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Report on 16th International Pigment Cell Conference

29 October - 3 November 1996
Anaheim, California

Transmitted by V. Hearing

The XVIth International Pigment Cell Conference was held from October 29th to November 3rd, 1996 at the Disneyland Hotel in Anaheim, California. Frank Meyskens was the Organizer of this meeting with Roger Bowers and Alistair Cochran serving as co-chairs of the Organizing Committee. Following are synopses of the various remaining Symposia, Workshops and Poster Discussions written by Chairs of those sessions that arrived after the publication of the last Newsletter (all of these reviews are also now posted on our Web Site). The Editor conveys a special thank you to all contributors of these summaries.

Symposium I: Economic and Societal Implication of Melanin and Melanogenesis by Shosuke Ito.

M Chedekel summarized the current situation regarding the commercial application of melanin and suggested its potential use as an antioxidant. G Imokawa presented data on the roles of endothelin-1 in mitogenesis and melanogenesis. He showed that extracts of M. chamomilla, an antagonist of ET-receptor binding-mediated signaling, inhibit UVB-induced pigmentation on human skin. P Autier reported their ongoing study exploring the possibility that sunscreen use might foster proliferation of pigmented lesions of the skin. Preliminary rsults on 109 children indicated that the use of sunscreen tends to increase nevi count. Finally, G Prota presented his view on cosmetic applications of melanin and melanogenesis with special emphasis on the application of dopa derivatives in hair dyeing.

Symposium II: Molecular Biology of Pigment Cells by Vincent Hearing.

This Symposium began with an elegant Keynote Address given by N Dracopali, who discussed mutations in genes that regulate the G1 checkpoint of the cell cycle and how a large number of familial melanomas are associated with such mutations. Phosphorylation of the RB (retinblastoma) protein is important to the regulation of the G1 checkpoint, and the ability of cyclin-dependent kinases to phosphorylate RB is inhibited by a family of proteins, including p16INK4a. Mutations in this p16INK4a gene have been identified in almost 50% of families with familial melanoma and these are thought to play roles in the generation of this type of melanoma.
M Schartl presented his work on molecular mechanisms which lead to melanocyte transformation by the Xmrk receptor tyrosine kinase using Xiphophorus fish as a model. Mutations in this gene can lead to overexpression of this kinase which in turn initiates transformation of the pigment cells. Xmrk is closely related to the EGFR (epidermal growth factor receptor). They have used differential display to determine changes in gene expression in cells transformed with mutant Xmrk oncogenes. H Yamamoto reported on their analysis of the evolution of developmental systems of pigment cells, using the tyrosinase gene as a model. Similarities in upstream regulatory sequences of the TRP (tyrosinase related protein) family suggest that these genes are coordinately regulated. Cloning of these genes from ascidians revealed only a single gene, which was most similar to TRP1 and TRP2 rather than

tyrosinase. K Toyofuku discussed his work on the importance of calnexin, a molecular chaperone, on the processing of tyrosinase, a step thought to be important to the regulation of melanogenic function of the enzyme. They have used cotransfection of calnexin and tyrosinase to examine the interactions, the results showing quite clearly that tyrosinase processing is markedly affected by coexpression of calnexin. DC Bennett discussed the cloning and mapping of a differentiation gene that regulates the state of differentiation of mouse melanoma cells. Transfection of this gene into B16 melanoma cells elicited increases in pigmentation and contact inhibition, along with decreases in tumor growth when inoculated into syngeneic mice. This gene was mapped to chromosome 14, and did not correlate with any known tumor suppressor gene or other cancer-related gene. Characterization of the function of this very important gene product awaits further study. S Porter reported on the regulatory sequences in far (15kb) upstream regions of the tyrosinase gene, and they are analyzing the mechanisms involved with those sequences. These sequences appear to be important to embryological development, particularly with respect to neural tube (optic cup) derived melanocytes. This Symposium was a fascinating insight into the varied molecular approaches being used to examine genetic regulation of pigment cell function and growth.

Symposium III: Melanoma Research: Basic and Applied by Frank Meyskens.

Six excellent papers comprised the content of this symposium. Cochran reviewed the current status of staging. Impressive data regarding the accuracy of sentinel node mapping were presented. Although large data bases have defined useful group predictions of outcome, increasingly sophisticated measurement of immunological and biochemical parameters is leading to the day when an individual's prognosis may be predicted with high accuracy. Such ability is likely to affect our post-surgical management of melanoma to a significant degree.

Three papers were concerned with manipulation of the melanin pathway. Y Mishima summarized his cummulate data about the use of neutron capture therapy; results continue to be encouraging. J Fruehauf reported on the cytotoxic action of busulfoxime (BSO), an inhibitor of GSH synthesis, on melanoma cells. BSO & busulfan was highly toxic to cells and cytotoxicity correlated to the melanin content of cells, suggesting that cells that have a higher oxidative stress (i.e. more active melanin synthesis pathway) are more sensitive to GSH depletion. E Link reported on elegant studies in mice and men that indicate that methylene blue is selectively taken up by melanoma cells. Based on these promising results a phase I/II study of Ab 211-labeled methylene blue is being planned. Finally, basic work on two important melanoma associated proteins were reported. Studies of ICAM-I in cytokine and hyperthermia treated cells in vitro (J Nakayama) showed that this molecule was differentially expressed. Elegant studies by MY Hsu demonstrate that melanocytes switch from E-cadherin to N-cadherin expression during melanonogenesis, which may be in part explain the biologic basis for invasive and metastatic potential. The symposium was a stimulating one in as much as new prospects for the management of melanoma based substitutive biological observations were raised.

Symposium IV: Photobiology of Melanocytes: Etiology and Prevention by Lisa Zeise.

This session had six well-presented papers; however, the presentations would be greatly enhanced if attributes of the light sources used were mentioned.

Nik Kollias: "Photobiology of Human Pigmentation" - The keynote speaker presented a clear, concise review of the literature published on human pigmentation and its regulation. A technique known as laser scanning confocal microscopy (LSCM), was described. Melanin is a good contrast agent and has an index of refraction of 1.7. LSCM utilizes this information for application in viewing actively pigmenting cells. This technique is exciting and will aid in the study of human pigmentation formation in vivo.

Yoko Funasaka: "The effect of ultraviolet B induced adult T cell leukemia-derived factor on survival and growth of human melanocytes" - Adult T cell Leukemia-Derived Factor (ADF), a human

homolog of thioredoxin, is induced by hydrogen peroxide and ultraviolet (UV) light, regulates gene expression, and scavenges reactive oxygen species to aid in protecting cells. This paper analyzed the effect of recombinant ADF (rADF) on normal human melanocytes and co-culture melanocytes and keratinocytes after UVB irradiation. ADF release was observed in keratinocytes but not melanocytes or fibroblasts after UVB irradiation. ADF was found to upregulate a-MSH induced DNA synthesis, to strongly induce melanocortin 1 receptor after 24 h, and to sustain survival of both keratinocytes and melanocytes.

Frank L. Meyskens Jr.: "Expression of NK-kB/IkB/c-Rel in human melanocytes and melanoma cells: changes in association and dissociation" - Redox control in melanocytes and melanoma cells was studied by quantifying the presence of NF-kB (p50), IkB, and c-Rel (p75) in basal cells and UVB stimulated systems. IkB and p50 were expressed more at the basal level in melanoma cells compared to melanocytes. UVB suppressed IkB levels but did not affect p50 levels in melanoma cells. In melanocytes, UVB increased both IkB and p50 levels. In contrast, basal p75 was increased in melanocytes and very low in melanoma cells. UVB also enhanced p75 in melanocytes; melanoma cells showed no difference. Hydrogen peroxide (H2O2), was generated with a glucose/glucose oxidase system and detected by chemiluminescence of luminal. Melanocytes and melanoma cells handled H2O2 differently as evidenced by time course measurements. The closing question posed was, "Is this difference in the handling of H2O2 due to the antioxidant ability of melanin?"

Mauro Picardo: "Alteration of antioxidants in normal melanocytes from patients with melanoma" - The role of free radicals in melanoma production was examined by the activities of superoxide dismutase (SOD) and catalase (CAT), the levels of vitamin E and ubiquinone, and the fatty acid pattern of cell membranes. Normal melanocytes and melanoma cells from the same patient were compared. CAT and SOD activities were higher in melanocytes. Melanoma cells exhibited lower CAT activity and a wide range of SOD activity. Melanoma cells had a higher concentration of arachidonic acid with respect to normal melanocytes. Levels of vitamin E were found to be inversely proportional to CAT activity. The ratio of vitamin E level to CAT activity was felt to correlate with antioxidant activity in cells. In melanoma patients, normal melanocytes were thought to exhibit an alteration in antioxidant pool, and thus, to exhibit increased sensitivity to oxidative stress.

Mayumi Fujita: "Activation of p53 is required for ultraviolet radiation-induced cell cycle arrest, apoptosis and BCL-2 regulation in melanoma cells"- Transcription of the p53 gene is involved in cell cycle arrest. To determine whether UV is involved in this gene mechanism both blocking and induction of p53 were examined. The former was studied using WM 35, the primary melanoma with functional p53, and transfection with the viable gene. Induction was studied by observing how UVB affected the cell cycle. UVB induced p53 expression and lead to cell cycle arrest. UVB also was observed to yield apoptosis in WM35 clones but not in p53 clones. By incorporating a temperature shift (38?C to 32?C), the conformation of p53 protein was changed from mutant to wild-type. Studies using WM 1617 and TS clones of p53 showed that UVB induced cell cycle arrest and apoptosis. Also, wild-type p53 was induced, p21 expression was increased which induced cell arrest. Thus, p53 is crucial for UVR induced cell cycle arrest and apoptosis in melanoma cells.

Ashok K Chakraborty: "Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by UVB"- It is demonstrated that UVB radiation stimulates increased expression of the proopiomelanocortin (POMC) gene which is accompanied by production and release of a a-melanocyte stimulating hormone and adrenocorticotropin by both normal and malignant human melanocytes and keratinocytes. The production and release of both peptides are also stimulated by dibutyryl cAMP and interleukin 1a but not by endothelin-1 or tumor necrosis factor-a. N-acetyl-cysteine, a precursor of glutathione, an intracellular free radical scavenger, abolishes the UVB-stimulated POMC peptide production and secretion. The conclusions were described and may be found in the following paper: AK Chakraborty et al. (1996) Same title, Biochim Biophys Acta 1313, pp. 130-138.

Symposium V: Melanogenesis and Pigmentary Disorders by James Nordlund.

R Boissy opened the session with a superb review of the embryology of the pigment cell and its migration from the neural crest. Mutations in the c-kit proto oncogene are responsible for piebaldism. The PAX and MITF genes, both encoding transcription factors, when defective are responsible for various forms of Waardenburg's syndrome. The receptor for endothelin causes a syndrome of piebaldism and megalocolon. The various forms of oculocutaneous albinism are caused by mutations in the tyrosinase gene (OCA-1), the p gene (OCA-2) or the TRP-1 gene (OCA-3).

M Mizoguchi studied the melanocytes of women with acquired facial dermal melanocytosis. This is a disorder that seems to have a predilection for oriental women and is characterized by formation of blue macules on the cheeks. She found in these lesions melanocytes in the lower dermis that seemed inactive but could be stimulated by various cytokines. She proposed that these melanocytes were embryological residue and were activated by cytokines during adult life to cause the syndrome.

RA Spritz presented information on the Hermansky-Pudlak syndrome (HPS). It is similar to albinism because the patients have marked pigmented dilution.
In addition they have a bleeding diathesis, pulmonary disease, colitis and liver dysfunction. By homozygosity mapping, he and his coworkers mapped the gene for HPS to chromosome 10q23.1-q23.3. Occasional patients have a disorder that does not map to this locus, an observation suggesting several forms of HPS.

J Fryer presented on mutations at splice sites as a cause of OCA 1. Many point mutations, frame shifts and similar mutations have been identified. Using a lymphocyte line and PCR, Fryer and his colleagues studied splice sites in one family with albinism. They found that in this family a splice site mutation at the 5' end of exon 3 caused the entire exon to be deleted and exon 2 was spliced to exon 4. A second splice site mutation was identified in exon 1.

V Hearing studied the copper binding sites of the tyrosinase related proteins. There are two copper binding sites on the tyrosinase enzyme, CuA and CuB. He showed that elemental copper bound to the enzyme and that both sites required copper binding for normal enzyme activity. Other divalent cations could not substitute for copper in these sites. They found that CuB seemed to facilitate binding at the CuA site. The other two tyrosinase related proteins, i.e., TRP-1 and TRP-2, did not bind copper under the conditions of these experiments and these latter proteins might depend on other metal cations for activity.

This symposium was superb and provided a molecular basis for understanding some of the many disorders of pigmentation.

Symposium VI: Comparative Developmental Biology of Pigment Cells by Roger Bowers.

Matsumoto (the keynote speaker) presented an overview of the molecular biology of fish pigmentation, in particular the medaka. Causes of albinism in this fish, as found by Y Hori and associates, are due to an insertion of transposon-like sequence in exon 1 of the tyrosinase gene (mutant "i") and due to deletion of the short frame in exon 3 (Mutant "i4"). Matsumoto's group have produced transgenic homozygous orange-colored variant medakas carrying the cloned mouse tyrosinase gene in which the fish exhibits wild type pigmentation. The gene is stable and follows Mendelian genetics.

S Frost-Mason presented an evolutionary perspective of vertebrate chromatophore development entitled "From 3 pigment cell types to 1". She presented strong morphological, histological, cell and molecular biological evidence that the epidermal melanocyte of the mammal and bird may have evolved in a convergent manner from the 3 chromatophore cell types (melanophore, xanthophore, iridophore) found in fish, amphibia and reptiles.

D Bennett discussed differential gene expression in her murine immortal melanocytes, in her newly derived murine melanoblast line and in her newly derived murine melanoblast precursor line. She compared transcription factors, melanosomal proteins and growth related genes in these 3 lines

and the results showed that not all express the same genes except for Pax3 and this difference may lead to a better understanding of cell differentiation and melanoma formation.

B Wehrle-Haller presented evidence that the early melanoblast migration is directed by localized steel factor. Migration is inhibited in mutant embryos that lack either steel factor (Steel, Sl) or its receptor (dominant white spotting, W). By studying various mutants that affect the presence or availability of steel factor, it was shown that the cytoplasmic domain of steel factor may have additional regulation functions for melanoblast migration not reflected in the COS cell system. The distribution of steel factor in the mutant will elucidate how steel factor regulates melanoblast migration and differentiation.

M Moody discussed the enhancement of the xanthophore lineage in guanosine-treated axolotl neural crest cells in vitro. Their results show that there is a specific developmental sequence which dictates where, when and what chromatophore type (black, yellow, white) differentiates. Axolotls can be treated with guanosine to suppress melanophore differentiation and simultaneously enhance xanthophore differentiation. Increasing one type of cell population is at the expense of the suppressed cell type population , suggesting transdifferentiation. This system may be a good model to study stem cell biology and transdifferentiation.

W Pavan presented results on genetic regulation of melanocyte patterning using 2 strains of piebald mice. Four loci are involved for the pattern difference. Chromosome 10 gene increased dorsal spotting and is probably steel factor, a conclusion supported by genetic and molecular biology analysis. The spotting pattern in the dorsal surface of the Mayer s/s mice is due to alteration in the normal function of the steel gene.

In posters related to this symposium and certainly no less important, T Fukuzawa et al showed that the melanization inhibiting factor in frog embryos was concentrated in the lateral and ventral skin and not in the dorsal skin at the external gill stage and that this changes as development proceeds. S Holder and G Thibaudeau presented evidence that axolotl neural crest cells from older embryos gave rise to more xanthophores than these same cells from younger embryos, that posteriorly located neural crest cells gave rise to more chromatophores that these same cells located in the anterior region and that guanosine treatment enhances xanthophore differentiation. R Kelsh and M Eisen characterized the colorless mutant in zebrafish and found that these embryos have essentially no melanophores and only a few normally pigmented xanthophores and iridophores. Any melanophores present are weakly pigmented and markers have shown that melanosomal related protein levels are low in these melanophores and that these cells do not migrate from their dorsal position in the embryo. K Mason et al showed that xanthine dehydrogenase is an excellent marker to identify differentiated xanthophores in axolotls. In another poster, they presented evidence that they have isolated a complete cDNA for axolotl TRP-1 and it is similar to its mammalian counterpart. A Masagaki and R Fujii presented evidence that shows the pigment pattern in pencilfish is changed at night by melatonin and this species may be used to study the action of b-melatonin and its analogs on melanophores. S Ali et al showed that nicotine caused fish apical melanophores to disperse their melanosomes whereas the basal melanophores aggregated their melanosomes. Frog melanophores dispersed their melanosomes due to nicotine as did the wall lizard. In another poster, they presented evidence in fish, frogs, toads and wall lizards that when histamine is bound to H1 receptors, it causes melanosome aggregation whereas when it is bound to H2 receptors, it causes melanosome dispersion. In a third poster, they showed that disinfectant phenolic compounds caused severe irreversible aggregation of scale in vitro fish melanophores. Hydroquinone was the most potent melanolytic agent. M Sugimoto and Hatayama presented evidence that nerve growth factor is involved in the regulation of the population of melanophores and in the density of adrenergic innervation in the medaka. Hirose et al showed that pigment cells in ascidians demonstrate a homology of chemical compounds but a difference in cell structures with higher vertebrate pigment cells and thus these chordates have a primitive form of pigment cell function and structure. R Morrison and Nagashima showed morphological evidence that the emergence of the embryonic pigment pattern in zebrafish is a highly dynamic process since the wild type adult pattern is quite different from the embryonic pattern. Okumoto et al presented evidence

that melanosome movement in melanophores is under indirect control of the actin-myosin system which is located in a radial array in these dendritic cells. H Ono et al showed that the mouse tyrosinase gene introduced into the medaka is integrated into the fish genome and is capable of germ line transmission. M Goda and R Fujii found dendritic chromatophores that contained blue organelles in both the epidermis and dermis of 2 species of callionymid fish. These blue chromatophores were termed cyanophores and their blue organelles were termed cyanosomes. N Oshima et al showed that prolactin caused pigment granule dispersion in erythrophores and xanthophores in the tilapia fish and that this response was seasonal in that it was greater in the spring and summer suggesting the involvement of prolactin in nuptial coloration. R Bowers et al presented evidence that IGF-II, EGF and insulin increased the number of in vitro adult highly differentiated avian primary culture melanocytes and that this was due to stimulation of migration from the feather piece onto the dish. Insulin also increased the viability of these cells. In a second poster, they showed that the number and migration distance of these same melanocytes can be doubled over control values by coating the dish with collagen type IV or fibronectin, suggesting that these cells still retain receptors for these ECMs from their embryonic

Workshop A: Extracutaneous Melanin, Melanocytes and Melanogenesis by Helene Z Hill.

The Workshop on extracutaneous melanin, melanocytes and melanogenesis began with an overview by RU Peter who pointed out that not all pigment cells in mammals derive from the neural crest. For example, the retinal pigment cells arise in the anterior neural tube. Pigment cells are found throughout the body in such varied sites as the meninges, peritoneum and blood vessels. In birds, pigment cells are prominent in the pericardium and in muscle. In fish, they are found in the lateral line among other sites. Extra-cutaneous melanomas in humans arise from many different primary loci. The functions of pigment are varied. It serves as camouflage, radiation protection and absorption, radical scavenging and as an anti-oxidant, mating signal and guidance for vasculature.

The first talk, entitled 'Melanin ~ the two-edged sword?' was by H Hill who studied mutation and survival in related cell lines that varied in pigment content. She found that induced eumelanin was photoprotective for mutations and survival but that constitutive melanin was only slightly photoprotective for survival for UVC and UVA but not for UVB nor a polychromatic lamp that resembled sunlight (FS20). In fact, albino melan-c cells were quite resistant to killing by FS20 compared to the pigmented melan-a and melan-b cells. DNA damage in the form of thymine dimers and 6-4 photoproducts appeared to be enhanced by pigment. In light of many conflicting reports in the literature concerning the role of pigment in light-induced damage to DNA, she emphasized that useful information regarding the role of melanin in the carcinogenesis of melanoma might only be gained by studying such biological endpoints as mutation and cellular transformation.

T Seikai described his studies performed of pigmentation abnormalities in flat fish. Ordinarily, the ocular side of these fish is hyperpigmented while the blind side is hypopigmented. The fish, a popular food item in Japan, are now produced by aquaculture. Under these conditions, there is a high incidence of hyperpigmentation on the blind side and hypopigmentation on the ocular side. Studies showed that irradiation of the blind side during metamorphosis inhibited mutation on this side. Nutritional factors during larval development are also important in the determination of pigmentation.

Prostaglandins are useful in the treatment of glaucoma. J Stjernschantz described the findings after chronic treatment of monkeys and patients in clinical trials with PGF2a, PGE2 and the FP receptor agonist latanoprost.

After 3 to 12 months there was an increase in pigmentation in irises of brown but not blue eyes. There was no pigmentation change in nevi or freckles. The effect was due to increase in tyrosinase, not TRP1, which resulted in an increase in eumelanin.

Workshop B: Dynamics of Invertebrate Pigment Cells by K Ranga Rao.

This workshop, organized by S Negishi, included presentations on diverse aspects of pigmentation in arthropods. Rapidly-reversible color changes due to pigment translocations within epithelial chromatophores are displayed by many crustaceans, and are regulated by neuropeptides called pigment-concentrating and pigment-dispersing hormones. The cellular mode of action of one of these peptides, red pigment concentrating hormone (RPCH), was the subject of a report presented by LEM Nery, MA Silva, and AML Castrucci. Their in vitro experiments with the erythrophores of the shrimp Macrobrachium indicate that the action of RPCH involves phosphoinositol degradation that induces Ca++/calmodulin complex formation and PKC activation.

Y Hasegawa and S Negishi have investigated the biochemical, cellular, and genetic basis of coloration in the terrestrial isopod, Armadillidium vulgare. The most common body color is black or grey, due to ommochrome containing chromatophores. Ultrastructural and biochemical studies indicate that albinism, as seen in the white phenotype, results from a defect in the synthesis or transport of the precursor before 3-hydroxykyneurenine in the ommochrome biosynthetic pathway.

A review of the hormonal control and pattern formation in insect pigmentation was presented by D Buckmann. Since chromatophores such as those in crustaceans are absent in insects, the latter are unable to display rapid color changes. Insects can undergo relatively slow color changes during the course of development or as morphological color adaptation. The role of environmental and endocrine factors, including the recent evidence for the involvement of neuropeptides, in the regulation of pigment synthesis was discussed. The characterization of the neuropeptides is in progress.

M Ashida presented biochemical and molecular evidence to establish that the insect prophenol oxidase is a protein homologous to arthropod hemocyanin. It appears likely that these molecules have originated from a common ancestral protein with a bi-nuclear copper cluster. Although time constraints in the workshop did not permit full disclosure of results, the activation of prophenol oxidase was reported to be triggered by minute amounts of microbial cell wall components and fungi--pointing to potential role as a defense mechanism.

Workshop C: Regulating Mechanisms of Melanocyte Proliferation by Zalfa Abdel-Malek.

An overview on the evolution of the methods for culturing normal human melanocytes was presented by Z Abdel-Malek. Cultured human melanocytes are an ideal in vitro model to investigate the regulation of human pigmentation. The first growth medium which allowed for the long term proliferation of human melanocytes relied on the use of tumor promoting phorbol esters and cholera toxin. Over the years, many investigators modified this initial procedure by replacing these artificial and toxic agents by physiologically relevant growth factors, most of which are synthesized by human keratinocytes and thus can function potentially as paracrine regulators of melanocytes. Such factors include basic fibroblast growth factor, leukotriene C4, hepatocyte growth factor, stem cell factor, endothelin-1 and a-melanocyte stimulating hormone. The observations that normal human melanocytes require several growth factors with different signaling pathways in order to proliferate in culture, suggested that mitogenic stimulation of these cells requires the crosstalk of different signal pathways. These pathways include the protein kinase C, cAMP/protein kinase A, and tyrosine kinase pathways.

The selected abstracts dealt with the role of: 1) microphthalmia-associated transcription factor (MITF), 2) extracellular matrix proteins, 3) oxidative damage, 4) cell cycle regulatory proteins, in normal melanocyte proliferation and differentiation.

M Tachibana presented data on the induction of melanocyte differentiation by MITF. Expression of this transcription factor in NIH/3T3 cells which constitutively express TRP-2 resulted in the expression of tyrosinase and TRP-1, and in a dendritic morphology. Data was also presented on two novel mutations of the MITF gene in individuals with Wardenburg Syndrome type 2 (WS2A) from two different families. WS2A is a dominantly inherited disease characterized by pigmentary abnormalities and deafness possibly related to loss of melanocytes from the stria vascularis of the inner

ear. The above two mutations result in proteins that lack sequence-specific DNA-binding activity, and ability to transactivate the tyrosinase promoter, but do not disrupt the function of the wild-type MITF protein. These results suggest that the WS2A phenotype is caused by loss-of-function of the two alleles of the MITF gene.

S MacNeil presented on the effect of extracellular matrix (ECM) proteins on cutaneous and ocular melanocytes. She stated that different ECM proteins from different sources (e.g. human dermal fibroblasts or microvascular endothelial cells) stimulate tyrosinase activity in cutaneous melanocytes. Fibronectin, in particular, stimulates tyrosinase activity in ocular melanocytes, and increases intracellular calcium.

The abstract that was to be presented by A Thody offered a new explanation for why melanocytes are more vulnerable to oxidative damage than keratinocytes or fibroblasts. In addition to their lower level of anioxidant enzymes, melanocytes seem to be capable of producing superoxide anion and nitric oxide. The production of superoxide anion by the xanthine oxidase/xanthine system was reduced in the presence of human fibroblasts or keratinocytes, but increased in the presence of human melanocytes or B16 melanoma cells. Additionally, B16 melanoma cells also produced superoxide anion and nitrous oxide following UVB irradiation.

Data from E Medrano's laboratory describing the role of cell cycle regulatory proteins in end-stage differentiation of human melanocytes was presented by M Haddad. Human melanocytes can be induced to reach end-stage differentiation by chronic treatment with high concentrations of cAMP inducers, such as cholera toxin. At this stage, melanocytes did not respond to the addition of fresh medium with significant pRb phosphorylation, expressed a low level of cyclin D1, high level of p27, and a moderately high level of p21. Unlike proliferating melanocytes in which MITF becomes highly expressed, downregulated, and then highly expressed again, irreversibly arrested melanocytes continuously express a high level of MITF.

A Platz described mutations in cell cycle regulatory genes in sporadic human melanoma tumors. In 26 metastases from 25 patients, 4 tumors had mutations in CDKN2, 2 had mutations in CDKN2B, 3 tumors had mutated p53, and 2 had mutations in N-Ras. In addition 34 patients, 8 had codon 61 mutations of N-Ras, 10 of 19 mutations were G-C/A-T or A-T/G-C transitions, and 2 were C-G/G-C transversions at sites of adjacent pyrimidines. These results suggest that these mutations are UV-induced, and support a role of UV in the etiology of human melanoma.

Workshop D: Biophysics and Chemistry of Melanin by Hal Swartz.

This workshop was organized by T Sarna. It brought together presentations and discussions of a wide spectrum of physical and chemical techniques which are helping to elucidate the nature of melanins. This is a most complex and difficult task because of the nature of the melanin molecule: it is a multi-functional polymer with many different and potentially important physical properties and chemical reactivities. The presentation by J Menter on electron transfer and photoprotective properties of melanins in solution focused on the polyquinoid nature of melanin which enables them to couple oxidation of electron donors with the reduction of electron acceptors. The presentation, as did many of the other presentations, emphasized the importance of the structure of the melanin and the particular conditions in determining the physical and chemical effect that are observed. In looking at a prototypic reduction, i.e., the reduction of ferricyanide, it was shown that melanin could either retard or accelerate the rate or reduction depending on the conditions. An important general principle that was noted is the importance and nature and extent of binding by melanin. The presentation also emphasized the important capability of melanin to affect electron transfer. These properties lead to some important photo chemical interactions as well as dark chemistry. The presentation by K Wakamatsu summarized some of the extensive work done by him in collaboration with S Ito. He reported on their microanalytical methods which make it possible to quantitate the amount of eumelanin and pheomelanin by means of analysis of partial degradation products. The presentation included a demonstration of the validity of their approach by methodology which enabled them to dissolve some

melanins completely. The presentation by R Peter focused on the redox state of enzymatically generated tyrosine melanin. He showed how very elegant results could be obtained using carbon 13 NMR and isotopic label precursors. With this technique he was able to quantitate the amount of oxidized and reduced subunits. M Eisner reported on EXAFS studies of chelated iron sites in natural and synthetic neuromelanins which have been carried out by an international group, including Drs Zecca and Crippa from Italy. It was pointed out that neuromelanins may have an important role in the understanding of Parkinson's Disease. The elegant EXAFS technique was demonstrated to be able to characterize the chelated iron sites in both synthetic neuromelanins and genuine substantia nigra. The results indicated some of the potential problems involved in the use of synthetic neuromelanins, especially if these do not fully reflect the chemical nature of neuromelanin as it is found in the human brain.

The last presentation was by H Swartz, who summarized results on the implications of the interactions of melanin with reactive species, based on extensive work done in collaboration with Drs Sarna, Nilges, and Pilas over a number of years. The capabilities of melanin to affect reactions by several different mechanisms was emphasized. Depending on the type of melanin and the conditions, melanin can play an important role by binding and changing the activity of both metal ions and organic molecules and thereby affect the amount of reactive species that are produced. It was emphasized here, as in the other presentations, of the need to take into account the effects of different types of melanin on the particular reaction or biological effect that is being assayed.

Overall, this workshop presented an excellent overview of the nature of melanin and indicated some of the remarkable progress that is occurring in understanding it.

Workshop E Vitiligo by David Norris.

This workshop of vitiligo covered topics related to the pathomechanisms of the development of vitiligo, and better approaches to repigmentation in vitiligo. D Norris (Univ Colorado) discussed the resistance of epidermal melanocytes to cytotoxic damage, proposing that intrinsic anti-apoptotic defenses mediated by proteins such as bcl-2 protect melanocytes from cytotoxicity induced by immunologic and inflammatory mediators and ultraviolet radiation. The environment of the epidermis is continually exposed to oxidative stress, ultraviolet radiation, cytokines, cytotoxic lymphocytes, and biochemical triggers of cell damage, and melanocyte survival is determined by a balance of survival signals and death signals. J Nordlund (Univ Cincinnati) discussed proposed etiologies for melanocyte destruction in vitiligo, and alleged that no current proposed mechanism was completely convincing, except for the hypothesis of intrinsic melanocyte defect. This inspired considerable discussion, with a common accord that the multiple possible mechanisms proposed in vitiligo might indeed by involved differentially in distinct subsets of patients. The problems in linking particular mechanisms to melanocyte damage in individual patients were acknowledged. A Taieb (Bordeaux Univ) demonstrated the usefulness of studying mechanisms of vitiligo in vitro in complex organotypic epidermal cultures, reporting that an intrinsic defect in melanocytes from vitiligo patients is not demonstrated in the absence of external stimuli, and concluding that an external trigger is needed for vitiligo. R van den Wingaard (Amsterdam Univ) reported that no differences in susceptibility to apoptosis were observed between melanocytes from normals compared to vitiligo subjects. Their work suggested that immunologic cell death of vitiligo melanocytes may be enhanced by changes in bcl-2 levels, which will be better defined in further investigation. They also confirmed reports that melanocytes resist induction of apoptosis triggered by binding the Fas receptor on the melanocyte plasma membrane. RK Tripathi (Univ Cincinnati) reported on genetic studies to determine whether the MITF (micropthalmia) genetic locus was linked to the development of human vitiligo. Even though this candidate gene is linked to other depigmentary problems, it was found to not be a genetic locus determining human vitiligo. W Westerhof (Amsterdam Univ) reported on the advantages of narrow-band UVR (311 nm) over typical PUVA therapy. In a large clinical trial, narrow band UVR was found to be more effective than PUVA and offered a number of advantages (safety, ease of treatment, fewer collateral changes).

Neither treatment was good for hand and foot vitiligo. Although there is continued progress on understanding basic mechanisms of melanocyte damage in vitiligo and although effective (although slow) treatments are available, we are not yet able to link breakthroughs in understanding the mechanism of this common disease with matched breakthroughs in treatment that are safe, rapid, and effective in all patients. Approaches to repigment hands and feet from endogenous melanocytes are still largely unsuccessful.

Workshop F: Control of Melanogenesis by John Pawelek.

Dr J Pawelek presented a summary of his work with Dr A Chakraborty in which it was shown that the Pmel17/Silver gene product has the ability to catalyze the polymerization of DHICA into DHICA-melanin, suggesting a potential role for this protein in vivo. He cautioned, however, that in the case of melanogenesis in vitro and in vivo enzymatic activities might not necessarily correspond, particularly since melanin intermediates are often a) unstable in vitro, spontaneously creating new potential substrates for the melanogenic factor in question, and b) recognized as substrates by more than one melanogenic protein in vitro. Dr H Kondoh presented his work with Dr Y Mishima on the role of TRPs in the control of eumelanogenesis. They showed that TRP-2 plays an important role on the content of DHICA-melanin in both eumelanin and mixed melanins, as well as preventing cell death by converting DOPAchrome to DHICA, which has less cytotoxicity than DHI. Dr F Solano presented work from his laboratory comparing TRP's from murine and human melanoma cells. They found that the three human melanoma lines had less DOPAchrome tautomerase activity than mouse B16 melanoma cells, and that the mouse enzyme appeared to contain Zn at its metal binding sites. Tyrosinase and TRP1 from all cell lines both showed DOPA oxidase activity. Dr M Miranda presented a spirited and fascinating overview of melanogenesis, tyrosinase expression, and reproductive differentiation in black and white truffles (Ascomycotina). His observations underscored the wide-spread uses that melanins have been put through by various life forms. Of particular interest was the observation that white truffles do not produce black melanins, yet they are tyrosinase positive. Dr H Chen summarized his work with Dr K Jimbow demonstrating, for the first time, the potential involvement of phosphatidylinositol 3-kinase activity in the sorting and transport of newly synthesized TRP-1 in melanogenesis.

Poster Session #2 Melanogenesis by John Pawelek.

Dr B Fuller discussed work from his laboratory on the regulation of tyrosinase in mouse melanoma cells and human melanocytes by PKC and PKA pathways. Using protein kinase inhibitors, evidence was obtained that PKC activity is not associated with stimulation of tyrosinase, rather it seemed to be a negative regulator of the melanogenesis pathway. Dr K Yasumoto presented his work with Drs Fuse and Shibahara on pigment cell-specific transcription of the tyrosinase family and MITF genes. Their results suggested that transcription of the TRP-2 gene is regulated in a different manner from that of the tyrosinase and TRP-1 genes. Further, they identified a melanocyte-type promoter of the MITF gene and are currently searching for the regulatory elements required for its pigment-specific expression. Dr Y Xu presented work on sorting of a melanosome membrane protein to both the endosomal and secretory pathways. They found that a major portion of the TRP-1 produced by melanocytic cells is secreted. Cell surface expression of TRP-1 was also detected. Dr M Furumura and co-workers used the technique of differential display to identify novel genes modulated during pheomelanogenesis. Several clones of cells were isolated that appeared to express genes that were regulated by agouti signalling protein, potentially opening new directions in the understanding of genetic regulation of pheomelanogenesis.

Workshop G: The "Blues" Symposium by Joseph Bagnara.

This workshop was organized by J Bagnara, J Bolognia and Y Hori in order to emphasize the reality that pigment cell researchers from very diverse areas deal with problems that are seemingly unrelated, but are in fact very similar. Blue coloration is a prime example of this fact. In his Introduction, Bagnara pointed out that blue colors among all the vertebrate groups have a physical basis and are truly "structural colors." With a few examples, he indicated that blue colors among the various vertebrates are related by either analogy or homology. As an example of the latter, it was shown that blue spots in some fishes are like the blue nevi of humans. A superb tone for the session was provided by C Bohren, an atmospheric physicist from Penn State, who, with unparalleled humor, poked holes into many of the physical misconceptions about blue coloration. "The physicists" were often foils for his humor. He emphasized the need for colorimetry in assessing blue colors.

The remainder of the session followed a phylogenetic approach and started with human cerulodermas. Blue nevi and mongolian spots were discussed by J Bolognia while Y Hori considered the Nevus of Ota and other nevi fuscocaeruli. A description of the nevi and treatments were presented. The results of ruby laser treatment were impressive. The blue colors of fish were discussed by R Fujii who emphasized the physical role of the reflecting platelet organelles of iridophores. He pointed out their function in light scatter, reflection, and thin-layer interference and explained how some of the respective hues of fishes could be achieved therein. A high point of his presentation was the novel demonstration of truly blue chromatophores (cyanophores) that contain a genuine blue pigment, as yet uncharacterized. P Fernandez presented numerous examples of blue coloration, either normal or "abnormal" among amphibians. He discussed the role of the dermal chromatophore unit in imparting both blue and green coloration. A high point of his presentation was his use of colorimetry to objectively describe skin colors through representation on a chromaticity diagram. R Morrison followed with an assessment of blue colors in several lizards, notably a scaly lizard, Sceloporus jarrovi. In this case, the role of thin-layer interference was emphasized. R also was given the task (but no time) to discuss blue colors of birds. He limited his words to bare patches of skin such as wattles. Here, blue coloration is attributed to structurally based events involving orderly arrays of extracellular collagen. W Quevedo concluded the formal presentations by considering the blue colors of mammals, notably those that occur as secondary sexual characteristics of adult male mandrills. He discussed the behavioral significance of the red, blue, and white pattern of the face and anogenital regions of such males and indicated that the "blue color depends upon a complex interplay of variable amounts of hemoglobin in dermal blood cells and immobile melanosomes of adjacent dermal melanocytes." Following the formal session, a brief free presentation from R Aquaron described a clinical manifestation of "blue ears" in patients with alkaptonuria who accumulate homogentisic acid. Altogether, the "Blues" symposium attracted a good audience and evoked lively discussion.

Workshop H: Biology and Biochemistry of Melanosomes by Seth J Orlow.

To kick off the session, Y Mishima gave an overview of the relationship between melanosomes and lysosomes. He reviewed data from his own lab on the transfection of genes encoding TRPs into amelanotic melanoma cells, as well as the data implicating coated vesicles in the trafficking of proteins to melanosomes. K Jimbow reviewed his lab's experience with identification of calnexin as a molecular chaperone implicated in the proper folding of tyrosinase in the endoplasmic reticulum as well as that of the small GTP-binding protein, rab7, in controlling trafficking of TRP-1 to melanosomes. Later in the session, P Gomez of Jimbow's group expanded on this latter subject. Rab7 was identified in 2-D gels of melanosomal proteins by overlay with radiolabelled GTP followed by partial sequence analysis and cDNA cloning. It colocalizes with TRP-1 to melanosomes. Melanoma cells transfected with a rab7 antisense construct show a more restricted perinuclear distribution of TRP-1, supporting the contention that rab7 may be involved in TRP-1 trafficking. K Araki spoke about the identification of rab3a, another small GTP-binding protein, with melanosomes both by

copurification as well as by immunoelectron microscopy. A protein which interacts with rab3a, namely Rabphilin-3A, was also present in melanosomes of B16 melanoma cells. In contrast, RabGDI was ubiquitously distributed in many subcellular components. C Sakai described the effects of recombinant agouti signal protein (ASP) on immortalized cultured murine melanocytes (melan-a cells). ASP counteracted MSH's stimulatory effects on these cells, but even in the absence of added MSH, ASP inhibited tyrosinase mRNA and protein levels and, to a lesser extent those of TRP-1 and TRP-2. Melanosomes in ASP-treated cells tended to be rounder, more like the shape of pheomelanosomes. Interestingly, ASP seemed to counteract even the stimulatory effects of cholera toxin, suggesting that it might act through an additional signal transduction pathway in addition to its role as a noncompetitive anatgonist of MSH. Finally, J Hammer discussed his research on the product of the murine dilute locus, aka myosin V. This unconventional myosin has calmodulin binding sites and may serve to link the melanosome to the cytoskeleton in a calcium-dependent manner. The protein is indeed associated with melanosomes, colocalizing with such bonafide melanosomal proteins as TRP-1. It was long thought that the defect in dilute mice was their inability to extend dendrites. Using antibodies to the melanocyte cell surface receptor c-kit, Hammer's group has now shown that there is nothing wrong with dendrite extension in dilute mice or cultured melanocytes derived therefrom. Rather, the problem appears to be due to an inability to translocate melanosomes from their perinuclear area of origin down through the dendrites from whence they can be transferred to keratinocytes.

Workshop I: Genetic Aspects of Albinism by Richard King.

This workshop focused on recent studies of human albinism and tyrosinase gene expression in the mouse. J Matsunaga reviewed their experience with tyrosinase gene mutations in tyrosinase-negative OCA in the Japanese population. Four mutations have been identified: R77Q, R278TER, DC310, and P431L. Affected individuals were homozygous for R77Q/R77Q (n=2) or DC310/DC310 (n=4), or compound heterozygous for two different mutations.
One individual was a compound heterozygote with R77Q on one allele and no detectable mutation of the homologous allele. Extensive evaluation of the promoter region of the tyrosinase gene on this allele, playing particular attention to the TDE region and the area of the (GA)n repeat did not reveal a mutation that would account for the loss of function associated with this allele. F Beermann evaluated the promoter of the tyrosinase gene using a tyrosinase-LacZ fusion gene in transgenic mice. Expression was found in several areas of the developing and the adult brain. Immunohistochemistry studies showed tyrosinase-specific bands in the brain and eye, although no enzyme activity was detected. The potential role of tyrosinase expression in the brain was discussed. JM Newton presented new data on the isolation of the mouse homologue (Moa1) of the human Ocular Albinism 1 (OA1) gene. The gene product appears to have six transmembrane regions and exists in two isoforms. The gene is expressed in the skin and eye of the neonatal mouse but only in the eye of the adult mouse. MSH and ASP had no effect on Moa1 expression. Analysis of tissue expression showed that the Moa1 protein co-segregated with TRP1 protein in the melanosomal-enriched fraction of pigmented tissue. W Oetting presented further data on the analysis of the P gene in human OCA2. Many silent and missense polymorphisms were found, as well as a large number of pathologic mutations. A screen on control individuals was used to establish the difference between a polymorphic and a pathologic mutation. The distribution of mutations in the gene was random and no functional domains were suggested by mutation distribution.

Poster Session #3: Biophysics and Chemistry of Melanin by Hal Swartz.

This interesting session was organized by P Riley. The session was well attended, indicating the attractiveness of such poster sessions. It consisted of oral presentations of the highlights of some the posters of most general interest that were included in the poster session on biophysics and chemistry of melanin. Z Abdel-Malek summarized the very interesting and important results on understanding the molecular mechanism of the effect of aMSH on UVB induced growth arrest. It was shown that the aMSH has an important effect on the kinetics but not the extent of apoptosis. The presentation by P Autier highlighted the complex interactions that occur between physical effects such as exposure to sunlight and human behavior. As a consequence of the increased reaction of individuals with certain skin types to UV, the subjects reduced the amount of exposure to sunlight and thereby their risk for malignant disease. Failure to take into account such behavioral changes could lead to erroneous interpretations of the relationship between exposure, practice predisposed to the induction of malignancy, and the amount of malignancy that is observed. N Kobayashi reported on the phenomenon of photoprotection by supranuclear melanin caps against DNA damage in normal human epidermis. This result suggested that appropriate positioning of melanin over the nucleus could account the observed differences of sun induced skin cancer in highly pigment races. The final presentation was given by T Sarna on behalf of the groups from Krakow and Medical College of Wisconsin. He summarized the complex and very important properties of melanin in both promoting and inhibiting autooxidation. In aggregate, the presentations at this poster session provided a stimulating and informative insight into the wide spectrum of effective approaches being used to relate the biophysical and chemical properties of melanin to human disease.

Poster Session #4: Pigment Cell Development and Dysfunction by Walter C Quevedo Jr.

This session revealed the narrowing gap between studies of the paraclinical aspects of melanocyte dysfunction (albinism, vitiligo, hypermelanism etc.) in humans and the basic studies on the cell and molecular biology of melanocyte development, pattern formation and regulation. Particularly promising were the reports of progress made toward characterizing the mechanistic basis for the generation of pigment patterns in animals. These findings, when integrated with the new information on life and death responses of melanocytes to growth factors that was reported in this session, should provide new insights into the origin of symmetry in the expression of several human hypopigmentary disorders. The broad range of vertebrate and invertebrate animals under investigation was striking as was the emerging evidence for evolutionarily conserved and divergent features of pigment cell development that makes each animal species, regardless of where it sits in the phylogenetic "tree", relevant to all of the others.

In a third poster, they showed that the in vitro melanocyte premature cell death induced by unchanged media or media supplemented with a-MSH/dopa was identical ultrastructurally with the premature melanocyte death found in 2 chicken pigment mutants. Cytochemistry evidence also supported this similarity of cell death processes in vitro and in vivo. In a fourth poster, evidence was presented that showed that these adult highly differentiated in vivo avian melanocytes responded to a-MSH via putative receptors and that c-AMP is the second messenger for MSH in chickens as it is in mammals.

1. Melanins and other pigments chemistry

(Comments by Prof. M. Peter)

Reports on the **Structures and Analysis** of melanins were dealing with IR spectroscopy (Bilinska), Transmission Electron-Microscopy (Donois and Surleve-Bazeille) and chemical methods (Ozeki et al.). Chemical modification of melanins was carried out in order to find better contrast agents for magnetic resonance imaging (Williams et al.).

Investigations of the **Properties of Melanins** continue to focus on redox chemistry, drug, and metal binding. Albino guinea pigs are more sensitive to ocular inflammations than pigmented animals due to the antioxidant effect of ocular melanin against excess of dispersed light (Bilgihan et al.). Protection against lipid peroxidation was also found in a study with albino and pigmented *Xenopus* (Corsaro et al.). A review on the free radical activity of melanins has appeared (Borovansky). The relevance of the chemical reactions of DA, 5-OHDA, and 6-OHDA with Fe^{3+} and with dioxygens for the pathogenesis of Parkinson´s disease was investigated (Kienzl et al.; Linert et al.). DA reacts with Fe(III) yielding an intermediate 1:1 complex, which decomposes releasing Fe(II) and the semiquinone, which reacts further under involvement of both Fe(III) and dioxygen. 6-OHDA is able to release iron as Fe(II) from ferritine without showing the necessity of forming an intermediate 1:1 complex. On the other hand, Fe(II) reacts in vitro in a Fenton type reaction with DA and H_2O_2 producing 5-OHDA and especially 6-OHDA. An explanation for the peculiarly toxic effects of manganese(II) is put forward in the second paper (Linert et al.). The interaction of fungal melanins with metals is covered in a paper by Fogarty and Tobin. **Drug Binding** was investigated with respect to uptake of compounds into melanin tissue. New results appeared with dimethylbenzanthracene and benzpyrene (Roberto et al.). Thiourea derivatives are incorporated into nascent melanin. It was now demonstrated that ^{14}C-thiourea accumulates in melanoma tissue of mice transplanted with B16 melanoma (Mårs and Larsson).

Investigations related to the **Biosynthesis** of melanins focus on the function (in particular cytotoxic) of precursors and new inhibitors or promotors of melanogenesis. Several new aspects on the cytotoxicity of intermediates of melanogenesis were described based on chemical approaches: Hydroxyl radicals ($^{\bullet}$OH) enhance initial oxidation of DA, dopa, and neurotoxic 6-OHDA and TOPA to semiquinones (Nappi et al.). Further oxidation to quinones is also enhanhed in case of DA and 6-OHDA, but apparently absent in case of dopa and TOPA. 6-OHDA is also generated from DA and $^{\bullet}$OH. It is concluded that OH radicals, when formed beyond the capabilities of cytoprotective mechanisms, not only rapidly oxidize catechol precursors but also generate of additional cytotoxic quinoid intermediates of eumelanin. Pavel and Smit suggest that melanogenic precursors can leak from melanosomes, in particular when melanogenesis is stimulated, and damage pigment cells through depletion of thiols and of *S*-adenosylmethionine which in turn leads to oxidative stress and diminished DNA methylation, respectively. Another theory concerning the onset of Parkinson´s disease is based on DA and/or oxidation products triggerd aptoposis which may be prevented by thiols but less likely by vitamins C or E (Offen et al.). Oxidation of 3,4-dihydroxybenzylamine by mushroom tyrosinase leads to 3,4-dihydroxybenzaldehyde, probably by the corresponding quinone methide intermediate (Sugumaran). Another paper has appeared dealing with the influence of β-alanine on catecholamine based cuticle colour in an insect (Wappner et al.). A new rôle of DHICA was discovered as a chemical messenger which stimulates nitric oxide production in macrophages (D´Acquisto et al.). **Inhibition of melanogenesis:** Kojic acid inhibits formation of pigmented products not only by inhibition of mushroom tyrosinase but also by reduction of intermediate quinones formed by residual enzyme activity from DL-DOPA, norepinephrine, or dopamine (Kahn). Novel melanin biosynthesis inhibitors are the plant secondary metabolites pauciflorine-A and -B from *Kopsia* (Kam et al.). Another very effective melanin biosynthesis inhibitor, melanoxazal [(E)-4-(2'-formyl-3'-hydroxybuten-1'-yl) oxazole], IC_{50} = 30.1 μg/ml with insect, 4.2 μg/ml with mushroom tyrosninase, was isolated from the fermentation broth of *Trichoderma* sp. (Takahashi et al.). NAD(P)H:(quinone acceptor) oxidoreductase reduces Leu- and Met-enkephalin-tyrosinase oxidation products, and dopachrome, but not *N*-acetyldopaquinone (Rescigno et al.). The enzyme decreases formation of melanin-like compounds from opioid peptides. Inhibition of melanin biosynthesis in fungi is an attractive target in plant disease control (Chumley). One report has appeared describing the **Degradation** of ocular melanin by cultured peritoneal macrophages (Hueber et al.). **Promotors of melanogenesis** were identified as a pyrazine derivative from the mushroom *Albatrellus* (Kawagishi) and as the anticancer strychnos alkaloids strychnopentamine and usambarensine which inhibit RNA synthesis and increase melanin synthesis (Bonjean et al.). McLeod et al. found that pro-opiomelanocortin-derived peptides stimulate tyrosinase in human melanocytes.

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2. Biology of pigment cells and pigmentary disorders

(Comments by Dr M. Picardo)

The definition of models suitable for studying the complex mechanisms of pigmentation is one of the tools of a biological approach to the pigmentary system. **Bessou et al.** (J.I.D. 107:684-688, 1996) have presented a new paper confirming the validity of the model proposed by the same group. The authors demonstrate that reconstructed skin can reproduce the in vivo phototype and that can be utilised to evaluate the response to UVB irradiation. The model seems to be promising to evaluate the role of melanocytes and keratinocytes in determining skin phototypes using allogenic reconstructs. **del Marmol** and co-workers demonstrated that cysteine deprivation promotes eumelanogenesis and that cysteine, glutathione and TRP1 are all important for the regulation of the balance between eu- and pheo-melanogenesis but that none of these factors seem to be predominant.

The understanding on the mechanisms controlling the balance between eu and pheo melanin is probably one of the most important points in the studies on the biology of pigmentation. Looking for the mechanisms controlling melanogenesis and hyperpigmentation, **Rungta at al.** report that exposure of melanoma cells to α-MSH induces the increase of tyrosinase mRNA and that the stimulation is independent of the protein synthesis; **Suzuki and co-workers** presented data, utilising the Northern blot analysis and determining the binding affinity of structurally similar melanotropic hormones, suggesting that α-MSH, ACTH and possibly ß-MSH are capable of a physiological role in regulating human pigmentation; **Chakraborty et al** have reported that UVB irradiation stimulates the production of POMC gene and the release of melanotropic hormones, possibly through a c-AMP dependent pathway and that an oxidative stress is involved in the mechanism. On the melanocortin receptors, **Hadley and co-workers** have published an interesting review and in the same issue of Pigment Cell Res. reported that cell specific melanocortin receptors are physiologic cell surface markers of epidermal melanocytes and keratinocytes.

The role of products secreted by keratinocytes in inducing hyperpigmentation have been evaluated by **Teraki et al.** The authors have demonstrated that endothelin 1, secreted by keratinocytes following UVB exposure, is a mitogen and melanogen for human melanocytes. Now they report that a similar mechanism could be considered in the hyper-pigmentation in seborrheic keratosis. The interactions between melanocytes and extracellular matrix protein is one of the most interesting argument in the biology of pigmented cells and several papers have been published on this matter in the last few months. The group of **S. MacNeil** have reported the possible role of extracellular matrix proteins in physiological regulation of melanocyte behaviour, including tyrosinase activity. On a similar argument, **Scott et al.** showed that attachment of human foetal and neonatal melanocytes to fibronectin prevent apoptosis. The integrin-dependent transducing signal seem to be the mechanism that suppresses apoptosis and the possible correlation with the expression of Bcl2 has been discussed. The importance of Bcl2 in maintaining melanocyte survival has been confirmed by the study of **Yamamura et al.** which reported an accelerated disappearance of melanocytes during hair cycle in Bcl2 deficient mice. The possible role of downregulation of CD44 expression, the major cell surface receptor for hyaluronate, in the behaviour of melanoma has been suggested by **Harwood and co-workers**. Studying the complex interactions between adhesion molecules and ECM

Danen et al. reported that adhesion to the components of basement membrane is reduced in proliferating melanocytes. In an interesting paper the group of **B. Gilchrest** have demonstrated, by confocal microscopy and with specific antibodies, an intimate contact between melanocytes and intraepidermal nerve endings. The presence of a structure similar to synaptic contacts was confirmed by EM studies and the data obtained sugggest that the nervous system may exert a tonic effect on melanocytes in human skin. **Grob et al.** have evaluated the relationship between immunodeficency and nevi reporting that in renal transplanted and in IIIV+ patients there is an increase of nevi number with respect to sex age and phenotype matched controls.
The interaction among UV exposure, free radical generation and skin response has been studied in different papers. **Romero-Graillet et al.** have presented stimulating data showing that exogenous nitric oxide donors stimulate cGMP and melanin synthesis. The authors concluded that NO and cGMP production is required for UVB-induced melanogenesis. The group of **J. Thody** reported that tyrosinase can utilise superoxide anion radical as a substrate for melanin synthesis and therefore can be considered a part of the scavenger system of the skin against free radical generation. The fact that free radicals can be considered among the biological transducers of UV irradiation has been confirmed by **Tebbe et al.** who reported that L-ascorbic acid can prevent the lipid peroxidation and he subsequent IL1 and IL6 secretion by human keratinocytes in culture. **Lavker & Kaidbey** have studied the correlation between the UVA spectrum and the cumulative damage in human skin. Evaluating both epidermal and dermal alterations, these authors reported that the shorter wavelengths (320-345) produce more significant epidermal modifications, whereas the broad spectrum of UVA was equally effective to producing dermal damage. Finally, **Hann and Lee** reviewed their casistic of 208 patients with segmental vitiligo and concluded that the clinical features of these patients differ from those of subjects with generalised disease. The authors have suggested that the difference could be correlated with different pathogenesis.

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Ultraviolet B radiation acts through the nitric oxide and cGMP signal transduction pathway to stimulate melanogenesis in human melanocytes. J Biol Chem. 271(45):28052-6, 1996.

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Regulation of tyrosinase mRNA in mouse melanoma cells by alpha-melanocyte-stimulating hormone. J Invest Dermatol. 107(5):689-93, 1996.

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Fibronectin suppresses apoptosis in normal human melanocytes through an integrin-dependent mechanism. J Invest Dermatol. 108(2):147-153, 1997.

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Binding of melanotropic hormones to the melanocortin receptor MC1R on human melanocytes stimulates proliferation and melanogenesis. Endocrinology. 137(5):1627-33, 1996.

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L-ascorbic acid inhibits UVA-induced lipid peroxidation and secretion of IL-α and IL-6 in cultured human keratinocytes *In vitro*. J Invest Dermatol. 108(3):302-306, 1997.

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Role of endothelin-1 in hyperpigmentation in seborrheic keratosis. Br J Dermatol. 135:918-928, 1996.

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Tyrosinase may protect human melanocytes from the cytotoxic effects of the superoxide anion. Exp Dermatol. 5(5):253-257, 1996.

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Atachment, speading and migration of melanoma cells on vitronectin: the role of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. Exp Dermatol. 5(6):308-315, 1996.

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Direct correlation between DNA repair capacity and metastatic potential of K-1735 murine melanoma cells. J Invest Dermaol. 108(1):3-6, 1997.

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Accelerated disappearance of melanocytes in bcl-2-deficient mice. Cancer Res. 56(15):3546-50, 1996.

4. Photobiology and photochemistry

(Comments by Dr M. d'Ischia)

Various papers on the photobiology of melanin pigmentation have appeared in the last months that seem worthy of comment. As an approach to dissect the complex regulatory mechanisms of melanization following UV irradiation in guinea pig skins, Maeda et al. (Photochem. Photobiol. 1996, Jul, 64:1, 220-223) examined the response of organ cultured skins to UV irradiation and different exogenous stimuli. In this system, phospholipases were found to have potent stimulatory properties on melanocytes and their activity, either directly or through an effect on keratinocytes.
The central role of keratinocytes as mediators of melanocyte response to UV stimulation is further supported in a relevant paper by Imokawa et al. (Biochem. J. 1996, 313 (Pt 2) 625-631). These authors showed that keratinocyte-derived growth factors produced by UVA-activated keratinocytes, and particularly granulocyte-macrophage colony-stimulating factor (GM-CSF), are important mediators of UVA-induced melanosis, as apparent from the marked increase in DNA synthesis in cultured human melanocytes elicited by a medium conditioned by UVA exposed human keratinocytes. Overall, these results provide an additional contribution to the understanding of the biological and biochemical mechanisms that concur to maintain melanocyte activity and melanin pigmentation in UV-stimulated epidermis. Among the other papers, mention goes to a review by Berardesca and Maibach (J Am Acad Dermatol, 1996 Apr, 34:4, 667-72) surveying racial differences in skin pathophysiology; a thoughtful article by Pavel and Smit (Sb Lek, 1996, 97:1, 29-39) examining the possible effects of intermediary products of the melanin pathway on the overall metabolic activity of pigment cells, and a paper by Bech-Thomsen and Wulf (Photoimmunol Photomed, 1996 Oct-Dec, 11:5-6, 213-8) evaluating the actual role of increased melanogenesis in the photoprotective response of skin to UV exposure and psoralen-UVA treatment.

- Bech-Thomsen N, Wulf HC .
Photoprotection due to pigmentation and epidermal thickness after repeated exposure to ultraviolet light and psoralen plus ultraviolet A therapy. Photodermatol Photoimmunol Photomed. 11(5-6):213-8, 1996.

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Racial differences in skin pathophysiology. J Am Acad Dermatol. 34(4):667-72, 1996.

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Granulocyte/macrophage colony-stimulating factor is an intrinsic keratinocyte-derived growth factor for human melanocytes in UVA-induced melanosis. Biochem J. 313(Pt 2):625-31, 1996.

- Maeda K, Tomita Y, Naganuma M, Tagami H .
Phospholipases induce melanogenesis in organ-cultured skin. Photochem Photobiol. 64(1):220-3, 1996.

- Pavel S, Smit NP .
Metabolic interference of melanogenesis in pigment cells. Sb Lek. 97(1):29-39, 1996.

- Stanford DG, Georgouras KE, Sullivan EA, Greenoak GE .
Skin phototyping in Asian Australians. Australas J Dermatol. 37(Suppl 1):S36-8, 1996.

5. Neuromelanins

(Comments by Marco d'Ischia)

In the present literature search a number of interesting papers are enlisted which deal with some basic issues relating to the nature, properties and functional significance of neuromelanin. By means of NMR techniques, Aime et al. (Adv. Neurol., 1996, 69: 263-270) provide a useful contribution to the structural characterization of human mesencephalic neuromelanin, with an eye to the iron content of this pigment, which is widely implicated as a potential etiological factor in degenerative diseases of the dopaminergic nigrostriatal tract. In pursuing their in vitro approach to the oxidative metabolism of catecholamines, Dryhurst and coworkers (Shen and Dryhurst, J. Med Chem. 1996, May 10, 39:10, 2018-2029) report the results of a study on the effect of L-cysteine on the oxidation of norepinephrine to melanin, as an extension of a similar investigation carried out on dopamine (Zhang and Dryhurst, J.Med.Chem. 1994, 37, 1084). As in the previous paper, the chemistry reported in this work revolves largely on the trapping of transient orthoquinones by the thiol compound to give adducts that are highly susceptible of intramolecular cyclization to give eventually benzothiazine derivatives with potential neurotoxic properties. If their biological relevance is confirmed, these studies would point to a considerable analogy between the aberrant oxidation chemistry of catecholamines in the central nervous system and the pheomelanin pathway in epidermal melanocytes. Hypotheses on the functional significance of neuromelanin and related pigments continue to add to the relevant literature. Smythies (Proc R Soc Lond B Biol Sci, 1996, Apr 22, 263: 1369, 487-489) revisits an early view on the origin of schizophrenia and degenerative diseases in light of recent biochemical data to postulate a function of Substantia nigra melanin as an intraneuronal scavenger of potentially toxic quinones and aminochromes produced during catecholamine oxidation. In a similar vein of thought, Borovansky (Sb Lek, 1996, 97:1, 49-70) provides an overview of the involvement of different types of melanins, including neuromelanin and soluble melanins, in various pathobiochemical processes, putting emphasis on the importance of methylation of reactive phenolic precursors as a means to divert them from toxic melanogenic reactions. Finally, by histochemical examination frequent abnormalities of the substantia nigra, often associated with nigral Lewy bodies, were demonstrated in patients with Alzheimer■s disease (Reyes et al., Panminerva Med 1996, Mar 38: 1, 8-14). Rarely, however, such abnormalities were severe in the absence of nigral Lewy bodies.

- BorovanskÆy J.
Free radical activity of melanins and related substances: biochemical and pathobiochemical aspects. Sb Lek. 97(1):49-70, 1996.

- Reyes MG; Faraldi F; Chandran R; Verano A; Levi AC.
Histopathology of the substantia nigra in Alzheimer's disease. Panminerva Med. 38(1):8-14 , 1996.

- Shen XM, Dryhurst G
Oxidation chemistry of (-)-norepinephrine in the presence of L-cysteine. J Med Chem. 39(10):2018-29, 1996.

- Smythies J .
On the functional of neuromelanin. Proc R Soc Lond B Biol Sci. 263(1369):487-9, 1996.

6. Genetics, molecular biology

(Comments by Dr F. Beerman)

- Aigner B, Besenfelder U, Seregi J, Frenyo LV, Sahintoth T, Brem G.
Expression of the murine wild-type tyrosinase gene in transgenic rabbits. Transgenic Research 5(6):405-411, 1996.

- Bassi MT, Incerti B, Easty DJ, Sviderskaya EV, Ballabio A.
Cloning of the murine homolog of the ocular albinism type 1 (OA1) gene - sequence, genomic structure, and expression analysis in pigment cells. Genome Research 6(9):880-885, 1996.
Shortened abstract : The mouse Oa1 gene encodes a putative protein of 405 amino acids displaying a high level of homology (78% identity, 87% similarity) to the human gene. The murine homolog shows six putative transmembrane domains, and the genomic organization is also conserved. Like its human counterpart, the expression pattern of Oa1, apart from the eye, is restricted to the epidermal melanocyte lineage and transcriptionally active at the same stage as most other tested melanosomal proteins (see also paper by Newton et al. 1996)

- Bernex F, Desepulveda P, Kress C, Elbaz C, Delouis C, Panthier JJ.
Spatial and temporal patterns of c-kit-expressing cells in W-lacz/+ and W-lacz/W-lacz mouse embryos. Development 122(10):3023-3033, 1996.

- Chen D, Guo JR, Gahl WA.
Rab GTPases expressed in human melanoma cells. Biochimica et Biophysica Acta - Molecular Cell Research 1355(1):1-6, 1997.

- Chen D, Guo JR, Miki T, Tachibana M, Gahl WA.
Molecular cloning of two novel rab genes from human melanocytes. Gene 174(1):129-134, 1996.

- Ermak G, Slominski A.
Production of POMC, CRH-R1, MC1, and MC2 receptor mRNA and expression of tyrosinase gene in relation to hair cycle and dexamethasone treatment in the C57BL/6 mouse skin. Journal of Investigative Dermatology 108(2):160-165, 1997.

- Ershov AV, Lukiw WJ, Bazan NG.
Selective transcription factor induction in retinal pigment epithelial cells during photoreceptor phagocytosis. Journal of Biological Chemistry 271(45):28458-28462, 1996.

- Graw J, Loster J, Neuhauserklaus A, Pretsch W, Schmittjohn T.
Molecular analysis of two new steel mutations in mice shows a transversion or an insertion. Mammalian Genome 7(11):843-846, 1996.

- Haffter P, Odenthal J, Mullins MC, Lin S, Farrell MJ, Vogelsang E, Haas F, Brand M, Vaneeden F, Furutaniseiki M, Granato M, Hammerschmidt M, Heisenberg CP, Jiang YJ, Kane DA, Kelsh RN, Hopkins N, Nüsslein-Volhard
Mutations affecting pigmentation and shape of the adult zebrafish. Development Genes & Evolution 206(4):260-276, 1996.
Summary (see also Kelsh et al. 1996): In a large scale screen for mutations affecting early development of the zebrafish, viable mutations were obtained which produce visible phenotypes in the adult fish. Among different other mutations, a group of mutations that failed to produce melanin was assayed for tyrosinase activity. Mutations in *sandy* (probably the c-locus homolog in zebrafish ?) produced embryos that failed to express tyrosinase activity. These are potentially useful for using tyrosinase as a marker for the generation of transgenic lines of zebrafish.

- Kelsh RN, Brand M, Jiang YJ, Heisenberg CP, Lin S, Haffter P, Odenthal J, Mullins MC, Vaneeden F, Furutaniseiki M, Granato M, Hammerschmidt M, Kane DA, Warga RM, Beuchle D, Vogelsang L, Nüsslein-Volhard C.
Zebrafish pigmentation mutations and the processes of neural crest development. Development 123. 369-389, 1996.
Summary: As part of a large-scale mutagenesis screen for embryonic/early lethal mutations in zebrafish, the group of Nüsslein-Volhard in Tübingen also isolated 285 mutations affecting all aspects of zebrafish larval pigmentation. In this paper, 94 genes were defined by complementation analysis. The authors classify the different phenotypes as acting on different levels of neural crest development and pigmentation (see Haffter et al., 1996).

- Klüppel M, Nagle DL, Bucan M, Bernstein A.
Long-range genomic rearrangements upstream of kit dysregulate the developmental pattern of kit expression in W-57 and W-banded mice and interfere with distinct steps in melanocyte development. Development 124(1):65-77, 1997.

- Lefur N, Kelsall SR, Mintz B.
Base substitution at different alternative splice donor sites of the tyrosinase gene in murine albinism. Genomics 37(2):245-248, 1996.
Summary: The classical albino (c) mutation in tyrosinase is caused by a C85S mutation. The c(2j) albino mutation at the mouse tyrosinase locus arose spontaneously in the C57BL/6 inbred strain and causes completeabsence of melanin synthesis. The authors now report that the mutation is different to c and caused by a R77L mutation. Apparently, on DNA, the mutation affects efficiency of splicing, mainly of one of the alternative splice donor sites.

- Marklund L, Moller MJ, Sandberg K, Andersson L.
A missense mutation in the gene for melanocyte-stimulating hormone receptor (MC1R) is associated with the chestnut coat color in horses. Mammalian Genome 7(12):895-899, 1996.

- Montoliu L, Umland T, Schütz G.
A locus control region at -12 kb of the tyrosinase gene. Embo Journal 15(22):6026-6034, 1996.
Comments: This paper supports further evidence for the importance of the enhancer/locus control region at -12 to -15 kb of the mouse tyrosinase gene. The two recent papers (Ganss et al., EMBO J. 13, 3083, 1994 and Porter and Meyer, Development 120, 2103, 1994) had described transgenic mice carrying tyrosinase minigenes and demonstrated the presence and necessity of the enhancer for high level, position-independent and copy-dependent expression of the tyrosinase gene. Here, the authors have choosen the YAC (yeast artificial chromosomes) technology to compare the expression of a wild type YAC (yielding wild type pigmentation, Schedl et al., Nature 362, 258, 1993) with YACs carrying several deletions in the mouse tyrosinase locus. Constructs in which the enhancer was deleted gave rise to much weaker expression and variable patterns of expression.

- Newton JM, Orlow SJ, Barsh GS.
Isolation and characterization of a mouse homolog of the x-linked ocular albinism (OA1) gene. Genomics 37(2):219-225, 1996.
Summary (see also above, Bassi et al. 1996): The mouse homolog of Oa1 (Moa1) was isolated as two isoforms from a melanoma cDNA library and predicted to encode proteins of 405 and 249 amino acids with six and two transmembrane-spanning regions, respectively. Interspecific backcross indicated that Moa1 is located much farther away from the pseudoautosomal region than its human homolog. In neonatal tissues, Moa1 RNA was detected in both skin and eyes by Northern hybridization and was not affected by the the albino mutation, or by the type of pigment synthesized, i.e., eumelanin vs pheomelanin. Expression of Moa1 RNA was not detected in embryonic tissues by Northern analysis or by in situ hybridization despite the active synthesis of ocular pigment by E16.5.

- Park KC, Park SK, Lee YS, Youn SW, Park BS, Kim KH, Lee ST.
Mutations of the tyrosinase gene in three Korean patients with type I oculocutaneous albinism. Japanese Journal of Human Genetics 41(3):299-305, 1996.

- Shioda T, Fenner MH, Isselbacher KJ.
Msg1, a novel melanocyte-specific gene, encodes a nuclear protein and is associated with pigmentation. Proceedings of the National Academy of Sciences of the United States of America 93(22):12298-12303, 1996.
Conclusion: In conclusion, msg1 encodes a nuclear protein, is melanocyte-specific, and appears to be lost in depigmented melanoma cells.

- Tief K, Hahne M, Schmidt A, Beermann F.
Tyrosinase, the key enzyme in melanin synthesis, is expressed in murine brain. European Journal of Biochemistry 241(1):12-16, 1996.

- Valverde P, Healy E, Sikkink S, Haldane F, Thody AJ, Carothers A, Jackson IJ, Rees JL.
The Asp84Glu variant of the melanocortin 1 receptor (MC1R) is associated with melanoma. Human Molecular Genetics 5(10):1663-1666, 1996.

- Wu M, Rinchik EM, Wilkinson E, Johnson DK.
Inherited somatic mosaicism caused by an intracisternal A particle insertion in the mouse tyrosinase gene. Proceedings of the National Academy of Sciences of the United States of America 94(3):890-894, 1997.

- Xu XL, Thornwall M, Lundin LG, Chhajlani V.
Val92Met variant of the melanocyte stimulating hormone receptor gene. Nature Genetics 14(4):1996.

- Yasumoto K, Yokoyama K, Takahashi K, Tomita Y, Shibahara S.
Functional analysis of microphthalmia - associated transcription factor in pigment cell-specific transcription of the human tyrosinase family genes. Journal of Biological Chemistry 272(1):503-509, 1997.
Summary: A further analysis on the role of the M box, the CATGTG motif and the microphthalmia transcription factor (MITF) in transcriptional regulation of tyrosinase,TRP-1 and TRP-2, analysed in the human genes. Amongst other results, the authors suggest from their data, that MITF is sufficient to direct pigment cell-specific transcription of the tyrosinase and TRP-1 genes but not the TRP-2 gene.

- Yoshida H, Hayashi S, Shultz LD, Yamamura K, Nishikawa S, Nishikawa S, Kunisada T.
Neural and skin cell-specific expression pattern conferred by steel factor regulatory sequence in transgenic mice. Developmental Dynamics 207(2):222-232, 1996.

7. Tyrosinase, TRP1, TRP2 and other enzymes

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

Two reports demonstrate a major role of specific PKC isoforms in the control of tyrosinase activity and pigmentation in murine melanocytes. PKC might regulate the constitutive expression of tyrosinase (Mahalingam et al, J. Cell. Physiol., 168, 549). Moreover, the kinase might mediate, at least partially, the melanogenic response to MSH (Park et al., Exp. Cell Res., 227, 70). This is a challenging observation, since most of us are used to assume that the melanogenic effect of MSH is mediated through the cAMP/PKA cascade, and there is a large body of evidence supporting this view (see for example Bertolotto et al., J. Cell Biol., 134, 747, abstract included in this issue). However, complex crosstalks between signaling cascades appear to be the rule, rather than the exception, and the work by Mahalingam et al, and Park et al. highlights the pertinence of reevaluating the mechanisms of MSH action.

On the other hand, several papers demonstrate a coordinate regulation of tyrosinase and TRP-1 on one hand, and TRP-2 on the other. Most extracellular signals affecting tyrosinase mRNA or protein levels exhibit a qualitatively similar effect on TRP-1. Conversely, TRP-2 is generally insensitive to these signals. In fact, few reports have shown a regulation of TRP-2, which appears to be a constitutive activity in those cell lines that express the protein. The implications of these observations are not clear, and the effects of varying the relative amounts of the melanogenic proteins on the overall rate of the melanogenic apthway and on the structure of the pigment will have to be assessed.

- Aigner B, Besenfelder U, Seregi J, Frenyo LV, Sahin-Toth T, Brem G.
Expression of the murine wild-type tyrosinase gene in transgenic rabbits. Transgenic Res. 5(6):405-11, 1996.

- Benathan M.
Modulation of 5-S-cysteinyldopa formation by tyrosinase activity and intracellular thiols in human melanoma cells. Melanoma Res. 6(3):183-9, 1996.

- Bertolotto C, Bille K, Ortonne JP, Ballotti R.
Regulation of tyrosinase gene expression by cAMP in B16 melanoma cells involves two CATGTG motifs surrounding the TATA box: implication of the microphthalmia gene product. J Cell Biol. 134(3):747-55, 1996.

- del Marmol V, Ito S, Bouchard B, Libert A, Wakamatsu K, Ghanem G, Solano F.
Cysteine deprivation promotes eumelanogenesis in human melanoma cells. J Invest Dermatol. 107(5):698-702, 1996.

- Halaban R, Bohm M, Dotto P, Moellmann G, Cheng E, Zhang Y.
Growth regulatory proteins that repress differentiation markers in melanocytes also downregulate the transcription factor microphthalmia. J Invest Dermatol. 106(6):1266-72, 1996.

- Jimenez M, Garcia-Carmona F.
Hydrogen peroxide-dependent 4-t-butylphenol hydroxylation by tyrosinase--a new catalytic activity. Biochim Biophys Acta. 1297(1):33-9, 1996.

- Longa SD, Ascone I, Bianconi A, Bonfigli A, Castellano AC, Zarivi O, Miranda M.
The dinuclear copper site structure of Agaricus bisporus tyrosinase in solution probed by X-ray absorption spectroscopy. J Biol Chem. 271(35):21025-30, 1996.

- Mahalingam H, Vaughn J, Novotny J, Gruber JR, Niles RM.
Regulation of melanogenesis in B16 mouse melanoma cells by protein kinase C. J Cell Physiol. 168(3):549-58, 1996.

- Mengeaud V, Ortonne JP.
PUVA (5-methoxypsoralen plus UVA) enhances melanogenesis and modulates expression of melanogenic proteins in cultured melanocytes. J Invest Dermatol. 107(1):57-62, 1996.

- Merimsky O, Shoenfeld Y, Baharav E, Altomonte M, Chaitchik S, Maio M, Ferrone S, Fishman P.
Melanoma-associated hypopigmentation: where are the antibodies? Am J Clin Oncol. 19(6):613-8, 1996.

- Park HY, Russakovsky V, Ao Y, Fernandez E, Gilchrest BA.
Alpha-melanocyte stimulating hormone-induced pigmentation is blocked by depletion of protein kinase C. Exp Cell Res. 227(1):70-9, 1996.

- Powers TP, Davidson RL.
Coordinate extinction of melanocyte-specific gene expression in hybrid cells. Somat Cell Mol Genet. 22(1):41-56, 1996.

- Ramon-Ros J, Rodriguez-Lopez JN, Carlos-Espin J, Varon R, Garcia-Canovas F.
Oxymetric and spectrophotometric study of the ascorbate oxidase activity shown by frog epidermis tyrosinase. Int J Biochem Cell Biol. 28(8):917-23, 1996.

- Rungta D, Corn TD, Fuller BB.
Regulation of tyrosinase mRNA in mouse melanoma cells by alpha-melanocyte-stimulating hormone. J Invest Dermatol. 107(5):689-93, 1996.

- Steiner U, Schliemann W, Strack D.
Assay for tyrosine hydroxylation activity of tyrosinase from betalain-forming plants and cell cultures. Anal Biochem. 238(1):72-5, 1996.

- Tief K, Hahne M, Schmidt A, Beermann F.
Tyrosinase, the key enzyme in melanin synthesis, is expressed in murine brain. Eur J Biochem. 241(1):12-6, 1996.

- Tief K, Schmidt A, Aguzzi A, Beermann F.
Tyrosinase is a new marker for cell populations in the mouse neural tube. Dev Dyn. 205(4):445-56, 1996.

- Vinayagamoorthy T, Rajakumar AR.
Stress relief protein modulation by calnexin. Sci. 793:479-84, 1996.

ANNOUNCEMENTS & RELATED ACTIVITIES

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

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

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

Keynote speakers: - Vincent J Hearing, PhD, Senior Investigator, National Cancer Institute, Bethesda, MD
- Yutaka Kawakami, MD, PhD, Visiting Scientist, Surgery Branch, National Cancer Institute, Bethesda, MD
- Richard L Sidman, MD, Bullard Professor of Neuropathology, Harvard Medical School, Boston, MA
- James H Wyche, PhD, Associate Professor of Medical Science and Associate Provost, Brown University, Providence,RI

The keynote speakers will provide the foundation for four important themes of the meeting:
1) The biogenesis and structure of melanosomes, 2) Novel approaches to the development of anti-melanoma vaccines and other treatments, 3) The origin, distribution, and functional significance of ocular melanin, 4) The nature and significance of programmed death (apoptosis) of normal melanocytes and melanoma cells.

Symposia, mini-symposia and workshops are being planned on these themes as well as others reflecting the broadest interests of the Society's members. A new addition will be the offering of one or two "Sunrise Course(s)" with an early morning session on each of three days. Each course will be designed to provide interested members with basic information, state of the art technical methods and materials, and up-to-date modes of access to literature and archival technical data central to a particular sub-field of pigment cell research. More detailed information about the potential courses will be provided in the Second Announcement of the meeting that will be distributed early in February, 1997. The social program, still tentative, will include a reception and banquet at the Brown University Faculty Club and an outing recapturing Rhode Island History. Travel stipends to attend the meeting will be available for members of our Society as outlined in the following section.

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

1997 **7th ESPCR Meeting: Bordeaux, France, 9 - 11 October**
Contact: Dr Alain TAEIB
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1999 XVIIth International Pigment Cell Conference: Nagoya Congress Center, Japan, October 30 - November 3

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Message from the President:

The ESPCR would like to thank and acknowledge Pharmacia & Upjohn for generous donations in support of the 7th Scientific ESPCR Meeting in Bordeaux and the invitation of the Council of the International Federation of Pigment Cell Societies (IFPCS) to their annual Board meeting, which will be hosted by the ESPCR in Bordeaux. We expect these financial contributions to be of great value for the organization of the meeting in all respects.

IFPCS Travel Stipends:

As part of our initiatives to promote international interactions, the International Federation of Pigment Cell Societies (IFPCS) has established IFPCS-sponsored travel grants for young scientists to visit and train in laboratories outside of their own country.

These travel grants will be awarded each year to highly qualified young scientists from each of the three regional Societies to visit other laboratories to learn specialized techniques and establish collaborations that will facilitate their own future research objectives. In addition to the importance of such training to the young investigators who receive these awards, this program should stimulate and foster interactions and collaborations between the international laboratories involved.

Each stipend will be for a maximum of $3,000 to support a visit for 2-3 months, and they will be competitive on an annual basis. Each regional Society will be entitled to award one IFPCS Travel Stipend per year.

We now invite young scientists from the ESPCR to apply for the IFPCS Travel Stipend 1997. The applicant should forward a single page, single spaced letter which states their laboratory of origin, the laboratory they wish to visit, the project overview, the specific reason for the visit, and the projected expenses (not to exceed $3,000). We also request a letter from the laboratory to be visited that they will accept the candidate if the application is successful.

The application should be sent to the Secretary of the ESPCR (Dr Stan Pavel) not later than June 1, 1997. The final designation of the Awardee will be made by an ad hoc Committee of the ESPCR Council before August 1, 1997.

Travel must begin in the calender year within which the IFPCS Travel Stipend is awarded but may continue into the following year. Awardees must send a summary of their expenses with appropriate receipts to the IFPCS Secretary/Treasurer (Dr Bengt S. Larsson) within two months following the conclusion of their travel.

The IFPCS would like to thank and acknowledge the following companies for their financial support of this programme:

Shiseido American Technocenter Sunstar, Inc.
Taisho Pharmaceutical Co., Ltd.
The Procter & Gamble, Co.
Unilever Research
Beiersdorf AG
.... others are pending.

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.

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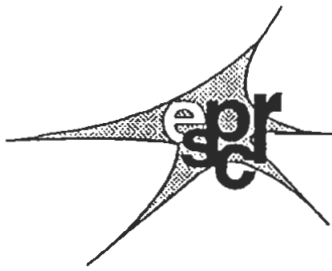

NEWS FROM THE IFPCS

INTERNATIONAL FEDERATION OF PIGMENT CELL SOCIETIES

ESPCR **PRESIDENT**, Vincent J Hearing, (Bethesda, USA);
JSPCR **VICE-PRESIDENT** - Yoshiaki Hori, (Fukuoka, JAPAN);
PASPCR **SECRETARY/TREASURER** - Bengt S Larsson (Uppsala, SWEDEN)

COUNCIL MEMBERS: Sally Frost-Mason, (Lawrence, USA); Yutaka Mishima, (Kobe, JAPAN); Shosuke Ito, (Toyoake, JAPAN); James J Nordlund, (Cincinnati, USA); Stan Pavel, (Leiden, NETHERLANDS); Giuseppe Prota, (Naples, ITALY)

The IFPCS Council met several times during the IPCC held in Anaheim this Fall, and, among many other actions (as outlined below), elected as Officers for the next 3 years, VJ Hearing (PASPCR) as President, Y Hori (JSPCR) as Vice-President and BS Larsson (ESPCR) as Secretary/Treasurer. On behalf of my fellow Officers, I would like to thank everyone for their confidence, and I can assure you that we intend to work extremely hard as a cohesive unit during these next 3 years to continue the growth, innovation and interactions initiated during the first 2 administrations under Profs Mishima and Prota. This now completes the rotation cycle among the regional Societies and we will strive to make the IFPCS even more active and efficient in fostering scientific exchange among our members. In addition to other Council members who have stayed on (Drs VJ Hearing, S Ito, BS Larsson, Y Mishima, J Nordlund, and G Prota), we would like to welcome our new Council members (Drs S Frost-Mason, Y Hori and S Pavel) and say goodby to departing Council members (RA King and PA Riley).

I would like to extend the thanks of the IFPCS to Drs. FL Meyskens, RR Bowers and AJ Cochran (Chairman and co-Organizers), as well as to their Organizing and Scientific Program Committees, for the outstanding job they did for the XVIth International Pigment Cell Conference (IPCC) held in Anaheim. The social and scientific program, as well as the Conference facility itself, was outstanding and a great success.

The **XVIIth IPCC** will be held in Nagoya, Japan from October 30th - November 3rd, 1999 under the chairmanship of Prof S Ito and I hope everyone will make plans to attend that meeting. We have seen the preliminary plans for that Conference and it promises to be an outstanding one; Prof Ito is planning a number of stimulating social and scientific events for that meeting and travel support should be available for Young Investigators in the various regional Societies who might want to attend. Details of the XVIIth IPCC will be forthcoming as the meeting approaches.

The **IFPCS is on the InterNet**; the address is: **http://lenti.med.umn.edu/paspcr/ifpcs.html**. From our home page you can access links to the InterPig DataBase, news of the upcoming XVIIth IPCC, summaries of the previous IPCC, home pages of the regional Pigment Cell Societies, and other pertinent information. We invite you to visit us at this site and enjoy the wealth of information there; I would like to thank WS Oetting for his time spent in maintaining this site at no expense to the IFPCS. Let us know how we might improve this Web site to make it a more valuable resource to your work.

The **Publications Committee** (Y Mishima, chair, JJ Nordlund, S Pavel) met with Peter Hartmann of Munksgaard regarding subscription prices and other publications issues. They were able to negotiate a very favorable subscription rate available only to members of the regional Societies; this $95 (USD) rate represents about a 70% discount over the standard rate of $324 (USD). We urge all of our members to subscribe to Pigment Cell Research through their regional Society to obtain this rate and support the journal. The Editorial Report given by J Matsumoto was excellent and the IFPCS Council expressed its appreciation to him for his outstanding job as the Editor.

The **Special Experts Committees** (G Prota, chair), initiated 3 years ago to foster interactions in specific subfields of pigment cell research, will be expanded over the next several years and should culminate with interim Reports, Discussion Groups and eventually with IPCC Symposia and Workshops. The topics and chairs of each Special Expert Committee is: Albinism - RA King; Biology of Melanoma - FL Meyskens; Development Biology - S Frost-Mason; Hypo/Hyperpigmentation - Y Mishima; Ocular/Extracutaneous Pigmentation - G Prota; Vitiligo - JJ Nordlund; InterPig DataBase - PA Riley. These chairs will be coordinating the activities of their groups and anyone who wishes to provide suggestions and comments to those groups can address them to the relevant chair.

As a new initiative, we are trying to generate a source of funds to sponsor **IFPCS Travel Awards**; these will be intended for Young Investigators from each of the 3 regional Societies to visit other laboratories (preferably in other countries) to learn specialized techniques and/or to establish collaborations. Such support will be for 2-3 months only and will be competitive on an annual basis; each Society will determine its own mechanism for soliciting and awarding such awards. We are hoping to establish these awards beginning in 1997 and further information about these will be forthcoming from your own Society.

Finally, we have established an ad hoc **Women Scientists Committee** (S Frost-Mason, chair, M Mizoguchi, DC Bennett) that will discuss women's and other minority issues in pigment research; that Committee will make recommendations to the IFPCS Council as to how we might help deal with any concerns noted and improve the lot of those affected.

To conclude, I am highly optimistic that the next 3 years will see continued outstanding growth and opportunity in our 3 regional Pigment Cell Societies. Our field of pigment cell research is becoming a more popular and interactive one every day and I would encourage each of you to become more active in your Society and recruit others in the field to join. I would welcome any suggestions and comments you might care to make as to how the IFPCS might function more efficiently to promote cooperation and collaborations between our members. I would like to take this opportunity to wish each of you a safe, healthy and prosperous New Year.

With best regards,

Vincent J. Hearing
President, IFPCS

Meeting Report - XIth JSPCR Annual Meeting

by Yasuo Kubota / Jiro Matsumoto

The XIth Annual Meeting of the JSPCR was held in Kawasaki, Japan from December 6th-7th, 1996. This meeting was organized by the Department of Dermatology, St. Marianna University School of Medicine with Prof Masako Mizoguchi as Chairman and Dr Yasuo Kubota as Secretary/General. The scientific program was composed of two Invited Lectures, one Special Lecture, five Research Seminars and 33 oral presentations. One of the major themes of this meeting was "Melanocyte differentiation and development" and most of the invited and special lectures were arranged along this line of thought.

Invited Lecture 1 "Segregation of melanocyte precursors and regulation of their fate during neural crest development" Dr James A Weston, Univ Oregon, addressed that mouse melanocyte precursors (MPs) segregate from the neural crest as that subpopulation enters a migration staging area, takes the dorsal pathway to distribute in the dermal mesenchyme and then reaches the epidermal layer. During their development, MPs transiently require the function of c-kit and its ligand SCF. He presented data relating the
expression of SCF mRNA in vivo by means of in situ hybridization.

Special Lecture "Life styles of pigment cells during embryogenesis and
postnatal life" Dr Nishikawa, Kyoto Univ, presented data on the cellular and molecular basis controlling the proliferation and differentiation of mouse melanocyte precursors. Based on his own and other groups' studies, he addressed that a number of molecules have been expressed from the very early phase of melanocyte differentiation, and that using such molecules as probes, melanocyte precursors have been easily distinguished from other cells of neural crest origin. He concluded that the life styles of melanocytes were essentially determined by the microenvironment where they resided. In this connection, Dr Kunisada, a member of Nishikawa's group, reported in his oral presentation that in transgenic mice bearing mouse SCF-DNAs fused with the human cytokeratin 14 promoter, epidermal melanocytes consecutively proliferate and the whole skin is heavily pigmented, suggesting the importance of SCF for growth, differentiation and motility of melanocytes. They also discussed the accumulation of mast cells in the dermis and epidermis of these hyperpigmented transgenic mice.

Invited Lecture 2 "Regulation of transcription in melanocyte differentiation and development" Dr Colin Goding, Marie Curie Research Inst, discussed the role of transcription factors in melanocyte development and differentiation based on his studies regarding identification and characterization of the sequences required for melanocyte-specific expression of the mouse TRP-1 and human tyrosinase promoters. He also discussed Brn-2 (POU domain transcription factor) and a bHLH-LZ protein associated with microphthalmia (Mi). The involvement of Brn-2 in melanocyte development was shown by deterioration of its function upon introduction of a double amino acid substitution to it. The ability of Mi for transcription activation was suggested to be regulated negatively by phosphorylation.

In a Research Seminar, Dr Tomohisa Hirobe, National Inst Radiological Sciences, summarized his recent findings on the proliferation and differentiation of mouse epidermal melanocytes following skin wound healing, indicating the combined roles of genetic and local tissue environmental factors. In combination with this seminar, Dr Rikako Furuya, Shiseido Research Center, reported her recent results on skin hyperpigmentation caused by UV irradiation as chronic effects, using melanocytes cultured from disaggregated epidermal cell suspensions of hairless mice (HR-1 x HR/De). Her conclusion was that the chronic effects of repeated UV exposures on melanocyte proliferation and differentiation were associated largely with changes in the nature of keratinocytes.

In another Research Seminar, Dr Saida, Shinshu Univ, presented his concept on "Malignant melanoma (MM) in situ" and emphasized its significance in clinical and basic oncology. He reported several cases of MM in situ appearing in the sole and the nail apparatus and proposed that the concept of MM in situ and the theory of its de novo origin should have great impact on the early correct diagnosis of MM and the design of basic research on human carcinogenesis.

With regard to new melanogenic inhibitors, several papers were read before this meeting; Dr Funasaka from Kobe Univ reported the inhibitory effects of DL-alpha-tocopherol having an antioxidant activity on melanogenesis. Dr Eiichiro Yagi, Pharmaco Science Research Lab, reported that an extract of Cola de caballo, a plant grown in the Andes district, has dual inhibitory effects for melanogenesis and inflammation induced by UVB irradiation. Dr Tomohiro Yokota, Kanebo Cosmetics Lab, reported that glabridin in hydrophobic licorice extracts showed an inhibitory effect on melanogenesis and inflammation. He also mentioned the relationship between the structure of glabridin and these two different inhibitory functions.

As to the intracellular localization of post-DOPAchrome melanogenesis, Dr Shinkichi Hatae, Mishima Research Inst for Dermatology, suggested that melanin polymer by DOPAchrome reaction at GERL and coated vesicles is produced by tyrosinase via DHI and that premelanosomes could polymerize melanin monomer, DHI, independently from tyrosinase.