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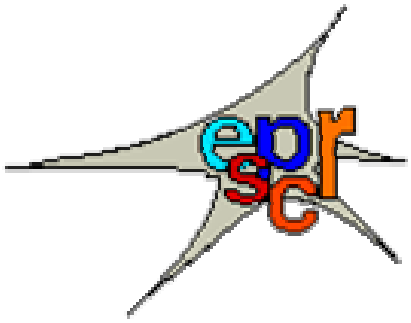

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

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HAPPY NEW YEAR 2008

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Bari 14-17 october 2007
"Pigment Cells and their Environment"

***Not available**

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Chairs: T.M. Lotti, A.Taïeb, M. Picardo

Symposium: Melanoma Therapy
Contributed by G. Ghanem, A. Bosserhoff

Two invited lectures and three oral contributions were presented at this session.
The first was from A. Spatz (presented on his behalf by G. Ghanem, Dr Spatz could not attend and sent his apologies to the audience) essentially describing the omics projects of the Melanoma Group of the European Organization for Research and Treatment of Cancer (EORTC). A supervised analysis focused on a gene profiling, identified highly discriminant signature for a group of 60 genes based on high to low risk for metastases (DMFS at 3 years). Among these, key genes involved in replication controlling pathways (Minchromosome Maintenance genes, MCM, and pituitary tumor transforming gene, hPPTG), DNA repair pathways and B-Raf Map kinase pathway. MCM-4 and 6 overexpression was an independant prognostic factor both for DMFS and overall survival. hPTTG gene encodes securin whose overexpression causes inhibition of chromatid segregation, aneuploidy, impairment of P53-mediated apoptosis, promotion of angiogenesis and induction of c-Myc expression. A second analysis addressed 231 genes involved in DNA repair, out of which 47 had significantly higher expression in the group of patients with poor prognosis. The large majority of these belong to all repair mechanisms with Topoisomese 2A as the most expressed. The latter is known to relieve DNA torsional stress and to confer resistance to alkylating agents. An association of 64 genes with the B-RAF mutation

V600E was found in 69 patients. One of these, CD63 expressed in malignant melanocyte and could alter cell motility and invasiveness, seems to play some role since it has been shown that its expression is associated with serine proteine kinase activity modulated by B-Raf mutation. Genetic aberrations of miRNA was found associated with Breslow thickness in 12 out of 159 tested and appears to be common in human cancer resulting in impairment of their function as posttranscriptional repressors. An interesting observation was made concerning the identified down-regulated genes out of which 58 were hypermethylated at the CpG promoter islands with 24 of the latter containing tumor suppressor genes.
The last part of the talk was dedicated to a discussion for a new classification for melanocytic tumors based on the latest advances in molecular pathology. Several arguments were shown to indicate at least three diseases: Solar melanomas with Elastolysis as helpful criterion, Acral melanomas with gains in chromosomes 5p, 11q and others with growth phase patterns.

The second invited lecture was presented by A. Bosserhoff about bone morphogenic proteins role in malignant melanoma progression.
BMP4 and BMP7 are strongly expressed by malignant melanoma cells and recent data showed that they play an important role in melanoma proliferation, invasion as well as vascular mimicry of malignant melanoma. BMPs, therefore, can serve as new attractive therapeutic target for therapeutic implication.
Interestingly, BMPs can also serve as diagnostic marker as recently it was shown that BMP7 protein expression in the tumor may predict differential disease outcome of melanoma patients.
Understanding of the molecular role of BMPs by e.g. finding target genes in melanoma will be the goal in the future.

The first oral contribution focused on the role of GnRH receptors in melanoma cell proliferation and invasion presented by P. Limonta.
The activation of GnRH receptors by an agonist (Zoladex) resulted in a reduced cell proliferation. In amore extensive study in a melanoma cell line model, the authors also observed a reduced migration in matrigel, a decreased expression of α_3 integrin, MMP-2 and VEGF by microarrays. Interestingly, the authors observed significantly less release of VEGF in the same conditions as well. This novel approach is proposed as an alternative hormonal therapy in melanoma.

The second oral contribution dealt with uveal melanoma management and was presented by C. Laculli. The experience and results of the Dept. of ophthalmology of the University of Foggia, Italy, were discussed. Their review of 414 uveal melanomas over the last 26 years led to the following conclusions: 1) plaque radiotherapy for medium or small size lesions is comparable to enucleation based on overall survival; 2) Surgery should be followed by brachytherapy in order to avoid relapses.

The third and last oral contribution reported a first approach to a particular delivery mechanism for an alkylating peptide, PSF (L-propyl-m-L-Sarcolysyl-L-p-fluorophenylalanine) presented by K. Dierickx. The drug showed a high affinity to cell membranes, including ghost RBCs and artificial membranes (e.g. liposomes) and can be transported as such by blood cells. The authors showed data supporting a protease-mediated release of active metabolites from membrane-bound PSF, in a panel of melanoma cell lines. Overexpressed proteases by tumor cells, including MMPs, are able to recognize the peptide moiety of the drug and liberate up to 4 metabolites that could be differentially inhibited by different protease inhibitor combinations. Furthermore, the bodydistribution of the radioactive drug showed significant accumulation in human melanoma tumor-bearing mice (amelanotic and pigmented), interestingly in spleen (supporting the binding to BC) and in the pancreas (supporting a protease-mediated drug delivery).

Session I: Melanins as biomaterial
Contributed by A. Napolitano, T. Sarna

The first session of the meeting was entitled "*Melanin as Biomaterial*" to put the emphasis on a new perspective from which melanin pigments are being considered in the last few years from the structural and physico chemical community that is as new class of polymers of natural origin possessing unique properties which may be exploited in the design of innovative high-tech functional materials. An interesting *casing model* for mixed melanogenesis was presented by S. **Ito** (IL11) based on a detailed analysis of the kinetic constants for the initial stages of eu- and pheomelanogenesis, and particularly the rates for *self cyclization vs cysteine addition of dopaquinone*. Cysteinyldopas formation, their oxidation to pheomelanin, and eumelanin formation were identified as the three fundamental steps of this pathway. Determinant factors were shown to be tyrosinase activity, cysteine levels and pH conditions. It was possible to conclude that pheomelanin is formed first and eumelanin is deposited on the preformed pheomelanin. At present only neuromelanin provided direct proof to this hypothesis. In invited lecture 12, D. **Peles** summarized recent results of a biophysical study of melanosomes that were obtained by the Duke group using advanced spectroscopic/imaging methods such as free electron laser photoelectron emission microscopy (FEL PEEM), mass spectrometry and optical spectroscopies. Their most interesting and intriguing result concerns differences in the *threshold surface ionization potential of eumelanosomes and pheomelanosomes*. Thus both melanosomes exhibit an ionization threshold at 282 nm; however, pheomelanosomes exhibit a second ionization threshold at much longer wavelength, i.e. 326 nm, suggesting that pheomelanosomes can be photooxidized by solar radiation that reaches the Earth surface. Using FEL PEEM the Simon group was able to demonstrate that neuromelanin granules have a heterogeneous structure, in which the core of the granule contains predominantly pheomelanin while the exterior part of the granules is mostly eumelanin in nature. In invited lecture 13, P. **Meredith** stressed the importance of *chemical disorder at the primary and secondary structural level of melanins* in defining the optical and photochemical properties of this biomaterial. Meredith summarized the current level of understanding with respect to the primary and secondary level structures in eumelanin, based on theoretical analysis and experimental studies, such as density functional theory calculations, optical emission and absorption spectroscopy and high resolution X-ray scattering. There is little doubt that the Meredith group will continue to play a major role in physicochemical characterization of melanin. In the oral presentation OP8 G **Zonios** showed results lying the basis for a non-invasive method for melanoma diagnosis based on the in vivo optical properties of melanin. Key elements were the exponential dependence of melanin absorption spectrum on the wavelength and the *differences in the absoprtion spectra* between melanin found in *dysplastic nevi and malignant melanoma*. Fluorescence spectroscopy was used as an approach to investigate the structural organization of eumelanin and the role of the protein coating in the optical and structural properties. In the presentation by G. **Perna** (OP9), a radiative recombination model characteristic of disordered materials was proposed to explain the features of the fluorescence spectra, particularly the red-shift and the line-narrowing effect of broad fluorescence bands with decreasing of the excitation energy. Fluorescence due to ensembles of large oligomer systems have been spectrally discriminated from that due to monomers and small oligomer systems. The spectra of synthetic and natural eumelanin have similar features, except for those due to the presence of proteins in natural eumelanins, as *proteins affect the structural organization of eumelanin by favoring small size aggregates*. In examining the effect of bonding mode and increasing molecular size on the absorption properties of oligomers of 5,6-dihydroxyindole A. **Pezzella** (OP10) showed that 1) no regular and predictable bathochromic shift occurs with increasing chain length; 2) a marked broadening of the absorption bands occurs when going from the monomer to tetramer ; 3) the mode of coupling of the monomer units is a critical structural parameter governing the absorption properties of indole oligomers. With regard to the central issue of why melanin is black it was

concluded that it is difficult to obtain a black chromophore by mixing oligomers of different chain lengths *in their reduced form*. Visible light absorption and significantly red-shifted HOMO-LUMO gaps can be achieved only by *oxidizing* oligomeric scaffolds.

Session II: Melanogenesis and its regulation
Ccontributed by C. Goding, C. Jiménez-Cervantes

Session II had 3 invited speakers and 8 selected presentations, which were chosen from the submitted abstracts. Two presentations were cancelled: one of the invited lectures and one oral presentation. The session was opened by a lecture of **Marie Dominique Gallibert** (CNRS, Rennes, France), reported her results on the multiple and critical roles of MITF on melanocyte differentiation and proliferation. Mitf, is a key regulator of many aspects of melanocyte and melanoma cell biology including survival, cell cycle entry and exit and differentiation. Not surprisingly therefore Mitf expression and function is regulated by a wide range of transcription factors and signalling pathways that control its transcription and post-translational modification. Dr Galibert reported a further interesting mode of regulation, namely that the presence or absence of a 6 aa alternative exon is regulated by MAPkinase signalling. She also reported a new stress-induced modification of the Usf1 transcription factor that can bind the same elements as Mitf. While the precise function of these novel regulatory events on Mitf and USF1 remain to be fully characterised, there is no doubt they will prove particularly important in the regulation of pigmentation and melanocyte biology. The second invited speaker was **José Carlos García-Borrón** (University of Murcia, Spain). He showed that the variants of MC1R known as RHC alleles and related to the red pigmentation phenotype have a partial loss of function upon hormonal stimulation. This partial loss of function was due to different causes in each case. R151C and R160W are partial loss of function MC1R variants because, on their way to the plasma membrane, they are partially retained in intracellular compartments. He showed by confocal microscopy images that R151C MC1R was clearly retained in the ER and R160W was retained between the ER and the Golgi apparatus. On the other hand, although D294H MC1R variant is correctly expressed in the cell surface, he showed that it does not couple to the signalling pathway neither it internalizes upon ligand binding, as the wild type form does. He demonstrated that the interaction with a new splice form of β-arrestin, the protein that promotes desensitization and internalization of the MC1R, was deficient for the D294H variant. In conclusion, he showed that both the proper exocytic and endocytic pathways of MC1R are necessary for efficient receptor function. The first oral short communication was presented by **Dr. Bellei** (San Gallicano Dermatological Institute, Rome, Italy). She showed that increasing evidence is accumulating pointing to a role of stress-activated P38 MAPK in the UV tanning response and in the regulation of melanocyte differentiation. Her group reported pharmacological evidence linking the effect of GSK3b with MAPK activation using B16 melanoma cells as a model. Three specific inhibitors of GSK3b increased the levels of several differentiation markers, including tyrosinase. Consisting with increasing differentiation, these inhibitors inhibited proliferation. The effects of the GSK3b were partially blocked by P38 selective inhibitors, but not by MAPK inhibitors. The second short oral communication was related to the MC4R function, presented by **Dr. Spencer** from the University of Bradford, UK. She presented some data suggesting a possible involvement of MC4R in the regulation of pigmentation. MC4R is a Gs protein coupled receptor with similar properties to MC1R. According to the authors, MC4R is expressed by epidermal melanocytes and keratinocytes, both at the mRNA and protein levels. Binding studies suggested a presence of several thousands of MC4R per cell and functional data suggest that these sites might be coupled to stimulation of differentiation. A short discussion followed because although these results are potentially interesting, they do not account for the lack of pigmentation phenotypes in knock-out mice and in human patients carrying dominant-negative loss of function mutations of MC4R. **Dr. Masoodi,** from the University of Bradford, UK, reported a study about the production of

prostanoids by melanoma cells and the effects of several lipid mediators on melanin production by normal melanocytes and melanoma cells. The authors identified the main lipid mediators within these cells and confirmed the presence of part of the enzymatic machinery required for this activity. By comparing several cell lines with different melanin content, the authors suggested and inverse correlation between prostanoic production and melanin concentration. **Rebecca Ginger** from Unilever, UK**,** spoke on the linkage between the putative K^+-dependent Na^{2+}/Ca^{2+} exchanger, SLC24A5 and melanogensis in mouse and human pigmentation. A whole genome association study of the genetic determinants of natural skin colour variation in South Asia identified non-synonymous SNPs in 3 genes that passed genome-wide significance. The strongest of these associations was with the pAla111Thr SNP in SLC24A5. Using siRNA mediated knock-down of endogenous SLC24A5, it was shown that this gene is required for pigmentation in cultured mouse and human melanocytes, consistent with the phenotype of the *golden* mutant previously described in zebrafish. Affinity-purified polyclonal antisera, validated by siRNA knock-down, suggest a novel *trans*-Golgi network localisation for this protein. Although the intracellular localisation of this protein has so far prevented direct measurement of its predicted exchanger function, site-directed mutagenesis of related family member SLC24A2 in a heterologous expression assay revealed that the residue altered by the SLC24A5 nsSNP is critical for K^+-dependent Na^{2+}/Ca^{2+} exchange activity in this protein. On the basis of high sequence homology this is likely to be true for all family members, including SLC24A5. **Dr. Passeron** (NIH, Bethesda, USA) reported the possible role of SOX9 in the UV-induced tanning response. Although its best known actions are in sexual determination and chondrogenesis, with mutations in SOX causing campomelic displasia and minor pigmentation defects, several lines of evidence suggest a role in the regulation of melanocyte biology. He showed that SOX9 is expressed in adult skin melanocytes *in vivo*. SOX9 expression correlates with basal pigmentation and increases rapidly in UVB-irradiated melanocytes. The cAMP elevating agent forskolin increases SOX9 expression and binding to the *MITF* promoter, and the induction of SOX9 in UV-treated cells is blocked by the PKA inhibitor H89. The authors also analyzed the molecular mechanisms of regulation of pigmentation by SOX9. SOX9 binds and activates the *MITF* promoter and increases *Tyrosinase* and *DCT* expression. Based on these data, the authors propose that pigmentation induced by UV radiation is at least partially dependent on SOX9. **Desmond Tobin** (University of Bradford, UK) presented the OP17, dealing with the occurrence and secretion of full length POMC from skin cells. It has been known for long that the skin is the major source of POMC and POMC-derived peptides for the control of pigmentation. POMC expression has been shown in melanocytes and keratinocytes which also express ACTH, β-MSH and β-endorphin peptides. Using a specific immunochemical assay designed to detect full length, unprocessed POMC, the authors showed that the prohormone is secreted by cultured skin cells. The pattern of POMC and derived peptides secreted by melanocytes and keratinocytes is different, with melanocytes secreting larger amounts of unprocessed POMC. The full length peptide appears to be biologically active on MC1R although the possibility of a partial processing in the extracellular medium that would account for this activity cannot be ruled-out. **Dr. Patrick Riley** (TIAS from London, UK) presented a study of the mechanisms of suicide inactivation of tyrosinase. Tyrosinases from all known biological sources exhibit a time-dependent decrease in their specific activity upon continued exposure to the substrate. This loss of enzymatic activity is irreversible and its molecular basis has remained elusive. The authors proposed a mechanism based on the changes of the oxidation state of copper ion cofactor present at the enzyme active site. The formation of one Cu (0) and one Cu (2+) would lead to the loss of one of the two cofactor atoms. This would lead to the irreversible inactivation without the binding of quinonic intermediates to nucleophilic groups of the protein.

Session III: Translational melanocyte research
Contributed by M. Böhm, J. Lambert and M. Picardo

The plenary session III had 2 invited lectures and 3 selected oral presentations.

M. Böhm presented alpha-MSH as a pleiotropic peptide regulator that does not only regulate melanogenesis but also the function of many other cell types. In particular, small molecular weight peptides derived from the C-terminus of alpha-MSH are emerging as novel therapeutic agents for the treatment of inflammatory and fibrotic human disorders. A protective role of MC1R has been recently been demonstrated in a mouse model of experimental bowel inflammation. In fibroblastic cells, moreover, alpha-MSH upregulated the expression of enzymes involved in cytoprotection against oxidative stress (e. g. SOD2, HO-1) thereby decreasing intracellular accumulation of ROS after oxidant challenge. Results from this kind of translational research may point towards unrecognized effector mechanisms and pathways of alpha-MSH also operational in pigment cells. **G. Imokawa** reviewed the complex role of MITF as a regulator of not only tyrosinase but also of the cell cycle and apoptosis machinery. Exposure of human melanocytes to UVB, ET-1 and MGF increased MITF expression. This effect was associated with increased CDK2 and c-KIT expression. On the other side, siRNA-mediated MITF gene knock-down or treatment with HDAC inhibitors downregulated MITF expression and led to G1 arrest and apoptosis. In the first of the next 3 short presentations, **D.J. Tobin** presented data emphasizing the role of ROS, ROS signaling and expression/activity of catalase during canities. In scalp hair melanocytes catalase expression /activity correlated inversely with donor age. Exogenous H_2O_2 induced higher expression of catalase in young donor cells than in old donor cells and this effect of ROS correlated with p38 phosphorylation and cell survival. Next, **O. Sorg** reported on the impact of chemical modification on the depigmenting effect of RA and retinaldehyde. As demonstrated by cell viability assays and melanin measurement of B16 melanoma cells as well as uptake and metabolism studies on BDV-II keratinocytes oxidation and esterification of RA increases the depigmenting capacity while lacking melanocytotoxicity. Finally, **S. Commo** finally presented a study on hair follicle melanocytes established from a patient with the R151C variant of the *MC1R* gene. Melanocytes displaying this *MC1R* variant had an impaired alpha-MSH-mediated increase in intracellular cAMP and lacked alpha-MSH-induced MITF and tyrosinase expression as compared to wild-type *MC1R* expressing follicular cells. In situ studies, however, failed to detect any difference in the expression and spatial distribution of several melanogenic enzymes and factors including tyrosinase, pMel17, MITF and TRP-l.

Session IV: Extracutaneous pigmentation
Chairs: by U. Schraermeyer and R. Cicero

Session V: Developmental biology of pigment cells
Contributed by S. MacNeil and L. Larue

This lively and diverse session had 3 Invited Lectures (Beerman, Larue and Goding) and three selected oral presentations (Guida, Pshenichnaya, Sviderskaya)

Invited lecture 22: Friedrich **Beermann** from ISREC (Epalinges, Switzerland) presented the work of his group on the Notch signaling pathway for hair pigmentation, melanocyte and RPE-development. It is widely accepted that this pathway is involved in diverse biological processes such as cell fate decisions or stem cell maintenance. A murine genetic approach was undertaken using RBP-J, Notch1 and Notch2 conditional knockout mice. Disruption of the Notch pathway by inactivating RBP-J in the melanocyte lineage using Tyr::Cre mice led to a severe coat color dilution. Similarly, hair graying was observed when Notch1 and/or Notch2 receptors were

ablated in melanocytes. This phenotype was proportional to the number of floxed Notch alleles, with the most pronounced effect seen in mice lacking both Notch1 and Notch2 in melanocytes. Interestingly, the phenotype seems to be directly correlated with the number of melanocyte stem cells. Similar experiments are currently performed on the RPE cells. The importance of the Notch pathway in this lineage will therefore be better understood. RPE cells are also pigmented are not derived from neural crest cells but form neuroectoderm. From the group of Lionel **Larue** "Non-classical melanocytes in the heart are involved in closure of the ductis arteriosus" we heard (Invited lecture 23) how the function of melanocytes in one non-pigmentary sites in the body was investigated. The bottom line question for these non-classical melanocytes is do they have any real function or are they just there by some sort of failed piece of embryological tidying up ? Lionel's group looked at melanocytes in the heart. Its not clear where they come from or if they have a function but a few of them are found in an area, the ductus arteriosus, which normally develops into the ligamentum arteriosum. The ductus arteriosus is composed of neural crest derived smooth muscle cells and a small number of melanocytes. It should develop into the ligamentum arteriosum which then prevents blood bypassing the lung as is the case during foetal development. Using a developmental biological approach to tackle the function of these melanocytes transgenic mice were produced in which there was an activated oncogenic form of beta catenin in tyrosinase producing cells. This abnormal form of beta catenin meant that the melanocytes could not attach properly and the net result for the mice seemed to be that they died prematurely between 8 and 18 weeks from major heart failure. Prior to death the mutant mice had massively expanded left atriums and left ventricles with much higher numbers of melanocytes present and a concomitant failure of the ductus arteriosus to development into the ligamentum arteriosum so that blood continued to bypass the lung as it would have done during foetal development. There were also less smooth muscle cells. The working hypothesis put forward was that the decrease in the number of smooth muscle cells and the increase in melanocytes affects the contraction of smooth muscle cells to produce the ligament necessary to allow the heart to mature. Lionel asked whether a similar problem might occur in foetal development in man but this is going to be a hard one to tackle without relevant clinical samples which are going to be very difficult to come by. Nevertheless these studies showed that interference with the development of these melanocytes did have dire consequences in the heart for these mice suggesting that the melanocytes are far from being passive bystanders. So too many melanocytes with too few smooth muscle cells leads to a bad outcome for cardiac development. Colin **Goding** as invited lecture 24 (MCRI, Oxted, UK) presented the work of his group on Transcription Regulation in the Melanocyte Lineage. Particularly, he presented the the Microphthalmia-associated transcription factor Mitf operating as a 'rheostat' to coordinate cell cycle entry and exit, differentiation, melanocyte dendricity, as well as melanoblast survival and melanoblast/melanoma migration/invasiveness. The main proteins involved in these cellular mechanisms are now discovered and were nicely described. The role of Tbx2 and Tbx3, as targets of Mitf, in melanomas progression was evoked rapidly at the end of the presentation. The regulation of Mitf was placed in context as a regulator and regulated protein. A coherent and exciting view of genetics and epigenetics events is rising in the melanoma field involving Mitf and the pou domain protein Brn2. **Dr G Guida (**Oral presentation 25) "Characterisation of the skin melanogenetic system of rana esculenta." Frogs can have dramatic differences in skin colour between the dorsal skin and the belly and like mammals they have a tyrosinase enzyme which is the key enzyme for melanin biosynthesis. However they can also show seasonal changes in skin colour. In this study the authors extracted skin tyrosinase from Rana Esculenta and cloned the gene and found that it was well conserved compared with other amphibian tyrosinases but with high levels of homology with respect to mammalian tyrosinases. It appears to consist of two bands of 63 and 67 kilodaltons when analysed by gel electrophoresis but the extracts of the rana esculenta tyrosinase only seemed to become activated when exposure to proteases like nagarse . Interestingly extracts could be activated to different levels depending on seasonal fluctuations. The next mystery was that post activation there was an apparent difference

in the molecular weights found-the enzyme complex now ran at 200 kilodaltons post treatment with nagarse suggesting the formation of an aggregate of tyrosinase. The ongoing conclusion from this work is that this hard to activate tyrosinase may be part of a complex whose regulation *in vivo* is as yet poorly understood. Irina **Pshenichnaya** Oral presentation 26 (ISREC, Epalinges, Switzerland) presented her work on the role of c-Myc in the melanocyte lineage. C-myc is known for a very long time to be regularly associated with transformation as an oncogene in various tumours including melanomas of poor prognosis. Deregulation of c-Myc is known to be a common event in cellular transformation. For instance, upregulation of this oncoprotein was shown in a variety of primary and metastatic cutaneous melanomas and associated with a poor prognosis. The function of c-Myc during melanocyte development and homeostasis was addressed using a conditional knock-out allele of c-Myc targeted specifically in melanocytes. Removal of both c-Myc alleles in melanocytes leads to a hair greying phenotype that is associated with a decrease in melanocyte number in the bulb of hair follicles. Preliminary data show that proliferation capacity of c-Myc deficient melanocytes is not affected in newborn animals. Moreover, using Dct::LacZ transgenic mice, we were able to detect a presence of melanocyte stem cells in adult hair follicles. Since the phenotype is already present at birth, the major effect of c-Myc deletion on the melanocyte lineage takes place during embryogenesis. Oral presentation 27: "Characterisation of possible neuronal subsets produced by postnatal mouse neural crest-like stem cells". **Dr Elena Sviderskaya**. In this very interesting talk Elena showed that three immortal lines of a pluripotent cell type established from neonatal mouse skin resembled neural crest stem cells in their capacity to differentiate into several cell types normally derived from the neural crest. Thus they produced melanoblasts and melanocytes and Schwann precursor cells. In this study she went further to look at the production of sensory neurones from these cells by exposing them to growth differentiation inducing factors-specifically neurotrophic factor 3, nerve growth factor and fibroblast growth factor 2. In response to these neurotrophic factors there were increased numbers of neuronal like types of cells ,bipolar and stellate or star shaped cells with long cytoplasmic processes. Their characterisation as neural-like was undertaken by immunostaining for substance P and she was able to show intracellular calcium signalling in response to tetrodotoxin and patch clamping of the cells showed sodium currents and receptors excitable by capsaicin and cinnamaldehyde. Thus these cells lines look to be a source of cells that can be pushed into postnatal neurone regeneration and are certainly worthy of further investigation.

Session VI: Genetics of pigmentation
Contributed by F. Beermann and E. Healy

The Session VI had 2 invited speakers and 3 selected presentations, which were chosen from the submitted abstracts.

The session was opened by a lecture of **Nick Mundy** (Cambridge, UK), who reported on evolutionary aspects of pigmentation and coloration. His studies had revealed that, in birds, mutations at the melanocortin receptor 1 locus are mainly responsible for melanin-based color variation. In 3 unrelated bird species, the bananaquit, the arctic skua and the snow goose, mutations are found in the *MC1R* gene at positions previously reported in human or mouse, indicating a convergent molecular evolution that underlies melanism in birds. Another pigmentation gene, ASIP (agouti signaling protein) is also expressed in birds, and a 90kb genomic deletion similar to the one described in mice is the cause of the yellow mutation in Japanese quail. However, the wide variation in the genetic control mechanisms of pigmentation across the animal kingdom was further exemplified by the *Mc1r* gene sequence of the red-ruffed lemur. Strikingly, this animal contained a E94K mutation which was characterized by increased pheomelanin synthesis (indicating reduced signaling), even though an increased activity would have been expected. This apparent paradox could be explained by a changing affinity to agouti

(ASIP), thus allowing pheomelanin synthesis despite an active Mc1r. The second lecture was presented by the co-chair, **Eugene Healy** (Southampton, UK), in which he outlined the difficulties of treating a variety of skin disorders, including pigmentation disorders, with topical therapies. The major barrier to the passage of exogenous drugs and therapeutic agents into skin is the stratum corneum, which generally prevents the passage of molecules greater than 500 daltons into normal skin. He reported on a new cell penetable peptide, polypseudolysine (PPL), which is capable of transporting alpha-melanocyte stimulating hormone and a peptide nucleic acid targeted against the translational start site of the tyrosinase gene into a variety of cells *in vitro*. Using confocal microscopy and live cell imaging, PPL was able to significantly enhance uptake of both cargo molecules, and as a result of PPL-mediated cellular entry the anti-Tyr PNA could inhibit melanin synthesis. Moreover, PPL significantly augmented the delivery of these two cargo molecules into skin *ex vivo*. The first selected presentation was given by **Geneviève Aubin-Houzelstein** (Paris, France). She reported on results obtained by conditional inactivation of the Notch pathway in melanocytes in mice (*Tyr::Cre, RbpJk*$^{flox/flox}$) characterized by hair graying. Having carefully dissected hairs and analysing melanocyte distributions along the hair follicle (using *Dct::lacZ* as a marker), an overall decrease in the number of Xgal stained melanocytes was obtained during the first (P8) and the second (P30) hair cycles. Furthermore, she reported on evidence indicating the presence of differentiated melanocytes in the bulge area, which might be explained by a premature and ectopic differentiation of the stem cell pool. The second talk was by **J. Latreille** (Neuilly-sur-Seine, France), who presented the results of a study investigating the relationship between melanocortin 1 receptor genotype and skin colour in 488 French middle-aged Caucasian women. Pigmentation of the skin on the inner forearm was assessed using spectrophotometry. Although 15 *MC1R* variants were detected, the 9 variants with a frequency greater than 1% were analysed in relation to the spectrophotometric values. The Asp84Glu, Arg151Cys, Arg160Trp and Asp294His variants were noted to be associated with lighter pigmentation. However, women with the Asp84Glu, Arg151Cys and Asp294His variants exhibited higher reflectance values across the entire 400 – 700nm spectrum, whereas those with an Arg160Trp variant demonstrated a more focal elevation at the end of the visible spectrum, indicating that individual *MC1R* variants may differ in their effects on melanin type and/or content in human skin. The session was ended by a presentation of **Laurence Denat** (Orsay, France). She had investigated the function of Brn2 in the melanocyte lineage using a transgenic approach. Wild type Brn2 (TS) as well as a mutant Brn2 (AA, with mutations in the POU domain) were linked to the tyrosinase regulatory sequences and used to generate *Tyr::Brn2AA* and *Tyr::Brn2TS* transgenic mouse strains respectively. In both situations, small changes in pigmentation were observed. The mutant Brn2 induced occurrence of a white belly spot and a reduced number of melanoblasts during embryogenesis, whereas the wildtype Brn2 showed some hyperpigmentation linked to an increase in melanoblast number. On a molecular level, both forms of Brn2 seem to repress Mitf-M. However, although the wild type form TS could activate Pax3 the mutant AA form could not, perhaps explaining the failure to increase pigmentation and proliferation.

Session VII: Pigmentary disorders: Animal models
Contributed by L. Montoliu, and M.V. Schiaffino

Session VII was conceived to discuss several aspects of melanocyte function, from development to cell biology to physiology, by means of animal models for different pigmentary disorders, including albinism, vitiligo and melanoma. The session had two invited speakers and 4 short presentations and was opened by **Dr. L. Montoliu (Madrid, Spain),** who discussed studies on the role of melanin and L-DOPA not only in vision, but also in hearing. In fact, mouse models of oculocutaneous albinism type 1 (OCA 1) were found to display a significant auditory defect, particularly at high frequencies, suggesting that, despite not generally observed, some degree of hearing impairment might actually represent a feature of human albinism as well. The results

arose several comments from the audience, ranging from statements that hearing problems were never reported in OCA, to opposite observations that evidence of hearing deficit exists non only in albinos, but even in individuals with fair skin. As take-on message, while clearly there is not a severe hearing impairment in human albinism, it is possible that some degree of it exists, which is not easily detected unless precise diagnostic analyses are performed. In light of this, the previously reported ocular albinism with late-onset sensorineural deafness (OASD; MIM 300650), associated to a deletion of the ocular albinism type 1 (OA1) gene, might represent a variant of OA1, in which high-tone sensorineural deafness in adulthood emerged since it was carefully searched. The second talk by **Dr G. Raposo (Paris, France)** brought the audience deeply into the cell biology of melanocytes. By an ultrastructural point of view, she illustrated recent studies on the biogenesis of melanosomes, which unravel surprising structural and functional modifications of the endosomal system in pigment cells, by taking advantage of the diverse phenotypes of mouse models of the Hermansky-Pudlak syndrome (HPS). Fascinating 3D electron-tomography analyses of early melanosomes revealed how intraluminal fibers are generated and an unexpected contiguity between endosomal tubules and melanosomal precursors, suggesting the presence of a direct exchange of material between the two compartments. The subsequent presentation was from **Dr. Delmas D. (Orsay, France),** who discussed the role of E-cadherin in epidermal melanocyte development. In melanoblasts, E-cadherin is first expressed just before their entry into the forming epidermis and is thereafter maintained during development and after birth. The importance of this cell-cell adhesion molecule in the epidermis was underlined by the phenotype of a mouse model in which E-cadherin is specifically deleted in the melanocyte lineage. Indeed, mutant mice displayed white bellies and paws, and heterogeneous hypo-pigmentation of the tails, due to reduction in the number and to the irregular distribution of melanocytes in the epidermis. **Dr. Y. Gauthier Y. (Bordeaux, France)** proposed an animal model to study the migration of melanocytes, a process that is essential to understand and promote repigmentation of vitiligo. The authors took advantage of black and white guinea pigs, which were (or were not) subjected to various treatments (phototherapy, topical treatments), known to stimulate marginal repigmentation in vitiligo patients. Marginal repigmentation was observed by means of tattooed lines, drawn in the achromic areas prior to treatments and parallel to the pigmented borders. The significant response to the treatment observed in the majority of the animals suggests that this animal model may provide a useful tool to investigate treatment modalities for vitiligo. **Dr. F. Rambow (Jouy en Josas, France)** presented an interesting animal model of spontaneous melanoma regression. The MeLiM swine model exhibits cutaneous melanomas which appear around birth, are clinically and histologically comparable to their human counterparts, yet mostly undergo complete spontaneous regression. Induction of regression is expressed by clinical features suggestive of an immune response against the tumor. By a transcriptomic analysis of the phase of regression, the authors were able to identify 1411 genes regulated during regression, mostly involved in cell cycle, apoptosis, migration, melanocyte-differentiation, and immune response. Finally, **Dr. G. Ranieri (Bari, Italy)** presented studies on the role of angiogenesis in tumour progression by means of the canine cutaneous mast cell tumour (CCMCT) model. The authors' pilot data suggest that angiogenesis and metastatic potential are associated with high malignancy grade of CCMCT and that poorly differentiated mast cells actively participate in this process through the release of VEGF, contained in their secretory granules.

Session VIII: Melanoma Biology
Contributed by S. Pavel and D.C. Bennett

This wide-ranging session contained two invited and five selected talks.

Dot Bennett (London) opened the session by discussing different pathways of cell senescence. Senescence is permanent cell growth-arrest following extensive proliferation or oncogene

activation. It is an important tumour-suppressor mechanism and the only established function of both p16 and ARF, products of familial melanoma gene *CDKN2A*. She discussed two collaborative projects on melanocyte senescence. In one, with Glenn Merlino et al. (NIH), evidence was found in a transgenic mouse melanoma model that Arf (mouse ARF), normally thought to act mainly via p53, is a stronger tumour suppressor than p53, and also contributes more significantly to melanocyte senescence than p53. In mouse melanocytes, Arf could promote proteasome-dependent degradation of E2F1, a transcription factor required to initiate the cell cycle. This could potentially mediate growth arrest and senescence. She also reported work with Veronique Bataille et al. (London), correlating mean, age-corrected leukocyte telomere length in a human population with numbers of benign naevi, postulating that the effect was actually on naevus size. In short, genetically-determined variation in telomere length is a factor in naevus numbers (or size), strongly suggesting that telomere shortening contributes to the senescence seen in naevi. The second presentation, by **Sheila Mac Neil** (Sheffield, UK), was about the influence of ibuprofen, a non-steroid anti-inflammatory drug (NSAID), on migration of melanoma cells. The research was inspired by observations that NSAIDs may reduce the risk of metastasis in some cancer patients. A second objective was to develop an ibuprofen-releasing hydrogel for topical application. The authors utilized three melanoma cell lines, in a scratch assay: an easy, low-cost and well-developed method to measure cell migration *in vitro*. The basic steps involve "scratching" a cell monolayer, capturing images at regular intervals of cells migrating to close the scratch, and comparing the images to quantify the cell migration rate. Migration of the melanoma cells could be accelerated by TNF-α. While the migration of non-stimulated cells was not detectably affected by ibuprofen, the authors found a significant inhibition of TNF-α-stimulated migration. They further examined the usefulness of ibuprofen sodium salt loaded into Pluronics F127 hydrogel to give slow release. Cell migration was somewhat reduced with the unloaded hydrogel; however the effectiveness of hydrogel loaded with 0.15 and 0.20% ibuprofen was significantly better. The authors concluded that the migration of melanoma cells can be up-regulated by the inflammatory mediator TNF-α, and that this stimulation can be inhibited by ibuprofen either in solution or when slowly delivered from a Plutronics F127 hydrogel. **Marcus Böhm** (Münster) talked about PACE4 (Paired basic amino-acid-cleaving enzyme 4), a protease able to process POMC to its derivative hormones and also to process some growth-factors, receptors and integrins. The authors found PACE4 to be commonly overexpressed in melanoma lines compared to melanocytes, and to be detectable in most melanomas by immunostaining. To test for a causal role in melanoma biology, exogenous PACE4 was overexpressed in a melanoma cell line with a low basal level. Upregulation of matrix metalloproteases 1 and 2 was seen (proteases associated with cell migration and invasion), together with increased cell invasiveness in culture. **Julia Soo** (London) described investigations of cell senescence in explant cultures from a range of primary human melanocytic lesions. Culture conditions involved keratinocyte feeder cells and several growth factors. Benign naevi are reported to be composed of senescent cells according to several markers; however when put into culture, only a few were static (failed to divide detectably); most benign and dysplastic naevi showed some proliferation before permanently stopping dividing. None were immortal. Most RGP and VGP melanomas proliferated for a period and then, surprisingly, also showed permanent arrest; very few RGP and only a third of VGP cultures were immortal. Naevus and melanoma cultures were stained for three markers of cell senescence; 100% of the arrested cultures were positive for all three, while growing cultures were negative or mostly negative. One conclusion, contrary to expectation, is that immortalization is a late event in melanoma progression and early melanomas are often not immortal. **Jan Borovansky** (Prague) reported research on whether there are differences in antioxidant defence between melanoma cells and others. The authors compared human and mouse melanoma cell lines with osteosarcoma, lung carcinoma and glioma cells. Total antioxidant status in cells and media was assayed by the Total Antioxidant Status Kit. Activities of several enzymes involved in antioxidant defence were also determined: catalase, glutathione peroxidase and superoxide

dismutase. Also the activities of two other enzymes (tyrosinase and γ-glutamyltransferase) which may affect redox balance were assessed, and melanin concentration was determined. Melanoma cells were found to have much higher total antioxidant status than other tumour cells (normal melanocytes were not tested). Most striking among the specific enzymes was higher catalase activity in the melanoma than the non-melanoma lines. There did not seem to be a connection with pigmentation. Two alternative interpretations of the high antioxidant status in melanoma cells are possible: (1) it may be primary and prevent oxidative imbalance, and (2) it may be a compensatory response to increased oxidative stress. The consensus among scientists in a subsequent discussion seemed to favour the second alternative, though further testing would clarify this. The last talk was by **Patrick Verrando** (Marseille, France). His group studied the ability of certain highly-invasive tumor cells to form capillary-like structures and matrix-rich patterned networks in three-dimensional culture, which mimic an embryonic vasculogenic network. This behaviour is called "vasculogenic mimicry" (VM) to stress the absence of endothelial cells or real angiogenesis. It appears uncommon, but possibly associated with worse prognosis. Molecular events that regulate VM may provide novel targets for therapy; hence the authors examined signalling pathways involved in VM by melanoma cells. It appeared that cAMP agonists inhibited the process, which recovered after removal of the agonists. There was some evidence that the EPAC (exchange protein activated by cAMP) pathway may play a role. EPACs are guanine-nucleotide exchange factors involved in a number of cellular processes such as the regulation of cell adhesion. Even in the presence of a PKA activator, an EPAC/GEM protein activator tended to inhibit VM. The results shed some light on signalling factors involved in VM.

Session IX: Melanoma Genetics
Contributed by P. Grammatico, C. Wellbrock

The plenary session IX had 3 invited lectures and three selected presentations, which were chosen from the submitted abstracts.

Paula **Ghiorzo** presented his work on Impact of CDKN2A founder mutations on Italian Families. **Fragnoli** presented his work on the Low-penetrance genes in melanoma susceptibility. Claudia Wellbrock showed that in human melanoma cells that are positive for a BRAF mutation, MITF expression is strictly dependent on BRAF. She demonstrated that V600E-BRAF is responsible for MITF expression and directly activates *MITF* transcription through BRN2 (N-Oct3). MITF regulates cell cycle progression downstream of BRAF and its transcriptional regulation by BRAF is required to ensure melanoma cell proliferation and survival. Surprisingly, although BRN2 expression is regulated through ERK, MEK inhibition did not affect MITF expression. This apparently contradictory finding could be explained by the fact that inhibition of ERK leads to an up-regulation of PAX3, which is also an activator of MITF expression, and compensates for the reduced function of BRN2. She suggested a model, in which a buffered regulation of the *MITF* promoter ensures that MITF protein levels are optimal for correct expression of cell cycle components such as CDK4 and CDK2 and survival factors such as BCL2, thereby favouring proliferation and survival over differentiation or apoptosis. E **Borneuf** presented his work on Genome-wide QTL discovery and candidate gene analysis in a swine model of cutaneous melanoma. Keith **Hoek** talked about the amplification of the *MITF* gene and its potential function as a 'lineage-addiction oncogene'. He confirmed an amplification frequency of the locus of approximately 17%, but also described samples that showed MITF amplification, which is restricted to MITF non-coding regions. He identified 3 different melanoma cohorts in DNA microarray analyses, which differed in their MITF expression pattern and also their metastatic potential. Importantly, MITF is not expressed in every cohort, thus questioning the 'addictive' requirement of MITF expression in melanoma. Cohort A expressed MITF, showed a neural crest phenotype, was susceptible to TGFbeta, highly

proliferative and had low motility. Cohort C had an invasive phenotype, was resistant to TGFbeta and did not express MITF (or possessed very low levels of MITF expression). This cohort was not very proliferative, and showed high motility. He suggested a 'phontype-switch' model, in which during melanoma progression cells can switch between the two different phenotypes in response to signals from the extracellular environment to eventually produce metastates. Laurent **Beuret** presented data on the HGF receptor MET and apoptosis protection. He showed that in melanoma cells and melanocytes MET expression is induced by alphaMSH through MITF. MITF binds to an E-box motif conserved in both the human and the mouse promoter and activates transcription from these promoters. Up-regulation of MET expression by cAMP results in increased responsiveness to HGF in melanocytes and melanoma cells and protects them from TRAIL or staurosporine induced apoptosis.

Session X: Photoprotection
Contributed by V.J. Hearing and G. Monfrecola

This session on Photoprotection began with 3 Invited Lectures, which were followed by 2 Oral Presentations selected from the abstracts submitted to the meeting.

Tad Sarna began the session by addressing the effects of photoaging on the photoprotective and antioxidant properties of melanosomes in the retinal pigment epithelium (RPE). He reviewed the physical properties of melanins, which exhibit several unique features including a distinct particle nature, strong optical absorption, very weak fluorescence and prominent electron, ion exchange and free radical properties. In human tissues, melanins could undergo photochemically-induced changes in their properties and biological functions, and the risk of such adverse changes could be most dramatic in the case of RPE melanosomes which have very slow rates of metabolic turnover. Their group studied the effects of photobleaching on various properties of melanosomes isolated from porcine and bovine RPE. Effects induced by intense visible light were monitored by ESR, UV-VIS absorption spectroscopy, and electron and atomic force microscopies. Photobleached melanosomes exhibited modified morphologies, reduced content of free radicals and decreased absorbance in the visible region. Compared to untreated melanosomes, photobleached melanosomes had decreased antioxidant efficiency and reduced metal-ion-binding capacity. Photobleaching stimulated the phototoxic potential of RPE melanosomes and their ability to photogenerate superoxide anions. Their study suggests that substantial photobleaching of RPE melanosomes, probably aggravated by aging, may significantly compromise their normal biological functions. **Vincent Hearing** presented an update on the studies of his collaborative group to determine the role of skin pigmentation in photoprotection against UV damage. They had previously shown that substantial differences in DNA damage caused by a single UV exposure occurred in skin with different levels of constitutive pigmentation. Their new clinical protocol assessed whether facultative pigmentation induced by repeated UV exposures is photoprotective in human skin. They examined 21 volunteers with type II – III skin, and irradiated 3 sites on the backs of each healthy subject at 100-600 J/m^2 every 2-7 days over a 4-5 week period; the 3 sites received different cumulative doses of UV (1900 J/m^2, 2900 J/m^2 or 4200 J/m^2) and were biopsied 1 day after the last exposure. They measured various biomarkers including pigment content assessed by Fontana-Masson staining, melanocyte function by expression of melanocyte-specific markers, DNA damage as cyclobutane pyrimidine dimers (CPD), nuclear accumulation of p53, apoptosis determined by TUNEL assay, and levels of p21 and Ser46-phosphorylated p53. Increases in melanocyte function and density, and in levels of apoptosis were similar among the 3 study sites exposed to different cumulative UV doses. Levels of CPD decreased while the number of p53-positive cells increased as the cumulative dose of UV increased. Those results suggest that pigmentation induced in skin by repeated UV exposures does protect against subsequent UV-induced DNA damage but not as effectively as constitutive pigmentation. **Alessandra**

Napolitano discussed her group's studies aimed at evaluating the contribution of the putative primary structural units to the chromophore of natural and synthetic pheomelanins. This was addressed by monitoring the pigment formation process using UV-Vis spectrophotometry and liquid chromatography/mass spectrometric analysis. On this basis, the chromophoric features of the intermediates detected were evaluated against the whole pigment chromophore. The major conclusions drawn were: I) 1,4-benzothiazines are indeed the early intermediates of pheomelanogenesis, but in contrast to the commonly held view, they may undergo substantial modifications prior to or after incorporation into the pigment backbone; II) benzothiazoles and 3-oxodihydrobenzothiazines are generated from benzothiazines during biosynthesis and moreover they are representative structural units in the final pigment; and III) factors such as zinc ions may affect the course of pigment formation favoring the 3-carboxylated benzothiazines from which trichochrome-related species are generated. Finally, she highlighted the potential of benzothiazine and derivative species as responsible for the enhanced photosensitivity of individuals with pheomelanic pigmentation. **Magdalena Gauden** reported on a collaborative study between the Chemical Physics Department of Lund University and the Organic Group of Naples Federico II University which focused on the femtosecond transient absorption spectroscopy of 5,6-dihydroxyindole (DHI), 2-carboxylic acid (DHICA) and related oligomers. The goal of the project was to gain a preliminary insight into the initial photophysical and photochemical changes accompanying the irradiation of eumelanin precursors. The main spectral changes within the very short time scale of the experiments were tentatively interpreted in terms of electron loss from a singlet excited state leading to cation species that would then evolve through different molecule-specific reaction channels. Further pursuit of these studies should provide new insights into the photochemical behavior of eumelanin precursors and their role in photoprotection. Finally, **Agatha Kurkot** reported on the in vitro and in situ expression and regulation of the transcription factors Nrf1 and Nrf2 in human skin cells. Nrf1 and Nrf2 regulate the expression of several phase II detoxifying enzymes, including HO-1, NQO1, NQO2 and gamma-GCS. Nrf1 and Nrf2 were found to be ubiquitously expressed in various skin cell types but were most abundant in melanocytes. UVB exposure led to a dramatic decline in the in vitro and in situ expression of Nrf1 and Nrf2 while αMSH was capable of counteracting this effect, including Nrf1/Nrf2-dependent HO-1 expression.

Session XI: Pigment cells and their environment
Contributed by Z. Abdel-Malek, M. Seabra

Session XII: Pigmentary disorders: Vitiligo
Contributed by A.Taïeb and M. Picardo

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Pr. A. Napolitano)

The fluorescence properties of natural and synthetic melanin pigments is continuing to attract interest from the theoretical view point as well as for application purposes. The dynamics of the fluorescence decay of synthetic eumelanin and related polymers were investigated (Nighswander-Rempel et al *Photochem Photobiol*.) by time resolved techniques providing evidence for a multiexponential behavior with decay times between 0.5 and 15 ns, whereas steady-state spectra for the polymer exhibited only two peaks. These features were interpreted as due to the presence of different oligomers within eumelanin.
The structural models for eumelanin protomolecules, based on tetramers consisting of four monomer units at different oxidation states in arrangements that contain an interior porphyrin ring were further investigated by Kaxiras and coworkers (*Biophys J*) by computational methodologies; a possible pathway of formation of the tetramers is proposed starting from the 2,7' dimer of 5,6-dihydroxyindole (DHI) which was found as the most stable of DHI dimers; also its dimerisation route to form the cyclic tetramer was hypothesized from considerations of the relative strength of the hydrogen bonds forming between the two facing dimers. Curiously enough in this same period, another paper (Pezzella et al, *J.Org. Chem*) dealing with the oxidative dimerization of DHI 2,7'-dimer by the peroxidase/ H_2O_2 system has appeared. Three novel tetramers were characterized in which the inner units are linked through unexpected 3,3'-, 4,4'-, and 2,3'-linkages; yet, no evidence for a cyclic tetramer or related species emerged from the experimental approach.
A plethora of reports on the mechanisms of inhibition of melanogenesis has appeared including products from plants such as galangin (Lu *et al.*), piceatannol (Yokozawa and Kim), kurarinol (Ryu *et al*) isopanduratin (Yoon *et al*) prenyl-substituted polyphenols from *Artocarpus heterophyllus* (Arung *et al*), from herbal extracts (oolong tea, *Angelica gigans*), from fungi (*Sparassis crispa* mushroom, Kawagishi *et a*l) as well as synthetic or semisynthetic leads (like tyrosyl gallate derivatives, Lee *et al,* feruloyltyramine, Efdi *et al*). This is a sign of the interest that this topic which is central for the treatment of pigmentary disorders is currently attracting.

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2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

A brief overview of the last works on melanocyte biology and pigmentary disorders, focusing on vitiligo and melanoma.

The paper of **Valtink and Engelmann** could be autoritatively placed between the basic cell biology and the medicine strategies. The main problem with the grafting approach is due to the use of potentially dangerous agents (serum, toxins, cancer-inducing factors) during the colture phase of the melanocytes. The authors propose here a colture methods able to allow the growth of the melanocytes without affecting both morphology and proliferative rate. The paper is relevant, useful, and clear both for bench activitiy and applied biology.

Jacobs by using an in vitro model and molecular approach provides new and relevant insights in the UV-mediated dendrites formation. The number of dendrites is crucial for the melanosome transfer and then for the most evident UV effect. Two key intermediates are evaluated: Rho-GTP-EP3 receptor (member of PGE2 receptors family) and PKCzeta kinase. The study indicates that PGE2, released by UV-stimulated skin, induces whithin few minutes (5 min) dendriticity through the activation of PKCzeta but not throught the activation of Rho-GTP. This is consistent with the occurrence of the EP3A1 expression by melanocytes whereas EP3B is associated with Rho-GTP. The work of Kacobs team takes also part to the understanding of the role of lipid metabolism in the pigmentation process.

It is already demonstrated that the direct cell-cell contact between melanocytes and keratinocytes depends on the expression of the calcium-dependent epithelium-specific cell adhesion molecule, E-cadherin. The paper presented by **Preeti et al.** suggest a more sophisticated role for calcium in the keratinocytes-melanocytes interection. The study presented focus on the role of calcium (Ca^{2+}) in keratinocytes-melanocytes recognition and melanin transfer. It is of interest that the physical keratinocytes-melanocytes interaction induces a release of Ca^{2+} from intracellular store in keratinocytes independently from the calcium concentration in extracellular medium. The mechanism proposed is a "receptor-ligand-like" interaction due to the demonstration that plasma membrane isolated from melanoma cells also induce the rise of Ca^{2+} but only in presence of membrane proteins in an active form.

A very interesting study from **Roberts et al**., proposed a model of human melanocyte-keratinocytes coculture system free from mitogenic factors to performe morphological studies of melanocytes dendriticity. By using this innovative approach they demonstrated an impaired increase of dendriticy following the activation of MC1R receptor by NDP-MSH in melanocytes expressing MC1R red hair color variants. The reduced capacity to change morphology was associated with a reduced induction of DCT in melanocytes expressing MC1R red hair color variants.

The paper from **Cardinali et al**., compared melanosome transfer promoted by KGF in light and dark keratinocytes. This study demonstrated an higher level of KGFR in light skin-derived keratinocytes that correlateted with an higher responsivity of light keratinocytes to KGF-induced up-take of fluorescente latex beads in these cells. Since in absence of KGF stimulation dark keratinocytes have an higher phagocytic activity authors concluded that light keratinocytes needs to activate alternative pathways in addition to the PAR-2-activated pathway to optimize the process of melanosome transfer.

Cotter and coworker reported a novel use of NAC for the prevention of UV-induced oxidative stress and damage in melanocytes. The data obtained in *in vitro* culture system demonstrated protection against UV-induced production of intracellular peroxide, formation of 8-oxoguanine and decrease of glutathione level. However, the protective effect of NAC *in vivo* was not completly confirmed. In fact, even if NAC administration delayed tumor onset, the incidente of malignant tumor was not statistically different in NAC-treated mice.

The immune involvement in the pathogenesis of the vitiligo is once time considered. The review of the **Kemp** group is a classical overview of the old and current evidences testifiyng for the immune-mediated damage. Another analysis of the immune aspects and apoptosis yes/not in vitiligo: the work of **Sanchez-Sosa** group. Flow cytometric of the phenotype of the circulating white cells and western blotting evaluation of the melanocyte-specific antibodies were carried out toghether with the analysis of apoptotic marker in melanocytes. The study becomes part of the debat on the apoptosis role in vitiligo melanocyte loss. The authors report an increased expression of apoptotic markers in residual melanocytes and a high level of intracellular vitiligo IgG. The pathogenetic role of the inflitrating IgG may be relevant even if, as already suggested by the authors, a dose-response penetration should be performed. The actual role of apoptosis is yet controversial and the different experimental approaches (cultured primary melanocytes, biopsy sections,…) take part to this confusion. Finally, at least for the vitiligo sub-section, the paper of **Cario-André** and coworkers. According to the investigative activity of the last years, the Bordeaux's team provides further evidence for the relevance of the melanocytorrhagy in vitiligo pathogenesis. Forthemore, the authors set-up an in vitro tridimensional model for the study of the potential inducers of melanocytes detachement.

The opposite site of pigmentary disorder is provided by the hyperpigmentation. Most of the current depigmenting agents act on melanin synthesis pathway. A new entry in this world is provided by the chalcone compounds isolated from Kaempferia Pandurata, as reported by **Hwang**. Dried rhizomes of Kaempferia Pandurata were frequently used in tropical area, including Indonesia and Thailand, for the treatment of several different diseases but not for hyperpigmentation disorders. On the basis of these preliminary data it is possible that Isopanduratin A inhibits tyrosinase activity and reduces the amount of the protein. This effect is probably due to the resorcinol-like structure.

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3. MSH, MCH, other hormones, differentiation

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4. Photobiology

(Dr. N. Smit)

In News and Views in the June issue of PCR Gaitonde and Ronai discussed the paper of Cui et al.in Cell. The role of p53 in the tanning response via induction of POMC/MSH in keratinocytes after UV irradiation provides an interesting new mechanism of how the skin protects itself against UV induced damage. As indicated by Gaitonde and Ronai the role of MSH in DNA repair may be a more direct mechanism of the protection provided by the upregulation of p53 protein in keratinocytes after UV radiation. The paper by Arad et al describes that both UV and thymine dinucleotides (pTT) increase melanin content in epidermal explant cultures. This may be suggestive of a role for DNA repair products (resulting from MSH binding to MC1R) being responsible for the tanning response.
Some other interesting discussion points about the role of p53 in the tanning response are raised by Barsh and Attardi in the NEJM.Considering the tanning response to be protective, the interest in artificial tanning may increase using drugs such as Melanotan-1 that act via activation of MC1R as described before in papers by Dorr et al (2000 and 2004). Also in the comments of Oren and Bartek in Cell another alternative is mentioned; the use of small molecules that stimulate p53 function.
In relation to the role of MSH and the MC1Receptor in DNA repair the paper by Garcin et al is of interest. Using HPLC coupled to tandem MS these authors could not confirm the effects of MSH on DNA repair of UV-B induced DNA photoproducts in a model of HaCat cells stably transfected with wild-type MC1R. It is rather surprising that the detection of the actual DNA damage products (e.g. CPDs and 6-4PPs) did not show the results that have been found earlier with anti CPD antibodies. More studies may be required using alternative or more physiological models (e.g. melanocytes with different MC1R variants) to demonstrate the role of MSH in DNA repair and identification of the DNA photoproducts by HPLC-tandem MS or other methods.
Different papers discus UVB and UVA as risk-factors for melanoma. Isaacson and Ramsay describe melanoma's that are present on the sole of the foot in black people where sun obviously does not play a role. The question is raised whether the (phaeo)melanin production in this localization could be relatively high and responsible for an increased risk of melanoma. On the other hand phaeomelanin production in tyrosinase-positive albinos does not seem to increase melanoma risk whereas occurrence of NMSC is very high among South African albinos. Grant considers UVA most important for melanoma development. In that case ozone depletion would not be responsible for the increase in melanoma risk. In the paper by Hocker et al mutational data from melanoma literature was analyzed. Non UVB changes accounted for most of the BRAF and NRAS variants. Evidence for participation of UVB was seen especially for the mutations in CDKN2A and TP53. In the paper by Benjamin et al the importance of BRAF, NRAS and INK4A/ARF mutations in melanoma models are also described. So far melanomas are induced in the animal models by UVB irradiation or treatment with DMBA as the carcinogen. In the paper by N.E. Thomas et al an association of BRAF mutations with early life UV exposure (0 to 20 years) was found and NRAS mutations appeared more in cases with high exposure at later age (50-60 years). A role for UVA in the induction of HGF by fibroblasts is described by Mildner et al. They conclude that a negative side effect of UV induced HGF production could be a decisive step in melanoma development.

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5. Neuromelanins

(Pr. M. d'Ischia)

Literature on Parkinson's disease research is ever expanding and new information is being gained on the etiopathogenesis of the disease. The present commentary will be focused only on those papers with a strict relevance to the subject of neuromelanin and pigmented neurones.
An interesting study by Beach et al. (2007) revealed a pronounced microglial reaction in normal aging substantia nigra correlating with the presence of extraneuronal neuromelanin deposits, which were abundant in older subjects but absent or rare in the younger ones. This observation suggests a pathologic process with age that may concur with specific neurodegenerative diseases, including Parkinson's disease.
Two papers provide further insights into the origin and biogenesis of neuromelanin by confirming the lack of expressed tyrosinase in human substantia nigra and locus coeruleus (Tribl et al., 2007) and highlighting the role of associated lipids in the process of pigment aggregation determining neuromelanin granule size (Dedov et al., 2007).
A detailed insight into the oxidation chemistry of norepinephrine as a model systenm to inquire into the origin of neuromelanin in noradrenergic neurons like those of the locus coeruleus is provided by Manini et al. (2007). Paris et al. (2007) propose the use of the aminochrome from dopamine as a model neurotoxin to study the neurodegenerative processes occurring in neuromelanin-containing dopaminergic neurons.
Finally, a paper by Hu et al. (2007) offers a complete list of human organelle reference datasets from large-scale proteomic studies and protein databases for seven lysosome-related organelles as well as the endoplasmic reticulum and mitochondria, for comparative organelle proteome analysis of relevance to neurodegenerative disorders, whereas an interesting study by Hong and Simon (2007) addresses the binding mechanisms of melanins toward metal ions, a crucial issue in relation to the possible etiopathogenetic role of neuromelanin in parkinson's disease.

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Abstract: Metal chelation is often invoked as one of the main biological functions of melanin. In order to understand the interaction between metals and melanin, extensive studies have been carried out to determine the nature of the metal binding sites, binding capacity, and affinity. These data are central to efforts aimed at elucidating the role metal binding plays in determining the physical, structural, biological, and photochemical properties of melanin. This article examines the current state of understanding of this field.

- Hu Zhang-Zhi, Valencia J.C., Huang H., Chi A., Shabanowitz J., Hearing V.J., Appella E., Wu C.
Comparative bioinformatics analyses and profiling of lysosome-related organelle proteomes. International Journal of Mass Spectrometry 259(1-3):147-160, 2007.
Abstract: Complete and accurate profiling of cellular organelle proteomes, while challenging, is important for the understanding of detailed cellular processes at the organelle level. Mass spectrometry technologies coupled with bioinformatics anal. provide an effective approach for protein identification and functional interpretation of organelle proteomes. In this study, the authors have compiled human organelle ref. datasets from large-scale proteomic studies and protein databases for seven lysosome-related organelles (LROs), as well as the endoplasmic reticulum and mitochondria, for comparative organelle proteome anal. Heterogeneous sources of human organelle proteins and rodent homologs are mapped to human UniProtKB protein entries based on ID and/or peptide mappings, followed by functional annotation and categorization using the iProXpress proteomic expression anal. system. Cataloging organelle proteomes allows close examn. of both shared and unique proteins among various LROs and reveals their functional relevance. The proteomic comparisons show that LROs are a closely related family of organelles. The shared proteins indicate the dynamic and hybrid nature of LROs, while the unique transmembrane proteins may represent addnl. candidate marker proteins for LROs. This comparative anal., therefore, provides a basis for hypothesis formulation and exptl. validation of organelle proteins and their functional roles.

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Oxidation Chemistry of Norepinephrine: Partitioning of the O-Quinone between Competing Cyclization and Chain Breakdown Pathways and Their Roles in Melanin Formation. Chemical Research in Toxicology 20(10):1549-1555, 2007.
Abstract: Aberrant oxidn. of norepinephrine via the transient o-quinone has been implicated as a crit. pathogenetic mechanism underlying the degeneration of noradrenergic cell bodies in the locus coeruleus in Parkinson's disease, the degeneration of noradrenergic nerve terminals in Alzheimer's disease and following transient cerebral ischemia, and the onset and progression of idiopathic vitiligo. An oxidative pathway of norepinephrine is also believed to account for the slow deposition of neuromelanin in pigmented neurons of the locus coeruleus. Remarkably, after extensive investigations spanning over several decades, there is still a lack of knowledge of the oxidn. chem. of norepinephrine beyond the classic cyclization route leading to aminochrome and lutin intermediates. The authors report herein that oxidn. of norepinephrine in the 50-500 □M concn. range with H2O2-dependent oxidizing agents, such as the Fenton reagent (Fe2+-EDTA/H2O2) and the horseradish peroxidase (HRP)/H2O2 system, leads not only to the known cyclization products, such as noradrenochrome and 5,6-dihydroxyindole, but also to a significant proportion of chain breakdown products, including 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, 3,4-dihydroxymandelic acid, and 3,4-dihydroxyphenylglyoxylic acid, which has never been described among the oxidn. products or metabolites of norepinephrine. Anal. of the brown melanin-like pigment obtained by oxidn. of norepinephrine with HRP/H2O2 gave pyrrole-2,3-dicarboxylic acid and pyrrole-2,3,5-tricarboxylic acid, diagnostic markers of 3-derived units in eumelanins. Comparison with ref. pigments prepd. by similar oxidn. of dopamine and 5,6-dihydroxyindole indicated that in the case of norepinephrine oxidative polymn. of indole units through the 2-position contributes only to a minor extent to melanin formation.
Overall, the results of this study provide a complete characterization of the oxidative chain fission pathways of norepinephrine, highlight 3,4-dihydroxyphenylglyoxylic acid as a novel possible metabolic product of this catecholamine, and yield an insight into norepinephrine-melanin, a putative component of locus coeruleus neuromelanin.

- Paris I., Cardenas S., Lozano J., Perez-Pastene C., Graumann R., Riveros A., Caviedes P., Segura-Aguilar J.
Aminochrome as a preclinical experimental model to study degeneration of dopaminergic neurons in Parkinson's disease. Neurotoxicity Research 12(2):125-34, 2007.
Abstract: Four decades after L-dopa introduction to PD therapy, the cause of Parkinson's disease (PD) remains unknown despite the intensive research and the discovery of a number of gene mutations and deletions in the pathogenesis of familial PD. Different model neurotoxins have been used as preclinical experimental models to study the neurodegenerative process in PD, such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and rotenone. The lack of success in identifying the molecular mechanism for the degenerative process in PD opens the question whether the current preclinical experimental models are suitable to understand the degeneration of neuromelanin-containing dopaminergic neurons in PD. We propose aminochrome as a model neurotoxin to study the neurodegenerative processes occurring in neuromelanin-containing dopaminergic neurons in PD. Aminochrome is an endogenous compound formed during dopamine

oxidation and it is the precursor of neuromelanin, a substance whose formation is a normal process in mesencephalic dopaminergic neurons. However, aminochrome itself can induce neurotoxicity under certain aberrant conditions such as (i) one-electron reduction of aminochrome catalyzed by flavoenzymes to leukoaminochrome o-semiquinone radical, which is a highly reactive neurotoxin; or (ii) the formation of aminochrome adducts with alpha-synuclein, enhancing and stabilizing the formation of neurotoxic protofibrils. These two neurotoxic pathways of aminochrome are prevented by DT-diaphorase, an enzyme that effectively reduces aminochrome with two-electrons preventing both aminochrome one-electron reduction or formation alpha synuclein protofibrils. We propose to use aminochrome as a preclinical experimental model to study the neurodegenerative process of neuromelanin containing dopaminergic neurons in PD.

- Tribl F., Arzberger T., Riederer P., Gerlach M.
Tyrosinase is not detected in human catecholaminergic neurons by immunohistochemistry and Western blot analysis. Journal of neural transmission. Supplementum (72):51-5, 2007.
Abstract: Catecholaminergic neurons of the primate substantia nigra (SN) pars compacta (SNc) and the locus coeruleus contain neuromelanin (NM) granules as characteristic structures underlying the pigmentation of these brain areas. Due to a phylogenetic appearance NM granules are absent in the rodent brain, but gradually become present in primates until they reach a maximal expression in humans. Although a possible mechanism of pigment formation may be autoxidation of the NM precursors dopamine or noradrenalin, several groups have suggested an enzymatic formation of NM mediated by tyrosinase or a related enzyme. Since tyrosinase mRNA is suggested to be expressed in the SN of mice and humans, we reinvestigated the expression of tyrosinase in the human SNc and the locus coeruleus at the protein level by immunohistochemistry and Western blot analysis, but could not detect tyrosinase in these brain regions.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

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  Identification of a tyrosinase (TYR) exon 4 deletion in albino ferrets (Mustela putorius furo). Anim Genet 38:421-423, 2007

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  A {beta}-Defensin Mutation Causes Black Coat Color in Domestic Dogs. Science, 2007 (online).
  Abstract: Genetic analysis of mammalian color variation has provided fundamental insight into human biology and disease. In most vertebrates, two key genes, Agouti and Melanocortin 1 receptor (Mc1r), encode a ligand-receptor system that controls pigment type-switching, but in domestic dogs, a third gene is implicated, the K locus, whose genetic characteristics predict a new component of the melanocortin pathway. Here we identify the K locus as beta-defensin 103 (CBD103) and show that its protein product binds with high affinity to the Mc1r, and has a simple and strong effect on pigment type-switching in domestic dogs and transgenic mice. These results expand the functional role of beta-defensins, a protein family previously implicated in innate immunity, and identify an additional class of ligands for signaling through melanocortin receptors.

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  Beta-Catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. Genes Dev 21:2923-2935, 2007.
  Shortened abstract: We show here that, contrary to expectations, stabilized beta-catenin reduces the number of melanoblasts in vivo and immortalizes primary skin melanocytes by silencing the p16(Ink4a) promoter. Significantly, in a novel mouse model for melanoma, stabilized beta-catenin bypasses the requirement for p16(Ink4a) mutations and, together with an activated N-Ras oncogene, leads to melanoma with high penetrance and short latency. The results reveal that synergy between the Wnt and mitogen-activated protein (MAP) kinase pathways may represent an important mechanism underpinning the genesis of melanoma, a highly aggressive and increasingly common disease.

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S*ee also News and Views by Barsh GS. How the dog got its spots. Nat Genet 39, 1304-1306, 2007.*

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A Melanocortin 1 Receptor Allele Suggests Varying Pigmentation Among Neanderthals. Science, 2007. (online)
Abstract: The melanocortin 1 receptor (MC1R) regulates pigmentation in humans and other vertebrates. Variants of MC1R with reduced function are associated with pale skin color and red hair in humans primarily of European origin. We amplified and sequenced a fragment of the MC1R gene (mc1r) from two Neanderthal remains. Both specimens have a mutation not found in ~3,700 modern humans. Functional analyses show that this variant reduces MC1R activity to a level that alters hair and/or skin pigmentation in humans. The impaired activity of this variant suggests that Neanderthals varied in pigmentation levels, potentially to the scale observed in modern humans. Our data suggest that inactive MC1R variants evolved independently in both modern humans and Neanderthals.

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SOX9 is a key player in ultraviolet B-induced melanocyte differentiation and pigmentation. Proc Natl Acad Sci U S A 104:13984-13989, 2007.
Abstract: SOX (SRY type HMG box) proteins are transcription factors that are predominantly known for their roles during development. During melanocyte development from the neural crest, SOX10 regulates microphthalmia-associated transcription factor, which controls a set of genes critical for pigment cell development and pigmentation, including dopachrome tautomerase and tyrosinase. We report here that another SOX factor, SOX9, is expressed by melanocytes in neonatal and adult human skin and is up-regulated by UVB exposure. We demonstrate that this regulation is mediated by cAMP and protein kinase. We also show that agouti signal protein, a secreted factor known to decrease pigmentation, down-regulates SOX9 expression. In adult and neonatal melanocytes, SOX9 regulates microphthalmia-associated transcription factor, dopachrome tautomerase, and tyrosinase promoters, leading to an increase in the expression of these key melanogenic proteins and finally to a stimulation of pigmentation. SOX9 completes the complex and tightly regulated process leading to the production of melanin by acting at a very upstream level. This role of SOX9 in pigmentation emphasizes the poorly understood impact of SOX proteins in adult tissues.

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Summary: They generated two novel hypomorphic Sox10 alleles, and mutant mice either expressed a Sox10 protein with a triple alanine substitution in the dimerization domain, or a Sox10 protein with a deletion in the central portion that we define as a cell-specific transactivation domain. With regard to melanocytes, phenotypic analysis revealed important roles for a functional dimerization domain and the newly defined novel transactivation domain in melanocyte and enteric nervous system development, whereas early neural crest development and oligodendrocyte differentiation were surprisingly little disturbed in both mutants.

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Adaptive variation in beach mice produced by two interacting pigmentation genes. PLoS Biol 5:e219, 2007.
Abstract: Little is known about the genetic basis of ecologically important morphological variation such as the diverse color patterns of mammals. Here we identify genetic changes contributing to an adaptive difference in color pattern between two subspecies of oldfield mice (Peromyscus polionotus). One mainland subspecies has a cryptic dark brown dorsal coat, while a younger beach-dwelling subspecies has a lighter coat produced by natural selection for camouflage on pale coastal sand dunes. Using genome-wide linkage mapping, we identified three chromosomal regions (two of major and one of minor effect) associated with differences in pigmentation traits. Two candidate genes, the melanocortin-1 receptor (Mc1r) and its antagonist, the Agouti signaling protein (Agouti), map to independent regions that together are responsible for most of the difference in pigmentation between subspecies. A derived mutation in the coding region of Mc1r, rather than change in its expression level, contributes to light pigmentation. Conversely, beach mice have a derived increase in Agouti mRNA expression but no changes in protein sequence. These two genes also interact epistatically: the phenotypic effects of Mc1r are visible only in genetic backgrounds containing the derived Agouti allele. These results demonstrate that cryptic coloration can be based largely on a few interacting genes of major effect.

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Abstract: Mammals generate external coloration via dedicated pigment-producing cells but arrange pigment into patterns through mechanisms largely unknown. Here, using mice as models, we show that patterns ultimately emanate from dedicated pigment-receiving cells. These pigment recipients are epithelial cells that recruit melanocytes to their position in the skin and induce the transfer of melanin. We identify Foxn1 (a transcription factor) as an activator of this "pigment recipient phenotype" and Fgf2 (a growth factor and Foxn1 target) as a signal released by recipients. When Foxn1 - and thus dedicated recipients - are redistributed in the skin, new patterns of pigmentation develop, suggesting a mechanism for the evolution of coloration. We conclude that recipients provide a cutaneous template or blueprint that instructs melanocytes where to place pigment. As Foxn1 and Fgf2 also modulate epithelial growth and differentiation, the Foxn1 pathway should serve as a nexus coordinating cell division, differentiation, and pigmentation.
see also comment by Barsh G, Cotsarelis G. How hair gets its pigment. Cell 130, 779-781, 2007.

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Activating mutation in a mucolipin transient receptor potential channel leads to melanocyte loss in varitint waddler mice. Proc Natl Acad Sci U S A 104:18321-18326, 2007.
Abstract: Transient receptor potential (TRP) genes of the mucolipin subfamily (TRPML1-3 and MCOLN1-3) are presumed to encode ion channel proteins of intracellular endosomes and lysosomes. Mutations in human TRPML1 (mucolipin 1/MCOLN1) result in mucolipidosis type IV, a severe inherited neurodegenerative disease associated with defective lysosomal biogenesis and trafficking. A mutation in mouse TRPML3 (A419P; TRPML3(Va)) results in the varitint-waddler (Va) phenotype. Va mice are deaf, exhibit circling behavior due to vestibular defects, and have variegated/dilute coat color as a result of pigmentation defects. Prior electrophysiological studies of presumed TRPML plasma membrane channels are contradictory and inconsistent with known TRP channel properties. Here, we report that the Va mutation produces a gain-of-function that allows TRPML1 and TRPML3 to be measured and identified as inwardly rectifying, proton-impermeant, Ca(2+)-permeant cation channels. TRPML3 is highly expressed in normal melanocytes. Melanocyte markers are lost in the Va mouse, suggesting that their variegated and hypopigmented fur is caused by severe alteration of melanocyte function or cell death. TRPML3(Va) expression in melanocyte cell lines results in high resting Ca(2+) levels, rounded, poorly adherent cells, and loss of membrane integrity. We conclude that the Va phenotype is caused by mutation-induced TRPML3 gain-of-function, resulting in cell death.

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In Vitro Expansion of Immature Melanoblasts and their Ability to Repopulate Melanocyte Stem Cells in the Hair Follicle. J Invest Dermatol, 2007 (online).
Shortened abstract: By co-culturing highly purified melanoblasts (MBs) with XB2 keratinocytes, we describe an efficient culture method that allows the expansion of immature MBs in vitro. These MBs are also capable of undergoing terminal differentiation into mature melanocytes (MCs) when differentiation is induced. Furthermore, by performing a hair-follicle reconstitution assay in which expanded MBs in a mixture of epidermal and dermal cells were grafted to reconstitute a hair follicle, we demonstrate that the expanded MBs retain their capacity to become incorporated into newly developed hair follicles and repopulate the MC stem cell population there.

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7. Tyrosinase, TRPs, other enzymes

(Pr. J.C. Garcia-Borron)

This search reflects the ever increasing interest in the identification of effective tyrosinase inhibitors from natural sources. Indeed, more than 40% of the papers referenced deal with this matter. Moreover, I have not included several other papers because they use crude extracts from natural sources, rather than well defined chemically pure compounds, which complicates the interpretation of the results. It remains to be seen what portion of this potential depigmenting panoply actually makes it to the clinical/cosmetic world. Interestingly, only one paper reports on a compound that activates melanogenesis (rosmarinic acid, Lee J, Kim YS, Park D., Biochem Pharmacol. 2007 Oct 1;74(7):960-8), which suggests that for some reason nature is richer in tyrosinase inhibitors than in melanogenic stimulators.

An interesting report adds new data to the issue of tyrosinase expression in the nervous system. In this case, Tribl and co-workers describe the lack of expression of tyrosinase in catecholaminergic neurons (Tribl F, Arzberger T, Riederer P, Gerlach M., J Neural Transm Suppl. 2007;(72):51-5).

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8. Melanosomes

(Prof. J. Borovansky)

Melanosome transport was investigated in several studies: According to *Hume et al* melanophilin is targetted to and/or stabilized on melanosomes by Rab27a, and then recruits myosin Va, which provides additional stability to the complex and allows melanosomes to transfer from microtubule to actin-based transport and achieve peripheral distribution. Similarly, *Sheets et al* proposed that cAMP-induced melanosome dispersion in Zebrafish depended on the actin-independent suppression of dynein by melanophilin and that melanophilin coordinates the early outward movement of melanosomes along microtubules and their later transfer to actin filaments. Melanosome motility in cultured fish RPE cells , particularly the role of myosin II, was traced using blebbistatin (myosin II inhibitor) and H-1152 (rho kinase inhibitor) by *Barsoum & King-Smith*. Myosin II and Rho kinase proved to be required for melanosome aggregation but not dispersion. In cocultures of human primary melanocytes with light and dark keratinocytes, KGF (keratinocyte growth factor) promoted melanosome transfer more to light than to dark keratinocytes which can be explained by an increased expression of KGF receptor on the light cells (*Cardinali et al*).

As for the **melanosome biogenesis** (and transport) *Watabe et al* demonstrated that the movement of early melanosomes in perinuclear area depends primarily on microtubules but not on the actin filaments, in contrast to the trafficking of tyrosinase and Pmel17 which depends on cytoplasmic dynein and its interaction with the spectrin/ankyrin system. *Harper et al* obtained new data with important implications for the site and mechanism of melanosomal fibril formation: These data indicate that Pmel17 follows a single biosynthetic route from the endoplasmic reticulum through the Golgi complex and endosomes to melanosomes . Only fragments encompassing functional lumenal determinants are present within the fibrils. Comparative proteomic analysis of two murine melanocyte lines differing in the degree of their melanosome maturation revealed that calreticulin was an essential molecule for the processing of tyrosinase and hence for melanocyte differentiation (*Kawase et al*).

Melanosome more or less detailed **description** under various pathological situations covered this time: a)Uneven accumulation of large pigment granules mainly in the medullary area of the hair shafts of two boys with Griscelli syndrome (*Celik et al*); b) Atypical pulmonary carcinoid with melanin pigmentation. In between tumour cells, S100 and HMB45 positive pigment cells containing melanosomes were found (*Goel& Addis*). c) Primary small cell malignant melanoma of the rectum: Tumour cells were positive for S100 and HMB45 and contained sparse but unambiguous stage II melanosomes (*Wang et al*). d) Compared to heterozygous (Rab38(cht/)(+) and wild mice , Rab38(cht/cht) melanosomes were smaller and fewer in the RPE cells, whereas in choroid melanocytes they were only smaller in size (*Brooks et al*). Also *Lopes et al* declared that loss of functional Rab38 in the chocolate mice caused reduced melanosome numbers in the RPE and in choroid melanocytes, but in the latter continuing low level of melanosome genesis gradually overcame the loss.

Functional consequences of RPE **melanosome photobleaching**, believed to occur with aging *in vivo*, were characterized in two studies: Protein extracted from isolated porcine RPE melanosomes showed signs of markedly increased carbonylation, both of associated actin and of endogenous melanosomal protein(s), in relation to the extent of melanosome photobleaching (*Burke et al*). Photobleached isolated melanosomes phagocytized by ARPE-19 cells made them more sensitive to light induced cytotoxicity (*Zareba et al*).

Also two **reviews** , that have appeared recently, are really worth reading (Raposo& Marks, Schallreuter).

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9. Melanoma experimental, cell culture

(Dr. R. Morandini)

Current protocols for normal choroidal melanocytes culture involve the supplementation serum, toxins and phorbol ester. Valtink (2007) describes a new protocol without these supplements. Since phorbol esters are known to act as tumor-inducing factors, their use in normal melanocyte culture should be considered carefully, especially when the cultures are established for subsequent transplantation purposes or would be used in studies on the transformation of normal melanocytes to melanoma cells. In the same way, at least for transplantation purposes, a serum supplementation of culture media should also be avoided, since serum may be a source of pathogens. In this study, Valtink used Melanocyte Growth Medium M2 (a commercially available serum-free medium), which was designed to support cutaneous melanocyte survival and proliferation in vitro without further supplementation with tumor inducers or toxins. A high recovery of melanocytes was obtained by the use of a sequential digestion method, using collagenase first to isolate RPE cells, and, secondly, dispase to isolate melanocytes.
In contrast to cutaneous melanocytes, choroidal melanocytes do not synthesise melanin in vivo, but they can do so in vitro.
Liu (2007) established a new method for the reconstruction of a tissue-engineered skin containing melanocytes by employing tissue engineering. The keratinocytes, melanocytes and dermal fibroblasts were isolated and purified from human foreskin biopsies. Then the cells were used to construct a tissue-engineered skin containing melanocytes. The results showed that the melanocytes could be detected in the basal layer of the constructed skin and the melanocytes showed dendritic morphology. Moreover, the constructed skins were used to repair the athymic mice skin defects. The method uses a mixture of collagen and fibroblast placed onto a 75 mm nylon mesh. After 4 days, a mixture of keratinocytes and melanocytes at a ratio of 20/1 was added. The collagen gel containing cells was incubated for 3 additional days and then braught up onto the air-liquid interface. The collagen gel could thus be kept ready for use for more than a week.

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D. Other tools and cell culture

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ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2008 66th Annual Meeting AAD
February 1-5, San Antonio (TX), USA

2008 Dermatologia Globale
April 23-26, Genoa, Italy

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2008 20th International Pigment Cell Conference (IPCC)
conjoined with
Vth International Melanoma Research Congress
May 7–12 Sapporo, Japan

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2008 International Investigative Dermatology (Joint Meeting of the ESDR, SID and JSID)
May 14-17 , Kyoto, Japan

Contact: E-mail: office@esdr.org
Web site: www.esdr.ch

2008 9th Congress of the European Society for Pediatric Dermatology
May 15-17, Athens, Greece

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2008 5th EADV Spring Symposium
May 22-25, Istanbul, Turkey

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2008 17th Annual Congress of the European Academy of Dermatology Venereology

September 17-21, Paris, France

Contact: EADV PARIS 2008 CONGRESS OFFICE:
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2009 6th EADV Spring Symposium

April 23 – 26, Bucharest, Romania

2009 Annual Meeting for the Society for Investigative Dermatology

May 6-9, Montreal, Quebec, Canada

Contact: Web site: www.sidnet.org

2009 39th Annual ESDR Meeting

September 9-12, Budapest, Hungary

2009 XVth Meeting of the ESPCR

September 20-23, Münster, Germany

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2009 18th EADV Congress

October 7-11, Berlin, Germany

2010 40th Annual ESDR Meeting

September 8-11, Helsinski, Finland

NEW MEMBERS

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

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