**Meeting report of the**
**19th International Pigment Cell Conference**
**September 18-22, 2005**

**Reston, Virginia**

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## Sunday, September 18, 2005

### Plenary Symposium 1 - 'Advancing the Frontiers'
*by John Pawelek*

Keynote Lecture 1: 'Genomics and Disorders of Human Pigmentation', Dr. FS Collins, National Human Genome Research Institute

Dr. Francis Collins summarized some of the major achievements of the Human Genome Project with the completion of its original goals in 2003. Some 30 mammalian genomes have now been sequenced. One of the most striking findings is the well-known remarkable similarity (98.9%) of the DNA sequences between humans and chimpanzees. There are now

many services and gene libraries available to scientists. Currently in the public repository are more than 22, 000 human loci and 23, 000 mouse loci. The Knock-Out Mouse Project has the goal of establishing knock-outs for all mouse genes. The ENCODE project is an encyclopedia of human DNA elements. The Molecular Libraries Roadmap has screening centers that can screen 100,000 small molecules for activity in enzymatic assays of your design. These molecules can be accessed in the new PubChem database. The Human Cancer Genome Project will sequence the genomes of 250 representative tumors from each of 50 tissue types. The International HapMap Project (www.hapmap.org) is designed to better characterize the single nucleotide polymorphisms (SNP's) in the human genome.

Keynote Lecture 2: Telomeres, Telomerase, Senescence and Cancer?, Dr. EH Blackburn, University of California, San Francisco

Dr. Blackburn summarized the role of telomerase in maintaining telomere length and lifespan extension in human cancer cells. One surprising finding was that net telomere lengthening and lifespan extension can be uncoupled, supporting the conclusion that telomerase plays roles in addition to the net lengthening of telomeric DNA. This came from an unexpected finding that a hairpin siRNA targeting human telomerase RNA rapidly inhibited growth of cancer cells without bulk telomere shortening and induced a novel gene expression that included suppression of genes implicated in angiogenesis and metastasis. The findings uncovered functions of telomerase in tumor growth and progression in addition to telomere maintenance.

Keynote Lecture 3: 'Frontiers in fluorescent Protein Imaging of Living Cells', Dr. J. Lippincott-Schwartz, National Institute of child Health and Human Development.

Dr. Lippincott-Schwartz detailed the use of fluorescent proteins such as green fluorescent protein (GFP) as molecular tags for following complex biological processes in living cells. Modified GFP's have been used as markers to track and quantify individual or multiple protein species, as probes to monitor protein-protein interactions, and as photochemically-modulatable proteins to highlight and follow the fate of specific protein populations within the cell. She focused on methods of kinetic microscopy involving photobleaching and photoactivation that are being used to monitor the appearance, location, movement, and degradation of GFP fusion proteins.

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**Plenary Symposium 2 - 'Hot Topics Symposium'**
*by Shosuke Ito, Stan Pavel, and Vincent J. Hearing*

Plenary Symposium 2 was co-chaired by the Organizers of the current and the two previous IPCCs: Shosuke Ito, Stan Pavel, and Vincent J. Hearing. The tradition of holding the ?Hot Topics Symposium? was introduced during the 17th IPCC held in Nagoya six years ago. The idea was to put together the best abstracts submitted with highly innovative contents that would be selected from all abstracts accepted for oral presentation in the various Symposia. It was an enjoyable yet (tough) uneasy job to anonymously choose the 5 best abstracts from the 15 already selected as #1 for each of the 15 symposia. Stan, Vince, and I were pleased to select the following 5 abstracts that will certainly advance further our knowledge on pigmentation and pigment cells. We take special delight in noting that the 5 abstracts chosen were from 5 widely divergent areas and that there was an excellent distribution of speakers internationally which shows the high quality of science being performed around the world.

The first presentation was given by Dr. Victor A. Canfield (Hershey, USA) who talked about the identification and characterization of zebrafish golden gene. This newly identified gene encodes a potassium-dependent sodium, calcium exchanger. Surprisingly, the human orthologue is involved in the regulation of constitutive pigmentation. It was shown that 30% variation in human pigmentation may be attributable to one of the alleles of the golden gene.

In the next presentation, Dr. Emi Nishimura (Hokkaido, Japan) discussed the mechanisms of hair graying which is everybody?s concern. She was able to demonstrate that hair graying is caused by defective self-maintenance of melanocyte stem/progenitor cells. In Bcl-deficient mice, hair turns gray with aging because melanocyte stem cells selectively die by apoptosis in the stem cell niche in dormant state. These processes are controlled by the melanocyte master transcription regulator Mitf.

In the following talk, Dr. Mitsunori Fukuda (Wako, Japan) discussed the roles of Slp- and Slac2- family proteins in melanosome distribution and maintenance of elongated shape of melanocytes. Among others, knockdown of endogenous Slp-2a, the most abundant of the Slp family, by siRNA caused a dramatically reduced number of melanosomes in the cell periphery of melanocytes (?peripheral dilution?) and a morphological change to a round shape. These processes mimicked those seen in Griscelli syndrome.

A happening took place at the beginning of the next talk by Dr. Guillaume Robert (Nice, France). A fire alarm went off, and everybody was forced to rush out of the building. After having watched fire engines arriving and firemen working, there was nothing to worry about. We returned to the room and resumed the symposium with a full audience. Dr. Robert discussed the roles of SPARC, Secreted Protein Acidic and Rich in Cysteine. SPARC expression is inversely correlated to E-cadherin expression in melanocytes and malignant melanoma cell lines. SPARC depletion leads to up-regulation of E-cadherin, and SPARC-null cells exhibit a marked decrease in their migratory and invasive phenotype, supporting a critical role for SPARC in malignant transformation of normal melanocytes.

Finally, Dr. James Grichnik (Durham, USA) closed the Symposium by addressing the most controversial topic among the five talks. He presented a hypothesis that melanomas are derived from stem cells and not through stepwise 'dedifferentiation' from melanocytes. Melanoma cell lines investigated revealed the presence of a subpopulation of small cells that were less pigmented and grew slowly to form colonies. These and other findings support the possibility of existence of melanoma tumor stem cells and have implications for the origin of melanoma.

Everybody found that the Hot Topics Symposium was successful as it was in Nagoya and Egmond aan Zee, and were ready for the Welcome reception with the 20 min's delay.

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**Monday, September 19, 2005**

**Plenary Symposium 3 - "Developmental Biology 1, Melanoblast/RPE - Specification, Development, Survival and Apoptosis (Waardenburg syndrome, Tietz syndrome)"**
*by Veronique. Delmas*

The symposium "Developmental Biology of Melanocytes" opened with two consecutive plenary lectures. The first was presented by E. Dupin who described the plasticity exhibited by the Neural Crest Cells (NCC) in vivo, and the presence of precursor cells in Neural Crest derivatives until late in development. Quail neural crest cells isolated at early migratory stages are heterogeneous with respect to their developmental potential, including lineage-committed cells as well as diverse pluripotent and oligopotent progenitors. The reversibility of differentiated cells (melanocytes or glia) to their pluripotent precursor can be induced in vitro by endothelin 3. Therefore, when subjected to appropriate stimulus, pigment cells can revert to their neural crest stem cell ancestors.

The second plenary lecture was delivered by H. Arnheiter who summarized a large body of work from his own lab and others on the essential role of MITF in pigment cell development. H. Arnheiter reported a novel and interesting way of regulating MITF activity by its association with the histone deacetylase HDAC proteins. The specific role of HDAC members in melanocyte development in vivo is under further investigation in his laboratory.

Next J. Lister addressed the function of the forkhead transcription factor, Foxd3 during zebrafish NCC fate specification and suggested that in culture Foxd3 can repress Mitfa promoter in a melanoma cell line.

From the same lab, K. Bismuth described the generation of a knock-in mouse whose Mitf gene encodes a non-phosphorylatable alanine instead of serine (S73). Surprisingly, the introduced mutation leads to preferential exclusion from the mRNA of the subexon 2b which encodes the mutated S73A residue. The predominant expression of MITF lacking exon 2b, along with a minor contribution of full-length MITF lacking a phosphorylatable S73, increases the numbers of differentiated melanocytes.

Several presentations described the function and the relationship/interconnection between the genes involved in the Waardenburg syndrome type 4, an auditory-pigmentary disorder which is characterized with the presence of the aganglionic megacolon. These genes are the endothelin receptor type B (EDNRB) gene, the endothelin 3 (EDN3) gene, or the SOX10 gene. S. Yokoyama described how Sox10 regulates the endothelin receptor type B in pigment cells. He reported that SOX10 can transactivate two of the four EDNRB promoters by different mechanisms; an Sp1-dependent mechanism for the conventional EDNRB promoter and by an Sp1-independent mechanism for the EDNRB?2 promoter. MK Lowenstein (L. Kos?s laboratory) described the generation of a new transgenic mouse line expressing the Endrb under the control of the Dct promoter which is able to rescue the hypopigmentation phenotype of the heterozygous Sox10 mutant, Sox10tmlWeg/+, but not the phenotype of Pax3 heterozygous mutants. These results suggest that Ednrb and Sox10 might interact specifically for proper melanocyte development.

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**Plenary Symposium 3 - "Developmental Biology 1, Melanoblast/RPE - Specification, Development, Survival and Apoptosis" (Waardenburg syndrome, Tietz syndrome)**
*by Lidia Kos*

In her plenary lecture, E. Dupin reviewed a series of experiments that demonstrate the initial heterogeneity of neural crest precursors. Avian neural crest precursors range from totipotent to lineage-committed cells, all with the potential to give rise to melanocytes. Some of these precursor cells exhibit stem cell properties and are dependent on the action of Endothelin 3 for their survival and proliferation. In vitro, already committed melanocytes maintain the capacity to respond to this factor by up-regulating a series of early neural crest markers and returning to a bipotential (melanocyte-glia) or pluripotent state. Dr. Dupin suggested that this plasticity might have some in vivo significance in cases such as nerve injury when melanocytes and glial cells might need to re-program to compensate for cell loss.

Very little is known about the regulation of the Endothelin 3 receptor, endothelin receptor b (EDNRB). S. Yokoyama et al showed that in human melanocyte cell lines, the transcription factor Sox10 was capable of transactivating the conventional EDNRB as well as the alternative EDNRB2 promoters. Sox10 could bind to two CA-rich regions in the conventional EDNRB promoter and its transactivation was synergistically enhanced by the binding of SP1 to a GC box. In further support of a possible interaction between Sox10 and Ednrb, M. K. Lowenstein et al. showed that the transgenic expression of Ednrb under the control of the DOPAchrome tautomerase promoter (Dct-Ednrb) was capable of rescuing the hypopigmentation phenotype of heterozygous mutant mice in which LacZ was inserted into the Sox10 locus (Sox10tm/Weg). The rescue happened as early as embryonic day 12.5 as more melanoblasts were observed in the trunk region of Sox10 mutant embryos carrying the Dct-Ednrb transgene when compared to those without the transgene. However, heterozygous mutant mice for both Sox10 and Ednrb did not exhibit an increase in their hypopigmentation phenotype when compared to either mutant alone, suggesting that at the genetic level these two genes do not act synergistically for the production of a normal coat color pattern.

The transcription factor MITF plays a critical role in all stages of melanocyte development, controlling cell determination, survival, proliferation and differentiation. In his plenary lecture, H. Arnheiter provided a series of genetic evidence in mice to highlight the importance of the tight regulation that MITF undergoes both at the transcriptional and post-translational levels that allows this factor to exert so many different functions. He focused specifically on the mechanism of gene regulation provided by the control of the acetylation state of nucleosomal histones mediated by histone acetyltransferases and histone deacetylases (HDACs). HDACs are expressed in various melanocyte cell lines and MITF overlaps with HDACs 1 and 4. A small domain in HDAC 4 can bind to MITF in vitro and this interaction resulted in the repression of MITF directed transcription of target genes. Dr. Arnheiter suggested that the pigmentation phenotypes of the many MITF mutant alleles could possibly be explained by their interactions with HDACs.

The other two talks further addressed mechanisms of MITF regulation. In vitro studies have shown that Kit signaling regulates MITF by phosphorylation at Serine 73 (S73), increasing both its transcriptional activity and its degradation. K. Bismuth et al. created a knock-in mouse in which S73 was substituted by a non-phosphorylatable alanine. The mutated codon lead to the exclusion of exon 2b from the mRNA, indicating that this region is part of an exonic splicing enhancer. Homozygous mutant mice displayed normal coat color pattern but neural crest cell cultures derived from mutant embryos gave rise to many more melanocytes when compared to wild type cultures, suggesting that MITF lacking exon 2b increases the proliferation and/or survival of precursors. J.A. Lister et al. presented evidence that in zebrafish, mitfa is negatively regulated by the transcription factor foxd3, an early neural crest marker. The zebrafish mitfa mutant nacre lacks melanophores but shows an increase in the number of iridophores. When foxd3 was knocked down by morpholinos, the number of iridophores was reduced in wild type animals but not in mitfa mutants, suggesting that in this pigment cell type foxd3?s effect is mediated via the repression of mitfa. This putative interaction seems to be direct as demonstrated by the ability of foxd3 to repress the activity of the mitfa promoter in vitro.

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**Plenary Symposium 4 - "Evolution and Development of the Pigmentary System"**
*by Randall L. Morrison*

Greg Barsh gave the Aaron B. Lerner lecture at the beginning of this session and discussed the coat color genetics of dogs. Agouti pigment type switching does occur in dogs and mahogany and mahganoid are involved as well. The dsk genes associated with dermal pigmentation also play a role as well and many of these genes seem to be acting through a Gq-mediated phospholipase C pathway. When double mutants between Dsk1 and MC1R were examined it was discovered that they promoted pigment type switching in the same direction. Dogs have a black coat that was thought to be caused by a dominant Agouti allele, but this was discovered not to be the case. Black coat color in German Sheperds was found to be caused by a recessive Agouti allele as is seen in other mammals. A genome scan of a Labrador x Greyhound cross identified a new locus responsible for pigmentation in dogs that they have called the ?K? locus. Variability of the K locus is responsible for the brindled and fawn color patterns seen in some dogs. K is also epistatic to Agouti which suggests this is novel interacting pathway regulating dog coat color.

Shin-Ichi Nishikawa gave the second keynote lecture in this session on the regulation of melanocyte stem cells by the stem cell niche. It is not well understood how the stem cell niche maintains the quiescence of melanocyte stem cells (MSCs). A Dct promoter was used to drive GFP expression which then acted as a marker for cell sorting of melanocytes. This allowed the isolation of specific populations of melanocytes. Differentiated cells were then depleted using an anti-c-kit antibody, leaving just the MSCs. These cells exhibit a down-regulation of a number of melanocyte specific markers. The bulge of the hair follicle is high in Wnt inhibitors produced by the melanocyte stem cells. Notch signaling is also active in MSCs. DAPT, a ?-secretase inhibitor, induces melanoblast cell death, but Hes1 over-expression acting through the Notch pathway can rescue cells treated with DAPT. Forced over-expression

of Hes1 also causes hair graying. These data suggest that Notch signaling is important for MSC maintenance in the stem cell niche.

Shigeki Shibahara gave the Makoto Seiji lecture to end the session and talked about stress responses in mice involving Mitf. The black-eyed white (bw) mouse is an Mitf mutant with a white coat and inner ear defects. The mutation is an insertion of a L1 element into the third intron of the gene. These mice exhibit some unexpected differences. Whole body plethysmyography demonstrated a changed ventilatory response, they have a low respiratory frequency and large tidal volume. There is apparently no altered activity of the neural crest derived oxygen sensing cells , but there is altered chemosensitivity in the brain. Lipocalin-type prostaglandin D2 synthase (L-PDGS) mRNA is not expressed in bw mice. Mitf siRNA decreases L-PDGS expression in B16 melanoma cells. L-PDGS is apparently regulated by Mitf by a cluster of E boxes. L-PDGS is known to be involved in sleep and pain perception. There are indeed behavioral differences in bw mice that include increased activity in the morning. The talk ended with the speculation that via chemosensing and regulation of L-PDGS that perhaps Mitf regulates murine quality of life!

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**Plenary Symposium 5 "Developmental Biology 2"**
*by F.Beermann*

The developmental biology session 2 consisted of 2 plenary lectures and 4 oral presentations.

Lionel Larue (Orsay, France) reported on the analysis of ß-catenin and melanocyte development. When a dominant-active ß-catenin was expressed in transgenic mice under control of tyrosinase regulatory elements, the resulting mice unexpectedly showed reduced pigmentation and a white belly spot. Apparently, nuclear ß-catenin thus inhibited melanoblast differentiation. Moreover, ß-catenin expressing cells immortalized efficiently due to silencing of the p16INK4a promoter, which contains binding sites for the Lef transcription factor. In consequence, the presence of the activated nuclear ß-catenin was sufficient to induce melanomagenesis in combination to an activated N-ras transgene.

Bill Pavan (Bethesda, MD, USA) introduced a mutagenesis screen in mice looking for interacting partners of the transcription factor Sox10. In the screen, he took advantage of a knock-in allele of Sox10 which leads to Sox10-deficiency but retaining Sox10-specific lacZ expression. This allowed to analyse 1. dominant enhancers of Sox10-mutant coat color phenotype, 2. changes in Sox10 embryonic expression pattern (as seen by lacZ staining) and 3. Sox10-mediated lethality. Several loci were identified and mapped to specific mouse chromosomes. Some mapped to known genes, as for example Gli3, which was not yet implicated as a coat color gene, whereas other mutations will most probably be new loci.

T.Kunisada (Gifu, Japan) reported on a mouse model for hair graying, the Mitfvit/vit mouse, which is characterized molecularly by a mutation in the DNA-binding domain of Mitf. Using transgenic expression of hepatocyte growth factor or stem cell factor, but not endothelin-3, the hair graying phenotype and the whitening of the mice could be suppressed suggesting that they might contribute to the self renewal of melanocyte stem cells in the niche.

S. Mirabal (Miami, FL, USA) generated Ednrb transgenic mice under control of the nestin regulatory sequences. These mice show a hypopigmentation phenotype including a belly spot. This phenotype could be rescued by inducing expression of the ligand for Ednrb, Edn3, in keratinocytes.

D.L.Silver (Bethesday, MD, USA) reported on molecular analysis of the belted mutation, which is caused by mutations in the ADAMTS20 gene (" a secreted disintegrin-like and and metalloproteinase"). The belted mutations appears to act synergistically with Pax3 and c-kit mutations, thus possibly modifying kit-receptor/kit-ligand interaction. Moreover, Adamts20

might affect melanoblast development by affecting processing/ expression of a chondroitin sulfate proteoglycan called versican.

I. Fernandez (Miami, FL, USA) studied the role of erbB3 receptors in melanocyte development, using mainly neural crest cultures. Using the ligand neuregulin in cultures, the number of Tyrp1- and Dct-positive cells increased suggesting that neuregulin, via erbB3 might act as a proliferation factor for melanoblasts in vivo.

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**Concurrent Session 1 - 'Genetics of Pigmentation'**
*Chaired by: Ian Jackson, Murray Brilliant and Richard Sturm*

Genetic studies of pigmentation have greatly benefited over the years by studies of model organisms. Indeed, the early work on genetics in a range of organisms has often been the study of pigmentary variation, and there is a rich source of information to be found among these variants. The speakers in this concurrent session spoke about genes in mammals ranging from mice through cats and dogs to cattle and humans.

Teresa Gunn (Cornell) previously showed that gene affected in mahogany mutant mice was the extracellular protein, attractin. At this meeting she described an attractin-related gene in the mouse genome, in which mutations produce the same dark coat and neurodegeneration phenotypes as mahogany. Further along the ?predator pathway?, Anna Schmidt-Kuntzel (NCI Frederick) described the identification of mutations in the Tyrp1 gene of cats. The chocolate mutation has two molecular defects; a mutation 5 bases inside an intron which results in the variable addition of 51 or 54 bases to the mRNA (and presumably an protein insertion of 17 or 18 amino acids) but also an Ala to Gly substitution in the signal sequence. It appears that even these two changes do not cause complete loss of function; there is a more severe colour phenotype, cinnamon, that is due to a nonsense mutation at codon 100. Maintaining the predator metaphor, Sheila Schmutz (University of Saskatchewan) discussed some coat colour variants of dogs, in particular the dilution phenotype seen in the Shar-Pei and Large Munsterlander breeds. The dilution appears to be due to mutation of the melanophilin gene, which underlies the leaden mouse mutation and some forms of Gricelli Syndrome in humans. The mutant dog gene contains only a synonymous change in the coding region, close to an intron, but RT-PCR reveals aberrant splicing. Also from University of Saskatchewan, Kim MacLean described cattle breeding data that suggest an association between MC1R genotype and traits desirable in meat production.

Moving on to human disease, Marjan Huizing (NIH) described her work screening Hermansky-Pudlak patients. There are, to date, seven genes that have been identified as mutated in HPS patients, and by screening the exons of these she identified mutations in 90% of a collection of 150 patients. Interestingly, mutations in these remaining 15 individuals could not be found, even after screening the human homologues of the genes affected in the additional nine or so mouse HPS models. Perhaps these remaining patients will provide mutation data on control elements of the genes. Tamio Suzuki (Nagoya University) described HPS patients in Japan. Mutation of the HPS1 gene appears to be the commonest form of the disease, and he has found a number of novel mutations. One previously-described splice site mutation was common in the population, most likely the result of a founder effect.

Two speakers addressed the genes responsible for normal variation in human pigmentation. Carolina Bonilla (Ohio State University) extolled the power of admixed populations for genetic association studies. A study using a series of ancestry informative markers on several admixed populations revealed a correlation between skin pigmentation and the contribution of African ancestry (indicating the multigenic nature of pigmentation). Several genes could be identified that showed significant contributions; including the known pigmentation genes, ASIP, TYR, OCA2 and MATP. Justin Graf (Queensland University of Technology) described in more detail polymorphisms of one of these, MATP. Two coding polymorphisms have been identified in the Caucasian population, and homozygosity for the rarer alleles of both show an association with dark hair and olive skin (although as these are the rarer forms the number of homozygotes in the population is small). However, he described a much commoner polymorphism upstream of

the coding region that is associated with skin colour variation, independent of hair and eye clour.

The session demonstrated once again the power of genetics to discover gene function, and the diversity of model organisms available to researchers.

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**Concurrent Session 2 - 'Biochemistry of Melanogenesis'**
*By Bryan B. Fuller*

Functional HPA Axis Homolog is Expressed by Melanocytes. A Slominski, B. Zbytek, M. Zmijewski, and J. Wortsman,

This paper, presented by Dr. Slominski, presented data on the expression in the skin of hormones and receptors typically found in the hypothalamo-pituitary-adrenal (HPA) axis. Results showed that human keratinocytes expressed corticotrophin releasing hormone (CRH) as well as the CRH-R1 receptor. The authors also found, as had been shown previously, that keratinocytes produce POMC (Pro-opiomelanocortin). Although one could envision an endocrine loop that involves the CRH induced production of POMC in keratinocytes, the authors found that CRH does NOT up regulate POMC in these cells. Further, no corticosteroids are produced in keratinocytes by CRH. Further studies showed that CRH could increase cAMP levels in human melanocytes and dermal fibroblasts and cause an increased production of POMC and ACTH. The importance of the CRH-R1 receptor in mediating this effect of CRH was demonstrated by blocking the CRH-R1 receptor with the specific antagonist, antialarmine, and showing that no POMC or ACTH was produced. Both melanocytes and fibroblasts were found to respond to either CRH or ACTH with enhanced production of corticosteroids although in fibroblasts, ACTH is the primary stimulator of corticosterone production.

A Novel 43 kDa Protein as a Negative Regulatory Componenet of Phenoloxidase-Induced Melanin Synthesis. M. Zhao, I. Soderhall, J.W. Park, Y.G. Ma, T. Osaki, C.H. Ha, C.F. Wu, K. Soderhall and B.L. Lee.

Phenoloxidase is widely distributed among animals, plants, and fungi, and is involved in many biologically essential functions. In arthropods, melanization plays an important role in defense reactions, such as wound healing, encapsulation, sequestration of microbes, and the production of toxic intermediates, that are speculated to kill invading microorganisms. In response to injury a melanization reaction occurs at the site of injury and the area wounded by invading microorganisms becomes blackened because of the de novo synthesis and deposition of melanin. In this presentation, the authors show that the activity of phenoloxidase and the production of melanin may be under negative regulatory control by a newly discovered protein (43kDa). The authors report the cDNA cloning of this protein from the mealworm and show that it has no homology to any known sequence. The protein is referred to as MIP (melanization inhibiting protein) and the authors have shown that a recombinant form of this protein can inhibit melanin synthesis in vitro. Further, if a double-stranded inhibitory RNA is injected in mealworm larvae to block the production of MIP, melanin synthesis increases. The mechanism of action of the 43kDa protein is not known.

Down-Regulated Mealnogenic Paracrine Cytokine Linkages in Hypopigmented Palmoplantar Skin?. J. Hasegawa, Y. Goto, H. Murata, M. Takata, T. Saida, and G. Imokawa.

Recent studies from Dr. Hearing?s laboratory have shown that at least one reason for the low melanocyte density in palmoplantar human skin (five times lower than that found in non-palmoplantar sites) may be that palmoplantar fibroblasts express high levels of dickkopf-1 (DKK1). This protein may then decrease melanocyte proliferation and pigmentation by inhibiting the Wnt signaling pathway. Further studies by Hearing?s group showed that transfection of DKK1 decreased melanocyte function, apparently through a ß-catenin–mediated regulation of MITF. In the paper by Dr. Hasegawa, results are presented that show a

decreased expression of melanogenic factors by keratinocytes and fibroblasts in palmoplantar skin. Immunohistochemical analysis revealed not only a decrease in the numbers of tyrosinase positive cells in palmoplantar skin, as would be expected, but also a decreased expression of SCF, ET-1, c-KIT and ET-R in palmoplantar skin. Interestingly, the authors present data that MITF levels are similar in palmoplantar and non-palmoplantar skin. Further, in contrast to published findings on DKK-1 levels in palmoplantar fibroblasts, the results presented by Dr. Hasegawa, show that the DKK-1 protein is not detectable in any palmoplantar or non-palmoplantar skin. This finding raises the question of the inhibitory role of DKK-1 in preventing proliferation and differentiation of melanocytes in palmoplantar skin.

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**Concurrent Session 3 - 'Intracellular Signaling'**
*by Hee-Young Park*

Mizutani et al. from Tokyo Women?s Medical College presented results that p38 activation is mainly responsible for UVB-induced increase in c-kit expression in human melanocytes. When human melanocytes were UVB (40-80 mJ/cm2) irradiated, the c-kit expression was increased at both gene and protein level at 12-24 hours after the irradiation. AP-2, the transcription factor c-kit, was concomitantly increased. Inhibitors of PKA (H-89), PKC (Calphostine), MAPK (PD98059), JNK (SP600125) and Akt (Akt inhibitor III) did not block UVB induced increase in c-kit. However, inhibitor of p38 (SB203580) blocked the UVB-induced increase the c-kit expression and partly inhibited the phosphorylation of MITF. Combined results suggest that UVB-induced increase in c-kit expression is mediated through p38 and once MITF is phosphorylated in part through p38 pathway then activated MITF would also participate in increasing the expression of c-kit.

Rouzaud et al. from NIH and U. Cincinnati College of Medicine examined how different isoforms of MC1R are regulated. Normally MC1R with amino acids numbers of 317 (MC1R317) is expressed on melanocytes. This group has identified MC1R 350, alternatively spliced form of MC1R317. Interaction with 125I--MSH was similar between two isoforms of MC1R. The melanin content correlated with MC1R317 level but inversely correlated with MC1R350 level. Over-expression of MC1R317 into melanocytes caused increase in tyrosinase expression where as over-expression of MC1R350 dampened the expression of tyrosinase. Results suggested that darker skins may have more of MC1R 317. Understanding of MCIR350 regulation may provide further insights in biology of melanocytes.

Bellei et al. from NIH presented results on how -catenin is degraded after UVA irradiation. UVA-irradiation of normal human melanocytes (8-16 and 32 J/cm2) decreased E-cadherin expression where as the expression of N-cadherin and -catenin remained unchanged. There was a slight down-regulation of -catenin and cytoskeleton was reorganized. Fractionation of cell lysates revealed that -catenin degradation primarily occurs in the cytoplasm, in the cytoskeleton and in the nucleus but not in the membrane fraction. Caspase 3 is the major protease responsible for the degradation of -catenin

Schepsky et al. from U. Iceland, U. Freiburg and UK reported that when MITF interacts with -catenin then the transcriptional complex is directed toward MITF specific genes. In a yeast two-hybrid system, -catenin co-immunoprecipitated with 35S-MITF and LEF-1. Over expression of MITF reduced -catenin-dependent gene expression. Conversely, over-expression of -catenin enhanced MITF-dependent gene expression. Therefore, MITF sequesters -catenin away from the -catenin target genes to MITF target genes.

Schuierer et al. from U. Regensburg examined RKIP expression in melanoma cell lines. RKIP (raf kinase inhibitor protein) is the physiological inhibitor of raf signaling pathway. It is increased in nevus, decreased in primary melanoma and dramatically reduced in metastasized melanomas. To determine mechanisms through which the expression of RKIP is reduced in melanomas, they examined hypermethylation of RKIP gene since RKIP promoter regions contained number of CpG islands. However, treatment of cells with demethylation agents did

not change RKIP expression. Transcription repressor Snail decreased the expression of RKIP. The stability of RKIP mRNA was also altered. Combination of transcription and mRNA stability contributes to the loss of RKIP in melanoma cells.

Ivanova et al. from Germany and Amsterdam University presented results that nitric oxide (NO) induces detachment of melanocytes through cGMP pathway. When normal melanocytes and vitiligo melanocytes were treated with NONOates, both types of melanocytes displayed detachment in the dose-dependent matter. Treatment with apoptosis inhibitor showed that the major part of detachment involved apoptosis. The NO-induced detachment was partly inhibited by cGMP inhibitor but not the apoptosis. Therefore, the NO may utilize cGMP pathway to detach melanocytes from extracellular matrix proteins such as fibronectin.

Smit et al. from Netherlands presented results on role of calcium/calcineurin/NFAT pathway in melanocytes. Calcineurin is a calcium/calmodulin dependent protein phosphatase. When this pathway is activated NFAT is translocated to nucleus and activates transcription. TPA activates this pathway. By comparing the genes affected by this pathway using microarry before and after TPA treatment and comparing between normal human melanocytes and melanoma indicate that genes are differentially regulated.

Seiberg et al. from Johnson and Johnson presented data that peptides SLIGRL, LIGL and RL induced skin darkening. All three peptides increased GTP-Rho activity and cytoskeleton reorganization. However, while SLIGRL stimulated ERK1/2, p38, AKT and IKB, LIGR did not induce phosphorylation of these kinases. They conclude that the shorter peptides LIGR and RL activate only a subset of the PAR-2 signaling pathways. These smaller peptides may be more desirable for skin pigmentation since limited pathway maybe activated, thus minimizing side effects.

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**Concurrent Session 4 - 'Innovative Technology'**
*By Miri Seiberg*

The innovative technology session provided four exciting talks on very different technologies; all have the potential to be used by many investigators in the future.

Dr. Kachhawa from the Medical College of Jodhpur, India, presented a comparative study of epidermal transfer techniques for the treatment of vitiligo. His novel technique, ECTT, was shown to be simpler and superior to the standard SGT (split thickness graft) technique. ECTT is an epidermal cell transfer technique where donor site is dermabraided, and the collected deeper layer- skin fragments are applied onto dermabraided vitiligo sites and bandaged. ECTT requires local anesthesia only, results in less damage and faster recovery of the donor sites, is suitable for large treatment areas, is less expensive and labor intense relative to SGT, and results in superior cosmetic results.

Dr. Kobayashi presented a collaborative research of three Japanese groups, on the internalization of c-kit in a clathrin-independent way. C-kit, an important player in melanocyte survival and migration, has been shown earlier to be internalized via a clathrin-dependent pathway. Using ligand-stimulated receptor assays, the team showed tyrosinase phosphorylation with 50% of c-kit remaining active at 10 minutes post stimulation, while internalization was documented as early as after 2 minutes. Localization studies showed that most of the activated c-kit was inside the cell. A hand held laser excitation system was presented, which was coupled to microscopy, and enabled visualization of GFP-conjugated proteins at the single molecule video imaging level. Using this system with clathrin pathway blockers or mutants, the early internalization of c-kit was documented, suggesting a clathrin-independent pathway that acts earlier than the clathrin-dependent pathway of c-kit internalization.

Dr. Yajima of Institut Curie, France, presented a transgenic system that enables to activate or inactivate a candidate gene in the melanocyte lineage at any time. A Cre-LOX system with an inducible tyrosinase promoter was used to create a transgenic line, when tyrosinase is coupled to an estrogen promoter and is activated by Tamoxifen. Titration of Tamoxifen dose and time was performed to avoid toxicity, and results were demonstrated by lac-z expression. Induction was demonstrated in vivo both in immature and mature melanocytes, with some ectopic expression in non-melanocyte cells. This transgenic system provides a research tool for identifying the role of candidate genes during melanocyte development and transformation.

Eduardo Ruvolo presented a collaborative study of Indian clinical facilities with the Johnson & Johnson imaging team. The study developed documentation of characteristics of melasma in Indian population using image analysis, and correlates these findings to clinical evaluations, using MASI scores. An integrated imaging system with an immobilized chin position, used polarized and cross-polarized lights to create images for lesion documentation, to enhance lesion borders and to measure darkness of lesion and no lesion sites. Diffuse reflectance spectroscopy documented the contribution of pigment, hemoglobin and deoxy-hemogblobin and dermal scattering to the visualized lesion color. A correlation with MASI scores was established, suggesting that this imaging system could be used as an objective tool to document melasma progression over time or treatment.

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**Concurrent Session 5 - ' Melanosome Structure and Function'**
*by Vijayasaradhi Setaluri*

That the biology of melanosomes is here to stay as major topic of pigment cell biology is demonstrated by its representation at the 19th International Pigment Cell Conference in a Plenary Symposium (Melanosome Biogenesis, Motility and Transfer) and a Concurrent Session (Melanosome Structure and Function). In addition to many excellent poster presentations, there were a total of ten oral presentations in these sessions. At the concurrent session chaired by Drs. Borovansky (Charles Univ., Czech Republic), Kidson (U. Cape Town, South Africa) and Setaluri (U. Wisconsin, USA) four abstracts on various aspects of melanosome structure and function- ranging from early events in melanosome biogenesis to factors that determine the fate of melanosomes transferred to keratinocytes- were presented.

It is becoming increasingly clear that despite shared sorting signals melanosomal membrane proteins follow different intracellular pathways to arrive at the melanosomal membrane. For example, the pathways that TYRP1 follows appear to be distinct from that followed by tyrosinase. Consistent with such notion, Vijay Setaluri's laboratory had earlier shown that newly synthesized TYRP1 interacts specifically with a cytoplasmic PDZ domain protein GIPC, suggesting a role fro such interaction in TYRP1 sorting. However, the exact site of interaction and the mechanisms of action of GIPC remained to be investigated. Kedlaya et al. (U. Wisconsin, USA) in their presentation showed data that suggest that TYRP1-GIPC interaction occurs at the endoplasmic reticulum (ER) and that this interaction is required for efficient export of TYRP1 from the ER. Additionally, using a combination of biochemical and cell biological methods, they also show that oligomerization of GIPC molecules, presumably on the cytoplasmic face of ER, is required for its action. They suggested that GIPC may cluster newly synthesized TYRP1 molecules on the ER for vesicular export similar to function of PDZ domain containing proteins in clustering cell surface receptors. After they are exported to trans-Golgi, a complex set proteins are required in orchestrating the assembly of melanosomal proteins into melanosomes. The discovery that defects in the biogenesis of lysosome-related organelles underlie a spectrum of human hypopigmentary disorders known as Hermansky-Pudlak Syndrome, made melanocytes derived from these patients a valuable cellular reagents for detailed investigation of events in melanosome biogenesis. In their presentation Helip-Wooley et al. showed that in adaptor complex-3 (AP3) defective HPS-2 cells, trafficking of tyrosinase but TYRP1 is affected. Additionally, these studies in Dr. Bill Gahl's laboratory (National Institutes of Health, USA) showed that the clathrin-binding domain protein HPS-3, which is defective in patients of the subtype HPS-3, binds to and colocalized with clathrin,

and that abnormal distribution of melanosomal proteins in HPS-1 and HPS-5 melanocytes can be corrected by expression of respective proteins. Although it is generally known that melanin biosynthesis closely resembles catecholamine metabolism and that melanocytes can synthesize catecholamines, it is not known whether tyrosinase plays a role in catecholamine metabolism in melanocytes. Data presented by Matsunga et al. (Tohoku University, Japan) suggested that notwithstanding its localization within the lumen of melanosomes, tyrosinase also controls cytosolic catecholamine metabolism. They showed that treatment of B16 melanoma cells with phenylthiourea enhanced the cytotoxic effect of dopamine (DA), but not L-DOPA, on these cells. This implicates a role for localization of tyrosinase in detoxifying DA. Matsunga et al. also reported that consistent with this requirement of transport of cytosolic DA into melanosomes, B16 melanoma cells express dopamine transporter (DAT) and it is localizes to lysosomes (and melanosomes?). While discovering novel function for melanogenic enzymes and melanosomes within melanocytes continues, factors that determine the patterns of distribution of melanosomes exported into keratinocytes have largely remained a mystery. This latter topic was the focus of Yoshida et al. Earlier cell culture observation by Dr. Raymond Boissy? laboratory (U. Cincinnati, USA) showed that distribution of melanin pigment within keratinocytes is determined not by factors intrinsic to the melanocyte or melanosome but by the recipient keratinocyte. Expanding on this, again Dr. Boissy?s group elegantly demonstrated this intrinsic function of keratinocytes in an in vivo human skin substitute system. First, they showed that the reconstituted keartinocytes, melanocytes and fibroblasts produce a skin substitute graft, on the backs of SCID mice, that recapitulates the pigment type of the donor skin. Then by mixing keratinocytes and melanocytes from different skin types they showed the pigment distribution in keratinocytes is determined by the pigment type of the skin from which keratinocytes.

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**Workshop - 'Genetics and Developmental Biology'**
*by William J. Pavan*

There was a joint workshop encompassing both the Developmental Biology & the Genetics interest Groups of the IFPCR. This workshop was hosted by Robert Kelsh, Hiroaki Yamamoto and Bill Pavan. The session began with an invited lecture by Keith C. Cheng, M.D., Ph.D, Associate Professor of Pathology, Biochemistry and Molecular Biology, and Pharmacology, Jake Gittlen Cancer Research Foundation Penn State College of Medicine. His presentation titled "Unexpected insight into human skin color from golden zebrafish" described recent studies involving the positional cloning of the gene mutated in the zebrafish mutant, golden. They found that the mutation resides in a member of the potassium dependent sodium, calcium exchanger gene family. Interestingly his group also went on to propose that polymorphisms associated with this gene may account for a large component of the genetic contribution to skin color variation in man.

Following the invited talk, four more talks were scheduled. Dr. Cooper from the University of Washington, Seattle, USA described evidence that Foxd3 and c-kit signaling cooperate to regulate melanogenesis. They propose that this may act through regulation of MITF transcription. Dr. Hou from the National Institutes of Health, Maryland, USA described a series of experiments to determine if MITF could rescue melanocyte differentiation in SOX10 mutant neural crest. He proposed that MITF alone is not sufficient to completely replace the need for SOX10 in mammalian melanocyte development. Dr. Kawa of St. Marianna University School of Medicine, Kawasaki, Japan, described experiments examining melanocyte differentiation in primary neural crest explant cultures from MITF mutant mice and by knockdown of Mitf in NCCmelb4M5. Results from her experiments suggest that Mitf plays a role in melanocyte survival in early developmental stages. Dr. Akiyama of Dept. Biology, Keio University, Yokohama, Japan described recent work on the Silky chicken which shows heavy pigmentation on internal organs. She proposes that increased signaling through the endothelin pathway early in melanoblast migration may be responsible for the increased melanization seen in these animals.

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**Tuesday, September 20, 2005**

**Sunrise Session 2 - 'Differentiated Functions of Melanocytes'**
*by William A. Gahl*

Dr. Stefana Petrescu laid the groundwork for the day?s talks by discussing how melanocytes perform their differentiated function, i.e., the production of melanin. The pathway requires melanogenic enzymes and structural proteins interacting within the melanosome. These subcellular organelles mature structurally and biochemically as they move centrifugally to the dendritic tips, where they are transferred to keratinocytes, providing pigment to hair, skin, and eyes. The copper enzyme tyrosinase plays the initial, crucial role in the synthesis of both eumelanin and pheomelanin; the enzyme?s egress from the endoplasmic reticulum is modulated by calnexin, a protein that fosters proper folding of tyrosinase molecules. Glycosylation is critical for folding, as demonstrated by aberrations due to glycosylation mutants; some OCA I mutations are defective in folding. Tyrosinase undergoes processing in the Golgi and eventually reaches the melanosome. Other melanogenic proteins include Tyrosinase-related Protein 1, Tyrosinase-related Protein 2, Pmel17, P, the OA1 receptor defective in ocular albinism, and Mart-1. Melanosomes mature as they move along microtubules for large distances and actin filaments for shorter distances, laterally near the nucleus and plasma membrane. Kinesins serve as motors binding a melanosome to the microtubule, and rab GTPases (e.g., rab27a) provide energy; when transfer to actin filaments is needed, specific proteins such as melanophilin connect with myosin Va on the actin filaments. The different types of albinism represent defects in various stages of the pathway described above. Hermansky-Pudlak Syndrome (HPS) represents a defect in the formation of lysosome-like organelles, which include not only melanosomes, but also platelet dense bodies and subsets of lysosomes. Hence, HPS patients have variable degrees of hypopigmentation plus a bleeding disorder. Seven different genes have been identified to cause autosomal recessive HPS; all are considered to be involved in vesicle (i.e., melanosome and platelet dense body) formation and trafficking, but only the ?3A subunit of adaptor complex-3 (whose deficiency causes HPS-2) has a defined role in this process. Chediak-Higashi patients? granulocytic cells display giant granules due to aberrant vesicle trafficking, and affected individuals suffer life-threatening infections, the hemophagocytic syndrome, and late neurological involvement. Griscelli syndrome patients have silvery hair and can have neurological impairment or the hemophagocytic syndrome. These inborn errors of pigment formation provide insights into the normal processes of melanosome formation and movement.

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**Plenary Symposium 6 - 'Differentiated Functions 1; Differentiated Functions of Melanocytes / Melanophores (Oculocutaneous albinism)'.**
*by Francisco Solano*

The session was opened by Prof. Richard King, one of the 3 co-chairs, and the first Plenary Lecture was given by Prof. Tomita, from Nagoya University, with the title ?Oculocutaneous albinism type 4 is one of the most common types of albinism in Japan?. Prof. Tomita introduced this lecture with basic definitions about what oculocutaneous albinism (OCA) is, its different types, and some historical facts. The first reported cases of OCA in human being were tyrosinase-negative, but pioneer work from Prof. Witkop introduced other types of OCA patient showing positive tyrosinase activity. The discover and characterization of other proteins essential for pigmentation allowed for a better classification, relating OCA2 to P protein, OCA3 to Tyrp1 and OCA4 to MATP. This last one is a serpentine protein with 12 transmembrane fragments found in the melanocyte membrane whose biochemical function is still not well-known, although it seems to be related to saccharide transporters, probably Glu-4 type.

Moving to statistics, he presented an updated list of Japanese patients with clinical phenotypes, doing special emphasis in OCA4, which in Japan is almost as frequent as OCA1 (from a total of 75 albinism patients, 38 were OCA1 but 30 were OCA4). The severity of the

symptoms is variable. The most common mutation is the D157N. He finally established some comparison to OCA4 in other countries, such as the cases described in Germany.

The second plenary lecture was given by Dr Jimbow, from Sapporo Medical University, with the title ?Melanogenesis cascade and biology of normal and abnormal pigmentation?. Prof. Jimbow offered to the audience a complete overview of melanogenesis mechanisms at cellular and molecular levels in 4 key steps. Most of the work he presented has been carried out in his wide research career, including (a) the study of proliferation of stem cells and the role of c-kit and endothelins, (b) the folding of tyrosinase and the use of castanospermine to explore tyrosinase maturation, interaction with chaperones and final activity, (c) the transport of tyrosinase and its related proteins to melanosomes and the crucial role of Rab7 in this vesicular process, and (d) assembly of tyrosinase and other melanosomal proteins to form the melanogenic complex and the final deposition of melanin, emphasizing the protective role of Trp1 as a protein which rescue melanocytic cells from tyrosinase- mediated cell death and in summary the synergistic role of Trp1 and Trp2 on tyrosinase melanogenic activity.

As scheduled, after the two plenary lectures, four communications were briefly orally presented. These communications were previously selected by the organizing committee on the basis of their high scientific interest. The first one was presented by Dr. Murray Brilliant with the title 'Gene polymorphism and human pigmentation'. His work tried to know the relative contributions of the main genes encoding melanin-related proteins to pigmentation of hair, eyes and human skin color. It is very likely the most extended study never made, involving 800 participants including African, Asian and European individuals genotyped at 48 polymorphisms in 17 genes. Polymorphisms in five genes, P protein MATP, MC1R, ASIP and DCT, account for 60-80% of the variance in skin and hair pigmentation. For eye color (blue, green, hazel, gray, brown, black), these genes account for only 39%, indicating that eye color is genetically the most complex pigmentation trait. Perhaps some other candidates, as Pmel17 or some Na+/H+ changers, should be added to the study. According to this data, in the near future it would be possible to predict the skin and hair color from a DNA sample by genotyping only a few polymorphic loci, with clear interest in forensic medicine and criminal investigation.

Secondly, Dr. S. Petrescu presented data about the traffic and maturation of tyrosinase, the key enzyme for melanogenesis. Some OCA 1 are related to changes in the hydrophobic region of this enzyme. She presented data using tyrosinase mutated at its C-terminal tail (soluble tyrosinase) to investigate the maturation process and the role of this fragment in the ER. Basically, she showed using this elegant approach that truncated tyrosinases are retained in the ER to be degraded through the proteasomal pathway. Interestingly, this form of tyrosinase interacts with BiP and calreticulin but not with calnexin as the native form of the enzyme does. However, N-glycosylation and mannose trimming is required for the degradation process. She defined soluble tyrosinase as an ERAD (Endoplasmic Reticulum Associated Degradation) substrate. All together, Dr. Petrescu demonstrated that mutations in the C-terminal region of tyrosinase cause a change in the specificity of chaperones interacting to the enzyme, giving calreticulin a novel role in these particular cases of OCA 1.

Thirdly, Dr. G.E. Costin reported some data about the effect of dopachrome tautomerase activity in the eumelanin/pheomelanin ratio in mouse melanocytes. Using C57Bl/6J non-agouti melanocytes and two well characterized Dct-mutant cell lines, slaty and slaty light, she showed that Dct activity is decreased approximately 3-fold and 28-fold respectively. Chemical analysis showed that both mutations increase pheomelanin and reduce eumelanin produced by those melanocytes in culture, in comparison to the non-agouti black phenotype. In turn, her results also demonstrated that the above mentioned mutations do not affect intracellular trafficking of the respective mutant proteins, but modelling studies indicate that the first mutation (R194Q) is located near or at the active site to alter the affinity of the enzyme for the substrate, and the second one (slaty light, G486R) may result in the sliding of the transmembrane domain towards the N-terminus of mutant Dct, affecting Dct function probably in the eumelanin complex. Taken together, the level of Dct activity seems to play a role in determining the preference for the eumelanin pheomelanin pathway in pigment biosynthesis.

Finally, Dr. Montoliu presented an interesting study under the title ?Gene expression profile analysis in normal retinal development in mammals: a comparative approach between albino and pigmented animals?. Albino patients (OCA 1) undergo some visual anormalities related to low number of photoreceptors and abnormal cellular conexions between retina and brain. The molecular mechanisms and interactions between lack of tyrosinase and appearance of these abnormalities are unknown. Dr. Montoliu presented that the defects can be mostly corrected in animal albino model inserted with functional tyrosinase transgene, indicating the role of tyrosinase gene product(s) in retina development. As very novel data on genes possibly related to albino abnormalities aside pigment absence, Dr. Montoliu discussed the possible role of Tia-1 overexpression in albino mice. This factor is overexpressed as a stress response in hypoxia , and he showed that tyrosinase induction reduces Tia-1 expression in albino background.

All presentations were followed by some brief questions and discussions on the particular aspects raised by the audience. A great number of researchers attending to the meeting participated in those minutes, with the only limitation of the time scheduled for this session.

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**Plenary Session 8 - 'Regulation of Pigmentation' Regulation of Melanocyte/Melanophore Function (constitutive pigmentation/environmental responses)**
*by Richard M. Niles*

'Defining the Role of Melanocortins and the Melanocortin 1 Receptor in Preserving Human Melanocyte Survival and Genomic Stability' Zalfa Abdel-Malik
There are extensive polymorphisms within the melanocortin-1 receptor (MC1R) gene. Some these are associated with red hair, poor tanning ability and increased susceptibility to melanoma. Dr. Abdel-Malik?s lab found that these particular alleles of the MC1R gene represent loss-of-function mutations in the MC1R gene. They render human melanocytes refractory to melanocortins and disrupt their normal response to UV radiation. Recently the lab has focused on the role of alpha-MSH as a survival factor that rescues human melanocytes from UV-induced apoptosis. This pathway involves, activation of Akt, increase in totl and phosphorylated of MITF and increased levels of Bcl2. DNA repair enzymes were increased, while ROS was decreased in alpha-MSH- treated cells. These effects were not seen in human melanocytes having loss of function MC1R genes. The lab is currently investigating the UV-radiation response in human melanocytes using DNA microarrays.

'Transcription and Signaling in Melanocytes and Melanoma' Colin Goding
The lab recently published that increased levels of MITF can inhibit prolifertion of some melanoma cells. However, Dr. Goding showed some unexpected results, that knock-down of MITF also led to decreased cell proliferation. This was accompanied by a decrease in the cyclin-dependent kinase inhibitor p21 and increase in p27. MITF knock-down cells also have a change in their shape, due to an altered distribution pattern of actin filaments. Dr. Goding concluded his talk by presenting a new model for the role of MITF. In this 'rheostat' model, the effect of MITF on growth and survival depends on the state of the melanocyte (melanoblast, mature melanocyte, stem cell, melanoma) and the relative level of MITF present in these cells.

'Upstream Stimulating Factor (USF-1) A Potent Stress-Response Transcription Factor', MD Galibert
USF1/2 are conserved transcription factors of the bHLH-leu-zip family. They bind to E-box DNA elements in target genes and recruit chromatin remodeling enzymes, interact with coactivators and members of pre-initiation complexes. Their transcriptional activity is dependent on post-translational modification. The lab previously found that USF-1 is phosphorylated by p38 MAPK. They showed that the tanning response involving the POMC, MC1R, Tyrosinase. TRP-1 and Dct genes is dependent on p38 activation of USF-1 initiated in response to UV-radiation-induced stress. Dr. Galibert recently discovered a second post-translational modification, termed M-USF-1, that occurs in response to distinct stress signals

such as UV radiation, viral infection, etc. M-USF-1 binds to E-box DNA as demonstrated by ChIP, but it could function as a dominant-negative regulator.

'Genetic Models of Human MC1R Variant Receptor Alleles for Pigmentation Phenotype and Cellular Function in Signal Transduction', Rick Sturm
The lab has quantified the contribution of individual MC1R alleles to pigmentary phenotypes in a large family of adolescent twins, partents and sibling. Four common and two rare alleles termed large R were strongly associated with red hair and fair skin. An additional 3 alleles designate small r had lower penetrance compared to the WT allele. Recent studies have examined the functional ability of MC1R variants to activate the cAMP pathway in transfectants of HEK293 stably expressing the gene. R associated variants showed agonist-induced cAMP levels and CREB phosphorylation. One of these variants, (D294H) showed severe impairment of the functional response. Cummulatively, these data indicate that these alleles are not complete loss of function receptors and are not equivalent. Additional studies examined the subcellular localization of the R receptors. Found that melanocytic cells expressing endogenous or ectopic receptor exhibited strong surface localization of WT and the D294H alleles, but markedly reduced surface expression of R151C and R160W R alleles. The r allele encoded MR1C receptors had normal or intermediate cell surface receptor levels.

'Melanocortin 1 Receptor Dimerization: Functional Consequences and Dominant-Negative Effects', J.C. Garcia-Borron
Through the use of co-immunoprecipitation of differentially epitope-tagged MC1R forms, dimeric and oligmeric forms of MC1R species were discovered. Dimerization occurred early during MC1R synthesis as revealed in studying MC1R mutants displaying intracellular retention and decreased plasma membrane expression. These mutants exerted a dominant-negative effect on WT MC1R. On the other hand, partial complementation of selected loss-of-function mutants was observed. WT MC1R did not exhibit this cooperativity, but co-expression of WT MC1R and a C-terminal deletion mutant yielded a form with higher affinity for agonist binding, but lower coupling efficiency than WT. Common natural diminished function alleles associated with red hair and increased melanoma risk, were able to heterodimerzie with WT MC1R. These results suggest that specific combinations of MC1R are associated with subtle changes in MC1R functional properties, indicating that the presence of mutant MC1R alleles may have consequences beyond those based on dosage effects and haploinsufficiency.

'Shorting and Trafficing of PMEL17 (GP100): Evidence for the Polarized Nature of Melanocytes', J.C. Valencia
Pmel is a constituent of the melanosomal matrix in melanocytes. It is also a target for immunotherapy in patients with melanoma. The lab has characterized the processing and trafficing of Pmel 17 via AP complexes. Pmel 17m AP1 and AP2, but not AP3 or 4 were detected in early melanosomes. Two forms for AP1 (AP1A and AP1B) are involved in epithelial cell-specific complexes involved in polarized sorting to the plasma membrane. The presence of AP1B in human melanocytes was confired by Q-RT-PCR, immunolabeling and in situ hybridization. Transfection of AP1 isoforms shown both a central area distribution for AP1A and a peripheral distribution for AP1B. AP1B is expressed in early stages of melanoma, while metastatic cells loose expression. Pmel17 is sorted to the plasma membrane regardless of AP1B expression. The results of this study suggest that Pmel17 is sorted to the melanosomes direclty or indirectly ghtough the plasma membrane. Presence of basolateral elements in melanocytes suggest the polarized nature of the melanocyte and suggest that loss of this polarization may be involved in malignant transformation and metastasis.

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**Concurrent Session 6 - 'Comparative Biology'**
*by Manfred Schartl and Manickam Sugumaran*

The Concurrent Session 'Comparative Biology' was composed of four presentations: (1) Diversification of melanin pigmentation caused by transposable elements in Medaka fish, (2) The Xmrk (Xiphophorus melanoma receptor kinase) is sufficient for induction of melanocyte migration, (3) Co-purification of DOPA chrome isomerase and quinone isomerase isolated from

Calliphora and (4) A search for genetic determinants of color variability in the panther chameleon.

The first topic was presented by M. Koga who has a long career on studies of transposons. He showed clear involvement of this element as one of the key steps yielding a wide variety of color mutants in Medaka fish. The juveniles of i b albino of this species are installed with small and varying sized melanophores, as a result of insertion of the Tol2 transposon in the promoter region of its tyrosinase gene. In as much as this DNA-based transposon moves within the gene in a cut-and-paste manner, the complete excision of this element from the tyrosinase gene is expected to recover the wild type phenotype. Even though this transposon is known to be inserted in particular positions of the gene, it leaves different footprint sequences behind upon excision, thus yielding various phenotypes with respect to melanin pigmentation. Koga indicated that the newly arising alleles are inherited in the Mendelian fashion and, at discussion, that in such revertants, pigmentation is similar between skin melanophore and pigment epithelium.

The second presentation was presented by M. Schartl who has first clarified the biochemical structure of the so-called "tumor gene" in platyfish, which was termed Xmrk later and is a bonafide oncogene by modern definition. The over-expression of Xmrk, a receptor tyrosine kinase of the EGFR family, is considered to be the critical step for the initiation and progression of melanomas in inter-species hybrids of the genus Xiphophorus through the signaling pathway depending on PI3 kinase, STAT5 and Ras/Raf/MAPK. Xmrk dependent signaling is responsible for increased proliferation, dedifferentiation and protection against apoptosis in 3D collagen matrices of melanoma cells. Based on these findings, Schartl further examined the signaling pathways associated with migratory behaviors of Xmrk transformed mouse melanocytes (melan-a) using 2D and 3D migration assays. The migration of these cells was shown to be dependent on the activities of the focal adhesion kinase FAK and the non-receptor tyrosine kinase fyn but not on the Ras/Raf/MAPK pathway. Thus, it becomes apparent that Xmrk induces migratory behavior characteristic to neoplastic phenotype in them through its signal transduction. In the second part of his talk Schartl presented a transgenic melanoma model in Medaka. Using the pigment cell specific MITF-promoter to drive Xmrk expression, transgenic Medaka develop pigmentation abnormalities ranging from hyper¬pigmentation to melanoma. In the discussion, the possibility that the MITF promoter in lower vertebrates functions equally in melanophore and in the xanthophore/erythrophore lineage was brought up.

The third topic was presented by M. Sugumaran, whose expertise is concentrated to the chemistry of phenoloxidase in arthropods. The hot issue of this presentation is the possible presence of a novel protein in Calliphora, which possesses the activities of both dopachrome isomerase and quinone isomerase. Thus far, extensive similarities are strongly suggested with regard to the biochemical transformation of melanization and sclerotization pathways in these organisms. A good example is given by quinone isomerase present in the sclerotinogenic pathway, since this enzyme converts N-acetyldopamine quinones formed by phenoloxidase to quinone methides as the dopachrome isomerase in mammalian melanogenesis. When the quinone isomerase is isolated from the hemolymph of this organism through various protein purification protocols, the dopachrome isomerase activities are also recognized together. Based on these findings, it is postulated that either these two activities are present on the same polypeptide chain or two different proteins are bound together. He emphasized that this observation should provide a chemical basis for a dual role of arthropod phenoloxidase in both melanization and sclerotization.

The final talk was given by R. L. Morrison who is attracted by the spectacular variation of pigmentary phenotypes in Madagascan Panther chameleon. This species is sexually dimorphic and the male vary heritably in skin pigmentation from one habitat to another, being predominantly blue in some localities (Nosy Be) whereas predominantly orange in others (Tamatave) with vertical bands of different combinations of blue and red or green and blue or green and orange etc. Some variant in another place (Ankaramy) has red spots on a pink background. In as much as skin coloration of these chameleons is formed by a very thick layer of five distinctive types of chromatophores arranged in a specific pattern within the upper

layer of the dermis, it is expected that such variation in pigmentation of this species may be associated with specific polymorphisms in the melanocortin 1receptor (MC1R) as observed with mammals, birds and reptiles. Search has now been made for a genetic basis determining locality-specific color patterns. At discussion, the possibility of diet effects was pointed out but denied based on the fact that all variant chameleons under captive are fed similarly with crickets.

The board of chairpersons, M. Schartl, M. Sugumaran and J. Matsumoto, was very much pleased with a large audience and active discussions.

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**Concurrent Session 7 - 'Chemistry and Physics of Melanins'**
*by Kazumasa Wakamatsu*

Dr. L. Hong of Duke University presented regarding the morphology and photoionization potential (IP) of RPE melanosomes and ocular lipofucin granules. Bovine and human RPE melanosomes were measured with SEM, AFM and photoelectron emission microscopy (PEEM). These morphology properties of these samples varies with tissue, age of samples and species. The IP of the samples were also determined using PEEM. Examination of single bovine RPE melanosomes suggests no correlations between the photoionization potentials (IP) and shape of melaosomes. They indicated that PEEM of two human lipofuscin samples (14-year and 76-year) reveal two IPS and the ratio of these two components was found to change with age.

Dr. Pezzella of Naples University presented on a new approach to the study the process of polymerization of 5,6-dihydroxyindoles (DHI) to melanin pigments, based on oxidation of the main dimers obtained by oxidation of the indoles under biomimetic conditions. In this way a restricted number of higher oligomers were isolated and characterized with respect to the structure and other properties, e.g. chiraloptical properties of 5,6-dihydroxyindole-2-carboxylic acid tetramers, providing new vistas on melanin pigments. The attention was particularly focused on the isolation and characterization by extensive 2D-NMR and mass spectrometry of a tetramer obtained by peroxidase/H2O2 oxidation of the 2,4? DHI dimer. The results of a pulse radiolytic investigation of the oxidation of 2,4? and 2,7? dimers of DHI carried out at Daresbury Laboratories in collaboration with Dr. Land were also presented. Evidence was obtained for the formation of two different semiquinone species for each dimer, which decayed with second order kinetics to quinonoid product(s) exhibiting absorption maxima at 530 and 550 nm. The symmetric 2,2? dimer on the other hand afforded a single semiquinone disproportionating at a much slower rate to give a quinone with absorption maximum at 580 nm, that is bathochromically shifted compared to those of the other dimmers. This would point to a better conjugation of the indole units in this latter quinonoid species. In the discussion that followed the presentation the mechanisms of the polymerization reaction were debated and some suggestions were given based on consideration of the structure of the oligomers so far isolated.

Dr Napolitano of Naples University presented the specific marker of pheomelanin given on the chemical degradation method. Red hair, fair skin and lack of tanning, which are associated with some loss of function mutations at the melanocrtin-1 receptor (MC1R), are recognized as risk factor for melanoma and other skin cancers. The loss of functions of MC1R cause the melanocyte to produce the pheomelanin. However, the similar red haired individuals do not exhibit the same erythemogenic responses and tanning capacities. This suggests that pheomelanin variants with different photoprotective and/or photosensitizing properties exist. On the chemical degradation of pheomelanin they used 1,3-thiazole-2,4,5-tricarboxylic acid (TTCA) and 6-(2-amino-2-carboxyethyl)-2-carboxy-4-hydroxybenzothiazole (BTCA) as the pheomelanin marker. From the data of 22 red haired individuals, BTCA was main product in the lowest MED and 5-days delayed pigmentation, while TTCA in higher MED values (mean value 67.5 mJ/cm2, $p < 0.001$). As a result, they suggested that the quantification of these markers would give the potent mean for routine prediction of high risk individuals.

The presentation by Dr. Ito with Drs. Wakamatsu, Kanavagh and Abdel Malek as coauthors was focused on the analysis of primary human melanocyte cultures established from 49 individuals (42 neonatal and 7 adult) exhibiting significant diversity of visual pigmentation. Identification and quantitation of the typical eumelanin and pheomelanin markers, pyrrole-2,3-5-tricarboxylic acid (PTCA) and 4-amino-3-hydroxyphenylalanine (4-AHP), by the chemical degradation of melanocytes allowed to determine the eumelanin and pheomelanin content for each melanocyte culture. The melanin content was also determined spectrophotometrically and the data on melanin pigmentation were correlated with the tyrosinase levels and activity as determined by the Pomerantz assay and with the mutations of MC1R gene. Data presented in the communication showed a good correlation between the spectrophotometric total melanin content and eumelanin plus pheomelanin content as determined by chemical degradation and between these data and the visual phenotype. Also, the eumelanin content showed a positive correlation with the levels of tyrosinase, while the relationship of the pheomelanin content with the visual phenotype was not straightforward. A most interesting aspect which was highlighted is that some MC1R loss of function mutations do not show a clear cut correlation with the chemical phenotype, hence mutations at this gene do not apparently alter the phenotype but may significantly affect the sensitivity of melanocytes to UV-induced damage. The discussion that followed the presentation pointed out aspects related to chemical analysis of melanin pigmented tissues and the possibility to introduce, in addition to the genotype, a ?chemotype? that is a classification of the pigmentary pathways as determined by analysis and quantitation of the resulting pigments was also considered.

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**Concurrent Session 8 - 'Photobiology'**
*By Helene Z. Hill*

Four papers were presented in this session that dealt with various aspects of exposure to mixed wavelength UV from solar simulators versus UVA. Experimental subjects ranged from cultured cells to human volunteers. Dr. Marrot from L?Oréal started off by describing the stress responses observed in cultured Caucasian melanocytes after exposure to solar simulated radiation (SSR) and UVA delivered at sub-lethal doses. The melanocytes arrested at G2/M with concomitant accumulation of GAD45 and XPC. Induction of p53 was less in the melanocytes than in fibroblasts. Heme oxygenase-1 was upregulated in melanocytes following both types of irradiation particularly if pigmentation had been induced.

Dr. Yamaguchi from the National Cancer Institute and colleagues looked at DNA damage in the form of pyrimidine dimers and 6-4 photoproducts, apoptosis and phosphorylation of p53 in African-American and Caucasian skin. Both types of DNA damage were similar in upper epidermis but reduced in lower epidermis in African-American skin. Furthermore, apoptosis was greater in the dark skin. These findings suggest that in more heavily pigmented skin, both protection against DNA damage and efficient removal of damaged cells are responsible for the lower rates of skin cancer in darker skin.

Dr. Briganti from Rome and his colleagues studied the interaction of pheomelanin and gap juction intercellular communication (GJIC) in cultured human keratinocytes. GJIC were reduced 2 hours following UVA exposure but were restored by 6 hours. When pheomelanin was present during the irradiation, the effects were enhanced. Induction of GSH in the cells before irradiation abrogated the effects, while depletion of GSH led to enhancement. It was concluded that pheomelanin can interfere with GJIC during sun exposure and high or repeated exposures could lead to long term effects on GJIC.

In the final talk by Dr. Wolberg from Hamburg, Germany, UVA responses were compared to those of a solar simulator (SSR) in suction blisters from volunteers. The two types of irradiation had dramatically different effects with SSR producing up regulation of tyrosinase, TRP1, MITF and p53 which were not seen after UVA although both types of irradiation resulted in tanning apart from IPD and PPD. It is clear that responses to solar radiation designed to

model natural exposure similar to holidays at the beach are quite different from responses that would result from UVA sun bed exposure.

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**Wednesday, September 21, 2005**

**Sunrise Session 3 - 'Pigmentary Disorders #2'**
*by Proshiela Manga*

Normal skin pigmentation is contingent upon four criteria. 1. Migration of melanoblasts from the neural crest to the epidermis and differentiation of these precursors to melanocytes. 2. Pigment synthesis in melanosomes. 3. Melanin transfer to and degradation in keratinocytes. 4. Regulation and survival of melanocytes after differentiation. Failure at any point results in either hypo- or hyperpigmentation.

1. Mutations in the gene encoding c-kit, MITF, Pax3, Sox 10, Endothelin 3 and the Endothelin 3 receptor prevent uniform establishment of melanocytes in the epidermis resulting in piebaldism and Waardenburg syndrome types 1-4. Waardenburg manifests with additional symptoms to the hypopigmentation, including heterochromia irides, sensori-neural hearing loss and aganglionic megacolon. 2. Insufficient melanin production in mature melanocytes is due either to failure of protein trafficking to melanosomes, as in Hermansky-Pudlak and Chediak-Higashi Syndromes, or due to mutations that render proteins non-functional, as in oculocutaneous albinism (OCA) types 1-4. The four forms of OCA all result in dysfunctional, either directly due to mutations in the tyrosinase gene (OCA1) or by disruption of protein folding and processing (OCA 2-4). 3. Failure to transfer melanosomes to the keratinocytes occurs due to an inability to transport and maintain melanosomes at the tips (Griscelli syndrome, mutations in Rab27, myosin Va). 4. Once a functional melanocyte has been established in the epidermis, cytokines are required to maintain these cells, which are slow-cycling and not readily replaced. Death of mature melanocytes results in depigmented patches characteristic of vitiligo. There has been much debate as to the precise etiology of this condition, which has both a genetic and autoimmune component.

Hyperpigmentary disorders can be similarly categorized. 1. Developmental hamartomas are thought to evolve from migrating melanocytes during embryogenesis. They present as at birth as congenital nevi, including nevus of Ota and nevus of Ito. Nevus of Ota and Ito are more common among Asians and females. 2. An epidermal inflammatory response can stimulate an increase in melanocyte activity and subsequent hyperpigmentation. This response is mediated by prostaglandins and leukotrines. Post-inflammatory hyperpigmentation is also present in patients with Incontinentia pigmenti, which is due to mutations in NEMO/IKK?, a protein vital for NF signaling. Increased pigmentation is due to the presence of melanophage. 3. Blockage of melanosome transfer can also result in hyperpigmentation as in Peutz-Jeghers syndrome, which results from mutations in the LKB gene that encodes a serine/threonine protein kinase. 4. Increased melanocyte numbers may also account for acquired hypermelanotic conditions such as lentigo senilis, which is common in photoaged skin and is accompanied by increased expression of Endothelin-1, the receptor ET-B-R and stem cell factor. Finally, a number of drug-induced cases of hyperpigmentation have been reported, including in response to bleomycin and fluorouracil. Epidermal hypomelanosis can be treated successfully with laser ablation, however this treatment modality, particularly in the case of congenital melanocytic nevi remains controversial because of the potential for malignancy.

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**Plenary Symposium 10 - 'Pigmentary Disorders #2, Disorders of Hyperpigmentation (Congenital and Acquired Hypermelanoses'**
*by Marjan Huizing*

Dr. Genji Imokawa showed that skin hyperpigmentation after UVB radiation and hyperpigmentation occurring in Lentigo Senilis (LS) occurs via different autocrine mechanisms, involving differential gene expression of the endothelin-1 (ET-1)/endothelin B receptor (ETBR) as well as stem cell factor (SCF)/SCF-receptor c-KIT cascades.

Dr. Masako Mizoguchi reported that the onset of the pigmented macules in ASDM (acquired symmetrical dermal melanocytosis) is caused by activation of pre-existing, immature melanocytes in these macules. This activation can occur through sunlight, estrogen and/or progesterone, inflammation, a hereditary deposition, surface interaction with elastin, or other unknown factors.

Dr. Hirofumi Aoki presented evidence that locally upregulated interferon-stimulated genes might induce migration of inflammatory cells to delayed pigmented lesions on dorsal skin. Factors excreted by inflammatory cells (such as SCF) can locally induce melanocyte proliferation and melanin synthesis. Anti-inflammatory drugs demonstrated suppression of pigment formation.

Dr. Michihiro Kono described that mutated ADAR1 (RNA specific adenosine deaminase gene) cause the autosomal dominant disorder Dyschromatosis Symmetrica Hereditaria (DSH). Patients with Dyschromatosis Universalis Hereditaria (DUH) or Acropigmentatio Reticularis (AR), both similar to DSH, did not carry any ADAR1 mutations, indicating that DUH and AR are different genetic entities.

Roman Garcia created a conditionally over-expressing Edn3 (Endothelin 3) mouse model, in which pigmentation was studied during embryogenesis and neonatal development with or without administration of oxycycline. Edn3 appeared to induce skin pigmentation.

Dr. Ganesh Diwakar showed increased expression of P-Erk (enhanced MAPK activation) and pigmentation genes (Tyr, Tyrp1, Dct, Mitf) in neurofibromin Nf1+/- mouse melanocytes. These effects were reversible by the Mek inhibitor PD98059. It is still unclear if haploinsufficiency of Nf1 affects melanocyte development and differentiation through direct effects of Ras activation or through other cellular mechanisms.

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**Concurrent Symposium 10 - 'Photobiology, Photoprotection/Photocarcinogenesis'**
*by G. Ghanem*

In this session, two plenary lectures and four talks have been presented.
Merlino et al. showed a human melanoma model with deregulated c-met pathway. This model transposed to mice with an inactive INK4a/ARF were susceptible to UVB but not UVA to develop melanoma. The aim of this work was to design a mouse model that can be extrapolated to human for further prevention strategy evaluation.

Young AR developed arguments based on in vivo data suggesting that individual susceptibility to DNA photodamage might be depending on other factors than on solely skin phototype.

Al Kaderaro et al. observed that UV induced damage of melanocytes bearing a loss-of-function MC1R is higher than in the one with a functional receptor. This prospective effect on DNA was significant in the presence of ?-MSH. The authors also observed a reduction in H2O2. Interestingly, they showed that functional MC1R is important to obtain a series of events including MITF phosphorylation, Heme Oxygenase-1 and P53 activation. They concluded that a non functional MC1R may increase the risk for a malignant transformation.

Hauser et al., from the same group as above, examined the rate of DNA repair in normal melanocytes with or without impaired MC1R function, after UV irradiation. The authors found a correlation between pigment content and CPD induction, H2O2 release and 8-O-dG

formation; indicating a protective role in these cells but not in hNM with non functional MC1R. These data support and complement the above stated work from the same team.

Noonan et al. presented a model for a UV induced melanoma in HGF/SF transgenic mice, similar to that occurring in man. They crossed these mice on C57BL/6 background with similar animals with a non functional MC1R (recessive yellow). By comparing wild type to transgenic mice after UVA, UVB and visible irradiation, the concluded that pheomelanin might not be involved in melanoma genesis but rather an aberrant signaling through MC1R.

Steinberg et al. described a novel mitochondrial deletion after a single UVB irradiation in an epithelial human cell line. The same could also be observed in different cell types as well. The authors suggested and showed evidence supporting the mechanism of such a deletion.

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*Concurrent Symposium 11 - 'Extracutaneous Pigment, Neuromelanin' (Oculocutaneous albinism, Parkinson's disease)*
*by T Sarna*

The symposium consisted of two plenary lectures and four oral presentations selected from submitted abstracts. It was co-chaired by D-N. Hu, M. Naoi and T. Sarna. Considering the scope of all presentations, the symposium was rather heterogeneous in nature touching on several different aspects of pigment research. The symposium was well attended and had lively discussion.

New methods for isolation and cultivation of conjunctival melanocytes, obtained from human donor eyes, were described by D-N. Hu, who also discussed differences in the cell morphology and the ability to form melanin in vitro, when compared with uveal melanocytes. The fact that conjunctival melanocytes are more similar to epidermal melanocytes is consistent with recent epidemiological studies, which indicate a significant increase in the annual incidence of conjunctival melanoma, coinciding with the trends seen in cutaneous melanoma. Haugarvoll et al. presented a paper, in which melanogenesis and melanosme transport and secretion was studied in a CD83 teleost leukocytes. It appears that these melanomacrophages, that are thought to participate in immune reactions and free radical trapping, represent a distinct melanin-producing DC population consisting of phylogenetically relict multifunctional cells. Anderson et al. discussed the pigment dispersion syndrome (PD), which is characterized by aberrant deposition of liberated iris pigment and often progresses to elevated intraocular pressure and pigmentary glaucoma. The authors utilized the DBA/2J mouse model to gain insight into the mechanism of the diseases. Results of the study strengthen the hypothesis that aberrant melanosomal processes contribute to the susceptibility of iris toward PD. The role of neuromelanin and neuromelanin precursors, particularly dopaminequinone, in initiating nigral neurons death cascade through oxidative stress, mitochondrial dysfunction and reduction in the activity of ubiquitine-proteosome system was discussed by M. Naoi.

The first plenary lecture, delivered by L. Zecca, was one of the Symposium highlights. The author characterized physicochemical properties of neuromelanin relevant for its postulated protective role in the substantia nigra and locus coeruleus, stressing the ability of neuromelanin to sequester redox-active metal ions. Although the synthesis of neuromelanin is a protective process since it removes potentially damaging excess of cytosolic catechols and decreases the level of free iron, neuromelanin can also be responsible for neurotoxicity. This could happen if extraneuronal neuromelanin, released by the dying neurons, activates microglia, which release several toxic factors and increase oxidative stress. As a result, a vicious cycle of chronic neurodegeneration occurs.

In the second plenary lecture, M.V. Schiaffino et al. discussed results of their study, which indicated that OA1, a glycoprotein, encoded by the gene responsible for ocular albinism 1, was a resident intracellular protein, containing multiple melanosomal/lysosomal sorting

signals, and functioned as a canonical G protein-receptor, capable of activating heterotrimeric G proteins and the associated signaling cascade.

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**Concurrent Session 12 - 'Developmental biology'**
*by Lluís Montoliu*

The session devoted to Developmental Biology was most interesting and gathered nearly all developmental biologists attending the IPCC meeting, either as speakers or among the audience. Itm included a few last minute changes, according to the original program, which are detailed below in this report. The session was chaired by L. Montoliu and E.K. Nishimura.

The first speaker was Bernier Wehrle-Haller (University of Geneva, Switzerland) who reported the last findings of his group on the relationship between the Kit-ligand dimerization, and its ER-export determinants, that appeared to be controlled by its transmembrane domains. The interaction of the Kitl protein with itself was assessed using elegant cute-edge fluorescence complementation assays. His results suggested that the transmembrane induced dimerization of Kitl in the ER was required for the efficient recognition and cell surface transport of Kitl dimmers by the ER export machinery. Next, Deborah Lang (University of Chicago, IL) presented the work of her laboratory about the role of Pax3 as a regulator for the differentiation of adult melanocyte stem cells. Her results indicated a dual role of this transcription factor, which initially triggers the melanogenesis cascade (by activating the expression of downstream Mitf) and, at the same time, prevents the expression of the Dct gene by covering the Mitf site found at the Dct promoter. This occupancy of the Mitf binding site by Pax3 is not released until Pax3 is removed by activated beta-catenin, thus maintaining an undifferentiated state and leaving the cell poised to differentiate in response to external stimuli. The third speaker was Aaron Thomas (University of California, Davis) who discussed the role of Mitf in the specification of avian melanoblasts. By decreasing the presence of Mitf by morpholinos they could show that Mitf is required to the cell-fate determination of migrating neural crests in their route to melanoblasts. Raising Mitf expression using recombinant plasmids was enough to differentiate neural crest cells to melanoblasts, in vitro. FoxD3 appears to control Mitf expression. Next, Robert Kelsh (University of Bath, UK) illustrated his most recent findings in the genetic regulation of iridophore development exploring new interactions between anaplastic lymphoma kinase (alk), a critical compound for iridophore fate specification in zebrafish. Another mutant, parade (pde) uniquely displays ectopic melanophores and iridophores. Unexpectedly, a double mutant alk-pde, having iridophores, suggested an alk-independent mechanism of generating this type of pigment cells. The fifth speaker was Robert Cornell (University of Iowa) who presented his work on a cation channel, trpm7, that appears to be needed for both differentiation and survival of embryonic melanophores in zebrafish and it is also being found and expressed in human melanoma cell lines. The trpm7 gene was identified as the cause of the touchtone mutation, in which all embryonic melanophores appeared pale, non-dendritic and prone to death. Other members of the TRPM family have a differential lower expression in metastatic melanoma cells, as compared to melanocytes, but this does not appear to be the case with trpm7. His results revealed a pathway critical for cell survival of melanocytes and possibly melanoma cells. Next, Stefano Bertuzzi (NINDS-NIH, Bethesda, MD), from Heinz Arnheiter?s lab, presented his results on the role of Mitf and Vax transcription factors in retinal pigment epithelium (RPE) specification and development. Mitf expression initiates dorsally at RPE whereas Vax expression determines the ventral aspect, with both functioning as mutual repressors of each other?s expression. The seventh speaker was Thomas Hornyak (NCI-NIH, Bethesda, MD) presented the microphthalmia-white mouse mutant model (Mitf Mi-wh/+) as an experimental model for human Waardenburg and Tietz syndromes, regarding its auditory defects. His experiments concluded that the hearing defect observed in these mice was associated with strial degeneration and lack of strial melanocytes in the early post-natal period. The observed rescue of otic melanocytes from these mutant mice with epidermal and dermal growth factors indicated how environmental factors may facilitate the selective survival of melanocytes in the hair follicles of these mutant mice. Finally, the session was concluded by a talk presented by Maria Wei (Universtiy of California, San Francisco) where

another two mouse mutants (pale ear and light ear), the experimental models for Hermansky-Pudlak Syndromes 1 and 4, respectively, were used to demonstrate that their corresponding gene products do not only regulate melanosoma biogenesis, but also play a developmental role in interfollicular melanocyte function.

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**Sunrise Session 4 - 'Malignant Transformation'**
*by Nelleke Gruis*

The sunrise session on malignant transformation aimed to provide an up-to-date view on the understanding of the molecular genetics of melanoma development as well as transcriptional and signaling mechanisms which underlie the malignant phenotype. In the first overview, provided by Dr. Nelleke Gruis from the Department of Dermatology at Leiden University, Netherlands, discrimination was made between genes and loci currently known to be involved in melanoma predisposition and progression. CDKN2A turned out to be the major melanoma susceptibility gene so far. In melanoma progression, oncogenes and tumor suppressor genes involved in three main cellular pathways: cell cycle regulation, DNA repair and receptor-mediated signal transduction play a role, and provide possible targets for therapeutic intervention.

The second presentation was by Dr. David Fisher from the Melanoma Program at Department of Pediatric Oncology at Dana-Farber Cancer Institute, Harvard Medical School. This talk focused on the MITF transcription factor which plays an essential role in melanocyte lineage development. MITF has been found to transcriptionally regulate expression of numerous components of the pigmentation/differentiation pathway in melanocytes. However, since its mutation affects melanocyte viability (rather than only pigmentation), studies have also focused on its potential role in regulating proliferation or survival. In addition to several transcriptional targets of MITF which play such proliferation/survival roles, the MITF gene itself was recently found to undergo genomic amplifications in 10-20% of primary melanoma specimens. These presentations thus provided an overview of molecular genetic as well as signaling/transcriptional pathways to melanoma tumorigenesis.

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**Plenary Symposium 11 - 'Melanoma 1, Senescence, Immortalization and Progression' (Nevi, Melanoma)**
*by A Bosserhoff*

Plenary Lecture by Meenhard Herlyn "Biology of Melanoma Progression"
Meenhard Herlyn focused on the progression model of melanoma describing the development from nevus to primary melanoma without competence to metastasize to vertical growth phase primary melanoma with competence for metastasis and, finally, to metastatic melanoma. Due to several new findings of his group and in the field this model may have to be adjusted. Melanocyte stem cells have been found in the hair follicle and it is no longer definite that melanoma originates from melanocytes or from melanocytic stem cells. Additionally, recent research led to the characterization of melanoma stem cells. These show high plasticity and can differentiate into melanocytic but also into mesenchymal cell types like fat cells or chondrocytes. These are also the cells in the tumor with high self-renewal capacity. Last but not least, the role of stromal cells should not be underestimated in melanoma development as they can play determining roles for progression and disease outcome.

Plenary Lecture by Estella Medrano "Cellular senescence and Chromatin Remodelers: Possible Mechanism-based Therapeutics for malignant melanoma"
Irreversible growth arrest in cellular senescence is thought to be a potent tumor suppressive mechanism. Estella Medrano could demonstrate this mechanism in primary melanocytes showing growth arrest due to diverse stress, however the cells stay viable over a long period

of time. This replicative senescence is controlled by the RB/p16/HDAC1 pathway whereas p53 is not involved in this process in melanocytes. Marker of this process are e.g. upregulation of expression of SPARC and downregulation of expression of MITF or cyclin E. Deregulation of p16 and/or RB is commonly found in melanoma possibly resulting in deregulation of induction of senesence. Additionally, Medrano presented that a critical balance of HDAC1 as chromatin remodeler is required maintaining cellular homeostasis and proliferation in normal cells, which is dysregulated in cancer cells.

"Senescence of human melanoma cells following activation of PKC and MAPK pathways" by PG Parsons
Parson et al. presented that treatment of B16 melanoma with PKC-activating ingenol ester PEP005 resulted in cure of mice. Treatment of human melanoma cell lines with TPA led to terminal growth arrest of sensitive cells after 24 hours with loss of telomerase activity whereas insensitive cells were resistant. Use of a PKC inhibitor (bisindolylmaleimide-1) confirmed the role PKC in this process. Microarray studies demonstrated the repression of E2F1 and E2F1-sensitive genes in the sensitive cell lines. PKC activation in the sensitive cells also resulted in activation of ERK1/2 which was required for growth arrest and cell cycle block. The resistance in several cell lines was possibly due to expression of H-rev107, an inhibitor of the MAPK pathway. The group suggested that PKC activation may play a role in the natural regression of melanocytic lesions.

"The cleavage of MITF by caspases plays an essential role in melanocytes and melanoma cell apoptosis" by L Larribere
Larribere et al. identified MITF (microphthalmia associated transcription factor) as a new substrate of caspases during apoptosis. MITF is known to play a key role in melanocyte development, survival and differentiation. Furthermore, an impact of MITF on melanoma development is speculated. It was presented that MITF processing by caspases mediates melanoma cell apoptosis. A non-cleavable form of MITF renders melanoma cells resistant to apoptotic stimuli. Additionally, the c-terminal fragment generated by caspase cleavage promotes caspase 3 activation, morphologic changes and increases in the sub-G1 population. This finding again points to the functional duality of MITF in survival and cell death that was postulated in several talks on this IPCC meeting.

"Stable overexpression of Smad7 in human melanoma cells inhibits their tumorigenicity in vitro and in vivo" by A Mauviel
Mauviel et al. investigated the effect of Smad 7 overexpression on melanoma cell properties to analyze whether autocrine effects of TGFß are essential for malignant melanoma cells. Smad 7 is known to inhibit Smad phosphorylation at the TGFß receptor. Smad 7 overexpression did not change the proliferative potential of cells. However, the capacity to invade Matrigel was strongly reduced, likely by downregulation of MMP-2 and MMP-9 secretion. Additionally, anchorage independent growth and tumor formation in nude mice was reduced. The data suggest that TGFß affects the tumor microenvironment as well as the tumor cells themselves by contributing to tumor aggressiveness.

"Further development of human skin xenografts towards modeling melanoma" by A Yoneta
Yoneta et al. present the development of a new skin graft model using skin reconstructs which reduce the problem of donor-dependent heterogeneity. Additionally, lentiviral vectors were applied, which result in stable expression of the transduced transgene. Using this model, melanocytes, keratinocytes and/or fibroblasts can now be stably transduced and then incorporated into the skin reconstruct. It was already demonstrated using this new model system that melanocyte proliferation is induced after transduction with ET-3 or bFGF.

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**Plenary Symposium 12 - 'The Malignant Phenotype'**
*by Dorthy Bennett and Zalfa Abdel-Malek*

In his keynote presentation, Jonathan Rees gave his perspective on pigmentary and non-pigmentary factors that influence the responses of skin to ultraviolet radiation (UV). The role of pigment in the UV response is well-established, however, other factors need to be considered, such as the MC1R genotype, which Rees and his co-workers found to be associated with freckling and hair color, but not necessarily with skin color, and to have a dosage effect. Expression of the wild type MC1R was associated with the highest ratio, while heterozygosity and homozygosity for alleles that reduce the function of the MC1R were associated with an intermediate and the lowest ratios of eumelanin to pheomelanin, respectively. Recently, Rees reported that eumelanin to pheomelanin ratios do not differ markedly in the skin of Asians vs. North Europeans, and that both eumelanin and pheomelanin were increased following UV exposure. These data refute a specific role for pheomelanin in the damaging effects of UV, and the usefulness of pheomelanin content alone, or eumelanin to pheomelanin ratio, in predicting the response to UV. One additional factor that needs to be considered is epidermal thickening, which confers photoprotection and is more pronounced in Northern European than Asian skin.

Dorothy Bennett gave the IFPCS Presidential lecture entitled 'the genetics of melanoma'. She reviewed evidence that melanoma development requires fewer events than for other solid tumors. Germline susceptibility to melanoma is partly determined by the CDKN2A locus, encoding two tumor suppressors, p16 and ARF. The main function of p16 is as an effector of senescence, particularly M0. p16/RB defects lead to M0 deficiency but then M1 senescence which is p53-dependent and associated with short telomeres. Additional blockage of p53 extends lifespan further, and leads to M2 (crisis), when cells divide and die. p16-deficient cultured human melanocytes exhibit delayed, p53-dependent senescence, and high apoptotic rate that can be inhibited by keratinocyte-derived survival factors. This raised the question: 'are moles the result of senescence in the skin'? A model for melanoma progression was presented. In the model, nevi arise through an activating mutation in BRAF or NRAS, leading to M0 senescence. Defects in p16 would lead to dysplastic nevi, M1 senescence and M2 crisis, then activation of hTERT leading to immortal RGP melanoma. Further mutations allow progression to invasive VGP melanoma. On testing the model, nevi did show markers of senescence, especially inability (6/6) to proliferate in culture. Phospho-CHK2 and phospho-p53ser20, part of the ATM-mediated DNA damage response, are possible markers of M1 and M2. Both were observed in most benign lesions but expression increased with progression. hTERT expression also increased with progression and was highest in VGP melanoma, while p16 was lost.

Robert Weinberg provided a stimulating and fast-moving talk, in the area of genetic changes required for the development of cancer. His group has published sets of genetic changes that can produce cancers from various normal mammalian cell types. Here he presented recent findings on human breast cancer and melanoma. He discussed changes found in breast cancer stromal cells (fibroblasts), as opposed to the cancer cells. When purified, these fibroblasts were abnormally able to stimulate angiogenesis and attract endothelial precursor cells, indicating either genetic or epigenetic changes in these cells, or that stromal cells could arise from tumor cells. He also discussed microarray analyses of genes overexpressed in metastatic cancer cells. In breast cancer these included TWIST, which is involved in normal epithelial-mesenchymal transitions in development. Genes apparently overexpressed in metastatic melanoma included Slug (SNAI2) and FOXC2, again developmental effectors. Interactions could be found; for example exogenous TWIST expression could upregulate SNAI2. Some of these findings appear in Nature Genetics, October 2005.

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**Plenary Symposium 13 - 'Melanoma 2 Genetics, Susceptibility, Epideniology, Etiology and Therapy (Melanoma)'**
*by Frank Meyskens*

The general theme of this session encompassed the genetics, susceptibility, epidemiology, etiology and treatment, with a predominant emphasis on the human malignancy. Two plenary lectures (Chin, Marais) and four competitive abstracts comprised this session.

Lynda Chin (Harvard) covered two major areas. She described a new transgenic model that involves an inducible ras in the setting of Ink4a/Arf? which resulted in an amelanotic melanoma phenotype. The majority of her talk was spent on describing a complex gene discovery program defined by programmable array-CGH. Many new potential targets of unknown function were identified.

Roge Marais (London) discussed the b-Raf system in detail including the various relationships between the ras-raf systems and among the a, b and c forms. Of considerable parenthetic interest was his description of b-Raf mutations in murine bronchiolalveolar lung lesions.

Frank Meyskens (UC Irvine) updated the evolving story on redox and the pathogenesis of melanoma. Abnormalities in redox regulation as related to NADPHoxidase, melanin conversion from an antioxidant to pro-oxidant, melanosomal disruption and mitochondrial mutations all seem to contribute to high levels of reactive oxygen species (ROS) in melanoma. A number of new preventive and treatment strategies were described based on this paradigm.

Sun Yang (also of UC Irvine) continued these themes and described the adaptive response to elevated levels of ROS which occurs including activation of many transcription factors and of the multifunctional protein apurinic/apyrinidic, endonuclease/redox effector factor-1(APE/Ref-1) which both assists in DNA repair as well as modulates the redox state of many transcription factors. Initial studies of an inhibitor of APE/Ref-1 were described with the polyphenoic antioxidant resveratrol being a lead compound.

Yutaka Kawakami (Tokyo) described the development of individualized immunotherapy by identification of highly immunogenic antigens using rapid serum IgG antibody screening or in vitro T-cell induction. Following this screen injection of dendritic cells based on reactivity to the individual immunogenic antigens produced induction of CD8+ CTL and regression of large untreated tumor located at a remote site. Based on these encouraging murine studies, a clinical phase I/II trial has commenced.

Finally, E Hacker (Australia) using the CDK4R24C/R24C/TPRAS mouse system demonstrated that a single dose of ultraviolet light radiation(UVR)induced metastaticic melanoma in neonatal mice but not in adult animals, even when multiple doses of UVR were administered. Detailed studies suggested that Ras activation was sufficient to predispose melanocytes to UVR-induced transformation but mutant Cdk4 was necessary for progression to large and/or aggressive metastatic lesions.

Overall these six presentations from four countries indicate that studies of human melanoma are alive and well and that a wide range of approaches are being used to offer better platforms for understanding the etiology and pathogenesis of the disease as well as designing new preventive and therapeutic treatments.

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**Concurrent Session 13 - 'Melanoma Basic Research'**
*by Faith Strickland*

Basic research conducted to understand the processes involved in transformation of melanocytes to malignant melanoma focuses on the steps involved in tumorigenesis: initiation, promotion and progression. The role of nuclear receptors in melanoma carcinogenesis was explored by Indra et al. The chemopreventive action of retinoids is mediated by heterodimerization between retinoid X receptor (RXR ???) and other receptors such as the vitamin D receptor (VDR) and peroxisome proliferators activated receptors (PPARS). Selective ablation of RXR? and PPARS in epidermal keratinocytes greatly enhanced nevi formation as well as increasing the numbers of papillomas and progression of papillomas to carcinomas in the two step chemical carcinogenesis protocol of DMBA initiation and TPA promotion. Nevi progressed to melanomas only in the RXR?-null animals and unlike the papillomas, malignant transformation of melanocytes was not blocked by treatment with

glucocorticoids. Their data suggested that a paracrine mechanism is involved in the formation of melanomas and that distinct mechanisms are involved in the formation of melanomas compared with carcinomas in their model. The BRAF-cyclin D1 pathway that controls p27Kip1 via Skp2 (an F-box protein) may contribute to deregulation of cell cycle progression in melanoma cells. BRAF regulates cell cycle control, Skp2 and MAPK activation. Mutations in BRAF led to increased Skp2, activation of MAPK signaling and subsequent proliferation (Bhatt et al.). Mutations in BRAF are found in many melanomas, however, Namkoong et al. reported that MAPK could also be activated in cells with wild type BRAF through overexpression of the metabotropic glutamate receptor 1 (Grm1). Nevertheless, overexpression of Grm1 alone is insufficient to bring about a fully transformed phenotype in melanocytes and requires additional growth factors to express continuous growth and anchorage independence (Shin et al.). Once cellular transformation and escape from growth control occurs, progression to metastatic disease requires further changes such as anchorage independence, expression of proteases to enhance migration, and freedom from growth control by the local tissue environment. The human HUGI-1 gene which has significant homology to the Drosophila tumor suppressor gene lethal (2)giant larval was found to be reduced or lost in melanoma cell lines and tumors and the loss was associated with the advancement of the disease stage (Bosserhoff et al.). HUG-1 expression downregulated MMP2 and MMP14 and increased E-cadherin thus, controlling melanocyte adhesion and migration. Beermann et al. used a transgenic mouse line expressing the melanocyte-targeted dominant activated human N-RasQ61K in combination with an absence of p16INK4a/19ARF to show that these mutations were sufficient to cause metastatic melanomas in 90% of the animals. Addition of FGF (bFGF, FGF2) had no effect on melanoma genesis, indicating that under conditions of major pathway dysregulation, additional growth factors may not be needed to induce melanoma progression. Proteins controlling cellular proliferation which are dysregulated in melanoma can be used as both markers for disease and potential therapy. Matsuzaki et al. reported a newly discovered protein FABP7 that controls proliferation and migration of melanoma cells in vitro and is expressed in high levels in both cell lines and melanomas isolated from tissues. Over half the patients with melanoma in their study also had serum antibody to FABP7 making this protein a potential marker for the disease. Finally, Miao et al reported creating a new class of metal cyclized melanotropin peptide analogues (CCMSH) that bind the melanocortin-1 receptor. These compounds can be used as radiopharmaceuticals for melanoma imaging and therapy. Taken together, the data presented at this session helped to shed new light on the complex interaction of pathways involved in regulation of cell growth and their role in melanoma development.

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**Concurrent Session 14 - 'Hypo- and Hyper Pigmentation'**
*by Jim Nordlund*

BK Goh talked on the loss of melanosome transfer accounts for guttate leukoderma in Darier's disease. Darier's disease is a genetic disorder in which the face, neck and trunk are covered with gritty, keratotic papules. There are mutations in a gene ATP2A2 that alters intracellular calcium homeostasis. In type 3 Asian skin guttate hypopigmented macules are observed. Electron microscopic studies from these macules demonstrate melanosomes are present in the dendrites but are not transferred to surrounding keratinocytes. The findings are consistent with dysfunction of calcium metabolism. It is not clear why these white macules are observed only in Asians with Darier's disease.

Hermansky Pudlak syndrome is caused by mutations in at least 7 different genes in humans. Protein products from normal genes form complexes (Westbroek et al.). Attempts to study binding of these proteins in fungi was not successful. Studies on proteins from normal human cells did show HPS proteins 5-3-6 form a complex. Such results permit additional investigations on complexing and binding of the various 7 HPS proteins.

Various species of chickens are hatched with pigmented feathers but lose color after molting. In the barred rooster, it is thought that toxic substances cause the barred appearance, possibly oxygen radicals. Glutathione was deficient in cells from the barred chicks.

Melanocytes in cultured were rescued by addition of catalase and glutathione, an observation that is consistent with oxygen toxicity as a cause of barring in chicken feathers.

Synergistic therapies for vitiligo was presented by A. Ramaiah. Standard therapies for vitiligo include PUVA, topical steroids and similar agents. bFGF is a known mitogen for melanocytes. A molecule derived from bFGF has been applied to depigmented skin and produced repigmentation in some patients. In combination with PUVA or other standard therapies, repigmentation was observed. The bFGF derivative might be a useful commercial product to treat vitiligo.

Information on a controlled study using Levamisole was presented by M. Ramam. Levamisole, an immune modulating medication, has been proposed as a medication for vitiligo. In a double blinded study, patients with vitiligo received either levamisole or a placebo. There was no observable difference between the treated and placebo group. Levamisole may not be effective for treating vitiligo.

Vitiligo can be treated with surgical transplants. Mac Neil et al. have developed a complex, expensive system for growing and amplifying keratinocytes and melanocytes into sheets that are life saving for treating patients with burns. The technique has potential for treating patients with vitiligo.

Repigmentation of patients with vitiligo is thought to depend on stem cell melanocytes in the niche are of hair follicles. L.M. Davids presented preliminary data to confirm this idea. However no studies have shown proliferation of melanoblasts in the niche area following treatment of PUVA. Results of this study have confirmed that single hair follicles can be obtained and maintained in culture. So far it has not been possible to demonstrate melanocyte proliferation in the niche area. These techniques will allow documentation of melanoblast behavior within the niche area following treatments such as PUVA.

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**Concurrent Session 15 - 'Immunology'**
*By I Caroline LePoole*

The melanocyte stands out for its melanin synthesis and because the organelles supporting this process make for a uniquely immunogenic cell type. Within the immunology session, abstracts were presented on immune responses to melanocytes and melanoma cells.
J van den Boorn presented a characterization of T cells that were derived from actively depigmenting skin biopsies of 7 vitiligo patients. Vitiligo T cells were found to be reactive mostly with MART-1 (up to 35% of isolated T cells) and tyrosinase, supporting intriguing similarities among vitiligo and melanoma. J Pawelek discussed the presence of LPHA expressing Langerhans cells (LC) in vitiligo epidermis, indicating that LC activation is apparent in actively depigmenting skin. IC Le Poole demonstrated that regulatory T cells were virtually absent from actively depigmenting vitiligo skin, contributing to the perpetuation of an immune response. LM Hopkins showed that several phosphorylated peptides are unique to cancer cells, describing some 10 candidate phophopeptides that may become part of future anti-tumor vaccines.

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**Concurrent Session 16 - 'Extracuetneous Pigmentation'**
*by William Oetting*

Baranova spoke on melanin synthesis in fat tissue from morbidly obese individuals. It was shown that expression of melanogenic proteins is upregulated in adipose tissue in morbidly obese individuals. Mason-Fontana staining of tissues confirmed the presence of melanin biosynthesis in these tissues. E. Greggio talked on Parkinson?s disease (PD). PD is characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars

compacta. They collected evidence of tyrosinase expression in the brain. There was a question if variation in tyrosinase activity was associated with variations in PD. They report that haplotype analysis of the tyrosinase gene did not exhibit any genetic association between tyrosinase activity and PD. A Gallone looked at melanin biosynthetic activity in the liver. Tyrosinase was shown to be present in Rana Esculenta L liver. The melanogenic system of the liver was further discussed. Using ultraviolet free electron laser photoelectron emission microscopy (UV-FEL-PEEM), WD Bush analyzed the oxidation potential of melanin in the Substantia nigra region of the human brain was analyzed. Analysis was also done with atomic fource microscopy and scanning electron microscopy.

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