears that the *erb-B* oncogene plays also an important role as a coordinator of oncogenes in different kinds of human neoplasia.

New implications for human tumor markers came up in the fish when the Anders´ team developed imitations of the controversially discussed accelerating increase of incidence of melanoma and other kinds of neoplasia in the changing human generations. Embryos or eggs in pregnant fish females, treated with X-rays or UV exhibited phenogenetically a so far unknown type of an oncodeterminant that is not a gene: This new oncodeterminant is intergenomicly mobile. It generates a uniform transformation of pigment cell accumulations that grow out to benign melanomas. The tumorous phenotype remains unchanged without any further carcinogenic treatment through the inbred generations and points to a chromosomal distribution of selfreplicating multipe copies of the oncodeterminants. When transferred by matings into ordinary benign and malignant melanoma outburst to anticipation at earlier inset ages and to increasing frequencies with increasing severity. Prospective benign ordinary melanomas grow out to malignancy. Fish embryos, equally endowed with the ordinary Mendelian capacity to develop malignant melanoma but equally strong protected from melanoma by a particular Mendelian suppressor show suppression of the suppressor by the extraordinary oncodeterminant.

The recent studies of Fritz Anders and his colleagues on neoplasia in fish follow the biological traces to molecularly identify the non-Mendelian elements coding for anticipation of the oncogene-suppressorgene machinery (Pigment Cell Research 7:433-450, 1994; Proc. 10th Internatl. Congr. Rad. Res. pp 592-596, 1996). These traces point to movable *Xiphophorus*-specific retrotransposons as was shown by Fritz Anders on the occasion of the 14th International Conference on Human Tumor Markers in Jerusalem (Journal of Tumor Marker Oncology 12:28, 1997).