XVIth IPCC (International Pigment Cell Conference) Program Summaries

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 Symposia, Workshops and Poster Discussions written by Chairs of those sessions who are PASPCR Members. The meeting was a great success as those of you who attended already know; we are indebted to the organizers for putting on such a good show on behalf of the PASPCR. *The Editor conveys a special thank you to all contributors of these summaries.*

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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.
N 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.
Y 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 (MSH induced DNA synthesis, to strongly induce melanocortin 1 receptor after 24 h, and to sustain survival of both keratinocytes and melanocytes.
FL Meyskens: "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 (H_2O_2), was generated with a glucose/glucose oxidase system and detected by chemiluminescence of luminal. Melanocytes and melanoma cells handled H_2O_2 differently as evidenced by time course measurements. The closing question posed was, "Is this difference in the handling of H_2O_2 due to the antioxidant ability of melanin?"
M 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.
M 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^{o}C to 32^{o}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.
AK 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 *alpha*-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 1-*alpha* but not by endothelin-1 or tumor necrosis factor-*alpha*. 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

J 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 (-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 days. In a third poster, they showed that the in vitro melanocyte premature cell death induced by unchanged media or media supplemented with (-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 (-MSH via putative receptors and that c-AMP is the second messenger for MSH in chickens as it is in mammals.

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 *alpha*-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 corrrespond, 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.

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, (C310, and P431L. Affected individuals were homozygous for R77Q/R77Q (n=2) or (C310/(C310 (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 #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 transription of the TRP-2 gene is regulated in a different manner from that of the tyrosinase and TRP-1 genes. Further, they idntified 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.

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

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.

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